The foot fault scoring system to assess walking adaptability in rats and mice: a reliability study

Foot fault scoring system reliability

Lucas Athaydes Martins^{1,2†}, Aniuska Schiavo^{1,2†}, Léder Leal Xavier^{2,3},
Régis Gemerasca Mestriner^{1,2,3*}

¹ Pontifical Catholic University of Rio Grande do Sul, PUCRS. Graduate Program in Biomedical Gerontology, School of Medicine, Porto Alegre, RS, Brazil.

²Neurorehabilitation and Neural Repair Research Group (NEUROPLAR). Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

³School of Health and Life Sciences. Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

† These authors contributed equally to this work.

*Corresponding Author:

Régis Gemerasca Mestriner, PhD
e-mail: regis.mestriner@pucrs.br
Address: Av. Ipiranga, 6681 – Building 12 / Room:104.
ZIP-code: 90619-900. Porto Alegre – RS, Brazil.
Phone: +55 51 33536031.
ResearchID: A-5911-2015.
ORCID: orcid.org/0000-0001-9837-1691

ABSTRACT

The foot fault scoring system of the ladder rung walking test is used to assess walking adaptability in rodents. However, the reliability of the ladder rung walking test foot fault score has not been properly investigated. This study was designed to address this issue. Two independent and blinded raters analyzed 20 rat and 20 mice videos. Each video was analyzed twice by the same rater (80 analyses per rater). The intraclass correlation coefficient (ICC) and the Kappa coefficient were employed to check the accuracy of agreement and reliability in the intra- and inter-rater analyses of the ladder rung walking test outcomes. Excellent intra- and inter-rater agreement was found for the forelimb, hindlimb and both limbs combined in rats and mice. The agreement level was also excellent for total crossing time, total time stopped and number of stops during the walking path. Rating individual scores in the foot fault score system (0 to 6) ranged from satisfactory to excellent, in terms of the intraclass correlation indexes. Moreover, we showed experienced and inexperienced raters can obtain reliable results if supervised training is provided. We conclude the ladder rung walking test is a reliable and useful tool to study walking adaptability in rodents and can help researchers address walking-related neurobiological questions.

Key Words: Walking, Locomotion, Rodentia, Reliability

1. INTRODUCTION

Walking adaptability can be defined as a complex sensory-motor function, qualified or required to control and coordinate various degrees of freedom in joints, in a variety of environmental contexts, or that interfere with locomotion [1-3]. Gait is influenced by the temporal and spatial integration of the cognitive and neuromusculoskeletal neural systems [4]. Moreover, the ability to adapt gait according to environmental context is a crucial aspect in maintaining body stability and preventing falls [5-8].

Whilst several studies into walking adaptability have focused on human biomechanics [2, 9, 10], animal models can usefully provide neurobiological insights at the cellular and molecular level [11-13]. For instance, the Ladder Rung Walking Test (LRWT) has been used to assess walking adaptability [14, 15] in unilateral ischemic injury in the motor cortex [12, 16]; spinal cord injury [17, 18]; dopaminergic depletion induced by 6-hydroxydopamine (a model of Parkinson's disease) [19]; neonatal white matter injury [20] and stress-related conditions [7, 13, 21].

The LRWT can assess walking patterns by using measures of inter-foot coordination, foot support, fore and hindlimb kinematics, step and gait cycles, gait speed, and the ability to adapt walking by applying a foot-fault score [16, 17]. The test provides measures of gait adaptability with emphasis in forelimb and hindlimb function by applying the foot-fault score [15]. The foot-fault score system is widely used in the literature since it requires only a hand camera and a minimally trained researcher to analyze the video and apply the foot-fault score [13, 14]. This method may avoid common pitfalls that occur when using reflective markers on the flexible skin of rodents [22, 23] and gives a measure of the success in adapting walking [7, 13].

The foot-fault score system is a 7-point category scale in which the quality and appropriateness of foot placement is judged by analyzing a video recording, frame-by-frame,

of rodents walking along a 1-meter long horizontal ladder. The rungs are arranged in a pattern that requires murine ability to adapt walking [14, 15]. However, to the best of our knowledge, this test has not been properly assessed regarding its intra-rater and inter-rater reliability and reproducibility, which is a source of uncertainty. Current studies usually elect a single rater to analyze all videos in an attempt to minimize bias, which is scientifically insufficient. The present study sought to provide scientific information regarding the external validity of the LRWT findings in rodents, thus contributing to advancements in the field of neurobiology of walking adaptability.

2. MATERIALS AND METHODS

We randomly select 40 video recordings of rodents from our lab database (20 recordings of Wistar CrlCembe:WI rats and 20 of C57BL/6JUnib mice), that performed the horizontal ladder rung walking test. At the time of the original experiments, the animals were provided by the Center for Experimental Biological Models (CeMBE) of the Pontifical Catholic University of Rio Grande do Sul. The animals were housed in cages each containing three to four rodents on a 12-hour dark-light cycle with food and water available *ad libitum*, at a temperature of 22 to 24 °C. The experiments were carried out in accordance with the National Council for Animal Control and Experimentation (Concea) and all the procedures were approved by the University Animal Ethics Commission (CEUA) under protocol numbers 15/00442 and 15/00475.

