Research paper

Adaptation of a cervical bilateral contusive spinal cord injury for study of skilled forelimb function

Camila Marques Freria, Lori Graham, Ali Azimi, Paul Lu

Keywords:
Spinal cord contusion
Bilateral cervical contusion spinal cord injury
Skilled forelimb function and forelimb behavioral assessment

ABSTRACT
We present an updated, clinically relevant model of moderately severe bilateral cervical level 6 contusive spinal cord injury (SCI) in the rat. This model is more clinically relevant than previous models due to its severity, yet animals readily survive the lesion. The C6 bilateral lesion is administered to Fischer 344 rats using the Infinite Horizons impactor adjusted to a 200 kdyne force with a 3.5 mm impactor head. The lesion results in loss of 60 ± 10% of the spinal cord area, including virtually the entire dorsal half of the spinal cord and complete interruption of the main corticospinal tract. Skilled forelimb performance declines by 60 ± 10% compared to the pre-operative baseline and deficits are sustained over time. This model is a substantial step closer to mimicking the most common level (cervical) and more severe form of SCI in humans and should provide a superior tool for assessing the likelihood that experimental interventions may promote motor recovery after SCI in humans.

1. Introduction

There remains a substantial need to develop superior models of spinal cord injury (SCI) to both understand cellular and molecular mechanisms associated with injury, and to test candidate therapies most accurately and reliably for human translation. Most humans sustain blunt force injuries that result in displacement of the vertebral column into the spinal canal, resulting in severe spinal cord compression that often permanently results in loss of function (Abuja et al., 2017). More rarely, individuals sustain bullet or knife wound injuries, but these account collectively for fewer than 17% of clinical cases (Farmer et al., 1998; Filho et al., 2014; Platt et al., 2021). A major advance in spinal cord injury model development was achieved with development of weight drop injury to the spinal cord in the 1970’s, the New York University weight drop model (NYU model) (1992) (Gruner, 1992). Subsequently, two different devices were developed to allow specific control of force application to the spinal cord with real-time readouts of impact kinetics: The Infinite Horizons impactor developed at in 2003 (Scheff et al., 2003), and the Ohio State University impactor device developed in 1987 (Bresnahan et al., 1987). These both became widely adopted, and the majority of recent publications in the SCI field utilize the Infinite Horizons device. These approaches delivered injuries that were sub-total in severity, allowing the testing of therapies that might not promote extensive anatomical repair, but could nonetheless screen for potentially translatable therapies. Several simple mechanical devices were also used to apply more severe injuries, including an aneurysm “clip compression model” described in 1978 (Rivlin and Tator, 1978) or simple compression of calibrated forceps around the spinal cord (Blight, 1991). These models were typically applied to the thoracic spinal cord, because rats could not survive bilateral injuries to the cervical spinal cord.

The development of models of cervical bilateral contusive SCI is important because the majority (57%) of human injuries occur at the cervical level, most frequently from a compressive mechanism (Van Den Berg et al., 2010; Wyndaele and Wyndaele, 2006). When surveyed, most patients with cervical level injuries report that their highest priority for functional recovery is upper extremity motor function (Anderson, 2004). The first bilateral cervical contusive SCI model was reported in 2009 (Anderson et al., 2009). Applied to Sprague-Dawley rats, this model resulted in injury to the majority of the corticospinal projection, and in long-lasting (to two months) deficits in grasp strength. Effects of the lesion on forelimb skilled motor function were not reported, and the vast majority of the cross-sectional area of the spinal cord was spared by the lesion (e.g., Fig. 5 in Anderson et al).

We were interested in developing a more severe, clinically relevant and survivable model of bilateral contusive SCI for the purpose of testing candidate translational therapies, such as neural stem cells. Because of
the importance of hand function to humans after SCI, we sought to develop a model that would result in complete interruption of the dorsal corticospinal projection in rats resulting in long-lasting functional deficits, together with more extensive parenchymal damage than the report of Anderson et al. (2009).

We now describe a bilateral C6 contusive SCI lesion model in Fischer 344 rats. This lesion is reproducible, eliminates ~60% of the spinal cord at the lesion level, including nearly the entire dorsal half of the spinal cord containing the corticospinal projection. Using a 3.5 mm impactor head and testing impact forces of 200, 225 and 250 kdynes, the lesion can be tuned to result in either 50%, or 85%, or permanent loss of skilled forelimb motor function, respectively. The lesion also results in persistent deficits of several parameters on the CatWalk device. Yet rats readily survive the injury without compromise to respiration. We believe that this is a reproducible and clinically relevant model for testing candidate therapies for human translation.

