Video Article

Calibrated Forceps Model of Spinal Cord Compression Injury

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Abstract

Compression injuries of the murine spinal cord are valuable animal models for the study of spinal cord injury (SCI) and spinal regenerative therapy. The calibrated forceps model of compression injury is a convenient, low cost, and very reproducible animal model for SCI. We used a pair of modified forceps in accordance with the method published by Plemel *et al.* (2008) to laterally compress the spinal cord to a distance of 0.35 mm. In this video, we will demonstrate a dorsal laminectomy to expose the spinal cord, followed by compression of the spinal cord with the modified forceps. In the video, we will also address issues related to the care of paraplegic laboratory animals. This injury model produces mice that exhibit impairment in sensation, as well as impaired hindlimb locomotor function. Furthermore, this method of injury produces consistent aberrations in the pathology of the SCI, as determined by immunohistochemical methods. After watching this video, viewers should be able to determine the necessary supplies and methods for producing SCI of various severities in the mouse for studies on SCI and/or treatments designed to mitigate impairment after injury.

Video Link

The video component of this article can be found at http://www.jove.com/video/52318/

Introduction

Animal models of SCI are valuable tools for assessing the efficacy of therapeutic paradigms designed to mitigate damage as a consequence of trauma to the spinal cord. Out of experimental necessity, these models must provide reproducible deficits in locomotor and sensory behaviors, be adjustable to produce injuries of differing severity, and demonstrate that injury severity correlates with the degree of neurological deficit observed. There are three main types of SCI with distinct features of injury: transection, contusion, and compression[1]. Briefly, a transection injury is a laceration to the spinal cord, a contusion injury arises from a brief, focal force applied to the dorsal spinal cord, and a compression injury occurs when an injurious force is applied to the spinal cord, and may also be referred to as a crush injury[2].

Complete transection injuries are clinically rare in humans[3], while contusion and compression injuries are more common. The compression injury produces an outcome similar to what is found in human SCI caused by, for example, tumor compression or other injurious compressive forces, and can be performed using a simple array of tools. Contusion and compression injuries are similar in that both are a compressive force and both have similar pathological features, such as cytoarchitectonic disorganization, and evoke similar endogenous responses to injury[1,4]. The contusion injury model usually applies this force to the dorsal spinal cord using a special apparatus in a manner similar to human cases of SCI resulting from an impaction of the spinal column[2,5,6]. In contrast, compression injuries can be generated by a variety of methods applying force dorsally or laterally. Methods of a compression injury include calibrated forceps[7], aneurysm clips[2], or placing a weight directly onto the spinal cord[8]. An advantage of the aneurysm clips is that they are able to provide different amounts of force[9]. The method of adding weights to the dorsal surface of the spinal cord directly[8] requires the weight to be in place for 10 min, drastically increasing the length of surgery and resulting in inconsistencies due to placement of the weight and movement due to the respiration of the animal. Due to the small size of mice, situating animals in specialized apparatuses designed for use in rats, such as impactors for contusion injuries, may be difficult or result in inconsistent injuries[7]. However, mice are available in a wide range of transgenic strains, unlike larger animals such as rats or rabbits that are very useful for SCI research.

The Plemel method of using calibrated forceps to compress the spinal cord generates a reproducible SCI with a high degree of correlation between injury severity and neurological deficit[7]. This surgical SCI model is generated using a pair of No. 5 Dumont forceps modified to be held apart at a defined distance by either metal epoxy or some other obstruction to prevent complete closure. This engineered spacing ensures that the forceps will always close to a certain width in multiple surgeries and by different users. The advantage of the Plemel method is that the materials to produce the calibrated forceps can be easily purchased and assembled in the laboratory without need of specialized equipment. These forceps can withstand multiple rounds of autoclaving and sterilization, and the lack of a separate, bulky apparatus streamlines surgeries.

In this video we demonstrate the surgical use of a pair of calibrated forceps on the mouse spinal cord to generate a compression injury. We also address unique concerns related to the care of spinal cord injured laboratory animals to improve their quality of life post-operatively and reduce mortality.