2.1 Ladder rung walking test

We used two LRWT apparatus, one for rats and another adapted for mice. Both consisted clear Plexiglas side walls (100 cm long and 20 cm high). The diameter of the metal rungs varied, being 3 mm for rats and 2 mm for mice. The minimum and maximum gaps between the rungs also varied, being from 1 to 5 cm for rats and from 0.5 to 2.5 cm for mice.

In both cases, the ladders were elevated horizontally 30 cm above the ground, with a neutral cage placed in the starting position and the animal's home cage placed at the opposite end of the ladder (Figure 1). The between-wall distance was adjusted leaving 1 cm wider than the size of rodent to prevent the animal turning around during the crossing [13, 14, 24].

----- Figure 1 here -----

Figure 1. Schematic illustration of the ladder rung walking test apparatus.

The pattern of the metal rungs demands different degrees of walking adaptability and can be used to vary the complexity of the test. A regular arrangement allows animals to learn the position of the rungs over training sessions and to anticipate limb placement (Figure. 1, Symmetrical Pattern). In irregular patterns, rungs are randomly repositioned in each trial to prevent the rodents learning the rung sequence. Thus, irregular patterns are more useful when studying walking adaptability (Figure. 1, Asymmetrical Pattern) [7, 13, 14]. In this study, only irregular rung patterns were analyzed.

In the test, the animals were placed at the beginning of the ladder, walked along it, adapting their foot placement on the rungs until reaching the home cage (Figure 1). While performing the test, we filmed the rodents using a camera (GoPro Hero 4, 12 megapixels). An acquisition rate of 240 frames per second (FPS) in a lateral view was adopted allowing a *post-hoc* frame-by-frame video analysis.

2.2 Foot Fault Scoring System

To assess the fore and hindlimb placement on the rungs, which requires precise and coordinated foot positioning as well as stride and inter-limbic coordination a quantitative foot fault scoring system [14] derived from a categorical analysis was used. In the system, a

frame-by-frame video recording analysis is performed to identify the steps in each limb and qualify foot placement using a 7-point category scale [14, 15] (Table 1). The score 0 is given when the limb did not touch the rung (missed a rung) and resulted in a fall (total miss). A fall is considered when the limbs fell between rungs and the animal's posture and balance are disturbed. Score 1 is given when the limb slipped off a rung and a fall occurred (deep slip). Score 2 is given when the limb slipped off a rung during weight bearing, but a fall did not occur and the rodent interrupts walking (slight slip). Score 3 is given when, before weight bearing the limb on a rung, the rodent quickly lifted and placed the foot on another rung (replacement). Score 4 occurs when the limb is clearly about to be placed on a rung, but the rodent quickly changes the feet placement to another rung without touching the first rung (correction). Score 4 is also given when the limb is placed on a rung, but the animal removes the foot and repositions it on the same rung. Score 5 is given when the limb is placed on the rung either using the wrist or digits for the forelimb or heel or toes for the hindlimb (partial placement). Finally, score 6 is given when the full body weight bearing is applied on a rung with the midportion of the foot (correct placement) (Table 1).

Table 1. Rating scale for foot placement in the LRWT.

Category	Type of foot misplacement
0	Total miss
1	Deep Slip
2	Slight Slip
3	Replacement
4	Correction
5	Partial Placement
6	Correct Placement

The score given in each category is then multiplied by the frequency of foot placements in the same category. Afterwards, the sum of all the categories provides the total combined score (sum of the forelimb plus the hindlimb scores). The fully explained video protocol and all technical details to apply the foot fault score were previously published by Metz and Whishaw (2009).

In this study the following outcomes in the LRWT were assessed for inter-rater and intra-rater agreement: Total Crossing Time, Number of Stops, Total Time Stopped, Scores 0 to 6 for forelimb, Total Score for forelimb, Scores 0 to 6 for hindlimb, Total Score of hindlimb and the Combined Total Score of limbs.

The skilled walking performance score (SWPS) was represented as a percentage of the maximum possible performance (100%) *. The number of cycles (NC) each rodent took to cross the ladder was multiplied by 6 (the maximum score for each cycle in the foot fault score system) and the resulting number was considered the maximum possible performance of each animal in a trial (100%). Then, during a trial, each cycle was rated according to the foot fault score system and the sum of the obtained scores provided the total score in the trial (TS). Finally, the SWPS was represented as a percentage of the maximum possible performance (100%) [7, 25], as follows:

$$SWPS = \frac{(TS * 100)}{(NC * 6)}, \text{ where:}$$

SWPS = skilled walking performance score

TS = total score in the trial

NC: number of cycles

6: maximum score for each cycle in the foot fault score system

2.3 Foot placement reliability between inter- and intra-rater

In order to assess inter- and intra-rater reliability, two independent and blinded raters (called I and II) analyzed 20 rat and 20 mice videos. Each video was analyzed twice by the same rater (80 analyses per rater). The videos were named randomly by another independent researcher (not involved in the analyses) to prevent raters I and II from perceiving half of the videos were the same. Thus, each video had a different number to ensure a blinded

reproducibility analysis. Rater I (Schiavo, A) was inexperienced in the foot fault score and received supervised training before starting data collection. Rater II (Martins, LA) had previous experience and publications using the LRWT [7, 13].

