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TITLE: DEVELOPMENT OF AN ANIMAL MODEL OF THORACOLUMBAR BURST
FRACTURE-INDUCED ACUTE SPINAL CORD INJURY

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14. ABSTRACT Background: Spinal cord injury (SCI) leads to permanent disability and has been increasingly observed in warfighters after introduction of more survivable vehicles (MRAP). Rodent research has led to many advances in SCI treatment, but successful clinical translation remains limited. Here we describe a large animal model of blunt acute traumatic SCI using a custom designed computer controlled spinal cord impactor. Methods: Thirteen female Yucatan miniature swine were subjected to spinal cord impact with a custom-made controlled spinal cord impactor and balloon compression. Neurological function was assessed for seven days after injury. Magnetic resonance imaging (MRI) and histology were performed on postoperative day one and seven respectively. Results: The custom spinal cord impactor delivered consistent, predictable, impacts to the spinal cord. MRI and histology showed a positive correlation between volume and severity of spinal cord injury and the impact force. Both the PTIBS and PNM scales also correlated with the target impact force. Conclusions: This novel and custom spinal cord impactor can reliably produce a gradient of ventral blunt SCI. This could prove to be a valuable tool to investigate SCI seen in burst fracture and other traumatic injuries, and may represent a useful intermediate step in evaluation of SCI treatments.					
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1. Introduction

Spinal cord injury (SCI) frequently leads to permanent disability following traumatic spine injury. A dramatic increase in blast related spinal burst fracture has been observed in recent armed conflicts (OEF/OIF), with many soldiers suffering debilitating SCI. Rodent research has led to many promising advances in SCI treatment, but successful clinical translation remains elusive. Many factors may contribute to this including inadequate animal models, and many researchers in the SCI community have called for improved large animal models of contusion SCI. Several large animal SCI models have been developed but most use static compression or transaction to create injury, not contusion. There are no models of ventral impact, which causes injury in burst fracture. Weight-drop, produces contusion but is imprecise and unpredictable. Therefore, we set out to develop and validate a large animal model of ventral contusion SCI that is seen in burst fracture using a custom-designed, controlled spinal cord impactor and sustained balloon compression.

2. Keywords

Spinal cord injury, spine trauma, burst fracture, large animal model

3. Accomplishments

Specific Aim 1 – Develop and complete proof of concept for a novel animal model of anterior (ventral) spinal cord injury following simulated vertebral body fracture in which the magnitude of primary injury: impulse load (contusion) + canal compromise (compression) can be controlled and titrated

We have successfully established the proof of concept of the large animal model of SCI in burst fracture. We performed 13 animal surgeries and created a variable degree of spinal cord injury with a combination of contusion and compression. The results of these surgeries are shown in figures 1-5.

Specific Aim 2 – Demonstrate ability to reliably and incrementally adjust contusion impulse load and compression magnitude and duration.

In order to accomplish this specific aim, we designed and built a custom spinal cord impactor specifically for large animal applications, which could be utilized in a ventral impact model. The impactor that we built was successful in delivering a gradient of force to the spinal cord in our animal experiments (figure 1). Furthermore, the variable force delivered to the spinal cord resulted in a predictable gradient of spinal cord injury observed in the animals. This was seen radiographically on post-operative MRI (figure 2), clinically via neurobehavioral testing (figure 3&4), and histologically (figure 5). We also demonstrated the feasibility of placing an inflatable balloon adjacent to the spinal cord to deliver a variable degree of sustained compression. This balloon was inflated during the animal surgeries, immediately following the contusion injury, and could be deflated at any point during the recovery period. We were able to produce severe, moderate, and mild spinal cord compression, and we showed, predictably, that the degree of

compression correlated with the neurobehavioral function of the animals during the recovery period.

