A simple and reliable behavioral analysis of locomotor function after spinal cord injury in mice

Technical note

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To establish a simple and reliable method to assess the behavioral function after spinal cord injury (SCI) in mice, the authors used an automated animal movement analysis system, SCANET. Two different SCI lesions were created in adult female BALB/c and C57BL/6 mice by transecting both the posterior columns and the left lateral and anterior funiculi or only the left lateral and anterior funiculi at T-8. Control mice underwent laminectomy only. The SCANET system consists of a cage equipped with two crossing sensor frames arranged at different heights, by which small (M1) and large (M2) horizontal movements and the vertical movement involved in rear- ing (RG) can be monitored. The authors assessed locomotor function by determining the M1, M2, and RG scores; to this end, they used the SCANET system and a previously established behavior test, the 21-point open-field Basso-Beattie-Bresnahan (BBB) Locomotor Rating Scale. The results indicated that the RG scores were significantly and consistently different between the spinal cord–injured and control mice, irrespective of the mouse strain or injury model, but that M1 and M2 scores were not. Moreover, there was a statistically positive correlation between the RG score and the BBB Scale score.

For the assessment of locomotor function after SCI, use of the SCANET system in behavioral analysis is simple and the method is highly reproducible. The analysis of vertical movement is useful for assessing the recovery of limb function in mice following thoracic hemisection.

KEY WORDS • locomotor function • spinal cord injury • mouse

THE ultimate goal in the treatment of SCI is to promote functional recovery. Studies in which investigators use animal models require a sensitive and reproducible method for assessing locomotor function after SCI. Well-known traditional methods for measuring functional recovery are the Tarlov Scale, in which a ratting system is used to assess open-field locomotion, and the inclined-plane test developed by Rivlin and Tator. Most other behavioral assessment methods are based on measurements of reflex responses and the kinematics of limb movements. Recently, the BBB Locomotor Rating Scale was developed for the assessment of open-field walking, and it has been used frequently in SCI experiments in rats.

To date, most experimental SCI models have been established in rats because the experimental techniques, including those involving devices for creating SCI lesions, have been best developed for application in the rat. In contrast, methods for assessing behavioral function have not been optimized for spinal cord–injured mice. Although the BBB Scale is a reliable and reproducible tool, it involves assessment of the movement of hip joints, forelimb–hindlimb coordination, and toe clearance, all of which are difficult to judge accurately in spinal cord–injured mice. Because of this, more than two trained examiners blinded to study parameters are required to rate function in mice when using the BBB Scale. Recently, Kuhn and Wrathall reported a method for analyzing functional outcome in mice after SCI by using videotape, but this method also requires two trained examiners blind to study parameters. Mice are extremely suitable for the molecular biological and immunological analyses of SCI because genetically manipulated knockout or transgenic mice are available and immunological data have been generated. Therefore, we require the development of simple and reliable methods for assessing locomotor function that are adaptable in mice after SCI.

An automated motion analysis system, SCANET
MV-10, was developed for measuring spontaneous motor activity in small animals. This system consists of a cage equipped with two crossing sensor frames at different heights. Using this system, three variables — small and large horizontal movements and vertical movement — can be monitored by one examiner without special training. This system has been used for assessing the side effects of various drugs in mice and rats.1,6,13 In this study, we used the SCANET system to assess locomotor function after thoracic hemisection injury in mice and demonstrated that the vertical movement can be an adequate index for the assessment of locomotor function in spinal cord–injured mice.

Materials and Methods

Spinal Cord Lesions

All animal experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals of Keio University School of Medicine. The BALB/c and C57BL/6 female (6-week-old) mice were purchased from SLC (Shizuoka, Japan) for this study. For the surgical procedures, anesthesia was induced by an intraperitoneal injection (0.25–0.30 ml of a solution consisting of 84% bacteriostatic saline, 10% sodium pentobarbital, and 6% ethyl alcohol). Under a microscope, the spinal cord was exposed by partial T-8 laminectomy without contusive injury. The dura mater was cut longitudinally and the posterolateral sulci, posterior rootlets, and posterior columns were identified bilaterally. Iridectomy scissors and a No. 11 scalpel blade were used to cut the spinal cord. Spinal cord injury was produced by transecting: 1) both the posterior columns and the left lateral and anterior funiculi (OH-SCI);10 or 2) was produced by transecting: 1) both the posterior columns and the left lateral and anterior funiculi (LH-SCI).11 Control mice underwent laminectomy only. The muscles were sutured using No. 4-0 nylon, and wound clips were used to close the skin. All procedures were performed under sterile conditions. The mice were returned to cages, which were furnished with a warmed bedding of pine shavings, allowed to recover from surgery, and given unlimited access to food and water. Bladder function was fully restored without manipulation. No antibiotic agents were required.

