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Bilateral Cervical Contusion Spinal Cord Injury in Rats

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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 (dICST) was partially spared, and dICST 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.

Keywords

Cervical injury; contusion; forelimb; corticospinal tract; digital flexors; grip strength

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

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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 Shrimsher and Reier (1992). They produced a C4-C5 midline/bilateral contusion by dropping a 10g weight from a height of 2.5cm 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), 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 176-201 kilodynes, 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 kilodynes, 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., submitted), 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 3-4 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 week 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 (100mg/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 & 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 kilodynes. The moderate lesion was one in which the force was preset to 250 kilodynes. 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 (5ml/100kg), Baytril (2.5mg/kg), and Buprenorphine (0.01mg/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 (2ml, 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 minutes 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.

Sensory Testing—To assess for changes in sensation or the development of mechanical allodynia, sensitivity to tactile stimulation was assessed using von Frey hairs applied to the plantar surface of the paws (both forepaws and hindpaws) and on the back of the neck/shoulder area girdling the SCI level. Von Frey hairs are used to detect changes in touch sensitivity, in response to mechanical stimulation, resulting from neural damage. The standard von Frey hairs are a set of 20 monofilaments in a linear scale of physical force. In order of increasing force, the different von Frey hairs are pressed against the skin being tested until the animal initiates an avoidance response. When testing the plantar surface of the paws, an avoidance response is represented by the animal withdrawing its paw quickly from the filament. When testing the girdle area of the SCI level, an avoidance response would be when the animal did any of the following: shook its neck/trunk, flinched, jumped to another area, vocalized, bit at the filament, or displayed other aggressive behaviors. The monofilament force at which an avoidance response was initiated was recorded. Testing was performed once per week, for one week prior to surgery and 8 weeks after surgery. Sensory testing was not performed on a day that GSM testing was performed.

Mini-ruby BDA tracing of CST projections

CST projections were traced by injecting mini-ruby/biotinylated dextran amine (BDA) into the sensorimotor cortex at 8 weeks post-injury. All of the rats received injections into the left cortex so that the integrity of the CST on the right side of the spinal cord could be assessed. Our general strategy is to trace CST projections from one hemisphere for two reasons. First, tracing CST projections from one hemisphere allows analyses of whether regenerative growth at the lesion site results in any disruption of the normal laterality of CST projections. Second, unilateral labeling provides a convenient way of determining which side is which after BDA staining after BDA staining and mounting.

For the BDA injections, animals were anesthetized as described above; the hair on the scalp was shaved and swabbed with betadine, and small holes were drilled in the skull over the sensorimotor cortex. A 10 μ l Hamilton microsyringe fitted with a pulled glass micropipette was used to inject tetramethylrhodamine and biotin conjugated dextran amine, 10,000 MW, lysine fixable (mini-ruby-BDA, Molecular Probes, Eugene, OR) at a total of 8 sites. Injection coordinates were 1.5 mm and 2.5 mm lateral to the midline at 1.0 mm anterior, centered on bregma, 1.0 mm posterior, and 2.0 mm posterior to bregma at a depth of 1 mm from the cortical surface, and 0.5 μ l of tracer was injected at each site (100 μ g/ μ l). After completing the injections, the scalp was sutured, and rats were placed on soft bedding on a water-jacketed warming pad at 37°C for 4 hours after surgery. Behavioral tests were not performed during the time period between BDA injections and perfusion.

Tissue Preparation

Rats survived for 18 days after BDA injections, after which they were killed humanely with an overdose of Euthasol® (195 mg/ml pentobarbital sodium and 25 mg/ml phenytoin sodium; Delmarva Laboratories, Inc., Richmond, VA). An 18 day survival interval was chosen in order to allow sufficient time for BDA transport to cervical and thoracic levels so as to detect CST axons that survived the injury. When deeply anesthetized, rats were perfused transcardially with cold saline followed by cold 4% paraformaldehyde (PFA) in 0.1 M sodium phosphate buffer (Na₂HPO₄), pH 7.4. The spinal cords were carefully dissected out, keeping the dorsal roots attached, and the tissues were post-fixed for 4 hours at 4°C in 4% PFA, rinsed for 1 hour in 0.1 M Na₂HPO₄, and then stored overnight at 4°C in 30% sucrose buffer. Tissues were immersed in TissueTek® (VWR International, West Chester, PA) and frozen using liquid nitrogen. The cervical dorsal roots were identified and used as reference points when preparing the tissue blocks for freezing. The injury site was identified visually and a block of tissue was cut that included two segments above and below the injury. In all cases, the lesion was contained entirely within the sample block. The portions of the spinal cord rostral and caudal to the injury block were frozen separately. Brains and spinal cords were embedded separately. Frozen tissue blocks were stored at -80°C until they were sectioned with a cryostat.

BDA staining

Injections of mini-ruby BDA allowed tract tracing based on either fluorescence (for the mini-ruby) or histochemistry (for BDA). We were mainly interested in confirming the interruption of CST axons and loss of innervation of cervical segments; thus, sections were stained histochemically because in our experience, histochemical staining better reveals fine terminal axon arbors.

For BDA histochemistry, the block of the spinal cord containing the lesion was frozen in OCT and sectioned in the horizontal plane at 20 μ m thickness using a cryostat. Alternate serial sections were collected free-floating in PBS for immediate use or thaw-mounted onto Bond-Rite slides (Richard Allan Scientific, Kalamazoo, MI) and stored at -80°C for future use. All free-floating sections (the complete collection of every other section from each case) were washed 2 times in 1X PBS and 0.1% Triton X-100, incubated overnight at 4°C with avidin and biotinylated horseradish peroxidase (Vectastain ABC Kit, Vector Labs, Burlingame, CA), washed again 2 times in 1X PBS, and then reacted with 0.6 mg DAB (DAB Substrate Kit, 3,3'-Diaminobenzidine tetrahydrochloride with nickel, Vector Labs, Burlingame, CA) in 0.45 M Tris buffer pH7.6. Sections were then rinsed in PBS and mounted onto gelatin coated slides (0.5% gelatin and 0.05% chromium potassium sulfate, Sigma-Aldrich, St. Louis, MO), air dried, dehydrated, and coverslipped with DPX mounting media (Sigma-Aldrich, St. Louis, MO). An advantage of this staining procedure was that axons were darkly stained and there was also light overall staining of gray matter, which allowed visualization of cytoarchitectural organization. Serial order was maintained during staining and mounting to facilitate analysis

of lesion extent and extent of spared tissue. Thus, for each case, there was one full set of every other 20 μ m section stained for BDA and mounted in serial order, and another set of every other section thaw mounted in serial order onto microscope slides. In each set, each section was separated by 20 μ m from the next in the series. In some cases in which the floating sections broke apart during staining, the second set of slide-mounted sections was also stained for BDA.

