

Introduction

Peripheral sensory cells are diverse, numerous, and a key component in the neural control of behaviour in gastropods. Prior work has been largely anatomical and spread across a variety of species (e.g., 1-6). This can limit progress on gastropod neuroethology because the distributions of sensory neurons, their modalities, and their connections to the central nervous system are not fully known in any one species.

Immediate goal: to describe the peripheral sensory neurons in *Lymnaea stagnalis*, including morphology, distributions and putative neurotransmitter profiles.

Long-term goal: to assign modalities and behavioural roles to the peripheral sensory neurons

Implications: increasing our understanding of the neuroethology of various behaviours in the snails and also providing a basis for comparative work in other gastropods.

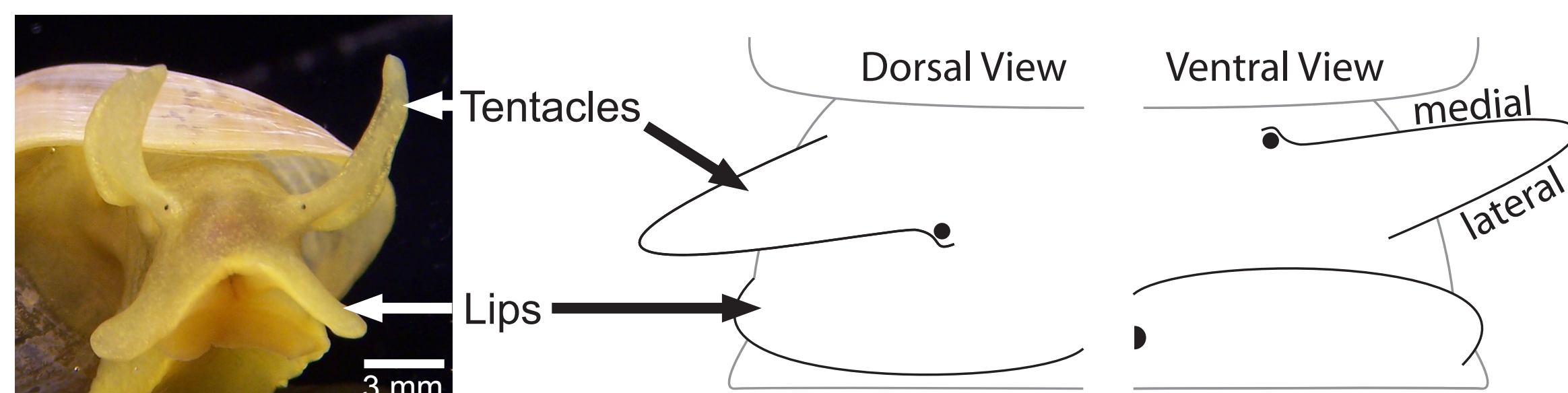
Methods

Immunohistochemistry: targeting either neurotransmitters or enzymes involved in neurotransmitter production.

- anti-tyrosine hydroxylase (monoclonal, Immunostar, Cat # 22941)
- anti-histamine (polyclonal, Immunostar, Cat # 22939)
- anti-nitric oxide synthase (polyclonal, Affinity Bioreagents, Cat# PA1039)

Histochemistry: FaGlu for catecholaminergic cells and NADPHdiaphorase (NADPHd) labelling for nitrergic cells

Direct stains: vital dyes that preferentially stain sensory cells



Lymnaea lips and tentacles processed for whole-mount confocal or transmitted light microscopy

* we use anaesthesia (7), a range of fixatives, and other treatments to promote complete labelling – previous reports have apparently been limited by incomplete labels due to penetration issues, poor fixation, etc.

Controls

Omitting primary antibodies eliminates labelling. FaGlu histochemical method shows similar labelling of catecholaminergic cells. Preincubation with histamine-BSA eliminates or reduces anti-histamine labelling. Omitting NADPH eliminates NADPH diaphorase labelling.

References

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3. Gobbeler, K. and Klusmann-Kolb, A. 2007. A comparative ultrastructural investigation of the cephalic sensory organs in Opisthobranchia (Mollusca, Gastropoda). *Tissue & Cell* **39**: 399-414.
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Catecholaminergic Putative Sensory Cells (PSCs)

