Invertebrate Color Vision and the Tuned-Receptor Paradigm

Recordings from invertebrate photoreceptors indicate that they are often sensitive to broad spectral bands.

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Newton's *Opticks*, published in 1704 and based upon work done in 1666, concludes with a series of queries or speculations for future investigation (*1*). Implicit in these speculations is a very modern notion about the nature of color vision. Consider queries 12 and 13:

> Do not the Rays of Light in falling upon the bottom of the Eye excite Vibrations in the *Tunica Retina*? Which Vibrations, being propagated along the solid Fibres of the optick Nerves into the Brain, cause the Sense of seeing. (Query 12)
>
> Do not several sorts of Rays make Vibrations of several bignesses, which according to their bignesses excite Sensations of several Colours, much after the manner that the Vibrations of the Air, according to their several bignesses excite Sensations of several Sounds? And particularly do not the most refrangible Rays excite the shortest Vibrations for making a Sensation of deep violet, the least refrangible the largest for making a Sensation of deep red, and the several intermediate sorts of Rays, Vibrations of several intermediate bignesses to make Sensations of the several intermediate Colours? (Query 13)

In these comments, Newton implicitly assumed that colors are seen because the organism selectively responds to (that is, the organism is tuned to) different portions of the visible spectrum. What remained to be determined was the precise nature of the response and its difference in quality from the stimulus.

That the implications of Newton's work were readily apparent can be seen in the "Principles of Vision" taken as postulates by Palmer in his *Theory of Colours and Vision*, published in 1777 (*2*):

> The superficies of the retina is compounded of particles of three different kinds, analagous [sic] to the three rays of light; and each of these particles is moved by his own ray. (Principle 1)
>
> These particles may be moved by the rays which are not analagous [sic] to them, when the intenseness of these rays exceeds their proportion. (Principle 6)

Palmer's principles precede by 25 years the equally explicit but better known statement of Thomas Young in 1802 (*3*):

> Now, as it is almost impossible to conceive each sensitive point of the retina to contain an infinite number of particles, each capable of vibrating in perfect unison with every possible undulation, it becomes necessary to suppose the number limited, . . . and that each of the particles is capable of being in motion less or more forcibly, by undulations differing less or more from a perfect unison . . . .

In this celebrated statement, Young was incorporating well-known and already ancient ideas about color vision into his novel and then controversial theory of light. These notions about the tuning mechanisms of color vision were refined and elaborated during the remainder of the 19th century, most notably by Maxwell and Helmholtz. They form the core of modern thinking about color vision as well as providing the basis of color photography and color television. A representative contemporary statement of this notion was given by MacNichol in 1964 (*4*):

> Investigations completed during the past two years have established that color vision in *vertebrates* [italics mine] is mediated by three light sensitive pigments segregated in three different kinds of receptor cells in the retina, and that one of these pigments is primarily responsible for sensing blue light, one for sensing green, and one for red. These findings solve one of the central problems of color vision: the nature of the primary receptors that discriminate light of various wave lengths.

Modern statements of this notion generally identify Palmer's and Young's "particles" as "light sensitive pigments." This identification involves a concept which is related to but distinct from the tuning notion. Another related concept is that more than one receptor is needed to subserve color vision. Information about color is not extracted from the response of one receptor but by comparing the relative responses of receptors which differ in their sensitivities to different spectral stimuli. As will be seen below, this latter concept is also distinct from the tuning notion.

Existence of a Paradigm

This brief recapitulation of the development of the tuning notion indicates that we are dealing with a scientific paradigm, in Kuhn's restricted use of the term (*5*). Kuhn's restricted definition of a paradigm is that it is a set of beliefs shared by members of a scientific community; the tuned-receptor concept has been so shared by workers in color vision since the time of Young. This paradigm is therefore one of the most ancient paradigms in contemporary science. Moreover, the underlying concepts were generalized to all sensory systems in the form of Müller's Doctrine of Specific Nerve Energies, which was developed by Müller and others during the 19th century (*6*).

Direct investigations of the characteristics of photoreceptors were technically impossible until recently. The tuned-receptor paradigm derived its support from indirect lines of analysis. During the last decade, however, advances in technique have made it possible to examine this paradigm directly, at the level of single photoreceptors. A substantial number of investigations have been conducted by numerous workers on the visual systems of a variety of species. The conclusion appears to be that the tuned-receptor paradigm is not universally valid: While vertebrate photoreceptors may be appropriately described by the paradigm, many invertebrate photoreceptors yield results that are not in accord with the paradigm. These results are not widely known outside the field of invertebrate color vision; the basic findings have been discussed within the field, but the implications of

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these findings have not been elaborated. My purpose in this article is to summarize the contraparadigmatic findings yielded by recent research on invertebrate photoreceptors. Such contraparadigmatic findings are not unique to the area of color vision. Workers in the area of gustation, for example, are generally agreed that the notion of receptors specifically tuned to the various aspects of taste (for example, sweet, sour) is not universally valid. Evidence on this point has been obtained from both vertebrate (*7*) and invertebrate (*8*) gustatory receptors.