Lesion quantification

All of the sets of stained horizontal sections from each animal were analyzed, from dorsal to ventral, using an Olympus IM80 microscope and the freely available ImageJ software. In every other section of the series (so every 4th section overall) the diameter of the spinal cord immediately rostral and caudal to the lesion was measured, the minimum width of spared lateral tissue at the center of the lesion was measured, and the rostro-caudal length of the lesion was measured. The percentage of lateral spared tissue *at the lesion epicenter* was determined by utilizing the measurements from the section containing the epicenter plus two sections immediately dorsal and two sections immediately ventral to the epicenter (n = 5 sections). For each section, the spared lateral tissue on each side of the lesion cavity was summed and then divided by the average diameter of the spinal cord rostral and caudal to the lesion and multiplied by 100, then the average of the 5 sections was calculated. That value was identified for each spinal cord and used in subsequent analyses. Additionally, the completeness of the lesion (dorsally, laterally, and ventrally) was visually assessed, as well as the extent of damage or sparing of the labeled CST.

Counts of BDA labeled CST axons

To determine the extent of damage to different components of the CST, cross-sections from the block of the spinal cord immediately caudal to the injury were stained for BDA. Using these sections, counts were made of the total number of BDA labeled axons in the dorsal part of the lateral columns (the dorsolateral CST) and the ventral column (the ventral CST). These counts were carried out in all animals that had received cervical injuries, and also in the rats that had received laminectomy only.

The density of labeled axons in the dorsal CST of the laminectomy controls was very high, making it impractical to count all axons. Thus, to estimate axon numbers in the dorsal CST of the laminectomy controls, images were taken at 126X initial magnification (a 63X objective magnified 2X) focusing on the cut edge of the section where the cut ends of BDA labeled axons could be visualized. We then counted the number of axons in a 5 $\times$ 5 μ m sampling frame, and calculated axon numbers per 25 μ m². We then determined the total area occupied by BDA stained axons in the dorsal column using NIH Image, and estimated the total number of axons that would be present in this area based on the axon numbers per 25 μ m². This strategy is conceptually identical to the method used to determine axon numbers in peripheral nerve, and is based on the fractionator principal in which axon numbers per unit area are determined in counting grids positioned in a systematic uniform random distribution across the total nerve cross-section (Larsen, 1988). Total axon numbers are estimated by multiplying the average axon count per unit area by the total cross-sectional area of the nerve (Larsen, 1988). Here, the total cross-sectional area is the area occupied by CST axons in the dorsal column. We were unable to count axons from systematic random counting frames because of the irregularity of the cut edge of the section, which made it impossible to photograph axons in some counting frames. Because of the lack of truly random sampling of the total area of the dCST, the values reported for CST axon numbers in the dorsal columns should be considered estimates. This caveat does not apply to the counts of BDA labeled axons in the dCST in sections caudal to the injury or to the axon counts in the dlCST and vCST because in these sites, the overall density of axons was low, making it possible to count all BDA labeled axons in the respective tracts.

Statistical Analysis

Grip Strength Meter—On each testing day, the maximal average force (in grams) exerted on the GSM by each forepaw at the point just before grip was released was calculated from four trials per paw per time point for each rat. Then, for each time point, the average force was determined for each paw for the rats in each group. A two-way ANOVA was performed to identify differences between groups (sham vs mild vs moderate; C5 mild vs C6 mild vs C7/8 mild; C6 moderate vs C7/8 moderate) and across time post-injury. The Bonferroni test was used for post-hoc analysis to correct for multiple comparisons. Scatterplot comparisons were created and analyzed with the JMP®7.0.1 Statistical Discovery Software program.

Body Weight and Residual Urine—For body weight and residual urine analyses, the average weight in grams was determined for each group at each time point. Then, a repeated-measures two-way ANOVA was performed to identify differences between groups and time post-injury. The Bonferroni test was used for post-hoc analysis to correct for multiple comparisons.

Von Frey hairs—For data presentation and analyses, the inverse of the strength of the von Frey hair monofilament that induced a paw withdrawal was calculated for each paw per rat. The values for each forepaw were then combined and the mean and 95% confidence interval were obtained. The same was done for the hindpaws. The responsiveness threshold data were then plotted over time. A two-way ANOVA was performed to identify threshold differences between forepaws and hindpaws and time post-injury. This was performed on data for all SCI animals as well as the sub-grouping of animals that received a mild contusion versus a moderate contusion. The Bonferroni test was used for post-hoc analysis to correct for multiple comparisons.

RESULTS

Bilateral contusion to the cervical spinal cord: Mild and Moderate

Our goal was to create bilateral cervical contusion injuries centered on the midline of the spinal cord that produced bilateral tissue damage and bilateral functional deficits, but which animals could survive and recover to the point that they could ambulate and care for themselves without experimenter intervention. In particular, we sought to define the force required to produce injuries of graded severity ranging from lesions that produced forelimb motor deficits that recovered (i.e. mild) to lesions that produced complete or nearly complete loss of forelimb motor function that persisted for the duration of the testing period (i.e. moderate).

In Experiment 1, the goal was to create mild injuries to evaluate impact on health and overall level of functional impairment. Rats received contusion injuries targeted to C5 with nominal force set to 200 kilodynes, which yielded actual force recordings ranging between 199-204 kilodynes (velocity = 123.8 ± 2.4 mm/s; dwell time = 0 sec). Upon dissection of the spinal cords we found that all but one of the lesions were in fact centered at C5; in the one exceptional case the lesion was centered at C6 (dorsal roots were used to determine spinal level). Because the rats in Experiment 1 did not exhibit severe impairments, the plan for experiment 2 was to create both mild and moderate lesions. Thus, 8 rats received contusion injuries targeted to C5 with nominal force set at 200kD (actual force was 200-219 kilodynes; velocity = 116.1 ± 5.7 mm/s; dwell time = 0 sec), and 5 received contusion injuries with nominal force set at 250kD (actual force was 255-275 kilodynes; velocity = 118 ± 5.3 mm/s; dwell time = 0). Upon dissection, we found that 4 of the animals that received mild contusions had injuries that were in fact centered at C5, but 3 were centered at C6 and 1 was centered at C7/8. Of the 5 that received moderate contusions, 2 were at C6, 2 were at C7, and 1 was C8. Because rats in Experiment 2 that received moderate injuries did not exhibit un-acceptable levels of impairment, in Experiment 3, 12 rats

received contusion injuries targeted at C5 with nominal force set at 250kD (actual force was 250-287 kilodynes; velocity = 120.3 ± 7.3 mm/s; dwell time = 0 sec) and 4 received injuries with nominal force set at 200kD (actual force was 208-231 kilodynes; velocity = 113 ± 1.7 mm/s; dwell time = 0 sec). Analysis of dissected spinal cords revealed that in all rats that received moderate force injuries, lesions were centered at C7. Of the rats that received mild force injuries, 3 of the lesions were centered at C7 and 1 was centered at C6. As a result of the variance in creating lesions at C5, we choose to report data from all animals. Thus, the overall analysis involves rats with mild (200kD) and moderate (250kD) injuries centered at 3 spinal levels: C5, C6, and C7/8. Table 1 indicates the number of animals in each final group based on injury level and severity.

