Video Article

A Tissue Displacement-based Contusive Spinal Cord Injury Model in Mice

Xiangbing Wu*[1], Yi Ping Zhang*[2], Wenrui Qu[1], Lisa B. E. Shields[2], Christopher B. Shields[2,3], Xiao-Ming Xu[1]

[1]Spinal Cord and Brain Injury Research Group, Stark Neurosciences Research Institute, Department of Neurological Surgery and Goodman and Campbell Brain and Spine, Indiana University School of Medicine

[2]Norton Neuroscience Institute, Norton Healthcare

[3]Department of Anatomical Sciences and Neurobiology, University of Louisville

*These authors contributed equally

Correspondence to: Christopher B. Shields at cbshields1@gmail.com, Xiao-Ming Xu at xu26@iupui.edu

Abstract

Producing a consistent and reproducible contusive spinal cord injury (SCI) is critical to minimizing behavioral and histological variabilities between experimental animals. Several contusive SCI models have been developed to produce injuries using different mechanisms. The severity of the SCI is based on the height that a given weight is dropped, the injury force, or the spinal cord displacement. In the current study, we introduce a novel mouse contusive SCI device, the Louisville Injury System Apparatus (LISA) impactor, which can create a displacement-based SCI with high injury velocity and accuracy. This system utilizes laser distance sensors combined with advanced software to produce graded and highly-reproducible injuries. We performed a contusive SCI at the 10th thoracic vertebral (T10) level in mice to demonstrate the step-by-step procedure. The model can also be applied to the cervical and lumbar spinal levels.

Video Link

The video component of this article can be found at https://www.jove.com/video/54988/

Introduction

The most common spinal cord injury (SCI) occurring in humans is a contusive SCI[1]. To investigate the mechanisms of injury and the various therapeutic strategies following SCI, a precise, consistent, and reproducible contusive SCI model in rodents is needed.

Many spinal cord contusive injury models with various injury-producing mechanisms have been used in experimental SCI research[2,3,4,5,6]. Three contusive SCI models – specifically, the weight drop-based New York University (NYU)/ Multicenter Animal Spinal Cord Injury Studies (MASCIS) impactor[3,6], the Ohio State University (OSU) impactor/electromagnetic SCI device (ESCID)[5,7], and the Infinite Horizon (IH) impactor[4,8] – are widely accepted in the SCI research field. The NYU/MASCIS impactor or an equivalent produces injury by dropping a fixed weight from different heights onto the target spinal cord to create multiple injury severities[3,6]. The OSU/ESCID causes injury by inducing tissue displacement[5,7]. The IH impactor produces injury by applying different forces to the spinal cord[4,8]. Each impactor uses a different velocity, which is an important parameter that influences the injury outcomes. The NYU/MASCIS apparatus generates velocities ranging from 0.33-0.9 m/s. The IH device has a maximum velocity of 0.13 m/s[4]. The OSU/ESCID impactor has a fixed velocity of 0.148 m/s[5]. Notably, the velocities of these models are lower than that observed in clinical velocities, which usually exceed 1.0 m/s[9].

Here, we introduce a novel displacement-based contusive SCI device, called the Louisville Injury System Apparatus (LISA), to produce SCI in mice with a high impact velocity[10]. This system includes a vertebral stabilizer, which firmly stabilizes the vertebra at the site of injury, allowing the production of a constant, reproducible SCI. The laser sensor of the device ensures the precise determination of tissue displacement and the resultant severity of the SCI. The velocity of the plunger at the point of contact with the spinal cord can be adjusted from 0.5 to 2 m/s. These injury parameters closely replicate traumatic SCI seen clinically.

Protocol

All surgical and animal handling procedures were performed as approved under the Guide for the Care and Use of Laboratory Animals (National Research Council) and the Guidelines of the Indiana University School of Medicine Institutional Animal Care and Use Committee.

1. Preparing the Animal and Performing the T10 Spinal Laminectomy

1. Sterilize the surgical instruments and metal spine stabilizer in an autoclave. Clean the surgical operating table. Warm a heating pad to 37 °C. Place the heating pad on the operating table and cover it with sterile surgical drapes. Use sterile technique throughout the operation.

