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Challenges facing quantification of rat locomotion along beams of varying widths

J K Madete[1*], **A Klein**[2], **A Fuller**[2], **R C Trueman**[2], **A E Rosser**[2,3], **S B Dunnett**[2], and **C A Holt**[1]
[1]School of Engineering, Cardiff University, Cardiff, UK
[2]Brain Repair Group, School of Biosciences, Cardiff University, Cardiff, UK
[3]School of Medicine, Cardiff University, Cardiff, UK

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Abstract: Optoelectronic motion capture systems have been widely used to investigate temporal gait parameters in humans and animals in order to understand function and behavioural attributes of different pathologies, e.g. Parkinson's disease (PD). The aim of the present paper was to investigate the practicality of utilising this system to investigate the effects of a unilateral 6-hydroxydopamine (6-OHDA) lesion on rat locomotion while walking on beams of varying widths (graduated, narrow, and wide). Temporal gait parameters of ten male Lister Hooded rats (five controls and five hemiparkinsonian) were observed using passive markers placed in locations that were representative of their four limbs and their body axis. The results demonstrate that marker-based motion capture can provide an effective and simple approach to quantifying temporal gait parameters for rat models of PD. They also reveal how the width of the path affects the locomotion in both experimental cohorts. Such measurements can be compared with human motion analysis to explore correlations between the animal model and human behaviour, which is an important step for translational medicine.

Keywords: Parkinson's disease, motion capture, gait analysis, 6-OHDA, motor behaviour

1 INTRODUCTION

Animal models of neurodegenerative diseases have been studied extensively to understand the underlying physiological features of the pathology and offer potential therapeutic solutions [**1**–**3**]. In order to develop and test new therapies, it is imperative to understand the functional adaptations of motor behaviour that occur in these diseases. Animal studies allow interpretation of the neurodegenerative effects of the condition and also help to assess the efficacy of rehabilitation and clinical intervention.

Animal gait studies are well established using different apparatus where data are acquired in either two-dimensional (2D; [**4**, **5**]) or three-dimensional (3D; [**6**]) space. As apparatus vary depending on the condition and behaviour being investigated, there is a need for a simple and practical 3D motion analysis protocol that can be modified according to the experimental conditions: e.g. Canu and Garnier (2009) studied the effects of unloading the hindlimb of rats by investigating gait on a runway and a ladder confined within Plexiglas walls [**6**], whereas Allbutt and Henderson (2007; [**7**]) investigated the effect of postural rotational asymmetry and motor dysfunction on a model of Parkinson's disease (PD) as they walked along an elevated narrow beam. Different apparatus may also enable the investigator to control the environment, for instance varying the walking speed by use of a treadmill [**8**–**10**] or the use of an enclosed space to control forward motion and avoid turning of the animal [**11**]. All these methods are devised to bring out behavioural features of neurodegenerative diseases affecting motor performance and to further understand brain pathologies.

Data acquisition techniques vary depending on the output and parameters under investigation. Both 3D and 2D methods of analysis have advantages and limitations depending on the type of application and

*Corresponding author: Research Office, Cardiff University, Queens Building, Cardiff CF24 3AA, UK.
email: Madetejk1@cf.ac.uk*

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required results. 2D analysis is the most common for rat locomotion studies because it does not necessarily need a camera at all, it is inexpensive, and it produces straightforward data that are relatively easy to analyse. An example of 2D motion capture is footprint analysis where ink is painted on the paws of the animal as it walks along a marked path [**12**–**14**]. The advantages of this approach are that a clear foot contact is visible, motion from all limbs can be analysed, and the results are reproducible. However, faint footprints of un-weighted limbs and smearing caused by toe drag are inevitable [**15**]. Recently, specially designed equipment to record animal locomotion has been employed, e.g. a novel pressure mat system used by Boyd *et al.* to monitor deviations in gait of rat models [**16**]. Another 2D method is the CatWalk method which has been found to improve paw contact analysis [**17**–**20**]. Nevertheless, recording is restricted to a chamber thus limiting versatility of the environment, e.g. motion analysis cannot be performed on a ladder or on a beam. The varying approaches to acquisition of animal gait patterns are still challenging and it is difficult effectively to reproduce the same environment or conditions when analysing human gait for comparative studies.

Three-dimensional kinematic techniques have been introduced to describe locomotion of animals in all three planes simultaneously. Data acquisition can include the use of high-speed video cameras [**21**, **22**] and attaching electromagnetic electrode systems to record muscle activation [**23**]. Motion analysis techniques provide powerful tools for objective analysis and classification of the effects of diseases such as arthritis and cerebral palsy in human patients [**24**–**27**]. The application of such techniques to enhance animal studies of motor function would provide a step change in the ability to quantify the effects of neurodegenerative disorders, allowing for greater scope in determining gait and postural parameters in terms of the amount and quality of the data recorded.

Marker-based analysis of animal locomotion is not widely used. This is because of the need to shave the animals to allow precise and accurate marker placement and the unavoidable artefact of large skin movement that may affect the results. However, previous studies have successfully used this method to investigated temporal and spatial parameters during gait as well as angular displacement of joints during locomotion in different apparatus [**1**, **6**, **8**, **28**, **29**]. Couto *et al.* (2008) introduced 3D marker-based motion analysis on a treadmill for spinal cord injured rat models where they shaved the rats and investigated motion of the fore and hind limbs [**29**]. Comparing the data of 2D and 3D analyses, Couto and colleagues concluded that 2D methods are acceptable and successful. Nevertheless, 3D analysis produces additional important variables, essential in determining foot progression of animals with spinal injury.