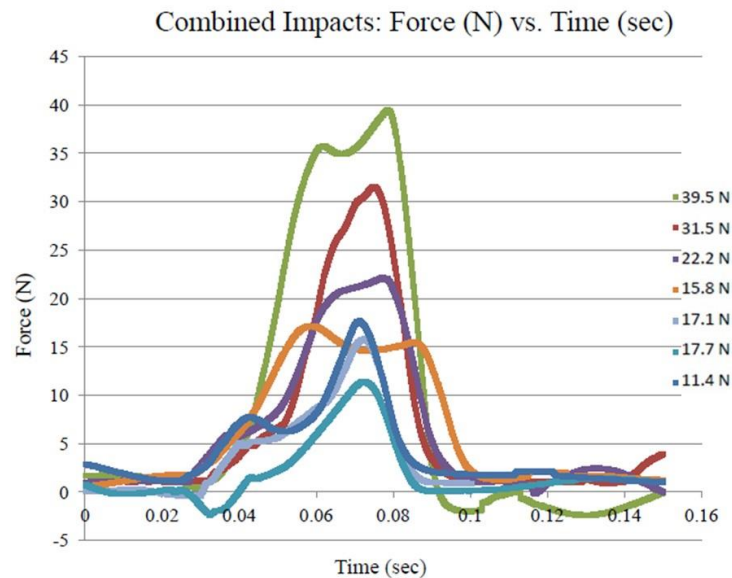


Figure 1. Composite graph of force over time for each of the successful spinal cord impacts performed. Note that the profile of the impacts is maintained at different impact energies and that the elapsed time for each impact is approximately 50 msec.

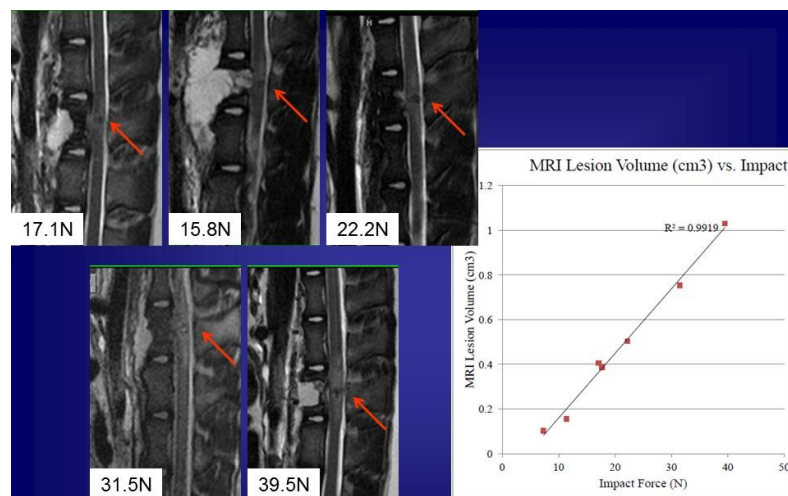


Figure 2. Post injury T2 weighted sagittal MRI showing a positive correlation between volume of T2 signal hyperintensity within the spinal cord and energy of spinal cord impact. This is also demonstrated in the graph on the right with an R-squared value of 0.9919, indicative of a strong linear correlation.

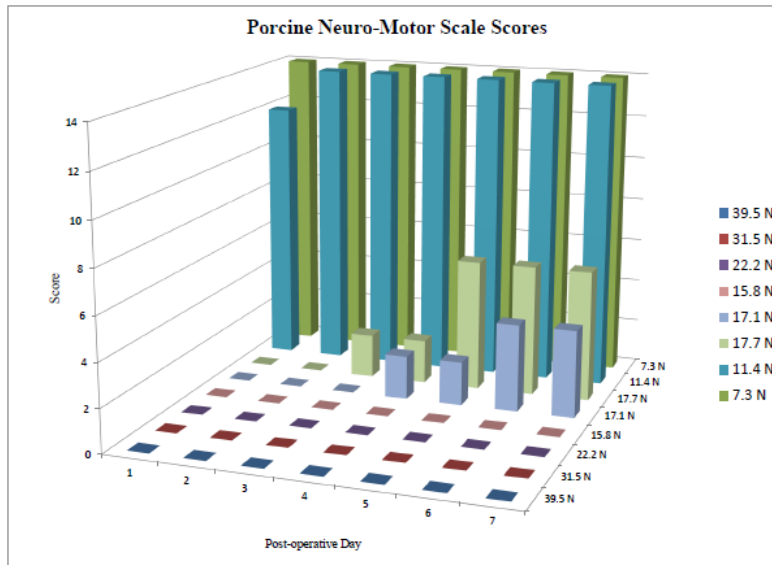


Figure 3. Bar graph showing the outcome of the Porcine Neuro-Motor (PNM) neurobehavioral analysis during the 7 day post-operative period. Animals with higher energy impact to the spinal cord had worse neurological function, as evidenced by the lower PNM scores. Some animals recovered some function after 3 to 4 days, likely due to spinal shock.

