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Article *in* Journal of Comparative Physiology · May 2002

DOI: 10.1007/s00359-002-0293-y · Source: PubMed

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Journal of Comparative Physiology A

DOI 10.1007/s00359-002-0293-y

Original Paper

Spectral sensitivity in a sponge larva

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Accepted: 2 February 2002 / **Published online:** 15 March 2002

Abstract. Cilia at the posterior pole of demosponge larvae are known to cause directional swimming, sometimes in response to light gradients, but so far neither the spectral sensitivity of, nor the molecular basis for, this response has been investigated. We exploited the fact that the larval cilia respond to sudden changes in light intensity, a shadow response, in order to determine the action spectrum of photosensitivity. Our results show that larvae of the haplosclerid sponge *Reniera* sp. respond most to blue light (440 nm), and have a smaller, secondary response peak to orange-red light (600 nm). These data suggest that the photoreceptive pigment in sponge larvae may be a flavin or carotenoid.

Keywords. Porifera - Sponge - Photoreceptor - Action spectrum - Visual pigments

Introduction

The ability to detect and use light as energy or as a signal came about early in the evolution of life on earth. Interest in the evolution of photoreceptor structure and photoreceptive pigments has produced a wealth of data on photoreceptors from bacteria to vertebrates (Wolken *1995* and references therein), with one noticeable omission: the Porifera. Despite the immense output of research on photoreceptors there is very little information about possible photoreceptive structures in sponges. Although sponges are

not known for their overt behavior because they lack nerves and cell junctions that would allow communication between cells (Pavans de Ceccatty *1974*; Mackie *1979*), it has been shown that some sponges respond to light by contracting their oscula (Reiswig *1971*), and it is well documented that many sponge larvae exhibit phototaxis (see Wapstra and van Soest *1987* for review).

Demosponge larvae are diploblastic, ciliated spheroids 0.1-1 mm in length (Bergquist and Sinclair *1968*; Brien *1973*; Wapstra and van Soest *1987*). They have an outer epithelial layer of monociliated cells and typically a solid center of amoeboid cells in an extracellular matrix of collagen (Fig. 1). The posterior swimming pole is often non-ciliated and is ringed by a row of cells that contain pigment and give rise to long cilia (Wapstra and van Soest *1987*). In some larvae the entire posterior pole is pigmented and others lack pigment altogether. The larvae often swim in a rotating or corkscrew fashion, due to the beating of the short cilia in metachronal waves, and directional swimming is conferred by the long cilia at the posterior pole (Woollacott *1993*).

