Effects Of Neuron Loss On The Development Of Neuropathic Pain Following Cervical Spinal Cord Injury

Katelyn Knox[1,3], Valerie Dietz[1,2], Sherilynne Moore[1], Nolan Roberts[1,3], Jennifer N. Dulin[1,4]

[1]Department of Biology, [2]Biology Graduate Program, [3] Biology Honors, [4]Texas A&M Institute for Neuroscience; Texas A&M University

Goal of this Study

We hypothesize that the loss or sparing of dorsal horn neuronal tissue is a reliable predictor of chronic sensory hypersensitivity in rodents after spinal cord injury.

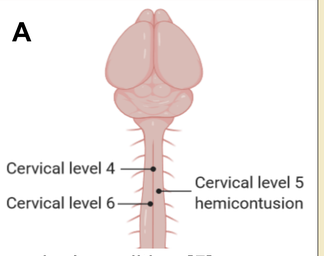
Background

- **Spinal cord injury (SCI)** is a traumatic event that frequently results in immediate and permanent loss of neurologic function, including the development of neuropathic pain at and below the level of injury.
  - SCI affects over 350,000 individuals in the US [1], and more than half of individuals with SCI experience severe or agonizing neuropathic pain [2,3].
  - The most common form of injury in humans is a contusion to the cervical spinal cord [4]. Cervical hemicontusion injury in rodents is an established model to study pain mechanisms [5,6]. This model focuses on sensory signaling neurons of the dorsal horn which are responsible for transmitting sensory signals from the periphery to the brain [7].

Methodology

Spinal Cord Injury Model

Unilateral cervical (C5) spinal cord hemicontusions were delivered to the right hemicord of adult, female C57BL/6 mice using an Infinite Horizon spinal impactor device (0.7 mm diameter probe, 40 kdynes, 2 s dwell). Laminectomy only surgeries were performed at C5 for the control (sham) surgical condition [5].



Mechanical Sensitivity Testing

Sensitivity of the forepaws and hindpaws to mechanical stimulation was assessed using the electronic von Frey system (Bioseb). Baseline testing was performed once weekly for 2 weeks and animals were tested weekly until 28 days post-injury (DPI).

Immunohistochemistry

Animals were sacrificed 4 weeks post-injury, and serial 20-µm transverse sections of cervical spinal cord were collected and used for immunohistochemical analysis. Primary antibodies against NeuN, GFAP, CGRP, collagen1α1 and DAPI were used. Tissue sections were imaged on a Nikon Eclipse fluorescent microscope.

Image Analysis & Statistical Analysis

Four regions of interest encompassing the dorsal and ventral aspects on both sides of the tissue section were drawn. Image J was used to hand count individual neurons expressing NeuN within each region as well as quantify the total volume.

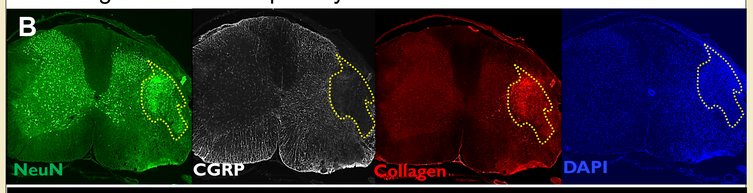


Figure 1. (A) Diagram of mouse brain and spinal cord to illustrate hemi-contusion at cervical level 5/6. **(B)** Representative image of the spinal cord lesion epicenter at 28 days post-cervical hemicontusion. Location of the lesion site is shown with dotted lines.