General health

Immediately following either a mild or moderate contusion, rats were significantly impaired and required considerable attention and care. Most rats were able to right themselves on the day following the injury and raise their heads to eat and drink, but were unable to move about in the cage. Rats exhibited flaccid paralysis of forelimbs, but considerable hindlimb movement, especially in the group with mild injuries. Within 1 or 2 days, rats with a mild contusion began moving their upper body significantly and used their hindlimbs in a variety of ways to move around their cage (i.e. crawling, scooting, hopping, etc). Animals with moderate contusions were at that level of impairment for 3 to 7 days. Recovery progressed so that rats were able to sit on their haunches or stand on their hindlimbs by about days 3-5 in the case of mild injuries and days 10-12 in the case of moderate injuries. At this stage, forelimb movement began to return, but the forepaws exhibited flaccid paralysis or spastic contractures.

To provide a quantitative measure of general health, animals were weighed just prior to injury and throughout the post-injury survival period. During the first week post-injury, animals in both contusion groups lost about 11-14% of their pre-injury body weight (Figure 1). A two-way ANOVA followed by a Bonferroni post-test demonstrated that body weight was significantly lower in comparison to control animals at 3, 4, 5, 6, 7, and 8 days post-injury ($F_{2,15}=1.751$; $p<0.05$). After 8 days, however, SCI animals began to gain weight and were no longer different from control animals.

Bladder function

Urine retention was monitored to define bladder dysfunction. Bladders were expressed 3 times per day (early morning, mid-afternoon, and early evening) for the first 6 days post-injury and the amount of urine collected from each animal at each time point was measured. Spinal cord injured animals that are unable to empty their bladders retain urine; thus the amount of retained urine is a measure of bladder dysfunction. The amount of urine collected each morning was analyzed. Surprisingly, urine retention was minimal; a two-way ANOVA revealed no significant difference at any of the time points between spinal cord injured animals and sham injured animals in the amount of urine collected (Figure 2). This is in striking contrast to what is seen after contusion lesions at the thoracic level (Keirstead et al., 2005). This discrepancy motivated a separate study to evaluate how bladder function is affected by lesions at cervical vs. thoracic levels (David et al, 2008) and David et al., in preparation.

Sensory testing

Paw sensation was assessed by touching the plantar surface of the fore- and hind-paws with von Frey hairs. Each von Frey monofilament is designed to deliver a specific amount of force when applied to the plantar surface of the paw with sufficient force to induce slight buckling. The force that elicited a paw withdrawal was designated the threshold for response. The inverse of that threshold was calculated for each paw per animal per testing time point. No response was calculated as 0. The normal inverse threshold for paw withdrawal was 0.2 ± 0.006 . After

cervical contusions, all rats exhibited a reduced sensitivity (i.e. less than 0.2) to von Frey hair stimulation of both fore- and hind paws (Figure 3). No animals developed greater sensitivity (i.e. more than 0.2) to mechanical stimulation in either forepaws or hindpaws (indicative of hyperalgesia). All animals regained some degree of mechanical sensation. However, recovery of forepaw sensation was much greater than recovery of hindpaw sensation. The force of the contusion had a significant impact on the recovery of responsiveness to mechanical sensation. Figure 3A shows the recovery pattern for rats with a mild contusion and Figure 3B for rats with a moderate lesion. Rats with a mild contusion exhibited less of a difference in the inverse response threshold between fore- and hindpaws. The difference was only significant at 52 days post-injury (two-way ANOVA, $F_{1,8}=2.31$, $p<0.0001$; post-hoc $p<0.05$). Rats with a moderate contusion, however, displayed a greater difference in the inverse response threshold between fore- and hindpaws and that difference began at 24 days post-injury (two-way ANOVA, $F_{1,8}=9.87$, $p<0.0001$; post-hoc $p<0.05$).

Differences between pre-injury (day -4) and post-injury (day 52) sensory thresholds were also analyzed. A two-way ANOVA revealed a significant difference in responsiveness between forepaws and hindpaws pre- and post-injury ($F_{3,1}=10.18$, $p<0.0001$), which was influenced by contusion severity. Post-hoc analyses demonstrated that, for the forepaws, there was no difference in the inverse threshold for response at 52 days post-injury compared to pre-injury regardless of mild or moderate contusion. For the hindpaws, however, both mild and moderate contusions significantly impaired the inverse response threshold. At 52 days post-injury, hindpaw responsiveness to mechanical sensation was significantly diminished in rats with mild or moderate contusions ($p<0.01$).

At-level hyperalgesia/allodynia was assessed by touching the region on the dorsal surface of the animals that girdled the injury site. None of the animals exhibited signs of sensitivity to stimulation or showed signs of pain (vocalization, biting, etc).

Lesion extent

Lesion extent was assessed both qualitatively and quantitatively by examining horizontal, serial sections that were processed for BDA histology, which lightly stains the gray matter enabling differentiation of the cytoarchitectural organization. Figure 4 shows representative images of mild (Fig. 4B) and moderate (Fig. 4A) contusions at C7. Figure 5 consists of low magnification serial sections spanning the entire dorso-ventral axis of the 2 cases shown in Figure 4. Note that in mild and moderate contusions the dorsal portion of the cord and the central gray matter was more severely damaged than the ventral portion of the cord. Moderate contusions tended to produce more lateral white matter damage. The extent of damage was variable across animals, but was clearly related to the severity of the contusion. Table 2 identifies whether dorsal column white matter or dorsal horn gray matter was spared in each animal. Both mild and moderate contusions produced 2 sub-groups of ventral gray matter damage. Complete GM damage refers to tissue damage to the entire ventral gray matter at the lesion epicenter. Partial GM sparing refers to partial sparing of the ventral gray matter, at the lesion epicenter. Twenty-four rats sustained a mild contusion at the C5 level and histology was available to evaluate on 19 of those rats. Of those 19, 13 had complete ventral GM damage and the other 6 had partial ventral GM sparing. Five rats sustained a mild C6 contusion, and all exhibited partial ventral GM sparing. Both rats that sustained a moderate C6 contusion had complete ventral GM damage. Fifteen rats received a moderate C7/8 contusion and histology was available to evaluate on 11 of those rats; 5 exhibited complete ventral GM damage and 6 exhibited partial ventral GM sparing. Four rats sustained a mild contusion at C7/8 and all exhibited partial ventral GM sparing. Hence, there is a degree of variability inherent to bilateral contusions in the cervical enlargement. When there was gray matter sparing, it was typically in the lateral/distal portions of the ventral horns.

