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Forelimb locomotor assessment scale (FLAS): Novel assessment of forelimb dysfunction after cervical spinal cord injury

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ABSTRACT

We describe here a novel forelimb locomotor assessment scale (FLAS) that assesses forelimb use during locomotion in rats injured at the cervical level. A quantitative scale was developed that measures movements of shoulder, elbow, and wrist joints, forepaw position and digit placement, forelimb–hindlimb coordination, compensatory behaviors adopted while walking, and balance. Female Sprague-Dawley rats received graded cervical contusions ranging from 200 to 230 ("mild," $n=11$) and 250–290 kdyn ("moderate," $n=13$) between C5 and C8. Rats were videotaped post-injury as they walked along an alley to determine deficits and recovery of forelimb function. Recovery of shoulder and elbow joint movement occurred rapidly (within 1–7 days post-injury), whereas recovery of wrist joint movement was slower and more variable. Most rats in all groups displayed persistent deficits in forepaw and digit movement, but developed compensatory behaviors to allow functional forward locomotion within 1–2 weeks post-injury. Recovery of forelimb function as measured by the FLAS reached a plateau by 3 weeks post-injury in all groups. Rats with mild contusions displayed greater locomotor recovery than rats with moderate contusions, but exhibited persistent deficits compared to sham controls. Reliability was tested by having seven raters (three internal, four external) from different laboratories, independently and blindly score videos of all rats. The multivariate correlation between all raters, all animals, and all time points ranged from $r^2=0.88$–0.96 ($p<0.0001$), indicating a high inter-rater reliability. Thus, the FLAS is a simple, inexpensive, sensitive, and reliable measure of forelimb function during locomotion following cervical SCI.

Introduction

In the last 4 years there has been a surge in the development and characterization of rodent cervical spinal cord injury (SCI) paradigms (Anderson et al., 2004, 2005, 2007; Velardo et al., 2004; Collazos-Castro et al., 2005; Pearse et al., 2005; Baussart et al., 2006; Gensel et al., 2006; Choo et al., 2007; Onifer et al., 2007; Schaal et al., 2007; de Rivero Vaccari et al., 2008; Sandrow et al., 2008). Expanding cervical SCI research is increasingly important as we learn from clinical epidemiology that the worldwide incidence of SCI ranges from 10.4 to 83 per million inhabitants per year (Wyndaele and Wyndaele, 2006) and that the proportion of individuals with cervical SCI ranges from 25% to 76%, depending on the country (Ackery et al., 2004). Regaining arm and hand function is the highest priority for people living with quadriplegia (Anderson, 2004) and the SCI population in general is receptive to the concept of incremental improvements. There is general consensus in the research field that the functional consequences of therapeutics that produce inter-segmental regeneration or plasticity will be easiest to test in cervical injury models because the distance of axonal growth that would be required to improve function is less than when assessing hindlimb function following injuries at thoracic levels.

There are many outcome measures available to assess forelimb impairments resulting from cervical SCI. Some examples include the grip strength meter (Anderson et al., 2004, 2005, 2007), food pellet reaching task (Whishaw and Pellis, 1990, Metz and Whishaw, 2000), cylinder task (Schallert and Lindner, 1990; Gharbawie et al., 2004), grooming task (Bertelli and Mira, 1993; Gensel et al., 2006), Montoya staircase (Montoya et al., 1991), horizontal ladder beam (Soblosky et al., 2001; Metz and Whishaw, 2002), and the sticker removal task (Schrimsher and Reier, 1992). Few studies, however, have assessed forelimb function during quadrupedal locomotion following cervical

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SCI in rodents. Part of the reason could be that there has not been a validated outcome measure to assess forelimb use during locomotion. The industry standard for evaluating deficits in hindlimb open-field locomotion in rats is the BBB scale (Basso et al., 1995), and a related scale has been developed for mice called the BMS scale (Basso et al., 2006). However, both of those were designed for and validated in animals with thoracic lesions and are inappropriate for assessing open-field locomotion in animals with forelimb impairments. One study has described a scale to evaluate deficits in forelimb function during open-field locomotion in rats (Cao et al., 2008). This scale was a modification of the BBB scale, had scoring categories ranging from 0 to 17, and was used along with a battery of other assessments to analyze the effect of administering a nogo-66 receptor antagonist peptide. This scale was only tested in animals subjected to a mild cervical lesion, which was a unilateral C3/4 lateral funiculus injury that produces very mild and transient forelimb locomotor deficits. Very recently, another scale was developed to evaluate forelimb deficits during open-field locomotion (Martinez et al., 2009). This scale was also a modification of the BBB scale, but it has a scoring range from 0 to 20 and it does not categorize behavioral recovery. This scale was only tested in rats subjected to partial, unilateral cervical spinal cord lesions. Thus, there have been no reports of a forelimb locomotor scale to assess deficits following bilateral injury at the cervical level.

