Behavioural Brain Research 311 (2016) 340–353

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

High-speed video gait analysis reveals early and characteristic locomotor phenotypes in mouse models of neurodegenerative movement disorders

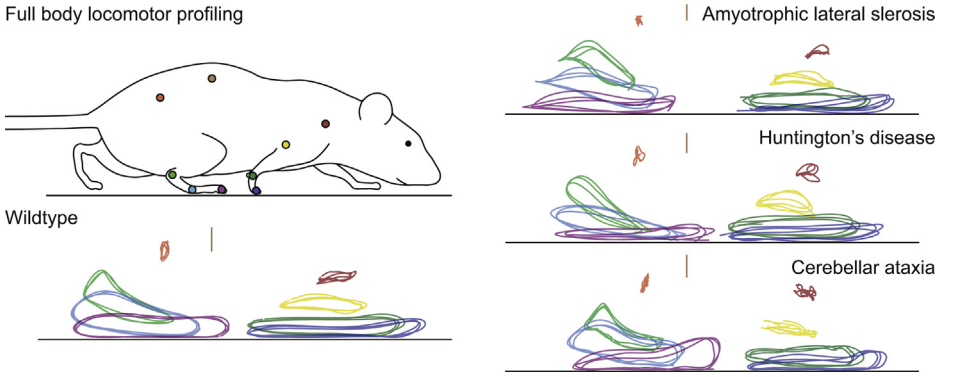
Daniel F. Preisig, Luka Kulic, Maik Krüger, Fabian Wirth, Jordan McAfoose, Claudia Späni, Pascal Gantenbein, Rebecca Derungs, Roger M. Nitsch, Tobias Welt*

Institute for Regenerative Medicine—IREM, University of Zurich, 8952 Schlieren, Switzerland

HIGHLIGHTS

- Full body high-speed video gait analysis increases specificity in motor testing.
- Comprehensive locomotor profiles are generated by a custom-built algorithm.
- Gait patterns of ALS, Huntington and cerebellar ataxia models differ substantially.
- Early and specific gait changes were found for each of the three models analyzed.
- Analysis of locomotor function is essential for the development of new therapies.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 25 April 2016
Accepted 26 April 2016
Available online 24 May 2016

Keywords:
Video gait analysis
Locomotion
Amyotrophic lateral sclerosis
Huntington's disease
Cerebellar ataxia
Neurodegeneration

ABSTRACT

Neurodegenerative diseases of the central nervous system frequently affect the locomotor system resulting in impaired movement and gait. In this study we performed a whole-body high-speed video gait analysis in three different mouse lines of neurodegenerative movement disorders to investigate the motor phenotype. Based on precise computerized motion tracking of all relevant joints and the tail, a custom-developed algorithm generated individual and comprehensive locomotor profiles consisting of 164 spatial and temporal parameters. Gait changes observed in the three models corresponded closely to the classical clinical symptoms described in these disorders: Muscle atrophy due to motor neuron loss in SOD1 G93A transgenic mice led to gait characterized by changes in hind-limb movement and positioning. In contrast, locomotion in huntingtin N171-82Q mice modeling Huntington's disease with basal ganglia damage was defined by hyperkinetic limb movements and rigidity of the trunk. Harlequin mutant mice modeling cerebellar degeneration showed gait instability and extensive changes in limb positioning. Moreover, model specific gait parameters were identified and were shown to be more sensitive than conventional motor tests. Altogether, this technique provides new opportunities to decipher underlying disease mechanisms and test novel therapeutic approaches.

* Corresponding author at: Institute for Regenerative Medicine—IREM, University of Zurich, Campus Schlieren, Wagistrasse 12, 8952 Schlieren/Zurich, Switzerland.
E-mail address: tobias.welt@bli.uzh.ch (T. Welt).

http://dx.doi.org/10.1016/j.bbr.2016.04.044
0166-4328/

1. Introduction

Neurodegenerative disorders of the central nervous system, depending on the structures involved, often affect movement and gait. In amyotrophic lateral sclerosis (ALS) muscle atrophy, paralysis and spasticity, due to spinal cord and motor cortex pathology, result in gait disturbances [1]. Damage to basal ganglia structures in Huntington's disease (HD) alternatively causes a hyperkinetic disorder (chorea) combined with a loss of voluntary movements (bradykinesia and rigidity) [2]. Disorders primarily affecting cerebellar structures, such as spinocerebellar ataxias, result in impaired balance and coordination (ataxic gait) [3].

To better understand the underlying pathophysiological mechanisms, several mouse models representing these neurodegenerative disorders have been established: (1) SOD1 G93A transgenic (tg) mice demonstrate ALS-like motor neuron loss and hind-limb paralysis [4]; (2) Huntingtin N171-82Q mice develop abnormal gait and behavioral abnormalities [5]; (3) Harlequin mutants bear a mutation leading to cerebellar degeneration and ataxic syndrome [6].

