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Original Articles

A Novel Porcine Model of Traumatic Thoracic Spinal Cord Injury

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Abstract

Spinal cord injury (SCI) researchers have predominately utilized rodents and mice for *in vivo* SCI modeling and experimentation. From these small animal models have come many insights into the biology of SCI, and a growing number of novel treatments that promote behavioral recovery. It has, however, been difficult to demonstrate the efficacy of such treatments in human clinical trials. A large animal SCI model that is an intermediary between rodent and human SCI may be a valuable translational research resource for pre-clinically evaluating novel therapies, prior to embarking upon lengthy and expensive clinical trials. Here, we describe the development of such a large animal model. A thoracic spinal cord injury at T10/11 was induced in Yucatan miniature pigs (20–25 kg) using a weight drop device. Varying degrees of injury severity were induced by altering the height of the weight drop (5, 10, 20, 30, 40, and 50 cm). Behavioral recovery over 12 weeks was measured using a newly developed Porcine Thoracic Injury Behavior Scale (PTIBS). This scale distinguished locomotor recovery among animals of different injury severities, with strong intra-observer and inter-observer reliability. Histological analysis of the spinal cords 12 weeks post-injury revealed that animals with the more biomechanically severe injuries had less spared white matter and gray matter and less neurofilament immunoreactivity. Additionally, the PTIBS scores correlated strongly with the extent of tissue sparing through the epicenter of injury. This large animal model of SCI may represent a useful intermediary in the testing of novel pharmacological treatments and cell transplantation strategies.

Key words: contusion; porcine; PTIBS; SCI

Introduction

Acute traumatic spinal cord injury (SCI) can result in the devastating and permanent loss of neurological function, for which there are currently no convincingly efficacious neuroprotective or neuroregenerative treatments. Over the past 30 years, international scientific research efforts to establish such treatments have been vigorous, and have produced a plethora of promising therapeutic strategies.[1–3] The exciting potential of these treatments has been demonstrated in *in vivo* animal models of SCI, the overwhelming majority of which utilize rats or mice.

The advantages of using rodents in such models of SCI are numerous. Methods for producing reproducible and controlled SCI are well described (e.g., New York University/ Infinite Horizon (IH) contusion impactors, clip compression, dorsal hemisection). A multitude of behavioral outcome measures are widely available (e.g., Basso, Beattie, Bresnahan [BBB] locomotor scale, cylinder rearing test, horizontal ladder test, catwalk), making the results interpretable and comparable across laboratories. Histological, biochemical, and molecular techniques for assessing the outcome of SCI and/or treatment are well established. In addition, such small animal models are relatively inexpensive and require basic housing facilities readily available to most researchers.

Despite these well recognized advantages, the SCI research community also acknowledges the frustrating reality that although many experimental treatments have shown great efficacy in these rodent models, the few that have gone into clinical trials for human SCI have failed to demonstrate convincing efficacy.[4] This disparity between the frequent reporting of neurologic benefits in rodent SCI models and the difficulty in establishing efficacy in human trials has a number of possible explanations, not the least of which is the heterogeneity of human SCI and the challenging nature of measuring neurological function in clinical trial settings.[5] However, at a more fundamental level, it is hard to deny the possibility that differences in size and anatomy and differences in the pathophysiological responses to injury might exist between SCI in rodents in human patients: differences that could significantly influence the

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clinical efficacy of therapies shown to be effective in rodent models.

With respect to size, there is a considerable difference between rodent and human spinal cords. Whereas the spinal cord diameter is ~8–9 mm in the average human with SCI weighing 70 kg, it is only 2–3 mm in the typical rat weighing 275–325 g (and even smaller in a mouse).[6] The length of the entire cervical spinal cord in a rat may be shorter than the length of the single C5-C6 segment within the human cervical spinal cord. These differences in size (both caliber and length) likely have implications in the translation of experimental therapies. The question is, whether, for systemically administered drugs (e.g., minocycline, riluzole) that are neuroprotective in rodent models of SCI: will that efficacy be achievable in a lesion that is volumetrically an order of magnitude larger? For biological therapies that are delivered directly to the spinal cord (e.g., anti-Nogo antibodies, chondroitinase ABC [ChABC], Rho antagonists), will their biodistribution within a much larger spinal cord be sufficient to reach their intended targets? For cellular transplantation therapies, will the extent of survival, migration, and integration within the width and rostrocaudal length of the rodent SCI lesion be achievable in a much larger human lesion simply by ''scaling up'' the cell transplant, and, if so, will the injection of a larger volume of a cell suspension contribute to local damage in the cord?[7] And for any of these therapies that might promote axonal regeneration and/or sprouting, will the precious few millimeters of axonal growth or plasticity observed within a rodent model be meaningful in a human spinal cord that is many centimeters longer?

The obvious differences in size and anatomy and potentially greater similarities in biological responses to injury between humans and higher order animals have prompted some investigators to explore the latter as models for the testing of novel SCI therapies. In a survey of >300 members of the SCI research community, the majority of respondents voiced the need for such intermediary studies before translating experimental therapies into human clinical trials.[8] Although strong arguments have been raised to justify primate models,[9] (Courtine et al., 2007), the high operational costs and the specialized facilities required make such models difficult to access for most researchers. Plausible alternatives to primates are therefore other large animal models, such as the dog, cat, sheep, and pig. Relevant biological and physiological differences may still exist between SCI in such quadruped animals and in humans, but at the very least, they have a more comparable spinal cord size than do the rat/mouse models. The costs associated with such large animal models are certainly higher than those with rodents, but are a fraction of that for primate studies.

In this study, we describe the development of a large animal model of SCI using Yucatan miniature pigs. Well-documented anatomical and physiological similarities between pigs and humans have made pigs useful as models in various research settings, including stroke,[10–12] traumatic brain injury,[13,14] and SCI.[15–19] Pigs are commercially available specifically for research purposes, and are relatively well mannered and easy to train. We propose that this porcine thoracic contusion SCI model may be a useful intermediary model that can be utilized by researchers who have access to basic animal care facilities and veterinary support.

Methods

All animal procedures were performed in accordance with the guidelines of the Canadian Council for Animal Care and approved by our institution's Animal Care Committee. All behavioral and histological analyses were performed by persons blinded to the biomechanical severity of the cord injury that was induced at the time of surgery (characterized by combinations of weight magnitude and drop height, which are themselves surrogates of mechanical energy).

Experimental groups

The development of this model occurred in two stages, with small modifications being made to the weight drop apparatus between the two. The first stage (Experiment 1) was a pilot experiment using the original design of the weight drop apparatus, which included a cylindrical guide tube through which the impactor was allowed to fall onto the cord. The impactor itself was made of stainless steel and had a spherical tip with a diameter of 9.53 mm[20] (Fig. 1). From a height of 50 cm, we dropped impactors weighing either 50 g ($n=7$) or 100 g ($n=5$), followed by an additional 100 g weight for 5 min of compression.

For the second stage, the cylindrical guide tube was replaced by a rail and linear bearing to minimize friction between the impactor and its guide, and the impactor tip was changed to a flat cylindrical shape with a diameter of 9.53 mm to prevent the spinal cord from being displaced laterally during and after impact (Figs. 2 and 3). The edges of the cylinder were rounded slightly so that there would not be a sharp edge striking the cord. The 50 g impactor weight was used exclusively, and drop heights of 5 cm, 10 cm, 20 cm, 30 cm, and 40 cm were tested ($n=5$ each), followed by 5 min of compression with the added 100 g weight. One additional animal group was included in this second stage: a 20 cm weight drop, but without the 5 min of compression ($n=5$). Table 1 lists these experiments and numbers of animals per group.

Surgical procedure

Female Yucatan miniature pigs (Memorial University of Newfoundland, Canada and Sinclair Bio-resources, Columbia, MO) weighing 20–25 kg were pre-anesthetized with an intramuscular (IM) injection of Telazol (4–6 mg/kg), xylazine (0.6 mg/kg), and atropine (0.02 mg/kg); endotracheally intubated; and maintained with isoflurane (2–2.5%) for the entire procedure. Pigs were given

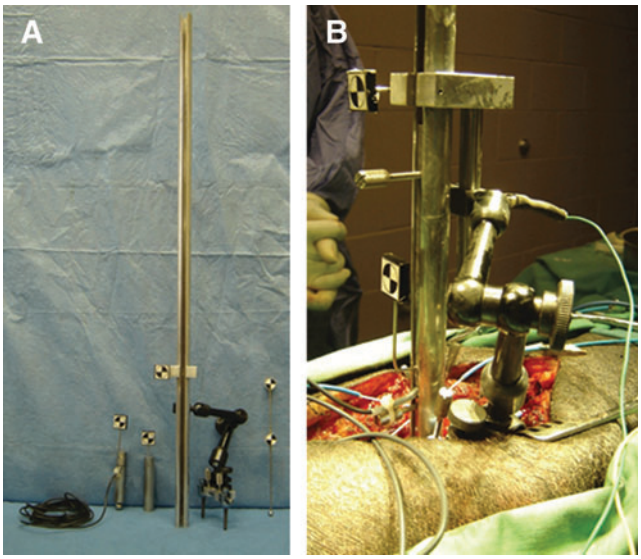

FIG. 1. Custom designed weight drop impactor 1. **(A)** (from left to right) The 50 g weight drop with spherical tip, static 100 g and the cylinder guide rail attached to the articulating arm. **(B)** Impactor mounted on the animals after weight drop injury. After the weight (50 g) was dropped, an additional 100 g static weight was added for 5 min.

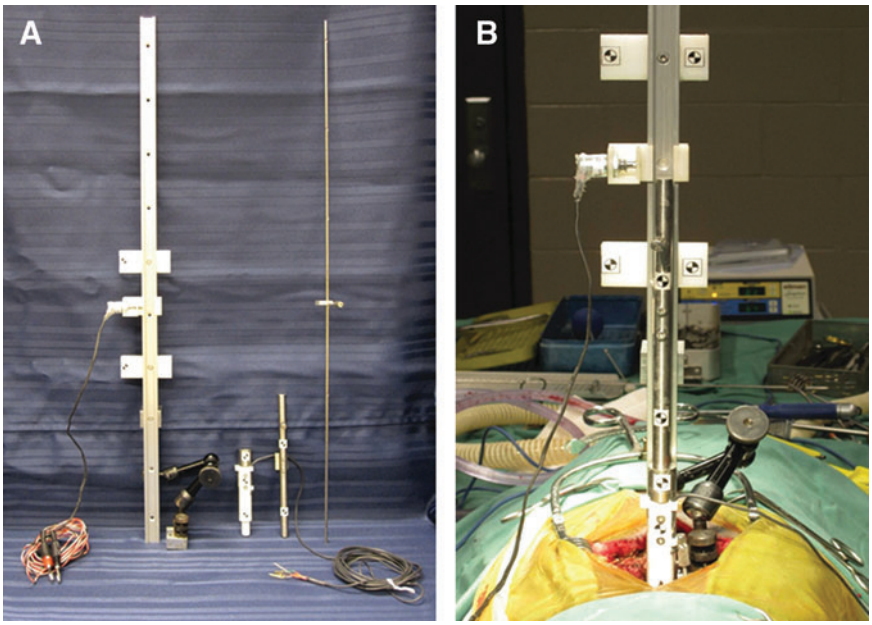

FIG. 2. Custom designed weight drop impactor 2. **(A)** Weight drop impactor. (From left to right) the guide rail attached to the articulating arm and trigger system, drop weight (50 g), static weight (100 g) and measure rod with pre-set distance. **(B)** Impactor mounted on the animals after weight drop injury. After the weight (50 g) was dropped, an additional 100 g static weight was added for 5 min.

an intravenous (IV) dose of cefazolin (15 mg/kg), and an IM injection of ketoprofen (3 mg/kg) and morphine (0.5 mg/kg) prior to surgery. Bupivicaine (1 mg/kg) was injected into the skin and subcutaneous tissue as a local anaesthetic. Prior to surgery, a urinary catheter (10 Fr, Jorgensen Laboratories Inc., Loveland, CO) was manually inserted for post-injury bladder drainage. Mechanical ventilation was maintained at 10–12 breaths/min and the tidal volume at 12–15 mL/kg (Veterinary Anesthesia Ventilator model 2002, Hallowell EMC, Pittsfield, MA). During the procedure, standard monitoring was performed for heart rate, respiratory rate, blood pressure, end tidal carbon dioxide, inspired and expired isoflurane levels, and oxygen saturation (pulse oximeter 8600V, Nonin Medical Inc., Markham, ON, and Cardell® MAX-12HD Veterinary Monitor, Paragon Medical Supply, Inc., Coral Springs, FL). Hydration was maintained with IV lactated Ringer's solution and the temperature was measured by a rectal temperature probe and maintained at 37.0–38.5°C with a heating pad (T/Pump, Gaymar Industries, Inc., Orchard Park, NY).

