Localization of the Violet and Yellow Receptor Cells in the Crayfish Retinula

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ABSTRACT Cellular identification of color receptors in crayfish compound eyes has been made by selective adaptation at 450 nm and 570 nm, wavelengths near the λ_{max}'s of the two retinular cell classes previously demonstrated. By utilizing earlier evidence, the concentration of lysosome-related bodies (LRB) was used to measure relative light adaptation and thus wavelength sensitivity in 665 retinular cells from six eyes. The observed particle distributions demonstrate the following. Both violet and yellow receptors occur ordinarily in each retinula. Of the seven regular retinular cells two (R_3 and R_4 using Eguchi's numbering [1965]) have mean sensitivities significantly greater to violet and less to yellow than the other five. The latter apparently comprise "pure" yellow receptors (R_1 and R_7) and mixed yellow and violet receptors (R_2, R_5, and R_6). Explanations of such ambiguity requiring two visual pigments in single retinular cells or intercellular coupling of adjacent neuroreceptors are apparently precluded by previous evidence. Present data imply alternatively some positional variability in the violet pair's location in individual retinulas. Thus R_3 and R_4 are predominantly the violet receptors but in some retinulas R_2 and R_3 or R_4 and R_5 (or rarely some other cell pairs) may be. The retinal distribution of such variations has yet to be determined. In agreement with intracellular recordings the blue and yellow cells here identified belong to both the vertical and horizontal *e*-vector sensitive channels.

Primary photoreceptor cells of the crayfish retina are known to be differentially sensitive both to the *e*-vector orientation of linearly polarized light and to wavelength. Two kinds of receptor units are present for each of these two visual parameters. Polarization sensitive cells are maximally responsive to one of two orthogonal *e*-vector directions (Waterman and Fernández, 1970); wavelength sensitive cells have λ_{max}'s either in the violet near 450 nm or in the yellow near 570 nm (Nosaki, 1969; Waterman and Fernández, 1970).

For *e*-vector discrimination the cellular mosaic in the retina is known for crayfish from microspectrophotometric measurements (Waterman et al., 1969) and for other decapod crustaceans from a variety of data (Shaw, 1966; Waterman and Horch, 1966; Eguchi and Waterman, 1968; Hays and Goldsmith, 1969). All seven regular retinular cells (R_1–R_7)[1] are sensitive to the direction of the polarization plane: R_1, R_4, and R_5 (using Eguchi's numbering [1965]) are maximally responsive to a horizontal *e* vector parallel to the animal's longitudinal axis. The other four R_2, R_3, R_6, and R_7 respond most strongly to a vertical *e* vector.

Because all regular retinular cells are involved in this function the two wavelength discriminating cell types must also be *e*-vector sensitive. Such full overlap of these two submodalities was demonstrated directly by intracellular recordings (Waterman and Fernández, 1970). The latter also showed that the yellow cells (λ_{max} average 594 nm) were about five times more commonly penetrated than the violet cells (λ_{max} average 440 nm). However, because no intracellular marking was done, the cellular localization of the color discriminating cells remained to be determined.

Consequently the present experiments were designed to discover this receptor mosaic in the crayfish. Particular interest centered on answering two questions. Do both violet and yellow cells occur regularly in the same retinula or are they segregated in different retinulas? Is there some systematic relationship between the *e*-vector and wavelength-discriminating components of the retina?

The cellular identification necessary to answer these questions was accomplished by the quantitative analysis of fine structural changes induced in the visual receptor cells by differential light adaptation at two appropriate wavelengths (Eguchi and Waterman, 1967). The technique is like that used previously to demonstrate the cellular basis of polarized light perception in the crab *Libinia* (Eguchi and Waterman, 1968). The present results prove that two particular cells (usually R_3 and R_4) of the seven in each ommatidium are the violet receptors, the other five have their λ_{max} at longer wavelengths and are the yellow receptors.

EXPERIMENTAL METHODS

Some initial comment on our technique of measuring relative responses (Eguchi and Waterman, 1967, 1968) may be appropriate in view of the complex and sometimes controversial nature of light adaptation (LA) and dark adaptation (DA). Previously we demonstrated quantitatively in the crab *Libinia* that several classes of organelles within the retinular cells showed statistically significant changes in their number per unit cross-sectional area with DA and LA. Their "concentrations" increased progres-

[1] Here we bypass the problem of whether R_8 in crayfish contributes to the rhabdom and is thus a primary photoreceptor (Parker, 1891; Eguchi and Waterman, 1966, 1973).

sively from their lowest to highest values in a sequence of four different adaptations tested: 17-h DA, 5-h DA, 5-h LA, and 17-h LA (Eguchi and Waterman, 1967).

From these results we reasoned that different concentrations of such cytoplasmic components arising in a controlled experiment can be interpreted as a difference in the cells' responses to light comparable in sign and relative magnitude. The coincidence of conclusions based on this working hypothesis with those derived from microspectrophotometry and intracellular recordings supports the rationale of our approach.

Response-energy curves have not yet been determined so that sensitivities cannot be quantitatively estimated (as discussed in detail by Shaw, 1969 *b*). Clearly such comparisons are highly desirable but cannot be obtained by our technique without extensive labor. Their achievement may well be stimulated by the kind of relative differences we have already measured.

The fact that our previous demonstration of quantitative correlations between adaptation and fine structural development was made in the crab *Libinia* might indicate that extrapolation to *Procambarus* in our present experiments demands justification. This we derive from the solid evidence that fine structural changes coupled with the state of adaptation like those we have documented quantitatively in *Libinia* occur in a wide range of arthropod photoreceptors (see Eakin, 1972, p. 662 ff. for review). Since such corroborative support ranges from larval mosquitoes (White, 1967, 1968) and an hemipteran (Burton and Stockhammer, 1969) to *Daphnia* (Rölich and Törö, 1965) and to spiders (Melamed and Trujillo-Cenóz, 1966), we feel justified in hypothesizing that it is valid for the much more closely related group of decapod crustaceans.

