

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

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

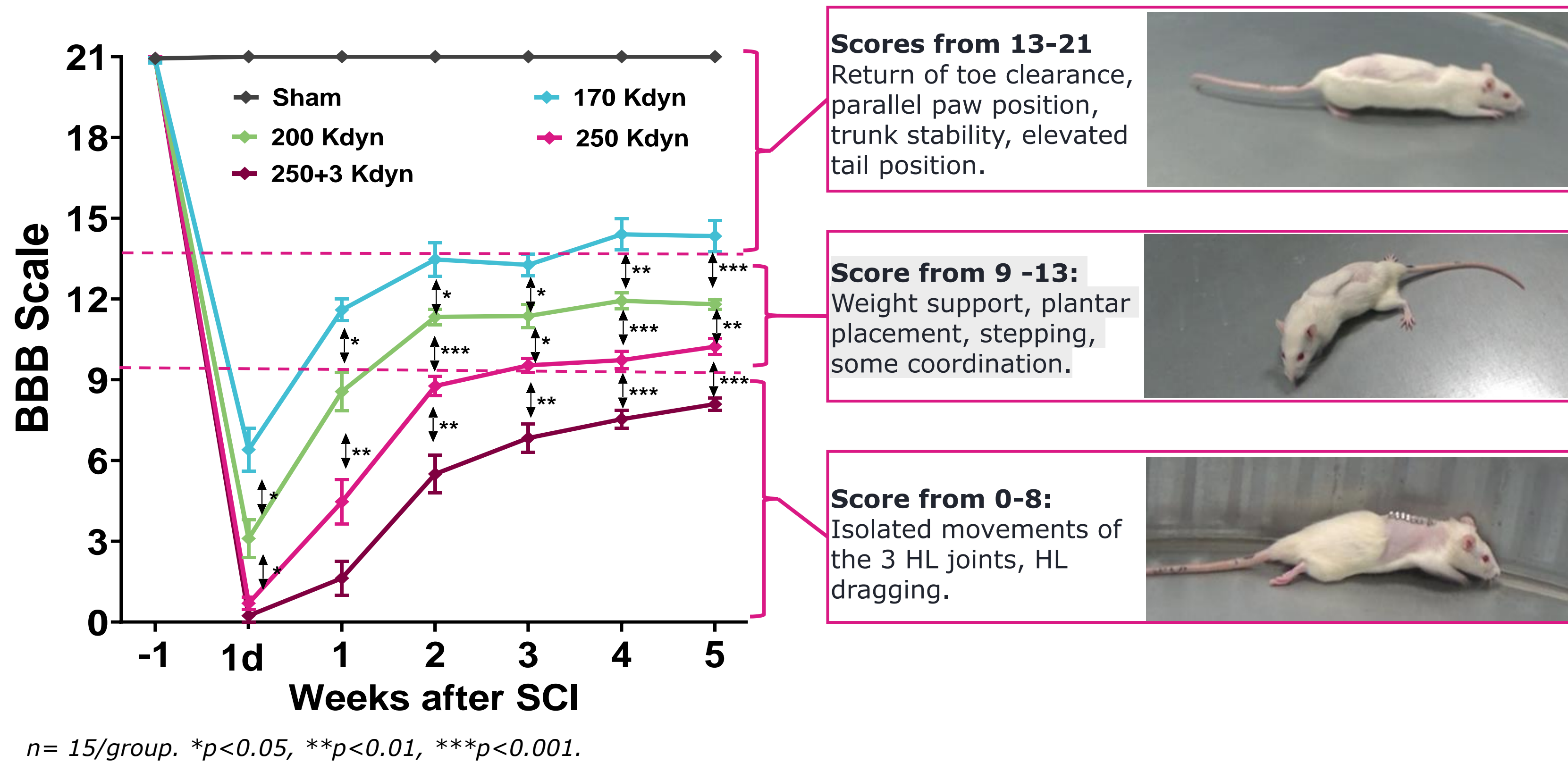
Therefore, in collaboration with the Christopher & Dana Reeve Foundation and Drexel University, PsychoGenics validated a graded thoracic (T8) SCI contusion lesion model.