Protocol

All animal procedures and animal care methods should be approved by the institution's Institutional Animal Care and Usage Committee (IACUC).

1. Surgical Preparation

1. Assemble all necessary surgical tools and reagents: forceps, vana scissors, roungeurs, retractor, scalpels, scissors, sutures, skin staples, Q-tips, sterile saline, surgical sponges, and isoflurane. Prepare and autoclave a complete pack of surgical tools prior to surgery. If performing surgeries on more than one mouse, prepare and autoclave one packet of tools per animal, or autoclave one packet of surgical instruments and sterilize with a tool sterilizer in between surgeries. Generally, one pack of sterilized tools can be used on up to 5 mice if a glass bead tool terilizer is used to clean tools in-between surgeries (after 5 animals the tools must be re-cleaned and sterilized before use). Please check with your local IACUC administrator for institution specific guidelines.
2. Sterilize the surgical field with 70% isopropyl alcohol wipes. Set up surgical drapes to ensure a sterile field is maintained during surgery.
3. Weigh each mouse prior to surgery. Administer a dose of 0.05 mg/kg body weight of buprenorphine, subcutaneously.
4. Anesthetize the animal by administering isoflurane at a dose of 4% in the induction chamber of isoflurane machine.
5. Once the animal is anesthetized, apply ointment to the eyes to prevent dehydration, set the animal on a heating pad at 37 °C, and ensure that the mouse's head is properly situated in the anesthesia cone. (Note: Use a heating source that will not cause thermal burns; *i.e.*, a circulating water blanket, hot water bottle, or equivalent.) At this point administer a dose of 2% isoflurane to the animal.
6. Shave the dorsal surface of the mouse, approximately 1 cm around the intended incision location.
7. Disinfect the incision site by washing with 70% isopropyl alcohol wipes, then with an iodine solution (10% Povidone-iodine, 1% available iodine). Repeat 3 times.

2. Dorsal Laminectomy

1. Before making an incision, ensure that the animal is properly anesthetized by checking for reflexes using the toe or tail pinch method.
2. Make an incision along the dorsal spine with scalpel and blade, and then check for reflexes again. Arch the back to help properly visualize landmarks, such as the boundaries between vertebrae.
3. Cut through the skin. Insert retractor to hold skin and fascia back from the spinal cord. Clear the tissue on either side of the spinal cord to expose the muscles covering the spine.
   NOTE: The surgeon should be familiar with the anatomical landmarks. For example, the inferior angle of the scapula corresponds with T7. The top of the natural curve of the mouse's spinal is T12 and may be used as a reference point.
4. With proper lighting, determine the space between the vertebrae. Find the posterior end of T10 and cut muscles and fascia perpendicular to the intervertebral disk space. Cut just enough to expose the spinous process and posterior lamina of T10.
5. Using a pair of fine tipped Dumont #5 forceps, remove some of the tissue from the lamina and spinous process to expose a small sliver of the spinal cord. When necessary, use tissue forceps to stabilize the spinal column.
6. Perform the laminectomy by inserting one side of a pair of small vana scissors along the dorsolateral side of the vertebra and just beneath the lamina.
   1. Make small, careful snips to cut through the lateral side of the vertebral lamina. Ensure that no pressure is applied to the spinal cord.
   2. Repeat on the other side.
   3. Apply gentle pressure to halt bleeding as necessary with a Q-tip or surgical sponge, taking care to not apply pressure to the spinal cord.
      NOTE: Prepare gel foam soaked in sterile saline in the event that bleeding needs to be controlled.
   4. Once the incisions have been made, lift off the dorsal aspect of the vertebra and gently clear any tissue attachments. Use appropriate means to control bleeding if necessary.