Behavioral Analysis

SCANET. The automated motion analysis system SCANET MV-10 (Toyo Sangyo Co., Ltd., Toyama, Japan) was developed for measuring spontaneous motor activity in small animals (Fig. 1). This system consists of a square cage (565 × 565 mm) equipped with two crossing sensor frames of 72 (x axis) × 72 (y axis) pairs of near-infrared beam sensors, spaced 6 mm apart and at right angles to each other. The beam sensors thus form two parallel horizontal grids set at different heights. A transparent Plexiglas cage (450 × 450 × 400 mm) for the mouse was centered inside the system. Each pair of sensors was scanned every 0.1 seconds to detect animal movement.

Two different variables of horizontal movements could be monitored by the lower sensors: small horizontal movements of 12 mm or more (M1; 1 U = 6 mm) and large horizontal movements of 60 mm or more (M2; 1 U = 6 mm). For instance, when a mouse traveled 84 mm, M1 and M2 showed 14 U, and when it traveled 48 mm, M1 and M2 showed 8 and 0 U, respectively. The upper sensors monitored the frequency of vertical movement caused by rearing (RG). Incomplete standing actions could be distinguished from RG movements by adjusting the upper sensor frame.

In preliminary experiments, we set both the upper and lower sensors at their lowest position: 3.75 and 8.25 cm, respectively. The lower sensor, however, did not detect the movements of the spinal cord–injured mice on Day 2. Therefore, we set the lower sensor grid at 2.25 cm above the floor of the cage and the upper sensor grid at 6.75 cm by putting a wooden board under the Plexiglas cage (Fig. 1).

To bring the mice to full alertness, they were transferred into a waiting cage under a light, 10 minutes before obtaining the measurement. Each mouse was then individually placed in the SCANET cage and its spontaneous locomotor activity was measured for 10 minutes. The locomotor scores for M1, M2, and RG could be monitored simultaneously. In this study, the scores for M1 and M2 represent the total distances of movement and the RG score represents the frequency of vertical movements in 10 minutes. The room temperature was maintained at 20 ± 2°C.

Basso-Beattie-Bresnahan Scale. Open-field walking was assessed using the BBB Scale as described by Basso, et al.2 This is a 21-point scale in which categories 0 to 7 measure isolated hindlimb joint movements during the early stage of recovery, categories 8 to 13 measure the frequency of stepping and coordination during the intermediate stage, and...
categories 14 to 21 measure paw rotation and dragging of the toes during the late-stage recovery. A locomotor score of 0 indicates no spontaneous left hindlimb movement, and a score of 21 represents normal locomotion.

Histological Analysis

For histological studies, animals were deeply anesthetized by inhalation of diethylether and killed by perfusion with 2% paraformaldehyde in 0.1 M PBS by intracardiac injection. The spinal cord was removed and postfixed with 2% paraformaldehyde in PBS for a few hours at room temperature. Tissue samples were immersed in 10% sucrose in PBS at 4°C for 24 hours, placed in 30% sucrose in PBS for 48 hours, and embedded in OCT compound (Tissue Tek; Sakura Fine Technical Co., Ltd., Tokyo, Japan). The embedded tissues were immediately frozen in liquid nitrogen and stored at −80°C until needed. Frozen sections of 10-µm spinal cord were cut on a cryostat in the axial plane and stained with hematoxylin and eosin.

Statistical Analysis

Statistical analysis was performed using the Statview software package (SAS, Cary, NC). Differences in mean locomotor scores were assessed using the unpaired t-test. The strength of correlation between the RG score and the BBB score was determined using the Pearson correlation coefficient.

