Journal of Visualized Experiments

Thoracic spinal cord hemisection surgery and open-field locomotor assessment in the rat

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE59738R1
Full Title:	Thoracic spinal cord hemisection surgery and open-field locomotor assessment in the rat
Keywords:	spinal cord injury; Hemisection; locomotion; Open-field; Hindlimb; rat; surgery
Corresponding Author:	Andrew Brown Universite de Montreal Montreal, Quebec CANADA
Corresponding Author's Institution:	Universite de Montreal
Corresponding Author E-Mail:	a.brown@umontreal.ca
Order of Authors:	Andrew R Brown
	Marina Martinez
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Montreal, Quebec, Canada

TITLE:

Thoracic Spinal Cord Hemisection Surgery and Open-Field Locomotor Assessment in the Rat

AUTHORS AND AFFILIATIONS:

Andrew R. Brown[1,2], Marina Martinez[1,2,3]

[1]Department of Neurosciences, Faculté de Médecine, Université de Montréal, Québec, Canada
[2]Hôpital du Sacré-Cœur de Montréal, Montréal, Québec, Canada
[3]Groupe de Recherche sur le Système Nerveux Central (GRSNC), Université de Montréal, Montréal, Québec, Canada

Corresponding author:
Marina Martinez (marina.martinez@umontreal.ca)

Email address of co-author:
Andrew Brown (a.brown@umontreal.ca)

KEYWORDS:

spinal cord injury, hemisection, locomotion, open-field, hindlimb, rat, surgery

SUMMARY:

The rat thoracic spinal hemisection is a valuable and reproducible model of unilateral spinal cord injury to investigate the neural mechanisms of locomotor recovery and treatment efficacy. This article includes a detailed step-by-step guide to perform the hemisection procedure and to assess locomotor performance in an open-field arena.

ABSTRACT:

Spinal cord injury (SCI) causes disturbances in motor, sensory, and autonomic function below the level of the lesion. Experimental animal models are valuable tools to understand the neural mechanisms involved in locomotor recovery after SCI and to design therapies for clinical populations. There are several experimental SCI models including contusion, compression, and transection injuries that are used in a wide variety of species. A hemisection involves the unilateral transection of the spinal cord and disrupts all ascending and descending tracts on one side only. Spinal hemisection produces a highly selective and reproducible injury in comparison to contusion or compression techniques that is useful for investigating neural plasticity in spared and damaged pathways associated with functional recovery. We present a detailed step-by-step protocol for performing a thoracic hemisection at the T8 vertebral level in the rat that results in an initial paralysis of the hindlimb on the side of the lesion with graded spontaneous recovery of locomotor function over several weeks. We also provide a locomotor scoring protocol to assess functional recovery in the open-field. The locomotor assessment provides a linear recovery profile and can be performed both early and repeatedly after injury in order to accurately screen animals for appropriate time points in which to conduct more specialized behavioral testing. The hemisection technique presented can be readily adapted to other transection models and species, and the locomotor assessment can be used in a variety

of SCI and other injury models to score locomotor function.

INTRODUCTION:

Spinal cord injury (SCI) is associated with severe disturbances in motor, sensory, and autonomic function. Experimental animal models of SCI are valuable tools to understand the anatomical and physiological events involved in SCI pathology, to investigate the neural mechanisms in repair and recovery, and to screen for efficacy and safety of potential therapeutic interventions. The rat is the most commonly used species in SCI research[1]. Rat models are low cost, easy to reproduce, and a large battery of behavioral tests are available to assess functional outcomes[2]. Despite some differences in tract locations, the rat spinal cord shares overall similar sensorimotor functions with larger mammals, including primates[3,4]. Rats also share analogous physiological and behavioral consequences to SCI that relate to humans[5]. Non-human primate and large animal models can provide a closer approximation of human SCI[6] and are essential to prove treatment safety and efficacy prior to human experimentation, but are less commonly used due to ethical and animal welfare considerations, expenses, and regulatory requirements[7].

Rat transection SCI models are performed by the targeted interruption of the spinal cord with a selective lesion using a dissection knife or iridectomy scissors after a laminectomy. Compared to a complete transection, partial transection in the rat results in a less severe injury, easier postoperative animal care, spontaneous locomotor recovery, and more closely models SCI in humans which is predominately incomplete with partial sparing of tissue connecting the spinal cord and supraspinal structures[8]. A unilateral hemisection disrupts all ascending and descending tracts on one side only, and produces quantifiable and highly reproducible locomotor deficits, enhancing exploration of the underlying biological mechanisms. The most prominent functional consequence of the hemisection is an initial limb paralysis on the same side and below the level of the lesion with graded spontaneous recovery of locomotor function over several weeks[9-12]. The hemisection model is particularly useful to investigate neural plasticity of damaged and residual tracts and circuits associated with functional recovery[9,11-18]. Specifically, hemisection performed at the thoracic level, i.e., above the spinal circuits that control hindlimb locomotion, is particularly useful for investigating changes in locomotor control. As a non-linear relationship exists between lesion severity and locomotor recovery after SCI[19], appropriate behavioral testing to assess functional outcomes is paramount in experimental models.

A comprehensive battery of behavioral tests are available to assess specific aspects of functional locomotor recovery in the rat[2,20]. Many locomotor tests do not provide reliable measures early after SCI as rats are too disabled to support their body weight. A measure of spontaneous locomotor performance that is sensitive to deficits early after injury, and does not require preoperative training or specialized equipment, is beneficial in order to monitor locomotor recovery for appropriate time points in which to supplement specialized behavioral testing. The Martinez open-field assessment score[10], originally developed for evaluating locomotor performance after cervical SCI in the rat, is a 20-point ordinal score assessing global locomotor performance during spontaneous overground locomotion in an open-field. Scoring is conducted separately for each limb using a rubric that evaluates specific parameters of a range

of locomotor measures including articular limb movement, weight support, digit position, stepping abilities, forelimb-hindlimb coordination, and tail position. The assessment score is derived from the Basso, Beattie and Bresnahan (BBB) open-field rating scale designed to evaluate locomotor performance after thoracic contusion[21]. It is adapted to accurately and reliably evaluate both forelimb and hindlimb locomotor function, allows for independent assessment of the different scoring parameters that is not amenable with the hierarchical scoring of the BBB, and provides a linear recovery profile[10]. Additionally, in comparison to the BBB, the assessment score is sensitive and reliable in more severe injury models[10,11,20,22]. The assessment score has been used to assess locomotor impairment in the rat following cervical[10,12] and thoracic[9] SCI alone and in combination with traumatic brain injury[23].

We present here a detailed step-by-step protocol for performing a thoracic hemisection SCI at the T8 vertebral level in the female Long-Evans rat, and for assessing hindlimb locomotor recovery in the open-field.

PROTOCOL:

The experiments described in this article were performed in compliance with the guidelines of the Canadian Council on Animal Care and were approved by the ethics committee at the Université de Montréal.

1. Thoracic hemisection surgery

1.1. Wear appropriate protective equipment (gloves, mask, and gown) to maintain an aseptic environment for surgery. Clean the surgical area with alcohol wipes, and place sterile surgical drapes over the surgical field. Sterilize surgical tools and place on the surgical field.

1.2. Anesthetize the rat under a mixture of isoflurane gas (3% induction, 0.5−3% maintenance) and oxygen (1 L/min). Confirm proper surgical anesthetic depth by verifying the absence of cutaneous and corneal reflex responses. Continuously monitor the rat during the entire procedure, and adjust the amount of anesthetic delivery as required to maintain surgical anesthetic depth.

1.3. Shave the dorsal trunk between the hip and the neck, place the rat on the surgical field, disinfect the incision site with alcohol wipes and proviodine solution, and maintain core body temperature at 37 °C using a feedback-controlled heating pad monitored by rectal thermometer.