With the animal prone on the operating table, the location of T10 was confirmed with a lateral radiograph. A 12 cm dorsal midline incision was made between T8 and T13. The spinous processes, laminae, and transverse processes of T9–T12 were exposed using electrocautery (Surgitron® Dual Frequency RF/120 Device, Ellman

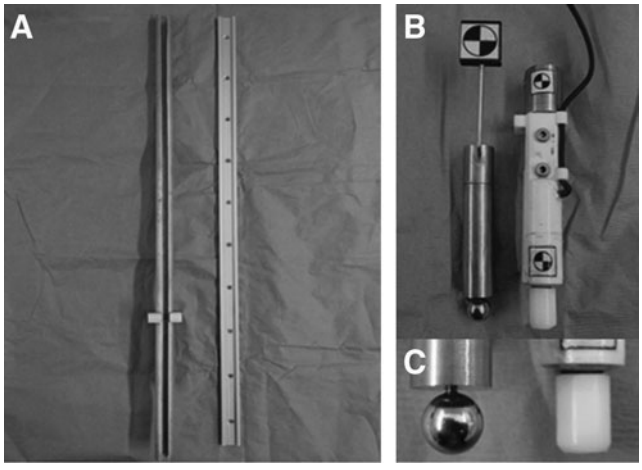

FIG. 3. Custom designed weight drop impactors. **(A)** Guide rail system: cylinder versus rail. **(B)** 50 g weight drops. Left: weight drop with spherical tip. Right: weight drop with flat tip **(C)** Close-up of weight drop tips.

Table 1. Experimental Groups

	Impactor weight	*Drop height*	*100 g compression + 5 min post-impact*
Experiment 1 - Pilot study using initial impactor	50 g ($n=7$) 100 g ($n=5$)[a]	50 cm	Yes
Experiment 2 - Study of various drop heights with refined impactor	50 g	5 cm ($n=5$)	Yes
		10 cm ($n=5$)	
		20 cm ($n=5$)	
		30 cm ($n=5$)	
		40 cm ($n=5$)	
		50 cm ($n=5$)	
		20 cm ($n=5$)	No

In the first experiment ($n=11$) we utilized a 50 or a 100 g impactor dropped from a height of 50 cm, followed by 5 min of compression. In the second experiment ($n=35$), the impactor system underwent some refinements and we evaluated a 50 g impactor dropped from various heights. The impact was followed by 5 min of compression in all groups except for one group (drop height of 20 cm).

[a]Animal numbers included one animal that died postoperatively

International, Oceanside, NY). Using anatomic landmarks, two 4.5×35 mm multi-axial screws (CD Horizon, Medtronic, Minneapolis, MN) were inserted into T9 and T12 pedicles. These are screws typically utilized in surgery of the human thoracic or lumbar spine. Because of the small size of the pedicles in the pig thoracic spine, for the second experiment we used 3.5×24 mm multi-axial screws (Select^{TM} Multi Axial Screw, Medtronic, Minneapolis, MN) that are designed for the human cervical spine. We used these to cannulate the T10, T11, and T12 pedicles. A titanium rod with a diameter of 5.5 mm (Experiment 1) or 3.2 mm (Experiment 2) was affixed to the screws with the locking caps, and served to not only rigidly fix the T10-11-12 segments during the weight drop, but also to secure the weight drop guidance system (described subsequently). A T10/T11 laminectomy was performed and widened to ensure that a circular window measuring at least 1.2 cm diameter was made to expose the dura and spinal cord.

The weight drop apparatus consisted of a guidance system (either a tube or rail) through or along which an impactor of a specific weight fell onto the exposed spinal cord from a defined height.[20,21] The guidance system for the impactor was secured rigidly to the pedicle screws, and by adjusting the articulating arm it was positioned directly over the laminectomy defect (Figs. 1 and 2). The guidance system was leveled to ensure that it was aligned vertically in both the coronal and sagittal planes. The impactor was then attached to the guidance system and the laminectomy defect was checked to ensure that it was wide enough for the impactor tip to fall freely onto the dura/spinal cord without striking adjacent bone. With this confirmed, the impactor was secured at the desired height. The tip of the impactor was instrumented with a load cell (LLB215, Futek Advanced Sensor Technology, Irvine, CA) to record the force at impact. After a final check to ensure that the guidance system was still vertical, mechanical ventilation was ceased to minimize movement of the animal, and the impactor was released to drop along the guidance system and strike the spinal cord. Immediately following the contusion injury, sustained compression was applied by placing a 100 g weight on top of the impactor for 5 min. Because the guidance system was rigidly fixed to the vertebrae, neither the weight drop force nor the sustained compression force applied were affected by the porcine subject's respiration or other motion. Following this, the weight drop apparatus was removed and the wound was closed in layers.

Twenty minutes prior to the weight drop injury, the animals were given an IM injection of hydromorphone (0.15 mg/kg). Postoperatively, the animals received diazepam (0.5–1.0 mg/kg) to calm them at recovery, as they were unable to stand. Subcutaneous morphine (0.25 mg/kg) and oral tramadol (1 mg/kg) were administered as analgesics. Enrofloxacin was administered IM once a day for 5 days as an antibiotic, and ketoprofen (3 mg/kg) was given IM once a day for 3 days as an anti-inflammatory agent. Animals were monitored frequently for the first 48 h. Typically, they were housed individually for the first 24 h, after which they were housed in pairs. The urinary catheter was removed 7–10 days after SCI, after which the animals were able to reflexively empty their bladders.

Biomechanical parameters

Load data were acquired with custom Labview (V8.6, National Instruments, Austin, TX) programs at 50 kHz then post-filtered and processed with custom Matlab (V2008b, The Mathworks, Matick, MA) programs with a two-way fourth-order Butterworth filter with 5 kHz low-pass cutoff frequency. High speed video (Phantom V9.1 camera, Phantom V9.0.640 software, Vision Research Inc., Wayne, NJ) was used to track quadrant markers rigidly attached to the impactor and guide rail for selected experimental groups (field of view: $\sim 50 \times 275$ mm, resolution: 240×1344, frames per sec: 5,000 to 5,500) (Figs. 1 and 2). The images were used to determine the impact velocity and the maximum dural displacement during injury, as described.[20] Prior to the actual weight drop, the location of the dorsal surface of the dura was identified with quadrant markers. Dural displacements were calculated based on measuring the distance of the quadrant markers on the weight drop relative to the location of the dura before, during, and after weight drop injury.

Behavioral recovery: The Porcine Thoracic Injury Behavioral Scale (PTIBS)

The pigs were delivered at least 3 weeks prior to the scheduled surgery. After acclimatization to the large animal facility, the animals underwent 1 h of training each day for 5 days to walk upon command up and down a rubber mat (width: 1.22 m, length: 5 m) without stopping. One week prior to injury, the animals were videotaped to establish the ''baseline'' locomotor behavior. This was captured on three high-definition camcorders positioned behind the animals so as to visualize the movements of the hindlimbs and rump as they walked away from the cameras. Videotaping of locomotor recovery resumed 1 week post-injury and continued weekly for 12 weeks in total.

From these observations, we formulated a scale that we named the ''Porcine Thoracic Injury Behavior Scale,'' or PTIBS. To develop the PTIBS, the natural progression in hindlimb function was analyzed and classified into 10 different stages, ranging from no active hindlimb movements (score: 1) to normal ambulation (score: 10). PTIBS scores of 1–3 are characterized by ''hindlimb dragging,'' scores of 4–6 reflect varying degrees of ''stepping'' ability, and scores of 7–10 reflect varying degrees of ''walking'' ability. A detailed description of each PTIBS score is listed in Figure 4, and the functional constituents of each score that distinguish one from another are listed in Table 2. A more detailed narrative of the behavioral assessment technique and how each PTIBS score was assigned is included as supplementary material (see online supplementary material at http://www.liebertonline.com/neu).

Once the scale was formulated, intra-observer and inter-observer reliability was measured using 154 short video clips containing various stages of recovery. Three observers independently assigned PTIBS scores for each video, and then 3 weeks later the videos were scrambled and distributed again for re-scoring by the same three observers. Intra-observer reliability was assessed by comparing each observer's two scorings of the same video, whereas inter-observer reliability was assessed by comparing the scores of the three different observers on the same videos.

Histological outcomes

Three months after injury, animals were euthanized with an IV overdose of sodium pentobarbital (120 mg/kg), after being sedated deeply with Telazol (4–6 mg/kg) IM. A 15 cm segment of thoracic spinal cord centered around the injury site was collected, postfixed in 4% paraformaldehyde (PF) for 48 h, and cryoprotected in 12, 22, and 30% sucrose. Spinal cord segments were divided into 1 cm blocks in length, frozen on dry ice, and stored at -80°C for later cryosectioning at 20 μm thickness.

White and gray matter sparing. A series of cross-sections through the SCI site 800 μm apart was stained with Eriochrome cyanide as described by Rabchevsky et al.[21] and counter-stained with neutral red. Images were then captured with a light microscope (Leica DM5000B, Leica, Concord, Ontario) using a 2.5x objective. The regions of spared white and gray matter were manually traced using ImageJ (National Institute of Health, Bethesda, MD). The epicenter was defined as the cross-section with the least amount of white matter sparing. Spared white matter was defined as areas that exhibited dense blue staining. Spared gray matter was defined based on the color of the stains and morphology. We defined intact gray matter as tissue containing normal gray matter cytoarchitecture with visible neutral red staining present. The percentage of spared white matter or gray matter was calculated by dividing the

Score	Description
1	No active hindlimb movements, with rump and knees on the ground
2	Active hindlimb movements, with rump and knees on the ground
3	Active, hindlimb movements, with "weight-bearing extensions" that lift the rump and knee transiently off the ground (hip joints are flexed but knee joints flexed and extended)
4	Active rhythmic hindlimb crawling with at least 3 reciprocating gait cycles (Crawling: L-R-L-R-L-R). Rump off the ground constantly and transient "weight bearing extensions".
5	The animal can take at least two steps (and up to six steps) with the rump and knee constantly off the ground during the steps. The knees do not fully extend. Hoof placement is a combination of dorsal and plantar. Balance while stepping is impaired.
6	The animal can take more than six steps with the rump and knee constantly off the ground. The knees do not fully extend. Hoof placement is a combination of dorsal and plantar. Balance while stepping is impaired.
7	The animal can take at least two steps (and up to six steps) with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired.
8	The animal can take more than six steps with the knees fully extended. Hoof placement is a combination of dorsal and plantar. Balance while stepping (walking) is impaired.
9	The animal can take more than six steps with the knees fully extended. Hoof placement is planter. Trunk imbalanced as the animal steps (walks).
10	The animal demonstrates grossly normal ambulation, with normal balance.

