Please cite this article as: Chuan-Wen *et. al.*, (2017). Contusion Spinal Cord Injury Rat Model, *Bio-protocol* 7 (12): e2337. DOI: 10.21769/BioProtoc.2337.

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

Contusion Spinal Cord Injury Rat Model

Chuan-Wen Chiu[1], Henrich Cheng[2, 3, *] and Shie-Liang Hsieh[1, 4, *]

[1]Genomics Research Center, Academia Sinica, Taipei, Taiwan; [2]Neural Regeneration Laboratory, Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan; [3]Center for Neural Regeneration, Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan; [4]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan

*For correspondence: hc_cheng@vghtpe.gov.tw; slhsieh@gate.sinica.edu.tw

[Abstract] Spinal cord injury (SCI) can lead to severe disability, paralysis, neurological deficits and even death. In humans, most spinal cord injuries are caused by transient compression or contusion of the spinal cord associated with motor vehicle accidents. Animal models of contusion mimic the typical SCI's found in humans and these models are key to the discovery of progressive secondary tissue damage, demyelination, and apoptosis as well as pathophysiological mechanisms post SCI. Here we describe a method for the establishment of an efficient and reproducible contusion model of SCI in adult rat.

Keywords: Spinal cord injury, Contusion, Rat, Demyelination

[Background] The spinal cord plays an important role in the interconnections between the brain and peripheral nerves. Severe SCI causes the loss of physiological functions and even paralysis or death (Singh *et al.*, 2014). After SCI, the microvascular hemorrhage with disruption of the blood-spinal cord barrier is followed by edema, ischemia, and the release of cytotoxic chemicals from inflammatory pathways (Oyinbo, 2011; Mothe and Tator, 2012). Secondary neurodegenerative events such as demyelination, Wallerian degeneration and axonal dieback occur in the non-permissive tissue environment. Contusion, a type of blunt injury in the spinal cord, mimics typical SCI in humans which is mainly caused by vehicle accidents, especially motorcycles. In contrast to the sharp SCI model such as the transection that provides an anatomical model for evaluating axonal regeneration, the contused spinal cord presents a preferable microenvironment for studying of pathophysiological mechanisms post injury (Young, 2002). Experimental induction of a contusive SCI in a rat model using the NYU-MASCIS (New York University-Multicenter Animal Spinal Cord Injury Study) impactor device has been validated as an analog to human SCI. Furthermore, a comparison between the rat model of SCI with human SCI shows functional electrophysiological and morphological evidence of similar patterns recorded in motor evoked potentials and somatosensory evoked potentials (SSEP) as well as high-resolution magnetic resonance imaging (Basso *et al.*, 1996; Metz *et al.*, 2000; Kwon *et al.*, 2002; Young, 2002). Here we describe a method with tips for construction of an efficient and reproducible contusion model of SCI in adult rat.

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

Materials and Reagents

1. Surgical blade #21 (DIMEDA Instrumente, catalog number: 06.121.00)
2. Chromic catgut (4/0) (UNIK, catalog number: CT134)
3. Nylon suture (3/0) (UNIK, catalog number: NC203)
4. Adult female Sprague Dawley (SD) rat (225-250 g)
5. Isoflurane (Halocarbon Laboratories, NDC12164-002-25)
6. 0.9% saline solution (TAI YU CHEMICAL & PHARMACEUTICAL, catalog number: RH1704)
7. Povidone-iodine solution (YING YUAN CHEMICAL PHARMACEUTICAL, catalog number: S-166)
8. Acetaminophen solution (CENTER Laboratories, catalog number: 19746)
9. Luxol fast blue stain kit (Abcam, catalog number: ab150675)
10. Hematoxylin and Eosin Stain Kit (Vector Laboratories, catalog number: H-3502)
11. Trimethoprim-sulfamethoxazole pre-mixed antibacterial solution (YUNG SHIN PHARM, catalog number: TRI-004)
12. Trimethoprim-sulfamethoxazole antibacterial injectable working solution (see Recipes)

Equipment

1. NYU-MASCIS weight-drop impactor with an alligator and the software
2. 2.5 mm tip of impactor for rat
3. Scalpel handle #4 (DIMEDA Instrumente, catalog number: 06.104.00)
4. Heating pad
5. Adson toothed forceps (DIMEDA Instrumente, catalog number: 10.180.12)
6. ALM self-retaining retractor (DIMEDA Instrumente, catalog number: 18.620.07)
7. MAYO HEGAR needleholder (DIMEDA Instrumente, catalog number: 24.180.16)
8. Littauer bone cutter (Stoelting, catalog number: 52167-80P)
9. Operating scissors (Shinetech, catalog number: ST-S114PK)
10. CMA/150 Temperature controller (CMA Microdialysis, model: CMA 150, catalog number: 600)
11. Dry sterilizer (Braintree Scientific, model: Germinator 500, catalog number: GER 5287-120V)
12. Surgical microscope (Carl Zeiss, model: Zeiss Stativ S3)
13. Table top anesthesia system (AM Bickford, catalog number: 61020)
14. EVA soft foam mat (Lee Chyun Enterprise, model: FM 600T)

