Defining Parameters in Automated Quantitative Gait Analysis for Evaluation of Progressive Neurodegeneration in Animal Models of Ataxia and Motor Coordination Impairments

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Introduction

Neurodegenerative disease is a broad term used to describe disorders which have as a common characteristic the progressive death of neurons and the 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 either movement, which in this case the disorders are called "ataxia", or with mental functioning and then it is referred to as "dementia". An increasing number of animal models for several neurodegenerative diseases which exhibit gait disorders have been reported in literature during the past 10 years. However, the lack of an objective and automated method to assess motor coordination and gait abnormalities is making the study of those models difficult, and sometimes the results given are difficult to interpret [1]. CatWalk® is a very promising tool providing an extensive number of gait parameters, however, it has been used so far mainly for modelling pain [2], sciatic nerve injury [3,4] and arthritis [5] but not so much for neurodegenerative diseases that affect locomotion, and more specifically cause ataxia. Therefore, our aim is to validate the system and identify those parameters that can more accurately describe ataxia. To that extend, based on a review of what kind of compounds have been used in literature to induce ataxia, we conducted a pilot study, where alcohol was used as a way to induce temporary ataxia and motor coordination impairments [6, 7].

Materials and methods

Eight adult male Wistar rats (~6 months old, Harlan, The Netherlands) were used, housed under standard conditions with 12:12 h reversed light: dark cycle, temperature 21ºC (±2) housed in Makrolon type IV-S cages (Tecniplast, Italy) with 2 rats/cage. Food and water were available *ad* libitum. Animals gait analysis was performed by using CatWalk XT 10.5 (Noldus Information Technology, Wageningen, The Netherlands). The apparatus consists of an enclosed corridor with a glass plate floor, and a goal-box at the end under which the home-cage of the animals can be put. The runway is illuminated from the ceiling with a red and green light. When the animals' paws have contact with the glass plate the light gets reflected and is captured by a high-speed video camera fixed 60 cm below the corridor. The width of the corridor was approximately set at approximately 8 cm to prevent the animals to turn back and interrupt their straight movement. The automatic detection settings were applied and the green intensity threshold was set to 0.12 and the camera gain to 15.84. The animals were trained for two days during their dark phase, under red light conditions. Before testing, cage-mates were separated for 30min in Makrolon type-III cages with bedding, water and food. This was done to prevent any effects of social interactions and potential hierarchical behaviour on the performance during the test. The animals were habituated to the food rewards and short separation before training and testing. First they were placed on CatWalk to run freely and move across the runway only from left to right. However, based on our previous experience with CatWalk we used an alternative schedule to test and train the animals on the apparatus. That is, each animal was let to have one run on CatWalk, followed by a single run of the next animal. Then the first animal returned on the apparatus for its second run and afterwards the second animal had the second run and so on. This procedure continued until at least 5 straight and with no interruption runs were acquired per animal. Also, the animals were motivated to traverse the corridor by using a food reward after entering their homecage underneath the goal box.

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After we established the baseline for all eight animals, two days later the rats were administered with 20% v/v ETOH with a dose of 1.5 g/kg through intraperitoneal injections. They were then tested again on CatWalk 10, 15, 20, 25 and 30 min after the injection. All static and dynamic gait parameters provided from CatWalk software were measured and analysed.

Results

Performance of the rats was determined compared to individual baselines. Data were analysed using the Student's paired t-test, after making sure that all data were normally distributed. Correlations between walking speed and individual gait parameters and between body weight and individual gait parameters were also calculated. Since we tested all parameters CatWalk software produces, we corrected for multiple comparisons by using the false discovery rate test (FDR).

