2-2021

Development of a method using retrograde neuronal tracers to neuroanatomically characterize respiratory neural circuitry

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Introduction

- There are over 270,000 Americans living with a spinal cord injury (SCI)
- The majority of SCI's are cervical
- Respiratory insufficiency is a common complication
  - Silencing & loss of phrenic motor neurons (PhMNs)
  - Loss of diaphragm function
- Significant contribution to morbidity & mortality
  - Increased risk of infection/pneumonia, pleural effusion, decreased cough reflex

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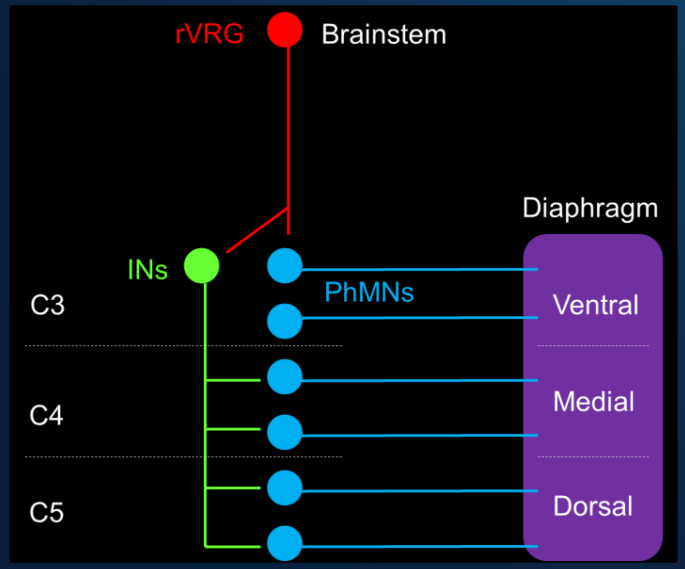
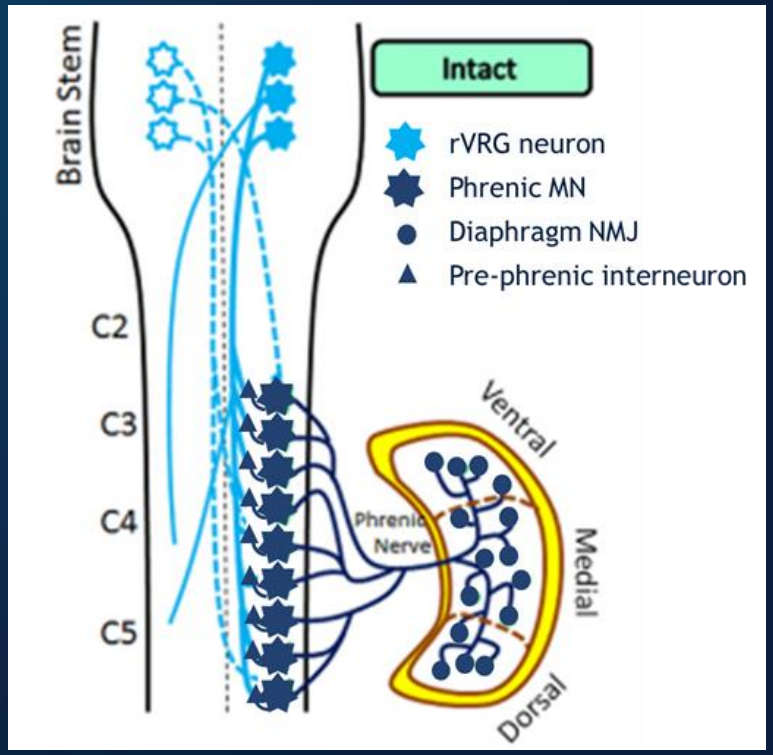
Introduction

Spinal cord has limited intrinsic capacity for recovery

- Several mechanisms
- Spinal interneurons contribute
- Little is known about pre-phrenic interneuron circuitry and remodeling





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Objectives & Hypothesis

- Research Question
  - Short term: How can we develop a method to further investigate the role and distribution of interneurons within the respiratory circuitry?
  - Long term: How is the respiratory circuitry remodeled following cervical SCI?

