Video Article

A Contusive Model of Unilateral Cervical Spinal Cord Injury Using the Infinite Horizon Impactor

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Abstract

While the majority of human spinal cord injuries occur in the cervical spinal cord, the vast majority of laboratory research employs animal models of spinal cord injury (SCI) in which the thoracic spinal cord is injured. Additionally, because most human cord injuries occur as the result of blunt, non-penetrating trauma (e.g. motor vehicle accident, sporting injury) where the spinal cord is violently struck by displaced bone or soft tissues, the majority of SCI researchers are of the opinion that the most clinically relevant injury models are those in which the spinal cord is rapidly contused.[1] Therefore, an important step in the preclinical evaluation of novel treatments on their way to human translation is an assessment of their efficacy in a model of contusion SCI within the cervical spinal cord. Here, we describe the technical aspects and resultant anatomical and behavioral outcomes of an unilateral contusive model of cervical SCI that employs the Infinite Horizon spinal cord injury impactor.

Sprague Dawley rats underwent a left-sided unilateral laminectomy at C5. To optimize the reproducibility of the biomechanical, functional, and histological outcomes of the injury model, we contused the spinal cords using an impact force of 150 kdyn, an impact trajectory of 22.5° (animals rotated at 22.5°), and an impact location off of midline of 1.4 mm. Functional recovery was assessed using the cylinder rearing test, horizontal ladder test, grooming test and modified Montoya's staircase test for up to 6 weeks, after which the spinal cords were evaluated histologically for white and grey matter sparing.

The injury model presented here imparts consistent and reproducible biomechanical forces to the spinal cord, an important feature of any experimental SCI model. This results in discrete histological damage to the lateral half of the spinal cord which is largely contained to the ipsilateral side of injury. The injury is well tolerated by the animals, but does result in functional deficits of the forelimb that are significant and sustained in the weeks following injury. The cervical unilateral injury model presented here may be a resource to researchers who wish to evaluate potentially promising therapies prior to human translation.

Video Link

The video component of this article can be found at https://www.jove.com/video/3313/

Protocol

1. Set up: Frame and Clamp Design for Holding the Animal

1. The frame and clamp to hold the animal was custom-designed to accommodate the Infinite Horizon (IH) Spinal Cord Injury Impactor.
2. The base of the frame is an aluminum platform cut into the following dimensions (30.2 cm x 20.3 cm x 1.3 cm) in order to fit into the table guide bracket that comes standard with the IH device (**Figure 1A**).
3. Four Flexaframe support foot plates (Fisher Scientific, Toronto, ON) are attached to the platform and eight Flexaframe support rods (Fisher Scientific, Toronto, ON, 30.5 cm) are assembled using eight Flexaframe support connectors (**Figure 1B**).
4. Two additional Flexaframe support connectors, attached to the middle two rods, house the custom made holds for the clamp (**Figure 1B**).
5. The angle by which the animal's spinal cord is rotated with respect to vertical is established by keeping one of the horizontal rod in place and varying the height of the other horizontal rod (**Figure 2**).
6. The clamp is 35.6 mm long, 25.4 mm high and with a 7.6 mm jaw designed to rigidly grab hold underneath the transverse process from C4 to C6 (**Figure 1C**). Additional details on the clamp design have been described previously by Choo *et al*., 2009.

2. Surgery

1. Male Sprague Dawley rats (Charles River Laboratories) weighing 300-350 g were anesthetized by isoflurane (4% for induction and 2% for maintenance) in oxygen (1 L/min).

2. Once the animals are under the plane of anesthesia, the animal is placed in a stereotaxic frame (Kopf, Tujunga, CA).
3. To minimize bleeding during the surgical procedure 0.4 mL of lidocaine (20 mg/mL; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) with epinephrine is injected intramuscularly around the surgical site in the dorsal neck region.
4. A 4-5 cm dorsal midline incision is made using a sterile scalpel (#15), starting from the base of the skull and extending caudally.
5. Sterile Adson forceps are used to bluntly dissect through the dorsal musculature to reach the spine, and a sterile Alm retractor (Fine Science Tools, North Vancouver, BC) is inserted to keep the muscles spread apart.
6. Using the #15 scalpel, the muscles overlaying the laminae of C4-C7 are scraped off, starting in the midline and sweeping them out laterally.
7. An incision with a sterile scalpel (#15) is made to the muscle attached to the transverse processes on both side of the spine in order to fit the clamp underneath the transverse processes of C4 to C6.
8. With a sterile fine tipped Friedman-Pearson Rongeur (Fine Science Tools, North Vancouver, BC), the left C5 lamina is carefully removed to visualize the dura and the spinal cord.
9. A rod with a diameter of 1.5 mm is slid under the arms to prop the animal up, thereby raising the spine slightly and facilitating clamp insertion.
10. Mount the jaw of the sterile clamp onto lateral transverse processes of C4 to C6 and tighten the screws.
11. Remove the Alm retractor.