Software

1. MAS 7.0 version
2. Microsoft Windows 98 operating system

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

Procedure

Ethical statement: Adult female Sprague-Dawley (SD) rats (225-250 g) were used in this protocol. All procedures involving animals were approved by the Animals Committee of Taipei Veterans General Hospital (permit numbers IACUC 2014-137 and IACUC 2015-253) and were in accordance with the Guide for the Care and Use of Laboratory Animals outlined by the National Institutes of Health.

1. All instruments (toothed forceps, tip, clamps, retractor, needle holder, scalpel handle and scissors) that touch the inside of the wound must be sterilized using the dry sterilizer.
2. Set up the PC and start the impactor program.
3. Place the rat into the induction chamber.
4. Turn on the airflow (1-1.5 L/min with isoflurane 5%) and then monitor the rat until recumbent.
5. Move the anesthetized rat from the chamber to the mask of anesthesia system and set the level of isoflurane to 1.5-2%.
6. Maintain the rat's core body temperature at 36-37 °C on a warming pad with an electrical temperature controller of the rectal probe.
7. Shave the thoracic area and apply the povidone-iodine solution on the shaved area.
8. Use the scalpel to make a longitudinal incision on the dorsal thoracic surface and dissect the paraspinal muscle to expose the vertebrae T7-T12.
   Note: The spinous process of T2 is longer than any of the others. Touch the position of T2 under the skin to determine the approximate position for operation with your finger (Figure 1A).
9. Use the retractor to gently pull the paravertebral muscles away from the spines. The laminectomy is performed with a Zeiss operating microscope (under 7.5x magnification). Cut and remove the bones of T8, T9 and partial T10 with the bone cutter and toothed forceps. This will expose the dorsal surface of the spinal cord without disrupting the dura. (Figure 1B)
   Note: To avoid hitting the vertebral bone with the tip of impactor, the bone must be removed to obtain more than 2.5 mm width because the diameter of the impactor tip is 2.5 mm.
10. Two stabilization clamps are used to immobilize the posterior spinous processes of the vertebrae T7 and T12 and to support the vertebral column during contusion.
11. Place the rat with the center of T8-T10 spinal cord under the tip (Figures 1C). The ground alligator of the impactor is placed on the muscle to form an electrical conductance between the tip and the spinal cord.
    Note: Using the toothed forceps push the middle of the clamped vertebral bone slightly in order to ensure the vertebral bone is clamped well and is stable (Figure 1D).
12. Place the tip at 0 mm and then lower down the rod to let the tip touch the dura of the spinal cord (Figure 1E, Left). When the tip touches the dura, the black box of the impactor will produce light and make a buzzing sound (Figure 1E, Right).