Label: anti-tyrosine hydroxylase immunohistochemistry

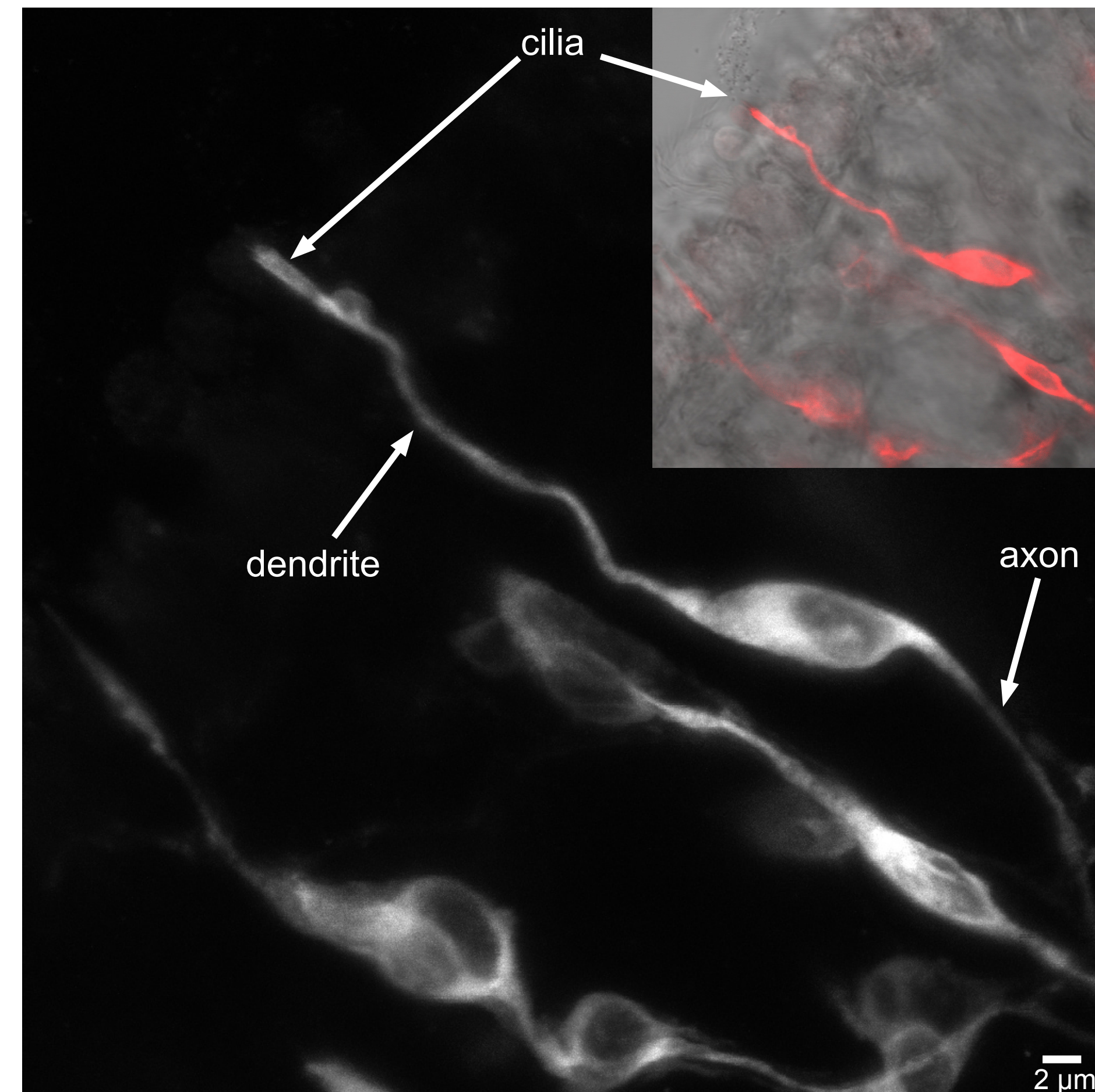


Fig 1. Putative ciliated bipolar catecholaminergic sensory cells. Inset: same view with transmitted light overlay to show ciliated epithelium.