2. **Use female young adult C57/6J mice at 10 weeks old for this study. Anesthetize each animal with an intraperitoneal (i.p.) injection of a ketamine (87.7 mg/kg) and xylazine (12.3 mg/kg) mixture. Confirm complete anesthesia by eliciting no response to a paw pinch-induced nociception stimulation.**
   1. Subcutaneously administer buprenorphine (0.01-0.05 mg/kg), an analgesic agent, and carprofen (5 mg/kg), a non-steroid anti-inflammatory drug.

3. Shave the hair over the thoracolumbar spine using an electric clipper. Scrub the skin with betadine solution and 70% alcohol wipes.
4. Apply ophthalmic ointment to the corneas to protect the eyes from drying during surgery.
5. With a scalpel, make a 1.5 cm midline skin incision on the back of the animal to expose the 9th to 11th thoracic vertebral laminae. Push the subcutaneous adipose tissue rostrally. Dissect the paraspinal muscles away from spinous processes and laminae, toward the lateral facets on each side.
6. Position the mouse on the U-shaped trough of the stabilizer (**Figure 2A** and **2B**). Bilaterally clamp the stainless steel arms beneath the exposed facets of the T10 vertebra (**Figure 4A**) and tighten using the thumb screws attached to the arms (**Figure 2A**).
7. Remove the T10 spinous process and lamina (laminectomy) using a micro-rongeur that exposes the dura mater overlying the spinal cord (**Figure 4B**).

2. Performing the T10 Contusion Injury Using the LISA Impactor

1. Turn the knob of the pressure regulator on the nitrogen tank to set the compressed nitrogen to 20 PSI or 138 kPa (**Figure 1A**) for this study. NOTE: The pressure is adjustable from 10-120 PSI. A higher pressure will result in a higher-velocity impact. The SCI device tip with a diameter of 1.2 mm is designed for mice, and the tip with a diameter of 2.2 mm is designed for rats. When changing from mice to rats, the larger-diameter tip can be formed by adding a ring to the metal tip (id 1.2 mm/od 2.2 mm). We used the 1.2 mm tip in this mice SCI study. Sterilize the SCI tip before use.
2. Turn on the computer to start the software. Push Button 1 (**Figure 1B**) to activate the impactor tip into a fully-extended position (**Figure 3A-1**). NOTE: The function of Button 1 is to manually activate or deactivate the pneumatic cylinder.
3. Place the U-shaped container with the mouse onto the stage (**Figure 2B**). Fix the stage in place by tightening the thumb screws of the mount (**Figure 2B**).
4. Under the "SET ZERO LEVEL" zone (green), set the zero level, with a laser sensor measuring the distance to the fully-extended plunger tip, by clicking the "START READING" button (**Figure 3A**). The distance will be shown in the "Range" parameter in this zone (**Figure 3A**). Click the "SET ZERO" button (**Figure 3A**), and a number in mm will appear in the "Zero" parameter box (*e.g.*, 8.951 mm, shown in **Figure 3A**).
5. Push Button 1 (**Figure 1B**) to withdraw the impactor tip (**Figure 3B-1**, indicated by an upper arrow) and unlock fastening Screw 1 (**Figure 2B**). Pull the screw to the right position (**Figure 3B-1**, indicated by a lateral arrow) to move the tip away from the laser beam path and turn the screw 90° clockwise to lock the screw.
6. Move the stage by adjusting the frontal and lateral micro-drivers (**Figure 1C**) to aim the laser beam onto the center of the exposed dorsal spinal cord. After the injury location is targeted, measure the tissue distance by clicking the "START READING" button under the "SET INJURY LEVEL" zone (blue) (**Figure 3B and 3B-1**).
7. **Slowly adjust the distance between the sensor and the spinal cord via the vertical micro-driver (Figure 1C) to reach the desired displacement parameter (*e.g.*, 0.500 mm, shown in the "Injury" parameter box) in the "SET INJURY LEVEL" zone (blue) (Figure 3B).**
   1. When the desired injury displacement is reached, record the tissue distance (*e.g.*, 8.451 mm, shown in the "Range" parameter box) (**Figure 3B**). Define the desired displacement (Injury) = tip distance (Zero) - tissue distance (Range) (**Figure 3B**). When the desired injury (*e.g.*, 0.500 mm tissue displacement) is reached (**Figure 3B**), click the "SET INJURY" button under the "SET INJURY LEVEL" zone to set the injury.