Damage to lateral and ventral white matter was even more variable. Quantification of the percentage of spared lateral tissue at the epicenter of the lesion is presented in Table 2, along with quantification of the maximum lesion length (see Fig. 4 for examples). Table 2 summarizes the measurements of the areas of tissue damage/sparing and if GSM recovery occurred for each of the 40 injured animals for which histology was available. In the table, rats are grouped according to injury severity (mild or moderate) and level of injury.

Corticospinal tract damage

To analyze how the contusion injuries impact the corticospinal tract (CST), the anterograde tracer (BDA) was injected into the left motor cortex to label the CST on the right side of the spinal cord. In rats, there are 3 contingents of CST axons. The main contingent descends in the ventral portion of the dorsal column (the dorsal CST). The next largest contingent of axons descends in the dorsal part of the lateral column (the dorsolateral CST). The dICST is most prominent at the cervical level. Both of these contingents descend contralateral to the cortex of origin. A smaller number of CST axons descend in the ventral column ipsilateral to the cortex of origin (the ventral CST).

In animals in which the overall number of BDA labeled axons was high, there were sometimes a few BDA labeled axons on the “wrong” side (that is, in the dorsal column or dorsal part of the lateral column ipsilateral to the cortex of origin, see for example Figure 6B). Ectopic projections of this sort are also seen in un-injured rats, and probably represent developmental errors. Nevertheless, we cannot exclude the possibility that some of these ectopic axons in injured rats are novel axonal projections formed in response to the injury.

Of the 40 rats that received a spinal cord injury and cortical tract tracing, 33 exhibited good-excellent BDA labeling of the CST, 3 exhibited light labeling, and in 4 rats, labeling was not adequate for assessment (see Table 3 for a summary). Assessments of CST labeling were carried out using horizontal sections from the block that included at least 1 segment above and below the epicenter, and using cross sections from blocks rostral and caudal to the block containing the lesion.

In all but one rat, the contusion injuries completely interrupted the main component of CST axons that descend in the dorsal column. Figure 6 illustrates this in horizontal sections through the injury epicenter (Fig. 6A). Rostral to the lesion, the dorsal CST is heavily labeled, BDA labeled axons are evident in the dICST, and there are extensive BDA labeled arbors in the gray matter (Fig. 6B). In the horizontal sections, labeled CST axons in the dorsal column rostral to the injury terminated in retraction balls characteristic of injured axons, and no labeled CST axons could be followed into the dorsal column caudal to the injury site. Complete interruption of the dCST was verified by the absence of BDA labeled axons in the dorsal column in cross-sections taken caudal to the injury (Figure 7A&B).

In keeping with the fact that the dorsal part of the lateral column was only partially damaged in most animals, BDA labeled axons in the dICST were partially spared in all but one of the rats. For example, Figure 6C illustrates BDA labeled dICST axons bypassing the lesion via the spared lateral column, and numerous BDA labeled axons are evident in the dICST in the cross sections taken caudal to the injury (Figure 7C). In some rats dICST axons located more medially (that is, near the dorsal gray matter) were clearly interrupted and ended in retraction balls that are characteristic of damaged axons, whereas labeled axons located more laterally were spared. In 4 rats, BDA labeling was too light to determine whether there were any spared dICST axons, although the dorsal part of the lateral column that would contain dICST axons was partially spared.

In one exceptional rat (Experiment 3 #17), a moderate number of BDA labeled CST axons were present caudal to the injury, which probably represent spared axons (see below for counts of spared axons in caudal segments). In three other rats, 1-2 BDA labeled axons were seen in the dorsal column caudal to the injury. These axons were not present in the first 1-2mm caudal to the injury but were seen further caudally, and could be seen in the cross-sections from the block caudal to the injury (Figure 7B, right arrow). We were unable to fully reconstruct these axons to determine their point of origin, but in two cases, these BDA labeled axons had a trajectory that suggested that they entered the dorsal column from the dorsal gray matter (not shown). Thus, these may represent axons that originated from the dICST, passed through the gray matter, and then entered the dorsal column. It was not possible to visualize BDA labeled axons in the ventral columns in the horizontal sections, but BDA labeled vCST axons could be seen in the cross sections caudal to the injury (Fig. 7D).

The CST mediates its function via axons that leave the descending tracts, enter the gray matter, and arborize extensively to terminate in the gray matter on neurons at the segmental level. Thus, we assessed arborizations of CST axons in the gray matter caudal to the injury. In all rats in which BDA labeling was adequate, BDA labeled CST arbors were seen in the gray matter caudal to the injury, although at much reduced density in comparison to what was seen in segments rostral to the injury. In many cases, it was obvious that the BDA labeled axon arbors originated from dICST axons that bypassed the lesion (Fig. 6D). Labeled axons could be seen streaming from the dICST into the gray matter caudal to the injury where they arborized extensively. BDA labeled arbors were also evident in the gray matter in cross sections taken caudal to the injury (Fig. 7E). In most rats, BDA labeled arbors were also detectable in the gray matter contralateral to the labeled tract (Fig. 7F), although at much lower density than on the side ipsilateral to the labeled tract. In many cases, these axons could be seen to cross the midline near the central canal (see Figure Fig. 7F). This suggests that most of the arbors contralateral to the labeled tract originate from spared dICST axons. We cannot exclude the possibility, however, that some originated from the un-crossed ventral CST that descends ipsilateral to the cortex of origin or were axons that had regenerated around the lesion from the cut axons in the dorsal column.

Quantitative assessment of CST components in cross-sections caudal to the injury

To provide a quantitative estimate of the sparing of different contingents of CST axons, we counted the number of BDA labeled axons in cross-sections taken just caudal to the block that had been sectioned in the horizontal plane. Counts were made in the dorsal columns, dorsal part of the lateral columns, and ventral columns in the rats with cervical contusion injuries. Counts of BDA labeled axons in the dICST and vCST were also carried out in the 4 laminectomy-only controls, and the number of BDA labeled axons in the main tract in the dorsal column was estimated as described in the Methods. Data from all animals is summarized in Table 3 and Figure 8. In the laminectomy control rats, large numbers of BDA labeled axons were present in the dorsal columns (estimated at $20,080 \pm 7,878$, mean $\pm$ standard deviation). The number of BDA labeled axons in the dICST ranged from 40-182 (125 ± 64); and the number of axons in the vCST ranged from 8-20 (13 ± 5). In rats with cervical contusions, the dCST was completely interrupted in all animals but one, with the exception of a very small number of BDA labeled axons (ranging from 1-3) in 9 cases. The number of BDA labeled axons in the dICST ranged from 0 (one case) to 230 (mean 59 in the rats with mild contusions and 28.8 in the rats with moderate injuries). The number of BDA labeled axons in the vCST ranged from 0 (15 cases) to 25 (mean 5.9 and 2 in mild and moderate contusion cases, respectively). Thus, the counts confirm the qualitative impression of complete destruction of the dCST, and partial sparing of the dICST and vCST depending on the severity of the injury.

