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Automated quantitative analysis to assess motor function in different rat models of impaired coordination and ataxia

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HIGHLIGHTS

- Design and validation of a CatWalk protocol based on repeated testing and rewards.
- Enriched housed rats show higher walking and swing speed and longer stride length.
- Ethanol-induced ataxia impairs gait and affects mainly the hind part of the body.
- A rat model for Spinocerebellar Ataxia type 17 shows coordination disturbances.

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ABSTRACT

Background: An objective and automated method for assessing alterations in gait and motor coordination in different animal models is important for proper gait analysis. The CatWalk system has been used in pain research, ischemia, arthritis, spinal cord injury and some animal models for neurodegenerative diseases.
New method: Our goals were to obtain a comprehensive gait analysis of three different rat models and to identify which motor coordination parameters are affected and are the most suitable and sensitive to describe and detect ataxia with a secondary focus on possible training effects.
Results: Both static and dynamic parameters showed significant differences in all three models: enriched housed rats show higher walking and swing speed and longer stride length, ethanol-induced ataxia affects mainly the hind part of the body, and the SCA17 rats show coordination disturbances. Coordination changes were revealed only in the case of the ethanol-induced ataxia and the SCA17 rat model. Although training affected some gait parameters, it did not obscure group differences when those were present.
Comparison with existing methods: To our knowledge, a comparative gait assessment in rats with enriched housing conditions, ethanol-induced ataxia and SCA17 has not been presented before.
Conclusions: There is no gold standard for the use of CatWalk. Dependent on the specific effects expected, the protocol can be adjusted. By including all sessions in the analysis, any training effect should be detectable and the development of the performance over the sessions can provide insight in effects attributed to intervention, treatment or injury.

1. Introduction

Neurodegenerative disease is a broad term used to describe disorders which have, as a common characteristic, the progressive death of neurons and their inability to be reproduced or replaced. Depending on the area of the brain where the nerve cell death takes place, neurodegeneration can cause problems with movement or mental functioning. Movement disorders are known as “ataxia” and cognitive disorders are referred to as “dementia”. In patients these two disorders are very often present at the same time (Bruni et al., 2004). Compared to movement disorders, cognitive aspects of dementia have been most extensively studied in rodents, resulting in a relatively limited understanding of movement disorders. In response to this limited understanding, an increasing number

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of animal models for movement disorders have been reported in literature during the past 10 years. However, proper interpretation of results is hindered by the lack of an objective and automated method to assess motor coordination and gait abnormalities (Wang et al., 2008). The CatWalk system can provide an extensive number of parameters that allows automated assessment of gait and locomotion. The concept of CatWalk is based on the classical footprint analysis test in which the animal's paws were dipped into non-toxic paint or ink and then had to walk along a paper-covered corridor leaving a track of footprints. However, compared to the inked-paw version which can give mainly information about the static gait parameters, the CatWalk system can provide information about distribution of the animal's weight across its feet, the maximum surface area of a foot touching the ground, the intensity of a print, and most importantly, the temporal relations of the animal's paws such as the stand duration, the swing, the walking speed and so on (Hamers et al., 2001; Vandeputte et al., 2010). Furthermore, with the inked footprint tracks, the body and paw angle measurements were often challenging to measure due to the fact that only the paws of the animals were dyed and therefore no information about the body center or the direction of the body axis could be analyzed (Carter et al., 2001). So far, the CatWalk system has been used mainly for modeling pain (Gabriel et al., 2009), sciatic nerve injury (Deumens et al., 2007; Bozkurt et al., 2008) and arthritis (Fu et al., 2012), but to our knowledge no work has been done on any rat model of Spinocerebellar ataxia.

Ataxia has been described as the loss of muscle coordination with symptoms of impaired interlimb coordination, irregular step patterns, instable stumbling walking path and increased step width (Ilg et al., 2007; Vinueza Veloz et al., 2015). These symptoms are however difficult to objectively capture in parameters. Therefore, we aimed to further validate the Catwalk system and identify those automatically generated parameters that can most accurately describe ataxia. In this study, we applied three different methods to obtain data of animals which have an altered gait and motor coordination pattern: (1) animals with different housing-conditions (enriched versus standard), (2) a temporally induced ataxia model after ethanol (ETOH) administration, and (3) a transgenic rat model for Spinocerebellar Ataxia type 17 (SCA17) (Kelp et al., 2013).

