



Increment Threshold and Purity Discrimination Spectral Sensitivities of X-chromosome-linked Color-defective Observers

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The goal of the study was to evaluate spectral opponency in nine X-chromosome-linked color-defective observers. The tasks included increment threshold spectral sensitivity on an achromatic background, heterochromatic flicker photometry, and colorimetric purity discrimination. With a task of heterochromatic flicker photometry, the anomalous trichromatic observers showed spectral sensitivity of the corresponding dichromat. The increment threshold spectral sensitivity and colorimetric purity discrimination data were analyzed using the concept of standard cone photopigment spectral sensitivities for normal and defective vision, and a model that postulates one cone-additive and two cone-antagonistic systems. The model incorporated a shift of the peak spectral sensitivity of the long-wavelength-sensitive (LWS) pigment (for protan observers) or of the middle-wavelength-sensitive (MWS) pigment (for deutan observers). Two dichromats and two anomalous trichromats did not show clear evidence of LWS vs MWS cone antagonism. Five anomalous trichromats showed such cone antagonism. Molecular genetic analysis of the opsin genes is presented for eight of the observers. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

There have been two enduring issues in understanding the color vision performance of X-chromosome-linked color-defective observers, their color matches and their chromatic discriminative ability. Color matching reveals two qualitatively different forms of X-chromosome-linked congenital defect, the protan and deutan defects. In a dichromatic form called protanopia and deuteranopia, respectively, the affected observer requires only two primaries for full spectrum color matching. Protanopia and deuteranopia have traditionally been regarded as a "reduction" form of color vision (von Kries, 1897, 1924). There are also trichromatic forms called protanomalous trichromacy (protanomaly) or deuteranomalous trichromacy (deuteranomaly). The color matches of protanomalous and deuteranomalous trichromats differ from each other and from those of normal trichromats. Anomalous trichromacy is traditionally regarded as an "alteration" system (von Kries, 1897, 1924). This finding implies that the defects involve alterations in the

absorption spectra of the normal long-wavelength-sensitive (LWS) and/or middle-wavelength-sensitive (MWS) cone photopigments.

The severity in discrimination loss of the protan and deutan defects varies considerably. The dichromatic form is regarded as more severe than the trichromatic form. Some anomalous trichromats have discrimination ability comparable to that of color-normal observers, others show color discrimination ability comparable to that of the corresponding dichromat (Chapanis, 1944; Wright, 1946; Regan *et al.*, 1994). Variation between pedigrees of anomalous trichromacy is large, but variation is minimal within a family pedigree. That is, both the qualitative and the quantitative extent of the defect are inherited (Pickford, 1967; Went & deVries-deMol, 1976; Pokorny & Smith, 1982).

The prevailing theoretical explanations of X-chromosomal color defect ascribed protan defects to a defect in LWS photopigment function and deutan defects to a defect in MWS photopigment function. The LWS and MWS visual photopigments were regarded as deriving from separate genes on the X-chromosome. Dichromacy resulted from a defect in one of these genes denying its expression or function; anomalous trichromacy resulted from an alteration in one of the genes resulting in an altered spectral sensitivity. The loss in chromatic

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discrimination in color defect reflected either the lack of function in dichromacy or a reduction due to a smaller separation of photopigments in anomalous trichromacy compared with normal trichromacy.

Explanations of the differences in chromatic discriminative ability among anomalous trichromats have been varied. They include the suppositions of variable separations in the photopigments (Alpern & Pugh, 1977), an independent neural loss mechanism (Jameson & Hurvich, 1956), and normal variability superimposed on an average loss (Pokorny & Smith, 1977). None of these explanations is entirely satisfactory. A complicating factor is that the classification of color defect is predicated on use of a 2 deg foveal field (Nagel, 1907). When the field extends to 10 deg or more, chromatic discrimination in the Rayleigh match is improved and the majority of 2 deg dichromats reveal residual chromatic discrimination.

It might be hoped that molecular genetic study of the photopigment opsins on the X-chromosome would provide an explanation of color defect. However, to date study of the genotype has raised as many questions as it has answered. The opsins for normal MWS and LWS photopigments lie in a tandem array on the X-chromosome (Vollrath *et al.*, 1988). There may be multiple copies of these genes and the factors controlling their expression are not yet delineated. The nucleotide sequences of the MWS and LWS opsin genes are very similar (Nathans *et al.*, 1986a). Only a few nucleotide changes differentiate whether the absorption spectrum will be that of an LWS or MWS photopigment (Neitz *et al.*, 1991b; Chan *et al.*, 1992; Merbs & Nathans, 1993; Asenjo *et al.*, 1994). Some variation in these nucleotides occurs naturally in the color-normal population (Winderickx *et al.*, 1992a; Neitz *et al.*, 1993). Hybrid genes (composed of parts of the normal opsin genes) may occur. The protan and deutan defects are correlated with deletions of the normal opsin genes and with a high frequency of hybrid genes (Nathans *et al.*, 1986b; Deeb *et al.*, 1992). As yet there is no precise correlation of genotype and phenotype. Thus, observers classified as anomalous trichromats may appear to have only one kind of opsin gene or may have a complement of normal genes in addition to hybrid genes.

The usual classification of X-chromosomal color defect is based on the Rayleigh match, a color match in which a 589 nm test field is matched to a mixture of 545 and 670 nm primaries. The mid-matching point is an indication of the photopigment sensitivities and the matching width is an indication of chromatic discrimination ability (Pokorny & Smith, 1977). Questions have arisen as to whether residual chromatic discrimination based on Rayleigh matching is indicative of multiple photopigments with $\lambda_{\max} > 535$ nm. We reasoned that spectral opponency may be revealed when multiple photopigments are present. We therefore assessed spectral opponency in color-defective observers. The task, increment detection on a white background, is commonly accepted to reveal spectral opponency (Sper-

ling & Harwerth, 1971; King-Smith & Carden, 1976). Chromatic mediation of the task was favored by using experimental conditions which minimize detection of luminance transients. We used a background-pedestal configuration and a raised cosine temporal profile. We evaluated sensitivity using both 2 and 10 deg test fields. These studies were complemented with measurement of spectral sensitivity using heterochromatic flicker photometry. We also searched for residual chromatic discrimination using a test of colorimetric purity discrimination. Brief descriptions of some of the data have appeared elsewhere (Miyahara *et al.*, 1994, 1995).

DATA ANALYSIS

Our approach to analysis of the data is based on the concept of a standard, normal trichromat, a standard deuteranomalous trichromat and a standard protanomalous trichromat. We recognize that opsin variations occur among color-normal observers and influence color matches (Sanocki *et al.*, 1994). However, for color-normal observers the average pigment separation is so large (*c.* 23 nm) that we do not expect minor variations to influence spectral opponency in the retina. The hybrid genes characteristic in anomalous trichromacy presumably result in lesser pigment separation. In this case opsin variations may have an effect on spectral opponency. The concept of a standard observer remains important since it establishes a baseline of expected opponency for evaluation of the data. If an observer with average pigment separation lacks spectral opponency, we can raise the issue of higher neural loss. As input spectral sensitivities for the visual photopigments, we used the LWS, MWS, and SWS cone fundamentals of Smith and Pokorny for normal trichromats and substituted the appropriate "anomalous" LWS' and MWS' spectral sensitivities for anomalous trichromats (DeMarco *et al.*, 1992).

We fit the data with equations that have been developed to describe chromatic discrimination (Miyahara *et al.*, 1993; Yeh *et al.*, 1993a,b), performance on Munsell color-based color vision tests (Smith *et al.*, 1993), flicker spectral sensitivity (Pokorny *et al.*, 1993) and temporal modulation sensitivity in silent cone substitution (Smith *et al.*, 1995). This simplified approach assumes that the cone types are subject to multiplicative adaptation (*vs* Hood & Greenstein, 1990; Shapiro *et al.*, 1992) before combining to form achromatic and spectral chromatic pathways. The achromatic system is based upon cell-types in the magnocellular pathway of the primate retina/lateral geniculate nucleus. The spectral chromatic system is based upon cell-types in the parvocellular pathway of the primate retina/lateral geniculate nucleus.

(L + M) Additive system

We assume an LWS and MWS cone-additive system forms the structure of the (L + M) additive system [Fig. 1(A)]. For an increment threshold task, we express the LWS cone and MWS cone-excitation levels produced by

the (L + M) adapting field in LWS and MWS trolands as L_W and M_W . If the LWS cone and MWS cone-excitation levels produced by the test light are expressed as ΔL and ΔM , and do not perturb the adaptation level, the output of the (L + M) system at the increment threshold can be written as

$$\log \Delta I = \log T_A - \log \left\{ p \frac{l(\lambda)}{l_{\max} V(\lambda)} \left(\frac{1}{1 + G_A L_W} \right) + (1 - p) \frac{m(\lambda)}{m_{\max} V(\lambda)} \left(\frac{1}{1 + G_A M_W} \right) \right\}, \quad (1)$$

where I is the retinal illuminance level in trolands, $l(\lambda)$ is the Smith and Pokorny (1975) LWS fundamental, l_{\max} is the Smith and Pokorny LWS fundamental at λ_{\max} (0.63721), $m(\lambda)$ is the Smith and Pokorny MWS fundamental, m_{\max} is the Smith and Pokorny M cone fundamental at λ_{\max} (0.39246), and $V(\lambda)$ is the luminous efficiency function modified by Judd (1951). G_A is a gain constant for the (L + M) system and T_A is the absolute threshold of the (L + M) system. The term p refers to the proportion of LWS cones in the (L + M) pathway. For the Judd observer $p = 0.6189$. According to this equation, the thresholds (in trolands) in the (L + M) system are regulated by two terms: the first the absolute threshold (T_A); and the second a gain control constant (G_A) characteristic of the (L + M) system.