General Form of the Results

The evidence to be considered here derives from studies of the spectral sensitivity of single photoreceptors obtained by the use of microelectrodes deployed so as to record the electrical activity of a single cell in response to spectral stimuli. It is useful to consider briefly the general form of the expectations that the tuned-receptor paradigm would provide before considering specific experiments in detail. Figure 1A illustrates the spectral sensitivity of a single receptor that would be consistent with the tuned-receptor paradigm. Such a receptor is maximally sensitive to a given wavelength and progressively less sensitive to other wavelengths. As already noted, color vision requires the presence of more than one receptor, each with its own peak wavelength; Fig. 1A illustrates the spectral sensitivity of a single such receptor. What is not consistent with the paradigm is the spectral sensitivity indicated by the curve in Fig. 1B. This contraparadigmatic receptor is more or less equally sensitive to a broad region of the spectrum; usually there are two small peaks and a shallow depression in between. For ease of discussion I use the term "alpha receptors" to describe tuned receptors and the term "beta receptors" to describe broad nontuned receptors (*9*). The spectral sensitivity of both types of receptors can be related to the absorption spectrum of the resident light sensitive pigments of the receptor. An alpha cell would be related to a single pigment; a beta cell might be related to two pigments. This question will be discussed again below. At this point, however, it is important to note that in this article I am concerned primarily with the form of receptor spectral sensitivities; current ideas about the underlying biochemical mechanism are compatible with either receptor type.

A visual system composed of either of these possible receptor types could mediate color discrimination. In either case, more than one receptor would be required. A single receptor generally cannot provide information about the spectral characteristics of the stimulus. Usually there is no specific receptor response to color. Rather, the response to any given wavelength can be matched by the response to an appropriate energy of another wavelength. There are a small number of exceptions to this rule (*10, 11*). These responses to different combinations of wavelength and energy are usually electrophysiologically indistinguishable at the level of the receptor and presumably are therefore indistinguishable to the central nervous system of the organism. This is known as the Principle of Univariance (*12*). Color discrimination must generally involve a comparison of the relative responses in a set of receptors. An array of paradigmatic receptors tuned to different portions of the spectrum will, as noted above, produce different relative receptor responses to lights of different wavelengths. But this is also true of a contraparadigmatic system. If an organism possesses more than one type of beta receptor, the relative responses in the array of receptors will necessarily vary across the spectrum. Nevertheless, contraparadigmatic receptors undoubtedly would involve a color vision system that is quantitatively different from a paradigmatic system. The details of such differences are beyond the scope of this article. An initial exploration of these questions has already been presented by Burkhardt (*13*). However, later in this article I will explore a difference between these two systems that might confer an evolutionary advantage on a species that utilized beta receptors.

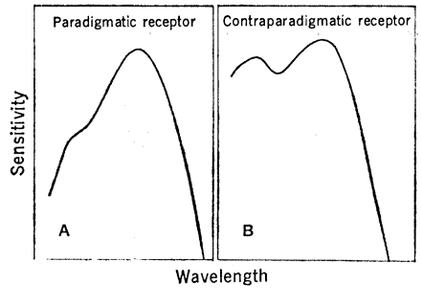

Fig. 1. (A) Typical photoreceptor spectral sensitivity function characteristic of a paradigmatic tuned receptor (alpha cell) which is sensitive to a narrow region of the spectrum. (B) Function characteristic of a contraparadigmatic nontuned receptor (beta cell) which is sensitive to a broad region of the spectrum. This figure indicates the general form of such spectral sensitivity functions. The actual peak wavelengths and the peak height differences (for beta cells) vary from cell to cell. See Tables 1 and 2 for specific values of these parameters.

Summary of the Literature

Microelectrode investigations of invertebrate photoreceptor spectral sensitivity have been carried out on 18 different species. The results of these investigations are summarized in Tables 1 and 2. To construct these tables I took the important data from the studies indicated and performed a number of manipulations and computations in order to put all of the results in a comparable form. Table 1 includes studies in which at least some beta cells were found; Table 2 includes studies in which only alpha cells were found. In some studies the investigators used more than one type of related organism. When there were no substantial differences among the results for related organisms, I combined the data. Where differences within species, due to caste (*14*) or season (*15*), for example, appeared to be of significance, the investigations were treated separately.

