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The Electrical Response of the Planarian Ocellus

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ABSTRACT The planarian ocellar potential (OP), an action potential evoked from the planarian ocellus by a light flash, was recorded with microelectrodes. OP amplitude, latency, and peak delay varied as a function of stimulus intensity and state of adaptation in a manner similar to the responses of other photoreceptors. Changes in the OP that occurred with different directions of incident light are described and attributed to screening effects of the ocellar pigment cells. The temperature coefficient (Q_{10}) of OP latency was 1.5; latency decreased continuously as temperature was increased to destructive levels. The energy of activation of the rate of OP formation was calculated to approximate 10 kcal. These findings suggest dependence of OP latency on ionic diffusion and of OP formation on a biocatalytic process.

INTRODUCTION

Investigations of the comparative physiology of visual receptors include extensive work on the highly organized eyes of arthropods and molluscs, but little is known of the more primitive eyes of members of lower phyla such as Platyhelminthes. It is important to determine the physiological limitations of the visual apparatus of planaria since this platyhelminth is commonly used in behavioral studies that involve the animal's response to visual stimuli. The planarian eye, or ocellus, is composed of pigment cells, in the shape of a cup, which enclose the distal ends of approximately 150 light-sensitive retinular cells (Press, 1959; Taliaferro, 1920). There are no synapses or cell bodies within the eyecup. Processes of the retinulae exit from the cup through a laterally directed aperture; the proximal ends of the retinular cells synapse directly in the neuropil of the cephalic ganglion (MacRae, 1964). Preliminary studies of the electrical activity of the planarian ocellus have been reported (Behrens, 1962; Brown and Ogden, 1965; Brown and Ogden, 1966).

It is the purpose of this paper to describe the ocellar potential (OP), an extracellular slow potential evoked in the planarian ocellus by light, and to describe the effect on this potential of (*a*) stimulus intensity and duration,

(*b*) direction of incident light, (*c*) polarized light, (*d*) light and dark adaptation, and (*e*) temperature.

METHODS

The improvisation of a method to restrain planaria was the major difficulty encountered in this study. The planarian ocellus "floats" on a loose syncytium of parenchyma; the eye can move even though the animal is immobilized. For these studies, the intact worm was placed in one of the small compartments formed by placing a single layer of cotton gauze on top of a moistened cotton pad. The animal was then covered with a thin, porous, artificial membrane (Pease, 1964) made by dipping a moisturized microscope slide into a 0.5% solution of Formvar (Ernest F. Fullam, Inc., Schenectady, N. Y.) in ethylene dichloride. Slight downward pressure applied uniformly to the edges of the membrane by a Perspex ring immobilized the animal. The membrane was easily penetrated by the microelectrode. This technique made it possible to obtain continuous recordings for as many as 3 hr. For those experiments in which an anesthetic was used to immobilize the preparation, a piece of the head containing the eyes was placed in 0.03% solution of MS-222 (Sandoz, ethyl M-amino benzoate) for 30 min. This was sufficient to reduce, but not abolish, movement; some mechanical restraint was also required.

The preparations were mounted on a hollow aluminum platform, cooled by circulating water. Experiments were conducted at 23°C unless stated otherwise. The preparation was kept moist by pumping an aerated solution to the pad of cotton on which the animal was placed. The bathing solution was similar to that used to culture planaria tissue in vitro (Murray, 1927), and consisted of the following molarities of salts 6.2×10^{-4} NaCl; 6.6×10^{-5} KCl; 7.7×10^{-4} $CaCl_2$; 1.7×10^{-4} $NaHCO_3$ (pH 7.4). Dextrose (2 mg/ml) was added to the medium.

Recording Methods The ocellar potential (OP) was recorded by means of 3 M KCl or sodium citrate–filled glass capillary micropipettes with tip diameters of approximately 0.5 μ, electrical impedance at 60 hz was 20–30 megohms. The microelectrode usually entered the superior margin of the eyecup aperture and was advanced ventromedially at a 45° angle by means of a hydraulic advancer. The reference electrode for differential recording was an electropolished platinum needle which penetrated the snout. The animal was grounded through the bathing solution. Potentials from the microelectrode were led to a capacity-neutralized cathode follower and a Tektronix 122 preamplifier; the reference electrode was led directly to the preamplifier. Potentials were displayed on a Tektronix 502A dual beam CRO for photographic recording. The frequency response of the system was approximately linear from 0.8 to 1000 hz. For DC recording, potentials were led directly from the cathode follower to the CRO.