1.4. Place ophthalmic ointment on the eyes to keep them hydrated and reapply throughout surgery as required.

1.5. Make a 2.5 cm incision in the skin overlaying the T6−T10 vertebrae with a scalpel. Retract the skin and superficial fat using blunt dissection scissors.

NOTE: The T6−T10 vertebral segments can be identified either rostrally by gentle palpation of the dorsal spinal segments from the base of the skull starting from the noticeable protuberance of the 2nd thoracic vertebra[24], or caudally by palpation of the most posterior floating rib which will induce movement in the 13th thoracic vertebrae.

1.6. Separate the paravertebral muscles inserting on the dorsal aspect of the T7−T9 vertebrae using blunt dissection scissors and a self-retaining retractor. Debride and clear any remaining tissue using fine forceps and cotton tipped applicators to expose the spinous processes and vertebral laminae.

NOTE: This, and the following steps are greatly aided by microscopic visualization (~5−15x).

1.7. Carefully cut the facets (zygapophysial joints) bilaterally on the T7 and T8 vertebrae with delicate bone trimmers. Cut the dorsal connective tissue between the T8 and T9 vertebral laminae superficially with a scalpel (1 mm depth) being careful not to injure the underlaying cord.

1.8. Remove the spinous process of the T8 vertebra with bone trimmers. With curved hemostatic forceps carefully clamped on the T7 spinous process, rotate the caudal end of the T8 laminae slightly rostrally (~20°), insert the bone trimmers under the T8 lamina, and make a midline cut extending along the lamina. Continue the laminectomy by repeating the cuts on the left and right side of the vertebral lamina medial to the transverse processes to expose the spinal cord.

NOTE: Be careful to remove all bone fragments created from the laminectomy.

1.9. Drip lidocaine (2%, 0.1 mL) in the exposed spinal canal and remove the dura overlaying the T8 spinal segment using fine forceps and iridectomy scissors. Repeat lidocaine administration to the exposed cord and identify the midline of the cord by visualization of a centre line created between the spinous processes extending between exposed T7−T9 vertebra.

NOTE: Along with the spinous processes on T7 and T9, the exposed dorsal root ganglia on T8 can also be used to aid identification of midline and a 30 G needle can be placed in the midline of the cord to aid with the subsequent hemisection.

1.10. Hemisect the spinal cord from midline towards the one side with a dissecting knife. Be careful not to cut through the anterior spinal artery on the ventral side (do not apply firm pressure to the vertebral body). Using iridectomy scissors, carefully cut through any remaining tissue on the lesioned side of the spinal cord to ensure the ventrolateral quadrant is appropriately transected.

1.11. Place sterile saline-soaked hemostatic sponge (~6 x 2 mm) in the exposed cavity above the spinal cord and suture the muscle layers (4-0 polyglactin 910). Next, suture the skin around the incision site.

1.12. Provide adequate analgesic (buprenorphine 0.05 mg/kg subcutaneous [s.c.]), antibiotic (enrofloxacin, 10 mg/kg s.c.), and replenish lost fluids with 5 cc lactated ringer's solution (intraperitoneal [i.p.]) immediately after surgery.

1.13. Remove the rat from anesthesia. Place the rat in a warm environment under a heating pad or lamp (~ 33 °C) until the animal is fully awake.

1.14. Provide supplemental analgesia daily over the first 3 post-surgical days and continually monitor for signs of pain, weight loss, improper micturition, infection, problems with wound healing, or autophagia.

2. Open-field testing procedure and locomotor performance scoring

2.1. Handle rats daily for 1 week and habituate them to the arena for two 5-min sessions prior to testing to acclimatize to being picked up, gently from the mid-trunk, while in the open-field and to ensure measurement reliability during testing.

2.2. Place a camera at ground level facing the circular plexiglass open-field arena to record testing sessions for offline analysis (30−60 frames/s minimum).

2.3. Begin video recording and place the rat in the center of the arena under dim light conditions to encourage locomotor activity.

2.4. Continue the testing session for 4 min to ensure an adequate amount of locomotor bouts for analysis. Pick up and replace rats in the center of the arena when they remain stationary for longer than 20 s to promote locomotion.

2.5. Score locomotor performance of the recorded testing session by completing the rubric provided in **Table 1** according to the parameters in the following subsections.

NOTE: It is helpful to score each parameter separately by repeated viewing of the recorded testing session using software that allows for variable playback speed and frame-by-frame analysis (e.g., VLC media player).

2.5.1. For articular limb movements, score hindlimb joint movements during spontaneous locomotion separately for the ankle, knee, and hip as either normal (more than half of the range of motion, awarded score = 2), slight (less than half of the range of motion, awarded score = 1), or absent (awarded score = 0).

2.5.2. For weight support, evaluate the ability of hindlimb extensor muscles to contract and support loaded body weight when the limb is on the ground separately for when the rat is stationary as well as during active locomotion. Award a score of 1 when weight support is present and a score of 0 when weight support is absent.

NOTE: Stationary weight support is deemed a perquisite for active weight support.

2.5.3. For digit position, evaluate the position of the hindlimb digits while the rat is stationary and during locomotion. Award a score of 2 when hindlimb digits are extended, spaced apart from one another, and tonic during locomotion in more than 50% of the testing period (considered normal). Award a score of 1 when digits remain predominantly flexed and a score of 0 when digits remain predominantly atonic.

2.5.4. For stepping, complete this parameter only if the rat can support its body weight during stepping. Evaluate stepping by rating the orientation of hindlimb paw placement at the time of initial contact and at lift off from the ground in addition to the fluidity of the swing phase during stepping.

NOTE: There are 3 scores for this parameter described in the following subsections separately evaluating: 1) the axial orientation of paw placement at limb contact (dorsal/plantar placement), 2) the longitudinal orientation of paw placement at initial contact and during lift (parallel to the body axis or rotated internally/externally), and 3) the quality of limb movement during swing (regular or irregular).

2.5.4.1. For the paw placement at limb contact, score the axial orientation of the paw placement at limb contact as 0 when dorsal placements occur in more than 50% of steps.

NOTE: Plantar placement is deemed a perquisite for scoring the orientation of paw at contact and lift (step 2.5.4.2), swing movement (step 2.5.4.3) and forelimb-hindlimb coordination (step 2.5.5).

2.5.4.2. For the paw orientation at limb contact and lift, award a score of 2 when the longitudinal paw and body axes are parallel and a score of 1 when the limb is rotated externally or internally, separately for both limb contact and lift.

2.5.4.3. For the swing movement, award a score of 2 when hindlimb joints move in a harmonious and regular way during swing and a score of 1 when jerky or spasmodic movements of the joints occur during swing.

2.5.5. For forelimb-hindlimb coordination, complete this parameter only if 4 consecutive steps occur during testing and if the limbs can actively support body weight. Award a score of 3 when coordination is consistent (>90% of steps), 2 when frequent (50–90% of steps), 1 when occasional (<50% of steps), or 0 when absent (0% of steps).

NOTE: Forelimb-hindlimb coordination is defined as a regular alternation in stepping between the hindlimb being scored and the forelimb on the same side of the body.

2.5.6. For tail position, evaluate the tail position during locomotion as either up (off the ground, awarded score = 1) or down (touching the ground, awarded score = 0).

NOTE: An elevated tail position during locomotion is an indicator of trunk stability in the rat. After hemisection, the tail is normally held close to or touching the ground as trunk stability is impaired.

2.5.7. Add the individual scores from each parameter to provide a total for each hindlimb of a maximum of 20 points.

NOTE: A score of 20 indicates normal locomotor performance. Scores <20 represent increasing amounts of locomotor impairment and a score of 0 indicates limb paralysis.

