The Components of the Visual System of a Dragonfly

PHILIP RUCK

From the Department of Biology, Tufts University, Medford, Massachusetts, and the Department of Zoology, University of Wisconsin, Madison, Wisconsin. Dr. Ruck's present address is the Department of Zoology, University of Wisconsin

ABSTRACT In this study of the electroretinograms of dragonflies (adults and nymphs) the objectives were to determine the number of classes of photoreceptors present in the visual system and to allocate these to particular morphological regions. There are probably five classes of photoreceptors present with peak sensitivities near 550, 530, 518, 420, and < 380 mμ. The dorsal ocelli contain two classes (518 mμ and < 380 mμ). The ventral (anterior) ommatidia of the adult compound eye contain at least two classes (near 518 mμ and < 380 mμ) and probably a third class (near 550 mμ). The dorsal ommatidia of the adult compound eye contain one class (420 mμ) and possibly another class (< 380 mμ). The compound eye of the nymph contains one class (530 mμ) and possibly another class (420 mμ).

INTRODUCTION

The visual system of a dragonfly includes at least four components: the compound eyes of the aquatic nymph, the dorsal and the ventral ommatidia of the compound eye of the adult, and the dorsal ocelli. Spectral sensitivity curves based on measurements of threshold or near threshold energies required to evoke an electroretinogram (ERG) are described here for each component.

Several years ago Mazokhin-Porshniakov (1959) applied a colorimetric procedure to a study of the ERG in *Libellula quadrimaculata.* His principal conclusions were as follows: (*a*) the compound eye of the nymph has one kind of photoreceptor with luminosity maximum at about 515 mμ; (*b*) the dorsal ommatidia of the compound eye of the adult have one kind of photoreceptor with luminosity maximum at about 420 mμ; (*c*) the ventral ommatidia of the compound eye of the adult have two kinds of photoreceptors with their respective luminosity maxima at 515 and 610 mμ. He did not report data for the dorsal ocelli.

The conclusions of the present study will be shown to differ in several respects from those of Mazokhin-Porshniakov. A critical evaluation of the

differences is difficult because both the experimental species and the experimental methods differ. A few words about the methodological differences are perhaps in order in this introduction. The response levels studied in the colorimetric procedure were all suprathreshold levels in which colorimetric equivalence was established between a monochromatic radiation and the one or more radiations, chosen as primaries, which were required to match it. A match was considered to be achieved when no response (no change in the ERG) was produced by the sudden substitution of a mixture of primaries for a pure radiation. Inherent in the colorimetric procedure is the simultaneous stimulation of more than one class of photoreceptors whenever more than one class are present in the eye under study. Simultaneous stimulation of two or more classes of photoreceptors introduces the possibility of functional interactions among the classes; for example, the responses of two classes may reciprocally inhibit one another, or one may inhibit the other although it itself is not inhibited in return, and so forth.

The response levels used in defining spectral sensitivity in the present study approached threshold levels. The energy required to evoke the threshold ERG from the dark-adapted eye was measured at each wave-length. One has a greater chance of isolating a receptor class at threshold than at levels above threshold. Consider two receptor classes, A and B, with overlapping spectral sensitivities. At some particular wave-length assume that the sensitivity of A is four times the sensitivity of B; *i.e.*, that the probability that A will absorb a given quantum is four times the probability that B will. Suppose that the threshold event represents the absorption of one quantum. The probability that it will occur in A is $\frac{4}{5}$; in B it is $\frac{1}{5}$. Considering a just suprathreshold event—the absorption of two quanta—the probability that both will be absorbed by A is $\frac{4}{5} \times \frac{4}{5} = \frac{16}{25}$, and the probability that both will be absorbed by B is $\frac{1}{5} \times \frac{1}{5} = \frac{1}{25}$. The probability that A and B will each absorb one is $\frac{8}{25}$. For three absorbed quanta, $\frac{64}{125}$ is the probability that A will absorb all three and $\frac{1}{125}$ the probability B will absorb all three. There is a probability of $\frac{60}{125}$ for mixed absorptions of the three quanta by both A and B. With increase in the number of absorbed quanta, the fractions of absorptions by A alone and by B alone decrease, while the fraction of mixed absorptions by A and B increases. One may conclude that the probability of isolating a receptor class increases as one approaches threshold levels of stimulation, and consequently that functional interactions among receptor classes decrease toward a minimum at threshold. The argument as applied to the ERG is a qualitative one since precise determinations of the relation between ERG threshold and single receptor cell thresholds are not available.

The range of wave-lengths over which there is high probability of isolating a receptor class will depend upon the degree of overlapping of its spectral sensitivity curve with that of another receptor class: the broader the overlap

the smaller will be the wave-length range of probable isolation, even at threshold. At wave-lengths where two spectral sensitivity curves intersect the probabilities of isolating either receptor class are least.

There is no *a priori* reason why the luminosity functions derived from colorimetry should be identical with the spectral sensitivity functions derived from measurements of threshold energies. The two procedures, when properly employed, are equally valid and each is capable of contributing a different kind of information.

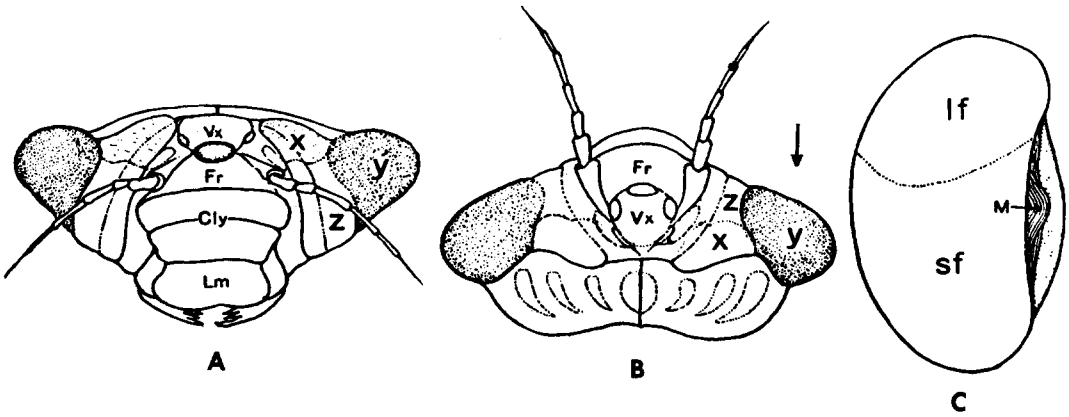

FIGURE 1. Head of *Libellula pulchella* (from Plate 17 of Lew, 1933). A and B, front and top views, respectively, of nymphal head. The Y region contains the functional ommatidia of the nymphal compound eye. The X region contains the presumptive tissue of the dorsal ommatidia and the Z region the presumptive tissue of the ventral ommatidia of the adult compound eye. C, side view of adult head; *lf*, dorsal ommatidia; *sf*, ventral ommatidia; *M*, vestige of the nymphal compound eye. (*Figure reprinted, by permission, from Lew, G. T., 1933, Entomol. America, 14, 41, Plate 17.*)

MATERIALS AND METHODS

The principal species of adult dragonfly which was used in these experiments is *Libellula luctuosa* Burmeister. It is abundant at local ponds during much of the summer and is easily netted. Some results on adults of *Sympetrum rubicundulum* Say and *Anax junius* Drury are included. The nymphs which were studied include *Libellula sp.*, *Sympetrum sp.*, *Leucorrhinia sp.* (Libellulidae), *Anax junius* (Aeschnidae), and *Gomphus sp.* (Gomphidae).