2.4 Statistical Analysis

Descriptive statistics were used to characterize the sample profile in the SWPS. The intraclass correlation coefficient (ICC) and the Kappa coefficient were employed to verify the accuracy of agreement and reliability in the inter-rater and intra-rater analyses of the foot fault scores. Agreement values in ICC greater than 0.75 were considered “excellent”; between 0.4 and 0.75 "satisfactory" and those <0.4 were considered "poor". When negative ICC values (difference between values greater than sample variance) occurred, the data were replaced by zero [26, 27]. The statistical analysis was performed using the software Statistical Package for the Social Sciences (SPSS) 20.0.

3. RESULTS

3.1 Inter-rater reliability for rat

The LRWT analyses in rat demonstrated rater I and II achieved an excellent agreement in the combined total score of limbs (ICC=0.938/p=0.0001). Regarding all the timed outcomes, the total crossing time (ICC=0.994/p=0.0001) and the total time stopped (ICC=0.992/p=0.0001) agreement levels were considered excellent, as were the variable number of stops (ICC=0.957/p=0.0001). Thus, the reliability between the total score for forelimb and hindlimb placement was shown to be excellent.

Furthermore, we analyzed the reliability among all scores described in the test, specifically, in the categories 0 to 6 for each of the limbs evaluated. For the forelimb, the data showed an excellent reliability in scores 0, 1 and 2, varying from ICC 0.839 to 1 (p=0.0001)

as well as for scores 5 and 6 (ICC 0.813 and 0.854, respectively / $p=0.0001$). However, for the forelimb scores 3 and 4, the raters obtained a satisfactory agreement (ICC 0.721 and 0.551, respectively / $p \leq 0.045$). Similarly, for the hindlimb excellent reliability was obtained for scores 0, 1, 3 and 4, with the ICC ranging from 0.889 to 0.931 ($p=0.0001$). The reliability for scores 2, 5 and 6 was also considered satisfactory (Table 2).

Table 2. Agreement between raters I and II regarding the outcomes obtained in the LRWT in rat.

Foot fault scoring in rat			
Outcome	ICC (IC95%)	Cronbach's Alpha	p Value
Combined Total Score	0.938(0.844-0.976)	0.938	0.0001*
Total Crossing Time	0.994(0.985-0.998)	0.994	0.0001*
Number of Stops	0.957(0.892-0.983)	0.957	0.0001*
Total Time Stopped	0.992(0.980-0.997)	0.992	0.0001*
Forelimb Placement			
Outcome			
Score 0	1(1-1)	1	0.0001*
Score 1	0.839(0.594-0.936)	0.839	0.0001*
Score 2	0.903(0.754-0.961)	0.903	0.0001*
Score 3	0.721(0.295-0.889)	0.721	0.004*
Score 4	0.551(0.000-0.822)	0.551	0.045*
Score 5	0.854(0.631-0.942)	0.854	0.0001*
Score 6	0.813(0.528-0.926)	0.813	0.0001*
Total Score	0.879(0.695-0.952)	0.879	0.0001*
Hindlimb Placement			
Outcome			
Score 0	0.889(0.719-0.956)	0.889	0.0001*
Score 1	0.931(0.826-0.973)	0.931	0.0001*
Score 2	0.593(0.000-0.839)	0.593	0.028*
Score 3	0.889(0.719-0.956)	0.889	0.0001*
Score 4	0.889(0.719-0.956)	0.889	0.0001*
Score 5	0.41(0.000-0.620)	0.41	0.456
Score 6	0.592(0.000-0.839)	0.592	0.029*
Total Score	0.931(0.826-0.973)	0.931	0.0001*

* Statistically significant difference.

The individual results for each animal in relation to SWPS are shown in Figure 2. In addition, the frequency of each score (1 to 6) for hindlimb and forelimb of each rodent is shown in Figure 3, 4 and 5.

Figure 2. SWPS obtained by Rater I and Rater II.

Figure 3. SWPS obtained at first (a) and second assessment (b) by Rater I and Rater II.

Figure 4. Frequency of scores in the foot fault score (%).

Figure 5. Frequency of scores in the foot fault score (%) in the first (a) and second assessment (b) by Rater I and Rater II.

3.2 Inter-rater reliability for mice

The inter-rater reliability score system for mice is shown in Table 3. We observed a strong agreement between the raters in the combined total score ($ICC=0.954/P=0.0001$), total crossing time ($ICC=1/P=0.0001$), number of steps ($ICC=0.922/P=0.0001$) and total time stopped ($ICC=0.998/P=0.0001$). In addition, the forelimb and hindlimb placement scores showed excellent agreement in the LRWT, with less consistency for forelimb placement (score 3) ($ICC=0.466/P=0.090$) and hindlimb correction (score 4) ($ICC=0.484/p=0.079$). Overall, the total scores for the forelimb ($ICC=0.925/p=0.0001$) and hindlimb ($ICC=0.919/p=0.0001$) placement between raters I and II showed strong agreement.

226

Table 3. Agreement between raters I and II regarding the outcomes (scores) recorded in the LRWT in mice.

