

## Video Article

# Controlled Cervical Laceration Injury in Mice

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Keywords: Medicine, Issue 75, Neurobiology, Anatomy, Physiology, Neuroscience, Immunology, Infection, Surgery, Nervous System Diseases, Diagnosis, Therapeutics, Surgical Procedures, Operative, Investigative Techniques, spine, spinal cord injury, SCI, mouse, laceration, stabilization, axonal regeneration, injury, mice, animal model, surgical techniques

Date Published: 5/9/2013

Citation: Zhang, Y.P., Walker, M.J., Shields, L.B.E., Wang, X., Walker, C.L., Xu, X.M., Shields, C.B. Controlled Cervical Laceration Injury in Mice. *J. Vis. Exp.* (75), e50030, doi:10.3791/50030 (2013).

## Abstract

Use of genetically modified mice enhances our understanding of molecular mechanisms underlying several neurological disorders such as a spinal cord injury (SCI). Freehand manual control used to produce a laceration model of SCI creates inconsistent injuries often associated with a crush or contusion component and, therefore, a novel technique was developed. Our model of cervical laceration SCI has resolved inherent difficulties with the freehand method by incorporating 1) cervical vertebral stabilization by vertebral facet fixation, 2) enhanced spinal cord exposure, and 3) creation of a reproducible laceration of the spinal cord using an oscillating blade with an accuracy of  $\pm 0.01$  mm in depth without associated contusion. Compared to the standard methods of creating a SCI laceration such as freehand use of a scalpel or scissors, our method has produced a consistent lesion. This method is useful for studies on axonal regeneration of corticospinal, rubrospinal, and dorsal ascending tracts.

## Video Link

The video component of this article can be found at <http://www.jove.com/video/50030/>

## Introduction

The availability of genetically modified mice is a powerful tool to identify the effects of specific genes that play a role in the mechanisms of SCI. Laceration SCI is an important model used to examine therapeutic agents or molecules that may provide effective treatment following this injury<sup>8</sup>. Fixation of the spinous processes during creation of the laceration injury in mice is imprecise due to the difficulty in grasping the thin and fragile spinous processes involved with maintaining spinal fixation<sup>5,11</sup>. Variability in the depth of the laceration of only 0.2 mm (10% of the diameter of the mouse spinal cord) causes misleading interpretation of data. The nature and extent of the spinal cord laceration lesion must be precisely defined<sup>10</sup>. To address this challenge, we have developed a novel technique consisting of vertebral stabilization and used fabricated blades attached to the Louisville Injury System Apparatus (LISA) to produce a laceration SCI<sup>7,14</sup>. This injury was created by using a sharp oscillating blade that avoided tissue deformation during the laceration process. The depth of the laceration was precise to an accuracy of 0.01 mm by using micro-drivers which control the laceration depth. Cutting blades are custom-made to specific shapes and widths to create the desired laceration contour<sup>9</sup>. We demonstrate 1) the method of cervical spine exposure, 2) the technique of vertebral stabilization using a bilateral facet fixation device, and 3) the creation of a cervical laceration injury using a vibrating blade.

## Protocol

### 1. Animal Preparation and Application of the Spine Stabilizer

The mouse cervical spine is concave ventrally as seen from the lateral view. The spinous processes from C3 to T1 are small and friable and, therefore, are not suitable for vertebral stabilization as commonly described<sup>3,4</sup>. We recommend that spine stabilization be performed by lateral facet fixation. The fixation device consists of a U-shaped metal channel to support the mouse and two adjustable stainless steel arms that clamp to each facet laterally. This provides excellent immobilization of the target vertebra. After spinal fixation, the spine is slightly elevated to flatten the cervical spine curvature to provide better exposure of the spinal cord.

1. Sterilize the following surgical instruments: 2-3 pairs of forceps, 2 pairs of microscissors, a 30 G needle, suture and needle holder, skin clips, and clip applicator. Disinfect the spine stabilizer. Anesthetize the mouse using an intraperitoneal cocktail of Ketamine/Xylazine (100 mg / 10 mg/kg). Shave the hair from the mouse's neck.
2. After skin cleansing with a povidone-iodine solution and 70% alcohol, move the mouse onto the operating table warmed with a heating pad. Cover the animal's eyes with ophthalmic ointment to prevent corneal drying.

