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Sensory adaptations in hydrothermal vent shrimps from the Mid-Atlantic Ridge

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Introduction

When discovered in 1985, *Rimicaris exoculata* Williams & Rona, 1986 and *Chorocaris chacei* (Williams & Rona, 1986) were assumed by most to be blind. Downwelling light at intensities useful for vision does not reach depths greater than 800-1300 m (rev. in Lakin et al., 1997), while the shrimps are found at depths of ~1700-3900 m. Nonetheless, *R. exoculata* possesses a dorsal organ with cellular, biochemical, and physiological characteristics of a retina that appears to be adapted to transduce the dim light emitted by the hydrothermal vents around which they feed (rev. in O'Neill et al., 1995).

We have also examined the morphology of the eyes of *R. aurantiaca*[1] Martin et al., 1997 (see Nuckley et al., 1996), *C. chacei* (see Lakin et al., 1997), *Mirocaris fortunata* (Martin & Christiansen, 1995), (see Kuenzler et al., 1997), and *Alvinocaris markensis* Williams, 1988 (see Wharton et al., 1997). Neurochemical analyses of the brain of *R. exoculata* are underway (Curra et al., 1996).

Electrophysiological recordings of concentration-dependent sulphide sensitivity from the antennal nerves of *Rimicaris* (Renninger et al., 1995) have provided insight into the mechanisms whereby vent shrimps might locate active hydrothermal systems.

Methods

Hydrothermal vent shrimps used in the studies described herein were collected during R/V *Atlantis II* cruise 129-6/7 to the Mid-Atlantic Ridge in May/June 1993 with DSV *Alvin* (see Lakin et al., 1997 for details). For studies of retinal anatomy, only mature specimens were examined. Visual morphology of juvenile bresiliids is described by Gaten et al. (this volume).

Upon arrival at the surface, the anterior portion of the cephalothorax containing the antennae, eyes, and/or brain was immediately dissected free from the shrimp, transferred to chilled fixative overnight, and prepared for light and electron microscopy. Dissection was done in daylight on the deck of the R/V *Atlantis II* (see Lakin et al., 1997 for details; O'Neill et al., 1995 for discussion of alternate fixation procedures). Samples for immunohistochemical analyses were prepared as described by Curra et al. (1996).

Electrophysiological recordings from excised antennal filaments of *Rimicaris* were made aboard R/V *Atlantis II*. Antennal nerve fibres were drawn into a suction electrode and those that displayed spontaneous action potential discharge and responded to tactile stimulation were judged to be in good physiological condition. Details are described by Renninger et al. (1995).

Results

I. Vision

Without exception, the eyes of each of the five species examined lack the eyestalks and dioptric apparatus used for directional form vision in surface decapod crustaceans (Figure 1). The eyes of freshly collected animals of each species have a characteristic white appearance that can be attributed to a white matrix of vesicle-filled cells within the retina.

1. Rimicaris and Chorocaris - In *R. exoculata* and *R. aurantiaca* the 'eye' is composed of a smooth cornea that is continuous with the dorsal carapace and is underlain by a bilobed retina that is fused at its anterior-most aspect. The retina extends posteriorly along the dorsum from its origin at the would-be position of the ancestral anterior eyes. Roughly 3200-3500 quasi-ommatidia, each composed of 5-7 photoreceptor cells, are embedded in a matrix of white

1 *Rimicaris aurantiaca*, also described by Vereshchaka (1996) as *Iorania concordia*, has been shown using molecular techniques to be a juvenile stage of *R. exoculata* (R. Vrijenhoek, T. Shank, and R. Lutz, pers. comm.; Dixon et al., this volume).