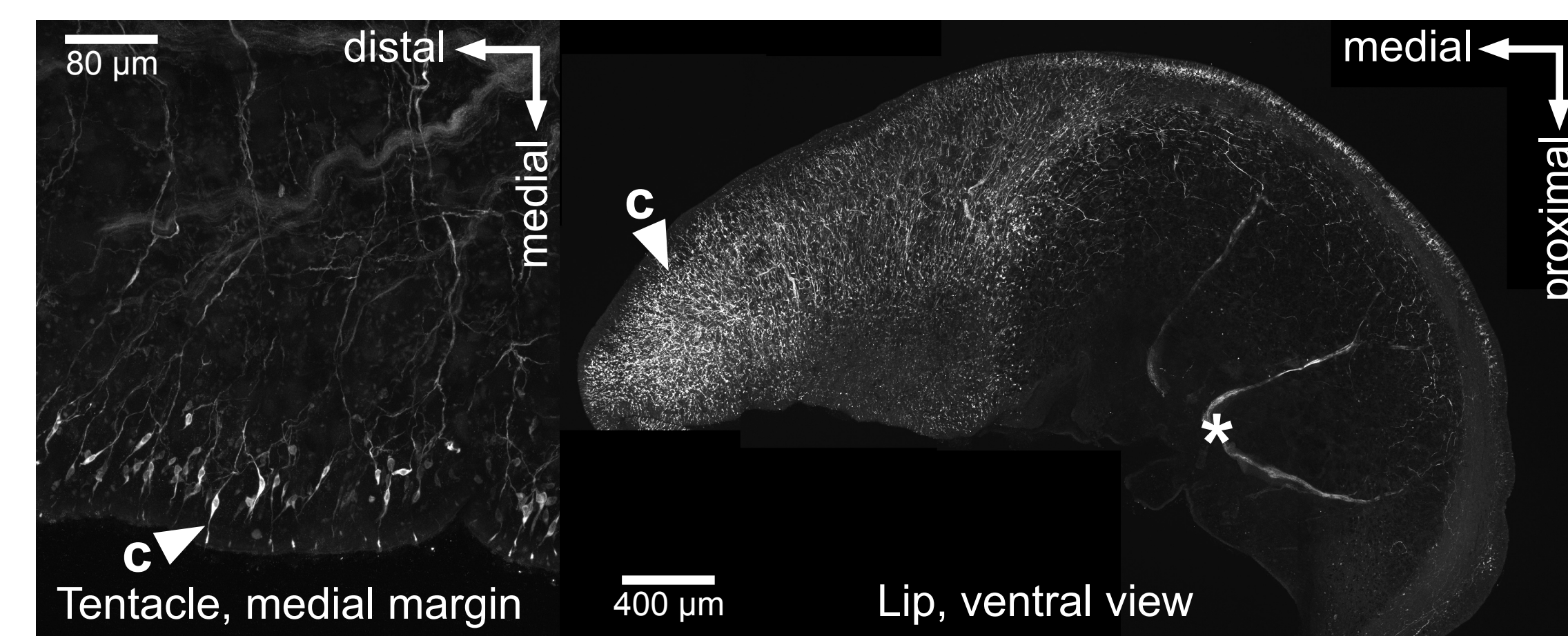


Fig 2. The catecholaminergic (c) PSCs have non-uniform distributions in the lips and tentacles; PSC axons join the branching innervation (*) of the lips and tentacles and likely extend centrally.

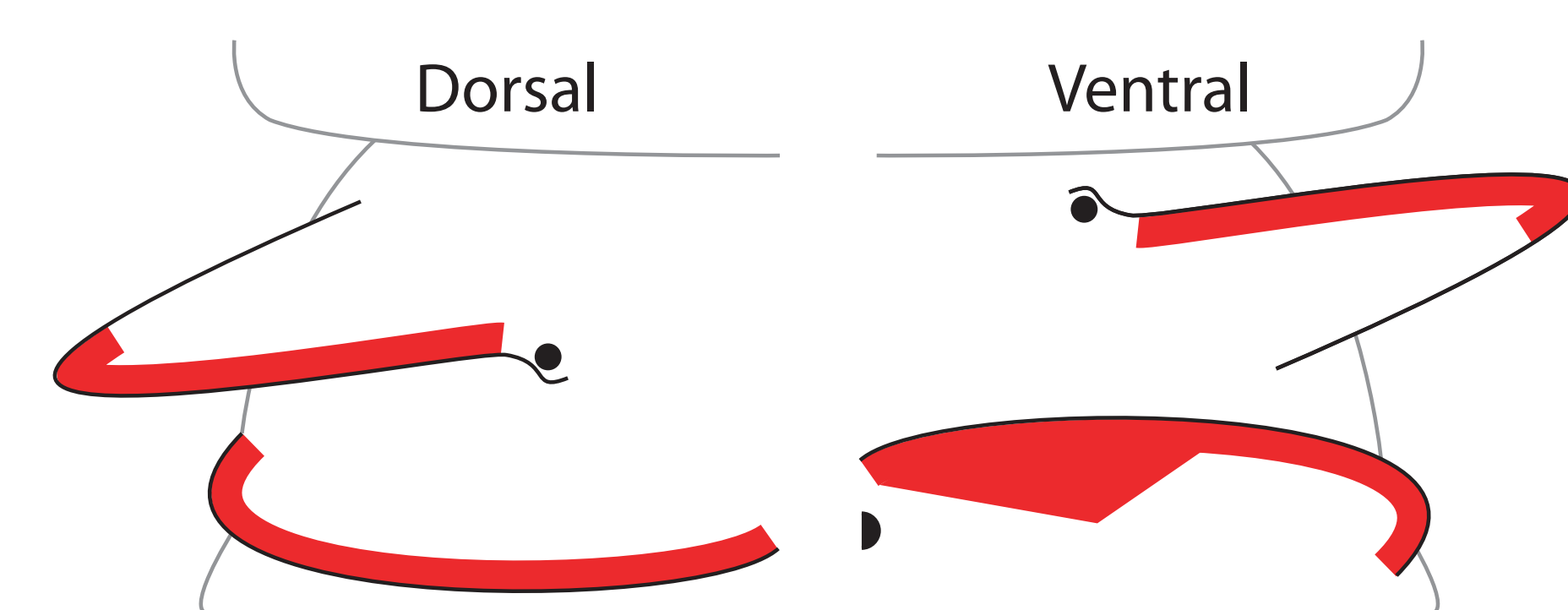


Fig 3. The catecholaminergic putative sensory cells were distributed along the margin and ventral medial surface of the lips, and along the medial and tip margins of the tentacles.

Histaminergic Putative Sensory Cells (PSCs)