8. Turn Screw 1 90° counterclockwise to unlock the screw, push the impact tip back into the laser beam trajectory (**Figure 3C-1**, direction indicated by an arrow), and lock Screw 1 by turning it 90° clockwise.
9. Click the Run button under the red "RUN EXPERIMENT" zone (**Figure 3C**) to execute the impact. The parameter boxes under this zone will show the injury time (s), the force (mV), the velocity (m/s), and the injury displacement (mm) (**Figure 3C**).
10. After all injury data are recorded and saved, remove the U-shaped trough with the mouse from the stage. Visually confirm the spinal cord injury under a surgical microscope (**Figure 4C**).
11. Suture the paravertebral muscles, superficial fascia, and skin using continuous suture with 3-0 silk (Henry Schein, 776-SK).
12. Inject the animal with 1 mL of 0.9% saline subcutaneously for hydration and place it onto a temperature-controlled pad until full consciousness has been regained. Place the mouse into a cage with accessible food and water.
13. For post-operative care, manually express the bladder until spontaneous bladder voiding returns. For analgesia, inject Buprenorphine (0.05-2.0 mg/kg, SQ) 8-12 h/day for 2 days. If urinary bladder infection occurs, inject Baytril (SQ, 5-10 mg/kg in 0.1 mL, 1 dose daily) for 7-10 days. If regional/systemic infection occurs, inject Gentamycin (SQ, 5-8 mg/kg, diluted in 1 mL sterile saline, every 8-12 h) for 4 days.
14. Remove the suture threads at 14 days post-SCI.
15. On the 42th day post injury, mice will be sacrificed by perfusion. After appropriate anesthesia as 1.2, they will be perfused with 30 ml (0.01M) phosphate buffered saline (PBS) and 30 ml 4% paraformaldehyde in 0.01 M PBS. One centimeter of the spinal cord including the lesion epicenter will be collected and processed for sectioning and histological analysis.

Representative Results

This device consists of five main components: (1) a body with an impactor tip (**Figure 1C**), (2) a computer with software (**Figure 1B**), (3) an electrical control box (**Figure 1B**), (4) a vertebral stabilizer (**Figure 2A**), and (5) compressed air for the pneumatic control system (**Figure 1A**). To induce precise tissue displacement, the system relies on a laser sensor to measure the distance between the fully-extended plunger tip and the targeted spinal cord dorsal surface. The software takes the 4-mm thickness of the tip into account due to the fact that the laser beam only reaches the reflecting surface of the impactor (**Figure 2B** and **Figure 3A-1**). There are two positions at which the plunger tip may be placed: (1) in the path of the laser beam (**Figure 3A-1**) or (2) in a lateral position away from the laser beam (**Figure 3B-1**). When the plunger is in the laser beam path (**Figure 3A-1**), it measures the distance from the impactor tip and monitors the velocity of the impactor tip during motion between

extension and retraction. When the plunger is in the lateral position away from the laser beam path (**Figure 3B-1**), the distance between the laser and the spinal cord is measured.

The stabilization of the T10 vertebra using our vertebral stabilizer is an integral component of the procedure (**Figure 2A**)[10,11]. Reliable distance measurements using the laser sensor depend on the stability of the target, which can be distorted if motion is present. To determine the accuracy and consistency of this system, 8 mice were subjected to 0.5-mm displacement injuries. These animals showed a displacement variability of ± 0.001 mm (± SD), indicating that the system is highly accurate and reproducible. **Figure 4** demonstrates the immobilized target vertebrae in the stabilizer (**Figure 4A**) and the exposed T10 spinal cord prior to (**Figure 4B**) and after (**Figure 4C**) contusion under a surgical microscope.

