Neuroanatomy of the *Hermissenda* central nervous system: GABA, FMRFamide, and histamine immunoreactivity

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Fig 1. The opisthobranch *Hermissenda crassicornis* can learn to associate light with a vestibular stimulus.

Introduction

The CNS and associated sensory organs of *Hermissenda crassicornis* have been extensively studied for their role in classical conditioning. The visual and vestibular systems are comprised of the eye and optic ganglion, and statocyst, respectively. In other opisthobranchs a variety of neurotransmitters are present in these systems (e.g., 1-4), however there has been no broad survey of putative neurotransmitter content in *Hermissenda*.

Immediate goal: to map locations of FMRF-, GABA-, and HA-immunoreactive cells in the CNS as well as visual and vestibular systems.

Long-term goal: determine the role of neurotransmitters in the animals' response to vestibular stimulation, and use to inform phylogenetic analyses.

Methods

Immunohistochemistry: targeting neurotransmitters.
- anti-GABA (polyclonal, Sigma, Cat # A-2052)
- anti-histamine (polyclonal, Immunostar, Cat # 22939)
- anti-FMRFamide (polyclonal, Immunostar, Cat # 20091)

Hermissenda central nervous system (excluding buccal ganglia) and associated sensory organs were processed for whole-mount epifluoresence and confocal microscopy.

Controls

Omitting primary and secondary antibodies confirmed specificity of secondary antibody labelling for all transmitters. Preincubation with GABA and HA conjugates eliminated anti-GABA and anti-HA labelling, respectively, in a dose-dependent manner.

GABA

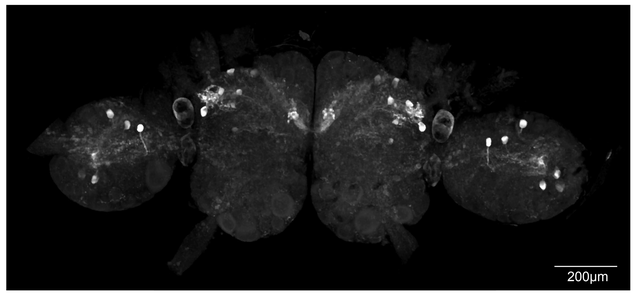


Fig 2. GABA-immunoreactive neurons in the CNS. In the cerebro-pleural ganglia, cells were distributed either in the anterior portion, or in a cluster at the border of the cerebropleural commissure. In contrast, several cells were distributed throughout the pedal ganglia.

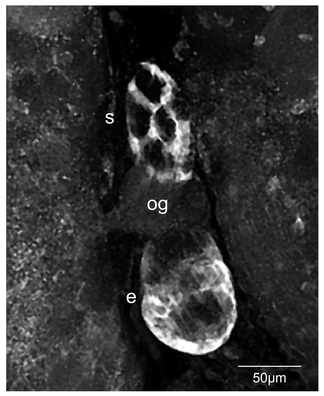


Fig 3. GABA-immunoreactivity in the visual and vestibular systems. The statocyst (s) showed consistent GABA-IR, appearing to border individual hair cells (see Fig 4 below). The eye (e) showed consistent GABA-IR, however in a pattern not consistent with previously described photoreceptor size or position (5).

Previous work indicates GABA is the hair cell transmitter in synapses with photoreceptors, and has localized GABA in hair cell axons and presumed terminal branches (6). In contrast, we did not find GABA in hair cells, the static nerve, photoreceptors, or the optic nerve and instead found GABA in a consistent pattern in the statocyst (which was eliminated with preadsorption controls).

FMRFamide

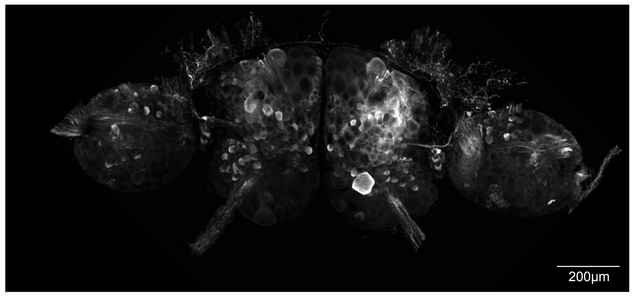


Fig 5. FMRF-immunoreactive neurons in the CNS. Labelling was widespread throughout the CNS, and included a single, large, asymmetric cell. Mapping of FMRF-IR CNS neurons was hindered by high background exhibited in all preparations.

