Dual Sensitivities of Cells in Wolf Spider Eyes at Ultraviolet and Visible Wavelengths of Light

ROBERT D. DEVOE

From the Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT Intracellular recordings have been made from visual cells in principal and secondary eyes of in vitro wolf spider preparations. The responses of all cells to all wavelengths of light were graded depolarizations; no hyperpolarizations or nerve discharges were seen. Cells in a secondary eye, the anterior lateral eye, had a maximum sensitivity in the visible at 510 nm and a secondary maximum, or shoulder, of sensitivity in the near ultraviolet at 380 nm. Cells in principal eyes, the anterior median eyes, all responded maximally both in the visible at 510 nm and in the ultraviolet at 360–370 nm or less. However, there was no typical ratio of ultraviolet to visible sensitivities; the differences in log sensitivities (log UV/VIS) varied from 3.3 to −0.5. Each principal eye had a population of cells with different ratios. These populations varied with the time of the year, possibly due to changes in light upon the animals. Chromatic adaptations of cells in anterior median (but not anterior lateral) eyes resulted in small, selective changes in spectral sensitivities, and there was some facilitation of responses from cells repeatedly stimulated. It is concluded that cells of secondary eyes contain only a visual pigment absorbing maximally in the visible, while cells of principal eyes probably contain variable amounts of both this pigment and one absorbing in the ultraviolet as well.

Some insects, such as bees, are known to see color, and they have different kinds of visual cells sensitive to different parts of the spectrum (Autrum and von Zwehl, 1964). Other insects, such as flies (Burkhardt, 1962), locusts (Bennett et al., 1967), and dragonflies (Horridge, 1969) have visual cells which are sensitive to more than one part of the spectrum. The ability of such animals to see color remains in doubt (Kaiser, 1968).

In another class of arthropods, the arachnids, there is behavioral evidence for color vision in spiders (Kästner, 1950; Young and Wanless, 1967) and in mites (McEnroe and Dronka, 1966, 1969). For spiders, however, there is little indication as to what such color vision might be due, since the available

electrophysiological evidence indicates that the visual cells of spiders are more like those of flies, locusts, and dragonflies than like those of bees. As measured using the electroretinogram (ERG), the spectral sensitivities of the principal eyes of spiders (two of the eight eyes; see later) peaked in both the ultraviolet (at 370–380 nm) and the visible (at 505–510 nm), while there was peak sensitivity in the secondary eyes (the other six eyes) only in the visible (DeVoe et al., 1969). Inasmuch as chromatic light adaptations failed to separate two populations of cells in any eye, it appeared that the dual sensitivities of the principal eyes in the ultraviolet and visible might be due to dual sensitivities of all cells in these eyes. The intracellular recordings of spectral sensitivities to be described here confirm this hypothesis, but themselves raise further problems of variability of spectral sensitivities at the single cell level. A preliminary report of this work has been made elsewhere (DeVoe and Small, 1970).

Spider Eye Nomenclature Most spiders have eight ocelli arranged in two rows of four each. The middle pair in the front row, the anterior median eyes, originates embryologically in a more anterior segment than do the other six eyes (Lambert, 1909), their structure is different, and they are called by the name of "principal" eyes. All six other eyes are called "secondary" eyes. In the principal eyes, the rhabdome-bearing part of the visual cell is distal to the cell body, whereas in the secondary eyes, the cell body is distal to the rhabdome and there is a tapetum. In all eyes, the visual cells narrow down proximally to form the axons of the (eight) optic nerve(s), and the synapses with second-order cells occur some millimeters away from the eyes in the optic lobes. A drawing of the visual system in the dissected spider is given in Fig. 1, which will be explained later. Further details of spider eye nomenclature and structure are given in a previous paper (DeVoe et al., 1969).

METHODS

Preparation of the Animal I began this work by inserting micropipette electrodes through small holes cut in the cuticle over the posterior median eyes of intact, restrained spiders. However, spiders are hydraulic animals capable of developing up to 40 cm Hg of blood pressure when aroused (Parry and Brown, 1959), and my spiders were certainly aroused by this surgery. The resulting copious blood flows, followed by speedily formed, impenetrable clots, made rapid electrode insertions mandatory and visual guidance of impalements impossible. Only one such impalement of a visual cell lasted long enough (16 min) to measure its spectral sensitivity.

Cells do survive at least 22 hr in a spider carcass immersed in a physiological salt solution (discussed later) and this is now the preparation of choice. The abdomen, legs, and palpi are cut off of anesthetized spiders. The carcass is glued upside down to a cork block with Tennessee Eastman Co. (Kingsport, Tenn.) 910 adhesive and is covered with physiological saline. Under saline, the chelicerae (with poison sacs) and

mouth parts are cut off to reveal the eyes, as shown in Fig. 1. The anterior median (*AME*) and anterior lateral (*ALE*) eyes are most accessible. The posterior median eyes (*PME*) are generally visible in the depth of the carcass, but are not well oriented for stimulation (see later).

Brief treatment of the eyes with the enzyme Pronase (Calbiochem, Los Angeles, Calif.) makes it possible for the electrode to pass through the otherwise refractory sheaths of connective tissue around the eyes. Eyes from one of the species used,

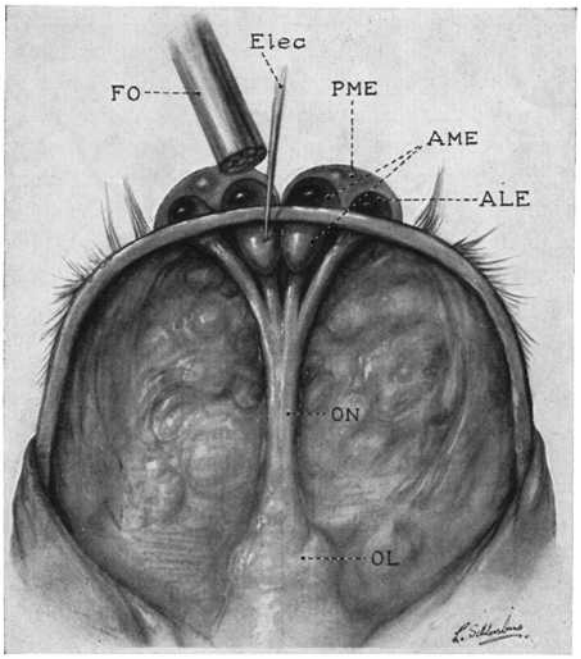

FIGURE 1. Schematic drawing of the dismembered wolf spider preparation, seen from above as it lies in physiological saline. The carcass is glued upside down with chelicerae and mouth parts removed, revealing the ventral aspect of the anterior eyes and the optic nerve. *AME:* anterior median eyes; *ALE:* anterior lateral eyes; *PME:* posterior median eyes (lens alone visible); *FO:* fiber optic bundle; *Elec:* micropipette electrode shown inserted into an anterior median eye; *ON:* optic nerves, containing contributions from all eight eyes; *OL:* optic lobes. The width of the carapace is about 4.5 mm. × 14.

Lycosa baltimoriana (from New Jersey), tolerated 5% (50 mg/ml) Pronase for 2 min, but those of *L. miami* and *L. lenta* (both from Florida) were unresponsive if treated for more than 1 min with 1% Pronase. The reasons for these species differences are unknown. Pronase treatments stopped spontaneous eye movements of the anterior median eyes, which alone have eye muscles (Scheuring, 1914).

All experiments were done at 20°–22°C.

Physiological Saline The wolf spider carcasses survived well in the following physiological saline, developed by Rathmeyer (1965) from analyses of the blood of a tarantula spider, *Eurypelma hentzi*: NaCl, 217 mM; KCl, 5 mM; $MgCl_2$, 1.1 mM; $CaCl_2$,

4 mM; $NaHCO_3$, 3 mM. For comparison, recent blood analyses of a different species of spider, *Cupiennius salei*, give the following ionic concentrations (in millimoles per liter): Na, 223; K, 6.8; Ca, 4; Cl, 258 (Loewe et al., 1970). The unaerated physiological saline has a pH of about 8.2, which is close to the pH of 8.05–8.22 found in vivo in *Cupiennius*.

