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# PHOTOPIGMENTS AND PSEUDO-PIGMENTS

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**Abstract**—The spectral properties of photopigments are often inferred from physiological measures of spectral sensitivity. From these measures pigments have recently been inferred with properties not corresponding to those of any pigment measured by spectrophotometry. It is proposed that in all these cases the properties of single pigments are not being measured; rather, they are pseudo-pigments resulting from a specific form of neural interaction involving several real, known, photopigments. These pseudo-pigments were accepted as being related to single, but unusual, pigments largely because they obeyed the commonly accepted but incomplete criteria for identifying single pigments. Starting from the essential features of a pseudo-pigment, an analysis is developed; the conclusion is that pseudo-pigments can be resolved into standard photopigments, as identified by spectrophotometry, and that these interact in a specified form. A method is described for identifying the number and $\lambda_{max}$'s of the photopigments involved in any physiological spectral sensitivity function, as well as certain details of the physiological interactions involved.

## (1) INTRODUCTION

Attempts have been made to infer the spectral properties of photopigments from the responses of cells at various levels of the visual system. Assorted criteria are used to determine whether the responses can be said to be driven by a single photopigment. If the responses meet these criteria, their spectral sensitivity is taken as equivalent to that of the photopigment. Using this approach several "new" photopigments have been identified from the responses of S-units and ganglion cells in the retinae of various cyprinid fish.

To choose a specific example, the absorption spectra of individual cones in goldfish have been measured by microspectrophotometry (Marks, 1963, 1965; Liebman and Entine, 1964; Harosi and MacNichol, 1974). The cone photopigments have their $\lambda_{max}$ respectively at about 455, 530 and 625 nm, and the spectra of all three are approximately as broad as those of other known photopigments. Records of the responses of single cones in the retina of the closely related carp have largely confirmed the spectrophotometric findings (Tomita, Kaneko, Murakami and Pautler, 1967).

However experiments on cells other than the receptors have produced contradictory findings. Thus, Spekreijse, Wagner and Wolbarsht (1972) concluded, from the spectral sensitivities of goldfish ganglion cells, that the long-wavelength pigment peaks at 650 nm and has a narrow spectrum. Several other reports have inferred the existence of a fourth, extremely narrow, pigment in addition to the three first described by Marks: Rushton (1965) and Naka and Rushton (1966a,b,c) reported one at 680–690 nm in the tench, Witkovsky (1967) presented some evidence for one at 660 nm in carp, and Daw and Beauchamp (1972) reported one at 670 nm in goldfish.

In spite of the disagreement with spectrophotometric findings the physiological data were accepted as correct estimates of photopigment spectra because the responses met certain criteria. For example, the response vs log intensity curves for the various test wavelengths were parallel, or the relative spectral sensitivity curves were unchanged by chromatic adaptation.

It has been suggested that these unusual pigments are all the result of interactions among responses derived from two or more of the directly measured pigments (Abramov, 1972; Sirovich, Abramov, Gordon and Levine, 1973). This paper analyzes the above suggestion and explores, in quite general fashion, the adequacy of the commonly used criteria; the conclusions are then specifically applied to some of the examples cited earlier.

Before we present a thorough and rigorous analysis of the problem, we will give an informal summary of our reasoning about and approach to, the unusual spectral sensitivities described above. In this summary we intend to stress the ideas at the cost of precision, but we will indicate where each part is dealt with in detail.

We consider data for which the response vs log intensity curves, for the various wavelengths, are parallel to each other (see Fig. 1). What are the implications of this? Assume first that the responses are indeed all due to a single photopigment. Let $\bar{R}$ characterize the response of the cell to a flash of monochromatic light of quantal intensity $I$ and frequency $\nu$. $\bar{R}$ is some function of the light absorbed by the photopigment at the given wavelength:

$$\bar{R} = \bar{R}(IA(\nu)), \tag{1.1}$$

where $A(\nu)$ denotes the effective fraction of incident quanta absorbed by the photopigment. For each stimulus, the intensity is simply scaled by some constant whose value depends only on $\nu$; since we usually deal with the logarithm of this product, each curve

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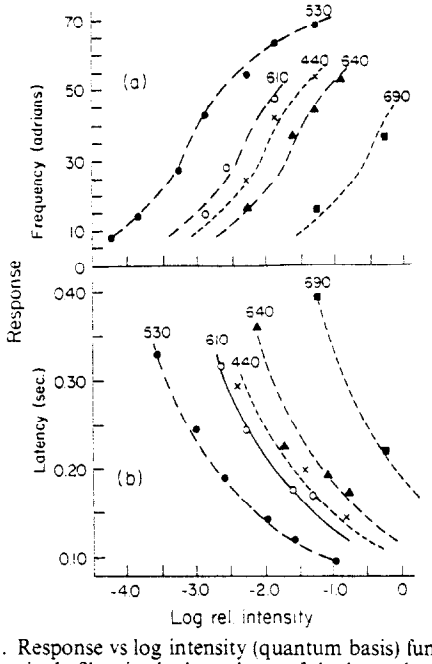


Fig. 1. Response vs log intensity (quantum basis) functions from a single fiber in the lateral eye of the horseshoe crab, *Limulus*. Stimuli were monochromatic lights; the wavelength is indicated next to each curve. (a) The response measure is frequency of spike firing (in adrians) in the initial portion of the response immediately following light onset. The same curve was slid along the abscissa to fit any given set of points. (b) The identical set of records as in the first graph, except that the response measure is latency from light onset to first spike. The same curve has been drawn through any set of points. (Unpublished data of C. H. Graham and H. K. Hartline, 1933.)

is merely translated along the logarithmic abscissa by an amount given by $\log A(\nu)$. In fact, the functional form (1.1) is both necessary and sufficient to give parallel response vs $\log I$ curves. (See Section 2, equation (2.4) and related treatment, for details.)

We now extend the argument to the case in which several pigments are involved. For simplicity, let us assume that the response is a function of two photopigments:

$$\bar{R} = \bar{R}(IA_1(\nu), IA_2(\nu)), \tag{1.2}$$

where the subscripts denote the individual pigments. If responses are indeed driven by two different photopigments and the data show parallel response vs log intensity curves, then the problem is to find a form of (1.2) that will have the same functional form as (1.1).

Consider the following specific form of (1.2):

$$\bar{R} = \bar{R}(\{\alpha[IA_1(\nu)]^p + \beta[IA_2(\nu)]^p\}^{1/p}) \tag{1.3}$$

where $\alpha$ and $\beta$ are constants, taking either sign, and giving the relative strengths of the inputs from the two pigments, and $p$ is some power. This form also gives the requisite parallel curves—i.e. it has the same functional form as (1.1). This can be seen by factoring out $I$ and rewriting (1.3) as follows:

$$\bar{R} = \bar{R}(I[\alpha A_1^p(\nu) + \beta A_2^p(\nu)]^{1/p}). \tag{1.4}$$

As in (1.1), all the terms to the right of $I$ (and which multiply it) are functions of frequency only and are constant for any given stimulus. Equation (1.3) can be generalized to any number of pigments, provided the response function's argument can be written in the form of (1.1).

Equation (1.3) is thus sufficient to meet the criterion of parallel response vs log intensity curves, even though more than one pigment is involved. In our terminology, this equation represents the response of a "pseudo-pigment". In fact, under minimal assumptions, we show that (1.3) is the necessary form. (See equation (2.12) and related discussion.)