Foot fault scoring in mice			
Outcome	ICC (IC95%)	Cronbach's Alpha	P Value
Combined Total Score	0.954(0.883-0.982)	0.954	0.0001*
Total Crossing Time	1(0.999-1)	1	0.0001*
Number of Stops	0.922(0.802-0.969)	0.922	0.0001*
Total Time Stopped	0.998(0.995-0.999)	0.998	0.0001*
Forelimb Placement			
Outcome			
Score 0	0.889(0.719-0.956)	0.889	0.0001*
Score 1	0.755(0.381-0.903)	0.755	0.002*
Score 2	0.699(0.239-0.881)	0.699	0.006*
Score 3	0.466(0.000-0.789)	0.466	0.090
Score 4	0.904(0.757-0.962)	0.904	0.0001*
Score 5	0.830(0.571-0.933)	0.830	0.0001*
Score 6	0.712(0.271-0.886)	0.721	0.005*
Total Score	0.925(0.812-0.970)	0.925	0.0001*
Hindlimb Placement			
Outcome			
Score 0	0.822(0.550-0.929)	0.822	0.0001*
Score 1	0.889(0.719-0.956)	0.889	0.0001*
Score 2	0.938(0.844-0.976)	0.938	0.0001*
Score 3	0.751(0.371-0.901)	0.751	0.002*
Score 4	0.484(0.000-0.796)	0.484	0.079
Score 5	0.622(0.046-0.850)	0.622	0.0001*
Score 6	0.764(0.405-0.907)	0.764	0.001*
Total Score	0.919(0.795-0.968)	0.919	0.0001*

* Statistically significant difference.

227

228 3.3 Intra-rater reliability for rat

229 Table 4 shows the intra-rater analyses in rat. We found excellent agreement in the
 230 combined total score, total crossing time, number of stops and total time stopped for both
 231 raters. Regarding score evaluation, rater I obtained excellent agreement in all the scores for
 232 the forelimb (ICC between 0.899 to 0.989 / p=0.0001). Rater II achieved excellent agreement
 233 in all scores for forelimb (ICC between 0.787 to 0.920), except for score 6, which was
 234 considered satisfactory (ICC=0.652 / p=0.13).

Table 4. Intra-rater agreement on outcomes in the analyses of the LRWT in rat.

Outcome	Rater I			Rater II		
	ICC (IC95%)	Cronbach's Alpha	P Value	ICC (IC95%)	Cronbach's Alpha	P Value
Combined Total Score	0.969(0.922-0.988)	0.969	0.0001*	0.950(0.875-0.980)	0.950	0.0001*
Total Crossing Time	0.993(0.982-0.997)	0.993	0.0001*	0.981(0.953-0.993)	0.981	0.0001*
Number of Stops	0.950(0.873-0.980)	0.950	0.0001*	0.915(0.786-0.966)	0.915	0.0001*
Total Time Stopped	0.806(0.509-0.923)	0.806	0.0001*	0.939(0.847-0.976)	0.939	0.0001*
Forelimb Placement						
Score 0	0.899(0.719-0.956)	0.899	0.0001*	0.919(0.796-0.968)	0.919	0.0001*
Score 1	0.889(0.719-0.956)	0.889	0.0001*	0.842(0.600-0.937)	0.842	0.0001*
Score 2	0.989(0.973-0.996)	0.989	0.0001*	0.877(0.688-0.951)	0.877	0.0001*
Score 3	0.978(0.944-0.991)	0.978	0.0001*	0.920(0.797-0.968)	0.920	0.0001*
Score 4	0.941(0.851-0.977)	0.941	0.0001*	0.849(0.618-0.940)	0.849	0.0001*
Score 5	0.948(0.869-0.979)	0.948	0.0001*	0.787(0.462-0.916)	0.787	0.001*
Score 6	0.905(0.761-0.963)	0.905	0.0001*	0.652(0.121-0.862)	0.652	0.13
Total Score	0.916(0.787-0.967)	0.916	0.0001*	0.875(0.685-0.951)	0.875	0.0001*
Hindlimb Placement						
Score 0	1(1-1)	1	0.0001*	1(1-1)	1	0.0001*
Score 1	1(1-1)	1	0.0001*	0.962(0.904-0.985)	0.962	0.0001*
Score 2	0.838(0.591-0.936)	0.838	0.0001*	0.829(0.567-0.932)	0.829	0.0001*
Score 3	1(1-1)	1	0.0001*	1(1-1)	1	0.0001*
Score 4	1(1-1)	1	0.0001*	0.904(0.758-0.962)	0.904	0.0001*
Score 5	0.992(0.980-0.997)	0.992	0.0001*	0.637(0.83-0.856)	0.637	0.16
Score 6	0.982(0.954-0.993)	0.982	0.0001*	0.810(0.519-0.925)	0.810	0.0001*
Total Score	0.988(0.970-0.995)	0.988	0.0001*	0.970(0.924-0.988)	0.970	0.0001*

* Statistically significant difference.

In relation to hindlimb agreement, rater I obtained a similar excellent degree of agreement to that for the forelimb, ranging from ICC 0.838 to 1 / $p=0.0001$. Whilst rater II achieved a lower agreement than rater I, the ICC was very good, ranging from 0.637 to 1, with only score 5 graded as satisfactory (ICC 0.637). Moreover, both raters obtained excellent intra-rater scores in the outcomes: combined total score, total crossing time, number of steps, total time stopped and total score for forelimb and hindlimb, ranging from ICC 0.806 to 0.993 for rater I and ICC 0.915 to 0.981 for rater II.

