The Spectral Sensitivities of Single Cells in the Median Ocellus of *Limulus*

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ABSTRACT The spectral sensitivities of single *Limulus* median ocellus photoreceptors have been determined from records of receptor potentials obtained using intracellular microelectrodes. One class of receptors, called UV cells (ultraviolet cells), depolarizes to near-UV light and is maximally sensitive at 360 nm; a Dartnall template fits the spectral sensitivity curve. A second class of receptors, called visible cells, depolarizes to visible light; the spectral sensitivity curve is fit by a Dartnall template with λ_{max} at 530 nm. Dark-adapted UV cells are about 2 log units more sensitive than dark-adapted visible cells. UV cells respond with a small hyperpolarization to visible light and the spectral sensitivity curve for this hyperpolarization peaks at 525–550 nm. Visible cells respond with a small hyperpolarization to UV light, and the spectral sensitivity curve for this response peaks at 350–375 nm. Rarely, a double-peaked (360 and 530 nm) spectral sensitivity curve is obtained; two photopigments are involved, as revealed by chromatic adaptation experiments. Thus there may be a small third class of receptor cells containing two photopigments.

INTRODUCTION

Receptor potentials have been recorded intracellularly from cells in the compound lateral eyes, median ocelli, and rudimentary eyes of *Limulus*. The spectral sensitivities of all these eyes have been investigated by examining the ERG and single eccentric cell responses in the lateral eye (Graham and Hartline, 1935; Wald and Krainin, 1963), the ERG in the median ocellus (Chapman and Lall, 1967; Wald and Krainin, 1963), and single cell responses in the rudimentary ventral eye (Millecchia, Bradbury, and Mauro, 1966). The spectral sensitivity curves obtained for lateral and ventral eyes agree fairly well (above 450 nm) with the difference spectrum of rhodopsin extracted from *Limulus* lateral eyes (Hubbard and Wald, 1960), and with the density spectrum obtained by microspectrophotometry in the ventral eye (Murray, 1966). However, the spectral sensitivity curve derived from median ocellus ERG measurements is bimodal, with a principal peak at about 360 nm and a secondary peak, 2–3 log units less sensitive, at 530–535 nm. On the basis of

experiments involving chromatic adaptation of the ERG, previous authors (Chapman and Lall, 1967; Wald and Krainin, 1963) concluded that the two peaks of sensitivity arise from the activity of two different populations of receptors, each containing a different photopigment. A preliminary investigation using intracellular microelectrodes (Nolte, Brown, and Smith, 1968) showed that this hypothesis is basically correct. Two principal types of receptor cells are found in the ocellus. One type responds with a depolarization to UV light and a small initial hyperpolarization to visible. The second type is complementary, responding with a depolarization to visible light and a small initial hyperpolarization to UV. For simplicity these receptors are called UV cells and visible cells, respectively. It was noted briefly in the earlier report that a small number of receptor cells seem to respond with a pure depolarization to all wavelengths. We provisionally call cells of this type "UV-visible cells." In this report we describe the spectral sensitivities of all three receptor cell types.

METHODS

Preparation and Recording A median ocellus was dissected away from the lens and carapace and pinned down in a perfusion dish. Artificial seawater (423.0 mM NaCl, 9.0 mM KCl, 9.27 mM $CaCl_2$, 22.94 mM $MgCl_2$, 25.5 mM $MgSO_4$, 2.15 mM $NaHCO_3$, 0.15 M Tris-SO_4 at pH 7.8) was flushed through the perfusion system at regular intervals to insure that the fluid level over the ocellus, and thus the light path to the ocellus, remained constant. All experiments were conducted at room temperature (about 21°C). Responses were recorded between an intracellular 3 M KCl-filled micropipette electrode and a 3 M KCl-agar bridge in the bath. Signals were fed through a solid-state, capacitance-compensated electrometer to an oscilloscope and a chart recorder. Current was injected through the microelectrode by means of a standard bridge circuit (Frank and Becker, 1964).

Optical System Light from a 150 w xenon arc was passed through a grating monochromator and focused by quartz and mirror optics onto the preparation. Light flashes of variable duration were produced by an electromechanical shutter and monitored by a photodiode. The intensity of the beam was controlled with neutral density filters (Inconel film on quartz). Various glass band-pass filters were usually added to minimize stray light. A second beam (from the same source) passed via another electromechanical shutter through Wratten neutral density filters and/or colored glass filters, and was combined with the first beam by a quartz beam splitter. This second beam was monitored by another photodiode. The radiant energy provided by the source was measured with a Yellow Springs Instrument Co. (Yellow Springs, Ohio) radiometer (Model 65).