It would appear from (1.3) that in order to find how many, and which, pigments are involved in some situation the precise form of the response function is required. However, this problem can be avoided by appealing to the relative spectral sensitivity of the response mechanism. Sensitivity is conventionally defined as the reciprocal of the intensity needed to evoke some constant criterion response. Since we are now dealing with sensitivity data we know that, for each stimulus, the intensity was found such that $\bar{R}$ was a constant. If $\bar{R}$ is constant, then,

$$I[\alpha A_1^p(\nu) + \beta A_2^p(\nu)]^{1/p},$$

in (1.4), is a constant. We can now define sensitivity, $\mathscr{S}(\nu)$, as equal to $1/I$ for constant response. Therefore,

$$\mathscr{S}(\nu) = [\alpha A_1^p(\nu) + \beta A_2^p(\nu)]^{1/p}. \tag{1.5}$$

(See equation (3.2) and related discussion.)

Since we have shown that spectral sensitivity data can be represented by (1.5), we can now enumerate the questions that must be answered:

(i) How many photopigments are involved? (Equation (1.5) was restricted to only two pigments purely for purposes of exposition.)

(ii) What is the shape of the absorbance function of each pigment, its wavelength of maximal absorption, and its density coefficient?

(iii) What is the value of the exponent $p$?

(iv) What is the relative strength of the neural coupling (e.g. $\alpha$, $\beta$) between the responses of the different spectral mechanisms?

In order to proceed with the solution of (1.5), we need some additional information about the nature of photopigment absorption spectra in general. We discuss pigment spectra in Section 3a (see equation (3.5)), and the specific method of solution in Section 3c. In brief, we assume (for the purposes of the method used) that the absorbance functions of visual photopigments have the same shape when plotted on a frequency axis (see equation (3.5)). From this, the sensitivity of a pseudo-pigment (possibly containing more than the two pigments in (1.5)) can be reformulated as the solution of the integral equation:

$$\mathscr{S}^p(\nu) = \int B(\sigma)A^p(\nu - \sigma)\,d\sigma \tag{1.6}$$

(see equations (3.7) and (3.8)).

That is, we attempt to represent sensitivity (raised to the $p$th power) as a sum of pigment absorption functions (each raised to the $p$th power) each centered at a frequency, $\sigma$, which is continuously distributed. The function $B(\sigma)$ provides the relative weights (with signature) to give to each of the distributed absorption functions.

$\mathscr{S}(\nu)$ is known and the form of $A(\nu)$ is assumed known (except for a density coefficient; see (3.4)). The

unknown is $B(\sigma)$, and as part of its solution the values of $p$ and the density coefficient are also found. In practice we expect $B(\sigma)$ to be composed of a small number of "delta functions", i.e. there will be prominent, narrow, "spikes" in the curve of $B$ against $\sigma$; the position of each spike indicates the wavelength of maximal absorption of each contributing pigment and the area under the spike gives the weight of that contribution.

The reader who wishes to avoid the detailed treatment in Sections 2 and 3 can turn directly to Section 4 in which we give the solutions of various sets of data. Finally, in Section 5 we discuss certain implications of our description of pseudo-pigments and in particular the effects of chromatic adaptation.

## (2) FORMULATING THE PROBLEM

The purpose of this section is to develop the general analytical form of a response function, based on previously accepted criteria for pigment isolation, and which meets all such tests for a single photopigment. For simplicity we shall deal only with response functions due to spatially uniform, brief, fixed-duration flashes of monochromatic light which may or may not be superimposed on a steady background.

### (a) *Responses driven by a single photopigment*

Recent investigations have revealed the possibility of coupling amongst photoreceptors (Baylor and Fuortes, 1970; Baylor and Hodgkin, 1973; Baylor, Fuortes and O'Bryan, 1971). This case will be treated later, and we term these "coupled photoreceptors", reserving the term "photoreceptor" for the uncoupled case.

Denote by $I$ the number of quanta contained in a monochromatic flash, of frequency $\nu$, and incident on the base of a photoreceptor. Further denote by

$$e = I(\nu)A(\nu) \tag{2.1}$$

the number of quanta effectively acting on the photoreceptor. $A(\nu)$ will be referred to as the "absorption function" although as we see in a moment it may not be strictly a measure of pigment absorption. It is a function of $\nu$, and has the dimensions of a cross-section. Note that $I$ refers only to the light flash, which is possibly superimposed on some steady background.

According to the Univariance Principle of Naka and Rushton (1966a), the response of a photoreceptor depends only on the rate at which quanta are captured. We restate this in the present framework as follows: The response, $r$, of a photoreceptor, is a function of time, $t$, and of $e$ (2.1); i.e.,

$$r = r(t; e(\nu)). \tag{2.2}$$

(We follow the convention that parameters, such as $e(\nu)$, are preceded by a semicolon.) The term "response" refers only to responses elicited by the light flash, and maintained response level, whether due to a background or not, will be ignored. The response, $r$, does not have to be a fixed function, but varies, in general, with receptor.

The quantity $e(\nu)$, defined by (2.1) will be referred to as "excitation", and we use this term instead of "effective capture rate". If, for example, response varies with locus of photon capture in the outer segment then Univariance is not valid in terms of effective capture rate (see also Rodieck, 1973). Nevertheless Univariance, (2.2) remains valid not only in this case but under much more general conditions if a properly defined excitation, (2.1), is employed (Sirovich, 1976). The resulting excitation is dependent on the molecular extinction function but since this generalization plays no role in the present analysis we do not go into these details. In this same vein we note that the presence of oil droplets (found in many avian and reptilian retinae) can also be incorporated in the treatment. The droplet generates a frequency dependent transmission coefficient multiplying the absorption function appearing in (2.2). Thus, a single photopigment could produce more than one type of excitation. Since this too, plays no role in our subsequent analysis, no further mention of this effect will be made.

Commenting on (2.2) we may say that in general the response of a photoreceptor depends on the time history, up to the moment of measurement, of the illumination on that receptor. Since, all physiological systems have "fading memory", sufficiently long interflash intervals allow us to regard the process as taking place anew. Further, by restricting attention to flashes of fixed duration, the entire time course of the response depends on the single quantity $e(\nu)$; i.e. the excitation furnished to the receptor by the flash. Therefore any two flashes (having the same duration) that give rise to the same value of $e$ (independent of the spectral content of the flashes) will produce responses with identical time courses. We may therefore conveniently eliminate time from our discussion by selecting for measurement some specific feature of the response. The characteristic feature chosen for analysis depends on the response (2.2) and we generically denote it by $\bar{r}$, where

$$\bar{r} = \bar{r}(e(\nu)) = \bar{r}(IA(\nu)). \tag{2.3}$$

Regarding (2.3) we see that $\bar{r}$ as a function of intensity, $I$, and frequency, $\nu$, is only a function of the single argument, $e = IA(\nu)$. In such case the function is said to have similarity form—a consequence of which is that coordinate systems may be found in which the function appears as parallel (or self-similar) curves. In particular if $\bar{r}$ is written as a function of $\ln I + \ln A(\nu)$, we see that $\bar{r}$ vs $\log I$ leads to parallel curves on each of which $\nu$ is held fixed. In the following we often use the term "self-similar" rather than the less precise "parallel" to depict this condition.

As an illustration of these ideas consider Fig. 1, which contains examples of previously unpublished data from the horseshoe crab, *Limulus*. (Collected in 1933 by C. H. Graham and H. K. Hartline, and kindly made available to us by H. K. Hartline.) The records are from a single fiber of the lateral eye and represent responses to various light frequencies. In Fig. 1a the characteristic feature is the initial firing frequency, while in Fig. 1b it is the latency to first spike. In both cases the curves, plotted vs log intensity, are parallel. Each set of plots determines the same absorption function, $A(\nu)$, obtained from relative distances between curves.

