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Original Paper

Methods and Advances in Behavioral Assessment of Spinal Cord Injury in Mice

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Abstract

Spinal cord injury (SCI) refers to the direct or indirect external factors that cause the spinal cord to be damaged, and various sensory, motor and sphincter disorders, abnormal muscle tone, and pathological reflexes appear in the corresponding segments of the injury. Behaviorology is for research. The responses made by individual animal groups and animal communities to adapt to changes in the internal and external environments, and behavioral assessment is one of the important means to judge the extent of spinal cord injury and measure the efficacy of treatment. At present, clinical SCI is still very different from animal models, so a complete and objective motor evaluation system is needed. This paper systematically evaluates the motor function evaluation methods and their respective advantages and disadvantages of various SCI mouse models, and publishes research The user selects appropriate models and evaluation methods according to the needs of experimental therapies.

Keywords

spinal cord injury, mouse model, animal behavior, BMS test, gait analysis

1. Introduction

Spinal cord injury (SCI) is a debilitating condition caused by direct or indirect external trauma to the spinal cord, leading to sensory, motor, and autonomic dysfunctions such as sphincter disorders, abnormal muscle tone, and pathological reflexes (Badhiwala, Wilson, & Fehlings 2019). In severe cases, SCI can lead to irreversible loss of sensory and motor functions, posing significant challenges to patients. Despite ongoing research, effective treatment strategies for SCI remain unavailable.

The use of animal models, particularly rodents, has been instrumental in advancing SCI research and exploring potential therapeutic approaches (Verma et al. 2019). Historically, rats were the preferred rodent model for SCI studies due to their size and suitability for behavioral testing (Chiu, Cheng, & Hsieh 2017). However, mouse models have become increasingly popular in recent years. Mice offer several advantages, including lower costs, ease of handling and breeding, high reproductive rates, and genetic engineering potential (Rosenthal & Brown 2007). These characteristics have established mice as an essential model for investigating SCI.

Behavioral assessments, which evaluate an animal's responses to environmental and physiological changes, are critical for assessing the severity of SCI and the efficacy of therapeutic interventions. Traditional behavioral assessment methods, such as the Tarlov scale, Basso, Beattie, Bresnahan (BBB) score (Basso, Beattie, & Bresnahan, 1995), grid walking test (Kunkel-Bagden, Dai, & Bregman, 1993), inclined plane test (Rivlin & Tator, 1977), and gait analysis (De Medinaceli, Freed, & Wyatt 1982), were initially designed for rats. When applied directly to mouse models, these methods often produced inconsistent or unreliable results, necessitating modifications or alternative approaches (Dergham et al., 2002; Jones et al., 2002). As mouse models became more widely used, researchers developed specialized behavioral assessment methods tailored to their unique characteristics (Sun et al., 2017). These newer methods have addressed previous limitations and are now widely accepted for studying SCI in mice.

This review highlights the behavioral assessment techniques for SCI in mice, discussing their development, advantages, and limitations. By providing a comprehensive overview, this article aims to guide researchers in selecting appropriate behavioral tools for studying spinal cord injury in mice.

2. Behavioral Assessment Techniques for SCI in Mice

2.1 The Inclined Plane Test

The inclined plane test (Rivlin & Tator, 1977), initially introduced by Alex S. Rivlin and colleagues in 1977, is a widely recognized method for evaluating motor function in rodent models of spinal cord injury (SCI). This test assesses the maximum angle at which an animal can maintain its position on an adjustable slope without sliding or falling. During the procedure, the angle of the slope is adjusted in increments of 5 °until the animal demonstrates stability for at least five seconds.