Results

Spinal Cord Lesions

Representative axial sections of SCI-induced lesions are shown in Fig. 2. In OH-SCI mice, the lesion showed obvious infiltration of inflammatory cells that had spread to both posterior columns, the left lateral and anterior funiculi, and the contralateral right gray matter by Day 7 postinjury (Fig. 2A). In LH-SCI mice, the cell infiltration was observed mainly in the left lateral and anterior funiculi (Fig. 2B). By Day 56 the OH-SCI lesion showed severe atrophy in the left portion of the spinal cord, characterized by a severe loss of white matter (Fig. 2C).

Time Course of Functional Recovery After SCI

The OH-SCI BALB/c mice characteristically showed bilateral flaccid hindlimb paralysis immediately after surgery. On Day 2 after injury, they displayed weight-supported plantar steps and consistent forelimb–hindlimb coordination in the right hindlimb, but no or only slight movements in the left hindlimb were observed (BBB Scale score < 6; Fig. 3A). On Day 7, most of the mice had recovered forelimb–hindlimb coordination considerably. The recovery of the OH-SCI BALB/c mice reached a plateau phase by Day 14 (BBB Scale Score 13.6 ± 0.5). By Day 56, they still rotated the left hindlimb externally (BBB Scale Score 14.3 ± 0.4). There were highly significant differences in the BBB Scale scores between the
OH-SCI BALB/c mice (14 animals) and control mice (17 animals) throughout the period of analysis (Fig. 3A). The mild-injury model of the LH-SCI BALB/c mouse characteristically showed flaccid paralysis in the left hindlimb immediately after surgery. By Day 2 post-SCI, these mice exhibited plantar steps in the left hindlimb. On Day 56, all the mice showed toe clearance in the left hindlimb (BBB Scale Score 16.3 ± 0.4). As with the OH-SCI mice, there were also highly significant differences in the BBB Scale scores between the LH-SCI BALB/c mice (eight animals) and control mice (17 animals) (Fig. 3A).

To determine if this SCI model could be applied to different strains of mice, we also created the OH-SCI lesion in OH-SCI C57BL/6 mice, in which no observable movements of the left hindlimb by Day 2 postinjury. Their recovery of left hindlimb movement was slower than that in the BALB/c mice. On Day 56, the OH-SCI C57BL/6 mice exhibited plantar steps but few could perform consistent forelimb–hindlimb coordination (BBB Scale Score 11.3 ± 0.5). There were highly significant differences in the BBB

Fig. 3. Locomotor activity following OH- and LH-SCI in adult BALB/c mice. A: The BBB Scale score. There were highly significant differences between OH-SCI or LH-SCI and control mice throughout the period of analysis. The BBB Scale scores measured in the OH-SCI mice were lower than those in the LH-SCI mice; however, there were no significant intergroup differences except on Days 2, 7, and 56. B: Small horizontal movements (M1). The M1 scores demonstrated in the OH-SCI mice were significantly lower than those in control mice throughout the period of analysis. Although the M1 scores in the LH-SCI mice were lower than those in control mice, there were no significant intergroup differences except on Day 28. There were no significant differences between the OH-SCI and LH-SCI mice except on Days 2, 4, and 14. C: Large horizontal movements (M2). The M2 scores obtained in the OH-SCI mice were significantly lower than those in control mice except on Day 7. Although the M2 scores of the LH-SCI mice were lower than those of the control mice, there were no significant intergroup differences except on Days 4 and 28. There were no significant differences between the OH-SCI and LH-SCI mice except on Day 2. D: Vertical RG movement. There were highly significant differences in the RG scores between OH-SCI and control mice throughout the period of analysis (p < 0.05 on Day 4 and p < 0.001 on other days; unpaired t-test). Bars represent means ± standard errors of the means. * p < 0.05; ** p < 0.01; *** p < 0.001 (unpaired t-test).