Canu *et al.* (2009) used a marker-based system to compare motion on a runway and on a ladder to investigate the support ability of the hindlimb of unloaded rats and their controls as they walked along the two apparatus [**6**]. In addition to temporal gait parameters they were able to calculate angular joint displacements, thus providing a more quantitative output. These studies demonstrated that marker-based 3D analysis is essential for quantifying temporal gait parameters, gait asymmetry, angular joint displacements, and it can be effectively used to determine features of instability and postural control in different environments.

Marker-based motion analysis has thus been used previously in animal gait studies; however, its application to compare the effect in unilateral nigrostriatally lesioned rats on locomotion along the graduated, narrow and wide beam has not been performed. The current study introduces a simple, quick and practical approach to marker placement where the animals do not need anaesthesia or have to be shaved. Useful movement data can be acquired for all limbs simultaneously together with video footage for subjective observation.

Gait disorders are a cardinal symptom in patients suffering from PD leaving them unable to adjust their walking patterns according to the demand of the situation with ease [**30**]. Patients with PD have reduced arm swing, shuffling of gait, reduced walking speed, shorter stride length, and an abnormal cadence [**30**, **31**]. This leads to increased gait variability and decreased executive function which may lead to falls and the condition known as 'freezing of gait' [**32**, **33**]. Therefore, PD patients walk with a more cautious gait exhibiting compensatory behaviour in an attempt to improve their stability [**34**]. Animal models of PD have been reported to exhibit characteristics similar to those found in patients, e.g. shorter steps and reduced toe clearance which reflects shuffling of gait in humans [**14**, **35**].

Introducing an element of dual tasking to human locomotion studies results in a detrimental effect on gait in terms of freezing, variability of gait and body asymmetry [**32**]. This phenomenon is difficult to replicate in animal models. Examples of human

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studies include walking while reciting words [**33**] or listening to concurrent music [**36**] at a self-selected pace. As these tasks can obviously not be applied to rats, the current study introduces this effect to the animal study by using beams of varying widths: the animals must adapt their gait to cope with (a) a narrow path and (b) a gradually narrowing path. The results were then compared with walking along a wide (control) beam which only directs their forward motion and allows them to walk freely otherwise. Consequently, it is hypothesised that marker-based optoelectronic motion analysis can be applied in a practical, non-invasive and simple way to determine the temporal and postural parameters of animal locomotion and that the output from such studies can be used to explore and quantify the observed gait differences for animal models of PD as compared with controls. Furthermore, beams of varying widths can be used to explore the effects of dual tasking for animal models of PD and for controls to quantify gait variability, asymmetry, and compensatory adaptations.

A novel protocol was developed to investigate animal locomotion of healthy control rats and of rats that have undergone a unilateral lesion of the right nigrostriatal medial forebrain bundle [**37**–**39**]. Motion capture took place while the rats were walking over-ground along a narrow, graduated, and wide beam recording the parameters swing time, stance time, stride length, speed, and cadence. The present study can be used for translational correlations comparing animal and human gait deficits in various pathologies and will also help to develop new protocols analysing rat motor function such as skilled forelimb movements (e.g. reaching-for-food movements).

2 MATERIALS AND METHODS

All procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986. Temporal parameters for ten male Lister Hooded rats were recorded as they walked across three differently shaped beams labelled graduated (GR), narrow (NR), and wide (WD). The animals were divided into two cohorts: five rats with a unilateral nigrostriatal lesion (PNL) and five control rats (CNL). The base of the beam had the same widths in all three settings. The ledges varied as follows: GR beam: see dimensions in Fig. 1; NR beam: 0.015 m × 1.65 m × 0.02 m; WD beam: 0.06 m × 1.65 m × 0.02 m. Both cohorts of animals were trained on the beams before the trial in order to habituate them to the environment.

All PNL rats were anesthetized with isoflurane (Abbott, Queensborough, UK) and were stereotactically injected with 6-hydroxy dopamine hydrobromide (3 μg/μl in 0.2 mg/ml ascorbic acid in 0.9 per cent sterile saline; Sigma, Poole, UK) into the right medial forebrain bundle using a 30-gauge cannula [**40**]. Lesion coordinates were set according to bregma and dura in mm [**39**]: 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.

Movement was measured and tracked into 3D using an optoelectronic camera system from Qualisis (Sweden) and Qualisys Track Manager software (Qualisys, Sweden). Adhesive spherical retro-reflective markers (2.5 mm in diameter) were used to identify positions of interest, with markers posi-