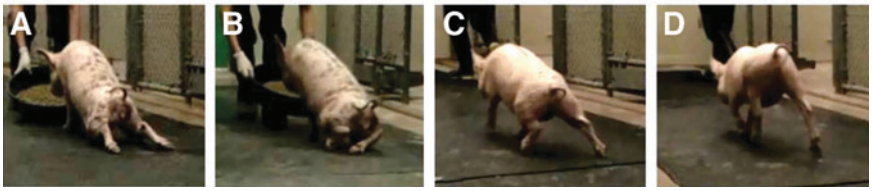

FIG. 4. Porcine Thoracic Injury Behavioral Scale (PTIBS). The PTIBS is a 10 point scale that describes various stages of hindlimb function. Scores of 1–3 describe varying degrees of ''dragging.'' Scores of 4–6 describe varying degrees of ''stepping.'' Scores of 7–10 describe walking behavior. Lower panel: In **A**, the animal's rump is off the ground but the knees are on the ground. In **B**, both the knee and rump are off the ground. In **C**, the animal is walking but with dorsal placement of the hooves. In **D**, the animal is walking with plantar placement of the hooves.

spared white or gray matter by the total area of the spinal cord on the given section. The cumulative spared white and gray matter was calculated by summing the percentage of spared white and gray matter on all the sections from 1.52 cm rostral to 1.52 cm caudal to the epicenter.[22,23] This provides a crude volumetric representation of the extent of damage through the injury site.

Immunohistochemistry. To assess axonal sparing around the injury site, three cross-sections 800 μm apart at rostral 4 mm, caudal 4 mm, and the epicenter were stained for neurofilament (i.e., nine sections per animal). The spinal cord tissues were first blocked by incubating in 10% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in 0.01M

Table 2. Functional Components of the PTIBS and Distinguishing Features Between Scores

PTIBS score	*Active hindlimb movements*	*Rump off ground*	*Knee off ground*	*Rhythmic crawling*	*Stepping partial knee extension*	*Stepping full knee extension*	*Hoof placements dorsal/plantar*	*Balance*
1	N	N	N	N	N	N	N	N
2	Y	N	N	N	N	N	N	N
3	Y	Transient	Transient	N	N	N	N	N
4	Y	Y	Transient	Y	N	N	N	N
5	Y	Y	$2 \le Y \le 6$ steps	Y	$2 \le Y \le 6$ steps	N	Both	N
6	Y	Y	$Y > 6$ steps	Y	$Y > 6$ steps	N	Both	N
7	Y	Y	Y	Y	$Y > 6$ steps	$2 \le Y \le 6$ steps	Both	N
8	Y	Y	Y	–	–	$Y > 6$ steps	Both	N
9	Y	Y	Y	–	–	$Y > 6$ steps	Plantar	N
10	Y	Y	Y	–	–	$Y > 6$ steps	Plantar	Y

The PTIBS scores follow the natural progression of hindlimb recovery, which was observed over a wide range of injury severities.
PTIBS, Porcine Thoracic Injury Behavior Scale.

Table 3. Biomechanical Parameters of Injury

	Groups	*Velocity (m/s)*	*Displacement (mm)*	*Force (N)*
Experiment 1	100 g at 50 cm	2.69 ± 0.06 (n = 4)	–	153.6 ± 13.2 (n = 4)
- Pilot study using initial impactor	50 g at 50 cm	2.67 ± 0.04 (n = 4)	–	62.5 ± 6.3 (n = 5)
Experiment 2	50 g at 40 cm	2.67 ± 0.03 (n = 5)	2.9 ± 0.4 (n = 5)	66.6 ± 7.8 (n = 5)
- Study of various drop heights with refined impactor	50 g at 30 cm	2.29 ± 0.01 (n = 5)	3.2 ± 0.3 (n = 5)	48.9 ± 0.8 (n = 5)
	50 g at 20 cm	1.83 ± 0.02 (n = 5)	2.1 ± 0.4 (n = 5)	36.4 ± 1.6 (n = 6)
	50 g at 10 cm	–	–	20.2 ± 2.0 (n = 3)
	50 g at 5 cm	–	–	11.1 ± 1.2 (n = 4)
	50 g at 20 cm no compression	–	–	29.7 ± 2.3 (n = 5)

A load cell within the impactor tip provides the force upon impact. In experiment #2, for the 20, 30, and 40 cm drop heights with compression, a high speed camera was utilized to measure impact velocity. The *n*/group represents the number of animals in which biomechanical data was available. For some animals, complete biomechanical data was not obtained because of technical issues. The *n*/group listed in this table is, therefore, not necessarily the same as the *n*/group used for the behavioral and histological analysis. (Values in mean ± SE.)

phosphate-buffered saline (PBS) (0.74% NaCl; pH: 7.4) with 0.1%Triton-X 100 for 30 min. Then, sections were incubated overnight at room temperature with rabbit polyclonal anti-NF200 (1:500; AbD Serotec, Raleigh, NC) diluted in PBS-Triton-X 100. The next day, sections were washed three times for 15 min with 0.01M PBS and incubated in Cy3-conjugated donkey anti-rabbit (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h. Finally the slides were rinsed again three times and cover-slipped using Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL). Images were then acquired using a fluorescent microscope (Leica DM5000B, Leica, Concord, Ontario) using a 20x objective. NF200 fluorescent immunoreactivity of the spinal cord sections were calculated using ImageJ (National Institute of Health, Bethesda, MD). To perform this semi-quantitative analysis of neurofilament immunoreactivity, an intensity threshold was applied, and the average neurofilament immunoreactivity was measured from each image.[24] The average neurofilament intensity values of the injured spinal cords were then normalized to the average neurofilament intensity of an uninjured spinal cord, and presented as a percent or ''percentage intensity.''

Statistical analysis

All statistics were calculated using SPSS 18.0 (IBM Corporation, Armonk, NY). The values are represented in means ± SEM. Mann–Whitney tests were utilized to compare the biomechanical data between the two groups. The Friedman test was used to establish PTIBS differences between groups over time, followed by the Kruskal–Wallis test to compare multiple groups. The intra-observer and inter-observer reliability was measured by obtaining the intra-class and inter-class correlation coefficient through reliability analysis. For establishing relationships between behavioral recovery (PTIBS) and histological outcomes, the Spearman correlation test was used to obtain the correlation coefficients, ρ.

Results

Animals

There were only two premature deaths in Experiments 1 and 2. One animal from the 100 g x 50 cm weight drop with compression group died from aspiration pneumonia within 72 h of injury. One animal from the 50 g x 20 cm weight drop with compression also died within 72 h of injury because of iatrogenic bladder perforation from the stylus of the urinary catheter. These animals were included only in the analysis of the biomechanical parameters of the injury (i.e., velocity, peak load).

Biomechanical parameters of the injury

Impact forces. Impact forces recorded during Experiments 1 and 2 are listed in Table 4. In Experiment 1, the 100 g group expectedly had significantly higher forces than the 50 g group (p = 0.014). In Experiment 2, the impact forces were significantly greater with the weight drop of 10 cm vs. 5 cm (p = 0.034), 20 cm vs. 10 cm (p = 0.025), 30 cm vs. 20 cm (p = 0.009), and 40 cm vs. 30 cm (p = 0.009). The impact forces were also significantly different between the 20 cm group with and that without compression (36.1 ± 1.8 N [n = 6] vs. 29.7 ± 2.3 N [n = 5], p = 0.022).

Impact velocities and dural displacements. Impact velocity was measured in Experiment 1 and in the 20, 30, and 40 cm weight drop groups in Experiment 2 (Table 4). As expected, the higher the starting position for the weight drop, the faster it was traveling as it struck the cord. The velocities were significantly different between the 20 cm and 30 cm groups (p = 0.014) and between the 30 cm and 40 cm groups (p = 0.009). Peak dural displacements were measured in the 20, 30, and 40 cm groups in Experiment 2. There were no significant dural displacement differences between any groups (p = 0.17).

Experiment 1: Pilot experiment (n = 11)

Behavioral outcomes. In the pilot study, we utilized the original weight drop apparatus, which consisted of a 50 g or 100 g impactor with a spherical tip. For both groups, this resulted in dramatic hindlimb impairments that improved very little over the subsequent 12 weeks. In the end, the 50 g group scored 2.4 ± 0.2 on the PTIBS and the 100 g group scored 2.5 ± 0.3 There was no statistical difference between the two groups (Fig. 5).

Histological outcomes. Both the 50 g and 100 g groups had severe parenchymal damage throughout the injury epicenter at 12 weeks post-injury. The representative images of the two groups are presented in Figure 6. Based on Eriochrome cyanine staining, the length of the lesion spanned ~12 mm on either side of the epicenter. In the sections from 1.6 mm rostral to 1.6 mm caudal to the epicenter, there was virtually no spared tissue at all (Fig. 6). There were no significant differences between the 50 g and 100 g groups with respect to the extent of white or gray matter sparing throughout the injury site (Fig. 7). At rostral 4.0 mm and caudal 4.0 mm, the average intensity of neurofilament immunoreactivity was ~20% of

Table 4. Summary of Biomechanical Parameters Reported for Contemporary Pig Compression or Contusion Spinal Cord Injury Models

Author	Animal breed, sex, weight, and age	Injury level and mechanism	Injury groups	Impactor tip shape and diameter (mm)	Impact area (mm^2)	Velocity (m/s)	Displacement (mm)	Peak force (N)	Peak pressure (MPa)
Lee et al., 2012 (present study)	Yucatan miniature Female 20–25 kg 16–20 weeks	T10/11 Extradural contusion + 100 g compression for 5 min (weight drop)	50 g, 5 cm	Circular (flat) 9.53	71	–	–	11.2	0.157
			50 g, 10 cm			–	–	20.4	0.286
			50 g, 20 cm			1.83	2.1	36.4	0.511
			50 g, 30 cm			2.29	3.2	49.0	0.688
			50 g, 40 cm			2.66	2.9	66.8	0.937
			50 g, 50 cm	Spherical 9.53		2.67	–	72.0	1.010
			100 g, 50 cm			2.69	–	115.0	1.614
Navarro et al., 2011	Gottingen & Libechov cross male and female 18–23 kg	T12 Extradural contusion (stepper motor, load control)	1.5 kg	Circular (flat) 5	20	0.03	–	9.8[b]	0.5[b]
			2.0 kg					14.7[b]	0.75[b]
			2.5 kg					19.6[b]	1.00[b]
Kuluz et al., 2010	Yorkshire female 5–7 kg 3–5 weeks	T7 Intradural contusion (controlled cortical impactor, displacement control)	Incomplete SCI 30 psi[a]	Circular (flat) 6	28	–	3	–	–
			Complete SCI 60 psi[a]				5		
			Complete SCI 80 psi[a]				8		
Zahra et al., 2010	Female Yorkshire 5–9 kg infant	C3-4 Intradural contusion (controlled cortical impactor, displacement control)	Complete SCI 80 psi[a] with 300 ms dwell	Circular (flat) 8	50	–	5	–	–
Skinner et al., 2009	Breed/sex/weight not specified, young adult	High thoracic extradural contusion	''Rapid'' caliper or clip compression	–	–	–	~50% of cord lateral diameter	–	–
Zurita et al., 2008	Minipigs female 20 kg Adult	T12,T13 Intradural contusion (clip compression)	Two-clip compression for 30 min	–	–	–	–	–	–
Bernards and Akers, 2006	Farm bred male and female 18–22 kg	T13 extradural contusion (weight drop)	25 g, 45 cm	Circular (flat) 10	79	–	–	–	–
Segler-Stahl et al., 1985	SS-1 miniature pigs 3 months	L3 Intradural contusion (weight drop)	25g, 20 cm	Circular (flat) 10	79	–	–	–	–

Authors underlined indicate that the model was evaluated with a behavioral recovery scale.
[a]For controlled cortical impactors, the pressure refers to an instrument setting to provide the desired impact velocity and is not a measure of force applied to the neural tissue.
[b]Peak loads and pressures were calculated from the reported peak compression masses (in kg), as measured loads were not reported.