3. Spinal Cord Injury

1. After the clamp is mounted on the animal, the animal is moved to the IH impactor.
2. The clamp is inserted into the metal holders on the two middle rods of the frame that have been secured at an angle of 22.5° off of horizontal (**Figure 2**).
3. The scissor jack that provides stable height adjustments, (VWR, Mississauga, ON) is raised up until the animal lays flat.
4. Ensure that the clamp is horizontal by placing a small cylinder level on top of the clamp and by tightening the screws. It is important that all the screws are tight and the set up is rigid without any movement.
5. The remainder of the procedure is conducted under a microscope (Leica MZ8).
6. Lower and aim the impactor tip (15 mm in diameter, with rounded-off edges) using the vertical adjustment knob and the two horizontal adjustment knobs on the IH impactor until the center of the impactor tip is hovering above the apex of C6 spinous process.
7. Once the impactor tip is centered, turn the y-axis adjustment knob one and two fifth turn (1.4 mm) to move the tip laterally to the left side and the x axis horizontal adjustment knob to move the impactor tip to the center of C5.
8. Lower the tip until it is just above the dura to verify that the impactor tip is aiming the lateral half of the grey matter.
9. Turn the vertical adjustment knob two turns to raise the tip 4 mm above the dura.
10. Ensure that the impact area is dry by using a cotton swab or stick.
11. Set the desired force to 150 kdyn on the program and click "Start Experiment" to trigger the impactor.
12. After the injury, the wound is closed in layers with 5-0 vicryl sutures. Buprenorphine (0.03 mg/kg SC, Temgesic; Schering-Plough Corporation, Kenilworth, NJ) and Saline (10 mL) is administered subcutaneously before and twice daily for two days after surgery. Animals are closely monitored twice a day for 2 weeks and one a week for 6 weeks post-injury.

4. Representative Results

Twenty Nine male Sprague Dawley rats (Charles River Laboratories) weighing 300-350 g were injured at a force setting of 150 kdyn. The impactor tip was aimed 1.4 mm lateral to midline, at an angle of 22.5° off of vertical. The mean actual force was 155.55 ± 0.73 kdyn. The mean displacement was 1512.72 ± 27.86 µm and the velocity was 120.24 ± 0.52 mm/s (**Figure 3**).

Behavior Outcome Measures

Functional recovery was assessed using the horizontal ladder test, cylinder rearing test, grooming test, and modified Montoya staircase test[1]. Animals were trained before injury and assessed at 2, 4 and 6 weeks post-injury. There were significant forelimb impairments sustained throughout the experimental period.

Horizontal Ladder test. Before the injury, animals made only 4.75 ± 0.73% errors on the ipsilateral forelimb while traversing across the irregularly spaced horizontal ladder. Following the injury, the animals demonstrated marked increases in the percentage of forelimb errors. The ipsilateral forelimb percent errors were 26.97 ± 2.92%, 26.23 ± 2.84% and 22.06 ± 2.05% at 2, 4 and 6 weeks post-injury, respectively (**Figure 4A**). Importantly, the forelimb impairment on this test was sustained for the 6 weeks.

Cylinder Rearing test. The percentage of ipsilateral forelimb (left + both) usage during exploration decreased significantly after SCI. Prior to the injury, the animals used the ipsilateral forelimb 75.12 ± 2.25%. After the injury the animals used the ipsilateral forelimb 8.59 ± 1.80% at 2 weeks, 14.25 ± 2.65% at 4 weeks and 11.76 ± 2.66% at 6 weeks (**Figure 4B**).