Two basic methods are used to determine the spectral sensitivity of photoreceptors. Action spectra provide the more useful results. An action spectrum is the sensitivity of the cell to different wavelengths, where sensitivity is the reciprocal of the energy (or often, the number of quanta) required to elicit a constant response. A response spectrum, on the other hand, presents the size of the responses to lights of equal energy and different wavelengths. Action spectra are usually invariant even though the criterion is changed [but see (*16*)], whereas response spectra generally change as the energy employed changes. This can be understood if one imagines the effect of stimulating an alpha receptor with monochromatic lights of solar intensities. Under such conditions, the receptor would be saturated and would give the same response to all wavelengths, even though it is really differentially sensitive. From the point of

view of the present discussion, response spectra provide less definitive results, since the response spectrum of an alpha cell could look like that of a beta cell. In all but three of the investigations shown in Tables 1 and 2, action spectra, or both action spectra and response spectra were obtained. In studies where both methods were used, the results shown are based principally upon the action spectra, but data from response spectra are included in the cell counts.

Alpha Cells

Tables 1 and 2 show the important characteristics of the alpha cells found in each investigation. In reporting the peak wavelength of each class of alpha cell, some investigators use values to the nearest nanometer (nm), others report a range of some 10 or 20 nm within which they are fairly confident that the peak wavelength occurs, and still others round the peak wavelengths off to the nearest 5 nm. In Tables 1 and 2 the peak wavelengths in the original reports have been rounded to the nearest 5 nm; this represents the usual limit of resolution obtainable in such experiments. Where the investigator indicated a range within which the peak might lie, the center of this range has been rounded to the nearest 5 nm. In some cases, the peak could not be determined because the stimuli available to the investigator yielded a maximum response at one end of the range of light stimuli available. The

Table 1. Summarized results of spectral sensitivity studies of invertebrate photoreceptors in which some beta cells were found as well as alpha cells. Bennett *et al.* (*27*), Burkhardt (*13, 42*), and Waterman and Fernandez (*21*) obtained action spectra and response spectra; all other investigators obtained action spectra only. Some investigators attempted to bleach beta cells selectively. Negative results were obtained by Autrum and Kolb (*18*), Burkhardt (*13, 42*), Horridge (*20*), McCann and Arnett (*28*), and Wasserman (*9*); positive results were obtained by DeVoe (*43*) and Nolte and Brown (*10*). See text for complete explanation.

Investigator	Organism	Material used	Alpha cells		Beta cells			
			Peak λ (nm)	No. of each class (%)*	Peak λ (nm)	No. of each class (%)†	Peak sensitivity difference (log units)	Change in spectral shape
Adolph (*16*)	*Limulus polyphemus* (horseshoe crab)	Lateral eye	525	100	≤ 380 and 525	100	0.0	No
Autrum and Kolb (*18*)	*Aeschna cyanea; A. mixta* (dragonflies)	Imagoes	460 495 520 535 550	2 29 44 5 6	355 and 420 355 and 460 355 and 495 355 and 520	3 5 6 2	−0.2 −0.2 −0.2 −0.1	
Autrum and Kolb (*18*)	*Aeschna cyanea; A. mixta* (dragonflies)	Larvae	355 495 520 535 615	3 9 74 9 3	355 and 410	3	0.0	Yes
Autrum and von Zwehl (*14*)	*Apis mellifica* (honeybee)	Drones	≤ 320 340 450 535	21 28 30 9	340 and 435 360 and 450	9 2	Uncertain Uncertain	Yes
Bennett *et al.* (*27*)	*Locusta migratoria* (locust)		None	0	430 and 515	100	0.0 to + 0.8	Yes
Bruckmoser (*24*)	*Notonecta glauca* L. (backswimmer)		350 420 565	15 18 67	370 and 565	See (*24*)	− 0.3 to 0.0	Yes
Burkhardt (*13, 42*)	*Calliphora erythrocephala* (blowfly)		None	0	350 and 450 350 and 490 350 and 520	14 70 16	0.0 to + 0.4 ≈ 0 ≈ 0	Yes
DeVoe (*43*)	*Lycosa miami; L. baltimoriana; L. lenta* (wolf spiders)	Anterior median eye	365 510	1 1	365 and 510	98	− 0.3 to + 3.3	Yes
Eguchi (*19*)	*Aeschna cyanea* (dragonfly)	Post-emergence	370 475 to 520	6 60	370 and 450	34	−0.1	Yes
Horridge (*20*)	*Anax junius; Libellula needhami* (dragonflies)		400 520	30 55	400 and 520	15	0.0	Yes
McCann and Arnett (*28*)	*Calliphora erythrocephala* (blowfly)		465	25	350 and 480	75	0.0	No
Nolte and Brown (*10*)	*Limulus polyphemus* (horseshoe crab)	Median eye	360 535	43 43	360 and 535	14	+ 2.0	No
Struwe (*25*)	*Heliconius numata* (butterfly)		None	0	360 and 490 370 and 540 390 and 460	14 72 14	+ 0.2 −0.2 −0.1	No
Wasserman (*9*)	*Limulus polyphemus* (horseshoe crab)	Lateral eye	525	90	375 and 525	10	−0.2	Yes
Waterman and Fernandez (*21*)	*Procambarus clarkii* (Girard) (crayfish)		440 595	18 77	440 and 595	5	Uncertain	Yes

* The number of alpha cells of each class is expressed as a percentage of all cells (both alpha and beta) investigated in each study. † The number of beta cells is also expressed as a percentage of all cells.

true peak might have occurred in the unmeasured region of the spectrum. Such results are indicated by the sign for less than or equal to, because the problem always occurred in the region of short wavelengths. The single cell peak wavelengths reported in Tables 1 and 2 vary from 320 to 615 nm. However, there is a tendency for them to cluster in two regions of the spectrum: 350 to 400 nm and 475 to 525 nm.

