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A bilateral cervical contusion injury model in mice: assessment of gripping strength as a measure of forelimb motor function

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Abstract

Here, we describe a bilateral cervical contusion model for mice. Adult female mice received graded bilateral contusion injuries at cervical level 5 (C5) using a commercially available impactor (the IH device). Three separate experiments were carried out to define conditions that produce impairments in forelimb function without unacceptable impairment of general health. A grip strength meter (GSM) was used to assess gripping ability as a measure of forelimb motor function; lesion size was assessed histologically by staining cross sections for H&E and GFAP. In Experiment 1, mice received injuries of 30 kilodynes (kdyn); these produced minimal deficits on grip strength. In Experiment 2, mice received injuries of 75 kdyn and 100 kdyn. Injuries of 75 kdyn produced transient deficits in gripping that recovered between 3–15 dpi to about 90% of control; injuries of 100 kdyn produced deficits that recovered to about 50% of control. In Experiment 3, none of the mice that received injuries of 100 kdyn recovered gripping ability. Histological assessment revealed graded injuries that ranged from damage limited primarily to the dorsal column (DC) to damage to the DC, grey matter, ventral column and lateral column. Most lesions filled in with a fibrous tissue matrix, but fluid-filled cystic cavities were found in 13% of the 100 kdyn injury group and a combination of fibrous-filled/fluid-filled cystic cavities were found in 22% and 38% of the 75 kdyn and 100 kdyn injury groups, respectively. There was minimal urine retention following cervical contusion injuries indicating preservation of bladder function. Our results define conditions to produce graded bilateral cervical contusion injuries in mice and demonstrate the usefulness of the GSM for assessing forelimb motor function after cervical contusions.

Keywords

Cervical injury; bilateral contusion; forelimb; digital flexors; grip strength; mouse; patterned hair loss, barbering

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INTRODUCTION

Spinal cord injury (SCI) continues to be a major cause of long term disability affecting millions of people world-wide. Indeed, a recent population survey by the Christopher and Dana Reeve Foundation (2009) indicates that approximately 1.275 million people in the USA report that they suffer from paralysis as a result of traumatic SCI. Traumatic SCI results from a variety of lesions including contusions, compressions, lacerations, and solid cord injury (Bunge et al., 1993). Importantly, injuries at cervical vertebral levels are common, representing over half of the total injuries (Norenberg et al., 2004). Cervical injuries are especially common consequences of sports injuries and water recreational activities, and these most commonly affect young individuals. Injuries at the cervical level produce functional impairments involving both upper and lower extremities; the impairment of upper extremity function can severely affect the ability to carry out daily activities that are critical for independent living and quality of life. Improvement of upper extremity function is considered to be the highest priority for individuals with injuries at the cervical level (Anderson, 2004). These facts have motivated a recent emphasis on the development of new models of cervical spinal cord injury, and methods to quantitatively assess forelimb impairments and recovery.

Currently, models of cervical contusion exist for rats but not mice. Although the rat contusion models of SCI (both at thoracic and cervical levels) may have greater clinical similarity due to cystic cavitation that is similar to the majority of human SCI cases (Kuhn and Wrathall, 1998, Jakeman et al., 2000, Ma et al., 2001, Basso et al., 2006), mice offer significant opportunities for genetic manipulation and provide a platform for proof-of-principle experiments involving manipulation of genes that are thought to be involved in responses to SCI. Accordingly, here we characterize a graded bilateral cervical contusion in mice and assess aspects of forelimb motor function.

MATERIALS AND METHODS

Experimental Animals

Experimental animals were female C57Bl/6 mice (Harlan, Inc., Indianapolis, IN) that were 7-8 weeks at the beginning of the experiment. This study was composed of 3 separate experiments completed in series, utilizing 77 mice (20 mice in Experiment 1, 25 mice in Experiment 2 and 32 mice in Experiment 3). Eight mice in Experiment 1; 6 mice in Experiment 2; and 16 mice in Experiment 3 died or were euthanized during or after SCI. After attrition due to all causes, a total of 47 mice (42 SCI and 5 sham controls) were included in the analysis (see Table 1).

Surgeries and Post-Operative Care

Spinal Cord Injury—The Institutional Animal Care and Use Committee of the University of California, Irvine, approved all procedures. Mice were initially anesthetized with Avertin (0.5 ml/20 g); when supplemental anesthesia was required, one-fourth of the original dose was given. Body temperature was maintained by placing mice on a water-circulating jacketed heating pad at 37 ± 0.5 C. The skin over the upper cervical area was shaved and cleaned with a Betadine solution. The skin was incised, and then the connective and muscle tissue were bluntly dissected to expose the fifth cervical vertebral body (C5). A C5 laminectomy was completed, taking care not to damage the spinal cord during the dorsal lamina removal. The size of the laminectomy (approximately 2 mm) was consistent between animals. Vertebral bodies rostral and caudal to C5 were cleared for spinal column stabilization. Special care was taken not to pinch the spinal cord during stabilization since the spaces between the cervical vertebral bodies are relatively large. The spinal column was clamped at approximately a 40° angle at C4 and C6 using forceps attached to the Infinite Horizon (IH) device platform (Precision Systems and Instrumentation, LLC). The impacting tip (1 mm diameter) was aimed

at the midline of the dorsal spinal cord at C5 to generate the bilateral contusion. Dwell time was set to 0 seconds. The IH device was set to deliver a force of 30, 75, or 100 kilodynes (kdyn). The actual impact force, spinal cord displacement, graph of time versus force, and graph of time versus displacement were recorded for each animal. The muscle was sutured with 5-0 chromic gut, and the skin was closed with 7-mm wound clips.

Post-operative Care—After surgery, mice were allowed to recover overnight in cages with Alpha-Dri bedding (Newco Distributors Inc.) placed on water-jacketed heating pads at 37°C. After recovering from the anesthetic, mice were group housed for the duration of the study four to five per cage. For 10-14 days after surgery, mice received subcutaneous injections of lactated Ringer's solution (1 ml/ 20g) for hydration, Buprenex (Buprenorphine, 0.05 mg/kg) for analgesia, and Baytril (Enrofloxacin, 2.5 mg/kg) for prophylaxis against urinary tract infections (UTIs). Nutri-cal® (1 ml; Henry Schein, Melville, NY) was administered orally twice a day for the first 10 days. Nutri-cal was also made freely available in their home cages for the first 10 days.

