Model development of a T8 rat contusion model: different lesion severities elicited distinct locomotor and sensory profiles

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INTRODUCTION

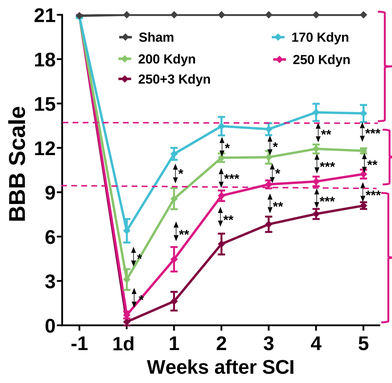
- Consistent, reliable preclinical spinal cord injury (SCI) models are critical to advance and understand the impact of potential therapeutics
- Establishing a SCI lab (for non-SCI academicians or industry) is challenging and expensive endeavor requiring specific surgical and behavioral assessment skills
- This can deter lab or industry sponsors and investors from entering the SCI space
- Establishing an SCI focused contract research organization (CRO) can address these issues by implementing standard, consistent injury models and reliably assessing outcomes may address some of these challenges
- This will ultimately decrease the cost and time to evaluate novel therapeutics with valid comparisons to other interventions
- **Therefore, in collaboration with the Christopher & Dana Reeve Foundation and Drexel University, PsychoGenics validated a graded thoracic (T8) SCI contusion lesion model.**

METHODS

- **Subjects & Surgeries.** Adult, female, Sprague Dawley rats (200-220 grams, Envigo, n=15/group) underwent laminectomy followed by T8 contusion SCI using the IH impactor with forces of 170, 200, 250 Kdyn, or 250 Kdyn with a 3-second dwell time. Measures of general health were recorded over time including body weight, bladder function recovery, urinary and health complications.
- **Functional recovery** was assessed over five weeks using standard locomotor tests: Basso, Beattie, Bresnahan locomotor rating scale BBB (Basso, Beattie, Bresnahan 1995), horizontal ladder test (Metz and Wishaw, 2009) and PsychoGenics' proprietary gait analysis system, NeuroCube® (NC). Changes in mechanical and thermal sensation were evaluated using Von-Frey and Acetone tests.
- **Sample Collection & Immunohistochemistry.** Plasma samples were collected at baseline, 1 day, 1 and 6 weeks post SCI, and cerebrospinal fluid was collected at 6 weeks post-SCI only. Furthermore, 3 cm sections containing the lesioned spinal cord at its center were cryoprotected, sectioned and stained with fluoromyelin to evaluate spinal cord atrophy, lesion size, and spared white matter.
- **Statistics.** Swing duration, acetone test, and immunohistochemistry data were analyzed via one-way ANOVA followed by Tukey's post-hoc test. All other data were analyzed with two-way RM ANOVA (group x time) followed by Tukey's post-hoc test.

RESULTS

Gross Locomotion Decreased with SCI Severity



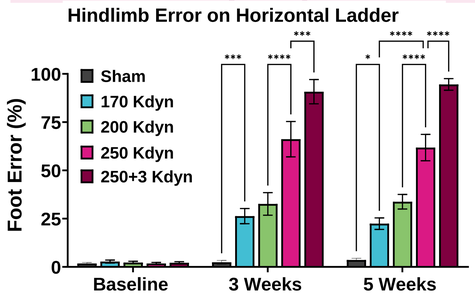
Scores from 13-21
Return of toe clearance, parallel paw position, trunk stability, elevated tail position.

Score from 9 -13:
Weight support, plantar placement, stepping, some coordination.

Score from 0-8:
Isolated movements of the 3 HL joints, HL dragging.

*n= 15/group. *p<0.05, **p<0.01, ***p<0.001.*

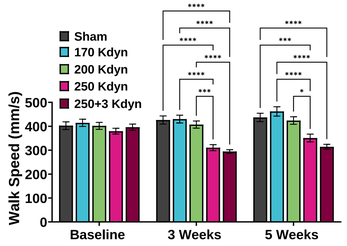
SCI Severity Impacted Sensorimotor Function



- Hindlimb footfall errors while crossing a horizontal ladder were quantified and represented as the percentage of the total number of steps.
- Notably, rats that do not plantar step were assigned a score of 100% error.