Tables 1 and 2 also show the numbers of alpha cells of different classes that were found in each study, the value for each class being expressed as a percentage of the total number of cells (both alpha and beta) investigated.

Beta Cells

The simplest way to describe beta cells is to show first where their peaks are located. Figure 1B indicates that even though the spectral sensitivities for beta cells are broad and not tuned to any particular region of the spectrum, they usually display two small peaks. Most investigators have recognized these two peaks and given estimates of their location, as shown in Table 1. The procedure of rounding to the nearest 5 nm employed with alpha cells was followed for beta cells. The peak wavelengths of beta cells exhibit a range and clustering that is similar to the wavelengths of the alpha cells.

Also shown in Table 1 are the numbers of beta cells of different classes, expressed as percentages of all the cells (alpha and beta) investigated. These percentages are based on the actual reports of cell counts or percentages in the original papers. However, there were some reports in which I was not certain whether the investigator was giving an example of a beta cell which represented an instance of a more general occurrence or whether he was giving a complete catalog. I am confident that the relative frequencies of the alpha cells are quite accurate. However the relative frequencies of beta cells among each other may be inaccurate and the proportion of all beta cells to the total number of cells is probably often underestimated. Not all investigators appear to attend to the contraparadigmatic beta cell finding as extensively as they attend to the paradigmatic alpha cell finding.

Another characteristic of beta cells that has generally been recognized as a parameter of the action spectrum is the difference in sensitivity of the two peaks. Table 1 shows this sensitivity difference in log units. A negative sign indicates that the short wavelength peak had a lesser sensitivity than the long wavelength peak; zero indicates that the two peaks were of equal sensitivity; and a positive sign indicates that the short wavelength peak had a greater sensitivity than the long wavelength peak. When a sensitivity difference is shown, it means that the investigator himself (either through a figure or through a quantitative statement in the body of the paper) gave some indication of this parameter's value. A result listed as "uncertain" indicates that I found no way to extract the information from the research report concerned. This sensitivity difference is a parameter of central importance to questions of color discrimination. A beta receptor with a sensitivity difference in excess of 1.0 log unit is not remarkably different from an alpha receptor. However, as Table 1 shows, this parameter usually has a value near zero.

Unlike an alpha cell, which can only become more or less sensitive, a beta cell can change the relative sensitivity of the two peaks. Sometimes this occurs during a single experiment on a single cell; in other cases the difference is between two or more cells with the same peak wavelengths. Table 1 shows whether or not such changes in spectral shape occurred. While a "Yes" in the table means that the investigator certainly gave some indication that a change occurred, a "No" does not necessarily mean that there were no changes, but that the investigator did not mention any such changes.

Bleaching is the reduction in concentration of a photosensitive pigment in a photoreceptor brought about by exposure to light. In the bleaching of a beta cell with long or short wavelength light, selective adaptation might occur so that there is a change in the relative sensitivities of the two peaks. In the legend of Table 1, "positive" means that the investigator was able to change the relative sensitivities of the peaks and "negative" means that he was not.

I have been conservative about classifying a cell as a beta cell. Figure

Table 2. Summarized results of spectral sensitivity studies of invertebrate photoreceptors in which alpha cells only were found. Mote and Goldsmith (*46*) obtained action spectra and response spectra; Nosaki (*15*) and Gogala (*44*) obtained response spectra only; all other investigators obtained action spectra only.

Investigator	Organism	Materials used	Alpha cells	
			Peak λ (nm)	No. of each class (%)*
Autrum and von Zwehl (*14*)	*Apis mellifica* (honeybee)	Worker	340	17
			430	23
			460	6
			530	54
DeVoe (*43*)	*Lycosa miami; L. baltimoriana; L. lenta* (wolf spiders)	Anterior lateral eye	510	100
Hillman *et al.* (*11*)	*Balanus amphitrite* (barnacle)		495	100†
			535	100
Gogala (*44*)	*Ascalaphus macaronius* Scop. (owl fly)		350	100
McReynolds and Gorman (*45*)	*Pecten irradiens* (scallop)		500	100
Mote and Goldsmith (*46*)	*Periplaneta americana* (cockroach)		365	46
			510	54
Nolte and Brown (*22*)	*Limulus polyphemus* (horseshoe crab)	Ventral eye	525	100
Nolte and Brown (*22*)	*Limulus polyphemus* (horseshoe crab)	Lateral eye	525	100
Nosaki (*15*)	*Procambarus clarkii* (crayfish)	Summer recordings	460	10
			610	90
Nosaki (*15*)	*Procambarus clarkii* (crayfish)	Winter recordings	560	100
Srebro (*23*)	*Limulus polyphemus* (horseshoe crab)	Lateral eye	525	100

* The number of alpha cells of each class is expressed as a percentage of all cells investigated in each study. † This is an unusual finding in that all of the cells in this eye can be made to switch their spectral sensitivity by appropriate adaptation.