Light Stimulation The OP was evoked by brief flashes from a Grass PS-2 (xenon) Photo-Stimulator, or by steps of light (10 msec— 2 sec) obtained from a glow modulator tube (Sylvania R1131-C); in the latter case, the beam was focused to a small spot by a 10 power microscope objective. Illuminance of the sources was measured with a photomultiplier (S-4 response), calibrated against a radiation standard (Electronic

Testing Laboratory, T-20). The peak illuminance of the 20 μsec electronic flash was 5000 lux; that of the glow modulator source was 1620 lux. For the studies of light adaptation, a tungsten source was used; the illuminance of this source was 5000 lux. The intensity of the sources was varied with neutral density filters, or by adjustment of the intensity settings of the Grass Photo-Stimulator.

RESULTS

Fig. 1 A shows the waveform of the OP evoked by a brief flash or light; the records were obtained with DC (top) and AC (bottom) coupled amplification. The amplitude of the potential shown here was unusually large (greater than 1 mv) which made it possible to obtain stable DC records. The majority of experiments, however, yielded OP's with amplitudes of 0.5–1.0 mv. Thus, most of the records to be shown required AC coupling in order to stabilize the base line. The OP was a simple monophasic potential of long duration (0.8–1.0 sec). The ascending negative limb had a minimum latency, with intense stimulation, of about 35 msec. The response decayed slowly to the base line, with a time constant of 0.3–0.4 sec. Unitary action potentials were never recorded from the eyecup; however, action potentials were detected occasionally when the electrode tip had passed through the eyecup into the presumed vicinity of the cephalic ganglion. On a number of occasions, the OP was observed to change polarity abruptly as the microelectrode was advanced through the eyecup. This phenomenon was not associated with a shift of DC potential, and has not been systematically investigated. Also, on occasion, the OP did not decay monotonically to the base line, but rather, a "step" appeared on the descending limb. This step, when present, was exaggerated with dark adaptation.

Effect of Stimulus Duration on the OP The OP showed no tonic component that was correlated with sustained exposures to light (glow modulator source). Also there was no evidence of an "off" component when a sustained light was extinguished. This is shown in Fig. 1 B. In this study there was little difference in the OP evoked by a 1.25 sec and a 40 msec stimulus. Free-living planaria, exposed to a 3 sec exposure of light of a similar intensity, respond by turning the cephalic end at both the onset and offset of the stimulus. The turns at "on" are by far more frequent than the turns at off (H.M. Brown, unpublished). Since it is not possible to correlate this behavioral response at off with the OP, it may reflect the activity of dermal photoreceptors. Fig. 1 B also shows that with stimulus durations less than the latency of the OP (35–400 msec, depending on intensity), the amplitude of the OP was reduced in size. This implies that Bloch's law (intensity × duration = constant response) might apply when the OP was evoked by stimuli with durations shorter than the OP latency.

Effect of Two Flashes on the OP In these studies two brief pulses of light, separated by a variable time interval, were presented. No OP was elicited by the second flash if the interval between flashes was less than about 240 msec. With the intensity used, an interflash interval of 50–60 msec or less resulted in potentiation of the OP. This effect, shown in Fig. 1 C, may represent a form of temporal summation since the light stimuli used did not elicit

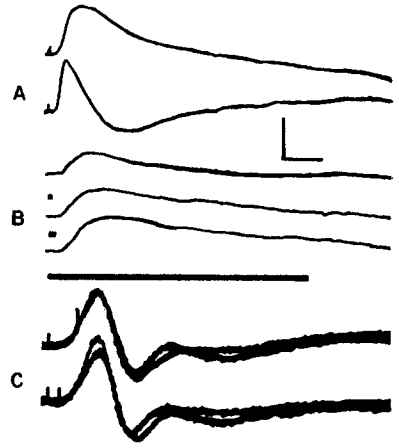

FIGURE 1 A. Ocellar potential (OP) evoked by a unit intensity xenon flash. The records shown were obtained with direct (top) and AC-coupled (bottom) amplification. Negativity of the microelectrode in the eyecup is shown as an upward deflection in this and all subsequent records. The brief upward deflection that precedes the OP is the response of a photocell used to monitor the light flash. Calibration, 2 mv, 200 msec.

FIGURE 1 B. Effect of stimulus duration on the OP. Durations of stimuli from the glow modulator source are indicated by the solid black line beneath each record. Top trace 0.025, middle trace 0.04, and bottom trace 1.25 sec. DC amplification. Calibration, 2 mv, 200 msec.