3.4 Intra-rater reliability for mice

Overall, the intra-rater reliability for mice was excellent for both raters (Table 5). For rater I, in the forelimb foot placement agreement for all the 7 scores were excellent (ICC 0.939 to 1 / $p=0.0001$). For rater II, the agreement was also excellent, varying between ICC 0.778 and 0.968 for scores 0 to 5. However, score 6 was considered satisfactory (ICC 0.488 / $p=0.077$). Regarding the hindlimb placement, similar results were found, with the raters only differing in score 6 (rater II obtained a lower ICC: 0.749 / $p=0.002$) (Table 5).

Table 5. Intra-rater agreement on outcomes in the analyses of the LRWT in mice.

Outcome	Rater I			Rater II		
	ICC (IC95%)	Cronbach's Alpha	P Value	ICC (IC95%)	Cronbach's Alpha	P Value
Combined Total Score	0.971(0.926-0.988)	0.971	0.0001*	0.963(0.906-0.985)	0.963	0.0001*
Total Crossing Time	1(1-1)	1	0.0001*	0.999(0.998-1)	0.999	0.0001*
Number of Stops	0.948(0.868-0.979)	0.948	0.0001*	0.774(0.429-0.911)	0.774	0.001*
Total Time Stopped	0.988(0.969-0.995)	0.988	0.0001*	0.985(0.963-0.994)	0.985	0.0001*
Forelimb Placement						
Score 0	1(1-1)	1	0.0001*	0.919(0.796-0.968)	0.919	0.0001*
Score 1	1(1-1)	1	0.0001*	0.778(0.440-0.912)	0.778	0.001*
Score 2	0.979(0.947-0.992)	0.979	0.0001*	0.829(0.568-0.932)	0.829	0.0001*
Score 3	0.979(0.948-0.992)	0.979	0.0001*	0.899(0.746-0.960)	0.899	0.0001*
Score 4	0.939(0.846-0.976)	0.939	0.0001*	0.956(0.888-0.982)	0.956	0.0001*
Score 5	0.982(0.965-0.995)	0.982	0.0001*	0.928(0.817-0.971)	0.928	0.0001*
Score 6	0.950(0.873-0.980)	0.950	0.0001*	0.488(0.000-0.797)	0.488	0.077
Total Score	0.978(0.944-0.991)	0.978	0.0001*	0.934(0.833-0.974)	0.934	0.0001*
Hindlimb Placement						
Score 0	0.919(0.796-0.968)	0.919	0.0001*	1(1-1)	1	0.0001*
Score 1	0.889(0.719-0.956)	0.889	0.0001*	0.889(0.719-0.956)	0.889	0.0001*
Score 2	0.978(0.945-0.991)	0.978	0.0001*	0.963(0.908-0.986)	0.936	0.0001*
Score 3	0.936(0.839-0.975)	0.936	0.0001*	0.821(0.548-0.929)	0.821	0.0001*
Score 4	1(1-1)	1	0.0001*	0.886(0.713-0.955)	0.886	0.0001*
Score 5	0.982(0.953-0.993)	0.982	0.0001*	0.861(0.649-0.945)	0.861	0.0001*
Score 6	0.958(0.895-0.983)	0.958	0.0001*	0.749(0.367-0.901)	0.749	0.002*
Total Score	0.950(0.873-0.980)	0.950	0.0001*	0.951(0.876-0.981)	0.951	0.0001*

* Statistically significant difference.

4. DISCUSSION

Studying walking adaptability in rodents is of importance to translational neuroscience, since the irregular distribution of the rungs in the walking path requires the animal's capacity to adjust its stride length, paw placement and control the center of mass. These adaptive motor control strategies are also found and widely studied in humans [28]. Rodents and humans perform some similar movements to protect an injured limb and/or prevent falls [29]. The ladder rung walking test fulfils the fundamental principles of walking adaptability such as pattern of rhythmic reciprocal limb movement; supporting body balance against gravity; and adapting locomotion in response to environmental challenges [1].

Metz and Whishaw created the ladder rung walking test in 2002 to assess forelimb and hindlimb stepping, placing, and coordination in models of cortical and subcortical injury. According to the authors, the test is a sensitive skilled task for assessing slight impairments of walking function and is useful when assessing functional recovery following brain or spinal cord injury and the effectiveness of rehabilitative therapies [14, 30]. Locomotion during the ladder rung walking test is known to depend on ascending and descending neural pathways, since accurately crossing the rungs requires finely adjusted motor control, balance, limb coordination and muscle control [7, 13, 14].

However, to determine the psychometric properties of behavioral tests it is essential to obtain reliable, consistent and scientifically valid findings [31]. Both, intra- and inter-rater agreement are important metrics to ensure reliability and reproducibility [32]. Here, we sought to assess intra- and inter-rater agreement in the foot fault score of the ladder rung walking test using two strains of rodents – Wistar rats and C57BL/6 mice. Two independent researchers (with and without previous experience using the test's scoring system) analyzed the videos. Our findings suggest the foot fault score system of the ladder rung walking test is a useful, reliable and consistent tool for studying skilled walking performance in rodents. We

also found excellent inter and intra-rater reliability for “total crossing time”, “number of stops” and “total time stopped”. The agreement measures provided by this study suggest data obtained by different research groups using the ladder rung walking test should be comparable [33] and encourage the use of the test in further studies.

The ladder rung walking test is an interesting option for researchers investigating neural mechanisms involved in the ability to adapt walking [7, 13, 15, 34]. Since the score reflects the animal’s ability to adapt limb placement and position in a contextual environment [14, 29], the foot fault score system is useful to study walking adaptability in rodents [7, 13]. Whilst traditional biomechanical models of walking analysis require expensive devices, constant animal handling for placing reflective markers and development of signal-processing routines [35, 36], the ladder rung walking test provides walking adaptability assessment using a fast, simple and inexpensive method.