So far we have considered responses of a single photoreceptor containing a single photopigment. The

above analysis also includes responses of any cell driven by one or more receptors all containing the same photopigment even if the individual receptors have different response functions. For, in the latter case the cell is driven by a single excitation, $e = IA(\nu)$, where $A(\nu)$ is the absorption function common to all the receptors; hence the response is again depicted by (2.2); further, $\bar{r}$ is given by (2.3); and, the spectral sensitivity of the cell is still proportional to $A(\nu)$. Clearly the individual responses of coupled photoreceptors containing a common photopigment is also covered by this argument.

It is of considerable importance to observe that the action of a single type of photoreceptor—i.e. responses driven by a single pigment—implies self-similar (parallel) response vs log intensity curves. And hence the lack of self-similarity implies that more than one photopigment (and hence more than one photo-receptor) is contributing to the response.

In certain instances in the literature one finds that the converse is also assumed to be true: if self-similarity is obtained, it is assumed that the response is due to a single photopigment. Such a strong conclusion, however, is unwarranted. Analytically such data indicate that $\ln I$, when plotted against $\bar{r}$, leads to a universal curve, $f(\bar{r})$, which is offset by a "constant" which depends only on frequency; i.e.,

$$\ln I = f(\bar{r}) + c(\nu).$$

On solving this for $\bar{r}$ (assuming that $\bar{r}$ is a monotonic function of $I$) we obtain the self-similar form,

$$\bar{r} = \bar{r}(I\mathscr{A}(\nu)) \tag{2.4}$$

where $\mathscr{A}(\nu)$ is the exponential of $c(\nu)$, and $c(\nu)$ is to be taken from the data, e.g. from Fig. 1. However, nothing in general can be said about $\mathscr{A}(\nu)$; in particular it need not have the form of a standard photopigment absorption function (which we have denoted by $A(\nu)$).

Summing up, we have shown that a self-similar form for $\bar{r}$ is a necessary and sufficient condition for parallel response vs log intensity plots. However, parallel response versus log intensity curves (i.e. a self-similar form for $\bar{r}$) by themselves do not imply that response is driven by a single photopigment, unless $\mathscr{A}(\nu)$ in (2.4) takes on the form $A(\nu)$.

A possible criterion for deciding when a response is determined by a single photopigment is based on the finding of Dartnall (1953) that the absorption (strictly "absorbance") functions of all visual pigments have approximately the same shape when quantal absorption is plotted against frequency of incident light. (For pigments absorbing maximally in the low frequencies, the spectra become systematically narrower—Liebman, 1972; Harosi and MacNichol, 1974—but this finding remains nonetheless a close approximation.) By this criterion, if a spectral sensitivity function matches the "standard shape" then it is presumed to be based on a single pigment. This particular criterion, match to a standard shape, was taken to be of minor importance by Naka and Rushton (1966a,b,c). They found parallel response versus log intensity curves, and concluded existence of a true single pigment ($\lambda_{max}$ = 680–690 nm) in spite of the fact that the spectrum of this "pigment" was very significantly narrower than those of pigments measured spectrophotometrically. Various other criteria have been proposed for identifying single pigments. Among these is one based on the effects of chromatic adaptation, discussion of which is deferred until later.

*(b) Responses driven by more than one photopigment*

We now consider the general form taken by the response function of a cell having afferents from several types of photoreceptors (we refer to this as a "compound cell"). This is necessary since, in most cases, responses are recorded from some stage beyond the receptors. Also we can consider responses of coupled photo-receptors having different absorption functions or cells receiving afferents from such photoreceptors.

We begin by assuming that the compound cell in question is visually isolated: i.e. only visual stimulation is present and all other stimuli are absent. In this case the cell's response which is time dependent, depends only on the responses of the different photoreceptor types. Recalling that we are restricting attention to monochromatic light flashes of fixed duration, the response of any one photoreceptor can be written as

$$r_i = r_i(t; IA_i(\nu)) = r_i(t; e_i(\nu)), \tag{2.5}$$

where the index, $i$, refers to the $i$th type photoreceptor, and runs through $1, \ldots, N$; $N$, the number of different excitations, will be taken as unknown. We denote the response of a compound cell, receiving afferents from various photoreceptors, by $R$. Therefore $R$ is a function of $r_i$, $i = 1, \ldots, N$; which is to say that $R$ is a function of $t$ as well as the time history of each of the responses $r_i(t; e_i)$, (2.5). However, since the duration of the light flash is fixed, $R$ can be regarded as an ordinary function of time, $t$, and the various excitation values, $e_i$:

$$R = R(t; e_1(\nu); e_2(\nu); \ldots; e_N(\nu)). \tag{2.6}$$

Using excitation instead of response in this formulation avoids discussing whether all photoreceptors have the same time course for their response.

As with a single photoreceptor, the time course of $R$ can be eliminated by regarding certain specific features of its time course. Although $R$ appears to be dependent upon the $N$ parameters ($e_i$, $i = 1, \ldots, N$), two parameters suffice, since $I$ and $\nu$ completely specify the "flash". (In principle, two features of the time course of $R$ should specify the response—in contrast to a single photoreceptor for which one property suffices.) In principle one should be able to "solve" for $I$ and $\nu$ in terms of these two features, although there is no guarantee that two such features may be found. For our purposes we need not go into this question in as much as in typical experiments only one such feature is usually considered: e.g. maximum amplitude of response (potential or firing rate, as the case may be), latency, average response, etc. In any case the time dependence is thus suppressed, and if the resulting form is generically denoted by $\bar{R}$, we can write

$$\bar{R} = \bar{R}(IA_1, \ldots, IA_N). \tag{2.7}$$

If the response function has the similarity form given by (2.4), and if several excitations are involved we can say that

$$\bar{R} = \bar{R}(I\mathscr{A}(\nu)) = \bar{R}(IA_1(\nu), \ldots, IA_N(\nu)). \tag{2.8}$$

This places a strong restriction on the form of $\mathscr{A}(v)$ if it is in fact a function of several absorptions, namely that

$$\mathscr{A}(v) = F(A_1(v), \ldots, A_N(v)), \tag{2.9}$$

where the function $F$ is homogeneous of degree 1. That is, for arbitrary $I$,

$$F(IA_1(v), \ldots, IA_N(v)) = IF(A_1(v), \ldots, A_N(v)) \tag{2.10}$$

(see Sirovich, 1976, for a proof).

In terms of $I\mathscr{A}(v)$, which we now term "equivalent excitation" and denote by $E$, we can write

$$E = F(e_1, e_2, \ldots, e_N),$$

where $F$ is still the same as in (2.10). This condition places a strong constraint on the function for it tells us that a knowledge of $F$ in the neighborhood of the origin determines $F$ everywhere. To see this, note that

$$F(e_1, \ldots, e_N) = \frac{1}{\delta} F(\delta e_1, \ldots, \delta e_N),$$

and then let $\delta$ tend to zero to demonstrate this property.

In order to further fix the form of $F$ we must make some specific assumption about it. For example, if $F$ is differentiable in its arguments at the origin,

$$E = \left(\frac{\partial}{\partial x} xE\right)_{x=0} = \frac{\partial}{\partial x} F(xe_1, xe_2, \ldots, xe_N)|_{x=0}$$

$$= \sum_{i=1}^{N} e_i \frac{\partial F}{\partial e_i} (0, 0, \ldots),$$

or, $E$ is linear in the $e_i$, or equivalently, that $\mathscr{A}(v)$ is linear in the $A_i(v)$. Rather than make such a restrictive assumption, we leave open the degree of differentiability. We merely assume that $F$ is differentiable at the origin in some power, $p > 0$; we need not restrict the value of $p$, which may be fractional. We can then write

$$E^p = \left(\frac{\partial}{\partial x^p} x^p E^p\right)_{x=0} = \frac{\partial}{\partial x^p} F^p(xe_1, \ldots xe_N)|_{x=0}$$

$$= \sum_{i=1}^{N} \alpha_i e_i^p,$$

where $\alpha_i$ are the differential coefficients and their precise form is immaterial; we shall refer to them as "coupling constants". Expressed in terms of absorptions, this states that

$$\mathscr{A}^p(v) = \sum_{i=1}^{N} \alpha_i A_i^p(v),$$

or,

$$\mathscr{A}(v) = \left\{\sum_{i=1}^{N} \alpha_i A_i^p(v)\right\}^{1/p}. \tag{2.11}$$

It should be noted that this form is homogeneous of degree 1, as the more general observation (2.10) already indicated.