REPRESENTATIVE RESULTS:

Reproducible lesions with a high degree of consistency can be generated with the hemisection technique. To assess and compare lesions sizes between experimental groups, the maximal area of the lesion as a percentage of the total cross-section of the spinal cord can be readily calculated with histological staining of spinal cord sections. **Figure 1** shows a representative lesion of the left hemicord and an overlay of the proportion of maximal lesion area shared between rats with a mean lesion size of 47.3% ± 4.0% of the cross-sectional cord area (n = 6).

[Place **Figure 1** here]

The primary consequence of the hemisection is an initial paralysis of the hindlimb on the side of the lesion during the first two to three postoperative days. Locomotor performance of the more affected hindlimb improves rapidly in the rat after hemisection over the first few weeks after injury. Small deficits in the opposite hindlimb are commonly observed initially after the hemisection that can reflect compensation for the more affected limb, or deficits resulting from a lack of postural stability, weight support, and consistent stepping. A large and persisting deficit in the opposite hindlimb would indicate a bilateral lesion extending into the opposing hemicord.

A sample scoring sheet for the performance of the rat shown in the associated video to this protocol, tested one week after a left side hemisection, is provided in **Table 1.**

[Place **Table 1** here]

The time course of representative changes in locomotor performance in the intact state and over the first five weeks after a left side hemisection in separate groups of rats (n = 6 per group) is depicted in **Figure 2.**

[Place **Figure 2** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Representative spinal lesions. (**A**) Microphotograph of a coronal spinal section at the lesion epicenter from a hemisected rat stained with cresyl violet (cell bodies, purple) and luxol fast blue (myelin, blue) indicating damage to the grey and white matter concentrated in the left hemicord. D, dorsal; V, ventral; L, left; R, right. Scale bar: 1 mm. (**B**) Schematic overlay of the shared proportion of maximal lesion area in a group of rats (n = 6). The location of the crossed corticospinal tract in the dorsal funiculus on the right side is shaded in black.

Figure 2: Representative time course of changes in hindlimb locomotor performance in the open-field in the intact state and for five weeks after a left side thoracic hemisection. Performance of the left hindlimb (**A**) is significantly impaired from intact values during the first three weeks after hemisection, and of the right hindlimb (**B**) during the first week after hemisection. Data are plotted as group mean ± standard deviation (SD; n = 6 per group). Statistical analyses were performed with Kruskal-Wallis non-parametric tests supplemented with Dunn's multiple comparison tests to assess group differences between time points. *$p <$ 0.05, ***$p < 0.001$.

Table 1: Sample scoring sheet. Open-field hindlimb locomotor assessment rubric with scores from performance of the rat in the associated video sample obtained one week following a left side hemisection. For each parameter, the scoring template is indicated in parentheses. I, internal; E, external; P, parallel; FL-HL, forelimb-hindlimb.

DISCUSSION:

A major strength of the hemisection technique is the selectivity and reproducibility of the lesion which leads to reduced variability in histological and behavioral phenotypes between animals[25]. In order to ensure a unilateral lesion at the appropriate spinal level, accurate identification of both the proper vertebral segment and spinal cord midline is critical. As there can be a tendency for the spinal cord to rotate in the direction of the cut during the hemisection procedure, it can be beneficial to stabilize the cord delicately with fine forceps placed on either side during the procedure. Placing the rat in a stereotaxic frame with the tail gently taped under light tension can help with stability and proper vertebral alignment during the procedure. A spinal clamp attached to the stereotaxic frame and a spinous process can also be used to enhance stability of the vertebral column, but we find that its presence can restrict access to the cord with surgical tools and requires awkward approach angles during the surgery. It is also essential to remove any bone fragments left in the spinal canal from the laminectomy as they can cause unwanted compression injury to the cord and promote secondary damage.

Rats should be constantly observed during the surgery to monitor necessary vital signs such as core temperature and breathing, as hypothermia is a leading cause of mortality both during anesthesia administration and initially after surgery. Regulation of core body temperature with a rectal probe and feedback-controlled heating pad can greatly avoid temperature complications. A pulse oximeter can also be used to monitor blood oxygenation and heart rate to regulate anesthetic depth. We find that fluid replenishment immediately after surgery with lactate ringer's solution warmed to body temperature results in a more rapid recovery time for

the rat to awaken after surgery, regain autonomic control of body temperature, and be able to drink and eat.

Post-surgical monitoring of the rat is essential after the hemisection surgery, especially for signs of improper micturition, pain, infection, weight loss, problems with wound healing, or autophagia. Consultation with veterinary staff for evaluation and treatment is crucial in situations of post-surgical complications. In particular, acute spinal shock or unintended bilateral lesions may interfere with micturition that can lead to potentially fatal infections. Carefully monitor the bladder of the rat after surgery and manually void three times per day if full by gentle pressure from the ventral side of the bladder descending caudally. We use female Long-Evans rats as they have a significantly shorter and straighter urethra than males that leads to a more rapid onset of an automatic urinary bladder, easier micturition, and lower rates of urinary tract infections[2]. Weights should also be monitored and a loss >20% from baseline warrants investigation into food and water intake. The teeth should be checked for malocclusion, the abdomen for ileus, and rats given appropriate supplementary fluids and nutrition such as hydrogel or a liquid diet. A cyst may rarely form under the incision site that can be drained safely with a syringe without complication in consultation with veterinary staff.

The Martinez open-field locomotor assessment procedure provides a simple technique that does not require any specialized equipment, preoperative training, or food deprivation of the animal to perform. The assessment can be performed as early as the animal recovers from anesthesia and can be used to screen animals for appropriate recovery indices (e.g., recovery of body weight support) when more rigorous and specific locomotor testing can be supplemented such as automated gait assessment of overground locomotion[26-28], kinematic analyses during treadmill locomotion[29-32], grid walking[33], and ladder rung walking[9,34]. Importantly, while the BBB scale has been shown to not be linear with locomotor recovery as scores tend to cluster around certain values[19], the Martinez open-field locomotor assessment provides a linear scoring profile during the recovery process[10]. To ensure reliable behavioral data, it is important to minimize the number of confounders during testing and analysis. To help reduce variability during testing, sessions should occur at the same time of day, in the same room, and by the same experimenter. The open-field assessment can be reliably performed over repeated sessions[9-12,23], but rats may become habituated to the environment over time and reduce their activity during testing resulting in an inadequate amount of locomotor bouts for analysis. To overcome immobility during testing, rats that remain stationary for longer than 20 seconds are picked up and replaced in the center of the arena to promote locomotion. Additionally, including a conspecific in the arena during testing that is marked for identification can help promote locomotor activity in the test rat. To ensure reliability in locomotor scoring two raters, preferably blinded, should conduct the analyses as previously described[10].

In conclusion, we describe methods for conducting a thoracic spinal cord hemisection in the rat and assessing spontaneous hindlimb locomotor performance in an open-field arena. Although a procedure for conducting lateral hemisections was described, the technique can be readily adapted to perform either dorsal hemisections[35], staggered alternating hemisections[36,37], or full transections[38] depending on the desired lesion location and amount of spared descending

supraspinal innervation. Importantly, the technique can also be used in larger animal models, including cats[39-41] and non-human primates[6,42] with comparable deficits observed between small and large animals, making it useful for investigating both the neurobiological mechanisms of recovery and for preclinical therapeutic testing.