Modified Montoya Staircase test. The number of pellets retrieved with the ipsilateral forelimb decreased dramatically after the injury. Prior to the injury, the animals collected 84.85 ± 2.88% of the food rewards. However, at 2, 4 and 6 weeks post-injury, the animals retrieved only 30.91 ± 4.03%, 28.94 ± 4.38% and 25.86 ± 3.09% of the pellets (**Figure 4C**).

Grooming test. There were dramatic decreases in the grooming scores after injury. After SCI, the ipsilateral grooming scores were 2 weeks: 2.00 ± 0.17, 4 weeks: 1.83 ± 0.17 and 6 weeks: 1.79 ± 0.11 (**Figure 4D**).

Histological Outcomes

White matter and Grey matter sparing. An example of a spinal cord injury at a force of 150 kdyn, angle of 22.5° and lateral aim of 1.4 mm is presented in **Figure 5**. The injury resulted in substantial damage to the grey and white matter on the ipsilateral side. Both corticospinal and rubrospinal tracts were injured and 23 out of 29 animals had parenchymal damage contained on the ipsilateral side. The longitudinal extent of the damage of the white and grey matter was 2400 rostrally and 2400 caudally (**Figure 6**). When adding the sections to provide a gross estimation of the "cumulative spread" of white and grey matter sparing (2000 µm rostral and caudal to the epicenter), the ipsilateral side had only 51.8% of the white matter spared and 39.7% of grey matter remaining compared to the contralateral side (**Figure 6**).

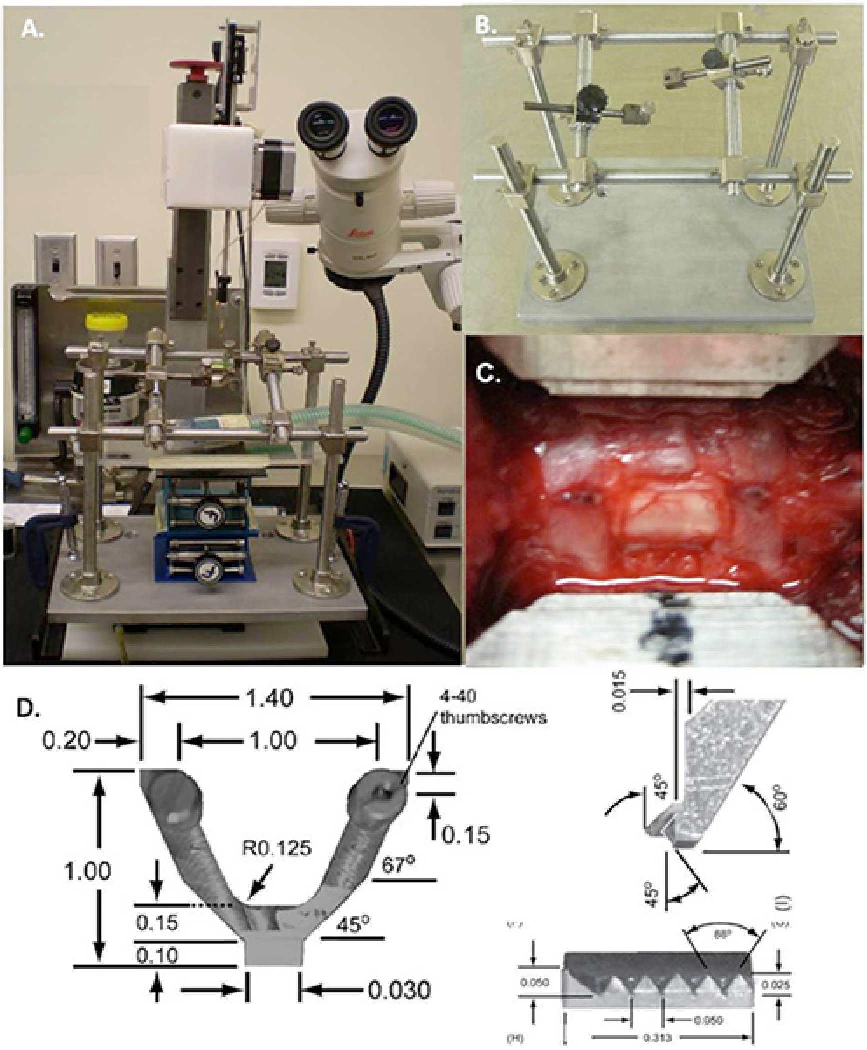

Figure 1. Infinite Horizon spinal cord injury impactor. A. Infinite Horizon impactor and overall set up. B. Frame set up. C. Close-up image of the clamp for holding cervical transverse processes. D. Specification (unit: inch) of the clamp. Suggested tolerance <0.002 in (Choo *et al*. 2009). Click here to view larger figure.