While we do not by any means claim that universal generality has been proved for this correlation of dark and light adaptation with the development of certain fine structural elements, the need for quantitative evidence in denying the presence of such a correlation is obvious. Yet in some widely quoted cases none whatever has been presented. Both in our *Libinia* data and in these *Procambarus* experiments careful measurements were essential before any conclusions of interest could be accepted or rejected.

In what follows, mature crayfishes (*Procambarus clarkii* [Girard]) collected in small ponds near Yokohama City University in Mutsuura were used as experimental animals. In preparation for adaptation, one eye of the animal was fixed in its normal position with dental cement and all appendages as well as the abdomen were secured with rubber bands to prevent their interference. Then the crayfish was clamped underwater and suspended but normally oriented in an aerated aquarium placed so that the light source would directly illuminate the central and lateral part of the retina of the immobilized eye. Black screens and diaphragms prevented stray light from reaching the eye.

After 12 h or more DA overnight the eye was illuminated for 6 h with monochromatic light of moderate intensity (2.1×10^7 quanta s^{-1} m^{-2}). This gave a light meter reading of about 2 lx which was estimated to be equivalent to the more discriminatory of the two intensity levels used previously for polarized white light in the *Libinia* experiments (Eguchi and Waterman, 1968). The quantal flux was measured with a photodiode calibrated against a standard lamp (Tomita et al., 1967).

The light source employed was a high pressure mercury arc lamp controlled with neutral filters and narrow band interference filters. Two Toshiba monochromatic filters with half band widths of 25 nm were used for adaptation, one centered at 450 nm, the other at 570 nm to correspond with the maxima of the previously determined spectral sensitivity curves for the two retinular cell types (Fig. 1). In addition UV and IR cut-off filters were placed between the source and the other filters.

Immediately after LA the affected compound eye was cut off and fixed for 2 h in 5% glutaraldehyde buffered at pH 7.4 by 0.1 M Sorenson's phosphate solution. Briefly washed in buffer the tissue was then postfixed for another 2 h in 1.5% osmium tetroxide similarly buffered at pH 7.4. Fixation was followed by dehydration through

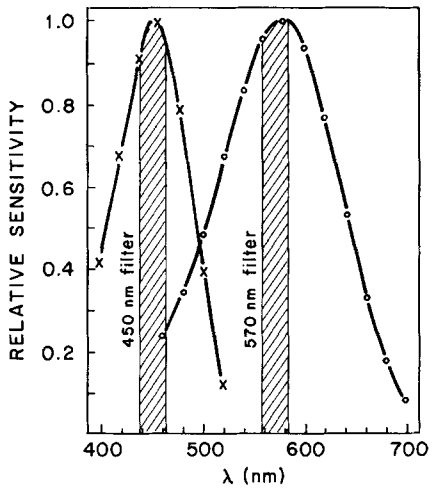

FIGURE 1. Strategy of selective adaptation used on the *Procambarus* retina. The relative spectral sensitivities of typical violet and yellow sensitive retinular cells (redrawn from Waterman and Fernández, 1970) are compared with the two narrow wavelength bands used for the adapting light.

an acetone series, transfer to propylene oxide, and embedding in epoxy resin. Sections were cut with a Porter-Blum MT-2 microtome (Ivan Sorvall, Inc., Newtown, Conn.) and stained with uranyl acetate and lead citrate. A JEM-100B electron microscope (JEOL USA, Medford, Mass.) was used to study the resulting preparations.

Cross sections were made of the proximal part of the retinular cell layer of the compound eye. Here the seven regular retinular cells could be readily identified by their relations to cell 1 as well as the four proximal cone processes and the directions of the cells' rhabdomere microvilli (Figs. 2 and 3).

The proximal region was chosen for study for several reasons. First the amount of retinular cell cytoplasm is small in the central and distal rhabdom region (Fig. 2 A). Hence a larger number of sections would be required there to characterize a given cell's cytoplasmic organelles. Second the retinula has a greater diameter more distally so that a whole retinular cross section could not conveniently be studied in one electron microscope plate. Finally the four crystalline cone processes are more