3. After induction of anesthesia (reached when the mouse does not respond to a tail pinch), make a posterior cervical midline skin incision from the occiput to the subcutaneous fat-pad of the lower cervical spine. Under magnification, perform a midline incision between the trapezius muscles at C2 and split the semispinalis capitis muscles. Identification of the submuscular fat pad facilitates dissection in the correct layer.
4. Extend the midline muscle dissection caudally to the T2 spinous process which serves as a reliable landmark. Cut the muscles attached to the T2 vertebra and remove the cartilaginous portion of the T2 spinous process.
5. Dissect the paraspinal muscles from the C2 through the T2 laminae using a pair of micro-scissors. Muscle dissection begins adjacent to the spinous processes and extends bilaterally to the facet joints. Separate the muscles immediately adjacent to the spinous processes and laminae (in the periosteal layer) to minimize bleeding. After the lateral facets are exposed, place the mouse on the U-shaped channel of the LISA stage.
6. Attach the stainless steel arms beneath the exposed facets bilaterally. Once the arms are in place, tighten the thumb screws of the steel arms to immobilize the spine. This maintains firm fixation of the target vertebra and provides excellent exposure. The arms can be adjusted to provide precise horizontal orientation of the spine.
7. Incise the ligamentum flavum between C5 and C6 to expose underlying dura. Between the interlaminar space, use a 30 G needle to create a small durotomy through which microscissors are placed to extend the durotomy. The spinal cord is now prepared to undergo the controlled laceration lesion.

## 2. Cervical Spinal Cord Laceration Using the LISA Device

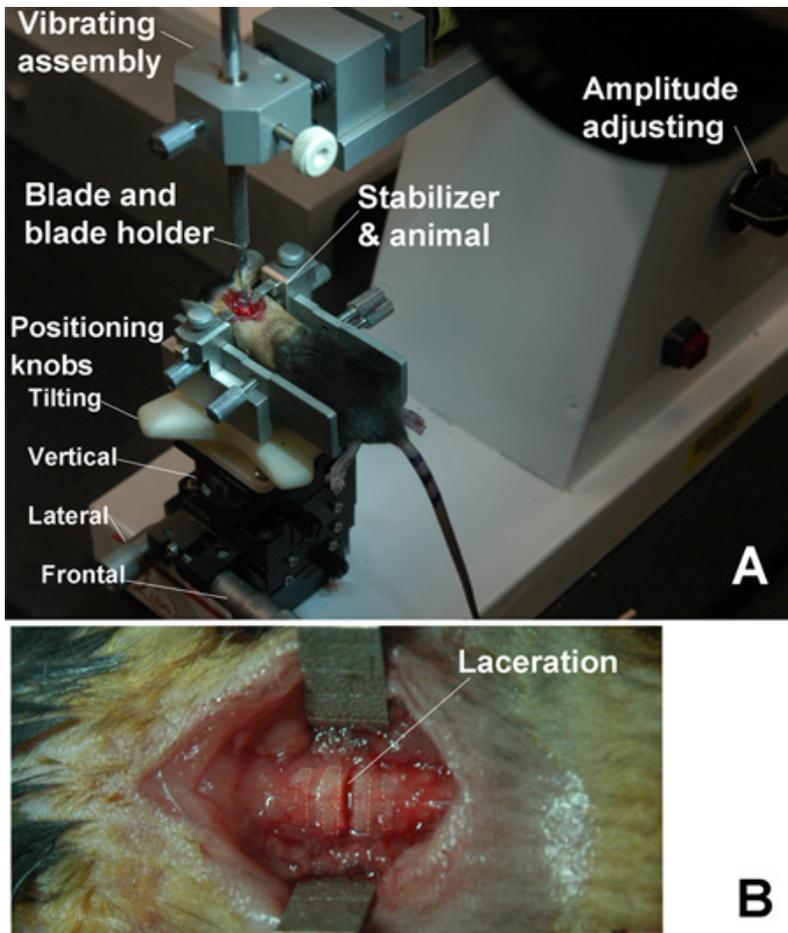
1. The width of the cervical spinal cord enlargement varies at different levels. Make a dorsal hemisection lesion at C5-6 using a 2.3 mm flat blade and set the amplitude of vibration to cover the entire width of the spinal cord. Blades are obtained from Fine Science Tools Inc. (Foster City, CA) and modified for spinal cord laceration. Maintain the amplitude of the blade oscillation at  $\geq 0.5$  mm, as lower amplitude levels will diminish the ease of cord laceration.
2. Position the spine stabilizer and mouse on the LISA stage. The blade is attached to the LISA with its position controlled by micro-drivers capable of three ranges of motion. Components of the LISA and their functions are described in **Figure 1**.
3. Power the blade-vibrating switch on. Under magnification, move the mouse so that the exposed spinal cord is positioned directly beneath the vibrating blade.
4. Elevate the stage supporting the mouse towards the oscillating blade. The "0" position is recorded when the blade barely touches the dorsal vein of the spinal cord. Measure the depth of the spinal cord laceration relative to the "0" position.
5. Elevate the stage position by micro-driver control: a 360° turn of the micro-driver knob elevates the stage by 0.25 mm. Thus, a 0.75 mm dorsal hemisection lesion is created by turning the micro-driver knob 3 times. The accuracy of the lesion is  $\pm 0.01$  mm. As the blade begins to lacerate the spinal cord, lubricate the surgical field with saline irrigation. The cutting depth of the spinal cord is controlled by the vertical micro-driver and is independent of visual guidance.
6. Once the predetermined depth has been reached, turn the vibrating switch off. Ideally, the oscillating blade is positioned in the lesion gap without evidence of tissue deformation. Lower the stage from the cutting blade and remove the blood and saline from the surgical field using cotton Q-tips. Hemostasis occurs spontaneously in  $< 1$  min.
7. Release the mouse from the spine stabilizer. Approximate the paraspinal muscles using 6-0 silk suture and close the skin wound using stainless steel Michel clips.