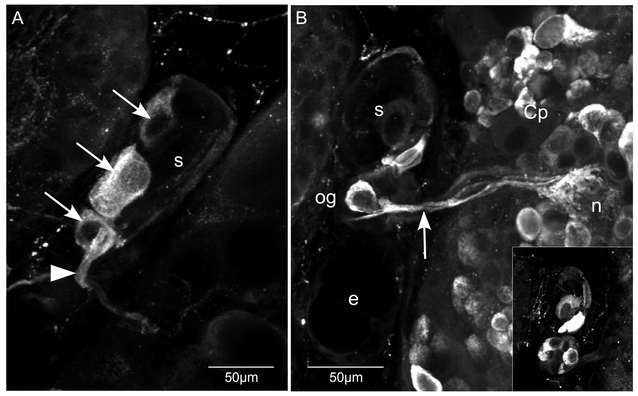
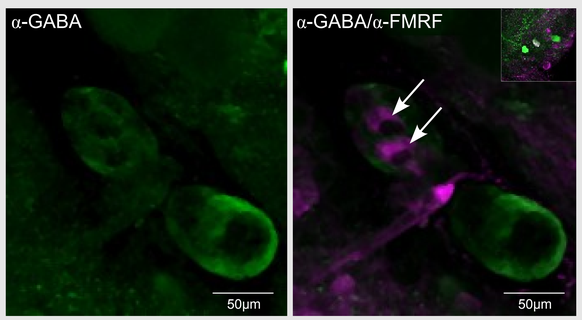


Fig 6. FMRF-immunoreactive cells in the vestibular and visual systems. **A**) Ventrally-located hair cells (arrows). FMRF-IR axon(s) at the base of the statocyst (s) were visible (arrowhead). **B**) A FMRF-IR optic ganglion (og) cell with associated axon (arrow) extending to the neuropil (n) of the cerebropleural ganglion (Cp). The eye (e) is labelled for orientation. Insert: dorsally-located hair cells and optic ganglion cells.



GABA-FMRFamide Double Label

Two views of the same statocyst (s) showing GABA-immunoreactivity alone (left) and double labelling of GABA and FMRFamide immunoreactivity (right). GABA-IR (green) borders individual FMRF-IR hair cells (magenta, arrows).
Insert: GABA-/FMRF-IR co-localization (arrowhead) of cells in the pedal ganglion to show labelling in the statocyst does not overlap, suggesting GABA is not in the hair cells.

Histamine

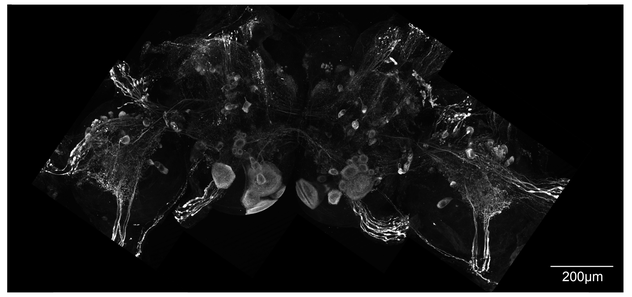
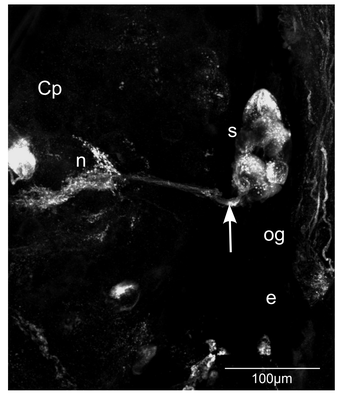


Fig 7. HA-immunoreactive neurons in the CNS. Labelling was widespread throughout the CNS, and included several giant cells (image from J. Thomson).

Fig 8. HA-immunoreactivity in the statocyst (s). Eight hair cells were consistently labelled, with HA-IR axons extending through the static nerve (arrow) into the neuropil (n) region of the cerebropleural ganglion (Cp). No labelling was observed in the optic ganglion (og) or the eye (e).



Results Summary

CNS: consistent patterns of histamine-immunoreactive and GABA-immunoreactive cells. Some FMRFamide-immunoreactive cells were also identified.
Statocyst: four consistently labelled FMRFamide- and eight histamine-immunoreactive hair cells and associated axons extended through the static nerve to the neuropil region of cerebropleural ganglia. GABA-IR was absent in hair cells.
Visual System: four consistently labelled FMRFamide-immunoreactive optic ganglion cells and associated axons extending to neuropil. GABA-IR apparently absent from photoreceptors.

Our results suggest both FMRF-amide and HA may be primary transmitters for gravireception, and additionally that FMRFamide is involved in visual transduction.

Our results are inconsistent with previous evidence concerning the presence of GABA in the visual and vestibular systems in *Hermissenda* (6), but are consistent with data from *Pleurobranchaea* (3).

Future Work

1. Further anatomical and biochemical studies to address the presence of GABA in the visual and vestibular systems.

2. Behavioural studies addressing the role various transmitters play in the behavioural responses to vestibular stimulation.

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