Electrical Recording Glass micropipettes were pulled from Pyrex tubing prefilled with small glass fibers and were filled with 2 M KCl by injection. Successful electrodes had resistances of 40–60 MΩ or greater. A Kopf hydraulic microdrive (David Kopf Instruments, Tujunga, Calif.) held the mounted micropipettes over the spider carcass, which was in a bath in a plastic chamber on a micrometer-driven x-y stage. The indifferent electrode was a chlorided silver wire placed in the bath with the carcass. The electrode leads ran to a unity-gain, negative capacity amplifier, which also contained an active bridge to measure the resistances of electrodes. Most cells were impaled by briefly causing the amplifier to oscillate, whereupon the micropipette tip was often found to be inside a cell. Resting potentials were measured by nulling them with a bucking potential applied through the indifferent electrode. In this way, resting potentials could be constantly monitored even while high gains were used to see small responses. The electrical signals from the eyes, as well as photocell signals of the light stimuli, were displayed on a Tektronix 502 oscilloscope (Tektronix, Inc., Beaverton, Ore.) and photographed with a Grass C-4 camera (Grass Instrument Co., Quincy, Mass.).

Optical Stimulation A previous paper (DeVoe et al., 1969) gives the complete details of the optical stimulator. Essentially, it consisted of a Bausch & Lomb High Intensity monochromator (Bausch & Lomb, Inc., Rochester, N.Y.) with a xenon light source and an exit slit of 1.5 mm (9.6 nm bandwidth), circular neutral density wedges (Eastman Kodak Co., Rochester, N.Y., Type M), and a Velmex shutter (Velmex Inc., Holcomb, N.Y.). To reduce stray light the following Schott, broadband, glass filters were placed between the light source and monochromator (Jenaer Glaswerk, Schott und Genoden, Jena, Germany): 350–372 nm, UG-1; 373–462 nm, BG-12; 463–532 nm, VG-6; 533–602 nm, OG-530; 603–650 nm, OG-590. A mercury arc lamp and a tungsten lamp provided ultraviolet light (at 365 nm) and visible-wavelength light, respectively, for chromatic adaptations.

All light beams were combined with beam splitters and were focused upon an ultraviolet-transmitting, fiber optic bundle of 0.5 mm diameter (American Optical Corp., Southbridge, Mass., type ULGM). The other end of this fiber optic bundle was placed in the saline close to the lens of the eye to be illuminated (*FO* in Fig. 1). Light left the fiber optic bundle under saline in a diverging cone of about 29° total included angle, and most of this light fell upon the lens. The refractive power of the lens in saline was probably much reduced from that in air (see Land, 1969). Hence, the light distribution upon the retina probably approximated that in the intercepted, diverging light beam.

To provide rapid spectral scans of cells, stepping motors were attached to the monochromator and wedge drives. These motors were programmed from prepunched paper tapes using conventional digital electronics. The paper tapes set the wedges at each wavelength to give approximately equal responses. The tapes were generated

after first determining the spectral sensitivities of a number of cells manually. Then, when a cell was isolated, its sensitivity was determined at 370 and 520 nm (near the peak sensitivities for anterior median eyes) and that tape was chosen which could most nearly elicit equal responses at all wavelengths. Each tape would cause the optical system to scan first from 350 to 640 nm in 20- (or 10-) nm steps and then back. Every sixth flash was a control (at 370 or 520 nm). After the first scan came a stimulus-response sequence at one wavelength, 370 or 520 nm, and after the second scan came a stimulus-response sequence at the other wavelength. Flashes could be given as rapidly as one every 2 sec, but in general interflash separations of 5–20 sec were used to avoid progressive light adaptation. All flashes were 100 msec long unless otherwise noted.

For calculation of spectral sensitivities, the responses at each wavelength were read off of film and corrected, if necessary, for drifts in the cell's sensitivity, as determined from the controls. The intensities at each wavelength needed for a criterion response (usually 4 mv) were then interpolated or extrapolated from stimulus-response relations recorded at 370 and/or 520 nm (see Results).

Calibrations The quantum fluxes were determined using a Reeder thermopile (Chas. M. Reeder & Co., Inc., Detroit, Mich.), which intercepted nearly all of the light emerging from the fiber optic bundle. The thermopile was calibrated in turn using an Eppley carbon filament lamp (The Eppley Laboratory, Inc., Newport, R.I.) whose calibration of total irradiance could be traced to the National Bureau of Standards. All light intensities are stated as quanta per second falling upon the eye. Although the 1 mm square receiver of the thermopile did not intercept all of the round, emerging beam from the fiber optic bundle, neither did the eyes, so the stated quantum fluxes are probably fair estimates of over-all retinal illuminations.

Calibrations were made at the beginning of the series of experiments below with one thermopile, and at the end of the series with another thermopile, a year and a half later. The two calibrations differed, both absolutely, due to breakage of some of the fibers in the fiber optic bundle, and relatively in the blue and ultraviolet, possibly due to differences between the thermopiles. The largest relative differences were 0.25 log units in quantum flux at 370–390 nm. There is no ready way to take these differences in calibrations into account, since some of them may have been occurring progressively during the course of the experiments. Because the initial calibrations were inherently more accurate (due to the larger fluxes available), they are used throughout this paper. However, fluxes in the ultraviolet and blue may be somewhat overstated (and the sensitivities thus understated).

Histological Techniques Animals were cut in half, fixed overnight in Bouin's solution, dehydrated in tetrahydrofuran, embedded in celloidin-paraffin (Machan, 1966), and cut into 10-μm-thick sections. The sections were usually stained with hematoxylin-eosin.

RESULTS

General Properties of Cells

Table I indicates the sizes of the eyes and visual cells for principal eyes (anterior median eyes) and a pair of secondary eyes (anterior lateral eyes) of

wolf spiders. Except as noted, these data come from sections of my animals. However, no corrections have been made for shrinkage during the histological preparation, so these dimensions are only approximate.

Table II summarizes the data on resting potentials from all impaled cells. In wolf spiders, it is possible to impale cells both in the eyes and in the optic nerves. In the anterior median eyes, 38 impaled cells survived an average of

TABLE I

SIZES OF EYES AND VISUAL CELLS OF WOLF SPIDERS

	Diameters of eyes	Numbers of cells*	Diameters of cells	Diameters of axons
	μm		μm	μm
Anterior median eyes	450–500	450	12–15 (Rhabdome-bearing distal ends) 20–23 (Cell bodies)	5–7
Anterior lateral eyes	250	145	15–27	5

* Data of Melamed and Trujillo-Cenoz, 1966.

TABLE II

SUMMARY OF DATA ON RESTING POTENTIALS FROM ALL IMPALED CELLS OF WOLF SPIDERS

	No. of cells impaled	Average minutes of impalement	Average initial resting potentials	Means of resting potentials averaged over impalement times
			mv	mv
Anterior median eyes				
Fully analyzed cells	38	55 (range 16–250)	50±15 (SD) (range 23–76)	48±3 (SE) (range 17–78)
Partially analyzed cells	83	10 (range 2–70)	43±12 (SD) (range 19–69)	41±2 (SE) (range 9–79)
Anterior lateral eyes				
Fully analyzed cells (all but one in eye)	7	33 (range 10–105)	43±7 (SD) (range 31–52)	49±5 (SE) (range 27–70)
Cells in optic nerve	14	—	41±16 (SD) (range 14–74)	46±5 (SE) (range 29–57)

55 min, long enough to make at least one spectral scan and to record at least one stimulus-response relation. These are called "fully analyzed cells." An additional 83 cells survived an average of 10 min, and had somewhat lower resting potentials. For the most part, the spectral sensitivities of these "partially analyzed cells" were determined at only two wavelengths, 370 and 520 nm. In all of these cells, resting potentials more often decreased than increased after impalement.

In the anterior lateral eyes, seven impaled cells survived long enough to

have their spectral sensitivities measured. Of these seven cells, six were within the eye and were from one animal. The seventh was an axon penetrated within the optic nerve just behind the eye in another animal. In addition, penetrations were made of 14 other axons farther back in the optic nerve. Table II shows that the resting potentials of cells in the anterior lateral eyes were about the same as those of cells in anterior median eyes.

Visual cells of both kinds of spider eye respond to light with slow depolarizations only. In the left-hand part of Fig. 2 are a number of responses of a cell in an anterior median eye to 1.5-sec flashes of light of 370 nm wavelength.