Anatomical Background The compound eye of the nymphal dragonfly is wholly or largely replaced at metamorphosis by the compound eye of the adult (Lew, 1933). In the genus *Libellula* (Fig. 1) the nymphal compound eye is represented in the adult by a functionless vestige at the postero-lateral margin of the adult compound eye. The adult compound eye contains two distinctly different kinds of ommatidia (Oguma, 1917), the dorsal ommatidia and the ventral ommatidia. A dorsal ommatidium contains four fully differentiated retinula cells while a ventral ommatidium

contains six. There are other differences as well: dorsal ommatidia are longer and larger in diameter; in cross-sections the rhabdomes of dorsal ommatidia are three-rayed while those of ventral ommatidia are circular; the density of the shielding pigments is greater in the ventral ommatidia.

Preparation of Experimental Animals Freshly captured animals were used exclusively. They were cooled to 8°C and then immobilized with tackiwax (Cenco). Adults and nymphs were treated alike except that a sheet of moist cotton was placed loosely over the hind parts of the nymphs to maintain high humidity about the rectal respiratory region. Animals were positioned so that the particular component of the visual system under study was the only one exposed directly to the stimulating light.

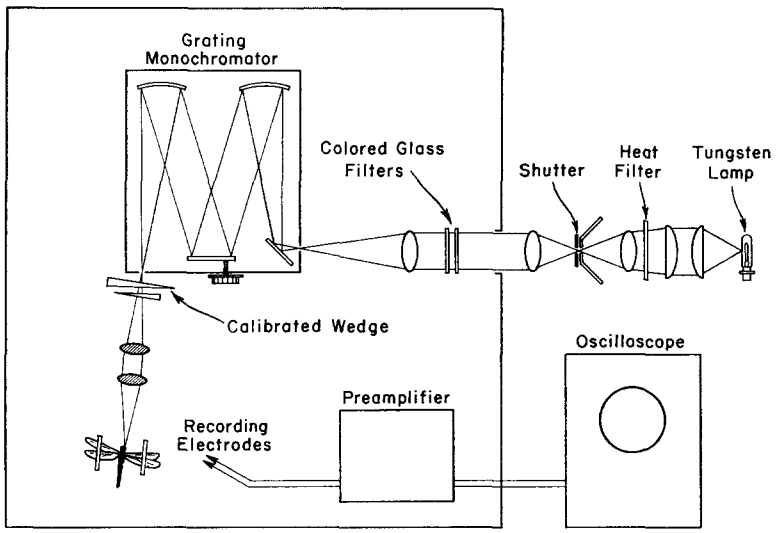

FIGURE 2. Optical stimulator.

This condition was achieved by masking all other components with aluminum foil. In the case of compound eyes, both in adults and nymphs, circular areas 1 mm or less in diameter were exposed to the stimulus. ERGs were recorded with a subcorneal electrode in the exposed part of the experimental eye and an indifferent electrode elsewhere in the body. In the case of adults the indifferent electrode was thrust through the frons in the midline until it made adequate contact with hemolymph. With nymphs the indifferent electrode was inserted just under the cornea of the dark eye. Both electrodes were electrolytically pointed stainless steel wires. These were led into the cathode follower input stage of a Grass P6 preamplifier. After positioning in the optical stimulator, the animals were dark-adapted for at least 45 minutes. This time was chosen on the basis of preliminary experiments designed to determine the period required to ensure stable conditions for threshold measurements.

The Optical Stimulator See Fig. 2. Monochromatic lights of 10 mμ band width from a Farrand grating monochromator were used. Combinations of Corning colored

glass filters were used to reduce stray light. A compensated neutral density wedge was used to control stimulus intensities. It had been calibrated at 20 mμ intervals from 380 to 640 mμ. Relative energies with the wedge removed from the system were determined from 380 to 640 mμ with the aid of an RCA phototube No. 917. The spectral calibration of the specific tube used was performed by RCA. Duration of test flash was either 0.1 or 0.125 second and the test flash repetition rate was one flash per 4 seconds. A Grass S4 stimulator was used to drive an electromagnetic shutter and to trigger an oscilloscope sweep.

An instrumental error of known direction, but of uncertain magnitude, affects measurements at wave-lengths 380 and 400 mμ on the adults of *Libellula luctuosa*. At these two wave-lengths a filter used to remove stray light of long wave-length (Corning filter 7-51) was inserted in the light path. This particular filter, and none of the others used at other wave-lengths, acquired a resistant surface film during the measurements on adults of *L. luctuosa*. Though all filters were cleaned with lens tissue at the outset of each experiment as routine, this film was not noticed until the end of the series of measurements. It was then removed with the aid of a polishing wheel. The energy calibrations which were used to compute spectral sensitivity at 380 and 400 mμ were made after removal of the film. The magnitude of the error introduced by the film is unknown but its direction is certain: since less light was transmitted by the filter during the experiments than during the energy calibrations, the computed sensitivities are too low. In the plots of spectral sensitivity, arrows directed upward from points at 380 and 400 mμ indicate the direction in which the true values lie. All the data from nymphs and from adults of *Sympetrum rubicundulum* and *Anax junius* were obtained after elimination of the above source of error. The error is disturbing where it is present, but it has very little effect on the principal conclusions of this study.

Experimental Procedure Two persons participated in the experiments. One made all settings of wave-length and test flash intensity. The other observed responses on the oscilloscope screen without knowledge of the wave-length and intensity settings. The criterion of threshold was the occurrence of five consecutive judgments by the observer that a response could be discriminated from perturbations of the sweep unrelated to the test flash. Amplitudes of threshold ERGs varied somewhat from eye to eye within the range, 50 to 100 μv. Polarity and wave-form of the threshold response were considered to be irrelevant in application of the threshold criterion. The response might take the form of an on-wave or an off-wave, or it might be positive or negative. If it was the threshold response it was scored. (A disadvantage of the threshold method is that response-energy functions in general have shallow slopes at threshold and consequently experimental error tends to be higher than at suprathreshold levels. One may reduce experimental error by measuring energies required to evoke constant responses at levels where the slopes of response-energy curves are steep. In so doing, however, one loses the merits of the threshold method which were stressed in the Introduction. Moreover, one may not be able to find a constant response at all at levels above threshold. The ventral ommatidia (Fig. 6) provide a case in point.)

