Experimental Neurology 220 (2009) 9–22

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Bilateral cervical contusion spinal cord injury in rats

Kim D. Anderson [a,d,*], Kelli G. Sharp [a,b], Oswald Steward [a,b,c,d]

[a] Reeve-Irvine Research Center, University of California at Irvine College of Medicine, USA
[b] Department of Anatomy and Neurobiology, Irvine, CA 92697-4292, USA
[c] Department of Neurobiology and Behavior, Irvine, CA 92697-4292, USA
[d] Department of Neurosurgery, Irvine, CA 92697-4292, USA

ARTICLE INFO

Article history:
Received 24 November 2008
Revised 3 June 2009
Accepted 17 June 2009
Available online 25 June 2009

Keywords:
Cervical injury
Contusion
Forelimb
Corticospinal tract
Digital flexors
Grip strength

ABSTRACT

There is increasing motivation to develop clinically relevant experimental models for cervical SCI in rodents and techniques to assess deficits in forelimb function. Here we describe a bilateral cervical contusion model in rats. Female Sprague–Dawley rats received mild or moderate cervical contusion injuries (using the Infinite Horizons device) at C5, C6, or C7/8. Forelimb motor function was assessed using a grip strength meter (GSM); sensory function was assessed by the von Frey hair test; the integrity of the corticospinal tract (CST) was assessed by biotinylated dextran amine (BDA) tract tracing. Mild contusions caused primarily dorsal column (DC) and gray matter (GM) damage while moderate contusions produced additional damage to lateral and ventral tissue. Forelimb and hindlimb function was severely impaired immediately post-injury, but all rats regained the ability to use their hindlimbs for locomotion. Gripping ability was abolished immediately after injury but recovered partially, depending upon the spinal level and severity of the injury. Rats exhibited a loss of sensation in both fore- and hindlimbs that partially recovered, and did not exhibit allodynia. Tract tracing revealed that the main contingent of CST axons in the DC was completely interrupted in all but one animal whereas the dorsolateral CST (dlCST) was partially spared, and dlCST axons gave rise to axons that arborized in the GM caudal to the injury. Our data demonstrate that rats can survive significant bilateral cervical contusion injuries at or below C5 and that forepaw gripping function recovers after mild injuries even when the main component of CST axons in the dorsal column is completely interrupted.

Introduction

Spinal cord injury (SCI) is a complex condition that impacts all aspects of an individual's life; the higher the level of the injury and the more severe the damage, the greater the impact on function. In the United States, it is now estimated that more than 50% of spinal injuries are to the cervical region (NSCISC, 2008). Cervical injuries impair both lower and upper extremity function, the latter of which severely limits a persons' ability to carry out the tasks required for daily living. Thus, it is not surprising that people living with cervical SCI report that regaining arm and hand function is of primary importance (Anderson, 2004). This fact motivates the development of new cervical injury models for SCI research and means to evaluate forelimb motor function that mimic what would be useful for humans.

The most common causes of human SCI are vehicular crashes or falls (NSCISC, 2008), which usually result in a contusive type of injury to the spinal cord. An important development has been the ability to produce standardized contusion injuries in rodent models, and there is a long history of research involving contusion spinal injuries to the thoracic region of the spinal cord, in both rats and mice. Far fewer studies have utilized contusion injuries to the cervical region of the spinal cord. The first model, and the only one for many years, was described by Schrimsher and Reier (1992). They produced a C4–C5 midline/bilateral contusion by dropping a 10 g weight from a height of 2.5 cm onto a Teflon impounder placed on top of the exposed spinal cord. They used this model again to study respiratory function (el-Bohy et al., 1998) and gene expression (Velardo et al., 2004) following cervical SCI in rats. Later, variations of the weight-drop technique were used to create C7 midline/bilateral contusions (Collazos-Castro et al., 2005), C4–C5 unilateral contusions (Soblosky et al., 2001; Gensel et al., 2006), and C2–C3 unilateral contusions (Baussart et al., 2006). All of the variations of that model relied on gravity to apply different amounts of force to the spinal cord.

More recently, studies have appeared that utilized the Ohio State University Electromagnetic SCI Device, which involves the electromagnetic displacement of a probe a short distance from the spinal cord (Pearse et al., 2005; Schaal et al., 2007; Choo et al., 2007; de Rivero Vaccari et al., 2008). So far, however, the OSU device is not produced commercially, limiting its general availability.

Accordingly, we have sought to develop standardized methods for producing spinal cord injuries at the cervical level using the commercially produced Infinite Horizons (IH) device (Scheff et al., 2003),

* Corresponding author. Reeve-Irvine Research Center, University of California, Irvine, 1113 Hewitt Hall, Irvine, CA 92697-1385, USA. Fax: +1 949 824 0057.
E-mail address: kanderso@uci.edu (K.D. Anderson).