FIGURE 1 C. Augmentation of the OP by a double pulse of light from the xenon source presented within the latent period of the response. Upper traces, response to a single flash (first artifact), and then test response to a pair of flashes (first and second artifact) photographically superimposed on a single film frame. Interflash interval, 70 msec. The second flash had no effect on OP amplitude. Bottom trace, interflash interval, 30 msec. Note increase in size of test response. Calibration, 0.5 mv, 100 msec. All flashes unit intensity.

a maximal response. Alternatively, the potentiated OP may be due to a photochemical effect and thus may be an expression of the Bunsen-Roscoe law.

The Effects of Stimulus Intensity on the OP Fig. 2 shows five CRO traces, photographically superimposed at the time of the experiment; stimulus intensity was approximately halved for each successive response. Peak delay and latency increased, and amplitude decreased linearly with a logarithmic

(log_2) reduction of light intensity. These effects are shown graphically in Fig. 3, the data for which were obtained from the same preparation. Each of these curves has about the same slope; this implies that there was no appreciable change in form, with change in light intensity, during the initial 100–150 msec of the OP. In most preparations the latency and amplitude of the OP varied linearly with the logarithm of intensity over a range of about 4 log_{10} units.

FIGURE 2. Effect of stimulus intensity on the OP. The five traces shown were photographically superimposed on the same frame of film during the experiment. The largest response was obtained with a xenon flash of unit intensity. Other traces were evoked with I = one-half, one-fourth, one-eighth, and one-sixteenth of this intensity. This study was carried out on a worm anesthetized with MS-222. Calibration, 10 msec, 0.5 mv.

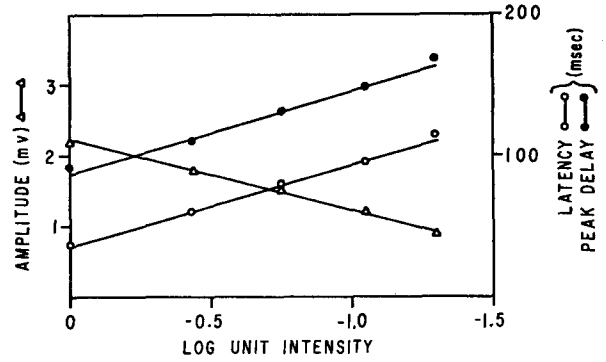

FIGURE 3. Graph of OP amplitude (left ordinate, triangles), latency (right ordinate, open circles), and peak delay (right ordinate, solid circles) as a function of the intensity (log_{10}) of flashes of light from the xenon source (abscissa). All measurements were obtained from the same preparation.

Directional Sensitivity of the Ocellus Planaria placed in a gradient of illumination move away from the light. On the basis of behavioral experiments, Taliaferro (1920) ascribed this negative phototaxic behavior to differential illumination of the photoreceptors in the two eyecups. Since light directed into the aperture of the ocellus was maximally effective in eliciting the phototaxic response, Taliaferro suggested that this orientation best exposed the lamellar surfaces of the photoreceptors. It was of interest to obtain direct physiological evidence of the effects of the intensity and direction of incident light. In the following study, the OP was used to evaluate ocellar sensitivity to light incident upon the ocellus from different directions. Sensitivity was

found to decrease as the stimulus was moved away from the axis of the aperture of the ocellus. When light was incident on the medial ocellar surface, it was necessary to raise the stimulus intensity by about 0.7–1 $\log_{10}$ unit to obtain a response of the same size as that evoked by light directed into the aperture.

To study the directional sensitivity of a planarian ocellus, the animal's eye was centered beneath a post which served as the axis of rotation of the stimulating light source. After the initial centering of the animal, the source was always equidistant from the eye, regardless of the angle of incidence of the light. This assured equal illuminance of the eye at all positions of the source.

Fig. 4 shows the effects on the OP of different orientations of the xenon flash light source. The inset, a diagrammatic planarian head, shows the four

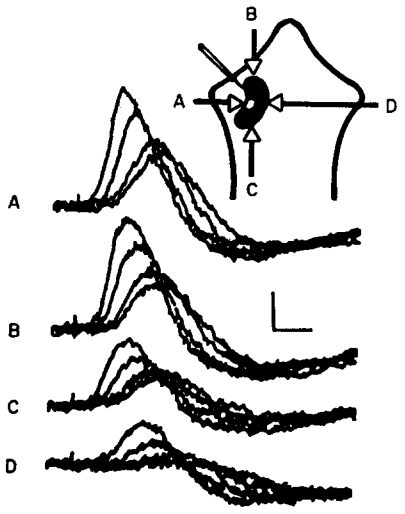

FIGURE 4. Directional sensitivity of planarian ocellus. Inset shows the four positions (A, B, C, D) of the xenon source and the position of the microelectrode with respect to the ocellus. Each of the four sets of superimposed traces (A, B, C, D) was obtained when the source was located at one of the positions shown in the inset. Responses to four light intensities were obtained at each position of the source. The top trace in each set was obtained at unit intensity; other traces were obtained with intensities of approximately one-half, one-fourth, and one-eighth unit intensity. Calibration, 100 msec, 0.5 mv.