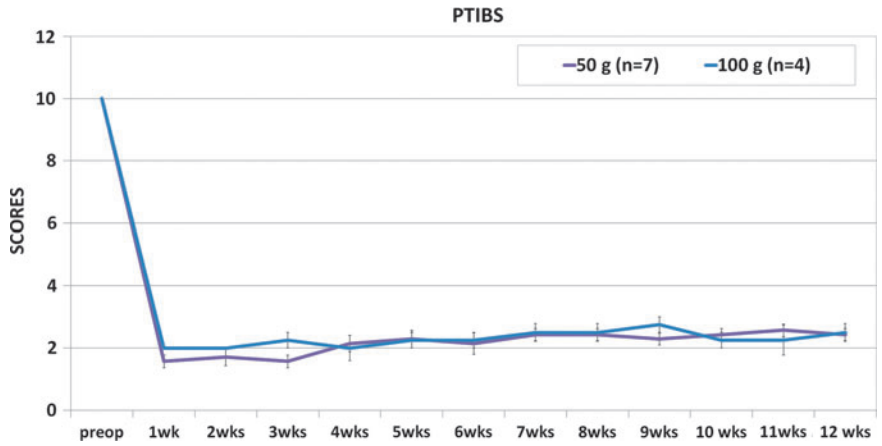

FIG. 5. Experiment 1 - Behavioral recovery on the Porcine Thoracic Injury Behavioral Scale (PTIBS). Impactors weighing either 50 g or 100 g were dropped from a height of 50 cm. At 12 weeks post-injury, the average PTIBS score for the 50 g and 100 g groups were 2.4 ± 0.2 and 2.5 ± 0.3, respectively. There was no significant difference between the two groups.

normal for both groups, and at the lesion epicenter it was ~18% in the 50 g group and 10% in the 100 g group (Fig. 8).

Taken together, these results suggested to us that the 50 g and 100 g weight drops from a height of 50 cm produced extremely severe injuries both histologically and functionally. Given that there was very little functional recovery in both groups and near complete destruction through the injury epicenter in both, we felt that further studies were warranted with lesser severities of injury.

Experiment 2: Effect of various drop heights (n = 30)

For the subsequent experiment, we utilized the 50 g weight with drop heights of 5, 10, 20, 30, and 40 cm ($n = 5$ each) (Fig. 2). All the drop heights had an additional 100 g static weight placed for 5 min of compression. One additional group of animals ($n = 5$) had just the weight drop contusion from 20 cm but with no compression, in order to determine the extent to which 5 min of compression affected the injury.

Behavioral outcomes. One week post-injury, all animals revealed striking impairments in hindlimb function (Fig. 9). In general, recovery occurred over 6–10 weeks and then plateaued. Twelve weeks post-injury, the 40 cm and 30 cm groups had PTIBS scores of 3.2 ± 0.4 and 3.0 ± 0.3 respectively, which is at the stage of ''dragging.'' The 20 cm group scored 3.8 ± 0.2, which was significantly higher than the 30 and 40 cm groups ($p = 0.013$ and $p = 0.008$). Between PTIBS scores of 3 and 4 the animals transitioned from ''dragging'' to ''stepping,'' and, therefore, the 20 cm group was at this interface. The 10 cm and 5 cm groups had PTIBS scores of 6.4 ± 1.3 and 9.0 ± 0.3, respectively, which were significantly different from each other ($p = 0.024$) and from the 20, 30, and 40 cm groups ($p < 0.001$ for all). The 10 cm group showed some stepping, but not consistently with full knee extensions (10 cm group: 6.4 ± 1.3), whereas animals in the 5 cm group were capable of executing weight-bearing plantar steps with full knee extensions (9.0 ± 0.3). PTIBS scores of both the 5 and 10 cm groups were significant higher than those for the 20, 30, and 40 cm groups ($p < 0.001$, Fig. 9A).

The effect of 5 min of compression was assessed by comparing the 20 cm drop height animals with and without compression (Fig. 9B). This revealed quite dramatic differences in both the rate and extent of recovery. The final average PTIBS score for the 20 cm with compression group was 3.8 ± 0.2, whereas for the group without compression it was 7.2 ± 0.9 ($p < 0.001$). In essence, with a drop height of 20 cm, the animals without compression achieved some level of walking, whereas the animals with 5 min of compression were at the earliest stages of stepping.

PTIBS scores for individual animals at 12 weeks after injury are depicted in Figure 10. With the exception of that between the 5 cm and 10 cm groups, statistically significant differences between all the groups occurred 6 weeks after injury and were maintained until

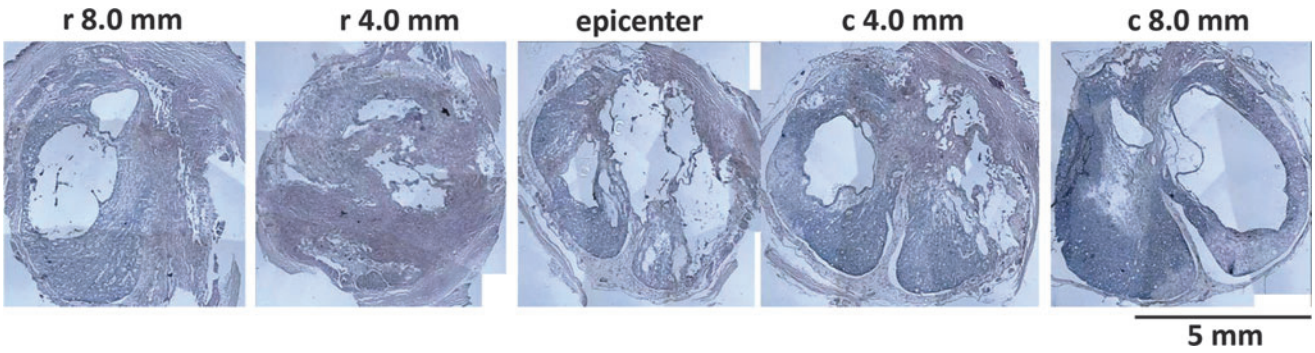

FIG. 6. Experiment 1 - Parenchymal damage through site of spinal cord injury (SCI). Considerable damage through the SCI site was observed in both the 50 and 100 g impactor groups of Experiment 1, particularly through the epicenter. This is a representative series of images from an animal injured with the 50 g impactor dropped from a height of 50 cm. (r = rostral, c = caudal, scale bar: 5 mm)

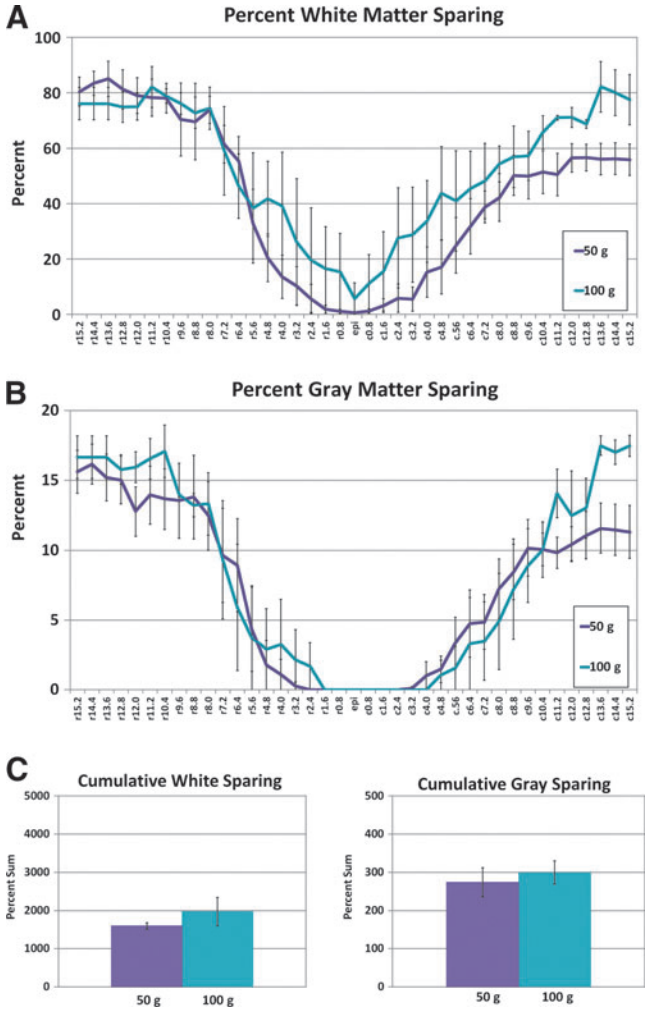

FIG. 7. Experiment 1 – white and gray matter sparing. Quantification of white and gray matter sparing was conducted on sections 800 μm apart after Eriochrome cyanide staining. The percentage of spared white matter and gray matter was calculated for each section (**A** and **B**). ''Cumulative sparing'' is the sum of percentage sparing from sections rostral 15.2 mm to caudal 15.2 mm spanning 30.4 mm **(C)**. There were no significant differences between the 50 and 100 g impactor groups with respect to white matter or gray matter sparing. (r = rostral, c = caudal)

12 weeks post-injury (Figs. 9A and 10). Between the two 20 cm groups with and without compression, the difference in PTIBS scores was significantly different starting from 1 week after injury, and this difference was retained for 12 weeks (Figs. 9B and 10).

Histological outcomes: white matter sparing. Representative images of 5 cm, 10 cm, 20 cm, 30 cm, 40 cm, and 20 cm without compression groups are shown in Figure 11. In general, the 5 cm and 10 cm groups had more white matter sparing compared with the 20 cm, 30 cm, and 40 cm groups. The rostral-caudal extent of the white matter damage was ~1.28 cm in the 5 cm group, 1.52 cm in the 10 cm group, 1.92 cm in the 20 cm group, 2.32 cm in the 30 cm group, and 2.64 cm in the 40 cm group. At the lesion epicenter, white matter sparing was ~50–60% in the 5 cm group, and 35–45% in the 10 cm group. For the 20 cm, 30 cm, and 40 cm groups, there was virtually no white matter spared from rostral 2.4 to caudal 2.4 surrounding the epicenter (Fig. 12A).

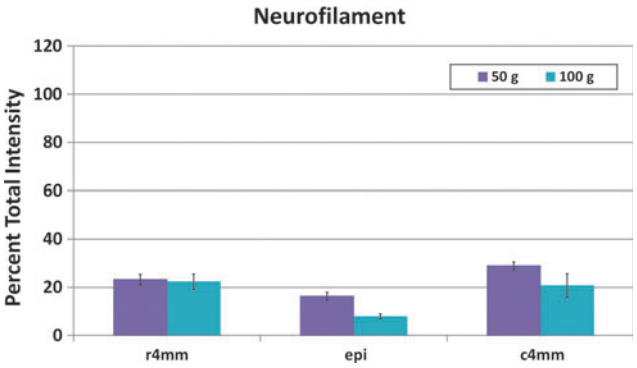

FIG. 8. Experiment 1 - neurofilament Immunohistochemistry. There were no significant differences between the 50 g and 100 g impactor groups with respect to neurofilament immunoreactivity at the injury epicenter and 4 mm rostral (r) and caudal (c) to it.