Here, we describe a novel forelimb locomotor assessment scale (FLAS) designed to evaluate impairments resulting from a midline cervical contusion injury in rats. The FLAS was designed to be a comprehensive measure of forelimb function during quadrupedal locomotion in rats subjected to graded, midline cervical injuries that could detect levels of function ranging from complete non-use to normal use. We developed this tool in rats subjected to a clinically relevant midline contusion injury of varying forces applied at different cervical levels, described in detail in the companion manuscript (Anderson et al., 2009). Our intent was to develop the FLAS as an inexpensive, reliable, and relatively simple tool that could be used across laboratories with a high degree of reliability.

Methods

SCI Surgery

Experimental animals were female Sprague-Dawley rats (from Harlan, Inc., San Diego, CA) that were 200–230 g at the beginning of each experiment and between 3 and 4 months of age. In two separate experiments, a total of 24 rats received a cervical spinal cord lesion surgery and 4 received sham surgery ($n=28$). All lesions were assessed via histology, as described in the companion paper (Anderson et al., 2009).

For surgery, rats were anesthetized with an intraperitoneal injection of ketamine and xylazine (100 and 10 mg/kg, respectively; Western Medical Supply, Inc., Arcadia, CA). Hair overlying the cervical vertebrae was removed by shaving, the skin was treated with betadine and incised, and the layers of muscle overlying the vertebral column were bluntly dissected. A dorsal laminectomy was then performed on the fifth, sixth, or seventh/eighth cervical vertebra (C5, C6, or C7/8), depending upon the study group. The impactor probe was centered over the exposed spinal cord and lesions aimed at the midline were created using an Infinite Horizons (IH) Impactor (Precision Systems & Instrumentation, Lexington, KY). The vertebral column was stabilized by clamping the vertebrae immediately rostral and caudal to the exposed spinal cord with stabilizing forceps. Two types of lesions were created, termed "mild" and "moderate," each with the dura mater left intact and with zero dwell time. The mild lesion was one in which the force of the impactor was preset to 200 kdyn. The moderate lesion was one in which the force was preset to 250 kdyn. The diameter of the head of the impactor probe was 3.5 mm, which was larger than the standard 2.5 mm probe. We used a larger diameter custom made impactor tip because the 2.5 mm tip that comes with the IH device was designed for thoracic contusions. The cervical enlargement is larger than the thoracic spinal cord and so a larger diameter probe is required to avoid narrow injuries at the midline. Sham-operated controls received a C5 dorsal laminectomy only.

After creating the lesions, the muscle was sutured in layers, and the skin was closed with wound clips. Post-operatively, rats received 5 ml per 100 kg of 0.9% saline, 2.5 mg/kg Baytril, and 0.01 mg/kg buprenorphine subcutaneously and were placed on a water jacketed warming pad at 37 °C overnight. For the first week post-injury, intensive animal care was administered each day. Saline (5 ml/100 kg), Baytril (2.5 mg/kg), and buprenorphine (0.01 mg/kg) were administered subcutaneously each morning. Bladders were manually expressed every day for the first week and residual urine was collected and weighed each morning prior to the administration of fluids. There was no noticeable impairment in voiding ability (i.e., bladders were empty or contained minimal urine when expressed). Body weight was measured every morning (also prior to the administration of fluids) for the first 8 days post-injury and once per week for the remainder of the experiment. Diet supplements (Fruit Loop cereal) and regular food pellets were placed on the floor of each cage to provide easy access for the rats. Nutri-cal (2 ml, Henry Schein, Melville, NY) was administered orally for the first week post-injury.

Behavioral testing

The Supplementary Materials contains step-by-step instructions for each of the components described below for acclimation, recording, administering, and analyzing the FLAS.

A. Acclimation

A simple procedure was used to acclimate rats to the testing environment (Supplementary Materials part A). This was performed 1 week prior to injury. On the 1st day, a clear plexiglass alley was placed in the center of an open, shallow enclosure (a kiddie pool as used when assessing animals with the BBB scale). The alley was 36.5 in. long, 4.5 in. wide, with sides that were 7 in. tall (see Supplementary Materials). Rats were placed in the enclosure in groups and were allowed to explore the alley for 30 min. A similar procedure was performed on the 2nd through 4th days, with the addition of a white back-board being placed behind the alley leaning against one side. This was done to emulate the environment during the videotaping. Subsets of animals were placed in the enclosure (cage-mates, animals housed in groups of four or five). Animals from each subset were placed individually at one end of the alley and allowed to run/walk to the other end for a total of 15 min. On the 5th day (i.e., the day prior to SCI surgery), the alley was placed on a counter (outside the enclosure) and each animal was videotaped while traversing the alley according to the videotaping instructions described below.

Techniques that were used to encourage animals to traverse the alley included putting a black box at the end of the alley toward which the animal was walking and placing sugary cereal and a buddy rat (from the same cage) in the black box. It was also helpful for the observer to stand at the end of the alley with the animal walking toward him/her.

Following injury, familiarity of the environment was reinforced by repeating the acclimation described above 2 days per week (non-testing days) for 15 min per day.

B. Video recording of behavior (Supplementary Materials part B)

On each testing day, rats were placed individually into the start end of the alley and videotaped as they moved to the darkened