The pressure of the compressed air controls the velocity of the impactor at the moment of injury. Our data demonstrate that the impact velocity is 0.81 ± 0.0345 m/s (mean ± SD) at a pressure of 138 kPa. The knob (**Figure 1B**) on the electrical box controls the duration of the tip-cord contact (dwell time) following the injury, and it can be adjusted between 0 and 5,000 ms. The tip-cord dwell time in most experiments is set at 0.32 ± 0.0147 s (mean ± SD) (**Figure 5**). Using this device, severity-dependent contusive injuries can be produced with tissue displacements of 0 mm (sham control), 0.2 mm (mild injury), 0.5 mm (moderate injury), and 0.8 mm (severe injury) in adult mice (**Figure 6**).
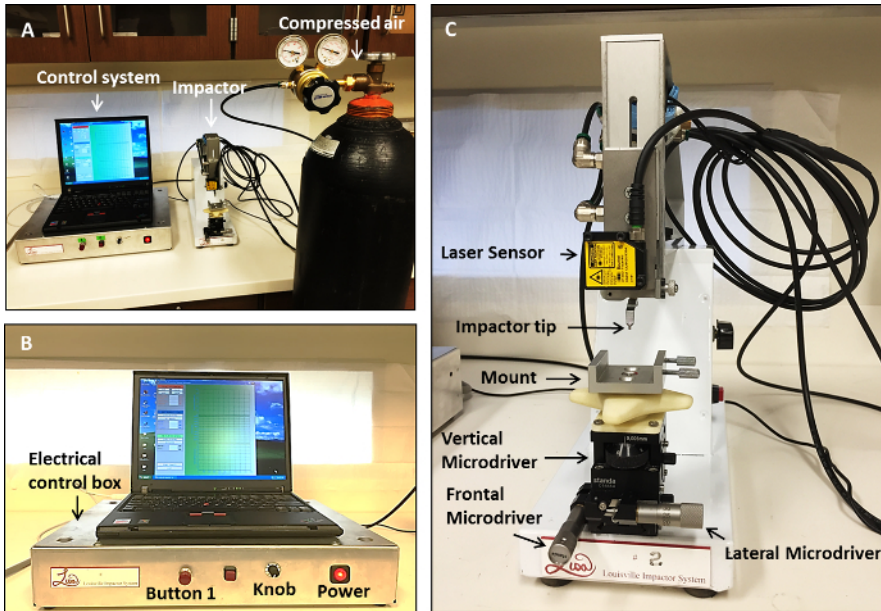

Figure 1: The Louisville Injury System Apparatus (LISA). (**A**) The system consists of an impactor, a control system, and a source of compressed air. (**B**) The control system includes a control box and a laptop computer. The software and control buttons of the control box allow the user to establish injury parameters. (**C**) The laser sensor is the key component of the device and measures the position of the injury target, the distance from the spinal cord to the sensor, and the injury velocity. The quick down-and-up movement of the impact tip is powered by compressed air. The location of the injury and the severity of the tissue displacement are adjusted by microdrivers, which control movement in three dimensions. Please click here to view a larger version of this figure.
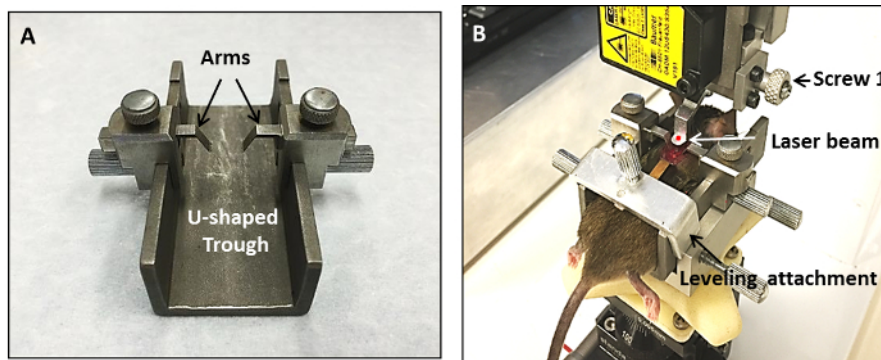

Figure 2: The stabilizer and Mouse Holder. (**A**) The spine stabilizer consists of a U-shaped trough and two metal arms to hold the mouse vertebra. (**B**) The stabilizer is then mounted on the impactor device. The red line indicates the laser beam path. Please click here to view a larger version of this figure.

