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Forelimb locomotor rating scale for behavioral assessment of recovery after unilateral cervical spinal cord injury in rats

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ABSTRACT

Background: Cervical spinal cord injury (SCI) models in rats have become increasingly useful because of their translational potential. The goal of this study was to design, develop and validate a quick and reliable forelimb locomotor rating scale for adult rats with unilateral cervical SCI injury.
New method: Adult female rats were subjected to a C5 unilateral mild contusion ($n = 10$), moderate contusion ($n = 10$) or hemisection injury ($n = 9$). Forelimb locomotion was evaluated before injury, four times during the first week (Days 2, 3, 4 and 7) and weekly for up to 8 weeks post-injury. Scoring categories were identified and animals were ranked based on their performance in these categories. The scale was validated for its usefulness by comparing animals with different injury models (dorsolateral funiculotomy C3/4), levels of injury (moderate contusion C4) and sex (male – moderate contusion C3/4) and also by correlating FLS scores with other established behavioral tests (grid walking and kinetic tests).
Results and comparison with existing methods: Forelimb performance on both the grid-walking and kinetic tests was positively correlated with the forelimb locomotor rating scale (FLS). Histological analysis established a positive correlation between the spared tissue and the observed FLS score. Our results show that the new rating scale can reliably detect forelimb deficits and recovery predicted by other behavioral tests. Furthermore, the new method provides reproducible data between trained and naïve examiners.
Conclusion: In summary, the proposed rating scale is a useful tool for assessment of injury and treatments designed to enhance recovery after unilateral cervical SCI.

1. Introduction

Spinal cord injury (SCI) affects 12,000 people every year, with the majority of these injuries occurring at the cervical level (NSCISC, 2008). To enhance the clinical relevance of animal studies, there has been a surge of interest in cervical SCI models in rodents to investigate mechanisms of injury and treatment strategies to enhance functional recovery. Although cervical SCI models are now being used extensively, rapid and reliable behavioral assessments that can capture forelimb function during open field locomotion are needed to provide a description of functional outcome.

Available techniques to assess motor and sensory deficits of the forelimb include: single pellet reaching (McKenna and Whishaw, 1999), tactile discrimination (Allred et al., 2008), grooming (Bertelli and Mira, 1993), grip strength (Anderson et al., 2005), horizontal ladder walking (Soblosky et al., 1997, 2001), limb preference (Shumsky et al., 2003), gait analysis (Hamers et al., 2001), forelimb step-alternation test (Khaing et al., 2012) and kinematic analysis (Metz et al., 1998). Most of these tests require pre-training, which is time consuming, and food or water deprivation, which can interfere with an animal's physiological and behavioral performance (Tucci et al., 2006; Jang et al., 2013; Yanai et al., 2004; Heiderstadt et al., 2000). Also, because many of these tests cannot be performed by animals in the early stages of recovery after injury, the full extent of deficits and early stages of recovery cannot easily be assessed. For example, gait analysis cannot be accurately performed on an animal that is unable to achieve weight support. Thus, there remains a need to develop tests that assess locomotor abilities in animals with forelimb deficits across the full range of behavior post-injury.

To this end, a few scales have been created to assess forelimb locomotor behavior after unilateral and bilateral cervical injuries in rodents. Our group has developed the Forelimb Locomotor Scale (FLS) for use in both surgical and contusive unilateral cervical injury (Cao et al., 2008; Sandrow-Feinberg et al., 2009). Martinez et al.

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(2009) created a modified BBB scale for use in surgical unilateral cervical injury. Anderson et al. (2009) created the Forelimb Locomotor Assessment Scale (FLAS) for use in a midline contusive cervical injury model. Each of these scoring systems has advantages and disadvantages. FLS was designed for high throughput studies to deliver a quick observational score that describes the forelimb's functional capability during locomotion. In the current study, we focused on validating the use of the FLS to assess early deficits and long-term recovery following several different types of cervical injuries including unilateral contusion, dorsolateral funiculotomy and hemisection injuries across commonly used cervical injury levels (C3–C5). We also correlated the FLS scores with grid walking and kinetic measurements of forelimb behavior, as well as with the amount of spared white matter at the injury site. To examine inter-rater reliability, we compared scores from novice raters to those from expert raters and the accuracy of live scoring with scoring from a digital video. By demonstrating the validity and reliability of our FLS score we propose that it can be easily employed in other laboratories.

2. Material and methods

2.1. Animals and groups

A total of 46 (female = 41, male = 5) adult (225–250 g) Sprague–Dawley rats sustained a unilateral cervical SCI of various types and severities. Some of these animals were used for scale development and all were used for scale validation (Table 1).