Label: anti-histamine immunohistochemistry

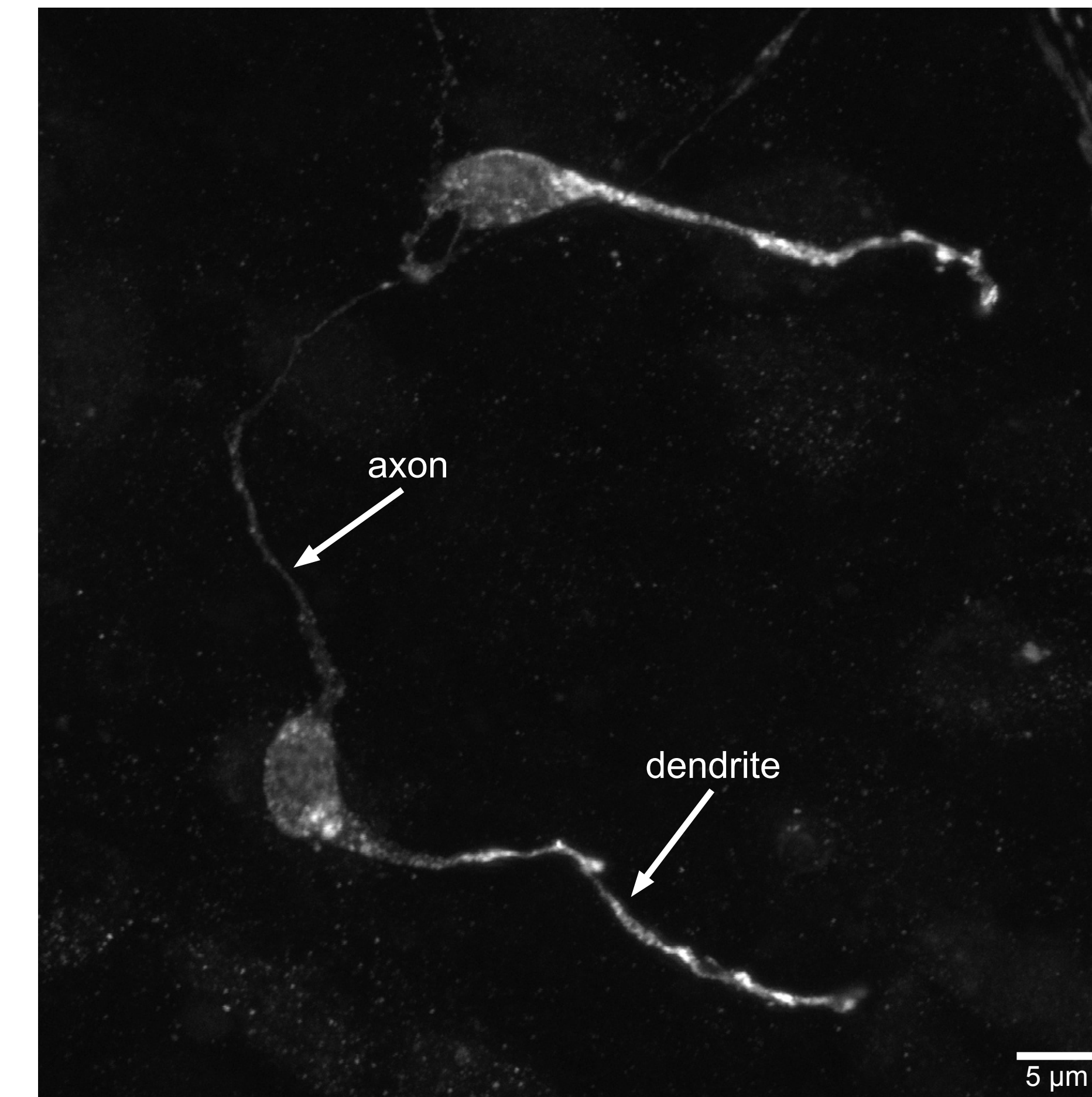


Fig 4. Putative ciliated bipolar histaminergic sensory cells. Cilia are not visible here, but sometimes label with both Dil (below) and with the histamine antibody.