Effect of different lesions on grip strength

Figure 9 illustrate the consequences of mild and moderate lesions at C5, C6, or C7/8 on forepaw grip strength measured by the GSM. Each graph illustrates the mean performance of all animals within a specific lesion group (level and severity). A baseline measure of grip strength was established during a two week training period prior to spinal cord injury (referred to as days -14 through -1 in all graphs; day 0 represents the day of spinal injury). On day -1 the mean force exerted by either forepaw was $154 \pm 7.4\text{g}$ (mean $\pm$ standard deviation) for all rats regardless of future stratification. These numbers are essentially identical to the values seen in previous studies (Anderson et al., 2005,2007). All rats failed to grip with either forepaw immediately following a mild or moderate contusion at C5, C6, or C7/C8 (Fig. 9).

All rats that received mild contusion injuries at C5 failed to grip for the first 3 weeks post-injury. After 3 weeks, there was a degree of spontaneous recovery of gripping in these rats (Fig. 9A). This reached a plateau at 5 weeks and the mean force was always less than 25% of pre-injury grip strength. There were no rats that received a moderate contusion at C5.

The diminished average grip strength exhibited during spontaneous recovery following injury could be a result of two behavioral patterns. One, for a particular rat, diminished average grip strength could come about because the rat failed to grip at all on some trials and gripped with normal strength on other trials. Two, the diminished average grip strength could be because grip strength for a particular rat was diminished on all trials. The latter was the case for all animals that displayed spontaneous recovery in our study. Specifically, when rats started to display recovered gripping ability they began gripping on all trials, but with greatly diminished strength.

Rats that sustained a mild contusion at C6 exhibited the same pattern of spontaneous recovery of gripping ability as the rats that sustained a mild contusion at C5, but to a lesser degree (Fig. 9C). Rats that sustained a moderate contusion at C6 failed to grip for the duration of the testing period (Fig. 9D).

Rats that sustained a mild contusion at the C7/8 interface displayed the greatest amount of spontaneous recovery of gripping (Fig. 9E). Those rats began to grip after 3 weeks post-injury, but there was a great amount of variability in the strength recovered. Rats that sustained a moderate contusion at C7/8 exhibited very minor recovery of gripping ability, and this was actually limited to 2 of the 14 animals in that group (Fig. 9F).

A two-way ANOVA comparing average grip strength (both forepaws combined) between **contusion groups (mild vs moderate vs sham)** over time post-injury, revealed that there was a significant effect on grip due to contusion force ($F_{2,31}=4385$, $p<0.0001$) and time ($F_{2,31}=209.6$, $p<0.0001$) and post-hoc analyses demonstrated that rats with a mild contusion displayed a statistically greater degree of grip strength recovery than rats with a moderate contusion ($p<0.05$) beginning at 39 days post-injury. Both groups were significantly impaired at all times post-injury compared to sham ($p<0.001$). Thus, lesion severity significantly influences grip strength recovery. Another two-way ANOVA was used to compare average grip strength between groups with a mild contusion at different levels of the spinal cord (C5 vs C6 vs C7/8) over time post-injury. There was a significant effect on recovered grip based on injury level ($F_{2,31}=26.5$, $p<0.0001$) and time post-injury ($F_{2,31}=308.9$, $p<0.0001$), however, post-hoc analyses revealed that this effect was only between the C5 and C6 groups and only at 53 and 55 days post-injury ($p<0.05$). A final comparison was made between the two groups with a **moderate contusion at C6 or C7/8** over time post-injury and no significant difference was found in regard to grip strength recovery.

There was a relationship between grip strength recovery and contusion force or percent of lateral spared tissue at the epicenter (Fig. 10A-B, respectively). Scatterplot comparisons demonstrated that there was a significant negative correlation between contusion force and grip strength recovery ($r^2=0.16$, $p<0.02$) and a significant positive correlation between the percent of lateral spared tissue at the epicenter and grip strength recovery ($r^2=0.38$, $p<0.0001$). After identifying trends within the data, it appears that lateral spared tissue and spared dICST fibers caudal to the lesion have a greater influence on partial GSM recovery than ventral gray matter sparing. However, at best this only accounts for 37% of animals with mild contusions.

DISCUSSION

Our goal in the present study was to develop a model of cervical spinal cord contusion using a commercially available device that is convenient to use (the IH device). We were able to define conditions that produced “mild contusions” that damaged the dorsal funiculi and most of the gray matter and moderate contusions that produced a larger lesion of the gray matter and that also injured lateral and ventral white matter. Neither lesion led to the development of hyperalgesia. Surprisingly, there was minimal urine retention, implying minimal bladder dysfunction. The animals required nursing care for several days following the injury; general health was acceptable with animals exhibiting only a very transient loss in body weight. Locomotion was severely impaired but recovered over time depending on injury severity as described in detail in the companion paper (Anderson et al., submitted). Forepaw gripping ability was impaired following injury, but gripping recovered spontaneously after mild injuries so that 13 out of 29 animals were able to grip and average grip strength recovered to less than 25% of preoperative control, depending on injury level and injury severity.

Considerations Regarding Cervical Contusions in Rats

Performing a contusion to the cervical spinal cord that produces bilateral tissue damage affects the rat's ability to eat and drink, thus affecting general health. Contusions ranging from 200-230 kilodynes using the IH device produced mild spinal cord tissue damage, and mild general health deficits. Many rats with mild injuries were able to function independently within 1-3 days post-injury. Contusions ranging from 250-290 kilodynes, on the other hand, yielded more significant general health deficits during the week immediately following injury. Most rats with moderate injuries were not able to move around the cage and required hand-feeding during the first week post-injury. Following that critical window, however, rats with moderate injuries regained the ability to move around and feed themselves independently. We observed no deficits in gross bladder function and no development of hyperalgesia, which are two important factors contributing to maintaining good health in this model. It is likely that more severe contusions (>300 kilodynes) in the cervical region would create impairments that would be unacceptable from an animal welfare standpoint.