RESULTS

1. *UV Cells*

(A) DEPOLARIZING RECEPTOR POTENTIAL Impaled cells had resting potetials of 35-60 mv, and time constants in excess of 100 msec, determined by

measuring the voltage change caused by a constant current pulse; this was true of all cell types in the median ocellus. Before measurements of light responses were made the preparation was allowed to dark-adapt for at least 30 min, or until large spontaneous "bumps" (Adolph, 1964; Dowling, 1968; Yeandle, 1958) were seen. The sensitivity (i.e. the size of the response to a given near-threshold stimulus) of a cell in this condition remains stable for several hours.

To find the cell's spectral sensitivity, the relation between stimulus intensity and response amplitude was determined at each wavelength. Three responses to a 50 msec flash were recorded at each intensity of a series covering response

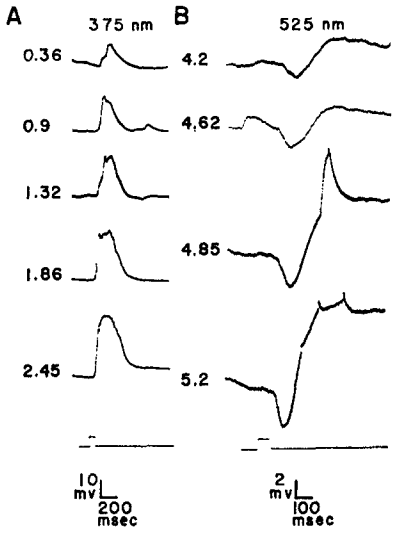

FIGURE 1. Response vs. stimulus intensity for a UV cell at two different wavelengths. The number to the left of each response is the stimulus intensity in log units. A, a near-UV stimulus elicits a depolarizing response whose amplitude increases with intensity. B, a visible stimulus elicits a small hyperpolarizing response whose leading edge becomes steeper with increasing intensity. Threshold for the hyperpolarizing response is almost 4 log units more intense than for the depolarizing response.

amplitudes from threshold to 20–25 mv (Figs. 1 A and 5 A). The average peak height of the response was plotted against log radiant energy, and a straight line was drawn by hand through each set of points (Fig. 2). The log radiant energy required to elicit 15 mv depolarization at each wavelength was determined from such plots. The spectral sensitivity curves obtained are nearly independent of the value of the criterion response, since the stimulus-response curves are nearly parallel (over the range of stimulus strengths used) at all wavelengths. Each value of log radiant energy was corrected to log relative number of photons by subtracting the log of the wavelength at which the measurement was made. Log relative spectral sensitivity at a given wavelength is then the negative of the log relative number of photons required to elicit a 15 mv depolarization at that wavelength. The sensitivity at a test wavelength was remeasured several times during each experiment. If any of

these control measurements differed from the original by more than 0.3 log unit, the data were discarded.

The averaged data, determined by this method, for the depolarizing receptor potential of nine UV cells are shown in Fig. 3. In these cells, we find a single peak of UV sensitivity at about 360 nm. This agrees with the wavelength of the principal peak of the ERG spectral sensitivity found by previous investigators (Chapman and Lall, 1967; Wald and Krainin, 1963). However, our data differ significantly from theirs in the following ways. The sensitivity of the whole ocellus at 450 nm, as measured by the ERG, is greater than that

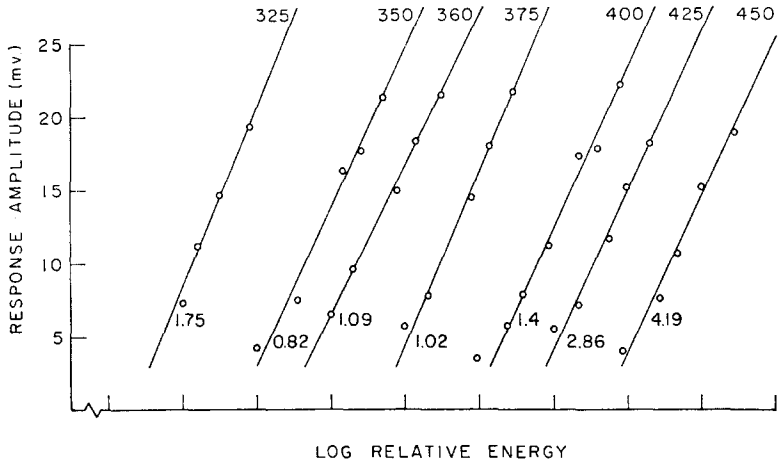

FIGURE 2. Amplitude of the depolarizing response of a dark-adapted UV cell as a function of intensity for several different stimulus wavelengths. Marks on the abscissa are 1 log unit apart. The number associated with each curve gives the relative intensity for the lowest point of that curve. Each point is the average of three responses.

