ON SOME MATHEMATICAL TECHNIQUES FOR THE ANALYSIS OF VISUAL SPECTRAL SENSITIVITIES

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ABSTRACT Starting from known spectral properties of visual photopigments and photoreceptors, a mathematical construction of neural response functions to visual stimuli is obtained. Included in this is a somewhat general derivation of the univariance principle. Temporal dependence of response on stimulus is included in the formulation.

Special attention is given to the case of flash stimuli and their resulting spectral sensitivities. This formulation is applied to certain physiological spectral sensitivity measurements that are at variance with known spectrophometric results. An analysis of the data in these cases suggests that they correspond to "pseudo-pigments" arising from the neural interaction of several photopigments. The method of analysis is constructive and identifies the λ-max's of the interacting photopigments. These are found to be in good agreement with existing spectrophotometric measurements.

INTRODUCTION

Visual sensitivity is well known to vary with the wavelength—or equivalently with the frequency[1]—of the incoming light. This dependency originates in the individual photoreceptors (i.e. rods and cones) of the eye and in particular in the photon absorption properties of the molecular photopigments. The functional dependence of sensitivity (or absorption) on light frequency has, to varying degrees, been investigated by spectrophotometry, physiology, and psychophysics (see Abramov, 1972, Abramov and Gordon, 1973a, and MacNichol et al. 1973, for surveys). From these diverse fields has evolved a more or less standard picture of the sensitivity dependence on frequency, both in regard to its shape (and hence the band characteristics of receptors) and to the loci of maximal sensitivity (see for example, Dartnall, 1962, and Lythgoe, 1972).

In recent years, starting with the experiments of Naka and Rushton (1966a, b, c) and continuing with those of Spekreijse et al. (1972), Daw and Beauchamp (1972), and Witkovsky (1967), a disturbingly consistent body of physiological data has emerged which contradicts the standard view of photoreceptor characteristics. In brief, these

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[1] For a number of reasons frequency, rather than wavelength, is the natural quantity to consider. However, the use of wavelength is well-entrenched in the literature, and we use it, expressed in nanometers, to designate the locus of maximal absorption, which, following the customary practice, we denote by λ-max.

investigators found evidence of unusually narrowly tuned photoreceptors having a maximal sensitivity relatively deep in the "red" or low-frequency spectrum, and well away from what was supposed possible on the basis of spectrophotometric findings. Since the aforementioned investigations are all physiological and describe recordings made beyond the receptor level, it is natural to inquire whether such unusual pigments are a result of neural interaction rather than some new type of photoreceptor.

In the above-mentioned investigations the resulting data were subjected to a variety of criteria before it was concluded that a new type of photoreceptor had been found. In what follows we intend to build up an analytical picture of visual response to light using only a few simple assumptions that meet the experimental criteria. This will then be used to reexamine the arguments establishing the existence of the new photopigments. From this it will be shown that the previous arguments were incomplete, and that the existence of new photopigments was not established. Moreover, the analysis indicates that a more probable explanation for these lies in the neural interaction of photoreceptors with known photopigments. In the present paper we lay emphasis on general analytical methods for dealing with such problems. A comparison paper, Sirovich and Abramov (1977), contains an extensive discussion of both experiments and physiological models.

PHYSICAL ASPECTS OF LIGHT ABSORPTION

As a point of departure we consider the various effects at work in photon capture by a photoreceptor. This we do in somewhat more detail and generality than is found in the standard treatments (see Dartnall, 1972 and 1957, and Rodieck, 1973).

The light-absorbing portion of a photoreceptor (rod or cone) is the outer segment (Fig. 1). The architecture is lamellar, as indicated, with the chromophores (light-absorbing portion of the pigment molecules) randomly oriented in planes parallel to the lamellae. A light quantum may only be absorbed when its electric vector lies roughly parallel to the chromophore.

With monochromatic light of frequency, ν, the light flux incident on the lamellae (expressed as quanta per unit time per unit area) moving to the right, parallel to the axis of an outer segment, is designated by $\hat{I}(x; \nu)$, where x measures the axial distance from the outer segment base. If we assume an arbitrary orientation for a chromophore (as would be the case in suspension), its capture cross-section for light of frequency, ν, is designated by $\hat{\epsilon}(\nu)$ (having dimensions of area) and referred to as the extinction function. Allowing for the possibility of more than one type of photopigment in an individual photoreceptor, we denote the extinction functions by $\hat{\epsilon}_i(\nu)$ and their respective concentrations by $n_i(x)$, $i = 1, \ldots, M$. If the photoreceptor cross-section is denoted by $a(x)$, the quantal flux passing a station, x, is $\hat{I}(x; \nu) a(x)$. The decrementing of this quantity is due to quantal absorption and to wall losses. Quantal absorption, or catch, $d\hat{c}$, in an increment dx, is given by

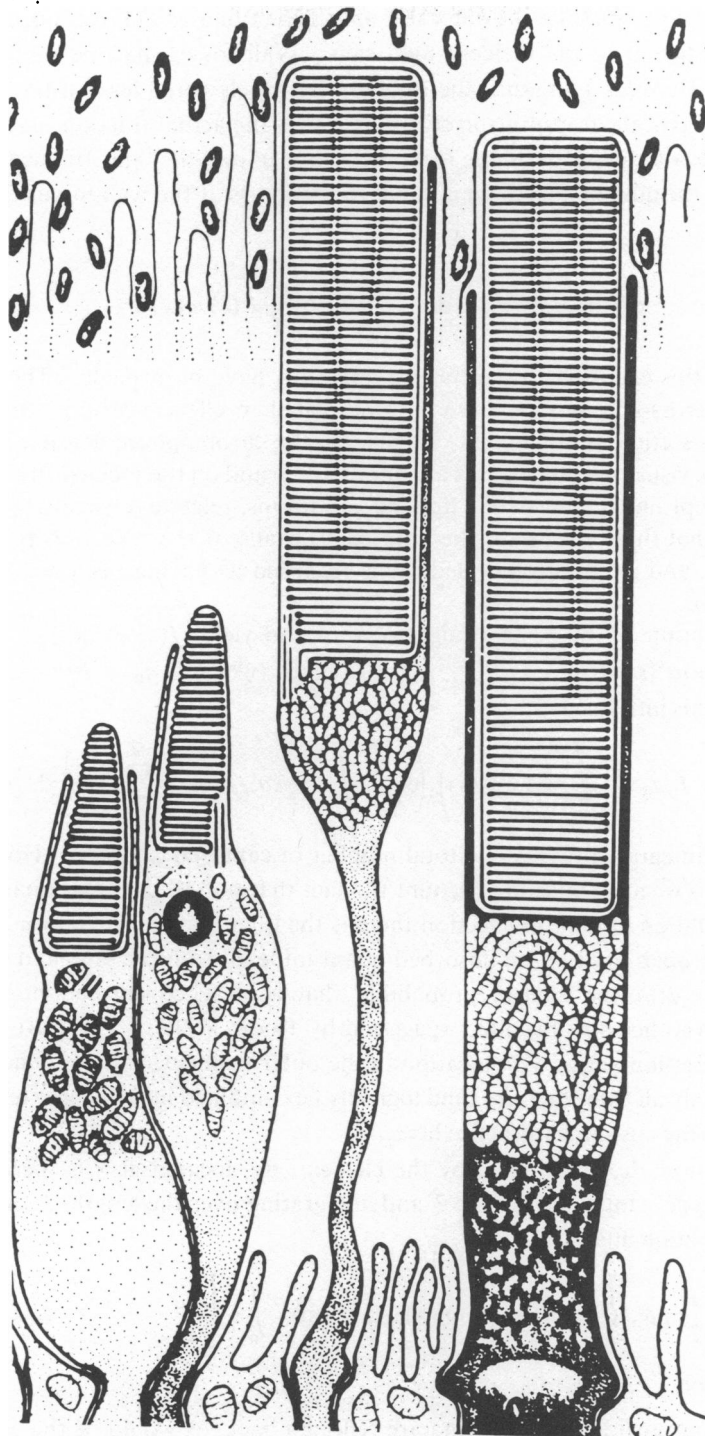$$d\hat{c} = 3/2 \, (\hat{I}a) \sum_{i=1}^{M} n_i(x) \, \hat{\epsilon}_i(\nu) \, dx \qquad (1)$$