Mice were monitored twice a day for general health, coat quality (indicative of normal grooming activity), mobility within the cage, and for signs of skin lesions on the paralyzed forelimbs or autophagia of the forepaws. None of the mice exhibited autophagia. To provide a qualitative measure of bladder function, the relative amount of urine expressed upon morning manual bladder expressions was annotated. Bladders were categorized as full or empty based on the relative amount of urine expressed. Bladders were manually expressed until they were found to be empty at the time of their morning expression. All mice regained the ability to empty their bladders as they were found to be empty within 3-10 days post injury, even with the more severe injuries. Bladders were still checked once a day through the duration of the study.

During the study, we noticed that some mice exhibited characteristic patterns of hair loss over the upper extremities and ventral surface of the chest in a girdling pattern (explained further below). Accordingly, a rating scale was developed to define the extent of hair loss.

Behavior Testing

Assessment of Grip Strength—Assessment of grip strength was conducted as previously described (Anderson et al., 2004) with slight modifications. Briefly, mice were handled for 1 week, followed by a 2-week handling and pre-training period, then a 2-week baseline grip strength assessment. A C-5 cervical contusion SCI was performed after the 5-week pre-training/baseline sessions. Grip strength was measured for 60-80 days post injury (dpi).

On week 1, mice were handled daily for five minutes each. Handlers wore leather gloves under latex gloves for the first week to allow mice to bite in order to allay their fear and aggression. After the first week of handling, the mice were no longer biting and the use of leather gloves was no longer necessary. In week 2, mice were trained to grip the bar of the Grip Strength Meter (GSM) [TSE-Systems (designer) and SciPro, Inc (distributor)] with both forepaws three times per week (10 trials/session) as described further below. Bar height was set at 3.5 mm so that as mice were gently pulled away, they remained suspended just above the surface, but did not drop extensively when they released the bar. Mice were also held by the nape of the neck in preparation for the taping of the forepaws to record the individual forepaw grip strength. Mice became accustomed to this restraint after 1 week. On week 3, mice were allowed to grip (10 trials/session) with both forepaws initially then with each individual forepaw by gently taping the opposing forepaw with non-stick surgical tape (Micropore™ surgical tape from 3M, Catalog Number 1530-0). The dimensions of the working piece of tape were approximately 0.5 × 0.75 inches to prevent the tape from hindering the pull by the opposite forepaw. On week

4 and 5, baseline grip strength data were collected for both forepaws and individual left and right forepaw. Grip strength data were collected for 60-80 days post injury (dpi).

Criteria for Assessing Grip Strength

Criteria and Procedures for GSM testing: To standardize the assessment of grip strength in mice we used the following procedures and criteria. (1) The duration of an individual testing trial was 2-3 seconds. A trial started as the mouse was placed in front of the GSM bar with all paws on the base of the GSM and was picked up by the base of the tail to be pulled across the bar (this was all in one motion). The trial ended when the mouse released the bar or failed to establish grip and landed back on the GSM platform. (2) The time interval between each trial was less than 10 seconds. (3) A positive grip was scored when the digits extended and then flexed upon contacting the bar followed by the digits being extended as the mouse released the bar and landed on the platform. A score of zero was given if force was generated when a clenched/closed forepaw engaged the bar or if the forepaw landed on the platform in a clenched/closed position. The same criteria were used when assaying gripping with both forepaws. When gripping by both forepaws was assessed, a score of zero was given if grip was only established by only one forepaw.

Trials per session: Four (4) criterion grips were collected per session per forepaw or forepaws (when using both forepaws), and no more than 15 trials per session were given per forepaw or forepaws. In each session, if the mouse did not grip within the first 10 trials then the mouse was given a zero for the 4 data points/session/forepaw(s). If the mouse gripped successfully within the first 10 trials, then testing was continued until 4 successful trials were executed or the 15 trials maximum was reached.

Mouse Pectoral Hair Loss Scale (mPHLS): A scale for scoring cervical SCI-related hair loss—We observed a characteristic pattern of hair loss in mice with moderate to severe cervical SCI (Experiments 2 and 3). The hair loss was first seen at about 3-7 days post-injury and progressed in a waxing and waning fashion over the survival interval (that is, hair grew back and was lost again). Hair loss was first seen along the ventral surface of the lower forelimbs and then progressed proximally along the upper forelimb and chest and caudally along the abdomen. The reasons for the hair loss are not known, but could be due to excessive grooming perhaps triggered by an abnormal sensation. Therefore, we developed the Mouse Pectoral Hair loss Scale (mPHLS) to annotate and report the pattern and the extent of the hair loss.

A 5-point scoring system was developed to describe the level of hair loss for each side of the ventral surface (arms/chest/abdomen). The hair loss was scored separately on each side because the pattern of hair loss was not always bilaterally symmetrical. Hair loss was defined as the absence of hair or less than/equal to a hair shadow. Level “0” = no apparent hair loss. Level “1” = hair loss in the medial forearm only. Level “2” = hair loss in the medial and lateral forearm. Level “3” = hair loss in the entire forelimb (distal and proximal) up to the shoulder. Level “4” = hair loss in the entire forelimb past the shoulder and extending toward the midline of the chest. Level “5” = hair loss in the entire arm, chest, and extending down toward the abdomen. For examples see Figure 9.

Immunohistochemistry

At the end of the study, mice were killed humanely with an overdose of Euthasol (0.1 ml/30 g) and perfused transcardially with 4% paraformaldehyde (PFA). Spinal cords and brains were dissected and postfixed in 4% PFA overnight. Brains and spinal cords were immersed in 27% sucrose for cryoprotection overnight. A 1-cm block (from the spinomedullary junction to ~T2) containing the injury epicenter as well as the brain and another ~1-cm block caudal to the injury

were frozen in Tissue-Tek® using dry ice and ethanol. The main block containing the injury epicenter was cut in cross section at 20 µm. Five sets of sections were collected with each set containing one section taken every 100 µm; each set contained approximately 80-100 sections and sections were mounted in order on microscope slides. A few sections from the most rostral and caudal ends of the block were lost. Sets of sections were stained for Hematoxylin/Eosin (H&E) and immunostained for glial fibrillary acidic protein (GFAP).

For H&E, slide mounted cross-sections of spinal cord tissue were washed in Phosphate Buffered Saline (PBS), dehydrated with graded ethanol, de-fatted in xylenes, and then incubated in Ehrlich's Hematoxylin Solution (0.65% hematoxylin, 1% aluminum potassium sulfate, 0.01% sodium iodate, 30% glycerin, 33% ethanol and 3% acetic acid) for 5 minutes. Slides were washed 3X in water for 5-minutes then incubated in an acid wash consisting of 1% hydrochloric acid in 70% ethanol for 10-15 seconds and washed 3X in water. Slides were then washed with 3% ammonium hydroxide, water, and 95% ethanol. Slides were then immersed in 0.1% Eosin Y, 0.01% phloxine, 0.4% acetic acid, and 80% ethanol, dehydrated through graded ethanol, cleared with xylenes and coverslipped with DPX.

