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Research report

Three-dimensional motion analysis of postural adjustments during over-ground locomotion in a rat model of Parkinson's disease

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ABSTRACT

Postural instability, a symptom of Parkinson's disease (PD) patients, leads to frequent falls and difficulty in forward motion during gait. These motor deficits are mainly caused by neurodegenerative processes in the brain leading to reduced levels of the neurotransmitter dopamine. Postural studies involving animal models of PD are mainly based on movement scores or descriptive approaches to discerning differences in behaviour or function. Our aim was to describe postural adjustments in a rat model of PD utilising a quantitative three dimensional motion analysis technique during gait to investigate the effects of unilateral dopamine depletion on rat locomotion while walking on beams of varying widths (wide, narrow and graduated). Tail orientation, limb positions on the beam, range of motion and kinematic waveforms of the *Roll*, *Pitch* and *Yaw* of male Lister Hooded rats were investigated using passive markers placed in locations that were representative of their body axis. Hemiparkinsonian rats moved on the wide beam with a significantly higher *Roll* range of motion coupled with a positively biased *Roll* kinematic waveform during one gait cycle. While walking on the narrow beam they displayed an increased use of the ledge and placed their tail towards the right. These results are brought about by the rats' inability to shift body posture using the impaired limb. Our data demonstrate that marker-based motion capture can provide an effective and simple approach to quantifying postural adjustments for rat models of PD.

1. Introduction

Understanding the functional motor adaptations that occur in Parkinson's disease (PD) is imperative to develop new therapeutic strategies [3,4,6,12,14]. Therefore, the development of new protocols refining the examination of motor deficits, e.g. postural instability, has become an important feature in neurodegenerative disease research.

Postural instability, defined as the inability to maintain body balance in a global reference system relative to the gravitational vector [26] is a common symptom of PD and contributes to an impaired forward motion and to the risk of frequent falls [10,18,22,23,27]. Maintaining the centre of gravity (COG) within the walking base (which is defined as the stride width) becomes more and more difficult for patients during the course of the disease [1,7]. To overcome those symptoms, PD patients exhibit compensatory movement patterns and strategies that develop in an attempt to improve stability [27]. Simple but biased methods for detecting postural instability coupled with clinical rating scales are typically included in standard clinical examination [20], e.g. in the Unified PD Rating Scale (UPDRS) where movement is described subjectively, rated by a neurologist from normal to severe and summed up to quantify the extent of the disease.

To investigate the clinical condition of PD, several animal models have been developed. The most commonly used rat model of PD is the unilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB; [21]). This injection exclusively targets the monoamine neurons within the MFB and causes selective loss of nigrostriatal dopamine (DA) neurons. The depletion of DA from striatal targets results in motor deficits that are similar to those in human PD [3,8,9,19,25]. The unilaterally lesioned (hemiparkinsonian) rats show motor deficits on the side contralateral to the lesion and the ipsilateral side serves as an internal control. Body weight bearing and body posture are compensated with the healthy ipsilateral side [16].

Previous studies of hemiparkinsonian rats have looked at motor impairments and their manifestations during walking and reaching by measuring body orientation and postural adjustments [5,13,16,17]. These studies have used two-dimensional (2D) video analysis to observe and evaluate movement patterns and force plates to measure COG displacements. Data were analysed based on a movement score or a descriptive approach to discerning

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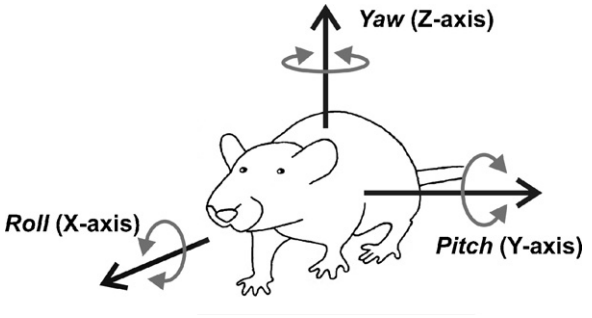

Fig. 1. Illustration of a rat showing the body axes representing body rotations in the three Cartesian axes in a global reference system relative to the gravitational vector: *X*-axis known as '*Roll*'; *Y*-axis known as '*Pitch*' and the *Z*-axis known as '*Yaw*'.

differences in behaviour or function [16]. None of the previous studies have investigated postural instability of hemiparkinsonian rats by means of three-dimensional (3D) motion analysis quantifying angular measurements (i.e. *Roll*, *Pitch* and *Yaw*) of the trunk during gait.

We previously developed a simple protocol to investigate gait patterns of hemiparkinsonian rats using marker-based optoelectronic motion analysis. In the present study, the same protocol was used, not to identify gait impairments, but to quantify and define normal posture and postural instability/adjustments in rats during locomotion along three different elevated beams (wide, narrow, and graduated). We investigated postural patterns in two groups of rats: hemiparkinsonian and healthy control rats. Body posture was analysed by measuring rotations in the three Cartesian axes: *X*-axis known as '*Roll*'; *Y*-axis known as '*Pitch*' and the *Z*-axis known as '*Yaw*' (see Fig. 1 and Table 1). Our study demonstrates that a novel application of marker-based optoelectronic motion capture can provide an effective and simple approach to quantifying postural instability in a rat model of PD and allows objective and unbiased calculation of body posture as the rats walk along the three different beams managing different levels of complexity.