different positions of the source (A, B, C, D) that were used during stimulation of the eye. The top set of superimposed traces (A) was obtained when the source was in position A. The first trace in set A was obtained with light of unit intensity; the second trace was obtained with one-half, the third trace with one-fourth, and the fourth trace of Fig. 4 A with one-eighth unit intensity light. The source was then moved to a new position and the same series of intensities was presented to the eye. The actual order of light positions was D, A, B, C; the intensity change was presented in ascending order at equal time intervals. These precautions were taken so that the results would be independent of changes in amplitude due to light adaptation or gradual deterioration of the preparation. The amplitude of the OP varied in a systematic manner with changes in the intensity and position of the source. This can be seen by comparing the top traces in each of the sets, A through D (each

obtained with the same intensity stimulus). The amplitude decreased as the source was changed to positions B, C, and D; the largest response was recorded when the source was in line with the eyecup aperture, and the smallest response was obtained when the source was 180° from the aperture. Also the latency of the OP increased as the amplitude decreased in a manner similar to that seen with changes in stimulus intensity alone (Fig. 3). These relationships can best be shown graphically.

Fig. 5 shows the amplitude (ordinate in top graph) and latency (ordinate in bottom graph) of the OP as a function of the logarithm ($\log_{10}$) of light intensity (abscissa) for four different positions of the stimulus source: A, B, C, and D (see inset, Fig. 4). The results shown in the top graph were plotted

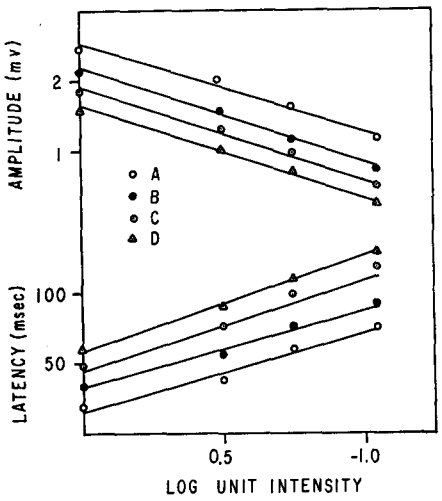

FIGURE 5. Graphs of the amplitude (top ordinate) and latency (bottom ordinate) of the OP as a function of the logarithm ($\log_{10}$) of intensity (abscissa) at four positions of the xenon light source A, B, C, D (see Fig. 4). At each position, the OP varied linearly with the logarithm of light intensity. Rotation of the source from A to D had about the same effect as a 1 log unit reduction in light intensity.

from data obtained during the experiment shown in Fig. 4. The latency plot was constructed from data obtained from a different preparation. Fig. 5 shows that the latency and amplitude of the OP varied consistently with the logarithm of intensity at each position of the source. Since the slopes of the curves were unchanged, these relationships were independent of the angle of incident light. The similarity of these plots to those shown in Fig. 3 suggests that the effect of directional light on OP amplitude results from differences in effective intensity, perhaps resulting from screening of the receptors by the pigment of the eyecup.

The plane of polarization of incident illumination did not appear to contribute to the directional sensitivity of the planarian eye. In a series of experiments, plane-polarized light was directed into the eye from the four directions illustrated in Fig. 4. Systematic alteration of the plane of polarization of incident light was without effect on the OP.

The Effect of Dark Adaptation on the OP Studies of the effects of dark adaptation on ocellar sensitivity were complicated by a tendency for the amplitude of the OP to diminish gradually during the experiment. The curves obtained represent a compromise between an optimal experimental procedure and a test series which could be completed rapidly. Ocellar sensitivity was significantly reduced for about 1 min following a single brief test flash. Fig. 6 illustrates the time course of recovery of OP amplitude following a single xenon flash of unit intensity (open circles) and 0.1 unit intensity (solid circles). In this experiment the ocellus was brought to a steady state of light adaptation by stimulating with a number of brief flashes at a rate of four per min. Then, following 90 sec dark adaptation, the ocellus was light-adapted with a single flash. The response to a subsequent test flash was recorded after a measured period in the dark. This procedure was repeated for each datum