For GFAP immunostaining, slide mounted cross-sections of spinal cord tissue were washed 3X in PBS, incubated in Blocking Solution [5% normal goat serum (NGS) in PBS] for 30 minutes followed by an overnight incubation with a 1:1000 dilution of a polyclonal rabbit anti-GFAP (Cat# Z0334; Dako North America, Inc., Carpinteria, CA) in Blocking Solution. The following day, sections were rinsed 3X in PBS then reacted with a 1:250 dilution of Alexa-Fluor 488 goat anti-rabbit (Molecular Probes, Inc., Eugene, OR) in Blocking Solution. Slides were then rinsed 3 times in PBS and coverslipped with Kaiser's mounting medium.

Lesion Quantification

GFAP immunostained cross-sections were used to calculate the percent of damaged/spared tissue at the lesion epicenter. Images of stained sections were captured using an Olympus IM80 microscope and measurements were carried out using NIH ImageJ software. Lesion epicenters were selected by identifying the section with the greatest amount of tissue damage. The lesion areas as well as the total area of the cross-sectioned spinal cords were traced. The percentage of lesion area at the epicenter was determined by dividing the total lesion area(s) by the total cross-sectional area of the section and multiplying that value by 100. Subtracting the lesion area from the total cross-sectional area of the section and multiplying that value by 100 yielded the percent of spared tissue. Lesion length was determined by counting the number of slices (each 100 µm apart) in which there was a clear lesion based on GFAP immunostaining.

Statistical Analysis

Grip Strength—On each testing day, the maximal average force (in grams) exerted on the GSM by each forepaw or both forepaws together at the point just before grip was released was calculated from four trials. When plotting individual mouse data (single forepaw grip strength or dual forepaw grip strength) the mean from each four trials plus or minus the standard deviation (SD) was plotted for each time point. When plotting combined data (single forepaw grip strength per group or dual forepaw grip strength per group), the mean plus or minus the standard error of the mean (SEM) was plotted. A two-way ANOVA was performed to identify differences between groups (sham vs 30 kdyn vs 75 kdyn vs 100 kdyn) and across days post injury. The Bonferroni test was used for post-hoc analysis to correct for multiple comparisons. Graphs were created using the Prism 4 for Mac (GraphPad Software, Inc., La Jolla, CA).

Lesion Area—Student's t test was used to compare lesion size, lesion length, and last day grip strength between groups. Comparisons were plotted as means plus or minus SEM.

RESULTS

Our goal was to create graded bilateral cervical contusion injuries in mice that produce bilateral tissue damage and bilateral functional deficits. It was important that the animals could survive and recover to the point that they could ambulate and care for themselves without excessive experimenter intervention. These types of injuries had not previously been done in mice at the cervical level, so in the first experiment, injuries of 30 kdyn were produced to evaluate the consequences of a mild injury on general health and forelimb motor use as measured by the grip strength meter (GSM). We then followed up with higher force injuries to determine the forces required to impair forelimb function (gripping strength) without unacceptable impairment of the ability of mice to ambulate, eat and drink independently in their home cages.

Experiment 1: 30 kdyn Injuries

One mouse died immediately after the bilateral contusion, 2 mice died from anesthetic complication and 5 mice were euthanized because the forceps used to clamp the vertebral bodies crushed the spinal cord while setting the animal up on the IH device platform. Thus, after surgical attrition due to all causes, 12 mice were available for post-injury analysis. All injuries were targeted at C5. Three control (laminectomy-only) mice were prepared. Upon dissection of the spinal cords, all of the injuries were found to be on target based on counts of vertebral bodies and dorsal roots. Supplemental Table 1 details the parameter outcomes of the 30 kdyn injuries.

The range of actual forces in these injuries ranged between 20-30 kdyn (mean = 24.60 kdyn $\pm$ 3.63 SD). The spinal cord displacement ranged between 105-176 μ m (mean = 131.6 μ m $\pm$ 26.30 SD). The velocity was 124 mm/s when the IH device detected a velocity. However, in some cases the IH device recorded a value of zero yielding a mean velocity of 27.56 mm/s $\pm$ 54.68 SD.

30 kdyn Injuries: General Health—After recovering from anesthesia, mice that received a 30 kdyn injury or a laminectomy-only did not show any apparent deficits in their ability to locomote around the home cage or evidence of problems with grooming, eating and drinking. Bladder expression at 1 dpi revealed no evidence of urine retention, and this was true throughout the survival period.

30 kdyn Injuries: Grip Strength Assessment—Grip strength of each forepaw was assessed individually before and after a 30 kdyn bilateral contusion (Figure 1). Baseline grip strength prior to injury ranged between 60-80 grams of force consistent with prior studies (Anderson et al., 2004). At 1 dpi, grip strength was about 25% lower than pre-injury baseline levels (50 vs 70 grams of force); a similar decrease in grip strength was seen following laminectomy-only (Figure 2). This could have been due to muscle injury produced by the laminectomy or perhaps some very limited trauma to the spinal cord. By 3 dpi, grip strength recovered to pre-SCI baseline levels in all mice. Dual forepaw grip strength was not assessed in Experiment 1.

Experiment 2: 75 kdyn and 100 kdyn Injuries

Since there was no significant deficit in grip strength in Experiment 1, we conducted a pilot study in which mice received graded contusion injuries from 50-100 kdyn to determine the amount of force needed to detect a deficit on grip strength one day after injury (Table 2). Deficits in grip strength were observed after a 70 kdyn or a 100 kdyn injury. Thus, In Experiment 2, we assessed the consequences of grip strength after a 75 kdyn or a 100 kdyn force injury. Supplemental Table 2 details the parameter outcomes of Experiment 2.

75 kdyn Injuries—All injuries were targeted at C5. At dissection, injuries in two cases were found to be off-target: in mouse #15 the laminectomy and injury was done at C6 and in mouse #17 the laminectomy and injury was done at C4. Mouse #5 and mouse #12 were laminectomy-only controls with laminectomies at C4 and C4/C5, respectively. One mouse died after a 75 kdyn bilateral contusion and 2 mice were euthanized because the forceps used to clamp the vertebral bodies crushed the spinal cord while setting the animal up on the IH device platform.

The range of actual forces in the 75 kdyn injuries ranged between 75-86 kdyn (mean = 79 kdyn $\pm$ 4.10 SD); spinal cord displacement ranged between 458-793 μ m (mean = 631 μ m $\pm$ 146.10 SD); velocity of the probe ranged from 122-127 mm/s (mean = 124 mm/s $\pm$ 2.06 SD). Supplemental Table 2 provides details for individual mice.

