Spectral Sensitivity of the Planarian Ocellus

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ABSTRACT   The ocellar potential (OP) of planaria was recorded using microelectrode techniques. The action spectrum and spectral sensitivity of the OP are described. Maximum OP sensitivity was found with 508 nm light. A moderate increase in sensitivity to blue light was observed. This is typical of many invertebrate photoreceptors and was shown, by selective chromatic adaptation, not to indicate the presence of a second pigment.

INTRODUCTION

It has been suggested that the ocellar potential (OP) of planaria is a relevant electrical correlate of visual function (Brown and Ogden, 1968). It was of interest to determine, by physiological means, the spectral sensitivity of the OP. Unlike a behavioral response, the OP provides a sharp criterion for the evaluation of the sensitivity of photoreceptors to different hues of light; dermal photoreceptors, which may affect the results of behavioral studies (Yoshida and Ohtsuki, 1966), should have no effect on the electrical activity of the ocellus.

OP action spectra and spectral sensitivity curves obtained from this study suggest that the OP is mediated by a single rhodopsin-like pigment, and generally confirm the results of behavioral studies (Marriott, 1958; Viaud, 1951).

METHODS

The method of recording action potentials from the eyecup of the planarian was described in detail in a previous paper (Brown and Ogden, 1968). For the present study, the light source was a shuttered 250 w, high pressure Hg-Xe arc lamp (Schoeffel L 250, Schoeffel Instruments, Westwood, N.J.) with an output of approximately 11,000 lumens. The beam of light was focused onto an electromagnetic shutter, then collimated and passed through interference and neutral density filters. The filtered light was reflected from a first surface mirror and focused to a 0.5 mm diameter spot which covered the ocellus. Small portions of the visible spectrum were isolated with Balzer interference filters (Filtraflex B-40) (Rolyn Co., Arcadia, Calif.) with approximately

255

40 % transmission at the nominal wavelength; half-intensity bandwidth (HIBW) of the filters was about 12 nm. The radiance of the source was controlled with Wratten neutral density films enclosed in glass, plain films, Perspex, and glass plates. Action spectra were obtained with equal energy stimuli. To obtain sensitivity curves, the energy of the stimulus light at various wavelengths was varied to elicit a constant amplitude response. A YSI radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) was used to determine the corrections necessary to obtain equal energy stimuli and to measure the energy used to elicit equal amplitude responses. The broad-band energy registration of the radiometer was calibrated against a standard of known radiant emittance (Electronic Testing Laboratories T-20). The sensitivity of the radiometer at specific wavelengths was calibrated against monochromatic light obtained from a quartz prism monochromator (Beckman DU); the latter was first calibrated against the isolated spectral lines of a low pressure mercury arc. The accuracy of the spectral calibration of the radiometer was checked by comparing the measured energy at different wavelengths with the calculated spectral irradiance of a 2200° K black body radiator, derived from Planck's equation.[1] There was good agreement between the calculated and measured energies.

Interference filters   The actual wavelength at which peak transmittance occurred (Table I, column 1) and the half-intensity bandwidth (HIWB) (column 2) of the interference filters were determined by measuring the transmission of the filters at 2 nm intervals in the Beckman spectrophotometer. The relative transmittance of the filters was estimated by graphical integration and normalized to 588 nm (column 3). These data, superimposed on the output characteristic ($E\lambda$ vs. λ) of the Hg-Xe source (column 4), yielded a first approximation of the stimulus energy at each wavelength (column 5).

Neutral Density Filters   The optical density ($-\log T$) of each of the filters was determined spectrophotometrically at 10 mμ intervals throughout the visible range; curves of the absorption characteristics of each filter were constructed. In general, the filters were 0.1 to 0.2 log units more opaque than their average nominal values at wavelengths shorter than 500 nm. The curves were used to determine the actual light energy required to elicit equal amplitude OP's and for the filter corrections to provide equal energy stimuli from each of the interference filters (colum 6, Table I). With the the filter corrections shown, 0.22 mw cm^{-2} of energy was obtained from each of the interference filters.