Though irrelevant to threshold determinations, variations in wave-form of ERGs

with change in stimulus wave-length did occur and were of great interest. Photographic records of many of the responses were made and will be described.

A number of selective adaptation experiments were carried out, the objective being to reduce through fatigue the responsiveness of particular receptor types and thereby to accentuate the responsiveness of other types. Constant background illumination of desired wave-length was provided by a separate light source together with a sequence of lenses, an interference filter, and a length of fiber optics. The adapting light was conveyed to the eye through the length of fiber optics which was mounted on a micromanipulator.

Spectral Sensitivity Curves Ordinates are the reciprocals of the relative numbers of quanta required to evoke the threshold ERG. Mean values of relative sensitivity are plotted together with the positive and negative limits of one standard deviation.

RESULTS AND DISCUSSION

The data will be related to two kinds of objective: (*a*) to determine the number of classes of photoreceptors present in the visual system; (*b*) to allocate the individual classes to particular regions of the visual system.

Each ERG of this study represents the simultaneously recorded responses of a large number of cells. Several restrictions were placed on the number of cells stimulated; (*a*) shielding from the stimulus all areas of cornea except the limited one selected for exposure; (*b*) choosing a threshold or near threshold ERG as a basis for spectral sensitivity measurements; (*c*) using narrow-band monochromatic stimuli. The first restriction ensures that the responding cells are stimulated by light which enters the eye through the exposed patch of cornea, but in compound eyes it does not ensure that all these cells belong to the immediately underlying ommatidia. A fraction of the light which enters may reach and stimulate outlying ommatidia. It will be helpful to distinguish between two categories of stimulation, direct and indirect. Both are produced by light entering an exposed patch of cornea, but direct stimulation occurs in the immediately underlying ommatidia, whereas indirect stimulation occurs elsewhere.

The second restriction above, namely that threshold or near threshold stimuli be used, acts to reduce the probability of indirect stimulation. Nevertheless the possibility remains and it must be considered for each component of the visual system.

Dorsal Ocelli A whole ocellus was the unit of study. The entire ocellar cornea was illuminated and the remainder of the head was shielded by aluminum foil. Indirect stimulation in this context is stimulation of cells in the adjacent compound eye by light which enters through an ocellar cornea. While such indirect stimulation may occur, it is of no consequence in recordings from ocelli. Recordings from a lateral ocellus are the same when the adjacent compound eye is illuminated simultaneously as when the ocellus alone is illuminated. This statement holds true up to levels of illumination at

least a million-fold above the ocellar threshold. Therefore the ERGs recorded from the ocellus represent responses of cells in the ocellus.

The spectral sensitivity curve of the dorsal ocelli has a peak at about 518 mμ, a valley near 410 mμ, and a rise into the ultraviolet beyond 380 mμ (Fig.

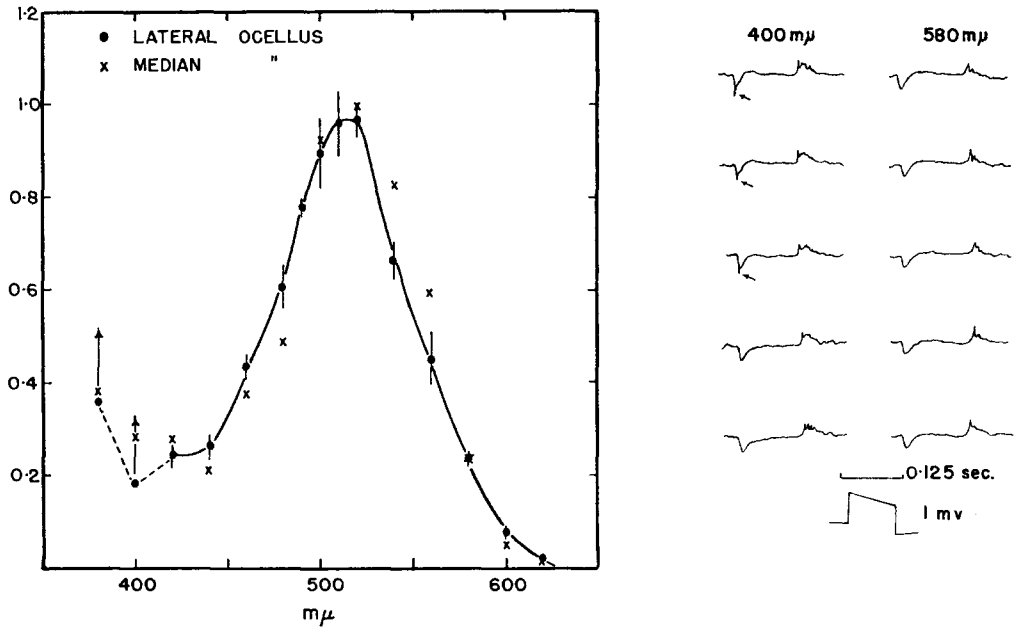

FIGURE 3. Left, spectral sensitivity of dark-adapted dorsal ocelli in *Libellula luctuosa*. Ordinate, reciprocal of relative number of quanta at threshold. Means and ± one standard deviation indicated for sample of twelve lateral ocelli. Data for one median ocellus included. (For explanation of arrows see Materials and Methods.) Right, ERGs of a lateral ocellus of *Libellula luctuosa*. Subcorneal electrode in ocellus *vs.* electrode in frons. Negativity of corneal electrode gives upward deflection. In each series the stimulus intensity was increased from bottom to top in equal steps of 0.24 optical density unit. At 400 mμ the off-effects are characteristically broader than at 580 mμ. The 400 mμ series acquires a sharp on-component (arrows) which is absent in the 580 mμ series. The transition between short and long wave-length responses fell between 400 and 420 mμ.

3). The valley is a transitional region in the sense that wave-forms of ERGs in response to stimulus wave-lengths shorter than 410 mμ differ from those in response to wavelengths longer than 410 mμ. The ERGs of Fig. 3 illustrate the wave-forms encountered using low stimulus intensities on each side of the transition. The two series of ERGs were matched as nearly as possible with respect to amplitude. Stimulus intensities increase stepwise from bottom to top, and the increments of increase are the same in both series. At 400 mμ the off-effects are broader than at 580 mμ, a slight but consistent difference. With

increase in stimulus intensity the positive (downward) on-effects acquire a sharp component (arrows) in the 400 mμ series which is not visible in the 580 mμ series. (This sharp component corresponds to component 2 in the nomenclature of an earlier study (Ruck, 1961).) A comparable component appears in response to stimuli of long wave-length, but its rates of rise and fall are less (*i.e.*, it is not so sharp) and higher stimulus intensities are required to demonstrate it.