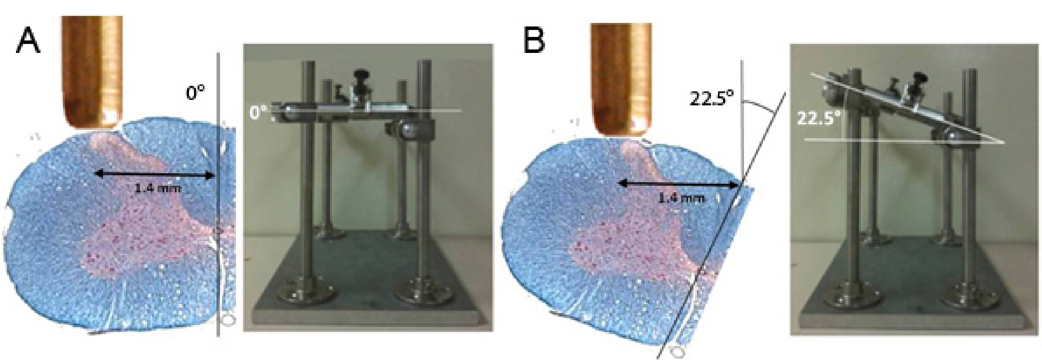

Figure 2. A. Illustration and corresponding frame set up to achieve spinal cord rotation of 0° (neutral) or B. 22.5° with the lateral aim of 1.4 mm.

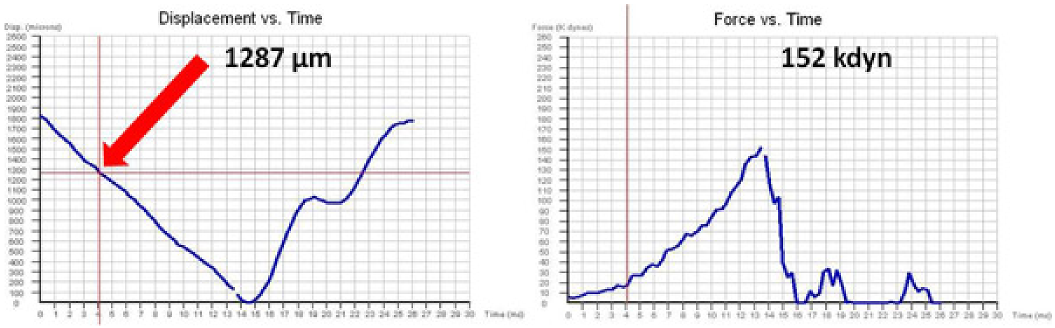

Figure 3. Representative force and displacement graphs for Infinite Horizon impactor. The arrow indicates the time that the impactor tip has reached 20 kdyn and the point when the recording of displacement is initiated. The actual force is read from the peak of the force vs. time curve and the corresponding displacement is calculated. The graphs below depict a typical 150 kdyn contusion, A. Displacement vs. time graph, B. Force vs. Time graph. These graphs show that the actual force achieved was 152 kdyn, and the impactor displacement into the cord was measured to be 1287 µm. Click here to view larger figure.