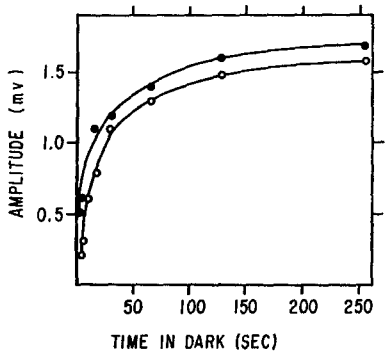

FIGURE 6. Graph of recovery of the OP in the dark following brief adapting flashes of unit intensity light (open circles) and 0.1 unit intensity light (solid circles) from the xenon source. The amplitude of the OP (ordinate) is plotted as a function of the interval of time in the dark (abscissa). Test stimulus, xenon source, unit intensity.

point shown in Fig. 6. The data were rejected if the steady-state responses significantly diminished in amplitude during the experiment. A comparison of the two curves shown in Fig. 6 shows that a very brief flash had a lasting effect on the absolute recovery level of the response. Even after 4 min of dark adaptation, the test response was smaller following a unit intensity adapting flash, than following a 0.1 unit intensity adapting flash. However, the shapes of the two recovery curves are quite similar. These data, plotted with a logarithmic time scale, yielded curves that were linear from 4–150 sec.

The analysis of dark adaptation was extended by using adapting lights of longer durations. For this purpose a tungsten source was used to light adapt the ocellus. The recovery of the OP following adapting exposures of 30 sec was determined using the same procedure outlined above. The analysis was limited to the first minute of dark adaptation; this permitted investigation of a full range of adapting intensities before there was an appreciable change in the control responses due to deterioration of the preparation or movement of the microelectrode.

Fig. 7 shows the amplitude of the OP, plotted as a function of time in the dark, following the adapting exposure. The relative intensity of the adapting exposure in $\log_{10}$ units is indicated beneath each of the curves. The growth of the response in the dark was more linear with the higher intensities of the adapting light.

Effects of Light Adaptation on the OP The amplitude and latency of the OP have been shown to vary in a systematic manner with changes in stimulus intensity (Figs. 2–5). Amplitude, but not latency, was also a function of background illumination. Fig. 8 A shows a set of photographically superimposed

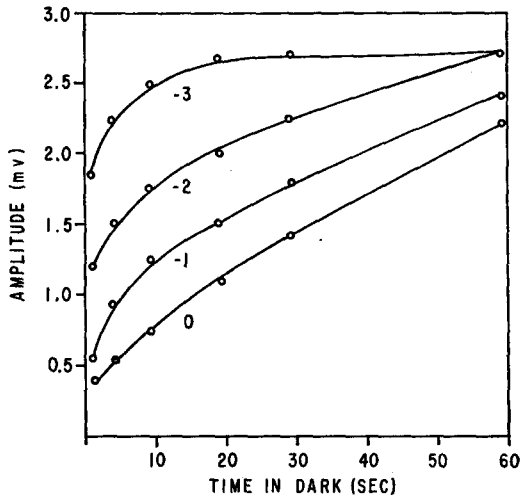

FIGURE 7. Graph of recovery of the OP in the dark following 30 sec light adaptation with tungsten source. The relative intensity of the adapting light in $\log_{10}$ units is shown below each of the recovery curves. Ordinate, OP amplitude. Abscissa, time in the dark. Test stimulus, xenon source, unit intensity.

traces obtained when the planarian eye was exposed to four different intensities of background light. The amplitude of the OP was reduced by approximately equal increments with each logarithmic increment in background illuminance; the latency of the OP was not markedly changed. With high levels of background light, it was difficult to determine OP latency with accuracy. Many experiments and a statistical treatment of the data were required to substantiate the lack of effect of background light on latency. The data from five preparations, studied with high amplification and high sweep velocity, are included in this analysis. Three records with stable base lines were selected for each level of background illumination. Latencies were measured and averaged. The results are shown in Table I. The means, variances, and rounded confidence intervals (mean $\pm$ 1 SD) are shown. The means of

the OP latency for the three lowest levels of background illuminance were approximately 40 msec; the overlap in the confidence intervals indicates that there was no statistical difference among them. The increase in the variance with background intensities greater than $-2 \log_{10}$ units reflects measurement difficulties.

The dependence of OP latency on stimulus intensity alone, and OP amplitude on both stimulus and background light intensity, is illustrated in Fig. 8 B. The OP shown in the top trace was evoked by a xenon flash of unit intensity in the presence of background illumination of $-1.6 \log_{10}$ units. The

TABLE I

AVERAGE OF THREE OP LATENCIES FROM FIVE PREPARATIONS UNDER THREE LEVELS OF BACKGROUND ILLUMINANCE (−4, −3, −2 LOG UNITS)

The mean, variance, and confidence interval (C.I., mean ± 1 SD) are shown for each level of background illuminance.

