Catecholaminergic peripheral neuroanatomy in the nudibranch *Tritonia diomedea*

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Introduction

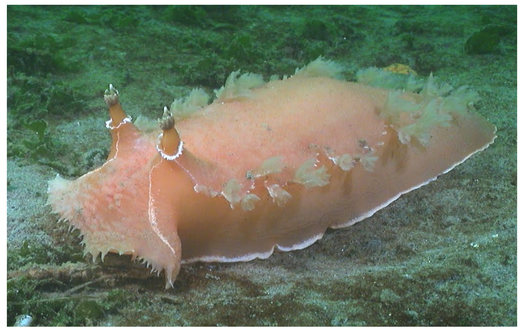

Fig 1. The nudibranch *Tritonia diomedea.*

Navigation in *Tritonia diomedea* (1,2) presents an opportunity to understand how multiple sensory cues are used in the neural control of behavior (3). The peripheral nervous system is thought to play a significant role in these (and many other) behaviours (4), yet we know little about the peripheral nervous system in *Tritonia*. We began our survey of peripheral neuroanatomy by focusing on catecholaminergic cells which previous studies have indicated are widespread in gastropods (4,5).

Immediate goal: To create a map of the peripheral catecholaminergic neuroanatomy in the nudibranch *Tritonia.*

Long-term goal: To determine the role of catecholaminergic peripheral sensory neurons in navigational behaviours.

What are catecholamines?

Catecholamines include the neurotransmitters dopamine, epinephrine & norepinephrine.

Tyrosine-hydroxylase (TH) is an enzyme involved in the production of catecholamines and thus anti-TH antibodies can be used to label putative catecholaminergic cells.

Oral Veil

Lateral Tip

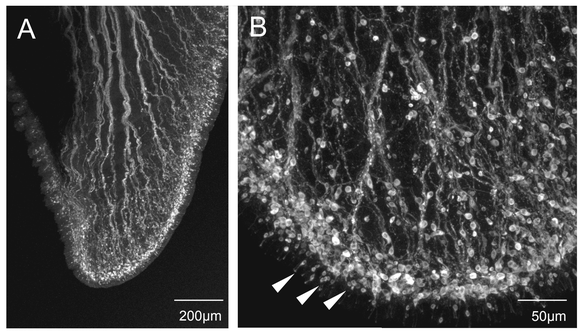

Fig 2. TH-immunoreactivity in the lateral oral veil tip.
A. Branched nerves underlie the sensory epithelium.
B. Higher magnification reveals numerous peripheral sensory cells (arrowheads) with dendrites in the epithelium and axons that presumably project centrally.

Medial Tip

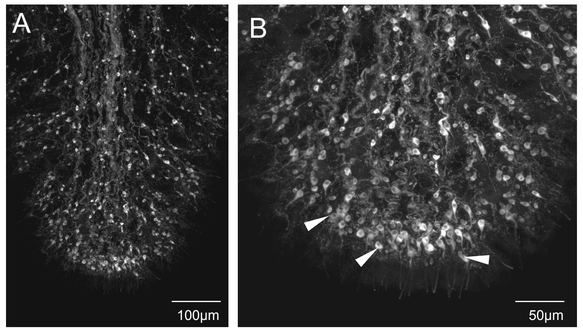

Fig 3. TH-immunoreactivity in the medial oral veil tips.
A. Branched innervation similar to the lateral tip.
B. Peripheral sensory cells in medial tips are also similar to the cells in the lateral tips (arrowheads).

Rhinophores

Spire

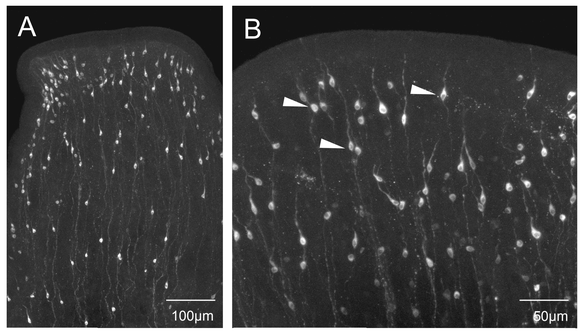

Fig 4. TH-Immunoreactivity in the rhinophore spire.
A. Peripheral sensory cells with separate but parallel processes occur in lower density than in the oral veil.
B. At higher magnification sensory cells appear similar to those found in the oral veil (arrowheads).

Tuft

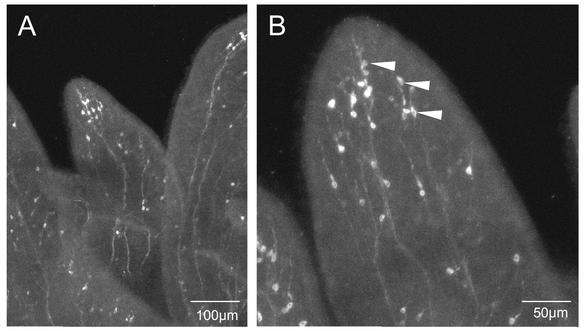

Fig 5. TH-immunoreactivity in rhinophore tufts.
A. Peripheral sensory cells concentrated at tuft tips.
B. Peripheral sensory cell processes in the tufts form a branching innervation, similar to the oral veil.