2. Materials and methods

2.1. Animals

Adult female Fischer 344 rats (Envigo) weighing 150-200 g (total \( N = 40 \)) were used for this study. F344 rats were chosen because they are a common breed for experimental studies in SCI, and because they are inbred and can be used for isogenic neural progenitor cell transplantation without immunosuppression (Lu et al., 2012). Females were used because post-operative care is simpler than male rats and because females exhibit less aggression than injured male rats. All experiments were conducted in strict accordance with NIH laboratory animal care and safety guidelines. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Department of Veterans Affairs (VA) San Diego Healthcare System. Animals had free access to water throughout the study and access to food during both surgeries and perfusions, animals were deeply anesthetized using a combination (2 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 g/ml), and ampicillin (3–5 mg/kg) and kept on heating pad for three days. Additionally, Nutri-cal direct oral feeding (2 ml, Henry Schein, Melville, NY) were given daily for 7 days following surgery. Following this time period, animals recovered to independent ambulation and were able to self-care; food pellets were placed at a lower level in the cage to facilitate animal access to nutrition for the remainder of the study period. Animals were perfused after completion of behavioral training at 15 weeks post-injury.

2.3. Behavioral assessment

Rats were habilitated to the Montoya staircase apparatus for 1 week (Montoya et al., 1991). Training was then begun for grasping of food pellets placed into each of the seven steps of the staircase device (Lafayette Instrument Co, Lafayette, Indiana). Training consisted of 2 rounds of 15 min each (total 30 min) daily for 5 days per week for 3 weeks prior to injury. Two food pellets (BioServ-F0042 45 mg sucrose) were placed into each step and the maximum number of food pellet eaten was 28, using both left and right forelimbs. For quantification, rats were allowed 15 min to retrieve and eat as many sugar pellets as possible from the left and right sides of the apparatus. The number of pellets eaten, displaced (including both eaten and dropped pellets) and the maximum level reached were recorded (Montoya et al., 1991). Accuracy was calculated as the percentage of pellets eaten per pellets displaced.

2.4. Catwalk

To capture fine locomotor changes we used an automatic digital footprint analysis system that provides quantitative information on limb recruitment pattern during locomotion across a transparent walkway. The rats were habituated on the CatWalk™ XT 8.1 system (Noldus Information Technology, Wageningen, The Netherlands) for 2 weeks before undergoing C6 bilateral contusion. CatWalk gait analysis was performed before lesion and 1- and 15-weeks post-injury. Each trial was repeated until three consecutive uninterrupted runs were recorded. Rats that exhibited prolonged stopping or that turned backward in the runway were considered to have failed the trial. All trials per time point were performed on the same day. The measurements taken were: support diagonal, support four paws, step cycle, stride length and swing speed.

2.5. Histology and immunohistochemistry

After completion of functional training, rats were deeply anesthetized and transectedally perfused with ice-cold saline and then 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. The spinal cords were then dissected out and were post fixed in 4% PFA overnight at 4°C. The spinal cords were then cryoprotected in a 30% sucrose solution for minimal 48 h at 4°C. Blocks of cervical spinal cord of 1.5 cm length

In a second experiment lasting 15 weeks, a moderate contusion with 200 kdynes force was chosen to test skilled forelimb motor function over an extended time-period (n = 12 lesioned animals) (Table 1). The injury was performed as described in the preceding paragraph at the 200 kdynes force.

Because the bilateral lesion model severely impairs locomotion and forelimb use, extensive animal care was required during the first post-operative week. Animals were given post-operative injections of lactated ringers solution (30–50 ml/kg), banamine (2.5–5 mg/kg), and ampicillin (3–5 mg/kg/) and kept on heating pad for three days. Additionally, Nutri-cal direct oral feeding (2 ml, Henry Schein, Melville, NY) were given daily for 7 days following surgery. Following this time period, animals recovered to independent ambulation and were able to self-care; food pellets were placed at a lower level in the cage to facilitate animal access to nutrition for the remainder of the study period. Animals were perfused after completion of behavioral training at 15 weeks post-injury.

| Table 1 | Experimental groups and animal numbers. |
|---------|----------------------------------------|
| Experiment | Total # of animals | Staircase training | Learned staircase | Not learned | Survived contusion | Die | Excluded | Behavioral test completed |
| Pilot     | 24          | 20          | 15          | 5          | 13          | 2       | 1       | 12          |
| Long-term | 16          | 16          | 12          | 4          | 9          | 3       | 0       | 9           |
| Total     | 40          | 36          | 27          | 9          | 22         | 5       | 1      | 21          |
centered around the contusion injury site were embedded in gelatin to provide additional support for retaining delicate strands of tissue and were sectioned in the horizontal plane on a cryostat set at 30 μm intervals. Gelatin embedded sections were serially collected in 24 wells plates containing tissue collection solution (TCS) as free-floating sections and stored at 4 °C.