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

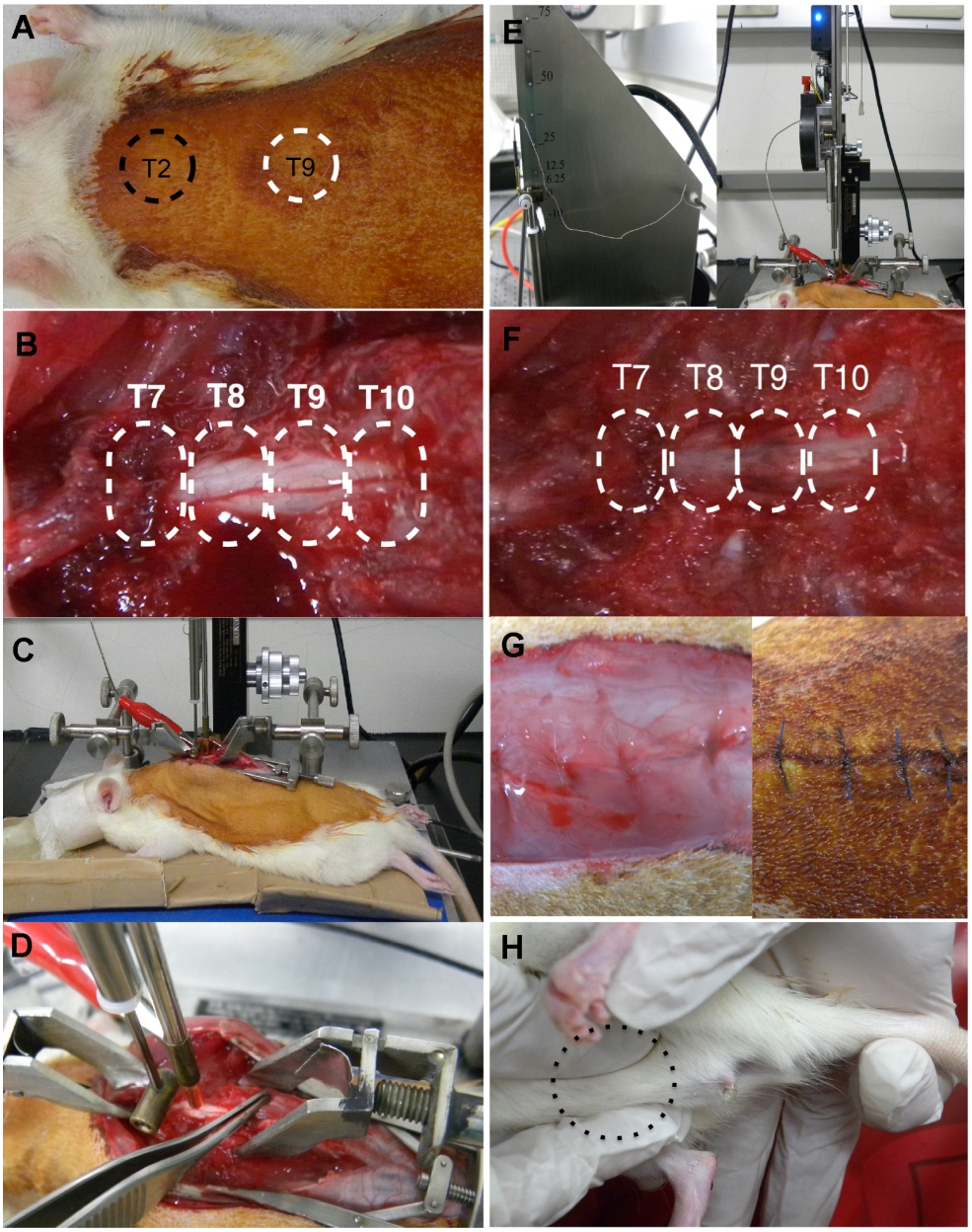


Figure 1. Laminectomy, impact site and animal care. A. The approximate positions of the 2nd thoracic spine (T2, black circle) and the 9th thoracic spine (white circle) shown on the dorsal thoracic surface. B. The spinal cord has been exposed by a T8-T10 laminectomy before contusion (white circles are vertebral locations). C. The rat placed at the center of the rod and the clamps was immobilized to show the spinous processes of the vertebrae T7 and T12. D. The toothed forceps pushed the middle of clamped vertebral bone slightly in order to ensure the vertebral bone is clamped well and stable. E. The tip was placed at 0 mm by an inserted pin; the tip can be raised up and held at specific heights (6.25, 12.5, 25 or 50 mm; Left). When the tip touches the dura, the black box of the impactor will produce light and buzzing sound (Right). F. The site of spinal cord with subdural hemorrhage on the T9 vertebral position after contused. G. The muscle was continuously closed with chromic catgut (Left), and the skin was interrupted suturing with nylon suture (Right). H. Holding the rat with one hand and gently squeezing the

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

bladder on the lower abdomen with the thumb and first two fingers of the other hand.