Whereas we observed satisfactory to excellent intraclass correlation indexes in rating individual scores (0 to 6), caution is necessary when using the foot fault score system. Individual scores present subtle differences that may confuse untrained raters. For example, differentiating between scores 3 (replacement) and 4 (correction) requires attention to identify whether the rodent touched the rung before completing paw placement. Moreover, in some situations, the rodent supports a single paw simultaneously on two rungs that are placed too close each other. This may cause confusion in scoring 5 (partial placement) or 6 (correct placement). In addition, rodents sometimes place their paw on the acrylic wall to help walking forward, a behavior that is not considered in the foot fault score system. Furthermore, the subtle differences between score 1 (deep slip) and 2 (slight slip) may cause uncertainty for untrained raters. Finally, the speed of the video recording may also change the perception of the raters during the gait cycle analysis [37]. Thus, the present results suggest experienced and inexperienced raters can get reliable results if appropriate training is

provided. We highly recommend the careful study of the article and videos previously published by Metz and Whishaw [14, 15] and supervised practice before using the foot fault scoring system.

Despite being originally designed for rats, the ladder rung walking test can be used in mice with some adjustments to the apparatus, namely a) the diameter of the rungs should be reduced to allow a proper grip and paw placement; and b) the minimal and maximal between-rung interval should be changed, as previously described [13, 24]. Our findings show these adaptations are valid to obtain reliable results in C57BL/6 mice and may be valid for other mice strains.

This study has some limitations. First, only two rodent strains were assessed. Anyway, the current findings provide evidence of the accuracy and reliability of the foot fault score in both Wistar rats and C57BL/6 mice. Second, we did not compare specific injury models. Despite which, all individual scores (0 to 6) in the foot fault score were found in the studied videos, which minimize this concern.

5. CONCLUSION

We conclude the foot fault score of the ladder rung walking test is a reliable and useful tool to study walking adaptability in rodents. Moreover, experienced and inexperienced raters can obtain reliable results if previous supervised training is provided. These findings are of importance for researchers working in the field of translational neuroscience and motor control and impact on the comparability of results obtained worldwide using the foot fault score in the ladder rung walking test.

ACKNOWLEDGMENTS

We thank Filipe Mello Medeiros and Rodrigo Orso for their valuable contribution in acquiring the video recordings.

REFERENCES

1. Balasubramanian CK, Clark DJ, Fox EJ. Walking adaptability after a stroke and its assessment in clinical settings. *Stroke Res Treat.* 2014;2014:591013. Epub 2014/09/26. doi: 10.1155/2014/591013. PubMed PMID: 25254140; PubMed Central PMCID: PMC4164852.
2. Ducharme SW, Liddy JJ, Haddad JM, Busa MA, Claxton LJ, van Emmerik REA. Association between stride time fractality and gait adaptability during unperturbed and asymmetric walking. *Hum Mov Sci.* 2018;58:248-59. Epub 2018/03/06. doi: 10.1016/j.humov.2018.02.011. PubMed PMID: 29505917.
3. Houdijk H, van Ooijen MW, Kraal JJ, Wiggerts HO, Polomski W, Janssen TW, et al. Assessing gait adaptability in people with a unilateral amputation on an instrumented treadmill with a projected visual context. *Phys Ther.* 2012;92(11):1452-60. Epub 2012/07/28. doi: 10.2522/ptj.20110362. PubMed PMID: 22836005.
4. Shimada H, Ishii K, Ishiwata K, Oda K, Suzukawa M, Makizako H, et al. Gait adaptability and brain activity during unaccustomed treadmill walking in healthy elderly females. *Gait Posture.* 2013;38(2):203-8. Epub 2012/12/26. doi: 10.1016/j.gaitpost.2012.11.008. PubMed PMID: 23266043.
5. Caetano MJD, Lord SR, Brodie MA, Schoene D, Pelicioni PHS, Sturnieks DL, et al. Executive functioning, concern about falling and quadriceps strength mediate the relationship between impaired gait adaptability and fall risk in older people. *Gait Posture.* 2018;59:188-92. Epub 2017/10/22. doi: 10.1016/j.gaitpost.2017.10.017. PubMed PMID: 29055270.
6. Esteves PO, Oliveira LA, Nogueira-Campos AA, Saunier G, Pozzo T, Oliveira JM, et al. Motor planning of goal-directed action is tuned by the emotional valence of the stimulus: a kinematic study. *Sci Rep.* 2016;6:28780. Epub 2016/07/02. doi: 10.1038/srep28780. PubMed PMID: 27364868; PubMed Central PMCID: PMC4929477.
7. Medeiros FM, de Carvalho Myskiw J, Baptista PPA, Neves LT, Martins LA, Furini CRG, et al. Can an aversive, extinction-resistant memory trigger impairments in walking adaptability? An experimental study using adult rats. *Neurosci Lett.* 2018;665:224-8. Epub 2017/12/13. doi: 10.1016/j.neulet.2017.12.017. PubMed PMID: 29229398.
8. van Ooijen MW, Heeren A, Smulders K, Geurts AC, Janssen TW, Beek PJ, et al. Improved gait adjustments after gait adaptability training are associated with reduced attentional demands in persons with stroke. *Exp Brain Res.* 2015;233(3):1007-18. Epub 2014/12/30. doi: 10.1007/s00221-014-4175-7. PubMed PMID: 25537466.
9. Hawkins KA, Clark DJ, Balasubramanian CK, Fox EJ. Walking on uneven terrain in healthy adults and the implications for people after stroke. *NeuroRehabilitation.* 2017;41(4):765-74. Epub 2017/09/28. doi: 10.3233/nre-172154. PubMed PMID: 28946584; PubMed Central PMCID: PMC5845824.
10. Lanini J, Duburcq A, Razavi H, Le Goff CG, Ijspeert AJ. Interactive locomotion: Investigation and modeling of physically-paired humans while walking. *PLoS One.* 2017;12(9):e0179989. Epub 2017/09/07. doi: 10.1371/journal.pone.0179989. PubMed PMID: 28877161; PubMed Central PMCID: PMC5587243.
11. Mestriner RG, Miguel PM, Bagatini PB, Saur L, Boisserand LS, Baptista PP, et al. Behavior outcome after ischemic and hemorrhagic stroke, with similar brain damage, in rats. *Behav Brain Res.* 2013;244:82-9. Epub 2013/02/14. doi: 10.1016/j.bbr.2013.02.001. PubMed PMID: 23403282.
12. Soleman S, Yip P, Leasure JL, Moon L. Sustained sensorimotor impairments after endothelin-1 induced focal cerebral ischemia (stroke) in aged rats. *Exp Neurol.* 2010;222(1):13-24. Epub 2009/11/17. doi: 10.1016/j.expneurol.2009.11.007. PubMed PMID: 19913535; PubMed Central PMCID: PMC2864515.
13. Wearick-Silva LE, Orso R, Martins LA, Creutzberg KC, Centeno-Silva A, Xavier LL, et al. Dual influences of early life stress induced by limited bedding on walking adaptability and Bdnf/TrkB and Drd1/Drd2 gene expression in different mouse brain regions. *Behav Brain Res.* 2019;359:66-72. Epub 2018/10/23. doi: 10.1016/j.bbr.2018.10.025. PubMed PMID: 30347225.