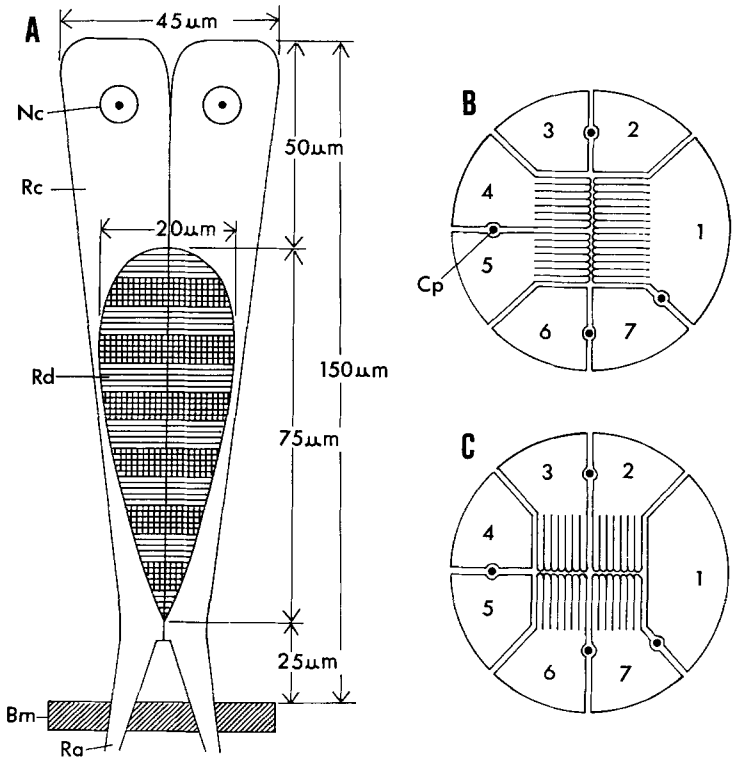

FIGURE 2. Diagram showing structural relations of the seven regular retinular cells (R_1–R_7) in a crayfish ommatidium. (*a*) Longitudinal section through the optical axis. The light enters from above via the crystalline cone stalk (not shown). Note that the actual number of alternating layers of orthogonal microvilli in the *Procambarus* rhabdom is about double those diagrammed. Typical dimensions for adult specimens are shown. (*b*) and (*c*) Cross sections through adjacent layers of orthogonal microvilli. In the central retina at least those from R_1, R_4, and R_5 are horizontally oriented while those in R_2, R_3, R_6, and R_7 are vertical relative to the animal's normal posture. Note that the double size of the rhabdomere of R_1 and the positions of two proximal processes of the crystalline cone stalk on either side of R_7 make unambiguous identification of the seven cells possible. The retinular cell numbering is that given by Eguchi (1965). Bm, basement membrane; Cp, proximal process of crystalline cone stalk; Nc, nucleus of retinular cell; Ra, retinular cell axon; Rc, retinular cell body; Rd, rhabdom.

evident in proximal sections which makes retinular cell identification more certain (Fig. 3).

In each cell the number of multivesicular bodies, lamellar bodies, and mixed vesicular lamellar bodies were counted per cytoplasmic area examined (Fig. 4). On the basis of previous evidence (Eguchi and Waterman, 1967, 1968; White, 1967, 1968) and general knowledge we will refer to these three types of particles collectively as lysosome-related bodies (LRB) until their functional nature is directly demonstrated.

As we have previously shown and discussed in some detail above the number of

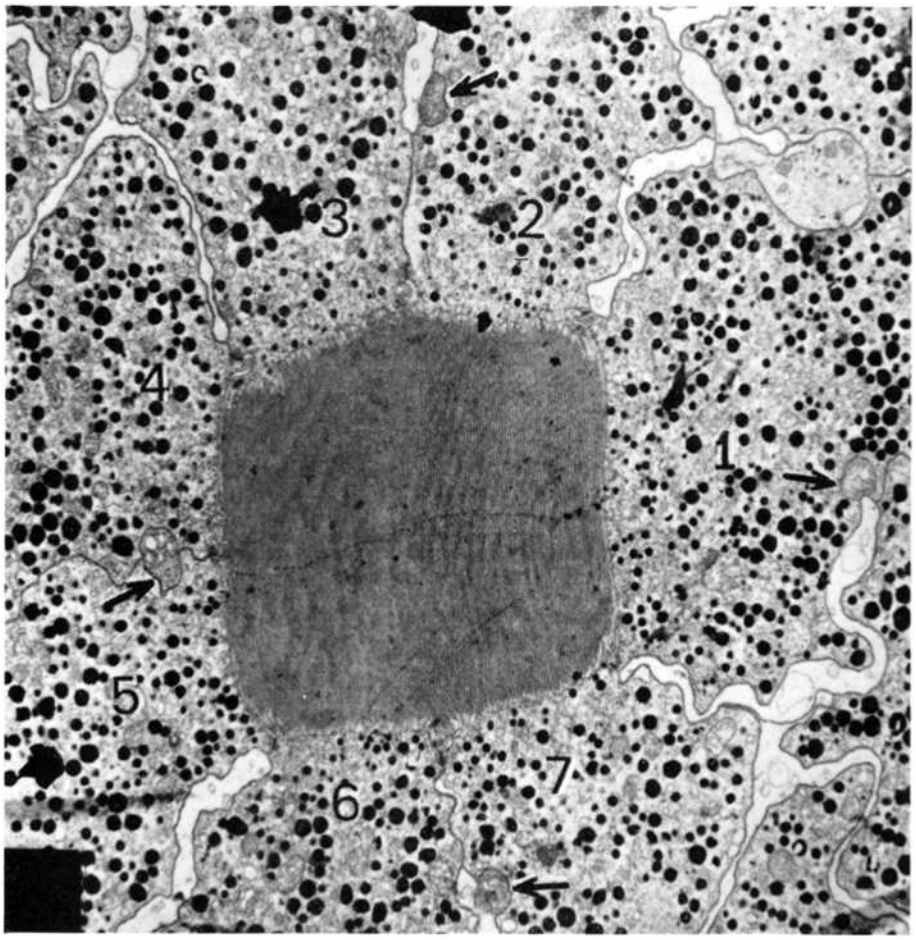

FIGURE 3. Electron micrograph of a crayfish retinula seen in a cross section like that diagrammed in Fig. 2 C. R_1-R_7 are numbered and the locations of the four proximal processes of the crystalline cone stalk are shown by arrows. × 6,200.

such intracellular organelles (as well as several other types not studied in the present work) increases significantly on LA. Therefore differential responses to the two wavelengths tested should appear as significantly different concentrations of LRB in the cells concerned. Analytical methods are described below along with the relevant data.