In addition, a series of transverse sections through the entire injury site were cut at 20 μm thickness for analysis of spared gray matter and white matter in the injury site (n = 4). Transverse sections of spinal cord 8 mm centered at lesion site were incubated in Eriochrome Cyanine (EC) for 30 min at room temperature, washed in deionized H₂O, then differentiated in 5% iron alum followed by borax-ferricyanide for 5–10 min.

A series of 1-in-6 sections was mounted on gelatin-coated slides for Nissl staining to study lesion morphology. The remaining sections were used for immunohistochromistry. Sections were washed in Tris-Buffered Saline (TBS) and then incubated for 1 h in TBS containing 5% donkey serum, and 0.25% Triton to block non-specific binding of antibodies. Sections were then incubated overnight at 4 °C in TBS containing 5% donkey serum, 0.25% Triton and primary antibodies against: (a) RFP rabbit polyclonal antibody (Rockland, 600–401–379, 1:5000) to amplify Td-Tomato signal for corticospinal tract axons; (b) NeuN guinea pig polyclonal antibody (Millipore ABN90, 1:1000) for mature neuronal nuclei; (d) GFAP mouse monoclonal antibody (MAB360, clone GA5, EMDMillipore; 1:1500) to label astrocytes. After primary antibodies, sections were incubated with 1:500 Alexa 488-, 594-, or 647-conjugated donkey secondary antibodies (Invitrogen, Thermofisher Scientific; 1:500) for 2.5 h at room temperature. The sections were then washed, mounted on uncoated slides, and coverslipped with Fluoromount-G (SouthernBiotech).

2.6. Image analysis

For assessment of lesion area, the proportion of lesion area relative to the spinal cord segment area that contains the lesion and spared tissue was measured and calculated to exclude error caused by difference in spinal cord sample size. A series of 1-in-6 Nissl-stained sections were imaged at 12.5× magnification using an Olympus BX53 microscope and CellSens imaging software (Olympus, Center Valley, PA, USA). The lesion area was identified as a region of disruption or loss of normal tissue parenchyma; this region was outlined and measured in ImageJ (version 1.38d; NIH). Then the spinal cord segment that contained the lesion area and the spared tissue was outlined and measured. The lesion area was divided by the spinal cord segment area to obtain the proportion of lesion area relative to the segment area. The proportion of lesion area from each section was added and then divided by the number of sections examined to obtain the average proportion of lesion area to spinal cord segment area.

For 3-D reconstruction of lesion volume, fluorescent images of GFAP labeled sections were captured at 100× magnification using a Zeiss LSM 880 Airyscan laser scanning confocal microscope. Tissue were auto-stitched in the XY planes to generate a single image and 6–8 images per rat (n = 9) were combined in the Z plane into a single image using Imaris (v9.2, Bitplane). Total lesion volume was determined in 6–8 serial sections 40 μm-thick spaced 240 μm apart.

For quantification of spared white matter, Eriochrome Cyanine (EC) was used to stain myelin. Single images at 10× were captured and auto-stitched using a Keyence BZ-X700 microscope. For each animal, the percentage of spared white and gray matter myelin relative to the total cross-section of the spinal cord was calculated using Image J software (version 1.38d; NIH) (n = 4).

2.7. Statistical analysis

In all quantification procedures, observers were blind to the nature of the experimental manipulation. Comparisons among groups were tested by ANOVA (JMP software) at a designated significance level of P < 0.05, followed by Fisher’s post-hoc tests between individual groups. Comparisons between two groups were tested by Student t-test (JMP software) at a designated significance level of P < 0.05. Data are presented as mean ± standard error mean (SEM). Catwalk gait analysis by Prism5 using one-way ANOVA with Bonferroni’s post hoc tests with statistical significance of p < 0.05.

