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Research report

Differential pattern of motor impairments in neurotoxic, environmental and inflammation-driven rat models of Parkinson's disease

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HIGHLIGHTS

- We compare neurotoxic, environmental and inflammatory Parkinson's models.
- These differentially impair the pattern, extent and stability of motor dysfunction.
- Models should be selected to maximise relevance of Parkinson's disease studies.

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ABSTRACT

One of the reasons proposed for the paucity of drug discovery for Parkinson's disease is the lack of relevant animal models of the condition. Parkinson's disease has been modelled extensively using the selective neurotoxin, 6-hydroxydopamine (6-OHDA). However, as this model bears little etiological resemblance to the human condition, there has been a drive to develop models with improved etiological validity. Two such models are those induced by the pesticide, rotenone, and the inflammagen, lipopolysaccharide (LPS). However, to date, these models have been poorly characterised in terms of their motor profiles and have never been directly compared to the more established models. Thus, the aim of this study was to characterise the behavioural profile of the rotenone and LPS models, and to compare them with the 6-OHDA model. Animals underwent baseline testing on the Stepping, Whisker, Corridor and Cylinder Tests of motor function. They were then grouped for unilateral intra-striatal infusion of 6-OHDA, rotenone or LPS. Motor testing continued for ten weeks after which the rats were processed for immunohistochemical analysis of nigrostriatal integrity. We found that, although all neurotoxins induced a similar level of nigrostriatal neurodegeneration, neither the rotenone nor LPS models were associated with amphetamine-induced rotation, and they were associated with significantly less pronounced and stable impairments in the spontaneous tasks than the 6-OHDA model. In conclusion, this study demonstrates key differences in the pattern of motor dysfunction induced by Parkinsonian neurotoxins which should be taken into consideration when selecting the most appropriate model for Parkinson's disease preclinical studies.

1. Introduction

Parkinson's disease is a progressive, neurodegenerative disorder characterised by the chronic and advancing loss of dopaminergic neurons from the substantia nigra pars compacta [8]. The cardinal features of the disease are a resting tremor, bradykinesia, rigidity and postural instability [33]. Despite its discovery over 50 years ago, levodopa remains the most effective treatment for the motor symptoms associated with Parkinson's disease. One factor that has been proposed to underlie the paucity of novel therapies for this condition is the lack of relevant animal models which recapitulate the human disease [22,40]. 6-Hydroxydopamine (6-OHDA) is used to induce the 'gold standard' model of Parkinson's disease

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which was first described by Ungerstedt in 1968 [36]. This model has been used extensively as it induces a rapid and pronounced dopaminergic neurodegeneration with the associated motor dysfunction [6]. However, one of the limitations of the 6-OHDA model is its poor etiological relevance to human Parkinson's disease which is thought to result from interplay between genetic and environmental factors. Therefore, there has been a drive to develop models with improved etiological validity for the human condition. Two of the main environmental factors associated with Parkinson's disease are exposure to agricultural pesticides [18,35] and infectious agents [9,15,38,5]. Both of these are now underpinning the drive to develop more etiologically relevant models of Parkinson's disease.

Rotenone is an organic pesticide, extracted from the roots of tropical plants belonging to the genera *Lonchocarpus* and *Derris*, that has been causatively linked to Parkinson's disease [35]. It is highly lipid soluble and freely crosses cellular membranes where it causes dopaminergic cell death through oxidative stress caused by inhibition of complex I of the mitochondrial electron transport chain [32,21]. Infection and the resulting inflammatory response (modelled in animals using the bacterial endotoxin, lipopolysaccharide (LPS)) also cause dopaminergic cell death through oxidative stress albeit indirectly through the production of pro-inflammatory cytokines and reactive oxygen species from immune cells such as microglia (reviewed in [23]). Both rotenone [4,1,34] and LPS [28] can be administered systemically to rodents to induce Parkinsonism. However, systemic rotenone is associated with significant systemic organ toxicity [10,20], body weight loss [2,13], and mortality rates of up to 50% [10,3], while systemic LPS has also been associated with 'sickness behaviour' [16]. Therefore, for these reasons, models have also been developed in which rotenone [26,25] or LPS [17] are injected directly into the rat nigrostriatal pathway.

Since these emerging intracerebral rotenone and LPS models have different inductive mechanisms compared to the 6-OHDA model, and this may have consequences for motor function, it is important to compare these to this gold standard model that has underpinned preclinical drug testing for several decades. Thus, the aim of this study was to characterise the behavioural profile of the rotenone and LPS models, and to directly compare them with the commonly-used 6-OHDA model.