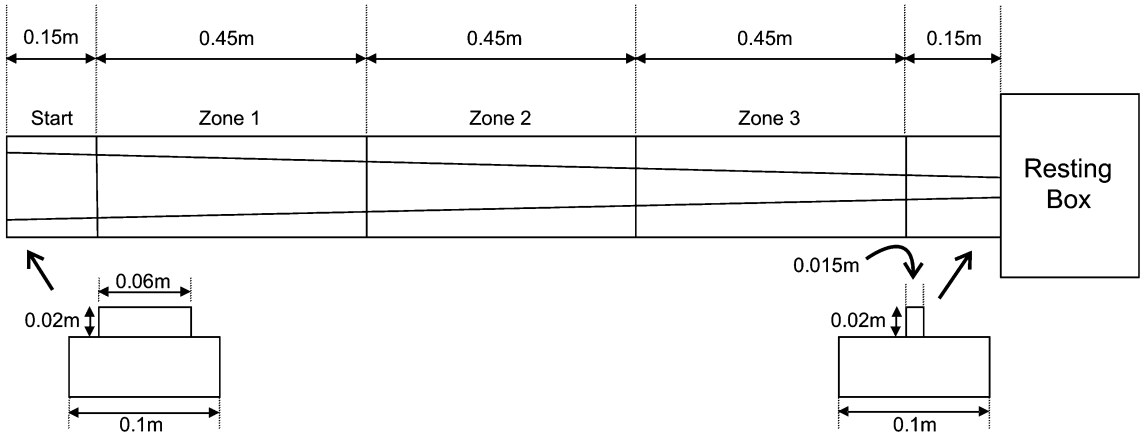

Fig. 1 Plan view of the GR beam showing the narrowing of the beam towards the resting box and the three zones used to calculate zonal speed

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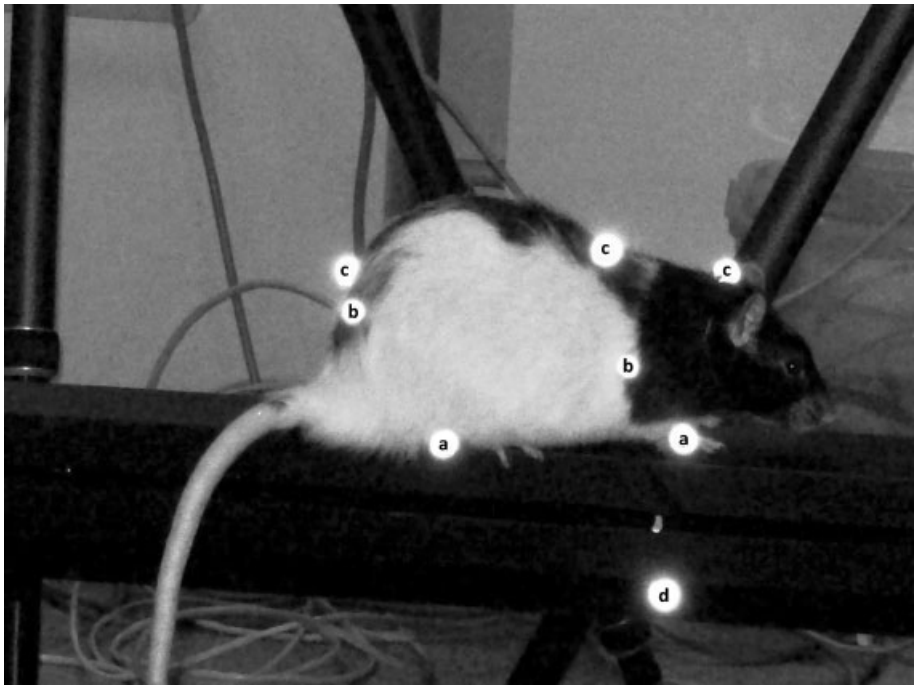

Fig. 2 Marker placement on the animal and the beam: (a) ankle markers, (b) appendicular skeletal markers, (c) spine and head markers, and (d) marker along the beam

tioned on the rats as shown in Fig. 2. Locations, which were representative of limb motion and allowed analysing the temporal and distance parameters, were chosen as follows: (a) above the ankles using cable ties since placing markers directly on the paws presented difficulties owing to the thick fur and skin movements as the animal was walking; (b) appendicular aspects of the rat skeletal structure; (c) along the mid-spine: and (d) along the length of the beam to identify relative motion of the rat and to illustrate the effect of varying beam width on animal motion. The method adopted was not to accurately determine joint rotation as animals would have required anaesthesia and shaving. The newly developed protocol is regarded to be a more practical and non-invasive approach determining quantitative and representative gait parameters based on observational studies using video [**41**].

Seven cameras (Qualisis, Sweden) were calibrated to cover the whole length of the 1.65 m beam and the surrounding areas. This enabled recording of the marker positions at a sample rate of 60 Hz, with the direction of the *X*, *Y*, and *Z* axes as shown in Fig. 3. The camera set-up allowed the collection of eight to ten gait cycles for each limb.

Animals were trained to walk along the beams starting with the GR beam where each animal was placed on the widest end, and subsequently walked along as the width gradually decreased into the resting box. The GR beam was then replaced with a NR beam using the same set-up. This procedure was then repeated for the WD beam as a control. 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. This provided the minimum number of steps that must be recorded to eliminate deviant kinematic curves [**42**]. Trials in which the animal stopped along the beam at some point before it reached the resting box were excluded from the results. Temporal gait parameters considered in this trial were swing time, stance time, stride length, speed, and cadence for each limb, with the definitions illustrated in Table 1.

The data were expressed as a mean $\pm$ standard deviation for each variable. A paired *t*-test ($p < 0.05$) was carried out to compare the differences between the PNL and CNL cohorts for each limb. A one-way analysis of variance (ANOVA) was used to investigate the effect of varying beam width on gait ($p < 0.05$) for both cohorts.