3. Spinal Cord Compression

1. Using roungeurs or laminectomy forceps, ensure that the lateral sides of the spinal cord are free of vertebral bone so that the calibrated forceps for the compression injury can situate on either side of the spinal cord. The arms of the forceps must be able to be placed within the epidural space on adjacent sides of the spinal cord and the tips of the forceps must be able to reach the floor of the vertebral canal.
   1. Ensure that visibility of the spinal cord is good.
2. Situate the calibrated Dumont #5 forceps approximately in the middle of the exposed segment of the spinal cord. Recall that the arms of the forceps must be placed within the epidural space on adjacent sides and the tips must touch the floor of the vertebral canal to generate reproducible injuries.
3. Carefully compress the spinal cord until the spacers connect. Hold in place for 15 sec.
4. Gently release the compressive force and remove the calibrated forceps from the spinal cord. Sterile saline should be used to regain homeostasis before the wound is closed.

4. Wound Closing

1. Carefully suture the muscle layer over the spinal cord, taking care to not disrupt or apply pressure to the spinal cord.
2. Use either sutures or staples to close the skin over the wound.
3. If using gas anesthetic, begin to taper/turn off the anesthetic.
4. Administer 0.1 ml of lactated ringers per 10 g body weight to help account for dehydration during the surgery and after the surgery when the animal is lethargic and recovering from the injury. The solution should be warmed to body temperature prior to injection.
5. Place the mouse in a bedding-free cage. The cage should rest on top of a heating pad (as described in 1.1.5) in a manner that allows half of the cage area to be on the pad, while the other half is resting on a RT counter, in order to give the mouse climate options once it is ambulatory. Take care to ensure that the “recovery cage” is located in a quiet environment. Closely monitor the animal until it has regained consciousness, at which time the mouse can be transferred to a regular cage with bedding.

5. Post-operative Care

1. After the surgery, administer a dose of 0.05 mg/kg body weight buprenorphine subcutaneously every 12 hr for the first 3 days after surgery, and then as needed to manage symptoms of pain.
2. Administer a dose of lactated ringers (0.1 ml per 10 g body weight subcutaneously) for the first 3 - 5 days after surgery. Give this dose if/when the animal begins to exhibit signs of dehydration outside of this initial time period.
3. Manually express the bladder of the animals using the Crede maneuver twice daily. Gently palpate the animal’s abdomen to locate the bladder, and then apply gentle downward pressure until the bladder is empty.
    1. If the bladder does not empty or the urine is bloody or cloudy, administer 50 mg/kg by body weight of Baytril to the animal via interperitoneal injection for 10 days.
4. Monitor animals for signs of autophagy, dehydration, and excessive weight loss (greater than 20% of body weight). If an animal experiences any of these symptoms please consult with a veterinarian immediately with regards to treatment options, or euthanize the animal in a humane manner following IACUC guidelines.
    1. Given that mobility may be limited right after surgery, take the necessary steps to make sure animals have access to food and water. Pre-packaged wet food, as well as hydrogel, can be made available to animals in these instances.

6. Assess Tissue Damage Resulting from Compression Injury

1. Anesthetize animal as described in **steps 1.4** and **1.5**. Check depth of anesthesia by toe pinch and monitoring corneal blink reflexes. When the animal is insensitive to stimuli, proceed to **step 6.2**.
2. Perform an intracardial perfusion[10].
    1. Expose the chest cavity and insert a needle into the left ventricle. Flush out existing fluids with 20 - 30 ml ice-cold phosphate buffered saline (PBS) followed by 15 - 25 ml of ice-cold 4% paraformaldehyde (PFA).
3. Remove the spinal cord.
    1. Cut the skin dorsal to the spinal cord and clear away any excess tissue surrounding the length of the spinal column.
    2. Excise spinal column and cut away any remaining tissue. The actual level of the laminectomy and injury can be confirmed by counting ribs.
    3. Use vanna scissors and forceps to displace small sections of the spinal column in a caudal-to-rostral direction. Continue to cut until the cord is exposed enough to allow safe removal. Visualization of the cutting location and process can be facilitated through the use of a stereoscope.
4. Place spinal cord tissue in 4% PFA. Allow tissue to post-fix in this solution for 24 hr at 4 °C.
5. Cryoprotect tissue by incubating in 30% sucrose for 24 hr at 4 °C.
6. Embed tissue in OCT. Briefly, take tissue from the 30% sucrose incubation and remove any excess solution. Place the tissue into a cryomold filled with OCT and incubate for 1 hr at 4 °C.
    1. Remove mold from 4 °C, confirm directional orientation of the tissue, put the mold in a shallow dish of 2-methylbutane (pre-chilled for 1 hr on dry ice) and allow OCT to completely solidify. Keep on dry ice if using immediately or store at -80 °C.
7. Cut the tissue into 20 µm sagittal sections using a cryostat. Mount tissue directly onto slides. Store at -20 °C until use.
8. Perform Hematoxylin and Eosin (H&E) stain.
    1. Briefly, rehydrate the tissue (5 min, 2 times), stain with Hematoxylin (2.5 min), and wash with water (1 min, 2 times).
    2. Incubate tissue in 50% and then 70% ethanol (3 min each), stain with Eosin (45 sec), and dehydrate by incubation in 90% (5 sec), 95% (5 sec), 100% ethanol (2 min), and isopropanol (2 min).
    3. Clear with xylene (5 min, 3 times).
    NOTE: H&E staining will vary with specific tissue thickness and conditions. Therefore, standardization is required before proceeding with experimental tissues samples.
9. Cover tissue with a thin strip of Permount (~100 µm) and coverslip. Press down on all sides of coverslip to ensure proper distribution of fluid. Let slides dry O/N.
10. Visualize tissue sections using a digital microscope and capture images using the accompanying software.