- Hypothesis
  - Pre-phrenic interneurons undergo changes in plasticity following SCI that allow their recruitment into new circuits with damaged rVRG axons to help drive respiratory recovery

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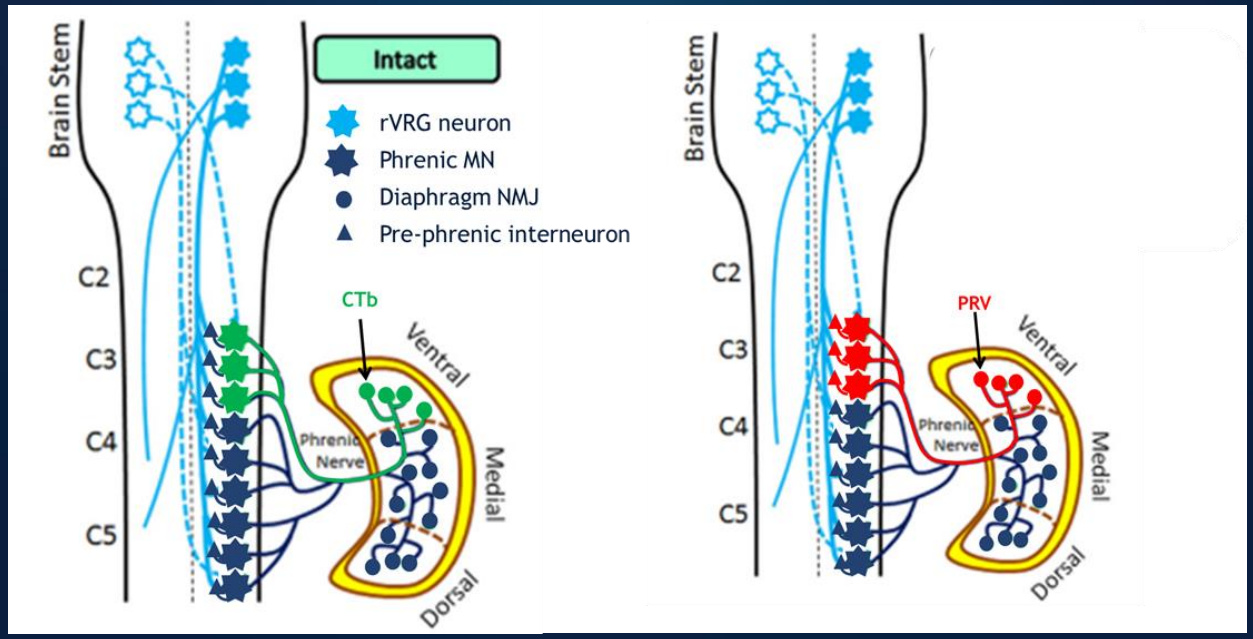
Approach: Validation of Method

- Rat model
- Injury model: C2 hemisection
- Fluorphore-conjugated neuronal tracers
- Sagittal SC tissue cuts – microscopy & quantification
- Examine normal distribution of pre-phrenic interneurons (intact SC)



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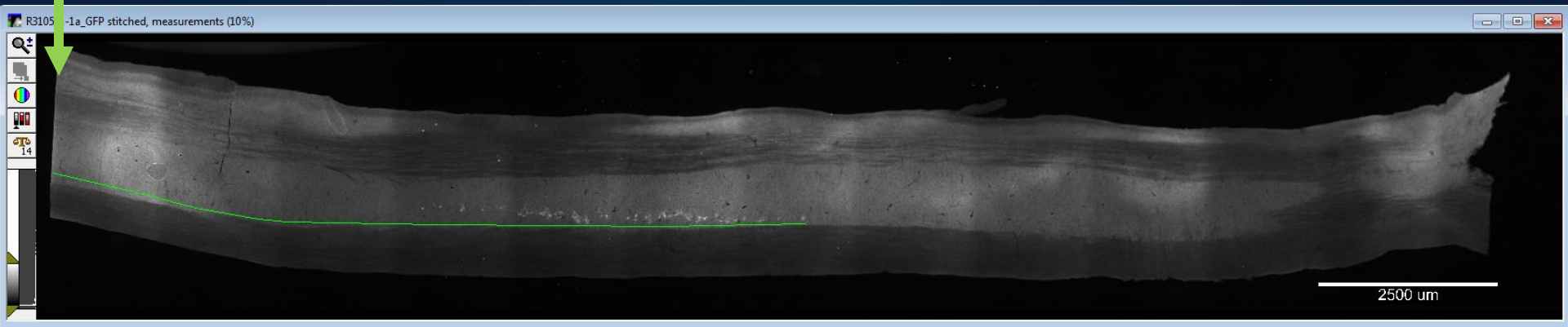
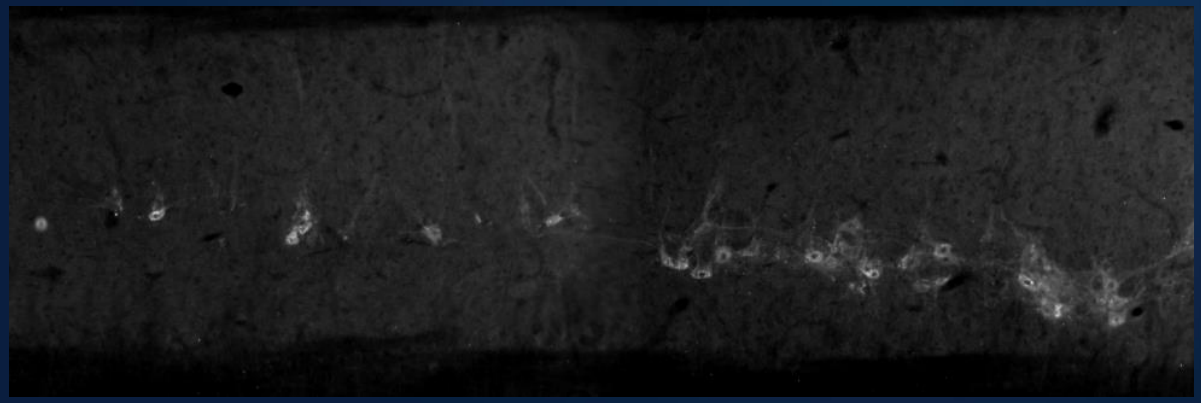
Analysis