It is known that housing conditions can influence the level of activity of animals. As has been reported in the literature, an enriched environment can affect motor function parameters (Lankhorst et al., 2001; van Meeteren et al., 2003; Glass et al., 2004; Spires et al., 2004; Gabriel, 2009) and can significantly enhance locomotion and motor coordination in many different cases such as spinal cord injured rats (Burke et al., 2007), Huntington's and Parkinson's disease animal models, as well as several psychiatric (animal models of schizophrenia) and neurodegenerative disorders (Laviola et al., 2008). The hypothesis behind this effect is that environmental factors can modulate experience-dependent plasticity and therefore influence the pathology and disease progression (Nithianantharajah and Hannan, 2006).

ETOH is known for having acute effects on motor function and coordination and inducing several neurobehavioral changes (Lai et al., 2007; Hansen and Pulst, 2013). On the other hand, chronic exposure to ETOH can have several effects attributed to a disturbed cerebellar activity (Forbes et al., 2013). ETOH has been demonstrated to influence animals' performance with the rotarod test, the aerial righting reflex (ARR) and a single cognitive performance task (the Morris water maze) (Novier et al., 2013). Using footprint analysis, only a few differences have been detected after ETOH administration such as a difference in the angle of foot placement and an overall change in paw overlap, whereas alterations in motor function were prominent (Forbes et al., 2013; Novier et al., 2013). Overall, literature findings support that ETOH has motor suppressant, ataxic or sedative effects that make it a valid method for modeling ataxia (Hansen and Pulst, 2013).

The third model used in this study is an animal model for SCA17. SCA17 is an inherited autosomal dominant neurodegenerative disease caused by an abnormal number of CAA/CAG repeats in the TATA-box-binding protein (TBP) gene which has, as a consequence, an expanded polyglutamine track in the TBP (Fujigasaki et al., 2001; Stevanin et al., 2008). This rat model shows a progressive phenotype including ataxia and impaired reflexes, reduced activity, loss of body weight as the disease progresses and early death (Kelp et al., 2013). Here we show for the first time results from this transgenic rat model for Spinocerebellar Ataxia tested on CatWalk.

For the purposes of this study we designed and validated a Catwalk protocol based on repeated testing and rewarding each run. This protocol aimed to minimize differences between animals due to motivation and walk speed through the CatWalk. Our goal was to examine and determine which of the large number of gait parameters provided by the CatWalk system are affected and are most accurate for evaluating locomotion impairments in different models. In the present study we used enriched housed conditions as well as acute and neurodegenerative animal models of ataxia to assess motor coordination impairments and as a secondary aim we analyzed performance over the three sessions to assess the effect of training. The possible learning effects were analyzed only for the enriched housed and the SCA17 models since the ETOH intoxication has an acute effect and repeated measures were not applicable.

2. Materials and methods

2.1. Animals

For experiments 1 and 2, 24 adult male Wistar rats (~6 months old, Harlan Laboratories, The Netherlands) that were available at our animal facility were used. They were kept under standard laboratory conditions with 12:12 h reversed light:dark cycle, temperature 21 °C (±2). All experiments reported here were performed with the permission of the Animal Ethics Committee ('Lely-DEC') and in full compliance with the legal requirements of Dutch legislation on the use and protection of laboratory animals. Food and water were always available ad libitum.

For experiment 3, we used six wild-type Sprague-Dawley male rats and nine TBPQ64 transgenic male rats (obtained from University of Tübingen, Germany). Housing conditions were as described for the other experimental groups. The transgenic males were tested at the age of 9 months when they had fully developed disease symptoms, but had not yet reached a humane endpoint (10 months of age based on published data concerning body weight, locomotion and general condition (Kelp et al., 2013)).

The use of two different strains provided us the opportunity to determine potential strain differences in the baseline levels of the CatWalk parameters.

2.2. The CatWalk system

Gait analysis was performed using CatWalk XT 10.5 system (Noldus Information Technology, Wageningen, The Netherlands). The apparatus consists of an enclosed corridor (width 8 cm to prevent the animals turning which would interrupt their straight movement) with a glass plate floor ($L \times W \times H$: $130 \times 68 \times 152\ cm^3$), and a goal-box at the end containing the home-cage of the animals (Fig. 1). The runway is illuminated from the ceiling with a red light and from the long edge of the glass plate with a green light (green intensity threshold 0.12 and camera gain 15.84). When the animals' paws make contact with the glass plate, the light is internally