An observer without knowledge of the wave-length setting on the monochromator has no difficulty assigning near threshold ERGs to the long or short wave-length series on the basis of qualitative differences in wave-form. There is only one transitional region between 380 and 640 mμ, namely the one at 410 mμ. It is concluded that the dorsal ocelli have two classes of photoreceptors, one with maximum sensitivity at about 518 mμ, the other with maximum sensitivity in the ultraviolet at a wave-length shorter than 380 mμ. A similar situation exists in the honeybee (Goldsmith and Ruck, 1958).

The position of the maximum at 518 mμ is most probably that of the absorption maximum of the responsible visual pigment. The retinulas of the ocellus are bounded by white reflecting material (Ruck and Edwards, 1964) which one would not expect to influence, as a colored shielding pigment might, the position of maximum sensitivity.

The Compound Eyes of the Adults

VENTRAL OMMATIDIA A small region of ventral ommatidia at the front of the head was chosen for study in all experiments. The facets of these ommatidia were at the level of the letter *M* in Fig. 1C but lay at the antero-medial boundary of the compound eye. These facets were exposed to the stimulating light through a hole, 1 mm in diameter, in a sheet of aluminum foil. The sheet of foil covered the remainder of the head. (The maximum dorsoventral dimension of the compound eye in an adult of *Libellula luctuosa* is approproximately 5 mm.)

The average spectral sensitivity curve measured in the anterior (ventral) ommatidia of twelve specimens of *L. luctuosa* (filled circles in Fig. 4) rises into the ultraviolet beyond 380 mμ, has a small hump in the vicinity of 420 mμ, a peak between 510 and 520 mμ, and a prominent shoulder near 550 mμ. Measurements have been made on anterior ommatidia of three specimens of *Sympetrum rubicundulum* and the results were similar to those from *L. luctuosa* except that no trace of a hump near 420 mμ appeared. One set of measurements from one dark-adapted specimen of *Sympetrum* is shown in Fig. 4 (the upper set of open circles). For the same specimen of *Sympetrum* a set of measurements made in the presence of constant background illumination with light of 606 mμ is included (lower set of open circles). Selective adaptation to light of 606 mμ causes a greater depression of sensitivity to light of long

wave-length than to light of short wave-length. Two such selective adaptation experiments have been performed on *Sympetrum* with essentially the same results. They suggest that there is a distinct class of ultraviolet receptor and at least one distinct class of receptor sensitive to the longer wave-lengths.

A comparison of ERG wave-forms at wave-lengths on either side of a region centered at about 410 mμ reinforces the view that there is a distinct class of

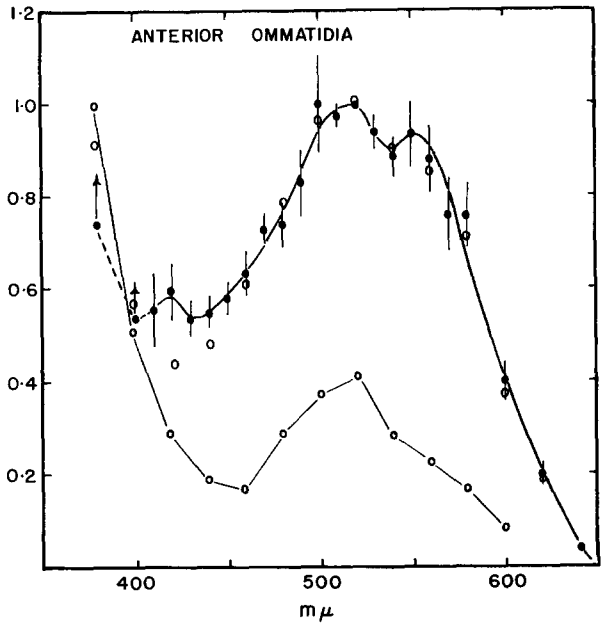

FIGURE 4. Spectral sensitivity of anterior (ventral) ommatidia in adult compound eye. Filled circles, *Libellula luctuosa*, twelve runs in twelve animals, dark-adapted; means and ± one standard deviation are indicated. Open circles, upper set is a series of measurements on a dark-adapted specimen of *Sympetrum rubicundulum*; lower set a series from the same specimen during selective light adaptation to 606 mμ. (For explanation of arrows see Materials and Methods.)

ultraviolet receptor. The threshold ERG in *L. luctuosa* at 400 mμ and shorter wave-lengths is a predominantly negative (upward) wave, whereas at 420 mμ and longer wave-lengths it is a predominantly positive wave (Fig. 5). With increase in stimulus intensity (from bottom to top in Fig. 5) the amplitude increases much more rapidly for equal energy increments at 400 than at 440 mμ. In addition the wave-form in the 400 mμ series is considerably simpler than at 440 mμ.

A series of ERGs from *Sympetrum* (the specimen which provided the measurements of Fig. 4) appears in Fig. 6. The wave-forms are not identical to those of *L. luctuosa* (Fig. 5) but are similar in several respects. A transition

in ERG wave-form occurs between 400 and 420 mμ. At 380 and 400 mμ the wave-form is simpler than at 420 mμ. A sharp, positive (downward) on wave and a subsequent steep, negative rise to a peak are characteristic of the 420 mμ series, and not of the series at 400 mμ.