ACKNOWLEDGMENTS:

This work was supported by the Canadian Institutes for Health Research (CIHR; MOP-142288) to M.M. M.M. was supported by a salary award from Fonds de Recherche Québec Santé (FRQS), and A.R.B was supported by a fellowship from FRQS.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Sharif-Alhoseini, M. et al. Animal models of spinal cord injury: a systematic review. *Spinal Cord.* **55** (8), 714-721 (2017).
2. Sedy, J., Urdzikova, L., Jendelova, P., Sykova, E. Methods for behavioral testing of spinal cord injured rats. *Neuroscience and Biobehavioral Reviews.* **32** (3), 550-580 (2008).
3. Butler, A. B., Hodos, W. *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. 139-152 (John Wiley & Sons, 2005).
4. Nudo, R. J., Masterton, R. B. Descending pathways to the spinal cord: a comparative study of 22 mammals. *Journal of Comparative Neurology.* **277** (1), 53-79 (1988).
5. Metz, G. A. et al. Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. *Journal of Neurotrauma.* **17** (1), 1-17 (2000).
6. Friedli, L. et al. Pronounced species divergence in corticospinal tract reorganization and functional recovery after lateralized spinal cord injury favors primates. *Science Translational Medicine.* **7** (302), 302ra134 (2015).
7. Talac, R. et al. Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies. *Biomaterials.* **25** (9), 1505-1510 (2004).
8. Kwon, B. K., Oxland, T. R., Tetzlaff, W. Animal models used in spinal cord regeneration research. *Spine.* **27** (14), 1504-1510 (2002).
9. Brown, A. R., Martinez, M. Ipsilesional Motor Cortex Plasticity Participates in Spontaneous Hindlimb Recovery after Lateral Hemisection of the Thoracic Spinal Cord in the Rat. *Journal of Neuroscience.* **38** (46), 9977-9988 (2018).
10. Martinez, M., Brezun, J. M., Bonnier, L., Xerri, C. A new rating scale for open-field evaluation of behavioral recovery after cervical spinal cord injury in rats. *Journal of Neurotrauma.* **26** (7), 1043-1053 (2009).
11. Martinez, M., Brezun, J. M., Zennou-Azogui, Y., Baril, N., Xerri, C. Sensorimotor training promotes functional recovery and somatosensory cortical map reactivation following cervical spinal cord injury. *European Journal of Neuroscience.* **30** (12), 2356-2367 (2009).
12. Martinez, M. et al. Differential tactile and motor recovery and cortical map alteration after C4-C5 spinal hemisection. *Experimental Neurology.* **221** (1), 186-197 (2010).
13. Leszczynska, A. N., Majczynski, H., Wilczynski, G. M., Slawinska, U., Cabaj, A. M. Thoracic Hemisection in Rats Results in Initial Recovery Followed by a Late Decrement in Locomotor

Movements, with Changes in Coordination Correlated with Serotonergic Innervation of the Ventral Horn. *PloS One.* **10** (11), e0143602 (2015).
14. Ballermann, M., Fouad, K. Spontaneous locomotor recovery in spinal cord injured rats is accompanied by anatomical plasticity of reticulospinal fibers. *European Journal of Neuroscience.* **23** (8), 1988-1996 (2006).
15. Garcia-Alias, G. et al. Chondroitinase ABC combined with neurotrophin NT-3 secretion and NR2D expression promotes axonal plasticity and functional recovery in rats with lateral hemisection of the spinal cord. *Journal of Neuroscience.* **31** (49), 17788-17799 (2011).
16. Petrosyan, H. A. et al. Neutralization of inhibitory molecule NG2 improves synaptic transmission, retrograde transport, and locomotor function after spinal cord injury in adult rats. *Journal of Neuroscience.* **33** (9), 4032-4043 (2013).
17. Schnell, L. et al. Combined delivery of Nogo-A antibody, neurotrophin-3 and the NMDA-NR2d subunit establishes a functional 'detour' in the hemisected spinal cord. *The European journal of neuroscience.* **34** (8), 1256-1267 (2011).
18. Shah, P. K. et al. Use of quadrupedal step training to re-engage spinal interneuronal networks and improve locomotor function after spinal cord injury. *Brain.* **136** (Pt 11), 3362-3377 (2013).
19. Schucht, P., Raineteau, O., Schwab, M. E., Fouad, K. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. *Experimental Neurology.* **176** (1), 143-153 (2002).
20. Metz, G. A., Merkler, D., Dietz, V., Schwab, M. E., Fouad, K. Efficient testing of motor function in spinal cord injured rats. *Brain Research.* **883** (2), 165-177 (2000).
21. Basso, D. M., Beattie, M. S., Bresnahan, J. C. A sensitive and reliable locomotor rating scale for open field testing in rats. *Journal of Neurotrauma.* **12** (1), 1-21 (1995).
22. Barros Filho, T. E. P. d., Molina, A. E. I. S. Analysis of the sensitivity and reproducibility of the Basso, Beattie, Bresnahan (BBB) scale in Wistar rats. *Clinics (Sao Paulo, Brazil).* **63** (1), 103-108 (2008).
23. Inoue, T. et al. Combined SCI and TBI: recovery of forelimb function after unilateral cervical spinal cord injury (SCI) is retarded by contralateral traumatic brain injury (TBI), and ipsilateral TBI balances the effects of SCI on paw placement. *Experimental Neurology.* **248**, 136-147 (2013).
24. Vichaya, E. G., Baumbauer, K. M., Carcoba, L. M., Grau, J. W., Meagher, M. W. Spinal glia modulate both adaptive and pathological processes. *Brain, Behavior, and Immunity.* **23** (7), 969-976 (2009).
25. Ahmed, R. U., Alam, M., Zheng, Y.-P. Experimental spinal cord injury and behavioral tests in laboratory rats. *Heliyon.* **5** (3), e01324 (2019).
26. Ham, T. R. et al. Automated Gait Analysis Detects Improvements after Intracellular sigma Peptide Administration in a Rat Hemisection Model of Spinal Cord Injury. *Annals of Biomedical Engineering.* **47** (3), 744-753 (2019).
27. Hamers, F. P. T., Koopmans, G. C., Joosten, E. A. J. CatWalk-Assisted Gait Analysis in the Assessment of Spinal Cord Injury. *Journal of Neurotrauma.* **23** (3-4), 537-548 (2006).
28. Neckel, N. D., Dai, H. N., Burns, M. P. A novel multidimensional analysis of rodent gait reveals the compensation strategies employed during spontaneous recovery from spinal cord and traumatic brain injury. *Journal of Neurotrauma.* 10.1089/neu.2018.5959, (2018).