This method enables the quantitative assessment of motor function by requiring animals to utilize both forelimbs and hindlimbs to maintain balance. Its advantages include simplicity, cost-effectiveness, non-invasiveness, high reproducibility, and consistency, making it a reliable tool for studying SCI-induced motor deficits. For example, Haojie Zhang et al. used incline experiments to verify that SCI mice can maintain a larger angle without falling after SS31 treatment (Zhang et al., 2023).However, some studies have reported that sham-operated mice occasionally exhibit a slight decline in performance shortly after surgery, rather than showing stability or improvement as anticipated (Pajoohesh-Ganji et al., 2010). These observations highlight potential limitations of the test and suggest that further methodological refinements may be needed to enhance its reliability and validity in mouse models of SCI.

2.2 Grid Walking Test

The grid walking test (Kunkel-Bagden et al., 1993) is a behavioral assessment method designed to evaluate the ability of spinal cord injury (SCI) animals to control hind paw placement. In this test, animals are placed on a horizontal or inclined grid and trained to navigate the grid in search of rewards, such as food, placed above it. During the task, key behavioral metrics are recorded, including the number of hind paw missteps, total steps taken, and the time required to traverse a specified distance.

Currently, the grid walking test remains a common tool for assessing hind paw placement ability and evaluating the extent of functional recovery in SCI animal models. Chenxi Zhang used grid walking to find that after EIPA treatment, the hind limb errors in mice occurred significantly less than those in the SCI group (Zhang et al., 2025). Its ability to measure subtle motor deficits makes it valuable for determining the effectiveness of therapeutic interventions in spinal cord injury research (Kijima et al., 2023). However, standardization of the method and further optimization may be necessary to enhance its reliability across studies.

While widely used, the grid walking test has certain limitations. One notable drawback is that thin grid lines can make it challenging to accurately identify and evaluate paw missteps. To address this issue, Ahdeat Pajoohesh-Ganji and colleagues proposed a refinement to the method in 2010 (Pajoohesh-Ganji et al., 2010). They suggested focusing on the "gripping type" movement, where the animal places its toes on the grid bars and simultaneously propels the hind limbs forward. This modification improved the test's sensitivity and accuracy in detecting motor impairments.

2.3 SCANET Automatic Animal Movement Analysis System

Since its introduction, the Basso, Beattie, and Bresnahan (BBB) score has become widely accepted and frequently used in spinal cord injury (SCI) research. However, as the importance of mouse models in SCI studies has grown, it has become evident that some aspects of the BBB scoring system, such as the evaluation of hip joint movements, forelimb-hindlimb coordination, and toe positioning, are difficult to assess accurately in mice. To address these challenges, Yuji Mikami and colleagues developed the SCANET automatic animal movement analysis system in 2002, a system specifically designed to improve the evaluation of motor function in SCI mice (Mikami et al., 2002).

The SCANET system consists of a square cage (565 x 565 mm) equipped with two sensors positioned at different heights, forming two parallel horizontal grids. One of the main advantages of this system is its ability to be operated by a single examiner without the need for specialized training, as the data collection and analysis are fully automated.

Experimental studies have demonstrated that the SCANET system is capable of accurately reflecting the degree of SCI and provides a reliable measure of motor function, particularly in models of thoracic hemisection SCI. Due to its precision, objectivity, and ease of use, the SCANET system has become a widely utilized method for assessing motor recovery in SCI animal models (Shinozaki et al., 2011).

2.4 Basso Mouse Scale (BMS) Test

With the widespread use of genetically engineered mice in spinal cord injury (SCI) research (Rosenthal & Brown 2007), the Basso, Beattie, and Bresnahan (BBB) score, along with its modified versions like the mBBB score (Li et al., 2006), has been widely applied to evaluate motor function recovery. However, due to significant differences between rats and mice in hindlimb movements, such as foot dragging, paw rotation, and tail positioning—key elements of the BBB score—certain components of the BBB and mBBB scales (Dergham et al., 2002; Gilad & Gilad, n.d.) are not fully applicable to mice. These limitations led Basso and colleagues to develop the Basso Mouse Scale (BMS) in 2006(Basso et al. 2006), a tool specifically designed to assess motor recovery in mice following SCI.