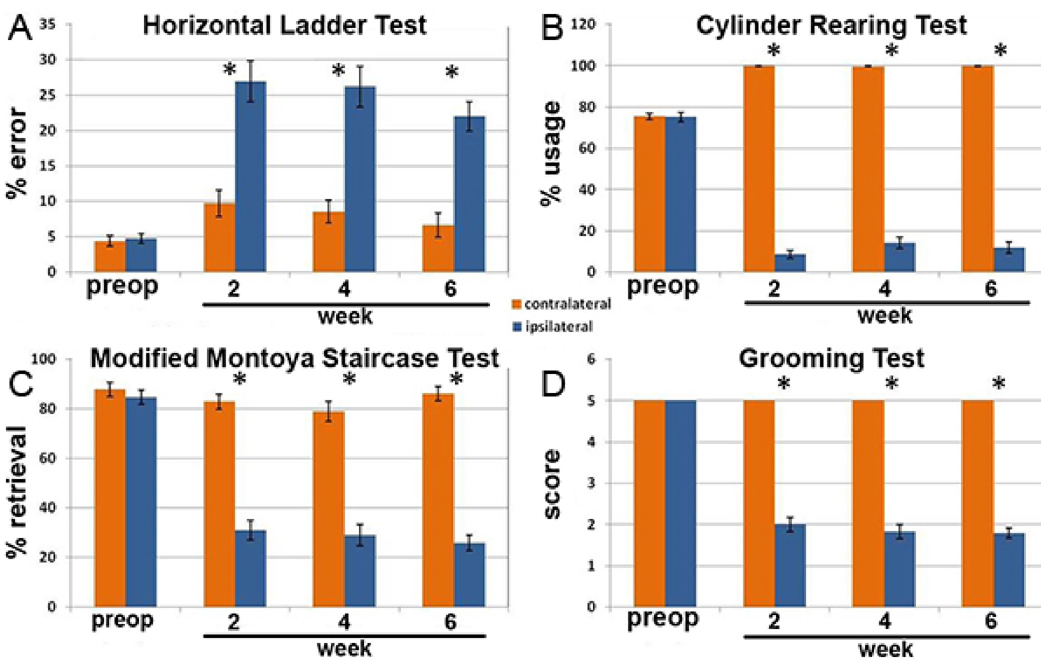

Figure 4. Behavioral assessments for force of 150 kdyn, impactor angulation of 22.5° and aim of 1.4 mm left of midline. A. Horizontal ladder test. B. Cylinder rearing test. C. Modified Montoya staircase test. D. Grooming test. The ipsilateral forelimb resulted in significant and sustained impairments compared to the contralateral forelimb.

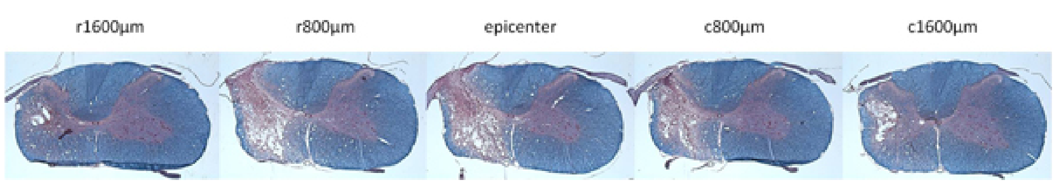

Figure 5. Representative images of the injured spinal cord from rostral 1600 µm to caudal 1600 µm.