FIGURE 1 The upper two receptors are schematics of cones, the lower of two rods. The parallel lines represent lamellae in which the chromophores are randomly oriented. Photons enter from left and move parallel to a photoreceptor axis.

The factor 3/2 appears since the outer segment chromophores are restrained to lie in a plane perpendicular to the incident light rays. Wall losses may be represented by $\hat{I} \alpha(a', \nu) da$. Here $\hat{I} da$ represents the quantal flux which would leave if the walls were transparent and α, the transmission coefficient, gives the actual fraction that leave. As indicated, the latter depends on the local wall slope a' and the light frequency, ν. The decrement in the quantal flux $d(\hat{I}a)$ is equal to the losses at the wall minus the absorption, i.e.

$$d(\hat{I}a) = \hat{I}\alpha(a', \nu) da - 3/2 \hat{I}a \sum_{i=1}^{M} \hat{\epsilon}_i(\nu) n_i(x) dx \qquad (2)$$

In obtaining this equation, several approximations have been made. The details of the receptor cross-section have been ignored and it in effect has been replaced by a circular cross-section of equal area. In general, the chromophore density, n, depends on time (since visual photopigments are photolabile) and on the incident flux history—however, except under very strong lighting conditions, relative few chromophores are bleached so that these effects can be ignored. Details of the reflection process have been ignored, and in effect a reflected ray is regarded as having been restored to the incident beam.

The integration of Eq. 2 is straightforward and yields $\hat{I}(x; \nu)$ $a(x) = \hat{I}_0 a_0$ exp$\cdot$ $\int_0^x \{\alpha(a'(s), \nu)a'(s)/a(s) - 3/2\sum_{i=1}\hat{\epsilon}_i(\nu)n_i(s)\} ds$ (where $I_0 a_0 = \hat{I}(0; \nu) a(0)$) and introducing this into Eq. 1:

$$d\hat{c} = \hat{I}_0 a_0 \frac{3}{2}\left(\sum_{i=1}^{M} n_i(x)\hat{\epsilon}_i(\nu)\right)\left(\exp \int_0^x \left\{\alpha a'/a - \frac{3}{2}\sum_{i=1}^{M} \hat{\epsilon}_i n_i\right\} ds\right)dx. \qquad (3)$$

This may be integrated to give the total number of captured photons. However, before doing this we must take into account the fact that not all captured quanta lead to eventual excitation. In this connection there is the quantum yield, $\gamma(\nu)$ (or efficiency), the relative probability that an absorbed quantum leads to bleaching, and the excitation efficiency $\phi(\nu)$, the relative probability that a bleached chromophore leads to excitation. Another possible effect suggested by Rodieck (1973, Ch. XB) is that the degree of excitation varies with location in the outer segment, and we denote this by $d(x)$. (Certainly all three factors could logically be combined into a single term; we are merely following customary practice here.)

The excitation, de, contributed by the element, dx, located at x is therefore $de = \gamma(\nu) \phi(\nu) d(x) d\hat{c}$. Introducing Eq. 3 and integrating over the length, l, of the outer segment we obtain finally,

$$e = \hat{I}_0 a_0\left(\frac{3}{2}\gamma\phi \int_0^l \left\{d(x)\left(\sum_{i=1}^{M} n_i(x)\hat{\epsilon}_i(\nu)\right)\exp \int_0^x \left[\frac{\alpha a'}{a} - \frac{3}{2}\sum_{i=1}^{M} \hat{\epsilon}_i n_i\right] ds\right\} dx$$

$$= I_0 A(\nu). \qquad (4)$$

(If a significant portion of the quanta are reflected back, as would be the case when a tapetum is present, l should be doubled). $I_0 = \hat{I}_0 a_0$ is the quantal flux at the base, and

we refer to $A(\nu)$ generically as the absorption, although it more specifically relates to excitation.

The form for e (Eq. 4) is quite elaborate and incorporates a number of effects beyond present measurement. Of importance to us here is that it has the form of a product of I_0 and a function of frequency only, which of course is a consequence of the linearity of Eq. 2. (Nonlinear effects would appear at high light intensities when the time variation of $n(x)$ enters.)

There seems to be no evidence that a typical photoreceptor contains more than one type of photopigment and we assume that only one type is present and for convenience write $n_0 = (1/l) \int_0^l n(x)\,dx$. What is known of the refractive properties of photoreceptors suggests that they behave as light funnels, at least to lowest order, and it is reasonable to assume that little of the incident light is lost at the walls, and we therefore set α to zero in the above. Also, in the absence of any information to the contrary, we may assume that photon capture produces identical excitation at all loci, i.e. we take $d(x) = 1$. On introducing these simplifying assumptions into Eq. 4 we obtain

$$e = I_0 A(\nu) = I_0 \phi(\nu) \gamma(\nu)(1 - \exp\{-(3/2)\hat{\epsilon}(\nu) n_0 l\}), \tag{5}$$

usually referred to as the effective quantal capture rate. The quantal efficiency has been found to be constant (≈ 0.68) over the visual spectrum and although less is known about the excitation efficiency, the indication is that it too is constant and probably near unity (see pp. 266–268 of Rodieck [1973] for references). We mention in passing that this author incorrectly places the coefficients ϕ and γ in the exponent of Eq. 5. We also note in passing that the effect of oil droplets, found in avian and reptilian retinae, can also be included in the treatment. The droplet generates a frequency-dependent transmission coefficient which multiplies the excitation in Eqs. 4 and 5. In this way a single photopigment can produce more than one type of excitation.

More generally light arriving at the photoreceptor[2] is a function of the frequency, ν, as well as time, t, $I(t) = \int \mathcal{I}(\nu, t)\,d\nu$, where $I(t)$ is the total quantal arrival rate at a_0 (photons per unit time) comprising all frequencies and $\mathcal{I}(\nu, t)$ is density of light intensity (on the frequency axis). The quantity which we called excitation is now given by $e(t) = \int \mathcal{I}(\nu, t) A(\nu)\,d\nu$, where the absorption function, $A(\nu)$, is defined by Eq. 3.