## 3. Animal Care

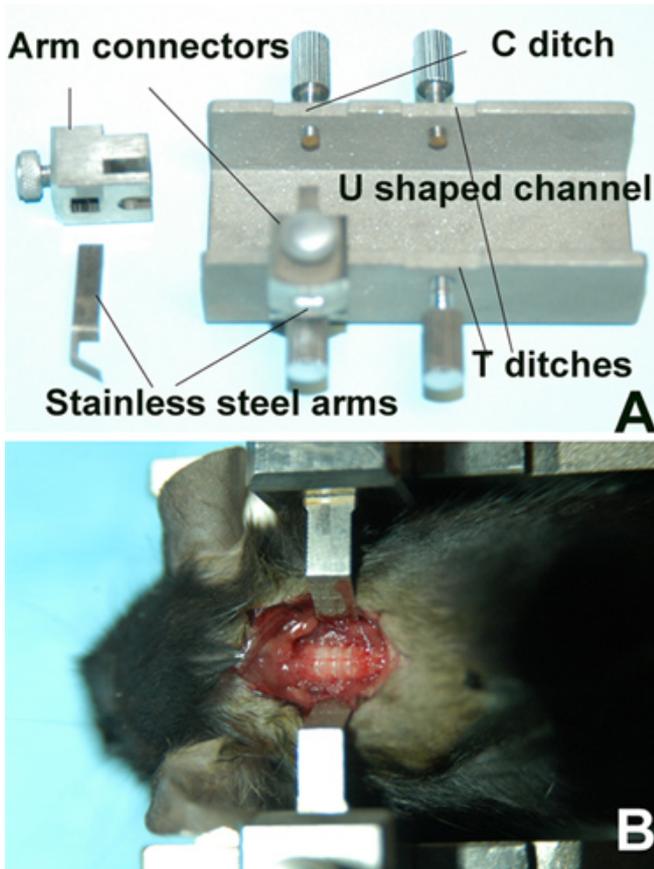
1. Subcutaneously inject a total of 1-2 ml saline to maintain adequate hydration and place the mouse in the recovery cage on a heating pad while regaining consciousness.
2. Provide water and soft food ad lib and administer analgesics for 48 hours post-operatively. There is no need for bladder care following dorsal hemisection of the spinal cord.

## Representative Results

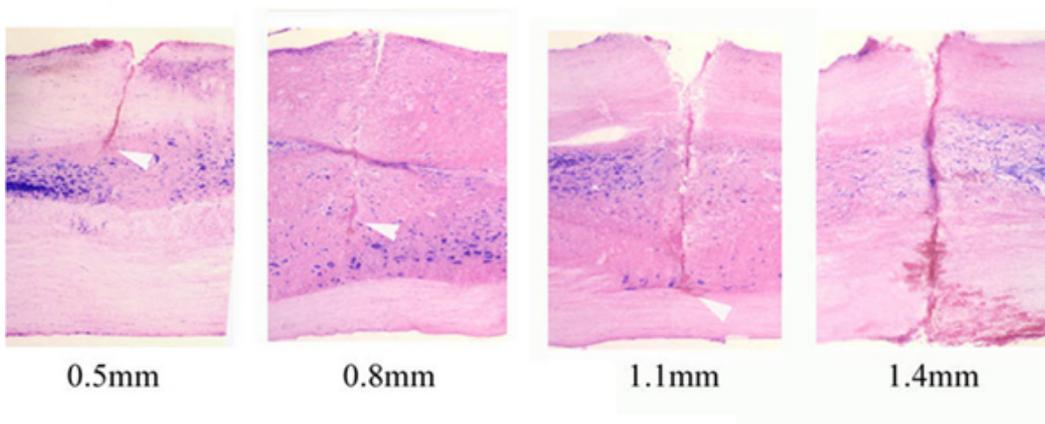
Immobilization of the target vertebra is of great importance in generating precise lesions of the mouse spinal cord. Our spine stabilization device overcomes the anatomical issues of short spinous processes and ventral lordosis of the mouse cervical spine. The cervical vertebrae are well exposed using our cervical spine stabilizer (**Figure 2**). Our mouse spine stabilizing device is a reliable technique to prepare the spine for cervical spinal cord procedures. The depth of the lesion using the LISA is accurate to 0.01 mm<sup>6,13</sup>. The precise laceration causes no contusion at the lesion/tissue interface (**Figure 3**). The precision of the dorsal hemisection lesions was demonstrated in C57BL/6 mice in a study on axonal regeneration in which a 0.9 mm deep laceration extended just beyond the central canal in each specimen confirmed by pathological sections of the spinal cord<sup>1</sup>. Locomotion of all of these animals recovered following this spinal cord laceration injury.