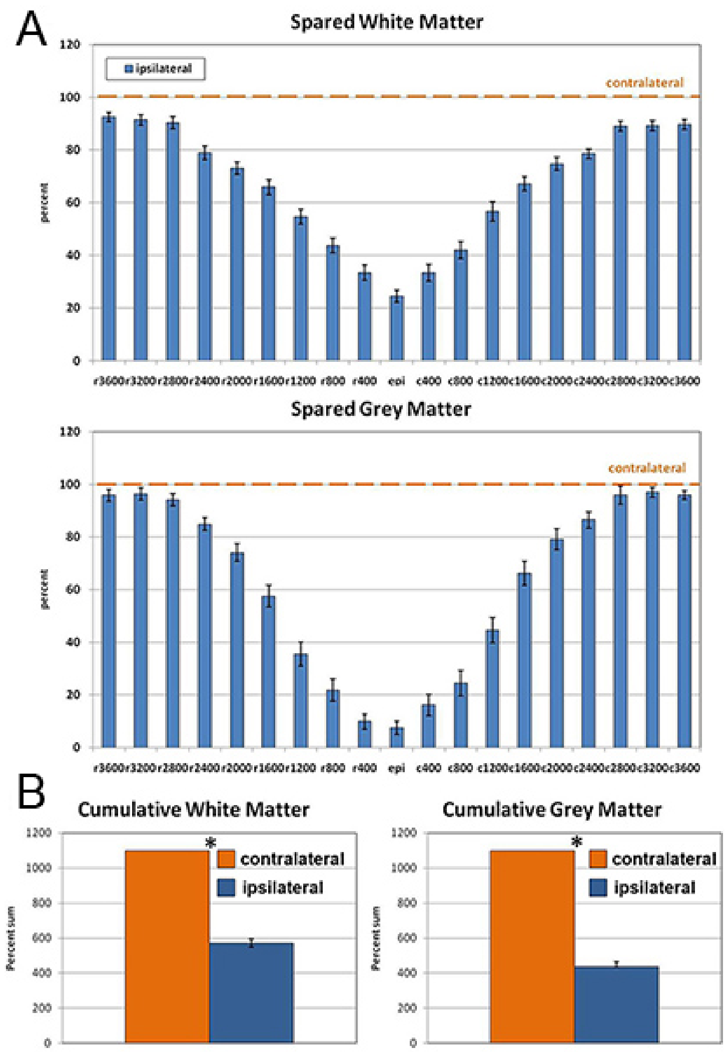

Figure 6. Histological assessments. A. Percent white and grey matter spared. Matter around 2000 µm of the epicenter of damage was largely spared. B. Cumulative white and grey matter spared within 2000 µm of the epicenter of damage. The ipsilateral side had significantly less spared white and grey matter compared to the contralateral side. Click here to view larger figure.

Discussion

In this paper, we describe a cervical unilateral contusion model using the Infinite Horizon (IH) impactor at a force of 150 kdyn, an angle of 22.5° off of vertical, and a lateral aim of 1.4 mm from the midline. With these settings, we were able to produce sustained behavioral deficits in the ipsilateral forelimb with parenchymal disruption contained largely to the ipsilateral side, where it appeared that considerable damage occurred to the regions where the rubrospinal, reticulospinal, vestibulospinal and corticospinal tracts would be expected to run. The development of this model occurred in a series of three experiments which established the optimal injury force, impact location off of midline, and degree of rotation. Firstly, we found that injury forces below 150 kdyn did not produce sufficient and sustained functional deficits. Additionally, with the impactor tip striking the cord vertically (i.e. with no rotation), we frequently observed spikes in the force vs. time curves, suggesting that the impactor tip was hitting the bone on the ventral side of the spinal canal. Many of these animals also did not have severe or sustained functional deficits. Consistent with this, the histological analysis damage in these animals with the sudden force spikes, revealed parenchymal damage that was both mild and very laterally placed within the spinal cord. We therefore rotated the animals such that the trajectory of the impactor was 22.5° off of the vertical midline. By having the impactor tip come in more perpendicular to the spinal cord, we resolved the problem of the tip striking the ventral floor of the spinal canal, but we observed considerable parenchymal damage on the contralateral side of the spinal cord. Finally, we aimed the impactor at three different distances off of midline, 1.0, 1.2 and 1.4 mm, using a 150 kdyn force and an angle of 22.5°. We observed that there were no behavioral differences between the 1.0, 1.2 and 1.4 mm settings, but as the target of the impact was moved laterally, it was more likely that the gross parenchymal damage could be contained on the ipsilateral side. We therefore arrived at our current unilateral contusion settings of a 150 kdyn injury delivered at 22.5° off of vertical, with the impactor tip aimed 1.4 mm to the left of midline.

The rationale for making cervical injury models available for the testing of SCI therapies is clear: the majority of individuals suffer cord injuries in the cervical spine, upper extremity function is paramount to these individuals, and clinical trials of novel neuroprotective or neuroregenerative interventions are increasingly focusing on cervical SCI patients in order to use segmental motor recovery as an outcome measure. Injury to the cervical spinal cord can occur via laceration, compression, or contusion. Amongst these injury models, contusion and compression injuries best represent the pathophysiological process observed in human SCI.[1,2,3] According to a recent survey of the SCI research community, 72% of the 324 respondents agree that contusion injury is the most clinically relevant injury model of SCI.[1]

Since Reginald Allen's description of the first experimental weight-drop device for generating spinal cord injuries in the laboratory setting[4], a number of contusion devices have been developed in an effort to optimize reproducibility and to generally simulate the pathology of human injury.[3] The New York University impactor uses electromechanical components to measure injury displacement and velocity during weight drop.[5,6] Here, injury severity is dictated by the height from which the weight is dropped. In contrast, in the Ohio State University (OSU) impactor and the multimechanism injury system designed by Choo *et al.* (2009), the maximum displacement of the spinal cord is determined, and the force imparted to the cord is then measured. The IH impactor is distinct in that the user dictates the force applied, and then the displacement is measured. While each of these systems (weight-drop versus displacement-control versus force-control) has its theoretical advantages, the relative ease of use, commercial availability and the availability of technical support from the manufacturer of the IH impactor have made it increasingly popular in recent years.

