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Quantitative assessment of locomotion and interlimb coordination in rats after different spinal cord injuries

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HIGHLIGHTS

- ► Different spinal cord injuries cause differential functional deficits.
- ► A new method to quantify interlimb coordination and gait disturbances in the rat is described.
- ► The quantitative assessment of locomotion discriminates spinal cord injuries of different severity.
- ► Subjective and objective methods combined provide a full description of locomotion.

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ABSTRACT

Animal models of spinal cord injury (SCI) are intended to mimic the main features of human spinal cord lesions, although sometimes it becomes a difficult task to find the right technique to discriminate the severity of the lesion as well as to assess different aspects of functional recovery. For this reason, we have used several functional methods to assess gross and fine locomotion deficits, as well as electrophysiological data to study the dysfunctions underlying the behavioral changes. Moreover, an extensive study based on the quantification of alternation and coordination parameters during gait has been done. Spinal cord injuries of varying severity (mild contusion, moderate contusion and hemisection) were performed at the thoracic level in adult rats that were followed-up for 6 weeks. Lesions resulting in similar scores in the open field test (i.e. mild contusion and hemisection) caused more marked differences in fine coordination when assessed by quantitative coordination analysis based on a digitized walking treadmill. In conclusion, gross and fine deficits can be detected using a battery of tests based on the performance of the animals during tasks of different difficulty. When used appropriately, they become useful tools to study functional recovery due to spontaneous plastic changes or to therapeutic interventions after SCI, as well as to test the effects of new therapies.

1. Introduction

The rat is widely used as a model of human spinal cord injury (SCI), specially spinal cord contusions, the type of lesion that has more clinical relevance and similarities with the lesions affecting humans (Majczyński and Sławińska, 2007). Deficits after SCI are especially noticeable below the lesion site, and affect motor and sensory systems, as well as autonomic functions. Most of the tests used to assess function after SCI are focused in assessing locomotion, especially of the hindlimbs. Several functional tests have already been described, but sometimes it is difficult to chose the right one to discriminate the deficits appearing in every injury and severity. Moreover, sometimes it is also difficult to interpret the obtained results, since animals present spontaneous improvements and compensations of their deficits after SCI, masking the real dimension of the deficits as well as the recovery. This occurs both in animals as in humans (Curt et al., 2008; Gulino et al., 2007; Majczyński and Sławińska, 2007).

Locomotion depends on a spinal network under the influence of supraspinal centers as well as the modulation of the afferent inputs (Majczyński and Sławińska, 2007). After the injury, descending motor tracts may become completely or partially disrupted, and the remaining elements of the network may adapt to the new circuitry to compensate the deficits. Most SCI imply the lesion of some white matter tracts, and depending on which are affected, the deficits can be more or less evident. For instance, while only a

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few preserved ventrolateral tract fibers are needed to allow weight support and plantar paw placement, dorsal tracts are required for skilled movements and coordinated actions of the distal musculature (Drew et al., 2004; Rossignol and Frigon, 2011).

The first aim of this work is to compare the appearance of functional deficits in different SCI. To achieve this objective several techniques are used, such as the BBB scale for open field locomotion, the narrow beam, the inclined plane and the walking track analysis. These tests give subjective and semi-quantitative results, but are fast and require short training of the researcher. Adding objectivity to this kind of methods is important to promote the comparison of results between different models, different treatments and different laboratories. The second objective was to perform a detailed quantitative assessment of the main features related to gait. In this case, the methods are more complex and time-consuming, requiring good training of the researcher. We have used the recordings obtained with the Digigait system to quantify different gait parameters, coordination and alternation. Using simple programs, we provide a novel analysis of locomotion, faster and more discriminative than others reported in the literature. Moreover, the treadmill used in the Digigait system can provide a fine control of speed, avoiding the effects of changing speed in the normal free walking or running of the animals during the analysis. Finally, electrophysiological studies were performed to assess the functionality of central and peripheral circuits, in order to determine the extent of damage below the injury and the functional consequences of SCI of different severity. All these results led to an objective evaluation of locomotor performance after different SCI in the rat, which may be useful when comparing new treatments or rehabilitation techniques aimed to improve motor function and locomotion.