(L - M) Spectral opponent system

Next we assume an (L - M) cone-antagonistic system whose output is described as the difference in LWS and MWS cone-excitation levels following gain control [Fig. 1(B)]. By the same logic as in the (L + M) system, the threshold of the (L - M) spectral opponent system is described as

$$\log \Delta I = \log T_R - \log \left\{ \left| \frac{l(\lambda)}{l_{\max} V(\lambda)} \left(\frac{1}{1 + G_R L_W} \right) - \frac{m(\lambda)}{m_{\max} V(\lambda)} \left(\frac{1}{1 + G_R M_W} \right) \right| \right\}, \quad (2)$$

where T_R is the absolute threshold of the (L - M) opponent system. According to Eqn (2), the thresholds (in trolands) for the (L - M) spectral opponent system are regulated by two terms: the first the absolute threshold (T_R); and the second a gain control constant (G_R) characteristic of the (L - M) spectral opponent system. The absolute value sign is a convenient way of expressing the thought that different cell-types may contribute to different lobes of an (L - M) spectral opponent system, but their adaptation is controlled identically.

S - (L + M) Spectral opponent system

We assume an antagonistic system between the SWS cones and the sum of LWS and MWS cones. In the normalization derived by Boynton and Kambe (1980), one S cone troland is equal to one photopic troland at the

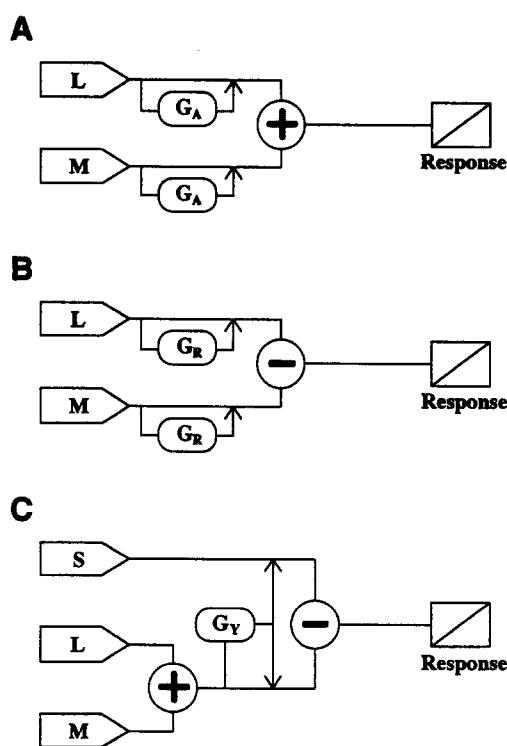


FIGURE 1. Schematic diagrams of (A) the achromatic system, (B) the L - M spectral opponent system, and (C) the S - Y spectral opponent system, of the model. S, M, L: S cone, M cone, L cone. G_A , G_R , G_Y : gain control. The signs (+ and -) denote addition or subtraction of the signals.

equal energy spectrum. The S cone troland has about 30-50 times larger value than the retinal illuminance level (photopic troland) near the short end of the visual spectrum because the SWS fundamental has a peak at 440 nm, whereas the luminous efficiency function keeps on decreasing toward shorter wavelength from 555 nm. Figure 1(C) shows a schematic diagram of the S - (L + M) spectral opponent system. We assume the same gain constant, G_Y in the SWS cone and the LWS and MWS cone portions of the S - (L + M) opponent system (Shapiro *et al.*, 1992). Given the troland definition and the broad spectrum adaptation, the gain is determined only by the luminance level [see Yeh *et al.* (1993a,b) for another interpretation of the S - (L + M) spectral opponent system]. We obtain:

$$\log \Delta I = \log T_Y - \log \left(\left| \frac{\bar{z}(\lambda)}{V(\lambda)} - 1 \right| \right) - \log \left(\frac{1}{1 + G_Y I} \right), \quad (3)$$

where T_Y is the absolute threshold of the S - (L + M) spectral opponent system. According to Eqn (3), the thresholds (in trolands) in the S - (L + M) spectral opponent system are regulated by two terms: the first the absolute threshold of the S - (L + M) spectral opponent system (T_Y); and the second a gain control constant (G_Y) characteristic of the S - (L + M) spectral

opponent system. The absolute sign is a convenient way of expressing the thought that different cell-types may contribute to different lobes of an S – (L + M) spectral opponent system.

Combination rule of the three systems

Equations (1)–(3) describe the threshold retinal illuminance level of the (L + M) system, the (L – M) spectral opponent system, and the S – (L + M) spectral opponent system, respectively. As a combination rule of these separate systems to determine psychophysical sensitivities, there are three possibilities: (A) the threshold is determined by the most sensitive system of the three; (B) probability summation; and (C) complete summation. Quick (1974) introduced a vector magnitude model that can approximate these summation rules. The vector magnitude model applied to the current systems can be written as

$$S(\lambda) = [S_A(\lambda)^n + S_R(\lambda)^n + S_Y(\lambda)^n]^{1/n}, \quad (4)$$

where $S(\lambda)$ is the final psychophysical sensitivity, $S_A(\lambda)$ is the predicted sensitivity for the (L + M) system, $S_R(\lambda)$ is the predicted sensitivity for the L – M spectral opponent system, and $S_Y(\lambda)$ is the predicted sensitivity for the S – (L + M) spectral opponent system. In case (A) where threshold is determined by the most sensitive mechanism, n assumes a large value in Eqn (4). If $n \geq 10$, the equation approximates closely the upper envelope of the sensitivities of the three systems. In case (B), probability summation is characterized by setting $1 < n < 6$. The special case of $n = 2$ is sometimes called the vector sum. In case (C), the linear sum of the three systems is described by Eqn (4) with $n = 1$.

Sperling and Harwerth (1971), King-Smith and Carden (1976), and Calkins *et al.* (1992) assumed that the test stimulus was detected by the most sensitive system in the increment threshold spectral sensitivity measurements. On the other hand, there are considerable data in the literature which suggest that temporal integration for incremental chromatic stimuli is determined by the vector sum of chromatic and achromatic systems (MacAdam, 1949; Guth & Lodge, 1973; Ingling & Tsou, 1977; Kranda & King-Smith, 1979; Noorlander *et al.*, 1980; Smith *et al.*, 1984; Lindsey *et al.*, 1986). Yeh *et al.* used the vector sum to combine two chromatic systems to predict chromatic discrimination (Yeh *et al.*, 1993a) and purity discrimination (Yeh *et al.*, 1993b) sensitivities. From previous studies, either no summation or the vector summation seemed plausible. However we considered the possibility of complete summation as well as no summation and vector summation in predicting spectral sensitivity functions.

Approach for color-defective observers

The equations described above are predicted on the psychophysical performance of a standard color-normal observer. To apply this approach to red–green color-defective observers, it is necessary to substitute the

appropriate photopigment spectral sensitivities for those of the standard color-defective observers.

The luminosity function can be considered as the representative spectral sensitivity function of the (L + M) system. Generally, the spectral sensitivity functions of dichromats and the corresponding anomalous trichromats (P and PA, D and DA) are indistinguishable when measured by absolute threshold (Alpern & Torii, 1968a, b), flicker photometric (Verriest, 1971) or electroretinographic (Jacobs & Neitz, 1993) techniques. Thus, we assume that the luminosity function of protan observers is represented by the MWS cone sensitivity and that the luminosity function of deutan observers is represented by the LWS cone sensitivity, and in Eqn (1) we set $p = 0$ for protan observers and $p = 1$ for deutan observers.

We assume that protan observers can be modeled as having a normal MWS and an anomalous photopigment, (LWS') in their cones. As the LWS' cone fundamental, we used $l'(\lambda)$ as tabulated in DeMarco *et al.* (1992). In the same way, we assume that deutan observers can be modeled as having a normal LWS and an anomalous photopigment, (MWS') in their cones. As the MWS' cone fundamental, we used $m'(\lambda)$ as tabulated in DeMarco *et al.* (1992). These tabulations are normalized to peak sensitivity and thus for the protan observers we substituted $l'(\lambda)$ for $l(\lambda)/l_{\max}$, and for deutan observers we substituted $m'(\lambda)$ for $m(\lambda)/m_{\max}$ in Eqn (2).

The threshold equation of S – (L + M) opponent system for normal observers [Eqn (3)] can be rewritten for color-defective observers by substituting the luminosity functions of the color-defective observers. For protans, we substituted the term $\{m(\lambda)/m_{\max}V(\lambda)\}$ for the spectral sensitivity of (L + M) in the S – (L + M) opponent system. For deutans, we substituted the term $\{l(\lambda)/l_{\max}V(\lambda)\}$ for the spectral sensitivity of (L + M) in the S – (L + M) opponent system.

