International Journal of Animal Behavior and Cognition, 2025, 1(1), 1-5
https://www.hillpublisher.com/Journals/ijabc/
ISSN Online:

Factors Influencing Neuromotor Function Assessment in Mice: mNSS and Grid Walk Tests

Xiaohui Li

Guangdong Country Garden School, Country Garden, Beijiao Town, Shunde, Foshan 528312, Guangdong, China.

How to cite this paper: Xiaohui Li. (2025). Factors Influencing Neuromotor Function Assessment in Mice: mNSS and Grid Walk Tests. *International Journal of Animal Behavior and Cognition*, 1(1), 1-5.
DOI: 10.26855/ijabc.2025.12.001

Received: September 7, 2025
Accepted: October 28, 2025
Published: November 14, 2025

***Corresponding author:** Xiaohui Li, Guangdong Country Garden School, Country Garden, Beijiao Town, Shunde, Foshan 528312, Guangdong, China.

Abstract

This study aims to evaluate the motor capacity of mice using standardized assessment methods, highlighting the influence of individual differences such as age, weight, and gender. The motor abilities of both male and female mice were comprehensively assessed by applying two behavioural tests—the Modified Neurological Severity Score (mNSS) and the Grid Walk test. The results indicated that age and weight were the primary factors affecting physical performance, while gender played a minor role. Furthermore, this study underscores the importance of considering additional variables, even under standardized testing conditions, as they may influence the outcomes of mNSS and Grid Walk assessments. Possible strategies to enhance the reliability and reproducibility of these behavioral tests are also discussed.

Keywords

Neurodegenerative diseases; mNSS; mice; genders; age

1. Introduction

Neuromotor function encompasses motor coordination, balance, reflex integration, and the involvement of the basal ganglia and spinal pathways. Clinically, neuromotor dysfunction is a common hallmark of traumatic brain injury and neurodegenerative diseases such as stroke and Parkinson's disease [1]. Therefore, developing appropriate animal models for mechanistic studies and therapeutic exploration is essential. Among these models, mice have become the predominant species due to their genetic manipulability, high neurobiological relevance, and relatively low maintenance cost. However, even under standardized procedures and controlled environmental conditions, researchers observe considerable individual variability in motor performance that cannot be solely attributed to experimental interventions. This raises important questions about the biological and ecological factors contributing to these differences.

Currently, two commonly used behavioural tests for assessing motor function in mice are the Modified Neurological Severity Score (mNSS) and the Grid Walking test. The mNSS comprehensively evaluates motor coordination, sensory function, reflexes, and balance, which is instrumental in brain injury studies [2]. In contrast, the Grid Walking test offers a more precise and quantitative measure of skilled locomotor ability by analysing footfall errors and is especially sensitive to spinal cord deficits [3]. While these methods have advanced our understanding of motor system function, the semi-quantitative nature of mNSS scoring introduces an element of subjectivity. Additionally, individual differences—such as sex and age—can influence behavioural outcomes. For instance, research has shown that adolescent and adult female rats exhibit more vigorous locomotor activity and heightened anxiety-like behaviors compared to males in open field (OF) and light-dark (LD) tests [3]. Environmental factors also play a role; studies indicate enriched environments can stimulate neuroplasticity and aid recovery from conditions like stroke [4].

The present study addresses these challenges through three primary objectives. First, we aim to refine and standardize the protocols for mNSS and Grid Walking tests to improve their reproducibility and objectivity. Second, we will systematically evaluate individual behavioral differences across repeated trials to identify consistent phenotypic subgroups. Third, we will explore possible biological correlates of these motor performance differences using physiological analyses. In parallel, we will review and integrate relevant literature to interpret these findings, offering insights into the potential mechanisms underlying the observed variability.

2. Materials and Methods

2.1 mNSS tests

2.1.1 Materials

The experiment used C57BL/6J mice, divided into three groups: three 6-week-old male mice, three 6-week-old aged 6 weeks, and three male mice aged 28 weeks. Experimental equipment included a balance beam (2 cm wide, 50 cm long), a foam box, and an electronic balance.