In order to sum up, and in anticipation of the following section, we find it convenient to define the notion of a pseudo-pigment. If parallel response versus log intensity curves are obtained and if the response is due to more than one excitation (loosely speaking, "photopigment"), we will say that the excitation is due to a pseudo-pigment. Furthermore, we have proven that the response function of a pseudo-pigment must take the form

$$\bar{R} = \bar{R}\left(I^p \sum_{i=1}^{N} \alpha_i A_i^p(v)\right). \tag{2.12}$$

This is true of the response for any cell in the visual system where self-similar curves are found.

The above analysis in no way constrains the form taken by $\bar{R}$. $\bar{R}$ could be linear in $I$ (e.g. responses of horizontal cells in goldfish for small amplitudes; Spekreijse and Norton, 1970):

$$\bar{R} = \bar{R}_0 I\left(\sum_{i=1}^{N} \alpha_i A_i^p(v)\right)^{1/p} \tag{2.13}$$

where $\bar{R}_0$ is just a constant. And in general the response, $\bar{R}$, can be any function of the intensity $I$.

The following should also be noted about responses, $\bar{R}$, of a compound cell. If the excitations, $e_i$, converging on the cell are near zero, the cell's response will be near zero (recall that we are ignoring maintained or spontaneous rates). In this case we can seek a differential approximation to the response function. If we make the same mild assumption we made earlier about the differentiability of the response function itself we obtain:

$$\bar{R} \sim I^p \sum_{i=1}^{N} \alpha_i A_i^p(v). \tag{2.14}$$

When the assumptions leading to (2.14) apply, similarity is obtained locally (i.e. for small excitations) even though it may be absent in the large. Under these same conditions the form of (2.14) also specifies the form of the response function. However, the mere fact that $\bar{R}$ is close to zero does not necessarily mean that the form in (2.14) applies. It may not apply either because, although $\bar{R}$ is close to zero, the excitations are not; or, because the response function does not have the differentiability properties necessary to obtain (2.14)—an example is provided by (2.13) if $p \neq 1$.

The form (2.12) constitutes a major theoretical result of this paper and it is worthwhile re-iterating some of its main points. If we are considering a pseudo-pigment, then the responses of the compound cell must be a function of the weighted sum of the excitations of the various receptor types converging on that cell. Each excitation appears in the same functional form, namely, raised to the $p$th power. We underline the fact that the power to which each excitation is raised must be the same. These restrictions hold only over the region in which the response vs log intensity curves are parallel.

We should emphasize that in the above analysis we made no assumptions about the shape of a photopigment's spectrum. However, if a cell's responses are driven by a pseudo-pigment and we wish to specify the various true photopigments involved, then we must make certain assumptions about the absorption spectra of these photo-pigments. The form (2.12) imposes no specific restrictions on $A_i(v)$. The restrictions must come from some other source such as a theory of pigment absorption spectra, or empirical observations, and so on. It should also be noted that (2.12) says nothing about the precise form of $\bar{R}$; clearly,

if all we are given is a cell's spectral *response* curve (i.e. the intensity, $I$, held fixed), we must know more about the form of $\bar{R}$ in order to resolve the responses into pigment absorption functions.

The ways in which a compound's cell's spectrum can be resolved into its component pigment spectra are to some extent dictated by the assumptions we choose to make about such spectra. The next section describes the details of one way to accomplish this.

## (3) RESOLUTION OF PSEUDO-PIGMENTS INTO PIGMENTS

### (a) *General method*

Most experiments leading to pseudo-pigments have sufficient data to produce sensitivity curves, where sensitivity, $\mathscr{S}$, is defined, in the usual way, as the reciprocal stimulus required to produce a criterion response. We will only deal with per cent of maximum sensitivity:

$$S(\nu) = \frac{\mathscr{S}(\nu) \times 100}{\max \mathscr{S}(\nu)} = \frac{\min I_{\bar{r}}(\nu)}{I_{\bar{r}}(\nu)} \times 100 \qquad (3.1)$$

where $\max \mathscr{S}(\nu)$ signifies the maximum of $\mathscr{S}(\nu)$ over the range of $\nu$, and the subscript $\bar{r}$ signifies that response is being held fixed. This we refer to simply as "sensitivity".

In the previous section we demonstrated that the response function of a pseudo-pigment must of necessity take on the form shown in (2.12). This still leaves open the functional form of the response function itself. However, due to the similarity form of the response function of a pseudo-pigment, the form of its sensitivity may be obtained without prior knowledge of the response function itself. Thus applying (3.1) to (2.12) we have that the sensitivity spectrum of a pseudo-pigment must have the form,

$$S(\nu) = \frac{\mathscr{A}(\nu)}{\max \mathscr{A}(\nu)} \times 100.$$

From (2.11) this can be written as,

$$S(\nu) = \left[ \sum_{i=1}^{N} \beta_i A_i^p(\nu) \right]^{1/p} \qquad (3.2)$$

where

$$\beta_i = \frac{\alpha_i \times 100^p}{\max \mathscr{A}^p(\nu)}.$$

The problem before us is now clear: Given that the sensitivity of a pseudo-pigment is found by experiment, then we must determine the right hand side of (3.2) to fit these data. This entails finding the number, $N$, of input pigments, the $N$ coupling constants $\beta_i$, the exponent $p$, and of course the $N$ individual absorption functions, $A_i$. The form of the effective absorption function is given by

$$A(\nu) = \delta(\nu)\phi(\nu)(1 - 10^{-k\epsilon(\nu)\ell n}) = \delta(\nu)\phi(\nu)f(\nu) \qquad (3.3)$$

in which $f(\nu)$ is the fraction of incident quanta which are absorbed by the photoreceptor; $\epsilon(\nu)$ is the molar extinction function, $n$ is the mean molar concentration of chromophores and $\ell$ is representative of the outer segment length. The two coefficients $\delta(\nu)$ and $\phi(\nu)$ measure the effectiveness of absorbed quanta. The quantum yield, $\phi(\nu)$, is the ratio of the number of bleached pigment molecules to the number of absorbed quanta. Over the visual spectrum this is found to be roughly constant for all photopigments (0.68; Dartnall, 1968). The excitation efficiency, $\delta(\nu)$, is that fraction of the bleached molecules which contributes to excitation; it too, is felt to be constant over the visual spectrum (e.g. Mitchell and Rushton, 1971a,b). Other possible features which can alter the form of $A(\nu)$, are discussed in Sirovich (1976); these are at most small effects, since (3.3) is known to approximate closely the behavior of a pigment *in situ*.