When sections from rostral 15.2 mm to caudal 15.2 mm were summed up to obtain a volumetric measure of white matter sparing, there was significantly more cumulative spared white matter between the 5 cm group and the 20 cm, 30 cm, and 40 cm groups ($p = 0.024$, $p = 0.016$, and $p = 0.008$, respectively). Also, there was more cumulative spared white matter in the 10 cm group when compared with the 20 cm, 30 cm, and 40 cm groups ($p = 0.016$, $p = 0.008$, and $p = 0.008$, respectively, Fig. 12C).

Between the 20 cm drop height groups with and without compression, there was no significant difference in cumulative white matter sparing through the entire lesion from 15.2 mm rostral and caudal to the epicenter. However, the longitudinal extent of the damage was less in the group without compression (1.52 cm versus 1.92 cm). At the lesion epicenter, white matter sparing was ~25% in the animals without compression, whereas there was almost no white matter sparing in the 20 cm group (Fig. 12B). The percentage of white matter sparing in the 20 cm group without the compression was significantly higher than in the 20 cm group with compression at rostral 17.6 mm, rostral 16.8 mm, from rostral 15.2 mm to rostral 13.6 mm, and from rostral 0.8 mm to caudal 3.2 mm (p values < 0.05) (Fig. 12B).

Histological outcomes: gray matter sparing. In general, the 5 cm and 10 cm drop height groups had more gray matter sparing than did the 20 cm, 30 cm, and 40 cm groups. The gross rostral-caudal extent of the gray matter damage for the 5 cm, 10 cm, 20 cm, 30 cm, and 40 cm drop height groups were 1.52 cm, 1.60 cm, 2.32 cm, 2.96 cm, and 2.88 cm respectively. Surrounding the epicenter, there was ~2.5% of gray matter spared in the 5 cm and 10 cm groups, and for the 20 cm, 30 cm, and 40 cm groups, there was no gray matter spared from rostral 3.2 mm to caudal 3.2 mm (Fig. 13A). Gray matter sparing was not different between the 5 cm and 10 cm groups, and not different among the 20, 30, and 40 cm groups.

There was significantly more cumulative gray matter sparing from rostral 15.2 to caudal 15.2 mm in the 5 cm drop height group than in the 20 cm, 30 cm, and 40 cm groups ($p = 0.024$, $p = 0.016$, $p = 0.008$, and $p = 0.008$, respectively). Also, there was significantly more cumulative gray matter sparing in the 10 cm group than in the 20 cm, 30 cm, and 40 cm groups ($p = 0.016$, $p = 0.008$, and $p = 0.008$, respectively).

For the comparison between the 20 cm drop height groups with and without compression, the longitudinal extent of the gray matter damage was less for the group without compression (1.68 cm vs.

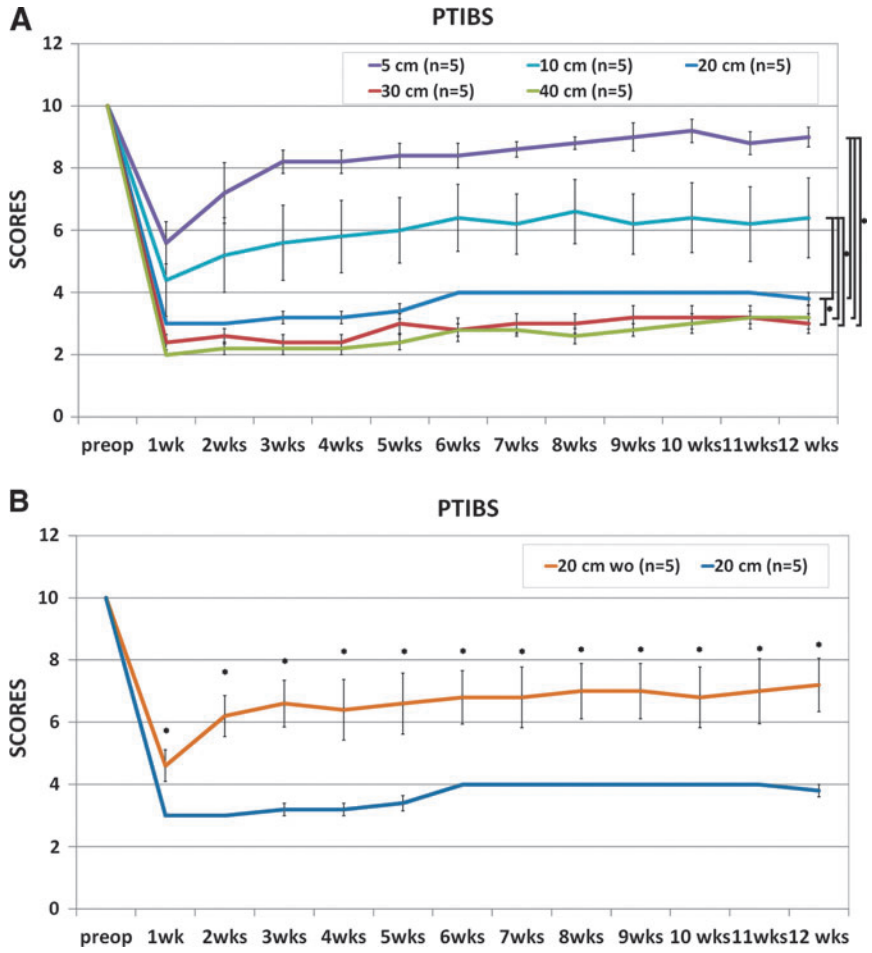

FIG. 9. Experiment 2 - behavioral recovery on the Porcine Thoracic Injury Behavioral Scale (PTIBS). In **A**, the PTIBS scores for animals subjected to drop heights of 5, 10, 20, 30, and 40 cm with 5 min of compression are depicted. Significant differences among animal groups in their final PTIBS scores at 12 weeks post-injury are depicted with an asterisk (*). In **B**, the PTIBS scores for the 20 cm drop height animals with and without the subsequent 5 min of compression are depicted. In animals with contusion and compression, the 12 week PTIBS score was 3.8 ± 0.4, as compared with 7.2 ± 0.9 for the animals with contusion alone (i.e., no compression). This difference was statistically significant ($p < 0.001$).

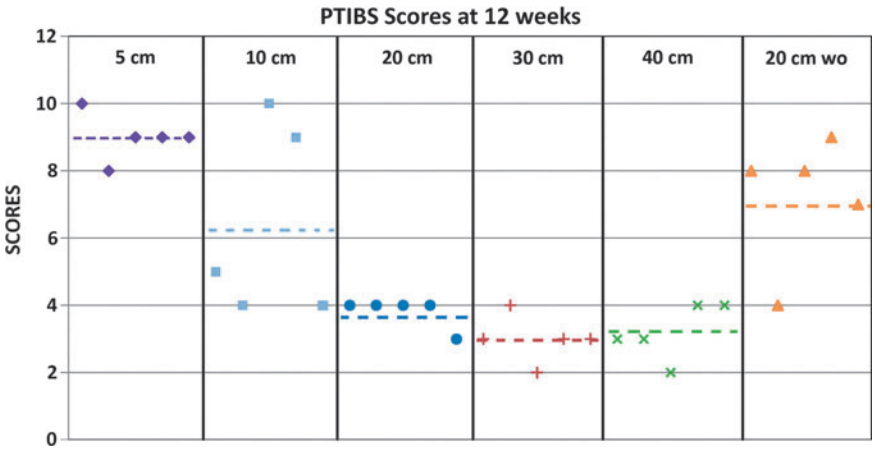

FIG. 10. Experiment 2 - Porcine Thoracic Injury Behavioral Scale (PTIBS) Scores at 12 weeks after injury. PTIBS scores for individual animals in 5, 10, 20, 30, and 40 cm, and 20 cm without compression 12 weeks after injury.

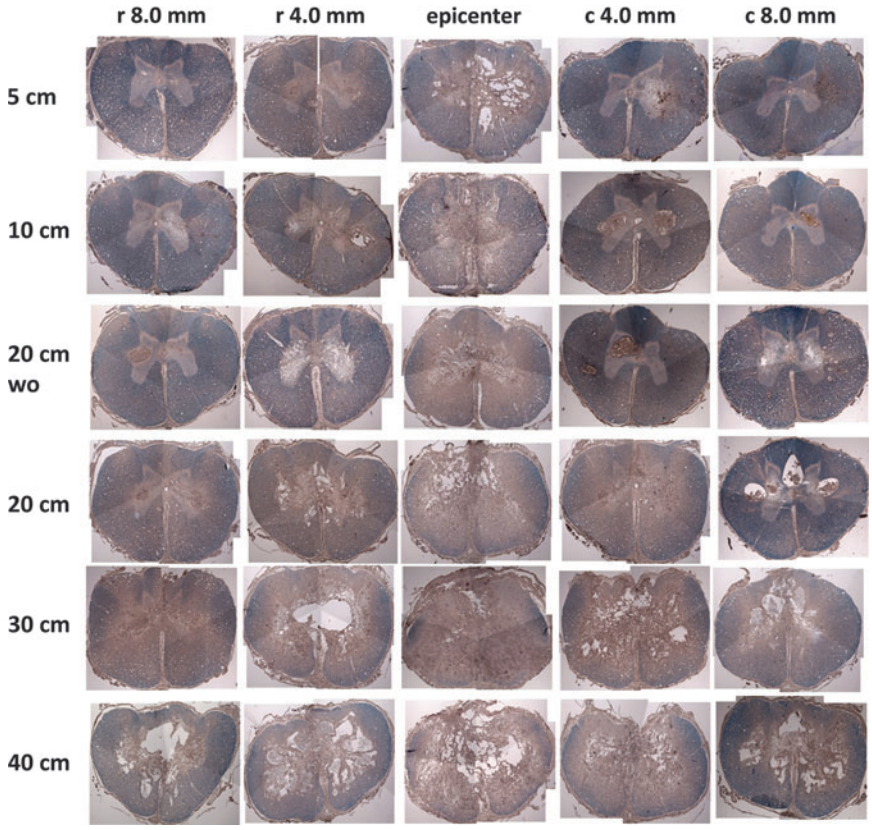

FIG. 11. Experiment 2 - parenchymal damage through site of spinal cord injury (SCI). For the animals subjected to weight drops of 20, 30, and 40 cm with subsequent compression, the epicenter was nearly completely devoid of intact white or gray matter. This resembled the epicenter of the animals with 50 cm weight drops. Damage through and around the epicenter was obviously less in the 5 and 10 cm weight drop groups, and in animals with the 20 cm weight drop without (wo) compression. (r = rostral, c = caudal, Scale bar: 5 mm) Representative Images of 5 cm, 10 cm, 20 cm wo, 20 cm, 30 cm, and 40 cm groups.

2.32 cm). However, on a section-by-section basis, there were no significant differences between the two groups in terms of percent gray matter sparing. Around the lesion epicenter, the percentage of gray matter spared was $\sim$2.5%, in the animals without compression, as compared with none in the animals with compression (Fig. 13B). There was no statistically significant difference in cumulative spared gray matter between the two 20 cm groups with and without compression (Fig. 13C).