	OP latencies		
	Log_{10} background I		
Ocellus	−4	−3	−2
	msec	*msec*	*msec*
1	40	42	30
2	38	40	44
3	46	38	56
4	38	40	30
5	40	36	40
Mean	41	39	40
Variance	11.25	5.25	118
C.I.	37–43	37–41	30–50

bottom trace, obtained with a flash of 0.1 unit intensity without background illumination, had about the same amplitude although the response latency was longer with reduced stimulus intensity.

Effect of Temperature on the OP As temperature was increased from 15°–23°C the amplitude increased and the latency and peak delay decreased (Fig. 9). At temperatures greater than 25°–27°C (as shown in the trace obtained at 30°C in Fig. 9) the amplitude diminished, but latency and peak delay continued to decrease until the potential was abolished (approximately 42°C). These temperature changes were reversible if the temperature was not raised above 30–32°C.

The amplitude of the OP is shown in the top graph in Fig. 10 as a function

of temperature. The temperature coefficient (Q_{10}) of OP amplitude between 14° and 24°C was calculated from the results of six experiments. The mean value was 1.75. The graph also shows the peak delay and latency of the OP as a function of temperature. Both decreased continuously as the temperature

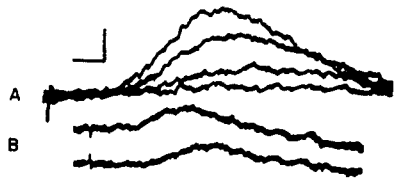

FIGURE 8 A. Effect of background illumination on the OP. Four traces were photographically superimposed at the time of the experiment. Responses to test flashes from the xenon source were evoked in the presence of background light of −1.0 (bottom trace) −2.0, −3.0, and −4.0 (top trace) $\log_{10}$ unit intensity. Calibration, 20 msec, 0.5 mv.
FIGURE 8 B. Effect of light adaptation on OP latency. The top trace (latency approximately 50 msec) was obtained with a flash of light from the xenon source of unit intensity in the presence of background illumination of −1.6 $\log_{10}$ units. The bottom response, without background light (latency approximately 80 msec), which has about the same amplitude, was obtained with 0.1 unit stimulus intensity. Calibration, 50 msec, 0.5 mv.

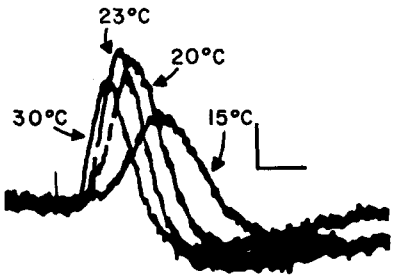

FIGURE 9. Effect oi temperature on the OP. The four traces shown were superimposed photographically during the experiment. The temperature of the whole animal was changed continuously from 15°–30°C. OP latency decreased and amplitude increased from 15°–23°C. At higher temperatures, both latency and amplitude diminished. Calibration, 100 msec, 0.5 mv. Test stimulus, xenon source, unit intensity.

was raised from 15°–26°C. Q_{10}'s calculated for the range of 14°–24°C, were 1.9 for peak delay and 1.5 for latency.

The Q_{10} values obtained in the physiologic range of 14°–24°C are less than 2.3; this suggests that the processes represented by the OP are not rate-limited by a conventional thermochemical reaction (van't Hoff rule). Additional evidence for this possibility was obtained when the reciprocal of the delay to the peak of the OP (a measure of the rate of formation of the peak of the response) was plotted against the reciprocal of temperature $(°K)^{-1}$. The

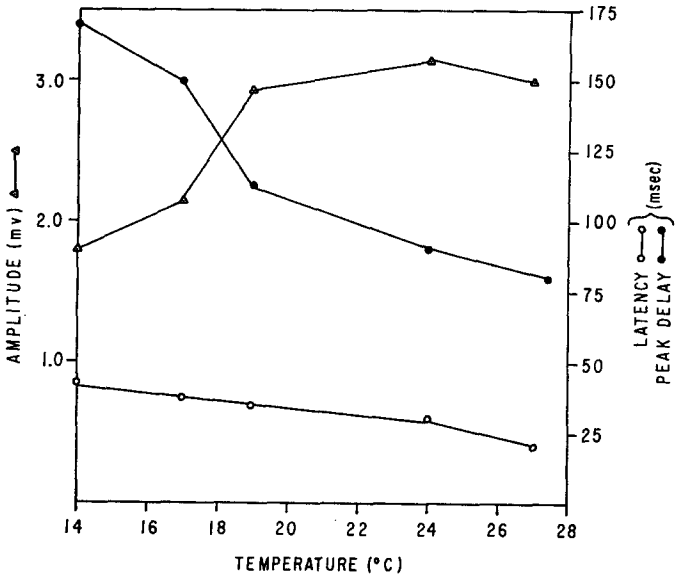

FIGURE 10. Graphs of the effect of temperature on the OP. The amplitude (left ordinate), peak delay, and latency (right ordinate) are represented as functions of temperature (abscissa). Test stimulus, xenon source, unit intensity.