3. Results

3.1. Initial training of staircase task before lesions

36 Fischer 344 rats that were trained to learn skilled pellet retrieval on the staircase task prior to placement of spinal cord contusions. Rats were trained 5 days per week for 3 weeks. 28 rats (77.8%) were able to successfully retrieve food pellets (Fig. 1) (Table 2). Among these 28 rats that were able to successfully retrieve food pellets, 1 rat retrieved only 5 food pellets, 14 retrieved 12–16 food pellets, and 13 retrieved 17–20 pellets (Fig. 1) (Table 2). The average number of food pellets retrieved (eaten) was 15.7 ± 0.6. The majority (96.2%) of rats were able to retrieve >12 food pellets from the first five stairs bilaterally (Fig. 1). A previous study cited a minimal criterion of 12 pellets retrieved as successful learning of staircase task (Pagnussat et al., 2009); on this basis, we selected those 27 rats that could retrieve 12 or more food pellets to proceed with the study and excluded the 9 remaining animals (Fig. 1).

The average number of food pellets retrieved for this cohort was 16.1 ± 0.4. The average number of food pellets displaced (but not necessarily eaten) among the rats that successfully learned the staircase task (after exclusion of 9 rats) was 17.4 ± 0.4. The high number of food pellets eaten among the food pellets displaced represents a very high accuracy of performance (92.5 ± 1.3%) (Table 2).

3.2. Lesion size generated by 200, 225 and 250 kilodyne lesions

Following baseline training to criterion performance, rats underwent C6 bilateral contusive SCI at either 200, 225 or 250 kdyne forces (N = 5 per injury force). Two rats died post-lesion, one in the 225 and one in the 250 kdyne group during the first post-lesion week. In addition, one rat was excluded from the 225 kdyne forced group due to misplacement of the impact probe and an inadequate lesion (Table 1). Surviving rats were perfused after 6 weeks 200 kdyne (n = 5); 225 kdyne (n = 3) and 250 kdyne (n = 4).

Histological analysis showed that the graded impact forces resulted in successively greater injury severities and proportionately greater parenchymal loss (Fig. 2). The proportion of lesion area relative to the spinal cord segment that contained the lesion area and spared tissue was assessed: 57.8 ± 2.7% in 200 kdyne group; 67.5 ± 2% in 225 kdyne group; 76.3 ± 2.1% in 250 kdyne group. Statistical analysis demonstrated that the overall group ANOVA yielded P < 0.01, with post-hoc
Fischer’s showing a significant difference of $P < 0.05$ comparing 250 kdyne injury to 225 kdyne, and $p < 0.001$ comparing 250 kdyne to 200 kdyne (Fig. 2D). In addition, comparison of 225 kydne and 200 kdyne yielded $p < 0.05$ (Fig. 2D). Notably, all of these lesion sizes were substantially greater in extent than previously reported by Anderson et al. (Anderson et al., 2009); see Discussion. All injuries completely disrupted the main dorsal corticospinal tract, which directly affects skilled forepaw use (García-Alías et al., 2009) (Fig. 3A-D).

### 3.3. Functional consequences of 200, 225 and 250 kilodyne lesions

All three groups exhibited substantial and persistent deficits in pellet retrieval on the staircase task (Fig. 3E). The deficits increased proportionately as force increased. In the 200 kdyne group, there was an approximate 80% reduction in performance for the first two weeks after injury, followed by recovery to a stable deficit of 50–60% subsequently. In contrast, both the 225 and 250 kdyne groups could not perform the task at all for the first 2 weeks after injury (Fig. 3E). Subsequently, the 225 kdyne groups exhibited partial recovery but with a persistent, 80% deficit compared to pre-lesion performance (Fig. 3E).

In contrast, the 250 kdyne group never recovered (Fig. 3E). Thus, this model provides a range of injury severities over which to test possible candidate therapies, with the 250 force resulting in a very severe and lasting deficit in skilled forepaw use; the 225 force also resulting in a severe and lasting deficit, but with slight improvement over time; and the 200 kdyne force resulting in approximately 50% functional recovery over 6 weeks. The latter force represents a moderate lesion severity, and one that might be appropriate for initial screening of candidate experimental interventions. We selected the 200 kdyne force for an additional, longer-term study to characterize additional aspects of lesion morphology and the persistence of skilled forelimb functional deficits over time.

### 3.4. Further study of 200 kdyne injury

12 of 16 rats learned the staircase task to criterion performance.