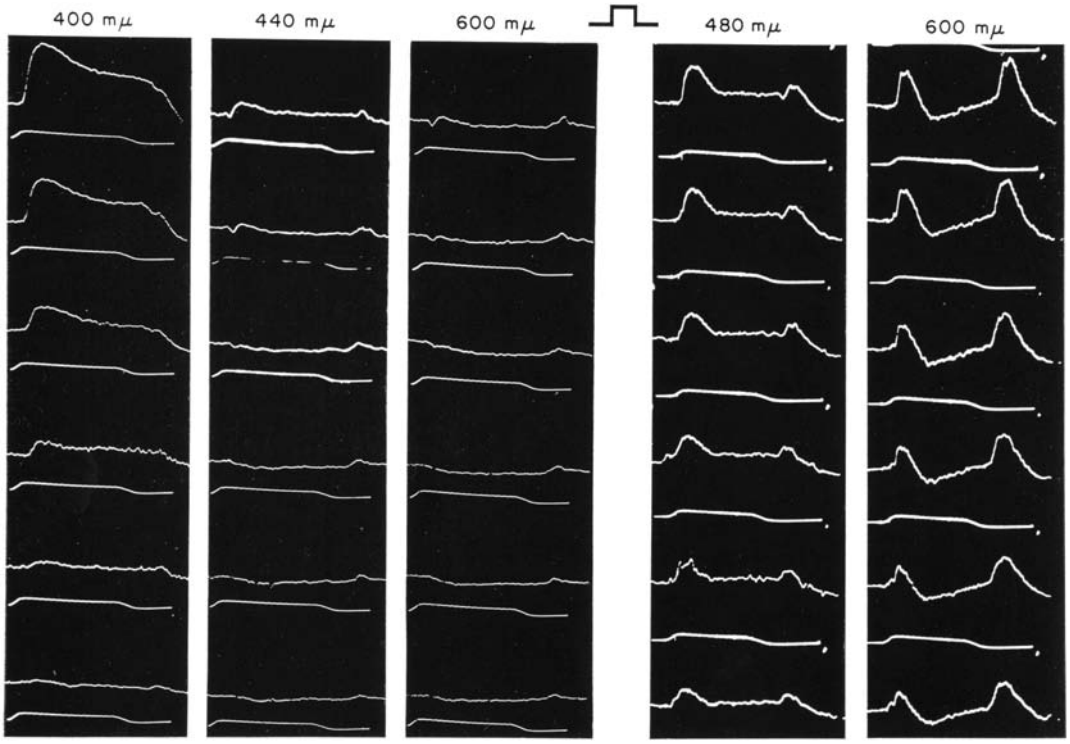

FIGURE 5. ERGs from anterior (ventral) ommatidia of a specimen of *L. luctuosa* recorded as in Fig. 3. Each of the three columns at the left begins (bottom) with a response near ERG threshold. Stimulus intensity in each column increases stepwise in equal increments of 0.24 optical density unit. The two columns at the right are approximately matched for equal amplitudes of the negative (upward) on-waves, and they begin (bottom) at somewhat higher stimulus intensities than the top members of the columns at the left. Again the stimulus intensity increased stepwise in equal increments of 0.24 optical density unit. Voltage calibration (top) is 400 μv.

The wave-form transition at about 410 mμ is common, then, to both the dorsal ocelli and the ventral ommatidia, and supports the conclusion that in both there is a distinct class of ultraviolet receptor.

The average spectral sensitivity curve for *L. luctuosa* (Fig. 4) has a small hump near 420 mμ. A fully satisfactory explanation for this hump is not yet available. Several interpretations of it are possible. (*a*) It may be largely a by-product of an error in energy calibrations. There is a recognized error (Materials and Methods) which contributes to abnormally low values for

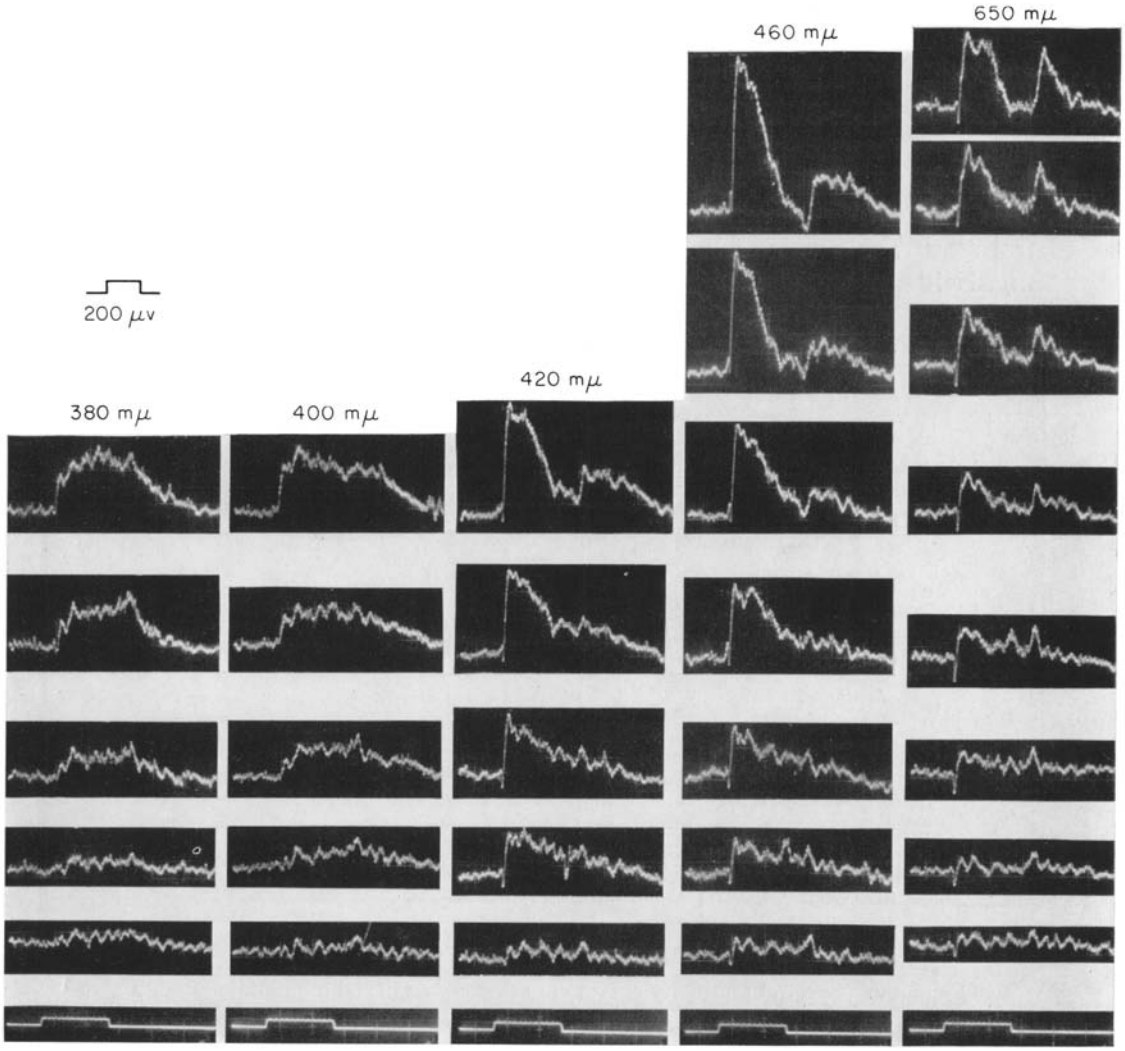

FIGURE 6. ERGs from anterior (ventral) ommatidia of *Sympetrum rubicundulum*. Recorded as in Fig. 3. Each of the five columns begins with a response somewhat above ERG threshold (bottom). Stimulus intensity increases stepwise in equal increments of 0.24 optical density unit from bottom to top in each column. Stimulus duration is 0.1 second.

the points at 400 and 380 mμ, and therefore to accentuation of the 420 mμ hump. It may be significant that the hump did not appear in *Sympetrum*, the measurements on which were made after elimination of the source of error. (*b*) The hump at 420 mμ in *L. luctuosa* may represent a contribution of photoreceptors in the dorsal ommatidia. According to this interpretation the electrode in the ventral ommatidia would record activity of indirectly stim-

ulated units in the dorsal ommatidia. (*c*) The hump at 420 mμ may be related to the filtering characteristics of shielding pigments in the ventral ommatidia. Available data do not permit a definitive interpretation of the hump at 420 mμ.