Conversion from retinal illuminance to sensitivity

The equations predict thresholds in retinal illuminance units (trolands). The retinal illuminance level can be converted to the logarithm of sensitivity ($-\log W/m^2/sr$) as follows:

$$\log S(\lambda) = -\log I + \log V(\lambda) + C, \quad (5)$$

where $S(\lambda)$ is the sensitivity ($W/m^2/sr$)⁻¹, I is the retinal illuminance (td), $V(\lambda)$ is the luminous efficiency function modified by Judd (1951), and C is a scaling factor determined from the calibrations.

Calculation of cone excitation to the adapting background

The excitation levels of the LWS, MWS, and SWS cones (L_w , M_w , and S_w) produced by the achromatic adapting field were determined by finding achromatic points for the (L – M), and the S – (L + M) systems to fit the TVR data to Eqns (1)–(3). The method was taken from Pokorny and Smith (1977). The theoretical achromatic point [“neutral point” in the paper of Pokorny and Smith (1977)] for the LWS and MWS cone sensitivities [used for the (L + M) system and for the (L – M) system]

is that wavelength for which the LWS and MWS cone sensitivity ratio is identical to the cone sensitivity ratio for the corresponding 4600 K adapting light in this experiment. For L_w and M_w , the following equation was solved:

$$\frac{\sum P_\lambda \frac{m(\lambda)}{m_{\max}} d\lambda}{\sum P_\lambda \frac{l(\lambda)}{l_{\max}} d\lambda} = \frac{m(AP)}{l(AP)}, \quad (6)$$

where P_λ is the monochromatic component of wavelength λ of the adapting field whose spectral radiant power distribution is $\{P_\lambda d\lambda\}$, $l(\lambda)$ and $m(\lambda)$ are the Smith and Pokorny (1975) LWS and MWS cone fundamentals, and AP is the achromatic point. The excitation levels of LWS cones and MWS cones (L_w , M_w) were determined using this wavelength in the calculation of cone trolands (Miyahara *et al.*, 1993; Yeh *et al.*, 1993a,b). By the same logic, the achromatic point for the S - (L + M) opponent system was obtained by solving the following equation:

$$\frac{\sum P_\lambda \bar{z}(\lambda) d\lambda}{\sum P_\lambda V(\lambda) d\lambda} = \frac{\bar{z}(AP)}{V(AP)}, \quad (7)$$

where $\bar{z}(\lambda)$ is one of the Judd color-matching functions (Judd, 1951) that is proportional to the S cone spectral sensitivity, $V(\lambda)$ is the luminosity function as modified by Judd (1951), and the other symbols are the same as Eqn (6). The AP computed this way was used to determine the excitation level of the SWS cones by the adapting field. Table 1 shows the AP s for normal, protan, and deutan observers.

GENERAL METHODS

Apparatus

The apparatus, a dynamic colorimeter has been described fully elsewhere (Yeh *et al.*, 1993a,b). Briefly, three mirror galvanometers were used to control the amounts of light from three of four channels which fed into two integrating spheres. The exit ports of the integrating spheres served as sources for a Maxwellian view system. Interference filters were used to determine the spectral characteristics of lights in mid-spectrum, broadband or cut-on dyed glass filters were used at the spectral extremes. The exit optics of the colorimeter were modified so as to allow circular test fields of up to 10 deg dia. A 2.4 mm artificial pupil was used. An ophthalmic lens could be placed adjacent to the artificial pupil to correct for any observer refractive error. A chin rest was used to stabilize the position of the observer's head.

The device was controlled by a Macintosh II computer and MacADIOS II digital input/output boards (GW Instruments). The mirror galvanometers were controlled

by 16-bit D/A converters (GW Instruments, GWI-DAC). Neutral density and chromatic filter wheels were also under computer control. The observer's responses were signaled by three switches which were read by the digital input port.

Calibrations

The spectral radiant power distributions of the chromatic and white lights were calibrated on the observer's side of the artificial pupil using a scanning spectroradiometer (International Light Inc., IL 1700 research radiometer with IL 781 spectroradiometer detector head). The spectroradiometer was calibrated for spectral sensitivity with a 500 W standard tungsten filament lamp (2856 K) of known spectral radiant exitance (Hoffman Engineering Corp., model HEC-500). The dominant wavelengths of the filtered lights were 458, 480, 500, 510, 529, 541, 559, 571, 580, 590, 610, 639, and 659 nm. The colorimetric purities of the chromatic lights ranged from 0.92 to 1.00. Minor perceptual differences in the continuous spectrum from the different colorimeter channels were corrected with mirrored shift filters, yielding a correlated color temperature of 4600 K for all channels.

The absolute radiance of 571 nm chromatic light was calculated from irradiance that was measured with a radiometer (EG and G model 550 photometer/radiometer) on the observer's side of the artificial pupil. The radiance of 571 nm light obtained this way was 0.068 W/m²/sr. The radiances of other chromatic and achromatic lights were calculated based on this value. In the increment threshold spectral sensitivity experiments, the sensitivity was calculated as the reciprocal of the threshold measured in radiance. The retinal illuminances of the achromatic lights were obtained from absolute illuminances (lx) measured with the EG and G photometer/radiometer on the observer's side of the artificial pupil. The value of C in Eqn (5) could be determined using the retinal illuminance (2.32 log td), the radiance (-1.17 log W/m²/sr), and the luminous efficiency (0.9455) of 571 nm light. The value of the scaling factor C obtained this way was 3.51.

The relative amounts of light as a function of the angular position of the mirror galvanometers were measured at the output port of one of the integrating spheres using a PIN silicon photodiode (Silicon Detector Corp.) and a current amplifier interfaced with the computer by a 12-bit A/D converter. Light output was measured for each of 110 angular galvanometer positions. Fourth-order polynomials were used to represent the relation between the computer output integer and the percentage of light at the output port of the integrating sphere for each galvanometer.

Observers

Observers were either laboratory personnel or individuals who responded to an advertisement asking for "red-green color blind people" on a campus bulletin board or in a campus computer news network. All

TABLE 1. Theoretical achromatic points for normal, protan, and deutan observers

Systems involved	Normal (nm)	Protan (nm)	Deutan (nm)
Achromatic and L - M	570	563	576
S - (L + M)	502	498	504

TABLE 2. Summary of color vision test results

Observer	Classification	Ishihara*	SPP2†	Neitz OT anomaloscope‡	Moreland anomaloscope§			FM 100
					2 deg	4 deg	8 deg	
EM	N	0	0	41	157–163	157–166	167–173	8
JK	PA	7	3	55–65	350–375	350–375	350	76
DK	EPA	8	1	0–50	0–300	220–300	300	91
RR	P	7	4	Full	Full	Full	Full	205
JH	DA	0	0	18	67	69–70	73	51
PM	DA	1	1	17–19	60–70	70	70	53
MM	DA	3	0	17–19	70	70	75	44
TJ	DA	4	5	10–24	50–80	55–65	60–80	218
WZ	DA	8	4	24–30	65–75	80	80–85	221
MT	D	7	3	Full	Full	20–370	175–250	214

*Number of failures in reading 10 RG plates.

† Number of failures in reading 5 RG plates.

‡The range of the reading is 0 (545 nm primary) to 73 (670 nm primary).

§The range of the reading is 0 (545 nm primary) to 427 (670 nm primary).

observers were tested for color vision using the Ishihara pseudoisochromatic plates, the Standard Pseudoisochromatic Plate tests II, the Farnsworth–Munsell 100-hue test, the Neitz OT anomaloscope, and the Moreland anomaloscope. The Moreland anomaloscope was used to test the Rayleigh match with 2, 4 and 8 deg fields. For the Rayleigh matches with different field sizes, the observer viewed the surface of an X-ray view box [900 cd/m², 4000 effective trolands (LeGrand, 1968), 5000 K] when he or she was not looking at the test to reduce the rod intrusion. Each observer was classified as N, PA, EPA, P, DA, EDA, or D, according to the 2 deg Rayleigh match. Table 2 shows a summary of color vision tests for all the observers. One of the authors (EM) served as a color-normal control; EM has superior chromatic discrimination and has observed in many experimental protocols. There were three protan observers and six deutan observers, all young males. We have organized the observers by diagnostic category arranged in order of increasing Rayleigh match width. There were three DA observers with narrow match widths (0–2 Nagel-units); their square root FM 100-hue test scores were near 7,

which is well within the spread of color-normal test scores (Verriest *et al.*, 1982). One PA and two other DA observers showed wider match widths (6–14 Nagel-units) with higher FM 100-hue test scores (square-root scores of 9–15) beyond that expected for age-matched color normals. The remaining observers included one EPA with a wide matching range, a P and a D observer with full 2 deg matching ranges. These observers showed square-root FM 100-hue test scores in the range 10–15.

EXPERIMENT 1: INCREMENT THRESHOLD SPECTRAL SENSITIVITY FUNCTIONS

Procedure

Figure 2 shows the spatial configuration of the stimuli. The background was a steady 200 td, 19 deg dia circular white field. Either a 2 or a 10 deg pedestal was used. The pedestal was a steady 800 td circular white field and was centered on the background. The test stimulus was a circular field of the same diameter as the pedestal, and when present was spatially superimposed upon the pedestal. The test stimulus was presented with a temporal profile of one trough-to-trough period of a 1 Hz raised cosine.