Figure 3: Method to Produce a Contusive SCI. (A-C) The Graphical User Interface (GUI) software with three injury parameters/zones are shown. (**A**, **A-1**) The green zone (SET ZERO LEVEL) calibrates the distance of the plunger tip. The red line indicates the laser beam path. (**B**, **B-1**) The blue zone is used to set the injury level (SET INJURY LEVEL). The impactor is lifted up and moved laterally to the right side to allow the laser beam to reach the dorsal surface of the spinal cord to set the zero level. The red line indicates the laser beam path. (**C**, **C-1**) Prior to impact, the tip is moved back onto the laser beam path to execute the injury (RUN). The injury parameters are under the red zone (RUN EXPERIMENT). The red line indicates the laser beam path. Please click here to view a larger version of this figure.
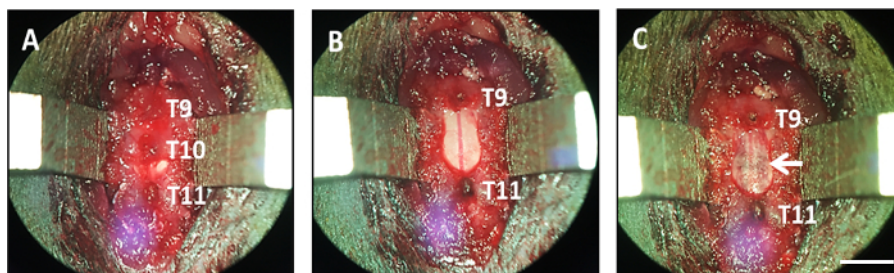

Figure 4: Injury Exposure and Assessment. (A) The metal arms of the spine stabilizer stabilize the T10 vertebra. (**B**) T10 laminectomy to expose the spinal cord, with the dorsal vessels clearly seen. (**C**) The impact-induced contusion (arrow) on the dorsal surface of the spinal cord confirms the injury. Scale bar = 2 mm. Please click here to view a larger version of this figure.
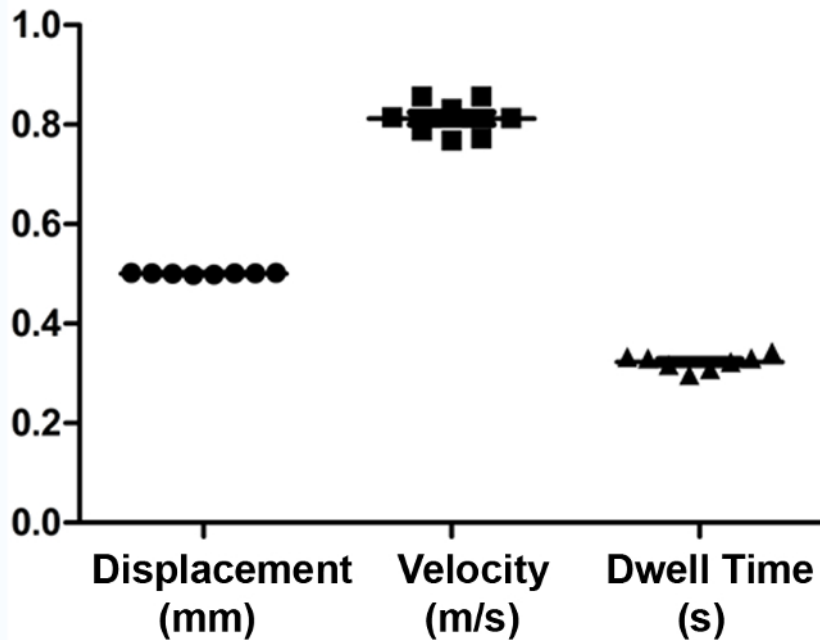
Figure 5: Injury Parameters. Consistent injury parameters include tissue displacement (mm), injury velocity (m/s), and tip dwell time (s). N = 8, Mean ± SD. Please click here to view a larger version of this figure.
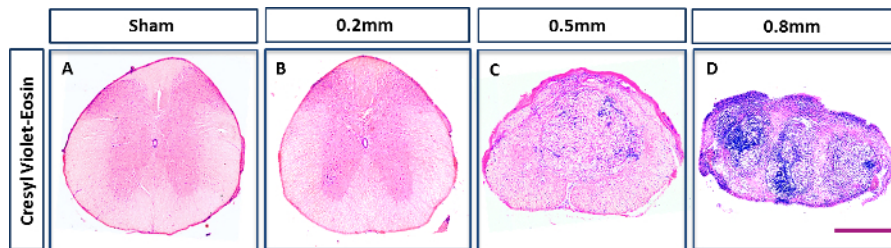