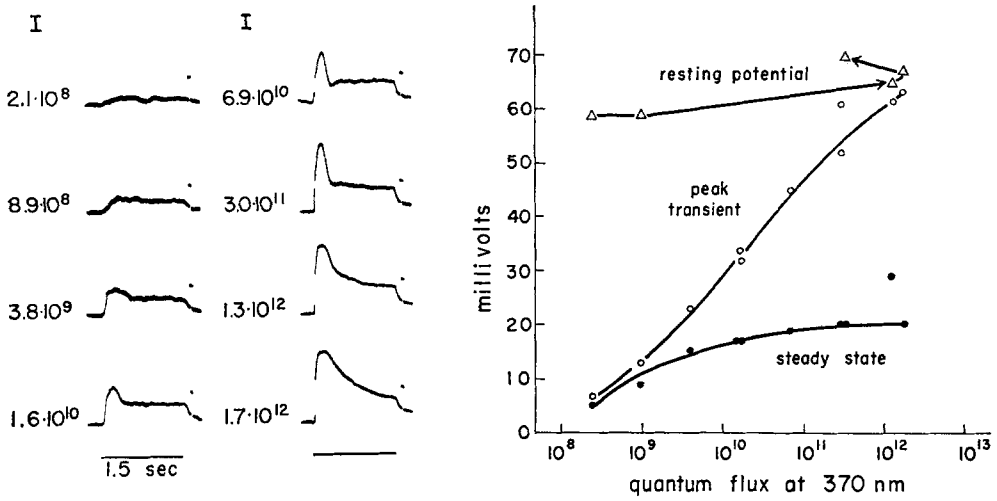

FIGURE 2. Slow-wave responses to light of a cell in an anterior median eye. *Left:* waveforms of responses to 1.5-sec flashes of 370 nm wavelength and intensities (in quanta per second incident on the eye) indicated are shown. The calibration mark at the end of each response represents 20 mv. *Right:* millivolts of response (ordinate) to 1.5-sec flashes of intensities given on the abscissa; data were taken from responses in the left-hand part of figure. Also shown are the resting potentials measured at the same times as the responses; the arrows indicate the order in which the data were taken. *Lycosa baltimoriana* was used.

The responses to 520-nm flashes are similar so far as I have seen. In these responses, there are no initial hyperpolarizations, at any wavelength, such as were reported to occur in median ocelli of *Limulus* (Nolte et al., 1968). Likewise, I have never seen repetitive nerve discharges, either in the eye or in the optic nerve, in the excised or in the intact preparation. Rather, the slow depolarizations to light are like those of the visual cells of most arthropods. At low intensities there appear to be slow potential fluctuations. At mid-intensities a sharp, initial rise occurs in the response. At high internsities the peak of the response approaches the resting potential, but does not reach or exceed it. This is shown on the right in Fig. 2.

Similar stimulus-response relations were recorded from all other fully analyzed cells. In general, however, saturating intensities were not used in order to avoid light adapting the cells. In anterior median eyes, stimulus-response relations at 370 and 520 nm had the same shapes (i.e., were parallel to each other) for each cell in 23 of 25 cells which survived long enough to be tested at both wavelengths. (The other two cells were deteriorating by the time of the second stimulus-response sequence.) The same applied in the anterior lateral eye: in the two of the seven cells which could be so tested, the stimulus-response relations at 370 and 520 nm were parallel. In none of these experiments on impaled cells were stimulus-response relations measured at wavelengths other than 370 and 520 nm; however, a wider range of wavelengths used previously with the ERG gave identical results (DeVoe et al., 1969). Therefore, it seemed valid to assume that stimulus-response relations recorded at only two (or one) wavelengths would be the same at other wavelengths as well and thus could be used at all wavelengths for calculation of spectral sensitivities (see Methods for details).

Spectral Sensitivities

ANTERIOR LATERAL EYES Previous measurements with the ERG of spectral sensitivities of secondary eyes, including anterior lateral eyes, indicated that these eyes probably contained only a single receptor type (DeVoe et al., 1969). The present results confirm this. The average spectral sensitivities of all seven fully analyzed cells of anterior lateral eyes are shown in Fig. 3. These have a maximum sensitivity at 510 nm in the visible and a submaximum at 380 nm in the ultraviolet. This submaximum is about $\frac{1}{8}$th of the maximum in the visible. The peak in the visible is narrower than a 510 nm nomogram curve (dashed line in Fig. 3 of this paper; Dartnall, 1953).

In addition to the above, a spectral sensitivity was measured from a cell in another secondary eye, the posterior median eye of an intact spider, as mentioned in Methods. This spectral sensitivity was the same as those of cells in anterior lateral eyes (Fig. 3) and those recorded from posterior median eyes using the ERG (DeVoe et al., 1969). Further study of the posterior eyes was not feasible in the present, upside-down, carcass preparation, since these eyes were now gazing into the bottom of the preparation chamber and so were difficult or impossible to illuminate through their lenses with the fiber optic.

ANTERIOR MEDIAN EYES Spectral sensitivities of anterior median eyes measured previously using the ERG were not the same from eye to eye or from experiment to experiment upon the same eye (DeVoe et al., 1969). The profusions of spectral sensitivities which have now been recorded from single visual cells in these eyes provide ample bases for such inconstancies. Fig. 4 illustrates only a few of the various spectral sensitivities found. The top

three curves are averages (and ranges) of three cells each, while the bottom curve is from one cell (the only one of its kind found). Between the extremes of the top and bottom curves, the sensitivity in the visible region of the spectrum ranges from about 0.1 log unit more sensitive to about 3.3 log units less sensitive than in the ultraviolet part of the spectrum. Intermediate curves represent intermediate differences in sensitivity.

In the visible, the sensitivities of all cells peak at about 510 nm. At wavelengths 440 nm and longer the spectral sensitivity curve has the same shape

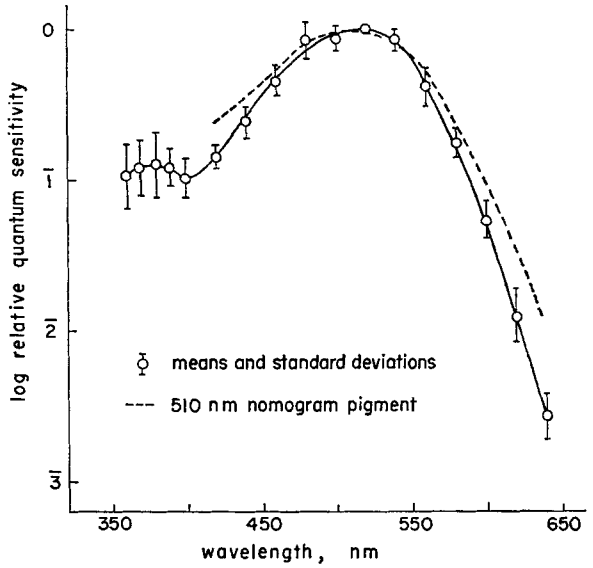

FIGURE 3. Average log spectral sensitivities of seven cells in dark-adapted anterior lateral eyes. The ordinate gives the −log relative quantum flux required to elicit 4-mv responses by 100-msec flashes of the wavelengths given on the abscissa. All data have been normalized to a log relative quantum sensitivity of 0.0 at 520 nm. Open circles give the average spectral sensitivities; vertical bars denote the standard deviations. The dashed line represents a 510 nm nomogram pigment (Darnall, 1953), which appears to be broader than the peak of spectral sensitivity. The solid line drawn by eye through the points has been used to draw the dashed curves through spectral sensitivities of cells in anterior median eyes in Figs. 4 and 7.

as in cells of the anterior lateral eye. This is shown by the dotted lines, taken from the full curve drawn through the points in Fig. 3. Presumably this means that a common spectral mechanism for sensitivity in the visible could be present in cells of both the anterior median and anterior lateral eyes.

In the ultraviolet, the shapes of all spectral sensitivity curves are again about the same, although there is some variability in the wavelength of peak sensitivity, which may lie at 370 nm or less. Because these shortest wavelengths were strongly absorbed by glass in the optical system, responses were small, and there are uncertainties in sensitivities at these wavelengths which

FIGURE 4. Examples of log spectral sensitivities of cells in dark-adapted anterior median eyes. In the top three curves, the circles represent averages from three cells each, together with the ranges of the data. The points in the bottom curve come from one cell, the only one of its kind found. Dashed portions of the curves are taken from the average spectral sensitivities of anterior lateral eyes in Fig. 3, where they fit. Solid curves have been drawn by eye through the remaining points. The bottom curve in the figure comes from a cell whose responses in the visible were small (about 1 mv) but unambiguous. The green and yellow filters used to reduce stray light at these wavelengths (480–540 nm) would have attenuated even the maximum available ultraviolet fluxes below the intensities needed to elicit even such small responses. Since the manufacturer states that the monochromator has only 0.1% stray light, it seems most improbable that stray UV light was responsible for the responses measured in the visible. Thus, had there been sufficient intensity at longer wavelengths with which to stimulate this cell, its responses in the red might have shown the further falloff seen in the upper curves.

may account for the variabilities in peak sensitivity. Alternatively, the lens of the eye may start to absorb at wavelengths around 350 nm, as does the lens of the median ocellus of *Limulus* (Nolte and Brown, 1969). The peaks of sensitivity in the ultraviolet do not appear to be closely related to the sub-

maximum in sensitivity in the ultraviolet in cells of the anterior lateral eye, since the latter submaximum occurs at a somewhat longer wavelength (380 nm) and is broader.