---

**Table 2**

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|
| Eaten   | 18| 18| 18| 15| 15| 20| 0 | 17| 16| 13 | 14 | 0  |
| Displaced|18 |18 |20 |19 |16 |21 |0  |18 |16 |16  |14  |0   |
| Accuracy (%) | 100 | 100 | 90 | 79 | 94 | 95 | 0 | 94 |100 |81  |100 |0   |

| Subject | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|
| Eaten   | 13| 15 | 15 | 17 | 0  | 13 | 13| 15| 17 | 18 | 0  | 19 |
| Displaced|15 |18 |17 |18 | 0  | 16 |14 |16| 17 |18  | 0  | 22 |
| Accuracy (%) | 87 | 83 | 88 | 94 | 0  | 81 |93 |94 |100 |100 | 0  | 86 |

| Subject | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|
| Eaten   | 17| 0  | 16 | 17 | 0  | 0  | 20| 5 |13  | 0  | 17 | 16 |
| Displaced|18 |0  |16 |19 | 0  | 0  | 21| 8 |13  | 0  | 19 | 18 |
| Accuracy (%) | 94 | 0  |100 |89 | 0  | 0  | 95 |63 |100 | 0  | 89 | 89 |

Fig. 2. C6 Bilateral Contusion morphology.

(A-C) Representative Nissl staining images show lesion extent in 200, 225, and 250 kdyn groups 6 weeks post-injury. Horizontal 35 μm-thick sections through or near central canal. Scale bar, 1 mm. (D) Comparison of the average proportion of lesion area to the spinal cord segment area containing the lesion in three groups: 200 ($n = 5$), 225 ($n = 3$), 250 ($n = 4$).

One-way ANOVA with post-hoc Fischer’s test, *$P < 0.05$; **$P < 0.001$. 

---

C.M. Freria et al.
and then received C6 bilateral moderate (200 kdyne) contusion injury. 3 rats (25%) died during the first week post-operatively. The remaining 9 rats survived the remaining 15 weeks of the study were tested weekly prior to perfusion (Table 1).

3.5. Moderately severe functional deficits stably persist for 15 weeks

Prior to lesion placement, rats achieved a mean accuracy score of 87.4 ± 2.1% (Fig. 4A-B) and could consistently reach level 5 of the staircase (4.8 ± 0.2) (Fig. 4C). In the first post-operative week, rats were unable to successfully retrieve food pellets. By week 2, they successfully recovered approximately 20% of their original performance, similar to findings in the first experiment. From week 3 and on, rats recovered to approximately 40% success, a level of performance that roughly mirrored the first experiment and was persistent for all 15 weeks of the study (Fig. 4).

We also measured several other parameters of performance in the staircase, including pellet displacement (including both pellets knocked off the staircase plus pellets consumed), pellet accuracy (# pellets eaten divided by pellets displaced), and staircase level reached (Fig. 4). On all these parameters, animals had severe initial deficits at week 1 after lesion, followed by partial and stable recovery to a moderate level of deficit (Fig. 4).

3.6. Deficits on the catwalk

Animals were trained on the Catwalk prior to injury and were reassessed 1- and 15-weeks post-injury. Non-injured rats showed regular, alternating step patterns (Fig. 5A). Contrarily, injured rats showed non-coordinated step pattern with abnormal footprint intensities on the CatWalk gait platform (Fig. 5B). Following SCI, subjects switched their cadence an adopted an abnormal walking pattern, using four paws simultaneously for support ($P < 0.05; P < 0.001$; Fig. 5C-D). These changes were accompanied by an enhanced step cycle, decreased fore-limb stride length, and reduced swing speed ($P < 0.01; P < 0.001$; Fig. 5E-G). These deficits remained stable for 15 weeks post injury.

3.7. Anatomical analysis: Severe loss of dorsal white matter, complete loss of gray matter, and partial sparing of ventral white matter

The 200-kdyne-contusion resulted in large lesion cavities that eliminated nearly all gray matter within the injury zone (Figs. 6 and 7). There was also extensive loss of white matter that was nearly complete in the dorsal half of the spinal cord with partial sparing in the ventral half of the spinal cord (Fig. 7 A-D). Quantification revealed ~40% sparing of total white matter relative to total cross-section at epicenter of this lesion model. This degree of parenchymal loss at 200 kdyne was extensive. Note that our study used a Fischer 344 rats of weighing

(minimum 12 successful retrievals per session) and then received C6 bilateral moderate (200 kdyne) contusion injury. 3 rats (25%) died during the first week post-operatively. The remaining 9 rats survived the remaining 15 weeks of the study were tested weekly prior to perfusion (Table 1).
C.M. Freria et al.

