# Selective Innervation of Upper and Lower Thoracic Spinal Segments by Medullary Raphe Neurons in Felines

*Laura Carlino, Sarah W. Ogburn, Michael F. Gowen, and Bill J. Yates*

Departments of Otolaryngology and Neuroscience, University of Pittsburgh, Pittsburgh, PA

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## INTRODUCTION

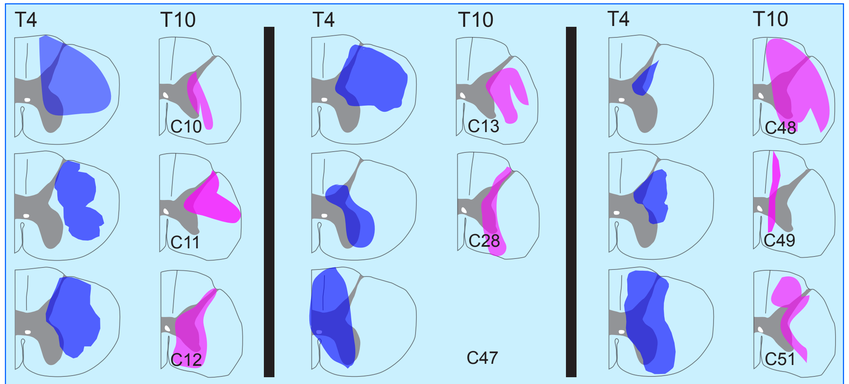
Vestibular stimulation elicits distinct changes in blood flow to the forelimb and hindlimb, showing that the sympathetic nervous system has the capacity to independently regulate blood flow to different body regions. While neurons in the rostral ventrolateral medulla that project to different levels of the spinal cord may contribute to this anatomical patterning of blood flow, other brainstem regions that influence sympathetic outflow could also elicit different patterns of blood flow in the upper and lower body.

In cats, neurons located in the raphe nuclei (particularly raphe pallidus) participate in cardiovascular regulation. These neurons exhibit cardiac-related activity, sometimes combined with 10-Hz activity, and raphespinal neurons that are excited by baroreceptor stimulation project to the intermediolateral cell column. However, the branching patterns of raphespinal neurons to the upper and lower thoracic spinal cord, and thus the propensity of neurons in these regions to independently regulate blood flow to different body regions, has not been determined.

In the present study, we determined whether different populations of medullary raphe neurons project to the upper and lower thoracic spinal cord by injecting the fluorescent dyes Fast Blue and Fluoro-Ruby into the vicinity of sympathetic preganglionic neurons in the T4 and T10 spinal cord, respectively.

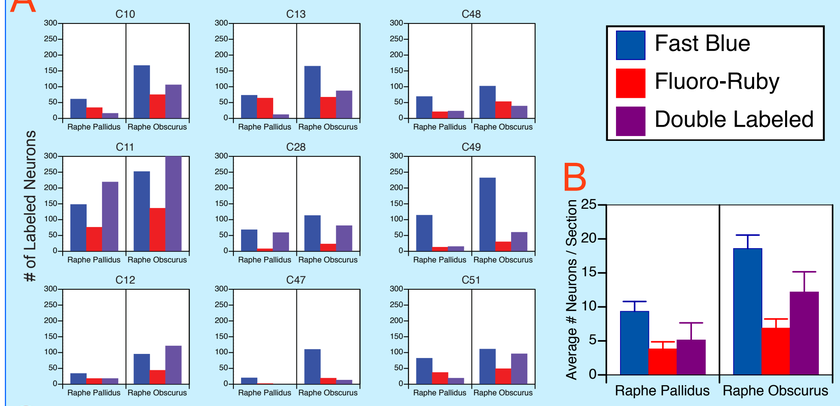
## METHODS

- Injections of 3% Fast Blue were made into the T4 spinal cord, and 5% Fluoro-Ruby was injected into the T10 spinal cord, using a 0.5 µl Hamilton syringe. The needle of the syringe was inserted through the dorsal surface of the right spinal cord, just medial to the dorsal root entry zone, to a depth of approximately 2 mm. Three injections, spaced 1 mm apart in the rostral-caudal plane, were made in an attempt to label the descending projections from the brainstem to the intermediolateral cell column across the rostral-caudal extent of the injected segment.
- The volume of each injection ranged from 150 µl (Animals C47, C48) to 500 µl (Animal C51). The typical injection volume was 250 µl. Large injections were included to ensure effective labeling of raphespinal neurons to a particular segment.
- Transverse spinal cord and brainstem sections were cut at a thickness of 40 µm using a freezing microtome, mounted serially, and observed using a photomicroscope.
- Since sections through injection sites often became shredded during histological processing, we estimated dye location by inspecting the area adjacent to each injection site. Dye permeated the area between injection sites, and was also present in the region just rostral and caudal to the injection sites.
- We counted the number of labeled cells in raphe pallidus and obscurus in ~8 sections through the rostral medulla.