Some additional comment on technique may be desirable here in view of another recently reported and potentially important method of discriminating spectral sensitivity of retinular cells (Gribakin, 1972). As applied by him to the compound eye of the honeybee this involved selective staining of rhabdomeres induced by long wavelength illumination ($\lambda > 480$ nm) of the retinal tissue during the second, osmic acid stage of fixation. Gribakin's technique promised such an attractive short cut to our obviously laborious procedure that we immediately tested it on crayfish eyes even though our experiments had been completed and their analysis was already well advanced.

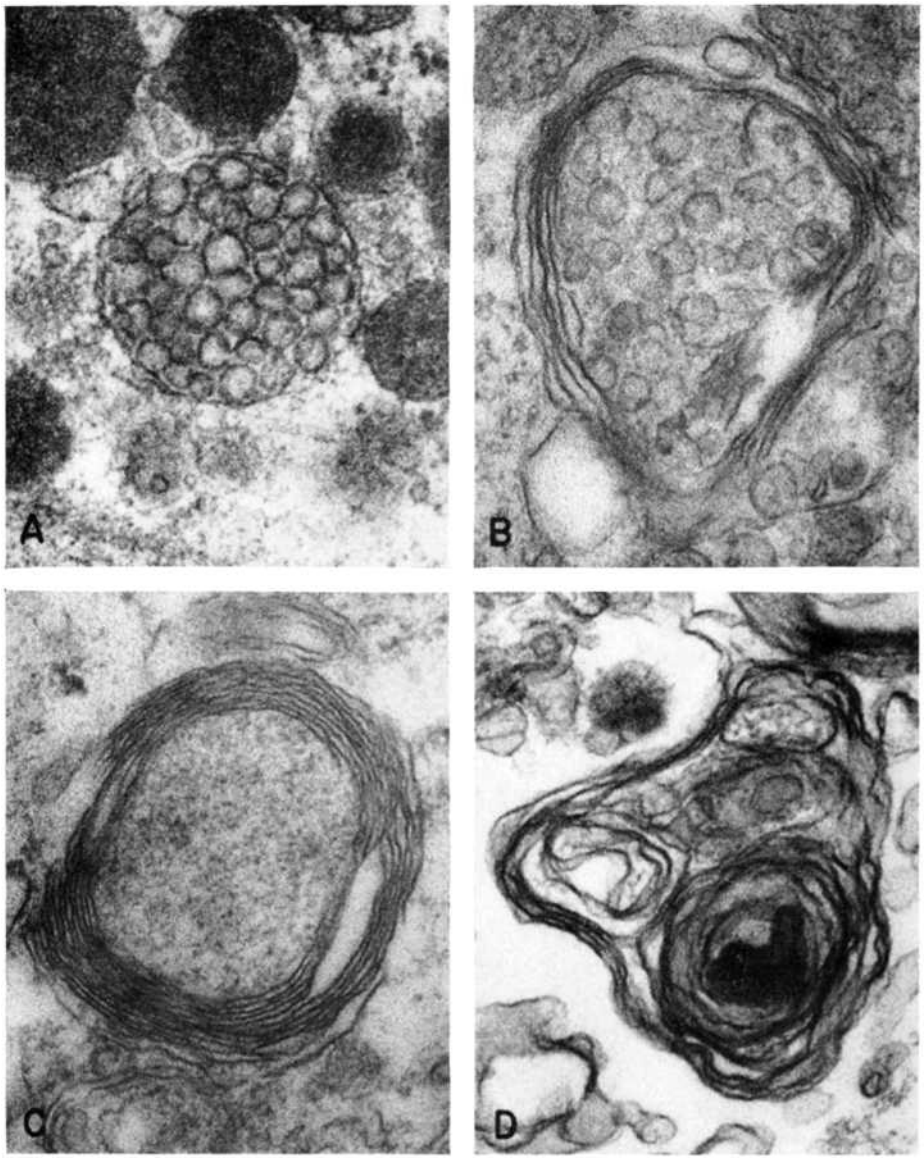

FIGURE 4. Typical LRB occurring in crayfish retinular cells (A-C). Their numbers per unit cross section of cytoplasm decrease with dark adaptation and increase with light adaptation. (*a*) Multivesicular body. (*b*) Mixed lamellar and multivesicular body. (*c*) Lamellar body. (*d*) Lamellar body present outside the retinular cells. × 20,000.

Unfortunately no differential results were obtained. However, the quality of our tissue fixation and embedding was not good. Hence we do not consider these initial efforts definitive; further work is required. Note, however, that even in the honeybee this selective staining technique may be ineffective in other hands than Gribakin's (Grundler, 1973).

Note also that our current method for measuring selective adaptation was not chosen *because* it requires extensive quantitative analysis. Originally a careful search was made in decapod crustacean eyes for a simple, directly identifiable indicator of adaptation like that reported by Gribakin (Eguchi and Waterman, 1967). We did not, however, find any qualitative, or indeed any readily identifiable quantitative changes in rhabdomeres with adaptation and hence fell back on the strong quantitative correlation we demonstrated for a number of the cytoplasmic particle types.

Pending the resolution of technical problems with the newer procedure, publication of the considerable body of data already obtained by our method seems appropriate.

RESULTS

A series of six adaptations were carried out on individual crayfish, three at 450 nm and three at 570 nm (Fig. 1). From the directly illuminated central retinal area of these eyes 95 retinulas have been quantitatively studied. Consequently the total data consist of lysosome-like body counts and areal measurements of the corresponding cytoplasmic cross sections for the 665 regular retinular cells involved (Table I). Of these LRB counts multivesicular bodies comprise 78% of the elements studied, mixed vesicular lamellated bodies 19% and lamellated bodies 3% (Fig. 4). Because the counts were generally too low to provide significant distributional data for the less frequent LRB types only the totals for all three have been analyzed below.