Experimental Neurology 360 (2023) 114275

4. Discussion

Our primary objective in this study was to generate a mid- to lower-level, clinically relevant, moderately severe to severe model of bilateral cervical contusive SCI. We chose to produce a lesion at C6 to impact forelimb grasping function, since this functional parameter is of greatest importance to humans that sustain cervical SCI (Ackery et al., 2004; Sekhon and Fehlings, 2001). At the same time, we sought to spare the CS spinal cord segment so that animals would retain biceps function and be able to ambulate and survive consistently and independently. Hemispiral spinal cord contusion was preferable for previous studies as this lesion model increases animals’ survival and requires less post-injury care (Dunham et al., 2010; Krisa et al., 2012; Brock et al., 2018; Lucas-Osma et al., 2022). However, one of the drawbacks of the hemiconcussion lesion is the difficulty in establishing whether the therapeutic interventions is indeed effective or whether the improvement is due to the collateral sprouting from remaining axons. In this study, at least 75% of animals survived the lesion with appropriate post-operative care. Notably, animals had functional deficits that were stable over time, reflecting the most common outcome of human SCI. A range of lesion severities are possible depending on the force of impact, with moderate recovery after 200 kdyne injury and no spontaneous recovery after 250 kdyne injury. Thus, the lesion model is useful for studying and screening a range of therapeutic interventions and mechanisms: for example, candidate therapeutics that enhance sprouting of spared axons are appropriate for the 200 kdyne model, while therapies that promote robust axonal regeneration might be appropriate for the 250 kdyne model.

Experimentally, not only a gradual increase in force but also size of rod impacting head cause a reproducibly increase graded loss of tissue and motor function in rats. Therefore, we reported severe lesion pathology using an impactor head of 3.5 mm compared to previous study that used 2.5 mm (Strotton et al., 2021).

While our study used the same impactor head size and force range as a previous study by Anderson et al. (2009), our outcomes were also more severe anatomically. This may have been because the rat strain we used, Fischer 344 rats, are smaller than Sprague Dawley rats, and our rats weighed 20–25% less than the subjects of the Anderson’s study. The advantage to use Fischer 344 rats since they are inbred strain that allows syngeneic transplantation of neural progenitor cells or other cells isolated from the same strain without immunosuppression. Another difference between studies is that we performed lesions on one spinal cord level lower than Anderson, and there may be a differential vulnerability of the spinal cord at different levels, although we are unaware of experimental evidence to support this possibility. In terms of functional outcomes, our study showed sustained locomotor impairment for 15 weeks post injury while previous study showed locomotor impairment for 1–2 days after mild contusion and 3–7 days after moderate contusion (Anderson et al., 2009). Additionally, to locomotor recovery, we used skilled forelimb function to measure the functional outcomes after lesion. Previous studies used grip strength (Anderson et al., 2009; Reinhardt et al., 2020; Strotton et al., 2021), horizontal ladder (Strotton et al., 2021; Reinhardt et al., 2020) or gait analysis (Reinhardt et al., 2020), which may not necessarily correlate with skilled forelimb grasping performance. Thus, we performed this study in part to clearly identify consequences of this lesion, at this spinal level, on a functional parameter of greatest relevance to humans. This function is mediated at least in part by the corticospinal tract in rats (Weidner et al., 2001; Welniarz et al., 2016; Whishaw et al., 1998); while rats with corticospinal lesions can perform several types of motor actions, humans with corticospinal tract lesions are permanently paralyzed. Thus, it was essential to examine this corticospinal-tract dependent function in the rat model.

Despite previous studies have developed cervical spinal cord contusion model in mice (Aguiar and Steward, 2010; Reinhardt et al., 2020), we rather choose rat model as their lesion pathology is more comparable to human patients. Rats and humans alike develop cysts cranial and caudal to the site of injury. Contrarily, following contusion SCI in mice, cells proliferate in the injury area keeping the opposing ends of the injured cords in contact and typically there is no formation of fluid-filled cysts (Ma et al., 2011).