RESULTS

In the studies described below, light stimuli of 50 msec duration were presented every 60 sec. Recovery of ocellar sensitivity following a single flash of

[1]

$$E_\lambda = \frac{C_1 \lambda^{-5}}{e^{c_2/\lambda T} - 1}$$

Where E_λ = energy at specified wavelength, λ is the wavelength in microns, e is the base of natural logarithms, c_2 = 14384 micron degrees, and T is the absolute temperature. c_1 (3.740^3 × 10^{10} μw × μ^4 × cm^{-2}) was set at 1 to obtain relative values.

light (0.22 mw cm^{-2}) was virtually complete in 75 sec. The shorter interflash interval used made it possible to study the complete wavelength series in both ascending and descending order before the condition of the animal changed appreciably. As described previously (Brown and Ogden, 1968), studies of the planarian ocellus may be complicated by gradual changes in the state of the preparation. To control for this, it was necessary to use a procedure which provided a continuous monitor of ocellar sensitivity. This was accomplished by the use of a "control" 508 nm flash 60 sec before each test flash. The ampli-

TABLE I

	Interference filters		Source	Stimulus	ND filters
					Log T (for stimulus E = 0.22 mw cm^{-2})
λ	HIBW	Relative T	Relative E	Relative E	
nm					
407	11	0.850	0.35	0.30	$\bar{1}.94$
426	10	0.765	0.39	0.30	0
444	12	0.970	0.48	0.47	$\bar{1}.81$
474	13	1.17	0.385	0.45	$\bar{1}.81$
494	11	0.820	0.35	0.30	$\bar{1}.94$
508	11	1.18	0.23	0.27	0
528	12	1.05	0.50	0.53	$\bar{1}.72$
554	9	0.725	1.10	0.80	$\bar{1}.63$
588	10	1.00	1.00	1.00	$\bar{1}.48$
624	11	0.975	0.42	0.41	$\bar{1}.90$
640	13	1.25	0.42	0.52	$\bar{1}.80$
682	9	0.780	0.55	0.43	$\bar{1}.90$

tude of the control response provided a check of the condition of the preparation; also, this procedure assured that the ocellus was in an equivalent state of dark adaptation at the time of each test flash.

The maximum OP obtained with equal energy stimuli (λ_{max}) was evoked by 508 nm light. The data points shown in Fig. 1, averages of OP amplitudes from five different preparations, are expressed as a percentage of the response amplitude obtained at the λ_{max} in each preparation. Vertical lines through the points represent the standard deviation from the five preparations. The OP showed the greatest variability in the blue end of the spectrum, but this was not sufficient to mask differences among the points. Each datum point was statistically different from the others at the 0.05 level of confidence (F test). The curve represented by the dotted line was drawn by eye.

The sensitivity curve (Fig. 2) (reciprocal of energy necessary to elicit a response of the same amplitude at each wavelength) was plotted from data obtained from eight different preparations. Although the inflections are steeper than those of the action spectrum, the curve has the same general
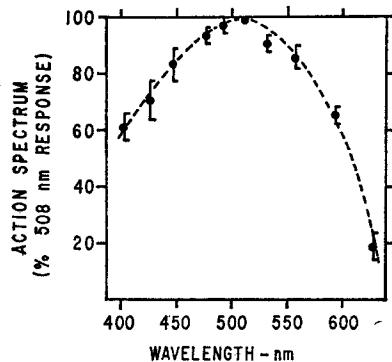
FIGURE 1. Action spectrum of planarian ocellus. Data points represent averages, from five different preparations, of OP amplitudes evoked by equal energy stimuli of various wavelengths, and are shown as a per cent of the maximum amplitudes obtained with 508 nm light. Vertical lines represent standard deviations.

form and the $E\lambda_{min}$ corresponds to the wavelength that elicited the maximal response with equal energy stimuli (508 nm). The curve obtained from the most sensitive eye was used to normalize data from each of the other eyes.