The observer adapted to the steady pedestal and background for 2 min. Then a detection threshold for the test stimulus was measured using two randomly interleaved staircases and a three-alternative temporal forced-choice method (Yeh *et al.*, 1993a,b). The mean amplitude of four reversal points with a 0.08 log unit step was taken as the threshold for each staircase and the mean of the two staircases was taken to represent the threshold. If an incorrect response occurred four times at the maximum amplitude stimulus, the staircase was terminated and recorded as no threshold measurable. It was possible to measure three to four thresholds in a 30 min session, without fatiguing the observer. Two thresholds were estimated for each stimulus and the mean of the two was taken as the final threshold.

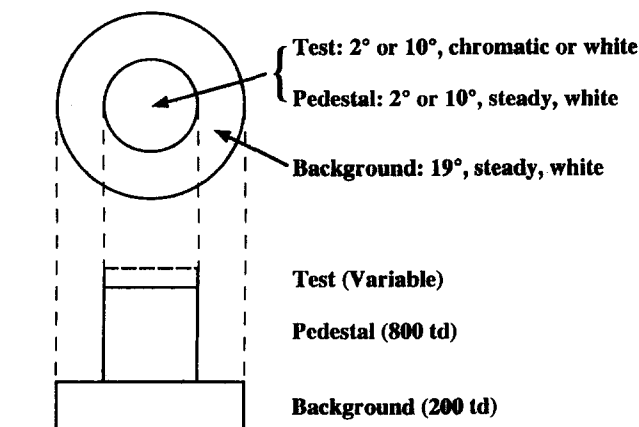


FIGURE 2. Spatial configuration of the stimuli for Expt. 1.

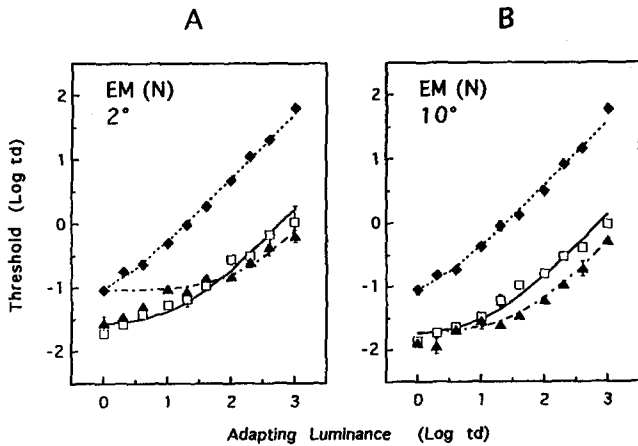


FIGURE 3. Results of TVR measurements of observer EM for (A) the 2 deg test field and (B) the 10 deg test field. \blacklozenge , Thresholds for the 4600 K test; \blacktriangle , thresholds for the 458 nm test; \square , thresholds for the 639 nm test. The error bars show the ranges of the two measurements. \cdots , least-squares fit of Eqn (1) to the 4600 K data; $-\cdots-$, least-squares fit of Eqn (3) to the 458 nm data; $---$, least-squares fit of Eqn (2) to the 639 nm data.

To determine the parameters of Eqns (1)–(3), threshold vs radiance (TVR) functions of three test stimuli were measured on color-normal observer EM. We used a test light of 4600 K for Eqn (1) since we assumed this test light to be detected exclusively by the (L + M) system. We used a test light of 639 nm for Eqn (2), and 458 nm for Eqn (3) since we assumed these test stimuli to be detected almost exclusively by the (L – M) spectral opponent and the S – (L + M) spectral opponent systems, respectively (Sperling & Harwerth, 1971; King-Smith & Carden, 1976; Calkins *et al.*, 1992). Inconel neutral density filters were inserted near the ophthalmic lens (OL1), thus reducing the retinal illuminance of the test, background, and pedestal at the same time. The retinal illuminances of the adapting field were 0.0, 0.3, 0.6, 1.0, 1.3, 1.6, 2.0, 2.3, 2.6, and 3.0 log td. For color-defective observers, time considerations precluded measurement of a full TVR function. We assumed that the three gain control parameters would be equivalent for all observers, normal or color defective and could be estimated from observer EM. The three threshold parameters were then determined individually for the color-defective observers. For this purpose thresholds were measured at three light levels, 2.0, 2.4, and 3.0 log td.

To determine spectral sensitivity functions, the background and pedestal were fixed at 3.0 log td, and thresholds were measured for all 13 chromatic lights plus the 4600 K achromatic light.

Results

TVR Functions. Figure 3 shows the TVR data of observer EM (N). Equations (1)–(3) were used to fit the TVR data; parameters were determined to minimize the mean of the squared residuals (MSR) on a logarithmic scale. (A) shows data (symbols) and fits (lines) for the 2 deg test stimuli, and (B) shows data (symbols) and fits (lines) for the 10 deg test stimuli. \blacklozenge – $---$ – \blacklozenge show results

for the 4600 K test light, \square – $---$ – \square show results for the 639 nm test light, and \blacktriangle – $---$ – \blacktriangle show results for the 458 nm test light. The error bars show the ranges of the two measurements. Parameter values for EM are given in Table 3. The threshold for the 4600 K light was about 0.5 log unit higher than for the other two test lights at the dimmest adapting field (pedestal and background), and increased up to a difference of more than 1.7 log units at the brightest adapting field. The 4600 K data showed linear behavior beginning at 1.0 log td adapting field, whereas the 639 and 458 nm data showed linear behavior above about 2.0 log td adapting field.

Figure 4 shows TVR data of the color-defective observers for the 2 deg test stimulus, and Fig. 5 shows TVR data for the 10 deg stimulus. Symbols and fits are as described for Fig. 3. For the protan and deutan observers, the gain constants (G_A , G_R , and G_Y) were assumed to be the same as for observer EM and the threshold parameters (T_A , T_R , and T_Y) were varied to fit the TVR data. All the TVR functions showed approximately linear increases. For the protan observers, TVR data for the 639 nm test could not be obtained due to the low sensitivity of these observers to long-wavelength lights. In these cases, the longest wavelength at which data could be collected was used: 610 nm for observer JK (PA); 580 nm for DK (EPA); and 590 nm for RR (P). For observers RR (P), TJ

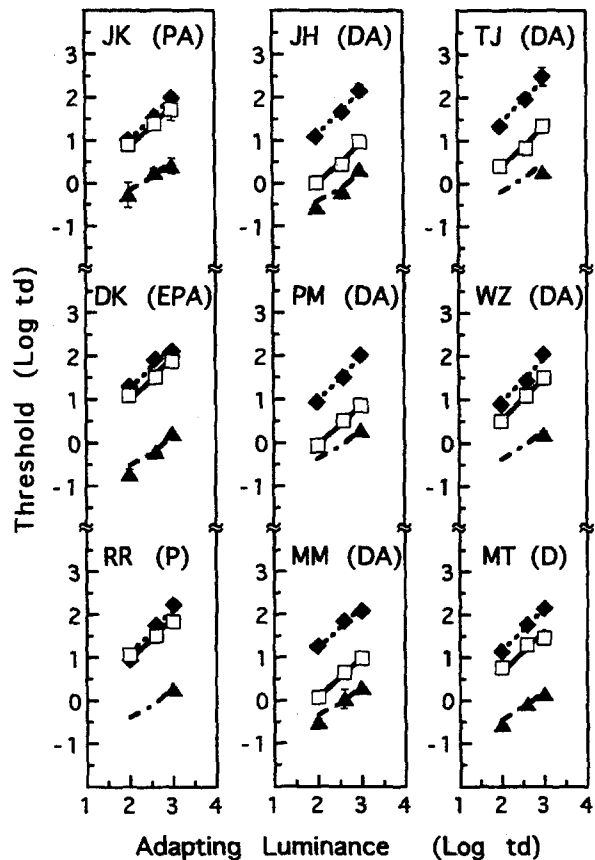


FIGURE 4. Results of TVR measurements for the color-defective observers for the 2 deg test stimuli. Symbols and lines are as for Fig. 3. See the text for further explanation of how Eqns (1)–(3) were adapted for color-defective observers.

TABLE 3. Parameters of the model

	2 deg test			10 deg test		
	G_A	G_R	G_Y	G_A	G_R	G_Y
	1.118	0.061	0.007	0.767	0.075	0.027

Observer	Classification	2 deg test			10 deg test		
		Log T_A	Log T_R	Log T_Y	Log T_A	Log T_R	Log T_Y
EM	N	-1.341	-1.482	0.311	-1.295	-1.667	-0.389
JK	PA	-1.073	-0.577	0.921	-0.868	-0.808	0.257
DK	EPA	-0.812	-0.720	0.574	-0.432	-0.970	-0.018
RR	P	-0.943	-0.549	0.706	-0.755	-0.744	0.140
JH	DA	-0.947	-1.285	0.666	-0.676	-1.316	0.199
PM	DA	-1.105	-1.324	0.735	-0.788	-1.315	0.210
MM	DA	-0.864	-1.195	0.756	-0.558	-1.254	0.239
TJ	DA	-0.657	-0.904	0.904	-0.438	-1.278	0.312
WZ	DA	-1.135	-0.728	0.732	-0.821	-0.858	0.127
MT	D	-0.909	-0.590	0.660	-0.779	-0.764	-0.008

(DA) PM (DA) and WZ (DA), TVR data were not obtained at 458 nm. For those observers, the absolute threshold for the S-(L+M) opponent system was varied to get the best fit to 458 and 571 nm for the increment spectral sensitivity data. The TVR functions for the color-defective observers were highest for the 4600 K test stimulus, lowest for the 458 nm test, and intermediate for the 639 nm test. Table 3 shows values of the threshold parameters for the color-defective observers determined by the fit for the 2 and 10 deg test stimuli, respectively.