METHODS

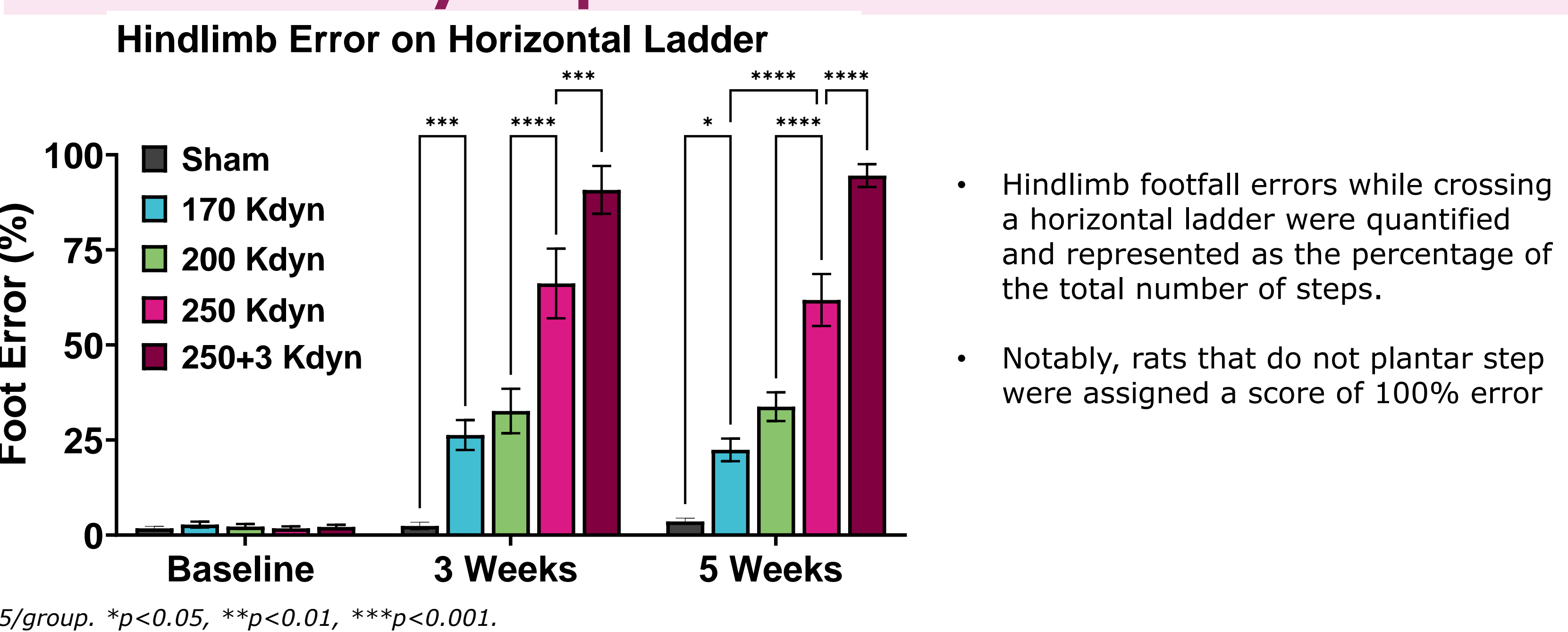
- Subjects & Surgeries.** Adult, female, Sprague Dawley rats (200-220 grams, Envigo, n=15/group) underwent laminectomy followed by T8 contusion SCI using the IH impactor with forces of 170, 200, 250 Kdyn, or 250 Kdyn with a 3-second dwell time. Measures of general health were recorded over time including 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