Variability of Spectral Sensitivities

All the measured spectral sensitivities of cells in anterior median eyes can be compared in terms of the ratios of sensitivities in the ultraviolet and in the visible. This is because these spectral sensitivities appear to consist of different proportions of ultraviolet sensitivity curves of one shape and (nearly constant) peak wavelength, and of visible sensitivity curves, also with constant shape and peak wavelength. The logarithms of the sensitivity ratios (that is, the differences in log sensitivity) are plotted in Figs. 5 and 6.

In Fig. 5 the data are plotted *versus* the date each experiment was done. Closed circles are from fully analyzed cells (as defined above). Open circles are from partially analyzed cells and show only the log ratios of sensitivities at 370 and 520 nm. (The open circles may be underestimates by as much as 0.2 log units, since maximum sensitivity in the ultraviolet was sometimes at less than 370 nm.) In Fig. 5 it can be seen, first, that there is a population of cells with different relative sensitivities in each eye, and, second, that the population means (through which the dashed line was drawn) varied from experiment to experiment. Such variations in population means might provide the explanation for the results obtained using the ERG, where relative spectral sensitivities of anterior median eyes also varied from experiment to experiment, even upon the same eye (DeVoe et al., 1969; the ERG presumably gave the mean response of the population of responding cells).

The variations in population means and ranges in Fig. 5 may have some relation to the way the animals were kept. At the beginning of the 1970 experiments, the animals then at hand were transferred from opaque cages (hardware drawers) with transparent tops, placed next to a window, into transparent cages with the same tops placed next to the same window. After this transfer, a somewhat abrupt change in population mean and spread took place and was maintained. This is made clearer in the frequency histograms in Fig. 6, where the total cell population, the 1969 cell population, and the 1970 cell population are depicted. The two year's populations are significantly different from each other ($P < 0.001$ using the t and F tests), and each is significantly different from the total population of all cells combined ($P < 0.001$ using the t test. The variance of the 1969 population is not significantly different from the variance of the total population [$P > 0.10$] using the F test.). These significant differences between the populations in the two years makes it unlikely that there is but one population of cell types in all eyes, but that insufficiently representative samples were recorded. Rather, it appears that some common influence may have been at work upon all the

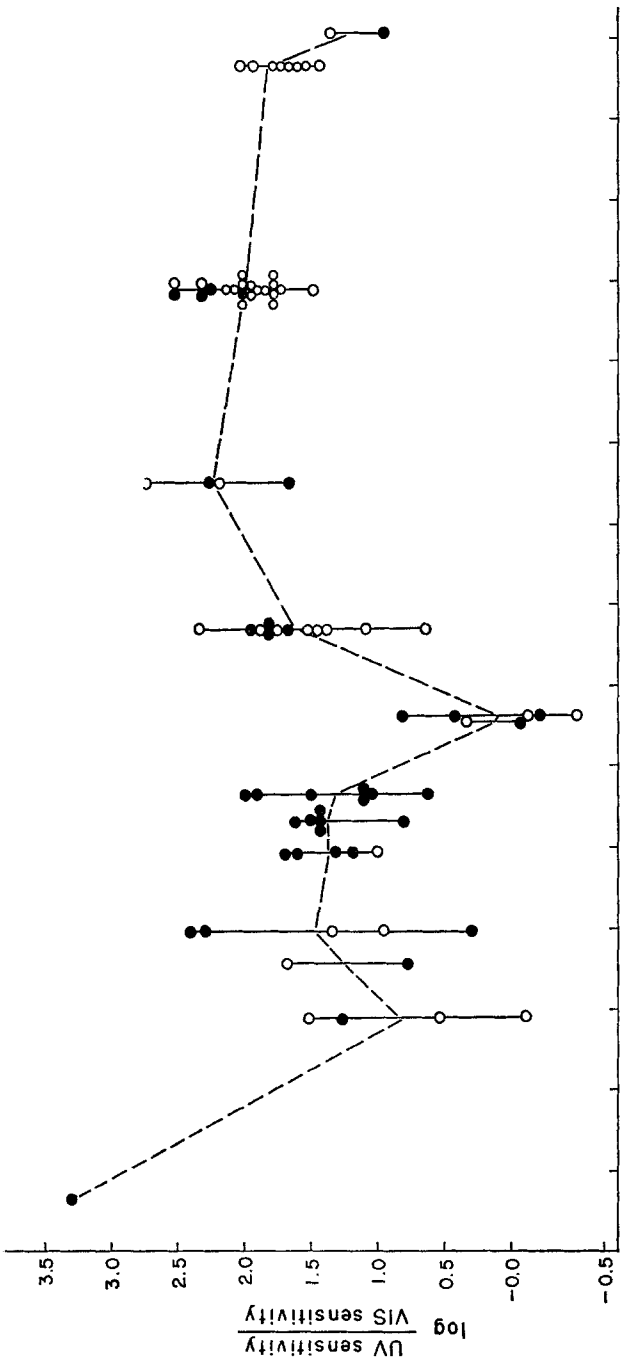

FIGURE 5. Relative ultraviolet and visible sensitivities of dark-adapted cells in anterior median eyes *versus* the date of the experiment. From such spectral sensitivity curves as in Fig. 4, the logarithm of the ratio of the sensitivity in the UV to the sensitivity in the visible is plotted for each cell on the ordinate *versus* the date of each experiment on the abscissa. Closed circles represent the 38 fully analyzed cells; open circles represent 49 partially analyzed cells. All cells impaled in a single experiment are joined by a vertical solid line. The mean log ratios from experiment to experiment are joined by the dashed line. A positive log ratio indicates that the cell was more sensitive in the ultraviolet (at 350–370 nm) than in the visible (at or near 520 nm), and vice versa. *Lycosa miami* collected in April 1969 were used for the experiments of June, August, early September, and the first one in December. *L. baltimoriana* collected in September 1969 were used in late September, November, late December, and January 1970. *L. lenta* collected in December was used in March. *L. miami* collected in May 1970 were used in May and August 1970. *L. baltimoriana* collected in August 1970 was used for the experiment of September 1970.