*n=15/group. *p<0.05, **p<0.01, ***p<0.001.*

Unbiased Quantification of Features of HL Locomotion Using NeuroCube®.



Walking Speed

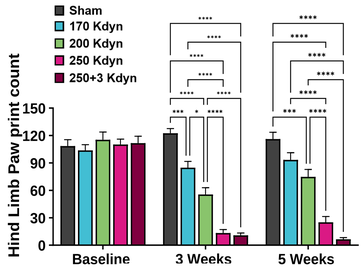
- Walking speed was not affected in rats with milder SCIS (170 Kdyn, 200 Kdyn).
- More severe SCI (250 Kdyn, 250 Kdyn + 3s) decreased walking speed compared to baseline, sham and mild SCI groups

*n= 15/group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

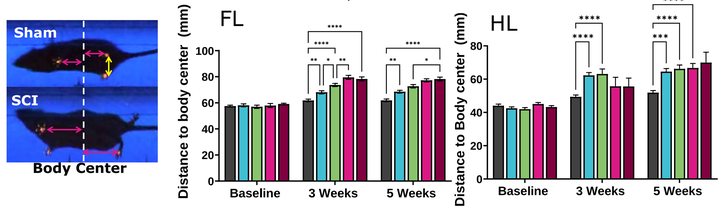
Frequency of Weight-Supported Steps

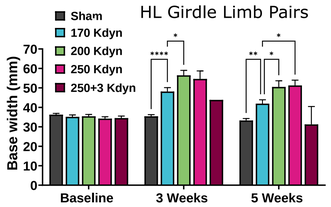
- Paw print count indicates the ability and frequency of SCI rats to take weight supported steps.
- HL paw print decreased as SCI severity increased.

*n= 15/group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*



Base of Support Across Ipsilateral and Girdle Limb Pairs

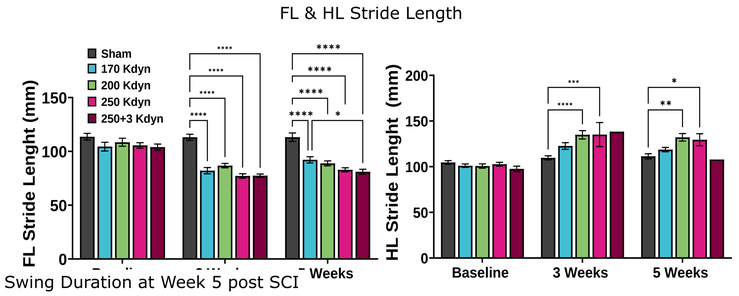


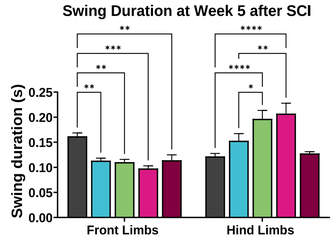


- Still image from video recording indicate Ipsilateral (pink) and girdle (yellow) measurements. Normally, the distance from the body center to the FL is ~60mm and ~40mm to the HL and body center. After SCI, this distance increases significantly.
- HL base of support increased in all SCI groups except for the most severe compared to baseline and sham
- Lack of HL stepping resulted in exclusion from analysis.

*Sham, 170 & 200kdyn groups: n= 15. 250 and 150+3s: n=1-5; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

Gait Geometry & Dynamics at Low-Walking Speed using NeuroCube®

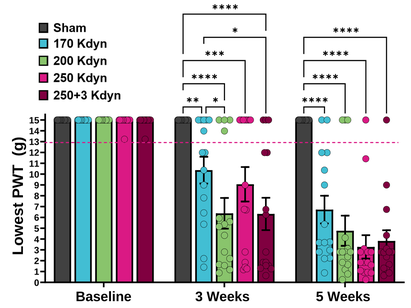




- HL step length increased, while FL stride length decreased in SCI rats as compared to Sham, with notable differences in the 200 and 250Kdyn groups.
- HL swing duration times were significantly increased and escalated with SCI severity in a graded manner. Conversely, FL swing duration decreased in SCI rats.

