The effect of animal weight on over ground locomotion in neurological diseases with a focus in CatWalk gait assessment.

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Editorial

Neurobehavioral tests, which assess animal function, are widely applied to evaluate treatment efficacy in various models of neurological diseases and in the progression of degenerative conditions (i.e. multiple sclerosis, degenerative cervical myelopathy). Nevertheless, despite the remarkable number of studies and articles published annually, results are failing to translate into humans. In our opinion, this discrepancy stems from poor standardization in behavioral protocols and their execution among researchers. Rodent locomotor analysis is increasingly the most prevalent form of neurobehavioral testing. As a result, the Automated Gait Analysis System, also known as CatWalk, has become the preferential assessment tool. According to the Noldus website, CatWalk has been cited 2 to 3 times more often than any other gait analysis system in the past 10 years. With this growth in popularity, we felt that an in depth analysis of the effect of body weight and speed on CatWalk parameters was warranted.

The CatWalk system (Noldus) is a computer-assisted video-based tool for the assessment of over ground locomotion, which simultaneously measures hundreds of gait parameters. It has the advantage of being an unforced neurobehavioral technique that best represents the animal's natural locomotor pattern. Importantly, it also allows researchers to quantify static and dynamic parameters, as specifically developed to study functional recovery in rodent models of spinal cord injury. Nonetheless, during the last few years its use has been expanded to other injury models, such as musculo-skeletal injury, traumatic brain, mitochondrial disease, neuropathic cancer, Huntington's Disease (HD), chronic pain, cerebellar cortex lesion, olivocerebellar degeneration, cerebral ischemia, and degenerative cervical myelopathy [1]. Taken together, this wide-scale adoption is indicative of Catwalk's sensitivity in assessing functional recovery following neural injury. For this reason, discussion of Catwalk limitations is especially important.

It is widely accepted that speed modifies over ground locomotion gait, not only at the experimental level, but also from a mathematical standpoint. Similarly, body weight also correlates with several CatWalk software parameter measurements. The effects of body weight and speed on Catwalk gait are being increasingly reported and thereby becoming especially relevant in the interpretation of results and final behavioural outcomes. Recently two studies, one in an animal model of hemiparkinson and the second in patients with early HD, have shown an association between brain metabolism and gait pattern as well as compensation. The average walking speed of hemiparkinsonian animals is significantly lower compared with sham animals, leading to reduced values in many parameters, such as swing speed [2,3]. In line with previously published studies, mice lacking PGC-1 (PGC-1$\alpha^{-/-}$), a transcriptional co-activator that regulates the genes involved in energy metabolism, had to be controlled for age with behavioural assessments that were not affected by body weight, considering that PGC-1$\alpha^{-/-}$ mice weighed significantly less than the PGC-1$\alpha^{+/+}$ controls at earlier time points [4]. These differences in body weight would dramatically affect the conclusions of the study. Other examples have been reported in rodent models of ischemia and spinal cord injury, where it has been clearly shown that body weight transiently drops after injury [5]. Yet, despite the evidence, few researchers consider body weight as an important parameter for the interpretation of their results. Recently, a 2014 study in non-injured animals showed that over 90% of CatWalk parameters were dependent on speed, many of which had a nonlinear correlation with speed. Additionally, in 2017 studying a mouse model of permanent focal ischemia (dMCAO) our laboratory demonstrated a correlation of body weight and speed over 20 and 25 CaWalk parameters at different time points and paws [6].

In certain animal models of central nervous system injury, as well as aging animal models, there are speed variations resulting from the disease progression or the normal aging of the animals that will affect the CatWalk variables. This is best demonstrated with neonatal models of ischemia, where the animals followed over a long period (weeks or months) will develop speed and body weight differences though normal aging processes. It is, therefore, important to calculate the correlation between body weight, speed and CatWalk parameters. To the best of our knowledge, only a few groups have considered whether

body weight and speed correlated with CatWalk parameters in rodents. However, some laboratories have started to customize their analysis, with the guidance of a statistician, for proper long-term data analysis. Instead of comparing one or two time-points with the classical t-test or one-way and two-way ANOVA, these studies demonstrate that models such as linear mixed model (LMM) or linear mixed-effects (LME) better suit long-term characterization for CatWalk.

In conclusion, we strongly believe that body weight and speed must be collected to distinguish non-relevant CatWalk parameters and thereby false positive readouts. It is also important to highlight the need for a clear and standardized experimental design, which incorporates thoughtful consideration of CatWalk parameters for comparable and reproducible results between studies/laboratories. Moving forward, it is clear that researchers should incorporate and control for differences in body weight and speed when using the CatWalk Automated Gait Analysis System.

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