The BMS test retains similarities to the BBB scale but incorporates adjustments tailored to the unique motor recovery patterns of mice. During the test, a single mouse is placed in an open field with a diameter of 36 inches and allowed to move freely for four minutes. Researchers observe and record various movements, including forelimb-hindlimb coordination during continuous locomotion, body stability, paw positioning, and tail posture. The scoring system consists of a primary and secondary scale, creating a semi-quantitative framework for evaluation. To ensure consistency, the assessment is conducted by two trained researchers who independently score the animal's performance.

The BMS test has proven to be a reliable and reproducible method, providing a more comprehensive evaluation of motor function recovery in SCI mice compared to its predecessors (Xu et al., 2023). It is now one of the most widely used scoring systems for SCI studies in mice (Lu et al., 2024). However, the method has inherent limitations. The scoring process is labor-intensive and heavily reliant on the training and collaboration of the researchers, making it somewhat subjective. Additionally, some parameters lack sensitivity, as the method primarily depends on visible events. Subtle recovery indicators, such as changes in gait speed or spatial distribution, may be overlooked, highlighting the need for further refinement to enhance the sensitivity and objectivity of the BMS test.

2.5 Gait Analysis

2.5.1 Footprint Analysis

Footprint analysis was first introduced by De Medinaceli et al. in 1982 as a method for evaluating the recovery of hindlimb motor function in animals (De Medinaceli et al., 1982). In this technique, the animal's hind feet are dipped in ink or a developer, and the animal is then allowed to walk across a paper surface, leaving a series of footprints. For example, Yu Xu used footprint analysis to analyze that the SCI+CHBP group performed better than the SCI group on the 28th day after injury (Xu et al., 2023). Initially used to assess locomotion in rodents, this method faced challenges, particularly due to the tendency of rodents to position their hind feet in overlapping contact with their forelimbs, which can cause ink overlap and hinder the accuracy of the analysis. Over time, the method was refined by introducing specialized walkways, which helped to reduce this issue and improve the reliability of the footprint analysis for evaluating motor function in SCI models.

2.5.2 CatWalk Analysis

The CatWalk analysis system is an automatic method for studying animal gait and has gained widespread acclaim in the academic community (Hamers et al., 2001). Developed by Frank Hamers and colleagues, CatWalk overcomes the difficulty of evaluating limb coordination due to the rapid movement of rodents. The system provides extensive data on motor function, including parameters such as the Regularity Index, Base of Support, Crossing Time, Stride Length, Contact Area, Print Area, Swing Duration, and the Duration of Tail and Abdominal Drags. Over time, additional parameters have been incorporated, including pressure estimates and measures related to limb coordination (Hamers, Koopmans, & Joosten, 2006).

CatWalk's primary advantage is its ability to assess a wide range of gait parameters with minimal human intervention, thereby reducing the influence of subjective bias. This leads to more reliable and consistent data. However, the system has its drawbacks, including low throughput, slow analysis, and the complexity and cost of the equipment, making it less accessible for some laboratories. Despite these limitations (Beare et al. 2009), the CatWalk analysis remains an invaluable tool for assessing the detailed coordination and recovery of motor function in SCI animals.

2.5.3 TreadScan Analysis

While the BMS scale, developed by Basso et al. in 2006, is widely used for evaluating motor function recovery in mice after SCI, it still exhibits limitations, such as subjectivity, sequentiality, and nonlinearity. Gait analysis provides an objective means to quantify motor behavior post-SCI. One significant advancement in this area was the development of the TreadScan system by Jason E. Beare and colleagues in 2009 (Beare et al., 2009).

The TreadScan system has proven to be highly sensitive for detecting behavioral disorders, particularly following mild to moderate SCI. Key advantages of the TreadScan system include: 1.Elimination of subjectivity in behavioral assessments. 2. High throughput, allowing rapid data collection. 3. The ability to provide objective, quantitative data on numerous gait parameters, including those related to injury. 4. The capability to collect and analyze data related to animal speed.

However, one limitation of the TreadScan system is that it requires the injured animal to be able to maintain weight-bearing movement of its hind limbs during the test, which may not be possible in severe SCI cases.