CONSTRAINTS ON NEURAL RESPONSE FUNCTIONS

In the absence of other stimuli the response of a neuron depends only on illumination for excitation. In particular if we denote the response of an isolated receptor by r we can write

$$r = r\{t; e(t')\} \tag{6}$$

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[2] It should be noted that we are speaking of photon arrivals at a photoreceptor and not the emission rates at the light source. The two are different not only because of geometrical effects but also because of transmission losses through the intervening medium, some of which are strongly frequency-selective. Unless otherwise stated, all light intensities refer to measurements at the photoreceptor, i.e. transmission corrections will be regarded as having been made.

The presence of curly brackets in Eq. 6 indicates that r is a functional of $e(t)$, for all $t' \leq t$: i.e. r in principle depends on the entire past history of the excitation $e(t)$. Also the photoreceptor may sensibly be supposed not to have an absolute sense of time and Eq. 6 is therefore translationally invariant in time.

Flash Stimulus

Without further information nothing more can be said about r in Eq. 6. As an example important for our investigation, we consider a stimulus that is a flash or pulse of mono-chromatic light. The pulse may be idealized by a delta function if its duration is less than the "complete summation time" (known as the Bunsen-Roscoe law in photo-chemistry and Bloch's law in psychophysics; see Abramov and Gordon, 1973b). Dis-cussion of stimulus duration may be conveniently avoided by restriction to a uniform stimulus of fixed duration. We write

$$g = \begin{cases} I^0 \, \delta(\nu - \bar{\nu})/t^0, & 0 \leq t \leq t^0 \\ 0, & t > t^0 \end{cases}$$

where I^0 is the quantal content of the flash incident on $a_0, \delta(\nu - \bar{\nu})$ is a delta function centered at frequency $\bar{\nu}$, and t^0 is the flash duration.

In this case

$$e = \begin{cases} I^0 A(\nu)/t^0, & 0 \leq t \leq t^0 \\ 0, & t \geq t^0 \end{cases}$$

By substituting into Eq. 6, the response can now be regarded as an ordinary function of time,

$$r = r(t; I^0 A(\nu)) = r(t), \tag{7}$$

which is completed specified by the parameter $I^0 A(\nu)$.

The time dependence can be also eliminated by taking an appropriate feature (functional) of $r(t)$. For the photoreceptor itself (since it gives rise to a slow poten-tial) one can consider $\bar{r} = \bar{r}(I^0 A(\nu)) = \max_t | r(t; I^0 A(\nu)) |$ where response away from the resting state is measured. For cells registering action (spike) potentials, a variety of other features suggest themselves, e.g. maximum spike rate, average spike rate, latency to first spike, etc. In general we regard $\bar{r}$ as generic for any well-defined feature. In brief, we see that in spite of the possible complexity in Eq. 6, restriction to uniform, fixed-duration flashes reduces the response to a one-parameter family of time-courses, which is further reducible to a consideration of any well-defined feature of the time-course.

Univariance

Naka and Rushton (1966a) enunciate the principle of univariance, "The signal from each cone depends only upon the rate at which it is effectively catching quanta; it does not depend on the associated wavelength." Our discussion suggests that this be modi-

fied to read "Photoreceptor response depends only on the time history of excitation" (with excitation possibly equal to capture rate). In other words, all stimuli having the same time histories $\int \mathcal{I}(\nu, t) A(\nu) \, d\nu$ independently of the spectral content of the illumination $\mathcal{I}(\nu, t)$ produce identical responses. It should be remarked that the preceding analysis constitutes a derivation of this principle.

In referring to the flash experiment, some cases in the literature stress the fact that receptor response cannot signal information on both frequency and intensity. Since $\bar{r}$ is a scalar, it conveys just one piece of information, so this is a fairly weak statement. Under general conditions we have shown $\bar{r}$ is of similarity form, i.e. $\bar{r}$ is a function of the product $I^0 A(\nu)$—excitation or quantal catch as the case may be—which is a more effective statement of the circumstances.

Response Functions with Multiple Inputs

Although the above discussion dealt with single photoreceptor response, it applies without change to the case of a cell receiving inputs from a single type of photoreceptor. And in particular to the response of a receptor "wired" to like photoreceptors. For in these cases the response depends on a single excitation and Eq. 7 still applies even though the individual photoreceptors contributing to it may have different time-courses.

In most investigations (and for all discussed in this paper), response recording is done at a level beyond the photoreceptors themselves. Admittedly this complicates matters, but some progress may be made by recognizing that it is unnecessary to discuss the detailed wiring leading to the test cell. For if there exist N photoreceptor types (*i.e.*, N different photopigments), then the response of a specific test cell depends at most on the excitation histories of these N photopigment types. Denoting the excitation histories by $e_i(t)$, $i = 1, \ldots, N$, the response of the test cell has the form

$$r = r\{t; e_1(t'), e_2(t'), \ldots, e_N(t')\}, \tag{8}$$

where again the curly brackets signify that the response is a functional of the time histories $(e_i(t'), t' \leq t)$ of all excitation types. On restricting attention to the fixed-duration monochromatic flash (Eq. 5), we obtain an ordinary function instead of Eq. 8;

$$r(t) = R(t; e_1, e_2, \ldots, e_N) = R[t; I^0 A_1(\nu), \ldots, I^0 A_N(\nu)], \tag{9}$$

where $A_i(\nu)$, $i = 1, \ldots, N$ refer to the N different absorption functions.

It is important to note that although R appears to depend on the N parameters, e_i, it depends on at most two, since ν and I^0 completely specify the flash. Therefore, in principle, two features of the time-course of r fully specify the response function. In virtually all cases in which time records are taken, the investigator at least tacitly assumes that one feature of the time-course is sufficient for its specification. The consequences of this are easily determined. For if this feature is denoted by $\bar{r}(I^0, \nu)$, the assumption that one feature fully determines the time-course implies that $r(t) =$

$R(t; \bar{r}(I^0, \nu))$. Comparing this with Eq. 9 indicates that we may also write

$$\bar{r} = \bar{R}(e_1, \ldots, e_N). \tag{10}$$

Photopigment Properties and Pseudo-pigments

Henceforth we restrict attention to the monochromatic flash experiment, and for simplicity we write I instead of $I^{0\cdot}$

Since $\bar{r}(IA(\nu))$ is in similarity form in the variable $IA(\nu)$, plots involving $\bar{r}$ and I have special properties. For example, experimental data are customarily plotted as $\bar{r}$ versus $\ln I$, in which case this form yields a family of parallel curves as sketched in Fig. 2, where ν is held fixed on each curve.

Each of the curves in Fig. 2 gives a "template" for the response function $\bar{r}$. Actually, study of $\bar{r}$ can be circumvented by introducing the sensitivity, $\mathcal{S} = 1/(I)_{\bar{r}}$, the reciprocal intensity required to elicit a criterion response, $\bar{r}$. (Hence the subscript, $\bar{r}$, to denote the criterion response held fixed.) For a single photopigment the criterion level is seen to be immaterial, although more generally, sensitivity can change with criterion level. Clearly the sensitivity can be derived from a plot such as Fig. 2. In this case $\mathcal{S}(\nu) \approx A(\nu)$, i.e., sensitivity and absorption are proportional.[3]

Any normalized form of the sensitivity is referred to as relative sensitivity and we fix matters by considering the percent of maximum sensitivity

$$S(\nu) = 100 \cdot \mathcal{S}(\nu)/\max_{\nu} \mathcal{S}(\nu) \tag{11}$$

and for simplicity refer to it as the sensitivity.