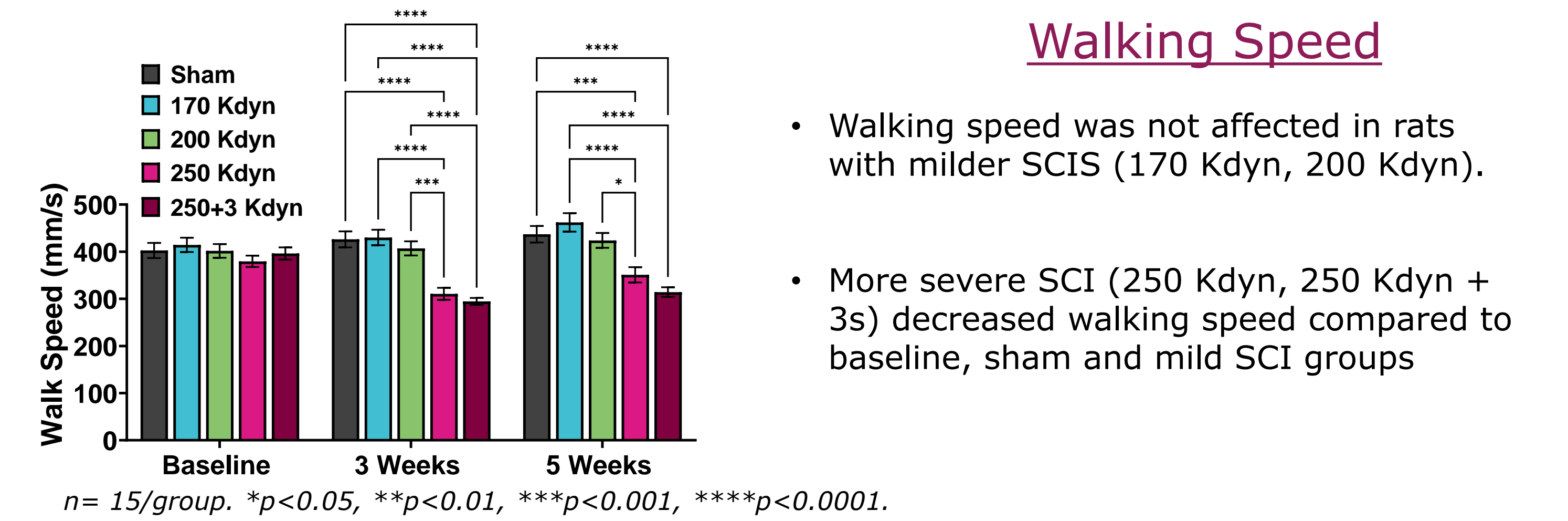
Gross Locomotion Decreased with SCI Severity



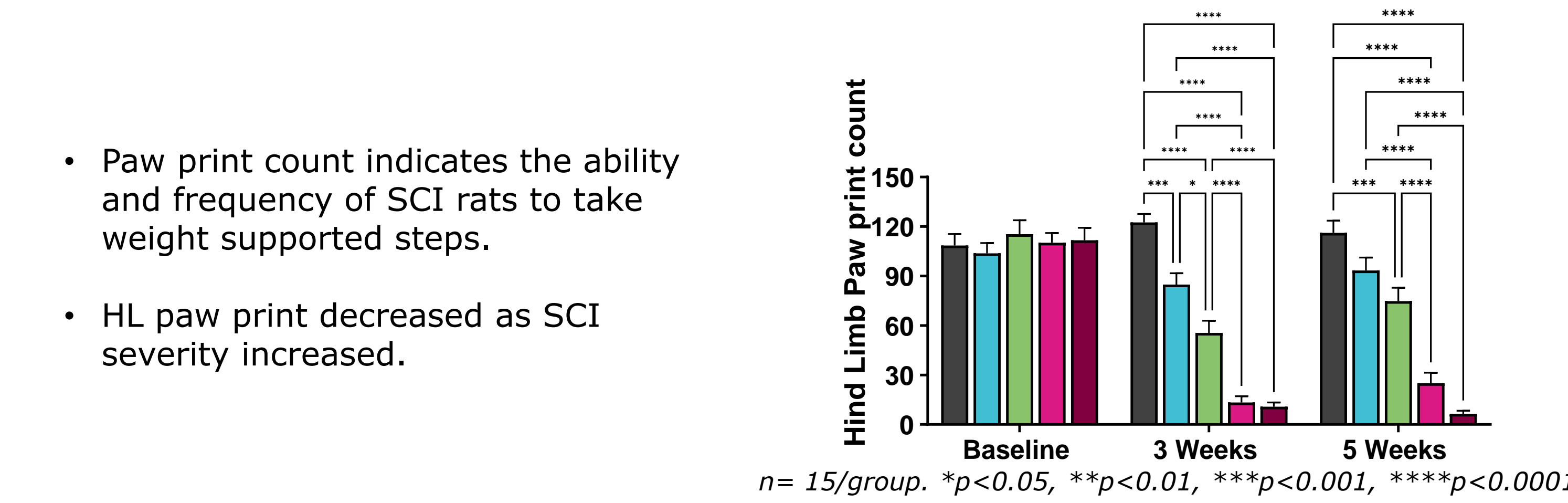
SCI Severity Impacted Sensorimotor Function