0014-4886/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2009.06.012

which produces injuries based on user-defined variables including force, displacement, dwell time, and velocity. Many studies have been published that utilized this model for thoracic injuries, but only two have so far been published that utilized the IH device for cervical injuries. One study created C5 bilateral contusions, ranging between 176 and 201 kdyn, and assessed sensory evoked potentials in the rat cuneate nucleus and somatosensory cortex (Onifer et al., 2007). The other study created unilateral C4 contusions of 200 kdyn, and assessed the effect of peripheral nerve grafting into the lesion site (Sandrow et al., 2008).

In this and the companion paper (Anderson et al., 2009), we use the IH device to produce bilateral injuries at different cervical levels, and quantitatively assess resulting deficits in forelimb motor function by measuring grip strength (this paper) and forelimb use during locomotion (the companion paper). We also assess the relationship between these functional outcome measures and anatomical variables including lesion size and corticospinal tract (CST) integrity. The companion paper also describes the development of a novel Forelimb Locomotor Assessment Scale (FLAS) designed to quantitatively assess impairments and recovery of forelimb use during locomotion after cervical contusion injuries.

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. A priori, our intention was to perform contusions at 1 spinal level, C5, and identify the forces necessary to produce mild and moderate lesions. The entire study was composed of 3 separate experiments, completed in series, utilizing 63 animals. A total of 7 out of 23 animals died or were euthanized during the 1st experiment. Two of those died from anesthesia complications during the SCI surgery and 1 died from anesthesia complications during the surgery for BDA cortical injections. Behavioral data were included in analyses for animals that survived until the cortical injection surgery. The remaining 4 started exhibiting autophagia of the toes on 1 or both hindpaws during weeks 5–6 post-injury, which necessitated sacrifice. Behavioral data for those animals were only included in analyses prior to the onset of euthanasia. A total of 5 out of 20 animals died in the 2nd experiment, all of which were a result of anesthesia complications during SCI surgery. A total of 6 out of 20 animals died in the 3rd experiment. Two of those were a result of anesthesia complications during SCI surgery and 4 during the surgery for BDA cortical injections. One animal died two days after the BDA cortical injection surgery without showing signs of illness on the day prior to death. Behavioral data were included in analyses for animals that survived until the cortical injection surgery. Thus, after attrition due to all causes, total animal numbers at the end of all experiments were: SCI, $n = 50$; Sham, $n = 4$.

For surgery, rats were anesthetized with an intraperitoneal injection of ketamine and xylazine (100 mg/kg 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 cervical vertebra (C5). Lesions were created at the spinal level of the laminectomy using the Infinite Horizons (IH) Impactor, (Precision Systems and 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 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 modified from the standard 2.5 mm diameter tip used for injuries to the thoracic cord. 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, significant 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). Importantly, there was never any 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 animals. Nutri-cal (2 ml, Henry Schein, Melville, NY) was administered orally for the first week post-injury.

Behavioral testing

A 3 week handling and pre-training procedure was used prior to SCI, in order to calm the rats and enhance reliability when testing, during which the animals were trained for all the tasks. Behavioral testing was then conducted for 8 weeks post-injury, as described below for the individual tasks.

Grip strength meter test

Reliable assessment of gripping ability requires that animals are accustomed to being held. Accordingly, the first week was limited to handling each animal for 5 min each day. Then, there were 10 training sessions during which animals were held around the midsection, facing the bar of the grip strength meter (GSM, designed by TSE-Systems and distributed by SciPro, Inc.), and one forearm was gently restrained by the experimenter. The animals were held parallel to the bar so that they did not reach at an angle during the trials. The hindlimbs were not allowed to touch any surfaces. When the unrestrained forepaw was brought into contact with the bar of the GSM, the animals reliably grasped the bar, and the animal was then gently pulled away from the device. The GSM then measured the maximal force before the animal released the bar. Our practice is to allow the rat to grip the bar fully and observe the way that the grip is established before pulling away. This is especially important in animals with spinal cord injuries to assure that the pull on the bar is not a result of spastic contracture of the digits rather than genuine gripping. *Our definition of spastic contracture is when the forepaw would become stiff, and the wrist was held in a flexed position, but no rhythmic spastic movements were observed.* When the animal's forepaw was drawn over the bar, the forepaw would passively hook onto the bar and the only force generated was the result of the stiffness of the forepaw as the experimenter pulled the animal away from the bar. Although a measurable force was recorded under these circumstances, *trials in which the recorded response was due to paw stiffness were scored as "0" force because the animal did not grasp and release the bar* (active grasping was easily detected through careful observation during the task). This decision was made because the force recorded by the GSM was generated by the experimenter and would not be an accurate reflection of paw function.

Each testing session assessed each forepaw separately four times. The handling and training took 3 weeks to complete, after which, surgery was performed as described above and GSM testing (4 trials/paw/session) was carried out 3 times per week for 8 weeks post-injury.