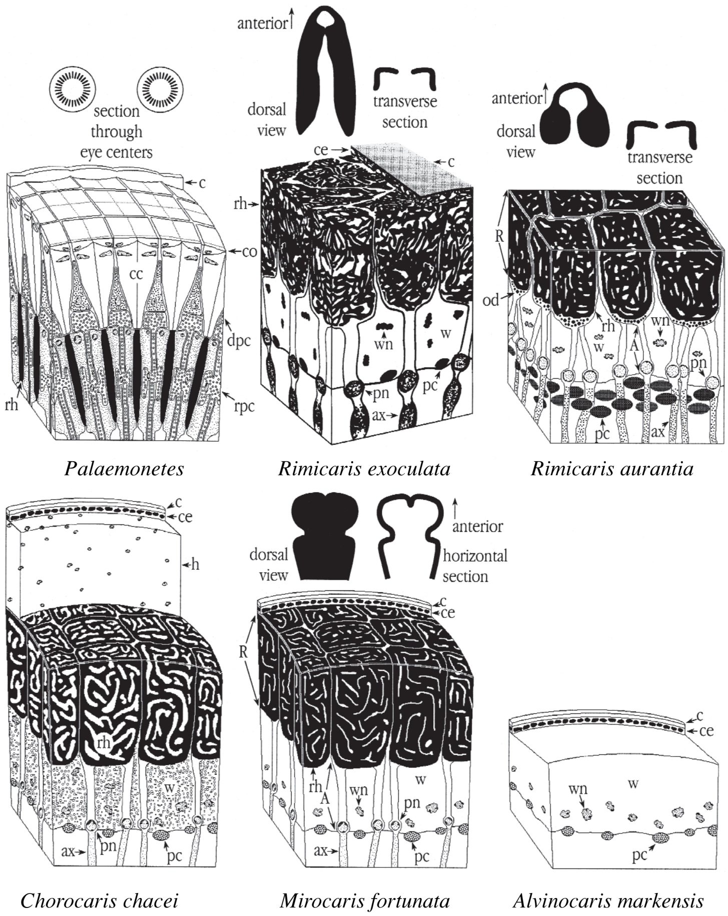

Figure 1. Summary anatomy of the eyes of the five hydrothermal vent species studied in comparison with that of the shallow-water caridean *Palaemonetes*. See text and Nuckley et al. (1996) for details. c - cornea, co - corneagenous cells, cc - cone cells, ce - corneal epidermis, dpc - distal pigment cells, rpc - reflecting pigment cells, rh - rhabdom (highlighted in black), w - white diffusing cell matrix, wn - nucleus of a white diffusing cell, pn - photoreceptor nucleus, ax - photoreceptor axon (containing screening pigment granules), pc - pigment cell (containing deep-brown screening pigment granules in living tissue), R - rhabdomeral segments of photoreceptors, A - arhabdomeral segment of photoreceptor (soma), od - oil droplets, h - hemocoel.

diffusing cell processes. By contrast, *Chorocaris chacei* has two prominent ovoid 'eyes', the corneas of which are fused with the surrounding carapace of the anterior extent of the cephalothorax, directly subjacent to the rostrum. Unlike *C. chacei*, the corneas of *C. fortunata* are fused along the midline to form a single window to the photosensitive membrane beneath it (Figure 1).

Photoreceptor cells of both *Rimicaris* and *Chorocaris* have hypertrophied rhabdomeral segments (R-segments) and severely attenuated arhabdomeral segments (A-segments, cell bodies). The proliferated rhabdom occupies 70-80% of the volume of the region of the retina containing the R-segments in *R. exoculata, R aurantiaca* and *M. fortunata,* and roughly 60% in *C. chacei* (Figures 1 & 2 A-D).

2. Alvinocaris - Eyes of adult *A. markensis* are atrophied, each possessing only an enlarged smooth cornea underlain directly by a white diffusing cell matrix with a degenerate retina. Retinal degeneration ranges from a few dozen disorganized photoreceptor cells to no recognizable neural retina. Pigment cells are also degenerate in size and number in the *A. markensis* eye (Figure 1).

II. Chemoreception

Suction electrode recordings from the nerves of all three antennal filaments of *R. exoculata* and *R. aurantiaca* revealed that the antennae respond to various stimuli (*e.g.,* mixtures of amino acids and homogenates of vent-specific bacteria, sodium sulphide) with trains of action potentials. Axons from the second antenna responded to sulphide (but not to pH) in a concentration-dependent fashion (Figure 2 E-G) with a predicted threshold in the micromolar range of sulphide concentration. Sensilla, that have open pores at their tips and are each innervated by 10-14 sensory dendrites, are distributed along the second antenna with a density that is 4-5 times greater than that found in a typical shallow-water caridean shrimp (*e.g.*, *Penaeus aztecus* Ives, 1891).