We proceed by adopting Dartnall's (1953) approximation that the extinction spectrum, $\epsilon_i(\nu)$, is, to good approximation, translationally invariant when plotted vs frequency. Analytically this says that

$$\epsilon_i(\nu) = \epsilon(\nu - \nu_i)$$

where

$$\lambda_i = 2\pi\, \nu_i$$

is the maximal absorption wavelength, and $\epsilon(\nu)$ is to be regarded as a fixed function. Then, since the coefficients $\delta(\nu)$, $\phi(\nu)$ are "constant" over the visual spectrum, we can state that

$$A_i = A(\nu - \nu_i) = \phi\delta f(\nu - \nu_i).$$

It is convenient to write

$$\epsilon(\nu - \nu_i) = \epsilon_m \bar{\epsilon}(\nu - \nu_i),$$

where $\epsilon_m$ is the maximum of $\epsilon$ and then to define the density constant by

$$\kappa = \tfrac{2}{3} n \ell \epsilon_m. \qquad (3.4)$$

We then obtain

$$A_i = A(\nu - \nu_i) = \delta\phi(1 - 10^{-\kappa\bar{\epsilon}(\nu - \nu_i)}). \qquad (3.5)$$

Substituting this into (3.2) our equation for sensitivity becomes

$$S(\nu) = \left\{ \sum_{i=1}^{N} \beta_i A^p(\nu - \nu_i) \right\}^{1/p}. \qquad (3.6)$$

Thus the problem of determining the $A_i$ in (3.2) is replaced by the determination of the maximal absorption frequencies, $\nu_i$, and the "density constant", $\kappa$ (which, in the absence of better information, we take as the same for all cone receptors; Liebman, 1972).

Therefore, for given sensitivity, $S(\nu)$, and extinction spectrum, $\epsilon(\nu)$, we must determine the following: $N$; $\beta_i$, $i = 1, 2, \ldots, N$; $p$ and $\kappa$. The choice of a specific form of extinction spectrum will be discussed in the next section. Several methods of attack suggest themselves and we pursue a method which is both general and direct.

For the time being it is convenient to regard both $p$ and $\kappa$ as known; then, raising both sides of (3.4) to the $p$th power,

$$S^p(\nu) = \sum_{i=1}^{N} \beta_i A^p(\nu - \nu_i). \qquad (3.7)$$

This is now a linear equation (forgetting that $p$ and $\kappa$ are unknown). Next we allow $N \to \infty$ and regard the possible photoreceptor types as being continuously distributed on the frequency axis. Equation (3.7) then takes the form

$$S^p(\nu) = \int_{\omega_0}^{\omega_1} B(\sigma)\, A^p(\nu - \sigma)\mathrm{d}\sigma. \qquad (3.8)$$

The discrete form (3.7) is recovered if $B(\sigma)$ (the coupling, or weighting function) takes the form

$$B(\sigma) = \sum_{i=1}^{N} \beta_i \delta(\sigma - \nu_i) \tag{3.9}$$

where $\delta(\sigma - \nu)$ represents the delta function located at $\sigma = \nu$.

The limits of integration, although in principle infinite, can sensibly be set to extend over the visible spectrum. This we have written as the interval $(\omega_0, \omega_1)$ and for purposes of discussion it is unnecessary to make definite the "exact" interval of the visible spectrum.

*(b) Data treatment*

To solve (3.8) we require, a reasonably well defined spectral sensitivity function, $S(\nu)$. In practice various different estimates are obtained and a method of averaging is required. In dealing with the spectral sensitivity of pseudo-pigments, as depicted by (3.2), we assume (and will enlarge on this in the Discussion) that the exponent is essentially a neural property and does not vary appreciably from preparation to preparation; however, the coupling coefficients, $\beta_i$, reflect the amount and quality of the convergence onto a compound cell and this coupling can vary from cell to cell. This being the case, the precise values of $\beta_i$ are of less interest to us than the values of $\lambda_{max}$ of the contributing photopigments, $A_i(\nu)$, and the exponent, $p$.

Denote by $S_i(\nu)$ a single determination of sensitivity. According to (3.2) and the above assumption, this has the representation

$$S_i^p(\nu) = \sum_{j=1}^{N} \beta_{ij} A_j^p(\nu). \tag{3.10}$$

To prevent unequal weight being given to any one $S_i(\nu)$, each data set is first normalized with respect to its own maximum. Denoting by $n$ the number of separate data sets, we take the arithmetic mean of (3.10):

$$\bar{S}^p(\nu) = \frac{1}{n} \sum_{j=1}^{n} S^p(\nu) = \frac{1}{n} \sum_{j=1}^{n} \sum_{i=1}^{N} \beta_{ij} A_j^p(\nu).$$

Then, writing

$$\bar{\beta}_i = \frac{1}{n} \sum_{j=1}^{n} \beta_{ij}$$

we have

$$\bar{S}^p(\nu) = \sum_{i=1}^{N} \bar{\beta}_i A_i^p(\nu) \tag{3.11}$$

which preserves the form of (3.2). (Unless otherwise stated, we shall henceforth deal only with averaged data, according to (3.11), but will suppress the overbar notation.)

It is worth noting that with the above assumptions we can average together *any* data for which we believe the exponent and contributing pigments to be the same—the individual sensitivity curves need not necessarily have any resemblance to each other.

Another form of average, which is commonly used, is the geometric mean which is the anti-log of the arithmetic mean of individual log sensitivity functions. This form is appropriate if we assume that the different curves merely differ by scale factors. In practice this form of average does not differ greatly from the methods in (3.11), since the numbers in question vary only over a limited range.

*(c) Specific procedures*

We now briefly discuss the methods used to solve (3.8); for further details see Sirovich (1976).

Regarding (3.8), $S(\nu)$ is known, and $A(\nu)$, the absorption function is known to within a density constant (3.4). The remaining unknowns are the exponent, $p$, and the weighting function, $B(\sigma)$, which incorporates information about the number of photopigments and their $\lambda_{max}$'s. Corresponding to each value of $p$ there exists a solution. Certain of these solutions can be discarded since they violate some of the assumptions in the formulation. Of the other solutions, a unique one is selected on the basis of certain criteria; that the solution, $B(\sigma)$, give rise to the minimal number of photopigments and that their $\lambda_{max}$'s conform to the spectrophotometric information we might have from the particular species.

The method is based on the Fast Fourier Transform (FFT) algorithm (Cooley and Tukey, 1965), which requires periodic functions. To meet this we suitably extend, in frequency space, the functions $S$ and $A$ in (3.8) to be periodic—in which case $B(\sigma)$ is also periodic. This is done in such a way as to minimize "aliasing" and "wrap-around" errors (Cooley, Lewis and Welch, 1967). Another requirement is that our functions be sampled on a mesh uniformly spaced in frequency. Since neither of the functions $S$ and $A$ are typically given in a continuous form they must be so extended: this is accomplished using third order spline fits (Ahlberg, Nilson and Walsh, 1967). These fits pass exactly through all data points in a smooth fashion.

An additional detail concerns the density coefficient, $\kappa$, in (3.4), and which enters into any pigment's absorption function, $A(\nu)$, as shown in (3.5). To solve (3.8) we assume $\kappa$ to have the same value throughout. We can also expect that the given spectral sensitivity function, $S(\nu)$, will, at very long wavelengths, be in asymptotic agreement with the contributing photopigment having the longest $\lambda_{max}$ (see Abramov, 1968, 1972, for applications of this argument). In brief, we find $\kappa$ by adjusting the density of the pigment with the longest $\lambda_{max}$ to fit $S(\nu)$ at long wavelengths.

Another feature of the general method of solving (3.8) follows from the fact that it is a Fredholm equation of the first kind (Pogorzelski, 1966). Such equations have one particularly unpleasant feature, namely that small rapidly oscillating changes in the sensitivity $S(\nu)$ in (3.8) can produce a large change in our solution for the coupling function $B(\sigma)$. Since experimental data can never be relied upon beyond some small error, this becomes a severe problem in the solution of (3.8). The difficulty is resolved by subtracting from $S(\nu)$ its small rapidly oscillating components, in a fashion such that $S(\nu)$ still lies within the error bounds under which it was obtained. See Sirovich (1976) for further details regarding this filtering procedure.