- 377 14. Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder
378 rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J*
379 *Neurosci Methods*. 2002;115(2):169-79. Epub 2002/05/07. doi: 10.1016/s0165-0270(02)00012-2.
380 PubMed PMID: 11992668.
- 381 15. Metz GA, Whishaw IQ. The ladder rung walking task: a scoring system and its practical
382 application. *J Vis Exp*. 2009;(28). Epub 2009/06/16. doi: 10.3791/1204. PubMed PMID: 19525918;
383 PubMed Central PMCID: PMCPMC2796662.
- 384 16. Antonow-Schlorke I, Ehrhardt J, Knieling M. Modification of the ladder rung walking task-
385 new options for analysis of skilled movements. *Stroke Res Treat*. 2013;2013:418627. Epub
386 2013/04/12. doi: 10.1155/2013/418627. PubMed PMID: 23577278; PubMed Central PMCID:
387 PMCPMC3610362.
- 388 17. Guo Y, Hu H, Wang J, Zhang M, Chen K. Walking Function After Cervical Contusion and
389 Distraction Spinal Cord Injuries in Rats. *J Exp Neurosci*. 2019;13:1179069519869615. Epub
390 2019/08/29. doi: 10.1177/1179069519869615. PubMed PMID: 31456646; PubMed Central PMCID:
391 PMCPMC6702777.
- 392 18. Sandner B, Puttagunta R, Motsch M, Bradke F, Ruschel J, Blesch A, et al. Systemic epothilone
393 D improves hindlimb function after spinal cord contusion injury in rats. *Exp Neurol*. 2018;306:250-9.
394 Epub 2018/02/07. doi: 10.1016/j.expneurol.2018.01.018. PubMed PMID: 29408734.
- 395 19. Faraji J, Metz GA. Sequential bilateral striatal lesions have additive effects on single skilled
396 limb use in rats. *Behav Brain Res*. 2007;177(2):195-204. Epub 2006/12/22. doi:
397 10.1016/j.bbr.2006.11.034. PubMed PMID: 17182115.
- 398 20. Ueda Y, Misumi S, Suzuki M, Ogawa S, Nishigaki R, Ishida A, et al. Disorganization of
399 Oligodendrocyte Development in the Layer II/III of the Sensorimotor Cortex Causes Motor
400 Coordination Dysfunction in a Model of White Matter Injury in Neonatal Rats. *Neurochem Res*.
401 2018;43(1):136-46. Epub 2017/08/02. doi: 10.1007/s11064-017-2352-3. PubMed PMID: 28762105.
- 402 21. Metz GA, Ng JW, Kovalchuk I, Olson DM. Ancestral experience as a game changer in stress
403 vulnerability and disease outcomes. *Bioessays*. 2015;37(6):602-11. Epub 2015/03/12. doi:
404 10.1002/bies.201400217. PubMed PMID: 25759985.
- 405 22. Bauman JM, Chang YH. High-speed X-ray video demonstrates significant skin movement
406 errors with standard optical kinematics during rat locomotion. *J Neurosci Methods*. 2010;186(1):18-
407 24. Epub 2009/11/11. doi: 10.1016/j.jneumeth.2009.10.017. PubMed PMID: 19900476; PubMed
408 Central PMCID: PMCPMC2814909.
- 409 23. Filipe VM, Pereira JE, Costa LM, Maurício AC, Couto PA, Melo-Pinto P, et al. Effect of skin
410 movement on the analysis of hindlimb kinematics during treadmill locomotion in rats. *J Neurosci*
411 *Methods*. 2006;153(1):55-61. Epub 2005/12/13. doi: 10.1016/j.jneumeth.2005.10.006. PubMed
412 PMID: 16337686.
- 413 24. Farr TD, Liu L, Colwell KL, Whishaw IQ, Metz GA. Bilateral alteration in stepping pattern after
414 unilateral motor cortex injury: a new test strategy for analysis of skilled limb movements in
415 neurological mouse models. *J Neurosci Methods*. 2006;153(1):104-13. Epub 2005/11/29. doi:
416 10.1016/j.jneumeth.2005.10.011. PubMed PMID: 16309746.
- 417 25. Altamentova S, Rumajogee P, Hong J, Beldick SR, Park SJ, Yee A, et al. Methylprednisolone
418 Reduces Persistent Post-ischemic Inflammation in a Rat Hypoxia-Ischemia Model of Perinatal Stroke.
419 *Transl Stroke Res*. 2020;11(5):1117-36. Epub 2020/03/07. doi: 10.1007/s12975-020-00792-2.
420 PubMed PMID: 32140998.
- 421 26. Bartko JJ. On various intraclass correlation reliability coefficients. *Psychological Bulletin*.
422 1976;83(5):762-5. doi: 10.1037/0033-2909.83.5.762.
- 423 27. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull*.
424 1979;86(2):420-8. Epub 1979/03/01. doi: 10.1037//0033-2909.86.2.420. PubMed PMID: 18839484.
- 425 28. Beauchet O, Herrmann FR, Grandjean R, Dubost V, Allali G. Concurrent validity of SMTEC
426 footswitches system for the measurement of temporal gait parameters. *Gait Posture*.
427 2008;27(1):156-9. Epub 2007/02/13. doi: 10.1016/j.gaitpost.2006.12.017. PubMed PMID: 17291765.