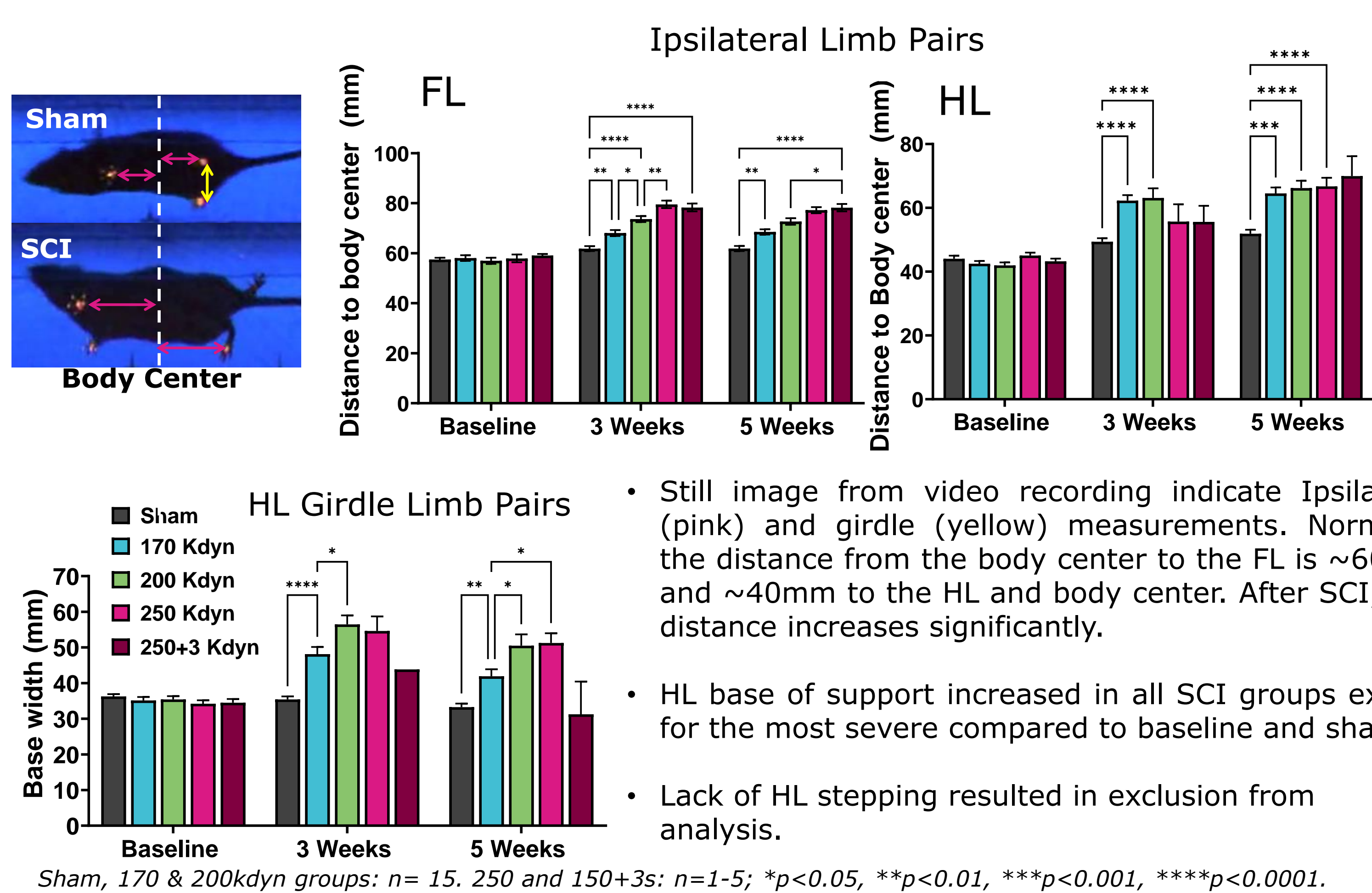
Unbiased Quantification of Features of HL Locomotion Using NeuroCube®.