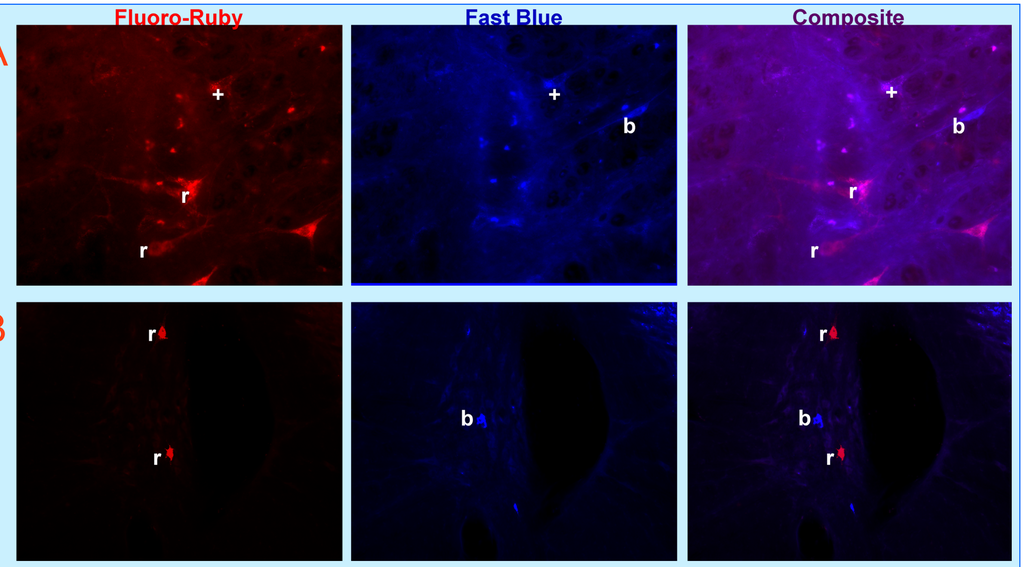



1 Injection sites resulting in the labeling of raphe neurons. These drawings reflect the cumulative area where dye was observed in sections neighboring the three injection sites. Dye was observed throughout the area between the injection sites, as well as rostral and caudal to the injection sites. For animal C47, although tracer was evident in the T10 segment, its distribution was difficult to characterize. As a result, the T10 injection site for this animal was omitted.

For most animals, both the T4 and T10 injection sites included the intermediolateral cell column. The only exceptions are animals C11 (T4 injection site) and C49 (T10 injection site).




3 A: Number of neurons in raphe pallidus and obscurus of each animal labeled selectively by injections of Fast Blue into T4 or Fluoro-Ruby into T10, or which were double-labeled by both tracers. B: Average number of raphe pallidus and obscurus neurons per section in all animals labeled by one or both dyes. Bars show mean ± SEM. Although double-labeled neurons were commonly observed in the medullary raphe nuclei, single labeled neurons were present in every animal, suggesting that some medullary raphe neurons have limited branching to particular spinal segments.




2 Photomicrographs of medullary raphe neurons labeled by the injection of Fast Blue into T4 and Fluoro-Ruby into T10. A: Photomicrographs of raphe obscurus from animal C11. A: Photomicrographs of raphe pallidus from animal C48. Immunofluorescence for Fluoro-Ruby, Fast Blue, and both fluorophors are shown in separate columns. Examples of neurons that selectively contain Fluoro-Ruby (r) and Fast Blue (b), as well as neurons that were double-labeled by both fluorophors (+) are indicated.

## Summary and Conclusions

- Although many neurons in the medullary raphe nuclei were double-labeled by multiple, large injections of Fast Blue into T4 and Fluoro-Ruby into T10, some neurons were selectively labeled by one dye. These data suggest that some raphespinal neurons have influences on a limited number of spinal cord segments.
- These findings are consistent with the hypothesis that some raphespinal neurons can regulate sympathetic outflow from the upper and lower thoracic spinal cord. Consequently, raphespinal neurons could contribute to the anatomical patterning of blood flow.