1A shows that the spectral sensitivity function of an alpha cell exhibits a shoulder in the short-wavelength region of the spectrum; this is a characteristic of the absorption of light by rhodopsin in solution (*17*) and is a genuine effect of a single-peaked photopigment. The shoulder is attenuated relative to the principal peak by approximately 0.6 to 0.7 log units. Therefore, when one looks at the short-wavelength end of the spectrum, there is a region of ambiguity wherein one is uncertain whether or not a cell is a beta cell. One could encounter a cell with a visible peak and an auxiliary ultraviolet shoulder wherein the shoulder was attenuated relative to the principal peak by (say) 0.4 to 0.5 log units. This could easily be the single pigment shoulder plus some experimental error. These methods involve a certain minimum of such error. It is rare that a difference of 0.2 log units is meaningful in such investigations. Therefore in constructing Table 1, I used the following criterion for declaring a cell to be a beta cell: when a peak occurred in the long-wavelength region of the spectrum and a peak of lesser sensitivity occurred in the short-wavelength region, the sensitivity difference had to be 0.3 log units or less in order for such a cell to be classified by me as a beta cell.

This means that beta cells are undoubtedly underrepresented in this article; this is in addition to the underrepresentation mentioned above that is due to investigators giving less attention to the reporting of beta cells than to the reporting of alpha cells. As I will show below, there is yet another factor that may have lead to an underrepresentation of beta cells in these tables.

Small Samples

One should be aware that the percentages given in Table 1 are subject to substantial sampling fluctuations. Rarely has a statistically large number of cells been investigated. In few of these experiments were more than 100 cells studied; in the majority of the experiments from 10 to 30 cells were studied. Under these circumstances, it would ordinarily be most appropriate to report the n rather than the percentages. However, this would make comparisons among the various reports very difficult. Speculations based upon these percentages which would otherwise appear to be attractive should be approached cautiously. For example, four different species of dragonfly in three different stages of development were investigated by three different sets of investigators (*18–20*). The differences in the proportions of alpha and beta cells found in these studies might tempt one to conclude that the differences are associated with development. Such differences may not be genuine.

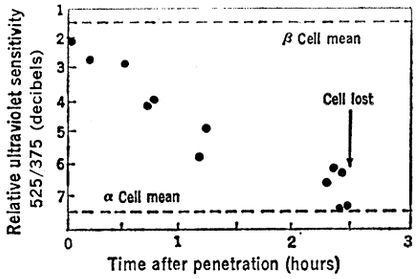

Fig. 2. Alteration of the spectral sensitivity of a beta cell. Ordinate is the relative sensitivity of this cell to visible (525 nm) and ultraviolet (375 nm) light. Units in this figure are in decibels which are equal to tenths of a log unit. Abscissa indicates time during the experiment from penetration to the ultimate and sudden loss of the cell. Each individual point represents a single determination of the cell's relative sensitivity to these two wavelengths.

Differences among Studies

The way in which the cells are categorized according to their peak wavelengths might also lead one to conclude that there are serious discrepancies among the results. In some instances, a subsequent study revealed that a parameter had a significance that was previously unrecognized. Thus Eguchi (*19*) found that the peak wavelength of the alpha cells in his studies could be varied over a substantial range by changing the angle of incidence of the light. He therefore grouped all such cells together, whereas Autrum and Kolb (*18*), in their earlier studies of dragonflies, did not take this factor into account; these workers described three subgroups within the range recognized by Eguchi.

Because of these general difficulties, it would be well to confine generalizations about differences within species or among related species to those investigations conducted by a single investigator using consistent criteria for classification and reporting. In such studies, the investigator should have recognized the factor in question during the experiment and have reported that the factor had a genuine effect. An example of this would be the difference in the peak wavelengths found by Nosaki (*15*) in the summer and winter recordings from crayfish. This is undoubtedly a genuine difference. Even here, however, Nosaki's failure to find alpha cells sensitive to light of short wavelength during the winter may not necessarily be valid because he found a low proportion of such receptors even in the summer.

Principle Conclusion

The data in Tables 1 and 2 indicate that the majority of studies of invertebrate photoreceptors reported the presence of both alpha and beta cells. In addition, most of the investigators found a higher proportion of alpha cells.