After alcohol administration the walking speed (cm/sec), the footfall patterns of the animals and the regularity index (% of regular step patterns) remained intact. While the main reason for the walking speed might be the large variation within the animals, we assume that the intact regularity index might be due to the light motivation of the animal to perform the task in order to receive their reward in the end of each run. However, some other static parameters such as the toe spread (fig. 1B), the print length and the print intensity were significantly affected and more specifically the hind paws. We also observed that all runs acquired under the influence of alcohol were identified as 'compliant' from CatWalk software (maximum speed variation of 50% was selected) suggesting that all treated animals despite their ataxic gait, they had a relatively steady and uniform walking speed compared to their baseline performance. In addition, the dynamic parameters measured by CatWalk, like the stand duration, the swing speed and the phase dispersion (Figure 1A, C and D) showed also a significant effects, which will be presented in a descriptive manner in relation to the potential application as read-outs for future studies associated with various neurodegenerative motor disorders. To that extend, in our future experiments, we aim to use a transgenic rat model for Spinocerebellar Ataxia type 17 [8], and monitor the disease progression over time.

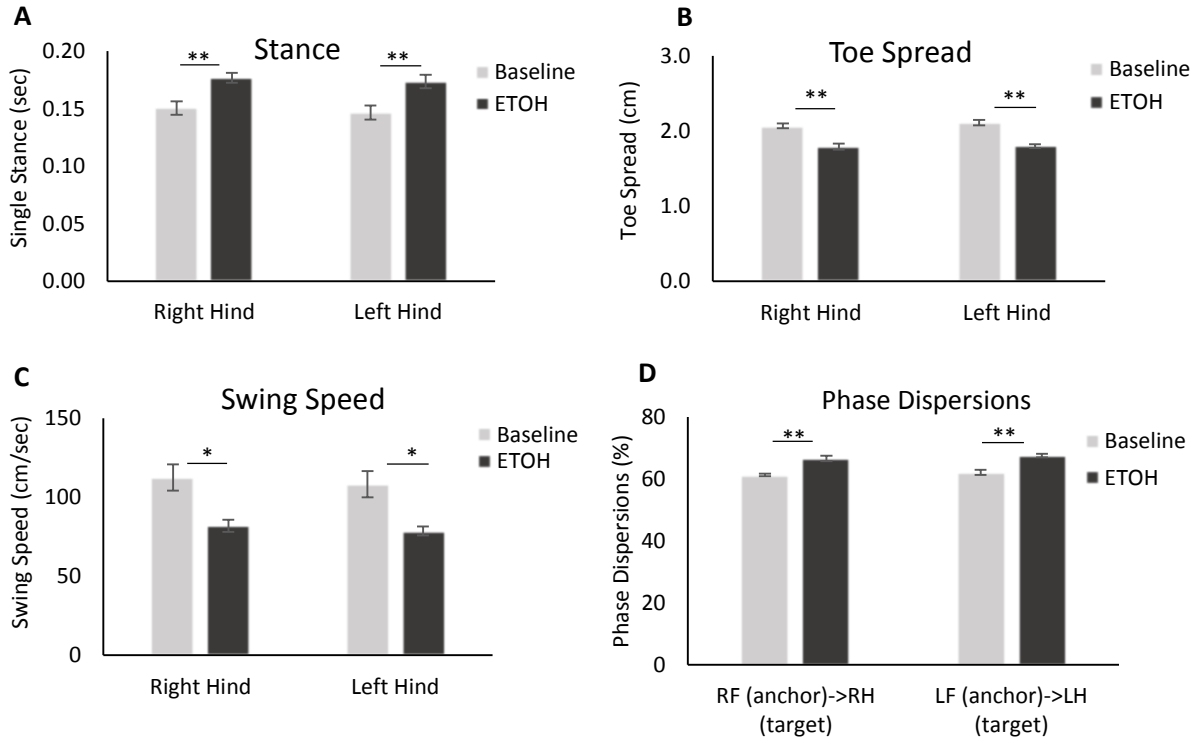

Figure 1: (A) The mean stance duration (s) of ground contact of both hind paws simultaneously and (B) the mean toe spread (cm) before and after alcohol administration. (C) The speed (distance unit/sec) of the paw during swing calculated by the formula: stride length/swing was significantly decreased for both hind paws. (D) The phase dispersions which describes the temporal relationship between placement of the lateral pair of paws within a step cycle and t is used as a measure of inter-paw coordination. The graph shows that the placement of the hind paws (affected) relative to the front paws (unaffected) were significantly delayed compared with the baseline.

Ethical statement

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 laboratory animals.

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