13. Raise the rod and hold the tip by the inserted pin at specific heights (included 6.25, 12.5, 25 or 50 mm). Manually pull the inserted pin to let the rod fall onto the exposed dura by gravity at T9 to produce a contusion injury.
    Note: The parameters for the impactor that can be recorded by the software are: impact velocity (Vi), cord compression distance (Cd), time (Ct), and rate (Cr = Cd/Ct). For instance, the impact velocity from a 50 mm height should be achieved in 0.98 m/sec. The compression distance should be around 2-3 mm by software display, if it is greater than 3 mm, that means the spinal cord is not clamped well. The Ct presents the time required for the rod to compress the spinal cord to the deepest point.
14. After contused, the subdural hemorrhage can be seen clearly under dura (Figure 1F).
15. Raise the tip and release the clamps gently from the vertebral column.
16. The wound is continuously closed with chromic catgut (4/0) for muscle, and interrupted suturing with nylon suture (3/0) for skin (Figure 1G).
17. Turn off the anesthesia system and then place the rat back to the cage which is on the heating pad.
    Note: The entire procedure takes about 1 h, and the rat should be woken up within 10-20 min after removing the isoflurane. Slightly press the paw of the rat, the reflex action of hind limb should not be present 24 h after SCI. In our experience, the mortality rate of rat is under 5%.
18. Monitor the rat and provide post-operative care by daily observation for signs of distress including weight loss, dehydration and bladder dysfunction. Provide 3 ml of sterile 0.9% saline subcutaneously for rehydration. Prophylactic antibiotics (Trimethoprim-sulfamethoxazole antibacterial injectable solution, 1:30 diluted in 0.9% normal saline, 2 ml/kg) are injected subcutaneously daily in 5 days post-surgery. The analgesic acetaminophen (65 mg/kg) is orally delivered if the rat shows the sign of self-mutilation. In addition, if the rat shows serious foot damage, we usually discard the rat.
19. The rat is taken care of in a conventional animal house and maintained at a 12-h light-dark cycle. Manual emptying of the rat's bladder is performed twice daily by squeezing the lower abdomen (Figure 1H).
    Note: Most rats show hematuria at 1-3 days after contused. If bloody urine does not empty well or a large residual volume of urine is left in the bladder after SCI, it might cause cystitis, infection, and even death.

Data analysis

1. Lesion volume
   SCI resulted in cavitation and demyelination, which expanded the extent of damage (Poon *et al.*, 2007). Luxol fast blue (LFB) and hematoxylin and eosin (H&E) stains were used to identify

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

cavities and myelinated white matter respectively. The staining procedures followed the manufacturer's protocols. Images were photographed from the rostral end to caudal end throughout the injury site at 2.5x magnification with a microscope camera. Contusion (50-mm height) caused the most of gray matter losing and few white matter sparing (Figure 2).

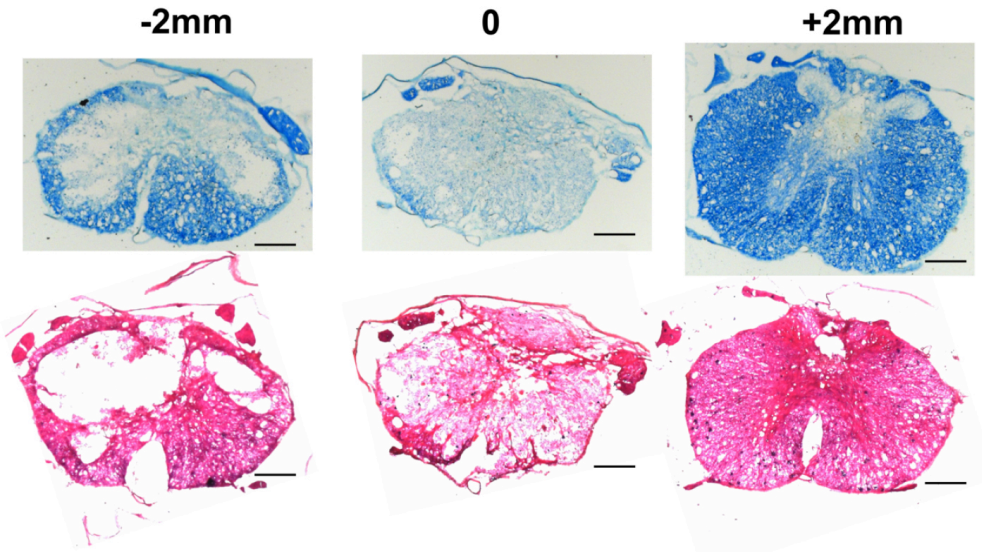


Figure 2. Histological staining at 6th week after SCI. One of the representative results showed that SCI rat by LFB staining (Upper) and the continued slides for H&E staining (Bottom) (-2 mm, 0 mm, and 2 mm; scale bars = 250 μm).