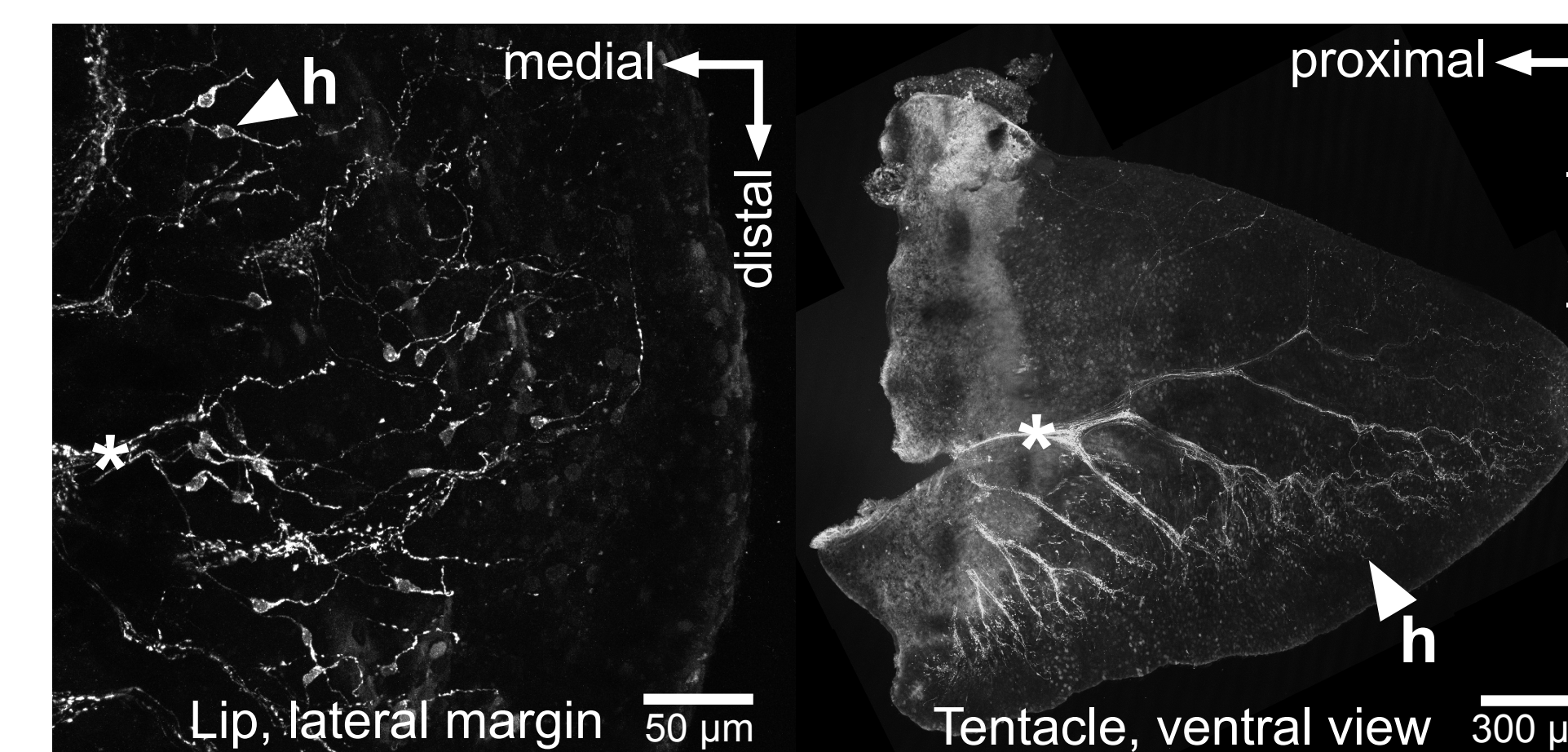


Fig 5. The histaminergic (h) PSCs have a non-uniform distribution in the lips and tentacles; PSC axons join the branching innervation of the lips and tentacles and likely extend centrally.

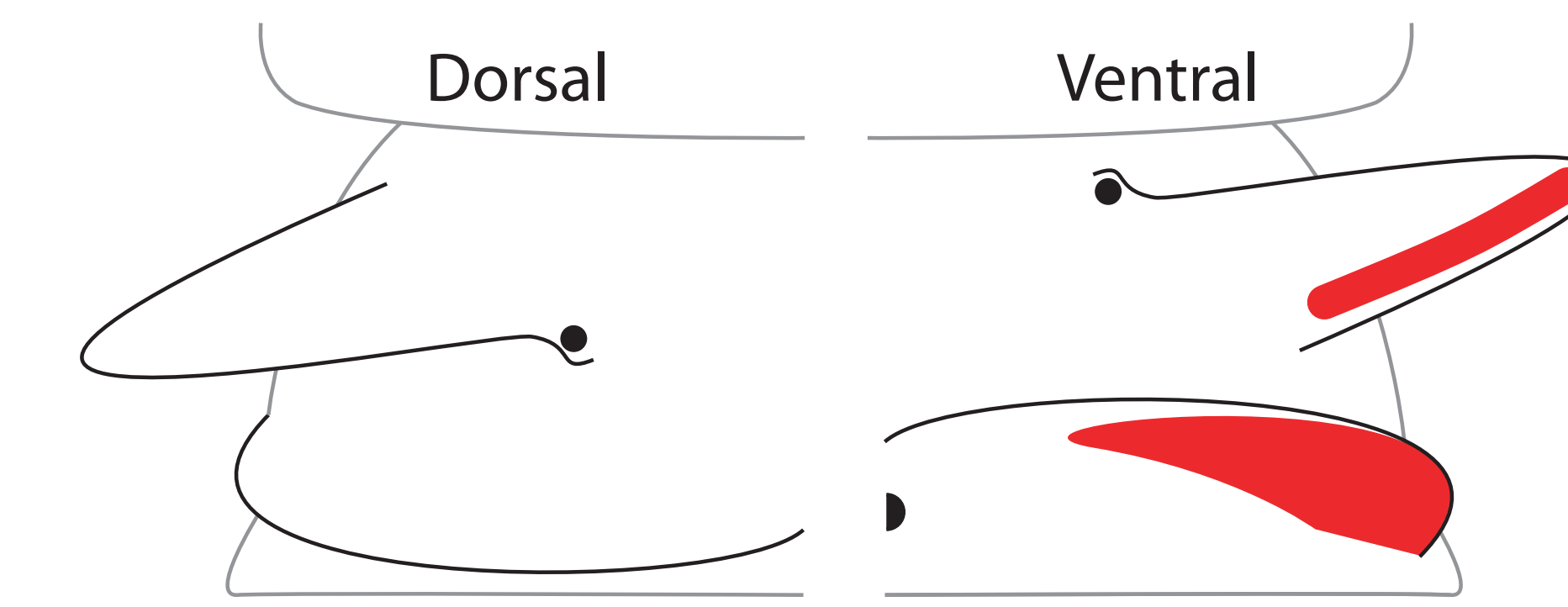


Fig 6. The histaminergic putative sensory cells were distributed along a ventral strip of the lateral tentacles and the ventral-lateral surface and margin of the lips.

Nitrergic Putative Sensory Cells (PSCs)

Labels: NADPH diaphorase histochemistry, anti-nitric oxide synthase (NOS) immunohistochemistry