From our discussion thus far we see that parallel $\bar{r}$ vs. $\ln I$ curves are a necessary condition for the presence of a single photopigment. And hence the violation of this parallel or self-similar property indicates the presence of more than one type of photopigment. In the literature one finds instances where this property is also taken to be a sufficient condition. That is, it is assumed that parallel $\bar{r}$ vs. $\ln I$ curves imply the existence of a single photopigment. This is in fact not true. For suppose parallel $\bar{r}$ vs. $\ln I$ curves (see Fig. 2): then this implies that there exist functions $f(\bar{r})$ and $g(\nu)$ such that $\ln I = f(\bar{r}) + g(\nu)$. Then since it is an experimental fact that $\bar{r}$ and I are monotonically related (there are exceptions), we can solve for $\bar{r}$ in terms of I,

$$\bar{r} = \bar{r}(I\mathcal{Q}(\nu)) = \bar{r}(E) \tag{12}$$

where $\mathcal{Q}(\nu)$ need not have any special properties. In particular it need not be the absorption function of a photopigment, but from the way in which it appears we refer to it as equivalent absorption or sensitivity, and $E = I\mathcal{Q}(\nu)$ by analogy will be called the equivalent excitation.

In the group of papers announcing the existence of unusual pigments, with one exception, the parallel $\bar{r}$ vs. $\ln I$ property was impicitly assumed. And in the Naka and

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[3] Actually the action spectrum is measured; however, since the quantum yield has been supposed a constant, it is the same as the absorption and we do not make the distinction.
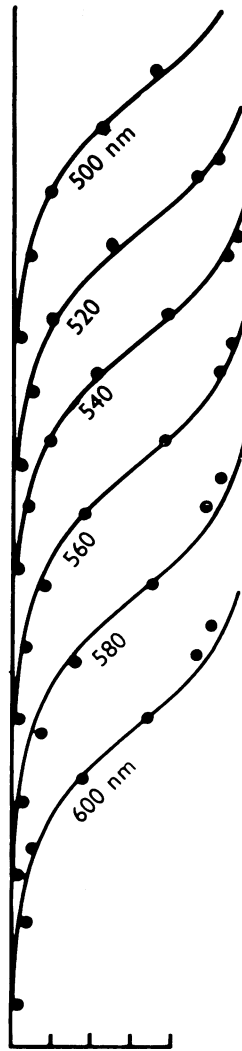
FIGURE 2 The ordinate represents response and the abscissa log intensity. A single curve has been slid to fit each set of data points (corresponding to fixed wavelength). (Redrawn from Naka and Rushton, 1966a).

Rushton papers (1966a, b, c) this property was demonstrated in some detail. On the basis of this they concluded that their sensitivity function, which we called $\mathcal{C}(\nu)$ above, was due to a new type of photopigment, although it differed from spectrophotometric measures in two essential ways: namely that it was much more narrowly tuned and that it peaked at a wavelength significantly higher than any known photopigment. Similar remarks apply to the investigations of the goldfish (Spekreijse et al., 1972). Fig. 3 contrasts the rhodopsin absorption function with the tench unusual pigment (peaking at 680 nm) found by Naka and Rushton (1966a) and the goldfish unusual pigment (peak-
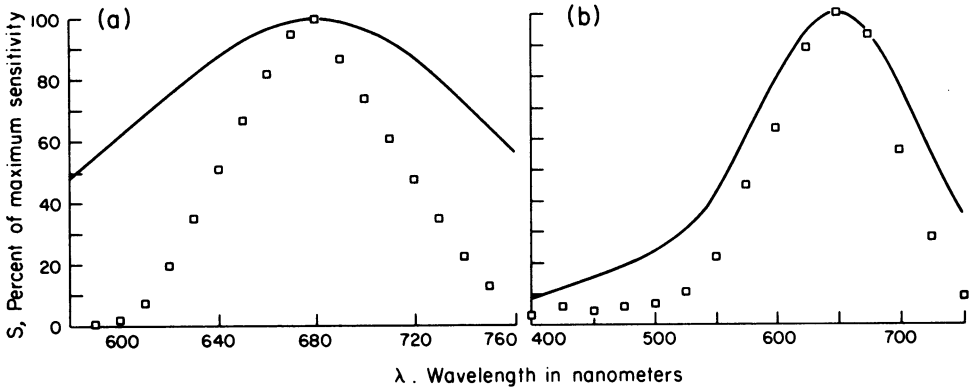
FIGURE 3 (a) Continuous curve is derived from a Dartnall nomogram centered at 680 nm and boxes represent averaged 680 nm pigment (tench) sensitivity of Naka and Rushton, 1966a, b, c. (b) Continuous curve is derived from a Dartnall nomogram centered at 650 nm and boxes represent average 650 nm pigment (goldfish) sensitivity of Spekreijse, et al. 1972.

ing at 650 nm) found by Spekreijse et al. (1972). As seen in both cases, the discrepancy is substantial.

Instead of accepting the existence of an unusual pigment, we now examine the possibility that an unusual photopigment is due to the interaction of several standard pigments. Combining Eqs. 10 and 12 gives

$$\bar{R}(IA_1(\nu), IA_2(\nu), \ldots, IA_N(\nu)) = \bar{r}(I\mathcal{C}(\nu)). \tag{13}$$

Since $\bar{r}$ is a monotonic function of its argument, there exists a function, say F, such that $I\mathcal{C}(\nu) = F(IA_1(\nu), \ldots, IA_N(\nu))$. Taking $I = 1$, $\mathcal{C}(\nu) = F(A_1, \ldots, A_N)$, and back-substituting, $F(e_1, \ldots, e_N) = F(IA_1, \ldots, IA_N) = IF(A_1, \ldots, A_N)$, so that F is homogeneous of degree unity. This places a strong restriction on the equivalent sensitivity, $\mathcal{C}(\nu)$, or alternately on the equivalent excitation $E = F(e_1, \ldots, e_N)$. For it tells us that a knowledge of F in the neighborhood of the origin determines F everywhere. To see this, note $F(e_1, \ldots, e_N) = (1/\delta)F(e_1\delta, \ldots, e_N\delta)$, and then letting δ tend to zero demonstrates this property. The form of F can be further fixed by assuming some analytic property of F. For example if F is differentiable in its arguments at the origin $E = [(\partial/\partial x)xE]_{x=0} = (\partial/\partial x)F(xe_1, \ldots, xe_N)_{x=0} = \sum_{i=1}^{N} e_i (\partial F/\partial e_i)(0, \ldots, 0)$, or that E is linear in the e_i or equivalently that $\mathcal{C}$ is a linear in the A_i. Rather than make such a restrictive assumption, we leave open the degree of differentiability and assume that F is differentiable in some possibly fractional power, $p > 0$, at the origin and leave open the value of p. Then, we can write $E^p = ((\partial/\partial x^p)x^p E^p)_{x=0} = (\partial/\partial x^p)F^p(xe_i, \ldots, xe_N)|_{x=0} = \sum_{i=1}^{N} \alpha_i e_i^p$ where the coupling constants α_i are the differential coefficients and their precise form is immaterial. Expressed in terms of absorptions, this states

$$\mathcal{Q}^p = \sum_{i=1}^{N} \alpha_i A_i^p \quad \text{or} \quad \mathcal{Q} = \left\{ \sum_{i=1}^{N} \alpha_i A_i^p \right\}^{1/p}. \tag{14}$$

Evidently $\mathcal{Q}$ in Eq. 14 is homogeneous of degree one.