An important complication of performing cervical contusions in rats is that there is variability in the amount of tissue damage.— This places a greater demand on the robustness of a therapeutic intervention in order to yield a statistically significant change. This variability is advantageous in regard to mimicking a model of human injuries because the extent of spinal cord damage in each person is different. The main disadvantage of this variability is that larger numbers of animals are required for statistical analyses than would be the case if the lesions were more reproducible.

Grip strength testing is an ideal outcome measure for evaluating flexor and extensor function of the digits. Advantages of this technique are that it is simple, reproducible, quantitative, allows independent assessment of each forelimb, allows assessment of the forelimbs independent of the hindlimbs, requires little expertise or subjective scales, is minimally stressful on the animals, and, importantly, is a measure of muscle functions that are likely to

be related to the corticospinal tract (for example, distal flexors). As with any behavioral task, there are simple rules that need to be followed to properly evaluate outcomes, as were described in the Methods section. This is particularly important regarding spastic contractures of the digits so as not to obtain false readings. There are other factors that can confound grip strength values. These include operational parameters (such as sampling rate, type of system, and trial angle), sensory impairments, and diet restriction-induced loss of body weight (Maurissen et al., 2003). It is important to use a standard protocol to limit user-dependent variability of operational parameters. Our laboratory has a standard protocol used by multiple evaluators across multiple studies. It may not be possible to compare exact force readings between different brands of grip strength meters; however, comparing patterns of behavior is feasible and comparing exact force readings between multiple units of the same brand is feasible. Sensory impairments in the absence of motor impairments can decrease grip strength force values (Maurissen et al., 2003). In the studies reported here, there was an initial loss of sensory response in the forepaws to mechanical stimuli, but there was significant recovery within 10 days post-injury. The recovery of gripping ability did not occur prior to that, in fact animals that exhibited grip recovery did not do so until approximately 3-4 weeks post-injury. Similarly, reduction of body weight in response to diet restriction has been shown to decrease grip strength values (Maurissen et al. 2003). The animals presented in the current study exhibited a drop in body weight only during the first week post-injury. After which, they regained weight and were indistinguishable from control animals. Again, recovery of gripping ability did not coincide with weight gain. Hence, there are several factors that can confound grip force readings, but, when controlled for, using a grip strength meter can be a sensitive, reliable, and useful assessment of gripping ability.

Effects of cervical contusion injuries on motor pathways

For any SCI model, it is important to know the degree to which the injury affects key pathways. Our anatomical analyses focused on the corticospinal tract (CST) because of its role in mediating voluntary motor function, especially forelimb motor function. It is generally believed that the CST mediates fine motor control, particularly for the forepaw (Castro, 1972; Kalil and Schneider, 1975; Whishaw et al., 1998), and more cortical fields influence forepaw functions as opposed to hindlimb functions (Li et al., 1990).

Both the mild and moderate contusion injuries completely destroyed the main contingent of CST axons in the dorsal CST but spared much or all of the dorsolateral CST and ventral CST. In addition, in all animals in which labeling was adequate, there were BDA labeled axon arbors in the gray matter caudal to the injury, indicating some degree of sparing of CST input to segments caudal to the injury.

There was no obvious relationship between the extent of sparing of the CST and gripping function by the ipsilateral paw. Indeed, the one animal with dCST sparing following a mild contusion at C6 also had partial sparing of the dorsal horn and partial sparing of ventral gray matter yet exhibited no recovery of gripping ability whereas other animals with complete lesions of the dCST did recover the ability to grip although at reduced strength. Considering the case with spared dCST axons that did not recover gripping ability, it is possible that the spared dCST axons projected to thoracic or lumbar regions rather than the cervical regions caudal to the injury. The recovery of gripping seen in animals with complete lesions of the dCST could be due to the spared axons in the dlCST because these clearly gave rise to collaterals that arborized in the gray matter caudal to the lesion.

There was a clear relationship between the degree of sparing of white matter at the injury site and recovery of gripping ability. Rats with less than 30% sparing showed very slight recovery in the case of lesions at C5 and no recovery following lesions at other levels. It is possible that the recovery depends on spared axons in the dlCST, but other descending pathways also could

be important including the rubrospinal tract, which descends in the dorsal part of the lateral column near the dICST. There is evidence that the rubrospinal tract can compensate for the CST to a certain degree following injury (Martin and Ghez, 1998; Raineteau et al., 2002). Also, spared CST fibers can influence behavioral recovery following injury (Kartje-Tillotson et al., 1987; Thallmair et al., 1998). Novel relays involving propriospinal pathways could also be important. For example, there is evidence that CST axons can sprout and form connections with propriospinal neurons creating novel alternate relays that bypass a spinal cord lesion (Bareyre et al., 2004).

In terms of hindlimb function, the cervical contusion injuries caused severe motor impairments for the first several days (how long depended on the severity of the injury). Interestingly, however, all rats recovered the ability to use their hindlimbs to get around in their cages and even to locomote for considerable distances (see companion paper). Thus, descending motor pathways that sub-serve hindlimb locomotor function must have been largely spared by the injuries. This is not surprising given that motor pathways important for hindlimb locomotor function are thought to descend primarily via the ventral column (Loy et al., 2002), and the ventral column was largely spared following contusion injuries at the cervical level.

Effects of cervical contusion injuries on sensory pathways

Both mild and moderate cervical contusion injuries impaired sensory function of both hindlimbs and forelimbs. This is not surprising given that the point of impact is the dorsal columns, which carries a major component of ascending sensory fibers that project to the dorsal column nuclei. In general, moderate contusions produced greater deficits than mild contusions immediately post-injury and hindlimb sensory function was more severely impaired than forelimb sensory function. Indeed, there was significant recovery of forelimb sensation over time and by 8 weeks post-injury there was no difference in response to mechanical stimulation compared to pre-injury. However, there was no recovery of hindlimb sensation. This may reflect the fact that primary sensory afferents enter the spinal cord and project both rostrally and caudally before terminating on second order neurons. These ascending and descending projections occur in the lateral margin of the dorsal gray matter, and may have been spared by the lesions resulting in some preservation of sensation in the forelimbs. Importantly, none of the animals exhibited hyperalgesia (vocalization, biting, etc. in response to sensory stimulation or handling). Also, only 4 of the 63 total animals exhibited autophagy. These are important factors in terms of animal welfare considerations.

We have demonstrated that it is feasible to perform mild and moderate midline contusions at cervical levels 5 thru 8 in adult rats, which yield graded bilateral tissue damage and behavioral deficits. This is a highly relevant model for studying the associated deficits and extent of natural recovery of arm and hand function following spinal cord injury. Recovery of forepaw gripping ability is influenced by the severity of the injury. Importantly, this model produces upper motoneuron damage (the corticospinal tract) and causes extensive damage to the gray matter that would produce lower motoneuron damage as seen in humans. As to whether grip recovery is more linked to white matter versus gray matter sparing, we do not feel that the current evidence warrants a conclusion either way. However, we intend to pursue this further in future studies, and would prefer not to suggest conclusions now based on insubstantial evidence.