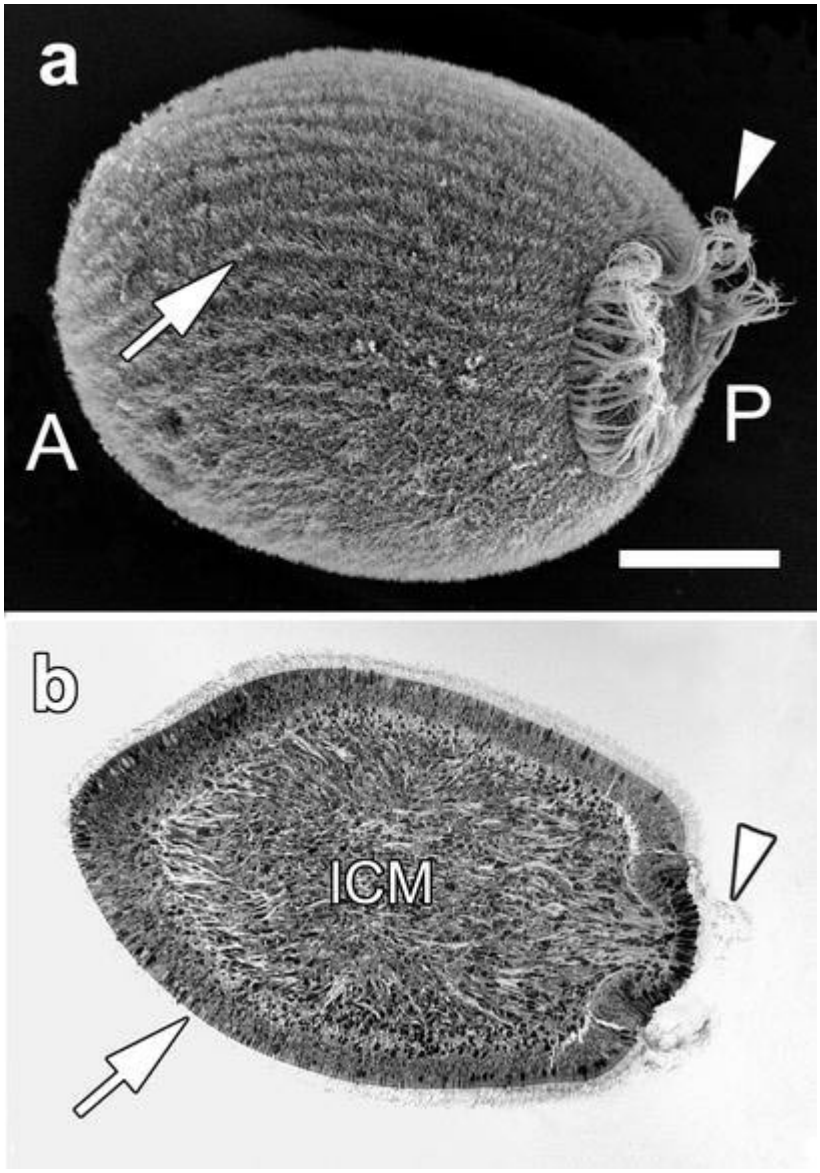

Fig. 1. Scanning electron micrograph (**a**) and epoxy section (**b**) showing the structure of the larva of the demosponge *Reniera* sp. The larva is ciliated at all but the anterior (*A*) and posterior (*P*) poles. The unciliated posterior pole is circumscribed by a ring of 120- to 150-µm-long cilia (*arrowheads*). Monocilitated epithelial cells form the outer layer (*arrows*), while the inner cell mass (*ICM*) contains amoeboid cells in a collagenous extracellular matrix. Scale bar, 100 µm

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Recently it was shown that sponge larval phototaxis is effected by the response of the long cilia at the posterior swimming pole to sudden increases and decreases in irradiance, a shadow response (Leys and Degnan *2001*). We have now determined that the wavelength of maximum sensitivity for the shadow response is in the blue light region

near 440 nm. Here we present the first action spectrum for spectral sensitivity in a member of the Porifera.

Materials and methods

Adult sponges of *Reniera* sp. (Demospongiae, Haplosclerida, Chalinidae) were collected on pieces of dead coral from Shark Bay on Heron Island Reef, Great Barrier Reef, and maintained in large containers of sea water at 28°C. Larvae that were released from the adults after 30 min in still water were collected by pipette and transported to the laboratory at Heron Island Research Station, Queensland, Australia, where they were kept in glass beakers containing 0.2-µm-filtered seawater (FSW) at 22°C away from direct light.

Larvae were pipetted into a 5-cm-diameter plastic Petri dish of FSW containing a new glass cover slip to which they adhered by their anterior end. White light (Olympus JCM 15 cold light source) was shone through a circular variable neutral-density filter (Edmund Scientific, Barrington, N.J.), and a narrow-band interference filter (Oriel Instruments, Stratford, Conn.) at approximately 20-nm intervals from 390 nm to 700 nm onto the larva (Fig. 2a). Light intensity was increased in steps of approximately 0.2 log density units that were marked on the rim of the variable neutral-density filter, until the long posterior cilia began to straighten and bend in response to the opening and closing of a shutter in front of the light source. The step at which this response occurred was considered to be the threshold intensity for the response of the cilia. The straightening or bending of the long posterior cilia was observed with a SZX 12 Olympus dissecting microscope equipped with a Panasonic CCD video camera and monitor. The irradiance at each neutral-density filter step with each narrow band interference filter was measured with an OceanOptics S2000 spectrophotometer in photons. The responses of 20 larvae were tested, and the mean relative sensitivity (inverse of threshold) was plotted for each interference filter. The experiment was conducted twice, once with the spectrophotometer calibrated to a known light source (OceanOptics LS1-CAL), with 7 interference filters, and once using relative irradiance and 16 interference filters. Both experiments gave identical results; the graph presented is from the second experiment.