2.1.2 Scoring System and Procedure

This experiment utilized the enhanced Modified Neurological Severity Score (mNSS) system developed by Chen et al. [5]. The scoring system consists of four major categories of neurological tests, with a total score ranging from 0 to 18 points. A higher score indicates more severe neurological impairment. The detailed procedure is as follows:

1) Body Weight Measurement

Each mouse was weighed with an electronic balance, and the weight was recorded for further analysis.

2) Motor Function Test

The motor function test includes the tail suspension and flat ground walking evaluations. In the tail suspension test, the mouse is gently lifted by the tail, suspending it in mid-air. Forelimb and hindlimb flexion are assessed individually: no flexion is scored as 0, while the presence of flexion is scored as 1 point. Additionally, head deviation is measured; if the deviation angle exceeds 10°, 1 point is given; otherwise, the score is 0. In the flat ground walking test, the mouse is placed on a flat surface and observed for 30 seconds. A straight-line gait is scored as 0, uncoordinated walking is scored as 1, circling to one side is scored as 2, and falling to one side is scored as 3 points.

3) Sensory Function Test

Sensory evaluation includes the visual/tactile placement and somatosensory tests. In the placement test, the experimenter gently touches the palm of the mouse's forepaw or approaches the table's edge near it. [6]A typical response, such as retraction or adjustment, is scored 0, while no reaction is scored 1 point. The mouse's forelimb is gently pressed against the tabletop for the somatosensory test. Resistance to this pressure indicates normal function and is scored 0, whereas the absence of resistance is scored 1 point.[7]

4) Beam Balance Test

The mouse is placed in the centre of a narrow wooden beam (2 cm wide, 50 cm long), and its behaviour is observed over 60 seconds. A stable and balanced posture receives 0 points. If the mouse grasps the edge of the beam, it is scored as 1 point. If one limb is suspended, the score is 2; if two limbs are suspended or the mouse rotates in place, 3 points are given. If the mouse attempts to balance but falls within 40 seconds, 4 points are provided; a fall within 20 seconds results in 5 points. If the mouse makes no attempt to balance and falls within 20 seconds, it is scored as 6 points.

5) Reflexes and Abnormal Movements

This section assesses several reflexes and abnormal motor responses. [8] The auricular reflex is tested by lightly touching the ear canal with a cotton swab—a head movement scores 0, while no reaction scores 1 point. The corneal reflex is checked by gently touching the cornea: blinking yields 0 points, and no blink response is scored as 1 point. For the startle reflex, an immediate noise stimulus is presented; a movement response is scored as 0, and absence of reaction as 1 point. Abnormal motor activity, such as epileptic seizures or muscle spasms, is also recorded; its presence scores 1 point, while its absence scores 0.

6) Interpretation of Total Score

The total mNSS score is calculated by summing the scores from all test categories, with a maximum of 18 points. Scores ranging from 13 to 18 indicate severe neurological impairment; scores between 7 and 12 represent moderate impairment; scores 1 to 6 suggest mild impairment; and 0 denotes normal neurological function.

2.2 Grid tests

2.2.1 Materials

This experiment used C57BL/6J mice, including three 6-week-old males, three 6-week-old females, and three 28-week-old males. The equipment included a horizontal grid apparatus, video recording devices, timing devices, and an electronic balance.

2.2.2 Experimental Procedure

Before testing, each mouse was weighed using an electronic balance, and the weight was recorded.

During the test, each mouse was gently placed at the starting point of the horizontal grid apparatus. Simultaneously, the video recording system and timing device were activated to record the mouse's movement for over 3 minutes. No human interference was allowed during the test to ensure unbiased behavioral observation.

Error Step Determination:

An error step was defined as a complete misplacement of the forelimb through the grid opening, confirmed by at least two consecutive video frames. The frequency of error steps was calculated by dividing the number of forelimb errors by the total number of steps taken during the test.