75 kdyn Injuries: General Health—Following a 75 kdyn contusion, mice were clearly impaired and required daily attention and care. One day after the injury, mice exhibited limited spontaneous locomotion but were able to right themselves and raise their heads to eat and drink. Given the clear deficits, however, as a precautionary measure, Nutrical™ and food pellets were left on the bottom of the cage on top of the bedding. About half the mice exhibited some locomotion with weight bearing by the hindlimbs, but movement was slow and labored and there was minimal use of the forelimbs. Other mice were incapable of weight support, and remained prone. Interestingly, there was evidence of some grooming in that the Vaseline used to cover the eyes during surgery was removed. Also, visible grooming behavior was noticed. This is in contrast to mice with more severe contusions (see below). In mice that exhibited slight or no weight-bearing, forelimbs appeared flaccid and paws were closed. Nevertheless, these mice were able to move about by propelling themselves with their hindlimbs. Within 5 days, mice were able to move about the cage readily, though mostly via hindlimb movements. Mice had more forelimb movements than at earlier post-lesion intervals, but forepaw weight-bearing was limited in about half of the mice. By 10 dpi, mice were able to locomote with weight-bearing movements on all limbs.

To provide a qualitative measure of general health, animals were weighed just prior to injury and five days post-injury. By 5 dpi, all mice were $\pm$ 1 g of their pre-injury weights, except in two cases; mouse #14 and mouse #23 were 2 g less than pre-injury levels. Laminectomy-only controls, mouse #5 and mouse #12, had no obvious deficits.

75 kdyn Injuries: Bladder Function—Mice that received a 75 kdyn contusion exhibited no urine retention the morning after the injury suggesting that there was a preservation of at least a reflexive bladder. Urine retention was never detected throughout the study in this group.

75 kdyn Injuries: Grip Strength Assessment—In Experiment 2, the testing procedure was changed in that grip strength of each forepaw was assessed individually before and after bilateral contusions as in Experiment 1, but we also assessed grip strength of both forepaws used together (dual forepaw grip strength). The individual forepaw grip strength of mice that received a 75 kdyn bilateral contusion is shown in Figure 3 and the dual forepaw grip strength is shown in Figure 4. At 1 dpi, most mice in the 75 kdyn injury group did not grip with individual forepaws, with the exception of 3 cases; mouse #16, mouse #19, and mouse #21 (Figure 3, Panels C, F, and G). In mice that were able to grip at 1 dpi, grip strength of individual forepaws was between 30 and 50 grams of force. Most mice began to grip between 3 and 14 dpi and gradually recovered grip strength between 5-14 dpi, to a plateau of approximately 40-70 grams of force. By the end of the testing period, the average individual grip strength was 60 grams of force, which is about 85% of pre-SCI baseline.

Dual forepaw grip strength in the baseline period prior to injury was approximately 120 grams of force (Figure 4). The pattern of change in dual forepaw grip strength was similar to the

individual forepaw grip strength. Again, most mice did not grip at 1 dpi, except mouse numbers 15, 16, 19 and 21 (Figure 4, Panels B, C, F, and G). For mice that could successfully grip with both forepaws at 1dpi, dual forepaw grip strength was between 60 and 100 grams of force. Most mice began to grip with both forepaws between days 3 and 12 dpi and gradually recovered dual forepaw grip strength by 15-30 dpi that was comparable to pre-SCI baseline levels, except mouse #14 and mouse #15. Dual forepaw grip strength was approximately the sum of the grip strength exhibited by each forepaw used independently.

100 kdyn Injuries—Again, the target level for all injuries was at C5. In mouse #9 the injury was off-target; laminectomy and injury were at C4. Three mice died immediately after the 100 kdyn bilateral contusion.

The actual forces in the 100 kdyn injuries ranged between 99-107 kdyn (mean = 104 kdyn $\pm$ 3.41 SD); spinal cord displacement ranged between 723-970 μ m (mean = 862 μ m $\pm$ 87.28 SD); velocity of the probe ranged from 120-127 mm/s (mean = 121 mm/s $\pm$ 2.23 SD). Supplemental Table 2 provides details for individual mice.

100 kdyn Injuries: General Health—Immediately following a 100 kdyn contusion, mice were severely impaired and required extensive attention and care. One day after the injury, most of the mice were unable to right themselves or maintain an upright posture. All mice exhibited flaccid paralysis of forelimbs, with forelimbs sometimes being held in a flexed position with paws closed. Mice exhibited considerable hindlimb movement, however, that allowed for some propulsion along the floor of the cage by scooting on the ventral surface with minimal if any forelimb use. Only one mouse in this group was able to stand and bear weight exclusively on its hindlimbs with minimal locomotion ability at 1 dpi. Many mice still had Vaseline on their face/eyes suggesting an absence of grooming. Because many mice were unable to right themselves on the day following the injury and raise their heads to eat and drink, Nutrical was given orally twice a day and also was provided in the cage alongside food pellets on top of the bedding. Mice received subcutaneous injections of Lactated Ringers solution for hydration and longer water bottle tips were used so that mice could more easily drink independently.

Within 5 days, recovery progressed so that all mice had more forelimb movement with increasing weight support. Grooming of the face was still somewhat compromised as the face on several mice was still not completely clean. The forepaws still exhibited flaccid paralysis or were held in a flexed position with paws closed. Approximately 50% of the mice had some weight-supported movements, while others still could only use their hindlimbs to move around the cage to feed and drink. By 10 dpi, 75% of the mice exhibited weight bearing by forelimb and hindlimb during locomotion, whereas the other 25% of the mice only had hindlimb weight-bearing locomotion, albeit some with abnormal movements.

To provide a qualitative measure of general health, animals were weighed just prior to injury and five days post-injury. By 5 dpi, all mice were $\pm$ 1 g of their pre-injury weights, except in two cases; mouse #7 and mouse #9 were 2 g less than pre-injury levels.

100 kdyn Injuries: Bladder Function—Two out of 8 mice in this group exhibited urine retention at 1 dpi. By 5 dpi, only one mouse exhibited some urine retention and by 10 dpi and thereafter, bladders were empty at the time of manual bladder expression. Thus, even in the case of severe contusion injuries at C5, there is preservation of at least reflex bladder function.