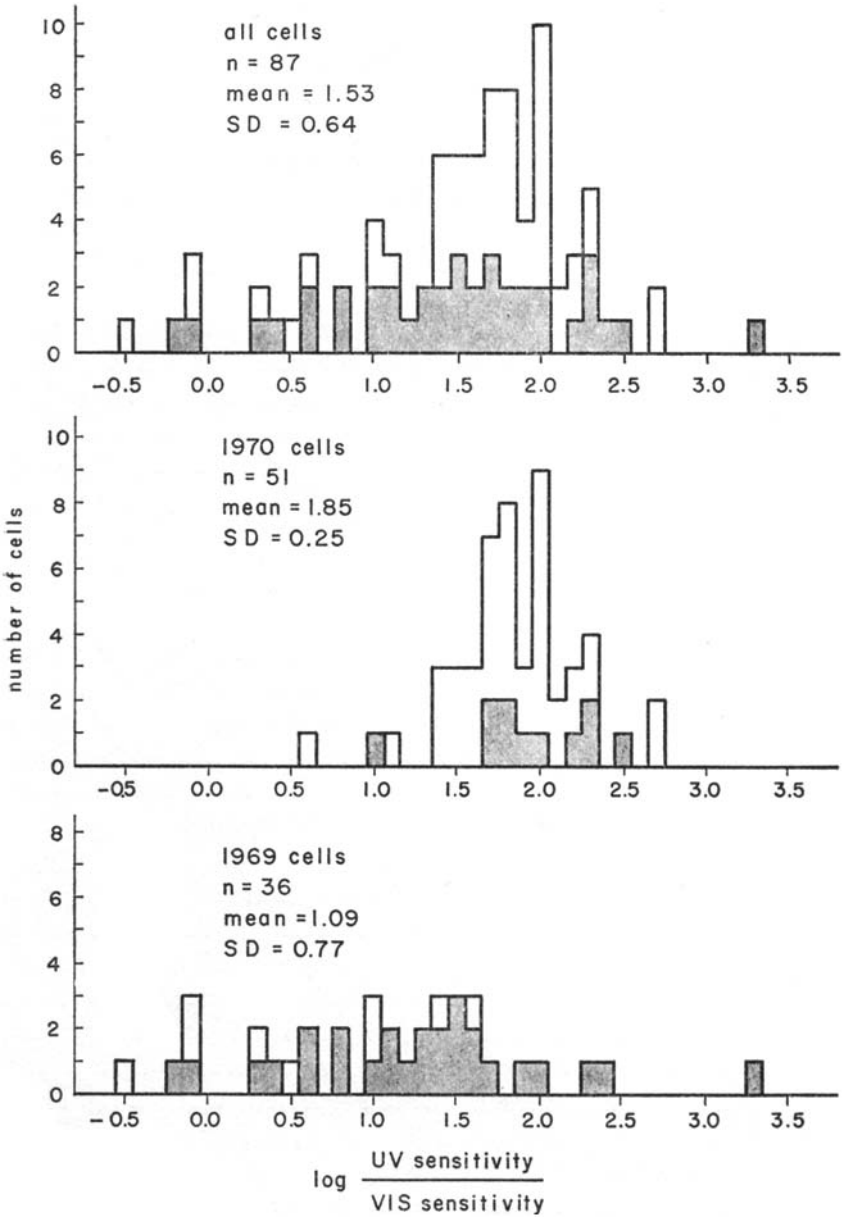

FIGURE 6. Frequency of occurence of cells from anterior median eyes with different log ratios of sensitivity in the ultraviolet to sensitivity in the visible. The data are taken from Fig. 5 and include the total cell population (top) as well as two year's populations: 1969 cells isolated during 1969 while animals were in opaque cages, and 1970 cells isolated during 1970 while animals were in transparent cages. Dark areas represent fully analyzed cells; open areas represent partially analyzed cells. The mean and standard deviation (SD) of the log ratio are given on the figure for each population of *n* cells.

caged spiders under the then prevalent conditions. The experiments illustrated in Figs. 5 and 6 utilized five different collections of spiders, and one of these collections is represented in both the 1969 and 1970 populations. Two collections made and used only in 1970 (from May 1970 on) fitted right into the progression established from animals maintained in the same cages over the fall and winter and used in January and March, 1970. Possibly the common influence could have been the light regimen the animals were subjected to, once caught and caged. However, this was not anticipated and so was not controlled.

Finally, inspection of Fig. 6 reveals that the 1970 population is distinctly unimodal, while the 1969 population is possibly bimodal due to the extreme relative spectral sensitivities recorded in December. The total population of cells is also unimodal, except for those few December cells. All told, it does not appear to be justified to distinguish spectrally different types of cells from these frequency histograms, as did Burkhardt (1962) for cells in flies' eyes and Bennett et al. (1967) for cells in locusts' eyes.

Light Adaptation of Cells

In order to see what effect, if any, light adaptation might have upon the spectral sensitivities of single cells, three of the longer-surviving cells were chromatically adapted with ultraviolet, yellow, or orange background lights. One cell from an anterior lateral eye was light adapted with both orange light ($\lambda > 550$ nm) and with ultraviolet light (365 nm). The shape of its spectral sensitivity curve (as opposed to its absolute sensitivity) did not change, either during or after these two chromatic adaptations.

With two chromatically adapted cells from the anterior median eye, it was different. Fig. 7 shows the results. The cell represented in the left part of the figure clearly underwent relative loss of sensitivity of visible wavelengths during adaptation with orange light. The sensitivity decreased by five times at 360 nm and by 16 times at 500 nm, so that the light-adapted curve resembles the bottom curve in Fig. 4. For technical reasons, light adaptation with ultraviolet light was not tried. The cell represented at the right in Fig. 7 survived the longest of all of the cells (250 min), so its spectral sensitivity in the dark could be measured after the adaptations with ultraviolet light and yellow light ($\lambda \geqslant 495$ nm). The intensities of the adapting lights were adjusted to reduce the sensitivities equally at 520 nm. What was then found was that ultraviolet adaptation reduced the relative sensitivity in the ultraviolet by 0.2 log unit, while yellow adaptation increased the relative ultraviolet sensitivity by 0.3 log units, compared to the dark-adapted cell.

After the yellow adaptation, the cell at the right was allowed to dark adapt. The course of dark adaptation was not followed, but for the period of 10–40 min in the dark a stable spectral sensitivity was recorded (open squares

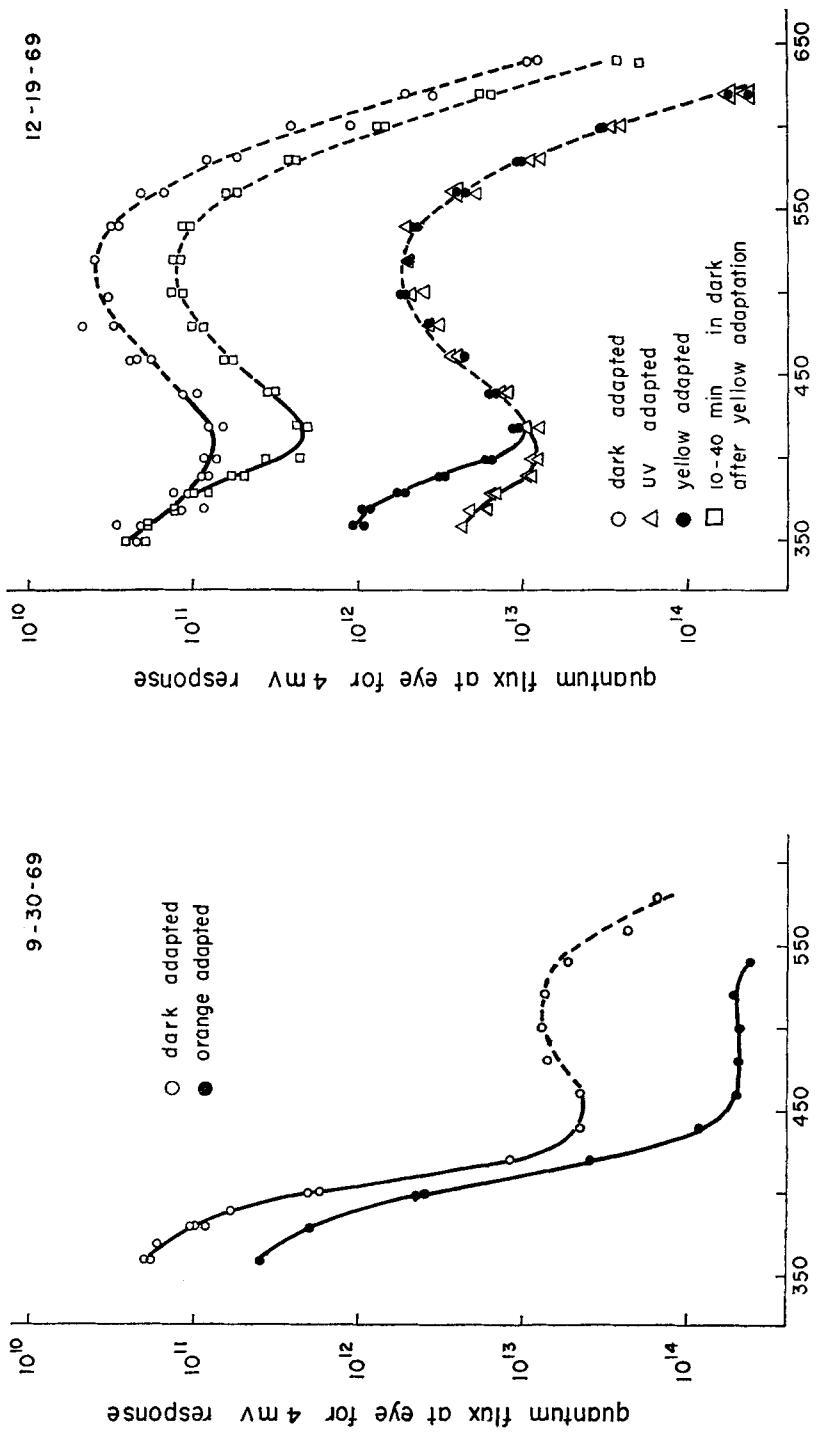

FIGURE 7. Spectral sensitivities of two cells in two different experiments when dark adapted and when chromatically adapted. The cell on the left was orange adapted using a tungsten light source with a Schott OG 550 filter (Jenaer Glaswerk Schott und Genoden). The cell on the right was yellow adapted using the same tungsten light source with a Schott GG 495 filter, and UV adapted using the 365 nm line of a mercury arc lamp isolated with a Wratten 18A filter (Eastman Kodak Co., Rochester, N.Y.). For this latter cell, the yellow and UV adapting lights were adjusted to decrease the sensitivities equally at 520 nm. Dashed lines in both parts of the figure are taken from the average spectral sensitivity of cells in anterior lateral eyes, Fig. 3, where it fitted. Both experiments were performed on *L. baltimoriana*.

