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Short communication

Day-to-day reliability of gait characteristics in rats

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ABSTRACT

The purpose of the present study was to determine the day-to-day reliability in stride characteristics in rats during treadmill walking obtained with two-dimensional (2D) motion capture. Kinematics were recorded from 26 adult rats during walking at 8 m/min, 12 m/min and 16 m/min on two separate days. Stride length, stride time, contact time, swing time and hip, knee and ankle joint range of motion were extracted from 15 strides. The relative reliability was assessed using intra-class correlation coefficients (ICC(1,1)) and (ICC(3,1)). The absolute reliability was determined using measurement error (ME). Across walking speeds, the relative reliability ranged from fair to good (ICCs between 0.4 and 0.75). The ME was below 91 mm for strides lengths, below 55 ms for the temporal stride variables and below 6.4° for the joint angle range of motion. In general, the results indicated an acceptable day-to-day reliability of the gait pattern parameters observed in rats during treadmill walking. The results of the present study may serve as a reference material that can help future intervention studies on rat gait characteristics both with respect to the selection of outcome measures and in the interpretation of the results.

1. Introduction

Assessment of gait characteristics is central in many studies on neurological disorders and the effects of different interventions in rat models (Amado et al., 2011; de Ruiter et al., 2007; Madete et al., 2011; Varejao et al., 2003). However, methods and systems used for gait analysis in rodents are diverse and with unclear reliability (e.g. observation based scoring, motion capture, foot print analysis) (Amado et al., 2011; Canu et al., 2005; de Ruiter et al., 2007; Dijkstra et al., 2000; Fu et al., 2012; Lakes and Allen, 2016; Madete et al., 2011; Simjee et al., 2007). Commercial motion capture systems offer feasible solutions for acquisitions of large amounts of gait data from which various parameters can be extracted (e.g. joint angles, step and stride length, step and stride time, contact and swing time).

In intervention-based studies, three parameters are of key importance for the validity of the interpretations: (1) the statistical significance of the observed change in variables, (2) the practical clinical significance of the observed change in variables, and (3) day-to-day reliability of the measured variables. The third parameter is required to evaluate whether any intervention induced difference exceeds the between-day variation in the investigated variable (Fleiss, 1986). The between-day variation originates from both the measurement errors (ME) of the used equipment and applied methodology and from the natural biological variation (Fleiss, 1986). In relation to gait analyses, the investigated variables contribute to the overall characterization of the gait pattern. When measured on two separate days, some characteristics will likely be better reproduced than others. This reproducibility of gait characteristics in humans has been quantified using intra-class correlation (ICC) (Henriksen et al., 2004; Stolze et al., 1998). One crucial issue related to between-day variation in motion capture studies is the position and reposition of external skin markers on anatomical landmarks on separate days. It is possible to reduce this variability with careful procedures, but it cannot be fully abolished (Deschamps et al., 2014; Long et al., 2010; Snider et al., 2011; Telfer et al., 2010). It is therefore relevant to quantify the day-to-day reliability in motion capture based gait characteristics of rats. While ICC quantifies the relative reliability and estimate how well two measurements result in the same value, the absolute

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reliability estimates the expected difference in absolute values between two measurements. An estimation of the absolute reliability enables a direct comparison between an intervention-induced difference between pre and post measures and the between-day variability of the variable in question.

The purpose of the present study was to investigate the day-to-day reliability of gait characteristics obtained by 2D motion capture of rats during treadmill locomotion at different speeds. The observations of this study will be useful in both the interpretation of previous studies and the planning of future studies using a test-retest design.

2. Method

2.1. Experimental animals

Twenty-six Sprague Dawley rats with an initial body mass of 437 ± 61 g were included. The experimental protocol was performed in accordance to the European Union Directive 2010/63/EU and was approved by the Danish Animal Experiments Inspectorate. The rats were housed in a room with 12 h light/dark cycle in a protected facility and food and water available ad lib. Prior to the experimental trials, the rats were familiarized to human handling and walking on a treadmill at various speeds (between 8 m/min and 16 m/min).