2. Methods

2.1. Animals

Ten adult male Lister Hooded rats were used in this experiment (Charles River, UK). These were the same rats as used for a previously published study [14]. Rats were housed in standard cages in groups of five animals in a temperature-controlled environment (23.0 ± 0.3 °C) on a 14 h light:10 h dark schedule. Food and water was provided *ad libitum*. The animals were divided into two cohorts: five rats with the unilateral nigrostriatal dopamine depletion (PNL) and five non-operated control rats (CNL). All PNL rats were anesthetized with isoflurane (Abbott, Queensborough, UK) and were stereotactically injected with 6-OHDA (3 μg/μl in 0.2 mg/ml ascorbic acid in 0.9% sterile saline; Sigma, Poole, UK) into the right MFB using a 30-gauge cannula [21]. Lesion coordinates were set according to bregma and dura in mm [11]: tooth bar – 2.3, anterior/posterior – 4.4, lateral – 1.0, dorso-ventral – 7.8. Injection volume was 3 μl and the injection rate was 1 μl/min. The cannula was left in place for 3 min before withdrawal, cleaning and suturing of the wound. All procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986.

Table 1
Definitions of *Roll*, *Pitch* and *Yaw*.

Roll	Rotation round an axis parallel to the beam, *x*-axis On the plane perpendicular to the beam, *z*–*y* plane Body rotation from left to right from a frontal view
Pitch	Rotation round an axis perpendicular to the beam, *y*-axis On the plane perpendicular to the beam, *z*–*x* plane Body rotation up and down the beam
Yaw	Rotation round an axis perpendicular to the beam, *z*-axis On the plane parallel to the beam, *x*–*y* plane Body rotation from left to right from an aerial view

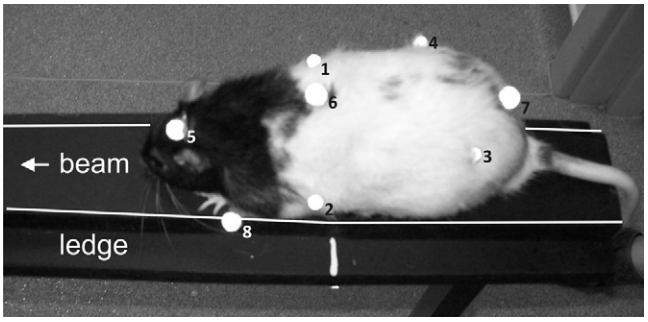

Fig. 2. Photograph of a rat with markers attached and ready to walk on the GR beam. Markers are located on the most lateral aspects of the appendicular skeleton (markers 1–4) and along the mid-spine (markers 5–7). Markers 1 and 2 are representative of the shoulder girdle and markers 3 and 4 are representative of the pelvis girdle. The left front ankle marker is also visible (marker 8; not used in this analysis).

2.2. Experimental design

Six weeks after the surgery, all animals were habituated to walk along three elevated beams. Marker based 3D digital video recordings were captured as described in detail by Madete et al. [14], and using the same rats. In brief, 3D Cartesian data of markers attached to the rat were acquired while they were walking along a wide (WD), a narrow (NR) and a graduated (GR) beam, as shown in Figs. 2 and 3, using an optoelectronic camera system (Qualisys, Sweden) and Qualisys Track Manager software (QTM, Qualisys, Sweden). The beams were equally divided into three sections called '*zones 1–3*'. Postural behaviour on the WD and NR beam is analysed for the middle zone (zone 2), whereas data from the GR beam are displayed for all three zones to acknowledge the decreasing width of the beam and the increasing difficulty of the task. Data collection for each beam was stopped when a minimum of three crossings with continuous walking was obtained. Analysis involved two trials from each animal that included at least five gait cycles. Trials in which the animal stopped before reaching the resting box were excluded from the analysis.

2.3. Measuring posture

Postural instability was quantified by calculating the rat's body displacement and orientation using Euler angles in a global reference system (GRS). A local coordinate system (LCS) was established using 3D data from 10 mm diameter, spherical and retro-reflective markers placed on appendicular parts of the rats' skeletal structure. Markers were attached to the trunk using double sided adhesive tape, and to

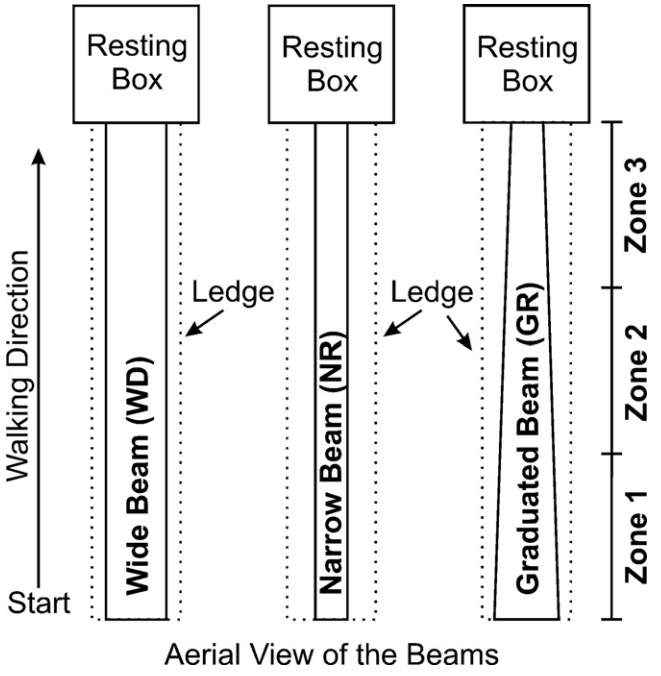

Fig. 3. Schematic view of the three different beams (wide = WD, narrow = NR and graduated = GR) used in this study. Dimensions and further details see Madete et al. [14].