## (4) RESULTS

We now apply the various methods described above to some specific sets of data, starting with the

680 nm "pigment" reported by Naka and Rushton (1966a,b,c), and which we assert to be a pseudo-pigment. Their data were obtained from horizontal cells (S-potentials) in the retinae of cyprinid fishes (mostly tench). In Fig. 2a (symbols) we have redrawn several of their estimates of this 680 nm spectral sensitivity function. We also know that each of these curves was obtained from a set of parallel response vs log intensity curves—thus they fulfill our prerequisites for being derived from a pseudo-pigment. Since there are relatively few points from any one cell and variability is high, we have averaged the results (following the method given earlier) to obtain a more representative form for analysis; this average is included in Fig. 2a as the smooth curve. In Fig. 2b we compare the average 680 nm curve with a smooth function (dashed curve) derived from the mean plot given by Marks (1963, 1965) for the spectrum of the long-wavelength cones in goldfish (also a cyprinid); its $\lambda_{max}$ is 625 nm, which is the longest value that has been found by spectrophotometry.

The average 680 nm function will represent $S(v)$ in equation (3.8). For a solution we must also specify $A(v)$; that is, we must decide on some standard photopigment that will in effect serve as the nomogram. The differences among the various standards that have been proposed are not significantly greater than the relatively slight narrowing of pigments with long $\lambda_{max}$. But this does place some limitations on the precision of the method of solution we have chosen here.

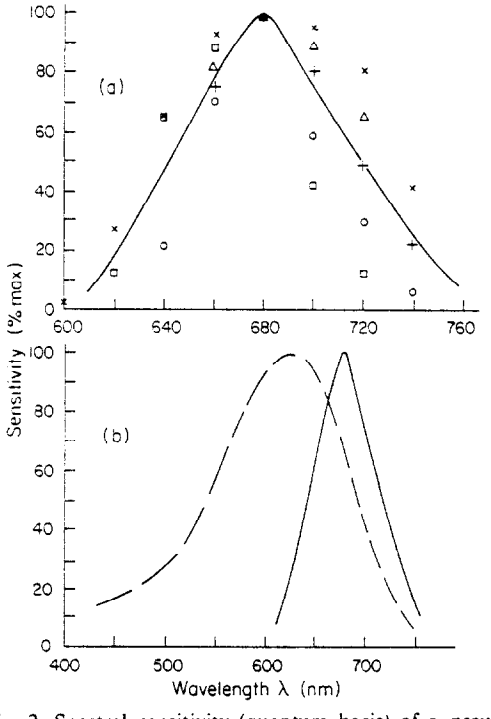


Fig. 2. Spectral sensitivity (quantum basis) of a pseudo-pigment peaking at 680 nm and recorded from S-potentials in the tench retina. (a) Symbols show sensitivities of several different cells—from Naka and Rushton (1966a,b,c). The smooth curve is an average (see text for details) of these sensitivities. (b) The average sensitivity of the 680 nm pseudo-pigment (solid line) is compared with the sensitivity of the long-wavelength cone pigment ($\lambda_{max}$ = 625 nm) in goldfish, as given by Marks (1963, 1965).

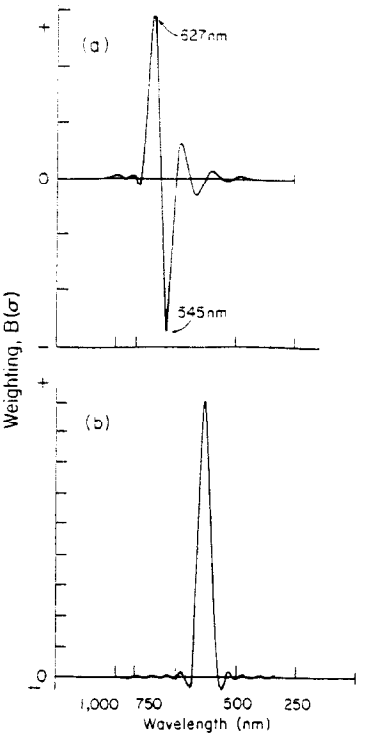


Fig. 3. (a) Weighting function, $B(\sigma)$, obtained from the solution of equation (3.8) for the 680 nm pseudo-pigment in Fig. 2. The $\lambda_{max}$'s of the two photopigments given by the solution are indicated. (b) Single delta function smoothed and filtered in the same way as the function in (a)—see text for details.

Since Naka and Rushton's pseudo-pigment peaks at so long a wavelength, the standard photopigment we chose for the analysis was the 625 nm curve given by Marks; it is slightly narrower than one generated by the Dartnall (1953) nomogram (see Fig. 2b). This curve we use for the extinction function, $\epsilon(v)$, which together with the density, $\kappa$, in (3.4) is then used to construct $A(v)$ in (3.8).

In Fig. 3a we show that weighting function, $B(\sigma)$, obtained from the "best" solution of the 680 nm pseudo-pigment. The abscissa is a frequency axis, although for convenience we have labelled it in nm. The ordinate gives the actual values of the weighting function, and, of course, is in units of reciprocal frequency. From this we would like to recover its discrete form (3.9) on which a reconstruction would be based.

In view of all the filtering and smoothing employed in our methods we cannot expect really sharp, narrow, "delta" functions. In fact in Fig. 3b we show the limit that can be achieved by our method; there we show a single delta function that has been filtered and smoothed in exactly the same way as the function in Fig. 3a. Keeping this limitation in mind, our interpretation of 3a is as follows: The function is composed of two delta functions, one positive and the other negative going; the remaining oscillations can be neglected. The positive one represents a photopigment with $\lambda_{max}$ = 627 nm, while the negative one has its $\lambda_{max}$ = 545 nm. The strengths of the delta functions, which are the coupling coefficients or $\beta_i$ in (3.6), are simply given by the signed area under each of these peaks. The exponent for this solution was 0.4

and the density, $\kappa$, (3.4) was found to be 0.35. (These values are discussed later.)

We now have all the information needed to construct a spectral sensitivity curve to compare with the original data. In carrying out the construction an additional step was inserted at this stage. We observe in Fig. 3a that the oscillations we neglected really only exist at wavelengths somewhat shorter than the peak of the second (545 nm) photopigment. This can in part be attributed to use of a standard pigment with a relatively narrow spectrum. We compensated for this in the construction by using the Dartnall (1953) nomogram to generate the spectrum of the 545 nm pigment and Marks' (1963, 1965) 625 nm pigment as the basis of the 627 nm component. The close agreement between the constructed curve and the original data will justify this procedure.

The parameter values from the best solution, shown in Fig. 3a, were used in equation (3.6) to construct our curve. The result is shown as the continuous plot in Fig. 4a, together with the original 680 nm data (symbols); also included is the equation with the precise values used in the construction. The spectral sensitivity of our pseudo-pigment, like the 680 nm curve, is very narrow and is essentially at zero for all wavelengths shorter than 580 nm. We had earlier said that, at very long wavelengths, the sensitivity of the long wavelength pigment should agree asymptotically with the data. In Fig. 4b we illustrate this, as well as the

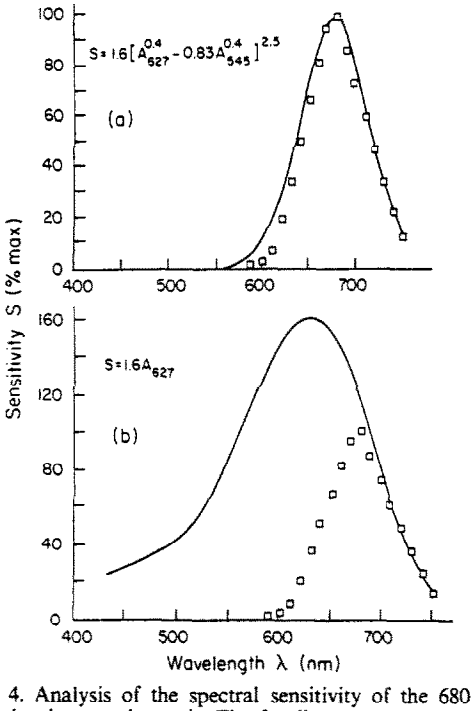


Fig. 4. Analysis of the spectral sensitivity of the 680 nm pseudo-pigment shown in Fig. 2; all curves are given as per cent of maximum sensitivity of this pseudo-pigment. (a) Symbols delineate the average spectral sensitivity of the 680 nm pseudo-pigment. The curve, generated by the inset equation, is the constructed pseudo-pigment obtained from analysis of the original data. (b) Symbols again denote the 680 nm pseudo-pigment. The curve, generated by the inset equation, shows the contribution of the long-wavelength pigment to the constructed pseudo-pigment in (a). Note the asymptotic agreement at long-wavelengths.