Of course, the "λ_{max}" of these studies is in fact the wavelength of peak transmission of the interference filter used. The actual λ_{max} of planaria could be 508 ± 10 nm. It was not possible to arrive at a statistically more significant estimate by fitting the data to Dartnall's nomograms; derived curves for 500 and 520 nm approximated the data no better than the theoretical curve for 508 nm.

One consistent feature of the sensitivity curves was the small increase in sensitivity at 426 nm. Selective chromatic adaptation experiments suggest that this did not result from the presence of two photopigments in the ocellus. Sensitivity curves from the blue end of the spectrum were obtained under
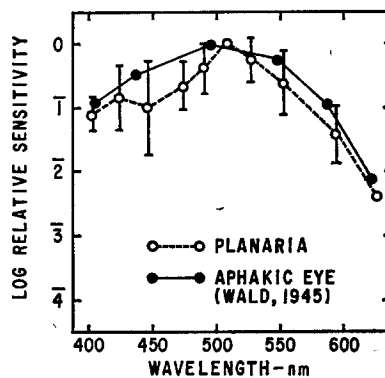

FIGURE 2. Spectral sensitivity of planarian ocellus. Sensitivity (reciprocal of energy required to elicit an OP of constant amplitude) is represented on the ordinate in $\log_{10}$ units (open circles). Data points and vertical lines represent the averages and standard deviations of data from eight preparations. The solid line represents the scotopic visibility curve of the aphakic eye, replotted from Wald (1945).

conditions of moderate adaptation with 407 nm light. The effect of the blue adapting light was to reduce sensitivity equally at all wavelengths. If a second pigment had been present, sensitivity at the shorter wavelengths might have been disproportionately reduced. The solid line in Fig. 2 is the scotopic visibility curve for the aphakic eye replotted from Wald (1945).

DISCUSSION

The action spectrum and spectral sensitivity of the planarian photoreceptors, obtained by physiological means, suggest the presence of a pigment that absorbs maximally at approximately 508 nm. The sensitivity curve of the OP was in general agreement with the form and $E\lambda_{min}$ of the scotopic visibility curve of the aphakic eye (Wald, 1945). This curve is known to parallel the absorption spectrum of rhodopsin. These lines of evidence suggest that the visual pigment in the planarian ocellus is a rhodopsin-like compound. The enhanced sensitivity of invertebrates to the blue region of the spectrum is well-documented (Milne and Milne, 1956), and the planarian appears to be no exception to this general finding. Wald (1945) has attributed this enhanced sensitivity to a lack of ocular media in simple eyes. Thus the small increase in blue sensitivity may reflect no more than lack of an ocular medium in planaria. Certainly the evidence presented makes it unlikely that this increase in sensitivity is due to a second photopigment. The $E\lambda_{min}$ found in the present study falls between those reported from behavioral studies conducted on planaria (475 nm, Viaud, 1951; 530 nm, Marriott, 1958). The discrepancy in these findings may be attributable to an unrecognized contribution by dermal photoreceptors to the behavioral response.

It is of interest to compare the amount of light required to elicit behavioral responses from planaria with that required to evoke an OP from the planarian ocellus. Marriott (1958) reported that the log threshold flux of 520 nm light required to elicit negative phototaxic behavior in planaria was approximately 4×10^4 quanta sec^{-1} eyespot^{-1}. In the present experiments, with the ocellus partially light-adapted, small OP's were obtained with 3×10^3 quanta flash^{-1} eyespot^{-1} of 508 nm light or 6×10^4 quanta sec^{-1} eyespot^{-1}. The lower thresholds of the behavioral studies probably result in part from more thorough dark adaptation. Also the dermal photosensitivity observed in planaria (Parker and Burnett, 1900) may lower their behavioral threshold. Marriott (1958) observed that an intact visual apparatus was required in order to demonstrate threshold responses from planaria. However, this may reflect the inability of planaria to display integrated phototaxic behavior when the head ganglion and the eyes are removed (Taliaferro, 1920; Ullyott, 1936); i.e., such planaria may be incapable of avoiding a beam of light within the amount of time allotted them.

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