For purposes of designation, we will say that a response function is due to a pseudo-pigment if it has similarity form (parallel $\bar{r}$ vs. ln I curves) and is due to more than one photopigment type. We have therefore demonstrated that a pseudo-pigment response must take on the functional form

$$\bar{R} = \bar{R}\left(I^p \sum_{i=1}^{N} \alpha_i A_i^p(\nu) \right). \tag{15}$$

The above analysis in no way constrains the functional form taken on by $\bar{R}$ in Eq. 15. In particular $\bar{R}$ for low intensities could for example be linear in I. We mention this in particular since some evidence for linearity at the horizontal cell level in gold-fish has been presented (Spekreijse and Norton, 1970).

DECOMPOSITION OF PSEUDO-PIGMENTS INTO PHOTOPIGMENTS

The analysis of the previous section indicates that the form of the equivalent absorption function is strongly constrained (Eq. 14). This in turn implies that the spectral sensitivity spectrum has the form

$$S(\nu) = \left\{ \sum_{i=1}^{N} \beta_i A_i^p(\nu) \right\}^{1/p} \tag{17}$$

where the β_i are constants. (Recall the convention that max $S(\nu) = 100$. Also we recall that the form taken by the absorption function is[4] $A(\nu) = \phi\gamma[1 - \exp\{-(3/2)n_0 l \cdot \hat{\epsilon}(\nu)\}]$ where we have used the simple form given in Eq. 5, ϕ and γ will be assumed constant since to the best of our knowledge no evidence exists for variation in either quantity. Setting $\hat{\epsilon} = \epsilon_0 \epsilon(\nu)$; max $\epsilon(\nu) = 1$, we set $\kappa = (3/2)n_0 l \epsilon_0$, and refer to it as the density constant. Since ϵ_0 is the cross-section of a chromophore, κ is to a rough approximation the number of chromophores in a filament having the length of an outer segment and the diameter of a chromophore. In the absence of better information we will assume that this is constant across photoreceptor types.[5]

In our discussion thus far we have, at least tacitly, assumed that the absorption function $A_i(\nu)$ is characterized by the position of the maximal absorption, μ, say. Ex-

[4]The absorption function is customarily written in the form $A(\nu) = \phi\gamma(1 - 10^{-(3/2)n_0 l \bar{\epsilon}})$, where $\bar{\epsilon} = 0.43\hat{\epsilon}$ is the decadic extinction coefficient. However, to keep the formalism simple we use the Napierian base.

[5]Additionally, since the constant is small compared to unity, $A \sim \phi\gamma\kappa\epsilon(\nu)$, and since relative rather than absolute sensitivity is considered, the precise values of κ is of minor importance, in as much multiplicative constants get absorbed in the weighting factors β_i. Although we mention this property of the calculation, no direct use of it will be made.

plicitly we can write $A_i = A(\nu, \mu_i)$, where μ_i is the maximal absorption frequency of the ith photoreceptor type. This in turn depends on the extinction coefficient's dependence on μ_i, i.e. $A(\nu, \mu_i) = \phi\gamma(1 - \exp[-\kappa\epsilon(\nu, \mu_i)])$.

The problem to be solved may now be clearly stated. Given the sensitivity (from experiment) and the left-hand side of Eq. 17, we must determine the number of photoreceptors, N, their maximal absorption frequencies, μ_i, $i = 1, \ldots, N$ (which then determines the A_i), N coupling coefficients, $\beta_{i,}$ an exponent p, and a constant κ. A method which immediately suggests itself is that of least squares, i.e. to determine the various constants by minimizing

$$\int \left\{ S(\nu) - \left[\sum_{i=1}^{N} \beta_i A^p(\nu, \mu_i) \right]^{1/p} \right\} d\nu \tag{18}$$

where the integration is over the visual spectrum. In doing this we must assume a sufficiently large value of N ($N = 4$ would suffice since there has been no demonstration of more than four pigments in any retina), and in addition we must supply the function of two variables $\epsilon(\nu, \mu)$, i.e. the extinction coefficient at all possible maximal absorption frequencies. Another method of attack emerges if we raise Eq. 17 to the power p and then assume that the photopigments are continuously distributed. Denoting the continuous coupling coefficient by $B(\mu)$ this gives

$$S^p = \int B(\mu) A^p(\nu, \mu) \, d\mu \tag{19}$$

$B(\mu)$ is therefore a weighting function, and is in principle determined by solution of the integral equation, Eq. 19.

As an approximation we introduce the observation of Dartnall (1953), that to good approximation $\epsilon(\nu, \mu)$ is translationally invariant

$$\epsilon(\nu, \mu) = \epsilon(\nu - \mu) \tag{20}$$

and hence $A(\nu, \mu) = A(\nu - \mu)$. In this regard it should be remarked that Dartnall's original observation applied to rod pigments and that for cone pigments there is a systematic narrowing for large λ-max, (Marks, 1965; Liebman, 1972; Liebman and Entine, 1968; Harosi and MacNichol, 1974). Fig. 4a contrasts the rod pigment extinction curve with a cone pigment curve (Munz and Schwanzara, 1967) and with the goldfish 625-nm curve obtained by Marks (1965). With the Dartnall curve as a reference, the percentage differences, based on root-mean-square differences, is only 5% for the Marks curve and 8% for the cone curve. Therefore although absorption curves narrow at higher λ-max the effect is relatively small and we will regard it as higher order. Since the absorption spectra under consideration peak at relatively high wavelengths, we will use Marks curve, shown in Fig. 4a, as the nomogram for the absorption function.

Introducing Eq. 20 into Eq. 19 yields

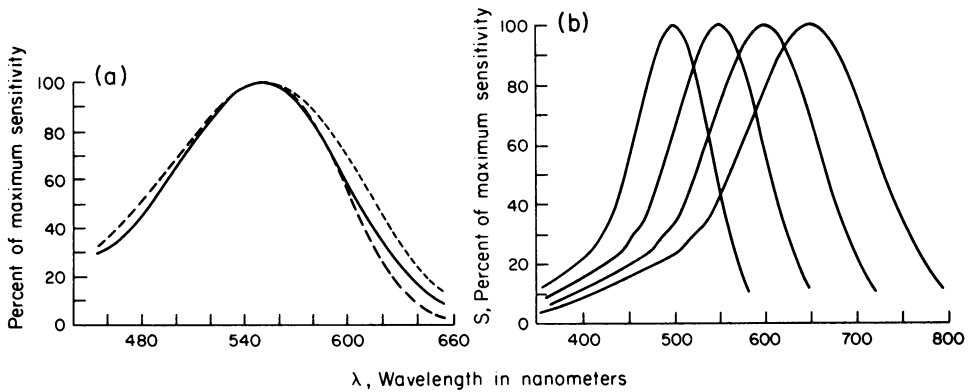$$S^p(\nu) = \int B(\mu) A^p(\nu - \mu) \, d\mu, \tag{21}$$

FIGURE 4 (a) Comparison curves. Continuous curve (———), Dartnall; short broken line (----), Munz and Schwarzara; long broken line (— — —), Marks. In drawing these curves Marks' data has been used as a nomogram and all curves drawn to peak at 550 nm. (b) Dartnall sensitivity curves plotted versus wavelength at different λ-max.

which has the appearance of being a Fredholm integral equation of the first kind (Pogorzelski, 1966) with convolution kernel. The relation to the original, discrete, form of the equation, (Eq. 17), is obtained by taking

$$B(\mu) = \sum_{i=1}^{N} \beta_i \delta(\mu - \mu_i) \tag{22}$$

where $\delta(\mu)$ is the Dirac delta function. Ideally the solution to Eq. 21 is a series of delta functions.

