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Research report

# The 6-OHDA mouse model of Parkinson's disease – Terminal striatal lesions provide a superior measure of neuronal loss and replacement than median forebrain bundle lesions

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## HIGHLIGHTS

- Generation of a reproducible mouse 6-hydroxydopamine lesion model.
- Discovery that terminal striatal lesions produce reproducible motor asymmetry when mice are challenged with D-amphetamine.
- Rotational deficits are directly correlated to the number of dopamine neurons lost.
- Transplants of immature dopamine neurons can effectively reverse rotational deficits in the terminal striatal lesion model.

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## ABSTRACT

Unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway produce side-biased motor impairments that reflect the motor deficits seen in Parkinson's disease (PD). This toxin-induced model in the rat has been used widely, to evaluate possible therapeutic strategies, but has not been well established in mice. With the advancements in mouse stem cell research we believe the requirement for a mouse model is essential for the therapeutic potential of these and other mouse-derived cells to be efficiently assessed.

This aim of this study focused on developing a mouse model of PD using the 129 P2/OLA Hsd mouse strain as this is widely used in the generation of mouse embryonic stem cells. Both unilateral 6-OHDA medial forebrain bundle (MFB) and striatal lesion protocols were compared, with mice analysed for appropriate drug-induced rotational bias. Results demonstrated that lesioned mice responded to D-amphetamine with peak rotation dose at 5 mg/kg and 10 mg/kg for MFB and striatal lesions respectively. Apomorphine stimulation produced no significant rotational responses, at any dose, in either the MFB or striatal 6-OHDA lesioned mice. Analysis of dopamine neuron loss revealed that the MFB lesion was unreliable with little correlation between dopamine neuron loss and rotational asymmetry. Striatal lesions however were more reliable, with a strong correlation between dopamine neuron loss and rotational asymmetry. Functional recovery of D-amphetamine-induced rotational bias was shown following transplantation of E13 mouse VM tissue into the lesioned striatum; confirming the validity of this mouse model.



## 1. Introduction

Parkinson's disease is a progressive neurological disorder that is characterised by a catalogue of movement impairments such as rigidity, tremor and bradykinesia [1,2]. The disease is primarily caused by the loss of the nigrostriatal dopamine pathway [1] and therefore highlights the importance of striatal dopamine on motor function.

Experimental evidence for the role of dopamine in the striatum, and its effects on motor function first came from dopaminergic

*Abbreviations:* MFB, medial forebrain bundle; PD, Parkinson's disease; VM, ventral mesencephalon.

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stimulation of the rat striatum, which resulted in marked changes in motor response [3]. The subsequent pioneering investigations on motor effects following unilateral dopamine depletion using 6-hydroxydopamine provided a unique way of analysing dopamine activity by measuring drug-induced motor function [4,5]. Under these conditions rats display rotational asymmetry with the degree of rotation being proportional to dopamine loss [6] and the direction of rotation being dependent on the dopamine agonist drug used [4,5].

Since its development, this behavioural model has been used extensively in PD research. It has provided a valuable tool to assess the potential of curative treatments by examining the attenuation of 6-OHDA-lesion-induced behavioural deficits. While most of these models have used rats, behavioural impairment has also been observed in dopamine-depleted mice [7–12]. With respect to rotational bias, the methods used to induce rotational behaviour in these studies vary considerably, with differences in lesion type, dopamine agonist drug, and drug doses used. For instance, the unilateral, 6-OHDA intrastriatal lesions used by Brundin et al. [7] produced low rotational asymmetry following 2.5 mg/kg amphetamine stimulation. In contrast, better rotations were reported by Barberi et al. [8] following striatal lesions and stimulation by 10 mg/kg amphetamine and apomorphine. While good rotations were observed by Barberi and colleagues, the drug doses used seem excessive, particularly with respect to apomorphine. This high-dose apomorphine-induced rotation suggests that no supersensitivity of post-synaptic dopamine receptors occurred, even though increased receptor binding has been previously reported following striatal 6-OHDA lesions in mice [13]. In fact, good rotational bias following 0.5 mg/kg apomorphine stimulation in striatal lesioned mice has been demonstrated, indicating that supersensitivity can be achieved and a good functioning mouse model can be produced [10]. In addition, over 95% of the mice in this study received reliable lesions unlike the study by Iancu et al. [9] which showed inconsistent lesions resulting in less than 50% of lesioned animals being used in behavioural tests. More recent studies have shown stable lesions following unilateral 6-OHDA administration which produce deficits in a variety of behavioural tasks [11,12]. However, whilst improvement is seen in the majority of these behavioural tests, reversal of the rotational bias following transplantation of E12.5 ventral mesencephalon tissue is not [14].