Larvae were preserved for scanning electron microscopy with cilia in the straight and bent position by adding a fixative cocktail containing 1% OsO_4 and 2% glutaraldehyde in 0.45 mol l^{-1} sodium acetate buffer at pH 6.4, with 10% sucrose in the final volume, directly to the dish on the microscope stage. Specimen preparation for epoxy sections and scanning electron microscopy was carried out as described in Leys and Degnan (*2001*). Photographs were captured digitally from the video recording of the larva using Dazzle Digital Video Creator II (Dazzle Incorporated, Freemont, CA).

Results and discussion

The sponge larval cilia respond to abrupt increases and decreases in irradiance by straightening and bending (Fig. 2b). Using this response we determined an action spectrum for *Reniera* sp. larvae which indicates that the larvae have a main peak of

sensitivity to light near 440 nm and a secondary peak of sensitivity to light near 600 nm (Fig. 2c).

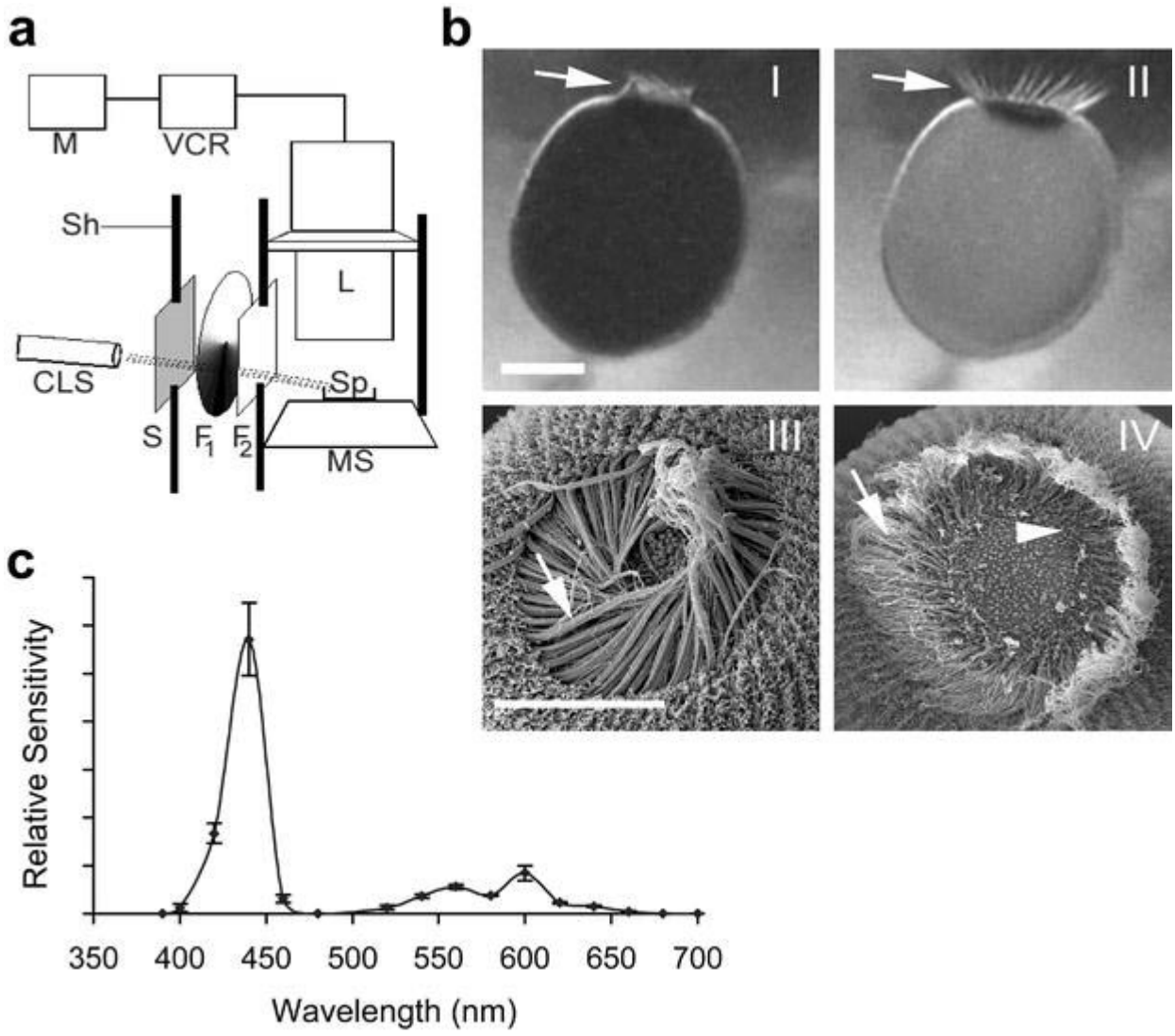

Fig. 2 a The experimental setup used to measure the response of the sponge larval cilia to light of different intensity and wavelengths. Cold white light (*CLS*) was shone through a variable neutral density filter (F_1) and a narrow band interference filter (F_2) onto the sponge larva (*Sp*) in a Petri dish on the microscope stage (*MS*) shield (*Sh*). A shutter (*S*) was opened and closed to generate abrupt increases and decreases in light intensity on the larva. Responses of the long posterior cilia were observed through the microscope lens (*L*) and monitor (*M*), and recorded on a video recorder (*VCR*). **b** Video recording of bending (*I*) and straightening (*II*) of the cilia (*arrows*) in response to closing and opening of the shutter respectively. Scanning electron micrographs show the position of the long cilia when the shutter is closed (*III*) and opened (*IV*). *Arrows* indicate the long posterior cilia; the *arrowhead* in IV shows the location of a ring of pigment-filled protrusions of cells adjacent to, and containing, the long posterior cilia. Scale bars, 100 µm. **c** Action spectrum of the response of the long posterior cilia to increases and decreases in light