3. Results

As shown in Figure 1, In the mNSS test, the body weights of the 6-week-old male mice were 22.1 g, 23.2 g, and 23.4 g. All three male mice had identical mNSS scores of 1, indicating minimal neurological impairment. The weights of the 6-week-old female mice were 17.5 g, 17.8 g, and 20.3 g, and they also showed mNSS scores of 1, consistent with minimal impairment. In contrast, the 28-week-old male mice had significantly higher body weights of 31.4 g, 35.5 g, and 38.4 g, accompanied by increased mNSS scores of 4, 5, and 5, suggesting that neurological deficits associated with age are more pronounced.

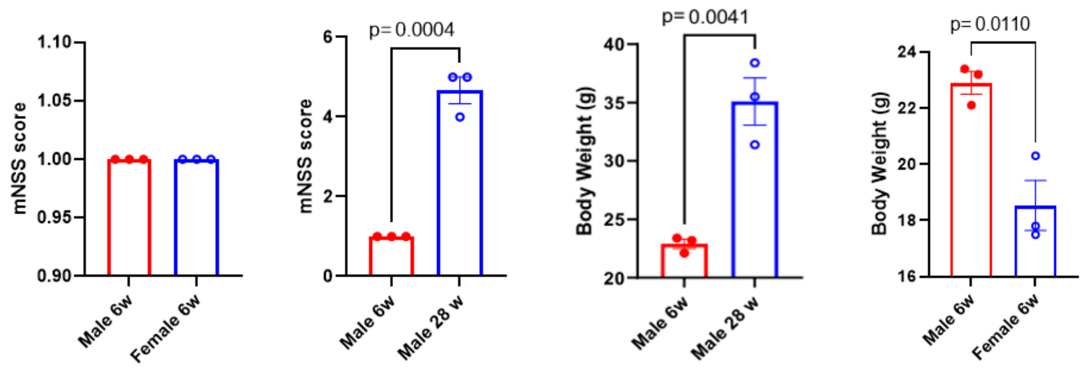

Figure 1. Difference in mNSS scores of mice (*p < 0.05).

Figure 2 shows that, In the Grid Walk Test, the 6-week-old female mice had error rates of 1.38%, 1.21%, and 1.32%. Similarly, the 6-week-old male mice showed error rates of 1.40%, 1.36%, and 1.05%. In contrast, the 28-week-old male mice displayed significantly higher error rates of 1.52%, 1.69%, and 2.27%. This suggests a decline in fine motor coordination and an increase in foot placement errors as the mice age.

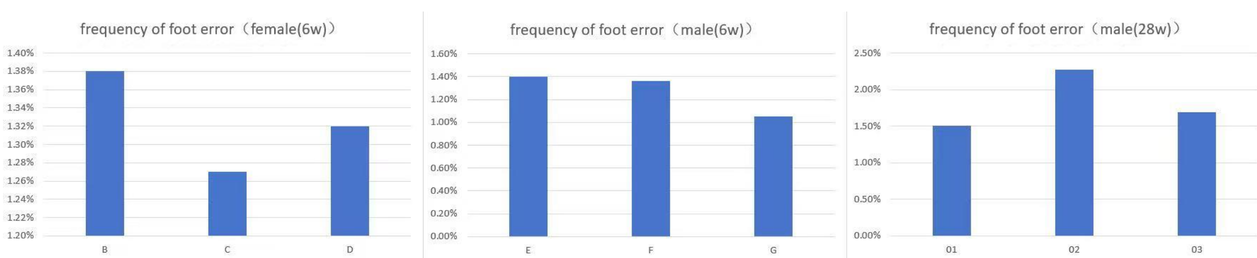

Figure 2. Comparison of total steps and misstep rate in mice.