It is clear from these reports that a standard protocol for developing good rotational bias following the unilateral 6-OHDA lesions in mice, which can be reversed following replacement therapy, has yet to be established.

This study addressed this issue by evaluating the suitability of the 129 P2/OLA Hsd mouse strain to exhibit a rotational bias following 6-OHDA lesioning. Here we provide a comprehensive, detailed assessment of lesion type, drug, and drug dose required, to produce a standard, reproducible model where rotational bias reflects both dopamine loss and replacement in the 129 P2/OLA Hsd mouse strain.

## 2. Materials and methods

### 2.1. Subjects

In vivo studies were conducted in young adult female mice of the 129P2/OLA Hsd strain (Harlan Olac, Bicester, UK). Animals were housed in groups of 4–6 mice/cage on a natural 12 h:12 h light dark cycle and with ad libitum access to food and water throughout. Foetal tissue for cell culture or grafts was derived from E13 foetuses (crown-rump length = 11 mm) obtained from pregnant female mice of the same strain. All studies were conducted in accordance with full ethical appraisal and licences under the UK Animals (Scientific Procedures) Act 1986.

### 2.2. Surgical procedures

#### 2.2.1. 6-Hydroxydopamine (6-OHDA) lesions

Adult female 129P2/OLA Hsd mice (~25–30 g), were anaesthetised using gaseous isoflurane (2–5% in 2:1 $O_2$:$N_2$), and received unilateral stereotaxic injections of 1 microlitre of 4 g/l of 6-hydroxydopamine hydrobromide (6-OHDA, Sigma) dissolved in physiological saline containing 0.01% ascorbic acid. Infusions were delivered over 1 min via a 30 gauge stainless steel cannula. Lesions were placed in either the right medial forebrain bundle ($A$ = −2.0 mm anterior to bregma, $L$ = −0.7 mm lateral to bregma, $V$ = –4.8 mm ventral to dura; with the incisor bar set at 0.0 mm relative to the interaural line) or the right mid-striatum ($A$ = 0.4 mm, $L$ = −1.8 mm, $V$ = −3.5; with the incisor bar set at 0.0 mm). The cannula was left in place for a further 2 min for diffusion, before being slowly removed and the wound cleaned and sutured.

After lesioning, mice were sutured and injected subcutaneously with 0.5 ml 0.9% saline/glucose solution to prevent dehydration. The drinking water was supplemented with paracetamol for the following 48 h and mice were carefully monitored post-surgery. All mice were allowed to recover for at least 10 days before behavioural testing commenced.

#### 2.2.2. VM tissue transplantation

VM tissue was dissected from E13 mouse embryos and a single cell suspension was prepared as previously described [15]. Briefly, pregnant donors were killed by decapitation under general anaesthesia, and the foetuses removed by caesarian section. The ventral mesencephalon was dissected and pooled from all donors in a litter. Dissected tissue was enzymatically digested using a solution of 0.1% trypsin (Worthington, Lakewood, USA) and 0.05% DNase (Sigma, Poole, Dorset), at 37 °C for 20 min. After two rinses in Hank's balanced salt solution (HBSS) the tissue was dissociated into a single suspension by mechanical trituration using a 200 microlitre Gilson pipette. Cell counts and percentage viable cells were assessed by trypan blue exclusion in a haemocytometer.

Tissue was grafted into the right neostriatum by stereotaxic injection of 500,000 cells in 4 microlitres at $A$ = 0.8 mm, $L$ = −1.8 mm, $V$ = −3.0 mm and −2.5 mm (2 microlitres at each depth) using a 10 microlitre Hamilton microsyringe over 4 min. A further 4 min was allowed for diffusion prior to syringe removal, and the wound was then cleaned and sutured. All animals were allowed to recover for at least 10 days before behavioural testing commenced.

### 2.3. Behavioural testing

#### 2.3.1. Amphetamine and apomorphine-induced rotations

Mice were placed in cylinders with a diameter of 11.5 cm and height of 14 cm, in a closed room to avoid any environmental disturbance, and allowed to habituate for 10 min before injection with a specific dose of drug. D-Amphetamine (Sigma) was delivered intraperitoneally, while apomorphine hydrochloride (Sigma) was delivered subcutaneously to the scruff of the neck.

Mice were monitored for either 90 min (D-amphetamine-induced rotations) or 48 min (apomorphine-induced rotations), due to the difference in the metabolism of the two drugs. Each subject was scored for full body rotations in 1-min intervals, every 6 min, and the net ipsilateral rotation (total right – total left 360° turns) per minute was used as the primary dependent variable. Mice were given at least 7 days to recover following each amphetamine dose and at least 4 days following each apomorphine dose to ensure that the drugs had been eliminated from the system prior to behavioural testing.