intensity shows the larvae are maximally sensitive to light near 440 nm, and somewhat sensitive to light near 600 nm. Sensitivity was measured in photons. Bars indicate standard error (see text for methods)

For photoreceptors containing one photoreceptor pigment it is typically assumed that the action spectrum corresponds directly to the absorption spectrum of the photoreceptive pigment (Foster *2001*). However, self screening or absorption of light by pigments in the cell can significantly alter the shape of the action spectrum, and there is some evidence that the correspondence of action spectra with absorption spectra is least reliable with blue light responses (Galland and Senger *1991*).

The sponge ciliary response to light near 440 nm appears to be a typical blue-light response because the bandwidth is extremely narrow. The unicellular alga *Euglena* also shows two regions of peak sensitivity, near 465 nm and near 630 nm, during photokinesis, and an additional region of sensitivity, at 490-500 nm during phototaxis (Wolken *1971*). The so-called 'A-band' at 465 nm has long been considered by researchers to be caused by a flavin or a carotenoid (Diehn *1969*; Checcucci et al. *1976*; Wolken *1995*). Recent reanalysis of the paraflagellar organ of *Euglena* by microspectrophotometry in situ using polarized light also shows two principal absorbance peaks, an A-band near 465 nm, and a B-band near 500 nm (James et al. *1992*). The B-band is best fitted by a rhodopsin spectral template, and isolation of all-*trans*-retinal from the unicellular alga now strongly implicates rhodopsin as the molecular photoreceptor (Gualtieri et al. *1992*). The B-band is also similar to the peak seen in the action spectrum for phototaxis the unicellular alga *Chlamydomonas* in which light sensitivity, though previously thought to have been a blue-light response caused by a carotenoid (Nultsch *1983*), is in fact brought about by a rhodopsin-like protein, chlamyrhodopsin (Foster et al. *1984*; Deininger et al. *1995*).