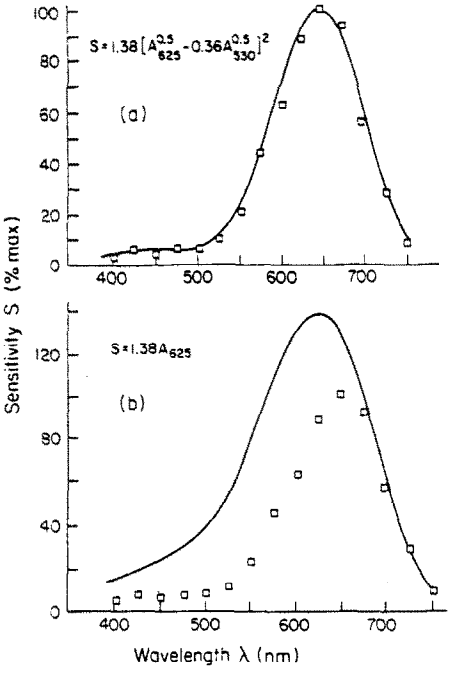


Fig. 5. Analysis of the mean spectral sensitivity of a 650 nm pseudo-pigment recorded from ganglion cells in the goldfish retina (Spekreijse *et al.*, 1972). All curves are given as per cent of maximum sensitivity of this pseudo-pigment. (a) Symbols show the mean sensitivity of the 650 nm pseudo-pigment. The curve, generated by the inset equation, is the constructed pseudo-pigment obtained from analysis of the original data. (b) Symbols again denote the 650 nm pseudo-pigment. The curve generated by the inset equation, shows the contribution of the long-wavelength pigment to the constructed pseudo-pigment in (a). Note the asymptotic agreement at long-wavelength.

appropriateness of the value we obtained for the density of the photopigments used in the above construction; the original data are shown together with the sensitivity of a 627 nm photopigment, with density of 0.35, and scaled according to this pigment's contribution to our constructed curve (see inset equation).

Another set of data of some interest is provided by Spekreijse, Wagner and Wolbarsht (1972) who measured the spectral sensitivity of ganglion cells in goldfish retinae. The curve (shown as symbols in Fig. 5a) is appreciably narrower than that of known photopigments and also peaks at a very long wavelength (650 nm); however, as with the 680 nm data, these data were claimed to represent the sensitivity of a single class of cones.

The goldfish data are interesting because sensitivity was measured for a response criterion close to ganglion cell threshold. The curve could be that of a pseudo-pigment if Spekreijse *et al.* (1972) had evidence to show that the spectral sensitivity function was indeed independent of the response criterion chosen (i.e. parallel response vs log intensity curves). However, since no information is given about this, we might still assume that the arguments leading to equation (2.14) could apply.

The data (symbols) in Fig. 5a were analyzed in exactly the same way as the 680 nm pseudo-pigment. The best solution of the goldfish data also showed two photopigments, one essentially the same as before

($\lambda_{max} = 625$ nm) and the other with $\lambda_{max} = 530$ nm; density was 0.25 and exponent, $p$, was 0.5. For the construction we used the same two standards used for the 680 nm curve. The construction is shown as the continuous curve in Fig. 5a together with the specific equation (inset). In Fig. 5b we show, as before, the asymptotic agreement of the long wavelength pigment in our construction.

## (5) DISCUSSION

### (a) *Pseudo-pigments*

We have argued that spectral sensitivity functions differing greatly from those of standard photopigments are most probably the result of interactions among receptors containing unlike photopigments.

Starting from the general form taken by a response function due to one or more photopigments (2.8), the property of parallel response vs log intensity curves leads to the similarity form shown in (2.12). This is especially noteworthy since the above property has been used by others (e.g. Naka and Rushton, 1966a,b,c; De Valois, 1965) as the major criterion for identifying responses driven by a single photopigment. A major goal of our investigation has been the resolution of a pseudo-pigment into its component photopigments. Fundamental to our analysis is the form

$$\mathscr{A}(\nu) = \left[\sum_{i=1}^{N} \beta_i A_i^p(\nu)\right]^{1/p}. \qquad (2.11)$$

To achieve this form we made a mild differentiability assumption. From our analysis of the 680 nm pseudo-pigment we can now indicate an *a posteriori* verification of this property. We start with the form $\bar{R} = \bar{R}(I\mathscr{A}(\nu))$, and with $\mathscr{A}(\nu) = \mathscr{A}(A_1, A_2)$, homogeneous of degree one. We also assume that $A_1$ and $A_2$ are now known—they are as obtained from our analysis of the 680 nm data. We can show that $\mathscr{A}(A_1, A_2)$ must have the form given by (2.11).

Imagine a series of experiments, involving monochromatic flashes, that will determine $\mathscr{A}(A_1, A_2)$. For example, we can vary $I$ and $\nu$ and hold $\bar{R}$ fixed (or, alternatively, hold $I$ fixed and record $\bar{R}$ varying with $\nu$). In any case, we determine $\mathscr{A}(A_1, A_2)$ on some curve in the $(A_1, A_2)$-plane traced out by $A_1(\nu)$ and $A_2(\nu)$ as $\nu$ varies. $\mathscr{A}(A_1, A_2)$, which is defined in the quadrant $A_1, A_2 > 0$, is now completely known, since the homogeneity property tells us that $\mathscr{A}$ varies linearly on rays from the origin ($\mathscr{A}(xA_1, xA_2) = x\mathscr{A}(A_1, A_2)$). The desired experiments are in fact contained within the experiments of Naka and Rushton (1966a,b,c). Since their response vs log intensity curves are parallel, we can choose any fixed value of $\bar{R}$ and obtain $\mathscr{A}(\nu)$. Our construction (Fig. 4a) shows that (2.11) is in close agreement with $\mathscr{A}(\nu)$, and hence is in close agreement with $\mathscr{A}(A_1, A_2)$ everywhere. This certainly lends justification to the differentiability assumption under discussion.

We next consider the specific photopigments indicated by our analysis. Spectrophotometric studies of the pigments in the goldfish retina all show that there are three cone pigments with $\lambda_{max}$'s at approx 450, 530 and 625 nm (see Introduction). Our analysis (Fig. 4a) of the data of Spekreijse *et al.* (1972) concurs with this; we find the 650 nm pseudo-pigment to be derived from pigments with $\lambda_{max}$'s at 530 and 625 nm respectively. For the density coefficient, $\kappa$, in (3.4) we obtained a value of 0.25. It is somewhat difficult to interpret this value since we do not really know if the pigments in these experiments with isolated retinae were at full concentration. However, if we assume that the effective length of the outer segment is about 25 $\mu$m, our value agrees fairly well with values obtained by Marks and by Liebman (Liebman, 1972). As far as tench is concerned, there are few published spectrophotometric data (Lythgoe, 1972). However recent microspectrophotometry shows that the cone pigments have their $\lambda_{max}$'s at approx 615, 532 and 450–455 nm (Dartnall and Liebman, personal communication). Our values of 627 and 545 nm do not agree with the spectrophotometry as closely as did our values from goldfish data; however, it should be noted that the data from tench (Fig. 2a) were much "noisier".