data points fit the Arrhenius equation (solid line, Fig. 11) reasonably well when the activation energy (*Ea*) was approximately 10 kcal mole^{-1}.

$$\text{Log}_{10}\,(\text{peak delay})^{-1} = \frac{Ea}{2.3R} \cdot \frac{1}{T}$$

(R, gas constant = 1.98 cal mole^{-1} degree^{-1}; T, temperature in degrees Kelvin). This value is in the range expected from enzymatically catalyzed biological reactions and the latency Q_{10} is in the range of values expected from the effects

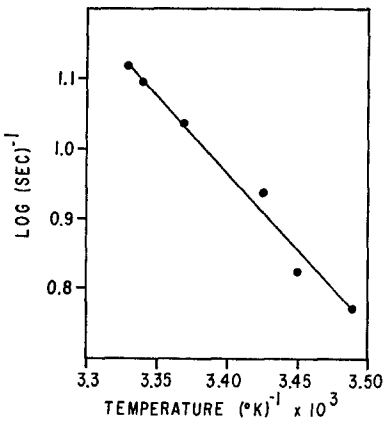

FIGURE 11. Graph of the logarithm of the reciprocal of OP peak delay as a function of the reciprocal of the absolute temperature of the preparation (solid circles). A plot of the Arrhenius equation with Ea = 10 kcal mole^{-1} is also shown (solid line).

of temperature on diffusion of electrolytes in water (Giese, 1957). Thus it may be suggested that the generation of extracellular current in the planarian eyecup is dependent upon an enzymatically catalyzed reaction. The magnitude of the OP latency and its Q_{10} are consistent with a dependence of latency on ionic diffusion over a considerable distance.[1]

DISCUSSION

The OP was detected only when the microelectrode tip was within the ocellus. Because the ocellus is composed of but two types of cells, retinular and pigment cells, the OP must represent extracellular current flux from one or the other, or a combination, of these cells. Anatomical evidence and evidence presented in this study indicate that the OP is generated primarily by retinular cells: (*a*) The fine structure of planarian retinulae is characteristic of photoreceptors that develop from infolding of plasma cell membranes (Eakin, 1965) and it has been suggested that the lamellated distal endings of the retinulae are the site of the visual photopigment (Press, 1959). (*b*) Movement of the stimulating light source away from the ocellus aperture reduced OP amplitude but did not alter its form. If pigment cells contributed substantially to the OP, such a procedure should cause a relative increase in the pigment cell contribution; this would probably alter OP form. The observation of Behrens (1962), that the OP of the dark-adapted ocellus has a step on the descending phase, was confirmed during the course of the present study. A similar step also appeared with temperature elevation. These findings suggest that the OP may contain a second small component, possibly contributed by the pigment cells.

The absence of a detectable OP outside the eyecup was not an unexpected finding. In an isotropic volume as small as the planarian head, action currents spread widely. Thus the entire head may be essentially equipotential for an extraocellar OP. If this were so, no OP would be recorded with both the reference and microelectrode outside the ocellus. OP inversion, which was only observed occasionally, cannot be explained satisfactorily from the existing data. When inversion occurred, the electrode appeared to penetrate a mechanical barrier. This was not associated with a sustained change in DC potential.

[1] The average displacement of a molecule down a concentration gradient may be related to diffusion time by the equation for linear diffusion, a simplified form of which is:

$$\bar{X}^2 = 2\,Dt \text{ (Setlow and Pollard, 1962)}$$

where $\bar{X}$ is the average displacement, D = diffusion coefficient, and t = time. The identity of a hypothetical "photoactivated molecule" is, of course, unknown. However, if one assumes that it is relatively large (molecular weight, 40,000–400,000), its diffusion coefficient would be in the range 1.0 to 8.0 $\times$ 10^{-7} cm^2 sec^{-1}. Such molecules would be expected to diffuse approximately 0.8–2.5 μ in the minimal latent period of 35 msec. The microvillar portion of the planarian photoreceptor measures approximately 5 $\times$ 35 μ (Wolken, 1958).

