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,

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