In comparing the effect of field size we noted no clear differences for the white and the 639 nm tests. However, for the 458 nm stimulus, all the observers showed lower thresholds for the 10 deg test light than for the 2 deg test light and the mean difference was 0.16 log unit. The decrease in threshold for the 458 nm test with increased test size is consistent with previous studies (Stiles, 1949; Kishto, 1970) and can be explained by two factors: the higher optical density macular pigment (Moreland & Bhatt, 1984); and perhaps the sparsity of S cones (Curcio *et al.*, 1991) in the central fovea.

Spectral Sensitivity Functions. Figures 6 and 7 show the spectral sensitivity data (symbols) for the 2 and 10 deg test stimuli, respectively. The error bars show the ranges of the two measurements. For all observers, there were no notable differences in the heights or shapes of the spectral sensitivity functions between the two stimulus sizes.

The normal control (EM) showed minimum sensitivity at the L/M achromatic point of the adapting field (see Table 1). This phenomenon is often termed the Sloan notch following Sloan's observation of a shoulder in brightness matching at this location (Sloan, 1928). As pointed out by Mollon (1991), the minimum might more properly be attributed to Stiles and Crawford (1933), since they first observed the phenomenon as a minimum

detection threshold on an achromatic adapting field. Five of the seven anomalous trichromats showed a Sloan notch. Two anomalous trichromats (EPA observer DK and DA observer WZ) and the two dichromats (Protanope RR and Deuteranope MT) did not show a Sloan notch.

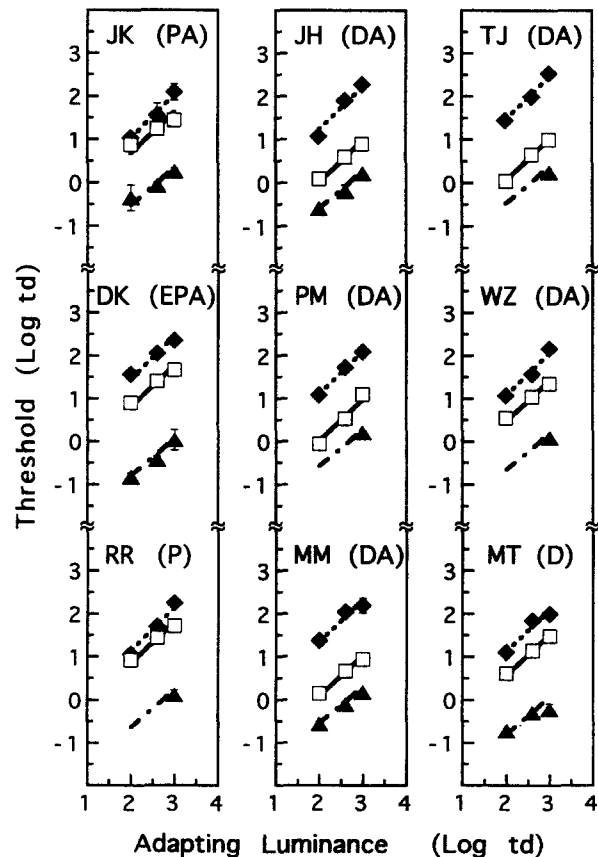


FIGURE 5. Results of TVR measurements for the color-defective observers for the 10 deg test stimuli. Symbols and lines are as for Fig. 3. See the text for further explanation of how Eqns (1)–(3) were adapted for color-defective observers.

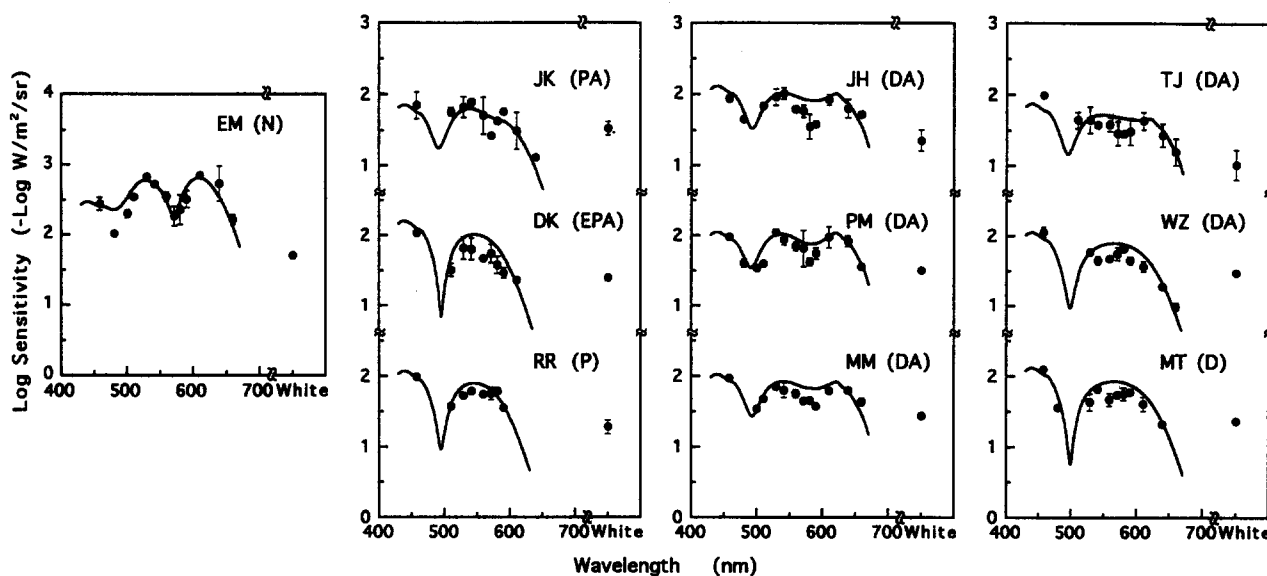


FIGURE 6. Increment threshold spectral sensitivity functions for the 2 deg test stimuli. The error bars show the ranges of the two measurements. The solid line is the prediction based on the fits of Figs 3 and 4.

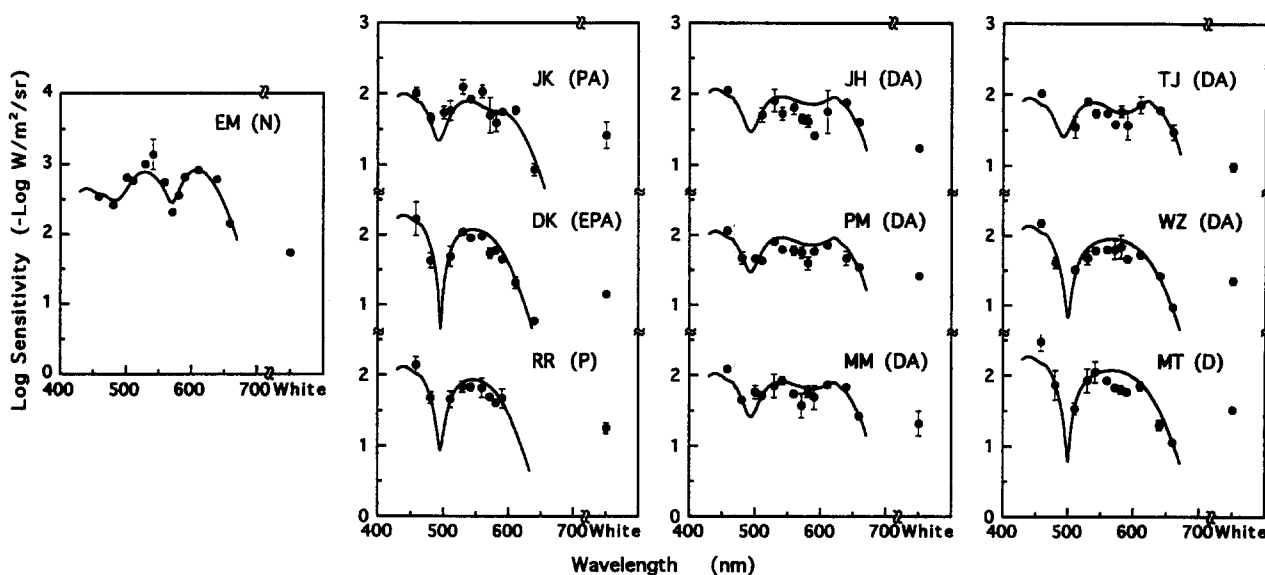


FIGURE 7. Increment threshold spectral sensitivity functions for the 10 deg test stimuli. The error bars show the ranges of the two measurements. The solid line is the prediction based on the fits of Figs 3 and 5.

For all the observers except JK, the sensitivity to the 4600 K test (the rightmost data point in Figs 6 and 7) was below the Sloan notch.