29. Fouad, K., Metz, G. A. S., Merkler, D., Dietz, V., Schwab, M. E. Treadmill training in incomplete spinal cord injured rats. *Behavioural Brain Research.* **115** (1), 107-113 (2000).
30. Thibaudier, Y. et al. Interlimb Coordination during Tied-Belt and Transverse Split-Belt Locomotion before and after an Incomplete Spinal Cord Injury. *Journal of Neurotrauma.* **34** (9), 1751-1765 (2017).
31. Alluin, O. et al. Kinematic study of locomotor recovery after spinal cord clip compression injury in rats. *Journal of Neurotrauma.* **28** (9), 1963-1981 (2011).
32. Martinez, M., Delivet-Mongrain, H., Leblond, H., Rossignol, S. Effect of locomotor training in completely spinalized cats previously submitted to a spinal hemisection. *Journal of Neuroscience.* **32** (32), 10961-10970 (2012).
33. Behrmann, D. L., Bresnahan, J. C., Beattie, M. S., Shah, B. R. Spinal cord injury produced by consistent mechanical displacement of the cord in rats: behavioral and histologic analysis. *Journal of Neurotrauma.* **9** (3), 197-217 (1992).
34. Soblosky, J. S., Colgin, L. L., Chorney-Lane, D., Davidson, J. F., Carey, M. E. Ladder beam and camera video recording system for evaluating forelimb and hindlimb deficits after sensorimotor cortex injury in rats. *Journal of Neuroscience Methods.* **78** (1-2), 75-83 (1997).
35. Bareyre, F. M. et al. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nature Neuroscience.* **7** (3), 269-277 (2004).
36. Courtine, G. et al. Recovery of supraspinal control of stepping via indirect propriospinal relay connections after spinal cord injury. *Nature Medicine.* **14** (1), 69-74 (2008).
37. van den Brand, R. et al. Restoring Voluntary Control of Locomotion after Paralyzing Spinal Cord Injury. *Science.* **336** (6085), 1182-1185 (2012).
38. Lukovic, D. et al. Complete rat spinal cord transection as a faithful model of spinal cord injury for translational cell transplantation. *Scientific Reports.* **5**, 9640-9640 (2015).
39. Wilson, S. et al. The Hemisection Approach in Large Animal Models of Spinal Cord Injury: Overview of Methods and Applications. *Journal of Investigative Surgery.* 10.1080/08941939.2018.1492048, 1-12 (2018).
40. Martinez, M., Delivet-Mongrain, H., Leblond, H., Rossignol, S. Incomplete spinal cord injury promotes durable functional changes within the spinal locomotor circuitry. *Journal of Neurophysiology.* **108** (1), 124-134 (2012).
41. Martinez, M., Delivet-Mongrain, H., Leblond, H., Rossignol, S. Recovery of hindlimb locomotion after incomplete spinal cord injury in the cat involves spontaneous compensatory changes within the spinal locomotor circuitry. *Journal of Neurophysiology.* **106** (4), 1969-1984 (2011).
42. Capogrosso, M. et al. A brain–spine interface alleviating gait deficits after spinal cord injury in primates. *Nature.* **539**, 284-288 (2016).

Figure 1

Click here to access/download;Figure;Figure1.tif

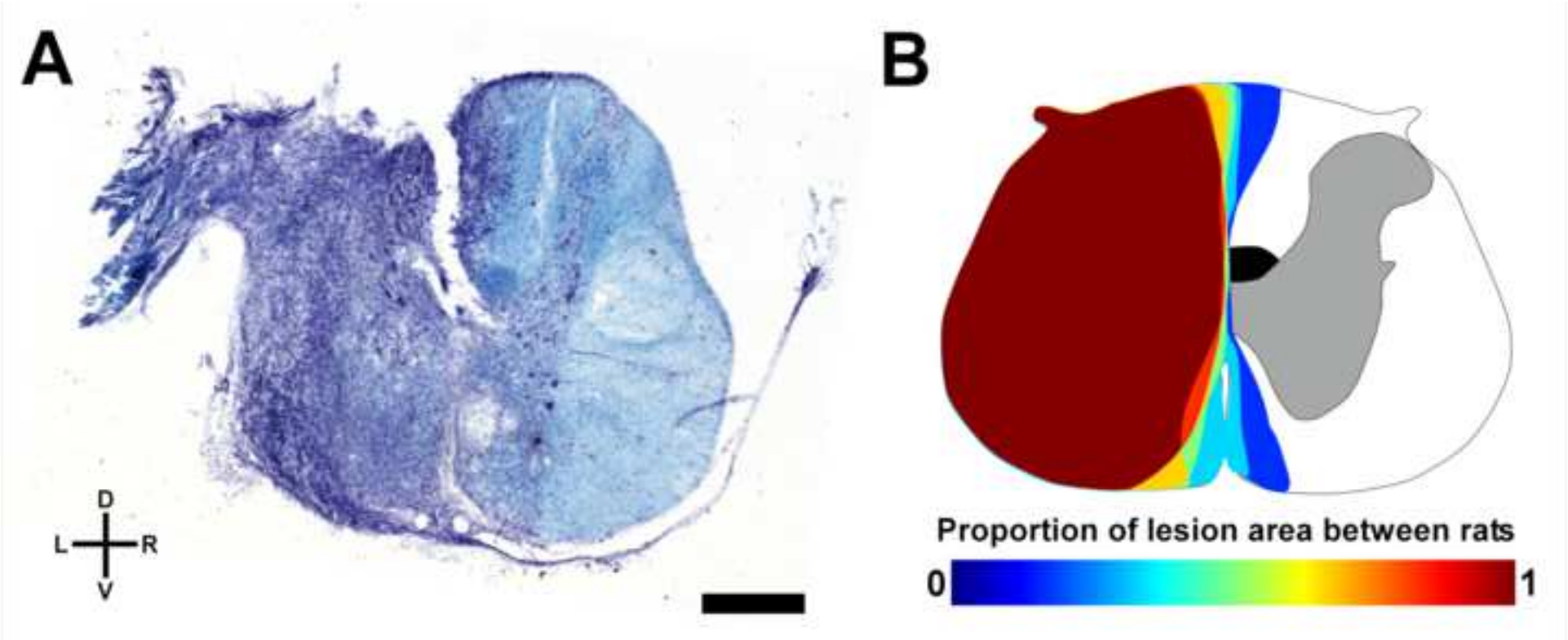

Figure 2

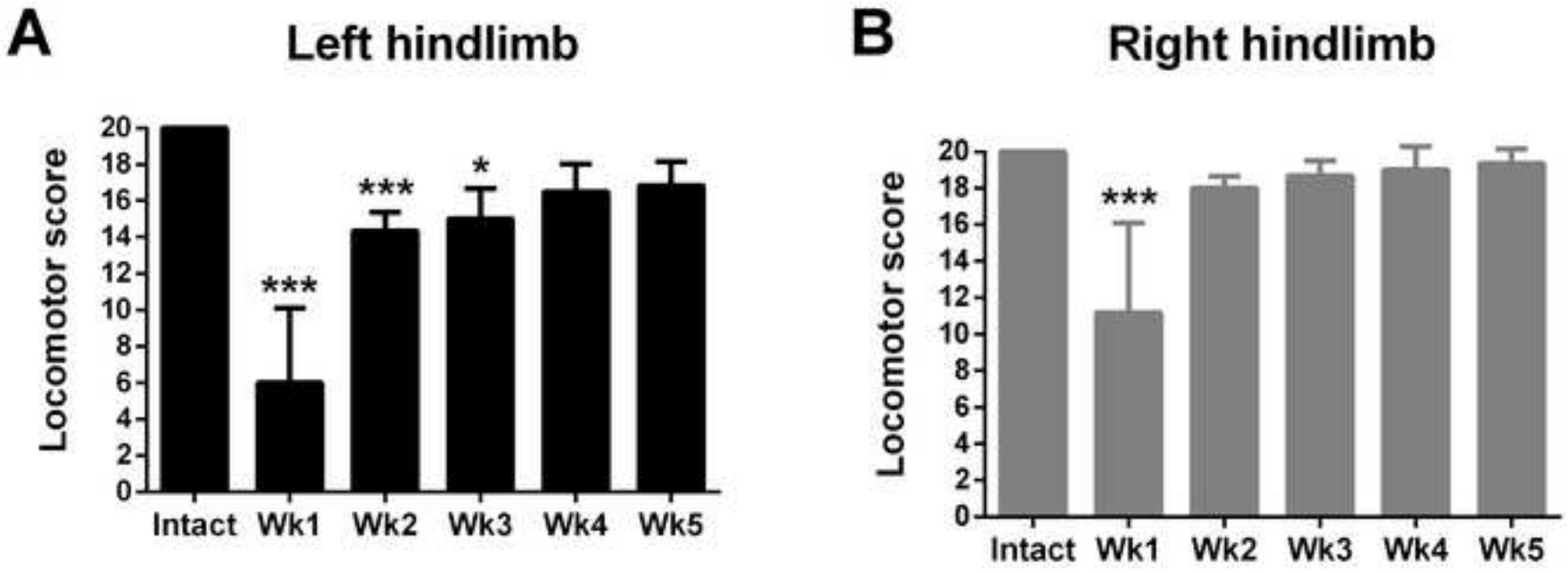

Table 1

Rat: Video sample **Day post-op:** 7 **Date:**

SCORE: Left Hindlimb: **4 /20**
Right Hindlimb: **16 /20**

	Articular movements of the hindlimbs					
	Left Hindlimb			Right Hindlimb		
	Hip	Knee	Ankle	Hip	Knee	Ankle
Absent (0)						
Slight (1)	1	1	1			
Normal (2)				2	2	2