of single UV cells, since at this wavelength the former measurements are affected by the visible cell population, while the latter are not. The sensitivity of the whole ocellus (measured by the ERG) at wavelengths shorter than 350 nm is less than that of single UV cells, and this difference is due to the fact that the former measurements were made with the ocellar lens in place, while the latter were not. The optical density of this lens increases rapidly for wavelengths shorter than 350 nm (P. K. Brown, personal communication; Nolte and J. E. Brown, unpublished observations); therefore the ERG sensitivity is decreased at these wavelengths. Our spectral sensitivity curve agrees well with a Dartnall template curve (Dartnall, 1953) for a pigment with λ_{max} at 360 nm.

That only one pigment is involved in the response is indicated by the results of chromatic adaptation experiments (Fig. 4 A); a background visible light ($\lambda > 480$ nm) shifts the spectral sensitivity curve along the sensitivity axis, but does not change its shape.

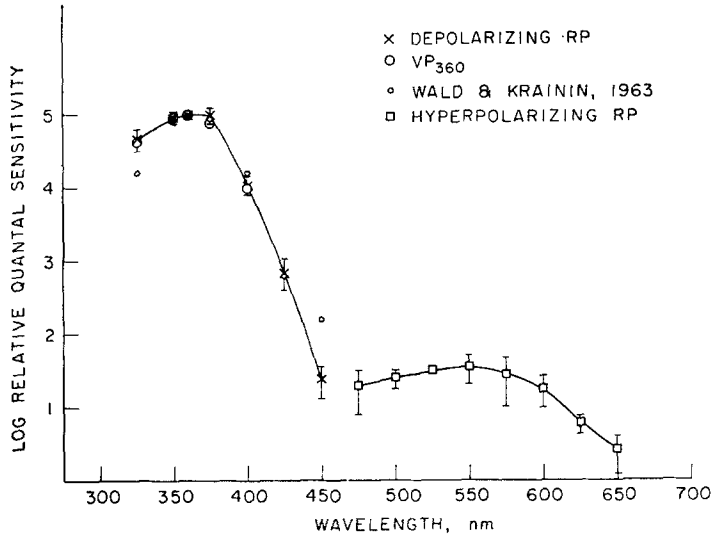

FIGURE 3. Spectral sensitivities of UV cells. Crosses indicate depolarizing responses (mean of nine cells), squares indicate hyperpolarizing responses (mean of six cells). Error bars are ±1 SD. The relative positions of the peaks of the two curves on the ordinate indicate that at the wavelength of maximum sensitivity for each type of response 3–4 log units more energy is needed to elicit a hyperpolarizing response than to elicit a depolarizing response. Large circles are points predicted by Dartnall's nomogram for a rhodopsin with λ_{max} at 360 nm. Small circles are points taken from the ERG spectral sensitivity curve of Wald and Krainin (1963).

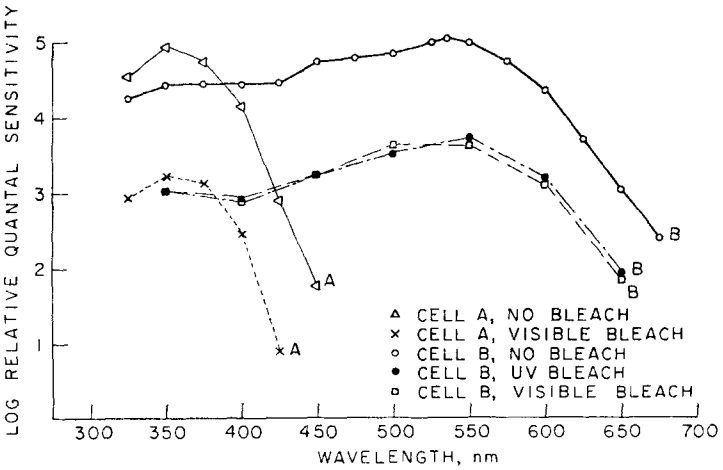

FIGURE 4. Chromatic adaptation experiments. A, a UV cell; triangles indicate spectral sensitivity curve for a dark-adapted cell, crosses indicate the values obtained in the presence of a visible ($\lambda > 480$ nm) adapting light. B, a visible cell; open circles indicate spectral sensitivity curve for a dark-adapted cell, filled circles indicate the values obtained in the presence of a UV (300–400 nm) adapting light, squares indicate the values obtained in the presence of a visible ($\lambda > 480$ nm) adapting light.