The response of the sponge larva to light near 600 nm (orange/red) cannot be explained by flavoproteins, which show no absorption over 500 nm. In *Euglena*, chlorophyll is thought to cause the secondary peak near 630 nm (reviewed in Wolken *1995*); the sponge larva presumably has an equivalent pigment that causes the response at this high wavelength.

While blue light responses are common in plants, fungi, and protists, they are not so usual in animals, except perhaps in the entrainment of developmental rhythms (Holmes *1991*). If a flavoprotein is presumed to be responsible for the spectral sensitivity demonstrated by *Reniera* sp. larvae to light near 440 nm, then *Reniera* sp. would be the only metazoan in which the primary photoreceptive pigment (that used for behavioral responses) does not involve a rhodopsin or rhodopsin-like protein. Action spectra for phototaxis and a shadow response in hydromedusae (Cnidaria) show maximal sensitivity to light of 480-530 nm (Arkett *1989*). Extraocular photoreceptors responsible for contractions in *Hydra* in response to blue light at 470 nm have recently been localized on the tentacles with an antibody to rhodopsin (Musio et al. *2001*). All these cnidarian photoresponses are

consistent in spectral location and shape with a rhodopsin-based photoreceptive system. Furthermore, photoreceptors in cephalopod molluscs and in various Crustacea are known to involve true rhodopsins (Yoshida *1979*; Crandall and Hillis *1997*; Kitamoto et al. *1998*). As a final point, the presence of retinal isomers in the cerebral ganglia of an ascidian suggests that urochordates also possess rhodopsin-like photoreceptor pigments (Kajiwara et al. *1990*). Thus, while our data do not permit an unequivocal identification of the molecular basis of photoreception in *Reniera*, they do not rule out the possibility of a rhodopsin-based system.

Our results provide the first report of spectral sensitivity in the Porifera. The action spectrum indicates that larvae of the demosponge *Reniera* sp. are maximally sensitive to blue light. Although other pigments or combinations of pigments (e.g., cytochromes, β-carotene, or short-wavelength-absorbing rhodopsins) could be responsible for this action spectrum, the extremely narrow bandwidth of the primary response to light near 440 nm suggests that the photoreceptor pigment may be a flavin or carotenoid. Furthermore, while this response could conceivably be produced by the filtering of light by pigment vesicles near the responsive cilia, no reasonable short-pass filter pigments that would produce this sort of spectral sensitivity are known to exist in animals. The sensitivity of the larva to longer wavelengths is likely the result of another pigment such as a pterin. Future work incorporating action spectroscopy for phototaxis with microspectrophotometry of the larval cilia is planned to resolve these issues.

Acknowledgements. We thank the director and staff at the University of Queensland Heron Island Research Station for use of facilities for conducting this work, and the Great Barrier Reef Marine Park Authority for permission to carry out research. The experiments conducted herein comply with the current laws of the country in which they were performed. This research was funded by an NSERC operating grant 222863-99 to S.P.L.