Of the 665 retinular cells studied LRB's were absent in more than half of them (361). This evidence for a low response level is not randomly distributed among the cell population. For the three violet-adapted eyes the numbers of zero counts are conspicuously smaller for R_3 and R_4 than in the other five retinular cells. In fact the probability estimated from χ^2 that these two classes are drawn from the same population is $<0.5\%$. This relation is reversed in the yellow-adapted eyes where the same two cells have considerably more zero counts than R_1, R_2, R_5, R_6, and R_7. The probability that the actual distribution of LRB's is the same in the two groups is $<1\%$.

Thus the seven regular retinular cells fall into two classes differing in their sensitivities to violet and yellow light. Quantitative testing of this hypothesis requires that the raw counts be adjusted for the unequal cytoplasmic areas surveyed in the various retinular cells. When this is done for the full 95×7 table of counts, the rank order of the seven retinular cells can be examined for each of the 95 retinulas.

In general these reinforce the impression gained from initial analysis of the zero count distributions just cited. However, testing with Kendall's coefficient of concordance (Siegel, 1956) indicates that these rankings are not in sufficient agreement to be individually reliable for each retinula. Clearly the many ties arising from numerous zero count entries are at least partly responsible for

TABLE I

BASIC DATA

RATIOS OF LRB COUNTS TO CYTOPLASMIC AREAS SURVEYED (μm^2)

		Counts		Retinular cells							
Adapt. λ	Crayfish	Retinulas	Cells	1	2	3	4	5	6	7	Row totals
V	1	14	98	3/694	8/579	16/553	15/579	5/510	7/580	1/518	55/4013
V	2	11	77	1/644	5/499	8/559	8/538	5/523	1/535	1/467	29/3765
V	3	15	105	9/582	11/410	21/583	21/480	12/458	12/535	3/447	89/3495
(450 nm)	Σ 1–3	40	280	13/1920	24/1488	45/1695	44/1597	22/1491	20/1650	5/1432	173/11273
Y	4	20	140	29/1642	29/1228	9/1067	10/1333	19/1301	23/1123	23/1228	142/8922
Y	5	10	70	16/725	8/555	6/589	5/598	6/545	8/732	12/599	61/4343
Y	6	25	175	23/1044	19/655	8/631	10/608	12/659	14/763	14/583	100/4943
(570 nm)	Σ 4–6	55	385	68/3411	56/2438	23/2287	25/2539	37/2505	45/2618	49/2410	303/18208
V+Y	Σ 1–6	95	665	81/5331	80/3926	68/3982	69/4136	59/3996	65/4268	54/3842	476/29481

this as are the low numbers of counts in the remaining retinular cells (average only 1.56/cell, maximum 8/cell).

However, if the rank orders are pooled by animals some firm conclusions may be reached from the two resulting 3 × 7 tables for the violet-adapted and yellow-adapted eyes (Table II). To begin with R_3 and R_4 in all three violet adapted eyes rank either 1 or 2 in the LRB concentrations they contain. In contrast R_3 and R_4 in all three yellow-adapted eyes have the least LRB's per unit area and hence rank sixth or seventh among the regular retinular cells.

The degree of correlation shown between the rankings provided by the three eyes in each of the two conditions (tested by the method of Kendall cited above) shows that the probability that they are not correlated is less

TABLE II

RETINULAR CELL RANK ORDERS (BASED ON MEAN ADJUSTED LRB COUNTS PER CELL BY ANIMALS; 1 RANKS THE MOST)

	Cells						
Animals	1	2	3	4	5	6	7
Violet-adapted							
1	6	3	1	2	5	4	7
2	6.5	4	1	2	3	6.5	5
3	6	4	2	1	5	3	7
Σ 1–3	18.5	11	4	5	13	13.5	19
Yellow-adapted							
4	4	2	6	7	5	1	3
5	2	3	6	7	4.5	4.5	1
6	3	1	7	6	5	4	2
Σ 4–6	9	6	19	20	14.5	9.5	6

than 1%. Such good agreement between replications indicates that a more detailed analysis of LRB density distributions should be profitable. Since the data are counts and their variability is not greater than that expected for a Poisson distribution, χ^2 methods are appropriate for this study. A number of null hypotheses have been tested by pooling the counts and adjusting them appropriately for the cytoplasmic areas involved.

If a 3 (eyes) × 7 (retinular cells) table of counts adjusted for mean areas is considered for each adapting wavelength (Table III) the following conclusions may be reached. When the LRB densities for the seven retinular cells are compared within each row of the table the χ^2 values differ strongly ($P < 0.5\%$) from random. Hence the various retinular cells were adapting differentially.

Testing counts for the cells against the row means proves that in R_3 and R_4 significant differences of opposite sign occur in the two series. These show that R_3 and R_4 are more sensitive to violet light and less sensitive to yellow than

TABLE III

ADJUSTED COUNTS FOR LRB

Adapt. λ	Crayfish	Counts: Retinulas	Counts: Cells	Retinular cells: 1	2	3	4	5	6	7	Row totals (mean counts/area)
Adjusted within rows											
V	1–3	40	280	10.90	25.97	42.75	44.37	23.76	19.52	5.67	24.71/1610.43
Y	4–6	55	385	51.86	59.74	26.16	25.61	38.42	44.71	52.38	43.28/2601.14
V+Y	1–6	95	665	63.99	85.81	71.92	70.26	62.18	64.14	59.19	68.0/4211.57
Adjusted between rows											Row totals (mean areas)
V	1–3	40	280	14.25	33.96	55.90	58.02	31.07	25.52	7.41	1610.43
Y	4–6	55	385	41.48	48.36	21.18	20.73	31.10	36.20	42.40	2601.14
V−Y	1–6	95	665	−27.23	−14.4	34.72	37.29	−0.03	−10.68	−34.99	2105.78

expected from the means of all seven retinular cells. In addition R_1, and R_7 have significantly fewer LRB's than expected in the violet adapted eyes. Therefore R_1 and R_7 are on the average less sensitive to violet light than the other five cells.