The principal peak of sensitivity in the ventral ommatidia lies between 510 and 520 mμ for both *L. luctuosa* and *S. rubicundulum* (Fig. 4). In the spectral sensitivity curves of both there is a fairly prominent shoulder near 550 mμ. Neither the peak nor the shoulder appears in measurements of sensitivity

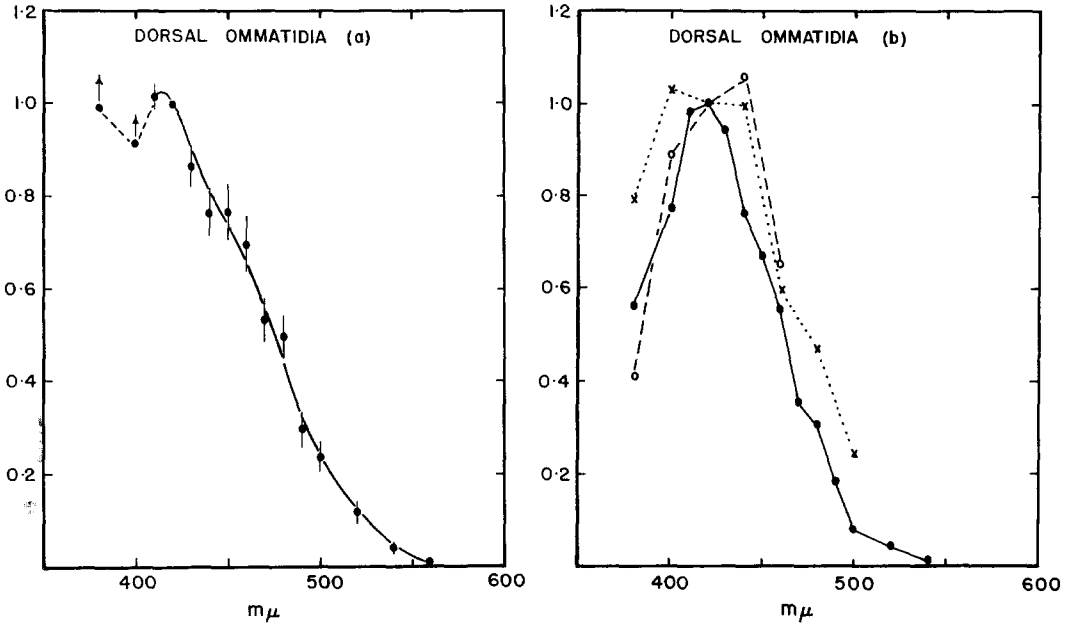

FIGURE 7. Spectral sensitivity curves of dark-adapted dorsal ommatidia of adult compound eye in *L. luctuosa*. (*a*) fifteen runs in fifteen animals; means and ± one standard deviation. (*b*) three runs on one unusual animal. (For explanation of arrows see Materials and Methods.)

of the dorsal ommatidia (Fig. 7), and therefore it may be concluded that they do not arise as a result of indirect stimulation of the dorsal ommatidia. That is, they are properties of the ventral ommatidia.

A simple interpretation of the peak and the shoulder is as follows: the ventral ommatidia contain the same class of receptors which is responsible for the peak near 518 mμ in the dorsal ocelli (Fig. 3), and another class of receptors, not present in the ocelli, which is maximally sensitive near 550 mμ. In support of this interpretation is the fact that ERG wave-forms at the longest wave-lengths to which the ventral ommatidia respond are distinctive and cannot be duplicated elsewhere in the spectrum. They are easily distin-

guished from wave-forms in the blue and green, and also from those in the ultraviolet. That is, there are two transitions in wave-form in the ventral ommatidia, the one near 410 mμ and the other, much less abrupt, at the long-wave end of the spectrum.

The two columns of ERGs from *L. luctuosa* at the right of Fig. 5 illustrate the differences in wave-form at 480 and 600 mμ at levels of stimulation somewhat above threshold. On-effect amplitudes are approximately the same at each level in both series, but off-effects are consistently larger in the 600 mμ series. Closer to threshold (second and third columns from left, Fig. 5) wave-form differences may not be detectable in *L. luctuosa*.

The ERGs of Fig. 6 are from the specimen of *Sympetrum* which provided the spectral sensitivity measurements of Fig. 4. The wave-form transition between 400 and 420 mμ has been pointed out previously. The wave-forms at 420 mμ are very similar to those at 460 mμ, but different from those at 650 mμ. For equal amplitudes of on-effect, the off-effects are in general larger in the 650 mμ series. Considering also that the increments of increase in stimulus intensity (from bottom to top) are the same in all columns, it is apparent that the *on-effect magnitude vs. stimulus intensity* function is less steep at 650 mμ than in the blue.

An alternative interpretation of the long-wave portion of the spectral sensitivity curve of the ventral ommatidia is that a single class of receptor underlies it, but that filtering action of retinal shielding pigments is responsible for the "peak and shoulder" characteristic. Thus each ommatidium receives direct light through its own facet and indirect light obliquely or transversely from its neighbors. The threshold response depends, presumably, upon the absorption of a fixed quantity of light energy and this quantity is made up of direct and indirect fractions. The indirect fraction may be expected to increase, relative to the direct fraction, in spectral regions where the shielding pigments are more transparent, and to be responsible for inflections, shoulders, and so forth in the action spectrum of a single class of photoreceptor.

This interpretation would be acceptable if the filtering action of the retinal shielding pigments in the ventral ommatidia were appropriately related to the observed sensitivity curve, and if the differences in ERG wave-form could be related causally to the filtering action of shielding pigments. This interpretation cannot be evaluated because no appropriate measurements have been made on the shielding pigments in either *L. luctuosa* or *S. rubicundulum*.

In earlier times one might have argued that however complex the filtering action of colored shielding pigments, the qualitative nature of the response arising from excitation of a single class of receptor should remain unchanged with change in stimulus wave-length; that the ERG obtained at one wave-length should be matched in wave-form at another wave-length by making an appropriate intensity adjustment. Goldsmith (1965), however, has recently

provided evidence that qualitative changes in wave-form of ERG can be brought about by action of colored shielding pigments alone in the eye of the housefly. His stated reason for this is that ERGs may vary in wave-form when the number only of identical excited receptor cells changes, and that colored shielding pigments in part determine the number of cells excited.