2.2. Spinal cord injury

All animals were subjected to surgical procedures that were performed in accordance with protocols approved by the Drexel University College of Medicine's Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines for the care and use of laboratory animals. Animals were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg) and a unilateral cervical laminectomy was performed to expose the spinal cord at the appropriate level. For contusion injuries, the vertebral column was stabilized by clamping the vertebral bodies immediately above and below the lesion level with forceps fixed to the base of an Infinite Horizon Impact Device (Precision Systems and Instrumentation, Lexington, KY). Animals were placed on the impactor and the custom-built 1.6 mm stainless steel tip was positioned over the right side at the lesion level such that the tip was immediately above the spinal cord midway between the medial dorsal vein and the lateral edge of the spinal cord. The impactor tip was lowered to 3–4 mm above the dorsal surface of the spinal cord and the field flooded with sterile saline up to the impactor tip. A moderate or mild contusion injury was created by an impact force of 200 kdyne (C200) and 100 kdyne (C100), respectively. After injury, animals were immediately released from the clamping forceps and muscle layers were closed with sutures and the skin incision was closed with wound clips (Krisa et al., 2011; Sandrow-Feinberg et al., 2009). For dorsolateral funiculotomy (DLF), a longitudinal incision was made in the dura to gain access to the spinal cord and the right dorsolateral funiculus was removed by gentle aspiration with a finely pulled glass pipette resulting in a lesion cavity that was 1.5–2 mm in length. For hemisection, the entire right half of the spinal cord was removed by aspiration, resulting in a cavity that was 2–3 mm in length. The dura was closed using a 10-0 suture, the overlying muscles closed with sutures and the skin incision closed with wound clips (Stackhouse et al., 2008). Animals were given ampicillin (100 mg/kg) and the analgesic buprenorphine (0.05 mg/kg) for 3 days post operatively.

2.3. Behavioral testing

After acclimation to the various testing apparatus over a 1-week period, baseline pre-injury scores for Forelimb Locomotor Scale, hindlimb open field test (BBB), grid walking, and kinetic tests were established for each animal. Details of each testing procedure and time-point are described below.

2.3.1. Forelimb open field test

2.3.1.1. Testing procedure. Post-injury, forelimb open-field testing was performed at Days 2, 3, 4 and 7 in the first week during development of the scale, 3 days post-injury during scale validation, and weekly thereafter for 8 weeks for all animals. Rats were placed in an enclosure (2.5 ft × 3 ft diameter) allowing the animal to move freely in the open field. The rats were observed and live scored for 4 min during testing. Forelimb behavior was also recorded by digital video with a minimum of three locomotor passes showing the affected limb. If the animal remained stationary for more than 15 s it was picked up (held at mid-trunk) and placed in the center of the field to reinstate locomotion. While observing the animal in the open field a score sheet was filled out by two observers (see Table 2). The score sheet was designed as a series of simple parameters so that the observations could be translated directly into a score that provided an accurate description of the animal's performance. Also, the use of the score sheet allowed the observer to record the movements as they occurred during testing. No scores were given while the animal was turning, rearing, defecating or urinating.

2.3.1.2. Scale development. Animals with mild contusion, moderate contusion and hemisection injuries at the C5 level were used for scale development. The categories were based on behavioral changes observed after unilateral cervical injury. The scoring order and the scale were determined by the consensus of several experienced BBB raters observing the behavioral changes and assessing typical pattern of recovery over time. Immediately after injury, the joint movements at all three forelimb joints (shoulder, elbow, wrist) were significantly affected and recovery in these joint movements was assessed as none, slight (less than 50% of normal range of motion) or extensive (more than 50%) during locomotion (FLS scores: 0–6). Following improvement in movements of two or all the joint movements, improvement in foot placement was observed. Plantar or dorsal placement of the foot with no, partial or full weight support was observed (FLS score: 7). The improvement further extended to dorsal stepping with partial or full weight support followed by occasional (less than 50%) to frequent (50–95%) to continuous (100%) plantar stepping (FLS scores: 8–11). Once continuous plantar placement was observed, focus was turned to the paw. Paw placement was scored as either parallel or rotated during locomotion (FLS scores: 11–13). Animals were required to have continuous plantar placement before toe clearance was assessed. Toe clearance was assessed to determine if the toe was raised above the ground and cleared during swing phase of locomotion. The paw, however, could be either parallel or rotated. Toe clearance in plantar-stepping animals was then assessed as being occasional (less than 50%), frequent (greater than 50%, but less than 99%) and continuous (100%) (scores: 14–17). A higher score was given to an animal with parallel paw placement and occasional clearance than to an animal with similar clearance but rotated paw. Animals with frequent or continuous toe clearance were often observed to have parallel paw placements. We did not include forelimb-hindlimb coordination, because this function is often spared except in very extensive unilateral injury models where the interlimb locomotion circuits can be affected quite variably depending upon the lesion type.