Representative Results

We performed a laminectomy on 12 mice (25 - 30 grams) as described above, followed by spinal cord compressions at 0.25 mm (n = 4), 0.35 mm (n = 4) and 0.55 mm (n = 4). We sacrificed animals at three (n = 6) and seven (n = 6) days post injury by intracardiac perfusion. The spinal cord was removed from the spinal column, and the tissue was prepared and processed as described above. Images of whole spinal cord were taken with a Leica EZ4 digital microscope and accompanying software. Images of spinal cord sections were taken at 2X magnification using an Olympus digital microscope and accompanying software.

We found that spinal cord compression produces an injury with the epicenter at the site of the compression (**Figure 1**). The effects of the injury extend several millimeters in the rostral and caudal directions. The severity of the injury increased as the distance between the spacers decreased (0.25 mm > 0.35 mm > 0.55 mm, **Figure 2**). Three days after compression there was blood at the epicenter of the injury and surrounding regions that was not present 7 days after injury. The 0.25 mm and 0.35 mm compressions produced a cavity, but not the 0.55 mm model. After 7 days, the dorsal and ventral white matter largely decreased in size at the epicenter, the grey matter organization was highly distorted, and cavitation was persistent. These cytoarchitectonical alterations are translated into motor and sensory alterations in animal behavior evaluated using appropriate tests such as the Basso Mouse Scale for Locomotor Function and the von Frey hair and ethyl chloride tests for sensory function as we demonstrated in previous publications[8].

