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

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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 agorizing neuropathic pain [2,3].
- The most common form of injury in humans is a contusion to the cervical spinal cord [4]. Cervical hemiconfut 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 hemiconfutions 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 kdyne, 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 per week 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, collagen Iα1, 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.

Ongoing Work

Correlation of Tissue Displacement Neurological Outcomes
- 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 Sensitivity.

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 the rats not sensitive animals in C5 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.

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References