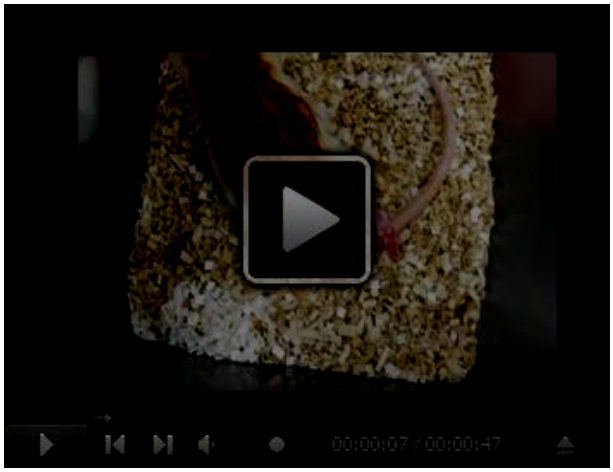
2. Locomotor score

The Basso, Beattie, and Bresnahan (BBB) open field score is used to evaluate locomotion of the hindlimbs (Basso *et al.*, 1996). Briefly, the rat was placed on the mat (size: 100 x 100 x 40 mm) and scored by BBB test from 0 (no observable hindlimb movement) to 21 (normal hind-limb movement) points. The low end of the BBB score (0-8) is characterized by each hind-limb joint movements, the intermediate (9-14) and high (15-21) are characterized by weight support, forelimb hindlimb coordination stepping, toe clearance, predominant paw position and tail position (for a detailed description of BBB scores, please see the reference by Basso *et al.*, 1996). Behavioral analyses were conducted and recorded using a video camera every week by both blinded examiners. The weekly scores of each hindlimb from examiners were averaged together to yield one score. Groups by different grades (or treatments) could be compared using a two-way analysis of variance (ANOVA) with Bonferroni's post hoc test. According to the study by Basso *et al.* (1996), the locomotor scores were greatest in the 6.25 mm group and lowest in the 50 mm group (please refer to step 13). The 6.25 mm group demonstrated the maximal functional recovery to near the normal locomotion within 3 weeks after contused. The 12.5 mm group recovered quickly from no or slight hind-limb joint movements to consistently stepping within 3 weeks. In contrast, the 25 mm group presented

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

slower improvement of locomotion after contused. The 50 mm group showed paralysis within 3-7 days and no weight-support recovery in the following 6 weeks. In addition, Mestre *et al.* (2015) and our previous study yielded similar results (Video 1) in 50 mm group of SD rat at 6th week after contused (Chiu *et al.*, 2016).

Video 1. Locomotor recovery in 50 mm group. The rat showed paralysis within 1-7 days and no weight-support recovery in the following 6 weeks.



Recipes

1. Trimethoprim-sulfamethoxazole antibacterial injectable working solution
   Trimethoprim-sulfamethoxazole, 1 vol
   Antibacterial solution diluted in 0.9% normal saline, 30 vol

Acknowledgments

This study was supported by the postdoctoral fellows program of Academia Sinica, grants of Ministry of Science and Technology, Taiwan (MOST 104-2314-B-010-012-MY3), and Taipei Veterans General Hospital (105V-E6-001-MY3-1). We also thank the Dr. May-Jywan Tsai for her help and Neural Regeneration Laboratory of Taipei Veterans General Hospital for providing experimental space and facilities.

References

1. Basso, D. M., Beattie, M. S. and Bresnahan, J. C. (1996). Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol* 139(2): 244-256.

bio-protocol www.bio-protocol.org/e2337 Vol 7, Iss 12, Jun 20, 2017
DOI:10.21769/BioProtoc.2337

2. Chiu, C. W., Huang, W. H., Lin, S. J., Tsai, M. J., Ma, H., Hsieh, S. L. and Cheng, H. (2016). The immunomodulator decoy receptor 3 improves locomotor functional recovery after spinal cord injury. *J Neuroinflammation* 13(1): 154.
3. Kwon, B. K., Oxland, T. R. and Tetzlaff, W. (2002). Animal models used in spinal cord regeneration research. *Spine* 27(14): 1504-1510.
4. Mestre, H., Ramirez, M., Garcia, E., Martiñón, S., Cruz, Y., Campos, M. G. and Ibarra, A. (2015). Lewis, Fischer 344, and sprague-dawley rats display differences in lipid peroxidation, motor recovery, and rubrospinal tract preservation after spinal cord injury. *Front Neurol* 6: 108.
5. Metz, G. A., Curt, A., van de Meent, H., Klusman, I., Schwab, M. E. and Dietz, V. (2000). Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. *J Neurotrauma* 17(1): 1-17.
6. Mothe, A. J. and Tator, C. H. (2012). Advances in stem cell therapy for spinal cord injury. *J Clin Invest* 122(11): 3824-3834.
7. Oyinbo, C. A. (2011). Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. *Acta Neurobiol Exp (Wars)* 71(2): 281-299.
8. Poon, P. C., Gupta, D., Shoichet, M. S. and Tator, C. H. (2007). Clip compression model is useful for thoracic spinal cord injuries: histologic and functional correlates. *Spine* 32: 2853-9.
9. Singh, A., Tetreault, L., Kalsi-Ryan, S., Nouri, A. and Fehlings, M. G. (2014). Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol* 6: 309-331.
10. Young, W. (2002). Spinal cord contusion models. *Prog Brain Res* 137: 231-255.