The results indicate that gender had little to no effect on performance in the mNSS and Grid Walk tests, with both male and female mice at 6 weeks showing similar scores and error rates. In contrast, age proved to be a significant influencing factor. Older mice (28 weeks), which also had higher body weights, demonstrated more pronounced deficits in neuromotor function, as indicated by elevated mNSS scores and increased error rates in the Grid Walk test. This finding suggests that age—and the associated changes, such as weight gain or neurodegenerative processes—may greatly influence the outcomes of behavioral assessments. Therefore, when interpreting motor function test results, particularly in studies related to neurodegeneration or age-related behavioral changes, age should be considered a critical factor.

Gender did not influence the mNSS and Grid Walk assessment systems; however, there is a stronger correlation between age and these evaluations. Additionally, both age and weight can impact the assessment results, with age being a key factor affecting the Grid Walk test.

4. Discussion

This experiment highlights the influence of various factors on motor function assessment in mice. Notably, age and body weight emerged as the primary variables affecting performance, while gender seemed to have a minimal impact. These findings contrast with those of Indra R. Bishnoi et al., who reported significant gender-related differences in behavior. One possible explanation for this discrepancy is the relatively small sample size of female mice in the current study, which may have limited the statistical power to detect subtle sex-based differences. Nevertheless, our results suggest that female mice may be equally suitable for behavioral experiments, and that aging represents a crucial and potentially underexplored area in neuroscience research.

Despite these insights, several limitations must be acknowledged that could impact the accuracy and generalizability of the findings. Firstly, the small sample size (n=3 per group) limits the representativeness of the data and hinders broader extrapolation. Secondly, the timing of the behavioral tests, which were conducted during the mid-light phase (ZT5–ZT7), may have influenced the outcomes. Although the environmental conditions were carefully controlled for consistency, circadian rhythms remain a confounding factor. As nocturnal animals, rodents typically exhibit a 30–40% reduction in basal activity during the daytime, especially around midday. Therefore, the spontaneous motor behaviors observed in this study may not fully represent the mice's capabilities during their active (dark) phase. Additionally, circadian fluctuations in neurotransmitters such as dopamine and serotonin could affect the results of cognitive and motor assessments. To address these issues, future studies should consider conducting parallel experiments at multiple circadian time points to ensure a more comprehensive behavioral profile.

A potential limitation of the study is the subjectivity in data recording, particularly in the Grid Walk test, where manual scoring might lead to errors. To improve accuracy, automated tracking systems using AI are recommended, providing frame-by-frame precision and enhancing objectivity in behavioral analysis.

In summary, while this study offers valuable preliminary insights into age- and weight-related effects in neuromotor assessments, future research should expand sample sizes, ensure balanced sex representation, and consider circadian influences. Additionally, using AI-assisted analysis tools could further enhance the reliability of behavioral neuroscience experiments.

References

[1] Schaar KL, Brenneman MM, Savitz SI. Functional assessments in the rodent stroke model. J Cereb Blood Flow Metab. 2010;30(7):1391-407.

[2] Han Y, Hao, Zhang, Zhang X, Hao. Modified Neurological Severity Score (mNSS). Chin J Neurol. 2021;54(2):148-51.

[3] Bishnoi IR, et al. Sex and age differences in locomotor and anxiety-like behaviors in rats: from adolescence to adulthood. Dev Psychobiol. 2021;63(3):358-71.

[4] van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci. 2000;1(3):191-8.

[5] Alam JJ, Krakovsky M, Germann U, Levy A. Continuous administration of a p38α inhibitor during the subacute phase after transient ischemia-induced stroke in the rat promotes dose-dependent functional recovery accompanied by increase in brain BDNF protein level. PLoS One. 2020;15(5):e0233073.

[6] Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab. 2012;32(9):1677-98.

[7] Hewett SJ, Jackman NA, Claycomb RJ. Interleukin-1beta in Central Nervous System Injury and Repair. Eur J Neurodegener Dis. 2012;1(2):195-211.

[8] Lambertsen KL, Finsen B, Clausen BH. Post-stroke inflammation-target or tool for therapy? Acta Neuropathol. 2019;137(5):693-714.