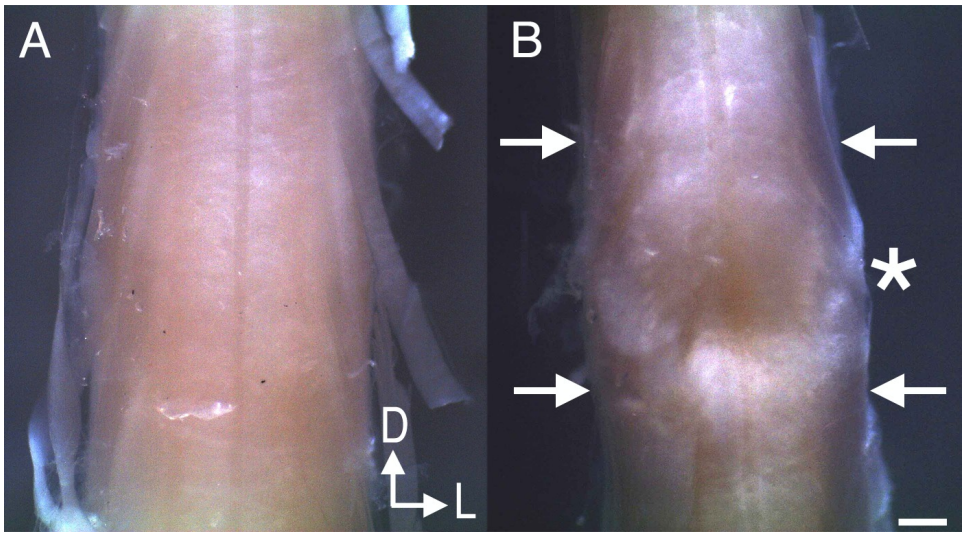

Figure 1. Representative images of the intact spinal cord both before and after injury. (A) Intact spinal cord. **(B)** Spinal cord after 0.35 mm compression. Arrows indicate border of injury. Asterisk indentifies epicenter of injury. D = Dorsal, L = Lateral. Scale bar: 0.50 mm. Please click here to view a larger version of this figure.