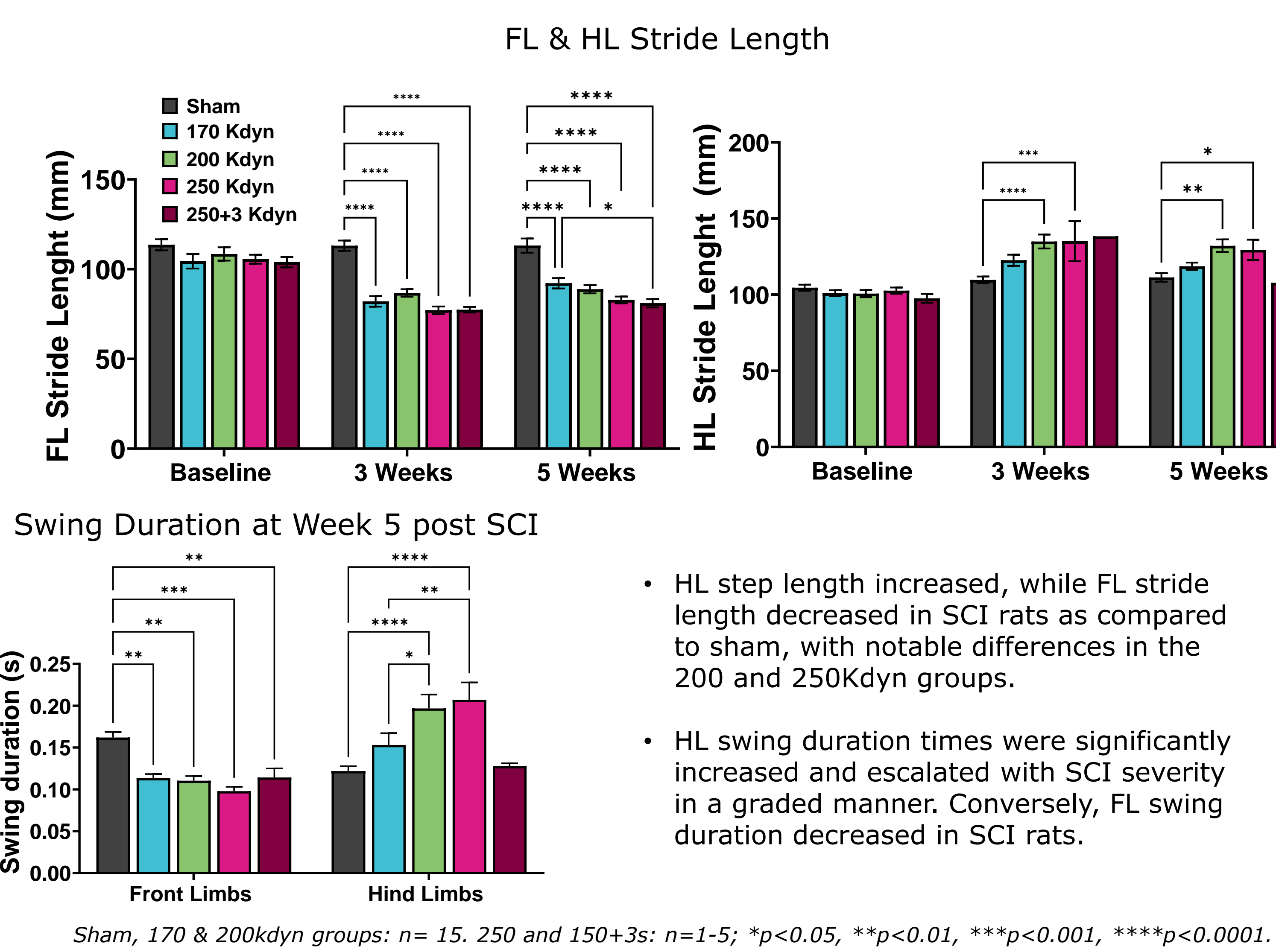
Frequency of Weight-Supported Steps



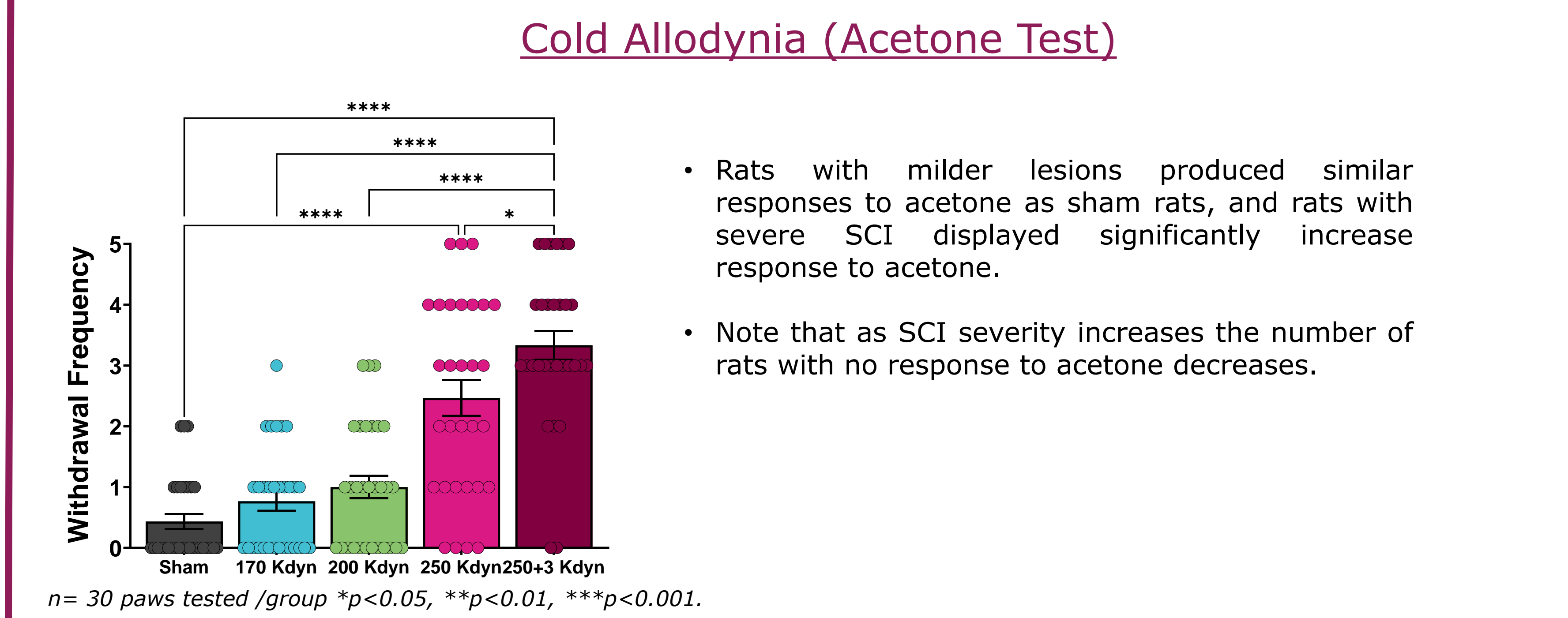