The solid lines represent the increment threshold spectral sensitivity functions for each observer predicted from the TVR data. The sensitivity prediction was obtained using the parameters of Table 3 in Eqns (1)–(3) and then using Eqn (5) to convert from increment threshold to sensitivity. As a combination rule of the three systems, we tried taking the upper envelope, the vector sum, and the linear sum. The average MSR for all the observers by the three combination rules were 0.020, 0.019, and 0.045 for the 2 deg test and 0.020, 0.021, and 0.057 for the 10 deg test stimuli. Thus, we used the vector

summation in this and in the subsequent predictions of the visual sensitivity functions. The interobserver range of the square residuals for the vector sum was 0.007–0.037. If the predicted $S - (L + M)$ opponent system threshold for 639 nm was < 0.20 log unit from the data point of 639 nm, we concluded that the observer did not have a functional $(L - M)$ spectral opponent system which could be measured with this increment threshold procedure. These observers were DK (EPA), RR (P), WZ (DA), and MT (D). In these observers, all predictions were made without the $(L - M)$ spectral opponent system.

Discussion

For all the observers and for both test stimulus sizes,

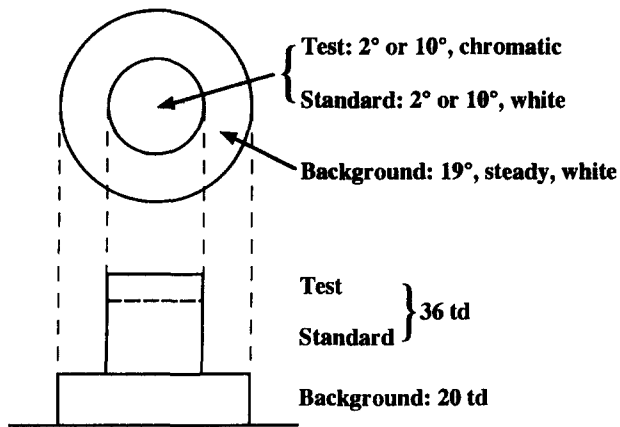


FIGURE 8. Spatial configuration of the stimuli of Expt 2.

the predicted sensitivity for the $S-(L+M)$ opponent system was higher than the predicted sensitivity for the $(L+M)$ system at 541 nm and at longer wavelengths. The higher predicted $S-(L+M)$ opponent system sensitivity than the predicted $(L+M)$ sensitivity around the Sloan notch means that the detection of the test stimulus at the Sloan notch was predominantly mediated by the $S-(L+M)$ opponent system, not by the $(L+M)$ system. Previous models of increment detection on white assumed that the test stimulus is detected by the achromatic system at the Sloan notch (Krandt & King-Smith, 1979; Calkins *et al.*, 1992; but see Kalloniatis & Harwerth, 1990). The $(L+M)$ system and the $(L+M)$ lobe of the $S-(L+M)$ opponent system have very similar spectral sensitivities > 520 nm. The detection of the test stimulus by the $S-(L+M)$ opponent system at the Sloan notch was evident in this experiment due to three factors: the use of red-green color-defective

observers who tend to have a larger region where detection is influenced by the $S-(L+M)$ opponent system than the normal control; the use of a pedestal and a background configuration; and the use of a cosine temporal waveform.

The effect of test wavelength varied between the color-normal and the color-defective observers. The separation between the 4600 K thresholds and the 458 nm thresholds varied from 1.6 to 2.3 log units in color-defective observers comparable to the data of the color-normal observer (2.0 log units). On the other hand, the separation between the white threshold and the 639 nm threshold differed greatly between the normal and the color-defective observers. Observer EM (N) showed 1.8 log units of separation between the white and the 639 nm threshold for both stimulus sizes. The four deuteranomalous observers (JH, MM, PM, TJ) showed separations ranging from 1.0 to 1.5 log units. According to the parameters of Eqn (2), the closer proximity (6 nm) of the theoretical photopigments for deuteranomalous would reduce the high luminance separation by 0.485 log unit to give an average separation of 1.28. A similar calculation for the protanomalous observer with a 10 nm proximity of the theoretical photopigments would reduce the high luminance separation by 0.635 (measured at 610 nm). This predicts a separation of 1.14. Thus Observer JK's separation of 0.65 showed a much closer overlap of TVR functions for 4600 K and 610 nm than predicted by DeMarco *et al.* (1992). Evaluation of the separation for extreme anomalous and dichromats is not appropriate. The use of Eqn (2) forces evaluation of parameters for an $(L-M)$ spectral opponent. However, we subsequently concluded that DK (EPA), RR (P), WZ (DA), and MT (D) did not show evidence for such activity. The assumption that their long-wavelength TVR thresholds are determined by the $(L-M)$ spectral opponent is thus

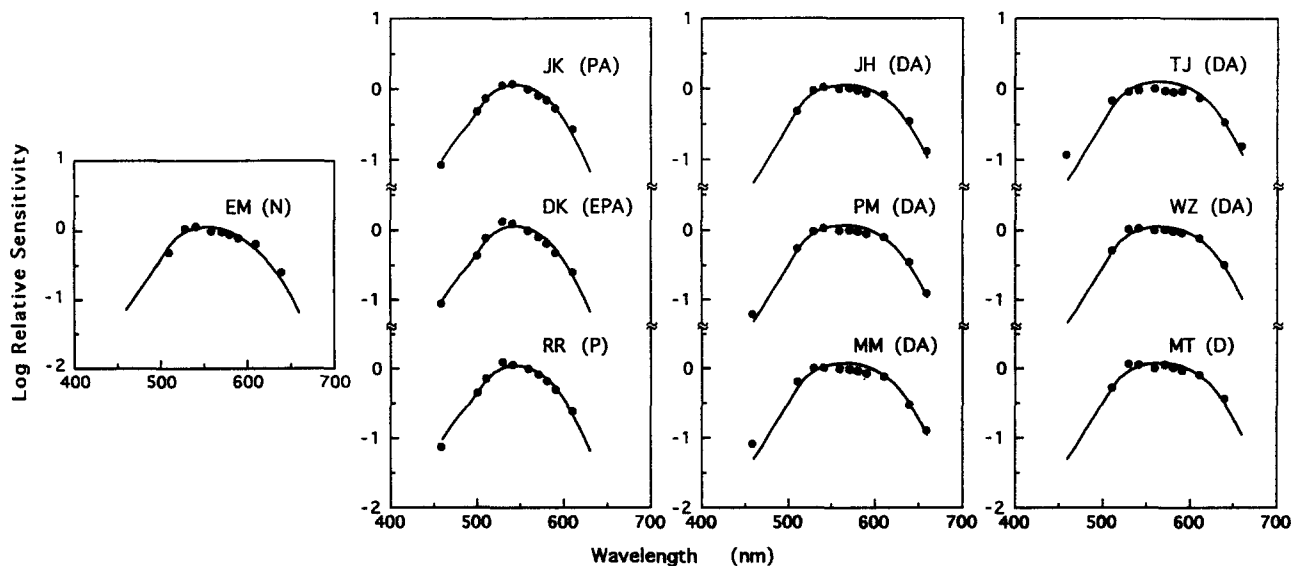


FIGURE 9. Relative spectral sensitivities by HFP for the 2 deg test stimuli. All the ranges of the two measurements are smaller than the symbol size. The solid line is the prediction based on the fits of Figs 3 and 4. The scaling of the prediction was adjusted to obtain the best fit to the data.

violated. It is likely that these increment thresholds were determined by an $S - (L + M)$ mechanism.

The current model constrains the relative heights of the LWS and the MWS lobes of the $(L - M)$ spectral opponent system. The heights of both lobes are predicted only by the excitation levels of the cones and their adaptation levels. Previous models of Sperling and Harwerth (1971) and of Calkins *et al.* (1992) allowed independent variation of the heights of the two lobes. Kranda and King-Smith (1979) found asymmetry (different heights) between the MWS and the LWS lobes. Boynton *et al.* (1964) reported asymmetry between the summation of red and green lights. The data of observers EM (N) and JK (PA) for the 10 deg test stimuli in this experiment would be fitted better with asymmetry of the two lobes. The previous models may describe the data well. However, they have three or four free parameters that are determined directly from the increment spectral sensitivity data. The fits of the current model do not require any free parameters.

EXPERIMENT 2: PURITY DISCRIMINATION THRESHOLDS

Procedure

There were two parts in Expt 2. The first part was measurement of sensation luminance (Kaiser, 1988) of the test stimulus. The second part involved purity discrimination threshold measurements in which the first step from white was measured with the sensation luminance of the test field fixed. Figure 8 shows the spatial configuration of the stimuli. A steady 19 deg dia 20 td circular white background was present. Superimposed on this background was a 2 or 10 deg test stimulus of 36 td composed of a mixture of 4600 K achromatic and a chromatic test stimulus.

The observer adapted to the background and the white standard light for 2 min. Heterochromatic flicker photometry (HFP) was used to equate the test stimulus to a 36 td, 4600 K standard light. We used counterphase sinusoidal temporal modulation at a frequency between 16 and 22 Hz and the method of adjustment. A median of three settings was used to represent the sensation luminance for each test light.