(b) HYPERPOLARIZING RECEPTOR POTENTIAL The spectral sensitivity was more difficult to measure in the case of the hyperpolarizing receptor potential, for two reasons. In order not to light-adapt the cell, the least intense stimuli which could elicit the response were used. At these light levels, the response was seldom larger than 2 mv, and so the signal-to-noise ratio was much lower than for the depolarizing response. Also, we found it more difficult to

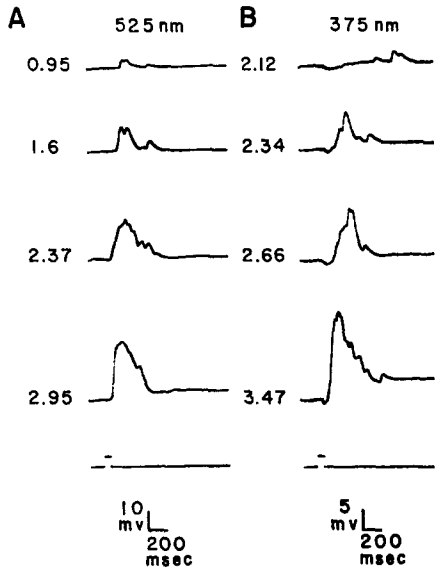

FIGURE 5. Variation of response of a visible cell with stimulus intensity for two different wavelengths. A, a visible stimulus elicits a depolarizing response whose amplitude increases with intensity. B, a near-UV stimulus elicits a biphasic response consisting of a small hyperpolarization, whose initial slope is graded with intensity, followed by a larger depolarization, whose amplitude is graded with intensity. Note that with near-UV stimuli, particularly at the highest intensity, membrane potential does not return quickly to the original base line; the tail of the depolarizing response is greatly prolonged. In this case, threshold for the hyperpolarizing response (at 375 nm) is at about 1 log unit greater intensity than that of the depolarizing response (at 525 nm).

obtain data within our criteria of stability; the cells tended to become less sensitive during the course of the experiment.

Since the response was frequently obscured by spontaneous bumps in a dark-adapted preparation, six to eight high-gain records of responses at each wavelength and intensity were averaged on film. Peak amplitude was found to be an unsatisfactory measure of response; over much of the spectrum, particularly in visible cells (see text below and Fig. 5 B), the depolarizing phase of the receptor potential occludes the later part of the hyperpolarizing response. The slope of the leading edge of the hyperpolarizing phase of the receptor potential was found to be a reasonably linear function of log intensity

over the range of stimulus intensities studied, and was therefore used as the response parameter. Log intensity vs. response curves were plotted and the spectral sensitivity was determined as for the depolarizing receptor potential. The averaged data for the hyperpolarizing response of six UV cells are shown in Fig. 3. On the average, the hyperpolarizing receptor potential is only seen with stimuli at least 4 log units more intense than those eliciting a threshold depolarizing receptor potential in the same cell. The spectral sensitivity curve displays a broad maximum at 525–550 nm.