The data reveal no consistent basis for the variable incidence of beta cells. In fact, the data show that in different investigations of the same organism the different investigators often do not agree on whether or not beta cells are present. For example, Nosaki (*15*) reports the presence of only alpha cells in the crayfish whereas Waterman and Fernandez (*21*) report a few beta cells. In the lateral eye of the horseshoe crab, both Wasserman (*9*) and Adolph (*16*) report the presence of beta cells while Nolte and Brown (*22*) as well as Srebro (*23*) report no beta cells.

Beta Cell Lability

I have already suggested that in Tables 1 and 2 the number of beta cells may be underestimated because of inadequate attention of investigators to the contraparadigmatic finding and because of my conservatism in constructing the tables. But these two factors alone do not account for the wide range of results in which the frequencies of beta cells range from 0 to 100 percent, nor do they account for the disagreements among investigators. As mentioned previously, investigators of beta cells have often reported changes in the relative sizes of the two peaks. Sometimes the changes occur during an experiment on a single cell; at other times differences among cells tested on different days are revealed. It is often difficult to determine from their reports whether investigators are referring to the former or the latter

when they discuss beta cell variability. However, some investigators specifically report that a single cell can vary during the course of the investigation and change from a beta cell to an alpha cell (*14, 18, 24*). There are also reports of changes from an alpha cell to a beta cell (*18, 24*); this appears to occur less frequently. The logic of this situation indicates that a beta cell can be mistaken for an alpha cell if the changes are of sufficient magnitude. An investigation of such changes is to a very high degree incompatible with the determination of a complete action spectrum. It takes time to measure all of the responses to all of the wavelengths and energies necessary to extract the spectral sensitivity of the cell. If the cell is changing during the course of an experiment, it will not repeatedly yield the same response to the same stimulus. In such a case, the investigator is in a difficult position. He can either discard the data as contaminated by excessive amounts of error, or he can report the data with a strong cautionary statement of the nature of the problem.

However, once an investigator has determined the spectral sensitivity function of at least a few stable beta cells, he can investigate this question directly by confining himself to a few stimuli which can be delivered over a short time interval and which are adequate to characterize the relative height of the two peaks.

Example from the Horseshoe Crab

I have obtained such intracellular recordings from the lateral eye of the horseshoe crab, *Limulus polyphemus*, and the results of one such experiment are indicated in Fig. 2. The methods employed have been described elsewhere (*9*). Figure 2 shows the sensitivity ratio of a cell at two characteristic wavelengths as a function of time during the experiment. These two wavelengths (375 nm and 525 nm) correspond to the two peaks of the beta cell action spectrum in this eye. The relative sensitivity of the cell at these two wavelengths can be determined in a few minutes, in contrast to the period of hours required to take a complete action spectrum. The relative sensitivity of a beta cell at these two wavelengths differs by 0.1 to 0.2 log units (1 to 2 decibels in Fig. 2) while an alpha cell differs by 0.7 to 0.8 log units (7 to 8 decibels in Fig. 2). Repeated measurements early in the experiment indicated that this was a beta cell; furthermore, the relative ultraviolet sensitivity gradually diminished so that the cell converted into an alpha cell over a period of hours. Dramatic changes in the overall condition of the cell were not observed during this time. I have observed similar spectral sensitivity changes during many experiments. Usually these changes are more rapid than those illustrated in Fig. 2; often they are so rapid that one loses confidence in the original results that indicated that the cell was originally a beta cell.

Because similar changes have been noted by other investigators it is evident that at least some alpha cells are really beta cells that have lost some of their light sensitivity. The limitations of the techniques employed make it impossible to determine the cell's spectral sensitivity quickly enough to determine if such changes occur in periods measured in minutes and suggest that some cells may lose certain light sensitive processes so rapidly that they appear to be alpha cells by the time the experiment begins and appear to remain so indefinitely.

Explanations of Beta Cells

Several hypotheses have been advanced concerning the nature of beta cells. It is necessary to consider the evidence for these hypotheses in order to evaluate the implications of the experiments described herein for the tuned-receptor paradigm. In some hypotheses, beta cells are treated essentially as artifacts that do not represent normal photoreceptor function. According to one hypothesis in particular, a beta cell occurs when one is simultaneously recording from two adjacent receptor cells, each of which is an alpha cell sensitive to a different portion of the spectrum (*14, 18, 19, 21, 25, 26*). Thus a beta cell occurs as an artifact when an electrode has penetrated through one alpha cell and protrudes into an adjacent alpha cell leaving a common hole between them. This hypothesis has been attractive because of its simplicity and because it provides a ready means of accounting for the changes in peak heights. Such changes would then represent changes in the relative viability of the two jointly penetrated cells.

However, direct evidence indicates that this hypothesis cannot account for all occurrences of beta cells. For example, many invertebrate receptor cells are sensitive to the plane of polarization of the incident light. Adjacent receptor cells are generally sensitive to different polarization planes. Therefore, if a beta cell were the result of a double puncture of adjacent receptors, one would expect that varying the plane of polarization would often produce systematic changes in the relative heights of the beta peaks. However, such effects do not occur (*27*).