*Sham, 170 & 200kdyn groups: n= 15. 250 and 150+3s: n=1-5; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

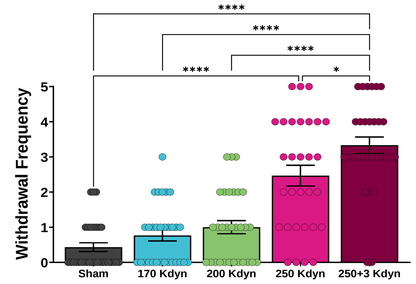
Neuropathic Pain Development after Graded SCI



Mechanical Allodynia (Von Frey test)

- All SCI severities decreased paw withdrawal thresholds to mechanical stimuli compared to baseline and sham.
- Importantly, hindpaw sensitivity was more pronounced with increased SCI severity.
- Some animals from each cohort did not show decrease in PWT, showing values of 13grms or higher.

*n= 15 *p<0.05, **p<0.01, ***p<0.001.*



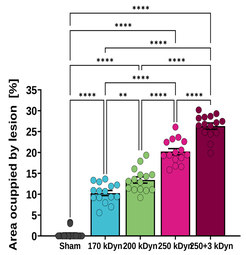
Cold Allodynia (Acetone Test)

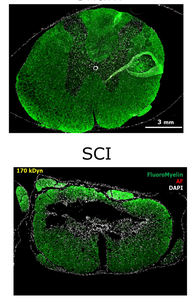
- Rats with milder lesions produced similar responses to acetone as sham rats, and rats with severe SCI displayed significantly increase response to acetone.
- Note that as SCI severity increases the number of rats with no response to acetone decreases.

*n= 30 paws tested /group *p<0.05, **p<0.01, ***p<0.001.*

Evaluation of Lesion and Spared Tissue

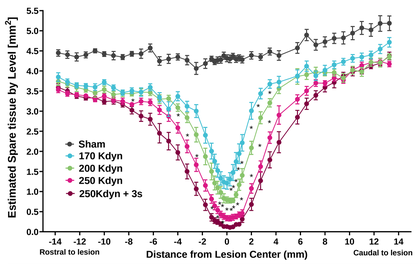
Lesion Area (%)



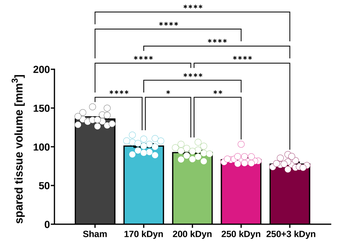


- A 3cm spinal cord segment containing the lesion epicenter was sectioned transversally and stained with flouromylein.
- As expected, lesioned area at the SCI epicenter increased with SCI severity.
- Similarly, the volume of spared whitematter decreased with SCI severity.
- Unlike lesion area, white matter sparing was not affected by the addition of a dwell time during the SCI surgery.

Spared Tissue Area by Level



Spare Tissue Volume



*n= 15/group. *p<0.05, **p<0.01, ***p<0.001.*

SUMMARY

The Psychogenics platform for thoracic SCI has the capability to run large studies, capturing many different aspects of recovery.

The data indicate that we can produce mild, moderate, and severe mid-thoracic SCI lesions, with consistent patterns of recovery reflecting lesion severity.

- Distinct locomotor profiles were produced, ranging from lack of HL weight support in the most severely lesioned cohorts to more subtle changes in walking parameters in less severely lesioned cohorts.
  - The BBB score, ladder test effectively detected significant differences in locomotion among the four SCI severity cohorts as well as with Sham controls.
  - The automated gait analysis provided valuable insights on finer details of locomotion produced by milder lesions.
- Distinct recovery profiles emerged depending on the modality of evoked sensory stimuli.
  - Responsiveness to cold stimuli increased with SCI severity
  - Hypersensitivity to mechanical stimuli was observed in all SCI groups compared to sham, and the incidence of mechanical allodynia increased with severity. Not all SCI rats developed hypersensitivity, even the most severely injured cohorts.

The battery of behavioral assessments available are sensitive to distinguish even small differences in lesion severity. The ability to detect small but functionally significant changes will improve the drug discovery pipeline for SCI therapeutics.

Since this SCI platform encompasses a broad range of functional recovery after SCI, it increases its usability in testing a wide range of drug applications.