References

Arkett SA (1989) Hydromedusan photophysiology: an evolutionary perspective. Nato ASI Series, Series A, Life Sciences 188:373-388

Bergquist PR, Sinclair ME (1968) The morphology and behaviour of larvae of some intertidal sponges. NZ J Mar Freshwater Res 2:426-437

Brien P (1973) Les Démosponges. In: Grassé P-P (ed) Traité de Zoologie. Masson, Paris, pp 133-461

Checcucci A, Colombetti R, Ferrara R, Lenci F (1976) Action spectra for photoaccumulation of green and colourless *Euglena*: evidence for identification of receptor pigments. Photochem Photobiol 23:51-54

Crandall KA, Hillis DM (1997) Rodopsin evolution in the dark. Nature 387:667-668

Deininger W, Kroger P, Hegemann U, Lottspeich F, Hegemann P (1995) Chlamyrhodopsin represents a new type of sensory photoreceptor. EMBO J 14:5849-5858

Diehn B (1969) Action spectra of the phototactic response in *Euglena.* Biochim Biophys Acta 177:136-143

Foster KW (2001) Action spectroscopy of photomovement. In: Häder D-P, Lebert M (eds) Photomovement. Elsevier, Amsterdam, pp 51-115

Foster KW, Saranak J, Patel N, Zarilli G, Okabe M, Kline T, Nakanishi K (1984) A rhodopsin is the functional photoreceptor for phototaxis in the unicellular eukaryote *Chlamydomonas.* Nature 311:756-759

Galland P, Senger H (1991) Flavins as possible blue light receptors. In: Holmes MG (ed) Photoreceptor evolution and function. Academic Press, London, pp 65-124

Gualtieri P, Pelosi P, Passarelli V, Barsanti L (1992) Identification of a rhodopsin photoreceptor in *Euglena gracilis*. Biochim Biophys Acta 1117:55-59

Holmes MG (1991) Photoreceptor diversity. In: Holmes MG (ed) Photoreceptor evolution and function. Academic Press, London, pp 65-124

James TW, Crescitelli F, Loew ER, McFarland WN (1992) The eyespot of *Euglena gracilis*: a microspectrophotometric study. Vision Res 32:1583-1591

Kajiwara S, Tamotsu S, Morita Y, Numakunai T (1990) Retinal isomers in the cerebral ganglion of the ascidian *Halocynthia roretzi*. Invert Reprod Dev 17:155-158

Kitamoto J, Sakamoto K, Ozaki K, Mishina Y, Arikawa K (1998) Two visual pigments in a single photoreceptor cell: identification and histological localization of three mRNAs encoding visual pigment opsins in the retina of the butterfly *Papilio xuthus*. J Exp Biol 201:1255-1261

Leys SP, Degnan BM (2001) Cytological basis of photoresponsive behaviour in a sponge larva. Biol Bull 201:323-338

Mackie GO (1979) Is there a conduction system in sponges? Colloques Int Cent Nat Res Sci Biol Spongiares 291:145-151

Musio C, Santillo S, Ferretti C, Robles LJ, Vismara R, Barsanti L, Gualitieri P (2001) First identification and localization of a visual pigment in *Hydra* (Cnidaria, Hydrozoa). J Comp Physiol A 187:79-81

Nultsch W (1983) The photocontrol of movement of *Chlamydomonas*. In: The biology of photoreception. Cambridge University Press, Cambridge, pp 521-540

Pavans de Ceccatty M (1974) Coordination in sponges. The foundations of integration. Am Zool 14:895-903

Reiswig HM (1971) In situ pumping activities of tropical Demospongiae. Mar Biol 9:38-50

Wapstra M, Soest RWM van (1987) Sexual reproduction, larval morphology and behaviour in demosponges from the southwest of the Netherlands. In: Vacelet J, Boury-Esnault N (eds) Taxonomy of Porifera. Springer, Berlin Heidelberg New York, pp 281-307

Wolken JJ (1971) Invertebrate photoreceptors. Oxford University Press, New York

Wolken JJ (1995) Light detectors, photoreceptors, and imaging systems in nature. Oxford University Press, New York

Woollacott RM (1993) Structure and swimming behavior of the larva of *Haliclona tubifera* (Porifera: Demospongiae). J Morphol 218:301-321

Yoshida M (1979) Extraocular photoreception. In: Autrum H (ed) Comparative physiology and evolution of vision in invertebrates. A. Invertebrate photoreceptors. Springer, Berlin Heidelberg New York, pp 581-640