- 428 29. Jacobs BY, Kloefkorn HE, Allen KD. Gait analysis methods for rodent models of osteoarthritis.
429 Curr Pain Headache Rep. 2014;18(10):456. Epub 2014/08/28. doi: 10.1007/s11916-014-0456-x.
430 PubMed PMID: 25160712; PubMed Central PMCID: PMC4180257.
- 431 30. Riek-Burchardt M, Henrich-Noack P, Metz GA, Reymann KG. Detection of chronic
432 sensorimotor impairments in the ladder rung walking task in rats with endothelin-1-induced mild
433 focal ischemia. J Neurosci Methods. 2004;137(2):227-33. Epub 2004/07/21. doi:
434 10.1016/j.jneumeth.2004.02.012. PubMed PMID: 15262065.
- 435 31. Souza ACd, Alexandre NMC, Guirardello EdB. Propriedades psicométricas na avaliação de
436 instrumentos: avaliação da confiabilidade e da validade. Epidemiologia e Serviços de Saúde.
437 2017;26:649-59.
- 438 32. Meseguer-Henarejos AB, Sánchez-Meca J, López-Pina JA, Carles-Hernández R. Inter- and
439 intra-rater reliability of the Modified Ashworth Scale: a systematic review and meta-analysis. Eur J
440 Phys Rehabil Med. 2018;54(4):576-90. Epub 2017/09/14. doi: 10.23736/s1973-9087.17.04796-7.
441 PubMed PMID: 28901119.
- 442 33. Hutchinson D, Hines S, Vijayaraghavan N, Sammond A, Metzler-Wilson K, Kuchera ML.
443 Interexaminer reliability study of a standardized myofascial diagnostic technique of the superior
444 thoracic inlet. J Bodyw Mov Ther. 2017;21(3):658-63. Epub 2017/07/29. doi:
445 10.1016/j.jbmt.2017.05.004. PubMed PMID: 28750981.
- 446 34. Metz GAS, Schwab ME, Welzl H. The effects of acute and chronic stress on motor and
447 sensory performance in male Lewis rats. Physiology & Behavior. 2001;72(1-2):29-35. doi:
448 10.1016/S0031-9384(00)00371-1.
- 449 35. Berryman ER, Harris RL, Moalli M, Bagi CM. Digigait quantitation of gait dynamics in rat
450 rheumatoid arthritis model. J Musculoskelet Neuronal Interact. 2009;9(2):89-98. Epub 2009/06/12.
451 PubMed PMID: 19516084.
- 452 36. Vrinten DH, Hamers FF. 'CatWalk' automated quantitative gait analysis as a novel method to
453 assess mechanical allodynia in the rat; a comparison with von Frey testing. Pain. 2003;102(1-2):203-
454 9. Epub 2003/03/07. doi: 10.1016/s0304-3959(02)00382-2. PubMed PMID: 12620612.
- 455 37. Spitz J, Put K, Wagemans J, Williams AM, Helsen WF. Does slow motion impact on the
456 perception of foul play in football? Eur J Sport Sci. 2017;17(6):748-56. Epub 2017/03/30. doi:
457 10.1080/17461391.2017.1304580. PubMed PMID: 28350233.

458

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5