Comprehensive analysis of motor and gait function is a cornerstone for the pre-clinical development of new therapies. Assessment techniques based on behavioral tests such as rotarod, grip strength or scoring systems are characterized by several limitations, including a restriction to a few specific aspects of locomotion, non-physiologic test conditions and a dependency on users and motivational factors. They often fail to fully capture delayed onset following treatment intervention (sensitivity) and progressive motor dysfunction (qualitative and quantitative changes). Therefore, more sophisticated techniques were needed.

Gait analysis is promising for the evaluation of motor deficits, as gait is a fundamental, physiological and unforced form of locomotion with direct clinical relevance. However, available systems focusing on ventral plane video gait analysis produce results that vary widely, fail to reproduce or even contradict previous findings [7–12]. This is not surprising, as many characteristic changes in limb positioning and movement dynamics in ALS and HD models can only be observed in the lateral plane. Lately, innovative systems including machine learning algorithms (NeuroCube®) and analysis of the lateral view (MotoRater, Locomouse) became available and were used to examine gait more thoroughly [13–17]. But the maximum potential of lateral plane videography has not yet been exploited, as analyses were restricted to single functional aspects of gait and a limited number of gait parameters in few models.

In this study we performed a comprehensive whole-body locomotion and gait analysis of the mentioned mouse models of neurodegenerative movement disorders using the MotoRater (TSE-Systems, Bad Homburg, Germany), a high-speed video tracking system [18]. This system films the spontaneous gait from three sides (ventral and lateral planes) to track bony landmarks.

We followed an exploratory approach (using 17 tracking points) to study the movement of limbs, trunk and tail in order to create a highly comprehensive locomotor profile and developed a custom-built algorithm, which converts and interprets the raw tracking data and calculates 164 objective spatial and spatiotemporal parameters describing locomotion. Based on this extensive dataset, we identified a small set of relevant tracking points and parameters for each model, which specifically describe gait disturbances, and may be used in following studies to assess potential therapeutic approaches with a higher throughput. Further, we examined effects of locomotion speed and body weight, as previous gait analysis studies pointed out their influence on many individual locomotor parameters [19–21,16]. Moreover, we compared the three models representing different human neurodegenerative gait disorders and observed specific and distinct motor profiles. Several gait changes observed in the three models correspond closely to the classical clinical symptoms described in these disorders.

Lastly, a higher sensitivity of this system compared to conventional motor tests was proven, revealing substantial locomotor deficits detectable prior to the onset of clinical impairments on conventional tasks.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the veterinarian authorities of the Canton of Zurich (animal license numbers: 109/2010, 183/2012, 229/2013), in compliance with Swiss law ("455.163 Tierversuchsverordnung" 2010) and international guidelines for care and use of animals.

All animals were held and housed 3–5 per cage under specific pathogen-free conditions in an air-conditioned and temperature controlled environment (22 °C ± 1 °C). 3 weeks after birth, tg animals were identified by standard PCR assessing the expression of the transgene or mutation and separated according to gender. Water and normal diet was provided ad libitum and a 12-h light-dark-cycle beginning at 7:00 am was maintained in the housing room.

As a model for ALS the widely used tg SOD1 G93A mouse line overexpressing a mutant human SOD1 gene was used. Male tg mice high copy number; B6.Cg-Tg(SOD1*G93A)1Gur/J were bred with C57BL/6J females.

As a model for Huntington's disease we used N171-82Q tg mice expressing the first 171 amino acids of the exons 1–3 of the human huntingtin gene with 82CAG repeats. Male tg mice (B6C3-Tg(HD82Gln)81Gschi/J) were bred with C57BL/6J females.

As a model of cerebellar ataxia, Harlequin (Hq) mutant mice, bearing an X-chromosome-linked proviral insertion in the first intron of the AIF gene, were used [6]. Male Hq/y mutant mice and wild-type (wt) (+/y) control were on a B6CBACa-A^{w-J}/A (B6CBA) background.

All mice were ordered from the Jackson Laboratory (Bar Harbor, Maine, USA), all experiments were performed in F1 generation animals and only males were used as sex differences in SOD1 and huntingtin tg mice were described [22,23].

2.2. Motor testing

Motor functions were assessed comparing conventional motor testing with high-speed video gait analysis. We recorded the animal gait, using the MotoRater (Fig. 1A), an advanced quantitative high-speed video system (TSE Systems, Bad Homburg, Germany) [13,18,24]. The mice were filmed moving freely over a transparent runway (Fig. 1B), at 200 frames per second. Video recordings from three different sides of the animal (below, left and right) were obtained simultaneously, using a mirror system (Fig. 1C/D).

2.2.1. MotoRater testing: preparation, recording and video tracking

To motivate the mice crossing the runway, a darkened area was created at the end (Fig. 1B) and all mice were acclimated to the setup. Fig. 1E shows analyzed landmarks overlying bony structures and joints, which were marked on the shaved skin with a white Edding© marker during an inhalational anesthesia using 3.5% Sevoflurane (Abbot, USA). SIMI Motion (SIMI Reality Motion Systems, Germany) software was used for motion-tracking and to acquire two-dimensional coordinates of three consecutive steps (Fig. 1F). Lateral points were tracked continually, while bottom points were only tracked in the first frame of paw contact for each step. This process worked semi-automatically, consuming about