Histological outcomes: neurofilament immunoreactivity. At the lesion epicenter, the average intensity of neurofilament immunoreactivity decreased with increasing height of the weight drop, with significant differences observed between groups (Fig. 14A). At the sections 4 mm rostral and caudal to the epicenter, there was greater neurofilament immunoreactivity (as one would expect moving away from the epicenter). Significant differences in the intensity of immunoreactivity were observed between the different drop height groups (Figure 14A). When neurofilament immunoreactivity was compared between the 20 cm drop height groups with and without compression, it was only at the epicenter that the 20 cm without compression group showed significantly greater intensity ($p < 0.001$, Fig. 14B). However, at rostral and caudal 4 mm, there were no statistical differences in the intensity of neurofilament immunoreactivity between the two groups. Representative images of neurofilament immunostaining and EC staining are shown in Fig. 15.

Correlation between histology and behavior. To determine the relationship between the histological damage of the spinal cord and the behavioral recovery, we utilized the 5 cm, 10 cm, 20 cm, 30 cm, and 40 cm drop height groups with compression from Experiment 2. The 20 cm drop height group without compression was excluded from this analysis. There was a negative correlation between the PTIBS scores at 12 weeks and the force imparted to the spinal cord at the time of injury (Fig. 16A). The coefficient value was $\rho = -0.714$ ($p < 0.001$). PTIBS scores were positively correlated to cumulative white and gray matter sparing (Fig. 16 B, C). The correlation coefficient values between PTIBS scores and spared white matter was $\rho = 0.888$ ($p < 0.001$) and gray matter was $\rho = 0.748$ ($p < 0.001$). Taken together, PTIBS scores were highly correlated with total (white and gray matter) spared tissue with a coefficient value of $\rho = 0.881$ ($p < 0.001$). The neurofilament immunoreactivity was also positively correlated to the PTIBS scores with a coefficient value of $\rho = 0.663$ ($p = 0.002$) (Fig. 16 D).

Reliability of the PTIBS

To test the inter- and intra-observer reliability of the PTIBS, 154 video clips were scored by three observers (two trained technicians and an undergraduate student) on two occasions, 3 weeks apart. The intra-observer correlation coefficients were 0.954, 0.992, and 0.972 for the three observers and the inter-observer correlation coefficients were 0.961, 0.927, and 0.918.

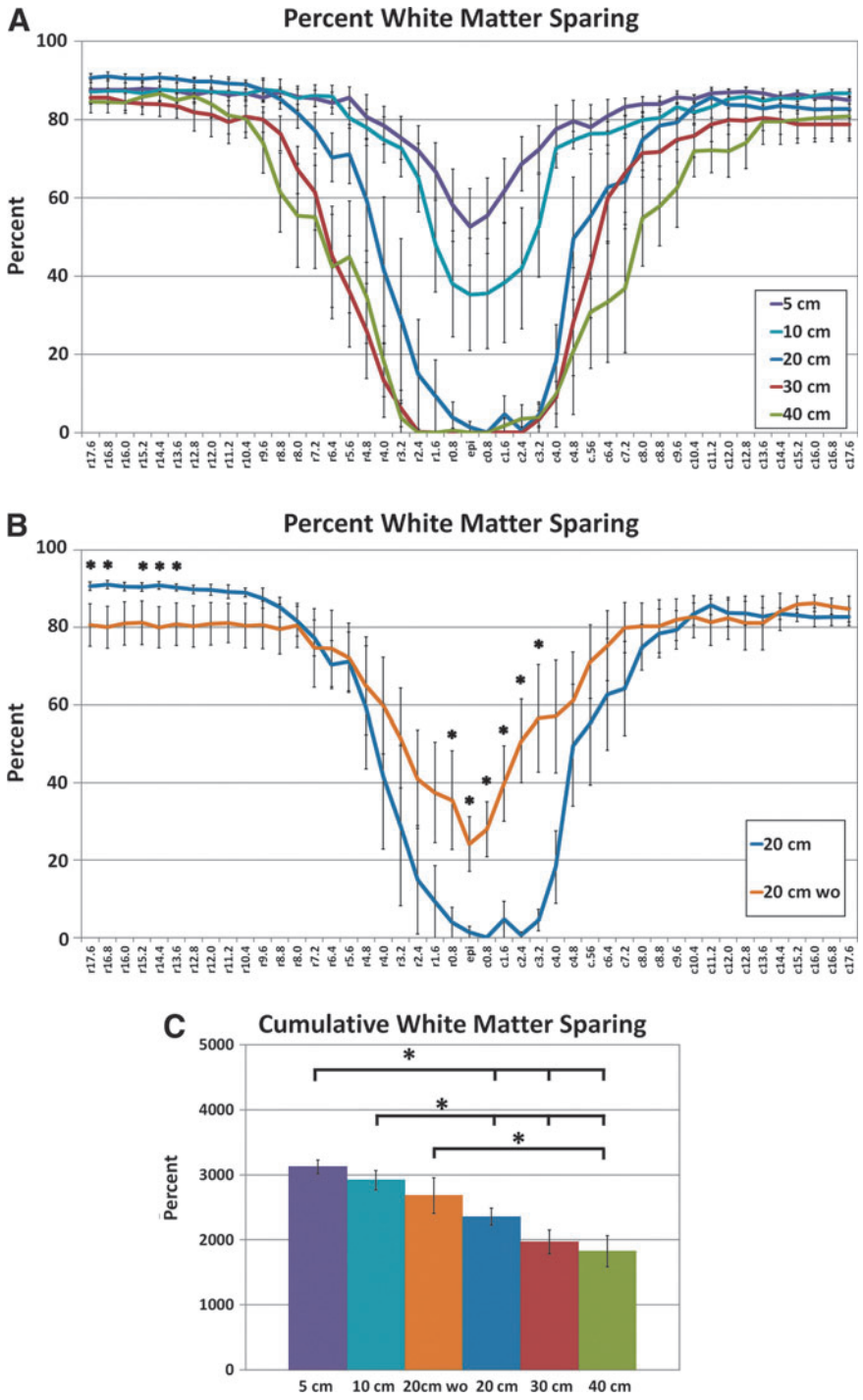

FIG. 12. Experiment 2 – white matter sparing. The quantification of white matter sparing is depicted for the 5, 10, 20, 30, and 40 cm groups with compression **(A)** and for the 20 cm with and without compression **(B).** Between the 20 cm drop height animals with and without compression, there were significant differences in percentage white matter sparing at various sections (+). Cumulative white matter sparing sections from rostral 15.2 mm to caudal 15.2 mm spanning 30.4 mm of spinal cord are shown in **C**, with significant differences denoted with an asterisk (*). (r = rostral, c = caudal)

Discussion

In this study, we describe a novel, clinically relevant contusion and compression porcine model of thoracic SCI. Using a custom-made weight drop device, a combination of contusion and compression was applied to the thoracic spinal cord of 20–25 kg pigs. To quantify behavioral recovery we developed the PTIBS. Scoring on the PTIBS correlated negatively with the biomechanical severity of injury, and positively with the extent of spared white matter, gray matter, and neurofilament staining through the injury site.

The varying degrees of injury severity that we employed in our model development allowed us to observe a wide spectrum of

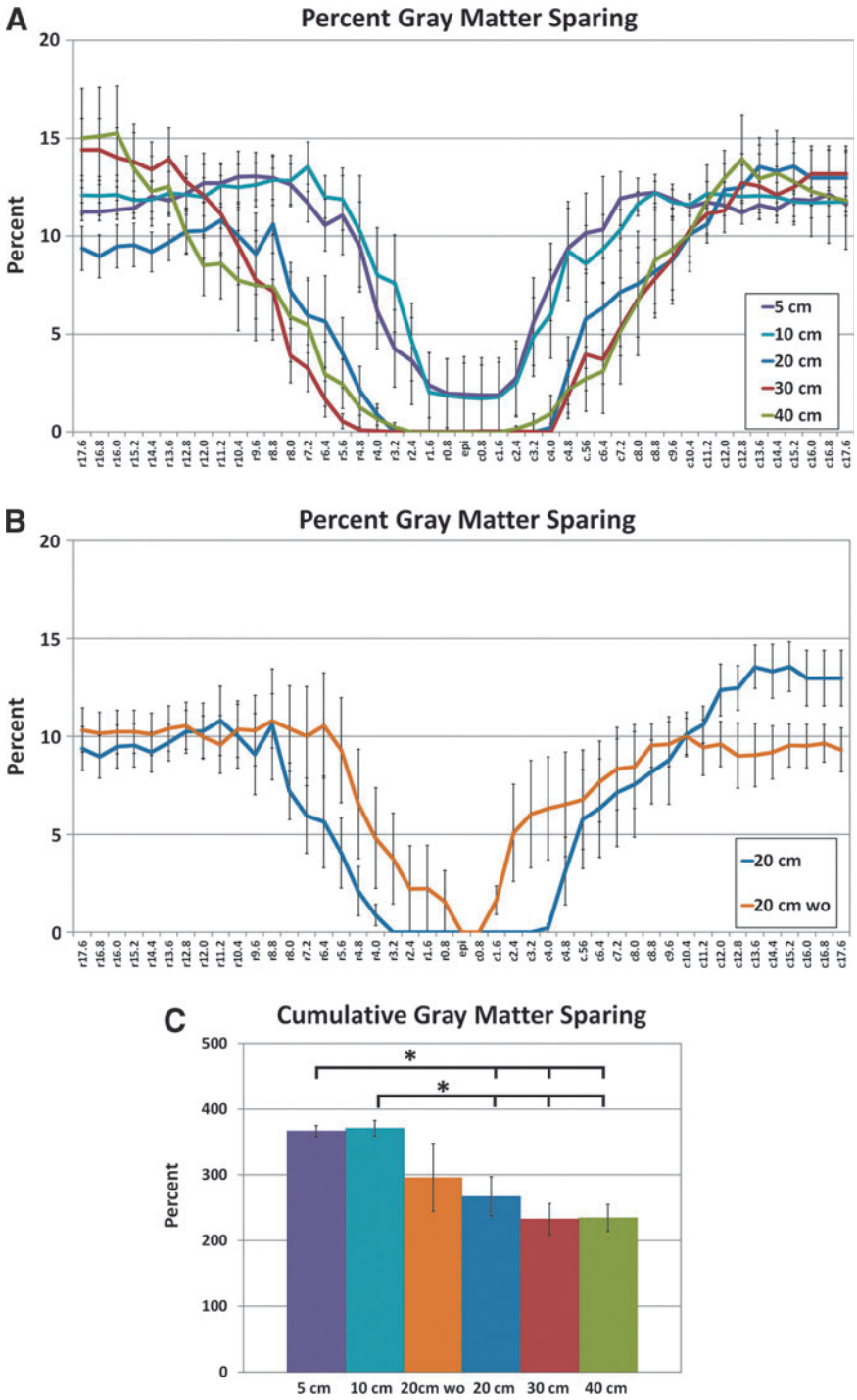

FIG. 13. Experiment 2 – gray matter sparing. The quantification of gray matter sparing is depicted for the 5, 10, 20, 30, and 40 cm groups with compression **(A)** and for the 20 cm with and without compression **(B).** Gray matter destruction through the epicenter was nearly complete in all animal groups except for the 5 and 10 cm drop height groups. Cumulative white matter sparing sections from rostral 15.2 mm to caudal 15.3 mm spanning 30.4 mm of spinal cord are shown in **C**, with significant differences denoted with an asterisk (*).(r = rostral, c = caudal)

behavioral recovery over time. In the future, we would propose a drop height of 20 cm with 5 min of compression as the injury severity to test further interventions. With this injury severity, the animals fairly consistently reached a PTIBS score of 4 (Fig. 10), at which point in time they had a rhythmic, reciprocating gait pattern and kept their rump off the ground, but were unable to take steps. With this PTIBS score of 4 as a ''control'' level of functional ability, improvements in recovery with a therapeutic intervention to the point where the animals could take some steps would be quite apparent and meaningful. A PTIBS score of 4 in control animals also made it possible to detect lower scores that could reflect the detrimental effects of an intervention, thus providing an assessment of risk.