on the right in Fig. 7) which was nearly identical in shape to that of the yellow-adapted cell. After 70–90 min in the dark, the spectral sensitivity returned to its original, dark-adapted shape (the absolute sensitivity had fallen, however, so these data are not drawn in Fig. 7). This delayed recovery of full sensitivity in the visible need not be due to cell damage, as a somewhat similar change in relative spectral sensitivity was seen earlier, using the ERG of an intact animal, upon going from a strongly to a weakly orange-adapted eye (DeVoe et al., 1969). On the other hand, the sensitivity in the visible recovered fully in 14 min in a cell in an anterior lateral eye, light adapted (with orange light) a bit more strongly than the cell at the right in Fig. 7. The cell at the left in Fig. 7 took about 2 min to return to nearly full sensitivity at 400 nm, but its recovery of sensitivity at longer wavelengths was not followed.

Facilitation

Ruck and Jahn (1954) found that ERG's of the crustacean *Ligia* became larger and faster if they were due to a second flash following about a second or more after a first of equal intensity. Ruck and Jahn called this phenomenon "facilitation." Since then, Machan (1966) has reported facilitation of the ERG from scorpion eyes, it may appear in responses from the barnacle eye (Stratton and Ogden, 1971), and I have now seen it in single cells of dark-adapted, anterior median eyes of spiders. Fig. 8 contains an illustration of facilitation during repeats of the same flash at 1–2–sec intervals. The amplitude of response tripled with time, the time to peak decreased by about a third, whereas the latency changed hardly at all. In other experiments, there seemed to be more facilitation at short intervals between flashes than at long intervals, and, as in *Ligia* (Ruck and Jahn, 1954), facilitation took time (undetermined) to decay completely.

There is no immediate explanation for facilitation. It is not an injury phenomenon, since it occurs both in the ERG of intact animals (scorpions and crustaceans) and in impaled cells (of spiders). However, it is ephemeral, as it may be present in a cell immediately after impalement, but not later. Neither its appearance nor its disappearance is accompanied by changes in resting potential. It does not depend on the stimulus wavelength. At one time I thought that perhaps electrolyte was leaking out of the micropipette tip and locally changing internal concentrations of ions in these rather small cells. If so, facilitation nonetheless occurred when I used micropipettes filled with potassium chloride or with potassium citrate or with sodium chloride. The presence of steady background illumination appears to abolish facilitation, but the resulting changes in membrane potential per se do not appear to be involved, as polarization of the cell membrane by passing current through

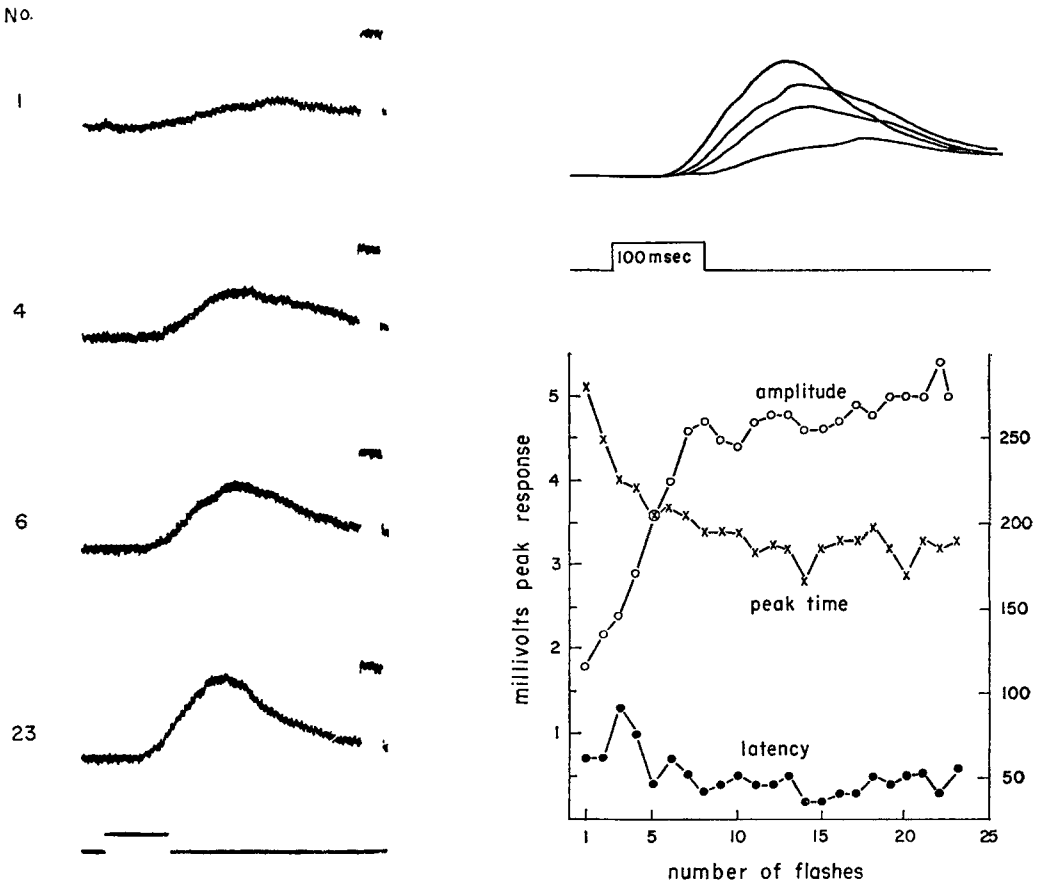

FIGURE 8. "Facilitation" of responses of a cell in an anterior median eye to repeated flashes. *Left:* responses of the cell to the 1st, 4th, 6th, and 23rd flash in a train of 100-msec, 520-nm flashes presented (by hand) every 1–2 sec. The bottom-most trace is a photocell record of the stimulus; the calibration marks at the ends of the other traces represent 5 mv. *Top right:* superimposed tracings of the four responses at the left, showing the changes in peak amplitudes and time due to facilitation. *Bottom right:* latencies of response, peak times, and peak amplitudes for responses to all 23 flashes in the train. The abscissa gives the position of each flash in the train, the left ordinate gives millivolts of peak amplitude, and the right ordinate gives milliseconds of latency and peak time (note that zero time on this right ordinate is below the graph and not drawn). *L. baltimoriana* was used.

the recording electrode is without effect. So far, facilitation has not been seen in cells of the anterior lateral eye.

DISCUSSION

The unexpected finding of this paper is the great variability of the spectral sensitivities of cells in anterior median eyes. There were often large differences

in the relative ratios of ultraviolet and visible sensitivities within the sampled population of cells from individual eyes, and the populations themselves seemed to change from animal to animal. The reason for either type of variability is unknown. Presumably the variation from population to population is not due to experimental trauma of the animal, since similar shifts in populations of cells can be inferred to have taken place in previous experiments using the ERG's of intact animals (DeVoe et al., 1969). The population shifts might have been related to changes in light regimen, as mentioned previously.

The variability of spectral responses from cells of spider eyes is closest to that seen in cells from the compound eye of the locust. In the locust, the ratio of blue to green sensitivities varies from 1:1 to about 3:1, with the distribution of ratios appearing to be trimodal (Bennett et al., 1967). Cells in locust ommatidia are too weakly coupled for this to result from variable coupling of spectrally different cell types (Shaw, 1969). Rather, variable ratios of two visual pigments were inferred (Bennett et al., 1967).