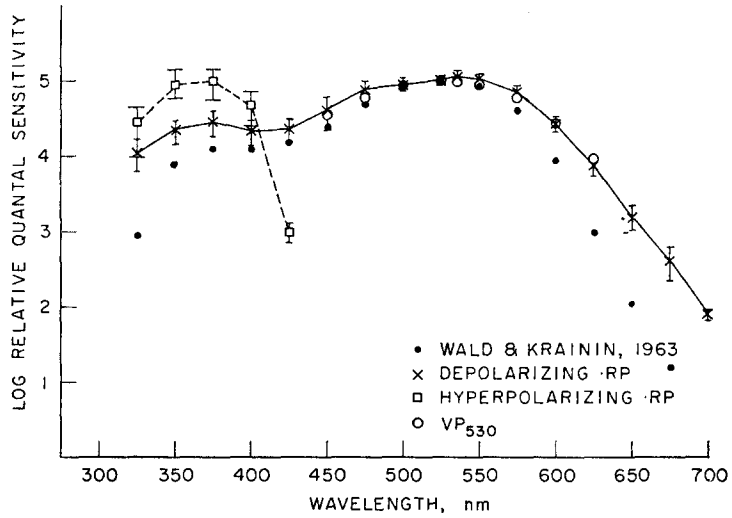

FIGURE 6. Spectral sensitivities of visible cells. Crosses indicate depolarizing responses (mean of nine cells), squares indicate hyperpolarizing responses (mean of four cells). Error bars are ±1 SD. The relative positions of the peaks of the two curves on the ordinate indicate that about the same energy can elicit a hyperpolarizing response at 375 nm or a depolarizing response at 525 nm. Large circles are points predicted by Dartnall's nomogram for a rhodopsin with λ_{max} at 530 nm. Filled circles are points taken from the ERG spectral sensitivity curve for the lateral eye (Wald and Krainin, 1963).

2. *Visible Cells*

(a) DEPOLARIZING RECEPTOR POTENTIAL The spectral sensitivities of visible cells were determined by the same methods used for UV cells. The averaged data for the depolarizing receptor potential of nine visible cells are shown in Fig. 6. We find a broad principal peak of sensitivity at 525–550 nm, which agrees with the location of the visible peak found by previous authors for the ERG (Chapman and Lall, 1967; Wald and Krainin, 1963). We also find a secondary peak in the near UV at 350–375 nm. The curve agrees well with that predicted by Dartnall's nomogram (Dartnall, 1953) for a visual pigment with λ_{max} 530 nm.

Visible-type cells respond with different waveforms to stimuli of different wavelengths, as shown in Figs. 5 and 7 B. After a response to UV light the membrane does not return quickly to the original resting potential; the last few millivolts of return are relatively slow. However, neither UV nor visible adapting lights change the shape of the spectral sensitivity curve (Fig. 4).

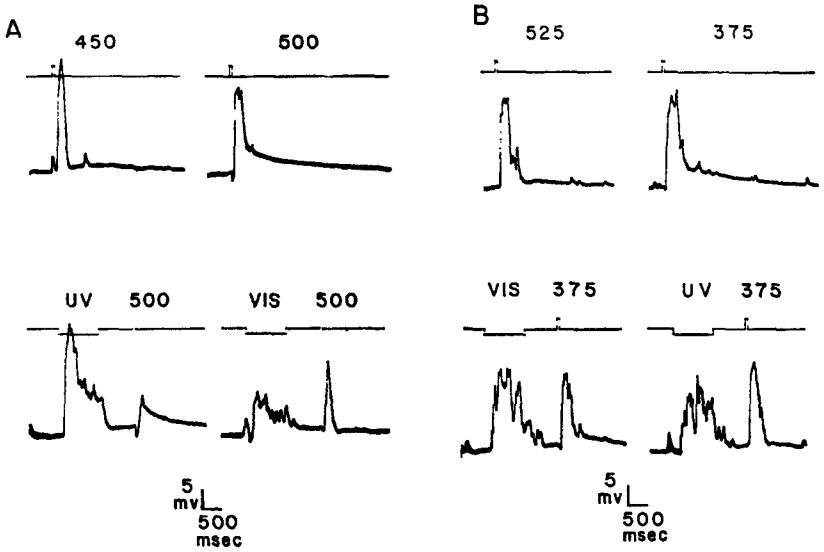

FIGURE 7. Wavelength-dependent waveform differences. A, UV cell. In the two upper traces, a 450 nm flash elicited a depolarizing receptor potential with a quickly decaying tail; a 500 nm flash elicited a biphasic receptor potential, with an initial hyperpolarizing phase and a later depolarizing phase with a slowly decaying tail. The two lower traces show that the response to a 500 nm flash can be dissected by appropriate chromatic adaptation; the same intensity 500 nm flash was used in both cases. After a UV (300–400 nm) adapting light, only a hyperpolarizing response and a slowly decaying depolarization occurred. After a visible ($\lambda > 480$ nm) adapting light only a depolarization with a quickly decaying tail occurred. B, visible cell. In the two upper traces, a 525 nm flash elicited a depolarizing receptor potential with a quickly decaying tail; a 375 nm flash elicited a receptor potential with a very small initial hyperpolarization and a slowly decaying tail. In the lower two traces, the components of the response to two identical 375 nm flashes are differentially affected by appropriate chromatic adaptation. After a visible ($\lambda > 480$ nm) adapting light, the initial hyperpolarization and the slowly decaying tail of the depolarization occur; after a UV (300–400 nm) adapting light they do not.