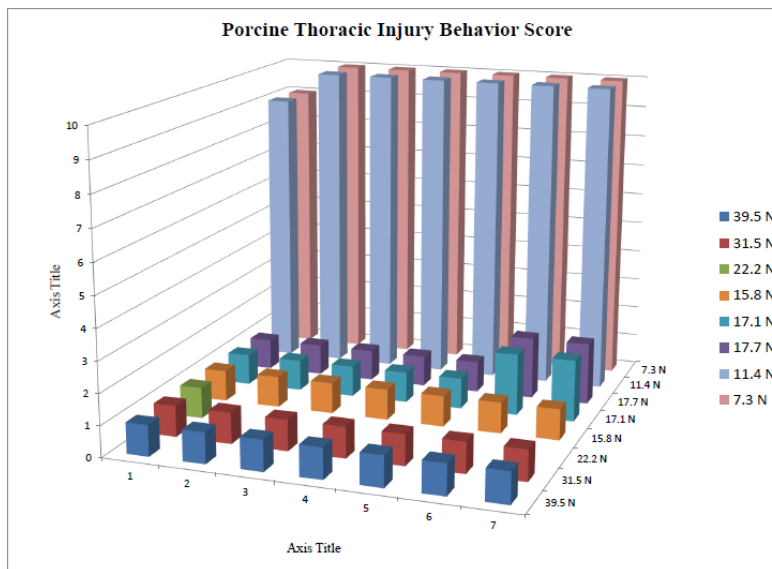


Figure 4. Bar graph showing the outcome of the Porcine Thoracic Injury Behavior Scale (PTIBS) neurobehavioral analysis during the 7 day post-operative period. Again, animals with higher energy impact to the spinal cord had worse neurological function, as evidenced by the lower PTIBS scores. Also, some animals recovered some function after 6 to 7 days, likely due to spinal shock.

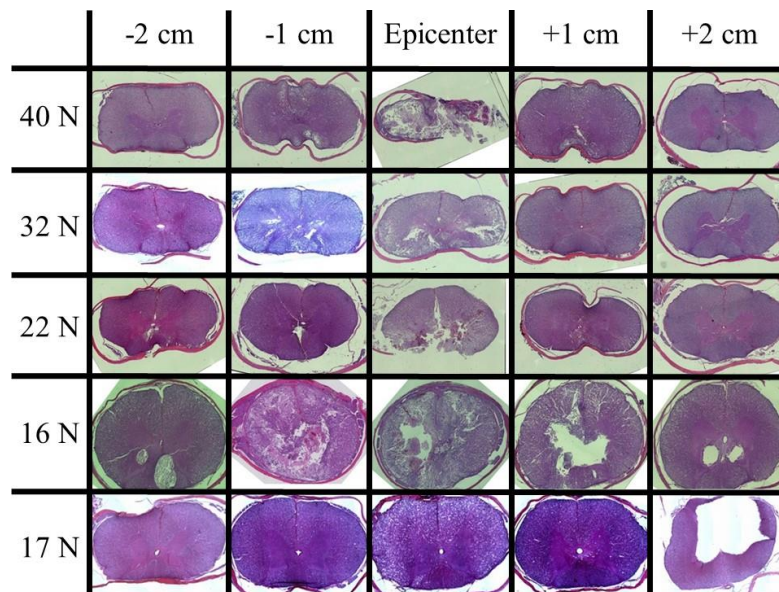


Figure 5. Chart showing the outcomes of histopathological staining of the spinal cord specimens. Of note is the increasing severity of spinal cord injury and the tissue disruption as the energy of impact increases. Injury also extends further from the epicenter as the energy of impact increases.

Specific Aim 3 – Develop and validate a postoperative assessment plan for this animal model.

For the post-operative assessment we relied on magnetic resonance imaging (MRI) of the spinal cord at day 1, neurobehavioral testing for 7 days, and histology after the animals were sacrificed. MRI has been used in clinical and animal studies to assess the severity of spinal cord injury. We found that the volume of abnormal T2 signal within the spinal cord directly correlated with the impact force and degree/duration of compression (figure 2). While we may explore other modalities of advanced imaging, including MRI, for future experiments, the sagittal T2 MRI will undoubtedly be a cornerstone of the post-injury assessment in this or any similar models.

We also performed histopathological staining following animal sacrifice at 7 days post-injury. We were able to show a reliable gradient in the severity of spinal cord injury as contusion force and degree/duration of compression increased. The histopathological analysis was complicated by delays with processing and digitally analyzing tissue, but we were indeed able to demonstrate the expected changes in injury severity (figure 5). In future experiments, we will add immunohistochemistry to show the relative degree of neuronal apoptosis, demyelination, inflammation, and other effects of spinal cord injury.