If the pooled counts of both series are analyzed the row χ^2 does not indicate rejection of the null hypothesis that R_1–R_7 all give the same response in this tally. Similarly χ^2's for the individual cells against the row mean show that none differ significantly. This proves that there is no evidence of differential adaptation between retinular cells in an ommatidium aside from that which is wavelength dependent. These various results are graphed as percentages in Fig. 5 A, B, C.

If a comparison is made between the adjusted counts for the two wavelength series two further points are demonstrated (Table III). First there is no significant difference between the mean LRB densities in the violet-

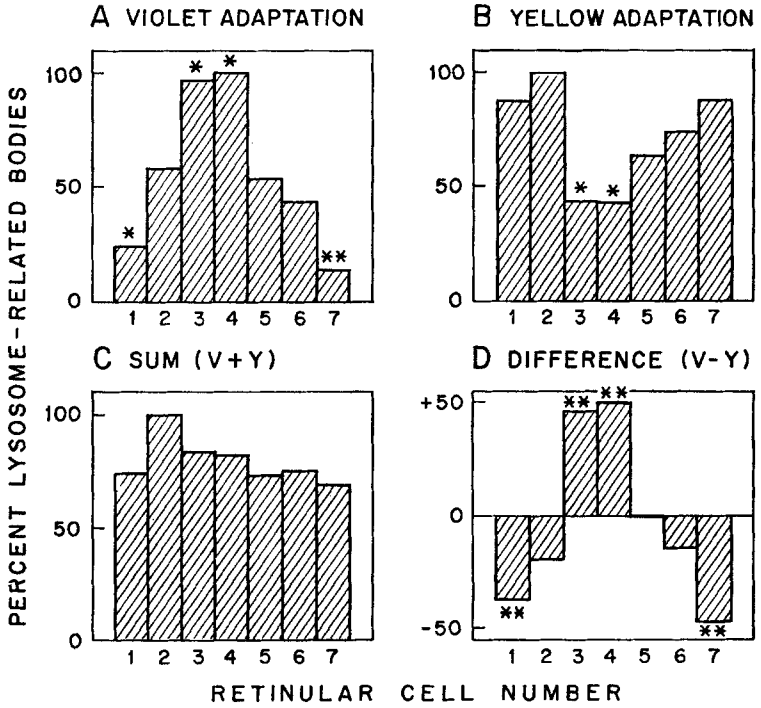

FIGURE 5. Relative distribution (percent) of LRB in the seven regular retinular cells of the crayfish retinula. (*a*) After 6 h adaptation at 450 nm. Mean data for 40 retinulas from three animals. (*b*) After 6 h adaptation at 570 nm. Mean data for 55 retinulas from three additional animals. (*c*) The sum of the distributions in A and B. (*d*) The difference between the distributions in A and B. Significant variations from the retinular mean estimated from χ^2's derived from the adjusted mean counts are indicated by asterisks: $*P < 0.05$, $**P < 0.01$. The original counts, cytoplasmic areas and relevant adjusted means are given in Tables I–III.

adapted and yellow-adapted eyes. Secondly instead of the previously emphasized two there are three relations shown between the different cells of the retinulas in the two series (Fig. 5 D). R_2, R_5, and R_6 were as sensitive to violet as they were to yellow light. R_3 and R_4 were significantly more sensitive to violet than to yellow light ($P < 0.5\%$) and R_1 and R_7 were significantly less sensitive to violet light than to yellow ($P < 0.5\%$).

In interpreting these relations we must recall that they are derived from pooled counts for 10–25 retinulas from each of the six eyes examined. As described above these totals demonstrate certain statistically significant differences in the cells' spectral sensitivities. However the LRB counts and the differences between them for the two test conditions are not great enough for the measurements for each of the 95 ommatidia involved to show statistically significant differences.

DISCUSSION

Using LRB densities as a measure of light adaptation and LA as a measure of wavelength sensitivity we may draw the following conclusions. Retinular cells 3 and 4 are violet receptor cells and must, therefore, correspond with the class of cells previously shown by intracellular recordings in the same crayfish species to have its λ_{max} around 470 nm (Nosaki, 1969; Waterman and Fernández, 1970). The remaining cells (R_1, R_2, R_5, R_6, and R_7 are yellow receptors.

Our present evidence for two major color receptor types is congruent not only with the data from intracellular recordings but also with those previously obtained from selective adaptation of the electroretinogram (ERG) (Goldsmith and Fernández, 1968; Wald, 1968). Wavelength sensitivities of higher order sustaining fibers in the optic nerve also fit in well with the above conclusions (Treviño and Larimer, 1970; Woodcock and Goldsmith, 1970).

In addition the data reported here show that the yellow receptors apparently fall into two subtypes, one comprising pure yellow receptors (R_1 and R_7) and the other yellow receptors also sensitive to violet (R_2, R_5, and R_6). However, analysis indicates that this subdivision most likely arises from some variability in violet cell localization from one retinula to another.

Abstractly there are several possible explanations for these three apparently broad band receptor units. For instance we might hypothesize that the cells involved contain both of the visual pigments which must be present separately in the violet cells and the pure yellow cells. There is precedent for such a "pigment mixture" interpretation in a number of insects (reviewed by Goldsmith, 1972). Against this possibility is the fact that intracellular recordings in *Procambarus* usually do not demonstrate such doubly sensitive cells. Of all units penetrated >95% gave relatively narrow band sensitivities indicative of a single photosensitive pigment (Waterman and Fernández, 1970).