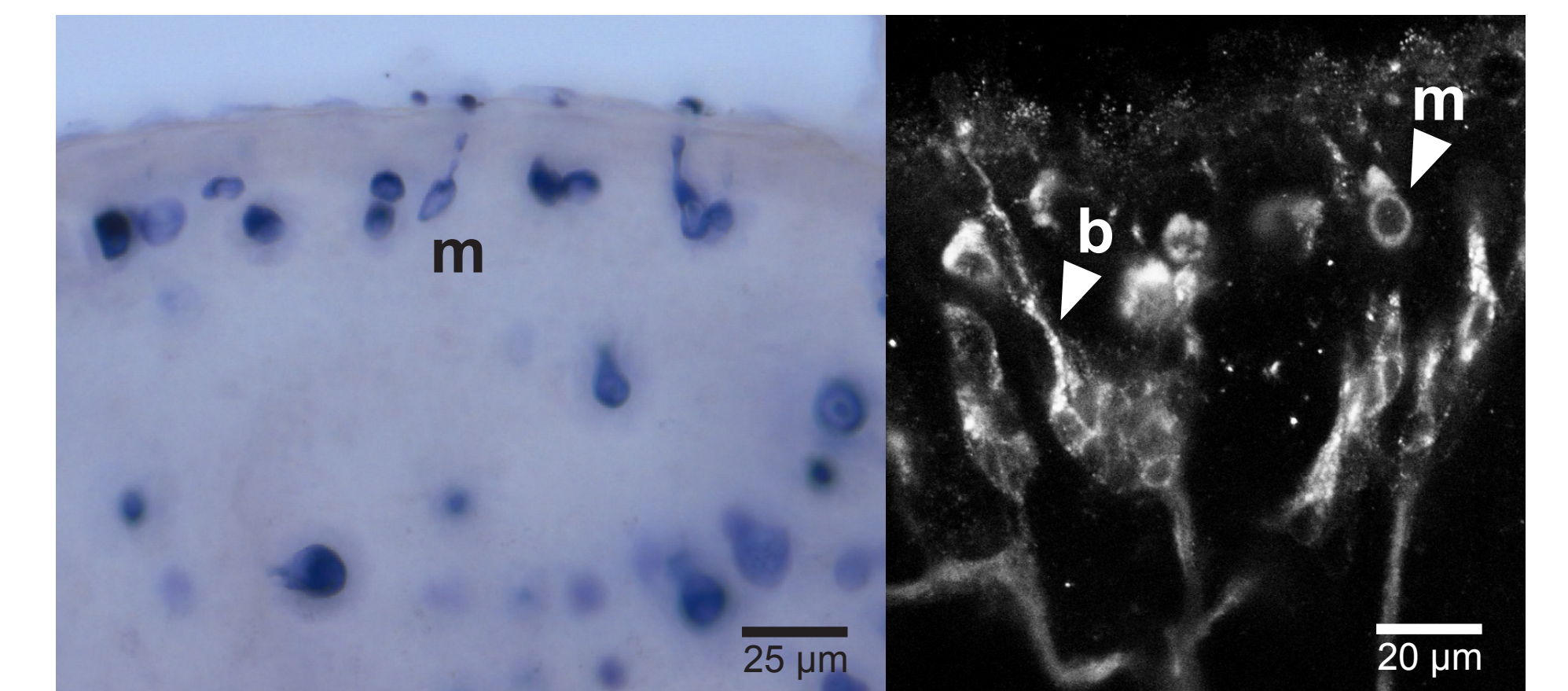


Fig 7. Putative monopolar (m) and bipolar (b) nitrergic sensory cells. NADPHd histochemistry labels only the monopolar cells; anti-NOS labels both.