Fig. 4. Locomotor activity following OH-SCI in adult C57BL/6 mice. A: The BBB Scale score. There were highly significant differences between SCI-treated and control mice throughout the period of analysis. B: Small horizontal movements (M1). There were no significant differences in the M1 score between SCI-treated and control mice after Day 7. C: Large horizontal movements (M2). There were no significant differences in the M2 score between SCI-treated and control mice after Day 7. D: Vertical movement of rearing. There were highly significant differences in the RG score between SCI-treated and control mice throughout the period of analysis. Bars represent means ± standard errors of the means. * p < 0.05; ** p < 0.01; *** p < 0.001 (unpaired t-test).
In this study, we created two SCI models in mice by transecting both the posterior columns, including the corticospinal tract, and the left lateral and anterior funiculi, including the rubrospinal tract (OH-SCI), or the left lateral and anterior funiculi (LH-SCI), at T-8. In the OH-SCI mice severe monoparesis in the left hindlimb was shown, but significant recovery in movements was observed in 2 weeks. Recovery in the LH-SCI mice was such that plantar steps in the left hindlimb were observed by Day 2 and toe clearance by Day 56. Although regeneration of severed neural pathways does not occur in adult mammals, some spontaneous recovery is mediated through compensatory mechanisms. Most descending neural pathways maintain a projection to the contralateral gray matter in addition to their major ipsilateral projection. These pathways can activate hindlimb motor neurons of the contralateral side, not only via commissural interneurons but also via their partial segmental crossing. The spared contralateral descending systems may contribute to the recovery of locomotor function after hemisection of the spinal cord in adult mice.

Previously established locomotor tests for spinal cord–injured animals, such as the Tarlov scale and the inclined-plane test, would not be adequate for application in SCI-treated mice, because it is very difficult to obtain accurate measurements in restless mice. Recently, the BBB Scale was applied to the assessment of functional outcomes in spinal cord–injured mice. Although the BBB Scale score correlates closely with the exploratory activity of animals with low motor ability, in the upper category of animals with low motor ability, in the upper category.
ry of the tool (≥ 13 points), the scale includes rather discrete aspects of movement that do not represent major improvements in an animal’s ability, and the sequence of recovery is often not related to the scaling hierarchy. In fact, it was difficult to determine a score in spinal cord–injured mice in the points of coordination and toe clearance, even when using videotape. Metz, et al., have suggested that second priority tests such as the grid walk and the narrow-beam tests should be used to assess animals showing high locomotor ability (BBB Scale score ≥ 13). In addition, the BBB Scale scores should be assessed by multiple blind examiners because differences in scores among examiners are often seen.

In this study, we used the automated motion analysis system SCANET MV-10 to assess locomotor function in an SCI mouse model. Using SCANET, the assessment of locomotor function is simple and highly reproducible. Motion is electronically detected, and the analysis can be performed by one examiner who has not been specially trained. In our experiments, the horizontal movements (M1 and M2 scores) observed in the OH-SCI BALB/c mice were significantly lower than those in the control mice. In C57BL/6 mice, however, the M1 and M2 scores were not significantly different when the OH-SCI-treated and control mice were examined, except in the first few days postinjury. Because C57BL/6 mice are wilder than BALB/c mice, the horizontal movements of the C57BL/6 mice with monoparesis in the hindlimb appear to be compensated for by the forelimbs and the contralateral healthy hindlimb. Therefore, the M1 and M2 scores may not be reliable indices for assessing long-term locomotor function in the hemisection SCI model. These results also indicate that strain variation should be considered in behavior analysis. In contrast, there were consistently significant differences in the RG scores between the SCI-treated and control mice regardless of the mouse strain or injury model. Because adequate muscular power in both hindlimbs is required for the vertical movement of RG, this activity, after hemisection, would not be fully compensated for by the contralateral hindlimb. In addition, SCI-treated animals have a diminished ability to maintain their balance. Analysis of our results suggests that the SCANET-determined RG score is useful for the assessment of limb function after thoracic hemisection SCI in mice.

Pearson correlation analysis revealed a strong positive relationship between BBB Scale scores greater than 9 and the RG scores in SCI mice regardless of the mouse strain or injury model. The scatterplots of these two parameters demonstrated that the RG scores could exhibit a wider range than the BBB Scale scores when representing the locomotor activity in mild SCI, especially in the range corresponding to high BBB Scale scores (≥ 13 points). The RG score, however, may be less sensitive than the BBB Scale for detecting recovery after severe SCI. To assess locomotor function in SCI-treated mice more accurately, it would be best to analyze the RG score together with the BBB Scale score.

Conclusions

Behavioral analysis in which the SCANET system is used is a simple and highly reproducible method for assessing locomotor function after SCI. The SCANET analysis of vertical movement is useful for assessing the recovery of limb function after thoracic hemisection SCI in mice.

References