In the future, we will use this clinically relevant low cervical bilateral contusive injury model to study corticospinal tract (CST) regeneration and skilled forelimb function recovery after neural stem cell transplantation and other potential therapeutic treatments. Although our studies demonstrate robust regeneration of CST after SCI, the injury models are either a small cervical dorsal column or dorsal quadrant lesion (Dulin et al., 2018; Kadoya et al., 2016; Kumamaru et al., 2019) or an upper-thoracic transection model (Koffler et al., 2019), which are less clinically relevant. The use of a clinically relevant model in combination with a reliable function test, such as a staircase task from this study, is
Fig. 5. Catwalk gait analysis.
(A) Digital footprints, 3D footprint intensities and Footfall patterns of non-injured rats showed regular footprint intensities and alternating step patterns on the CatWalk gait platform (B) Digital footprints, 3D footprint intensities and Footfall patterns of injured rats reveal non-coordinated step pattern and abnormal footprint intensities on the walking platform. (C) Diagonal support was the percentage of using 2 diagonal paws (LF-RH, RF-LH). (D) Four support was the percentage of using 4 paws simultaneously for support. (E) Step cycle was the time between two consecutive initial contacts of the same paw. (F) Stride Length was the distance from the toe of paw at the starting position to the toe of the same paw at ending position. (G) Swing speed was the speed in which two consecutive paw placements of the same paw is not in contact with the walkway. Graphs were analyzed using one-way ANOVA with Bonferroni post-tests *p < 0.05; **p < 0.01 and ***p < 0.001. Data are mean ± SEM (n = 12).
Fig. 6. Contusive injury morphology. (A-H) GFAP and NeuN double labeling in every 6th section reveals lesion morphology and neural tissue loss across the dorso-ventral axis of the spinal cord 3.5 months post-injury. This series sections start from most dorsal spinal cord (A) and end in the most ventral cord (H). Sections are in the horizontal plane with rostral towards the right and caudal left. Scale bar: 1 mm.

Fig. 7. Lesion area after lower-cervical spinal contusion in rats. A- Serial transversal sectioning every 400 μm from lesion site labeled with erichrome cyanine (EC, blue) 2 weeks post-injury. B- Quantitative analysis of the total spared area 2 weeks post SCI (n = 4). C- Quantitative analysis of spared gray matter after SCI (n = 4). D- Quantitative analysis of spared white matter after SCI (n = 4). E- Three-dimensional reconstruction of the lesion site 15 weeks post-injury generated by serial horizontal spinal cord images labeled with GFAP. F-Quantitative analysis of the total lesion volume (n = 9) and (G) lesion area (n = 9). Scale bar: (E)1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
important to assess skilled forelimb function recovery after potential therapeutic treatments.

Author contributions

Conceptualization and designed research, C.M.F and P.L.; performed research, C.M.F; L.G; A.A.; Contributed reagents/analytic tools; P.L; analyzed data; C.M.F; A.A.; wrote the paper C.M.F and P.L.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgements

The authors acknowledged the support of the Veterans Affairs (I01RX00264-01A2 to P.L.); the Gordon Masfield Consortium for Spinal Cord Injury Research (5I50RX001706-06 to P.L); Wings For Life therapeutic treatments.

References

Dulin, J.N., Adler, A.F., Kumamaru, H., Poplawski, G., Dulin, J.N., Strobl, H., Takashima, Y., Biane, J., Conner, J., Zhang, S.-C., Tuszynski, M.H., 2019. Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. J. Neurosci. 39, 2971–989. https://doi.org/10.1089/neu.2018.0297.

Koffler, J., Zhu, W., Lu, P., Rosenzweig, E.S., Kadoya, K., Tuszynski, M.H., 2020. Regenerating corticospinal axons innervates stereotypically appropriate neurons within neural stem cells grafts. Cell Rep. 26. https://doi.org/10.1087/cellrep.2019.01.3999-2399.

Lu, P., Wang, Y., Graham, L., Michele, K., Gao, M., Wu, D., Brock, J., Blesch, A., Rosenzweig, E.S., Havton, L.A., Zheng, B., Conner, J.M., Marsala, M., Tuszynski, M.H., Lu, P., 2011. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 150, 1264–1273. https://doi.org/10.1016/j.cell.2012.08.020.

Lucas-Osma, A.M., Schmidt, E.K.A., Vavrek, R., Bennett, D.J., Foad, K., Fenrich, K.K., 2020. Rehabilitation training improves skilled forelimb motor function after cervical unilateral contusion injury in rats. Behav. Brain Res. 26, (422), 113731 https://doi.org/10.1016/j.bbr.2021.113731.

Ma, M., Basso, D.M., Walters, P., Stoken, B.T., Jakeman, L.B., 2011. Behavioral and histological outcomes following graded spinal cord contusion injury in the C57BL/6 mouse. Exp. Neurol. 169, 239–524. https://doi.org/10.1016/j.expneurol.2001.7679.

Montoya, C.P., Campbell-Hope, J.L., Pemberton, K.D., Dunnett, S.B., 1991. The ‘staircase test’ a measure of independent forelimb reaching and grasping abilities in rats. J. Neurosci. Methods 36, 219–228. https://doi.org/10.1016/0165-0270(91)90048-5.