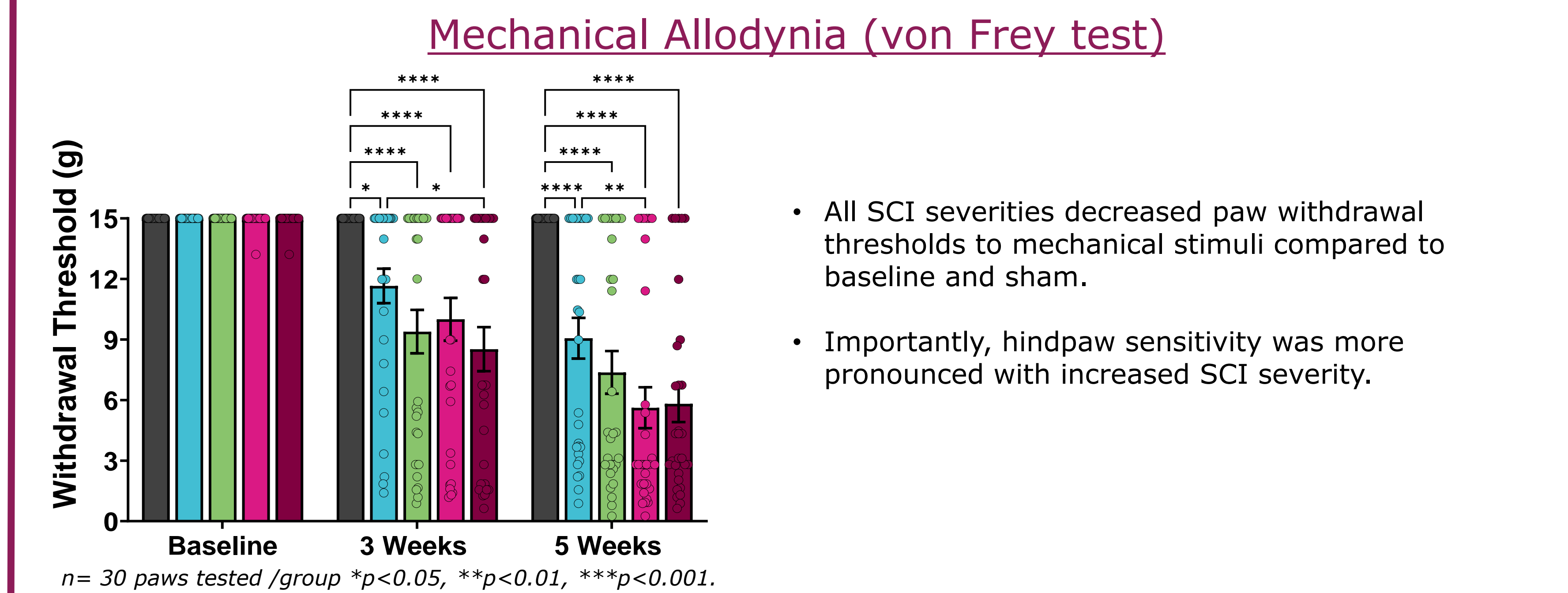
Base of Support Across Ipsilateral and Girdle Limb Pairs