Finally, we used two validated neurobehavioral scores for assessing the clinical severity of spinal cord injury in the Yucatan miniature swine. Both scales also showed a direct correlation with contusion force and degree/duration of balloon compression and severity of injury (figure 3&4). However, it is likely that our short recovery time (7 days post-injury) caused us to overestimate the severity of spinal cord injury and did not allow for a period of “spinal shock” to abate. Most of the animals in our study showed signs of complete (ASIA A) or no (ASIA E) spinal cord injury. There were very few animals in the incomplete (ASIA B-D) category. This may be due

to a period of “spinal shock” during which the injury appears more severe than it is. Allowing for a longer recovery would get past the period of “spinal shock” and potentially give a more accurate representation of the true injury. Furthermore, because the scales we used assess motor function, other modalities, such as sensory function, bowel/bladder function, and coordination, were not directly assessed. Other methods to assess these spinal cord functions will be used in future experiments.

Specific Aim 4 – Utilize this validated model to address future specific aims.

We have identified several future aims that we will address using the model that we successfully validated in the experiments under this grant. The first of these aims will be to improve upon our current model with additional technologies and methods, some of which are described above. In future experiments we will utilize a longer post-injury recovery period to allow for recovery from spinal shock. We will also enhance our post-operative assessment using advanced MRI modalities that can map white matter tracts within the spinal cord, and we will perform additional immunohistochemical staining to further define the degree of spinal cord injury.

We will also seek to further validate the model by defining the role of sustained compression in burst fracture induced spinal cord injury. We will use a larger group of animals to determine if there is a critical threshold for degree and duration of compression, which could help to inform the clinical treatment of these injuries.

We will also use this model to investigate physiological factors of spinal cord injury and various treatment modalities intended to limit SCI in clinical situations. Included among these treatments are hemodynamic support, membrane stabilization, and neuronal regeneration. We will investigate the effects of various vasoactive medications on injured tissue in the spinal cord and we will test therapies that have shown promise in small animal laboratory experiments.

4. Impact

Impact to principal discipline: In these experiments we have successfully demonstrated the feasibility of a ventral model of thoracolumbar spinal cord injury (contusion and compression) in a large animal species. This is the first such model of this type of injury. In developing this model, we have designed, built and tested the first fully automated and precision controlled spinal cord impactor. This model and impactor could be used in the future to further define the pathophysiology of spinal cord injury, and is the only model that could be used to investigate ventral thoracolumbar impacts. These types of injuries are often seen in blast events and other traumatic events. We will further define this model and impact device to develop a highly reliable and predictable model of these ventral thoracolumbar injuries.

Impact on other disciplines: The technology of our impactor could be modified with relatively simple changes and be adapted for other purposes. These could include brain injury studies and other models of spinal cord injury, i.e. dorsal contusion, cervical spine, etc. This impactor is the only large animal impactor that reproduces the events of acute contusion type spinal cord injury.

Impact on technology transfer: It is not clear at this point whether any technology developed in our model will be transferred for any other purposes. However, the techniques and procedures

we have developed may be used by other investigators to develop other large animal models of spinal cord injury.

Impact on society beyond science and technology: This project will have minimal impact outside of science and technology.

5. Changes/Problems

Changes in approach: As this model is the first of its kind, and this project was designed to demonstrate the feasibility of the model, we have had to make several changes during our study in order to successfully develop the model. These changes have primarily been related to our surgical techniques, custom equipment, and post-operative animal monitoring and care. Because of the novelty of this model, there was significant trial and error in the early stages. We made several modifications to our surgical technique and custom designed equipment to accommodate unforeseen challenges during the surgical experiments. These were mainly minor technical changes including the target vertebra (L2 vs. L1), design of retractor blades, methods to control epidural bleeding, and methods to place and constrain the balloon catheter. At the advice of our veterinary colleagues we also made some minor changes to the post-surgical animal care. The primary analgesia was changed from fentanyl patch to IM buprenorphine because the patches did not stick to the animal hide. We also performed routine skin care, bladder expression, and bowel regimen (glycerin suppository) when necessary. None of the changes represent a significant departure from our initial proposal, but they are necessary modifications to develop and refine this novel model and custom spinal cord impactor.