From a technical perspective, we should note that significant modifications were made in the method for clamping the animals and securing them prior to impact (**Figure 1**). To improve upon the consistency of our injuries and to accommodate the unique anatomy of the cervical spine, we stabilized the animals with a custom-built clamping system that firmly grasps the transverse processes of the cervical spine.[7] As the clamps that are provided with the IH impactor are intended to hold the spinous processes within the thoracic spine, we felt that they were not as well suited for the much smaller spinous processes of the cervical spine. The frame and clamping system holds the animals very rigidly during the impact, with virtually no 'slippage' between the clamp and the spine.[7] The clamping device is relatively easy to use and apply to the spine. A number of trainees and technicians in the laboratory have utilized it with consistent success. Additional dissection of the soft tissues more laterally off the dorsal aspect of the cervical spine is however needed in order to grasp the transverse processes, and bleeding can be encountered in doing so. Hemostasis is typically achieved by simply applying gentle pressure with a small piece of surgical sponge. Additionally, the clamp is designed specifically for animals in the 300-350 gm weight range, and would need modification to accommodate smaller animals (although this could likely be achieved with spacers lodged between the two arms of the clamp).

With respect to the intended target of the injury, we aimed to injure both the corticospinal (CST) and rubrospinal (RST) tracts of the ipsilateral side only, as these both play a role in forelimb function in rodents.[8] In our study, functional deficits were assessed with the horizontal ladder test, cylinder rearing test, grooming test and Montoya staircase test. Both the horizontal ladder test and cylinder rearing are valuable assessments after cervical injury models.[8,9,10,11] The horizontal ladder test forces the animals to use both their injured and uninjured forelimbs to get across the ladder, and hence, the test measures the compensatory and adaptive function of the forelimb. During the pre-injury training, animals typically will "grab" or place their forepaw on the bars with their digits while crossing the ladder. After severe or moderate cervical unilateral contusions, most of this motor function is abolished, and animals are no longer able to consistently place or grasp the rungs.[2,12] The cylinder rearing test examines recovery naturally by analyzing voluntary forelimb usage. Typically, the use of the injured forelimb while exploring is dramatically reduced after injury. The loss of these functions are likely related to a combination of both axonal disruption and to the eradication of motor neurons at the lesion epicenter, which innervate such muscles as the deltoid, biceps, extensor carpi radialis longus and the extensor carpi radialis brevis muscles.[13] The grooming test, like the cylinder rearing test, examines the gross natural behavior of the animals. The modified Montoya staircase evaluates the digit functions, or fine control, of the forelimbs. Surprisingly to date, there is only one study that has utilized the modified Montoya's staircase test in cervical SCI.[14] Together, these tests evaluate both the fine and gross components of the overall forelimb functions.