2. Materials and methods

2.1. Laboratory animals

Adult female Sprague Dawley rats (8 weeks old; 250–300 grams) were housed with free access to food and water, at a room temperature of 22 ± 2 °C under a 12:12 h light-dark cycle. All experimental procedures were approved by the Ethics Committee of our institution, and followed the European Communities Council Directive 86/609/EEC. Researchers involved in the different assessments were initially blinded to the injury received by the animals.

2.2. Surgical procedure

Operations were performed under pentobarbital anesthesia (50 mg/kg i.p., Sigma–Aldrich), and after subcutaneous injection of buprenorphine (0.05 mg/kg, Buprex, Schering-Plough) near the incision site. In two groups of rats, after dorsal laminectomy of T8–T9, the spinal cord was contused at the T8 level using the Infinite Horizon Impactor device (Precision Scientific Instruments, Lexington, UK), applying a force of 100 kilodynes (kdyn; group 100 kdyn, n = 10) or 200 kdyn (group 200 kdyn, n = 10). Data from displacement and real force applied were collected for each contusion. In another group of animals a hemisection was performed at T8 level using a thin scalpel to cut only the right part of the spinal cord (hemisection group, n = 10). To ensure that the spinal cord was completely transected in the right side, a thin needle was introduced twice until touching the ventral surface of the vertebral channel. After the injury, the wound was sutured with 5/0 silk thread in the muscular plane and small surgical clips in the skin, and disinfected with povidone iodine. Animals were rehydrated and kept in a warm environment until full recovery from anesthesia. Bladders were expressed twice a day until reflex voiding of the bladder was re-established. To prevent infection, amoxicillin (500 mg/l) was given in the drinking water for one week. No additional analgesia was administered during the follow-up.

2.3. Functional evaluation of locomotion

All animals were tested before surgery and at 42 days thereafter, except for the BBB test that was performed at 3 days post operation (dpo) and then weekly. Since preoperative values did not differ between groups, for convenience all values were considered together and represented as the control "intact group".

2.3.1. Open field locomotion test

Locomotor hindlimb function was assessed using the Basso, Beattie and Bresnahan (BBB) rating scale (Basso et al., 1995). Briefly, the BBB scale consists of an ordinal scale from 0 points (no discernable hind limb movement) to 21 points (consistent, coordinated gait with parallel paw placement of the hind limb and consistent trunk stability). For measuring locomotor recovery, one animal at a time was allowed to walk freely inside a circular plastic tray (90 cm diameter × 24 cm wall height) for 5 min, and two examiners observed the hindlimbs movements of the rat. The final score of each animal was the mean value of both examiners.

2.3.2. Inclined plane test

This test measures the ability of the animals to maintain their position in an inclined plane for at least 5 s. The angle of the surface was progressively increased (starting from a flat surface, 0°), until recording the maximum angle supported by the animal that was scored as the outcome measure. Three trials were done to obtain the average value for each animal and day.

2.3.3. Beam test

For this test, a dark tunnel was designed (tunnel dimensions: 7 cm width, 13 cm height, 40 cm length) to allow the animals walking along an elevated beam (2.5 cm width, 2 cm height, 2 mm separated from the ground to allow the placement of a paper sheet to print the inked paws). The hindpaw plantar surfaces were inked while the animal was gently subjected with a cotton cloth by the researcher. Then, the rat walked along the beam so only the missteps were recorded by ink prints on the paper placed under the beam. Three consecutive runs were performed, and the left and right missteps done in the first 30 cm were counted in each run. The mean of the three values was considered as the result for the animal in each testing day.

2.3.4. Walking track and foot print analysis

It was carried out to assess the recovery of locomotor function and changes in the normal gait posture, adapting the original technique set to study sciatic nerve lesions (De Medinaceli et al., 1982). The plantar surface of the rat paws was painted with red ink for forepaws and blue ink for hindpaws, and the rat left to walk along a corridor of 40 cm × 8 cm × 10 cm with a white paper on the base. Distances between forepaw prints (forelimb stance width) and between hindpaw prints (hindlimb stance width) were measured with a precision device in order to assess the base of support (García-Alías et al., 2010).

2.4. Analysis of locomotion using treadmill tests

Gait video images were obtained with the Digigait Imaging system (Mouse Specifics Inc., Boston, MA) as previously described (Vincelette et al., 2007). Briefly, a high-speed video camera mounted below a transparent treadmill belt captured ventral images of the animal. The images were automatically digitized at 140 frames per second, and a minimum of 7 s of continuous gait were recorded, enough to provide around 10 sequential step cycles.