With regard to the ventral eye of the dragonfly, the available data do not exclude rigorously either the hypothesis that two classes of receptors underlie the peak between 510 and 520 mμ and the shoulder near 550 mμ, or that only one class of receptor underlies both. The "two class" hypothesis, however, has preferred status at the present level of investigation. The similarities in peak positions near 518 mμ in ventral ommatidia and dorsal ocelli, the same region of wave-form transition centered near 410 mμ, and the same rise of sensitivity into the ultraviolet suggest that two classes of receptors are common to the ventral ommatidia and the dorsal ocelli. Because the dorsal ocelli have no shielding pigments between retinulas, the peak at 518 mμ probably corresponds to the absorption maximum of the underlying visual pigment. Even though the ventral ommatidia are densely bounded by shielding pigments, they have a peak at about the same place. Perhaps the reason for this is that the fraction of incident light which is transmitted by the pigment sleeves is so attentuated that it has little influence on threshold measurements. (In other words, the presence of an opaque pigment sleeve might have as little influence on the positions of maxima in a spectral sensitivity curve as the total lack of a pigment sleeve.) Proceeding on the assumption that the peak at 518 mμ is approximately the absorption maximum of a visual pigment, the shoulder near 550 mμ and the associated change in ERG wave-form at long wave-lengths would, on the "one class" hypothesis, be attributable to a colored pigment screen with heightened transmission in the green to red region of the spectrum. One would predict a steeper slope of the *response amplitude vs. stimulus energy* function at long wave-lengths. That is, the effectiveness of indirect stimulation increases when transmission of shielding pigment increases. Actually, it is rather the reverse effect which occurs in *Sympetrum* (Fig. 6). The increase in response amplitude per increment of stimulus energy is less at 650 than at 460 mμ.

DORSAL OMMATIDIA A circular area of facets approximately 1 mm in diameter was chosen at about the center of the dorsum of the eye. The spectral sensitivity curve representing fifteen specimens of *L. luctuosa* (Fig. 7 *a*) has a crest or shoulder of sensitivity at about 420 mμ, and a rise of sensitivity into the ultraviolet beyond 380 mμ. One animal (Fig. 7 *b*) showed a peak of sensitivity in the vicinity of 420 mμ and no rise of sensitivity into the ultraviolet.

Spectral sensitivity measurements have been made on dorsal ommatidia of

one specimen of *Anax junius* and two specimens of *Sympetrum rubicundulum*. In these three cases there was a peak in the vicinity of 420 mμ but no rise into the ultraviolet; *i.e.*, they belonged to the category of Fig. 7 *b* rather than to that of Fig. 7 *a*.

One may conclude that a population of receptors with maximum sensitivity at about 420 mμ is present. There may be another population with maximum sensitivity in the ultraviolet but additional experiments will be essential to prove or disprove this. The rise of sensitivity into the ultraviolet could conceivably be a property of cells in the ventral ommatidia which were stimulated indirectly by light transmitted through the dorsal ommatidia. (One may exclude the reverse proposition, namely that the ultraviolet sensitivity recorded in the ventral ommatidia is a property of the dorsal ommatidia, because the sensitivity of the ventral ommatidia at 380 mμ was generally more than tenfold higher than that of the dorsal ommatidia.)

The pigmentation of the dorsal ommatidia differs from that of the ventral ommatidia. There is scarcely any pigment extending the lengths of the retinulas. These appear light yellow. The primary iris pigment surrounding the crystalline cones, however, is dense and of the same general appearance as the corresponding pigment in the ventral ommatidia.

The Compound Eyes of the Nymph

Twelve individual spectral sensitivity curves were obtained from the dark-adapted eyes of twelve animals, eight belonging to the family Libellulidae, three to the Aeschnidae, and one to the Gomphidae. In all these there was a single peak near 530 mμ and a moderately low shoulder or plateau on the short wave-length side (Fig. 8). In four of the animals, two *Anax junius*, one *Libellula sp.*, and one *Sympetrum sp.*, the spectral sensitivity curves were redetermined in the presence of constant illumination of 606 mμ. This selective light adaptation reduced the sensitivity to long wave-lengths more than to short wave-lengths, and a new maximum appeared in the region between 410 and 435 mμ. Moreover, the wave-forms of ERGs differ qualitatively depending upon the wave-length of the stimulus. ERGs from a nymph of *Anax jumius* are shown in Fig. 8. The rates of rise of the simple, cornea-negative ERGs are greater in response to long wave-lengths than to short. The differences, though relatively slight, are consistent and an observer can identify long or short wave-length responses without knowledge of the wave-length setting on the monochromator. It is concluded that two different classes of photoreceptors contribute to the responses recorded from the compound eyes of nymphs.

It must be kept in mind, however, that the presumptive tissue of the dorsal ommatidia of the adult lies adjacent to the nymphal eye (Fig. 1), and that

the dorsal ommatidia of the adult have a receptor maximally sensitive near 420 mμ. It is not yet known whether the incompletely differentiated dorsal ommatidia are light-sensitive or, if so, whether their responses can be recorded with an electrode in the nymphal eye. However, in all the measurements on the nymphal eye, all other parts of the head were masked with foil and care

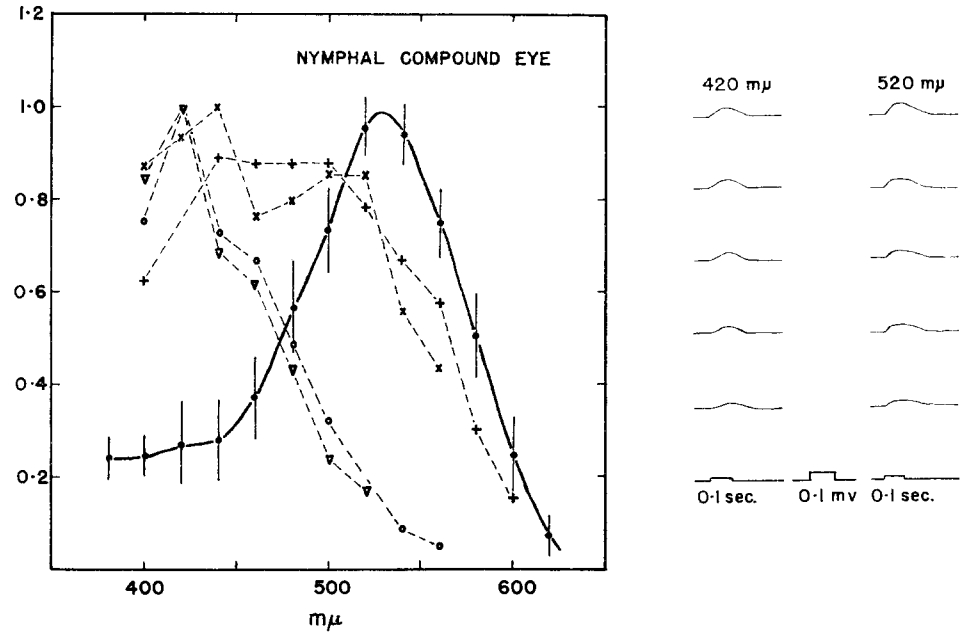

FIGURE 8. Left, spectral sensitivity of compound eye of nymphs. Solid circles, twelve runs in twelve nymphs, eight from the family Libellulidae, three from Aeschnidae, and one from Gomphidae; means and ± one standard deviation indicated; all nymphs dark-adapted. Dashed curves, spectral sensitivities of four nymphs during selective light adaptation to light of 606 mμ. These nymphs included two *Anax junius*, one *Libellula* sp., and one *Sympetrum* sp. Right, ERGs from nymph of *Anax junius*. Stimulus intensities increase stepwise in equal increments of 0.24 optical density unit from bottom to top in both series. The two series were recorded during light adaptation to 606 mμ. The rate of rise of the on-wave is greater in the 520 mμ series.

was taken that the axis of the stimulating light beam was not aligned with presumptive adult eye tissues. Specifically, the axis of the stimulating beam coincided with that of the arrow drawn above the compound eye of the nymph in Fig. 1B.