100 kdyn Injuries: Grip Strength Assessment—The individual forepaw grip strength in mice that received a 100 kdyn bilateral contusion is shown in Figure 5 and the dual forepaw grip strength is shown in Figure 6. At 1 dpi, none of the mice gripped the bar successfully. This

individual forepaw grip strength deficiency persisted for 3-14 days. By 14-30 dpi, individual forepaw grip strength reached a plateau at 30-60 grams of force, with the exception of mouse #4 and mouse #10 (Figure 5, Panels B and G).

A similar pattern of deficit and recovery was seen in the data from dual forepaw grip strength (Figure 6). Although mouse numbers 6, 7, and 9 gripped with their individual forepaws (Figure 5, Panels C, D and F), their dual forepaw grip strength was erratic (Figure 6, Panels C, D and F). Mouse #4 and mouse #10 did not grip successfully with both forepaws after the injury so that their dual forepaw grip strength was 0 (Figure 6, Panels B and G). In mouse numbers 2, 8 and 11 (Figure 6, Panels A, E, and H), dual forepaw grip strength plateaued by 30 dpi to 80 to 120 grams of force.

Combined Grip Strength Data: Experiment 2 (Sham, 75 kdyn and 100 kdyn Injury Groups)—The combined grip strength data from Experiment 2 is shown in Figure 7. The individual forepaw grip strength assessment is shown in Panel A and the dual forepaw grip strength assessment is shown in Panel B. Values represent the mean plus or minus the SEM per injury group.

Two-way ANOVA comparisons with **individual forepaw grip strengths** (100 kdyn Left Forepaw vs 100 kdyn right forepaw vs 75 kdyn Left Forepaw vs 75 kdyn Right Forepaw vs Sham Laminectomy Control Right Forepaw vs Sham Laminectomy Control Left Forepaw) over time (dpi), revealed that a significant effect due to contusion force ($F_{5, 864} = 5.21$, $p < 0.0013$) and time ($F_{27, 864} = 27.64$, $p < 0.0001$). Bonferroni post-hoc tests demonstrated no significant differences between left and right forepaw grip strength within each group (100 kdyn Left Forepaw vs Right Forepaw; 75 kdyn Left Forepaw vs Right Forepaw; and Sham Laminectomy Control Left Forepaw vs Right Forepaw).

Two-way ANOVA comparisons with **dual forepaw grip strengths** (100 kdyn Both Forepaws vs 75 kdyn Both Forepaws vs Sham Laminectomy Control Both Forepaws) over time (dpi) revealed a significant related to contusion force ($F_{2, 432} = 8.54$, $p < 0.003$) and time ($F_{27, 432} = 13.56$, $p < 0.0001$). Bonferroni post-hoc tests demonstrated no significant differences on the dual forepaw grip strength between the 75 kdyn injury group and the Sham Laminectomy Controls at any time point (dpi). However, the dual forepaw grip strength of the 100 kdyn injury group was significantly less than the dual forepaw grip strength of either the 75 kdyn injury group or the Sham Laminectomy Control at virtually every dpi (p values ranged from 0.05 to 0.001 with Bonferroni correction).

Experiment 3: Repeat of 100 kdyn Injuries

Experiment 3 repeated the assessment of the consequences of a 100 kdyn contusion injury. The outcomes parameters of Experiment 3 are listed in Supplemental Table 3. Seven mice died immediately after the 100 kdyn bilateral contusion and 9 mice were euthanized because the forceps crushed the spinal cord while on the IH device platform; thus 16 mice remained for Experiment 3 (Table 1). Targeted injuries were at C5. At dissection, all injuries were found to be centered at C5.

The range of actual forces (excluding one outlier, mouse # 6) in the 100 kdyn injuries ranged between 99-117 kdyn (mean = 106 kdyn $\pm$ 8.73 SD); spinal cord displacement ranged between 781-864 μ m (mean = 781 μ m $\pm$ 88.08 SD); velocity of the probe ranged from 117-127 mm/s (mean = 121.8 mm/s $\pm$ 4.46 SD).

Repeat 100 kdyn Injuries: General Health—After recovering from the anesthesia, mice that received a 100 kdyn injury again showed major deficits in their ability to locomote around their home cage as in Experiment 2. At 1 dpi, grooming of the face was severely compromised

because Vaseline was still present around the eyes. Most mice were not able to right themselves on the day following the injury or raise their heads to eat and drink. Thus, Nutrical™ and food pellets were provided on top of the cage bedding and longer water bottle tips were used so that mice could reach more easily to drink independently. Forelimbs were flaccid and forepaws were closed. Some mice were able to propel themselves with their hindlimbs, and a few were able to locomote somewhat using their hindlimbs with no weight support by forelimbs. By 5 dpi, approximately 50% of the mice had recovered some locomotion ability with increasing weight-supported movements (scooting with hindlimbs, wheel-barreling and limited forepaw weight-support), while others still could only propel themselves with their hindlimbs. There was evidence of grooming in that faces appeared cleaner than at 1 dpi. The forepaws still exhibited flaccid paralysis or were held closed. By 10 dpi, mice exhibited increased locomotion and limited weight bearing by the forepaws. A shift in weight support toward the hindlimbs was more evident in these repeat 100 kdyn injuries as mice would spread their hindlimbs when grooming or eating. Body weight measurements taken at 5 dpi revealed that mice lost 1-4 g of their pre-injury weight. Over time, however, body weight recovered to pre-operative control levels.

Repeat 100 kdyn Injuries: Bladder Function—Seven out of 16 mice had full bladders at the time of manual expression at 1 dpi, indicating some bladder function deficits. By 5 dpi, only 2 of the 16 mice had urine retention indicating preservation of at least reflex bladder function. By 10 dpi and thereafter, none of the mice exhibited urine retention.

Repeat 100 kdyn Injuries: Grip Strength Assessment—Immediately following the contusion injury, none of the mice gripped the bar successfully with either individual forepaw or with both forepaws together (Figure 8). Two out of 16 mice exhibited limited recovery of gripping by the individual forepaws (Panel A). Grip strength reached a plateau between 14-30 dpi. There was no recovery of dual gripping by both forepaws, however (Panel B). Again, the GSM data indicate that functional deficits in Experiment 3 were more severe than in the 100 kdyn injury group in Experiment 2.

Mouse Pectoral Hair Loss Scale (mPHLS)

In Experiment 2, we noticed hair loss in the forearms, forelimbs, and on the ventral side of mice with 75 kdyn and 100 kdyn injuries. Although this hair loss appeared 1-2 weeks after injury, we initially did not notice a pattern. However as Experiment 2 progressed, we began to notice the pattern of the hair loss that let us to design the Mouse Pectoral Hair Loss Scale (mPHLS) (Figure 9 and Materials and Methods section). Panel A illustrates the mPHLS scale. The hair loss was seen on the distal forearms (Levels 1 and 2) and progressed through the entire forearm (Level 3), chest (Level 4), and abdomen (Level 5). Panel B depicts examples of hair loss after a 100 kdyn contusion injury using the mPHLS.