Delays and actions to resolve them: The biggest delay in our initial experience involved the analysis and data processing of our pathology slides. Our lab has extensive experience with harvesting and processing spinal cord tissue from many rat models that we have developed. However, the amount of tissue obtained from the pig spinal cords presented a challenge in processing, staining, and analyzing all of the specimens. We especially encountered difficulty when attempting to digitize and analyze the tissue samples. The digital files were so large that they took an unexpectedly large amount of time to process and analyze. Indeed we ultimately had to enlist the expertise of a colleague in our department who helped develop a custom computer program to perform quantitative analysis of the tissue slides for signs of spinal cord injury.

Expenditures: No changes.

Changes in use of animals: No changes.

6. Products

Publications

Journal publications

- 1) **Petteys RJ**, Spitz SM, Snyder R, Rice RA Syed H, Goodwin CR, Sarabia-Estrada R, Sciubba DM, Freedman BA: Design and development of a controlled spinal cord impactor for use in large animal models of acute traumatic spinal cord injury. **JNS: Spine**: submitted on-line, under review.
- 2) **Petteys RJ**, Spitz SM, Snyder R, Rice RA Syed H, Goodwin CR, Sarabia-Estrada R, Sciubba DM, Freedman BA: Development of a novel large animal model of acute traumatic ventral spinal cord injury. **Spine (Phila. 1976)**: submitted on-line, under review.

Conference presentations

- 1) **Petteys RJ**, Spitz SM, Rice RA Syed H, Sarabia-Estrada R, Sciubba DM, Freedman BA: Design and development of a controlled spinal cord impactor for use in large animal models of acute traumatic spinal cord injury. *Military Health Systems Research Symposium*, August 19, 2014, Fort Lauderdale, Florida.
- 2) **Petteys RJ**, Spitz SM, Rice RA Syed H, Sarabia-Estrada R, Sciubba DM, Freedman BA: Development of a novel large animal model of acute traumatic ventral spinal cord injury: initial experience. *Military Health Systems Research Symposium*, August 2014, Fort Lauderdale, Florida.
- 3) **Petteys RJ**, Spitz SM, Rice RA Syed H, Sarabia-Estrada R, Sciubba DM, Freedman BA: Design and development of a controlled spinal cord impactor for use in large animal models of acute traumatic spinal cord injury. *Congress of Neurological Surgeons Annual Meeting*, October 2014, Boston, Massachusetts.
- 4) **Petteys RJ**, Spitz SM, Rice RA Syed H, Sarabia-Estrada R, Sciubba DM, Freedman BA: Development of a novel large animal model of acute traumatic ventral spinal cord injury: initial experience. *Congress of Neurological Surgeons Annual Meeting*, October 2014, Boston, Massachusetts.
- 5) **Petteys RJ**, Spitz SM, Sarabia-Estrada R, Syed H, Rice RA, Sciubba DM, Freedman BA: Validation of a novel animal model of thoracolumbar burst fracture-induced spinal cord injury – the impactor study phase. *Orthopedic Research Society Annual Meeting*, March 2015, Las Vegas, Nevada.
- 6) **Petteys RJ**, Spitz SM, Snyder R, Sarabia-Estrada R, Sciubba DM, Freedman BA: Development of a novel large animal model of acute traumatic ventral spinal cord injury: initial experience. *14th Annual AO Spine North America Fellows Forum*, April 1, 2016, Banff, Alberta, Canada.

Technologies and techniques: None

7. Participants & Other Collaborating Organizations

Individuals working on the project

Pis

Brett A. Freedman, MD, LTC MC

Dr. Freedman supervised all aspects of this project including design, execution, and data analysis.

Daniel M. Sciubba, MD

Dr. Sciubba supervised all aspects of this project including design, execution, and data analysis.

Key Personnel

Name: Rory J. Petteys, MD

Role: Lead investigator

Identifier:

Person months worked: 24

Contribution: Dr. Petteys performed all animal experiments and supervised all data collection. He contributed to the animal care and acquisition and analysis of MRI and histological data.

Funding Support: N/A

Name: Rachel Sarabia-Estrada, DVM, PhD

Role: Assistant investigator

Identifier:

Person months worked: 6

Contribution: Dr. Sarabia-Estrada assisted with all aspects of animal care and supervised the preparation and analysis of histological data.

Funding Support:

Name: Steven M. Spitz, MD

Role: Assistant investigator

Identifier:

Person months worked: 6

Contribution: Dr. Spitz assisted with all animal experiments and helped supervise animal care and data collection.

Funding Support:

Change(s) in active/other support of Pis / key personnel

No changes

Other Organizations Involved

None

8. Special Reporting Requirements

9. Appendices