3 RESULTS

The temporal parameters were determined for comparison of the PNL and CNL cohorts on the GR, NR and WD beams (Table 2). The main observations comparing the PNL cohort with the CNL were as follows: the PNL cohort showed

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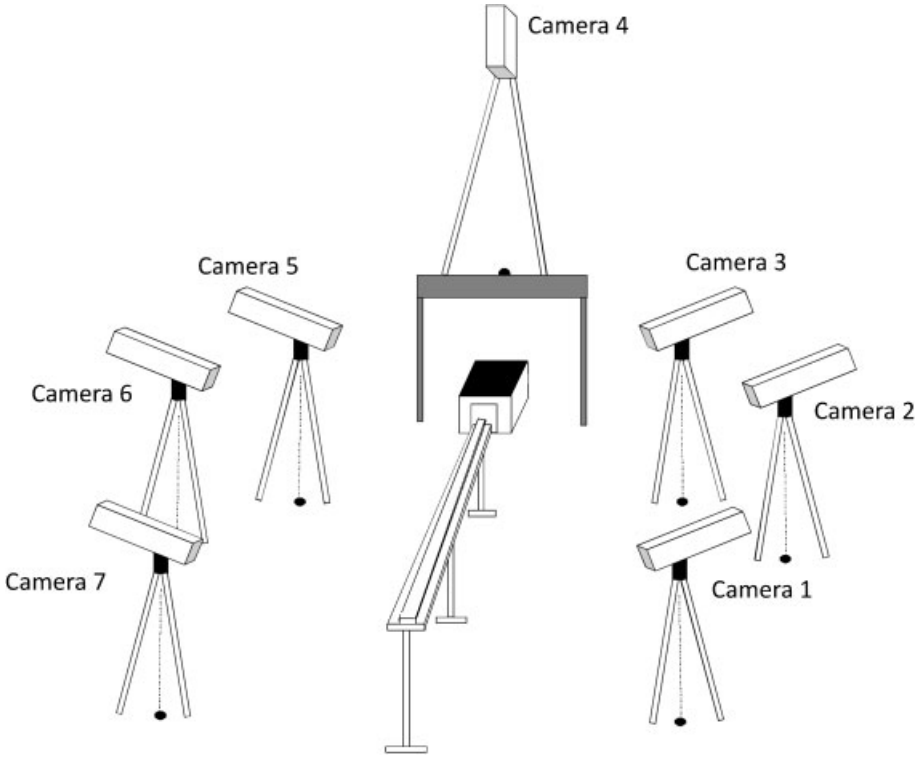

Fig. 3 Camera position showing axes direction, orientation of the seven cameras used, and position of the beam relative to the cameras

Table 1 Parameters evaluated during motion analysis

Swing time	Time it takes the rat to start lifting the limb, until it once again makes contact with the beam
Stride length	The distance between heel strike and the next heel strike of the same limb
Speed	Calculated by taking the length of the beam and the average time it takes to walk along it
Cadence	The number of steps per minute calculated from the swing time and stance time
Stance time	Time the limb was in contact with the beam to the time the limb was lifted
Zonal speed	Calculated by taking the length of each zone and the average time it takes to walk along it

Table 2 Kinematic parameters comparing the PNL (lesioned) and CNL (control) cohorts on the GR, NR and WD beams (RFL = right forelimb, LFL = left forelimb, RHL = right hindlimb, LHL = left hindlimb). The results are expressed as mean ± standard deviation (SD)

		Graduated beam (GR)		Narrow beam (NR)		Wide beam (WD)	
Parameter	Limb	CNL ($n = 5$)	PNL ($n = 5$)	CNL ($n = 5$)	PNL ($n = 5$)	CNL ($n = 5$)	PNL ($n = 5$)
Stance time (s)	RFL	30.49 ± 7.73	(36.46 ± 8.49)¤	24.93 ± 8.80	32.32 ± 7.03	29.21 ± 7.84	28.27 ± 8.05
	LFL	29.67 ± 8.07	(36.80 ± 10.22)¤	24.20 ± 4.76	26.76 ± 11.57	26.69 ± 7.74	30.96 ± 9.33
	RHL	31.96 ± 8.88	41.30 ± 13.55	23.00 ± 10.04	(32.49 ± 5.53)¤	25.11 ± 7.63	34.02 ± 8.09
	LHL	27.37 ± 4.38	35.32 ± 9.22	18.27 ± 7.87	28.70 ± 2.30	28.89 ± 9.82	29.69 ± 10.58
Swing time (s)	RFL	8.84 ± 0.66	10.67 ± 5.16	10.57 ± 4.10	8.10 ± 0.99	10.70 ± 1.08	(9.37 ± 1.10)¤
	LFL	9.12 ± 1.16	9.75 ± 1.48	12.84 ± 1.91	(10.30 ± 2.02)¤	10.47 ± 0.82	(9.98 ± 1.48)¤
	RHL	10.61 ± 3.40	11.19 ± 3.01	13.40 ± 2.31	11.85 ± 3.12	12.54 ± 1.85	(12.27 ± 2.89)¤
	LHL	10.93 ± 1.37	11.18 ± 2.27	10.53 ± 0.51	9.40 ± 1.66	14.02 ± 3.32	(13.14 ± 2.38)¤
Stride length (mm)	RFL	74.68 ± 13.92	63.65 ± 13.18	86.14 ± 14.37	(63.82 ± 12.27)¤	98.84 ± 14.73	(75.35 ± 16.45)*
	LFL	67.30 ± 11.13	64.43 ± 14.37	79.91 ± 2.72	(58.67 ± 12.19)*	96.24 ± 9.618	(75.07 ± 19.58)¤
	RHL	74.10 ± 13.29	77.92 ± 30.96	86.29 ± 12.14	75.11 ± 22.25	98.32 ± 14.57	101.98 ± 34.16
	LHL	87.85 ± 17.00	(65.54 ± 13.27)*	76.72 ± 23.09	81.19 ± 53.55	97.71 ± 14.75	78.21 ± 18.75)¤
Cadence (steps per min)	RFL	7.84 ± 1.45	(6.42 ± 0.66)¤	8.72 ± 1.71	7.63 ± 1.54	7.75 ± 1.46	8.25 ± 1.70
	LFL	7.99 ± 1.55	6.66 ± 1.31	8.20 ± 1.01	8.56 ± 2.32	8.37 ± 1.79	7.55 ± 1.43
	RHL	7.17 ± 1.06	(5.94 ± 1.22)¤	8.72 ± 2.37	6.83 ± 0.74	8.13 ± 1.24	(6.57 ± 0.84)*
	LHL	7.89 ± 0.81	6.60 ± 1.12	11.20 ± 3.48	8.57 ± 0.283	7.23 ± 1.48	7.23 ± 1.47
Speed (ms^{-1})		10.88 ± 2.92	(6.70 ± 2.03)*	11.90 ± 2.42	8.51 ± 3.20	15.93 ± 2.42	(9.87 ± 3.24)*