The few cases (<5%) observed with double peaks were transient and vari-

able (Waterman and Fernández, 1970, p. 163) so that their explanation most likely depended on temporary accidents of electrode tip location. Actually if three out of seven retinular cells contain mixtures of two pigments more than 40% of randomly penetrated retinular cells should yield double peaks. This has certainly not been the case (Nosaki, 1969; Waterman and Fernández, 1970).

Another possible explanation suggested particularly by the LRB concentrations in violet-adapted retinulas (Fig. 5 A) is that R_2, R_5, and R_6 contain the λ_{max} 570-nm pigment but are sufficiently strongly coupled electrically (Shaw, 1969 *a*; review by Fuortes and O'Bryan, 1972, p. 293) to R_3 or R_4 (containing the 470-nm λ_{max} pigment) that mixed sensitivity would ensue. But again this seems unlikely since such hypothesized coupling should yield corresponding mixed wavelength sensitivity responses in intracellular recordings.

In addition strong differential sensitivity to *e*-vector direction is shown by both violet and yellow cells in *Procambarus* (Waterman and Fernández, 1970). This would be impossible if significant coupling between adjacent cells included pairs with orthogonal microvillus directions (Shaw, 1969 *a*). Also for intercellular electrical coupling to have the required effect some further untested assumptions would have to be made concerning the way in which LA results in increased densities of LRB. However, the possibility of coupling cannot be completely dismissed without further study. Thus the time scale on which interactions might have occurred in our experiments (6 h) is much greater than that on which intracellular responses are measured (usually less than 1 s). Whether this might have some effect is not yet known.

A third and most likely explanation of the apparent double sensitivity of R_2, R_5, and R_6 could depend on variability of the cellular pattern from one retinula to another. This sort of thing has been reported with regard to the numbers of yellow-green and blue receptors in the ommatidia of honeybee workers (Gribakin, 1972).[2] Thus if the predominant location of the violet receptors in *Procambarus* is in cells R_3 and R_4, but if in some retinulas R_2 and R_3, R_4 and R_5, or R_6 are instead occasional violet receptor pairs, our results could be accounted for. Of course such variation could either be random or dependent on some pattern (e.g., Kunze, 1968; Bohn and Täuber, 1971; Trujillo-Cenóz and Bernard, 1972). Our data cannot discriminate this point.

Good evidence for distributed variability in retinular cell patterns in the crayfish retina can be derived from our data for the three eyes adapted at 470 nm. The relevant information is the rank order of the seven retinular cells' sensitivities to violet light. Of the 41 retinulas studied 8 are uninformative since 6 or 7 of their cells had zero LRB counts. Study of the rank orders of the remaining 33 supports the following analysis.

Our pooled data (Fig. 5 A) show that on an average there are two retinular

[2] See above under Experimental Methods for comments on the technique used by Gribakin.

cells in each ommatidium which are significantly more sensitive to violet light than the other five R's. Again on the average these cells are specifically R_3 and R_4. But the variability in violet sensitivity indicate by Fig. 5 D shows that other retinular cells significantly but less frequently are also short wavelength receptors. In these less common retinular patterns either one or both 470-nm units could be shifted from the regular locations. Also the shifts could be made by one or both members of the usual pair. As a result the new pair could be either contiguous or disjunct.

Pair analysis of seven sectors of a circular cross section shows that there are 21 possible in all (Table IV). Seven of these are contiguous pairs and 14 are disjunct pairs. Consequently in the 33 rank orders in our data we could expect 11 contiguous pairs and 22 disjunct if the pairs were randomly distributed.

Examination of the violet-adapted retinulas shows that the ratio of contiguous pairs most sensitive to 470 nm to disjunct pairs of the same sort is 20:13, quite distinct from random ($\chi^2 = 11.04$, 1 df). Thus we may conclude that the two violet cells occur as contiguous pairs whether they are R_3 and R_4 or any of the other five possibilities. This is consonant with the following ex-

TABLE IV
PAIR ANALYSIS

	Total		Contiguous		Disjunct	
Cell pairs	no.	Counts*	no.	Counts*	no.	Counts*
1, 2	1	0	1	0	—	
1, 3	2	2	—		1	2
1, 4	3	0	—		2	0
1, 5	4	0	—		3	0
1, 6	5	1	—		4	1
1, 7	6	0	2	0	—	
2, 3	7	6	3	6	—	
2, 4	8	0	—		5	0
2, 5	9	1	—		6	1
2, 6	10	2	—		7	2
2, 7	11	0	—		8	0
3, 4	12	9	4	9	—	
3, 5	13	2	—		9	2
3, 6	14	3	—		10	3
3, 7	15	0	—		11	0
4, 5	16	5	5	5	—	
4, 6	17	2	—		12	2
4, 7	18	0	—		13	0
5, 6	19	0	6	0	—	
5, 7	20	0	—		14	0
6, 7	21	0	7	0	—	
Σ	21	33	7	20	14	13

* Counts = number of times a given pair ranked 1, 2 in violet sensitivity for its retinula.

perience during intracellular recording. Violet cells were penetrated significantly less frequently than the cells with a yellow λ_{max} but when one violet unit was found another was not uncommonly entered nearby (Waterman and Fernández, 1970, 162).