2.2. Experimental setup and protocol

The present study consisted of two experimental sessions on two days separated by 1–3 days and each session was conducted during the 12 h dark period. During each session, the rats walked on a treadmill (Exer 3/6 treadmill, Columbia Instruments, Columbus, Ohio, USA) with transparent plastic glass on both sides and an electrical grid at the end of the treadmill. The experimental setup was forced treadmill walking where an electrical grid at the end of the treadmill kept the rats walking on the treadmill. Before the actual testing the rats were familiarized to the task on two separate days. All rats easily learned the task and recordings were done during relative continuous walking (see below).

Five small (4.5 mm diameter) reflective markers were attached to the left hind limb with eyelash adhesive after careful shaving of the skin. The markers were positioned on the following anatomical landmarks: spina iliaca anterior superior (iliac crest), trochanter major (hip joint), epicondylus lateralis femoris (knee joint), lateral malleolus (ankle joint), and head of the fifth metatarsal (paw) (Fig. 1). During each session, the rats walked for 45 s on the treadmill at three different speeds (low: 8 m/min = 13.3 cm/s, medium: 12 m/min = 20 cm/s, and high: 16 m/min = 26.7 cm/s). One infrared camera (Qualisys Oqus310) was placed perpendicular to the side of the treadmill and the walking direction of the rat at a distance of 60 cm. The camera was operating at 120 Hz and recorded the two-dimensional positions of the reflective markers.

2.3. Data analysis

Sagittal plane hip, knee, and ankle angles were calculated based on the marker position using custom made scripts in Matlab (MatWorks R2011b). In order to establish the gait cycle (defined as the time from left hind leg toe on to the subsequent toe on), toe on of the left paw was identified as the time point of the reversal in horizontal movement (i.e. the shift from forward to backward movement) of the paw marker's x-coordinate. To eliminate any incorrect step events where the rats sat down on the belt and subsequently jump forward to catch up with the treadmill, only gait cycles with duration of a critical length were accepted. Fifteen gait cycles were randomly selected out of the total number of accepted cycles.

Fig. 1. Position of reflective markers.

$$\textit{Critical length} = \textit{mean gait cycle} + 0.10 \cdot (\textit{mean gait cycle} + SD)$$

The following variables were extracted from each stride and averaged across the fifteen strides for each rat: stride length, stride time, contact time, swing time, and hip, knee, and ankle joint angle range of motion (ROM).

2.4. Statistics

The statistical analyses were completed with n = 26 for 8 m/min and 12 m/min and n = 24 for 16 m/min (at this speed, data from n = 2 were discarded due to insufficient quality).

Quantification of the relative and absolute reliability of the measured variables followed the procedure described in details elsewhere (Henriksen et al., 2004). The relative reliability was measured by ICC(1,1), assuming that all within-subject variability to be ME and by ICC(3,1) where the within-subject variability is split into a systematic effect and a random error of measurement. ICCs calculations required that the amount of error of measurement was uncorrelated to the size of the measured value. This was tested by calculating the correlation between the absolute difference between measurements and individual test-retest mean values using Pearson's correlation coefficient. A high correlation coefficient significantly different from zero would indicate the presence of heteroscedasticity and misleading ICC values. ICC between 0.4 and 0.75 was considered to represent fair to good reliability and ICC above 0.75 was considered to represent excellent reliability (Fleiss, 1986). The absolute reliability was calculated as the square root of the mean square error term obtained from a two-way ANOVA with speed and session as independent factors. This value was termed the measurement error (ME) and expressed in the original data unit. Level of significance was set at 5%. All statistical calculations were performed in SPSS (IBM SPSS Statistics, version 24, 2016, US) and Sigmaplot (Systat Software, Inc. 2014, version 13.0, Germany).

3. Results

Descriptive statistics and test-retest difference of the investigated variables are presented in Table 1. There was a significant