*Significant difference ($p < 0.05$).
¤Significant trend ($0.04 < p < 0.1$).

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(a) longer stance time for all limbs on the GR and NR beam;
(b) longer swing time for all limbs on the GR beam;
(c) shorter swing time for all limbs on the NR and WD beams;
(d) smaller stride length for all forelimbs on the GR, NR and WD beams;
(e) lower cadence for the hindlimbs for all three beams; and
(f) slower walking speed for the three beams.

Although these observed differences were not found to be significant, they showed a significant trend ($0.05 < p < 0.1$). The observations that were found to be significantly different ($p < 0.05$) were:

(a) slower walking speed along the WD beam;
(b) smaller stride lengths for the left (impaired) forelimb on the NR and WD beams, and for the left (impaired) hindlimb on the GR beam.

Table 2 also discloses the comparison of the left (impaired) and right (healthy) sides for the PNL cohort. The impaired left side displayed (a) a shorter hindlimb stance time on all three beams, (b) a shorter hindlimb swing time for the GR and NR beam, and (c) a higher hindlimb cadence on all three beams.

For the current study, the effects of a wide, narrow beam and graduated beam on walking were also analysed by evaluating the changes in speed as the animal walked through three different zones.

In general, the CNL cohort was faster than the PNL cohort on all three beams, see Table 2. However, the zonal speed of the CNL cohort on the GR beam increased between zone 1 and zone 2, and then decreased in zone 3 before entering the resting box. On the NR beam their speed increased from zone to zone, whereas on the WD beam the speed increased between zone 1 and zone 2 and remained relatively constant in zone 3. The zonal speed of the PNL cohort increased from zone to zone on the GR and the NR beam, but decreased between zone 1 and zone 2 while walking on the WD beam. Significant differences were observed between the PNL and the CNL cohorts in zone 2 of the GR beam, in zone 3 of the NR beam and in zone 2 and 3 of the WD beam (see Table 3).

4 DISCUSSION

This study presents quantitative assessment of 3D temporal gait parameters and speed during over-ground locomotion of healthy and hemiparkinsonian Lister Hooded rats. The comparison of lesion and control animals indicates that animal motion capture can provide a measure of different behaviour and functional characteristics of all four limbs. Five different variables were tested for significant differences between the two cohorts; body asymmetry was quantified between the impaired and the healthy side within the PNL cohort and the effects of varying the width of the beam on speed was also measured.

Numerous methods have been suggested to study locomotion of rats with PD lesions, both 2D and 3D. However, few were performed with the intention to correlate motor performance of patients with their respective animal models. For this reason, the current study was established using 3D motion capture techniques to evaluate functional aspects of PD during over-ground walking on three different elevated beams.

The PNL animals with unilateral dopamine depletion show impairments that are homologous to PD patients [**35**, **43**] and present the motor deficit on the side contralateral to surgery, in this case the left side. For the current study, expected asymmetry was observed and quantified for the PNL animals, affecting stance and swing times as well as cadence.

Speed of movement along the beams has also been shown to be slower following lesion surgery, caused by delayed initiation and processing of motion [**44**]. In the current study the average crossing speeds of the three beams was found to be lower for the PNL cohort.

The present results confirm previous studies [**35**, **41**, **45**] as the PNL cohort walks more slowly with a longer stance time and swing time indicating that the animals consistently remain in stance for a

Table 3 Zonal speed for both experimental groups on the GR, NR, and WD beam. The results are expressed as mean ± standard deviation (SD)

	GR beam (speed in ms^{-1})		NR beam (speed in ms^{-1})		WD beam (speed in ms^{-1})	
Zone	CNL	PNL	CNL	PNL	CNL	PNL
1	10.04 ± 3.43	6.27 ± 3.36	12.16 ± 1.39	8.36 ± 4.50	12.78 ± 6.13	13.65 ± 4.54
2	12.39 ± 2.83	(8.65 ± 3.93)*	13.35 ± 2.93	9.76 ± 4.20	20.30 ± 4.16	(11.55 ± 5.37)*
3	9.80 ± 1.60	9.04 ± 4.10	13.98 ± 1.75	(9.64 ± 1.25)*	19.06 ± 2.84	(12.68 ± 5.31)*

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longer period of time in all four limbs. This results in a lower cadence and a shorter stride length. The latter was significantly shorter in the PNL cohort for the impaired forelimb on the NR and WD beams. The impaired hindlimb on the GR beam ($p < 0.05$) also had significantly shorter strides. PNL animals have a reduced ability to move the body forward using the impaired limb; they stay in stance for a longer period of time to allow the non-impaired limbs to move the body forward when entering the swing phase.