In cells in anterior median eyes of wolf spiders, on the other hand, the ratios of UV to VIS sensitivity vary from 1:4 to 2000:1. However, there is no direct evidence either for or against the possibility that this much larger variability could be due to variable coupling between cells. Although coupling between cells has not been tested, it seems unlikely. In the bee, coupling has been argued to occur by tight junctions between adjacent rhabdomeres in this animal's fused rhabdome (Shaw, 1969). Retinal cells in anterior median eyes of wolf spiders are "commonly" ensheathed in pigment cells from the tips of their open-type rhabdomes to their bases, although there are "small zones" where the rhabdomeres lie in "close proximity" (Melamed and Trujillo-Cenoz, 1966). "Rarely", small, nervelike processes of unknown origin have been seen in apical contact with rhabdomeres (Melamed and Trujillo-Cenoz, 1966). In the web-spinning spider *Zygiella*, similar processes of unknown termination arise from the bases of the retinal cells (W. Edwards, personal communication). Possibly, coupling between cells could occur via such processes, but the strength of coupling, if any, remains to be determined.

However, if cells are coupled one to another, then every cell sensitive in the visible must be coupled to one sensitive in the ultraviolet, and vice versa, since I have not seen either type alone (with the possible exception of the cell depicted at the bottom of Fig. 4). Likewise, measurements of spectral sensitivities with massed responses (the ERG) have not revealed two cell populations separable by chromatic adaptations (DeVoe et al., 1969). On this line of reasoning, the variability of relative spectral sensitivities from cell to cell and from experiment to experiment could either be due to variations in strength of coupling, or to numbers of cells of each type coupled to each other.

Alternatively, there might be mixtures of two visual pigments in visual

cells, as postulated for locust visual cells (Bennett et al., 1967). Changes in the mixtures could provide a basis for the variability of spectral sensitivities observed. As with coupling between cells, there is again no direct evidence for or against this possibility. However, it is valuable here to compare the spectral sensitivities of cells from the two types of spider eyes, principal eyes (anterior median eyes) and secondary eyes (all other eyes). The results which have been presented above indicate that there is probably one spectral type of cell in anterior lateral eyes (and other secondary eyes), and that these cells could well contain only a single visual pigment. The many different types of cells in anterior median eyes are also similar in having spectral sensitivities in the visible like those of cells in anterior lateral eyes, and in having sensitivities in the ultraviolet which all appear to be derived from a common curve (except for unexplained differences in the wavelengths of peak sensitivities). It is not unreasonable to suppose that if there is a single visual pigment in cells of anterior lateral eyes, this same pigment (or something very much like it) is also present in cells of anterior median eyes. The sensitivities of cells in anterior median eyes in the ultraviolet might then be due to a second, ultraviolet-absorbing pigment, or to something else (see later discussion).

By way of illustration, Fig. 9 provides a comparison of spectral sensitivities of cells in anterior median and anterior lateral eyes with difference spectra of visual pigments extracted from frontal eyes of owl flies (Hamdorf et al., 1971) and from house flies (Marak et al., 1970), respectively. In addition, there is shown the absorbance of screening pigments from anterior lateral eyes of wolf spiders (Strother and Casella, 1972), about which more will be said later. The insect visual pigment absorbances give a fair fit to the spectral sensitivity data from the spider, and this is about all that can be expected given the species (and class) differences. (The goodness of fit to the ultraviolet-absorbing pigment is somewhat arbitrary, because it obviously depends on which spectral sensitivity curves from, e.g., Fig. 4, are chosen for comparison. Those curves which peak in the ultraviolet at, say, 370 nm would fit the insect pigment much more poorly.) Nonetheless, the extracted visual pigments and the spectral sensitivities are not so dissimilar as to exclude the absorbances of two visual pigments being the determinants of the spectral sensitivities.

If there are mixtures of two visual pigments in visual cells of anterior median eyes, there are then a number of ways that the great variability of spectral sensitivities of these cells could result. For one thing, the proportions of the two pigments in the mixtures might vary, as is postulated to occur amongst cells in the locust eye (Bennett et al., 1967). Seasonal, light-dependent variations in mixtures are known to occur in fishes whose retinas contain both rhodopsin and porphyropsin (vitamin A_1– and vitamin A_2–based pigments, respectively). In the darker winter months and/or in fish kept in the dark, the

proportion of porphyropsin increases, while in the brighter summer months and/or in fish kept in the light, the proportion of rhodopsin increases (Dartnall et al., 1961; Bridges, 1965). Similar seasonal variations might underlie changes in the ratios of ultraviolet to visible sensitivity seen in the spider, where the longer wavelength sensitivity (in this case at 510 nm) likewise increased in the early winter, albeit briefly. However, any determinative effects

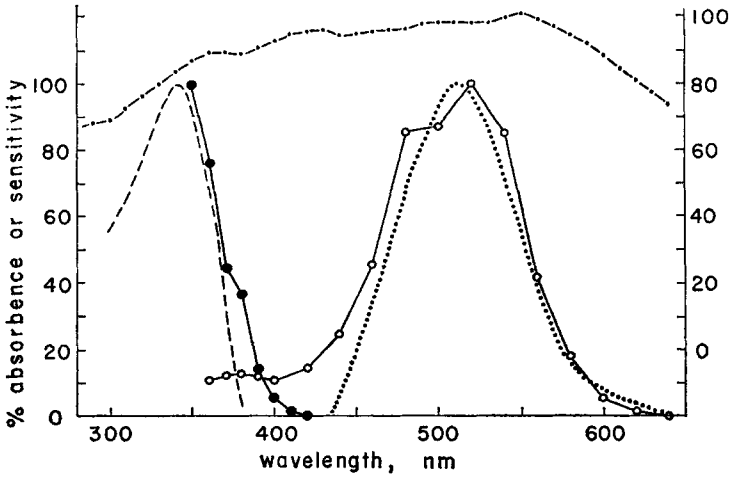

FIGURE 9. Comparisons of recorded spectral sensitivities with screening pigments and with insect visual pigments. The closed circles represent the normalized spectral sensitivity, on a linear scale, of cells in the anterior median eye (taken from the next to bottom curve, Fig. 4). The points at wavelengths longer than 420 nm are not shown. The open circles represent the normalized spectral sensitivity, on a linear scale, of cells in the anterior lateral eye (taken from Fig. 3). The dashed line represents the normalized difference spectrum of the ultraviolet-absorbing, extracted visual pigment of the owl fly frontal eye, absorbing maximally at 345 nm (redrawn from Hamdorf et al., 1971). The dotted curve represents the normalized difference spectrum of an extracted visual pigment from the house fly, absorbing maximally at 510 nm (redrawn from Marak et al., 1970). The left-hand ordinate, in per cent absorbance or sensitivity, refers to the above four curves. The dot-dashed line represents the normalized absorbance of shielding pigments in the anterior median eye of wolf spiders (redrawn from Strother and Casella, 1972). For clarity, this curve is given in the same scale as the other curves, but is shifted upwards and corresponds to the right-hand ordinate.

of light upon spectral sensitivities of spiders were not anticipated and so not controlled, nor were the experiments extended enough in time to be certain that the variabilities could have been cyclic with the seasons.

Secondly, the two hypothetical visual pigments might have variable efficiencies for exciting the cell membrane. In the fly, the absorbances of the rhabdomeres, measured microspectrophotometrically (Langer, 1966), are not directly proportional to the spectral sensitivities, measured with intracellular electrodes (Burkhardt, 1962), although they agree well with the behaviorally

determined spectral sensitivities (Kaiser, 1968). Moreover, sensitivity falls more in the ultraviolet than in the visible in carotenoid-depleted flies (Goldsmith and Fernandez, 1966). Since Marak et al. (1970) claim to have isolated the visible-absorbing pigment alone, the above results might mean that two visual pigments in fly visual cells have differing and differentiable abilities to excite the cells. If something similar occurred in visual cells of spiders, it might also explain the small, "selective" changes in spectral sensitivities seen in chromatically adapted cells. However, such selective adaptations were not seen in visual cells of the fly itself (Burkhardt, 1962) and, in general, chromatic adaptations of single visual cells have given variable results (Autrum and Kolb, 1968; Nolte and Brown, 1969; Wasserman, 1969).