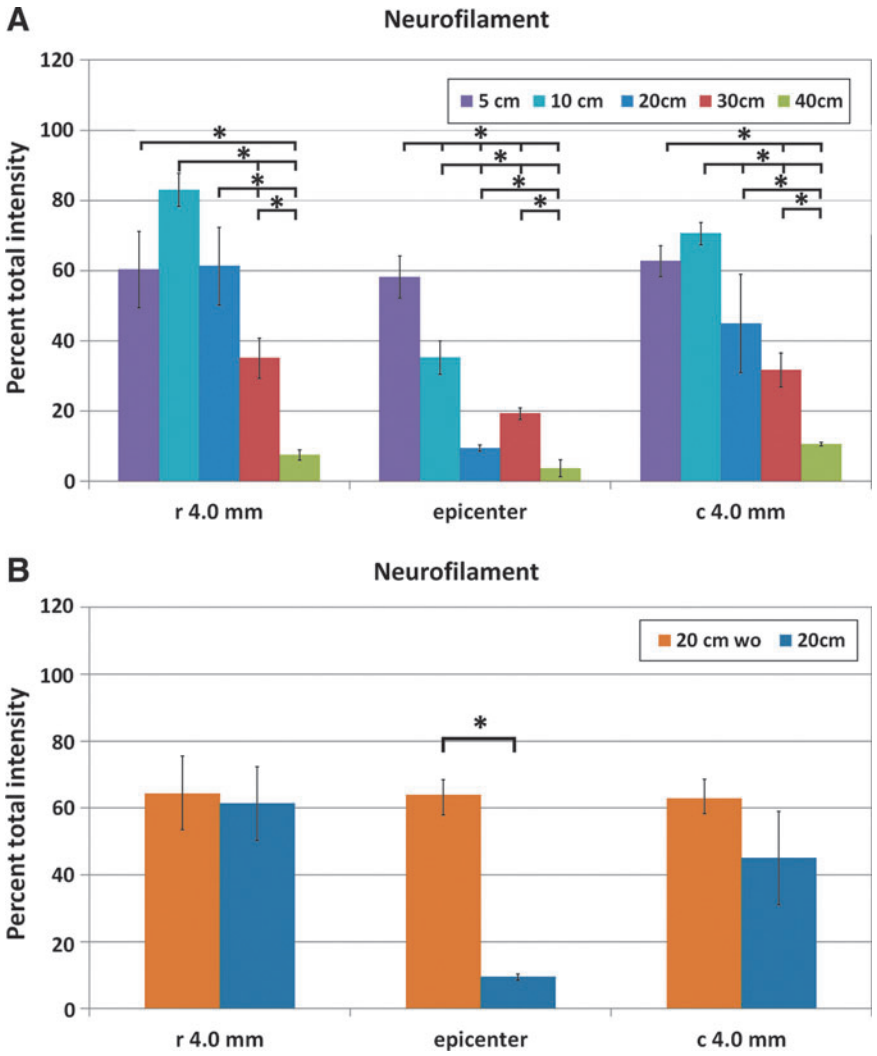

FIG. 14. Experiment 2 - Neurofilament immunohistochemistry. Neurofilament immunoreactivity at the injury epicenter and 4 mm rostral and caudal to it is depicted for the 5, 10, 20, 30, and 40 cm drop height animals with compression (**A**). There were no significant differences among the groups at any of the sections. In **B**, the 20 cm drop height animals with and without compression are depicted. Significantly more neurofilament immunoreactivity was observed at the injury epicenter in the animals without compression ($p < 0.001$). (r = rostral, c = caudal)

We conducted a power analysis using the 12 week post-injury PTIBS scores from the 20 cm weight drop with 5 min of compression. With a standard deviation of 0.447 (which was the standard deviation observed in the PTIBS scores for this 20 cm weight drop with 5 min of compression group), to detect a difference of 1 on the PTIBS scale with an α of 0.05, we would need four animals per group for a power of 80%, and five animals per group for a power of 90%. This would lend itself to a fairly feasible study of 8–10 animals (4–5 per group) when testing a specific intervention. To detect an anticipated difference of two points on the PTIBS scale would obviously require fewer animals.

In the context of modeling SCI, the pig spinal cord shares a number of relevant anatomical features with that of humans. First, there is the issue of sheer size/caliber. Whereas the rat spinal cord is typically only 2–3 mm in width, the pig cord at T10/11 is $\sim$7 mm (Fig. 17) and is, therefore, much more similar to the human spinal cord, which is typically 8–9 mm across.[6] Although this may seem rather simplistic, the issue of size is likely to be of particular relevance to the biodistribution and subsequent efficacy of locally delivered therapies such as cellular transplants, ChABC, or Rho antagonists.[1,2] The pig spinal cord (like the human spinal cord) is surrounded by a prominent layer of cerebrospinal fluid (CSF) (Fig. 16), making the pig a more clinically relevant animal model to test the biodistribution and effect of therapies applied extradurally or infused intrathecally. This CSF compartment in pigs is large enough to insert catheters for biochemical and physiological monitoring,[20,21,25,26] which has particular translational relevance, given that CSF monitoring can be also be done in human SCI patients.[27,28] The vascular supply to the thoracic spinal cord is similar to that of humans, and has therefore made the pig a widely used model of ischemic thoracic paraplegia secondary to thoracoabdominal aortic aneurysm surgery.[29,30] Given that ischemia is likely a component of secondary injury after traumatic SCI, similarities between humans and pigs in the vascular supply to the spinal cord are desirable.

There are numerous challenges associated with modeling a clinically relevant injury for which the mechanical parameters are often unknown and highly variable, as is the case in human SCI. We

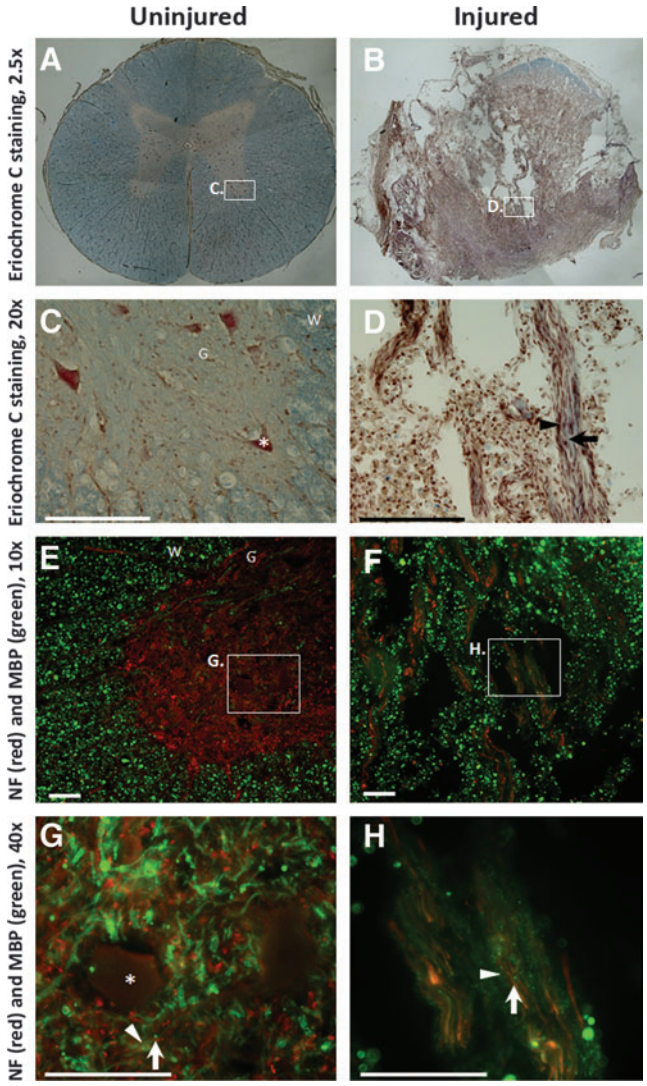

FIG. 15. Representative images of uninjured and injured spinal cords with Eriochrome cyanide (EC) staining and immunohistochemistry. A section of an uninjured animal at T10 is on the left column, whereas the epicenter of an animal injured with a 20 cm weight drop and compression is on the right column. Uninjured (**A**) and injured (**B**) spinal cord at T10 2.5x magnification. Uninjured (**C**) and injured (**D**) gray and white matter with EC staining at 20x magnification. There is a dramatic loss of axons, myelin, and cell bodies in the injured spinal cord compared with the uninjured spinal cord. Uninjured (**E**) and injured (**F**) white and gray matter with neurofilament (NF: red) and myelin basic protein (MBP: green) expression at 10x magnification. Uninjured (**G**) and injured (**H**) high magnification image of axons (red) surrounded by myelin (green) in the gray matter (40x). There are several spared myelinated axons in the gray matter after injury. (G: gray matter, W: white matter* cell body, arrowhead: myelin, arrow: axon, scale bar: 100 μm)

have attempted to advance this aspect of injury modeling by providing measures of impact force, dural displacement, and the range of impact severities. As shown in Table 5, pig models of traumatic SCI have been described previously, utilizing a variety of biomechanical parameters such as compression area, impact velocity, cord displacement and peak load.[15,16,19,31–34] With further, future, development of injury devices that can provide and measure key biomechanical inputs and outcomes and further correlate these with histological and biochemical values, the pig's size will likely enable a more accurate representation of (and allow a more comprehensive study of) human SCI biomechanics.

Most recently, Navarro et al. described a graded (1.5, 2.0, and 2.5 kg) compressive SCI model using adult miniature pigs,[19] and a comparison of the biomechanical parameters utilized and behavioral scoring is warranted. Navarro et al. used an impact velocity approximately two orders of magnitude lower than that which was used in our study (Table 5). Rat SCI models utilizing the Ohio State University (OSU) and IH impactors typically use impact velocities of 0.08 - 1.0 m/sec,[35,36] and cadaver models of spinal burst fractures suggest that human SCIs may occur at impact speeds > 1 m/sec.[37–39] Although Navarro et al. did not report a measured impact load, it is likely that the dynamic component was minimal, because of the low velocity of impact. When the nominal injury load is estimated as a static force from the applied mass (See Table 5), the injury severity groups at 1.5 kg and 2.0 kg were most similar to the dynamic peak impact force measured for our 5 cm drop height group (measured during the injury event), whereas their 2.5 kg group was most similar to the peak impact force of our 10 cm drop height group. Comparing the applied peak pressure, to account for the difference in impactor diameter between the two models, the 1.5, 2.0, and 2.5 kg groups of Navarro et al. are most similar (but still considerably different) from our 20, 30, and 50 cm drop height groups, respectively (Table 5). Navarro et al. also did not report a dural or spinal cord displacement from their experiments, and we were able to record these values in some of our animal groups in the present study.

A number of aspects of our pig model warrant discussion. The first is animal care. In general, veterinary care is demanding during the first 24–48 h. However, we had only two deaths in our entire series, and good survival through to the final endpoint of 12 weeks for all other animals. Hence, we feel that the animal care challenges are reasonable, and could be successfully managed by other researchers who have a large animal facility.

