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SHORT COMMUNICATION

THE EFFECT OF SCREENING PIGMENT MIGRATION ON SPECTRAL SENSITIVITY IN A CRAYFISH REFLECTING SUPERPOSITION EYE

BY KIM P. BRYCESON*

Department of Neurobiology, Research School of Biological Sciences, Australian National University, Canberra City, ACT 2600, Australia

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In crayfish, there is some evidence that visual pigments in the eye affect the spectral sensitivity, both at the receptor level (Goldsmith & Fernandez, 1968; Kong & Goldsmith, 1977) and in the optic fibres (Woodcock & Goldsmith, 1970). However, there is a discrepancy between the absorption spectrum of rhodopsin as measured by microspectrophotometry (λ_{max} is 530–550 nm, Goldsmith, 1978) and published measurements of spectral sensitivity [e.g. electroretinogram (ERG) peaks of 565–570 nm (Kennedy & Bruno, 1961) and intracellular recordings of 556 nm and 600–620 nm (Nosaki, 1969) and 538–634 nm (Waterman & Fernandez, 1970)]. Woodcock & Goldsmith (1970) suggested that sensitivity maxima in the orange and red were due to the non-alignment of the stimulating beam with the optical axis of the cell, thereby allowing light to filter obliquely through red and brown proximal screening pigment surrounding the rhabdom. Goldsmith (1978) showed (theoretically) that the red–brown proximal screening pigments of crayfish and lobster are indeed capable of shifting the effective absorption of the rhabdoms enough to account for the discrepancy between the absorption peak of the visual pigment and the published measurements of spectral sensitivity. However, the effect on spectral sensitivity of the migration of the proximal screening pigment has not been documented.

The present work shows for the first time that there is a diurnal variation in spectral sensitivity in the Australian freshwater crayfish *Cherax destructor* (Clark, 1936), which can be directly related to the migration of the proximal screening pigment.

The intracellular recording techniques of Bryceson & McIntyre (1983) for crayfish were used to measure the dark-adapted (DA) intensity/response function and spectral sensitivity over a 24-h period in the same animal. Animals were kept in shallow-water tanks on a light regime of 14 h light: 10 h dark and were of approximate carapace length 5 cm; those with visible corneal damage or milky or opaque eyes were

*Present address: Australian Plague Locust Commission, Department of Primary Industry, Barton, Canberra City, ACT 2600, Australia.

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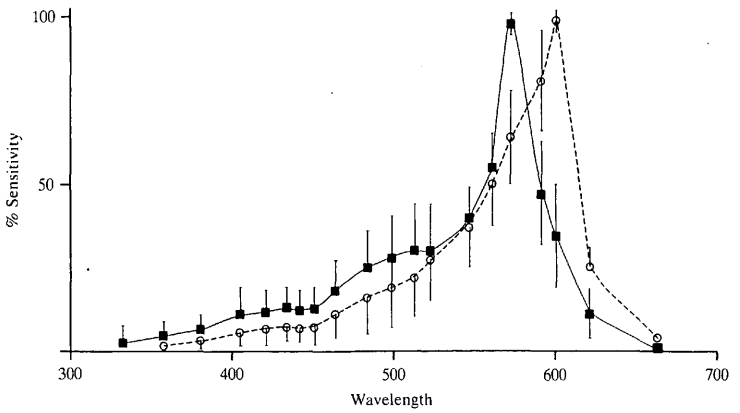

Fig. 1. Dark-adapted (DA) spectral sensitivity curves. Solid line, night; broken line, day. Error bars show standard deviation, $N = 12$.

discarded. The results of Bryceson & McIntyre's (1983) investigation on screening pigments in *Cherax* were used for correlating spectral sensitivity to screening pigment position.

The spectral sensitivity of a cell is defined as the reciprocal of the number of photons which elicits a constant response at each wavelength. This was obtained by recording responses of the cell to (calibrated) isoquantal flashes of light of known wavelength from a point source subtending an angle of 0·8° at the cornea and positioned on the optical axis of the cell (the direction giving a maximum response). The Schott (DIL) spectral filters ranged from 333 nm to 663 nm at approximately 20-nm intervals. To ensure that a rhodopsin–metarhodopsin conversion (Cronin & Goldsmith, 1982) was not affecting the result, the response values for each wavelength during two spectral runs in opposite directions were examined for differences. Runs with large differences were discarded since this indicated a rhodopsin–metarhodopsin conversion; for those with very small differences, the two runs were averaged and then converted to relative sensitivity by the use of the measured intensity/response function for that cell, normalized to a percentage of the maximum response on axis. This normalization of the intensity/response function enabled comparisons to be made between cells of different animals.

The normalized results from 12 animals (Fig. 1) show spectral sensitivity curves with a narrow bandwidth (see below) and a peak DA sensitivity (λ_{max}) during the day lying between 590 nm and 600 nm. At night this is shifted to the left in the DA state to a λ_{max} of 572 nm with a small secondary peak at 420–435 nm. These λ_{max} values are in general agreement with previously published results for *Procambarus* (Nosaki, 1969; Woodcock & Goldsmith, 1970) and *Orconectes* (Goldsmith & Fernandez, 1968), which are slightly red-shifted from the absorption peak of rhodopsin (see below). Difficulty was experienced in obtaining good spectral sensitivity runs during

the day because the maximum intensity of the monochromatic light was insufficient (Fig. 2). This difficulty was expected since it is known that the absolute sensitivity of this reflecting superposition eye is low during the day ($3{\cdot}17\times10^{10}$ peak axial photons $cm^{-2}s^{-1}$ are required to produce a 50 % maximum response) and increases at night (2×10^{8} peak axial photons $cm^{-2}s^{-1}$ gives a 50 % maximum response) (Bryceson, 1986).