### (b) *Model of a pseudo-pigment*

(i) *Model.* A pseudo-pigment is the result of a specific sort of interaction between responses driven by different photopigments. As we have shown, this interaction must be describable by equation (2.12). However, our analysis has not been based on any specific physiological system. The particular form we state, (2.12), can be realized in many ways. We shall present here a network with the requisite properties, but we must emphasize that it is not the only one that agrees with our analysis.

In Fig. 6 we show a simple model of a pseudo-pigment derived from two photopigments, $A_1$ and $A_2$. The boxes in the figure represent different stages at which the various components, explicit or implicit, of (2.12) might be realized; each stage does not necessarily represent a single anatomical entity. We shall deal first with a simplified form that excludes the portions drawn with dashed lines.

The "photopigments" stage needs no comment here except to reiterate that we assume these pigments to

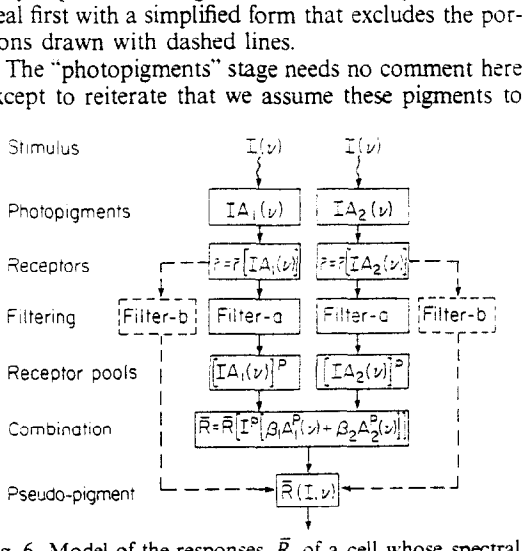


Fig. 6. Model of the responses, $\bar{R}$, of a cell whose spectral properties are those of a pseudo-pigment. The boxes denote different stages, as labelled, from light transduction to responses of the given cell; they do not necessarily each refer to a single anatomical entity. The forms and equations in the boxes are all readily related to equation (2.12). $I$ and $\nu$ denote intensity and frequency of light, and $A_i$ denotes the absorption coefficient of a photopigment. The nature of the filters, and other details, are given in the text.

have approximately standard spectra, such as might be generated from the Dartnall (1953) nomogram. The "receptors" stage does not specify any particular form for the intensity–response function, except that it be in the similarity form (2.3). Recordings from single receptors have indicated that their responses, $\bar{r}$, are best described by the following:

$$\bar{r} = \frac{gIA(v)}{IA(v) + k}, \tag{5.2}$$

where $g$ and $k$ are constants (e.g. Baylor and Fuortes, 1970; Grabowski, Pinto and Pak, 1972; Fain and Dowling, 1973; Baylor and Hodgkin, 1973). Of course, this function has the similarity form we require.

We shall ignore for the moment the "filtering" stage except to note that some form of filtering must of necessity be included—the bandwidth of any real system is limited. The importance of this stage will become apparent when we deal with chromatic adaptation.

We now deal with the "receptor pools" and "combination" stages. It is perhaps artificial to separate these stages, as shown in Fig. 6; they may in fact occur together. The only essential requirement of our analysis is that the pigment terms, $A(v)$, be each raised to the $p$th power prior to combination; this is a mathematical requirement and does not necessarily imply that there exists a specific location at which a power function operates. Even for $p \neq 1$, our formulation does not contradict completely linear stages; for example, individual receptors could be linear over large ranges of intensity (which is implicit in (5.2)) and the pseudo-pigment could be linear (as in (2.13)). The algebraic summation of the responses of different groups of receptors is supported, for goldfish, by Spekreijse and van den Berg (1971) and Levine and Abramov (1975). Furthermore, Easter (1968) and Levine and Abramov (1975) had concluded that when responses of a ganglion cell were driven solely by receptors all containing the same photopigment, the responses of the pooled receptors were approximately proportional to the square root of intensity.

(ii) *Chromatic adaptation.* We must now consider how the model in Fig. 6 would operate when chromatic adaptation is used to desensitize differentially some of the spectral mechanisms associated with a given cell. The question is why, in many cases, this isolates a pseudo-pigment rather than isolating responses due just to one photopigment. However, the spectral properties of at least some cells (that in our terms appear to be pseudo-pigments) remain the same regardless of adapting wavelength—only absolute sensitivity is changed; this was taken as evidence that the unusual sensitivity curves of these cells represented a true but strange pigment (Daw and Beauchamp, 1972). Moreover, Naka and Rushton (1966a,b,c) occasionally used chromatic adaptation to isolate their 680 nm pseudo-pigment.

Consider the following case of chromatic adaptation: pigment $A_2$ is absolutely more sensitive than $A_1$ to the adapting wavelength. Now assume that adaptation occurs at any stage prior to combination of responses due to $A_1$ and $A_2$. In this case the contribution of $A_2$ to the pseudo-pigment will be reduced, and, in the limit, such adaptation will isolate responses due just to $A_1$. Since this is not always observed, we postulate that this adaptation occurs after combination.

A remaining difficulty is that individual receptors maintain a level of hyperpolarization for the duration of a stimulus (Tomita, 1970). Consider the responses of the network in Fig. 6 to the steady adapting light mentioned above. The receptors containing $A_2$ will operate at some high level on their response function, while the other receptors (with $A_1$) are at a low level. Now, in response to a test flash, the magnitudes of the two inputs to the pseudo-pigment's summation point can no longer be the same as before adaptation; i.e. the spectral sensitivity of the pseudo-pigment would depend on the wavelength and intensity of the adapting light. To circumvent this problem we postulate that "Filter-a" in Fig. 6 represents a high-pass, linear, filter (allowing only transient responses to pass). However, steady-state information is required at or beyond the combination stage, otherwise a continuous adapting light would not reduce sensitivity throughout the time it was present. We allow for this by the pathway through "Filter-b" (dashed lines in Fig. 6). This filter is a low-pass filter that excludes transient responses; it provides some form of the tonic signal needed to change over-all sensitivity of the pseudo-pigment.

(iii) *Implications of the model.* Assume that something like (5.2) applies at the receptors. Then, for brief flashes of light, the response versus log intensity curves measured from the pseudo-pigment stage will be parallel. Even if the flashes are superimposed on an adapting background the same will still be true, until one or other of the receptor types starts approaching its response saturation level. At that point the spectrum of the pseudo-pigment will begin to distort, since the transient input (through Filter-a) then becomes dependent on the background level.

Now consider stimulus flashes of relatively long duration. If we examine the response, $\bar{R}$, during the initial portion of the stimulus, the conclusions in the above paragraph apply. But at later times during the stimulus the effect of Filter-b will become apparent; i.e. the spectral sensitivity of $\bar{R}$ must change. This becomes obvious if we think of pseudo-pigments of the type analyzed earlier in which the responses from $A_2$ are subtracted from those due to $A_1$: the $\lambda_{max}$ of $\bar{R}$ will probably lie outside the $\lambda_{max}$'s of $A_1$ and $A_2$. However the responses from each Filter-b must be combined in some non-opponent fashion in order to establish chromatic adaptation. Therefore the $\lambda_{max}$ of $\bar{R}$ will shift, as time evolves, to a position not outside the interval of the $\lambda_{max}$'s of $A_1$ and $A_2$. In fact if the stimulus is long enough and if $\bar{R}$ is from a cell capable of some tonic response, then the steady-state responses will reflect only the spectral sensitivity as observed through Filter-b.

These, and other implications of the model are open to experimental verification and this is under way.

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