In Experiment 3, we utilized the mPHLS to analyze hair loss throughout the post-injury period (Figure 10). Hair loss was scored separately on each side. Figure 10 Panels A-C illustrate hair loss in individual mice and Panel D illustrates the average scores. In most cases, hair loss first appeared 3-7 dpi and progressed over a period of a few days.

Interestingly, on some testing days, hair had re-grown so that the mPHLS score was “0”, but hair loss was again apparent on subsequent testing days. Average mPHLS score plateaued between 12-16 dpi at a score of 2-3 which was maintained through out the study (Panel D). A two-way ANOVA revealed that there was no significant difference in the pattern of hair loss between sides, but there was a significant effect of time ($F_{27, 810} = 35.01, p < 0.0001$).

Lesion Size

Tissue samples from Experiment 1 were lost due to freezer malfunction; thus only tissues from Experiment 2 and 3 were analyzed histologically. Figure 11 illustrates 2 representative examples of lesion epicenter sizes from a 75 kdyn and 100 kdyn injury. GFAP immunoreactivity is shown in Panels A, B, E, and F and H&E stains are shown in Panels C, D, G and H. Mouse #17 from Experiment 2 is illustrated in Panels A and C; mouse #16 from Experiment 2 is illustrated in Panels B and D; mouse #6 from Experiment 2 is illustrated in Panels E and G; and mouse #26 from Experiment 3 is illustrated in Panels F and H. Lesion epicenter size and the total cross-sectional area are shown with the traces used to calculate the percent lesion size.

The lesions in mice that received a 75 kdyn contusion injury resulted in damage to the dorsal column including the dorsal corticospinal tract (dCST) and extended through the central grey matter. Lesions in mice that received a 100 kdyn contusion injury were larger, extending ventrally past the central canal and laterally to include the dorsal part of the lateral column, which contains the dCST, and the medial part of the lateral column.

It has previously been noted that there is minimal cystic cavitation at lesion sites at thoracic levels in mice; instead the lesion fills in with a connective tissue matrix (Zhang et al., 1996, Kuhn and Wrathall, 1998). There were, however, cystic cavities in several of the C5 lesion sites. In the 75 kdyn injury group, 7/9 had lesions filled with a connective tissue matrix; 0/9 mice had large cystic cavities at the lesion site; 2/9 mice had lesions with both cystic and matrix components. In the 100 kdyn injury group of Experiment 2, 5/8 mice had lesions filled with a connective tissue matrix; 1/8 mice had large cystic cavities at the lesion site; 2/8 mice had lesions with both cystic and matrix components. In the 100 kdyn injury group of Experiment 3, 7/16 mice had lesions filled with a connective tissue matrix; 2/16 mice had large cystic cavities at the lesion site; 7/16 mice had lesions with both cystic and matrix components. Thus, fluid-filled cystic cavities were found in 13% (3/24) of the 100 kdyn injury group. Whereas, a combination of fibrous-filled/fluid-filled cystic cavities were found in 22% (2/9) of the 75 kdyn injury group and 38% (9/24) of the 100 kdyn injury group.

We calculated the size of the lesion at the epicenter as a percentage of the total cross-sectional area of the spinal cord; these values are shown in Figure 12, Panel A. In the 75 kdyn injury group the lesions averaged $8.75\% \pm 2.99\%$ SEM of the total cross-sectional area. In the 100 kdyn injury group of Experiment 2 the lesions averaged $18.28\% \pm 1.78\%$ SEM of the total area. In the 100 kdyn injury group of Experiment 3 the lesions averaged $24.70\% \pm 1.82\%$ SEM of the total area. Statistical comparisons revealed significant differences between the 75 kdyn injury group and the 100 kdyn injury group from Experiment 2 ($p < 0.0144$) and between the 75 kdyn injury group and the 100 kdyn injury group from Experiment 3 ($p < 0.001$). Additionally, there was a significant difference between the 100 kdyn injury groups in Experiment 2 and 3 ($p < 0.0393$). There were no significant differences in lesion lengths between the groups; for all groups, lesions were approximately 1.25 mm in length (Figure 12, Panel B).

Panel C of Figure 12 illustrates the relationship between lesion size in individual mice and final gripping ability. All mice with lesions less than 10% were able to grip on the last day of testing with a force of 45g or more. About half of the mice with lesions between 10 and about 25% were able to grip on the final day of testing with a force of about 25g or more; the other half did not grip at all with one or both forepaws. None of the mice with lesions greater than 25% were able to grip on the last day of testing day. Panel D of Figure 12 illustrates the averaged left and right forepaw grip strength on the last testing day. Again, the striking feature of the data is that mice either were able to generate force of about 25g or more or they did not grip at all.

In considering these data, it is important to note that mice can receive a score of zero even though force is generated if the paw lands on the bar in a fixed flexed position or if the paw remains flexed after release. This scoring rule was adopted to eliminate scores generated by dragging a spastic clenched forepaw across the bar. Some of the mice with average scores of zero fell into this category. Most, however, simply failed to grip at all, especially mice that received 100 kdyn force injuries.

DISCUSSION

The present results demonstrate the feasibility of producing graded bilateral cervical contusion injuries in mice and show that the GSM provides a useful measure of deficits and recovery of forelimb function after cervical contusion injury. Importantly, although impaired immediately after the injury, mice recovered to the point that they could move about their cages, eat and drink independently, and maintain themselves even after the more severe injuries. Because the GSM provides an absolute measure of grip strength that is remarkably constant across animals, deficits and recovery can be measured for each forepaw independently as well as assessing the combined grip strength of both forepaws used together. There were greater and longer lasting impairments in both measures with larger force injuries. Urine retention was minimal following cervical contusion injuries indicating preserved bladder function as has also been reported following cervical contusion injuries in rats (Anderson et al., 2009). Finally, we observed a characteristic pattern of hair loss that was apparent within 1 or 2 weeks after SCI in the 75 kdyn and 100 kdyn injury groups. This hair loss may be due to excessive grooming triggered by abnormal sensations. In what follows, we discuss each of these findings in turn.