The current study also evaluates the effect of dual tasking (gradually changing the beam width whilst walking along the beam) on both cohorts. Zonal speed was calculated for three different zones of the beams to investigate the effect of a graduating walkway on locomotion ability. The results disclose that the lesioned animals accelerated on the GR and the NR beam, possibly dealing with the decreasing path width and trying to reach the other end of the beam and the safe resting box more quickly. The CNL cohort was able to adapt their gait to cope with the varying beam widths at all times. Comparison of zonal speed between the two cohorts displays a significantly slower speed in zone 2 for the PNL animals on the GR beam (the GR beam is supposed to be the most difficult one as a constant adaptation of gait is required); zone 2 is the area of the beam where the 'graduating' of the path width begins indicating that the PNL group took longer to adjust their speed as the beam narrowed. However, on the NR beam the significant difference occurs in zone 3; here the PNL animals decelerated more than the CNL animals to allow for adjustments of their walking pattern.

Hemiparkinsonian rats show typical symptoms of Parkinson's disease [**14**, **35**, **46**]: not only is the rats' gait severely impaired, but also other motor deficits are obvious such as cataleptic behaviour [**47**] and motor coordination deficits [**4**]. Although the PNL rats walk more slowly over the beam than their healthy counterparts, their speed varies between the zones. This might be part of compensatory mechanisms to overcome some of the motor deficits. Similar behaviour can be observed in patients with shuffling gait but high cadence [**36**]. After a period of slow initiation of motion, a higher cadence/speed is needed to overcome freezing and bradykinesia.

This study enables the quantification of animal motion in terms of the ability to adjust their gait to cope with the different beam widths, thus introducing an element of dual tasking. For example, the zonal speed varied as the animals adjusted their speed to compensate for the change in beam width. Therefore, the present protocol can successfully quantify compensatory behaviour, gait variability, and deficits in executive function in a group of hemiparkinsonian rats allowing future correlation of motor performance with patients' data.

These results demonstrate that marker-based optoelectronic motion capture can provide an effective and simple approach to quantifying temporal gait parameters for rat models of PD. Animal motion capture can thus provide a practical and powerful tool for validating these models. The new protocol will also help to identify gait deficits and classify them objectively. The results of this explorative study demonstrate functional characteristics of PD in terms of longer stance time, longer swing time, smaller stride length and less steps per minute. They also reveal how the width of the beam affects the locomotion in both experimental cohorts. Furthermore, the findings allow effective and detailed analysis of motor deficits in an animal model of PD. Such animal measurements can then be directly compared with human measures to explore correlations between patients and animal behaviour. This will help to translate animal research into potential therapies for many different pathologies affecting motor performance.

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REFERENCES

1 **Garnier, C., Falempin, M.,** and **Canu, M. H.** A 3D analysis of fore- and hindlimb motion during locomotion: comparison of overground and ladder walking in rats. *Behav. Brain Res.*, 2008, **186**(1), 57–65.

2 **Brooks, S. P.** and **Dunnett, S. B.** Tests to assess motor phenotype in mice: a user's guide. *Nat. Rev. Neurosci.*, 2009, **10**(7), 519–529.

3 **Cenci, M. A., Whishaw, I. Q.,** and **Schallert, T.** Animal models of neurological deficits: how relevant is the rat? *Nat. Rev. Neurosci.*, 2002, **3**(7), 574–579.

4 **Metz, G. A.** and **Whishaw, I. Q.** The ladder rung walking task: a scoring system and its practical application. *J. Vis. Exp.*, 2009 (28), 1204. doi: 10.3791/1204.

5 **Whishaw, I. Q., Li, K., Whishaw, P. A., Gorny, B.,** and **Metz, G. A.** Distinct forelimb and hind limb stepping impairments in unilateral dopamine-depleted rats: use of the rotorod as a method for the qualitative analysis of skilled walking. *J. Neurosci. Methods*, 2003, **126**(1), 13–23.

6 **Canu, M. H.** and **Garnier, C.** A 3D analysis of fore- and hindlimb motion during overground and

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ladder walking: comparison of control and unloaded rats. *Expl. Neurol.*, 2009, **218**(1), 98–108.

7 **Allbutt, H. N.** and **Henderson, J. M.** Use of the narrow beam test in the rat, 6-hydroxydopamine model of Parkinson's disease. *J. Neurosci. Methods*, 2007, **159**(2), 195–202.

8 **Canu, M. H., Garnier, C., Lepoutre, F. X.,** and **Falempin, M.** A 3D analysis of hindlimb motion during treadmill locomotion in rats after a 14-day episode of simulated microgravity. *Behav. Brain Res.*, 2005, **157**(2), 309–321.