Finally, even if there is a two-pigment mixture in spider visual cells, perhaps some of the apparent variability in their spectral sensitivities might nonetheless arise only because the spectral distribution of light within the eye is different from that calibrated in the incident light. Of the ways that this could occur, fluorescence has not been observed within wolf spider eyes (Young and Wanless, 1967; DeVoe et al., 1969). Ocular media would probably scatter ultraviolet more than visible light, but the degree of scatter would vary from the reciprocal fourth power of wavelength for very small scattering particles (Rayleigh scattering) to the reciprocal second power of wavelength (Mie scattering) for cellular particles commensurate with the wavelength of light (Koch, 1961). Thus, between 2.2 and 4.5 times as much 350 nm light would be scattered as 510 nm light. Even if such scatter were to result in some cells receiving up to 4.5 times more ultraviolet light than expected from the calibrated light source, such scatter could hardly account for the 100:1 variability in UV/VIS sensitivity ratios observed in cells from one eye alone. Direct evaluation of scatter, as by small displacements of light incident upon the eye, was not technically possible, however, Finally, the absorbance of the screening pigment in anterior median eyes (Strother and Casella, 1972: curve reproduced in Fig. 9 of this paper) is uniform to within 20% from 330 to 620 nm. Thus, it would minimally influence relative spectral sensitivities of cells even if it migrated from its dark-adapted position at the base of the rhabdomes to its light-adapted position(s) between or even proximal to the rhabdomes (Scheuring, 1914). On this basis, the delayed recovery of sensitivity in the visible after light adaptation of the cell in Fig. 7 was probably not due to movements of shielding pigments, as proposed earlier (DeVoe et al., 1969). There was no delayed recovery of sensitivity in the ultraviolet, yet this sensitivity as well as that in the visible would have been reduced by movements of the essentially neutral shielding pigments. This neutrality also precludes these particular screening pigments from serving in a schema of radiationless-energy transfer of ultraviolet light to a visible-absorbing pigment, as proposed previously (DeVoe et al., 1969). Some cells had too little sensitivity in the visible for this schema to be tenable any longer, anyway.

This investigation was supported by a United States Public Health Service Research Grant EY-00008 from the National Eye Institute.

I would like to thank the superintendent of the Everglades National Park, Florida, for a permit to collect spiders used in some of this work and Dr. R. J. W. Small for assistance during some of the experiments.

Received for publication 5 April 1971.

REFERENCES

AUTRUM, H., and G. KOLB. 1968. Spektrale Empfindichkeit einzelner Sehzellen der Aeschniden. *Z. Vergl. Physiol.* **60**:450.

AUTRUM, H., and V. VON ZWEHL. 1964. Die spektrale Empfindichkeit einzelner Sehzellen des Bienenauges. *Z. Vergl. Physiol.* **48**:357.

BENNETT, R. R., J. TUNSTALL, and G. A. HORRIDGE. 1967. Spectral sensitivity of single retinula cells of the locust. *Z. Vergl. Physiol.* **55**:195.

BRIDGES, C. D. B. 1965. Variability and relationships of fish visual pigments. *Vision Res.* **5**:239.

BURKHARDT, D. 1962. Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. *Symp. Soc. Exp. Biol.* **16**:86.

DARTNALL, H. J. A. 1953. The inteperetation of spectral sensitivity curves. *Brit. Med. Bull.* **9**:24.

DARTNALL, H. J. A., M. R. LANDER, and F. W. MUNZ. 1961. Periodic changes in the visual pigment of a fish. *In* Progress in Photobiology. B. C. Christensen and B. Buchman, editors. Elsevier Publishing Co., Amsterdam.

DEVOE, R. D., and R. J. W. SMALL. 1970. Spectral sensitivities of single visual cells in eyes of wolf spiders. *Biophys. Soc. Annu. Meet. Abstr.* 16.

DEVOE, R. D., R. J. W. SMALL, and J. E. ZVARGULIS. 1969. Spectral sensitivities of wolf spider eyes. *J. Gen. Physiol.* **54**:1.

GOLDSMITH, T. H., and H. R. FERNANDEZ. 1966. Some photochemical and physiological aspects of visual excitation in compound eyes. *In* The Functional Organization of the Compound Eye. C. G. Bernhard, editor. Pergamon Press Ltd., Oxford.

HAMDORF, K., J. SCHWEMER, and M. GOGALA. 1971. Insect visual pigment sensitive to ultraviolet light. *Nature (London).* **231**:458.

HORRIDGE, G. A. 1969. Unit studies on the retina of dragonflies. *Z. Vergl. Physiol.* **62**:1.

KAISER, W. 1968. Zur Frage des Unterscheidungsvermögens für Spektralfarben: eine Untersuchung der Optomotorik der königlichen Glanzfliege *Phormia regina* Meig. *Z. Vergl. Physiol.* **61**:71.

KÄSTNER, A. 1950. Reaktion der Hupfspinnen (Salticidae) auf unbewegte farblose und farbige Gesichtsreize. *Zool. Beitr.* **1**:12.

KOCH, A. L. 1961. Some calculations on turbidity of mitochondria and bacteria. *Biochim. Biophys. Acta.* **51**:429.

LAMBERT, A. E. 1909. History of the procephalic lobes of *Epeira cinerea*. A study in arachnid embryology. *J. Morphol.* **20**:413.

LAND, M. F. 1969. Structure of the retinae of the principal eyes of jumping spiders (Salticidae: Dendyphantinae) in relation to visual optics. *J. Exp. Biol.* **51**:443.

LANGER, H. 1966. Spektrometrische Untersuchung der Absorptionseigenschaften einzelner Rhabdomere im Facettenauge. *Verh. Deut. Zool. Ges. Jena.* **1965**:329.

LOEWE, R., R. LINZEN, and W. VON STACKELBERG. 1970. Die gelosten Stoffe in der Hämolymphe einer Spinne, *Cupiennius salei* Keyserling. *Z. Vergl. Physiol.* **66**:27.

MACHAN, L. 1966. Studies on structure, electroretinogram and spectral sensitivity of the lateral and median eyes of the scorpion. Dissertation. University of Wisconsin, Madison, Wis. University Microfilms, Inc. No. 66-5920.

MARAK, G. E., G. J. GALLIKY, and R. A. CORNESKY. 1970. Light-sensitive pigments in insect heads. *Ophthalmic Res.* **1**:65.

MCENROE, W. D., and K. DRONKA. 1966. Color vision in the adult two-spotted spider mite. *Science (Washington).* **154**:782.

MCENROE, W. D., and K. DRONKA. 1969. Eyes of the adult female two-spotted spider mite, *Tetranychus urticae*. II. Behavioral analysis of the photoreceptors. *Ann. Entomol. Soc. Amer.* **62**:466.

MELAMED, J., and O. TRUJILLO-CENOZ. 1966. The fine structure of the visual system of *Lycosa* (Araneae:Lycosidae). I. Retina and optic nerve. *Z. Zellforsch. Mikrosk. Anat.* **74**:12.

NOLTE, J., and J. E. BROWN. 1969. The spectral sensitivities of single cells in the median ocellus of *Limulus*. *J. Gen. Physiol.* **54**:636.

NOLTE, J., J. E. BROWN, and T. G. SMITH JR. 1968. A hyperpolarizing component of the receptor potential in the median ocellus of *Limulus*. *Science* (*Washington*). **162**:677.

PARRY, D. A., and R. H. J. BROWN. 1959. The hydraulic mechanism of the spider leg. *J. Exp. Biol.* **36**:423.

RATHMEYER, W. 1965. Neuromuscular transmission in a spider and the effect of calcium. *Comp. Biochem. Physiol.* **14**:673.

RUCK, P., and T. L. JAHN. 1954. Electrical studies on the compound eye of Ligia occidentalis Dana (Crustacea:Isopoda). *J. Gen. Physiol.* **37**:825.

SCHEURING, L. 1914. Die Augen der Arachnoideen. II. *Zool. Jahrb.* **37**:369.

SHAW, S. R. 1969. Interreceptor coupling in ommatidia of drone honeybee and locust compound eyes. *Vision Res.* **9**:999.

STRATTEN, W. P., and T. E. OGDEN. 1971. Spectral sensitivity of the barnacle, *Balanus amphitrite*. *J. Gen. Physiol.* **57**:435.

STROTHER, G. K., and A. J. CASELLA. 1972. Microspectrophotometry of insect visual screening pigments. *J. Gen. Physiol.* In Press.

WASSERMAN, G. S. 1969. *Limulus* receptor action spectra. *Vision Res.* **9**:611.

YOUNG, M. R., and F. WANLESS. 1967. Observations on the fluorescence and function of spiders' eyes. *J. Zool.* (*London*). **151**:1.