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Figure 1. Body weight temporarily decreases following cervical contusions, but returns to normal values within two weeks post-injury. (Error bars represent standard deviation; * $p<0.05$).

Figure 2.

Lack of gross bladder dysfunction following cervical contusions. Bladders were expressed 3 times per day and the amount of urine expressed each morning was collected and weighed. Urine retention was used as a measure of gross bladder dysfunction. Error bars represent standard deviation.

Figure 3.

Contusions do not produce hypersensitivity to mechanical sensory stimulation. Graphs represent the mean ($\pm$ 95% confidence intervals) inverse response threshold of Von Frey hair stimulation to the forepaws and hindpaws of mildly (A) and moderately (B) contused rats. All animals are initially insensate to the stimuli or unable to initiate a withdrawal due to motor impairment. Sensation and/or the ability to withdraw the forepaws improves with time post-injury, as opposed to the hindpaws (* p <0.05; ** p <0.001). The extent of recovered sensation is related to the severity of injury.

Figure 4.

Representative tissue damage resulting from mild or moderate contusions at C7 and calculation of spared lateral tissue. A-C, horizontal sections through a moderately contused spinal cord. D-F, horizontal sections through a mildly contused spinal cord. Sections through the dorsal column are shown in panels A and D; sections through the central canal (cc) are shown in panels B and E; and sections through the ventral column are shown in panels C and F. Percent of spared lateral tissue was calculated as follows: $(c+d)/[(a+b)/2] \times 100 = \% \text{ spared}$, where $(a+b)/2 = \text{control tissue}$ and $(c+d) = \text{spared lateral tissue}$. Scale bar represents 1 mm for all panels.

Figure 5. Distribution of tissue damage across the entire dorso-ventral axis of the spinal cord. A, mild contusion at C7. B, moderate contusion at C7. All sections are in the horizontal plane with rostral towards the right and caudal towards the left. Scale bar represents 1 mm for all panels. Starting in the upper left corner, sections progress down each column from ventral to dorsal.

Figure 6.

Contusion-induced corticospinal tract damage. A, mild and moderate contusions produce complete disruption of the labeled dCST caudal to the lesion. B, the dCST and dlCST retain heavy BDA-labeling and gray matter arborization rostral to the lesion. C, spared BDA-labeled dlCST axons bypass the lesion in spared lateral white matter. D, spared BDA-labeled dlCST axons (right arrow) arborize into the gray matter caudal to the lesion (lower arrow). Scale bar represents 500 μm for B-D.

Figure 7.

Corticospinal tract labeling caudal to the lesion. A, low magnification image of the spinal cord >2mm caudal to the lesion. Notice the absence of BDA-labeled dCST axons in the dorsal column (DC). LC = lateral column; VC = ventral column. B, high magnification image of the DC in A. Right arrow points to a rare BDA-labeled dCST axon caudal to the lesion. C, high magnification image of the LC in A. There are numerous BDA-labeled dICST axons caudal to lesion. D, high magnification image of the VC in A. Arrows point to spared vCST axons. E, BDA-labeled dICST arbors within the gray matter on the side ipsilateral to the labeled tract. F, BDA-labeled dICST arbors within the gray matter on the side contralateral to the labeled tract. Scale bar represents 50 μ m for B, E, and F and 100 μ m for C-D.

Figure 8.

The number of BDA-labeled axons caudal to the lesion are influenced by the severity of the contusion. For each animal group (mild n=24, moderate n=12, sham n=4), the mean number of BDA-labeled axons counted for each component of the CST (dCST, dlCST, vCST) are represented. Error bars represent standard deviation.

Figure 9.

Grip strength recovery. A, grip strength in all rats with a mild contusion. B, gripping ability in sham-injured rats is not impaired C, grip strength in rats with a mild contusion at C6. D, grip strength in rats with a moderate contusion at C6. E, grip strength in rats with a mild contusion at the C7/8 interface. F, grip strength in rats with a moderate contusion at the C7/8 interface. Error bars represent standard error of the mean.

Figure 10.

Grip strength recovery is influenced by the force of the contusion and the percent of lateral spared tissue at the epicenter. A, scatterplot showing the negative correlation between contusion force and mean grip strength at the end of the experiment. B, scatterplot showing the positive correlation between the percent of spared lateral tissue at the lesion epicenter and mean grip strength at the end of the experiment.

Table 1

Final number of animals in each group

	200-230 kilodynes (mild)	250-290 kilodynes (moderate)	Sham	TOTAL
C5	24 (5 no histology)	0	4	29
C6	5	2	0	7
C7/8	4 (1 no histology)	15 (4 no histology)	0	19
TOTAL	33	17	4	54

Table 2
Comprehensive description of spinal cord damage and consequent grip strength behavior.

MILD	Force (kDynes)	DC WM sparing	DH GM sparing	Ventral GM sparing	% lateral sparing at epicenter	Maximum lesion length (mm)	GSM recovery
exp 2 #7 C5	200	Y, on R	Y, on R	N	25.6	3.6	N
exp 2 #6 C5	202	N	N	N	35.5	3.7	N
exp 2 #5 C5	205	Y	Y	N	38.3	3.1	N
exp 2 #3 C5	200	N	N	N	12.5	8.6	N
exp 1 #15 C5	199	N	N	N	29.3	3.2	Y, R&L
exp 1 #17 C5	201	N	N	N	28.7	4.3	Y, R only
exp 1 #19 C5	201	N	N	N	20.4	4.9	N
exp 1 #22 C5	201	N	N	N	25.5	3.4	N
exp 1 #18 C5	204	N	N	N	17.7	7.0	Y, L only
exp 1 #12 C5	199	N	N	N	27.9	3.7	N
exp 1 #10 C5	201	N	Minor on R	N	26.9	6.0	Y, L only
exp 1 #5 C5	203	N	N	N	13.0	4.1	N
exp 1 #1 C5	205	Y	Y	N	46.1	3.2	Y, R&L
exp 1 #13 C5	202	N	N	Y, on R	41.1	4.1	Y, R&L
exp 1 #21 C5	202	Y, on R	Y, on R	Y, on R	33.6	5.4	Y, R only
exp 1 #7 C5	198	N	N	Y, on R	21.0	5.3	N
exp 1 #6 C5	203	Y	Y	Y, on R	47.3	7.7	Y, R&L
exp 1 #3 C5	200	N	Minor on R	Y, on R	10.6	5.0	N
exp 1 #2 C5	200	Y, on R	Y, on R	Y, on R	34.3	3.3	Y, R&L
exp 2 #11 C6	208	N	N	Y, on L	11.2	3.8	N
exp 2 #9 C6	219	N	N	Y, on L	28.7	3.6	N
exp 2 #4 C6	207	N	N	Y, on R	29.1	3.5	N
exp 1 #14 C6	202	N	N	Y, on R	33.2	3.8	Y, L only
exp 3 #17 C6*	231	Y	Y, on R	Y, on R	42.7	2.6	N
exp 3 #16 C7/8	208	N	N	Y, on R	22.3	4.0	N
exp 3 #18 C7/8	211	N	Minor on R	Y, on R	37.0	3.3	Y, R only
exp 2 #12 C7/8	205	N	Minor on R	Y, on R	42.8	3.1	Y, R&L