	Weight support	
	Stationary (1)	Active (1)
Left Hindlimb	1	
Right Hindlimb	1	1

	Digit position			Stepping								
	Flexed (0)	Atonic (1)	Extended (2)	Paw placement at initial contact				Paw orientation during lift off			Swing Movement	
				Dorsal (0)	Plantar							
					I (1)	E (1)	P (2)	I (1)	E (1)	P (2)	Regular (2)	Irregular (1)
Left Hindlimb	0			0								
Right Hindlimb			2				2		1		2	

	FL-HL coordination (left side)	FL-HL coordination (right side)
Absent (0)		
Occasional (1)		1
Frequent (2)		
Consistent (3)		

Tail position	
Up (1)	
Down (0)	0

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials_Brown_Martinez.xls

Name of Material/ Equipment	Company
Baytril	CDMV
Blunt dissection scissors	World Precision Instruments
Buprenorphine hydrochoride	CDMV
Camera lens	Pentax
CMOS video camera	Basler
Compressed oxygen gas	Praxair
Cotton tipped applicators	CDMV
Delicate bone trimmers	Fine Science Tools
Dissecting knife	Fine Science Tools
Dumont fine forceps (#5)	Fine Science Tools
Ethicon Vicryl 4/0 Violet Braided FS-2 suture (J392H)	CDMV
Feedback-controlled heating pad	Harvard Apparatus
Female Long-Evans rats	Charles River Laboratories
Gelfoam	CDMV
Curved hemostat forceps	Fine Science Tools
Hot bead sterilizer	Fine Science Tools
Hydrogel	70-01-5022
Isofluorane	CDMV
Lactated Ringer's solution	CDMV
Lidocaine (2%)	CDMV
Needle 30 ga	CDMV
Open-field area	Custom
Opthalmic ointment	CDMV
Personal computer	
Physiological saline	CDMV
Proviodine	CDMV
Rodent Liquid Diet	Bioserv
Scalpal blade #11	CDMV
Self-retaining retractor	World Precision Instruments
Vannas iridectomy spring scissors	Fine Science Tools
Veterinary Anesthesia Machine and isofluarane vaporizer	Dispomed
VLC media player	VideoLAN

Catalog Number	Comments/Description
11242	
503669	
C31204TH	12.5-75mm, f1.8, 2/3" format, C-mount
acA2000-165uc	2/3" format, 2048 x 1088 pixels, up to 165 fps, C-mount, USB3
108703	
16109-14	
10055-12	
11254-20	
111689	
55-7020	
Strain code: 006	225-250g
102348	
13003-10	
18000-45	
Clear H20	
118740	
116373	
123684	
4799	
	Circular Plexiglas arena 96 cm diameter, 40 cm wall height
110704	
	With USB3 connectivity to record video with the listed camera
1399	
4568	
F1268	
6671	
14240	
15002-08	
975-0510-000	
	videolan.org/vlc

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:	Thoracic spinal hemisection surgery and open-field locomotor assessment in the rat.
Author(s):	Andrew R. Brown and Marina Martinez

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☑ Standard Access ☐ Open Access

Item 2: Please select one of the following items:

☑ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Marina Martinez
Department:	Neurosciences
Institution:	Université de Montréal
Title:	Assistant Professor
Signature:	
Date:	January 23rd, 2019

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

Université de Montréal
HÔPITAL DU SACRÉ-CŒUR DE MONTRÉAL
CENTRE DE RECHERCHE
HSCM Doués pour la vie
Groupe de recherche sur le systeme nerveux central
GRSNC

Vineeta Bajaj, PhD *Review Editor*
Ronald Myers PhD*, Science Editor*
JoVE

Montréal, April 1st, 2019

Subject: Resubmission for JoVE59738

Dear Drs. Bajaj and Myers,

We would like to thank the reviewers for their helpful suggestions regarding our manuscript and the editors for your interest regarding the manuscript for publication in *JoVE*. We were encouraged by reviewer responses indicating that the manuscript is of interest to the readership of the journal. We have incorporated reviewer suggestions in a revised manuscript and would like to present a point-by-point reply to the editorial and reviewer comments below. Editorial and reviewer comments are indicated in blue while author responses and indicated in black. Revised text is indicated in red in this letter and by track changes in the revised manuscript.

Please note that all references from deleted text in this revision have been removed and that line numbers reflect those showing "All markup" with track changes in Microsoft Word in the revised manuscript. We have also included a clean version of the revised manuscript with all changes incorporated for clarity.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We have proofread the manuscript.

2. Please mention how proper anesthetization is confirmed.

We have added mention of how proper anesthetization is confirmed in the revised manuscript. Lines 123-127 now read:

"1.2 Anesthetize the rat under a mixture of isoflurane gas (3% induction, 0.5-3% maintenance) and oxygen (1 L/min). Confirm proper surgical anesthetic depth by verifying the absence of cutaneous and corneal reflex responses. Continuously monitor the rat during the entire

procedure, and adjust the amount of anesthetic delivery as required to maintain surgical anesthetic depth."

3. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

We have revised the manuscript to have all sections of the protocol written in the imperative tense. The subsections of section 2.5 (2.5.1-2.5.7) provide a thorough description of how to score each parameter of the locomotor assessment rubric. In order to provide a comprehensive description to readers, we initially had a portion of these sections in the declarative tense. In this revision, we have edited those subsections for the imperative tense and have used notes where additional declarative sentences are important for comprehension and the ability to properly replicate the techniques described.

4. Please do not abbreviate journal titles.

We have included full journal titles in the reference section in the revised manuscript.

Reviewers' comments:

Please note that novelty is not a requirement for publication and reviewer comments questioning the novelty of the article can be disregarded.

We acknowledge that novelty is not a requirement for publication and that all reviewers do mention the utility and value of this paper, despite novelty, in demonstrating to readers how to carry out the techniques described that have not previously been a focus of a *JoVE* article.

Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please revise the manuscript to thoroughly address these concerns. Additionally, please describe the changes that have been made or provide explanations if the comment is not addressed in a rebuttal letter. We may send the revised manuscript and the rebuttal letter back to peer review.

We have revised the manuscript in order to address reviewer concerns and describe changes in the manuscript that have been made in response to reviewer comments as well as document cases in this letter, including a rationale, for where we elected not to address changes in the revised manuscript.

Reviewer #1:

Manuscript Summary:

This paper is a follow-up to a recent study published in the Journal of Neuroscience. As mentioned in the abstract and introduction, there are different spinal cord injury (SCI) models, including contusion, compression and transection. Among these different models, transection is highly reproducible and allows for assessment of therapies and treatments aimed at promoting functional recovery of spared ascending and descending pathways. The paper describes in details the protocol to perform a thoracic hemisection. It also details a new motor scale to assess gross motor recovery after SCI. In summary, the protocol is well described and could be useful for trainees in the SCI field.

We thank the reviewer for their comments and have incorporated all of them in our revised manuscript.

Major Concerns:

Introduction
Line 60: the statement about the mouse should be removed or rephrased, as it suggests in the current form that there is axonal regeneration in the mouse following SCI in contrast to the rat.

We have removed this statement in the revised manuscript for clarity. Our intention was to highlight the cyst formation that occurs in rat models rather than imply axonal regeneration in the mouse.

There are economical and ethical reasons that could be highlighted to explain the popularity of the rat as an animal model of SCI.