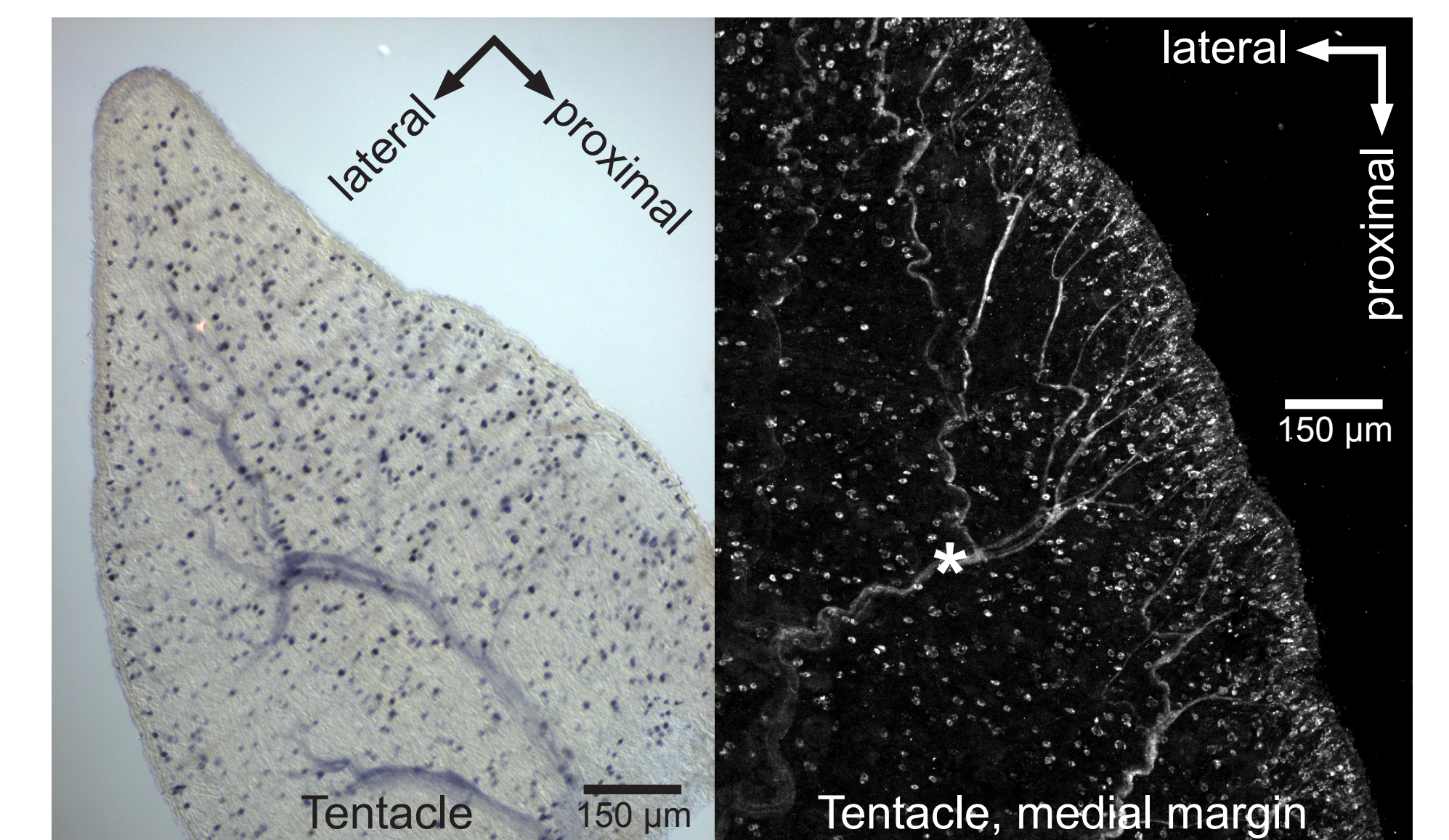


Fig 8. The monopolar nitrergic PSCs (dots) have a largely uniform distribution in the lips and tentacles; the bipolar nitrergic PSCs were found along the medial tentacle margin; their axons join the branching innervation (*) of the lips and tentacles and likely extend centrally.

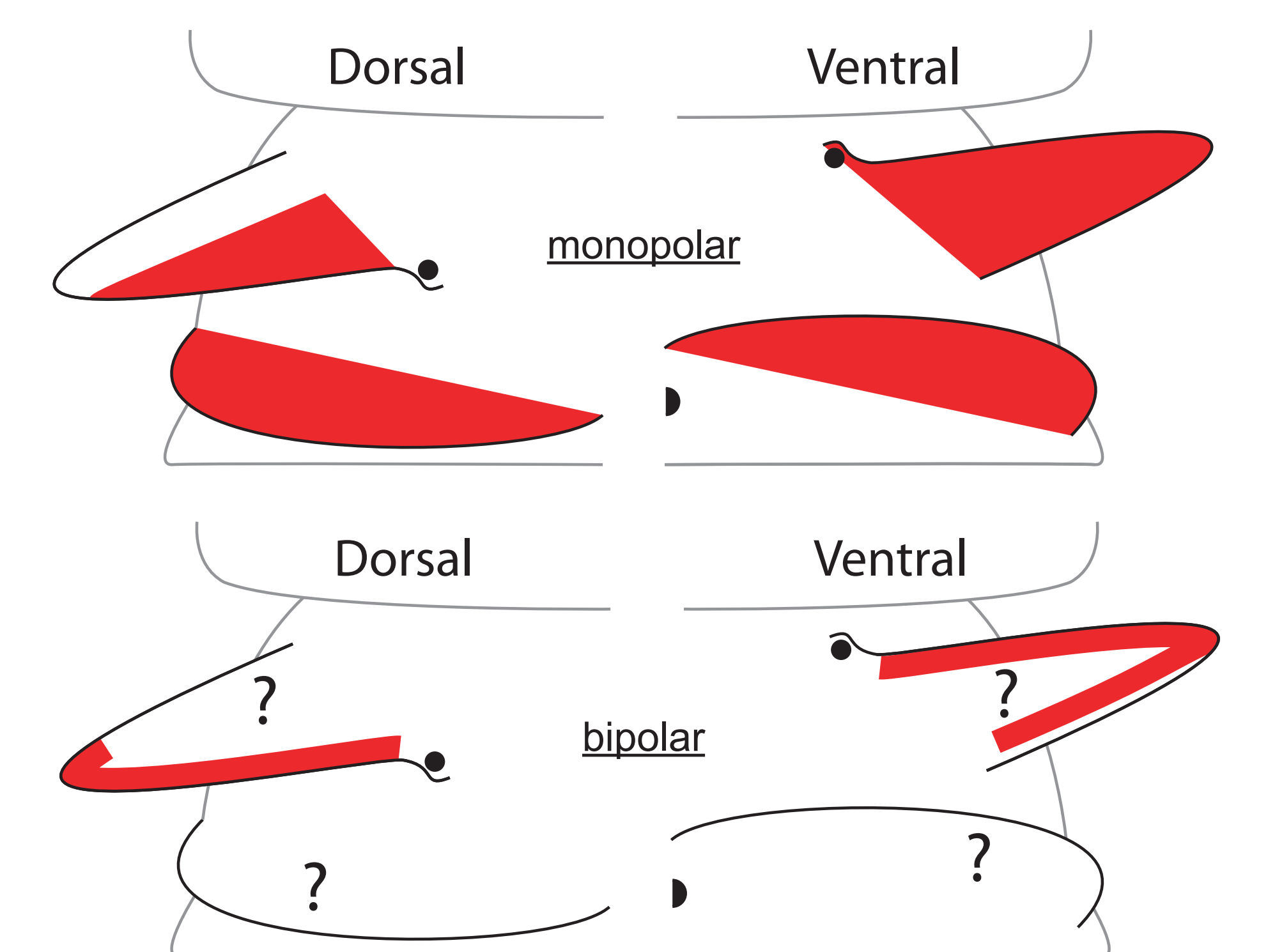


Fig 9. The monopolar nitrergic putative sensory cells were distributed over all lip and tentacles surfaces except for the lateral-dorsal tentacle. The bipolar nitrergic putative sensory cells were found along the medial tentacle margins, but labelling has been too capricious to determine distributions.