MODERATE	Force (kdynes)	DC WM sparing	DH GM sparing	Ventral GM sparing	% lateral sparing at epicenter	Maximum lesion length (mm)	GSM recovery
exp 2 #19 C6	259	N	N	N	23.3	4.8	N
exp 2 #18 C6	275	N	N	N	18.0	4.4	N
exp 3 #6 C7/8	252	N	N	N	28.2	3.2	N
exp 3 #14 C7/8	276	N	N	N	28.8	3.8	N
exp 3 #10 C7/8	267	N	N	N	32.7	3.8	N
exp 2 #15 C7/8	272	N	N	N	26.0	3.8	N
exp 2 #13 C8 ⁺	255	N	N	N	31.8	4.2	Y, R&L
exp 3 #9 C7	287	N	N	Y, on R	13.1	4.0	N
exp 3 #7 C7	250	N	N	Y, on L	36.8	3.4	N
exp 3 #8 C7	267	N	N	Y, on R	25.1	4.1	N
exp 3 #13 C7	264	N	N	Y, on R	31.1	3.4	N
exp 3 #2 C7	283	N	N	Y, on R	30.9	3.0	N
exp 2 #14 C7 [#]	255	Y	Y	Y, on R	34.5	3.3	Y, R&L

GM = gray matter; DC = dorsal column; WM = white matter; DH = dorsal horn; GSM = grip strength meter; Y = yes; N = no; R = right; L = left

* outlier = excluded from analyses due to low force, little damage, dCST sparing caudally — no GSM recovery

⁺ outlier = excluded from analyses due to high force, severe damage — yes GSM recovery

[#] outlier = excluded from analyses due to high force, little damage — yes GSM recovery

Table 3
Comprehensive description of BDA-labeled CST axons **caudal** to the injury

MILD	BDA labeling of CST	dCST-I (right)	dCST-C (left)	dlCST-I (right)	dlCST-C (left)	vCST	GM arbor-I	GM arbor-C	GSM recovery
exp 1 #1	Good	2	0	29	0	6	Few	Very few	Y, R&L
exp 1 #2	Good	0	0	115	0	0	Y	Y	Y, R&L
exp 1 #3	N/A	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N
exp 1 #5	N/A	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N
exp 1 #6	Good	3	0	230	0	28	Y	Y	Y, R&L
exp 1 #7	Good	0	0	0	0	0	Y	Y	N
exp 1 #10	Good	0	0	13	0	4	Y	Y	Y, L only
exp 1 #12	Light	1	0	4	0	1	Y	Y	N
exp 1 #13	N/A	N/E	N/E	N/E	N/E	N/E	N/E	N/E	Y, R&L
exp 1 #14	Good	0	0	41	0	3	Y++	Y+	Y, L only
exp 1 #15	Good	1	0	50	0	2	Y++	Y+	Y, R&L
exp 1 #17	Good	0	0	5	0	0	Y++	Y+	Y, R only
exp 1 #18	Good	0	0	N/E	N/E	7	Y	Y	Y, L only
exp 1 #19	Good	0	0	92	0	4	Y	Very few	N
exp 1 #21	Good	1	0	115	0	4	Y	Few	Y, R only
exp 1 #22	Good	0	0	51	0	0	Few	Very few	N
exp 2 #3	Good	0	0	11	0	17	Y	Y	N
exp 2 #4	Good	0	0	21	0	0	Y	Y	N
exp 2 #5	Good	0	0	58	0	6	Y	Very few	N
exp 2 #6	Good	0	0	163	3	10	Y++	Y	N
exp 2 #7	Good	0	0	29	0	6	Few	Very few	N
exp 2 #9	Good	0	0	51	0	13	Few	Very few	N
exp 2 #11	Good	1	0	61	4	6	Y	Y	N
exp 2 #12	Good	0	0	41	0	0	Y	Y	Y, R&L
exp 3 #16	Good	0	0	4	0	0	Y	Very few	N
exp 3 #17	Good	90	0	54	0	21	Y++	Y+	N
exp 3 #18	Good	0	0	5	0	0	Y	N	Y, R only

MODERATE	BDA labeling of CST	dCST-I	dCST-C	dCST-I	dCST-C	vCST	GM arbor-I	GM arbor-C	GSM recovery
exp 2 #13	Good	0	0	9	0	0	Y	Y	Y, R&L
exp 2 #14	Good	1	0	22	0	1	Y	Y	Y, R&L
exp 2 #15	NA	N/E	N/E	N/E	N/E	N/E	N/E	N/E	N
exp 2 #18	Good	0	0	0	0	6	Few	Very few	N
exp 2 #19	Good	0	0	7	0	1	Few	Very few	N
exp 3 #2	Good	0	0	97	0	0	Few	Very few	N
exp 3 #6	Good	0	0	30	0	0	Y++	Few	N
exp 3 #7	Good	0	0	31	0	0	Y	Few	N
exp 3 #8	Good	1	0	40	0	0	Y+	Y	N
exp 3 #9	Light	0	0	4	0	0	Y+	Few	N
exp 3 #10	Light	0	0	12	0	0	Y+	Y	N
exp 3 #13	Good	3	0	8	0	0	Y	Few	N
exp 3 #14	Good	0	0	85	0	16	Y++	Y	N

SHAM	BDA labeling of CST	dCST-I	dCST-C	dCST-I	dCST-C	vCST	GM arbor-I	GM arbor-C	GSM recovery
exp 2#1	Good	25,608	4	111	3	13	Y+++	Y++	No deficit
exp 2#20	Good	8,619	5	40	5	8	Y+++	Y+	No deficit
exp 3#19	Good	21,217	20	167	0	20	Y+++	Y++	No deficit
exp 3#20	Good	24,875	23	182	0	11	Y+++	Y++	No deficit

CST = corticospinal tract; dCST = dorsal CST; dCST = dorsolateral CST; vCST = ventral CST; I = ipsilateral side of the labeled tract; C = contralateral side of the labeled tract; GSM = grip strength meter; N/A = not adequate staining; N/E = not evaluated