Also, both UV and visible responses recover at the same rate following adaptation to either UV or visible light (Fig. 8 A). UV adapting lights tend to diminish the wavelength-dependent waveform differences (Fig. 7 B).

(b) HYPERPOLARIZING RECEPTOR POTENTIAL The averaged data for the hyperpolarizing receptor potential of four visible cells are shown in Fig. 6.

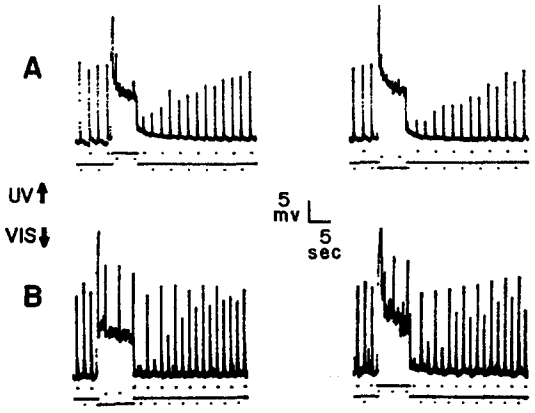

FIGURE 8. Recovery of response after chromatic adaptation. The lower trace of each pair is a light monitor; upward deflections indicate 375 nm stimuli, and downward deflections indicate visible ($\lambda > 480$ nm) stimuli. A, visible cell. After adaptation to UV or visible light, the responses to both UV and visible stimuli are depressed, and both recover at the same rate. B, UV-visible cell. After adaptation to visible light, the response to visible stimuli is depressed, while the response to UV stimuli is unaffected. After adaptation to UV light, the response to UV stimuli is depressed much more than that to visible stimuli.

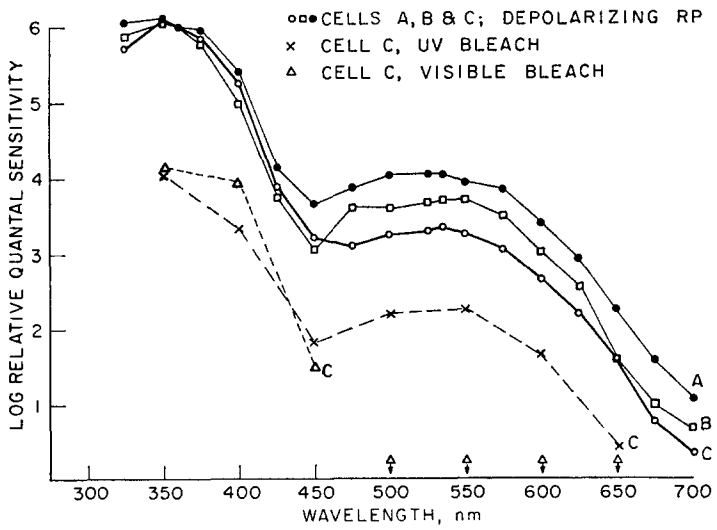

FIGURE 9. Spectral sensitivity of UV-visible cells. Continuous lines indicate the spectral sensitivity curves of three different cells. Interrupted lines indicate the spectral sensitivity of one of these cells during chromatic adaptation. The crosses indicate values obtained in the presence of a UV (300–400 nm) adapting light. Triangles indicate values obtained in the presence of a visible ($\lambda > 480$ nm) adapting light. With this visible adapting light present, the sensitivity was too low to measure for wavelengths longer than 450 nm.

There is a single peak of spectral sensitivity at 350–375 nm. Absolute threshold intensities for the depolarizing and hyperpolarizing receptor potentials of visible cells are approximately the same.

3. *UV-Visible Cells*

A small percentage (on the order of 5–10%) of the cells encountered did not respond with a hyperpolarizing receptor potential to any wavelength, but responded to all wavelengths with a depolarizing receptor potential. The spectral sensitivity curves of three such cells are shown in Fig. 9. All three cells were maximally sensitive at 350–375 nm, and had a second broad peak of sensitivity at 525–550 nm. The results of a partial bleaching experiment on one of these cells are also shown in Fig. 9. A visible ($\lambda > 480$ nm) adapting light decreased UV sensitivity by 2 log units and visible sensitivity by more than 3 log units; conversely, a UV (300–400 nm) adapting light decreased visible sensitivity by 1 log unit and UV sensitivity by 2 log units. Also, sensitivities to different wavelengths recovered at different rates after light adaptation, depending on the color of the adapting light (Fig. 8 B).