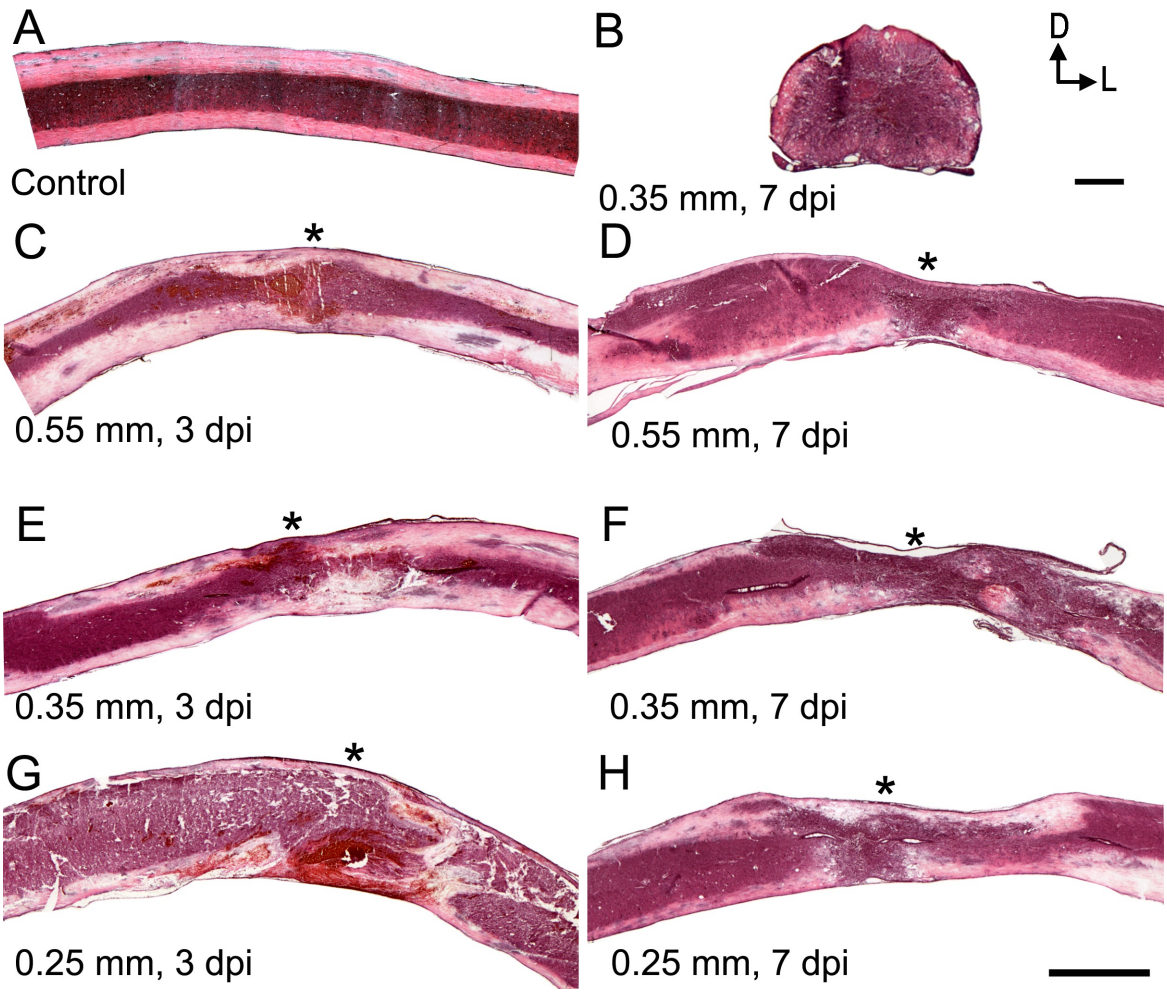

Figure 2. Representative images of mouse spinal cord before and after compression injury at varying compression widths. (A) Sagittal section of control spinal cord. **(B)** Coronal section at epicenter of a 0.35 mm SCI 7 days post compression injury (dpi). **(C,E,G)** Sagittal sections of spinal cords 3 dpi to a width of 0.25, 0.35 or 0.55 mm. **(D,F,H)** Sagittal sections of H&E stained spinal cords 7 dpi to a width of 0.25, 0.35 or 0.55 mm. Asterisk indentifies epicenter of injury. All sections stained with H&E. D = Dorsal, L = Lateral. Scale bar: 1.25 mm. Please click here to view a larger version of this figure.

Discussion

The choice of an SCI model is important in designing experiments to determine the efficacy of treatments for human cases of SCI. Such experiments require an animal model that is highly reproducible to limit variability that may result in inconclusive data. They should also be of clinical relevance to accurately evaluate the human condition they are modeling. To that end, choosing a compressive or contusive injury over a transection is more clinically relevant[3]. However, impactors and weight drop apparatuses for contusion injuries require the use of expensive and complicated machinery. In contrast, the calibrated forceps model of SCI utilizes modified forceps that are easy to assemble from common laboratory materials, and the surgery requires only one additional step after a standard dorsal laminectomy to expose the spinal cord. However, one drawback of using this method is that the compressive force is always applied laterally rather than dorsally, as is most often seen in human clinical cases of SCI[9], and compressive injuries generated using the method affect a greater rostral-caudal extent of tissue than contusion models[1,2]. This model has been demonstrated by the originators of the technique, and us, to generate reproducible SCI[7,11], and is well suited to the size of mice. Furthermore, this injury model allows for animals to be evaluated after surgery and therapeutic treatments using a multitude of behavioral tests, such as the Basso Mouse Scale for Locomotion and the von Frey hair test, to verify that a cohort of animals share the same injury severity and neurological deficits[7,11-13]. These same techniques can also be used to evaluate the efficacy of treatments administered to animals during investigative studies, fulfilling the general criteria for animal models used to evaluate therapies for SCI[2,7].

The method of producing the calibrated forceps for the injury model is simple and can be accomplished with a variety of different methods. We have used the spacer method[11], as published by Plemel[7], and have also modified forceps using a small screw, which not only provides an easier method for creating the compression device, but also allows for versatility in adjusting the final compression width, of benefit for comparative studies. The range of options in creating the forceps is nearly unlimited so long as the spacer(s) provide a stable means of always closing the forceps to the same distance and can withstand autoclaving and sterilization. The surgical methods described within this video are highly reproducible across users, however it is necessary that care be taken when performing the laminectomy and suturing the animal after the

procedure has been performed so that the spinal cord does not suffer any additional compressive forces that may increase the injury severity and confound future experiments.

With proper training and practice, the calibrated forceps model of compression injury is well suited for performing SCI in mice that mimic clinical cases observed in humans[2,3,7]. Due to the ease of creating forceps, producing mice of differing degrees of injury severity can be easily done. This will be of great benefit for observing genetic effects on SCI of differing degrees of severity in transgenic mice as well as evaluating the efficacy of stem cell transplantations in mice. The majority of studies in the literature have been performed on rats due to their size, which generally makes surgeries easier to perform. However the method published by Plemel *et al.*[7] and described by us in this video should enable SCI to be performed on mice with great ease and reproducibility.

Disclosures

The authors have nothing to disclose.

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