Results Summary

Using anti-tyrosine immunohistochemistry, we found numerous cells we judge to be peripheral sensory cells, based on putative dendrites that penetrate the epithelia of the cephalic sensory organs.

Oral Veil: We found TH-immunoreactive putative sensory cells both in the lateral (Fig. 2) and medial (Fig. 3) oral veil tips. Distribution in lateral and medial tips was similar. Overall cell body density was greater in the oral veil in comparison to the rhinophores.

Rhinophores: We also found TH-immunoreactive sensory cells in the spires (Fig. 4) and tufts (Fig. 5) of the rhinophores. Projecting processes were separate but parallel in the spires, whereas processes formed a branching innervation in the tufts. TH-immunoreactive cells were concentrated at the tip of the tuft. In addition, two types of bipolar neurons were observed in the rhinophore ganglion (Fig. 6).

Implications

Our map of putative catecholaminergic peripheral sensory cells will facilitate experiments manipulating catecholamine pharmacology, thus helping us to discover:

1) these cells' role(s) in navigation behaviour in *Tritonia*

2) the modality (chemosensation or mechanosensation) of this sensory cell type found in all gastropods.

Furthermore, our discovery of two cell types in the rhinophore ganglion warrants further study of their function (e.g., sensory interneurons, retraction motor neurons, etc.).

Acknowledgements

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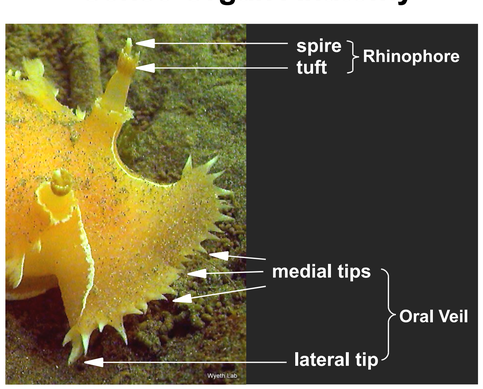
Sense Organ Anatomy



Methods

Immunohistochemistry: targeting catecholaminergic cells

- 1º antibody: mouse anti-tyrosine hydroxylase (Immunostar)
- 2º antibody: goat anti-mouse Alexa Fluor 555 (Invitrogen)

Labelling was documented with whole mount confocal microscopy. To improve labeling, we applied collagenase and flattened tissue in anaesthetic (6) prior to fixation in paraformaldehyde. Tissues were dehydrated and cleared.

Controls
Primary and secondary antibodies were separately omitted and confirmed secondary antibody specificity and the lack of cell auto fluorescence respectively.

Rhinophore Ganglion

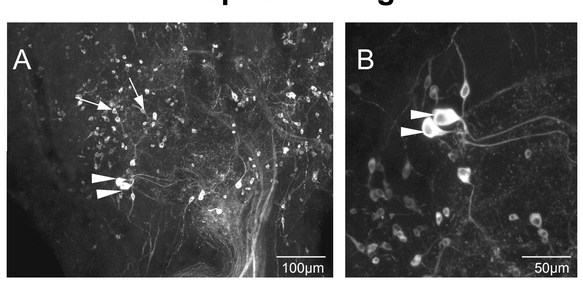


Fig 6. Rhinophore ganglion. A. Two cell types, with large (~20 µm, arrowheads) and small (~10 µm,arrows) somata.
B. A closer view of 2 large cells (arrowheads).

References

1. Wyeth, R.C. and A.O.D. Willows. 2006. Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, *Tritonia diomedea.* J. Exp. Biol. 209: 1441-1453.

2. Wyeth, R.C. and A.O.D. Willows. 2006. Field behaviour of the nudibranch mollusk *Tritonia diomedea.* Biol. Bull. 210: 81-96.

3. Murray, J.A., J. Estepp and S.D. Cain. 2006. Advances in the neural bases of orientation and navigation. Integr. Comp. Biol. 46: 871-879.

4. Wyeth, R.C. and Croll, R.P. 2011. Peripheral sensory cells in the cephalic sensory organs of *Lymnaea stagnalis.* J. Comp. Neurol. 519: 1894-1913.

5. Croll, R.P. 2001. Catecholamine-containing cells in the central nervous system and periphery of *Aplysia californica.* J. Comp. Neurol. 441: 91-105.

6. Wyeth, R.C., R.P. Croll, A.O.D. Willows, and Spencer, A.N. 2009. 1-Phenoxy-2-propanol is a useful anaesthetic for gastropods used in neurophysiology. J. Neurosci. Methods 176: 121-128.