Survival after injury and degree of impairment of overall function

A substantial number of mice died or were euthanized during or soon after the surgical procedure to create the contusion injuries. This was especially true for the higher force (100 kdyn) injuries. In some cases mice were euthanized because the forceps used to clamp the spinal cord caused obvious injuries, making the animals unsuitable for inclusion in the experiment. In other cases mice died shortly after the injuries due to respiratory complications. Contusion injuries at the cervical level have the potential of disrupting respiratory function, which may interact with the anesthetic. Possibly, survival could be improved if mice were artificially respired during the procedure. Doing this in mice at the same time that they are positioned in the IH clamping system is technically challenging, however. Using inhalant rather than injectable anesthetics may also reduce respiratory complications after the contusion. These adjustments may be especially worthwhile for experimental animals that are in limited supply (for example, genetically modified mice).

In terms of general health after the contusion injury, the mice were severely impaired for the first few days after the more severe injuries, and required daily monitoring and care. Special care was taken to ensure that mice were able to drink and eat. Within a few days, however, mice had recovered to the point that they could get around their cages primarily using their hind limbs, feed themselves, drink, and generally maintain themselves independently.

Previous studies have shown that contusion or crush injuries at the thoracic level produce impairment in bladder function in both mice and rats (Mure et al., 2004, Leung et al., 2007, Urakami et al., 2007). Interestingly, there was minimal urine retention following cervical injuries in the present study and a similar lack of urine retention was observed following cervical contusion injuries in rats (Anderson et al., 2009). Importantly, the lack of urine retention means that there is minimal risk of bladder infection. Indeed, in other studies involving transection injuries, we have found that both male and female mice show minimal urine retention following dorsal hemisection injuries at C5, which means that mice of either sex can be used for cervical SCI experiments. This is of major importance because human

spinal cord injuries are much more common in males than females; being able to study SCI in male rodents is a considerable advantage. Ongoing studies in rats using a bilateral contusion model indicate that there may be a tract or circuit that is spared when contusion injuries are at the first thoracic (T1) level or above (David and Steward, In Preparation).

Grip strength after the bilateral cervical contusion

Previous studies of forepaw gripping in mice using the GSM have assessed deficits and recovery following unilateral injuries that affect one forepaw (Anderson et al., 2004, Blanco et al., 2007). Because the grip strength values are so consistent from animal to animal, the GSM can also be used to measure deficits in each forepaw following bilateral cervical contusion injuries. We show here that following injuries of moderate severity (Experiment 2, 75 kdyn), grip strength of both individual forepaws was compromised initially, but recovered to very near pre-SCI levels by 30 dpi. In the first experiment involving 100 kdyn injuries (Experiment 2), grip strength recovered partially to a level that was about 50% of pre-injury baseline. This is an ideal setting in which to assess interventions that might incrementally improve function. However in the second experiment involving 100 kdyn injuries (Experiment 3), lesions were significantly larger and gripping function did not recover. The reason for the difference in lesion severity is not clear. There were no significant differences in the injury parameters recorded by the IH device for the two groups. It is possible that the differences are due to differences in the surgical preparation, laminectomy size, etc. Also, it is possible that slight differences in the rostro-caudal location or extension of the lesion might affect the behavioral outcome. In any case, the differences highlight the importance of internal controls for each experiment rather than relying on historical controls.

For previous studies involving unilateral lesions it was important to test each forepaw separately. This requires extensive pre-training for the animals to become accustomed to having their paws taped. Here, lesions produced bilateral deficits, and so we also assessed grip strength of both forepaws used together. This assessment requires minimal pre-training and is thus more convenient. The combined grip strength of both forepaws was approximately the sum of the grip strength of the individual forepaws tested independently. Thus, for experiments that do not require independent testing of each forepaw, testing gripping by both forepaws may provide a convenient measure of overall forelimb motor function.

One interesting aspect of gripping performance is that some mice grip on some days and fail to grip on others even when given multiple opportunities. Since the “off” days occur asynchronously across the group, the zero scores on some days draw down the group average. Indeed, as is evident from the graphs of gripping of individual mice, some mice do not grip at all on one day yet grip strongly on the next. The lack of gripping on a particular day clearly does not reflect an actual inability to grip, and on and off gripping occurs in animals that are well accustomed to the task. Thus, the reason for the lack of gripping on particular days is not clear. It is important to note, however, that even when the days with zero scores are excluded, average grip strength is still below pre-operative baseline.

Lesion size and pathology

In previous studies, it has been noted that there is minimal cavitation following either crush or contusion injuries in mice (Zhang et al., 1996, Kuhn and Wrathall, 1998, Nishi et al., 2007). All these studies involved injuries at thoracic levels, however. Here however, we observed cavitation at the C5 lesion site in several mice. This may reflect a difference in the histopathological response at cervical level versus thoracic levels.

The lesions we observed ranged from the damage restricted primarily to the dorsal column including the dCST and underlying grey matter to damage that involved the dCST, dICST,

grey matter and some lateral white matter. The dCST and the dlCST are the descending motor tracts involved in voluntary motor activity. Additionally, the motor pools that control forepaw grip strength extend from C5-C8. Thus, the deficits in forelimb motor function that we observed could be due either to loss of descending motor inputs or damage to lower motoneurons

Patterned pectoral hair loss after cervical contusion injuries

An unexpected observation was a characteristic pattern of hair loss, for which we designed a scale. We have coined this scale the Mouse Pectoral Hair Loss Scale (mPHLS) because of the location of hair loss. Normal mice exhibit barbering behavior resulting in hair loss around the face (around the eyes, mouth, and whiskers) and on the dorsal surface of the back and neck (Kurien et al., 2005, Kalueff et al., 2006). This may occur via self-barbering or barbering by cage mates when mice are group-housed. The pattern of hair loss seen here, however, is different from what has been reported previously in normal mice in that it occurred along the ventral surface of the lower forelimbs and then progressed proximally along the upper forelimb and chest and caudally along the abdomen. Importantly, the extent of hair loss was greater in mice with more severe injuries. Mice in the present study were group-housed, and so we cannot exclude the possibility that the hair loss was due to barbering by cage mates. It seems more likely, however, that the hair loss was due to self-barbering, perhaps as a result of excessive grooming. It is noteworthy that the hair loss occurred first in approximately the distribution of the sensory afferents that enter the spinal cord at C5. This fact invites the speculation that the barbering is in response to some abnormal sensation the mice are experiencing. It will be of interest in future studies to incorporate sensory assessments to address this question. Whatever the cause, the hair loss is a characteristic phenomenon after cervical contusion injury in mice and the mPHLS provides a convenient means to quantify the degree of hair loss.

In conclusion, these results indicate that cervical contusion injuries of increasing forces using the IH device are feasible in mice and that the GSM and the mPHLS are reliable tools for assessing forelimb strength, and perhaps for assessing altered sensation after cervical SCI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Individual forepaw grip strength before and after a 30 kdyn bilateral contusion injury at C5. Panels A-I represent mouse numbers 2, 3, 5, 6, 8, 9, 10, 15 and 17 from Experiment 1, respectively. Open circles with dashed lines represent the grip strength of mice when pulling the bar with their left forepaw. Filled circles with solid lines represent the grip strength of mice when pulling the bar with their right forepaw. Data represents mean $\pm$ standard deviation.