Results

Development of Mechanical Sensitivity by 28 Days Post Injury

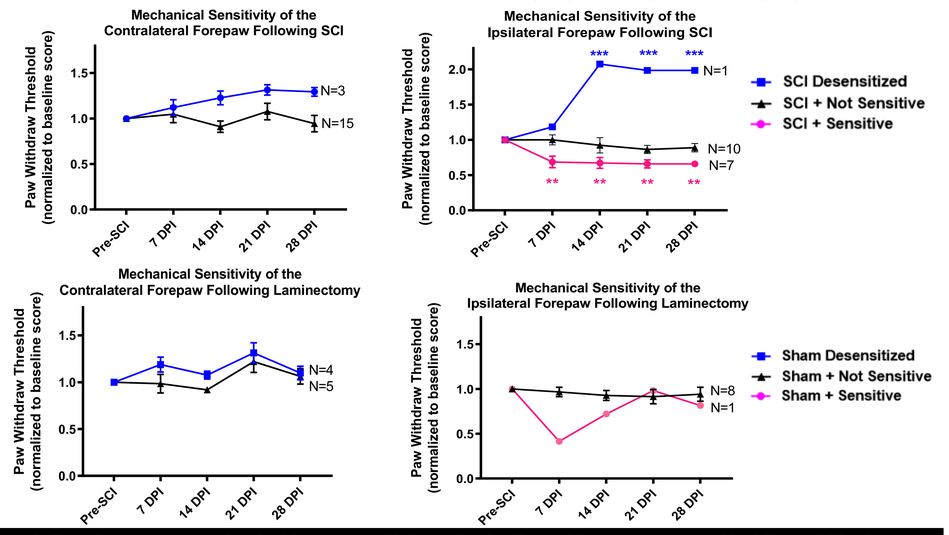


Figure 2. Development of mechanical hypersensitivity of the ipsilateral forepaw occurred in 38% of SCI mice.
Mechanical sensitivity data for N=18 SCI mice (top) and N=9 laminectomy (bottom) at 7, 14, 21, or 28 days post-SCI, normalized to baseline scores. Animals were stratified into pain groupings if they scored above/below their normal range 3 out of 4 time points. Normal range was determined by calculating baseline score +/- standard deviation for each animal independently. Mean ± SEM, **p<0.01, ***p<0.0001 by two-way ANOVA + Sidak's multiple comparisons test.