We agree and have indicated this in the manuscript on lines 61-64:

"Non-human primate and large animal models can provide a closer approximation of human SCI[6] and are essential to prove treatment safety and efficacy prior to human experimentation, but are less commonly used due to ethical and animal welfare considerations, expenses, and regulatory requirements[7]."

Moreover, it would be important to highlight the advantage of the new motor scale presented with respect to the problems of sensitivity and reproducibility previously reported for the BBB scale with severe injury.

We agree and have expanded on this point in the revised manuscript. Lines 97-103 have been revised to read:

"The assessment score is derived from the Basso, Beattie and Bresnahan (BBB) open-field rating scale designed to evaluate locomotor performance after thoracic contusion[21]. It is adapted to accurately and reliably evaluate both forelimb and hindlimb locomotor function, allows for

independent assessment of the different scoring parameters that is not amenable with the hierarchical scoring of the BBB, and provides a linear recovery profile[10]. Additionally, in comparison to the BBB, the assessment score is sensitive and reliable in more severe injury models[10,11,20,22]."

Minor Concerns:

Protocol
Line 166: I guess the authors mean the spinous process of vertebrae 8 and not the DRG to identify the midline.

In this note for the protocol, we intended to mean the location of DRG on either side can be used in conjunction with the spinous processes of adjacent vertebrae to help identify the midline. We have revised this to now read (Revised manuscript line 172):

"Note: Along with the spinous processes on T7 and T9, the exposed dorsal root ganglia on T8 can also be used to aid identification of midline and a 30 gauge needle can be placed in the midline of the cord to aid with the subsequent hemisection."

Line 197: The following statement is unclear: 30-60 frames/sec minimum with a shutter speed at least double the frame rate. Can the authors simply state that a frame rate of 30-60 frames/s minimum should be used to monitor movements?

We have edited this sentence to simply state that a frame rate of 30-60 frames/s minimum should be used as suggested by the reviewer. Lines 203-204 in the revised document now read:

"2.2 Place a camera at ground level facing the circular Plexiglas open-field arena to record testing sessions for offline analysis (30-60 frames/s)."

Line 246: It is likely plantar paw placement and not dorsal.

We have corrected this statement in the revised manuscript. Lines 274-275 in the revised manuscript now read:

"Note: Plantar placement is deemed a perquisite for scoring the orientation of paw at contact and lift (2.2.4.2), swing movement (2.5.4.3) and forelimb-hindlimb coordination (2.5.5)."

Table
Page 19: Typo: Precision not "Presision" in Table 1.

We have corrected this typo in the updated materials list excel attachment.

Reviewer #2:

Manuscript Summary:
The authors have reported an approach to performing hemi-section SCI rats and assessing it using their existing locomotor scale. While the technique mentioned is not novel the study does add value to existing literature.

We thank the reviewer for their comments and have incorporated most suggestions in the revised manuscript.

Major Concerns:

There are several major concerns. Most importantly, the authors have overlooked existing locomotor scales available and already used in studies (interventional and behavioral) to assess hemi-section injuries. Authors should address the following to make the presented work concise and all inclusive.

We appreciate that alternative interventional and behavioural assessments are available for hemisection injury models. In this manuscript we focus on a thoracic hemisection injury and ordinal hindlimb locomotor assessment in the open-field in the rat. To keep the manuscript concise, we have not included a comparative discussion on other locomotor assessment techniques. We do state that a comprehensive behavioural test battery is available to assess locomotor function in spinal cord injury models in the introduction (lines 85-86). Additionally, in the discussion we refer to more rigorous and specific locomotor testing that can be performed either once an appropriate recovery time point has been reached or in conjunction with the locomotor assessment. Moreover, we also refer to another established protocol for hindlimb locomotor assessment in the open-field in the rat (the BBB scale) and compare that scale with the one presently described to make the presented work concise and inclusive.

1)Introduction: Para 2: Non reproducible contusion SCI claim is invalid

We appreciate this comment and have removed the sentence from the revised manuscript. We had not intended to indicate that contusion models were non reproducible, but rather that they can result in increased variability in lesion size and functional outcomes in comparison to transection models. We have removed the sentence to avoid any potential confusion on the matter.

2)Section 1.13: Does animal need bladder expressions?

In the vast majority of cases animals do not require bladder expressions. In rare cases during acute spinal shock or unintended bilateral lesions, manual bladder expression may be required. We include mention of this in the discussion on lines 409-415:

“In particular, acute spinal shock or unintended bilateral lesions may interfere with micturition that can lead to potentially fatal infections. Carefully monitor the bladder of the rat after surgery and manually void three times per day if full by gentle pressure from the ventral side of the

bladder descending caudally. We use female Long-Evans rats as they have a significantly shorter and straighter urethra than males that leads to a more rapid onset of an automatic urinary bladder, easier micturition, and lower rates of urinary tract infections[2].”

3) Section 2.1: Claim that no re-trains does not hold true then?

We are unsure whether the reviewer was referring to either pre-training or repeated testing in the task and provide responses for both cases:

The handling and acclimatization steps described in section 2.1 are for familiarizing rats to being handled by experimenters and to acclimatize them to the testing environment rather than reflecting pre-training in the task prior to testing. These steps could be avoided, but they are standard procedures in all behavioral testing to help reduce measurement reliability.

In terms of repeated testing viability, we show here that repeated testing is readily amenable at weekly intervals over the first 5 weeks after hemisection. Other studies have demonstrated that this can be extended for at least 6 (reference #23 in revised manuscript) and 8 weeks (references #10-12). We therefore strongly contend that repeated testing viability with this task does indeed hold true.

4) Section 2.4: Animals picked from tail or mid-trunk?

Animals are picked up gently from the mid-trunk. We have revised the manuscript to mention this. Lines 199-201 now read:

“2.1 Handle rats daily for 1 week and habituate them to the arena for 2 5-min sessions prior to testing to acclimatize to being picked up, gently from the mid-trunk, while in the open-field and to ensure measurement reliability during testing.”

5) Section 2.5: How many raters?

Two raters, agreeing on a score, are optimal for the assessment. We have updated the manuscript to reflect this point. Lines 439-441 in the revised manuscript now read:

“To ensure reliability in locomotor scoring two raters, preferably blinded, should conduct the analyses as previously described[10].”

5) Discussion first sentence needs referencing

We have added a reference to this sentence in the revised manuscript (reference #25).

6) Line 356: Does animals chew their feet?

We do not observe autophagia of the foot at the time points observed in this manuscript. In very rare cases, at long post-injury time points, autophagia of the foot has been observed and we have

included mention of this in the paragraph on post-surgical monitoring and complications in the protocol and discussion of the reviewed manuscript.

Lines 193-195 now read: "Provide supplemental analgesia daily over the first 3 post-surgical days and continually monitor for signs of pain, weight loss, improper micturition, infection, problems with wound healing, or autophagia."

Lines 406-409 now read: "Post-surgical monitoring of the rat is essential after the hemisection surgery, especially for signs of improper micturition, pain, infection, weight loss, problems with wound healing, or autophagia. Consultation with veterinary staff for evaluation and treatment is crucial in situations of post-surgical complications."

7)Line 360: Does that mean the the surgical post care will be different if males were used?

While the principles of surgical post care will be the same between females and males, manual bladder expression in males can potentially require a more involved process that can be addressed in consultation with veterinary staff. In lines 412-415 of the manuscript we mention the benefits of using female rats on this point:

"We use female Long-Evans rats as they have a significantly shorter and straighter urethra than males that leads to a more rapid onset of an automatic urinary bladder, easier micturition, and lower rates of urinary tract infections[2]."

General comment: Lacks information on how the rater was trained and how repeatable the scoring is among various raters.