Figure 2.

Combined individual grip strength data from sham operated mice (black; n=3) and mice that received a 30 kdyn bilateral contusion injury at C5 (green; n=9) in Experiment 1. Open circles with the dashed line represent the grip strength from mice that pulled the bar with their left forepaw. Closed circles with the solid line represent the grip mice that pulled the bar with their right forepaw. Data represents mean $\pm$ standard error of the mean.

Figure 3.

Individual forepaw grip strength before and after a 75 kdyn bilateral contusion injury at C5. Panels A-I represent mouse numbers 14, 15, 16, 17, 18, 19, 21, 22, and 23 from Experiment 2, respectively. Open circles with dashed lines represent the grip strength of mice that pulled the bar with their left forepaw. Filled circles with solid lines represent the grip strength of mice that pulled the bar with their right forepaw. Data represents mean $\pm$ standard deviation.

Figure 4.

Dual forepaw grip strength before and after a 75 kdyn bilateral contusion injury at C5 from individual mice. Panels A-I represent mouse numbers 14, 15, 16, 17, 18, 19, 21, 22 and 23 from Experiment 2, respectively. Data represents mean $\pm$ standard deviation.

Figure 5.

Individual forepaw grip strength before and after a 100 kdyn bilateral contusion injury at C5. Panels A-H represent mouse numbers 2, 4, 6, 7, 8, 9, 10, and 11 from Experiment 2, respectively. Open circles with dashed lines represent the grip strength of mice that pulled the bar with their left forepaw. Filled circles with solid lines represent the grip strength of mice that pulled the bar with their right forepaw. Data represents mean $\pm$ standard deviation.

Figure 6. Dual forepaw grip strength before and after a 100 kdyn bilateral contusion injury at C5 from individual mice. Panels A-H represent mouse numbers 2, 4, 6, 7, 8, 9, 10, and 11 from Experiment 2, respectively. Data represents mean $\pm$ standard deviation.

Figure 7.

Combined grip strength data from Experiment 2 illustrating individual forepaw grip strength (Panel A) and dual forepaw grip strength (Panel B) from the Sham Laminectomy Control group (black; $n = 2$), 75 kdyn injury group (blue; $n = 9$) and the 100 kdyn injury group (red; $n = 8$). In Panel A, the open circles with the dashed lines represent the left forepaw grip strength and the filled circles with the solid lines represent the right forepaw grip strength per group. In Panel B, black, blue, and red lines represent the grip strength of both forepaws pulling from the Sham Laminectomy Control group, 75 kdyn injury group, and the 100 kdyn injury group, respectively. Data represents mean $\pm$ standard error of the mean.

Figure 8.

Combined grip strength data from Experiment 3 illustrating individual forepaw grip strength (Panel A) and dual forepaw grip strength (Panel B) from the 100 kdyn injury group ($n = 16$). In Panel A, the red open circles with the dashed red lines represent the left forepaw grip strength from the 100 kdyn injury group. Red filled circles with the solid red lines represent the right forepaw grip strength from the 100 kdyn injury group. In Panel B, the red solid circles with red solid lines represent the dual forepaw grip strength from the 100 kdyn injury group. Data represents mean $\pm$ standard error of the mean.

Figure 9.

The Mouse Pectoral Hair Loss Scale (mPHLS). Panel A illustrates the mPHLS on mouse drawings displaying the mouse's Right Ventral Side (RVS) and Left Ventral Side (LVS). Each ventral side is scored independent of each other. Level "0" = no apparent hair loss. Level "1" = hair loss in the medial forearm only. Level "2" = hair loss in the medial and lateral forearm. Level "3" = hair loss in the entire forelimb (distal and proximal) up to the shoulder. Level "4" = hair loss in the entire forelimb past the shoulder and extending toward the midline of the chest. Level "5" = hair loss in the entire arm, chest, and extending down toward the abdomen. Panel B illustrates mouse examples of hair loss after 100 kdyn contusion injuries at C5 using the mPHLS.

Figure 10.

Representative examples of hair loss using the mPHLS in mice that received a 100 kdyn injury in Experiment 3. Panels A, B, C represent the hair loss in mouse #10, mouse #20 and mouse #17, respectively. Panel D represents the combined hair loss data from Experiment 3. Open circles with the dashed line represent the Left Ventral Side (LVS) and the solid circles with the solid line represent the Right Ventral Side (RVS). In Panels A-C, data represents individual recordings and in Panel D, data represents the mean $\pm$ SEM.

Figure 11.

Representative examples of lesion size at the lesion epicenter from the 75 kdyn and 100 kdyn injury groups in Experiments 2 and 3 using GFAP immunostained tissue (Panels A, B, E, and F) and H&E stained tissue (Panels C, D, G, and H). Mouse #17 from Experiment 2 is illustrated in Panels A and C; mouse #16 from Experiment 2 is illustrated in Panels B and D; mouse #6 from Experiment 2 is illustrated in Panels E and G; and mouse #26 from Experiment 3 is illustrated in Panels F and H. Scale bar = 250 μ m.

Figure 12.

Comparisons of lesion size and lesion length at the lesion epicenters against increasing injury forces (Panel A and B) and final grip strength measurement per group (Panel C and D) from Experiments 2 and 3. Data is plotted as individual cases with means $\pm$ standard error of the mean. Student's t-test was used for statistical analysis: * = $p<0.0144$; ** = $p<0.0393$; *** = $p<0.001$.

Table 1

Final number of mice used per group in each experiment.

Group	30 kdyn	75 kdyn	100 kdyn	Sham	TOTAL
Exp. # 1	9	0	0	3	12
Exp. # 2	0	9	8	2	19
Exp. # 3	0	0	16	0	16
TOTAL	9	9	24	5	47

Table 2

Experimental outcomes recorded from a pilot experiment 1 day after a bilateral contusion at C5 of 50-100 kdyn with the IH device.

Mouse Number	Level of Laminectomy	Level of Injury	Targeted Injury (kdyn)	Actual Injury (kdyn)	Weight Support on Forepaws	Coordinated Movements	Bladder Full Next Morning	Able to Grip the GSM
1	C5	C5	50	44	Yes	Yes	No	Yes
2	C5	C5	50	47	Yes	Yes	No	Yes
3	C5	C5	70	69	Minimal	Minimal	No	Minimal
5	C5	C5	100	100	No	No	Yes	No