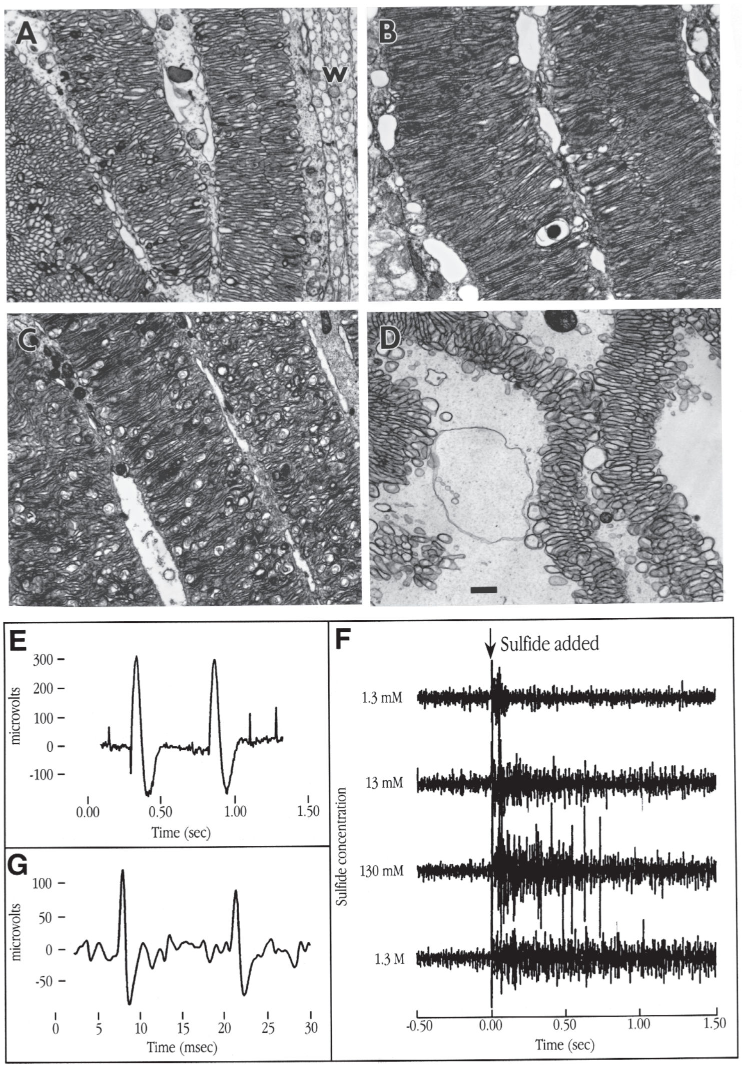

Figure 2. A-D: Ultrastructure of the rhabdoms of the four bresiliid species with hypertrophied retinas. A. Rhabdom of *Rimicaris exoculata* is surrounded by a matrix of vesicle-filled white diffusing cell processes (w). B. *Rimicaris aurantiaca*. C. *Mirocaris fortunata*. D. *Chorocaris chacei*. Bar in D represents 0.7 µm in A-D. E-G: Neurophysiological recordings from *Rimicaris*. E. Electrocardiographic (large) and electromyographic (small) signals recorded with an extracellular electrode placed over the dorsal eye in a position normally used to record electroretinographic responses. F. Fast sweep of single action potentials (spikes) recorded from the lateral antennal nerve. The two different spike amplitudes represent activity from two active fibres. G. Recordings from a bundle of nerve fibers in the lateral antennal nerve showing massed action potential generation in response to the application of different concentrations of sulphide (Na_2S). The initial responses (at arrow) appear to be tactile responses to the drop of test solution merging with the bathing solution (*Limulus* Ringer's solution - see Renninger et al., 1995).