Figure 6: Histologic Assessment. Representative cross sections of spinal cords, stained with Cresyl Violet and Eosin, show displacement-severity-dependent injuries following (**A**) sham (0 mm), (**B**) mild (0.2 mm), (**C**) moderate (0.5 mm), and (**D**) severe (0.8 mm) contusive SCIs at T10 using the LISA device. Images were taken at the injury epicenter. Scale bar = 500 µm. Please click here to view a larger version of this figure.

Discussion

In 1911, Allen described the first weight drop model using a fixed weight to induce injuries on the exposed spinal cords of dogs[12]. Similar weight drop models have been developed based on the Allen model, including the NYU/MASCIS impactor[3,6,13,14]. In addition to the weight drop model, other SCI devices have been created. The OSU/ESCID[5,7] model uses a tissue displacement mechanism to control injury severity, and the IH model[4,8] uses force to create a gradable SCI. In these systems, vertebral stabilization is obtained by clamping the spinous processes rostral and caudal to the injury site. These devices utilize low injury velocities, specifically 0.33 - 0.9 m/s (NYU/MASCIS), 0.148 m/s (OSU/ESCID), and 0.13 m/s (IH). Stabilizing the rostral and caudal spinous processes may cause spine flexibility and spine movement during the impact, which may affect injury accuracy.

The LISA method attempts to overcome the shortcomings of existing models, particularly in regard to spine instability and low injury velocity. This method uses bilateral facet stabilization and avoids movement artifacts associated with the injury. This device utilizes a high-impact velocity that can be set between 0.5-2 m/s[11,15]. The laser sensor is more advanced than the Ling Vibrator used in the ESCID model and precisely measures the distance from the surface of the spinal cord without requiring any tissue contact. The model was originally developed to produce a rat SCI, and it has now been adapted to produce SCI on mice and on non-human primates[16], with modifications.

Spine stabilization reduces variability in all experimental SCI methods, particularly in tissue displacement models. The laser distance sensor determines the magnitude of tissue displacement of the spinal cord during respiratory movements. It is important that the point of the spinal cord on which the laser is focused should be the identical point struck by the impactor. This step is accomplished during the calibration step (**Figure 3**), when the impactor tip and the laser beam are aligned. A potential weakness of this model is that the magnitude of tissue displacement is measured from the dural surface. Although the thickness of the dura constitutes a negligible difference between animals, significant variability may exist in the subarachnoid space filled with cerebrospinal fluid (CSF). Variability in injury outcomes may occur when producing a very mild contusion injury using a small tissue displacement. Overall, the consistency of the injury is mainly dependent upon the accuracy of tissue displacement and also upon the velocity and tissue contact time of the plunger.

The range of tissue displacement is wide (accuracy: 0-10 ± 0.005 mm). Based on previous pilot data and published information in rodents and non-human primates, a displacement of 20% of the anteroposterior diameter of the SC yields a mild SCI, a 30-40% displacement produces a moderate SCI, and a >50% displacement produces severe SCI at a 1 m/s velocity. There will be slight differences depending on the animal species. The dwell time is adjustable from 0 to 5 s using a time relay. In our study, the dwell time was set at 300 ms. This can be easily adjusted to replicate the dwell times of other SCI devices, including the NYU and IH models.

In summary, we have developed a displacement-based model of contusive SCI in adult mice. The model uses a U-shaped stabilizer to stabilize the bilateral spinal facets, avoiding the cord movement artifacts associated with the laser-guided measurement of the cord surface. This model can produce high-velocity cord injuries from 0.5-2 m/s. The laser sensor is more accurate than the conventional method to determine the velocity and the distance to the impact surface. The model can produce injuries of the spinal cord at all levels, from mild to severe. When modified, this device can also produce injuries in rats and large animals such as non-human primates.

Disclosures

Christopher B. Shields, M.D. has ownership of the Louisville Injury System Apparatus (LISA) produced by Louisville Impactor System, LLC.

Acknowledgements

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