In the purity discrimination part of Expt 2, the 36 td white standard light was decremented with a single cycle of peak-to-peak raised cosine, while the test stimulus was incremented with a single cycle of trough-to-trough cosine with a period of a 1 Hz. The total sensation luminance of the standard and the test lights remained fixed while the chromaticity was changed. The first step from the white was measured using temporal forced choice and two randomly interleaved staircases, as described for Expt 1. Three to four purity discrimination steps could be estimated per session without fatiguing the observer. Two steps were estimated for each stimulus and the mean of the two was taken as the final first step from white.

Results

HFP. Figures 9 and 10 show the results of HFP measurements for the 2 and 10 deg test stimuli, respectively. The data represent normalized spectral sensitivity. All the ranges of the two measurements are smaller than the symbol size. The data showed similar shapes for the 2 and for the 10 deg test. Observer EM (N) showed a curve with one broad peak at 550 nm. The protan observers, with $p = 0$, (JK, DK, and RR) showed somewhat narrower curves with peaks at 540 nm. The deutan observers (JH, TJ, MM, PM, WZ, and MT), with $p = 1$, showed curves with one broad peak at 560 nm. The solid lines in Figs 9 and 10 are the spectral sensitivities

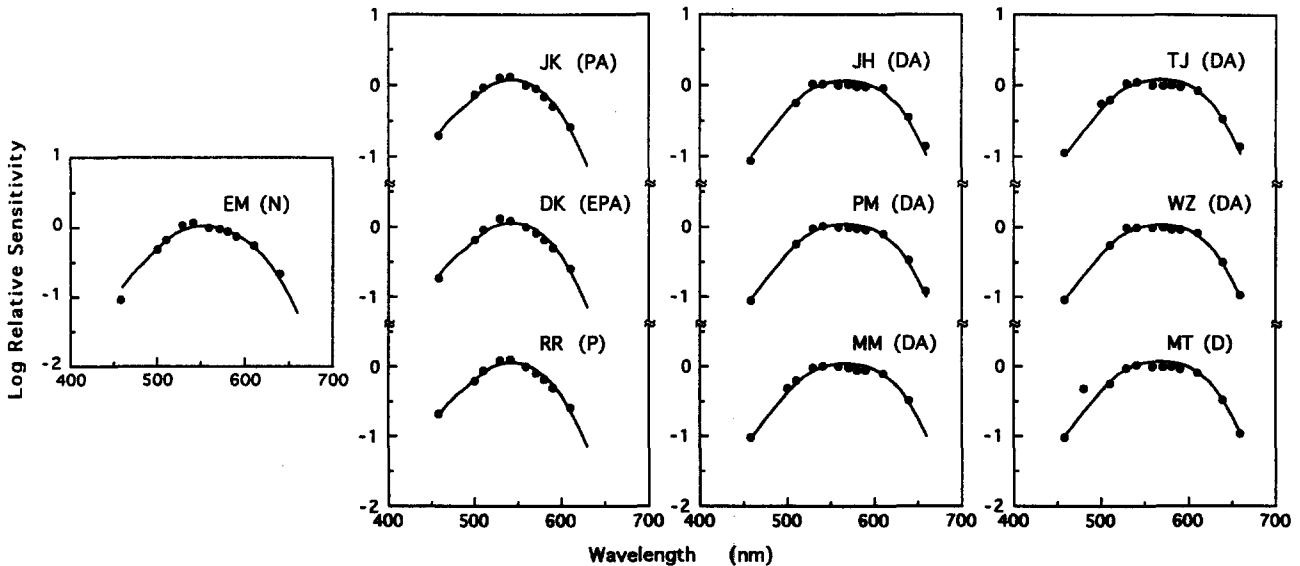


FIGURE 10. Relative spectral sensitivities by HFP for the 10 deg test stimuli. All the ranges of the two measurements are smaller than the symbol size. The solid line is the prediction based on the fits of Figs 3 and 5. The scaling of the prediction was adjusted to obtain the best fit to the data.

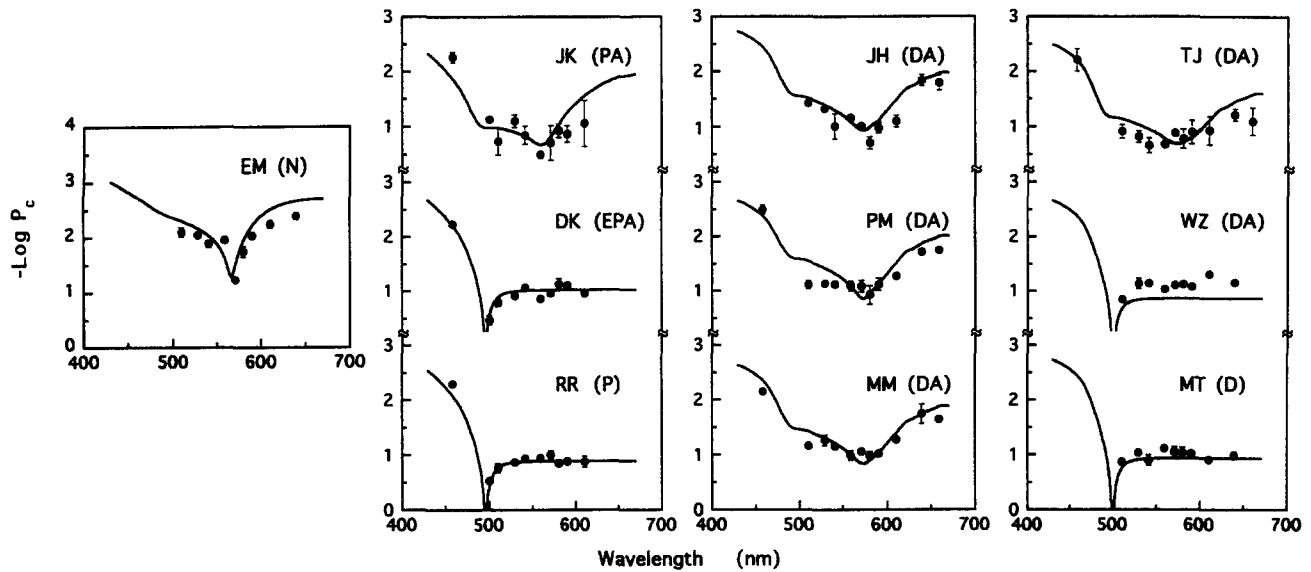


FIGURE 11. Purity discrimination thresholds from white plotted as the negative logarithm of the threshold colorimetric purity for the 2 deg test stimuli. The error bars are the ranges of the two measurements. The solid line is the prediction based on the fits of Figs 3 and 4.

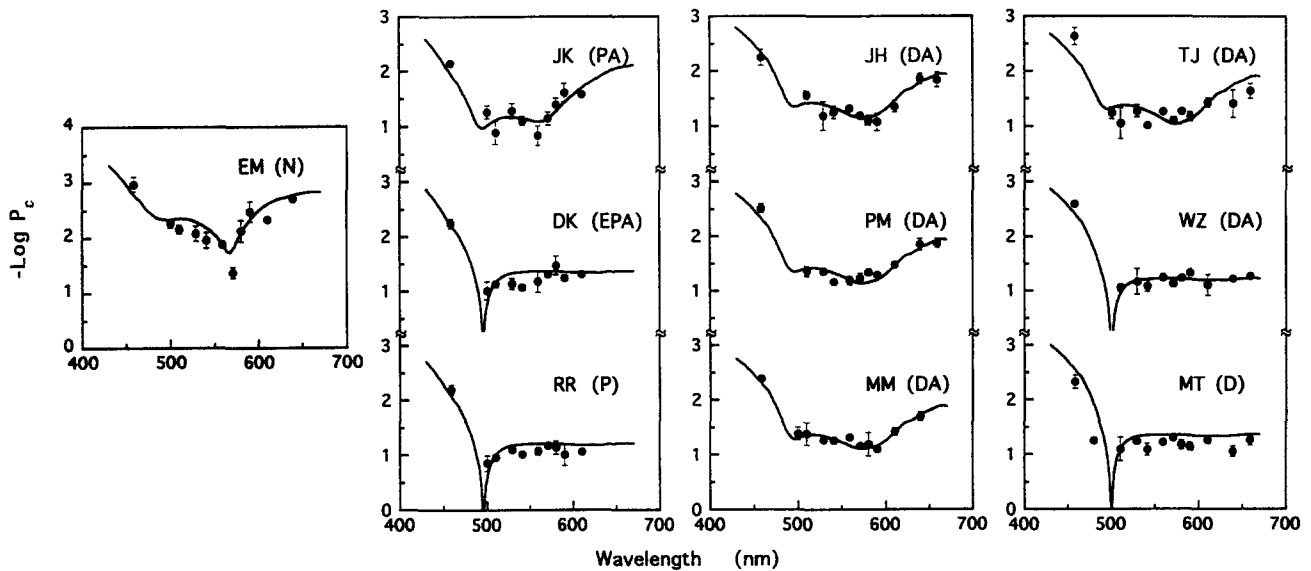


FIGURE 12. Purity discrimination thresholds from white plotted as the negative logarithm of the threshold colorimetric purity for the 10 deg test stimuli. The error bars are the ranges of the two measurements. The solid line is the prediction based on the fits of Figs 3 and 5.

using Eqns (1) and (5). Since HFP data are normalized to unity, we allowed a free scaling parameter to obtain the best fit of the line and the data. The mean residuals ranged from 0.001 to 0.020.