If we examine the distributions of contiguous pairs in the 33 retinulas only three of the seven possible locations occur: R_2, R_3; R_3, R_4; and R_4, R_5 (Table IV). As we would expect from the pooled counts (Fig. 5 A) R_3 and R_4 rank most frequently (9 out of 20) as the first pair in violet sensitivity. No first ranking pairs occurred at locations R_1, R_2; R_1, R_7; R_5, R_6; or R_6, R_7. If we compare the observed (20:0) with expected (8.57:11.43) ratios of contiguous pairs 3, 4, and 5 with contiguous pairs 1, 2, 6, and 7 (Table IV) we can conclude that the observed distribution represents a highly significant difference from random (χ^2 = 26.67, 1 df). These analyses clearly confirm that although the two short wavelength receptors are typically R_3 and R_4 there are a significant number of cases where they are in the alternative locations of R_2, R_3 and R_4, R_5. The distribution of the relatively few first ranking disjunct pairs is random (Table IV).

Previous intracellular recordings yielded lower percentages of violet receptors than our present selective adaptation data. The latter indicate as we have seen two cells per retinular (28% of the receptor retinular cells). Thus Nosaki (1969) reported 10% violet cells which would average less than one per retinula. But Waterman and Fernández (1970) found 19% violet cells along with evidence that this figure might be lower than the actual fraction present.

The present localization of the wavelength sensitive cells in the crayfish retinula is interesting in that it clearly excludes R_1 as a violet receptor. This had previously been suggested to be *the* short wavelength unit because R_1 is special in having a rhabdomere about twice the size of R_2-R_7 and because the fraction of violet receptors seemed from the earlier data to be 10–19% (Waterman and Fernández, 1970, p. 170). Note also that the identification of R_1 and R_7 as pure yellow receptors "centers" this color channel in a diagonal position vis-à-vis R_3 and R_4 as the violet receptors. With regard to polarized light R_3 and R_7 belong to the channel most sensitive to vertical *e* vector and R_1 and R_4 to the horizontal one.

This relation between the sensory channels mediating *e*-vector directional sensitivity and wavelength sensitivity is a matter of some interest (Waterman, 1966; Horridge, 1967). Intracellular recordings showed that both violet and yellow retinular cells are *e*-vector discriminating. Different cells in both channels showed maximum responses to either vertical or horizontal *e*-vector directions relative to the normal spatial position of the animal (Waterman and Fernández, 1970). The present color cell localization is consistent with these facts (Fig. 6).

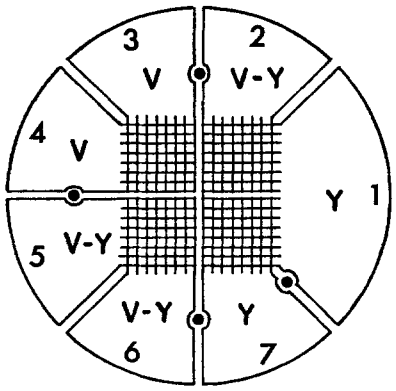

FIGURE 6. Diagrammatic summary of the relations between *e*-vector and wavelength sensitivites in the *Procambarus* retinula. There are two violet receptor cells (usually R_3 and R_4 but sometimes R_2 and R_3 or R_4 and R_5) and five yellow receptor cells (R_1 and R_7 always belong in this category). R_2, R_5, and R_6 statistically appear to have mixed violet and yellow sensitivity but the available evidence shows this must be due to some variation in violet cell location in different retinulas. Note that R_3 and R_4 as well as pure yellow receptors R_1 and R_7 occur in both the horizontal and vertical *e*-vector channels.

According to the theoretical analysis of Snyder (1973) the high degree of polarized light sensitivity shown by compound eyes like those of *Procambarus* (Waterman and Fernández, 1970) depends on two major factors. One is the presence of alternating layers of orthogonal microvilli which make up the rhabdom; the other is the proviso that wavelength sensitivity is the same for the pairs of cells with alternating rhabdomeric layers.

The predominent cellular pattern here reported should fulfill these optimal requirements. The alternating orthogonal microvilli of R_3 and R_4 with λ_{max} in the violet as well as those with λ_{max} in the yellow for the pairs R_5–R_6, one-half of R_1–R_2 and one-half of R_1–R_7 provide four paired elements of the right sort. At least at the primary receptor level absolute sensitivity, *e*-vector discrimination and wavelength discrimination all appear to be well provided for in a single ommatidium.

Clearly from the point of view of visual information processing, detailed retinal patterns must be further worked out. In addition both optical pathways upstream from the retina as well as neural pathways downstream need major attention. Thus the specific dioptrics of the crayfish eye have yet to be carefully studied. The visual fields of individual retinular cells and the extent to which they are shared within and between ommatidia are obviously important (Shaw, 1969 *c*).

This is generally true of eyes like *Procambarus*' which Exner called of superposition type (1891); the optics of such photoreceptors have been the subject

of considerable recent controversy (e.g., Horridge, et al., 1972; Kunze, 1972). Clearly the relations between the optical stimulus pattern and the receptor cell mosaic are crucial.

More centrally the wiring diagram of the afferent pathway and its functional significance also must be worked out in detail. Only a beginning has been made in the connectivity analysis (Rutherford and Horridge, 1965; Hámori and Horridge, 1966 *a*, *b*).

We know already from the far more thoroughly studied compound eye of dipteran insects that convergence of afferent axons can obliterate sensitivity in second-order neurons which is clearly present in the primary photoreceptors (e.g., differential responses to *e*-vector direction [Scholes, 1969; McCann and Arnett, 1972]). In this case even the primary cell's axon can lose the discrimination present in the soma of the retinular cell (Smola and Gemperlein, 1972).

Conversely appropriately organized neural processing systems can circumvent the deleterious effects of a low resolution optical system (Reichardt, 1965; Trujillo-Cenóz, 1965; Kirschfeld, 1967, 1973).

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