Other studies have also described cervical contusion models, which have typically been devised with some modification to a pre-existing thoracic contusion device.[2,12,15,16,17] Dunham *et al*. (2011), Popovich *et al*. (2010) and Sandrow *et al*. (2008) all utilized the IH impactor. Dunham *et al*. (2011) characterized the injury model using 100, 200, and 300 kdyn by evaluating the cylinder rearing test, Catwalk gait analysis, vermicelli handling test and horizontal ladder test. Popovich *et al*. (2010) injured the cervical spinal cord at a force of 175 kdyn and observed the functional outcomes using the incline plane test, cylinder rearing test and the BBB test. Sandrow *et al*. (2008) used a force of 200 kdyn and resultant displacements of 1.6 to 1.8 mm and then assessed behavioral outcomes with the forced locomotion test, forelimb open field locomotion, grip strength test and grid walk test. Previous work from our laboratory used the Ohio State University impactor at 1.5mm displacement to test unilateral cervical contusions (with a maximum peak force of 200 kdyn).[18] Gensel *et al.* (2006) used a MASCIS/New York University impactor using 10 g at 6.5 mm and 12.5 mm height and assessed behavioral outcome with the grooming test, horizontal ladder test, cylinder rearing test and Semi-Automated Walkway test (Catwalk gait analysis). Soblosky *et al*. (2001) used a modified Allen's weight drop device (10.5 g) to injury animals at 5.00, 2.50 or 1.25 mm heights on a 25.0° angle, and evaluated the horizontal ladder test and cylinder rearing test to assess behavioral recovery. It is difficult to compare our injury model to the study by Sandrow *et al*. (2008), since none of functional tests overlap with our current study. When we compare the functional outcomes to other studies, our current IH injury model is generally less severe to the 300 kdyn group from Dunham *et al*. (2011), but more severe compared to other cervical unilateral contusion reports. The animals in our injury model were not able to perform the functional tests until two weeks after injury whereas other injury models commence functional tests starting 1 week after injury. For the horizontal ladder test, Soblosky *et al*. (2001) reported the total number of slips without the total number of steps. The ipsilateral percent error at 6 weeks post-injury for the our current model was about 25 %, compared to study by Lee *et al*. (2010) and Gensel *et al*. (2006), which reported errors in the range of 10 - 15 % and Dunham *et al*. (2011) that reported 40 % error for the 300 kdyn group and 20 % for the 100 and 200 kdyn groups. For the cylinder rearing test, Popovich *et al*. (2010) reported the duration of rearing. Our 15-20 % of ipsilateral forelimb usage for the 22.5° angle was comparable to that reported with a 5.0 mm weight drop.[12] Comparing the behavioral deficits that resulted from the NYU impactor, Gensel *et al*. (2006) reports a complete abolishment of ipsilateral forepaw use for the 12.5 mm height. Dunham *et al*. (2011) reported about 5, 10 and 20 % ipsilateral forelimb usage for 100, 200 and 300 kdyn animals. For the grooming test, our animals scored less than the injury groups reported by Gensel *et al*. (2006).

Histologically, the injury model presented here generally induces greater parenchymal damage as compared to other cervical hemicontusion injury models, but less than the damage reported by Popovich *et al.* (2010). The rostral and caudal extension of our injury was 4.8 mm, as compared to 8.0 mm in Popovich *et al*., 4.0 mm in Lee *et al*. and 3.6 mm in Gensel *et al*. studies using the IH impactor, OSU impactor and NYU impactor, respectively.[2,18] At the lesion epicenter, we found about 20% grey matter spared in our injury model, as compared to Lee *et al*. (2010) at 10%, Gensel *et al*. (2006) at 20-50%, and Soblosky *et al*. (2001) at 31-99%. For white matter sparing, the injury model presented here left about 20% tissue remaining at the epicenter compared to 30% in Lee *et al*., (2010), 5 - 10% in Gensel *et al*., (2006) and 18 - 62% in Soblosky *et al*., (2001) at the lesion epicenter. In the study, by Lee *et al*., (2010) the rubrospinal tract suffered significant injury, but the corticospinal tract often appeared intact. Popovich *et al*. (2010) reported complete abolishment of the two tracts. Gensel *et al*. (2006) reported partial damage to the corticospinal tract and complete destruction of the rubrospinal tract for both height settings. Soblosky *et al*. (2001) report partial damage to the rubrospinal tract, but no injury to the corticospinal tract. These reports further enforce the importance of injuring both descending tracts in order to produce sufficient functional deficits.[19] It is also worth to note that in Popovich *et al*. (2010), Gensel *et al*. (2006) and Soblosky *et al*. (2001), injuries also extended to the contralateral side. The importance of this issue of extension to the opposite side in our model is debatable, given that there were no behavioral differences between the injuries aimed 1.0, 1.2, and 1.4 mm off of midline (unpublished data), but it would be desirable contain the injury to ipsilateral side, since we used the contralateral side as the 'uninjured' control. While there was, in some animals, some crossover of parenchmyal damage to the contralateral side, this was minimal. When comparing the representation of white and grey matter damage as either a ratio between ipsilateral and contralateral sides, or as the absolute extent of damage, there was virtually no difference (unpublished data).

In conclusion, we report the development of a unilateral contusion injury, and hope to provide sufficient detail about its development and technique. Others who wish to study preclinical SCI therapies may employ such a model, using an impactor device that is widely available (the Infinite Horizon impactor). We are currently utilizing the model to evaluate neuroprotective interventions, with the hope of providing important preclinical evidentiary support for specific treatments prior to human translation.

Disclosures

No conflicts of interest declared.

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