Other evidence that at least some beta cells are in fact unitary photoreceptors comes from experiments in which the dye, Procion yellow, was used to mark the recorded cell (*28*). Procion yellow does not pass through cell membranes although it does pass through electrically tight junctions (also known as gap junctions) (*29*). Intracellular injections of Procion yellow therefore reveal the structure from which the recording was obtained; if one were really recording from two cells, the Procion yellow would be expected to distribute itself between the two cells. Moreover, even if there were not a puncture but rather one were recording from a cell that was coupled by way of tight junctions to another cell, Procion yellow would be expected to pass through such tight junctions and reveal the connectivity. An experiment with Procion yellow that was conducted on the photoreceptors of the blowfly indicated that beta cells can be single cells without extensive relationships to other cells (*28*). Blowflies appear to have open rhabdomeres and show no evidence of tight junctions; an analogous experiment on a species with tight junctions would produce less definitive results. Of course, it is possible that the dual-puncture hypothesis is occasionally correct, but the probability of successful recordings being obtained under such circumstances seems very slight. In addition, one would expect such cells to be differentially coupled to the tip of the electrode so that the electrode would record different waveforms because of the passive electrical effects of the intervening membranes. This should produce specific effects of color on the response waveform. In general, such effects have not been observed [however see (*10, 11*)]. This is not to say that a microelectrode cannot suddenly shift from one receptor cell to another; there is direct evidence suggesting that such shifts do sometimes occur (*21*). However, the occurrence of such shifts

is not an adequate demonstration of the notion that all beta cells are necessarily produced by such shifts.

According to another hypothesis, the accessory pigments that shield and optically isolate the receptors play a role in spectral sensitivity by allowing the passage of light of certain wavelengths more than light of other wavelengths. In general this hypothesis will not serve to explain the existence of beta cells. A substantial amount of information is now available about such screening pigments (*30*). It seems clear that "windows" in these screening pigments are generally effective for light of long wavelengths in a region of the spectrum that is outside of the present range of interest. Except for a few species [for example, see (*15, 21*)], invertebrates are typically sensitive to ultraviolet light and not to light of long wavelengths. The windows in screening pigments, when they occur, cannot in general influence the double peaks found in the investigations described here.

A third hypothesis that might be offered is self-screening by the visual photopigment itself. If a visual pigment of sufficient optical density existed in a receptor, self-screening would produce spectral sensitivity changes toward the form characteristic of a beta cell (*31*). However, the optical densities required to produce such self-screening effects are quite large. That is, if a cell contained only one visual pigment, such a conversion of its spectral sensitivity would require an optical density of at least 1.0. Even then, the distal portion of the receptor would not be subject to much self-screening. There seems to be no evidence that this generally occurs, although in one specific instance (*32*) such substantial densities of visual pigment were reported.

Bleaching Experiments

The only remaining hypothesis is the one mentioned at the outset of this article; namely, that beta cells contain two photopigments located equivalently with regard to membrane activation. Under the terms of this hypothesis, one would expect that it should be possible to bleach these photopigments selectively by illumination with monochromatic light of an appropriate wavelength. Table 1 shows that the results of such bleaching experiments, where they were attempted, were mostly negative. That is, a few investigators were able to depress one or the other peak of a beta cell by illuminating it with appropriate light, but most were unable to alter selectively the relative heights of the two peaks. The experiments only resulted in a depression of the overall sensitivity of the cell through neural adaptation.

However, recent research into the dynamics of photoreceptors shows that the bleaching of substantial quantities of photopigment can require much more light than the amount that produces an easily demonstrable neural adapting effect (*33*). The bleaching of 50 percent of the visual pigment would produce a 0.3 log unit change in the absorbing ability of the pigment. But in such situations, neural adaptation occurs which is orders of magnitude greater than the adaptation due to bleaching. Thus, a light that produces a change in the concentration of visual pigment that is detectable by electrophysiological methods (that is, a 50 percent or 0.3 log unit change) requires the use of a very intense test light in order to determine the resultant spectral sensitivity function. When studying spectral sensitivity, one discards most of the light by filtering or by passing the light through a monochromator. The maximum energies therefore available to an investigator of spectral sensitivity are far smaller than the maximum energies available to an investigator interested in photoreceptor dynamics only. The negative results in bleaching experiments probably represent examples in which the adapting light, although as intense as it could be without eliminating the electrical response to a monochromatic test light, was too weak to produce detectable bleaching.