9 **Marcuzzo, S., Dutra, M. F., Stigger, F., do Nascimento, P. S., Ilha, J., Kalil-Gaspar, P. I.,** and **Achaval, M.** Beneficial effects of treadmill training in a cerebral palsy-like rodent model: walking pattern and soleus quantitative histology. *Brain Res.*, 2008, **1222**, 129–140.

10 **Pereira, J. E., Cabrita, A. M., Filipe, V. M., Bulas-Cruz, J., Couto, P. A., Melo-Pinto, P., Costa, L. M., Geuna, S., Mauricio, A. C.,** and **Varejao, A. S.** A comparison analysis of hindlimb kinematics during overground and treadmill locomotion in rats. *Behav. Brain Res.*, 2006, **172**(2), 212–218.

11 **Coulthard, P., Pleuvry, B. J., Brewster, M., Wilson, K. L.,** and **Macfarlane, T. V.** Gait analysis as an objective measure in a chronic pain model. *J. Neurosci. Methods*, 2002, **116**(2), 197–213.

12 **Brown, M.** and **Taylor, J.** Prehabilitation and rehabilitation for attenuating hindlimb unweighting effects on skeletal muscle and gait in adult and old rats. *Arch. Phys. Med. Rehabil.*, 2005, **86**(12), 2261–2269.

13 **Fan, L. W., Tien, L. T., Mitchell, H. J., Rhodes, P. G.,** and **Cai, Z.** Alpha-phenyl-n-tert-butyl-nitrone ameliorates hippocampal injury and improves learning and memory in juvenile rats following neonatal exposure to lipopolysaccharide. *Eur. J. Neurosci.*, 2008, **27**(6), 1475–1484.

14 **Klein, A., Wessolleck, J., Papazoglou, A., Metz, G. A.,** and **Nikkhah, G.** Walking pattern analysis after unilateral 6-OHDA lesion and transplantation of foetal dopaminergic progenitor cells in rats. *Behav. Brain Res.*, 2009, **199**(2), 317–325.

15 **Wang, H., Spinner, R. J., Sorenson, E. J.,** and **Windebank, A. J.** Measurement of forelimb function by digital video motion analysis in rat nerve transection models. *J. Peripher. Nerv. Syst.*, 2008, **13**(1), 92–102.

16 **Boyd, B. S., Puttlitz, C., Noble-Haeusslein, L. J., John, C. M., Trivedi, A.,** and **Topp, K. S.** Deviations in gait pattern in experimental models of hindlimb paresis shown by a novel pressure mapping system. *J. Neurosci. Res.*, 2007, **85**(10), 2272–2283.

17 **Deumens, R., Jaken, R. J., Marcus, M. A.,** and **Joosten, E. A.** The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. *J. Neurosci. Methods*, 2007, **164**(1), 120–130.

18 **Koopmans, G. C., Deumens, R., Brook, G., Gerver, J., Honig, W. M., Hamers, F. P.,** and **Joosten, E. A.** Strain and locomotor speed affect over-ground locomotion in intact rats. *Physiol Behav.*, 2007, **92**(5), 993–1001.

19 **Hamers, F. P., Lankhorst, A. J., van Laar, T. J., Veldhuis, W. B.,** and **Gispen, W. H.** Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. *J. Neurotrauma*, 2001, **18**(2), 187–201.

20 **Vrinten, D. H.** and **Hamers, F. F.** 'CatWalk' automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing. *Pain*, 2003, **102**(1–2), 203–209.

21 **Fu, S. C., Chan, K. M., Chan, L. S., Fong, D. T.,** and **Lui, P. Y.** The use of motion analysis to measure pain-related behaviour in a rat model of degenerative tendon injuries. *J. Neurosci. Methods*, 2009, **179**(2), 309–318.

22 **Varejao, A. S., Cabrita, A. M., Meek, M. F., Bulas-Cruz, J., Gabriel, R. C., Filipe, V. M., Melo-Pinto, P.,** and **Winter, D. A.** Motion of the foot and ankle during the stance phase in rats. *Muscle Nerve*, 2002, **26**(5), 630–635.

23 **Gorska, T., Zmyslowski, W.,** and **Majczynski, H.** Overground locomotion in intact rats: interlimb coordination, support patterns and support phases duration. *Acta Neurobiol. Exp. (Wars.)*, 1999, **59**(2), 131–144.

24 **Jones, L., Beynon, M. J., Holt, C. A.,** and **Roy, S.** An application of the Dempster-Shafer theory of evidence to the classification of knee function and detection of improvement due to total knee replacement surgery. *J. Biomech.*, 2006, **39**(13), 2512–2520.

25 **Jones, L., Holt, C. A.,** and **Beynon, M. J.** Reduction, classification and ranking of motion analysis data: an application to osteoarthritic and normal knee function data. *Comput. Methods Biomech. Biomed. Engng*, 2008, **11**(1), 31–40.

26 **Narayanan, U. G.** The role of gait analysis in the orthopaedic management of ambulatory cerebral palsy. *Curr. Opin. Pediatr.*, 2007, **19**(1), 38–43.

27 **Watelain, E., Dujardin, F., Babier, F., Dubois, D.,** and **Allard, P.** Pelvic and lower limb compensatory actions of subjects in an early stage of hip osteoarthritis. *Arch. Phys. Med. Rehabil.*, 2001, **82**(12), 1705–1711.