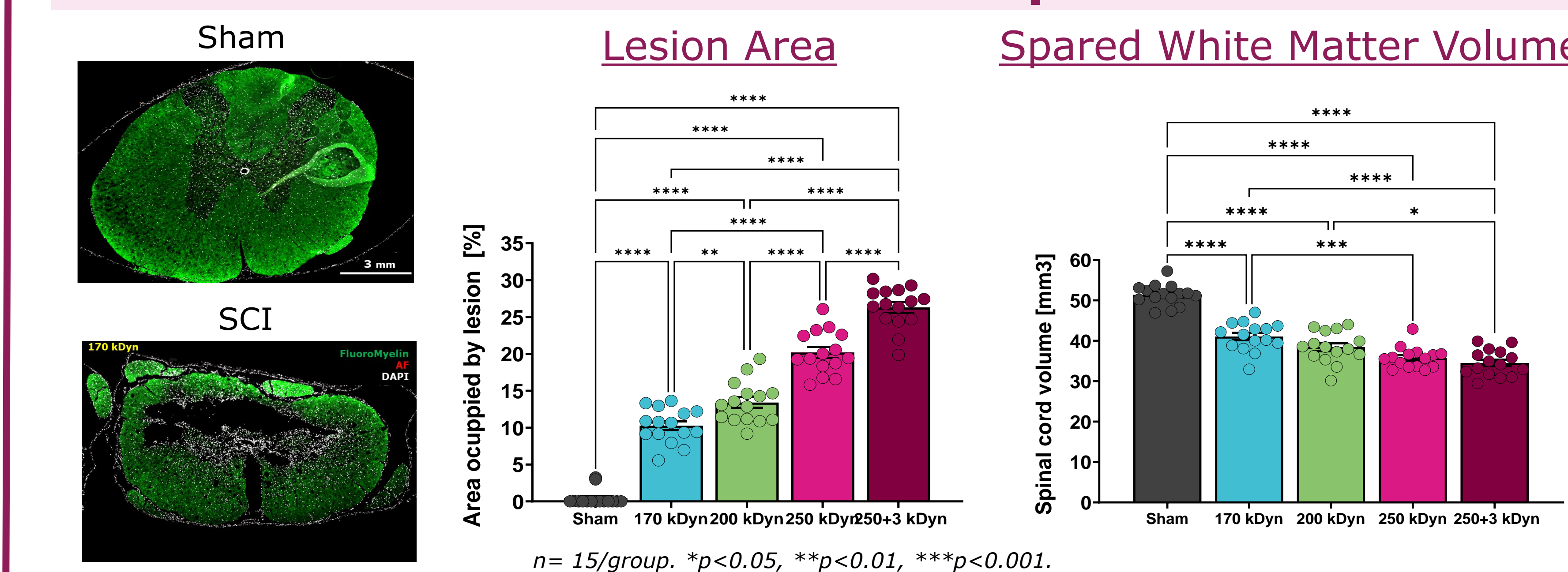
Gait Geometry & Dynamics at Low-Walking Speed using NeuroCube®



Neuropathic Pain Development after Graded SCI



Evaluation of Lesion and Spared Tissue



- A 3cm spinal cord segment containing the lesion epicenter was sectioned transversally and stained with fluoromyelin.
- 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.

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.