Rostral
C2

Caudal

Example of distance measurements: C2 to last labeled cell
(also recorded C2 to first labeled cell)





Higher resolution of CtB
labeled cells

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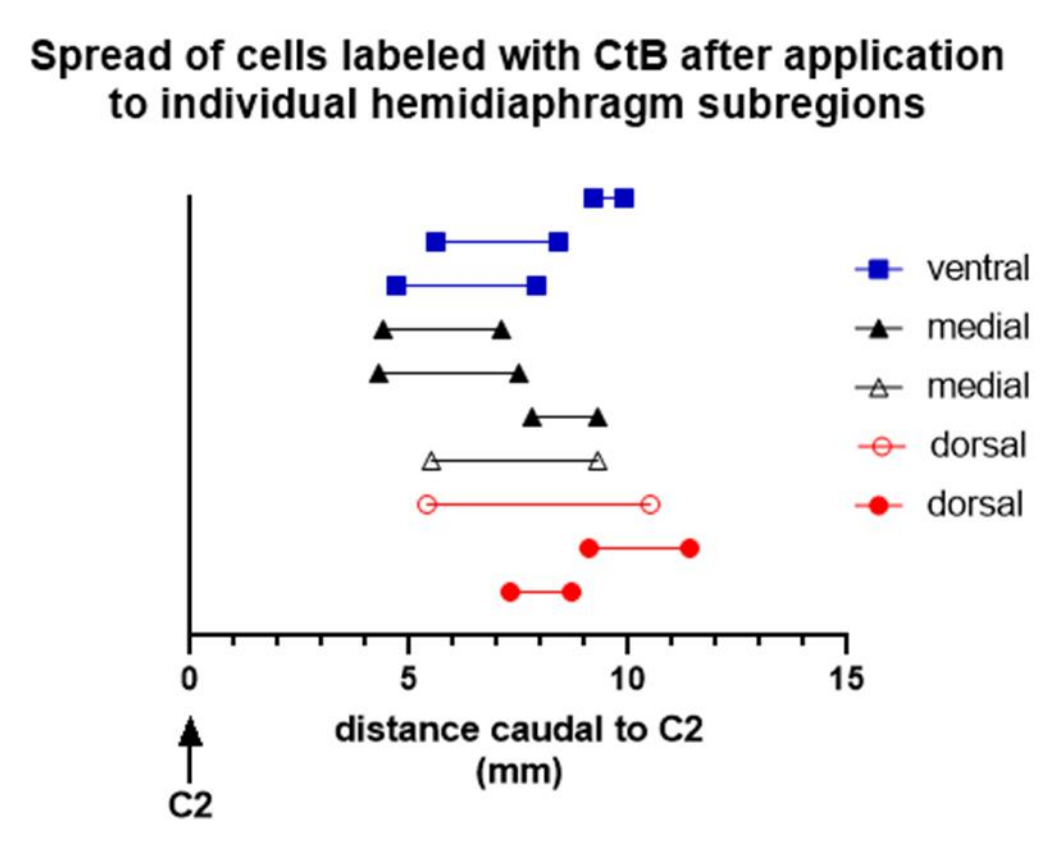
Results



- Each line is from one sagittal section
- Some animals had more than one section with labeled cells

Animals:
Ventral n=1 → C3
Medial n=2 → C4
Dorsal n=2 → C5

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Conclusions

- Retrograde neuronal tracers can be successfully used to label and characterize the neurons involved in the respiratory circuitry
- Innervation of the hemidiaphragm is distributed topographically
- Issues with keeping tracers within the borders of each subregion can be addressed by ensuring the diaphragm is dry before application

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Future Directions

- Apply method to SCI model
- Examine changes following injury
- Compare number of interneurons involved in the circuitry of each hemidiaphragm subregion in injured vs. uninjured animals
- Can this process be augmented?

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Acknowledgements

Thank you!

Lepore Lab

- Angelo Lepore
- R. Vivian Allahyari
- Eric Brown
- Nicolette Heinsinger
- Lan Cheng
- Biswarup Ghosh
- David Jaffe