Comparisons with the Work of Mazokhin-Porshniakov

Mazokhin-Porshniakov concluded that the ventral ommatidia of the compound eye of the adult of *Libellula quadrimaculata* have dichromatic color vision

within the spectral range from about 490 to about 660 mμ. The two classes of photoreceptors responsible were concluded to have their maximum luminosities at about 515 and 610 mμ. All wave-lengths less than about 490 mμ were stated to produce identical effects on the ERG provided that stimulus intensities were adjusted appropriately. Figs. 5 and 6 of this paper indicate that the latter statement cannot be true for *Libellula luctuosa* and *Sympetrum rubicundulum*. There is a distinctive class of ultraviolet-sensitive photoreceptor in the ventral ommatidia of the adult (and also in the dorsal ocelli, which Mazokhin-Porshniakov did not study).

For the ventral ommatida peaks near 515 mμ are common to the two studies, but the shoulder near 550 mμ in the present study and the peak at 610 mμ in Mazokhin-Porshniakov's study are widely separated. Considering the differences in the species studied it is unwise to make too much of either apparent agreement or disagreement. One should recognize the possibility, however, that the two quite different methods, if applied to the same species, might produce peaks in different spectral locations. In Mazokhin-Porshniakov's colorimetric method the eye is exposed for a time to a monochromatic radiation, say 540 mμ, and then abruptly this radiation is replaced by a mixture of two radiations, say 430 plus 640 mμ. Absence of change in the ERG accompanying such a substitution signifies a colorimetric match. A characteristic feature of this method is simultaneous excitation of two or more classes of photoreceptors when such are present. It is entirely conceivable that the response of one class can suppress or inhibit the response of another class. Inhibitory interactions, if present, could lead to a displacement away from one another of peaks in curves such as those which Mazokhin-Porshniakov has presented.

Inhibitory interactions of relevance to mechanisms of color vision are known in the retinae and in the lateral geniculate nuclei of vertebrates (for summary, see MacNichol, 1964). They are not yet known in insects but may be found, if sought, in studies of single unit responses of the eye or optic ganglia or even in the ERG. The latter includes in many insect eyes components of primary receptor cell origin as well as of postsynaptic origin.

For the dorsal ommatidia of the adult compound eye, Mazokhin-Porshniakov's and the present study agree that there is a photoreceptor present which has maximum sensitivity at about 420 mμ. Mazokhin-Porshniakov concludes that it is the only photoreceptor present, whereas the data of this study suggest that a conclusion on this point had better be reserved until the spectral region between 300 and 400 mμ can be explored more adequately.

In the case of the compound eye of the nymph, Mazokhin-Porshniakov stated that only one primary monochromatic radiation presented at appropriate intensity was required to match any ERG evoked by any other mono-

chromatic radiation. The data of Fig. 8 indicate that this is not true for the nymph of *Anax junius* nor for three nymphs of the genera *Libellula* and *Sympetrum*. Mazokhin-Porshniakov reports a single peak at about 515 mμ for *Libellula quadrimaculata*. In the present study the peak at about 530 mμ represents an average position for twelve nymphs. Of these twelve the individual peak which was furthest to the left fell at 520 mμ and was derived from one specimen of *Gomphus sp.* All other peaks were grouped quite closely about 530 mμ. The peak which appears at shorter wave-lengths following selective adaptation to 606 mμ (Fig. 8) represents the activity of either a second population of photoreceptors in the nymphal compound eye, or the activity evoked by indirect stimulation of the presumptive tissue of the adult dorsal ommatidia.

ERG and Metamorphosis

Autrum and Gallwitz (1951) found that the ERG of the nymphal compound eye differs in wave-form, rate of dark adaptation, and flicker fusion frequency from the ERG of the adult compound eye. The differences were related to a progressive movement during development of the synaptic layers of the optic ganglion close to the ommatidial layer. They appear to have made the tacit assumption that the same ommatidia and the same units of the optic ganglion were involved in production of the components of nymphal and adult ERGs. This assumption clearly requires modification. Consequently, their conclusions concerning the effects on the ERG of movements of the optic ganglion may require modification.

I would like to acknowledge with gratitude the assistance of my wife and of Dr. Melvin L. Goldstein who were the observers of threshold responses.

This work was begun at Tufts University with support from NIH Grant E-947 and was continued at the University of Wisconsin with support from NSF Grant GB-127.

Received for publication, May 14, 1965.

REFERENCES

AUTRUM, H., and GALLWITZ, U., 1951, Zur Analyse der Belichtungspotentiale des Insektenauges, *Z. vergleich. Physiol.*, **33**, 407.

GOLDSMITH, T. H, 1965., Do flies have a red receptor? *J. Gen. Physiol.*, **49**, 265.

GOLDSMITH, T. H., and RUCK, P. R., 1958, The spectral sensitivities of the dorsal ocelli of cockroaches and honeybees, *J. Gen. Physiol.*, **41**, 1171.

LEW, G. T., 1933, Head characters of the Odonata with special reference to the development of the compound eye, *Entomol. America*, **14**, 41.

MACNICHOL, E. F., JR., 1964, Retinal mechanisms of color vision, *Vision Research*, **4**, 119.

MAZOKHIN-PORSHNIAKOV, G. A., 1959, Colorimetric study of vision in the dragonfly (*in Russian*), *Biofizika*, **4**, 427; (in English translation), *Biophysics*, **4** (4), 46.

OGUMA, K., 1917, A histological study on compound eyes of dragonflies, *Entomol. Mag.* (*Tokyo*), **3**, 101.

RUCK, P., 1961, Electrophysiology of the insect dorsal ocellus. I. Origin of the components of the electroretinogram, *J. Gen. Physiol.*, **44**, 605.

RUCK, P., and EDWARDS, G. A., 1964, The structure of the insect dorsal ocellus. I. General organization of the ocellus in dragonflies, *J. Morphol.*, **115**, 1.

The following reviews are recommended for providing critical commentary on knowledge of color vision in insects:

BURKHARDT, D., 1964, Color discrimination in insects, *Adv. Insect Physiol.*, **2**, 131.

GOLDSMITH, T. H., 1964, The visual system of insects, *in* The Physiology of Insecta, (M. Rockstein, editor), New York, Academic Press, Inc., **1**, 397.