Cell Counting of Neurons by Quadrants in Cervical Spinal Cord

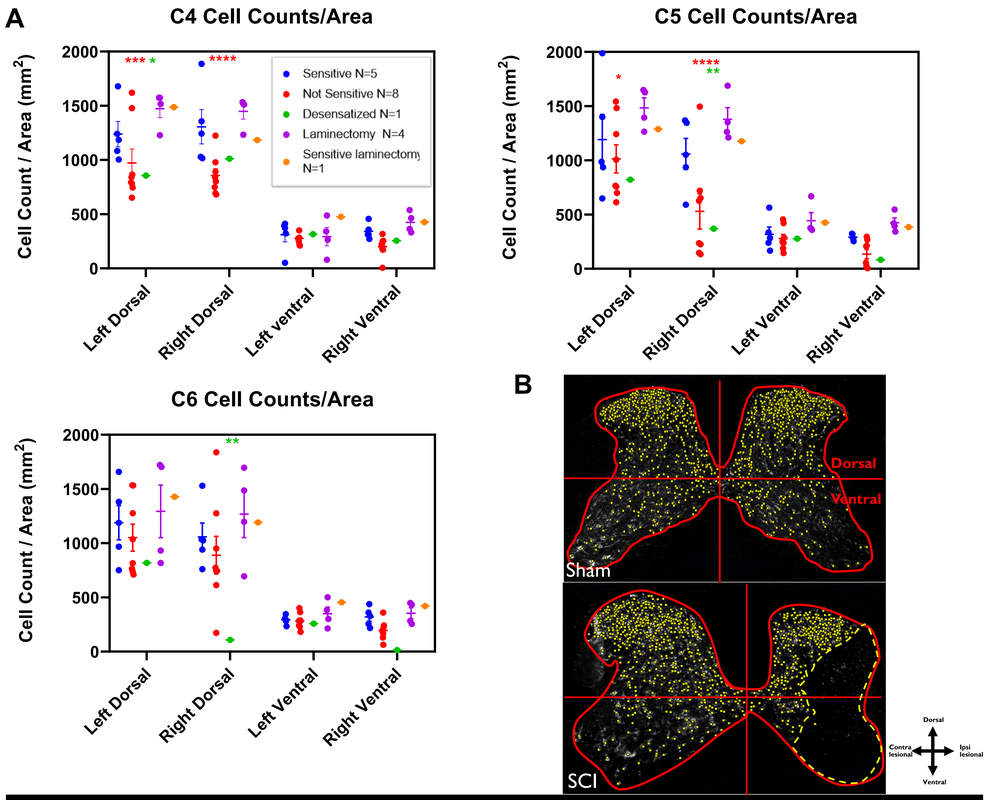


Figure 3. Quantification of neuronal sparing in dorsal and ventral regions across pain states. (A) Quantification of neuronal sparing at C4, C5, and C6. Cell counts were normalized to area and grouped into pain states based on mechanical sensitivity data (data shown in fig 2). Mean ± SEM, N=5 SCI sensitivity, N=8 SCI not sensitive, N=1 SCI desensitized, N=4 laminectomy, N=1 laminectomy sensitive, * p<0.05, **p<0.01, ***p<0.0001, ****p<0.00001 by two-way ANOVA + Dunnett's multiple comparisons test using laminectomy as comparison control. **(B)** Transverse spinal cord tissue from laminectomy (top), and SCI (bottom) animals with representative cell counting and quadrant regions of interest. Contusion area outlined in yellow dotted line.

Ongoing Work

Correlation of Tissue Displacement Neurological Outcomes

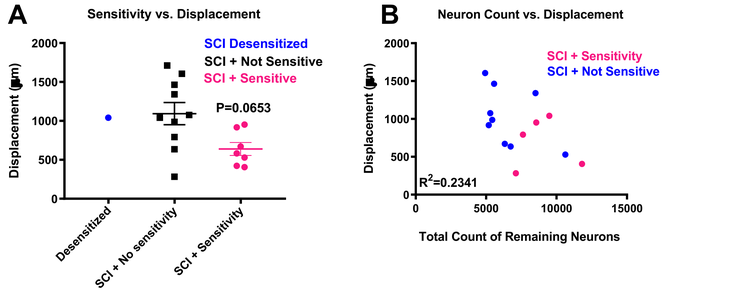


Figure 4. Lesion analysis using tissue displacement and total neuronal counts.
(A) Lower tissue displacement at time of impact in animals with post-SCI ipsilateral forepaw mechanical sensitivity. Mean ± SEM by one-way ANOVA + Tukey's multiple comparisons test.
(B) There is no clear correlation between the displacement value and the number of remaining neurons by Pearson r correlation analysis. N=5 SCI + Sensitivity, N=9 SCI + Not Sensitive.

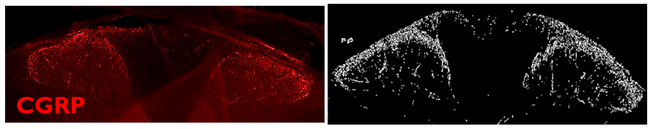


Figure 5. CGRP quantification by laminae. Calcitonin gene-related peptide (CGRP) staining localized in dorsal horn will be quantified using ImageJ. CGRP+ pixels were autothresholded (right) and the thresholded pixels will be quantified for spinal levels C4-C6.

Conclusions & Future Work

- This injury model produced development of neuropathic pain of the ipsilateral forepaw in 38% of injured mice.
- Overall, there was a significant loss of neurons in the left and right dorsal regions of not sensitive animals in C4 and a significant loss in right dorsal region at the C5 lesion epicenter for not sensitive animals when compared to the laminectomy and sensitive animals.
- Lower tissue displacement value is predictive of the development of mechanical sensitivity.
- Gaining a better understanding of the anatomical basis underlying the variability in development of sensory dysfunction after SCI will lead to the development of more detailed understanding of pain mechanisms and neural circuits.

Acknowledgments

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