The second consideration is the reproducibility of the behavioral outcome measures. We developed the PTIBS based on the recovery patterns of animals injured with a wide range of injury severities (weight drops of 5, 10, 20, 30, 40, and 50 cm). This provided direct observation of a spectrum of behavioral recovery spanning from no active hindlimb movements to normal walking, from which we developed the PTIBS. The PTIBS was developed to provide a practical, reproducible, and responsive instrument for measuring the hindlimb functional status of the animals. With a number of trained technicians and undergraduate students, we found that the intra-observer and inter-observer correlation coefficients were high, indicating that the scale is relatively easy to learn and apply with high reliability. The correlation between the PTIBS scores and the impact force recorded at the time of injury indicated that the behavioral recovery as measured by PTIBS was well predicted by the biomechanical severity of injury. The relationship between histology and behavior was confirmed by the close correlation between the PTIBS scores and the extent of white matter and gray matter sparing through the injury site. Taking into account all of these considerations, we feel that the PTIBS is a reasonably reproducible method for measuring behavioral recovery in this porcine model of thoracic SCI. It should be mentioned that other behavioral scales have also been developed for measuring hindlimb performance in large animals of SCI, including pigs.[15,18,19,40,41]

It is acknowledged that our primary objective was to develop a model in which we could describe the biomechanical parameters,

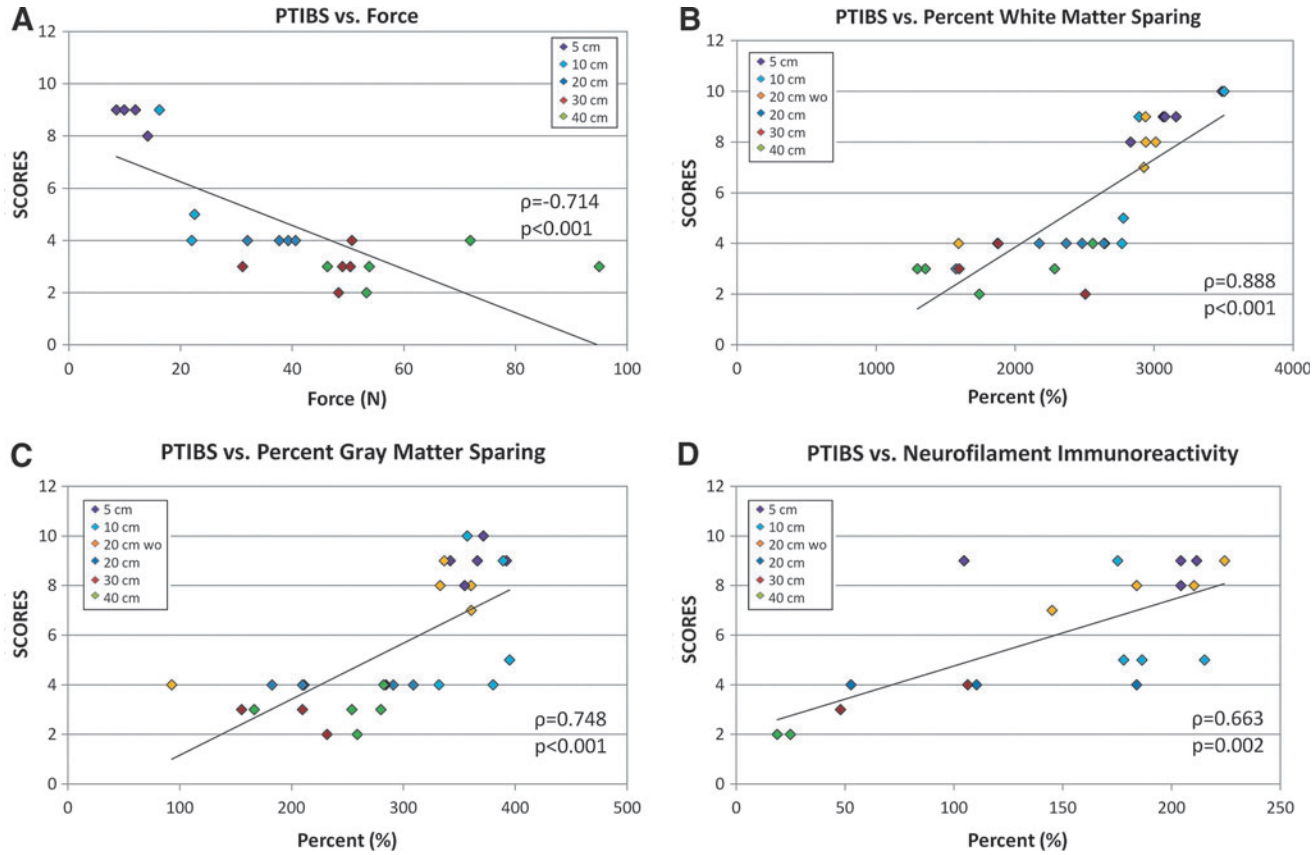

FIG. 16. Correlation between Porcine Thoracic Injury Behavioral Scale (PTIBS) scores and biomechanical injury severity and PTIBS scores with histological measures. The final, 12 week post-injury PTIBS scores were plotted next to impact force **(A)**, cumulative white matter sparing **(B)**, cumulative gray matter sparing **(C)**, and cumulative neurofilament immunoreactivity **(D)**. As expected, the greater the impact force (as measured by the load cell in the impactor tip), the lower the PTIBS scores **(A)**. There was a strong correlation between the extent of cumulative white matter and gray matter sparing through the injury site and the PTIBS scores. Cumulative neurofilament immunoreactivity was calculated as the sum of the three sections measured (epicenter and 4 mm rostral/caudal). It also revealed a strong correlation with PTIBS scores.

the progression of behavioral recovery, and the extent of parenchymal damage to the spinal cord. The histological measures we employed (white and gray matter sparing and neurofilament immunoreactivity) are relatively crude measures of parenchymal damage, and further, more detailed analyses are warranted to address specific research questions, such as the extent of demyelination, the actual number of spared axonal profiles, and the degree of glial scarring. These will be particularly relevant in the future when employing the model for testing interventions such as cell transplantation.

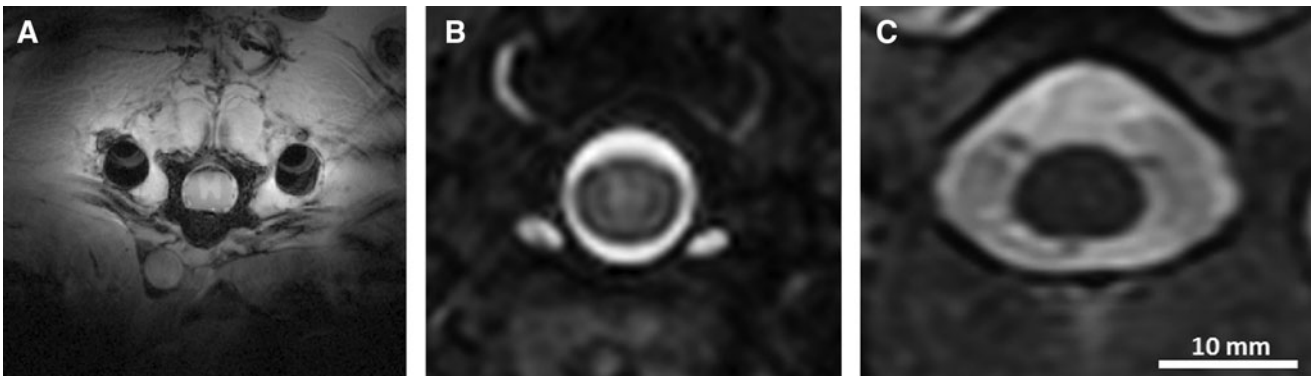

FIG. 17. Magnetic resonance imaging of a Sprague–Dawley rat **(A)** vs. Yucatan miniature pig **(B)** vs. human **(C)** at T10. The width of the porcine spinal cord is close to a human spinal cord and a prominent layer of cerebrospinal fluid (CSF) surrounds the porcine and human spinal cord. The transverse diameter of the rat, pig, and human spinal cord on these sections are 3.0, 7.0, and 8.2 mm. The scale bar (applicable to all three sections, is 10 mm).

Another limitation in our model is the age and weight of our animals. The Yucatan miniature pigs that are utilized in this study are between the ages of 5 and 6 months and weigh 20–25 kg; at sexual maturity at ~10 months of age, the animals can exceed 50 kg, which would make them extremely difficult to physically manage for their behavioral testing. It is acknowledged that as our pigs are ''adolescents;'' therefore, their recovery potential may be greater than that of an adult animal. However, from a translational standpoint, we still feel that it would be informative if an intervention were to induce behavioral recovery in this model.

The duration of sustained compression is also worth commenting on. We observed significant differences in both the histological extent of damage and the recovery on PTIBS between the animals receiving contusion versus contusion plus 5 min of compression. In rodent SCI models that employ clip compression, the duration of compression is typically 1 min. We somewhat arbitrarily chose to extend this period of compression to 5 min instead of 1 min in our pig model, but we do recognize that sustained compression in a human SCI patient can last many hours (to days). This is one element of injury that is difficult to model accurately in animals. From an experimental standpoint, keeping sustained compression on the animal for a long period of time does inflict a more prolonged surgical insult to the animals, which we are wary of.

Following the weight drop confusion at 20 cm, whether or not 5 min of static compression was added afterwards did result in a dramatic difference in the functional scores, as early as 1 week injury. Whereas we attribute much of this difference in behavioral outcome to the 5 min of compression, it should also be noted that the peak load measured during the actual weight drop impact was also significantly lower in the animals in the 20 cm without compression group (36.1N vs. 29.7N, excluding the one animal in the 20 cm with compression group that died). This could have then also contributed to the improved PTIBS scores of the 20 cm without compression group. It is unclear to us why there was a significant difference in the peak loads for these two groups; in theory, these should have been the comparable, given that the impactor device and drop height were the same. There might have been slight differences in impact velocity and impact velocity was not measured in the 20 cm without compression group. The significant difference in PTIBS scores (and subsequently described histology) between the 20 cm groups with and without compression must therefore be interpreted as the end result of both sustained compression and some differences in impact force. However, we believe that the improved behavioral recovery in the 20 cm without compression group was mainly because of the lack of 5 min of compression. The PTIBS scores in this group were comparable to (if not actually a bit better than) the PTIBS scores for the 10 cm drop height group with 5 min of additional compression, even though the latter had a lower peak load (20.2 N) and, therefore, a ''lighter'' contusion injury.

Here we present a graded porcine SCI injury model that was characterized at various injury severities with an element of contusion and compression that occurs most commonly clinically.[8] This large animal injury model may have a role as an intermediary testing platform for novel SCI treatment strategies such as cell transplantation and neuroprotective drugs. The larger size of the spinal cord and the CSF space around the spinal cord confers an advantage to the porcine model over rat or mouse models for experimental treatments delivered intrathecally or extradurally, where the biodistribution to and within the injured spinal cord are important therapeutic considerations. These issues notwithstanding, we would not argue against the role of mouse and rodent models as mainstays of pre-clinical research on novel SCI therapies, because of their low cost, widespread availability, and established technical and analytical methodology. However, for the handful of therapies being seriously considered for human clinical trials, we are of the opinion that important and relevant questions can be addressed in a large animal model such as ours. We contend that this could improve the chances of successfully translating experimental SCI treatments into clinically efficacious therapies for individuals with this devastating injury.

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Author Disclosure Statement

No competing financial interests exist.

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