It is important to note that Eq. 21 is not self-contained since both the density constant κ and the exponent p are unknown. Since this indicates a possible lack of uniqueness, the following two relatively self-evident criteria are used to fix the solution: (a) The number of photopigments, N, should be minimal. (b) The maximal absorption frequencies should conform to spectrophotometrically observed values.

ASYMPTOTIC CONSIDERATIONS

Since the largest λ-max found from spectrophotometry is typically less than 630 nm and since the λ-max for the pseudo-pigments under discussion are typically larger than 650 nm, asymptotic considerations apply. Fig. 4 displays a series of pigment absorption functions at different maximal absorption frequencies (note that plots are on a wavelength abscissa). From these we see that the fall-off at low frequencies (large wavelength) is relatively fast. This, coupled with the observation that λ-max is less than 630 nm for a photopigment and λ-max is 650 nm or more for the pseudo-pigments under discussion, implies that the coupling coefficient for the longest wavelength pigment in Eq. 17 is relatively large, so that for low frequencies $S(\nu) \sim \beta^{1/p} A(\nu - \bar{\nu})$, where $\bar{\nu}$ represents the maximal absorption frequency of the longest wavelength pigment. In other words, a pseudo-pigment should be asymptotically
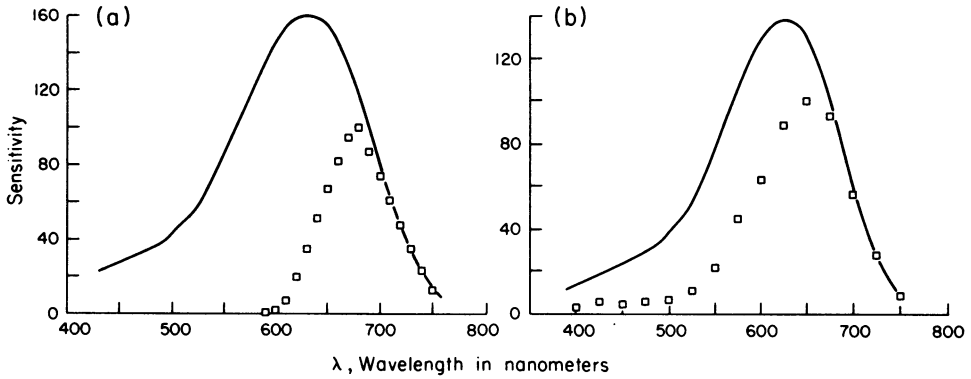
FIGURE 5 (a) Boxes represent data points from Naka and Rushton, 1966a, b, c and the continuous curve a Dartnall spectrum at λ-max = 627 nm. The relative weighting corresponds to Eq. 24, as explained in the text. (See Fig. 7a). (b) Boxes represent data points from Spekreijse et al., 1972, and the continuous curve a Dartnall sensitivity of λ-max = 625 nm. The relative weighting corresponds to Eq. 24, as explained in the text. (See Fig. 7b).

coincident with a real photopigment absorption curve. Anticipating our later results, this is indicated in Fig. 5 for the pseudo-pigments found by Naka and Rushton (1966a, b, c) and Spekreijse et al. (1972). (A graphical construction indicating a similar property is shown for the Naka-Rushton pseudo-pigment in Fig. 7b of Abramov [1972]).

Denoting the maximal absorption frequency of the pseudo-pigment by ν^*, we have $\beta^{1/p} \sim 100/A(\nu^* - \bar{\nu})$ and hence

$$S(\nu) \sim 100 \cdot A(\nu - \bar{\nu})/A(\nu^* - \bar{\nu}) \tag{24}$$

(since we set $\max S(\nu) = S(\nu^*) = 100$). As mentioned earlier the density constant κ is small, and therefore from the rapid fall-off at low frequencies

$$S(\nu) \sim \frac{100\phi\gamma\kappa\epsilon(\nu - \bar{\nu})}{A(\nu^* - \bar{\nu})} = \frac{100\kappa\epsilon(\nu - \bar{\nu})}{1 - \exp[-\kappa\epsilon(\nu^* - \bar{\nu})]} \tag{25}$$

If $\bar{\nu}$ is known, κ is easily estimated by the solution of this relation. To obtain $\bar{\nu}$ we again make use of the smallness of κ to find an approximate solution by solving Eq. 21 for $\kappa = 0$. In principle a solution is found for each p and then a best solution is chosen according to the above-mentioned criteria.

The solution for $\kappa = 0$ determines $\bar{\nu}$ and this in turn determines a new value of κ from Eq. 25. This in effect is the start of an iterative procedure for the solution to the integral equation, Eq. 21. In subsequent stages of the iteration an improved version of the density calculation is used, based on the form of Eq. 17.

Solution of the Integral Equation

To solve the integral equation, we first observe that the limits of integration can be sensibly set to extend over the visible spectrum (ν_0, ν_1). (In the actual calculation this is made to correspond to 250–850 nm with the cis peak elimated. The support of

$A(\nu)$ lies in the interval $(-L, L)$, i.e. $A(\nu) = 0$ for $|\nu| > L$, (in the actual calculation L corresponds to 400 nm). The sensitivity $S(\nu) = 0$ for $\nu \neq (\nu_0, \nu_1)$ and we seek a solution for the weighting function, $B(\nu)$, with the same property.

Next setting $V = 2L + \nu_1 - \nu_0$ we extend S and A to be V-periodic, which implies that B is also V-periodic. Then we can write, $S^P(\nu) = \int_{\nu_0}^{\nu_1 + 2L} A^P(\nu - \mu) B(\mu) \, d\mu$, where the assumption that $B(\nu) = 0$ for ν belonging to $(\nu_1, \nu_1 + 2L)$ has been used. (This must be confirmed a posteriori.) The reason behind the use of the extended interval is now clear. For from this choice of the interval we have avoided "wrap-around" error, i.e. no additional contribution in the fundamental range $(\nu_0, \nu_1 + 2L)$ arises from the periodicity.