We appreciate this comment and refer the reviewer to reference #10 in which detailed information on how raters are trained and inter-rater variability measures are provided. We have revised the manuscript to indicate this on lines 439-441:

"To ensure reliability in locomotor scoring two raters, preferably blinded, should conduct the analyses as previously described [10]."

Overall: The lesion in Figure 1 seems to have extended on contralateral side as well...

We included the photomicrograph (panel A) and overlay of the lesion extent (panel B) in Figure 1 to show a representative result of the hemisection procedure in a group of 6 rats in order to demonstrate the expected variability that can be obtained with this technique (in this sample, reflecting 47.3 ± 4.0 % of the cross-sectional spinal cord area). The lesion from the rat in (A) is also depicted as the dark blue area in (B) and does slightly extend to portions of the dorsal and ventral white matter on the right side (please note that spinal grey matter is also coloured in purple in the figure and is not resultant from the lesion). We show this in order to provide a representative sample that can be expected instead of an idealized result that would not be

achieved in 100% of cases. In the revised manuscript we have amended the figure legend to reflect that the damage is concentrated to the left hemicord.

The legend for Figure 1 now reads:

"**Figure 1.** Representative spinal lesions. (A) Microphotograph of a coronal spinal section at the lesion epicenter from a hemisected rat stained with cresyl violet (cell bodies, purple) and luxol fast blue (myelin, blue) indicating damage to the grey and white matter concentrated in the left hemicord. D, Dorsal; V, Ventral; L, Left; R, Right. Scale bar: 1 mm. (B) Schematic overlay of the shared proportion of maximal lesion area in a group of rats (n = 6). The location of the crossed corticospinal tract in the dorsal funiculus on the right side is shaded in black."

No figures for Forelimbs are provided

The thoracic hemisection model described in this manuscript does not cause functional deficits in the forelimbs. Forelimb motor performance assessed either using the open-field scoring system described in this manuscript, or during ladder crossing, is not affected in this hemisection model as we have previously shown (reference #9). As such, we elected to only provide a figure of a representative recovery profile for the hindlimbs.

The scoring does not seem to track recovery the way it was claimed.

We are uncertain as to what the reviewer is asking. In our representative data in Figure 2 we show sample recovery time points in the intact state and for the first 5 weeks after thoracic hemisection at weekly intervals to provide an overview of the recovery process using the lesion model and scoring scale described in the manuscript. In order to track recovery more closely, additional testing time points with shorter testing intervals could be performed as a plateau in recovery occurs for the affected hindlimb approximately 3 weeks after injury as indicated in Figure 2.

Minor Concerns:

Referencing overall

We are not sure what this comment is specifically referring to. If it is with respect to using abbreviated journal titles, as indicated by the Editors, we have used full journal names in the revised manuscript. Additionally, we have added extra citations in the revised manuscript.

Not discussing other similar locomotor scales

We do agree with the reviewer that while there are other locomotor scales available to assess forelimb and hindlimb motor function using both qualitative and quantitative techniques, in a variety of species and spinal injury models, we respectfully focus our discussion in the

manuscript to ordinal locomotor scales that visually evaluate hindlimb locomotor performance in the rat following a thoracic hemisection. In our manuscript we do discuss the BBB scale which is commonly used to assess hindlimb locomotor performance in an open-field arena in the rat after thoracic spinal injury. Additionally, an extensive discussion comparing our scale with the BBB can be found in the original paper (reference #10).

Reviewer #3:

The manuscript describes procedures related to the hemisection of the spinal cord in rat, the technique used in numerous laboratories and the open-field locomotor assessment, so the novelty presented in this paper is relatively low. Basing on my personal experience with this technique I believe that the article potentially is useful for researchers interested in this type of experiments.

We thank the reviewer for their comments and have incorporated most suggestions in the revised manuscript.

Authors should also refer to a problem of age of operated animals and describe in more details techniques of the locomotion and muscle activity analyses.

We are unsure as what "a problem of age of operated animals" in this comment refers to as all experiments described are conducted in young adult rats. There are certainly different surgical, post-operative care, and behavioural assessment considerations required for neonatal or very young rats. In addition, any surgical intervention can lead to more complications, reduced functional recovery, and special consideration in very old rats. However, the techniques described in this manuscript apply to all adult rats commonly used in research.

With respect to the comment on "locomotion and muscle activity analyses", this manuscript does not speak to those techniques and instead refers to locomotor assessment scored with a rating scale based on visual assessment of video-recorded testing in an open-field arena. We do agree that such other techniques can provide valuable insight on functional locomotor recovery in spinal cord injury models, and mention in the discussion starting from line 423 in the revised manuscript that the assessment scale described presently has a great potential to be used in order to screen animals for appropriate recovery indices in order to complement with more rigorous and specific locomotion testing techniques at appropriate time points. To expand on this point, we have incorporated suggestions below on additional complementary techniques, described in our next commentary that can be performed in the revised manuscript.

Potentially, some sample papers (not cited) focused on these topics will be interested for authors of the manuscript:
Automated Gait Analysis Detects Improvements after Intracellular Peptide Administration in a Rat Hemisection Model of Spinal Cord Injury. Ham TR, Farrag M, Soltisz AM, Lakes EH, Allen KD, Leipzig ND.
A novel multidimensional analysis of rodent gait reveals the compensation strategies employed

during spontaneous recovery from spinal cord and traumatic brain injury. Neckel ND, Dai HN, Burns MP.
Effect of acute lateral hemisection of the spinal cord on spinal neurons of postural networks. Zelenin PV, Lyalka VF, Orlovsky GN, Deliagina TG.
Interlimb Coordination during Tied-Belt and Transverse Split-Belt Locomotion before and after an Incomplete Spinal Cord Injury. Thibaudier Y, Hurteau MF, Dambreville C, Chraibi A, Goetz L, Frigon A.
Time-related changes of motor unit properties in the rat medial gastrocnemius muscle after the spinal cord injury. II. Effects of a spinal cord hemisection. Celichowski J, Kryściak K, Krutki P, Majczyński H, Górska T, Sławińska U.
Mechanism of Restoration of Forelimb Motor Function after Cervical Spinal Cord Hemisection in Rats: Electrophysiological Verification. Takeuchi T, Takahashi M, Satomi K, Ohne H, Hasegawa A, Sato S, Ichimura S
Forelimb muscle plasticity following unilateral cervical spinal cord injury. Gonzalez-Rothi EJ, Armstrong GT, Cerreta AJ, Fitzpatrick GM, Reier PJ, Lane MA, Judge AR, Fuller DD.

We thank the reviewer for providing additional topics of interest on specialized techniques involving gait assessment, physiological recordings of spinal neurons and muscle fibers, and assessment of muscle atrophy in hemisection and other spinal cord injury models. In the revised manuscript we have included a selection of these papers focusing on the behavioural assessment of locomotion in our discussion of other locomotor testing methodologies that can be used to complement the assessment scale described in the manuscript (references #1,2,4 from above).

Lines 423-427 of the revised manuscript now read:

"The assessment can be performed as early as the animal recovers from anesthesia and can be used to screen animals for appropriate recovery indices (*e.g.*, recovery of body weight support) when more rigorous and specific locomotor testing can be supplemented such as automated gait assessment of overground locomotion[26-28], kinematic analyses during treadmill locomotion[29-32], grid walking[33], and ladder rung walking[9,34].

Yours sincerely,

Marina Martinez, Ph.D.
Professeure sous octroi adjointe
Université de Montréal, Département de Neurosciences
Hôpital du Sacré-Coeur de Montréal, Montréal, QC, Canada
Groupe de recherche sur le système nerveux central (GRSNC)
2960, Chemin de la Tour
Montréal, Québec, H3T 1J4
Tel: 514-343-7046
marina.martinez@umontreal.ca