Pigment Extractions

The hypothesis that beta cells are the result of two distinct photopigments in one cell carries with it the auxiliary proposition that it should be possible to extract the underlying pigments. Pigment extraction was difficult until recently. However, there are now reports of such extractions. In the lateral eye of *Limulus*, alpha cells sensitive to visible light only (*22, 23*) and beta cells sensitive to visible and ultraviolet light (*9, 16*) have been reported. There are no reports of ultraviolet-sensitive alpha cells in this eye. The existence of beta cells therefore would imply the existence of two pigments in this eye, including one that was sensitive to ultraviolet light; the reports of the alpha cells would imply the existence of a single pigment sensitive to visible light. Although, until recently, only a single pigment sensitive to visible light had been extracted from this eye (*34*), Benolken *et al.* (*35*) have now succeeded in extracting two photopigments from the lateral eye of the horseshoe crab, one sensitive to light of short wavelengths, the other to light of long wavelengths. While these results are consistent with the hypothesis that the beta cells in this eye are the result of two photosensitive pigments located within a single receptor cell, they are not, by themselves, proof of this hypothesis because they are also consistent with the different pigments being located in different cells.

Advantages of Beta Cells

The evidence suggests that there is a substantial population of beta receptors in invertebrates and that their frequency has previously been underestimated. It is therefore of some interest to inquire into the selection pressures that would favor organisms with beta cells. A possible answer to this question might be found by considering visual acuity. Invertebrates have relatively few receptor units in their eyes [of the order of 100 to 30,000 (*36*)]. This produces extremely coarse visual resolution compared to the resolution available to the human eye, which has some 100 million receptor cells and 1 million optic nerve fibers. Therefore, invertebrates are constrained not to reduce their visual acuity further. On the other hand, many invertebrates are exposed to a demand that they process information only present in ultraviolet light [for example, patterns in flower petals (*36*, chap. 11)].

Consider now an invertebrate having only alpha cells and assume that 50 percent of these alpha cells are sensitive to ultraviolet light and the other 50 percent are sensitive to visible light. Under conditions of illumination where ultraviolet and visible light are present, such an organism would not suffer any substantial loss in acuity. The range of reflectances of real objects is rarely confined to just one narrow band of the spectrum. Such an organism would not be exposed to contradictory requirements if ultraviolet light were always present. However, there are conditions of illumination in which most or all of the ultraviolet light is absorbed by some material. Such conditions occur under the surface of the

water (*37*) and in air when the sun's altitude is 8 degrees or less above the horizon (*38*). Also, moonlight appears to be somewhat deficient in ultraviolet energy (*38*, pp. 198–199). If ultraviolet light were largely absent, organisms with only alpha cells would suffer a 50 percent reduction in their acuity since half of the units of the retinal mosaic would be inactive. On the other hand, organisms that had only beta cells or predominantly beta cells would suffer little or no loss of acuity; every beta receptor would be capable of responding to every band of illumination. Under such conditions of restricted spectral illumination, all receptors would still be capable of responding. Organisms that must be active under varying conditions of illumination would be favored by the presence of beta cells. This might include amphibious organisms, organisms that are active around dawn or dusk, and organisms found at high latitudes. Organisms that are active during the day and night might also be included.

Earlier it was noted that beta cells are not incompatible with color vision. Burkhardt (*13*) showed how even a single family of beta cells could subserve color vision through slight variations in the relative heights of the two peaks. Thus a beta cell receptor system would maintain acuity in restricted illumination and would also provide the capacity for color discrimination under appropriate illumination. The advantages of such a system should favor the survival of organisms in possession of beta receptors. An entirely alpha receptor system would force some invertebrates to sacrifice acuity for improved color discrimination. More comprehensive information than is now available would be required to test this hypothesis; it would be especially interesting to have information about the visual acuity of these organisms when they are exposed to illumination representing the whole spectrum or only parts of the spectrum. There appear to be some intrinsic differences in the resolution of spectrally distinct receptor types (*28*); these differences may complicate the results of experiments on invertebrate color vision.

Conclusion and Suggestions

The resistance to abandoning an established paradigm has nowhere been better expressed than by Helmholtz in the preface to his *Handbook of Physiological Optics* (*39*). Helmholtz said: "I have proceeded on the conviction that law and order *even if they are not fundamentally sound* [italics mine] are better than contradictions and lawlessness." This general attitude is common in scientific research and leads investigators to adhere to a paradigm even when there is evidence that is incompatible with the paradigm. There is substantial justification for this preference because all fields of science are continually confronted by unresolved problems. Undoubtedly the tuned-receptor paradigm has served vision research quite well for centuries. However, the evidence available from studies of invertebrate photoreceptors indicates that a novel yet orderly alternative description of photoreceptor spectral sensitivity is required by the data. We should therefore recognize the necessity to modify our future approach to studies in this area.

It would be helpful if investigators of this fundamental problem of vision were to use the method of multiple working hypotheses (*40*) so that strong inference (*41*) might be brought to bear upon the extent and function of these two different modes of color vision. Specifically, future investigations on receptor spectral sensitivity should be designed so that the hypothesis of nontuned receptors is deliberately entertained. Moreover, studies of invertebrate vision in general should be designed to be sensitive to differences in function implicated by these two modes of color vision.

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