To solve the integral equation we make the problem discrete. Introducing the increment $\Delta\nu = V/N$ we write, $S_j^P = S^P(\nu_0 + j\Delta\nu)$, $A_j^P = A(j\Delta\nu)$, $B_j = B(\nu_0 + j\Delta\nu)$. From periodicity $B_{j+N} = B_j$ and similarly for S_j^P and A_j^P. On taking the difference approximation to Eq. 21, we obtain $S_j^P = (V/N)\sum_{k=1}^{N} A_{j-k}^P B_k$. Introducing the discrete Fourier transform

$$A_j^P = \sum_{n=1}^{N} a_n \exp\left(-\frac{2\pi i}{N} jn\right), \quad a_n = \frac{1}{N}\sum_{j=1}^{N} A_j^P \exp\left(\frac{2\pi i}{N} jn\right) \tag{27}$$

$$B_j = \sum_{n=1}^{N} b_n \exp\left(-\frac{2\pi i}{N} jn\right), \quad b_n = \frac{1}{N}\sum_{j=1}^{N} B_j \exp\left(\frac{2\pi i}{N} jn\right) \tag{28}$$

$$S_j^P = \sum_{n=1}^{N} \Phi_n \exp\left(-\frac{2\pi i}{N} jn\right), \quad \Phi_n = \frac{1}{N}\sum_{j=1}^{N} S_j^P \exp\left(\frac{2\pi i}{N} jn\right) \tag{29}$$

Multiplying Eq. 26 by $(1/M)\exp(2\pi ijn/N)$ and summing on j we obtain

$$\Phi_n = (1/N)\sum_{j=1}^{N} S_j^P \exp(-2\pi ijn/N)$$

$$= (V/N)\sum_{j=1}^{N} A_{j-k}^P \exp([2\pi i/N]jn) \left(1/N \sum_{k=1}^{N} B_k\right)$$

$$= \sum_{k'=1}^{N} A_{k'} \exp(-2\pi ik'n/N) \sum_{k=1}^{N} B_k \exp(-2\pi ikn/N) = Va_n b_n$$

where $j - k = k'$ has been introduced and periodicity employed. Solving for b_n and substituting into Eq. 28 gives $B_j = (1/V)\sum_{n=1}^{N} (\Phi_n/a_n)\exp(2\pi ijn/N)$.

This in principle solves the problem since a_n and Φ_n are explicit from Eqs. 27 and 29, respectively. In the actual calculation of the mesh division, N was taken to be a power of 2 and the Cooley-Tukey (1965) fast Fourier transform algorithm used to carry out the computation indicated by the above analysis. Use of this algorithm requires sampling of data points on a uniform mesh. To accomplish this, all data sets were fit by third-order splines (Ahlberg et al., 1967). This gave smooth curve fits passing through the data points and permitted data sampling at arbitrary frequency values.

Filtering and Smoothing

Thus far we have glossed over a serious difficulty in the analysis which emerges from the fact that Eq. 21 is a Fredholm equation of the first kind (Pogorzelski, 1966). Such equations present difficulties since the integral operator has an eigenvalue spectrum which accumulates at the origin. This has the effect that small changes in the data, $S(\nu)$, can produce large changes in the solution. In as much as experimentally gathered data lie within an error bound this can seriously impair the calculation.

The periodicity imposed on the problem allows us to point out explicitly this difficulty. First note that the functions $\phi_n = \exp(2\pi in\mu/V)$ are eigenfunctions of the integral operator, i.e.

$$\int_{\nu_0}^{\nu_0+V} A^p(\nu - \mu)\phi_n(\mu)\,d\mu = \phi_n(\nu) \int_{\nu_0}^{\nu_0+V} A^p(\mu)\exp(-2\pi in\mu/V)\,d\mu = \phi_n\lambda_n,$$

(30)

λ_n giving the corresponding eigenvalues. Hence if we expand $B(\nu)$ and $S^p(\nu)$ in Fourier series

$$\left.\begin{array}{l} B(\nu) = \sum_n \beta_n\phi_n, \beta_n = (1/V) \int_{\nu_0}^{\nu_0+V} \phi_n^*(\nu)B(\nu)\,d\nu \\[2em] S^p(\nu) = \sum_n \sigma_n\phi_n, \sigma_n = (1/V) \int_{\nu_0}^{\nu_0+V} \phi_n^*(\nu)S^p(\nu)\,d\nu \end{array}\right\}$$

(31)

(the summations are over all integers and the asterisk denotes the complex conjugate) Equation 21 reads $\sum_n \sigma_n\phi_n = \sum_n \lambda_n\beta_n\phi_n$. Solving $\beta_n = \sigma_n/\lambda_n$, and the solution is

$$B(\nu) = \sum_n (\sigma_n/\lambda_n)\phi_n$$

(32)

It is clear from the expression for the eigenvalues, λ_n, (30), that $\lambda_n \to 0$, $|n| \to \infty$. On the other hand the Fourier coefficients σ_n, in the expansion of $S^p(\nu)$, for $|n|$ large, correspond to rapidly oscillating small amplitude contributions to the data, i.e. "noise." Yet the solution, Eq. 32, since $\lambda_n \to 0$, amplifies this noise, which is clearly incorrect. A truncation in the series for $S^p(\nu)$, Eq. 31, is indicated, and we develop this in a moment. First though, we note that the analysis leading to the solution, Eq. 32, is itself also a practical method of solution, and is in a sense an exact method. (This is because all data have been transformed to third-order splines, which are easily integrable.) In spite of this, the discrete method outlined earlier proves more efficient in an actual solution. The main reason for this is that the fast Fourier transform algorithm is extremely fast by comparison. Since in any calculation the integral equation must be solved for a sequence of values of p and κ before an acceptable solution is found, the time of calculation becomes an important factor. In any case since the mesh division was chosen reasonably large ($N = 2^8, 2^9$) and since only a

few Fourier components figure in the calculation (roughly 20 and their conjugates), the discrete and continuous solutions are virtually identical.

A criterion for the filtering of the noise from the data may be obtained from the measure of experimental error, ϵ. Let M be the minimal integer such that $|\hat{S}^p(\nu) - \sum_{|n| < M} \sigma_n \exp(2\pi i n \nu / V)| < \epsilon$. Denoting the filtered data by $\hat{S}^p$,

$$\hat{S}^p = \sum_{|n| < M} \sigma_n \exp(2\pi i n \nu / V). \tag{33}$$

The sharp cutoff itself introduces a noisiness or rippling in the curve and this in turn should be smoothed. As a smoothing procedure we consider a moving average over a "window" Δ, which we leave unspecified for the moment. $\langle F \rangle = (1/\Delta) \int_{\nu - \Delta/2}^{\nu + 1/2\Delta} \cdot F(\mu) \, d\mu$. Introducing the filtered form of the data (Eq. 33) into this expression

$$\langle \hat{S}^p \rangle = \frac{1}{\Delta} \int_{\nu - \Delta/2}^{\nu + \Delta/2} \hat{S}^p(\mu) \, d\mu = \sum_{|n| < M} \sigma_n \frac{V \sin(\pi n \Delta / \nu)}{\pi \Delta n} \exp(2\pi i n \nu / V)$$

Since the filtering process terminates the series at $|n| = M$, it introduces a natural rippling of wavelength V/M and this then is the natural window over which to average. Therefore taking $\Delta = V/M$ gives

$$\langle \hat{S}^p \rangle = \sum_{n = -M}^{M} \left(\frac{M}{\pi n} \sin \frac{\pi n}{M} \right) \sigma_n \exp(2\pi i n \nu / V) \tag{34}$$

Henceforth in speaking of the data we shall mean the filtered and smoothed form (Eq. 34), and for simplicity we drop the symbols indicating these operations.