It is possible that inversion occurred when the electrode passed out of the eyecup into the vicinity of the retinular cell fibers, but direct evidence of the location of the electrode is lacking. Unitary action potentials were never recorded from within the ocellus; on occasion they were recorded from a region presumed to be the cephalic ganglion. These units were spontaneously active and their firing rate was never altered by light stimulation. The possibility that the retinular fibers may generate impulses cannot be directly refuted; however, transmission from ocellus to brain need not involve impulse generation in planaria. The distance involved is only about 50 μ (MacRae, 1964). The retinular fibers are 0.5–1 μ in diameter, and should have a space constant in excess of 150 μ. Thus electrotonic conduction could adequately account for central transmission.

The OP is a phasic slow potential; it is not sustained during illumination, and no signal was seen at the cessation of illumination. In form, the OP resembles the photoreceptor potentials of the starfish ocellus (Hartline, Wagner, and MacNichol, 1952), the pulmonate snail (Wolbarsht and Gillary, 1966), the silkworm pupa (Eguchi, Naka, and Kuwabara, 1962), and the tadpole (Crescitelli and Nilsson, 1966). Like all other photoreceptor potentials, the amplitude of the OP was proportional to the logarithm of light intensity, and its latency varied inversely with stimulus intensity. The OP probably follows simple photochemical principles such as the Bunsen-Roscoe law for stimulus durations shorter than the response latency. Also, the effects of light adaptation and the time course of dark adaptation were qualitatively similar to those reported for higher forms.

The planarian ocellus, under the conditions of this study, was insensitive to the plane of polarization of incident light. Waterman and Horch (1966) have suggested that regularly oriented pigment molecules in a receptor cell can function as a dichroic analyzer capable of detecting plane-polarized light. If this were true in planaria, it would not be recognized under the conditions of these experiments. Planarian retinulae radiate in many directions from the axis of the ocellus. Unicellular recordings would be required to establish the ability of individual cells to detect the plane of polarization of incident light.

Temperature had a pronounced effect on the amplitude, latency, and form of the OP. As temperature was raised, latency and peak delay were reduced and the amplitude was increased. These results are similar to those obtained with intracellular microelectrodes from the receptors of *Limulus* (Borsellino, Fuortes, and Smith, 1965), but unlike the results obtained from leech photoreceptors. Walther (1966) found the Q_{10} of the leech photoreceptor potential latency to be between 2 and 3. The effect of temperature on latency was nonlinear and response amplitude was little affected by changes in temperature. As the temperature was raised in the present study, a differential effect on OP amplitude and latency was observed; amplitude began to decrease only when

the temperature was raised above 27°C, but OP latency decreased continuously until the OP was abolished. This suggests that the OP is dependent upon two distinct mechanisms. A similar proposal has been made for the retinal action potential of the arthropod eye (Wulff, Fry, and Linde, 1955). The dissimilar Q_{10}'s of OP latency and amplitude found in the present study are in accord with this concept. The low activation energy of the OP peak delay suggests that OP generation is dependent on a biocatalytic process. On the other hand, the latency of the OP was long and latency Q_{10} was low, in a range characteristic of diffusion. These findings suggest that a reaction involving diffusion may precede the changes in receptor membrane conductance responsible for the action currents of the OP. The observation that OP latency continuously shortens as temperature is raised to destructive levels is in keeping with this suggestion.

The findings of the present study may contribute something to the interpretation of behavioral experiments in which light stimuli are used. Taliaferro (1920) noted that light directed into the ocellar aperture was maximally effective for the elicitation of negative phototaxic behavior; he concluded that such illumination was most effective because it maximally exposed the lamellar surfaces of the retinular cells. From the present study, it appears that light is most effective from this direction simply because there is less masking by the pigment cells of the eyecup. Furthermore, recent studies of the fine structure of the planaria eye (Press, 1959; Röhlich and Török, 1961; Wolken, 1958) show that light directed into the aperture, along the axis of the eyecup, would actually be parallel to the microvillar surfaces of the retinular cells.

Several behavioral studies purport to show that planaria are capable of learning; i.e., that planaria can be classically conditioned. The interpretations of these studies have been criticized because the effects of variables such as light intensity, light and dark adaptation, and temperature were not given adequate consideration (Brown and Beck, 1964; Brown, Dustman, and Beck, 1966 *a*, *b*; Brown, 1967 *a*, *b*; VanDeventer and Ratner, 1964). The use of certain light parameters in conditioning trials has been shown to lead to altered phototaxis (Brown et al., 1966 *a*) mistakenly attributed by some to "learning." Also it has been suggested that the use of electric shock in conditioning trials may sensitize planaria to light (Brown et al., 1966 *b*). The present study has demonstrated the feasibility of direct investigation of planarian photoreceptors. However, additional studies, under the same conditions used in behavioral experiments, would be helpful in the interpretation of published behavioral data.

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