III. Central Nervous System

Brain atlases reveal that the basic topography of the decapod brain is preserved in the MAR shrimp, but the point of insertion of the optic nerves has assumed a more dorsal-posterior position in the vent shrimp relative to *Palaemonetes*. Immunohistochemical analysis of the retina and brain of *R. exoculata* suggests that the photoreceptors and their axons contain a histamine-like neurotransmitter, and that the axons of the optic nerve terminate in the first optic neuropil (lamina) of the brain (Curra et al., 1996). Overall, free amino acids were about 3 times more concentrated (per mg total protein) in the brain extracts of the deep-sea shrimp compared to those in brains of the grass shrimp *Palaemonetes pugio* Holthuis, 1949. The sulphur-containing amino acid methionine was 7.5 times more concentrated, but the concentration of taurine, another sulphur-containing amino acid, was similar in the two species. Alanine was 7 times more concentrated in the brain extracts of the MAR shrimp. The neuroactive amino acids aspartate and glutamate were each roughly 3 times more concentrated in the extracts from the deep-sea shrimp, while γ-aminobutyric acid (GABA) was about 4.5 times more concentrated. By contrast, the concentration of free glycine was unusually low in brain extracts of the vent shrimp, only 0.3 times that found in brain extracts of *P. pugio*.

Discussion

The eyes of *Rimicaris* and *Chorocaris* have no dioptric apparatus to focus light onto distinct receptors, a modification also common to other deep-water crustaceans (rev. in Lakin et al., 1997). Rather, light enters the retina through a smooth cornea with a wide acceptance angle, traverses an unpigmented blood space where the cones would have been in the plan for the standard decapod eye, and then falls directly upon a massive array of photosensitive membrane surrounded by white diffusing cells and a relative paucity of distal screening pigment (Figures 1 & 2 A-D). Increased rhabdomeral volume density is a common adaptation in mesopelagic crustaceans that live in environments with bioluminescence (rev. in Lakin et al., 1997; see also Nuckley et al. 1996 for a comparison of the *Rimicaris* eye with that of *Palaemonetes)*.

The modifications in the design of the eyes of *Rimicaris* and *Chorocaris* suggest that the eyes represent an adaption to the extremely dim light emitted from the orifices of the black smoker chimneys around which they live. *Rimicaris* and *Chorocaris* have sacrificed the spatial resolution of their ancestral compound eye for absolute sensitivity and their eyes now function, presumably, simply to detect the intensity and perhaps direction of the vent light. This idea is supported by the fact that *Alvinocaris, Chorocaris* and *Rimicaris* larvae have eyes on stalks with ommatidia displaying clear remnants of the dioptric apparatus characteristic of caridean compound eyes (Gaten et al., this volume).

Despite the fact that the eye of *R. exoculata* contains a rhodopsin-like pigment whose absorbance spectrum peaks at 500 nm (rev. in O'Neill et al., 1995) we were unable to record light-evoked responses from the eye of *Rimicaris* (presumably a result of blinding illumination with *Alvin's* operating lights; Figure 2E). Johnson and his colleagues (1995), found that the spectral sensitivity of the electroretinogram for a single juvenile *R. exoculata* matches roughly the absorbance spectrum of *Rimicaris* rhodopsin.

Degeneration of the retina of *A. markensis* suggests that the light available in its niche of the hydrothermal vent environment is too dim to be of significant use. The eyes appear to have evolved beyond the 'quit point' for exploitative proliferation of the retina, and it would appear that the retina is now being selected against in successive generations (Figure 1).

The sulphide-concentration dependence of antennal responses (Figure 2F-G) suggests that *Rimicaris* might be able to detect sulphide gradients occuring naturally in the near field (tens of meters) of the hydrothermal vents. In addition, increased sensilla density suggests enhanced chemosensory capability in *Rimicaris*.

Neurochemical analysis of the brain of *R. exoculata*, especially the differences in the relative concentrations of GABA and glycine, suggests that the neurochemical organization of the brain of *R. exoculata* is different from that of its surface relative *Palaemonetes pugio*.

Acknowledgements

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