In the reflecting superposition eye of *Cherax* there are four screening pigments – the distal reflecting pigment, the dark distal screening pigment, the red proximal screening pigment and the yellow proximal reflecting pigment. Special attention was paid by Bryceson & McIntyre (1983) to the effects of ambient light changes and inherent biological rhythms on the migration of these pigments. Of the four pigments, the distal reflecting pigment (found surrounding the distal ends of the cones) and the proximal reflecting pigment (which forms a cup around the base of each rhabdom) do not migrate, their positions remaining constant under different adaptation states and times of day. The distal and proximal screening pigments, however, do migrate. Distal screening pigment migration is dependent solely on ambient light levels, the pigment moving from the dark-adapted position (around the distal ends of the cones) into the clear zone on light adaptation. Proximal screening pigment migration is more complex and is dependent on both ambient light levels and a circadian rhythm, the pigment only reaching its fully dark-adapted position (below the basement membrane) at night.

The screening of the rhabdom during the day by the proximal screening pigment whatever the position of the distal screening pigment, means that the intensity and wavelengths of light available to the rhabdoms are substantially different to those at night. The shift from 600 nm to 572 nm in the intracellularly recorded day *versus* night spectral sensitivity in *Cherax* can be qualitatively attributed to the red proximal screening pigment around the rhabdom during the day, and the absence of it in the DA state at night. As the proximal reflecting pigment around the base of the

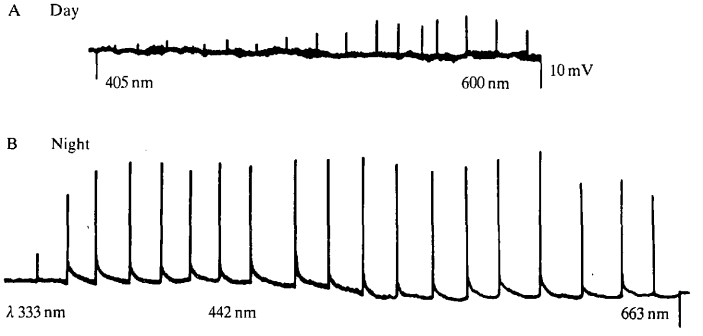

Fig. 2. Intracellularly recorded responses to monochromatic light flashes ranging from 333 nm to 663 nm. (A) Dark-adapted, day; (B) dark-adapted, night.

rhabdoms is non-migratory in *Cherax* (Bryceson & McIntyre, 1983) its effects on spectral sensitivity will be the same in all adaptation states and may be the cause of the slightly red-shifted spectral sensitivity curves during both day and night (Kong & Goldsmith, 1977).

Similarly, it is possible that the non-migratory distal reflecting pigment, which forms multilayer reflecting structures around the distal parts of the cones (Bryceson, 1981), may be the cause of the unusually narrow bandwidth of the spectral sensitivity curves. The physical optics of multilayer reflecting structures is outlined in Huxley (1968); essentially, however, there is only a narrow range of wavelengths over which constructive interference occurs at the surfaces of these structures; light not reflected is transmitted. In *Cherax* such transmitted light does not contribute to image formation (Bryceson & McIntyre, 1983) and the multilayers may therefore act as a selective filter modifying the spectral sensitivity of the underlying receptors.

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REFERENCES

BRYCESON, K. P. (1981). Focusing of light by corneal lenses in a reflecting superposition eye. *J. exp. Biol.* **90**, 347–350.

BRYCESON, K. P. (1986). Diurnal changes in photoreceptor sensitivity in a reflecting superposition eye. *J. comp. Physiol.* **158**A, 573–582.

BRYCESON, K. P. & MCINTYRE, P. (1983). Image quality and acceptance angle in a reflecting superposition eye. *J. comp. Physiol.* **151**, 367–380.

CLARK, E. (1936). The freshwater and land crayfishes of Australia. *Mem. natn. Mus. Vict.* **10**, 5–58.

CRONIN, T. W. & GOLDSMITH, T. H. (1982). Quantum efficiency and photosensitivity of the rhodopsin metarhodopsin conversion in crayfish photoreceptors. *Photochem. Photobiol.* **36**, 447–454.

GOLDSMITH, T. H. (1978). The spectral absorption of crayfish rhabdoms: Pigment, photoproduct and pH sensitivity. *Vision Res.* **18**, 463–473.

GOLDSMITH, T. H. & FERNANDEZ, H. R. (1968). Comparative studies of crustacean spectral sensitivity. *Z. vergl. Physiol.* **60**, 156–175.

HUXLEY, A. F. (1968). A theoretical treatment of the reflexion of light by multilayer structures. *J. exp. Biol.* **48**, 227–245.

KENNEDY, D. & BRUNO, M. S. (1961). The spectral sensitivity of crayfish and lobster vision. *J. gen. Physiol.* **44**, 1089–1102.

KONG, K. L. & GOLDSMITH, T. H. (1977). Photosensitivity of retinular cells in white-eyed crayfish (*Procambarus clarkii*). *J. comp. Physiol.* **122**, 273–288.

NOSAKI, H. (1969). Electrophysiological study of colour encoding in the compound eye of crayfish *Procambarus clarkii*. *Z. vergl. Physiol.* **64**, 318–323.

WATERMAN, T. H. & FERNANDEZ, H. R. (1970). E-vector and wavelength discrimination by retinula cells of the crayfish *Procambarus*. *Z. vergl. Physiol.* **68**, 154–174.

WOODCOCK, A. E. R. & GOLDSMITH, T. H. (1970). Spectral responses of sustaining fibres in the optic tracts of crayfish (*Procambarus*). *Z. vergl. Physiol.* **69**, 117–133.