2.6 BLG Comprehensive Scoring Method

The BMS scale is a widely used tool in SCI research, but to enhance its sensitivity and provide a more quantitative evaluation, researchers have explored integrating additional tests to assess motor function. One such method is the BLG comprehensive scoring system, proposed by Ahdeat Pajoohesh-Ganji et al. in 2010 (Pajoohesh-Ganji et al., 2010). This system combines the BMS score with modified versions of grid walking and ladder climbing tasks, addressing some of the limitations seen in traditional testing methods. For example, in the improved grid walking and ladder climbing tests, instead of counting steps, the focus is placed on the "grip type," where the animal places its toes on the horizontal bar and pushes the hind limbs forward simultaneously, thus measuring grip strength and motor function more accurately. The BLG scoring system converts these grip scores into an ordinal scale, which is then added to the BMS score to provide a more detailed and comprehensive motor function assessment. Literature suggests that the BLG score offers better differentiation between injury levels and lower variability compared to individual tests. The system has several notable advantages, including: 1. High reliability between raters. 2. Low variability between different experimental groups. 3. Strong correlation with injury severity. 4. Improved separation between injury groups, enhancing the sensitivity of recovery assessments. 5. Significant correlation with tissue preservation, allowing for a more comprehensive understanding of recovery.

The BLG comprehensive scoring method is thus considered an important advancement in the field, providing a more nuanced and reliable evaluation of motor function in SCI research (Zhou et al., 2024).

3. Challenges and Future Directions

At present, there are many problems between the behavioral methods of SCI mouse models. Even the commonly used BMS scoring method has inherent problems such as strong subjectivity, cumbersome scoring, and data nonlinearity. In particular, strong subjectivity is an inevitable problem for many behavioral methods. Secondly, most behavioral assessments of lower limb motor function are easy to observe items such as joint movement and whether the foot can step on the ground, and the assessment of subtle movements of the lower limbs is also lacking. Finally, due to differences in behavioral assessments in various laboratories and differences in evaluators, behavioral standardization has not been achieved, which is also a shortcoming of behavioral assessments.

4. Conclusion

Nowadays, there are many research methods for studying SCI mice, and behavioral methods are an indispensable one. There are many behavioral methods for SCI mouse models, but the main ones used by people are mainly several classic behavioral methods such as inclined plane experiment, grid walking, open field experiment, BMS method, gait analysis, etc. MSharif-Alhoseini et al. searched 2870 literatures in 2016 and extracted data showing that almost all of them involved the hind limbs of mice (Sharif-Alhoseini et al., 2017). In addition, several other behavioral assessment methods were used to supplement the BMS method. For example, Ahdeat Pajoohesh-Ganji et al. developed the BLG joint scoring method based on the BMS method, supplemented by improved grid walking and improved ladder climbing experiments. The main development trend of gait analysis will be closely related to the development of computers, such as more intelligent automatic analysis systems and more complete data receiving systems. In addition to behavioral studies on hindlimb motor function, many scholars have begun to develop animal forelimb scales. For example, Karen-Amanda Irvine et al. developed an IBB assessment scale in 2014 to quantitatively evaluate the forelimbs (Irvine et al., 2014), and Anita Singh et al. developed the FLS scoring scale in 2014 (Singh et al., 2014).

The evaluation of recovery after spinal cord injury in experimental animals is no longer limited to behavioral methods, but has begun to use a variety of methods, such as behavioral combined with imaging (Stivers et al., 2017), such as magnetic resonance imaging, for comprehensive evaluation, and biology combined with behavior, or biology, behavior and electrophysiology for comprehensive evaluation. For example, Gracee Agrawal et al. conducted an electrophysiology combined with the BBB method to evaluate rats with different degrees of contusion severity in 2010 (Agrawal et al., 2010), confirming that these are two different but complementary measures. Nowadays, single evaluation methods are gradually decreasing, and more and more people are choosing several behavioral methods for comprehensive evaluation.

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