Results Summary & Future Work

We have evidence for 4 types of putative sensory cells

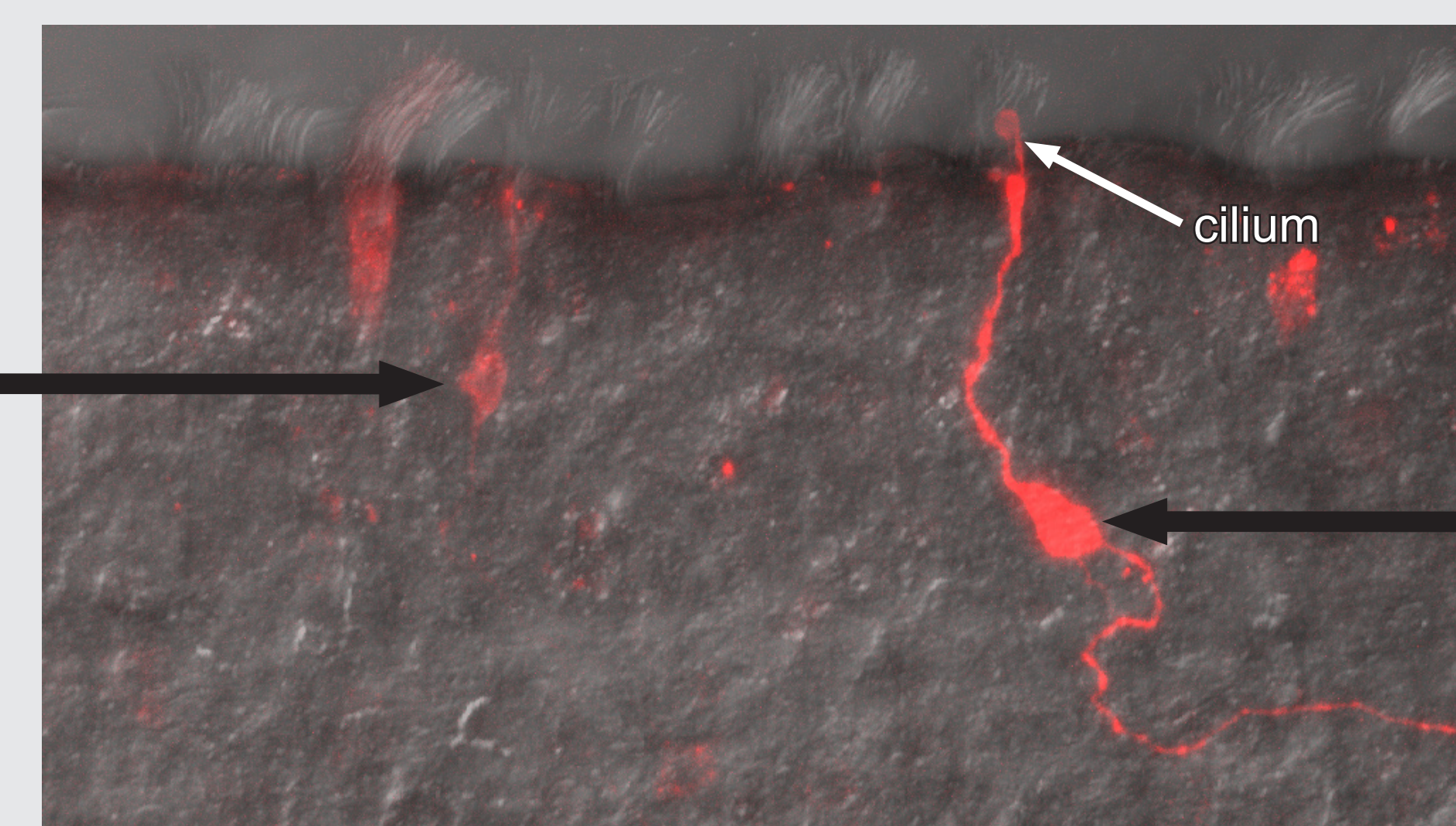
- Catecholaminergic bipolar sensory cells
- Histaminergic bipolar sensory cells
- Nitrergic bipolar sensory cells
- Nitrergic monopolar sensory cells

Next Steps: functional studies to test sensory roles for the cells and determine modalities - chemosensory or mechanosensory. E.g., optical recording from tentacle sensory cells loaded with either calcium or voltage sensitive dyes.

Vital staining of catecholaminergic and histaminergic PSCs further suggests a sensory role for these cells.

The dimly labelled Dil PSCs appear to correspond to the catecholaminergic cells

- similar sizes
- similar shapes
- distribution of the Dil cells is a subset of the catecholaminergic cells



The brightly labelled Dil PSCs appear to correspond to the histaminergic cells

- similar sizes
- similar shapes
- distribution of the Dil cells is a subset of the histaminergic cells

Fig 10. Immersing the live animal in a dilute Dil solution labels two types of sensory cells that appear to correspond to the catecholaminergic and histaminergic PSCs. Preferential labelling of these cells indicates they are exposed to the external environment, supporting a sensory role.