RESULTS AND DISCUSSION

The methods discussed in the previous sections have been applied to two cases. Each of these cases pertain to measurements of sensitivity spectra made at locations beyond the receptor stage in the visual pathway. In the case of the tench, Nake and Rushton (1966a, b, c), recorded data at the horizontal cell level. In the other instance, that of the goldfish, Spekreijse et al. (1972) took records at the retinal ganglion cell level. Both sets of data are exhibited in Fig. 3.

In each instance the result of solving Eq. 21 is the spectral weighting function $B(\nu)$, the exponent p, and the density constant κ. The solution for this quantity, κ, has been discussed in the previous section (see especially Eqs. 24 and 25 and the related discussion), as have the criteria for the determination of p. Plots of this solution for the tench and goldfish data are shown in Fig. 6a, and b. In order to draw conclusions from these curves it is first necessary to be able to identify a delta function. That is, after the filtering and smoothing, a delta function loses its sharpness. To see this we first observe, that under the requirement to be V-periodic, a delta-function located at
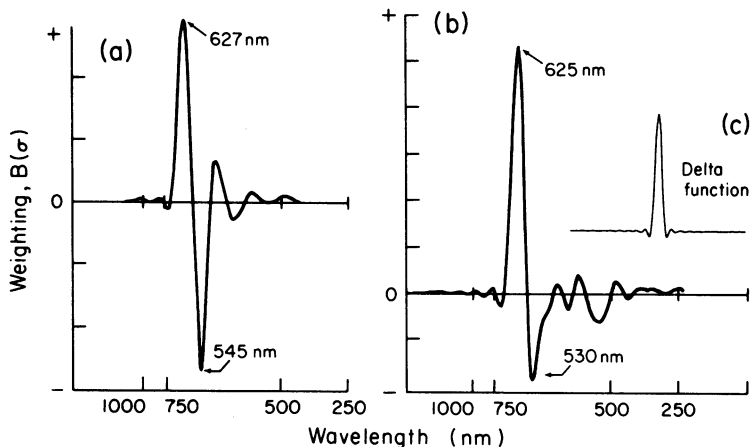
FIGURE 6 (a) The weighting function $B(\sigma)$ as determined for the tench data. The peak is at 627 nm and the trough at 545 nm. The area under the peak is 1.21 and under the trough is -1. (b) The weighting function $B(\nu)$ as determined for the goldfish data. The peak is at 0.625 nm and the trough at 530 nm. The area under the peak is 1.17, and under the trough is -0.42. (c) The delta function (Eq. 35) for $M = 20$.

$\nu = \nu_0$ has the form $\delta(\nu - \nu_0) = (1/V) \sum_m \exp [i(\nu - \nu_0)m\pi/V]$, which under filtering and smoothing becomes

$$\langle \delta(\nu - \nu_0) \rangle = \frac{1}{V} \sum_{|m|<M} \left(\frac{M}{\pi m} \sin \frac{\pi m}{M}\right) \exp [i(\nu - \nu_0)m\pi/V]. \qquad (35)$$

Fig. 6c depicts the shape that Eq. 35 takes on (for $M = 20$; roughly, the value used).

With this in mind, the spectral weighting function of Fig. 6a is interpreted as being due to two opponent photopigments, with λ-max at 627 nm and 545 nm and the respective weights (lobe areas) 1.21 and -1. (Neglect of the smaller peaks in Fig. 6a and b will be commented on later.) In this case an exponent of $p = 0.4$ and a density coefficient of $\kappa \approx 0.81$ are found. Similarly, the goldfish spectral density, $B(\nu)$, depicted in Fig. 6b, is interpreted as being due to two opponent photopigments having λ-max at 625 nm and 530 nm, and respective weights of 1.17 and -0.42. The exponent now is $p = 0.5$ and the density coefficient $\kappa \approx 0.58$.

These and the previous values for p and κ are within experimental range when such results are available. An extensive comparison with experiment is given in an associated paper, Sirovich and Abramov (1977),[6] and further discussion is not deemed necessary here. We only point out that the values for λ-max in the case of the goldfish, Fig. 6b, agree well with the microspectrophotometric findings of Marks (1963, 1965), Liebman and Entine (1964), and Harosi and MacNichol (1974), who found photopigments

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[6]As a cautionary remark we note that the present paper used the mathematically natural exponential notation. Sirovich and Abramov, (1977), on the other hand, use traditional decadic base notation. Therefore the values of κ mentioned here are larger by a factor log $10 \approx 2.3$ (See footnote 4).

peaking at 530 nm and 625 nm (and also a third pigment at λ-max = 450 nm). Only limited spectrophotometric data for tench is available: see Sirovich and Abramov (1977) for further discussion.

The severest test of the above solutions comes in reconstructing the sensitivity spectra from their solutions. More specifically we return to the discrete form of the sensitivity function given by Eq. 17 and substitute in it the values for the β_i, λ-max, and p just found; and plot this in frequency versus the corresponding experimental data. On doing this, excellent agreement is obtained except at the relatively low wavelengths. This was to be expected since Fig. 6a, and b shows additional rippling at the low wavelengths. However, it is also known that the Dartnall translational invariance property of sensitivity curves is only an approximation, and that sensitivity curves centered at higher wavelengths are narrower than those at lower values. The solution of the integral equation exhibits this effect by producing the low-amplitude ripples at the low wavelengths. To take this into consideration in the construction, we use a rhodopsin nomogram, i.e. Dartnall's nomogram (1953), for relatively low-wavelength pigments and Marks' (1963, 1965) 625-nm pigment as a nomogram for long-wavelength pigments. The results are shown in Fig 7a and b and the close agreement between the constructed curves and the original data lend strong support to the methods developed here.
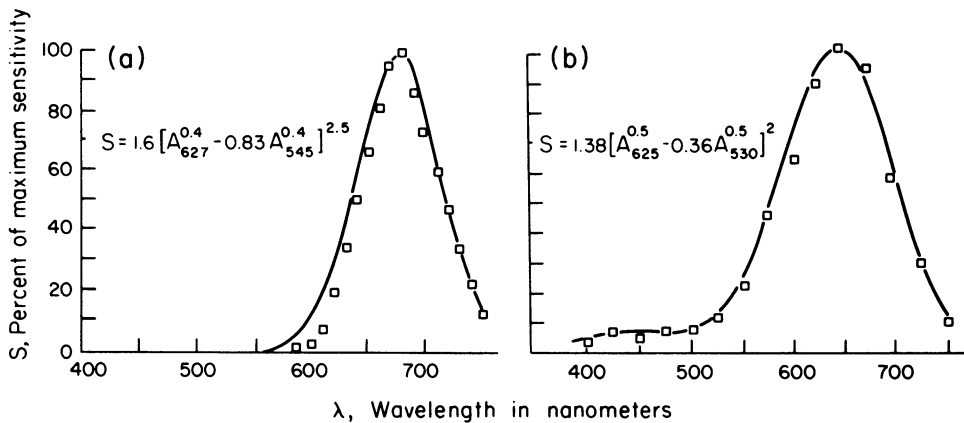
FIGURE 7 Reconstructed spectral sensitivities, based on Eq. 17 and solved for constants. All curves are presented as percent of maximum sensitivity. (a) Symbols represent the average spectral sensitivity of the tench 680-nm pseudo-pigment. The curve is generated by the inset equation. (b) Symbols represent the average spectral sensitivity of the goldfish 650-nm pseudo-pigment. The curve is generated by the inset equation.

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