2. Methods

2.1. Animals

All procedures were approved by the Animal Care and Research Ethics Committee of the National University of Ireland, Galway, were completed under licence by the Irish Department of Health and Children and the Irish Health Products Regulatory Authority, and were carried out in accordance with European Union Directive 2010/63/EU and S.I. No. 543 of 2012. Animals were housed in groups of four per cage, on a 12:12 h light/dark cycle, at 19–23 °C, and at humidity levels maintained between 40 and 70%. Throughout the experiment, rats were allowed water *ad libitum* and were fed 15–20 g of standard rat chow each per day to maintain their body weight at ~90% of free feeding weight (to ensure they were motivated to perform the food-motivated Corridor Test). All behavioural testing and quantitative immunohistochemistry was completed blind to the treatment of the rats.

2.2. Experimental design

Twenty-four young adult male Sprague Dawley rats were used in this experiment (weighing 250 ± 10 g at start of baseline testing). All rats underwent habituation to the Corridor, Stepping and Whisker tests. They were then performance-matched to receive unilateral, 4 site, intra-striatal infusion of 6-OHDA (4 × 7 μg; $n = 8$), LPS (4 × 5 μg; $n = 8$) or rotenone (4 × 0.9 μg; $n = 8$). Doses were chosen to induce a similar level of nigrostriatal neurodegeneration based on our previous studies [26,39,17] and unpublished work). Behavioural testing resumed the day after lesion surgery and continued for 10 weeks during which time amphetamine-induced rotation was also assessed (at 3 weeks post-lesion). The animals were then sacrificed *via* transcardial perfusion-fixation and their brains were used for *post mortem* assessment of nigrostriatal neurodegeneration *via* quantitative tyrosine hydroxylase immunohistochemistry.

2.3. Surgery

Unilateral 6-OHDA, LPS or rotenone infusion was conducted under isofluorane anaesthesia (5% in O_2 for induction and 2% for maintenance) in a stereotaxic frame with the nose bar set at −2.3 mm. The striatum was lesioned by infusion at 4 points along its rostro–caudal axis at the following stereotaxic coordinates (in mm): AP + 1.3, ML ± 2.7; AP + 0.4, ML ± 3.1; AP − 0.4, ML ± 4.3; AP − 1.3, ML ± 4.7 (from bregma) and DV − 5.0 below dura. All infusions were completed in a total volume of 3 μl at a rate of 1 μl/min with a further 2 min allowed for infusion.

2.4. Behavioural testing

The Stepping Test of forelimb akinesia was completed as previously described [27]. Briefly, the rat was held with both hindlimbs and one forelimb restrained. The body of the rat was held in a horizontal position with the unrestrained forelimb on the table top. The rat was then guided across the table top at a steady pace (90 cm in 5 s) and the number of adjusting steps made by the free forelimb in the forehand and backhand directions was counted. This was completed on both the ipsilateral and contralateral sides of the rat's body. The Whisker Test of sensorimotor integration was completed as described previously [31]. Briefly, the rat was held with both hind limbs and one forelimb restrained. The number of vibrissae-elicited forelimb placings made by the unrestrained forelimb was counted when the rat's whiskers were brushed against the side of a table top 10 times. This was completed on both the ipsilateral and contralateral sides of the rat's body. The Corridor Test of contralateral neglect was completed as previously described [7,11]. Briefly, the rat was placed into a long corridor and was allowed to freely retrieve CocoPops® from pots plated at intervals on the left and the right-hand sides of the corridor. Trials were deemed completed once the animal made a total of 20 retrievals or after the trial time of 5 min had elapsed. The numbers of retrievals made from both ipsilateral and contralateral sides of the rat's body were counted. The Cylinder Test of forelimb use asymmetry was used as previously described [31]. Briefly, the rat was placed into a clear cylinder for 5 min and the number of times it touched the side walls with the contralateral paw was expressed as a percentage of the total touches made. Rotational behaviour induced by a single injection of D-amphetamine (2.5 mg/kg i.p.) was assessed at 3 weeks post-surgery. Rotation was expressed as net ipsilateral turns/min over 60 min post-injection.

2.5. Immunohistochemistry

On Day 70, 10 weeks post neurotoxin injection, rats were sacrificed by terminal anaesthesia (50 mg/kg i.p.) and transcardially perfused with 100 ml of heparinised saline followed by 150 ml of 4% paraformaldehyde overnight. Extracted brains were then post-fixed in 4% paraformaldehyde overnight and cryoprotected in 25% sucrose plus 0.1% sodium azide solution. Serial coronal sections (30 μm) were cut using a freezing stage sledge microtome, and a