Pagnussat, A.S., Michaelson, M.S., Achaval, M., Netto, C.A., 2009. Skilled forelimb reaching in Wistar rats: evaluation by means of Monta Nova staircase test. J. Neurosci. Methods 177, 115–121. https://doi.org/10.1016/j.jneumeth.2008.10.001.

Platt, A., Dafoysto, M.H.E., Lee, M.J., Herman, M.H., Ramos, E., 2021. Gunshot wounds to the lumbar spinal cord: experimental review and meta-analysis. Global Spine J. https://doi.org/10.1016/j.gjo.2021.121958.82196211030875.

Reinhardt, S., Strehl, K., Satkundunardrahaj, A., Antje, Kroener, A., 2020. Bilateral cervical spinal cord injury mouse model: a mouse model to evaluate sensorimotor function. Exp. Neurol. 331, 113381 https://doi.org/10.1016/j.expneurol.2020.113381.

Rivlin, A.S., Tator, C.H., 1978. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg. Neurol. 10, 38–43. https://doi.org/10.1097/00002517-199810000-00006.

Filho, T.E.P., De B., Cristante, A.F., Marcon, M.R., Ono, A., Bilhar, R., 2014. Gunshot injuries in the spine. Spinal Cord 52, 504–510. https://doi.org/10.1038/sc.2014.56.

Kadoya, K., Lu, P., Rosenzweig, E.S., Kadoya, K., Tuszynski, M.H., 2019. Brainstem to spinal cord transection using a modified ligation procedure. J. Neurotrauma 36, 193. https://doi.org/10.1089/neu.2018.0297.

Koffler, J., Zhu, W., Lu, P., Rosenzweig, E.S., Kadoya, K., Tuszynski, M.H., 2020. Regenerating corticospinal axons innervates stereotypically appropriate neurons within neural stem cells grafts. Cell Rep. 26. https://doi.org/10.1087/cellrep.2019.01.2399.

Koffler, J., Zhu, W., Qu, X., Patlas, J., Dulin, J.N., Brock, J., Graham, L., Lu, P., Rosenzweig, E.S., Tuszynski, M.H., 2019. Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. J. Neurosci. 39, 2971–2989. https://doi.org/10.1087/neu.2018.0297.

Rivlin, A.S., Tator, C.H., 1978. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg. Neurol. 10, 38–43. https://doi.org/10.1097/00002517-199810000-00006.

Schell, S.W., Rabchewsky, A.G., Gugacca, I., Main, J.A., Lumpp, J.E., 2003. Experimental modeling of spinal cord injury: characterization of a force-defined injury device. J. Neurotrauma 20, 179–193. https://doi.org/10.1089/neu.2003.10.1793.

Sekhon, L.H., Fehlings, M.G., 2001. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine (Phila Pa 1976) 26, S2–S19. https://doi.org/10.1097/00005834-200106000-00002.

Strotton, M.C., Bodey, A.J., Wanelik, K., Hobbs, C., Rau, C., Bradbury, E.J., 2021. The spatiotemporal spread of cervical spinal cord contusion injury pathology revealed by 3D in-line phase contrast synchrotron X-ray microtomography. Exp. Neurol. 336, 113529 https://doi.org/10.1016/j.expneurol.2020.113529.

Van Den Berg, M.E.L., Castellote, J.M., Mahillo-Fernandez, I., De Pedro-Cuesta, J., 2010. Incidence of spinal cord injury worldwide: a systematic review. Neuroepidemiology 34, 184–192. https://doi.org/10.1159/000279035.

Weidner, N., Nee, A., Salimi, N., Tuszynski, M.H., 2001. Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. Proc. Natl. Acad. Sci. 98, 3513–3518. https://doi.org/10.1073/pnas.051627998.

Weisniter, Q., Usatit, I., Roze, E., 2016. The corticospinal tract: evolution, development, and human disorders: corticospinal tract human disorders. Dev. Neurobiol. 77, 810–828. https://doi.org/10.1002/dneu.22455.

Whishaw, I.Q., Gorny, B., Sarnia, J., 1998. Paw and limb use in skilled and spontaneous reaching after pyramidal tract, red nucleus, and combined lesions in the rat: behavioral and anatomical dissociations. Behav. Brain Res. 93, 167–183. https://doi.org/10.1016/S0166-4388(97)00152-6.

Wyndaele, M., Wyndaele, J.-J., 2006. Incidence, prevalence, and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord 44, 523–529. https://doi.org/10.1038/sj.sc.3101895.