DISCUSSION

Depolarizing Receptor Potential

UV CELLS As mentioned above, the peak of the spectral sensitivity curve presented here for the depolarizing receptor potential of UV cells is in agreement with that obtained by earlier investigators from ERG measurements (Chapman and Lall, 1967; Wald and Krainin, 1963). The ERG data show a higher sensitivity at 450 nm, but at this wavelength the visible sensitivity of the second population of receptors becomes appreciable. The ERG data also show a lower sensitivity at wavelengths shorter than 350 nm, but this is explained by the high absorption of the ocellar lens at these wavelengths. The lens was intact for all ERG measurements but was removed in our single cell recordings.

A single cell spectral sensitivity curve with a single peak in the near UV can be explained by assuming that the cell contains a photopigment with an absorption curve similar to this spectral sensitivity curve. However, there are at least two other explanations which must be considered before accepting this hypothesis. The first is that the normal β-band absorption of a rhodopsin photopigment with λ_{max} in the visible accounts for the UV sensitivity, and that a screening pigment blocks all visible wavelengths; this would produce a spectral sensitivity curve with a single peak in the near UV. The second is that some component of the receptor cell fluoresces under UV irradiation, and this fluorescence acts on a photopigment with λ_{max} in the visible; here again, a visible-blocking screening pigment could result in a sensitivity curve with a single peak in the UV.

There are a number of objections to the latter two hypotheses. The first and most direct is that no screening pigments of any sort are seen when one looks down on the receptors. All that is seen is a white reflecting layer (presumably guanine), and this layer has been found to lie mainly proximal to the receptor layer; relatively few pigment cells are found surrounding the distal ends of the receptor cells (C. Jones, unpublished observations). Also, any such scheme would necessarily make the cell less sensitive than it would be without the screening pigment, since either a less than maximally sensitive absorption band of the photopigment or a fluorescence emission efficiency of less than one would be involved in excitation. Actually, UV cells tend to be more sensitive than visible cells by 1 or 2 log units. Thus it seems most reasonable to assume that the UV sensitivity of UV cells is due simply to a photopigment absorbing maximally in the near UV.

The fact that the measured spectral sensitivity fits a Dartnall template curve suggests that the UV-sensitive photopigment, like other photopigments, has retinene as its chromophore.

Several near UV-sensitive receptors have been studied in insects (Autrum and vonZwehl, 1962; Autrum and vonZwehl, 1964; Bruckmoser, 1968; Goldsmith, 1960; Hasselman, 1962; Walther and Dodt, 1959). Unfortunately, although a retinene-based photopigment has been extracted from insects (Goldsmith, 1958), no such UV-sensitive pigment has been found. However, Goldsmith et al. (1964) found that UV sensitivity in carotenoid-deprived houseflies declined at least as much as visible sensitivity; thus there is corroborative evidence that retinene-based UV-sensitive photopigments exist in some invertebrate eyes.

VISIBLE CELLS The spectral sensitivity curve of median ocellus visible cells agrees well with Dartnall's template curve for a photopigment with λ_{max} 530 nm (Fig. 6). Our single cell preparations are somewhat more sensitive on both sides of λ_{max} than one would expect on the basis of lateral eye ERG data, assuming that visible cells contain the same photopigment as lateral eye retinular cells (Fig. 6). The discrepancy on the short wavelength side can be explained by the absorption of the lens and cornea, as in the case of the median ocellus UV cells. The discrepancy on the long wavelength side has no obvious explanation. However, the agreement of our measurements with Dartnall's curve gives us some confidence in our values.

We believe that the wavelength-dependent waveform differences found in visible cells (Figs. 5 and 7 B) arise in the following way. A pigment absorbing maximally in the visible principally determines the cell's sensitivity throughout the spectrum. Nonetheless, a UV-absorbing pigment affects the waveform of the response to UV light without appreciably contributing to the amplitude of the depolarization. That is, the response of a visible cell to UV stimuli consists of a normal depolarizing receptor potential (arising via absorption of photons by the $\lambda_{max} = 530$ photopigment) superimposed on a bipha-

sic receptor potential with a small slow depolarizing phase (arising via the λ_{max} = 360 photopigment). Two kinds of experiments indicate that a single pigment dominates the sensitivity of the cell: the shape of the spectral sensitivity curve is the same in any color adapting light (Fig. 4) and the rate of recovery of response amplitude after light adaptation is independent of the color of the adapting light (Fig. 8 A).