28 **Metz, G. A., Dietz, V., Schwab, M. E.,** and **van de, M. H.** The effects of unilateral pyramidal tract section on hindlimb motor performance in the rat. *Behav. Brain Res.*, 1998, **96**(1–2), 37–46.

29 **Couto, P. A., Filipe, V. M., Magalhaes, L. G., Pereira, J. E., Costa, L. M., Melo-Pinto, P., Bulas-Cruz, J., Mauricio, A. C., Geuna, S.,** and **Varejao, A. S.** A comparison of two-dimensional and three-dimensional techniques for the determination of hindlimb kinematics during treadmill locomotion in rats following spinal cord injury. *J. Neurosci. Methods*, 2008, **173**(2), 193–200.

30 **Moreau, C., Defebvre, L., Bleuse, S., Blatt, J. L., Duhamel, A., Bloem, B. R., Destee, A.,** and

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Krystkowiak, P. Externally provoked freezing of gait in open runways in advanced Parkinson's disease results from motor and mental collapse. *J. Neural Transm.*, 2008, **115**(10), 1431–1436.

31 **Schaafsma, J. D., Giladi, N., Balash, Y., Bartels, A. L., Gurevich, T.,** and **Hausdorff, J. M.** Gait dynamics in Parkinson's disease: relationship to Parkinsonian features, falls and response to levodopa. *J. Neurol. Sci.*, 2003, **212**(1–2), 47–53.

32 **Plotnik, M., Giladi, N.,** and **Hausdorff, J. M.** Bilateral coordination of walking and freezing of gait in Parkinson's disease. *Eur. J. Neurosci.*, 2008, **27**(8), 1999–2006.

33 **Woollacott, M.** and **Shumway-Cook, A.** Attention and the control of posture and gait: a review of an emerging area of research. *Gait Posture*, 2002, **16**(1), 1–14.

34 **Latt, M. D., Menz, H. B., Fung, V. S.,** and **Lord, S. R.** Acceleration patterns of the head and pelvis during gait in older people with Parkinson's disease: a comparison of fallers and nonfallers. *J. Gerontol. A Biol. Sci. Med. Sci.*, 2009, **64**(6), 700–706.

35 **Metz, G. A., Tse, A., Ballermann, M., Smith, L. K.,** and **Fouad, K.** The unilateral 6-OHDA rat model of Parkinson's disease revisited: an electromyographic and behavioural analysis. *Eur. J. Neurosci.*, 2005, **22**(3), 735–744.

36 **Brown, L. A., de Bruin, N., Doan, J. B., Suchowersky, O.,** and **Hu, B.** Novel challenges to gait in Parkinson's disease: the effect of concurrent music in single- and dual-task contexts. *Arch. Phys. Med. Rehabil.*, 2009, **90**(9), 1578–1583.

37 **Dowd, E.** and **Dunnett, S. B.** Comparison of 6-hydroxydopamine-induced medial forebrain bundle and nigrostriatal terminal lesions in a lateralised nose-poking task in rats. *Behav. Brain Res.*, 2005, **159**(1), 153–161.

38 **Sinclair, S. R., Fawcett, J. W.,** and **Dunnett, S. B.** Delayed implantation of nigral grafts improves survival of dopamine neurones and rate of functional recovery. *Neuroreport*, 1999, **10**(6), 1263–1267.

39 **Kirik, D., Rosenblad, C.,** and **Bjorklund, A.** Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. *Exp. Neurol.*, 1998, **152**(2), 259–277.

40 **Ungerstedt, U.** 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur. J Pharmacol.*, 1968, **5**(1), 107–110.

41 **Miklyaeva, E. I., Martens, D. J.,** and **Whishaw, I. Q.** Impairments and compensatory adjustments in spontaneous movement after unilateral dopamine depletion in rats. *Brain Res.*, 1995, **681**(1–2), 23–40.

42 **Duhamel, A., Bourriez, J. L., Devos, P., Krystkowiak, P., Destee, A., Derambure, P.,** and **Defebvre, L.** Statistical tools for clinical gait analysis. *Gait Posture*, 2004, **20**(2), 204–212.

43 **Whishaw, I. Q., Suchowersky, O., Davis, L., Sarna, J., Metz, G. A.,** and **Pellis, S. M.** Impairment of pronation, supination, and body co-ordination in reach-to-grasp tasks in human Parkinson's disease (PD) reveals homology to deficits in animal models. *Behav. Brain Res.*, 2002, **133**(2), 165–176.

44 **Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N.,** and **Cenci, M. A.** Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur. J. Neurosci.*, 2002, **15**(1), 120–132.

45 **Pinna, A., Pontis, S., Borsini, F.,** and **Morelli, M.** Adenosine A2A receptor antagonists improve deficits in initiation of movement and sensory motor integration in the unilateral 6-hydroxydopamine rat model of Parkinson's disease. *Synapse*, 2007, **61**(8), 606–614.

46 **Miklyaeva, E. I., Castaneda, E.,** and **Whishaw, I. Q.** Skilled reaching deficits in unilateral dopamine-depleted rats: impairments in movement and posture and compensatory adjustments. *J. Neurosci.*, 1994, **14**(11 Pt 2), 7148–7158.

47 **Klein, A.** and **Schmidt, W. J.** Catalepsy intensifies context-dependently irrespective of whether it is induced by intermittent or chronic dopamine deficiency. *Behav. Pharmacol.*, 2003, **14**(1), 49–53.

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