**Figure 1.** (A) The mouse in the spine stabilizer placed on the LISA stage. The vibrating blade is directed toward the spinal cord to be lacerated. Micro-driver controls are located beneath the stage and are designed to position the mouse in the appropriate site. The vertical micro-driver controls the lesion depth, and the tilting knob controls the horizontal plane of the spinal cord to prevent angulation of the laceration. The on-off switch controls the vibration motor, and another knob adjusts its amplitude. (B) A 0.75 mm dorsal hemisection laceration lesion cut beneath intact lamina arches.



**Figure 2.** (A) The mouse spine stabilizer consisting of a U-shaped channel and two arms and connectors. The mouse is placed in the C trough used for cervical SCI and in the T trough for thoracic SCI. (B) The cervical spine is fixated by placing the arms under the lateral facets and then locking the thumb screws. The dura is exposed between the laminae of C5-6, C6-7, and C7-T1 without any removal of bone.



**Figure 3.** Four dorsal spinal cord lacerations at depths of 0.5, 0.8, 1.1, and 1.4 mm observed in the sagittal view (cresyl-violet and eosin stain) depicting the high degree of precision using this technique.

## Discussion

Vertebral stabilization prior to laceration injuries to the spinal cord has been obtained by fixation of the spinous processes. Both the cervical spine lordotic curve and attachment of clamps to the friable short cervical spinous processes from C3 through T1 in the mouse prevent effective spine stabilization. Furthermore, use of a razor blade or microscissors utilized under manual control causes significant tissue deformation that creates variability in the depth of the lesion<sup>6</sup>. This may lead to the misinterpretation of data particularly when axonal regeneration of specific pathways is studied. For example, spared dorsal corticospinal axons may be misinterpreted as regenerated axons if the dorsal corticospinal tract was not completely transected at the time of lesioning. These challenges can be overcome by using a spine stabilization device with fixation to the facets at a single level and precise lesioning of the spinal cord. Additionally, using a high frequency oscillating blade produces a sharp laceration without crushing or contusing the adjacent spinal cord. This method has been used to produce spinal cord laceration injuries in rats<sup>9,12,14</sup>.

with subsequent modifications to produce thoracic spinal cord lacerations in mice<sup>6</sup>. In the present communication, we describe the method of creating reliable cervical laceration lesions in the mouse.

Insofar as the anteroposterior diameter of the spinal cord is <2 mm in the mouse, precise depths of the laceration lesion are vital in creating a reliable experimental model. Minimal variability in the lesion depth will significantly alter results of experiments assessing axon regeneration as well as volumetric and behavioral studies. The accuracy of the lesion depth using this method is  $\pm 0.01$  mm because we used high precision micro-drivers to control the position of the cutting blade. This method has reduced the inconsistency inherent in other models of creating a laceration SCI. This method is particularly useful in studying axonal regeneration of the long spinal cord pathways located in the dorsal half of the spinal cord, such as the corticospinal tract, the rubrospinal tract, and the dorsal ascending tract. With this method, these fiber tracts can be completely and reliably transected. In this respect, errors of data interpretation are minimized, thereby improving reliability of reporting of experimental studies on SCI.

In summary, we have described a novel technique to create a reproducible *in vivo* model of cervical spinal cord laceration injury in the mouse. This technique is based on spine stabilization by fixation of the cervical facets and laceration of the spinal cord using an oscillating blade. Using this method in a dorsal thoracic spinal cord laceration model in mice<sup>6</sup>, we demonstrated a tight correlation between the laceration depth, histology, and behavior recovery. Such a technique has also been found to be reliable by several other laboratories<sup>2,12</sup>.

## Disclosures

1. Several authors (YPZ, XMX, CBS) have a financial interest in the Louisville Impactor System, Inc.
2. The authors, Yi Ping Zhang, Lisa B. E. Shields, and Christopher B. Shields, are employees of Norton Healthcare, Louisville, KY. Other authors are employed by Indiana University, Indianapolis, IN.
3. The authors did not receive funding from any company that produce reagents or instruments utilized in this article.

## Acknowledgements

The development of this device was supported by the LISA Co., Louisville, Kentucky. We also acknowledge the ongoing support of Norton Healthcare, Louisville, KY to CBS, and NIH NS050243, NS052290, and NS059622 to XMX.

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