Two lines of evidence indicate that the waveform differences arise from the superimposition of a depolarizing receptor potential and a biphasic receptor potential. First, the slowly decaying waveform is seen at precisely those wavelengths and intensities where an initial hyperpolarization is also seen. Thus in UV cells, at wavelengths long enough and intensities great enough to evoke a hyperpolarizing response, depolarizing receptor potentials have a slowly decaying component (Fig. 7 A). Second, the proposed components of such slowly decaying responses can be relatively independently affected by appropriate adapting lights. Fig. 7 A shows that in a UV cell, a UV adapting light decreases the amplitude of a response to a 500 nm stimulus but does not affect its slow decay; a visible adapting light abolishes the slow decay of a response to the same stimulus, while leaving its amplitude relatively unaffected. Conversely, Fig. 7 B shows that for the response of a visible cell to a 375 nm stimulus, a visible adapting light selectively affects the amplitude, while a UV adapting light selectively affects the slow decay.

This superimposed slow depolarization may add slightly to the amplitude of the response and hence introduce an error into the determination of spectral sensitivity (in the direction of too great a sensitivity) at the wavelengths involved. However, this error must be small, since adapting lights capable of eliminating the slow depolarization do not noticeably affect the shapes of spectral sensitivity curves (Fig. 4).

DOUBLE-PEAKED SPECTRAL SENSITIVITY CURVES The infrequently encountered recording situation which results in a double-peaked spectral sensitivity curve admits of two explanations: either we record from two cells of opposite types at the same time by artificially coupling them with the micropipette, or we record from one cell containing two pigments. Recording from two cells at once was proposed by Autrum (Autrum and von Zwehl, 1964) as the reason he found double-peaked spectral sensitivity curves in honeybees, but in his preparation there was never a consistent relationship between the heights of the two peaks. We always find the UV peak to be about 2.5 log units higher than the visible peak. Also, injured median eye receptors often fail to develop bumps when dark-adapted, so it might be expected that if UV-visible cells were really two cells, one of the two might sometimes be injured and hence not develop bumps. This has not been our experience. All dark-adapted UV-visible cells have shown increased rates of bumping in response to weak stimuli of any color. Finally, if we were recording from two

cells one would expect the UV cell to show the very slow decay following bright UV stimuli which is characteristic of UV cells (Nolte et al., 1968), but we have never seen this occur.

We therefore favor the explanation that UV-visible cells do contain two photopigments. Single cells containing two active photopigments have apparently been found in the locust (Bennett, Tunstall, and Horridge, 1967). On the other hand, on one occasion we found it possible to differentially change the UV and visible responses of a UV-visible cell by moving the microelectrode. This indicates that we may after all have been recording from two spatially separate patches of membrane. A final decision between the alternatives awaits the completion of experiments with dye-filled electrodes. At the present time all that can be said with certainty is that two pigments are involved in such recording situations, as shown by chromatic adaptation experiments (Figs. 8 B and 9).

Hyperpolarizing Receptor Potentials

The spectral sensitivity curve for the hyperpolarizing receptor potential of visible cells is virtually superimposable on that for the depolarizing receptor potential of UV cells. Also, the curve for the hyperpolarizing receptor potential of UV cells fits that for the depolarizing receptor potential of visible cells. We take this as strong evidence that the pigments involved in the generation of depolarizing receptor potentials are involved in the generation of hyperpolarizing receptor potentials. It was found that threshold for the depolarizing receptor potential occurred in UV cells at a light level about 2 log units less intense (at 360 nm) than in visible cells (at 535 nm). Threshold for the hyperpolarizing receptor potential of UV cells was at a level about 3–4 log units more intense than for the depolarizing receptor potential. Threshold for the hyperpolarizing receptor potential of visible cells was at about the same level as for the depolarizing receptor potential. Thus for either cell type a stimulus about 2 log units more intense than that needed to produce a depolarizing receptor potential could produce a hyperpolarizing receptor potential in an opposite type cell. This provides weak evidence for two speculations: one, that the hyperpolarizing receptor potentials of both UV and visible cells are produced by the same type of mechanism; two, that the hyperpolarizing receptor potential depends somehow on the excitation of opposite type cells (rather than on, say, the presence of two pigments in each cell).

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