The lines for EM represent a proportion of L cones, $p = 0.6189$, those for protan observers represent $p = 0$ and those for deutan observers $p = 1$. We asked if the fit could be improved by allowing p to vary as a free parameter. For EM, the optimal values of p are 0.774 for 2 deg and 0.711 for 10 deg. The fit improvement for EM is significant only for the 2 deg data. The fits could not be improved for color-defective observers. Here we allowed the value of p to vary and used LWS' as the second

photopigment of protans and MWS' as the second photopigment of deutans. Although some of the color-defective observers show values of p other than 0 for protans and 1 for deutans, the improvement in the fit was not significant.

Colorimetric Purity Discrimination. Figures 11 and 12 show the data (symbols) for colorimetric purity discrimination for the 2 and 10 deg test stimuli, plotted as the negative logarithm of the colorimetric purity discrimination threshold. The error bars show the ranges of the two measurements. There were no notable differences in purity discrimination between the two stimulus sizes. Observer EM (N) shows a purity

discrimination function typical of the classical literature, with a sharp minimum at 571 nm (Wright, 1946). The data of the color-defective observers are also typical of the literature (Nelson, 1938; Chapanis, 1944; Wright, 1946). The dichromatic observers showed a maximum purity discrimination sensitivity at the shortest wavelength measured (458 nm), showed a minimum at 500 nm or threshold was unmeasurable, and the discrimination thresholds at 529 nm and above were almost constant. The anomalous trichromatic observers showed a maximum discrimination sensitivity at 458 nm, some of them showed a shallow minimum near the expected wavelength of the Sloan notch, and the variability of the shapes of the purity discrimination curves for the five DA observers was large.

The solid lines in Figs 11 and 12 are predictions using Eqns (2) and (3). For observers DK (EPA), RR (P), WZ (DA), and MT (D), however, only Eqn (3) was used. The predicted purity discrimination thresholds (in trolands) were converted to the colorimetric purities by the equation:

$$P_C = \frac{L_\lambda}{L_\lambda + L_w}, \quad (8)$$

where P_C is colorimetric purity, L_λ is the luminance of a spectral light, and L_w is the luminance of the white light. Then the logarithms of the reciprocals of the colorimetric purities were taken to match the unit of the data points. To compensate for the difference between sensation luminance and the photometric $V(\lambda)$, the logarithm of $m(\lambda)/m_{\max}/V(\lambda)$ was added to the raw prediction for the protan observer. For the same reason, the logarithm of $l(\lambda)/l_{\max}/V(\lambda)$ was added in predictions for the deutan observers. In predicting the purity discrimination for the 10 deg test, the logarithm of $V_{10}(\lambda)/V(\lambda)$ was added to the predicted values. $V_{10}(\lambda)$ is the CIE 10 deg y function (CIE, 1964) for all the types of the observers. The mean residuals ranged from 0.005 to 0.079. The residuals for the purity data were similar as for the increment spectral sensitivity data. A few isolated data sets show higher residuals (WZ, 2 deg; and MT, 10 deg).

The colorimetric purity data can equally well be plotted in chromaticity space. In this format they describe nearly 75% of a chromaticity figure (Yeh, 1991; Regan *et al.*, 1994) measured from a starting chromaticity of 4600 K. In color-normal observers the data are often described as a chromaticity ellipse. In color-defective observers the ellipse is elongated in anomalous trichromats and may degenerate to two parallel lines in dichromats.

MOLECULAR GENETIC ANALYSIS

The color-normal observer and eight of the nine color-defective observers consented to molecular analysis of their opsin genes. This analysis was performed by Maureen Neitz of the Medical College of Wisconsin, with the assistance of Alla Grishok.

Methods

Genomic DNA was extracted from blood samples obtained from each observer, and used in the polymerase chain reaction (PCR) to amplify specific segments derived from the X-linked visual pigment genes. The PCR products were subject to asymmetric PCR, and direct DNA sequence analysis. The primers, conditions for PCR, asymmetric PCR and DNA sequence analysis were as described by Neitz *et al.* (1995b). Briefly, gene fragments containing exons 4 and 5 from long-wave or middle-wave genes were first amplified separately using primers selective for a long- or a middle-wave exon 5. Thus, the nucleotide sequences of exon 4 associated with either a long- or a middle-wave exon 5 were determined. A second DNA fragment containing exons 3 and 4 was then amplified for each observer using primers selective for a long- or a middle-wave exon 4. Thus the nucleotide sequences of exon 3 associated with long- or middle-wave exon 4 were determined. Exon 2 of the X-linked pigment genes was not examined in this study.

Results

The results of the genetic analysis are summarized in Table 4. M4 or L4 denotes middle- or long-wave exon 4 sequence, and M5 or L5 denotes middle- or long-wave exon 5 sequence, respectively. Position 180 of exon 3 is an allelic site in normal genes. Serine at position 180 of the opsin is indicated by ser180 and alanine by ala180. Normal observer EM has genes for LWS and MWS opsins, including two types of M opsin genes, one with alanine and one with serine at position 180 (denoted as L4L5ser180, M4M5ala180 and M4M5ser180).

Protanomalous observer JK showed two types of M opsin gene, one with alanine and one with serine at position 180 (denoted as M4M5ala180 and M4M5ser180). The extreme protanomalous observer DK and protanope RR showed only one X-linked opsin gene sequence with alanine at position 180 (denoted as M4M5ala180).

Deuteranomalous observers JH, MM, PM and TJ all have normal L opsin genes. The L opsin gene for JH and MM showed serine at position 180 (denoted as L4L5ser180) and that for PM showed alanine at position 180 (denoted as L4L5ala180). Observer TJ showed two types of L opsin gene, one with alanine and one with serine at position 180 (denoted as L4L5ala180 and L4L5ser180). These observers also showed hybrid genes which combined exon 4 of the normal M opsin gene and exon 5 of the normal L opsin gene with alanine at position 180 (denoted as M4L5ala180). Deuteranopic observer MT showed one type of L opsin gene with alanine at position 180 (denoted as L4L5ala180). Additionally three of the deutan observers (JH, MM and MT) showed M opsin genes (denoted as M4M5ala180).

Discussion

Exon 5 of the X-linked visual pigment genes shows amino acid differences that produce the spectral difference between long-wave and middle-wave sensitive

TABLE 4. Summary of molecular genetic analysis

Observer	Phenotype	"L" genes*	"M" genes*
EM	N	L4L5ser180	M4M5ala180 M4M5ser180
JK	PA		M4M5ala180 M4M5ser180
DK	EPA		M4M5ala180
RR	P		M4M5ala180
JH	DA	L4L5ser180 M4L5ala180	M4M5ala180
MM	DA	L4L5ser180 M4L5ala180	M4M5ala180
PM	DA	L4L5ala180 M4L5ala180	
TJ	DA	L4L5ser180 L4L5ala180 M4L5ala180	
MT	D	L4L5ala180	M4M5ala180

*Indicated are the deduced gene structures found in each observer. M4 or L4 denotes middle- or long-wave exon 4 sequence, and M5 or L5 denotes middle- or long-wave exon 5 sequence, respectively. Serine encoded at position 180 of the opsin is indicated by ser180 and alanine by ala180.

pigments (Neitz *et al.*, 1991b; Chan *et al.*, 1992; Merbs & Nathans, 1993; Asenjo *et al.*, 1994). Thus, exon 5 should determine the class of photopigment opsin as L or M. The protan observers showed only M opsin genes. The deutan observers showed genes for L photopigments but additionally three observers (JH, MM, and MT) showed genes for M photopigments. This finding is common in the literature and remains unexplained. It is usually assumed that these M genes in deutan observers are defective or unexpressed (Neitz *et al.*, 1991a; Winderickx *et al.*, 1992b).

Exons 2, 3 and 4 of the visual pigment genes show amino acid differences that produce small spectral shifts, and can be thought of as producing subtypes of long-wave or middle-wave pigments (Neitz *et al.*, 1991b, 1995b; Chan *et al.*, 1992; Merbs & Nathans, 1993; Asenjo *et al.*, 1994). Amino acid differences in exon 2 produce small spectral shifts of < 2 nm. At exon 3, the serine/alanine amino acid difference at position 180 of the opsin produces a shift of 5–7 nm in long-wave pigments, and 2–4 nm in middle-wave pigments (Neitz *et al.*, 1991b, 1995a; Merbs & Nathans, 1993; Asenjo *et al.*, 1994). Amino acid substitutions in exon 4 produce spectral shifts on the order of 0.5–4 nm.

The M genes of PA observer JK showing only a difference at position 180 is thus consistent with a 2–4 nm separation of his visual photopigments. The multiple L opsin genes of the deuteranomalous observers JH, MM, PM, and TJ are consistent with a possible 5–7 nm separation for groups of L opsin genes. The finding of the alanine at position 180 in deuteranopic observer MT might suggest a spectral sensitivity shifted 2.5 nm from the average deuteranopic sensitivity. However, spectral

sensitivity in this observer was well described under all conditions by the LWS fundamental of normal trichromats.

GENERAL DISCUSSION

A model to predict increment thresholds of normal trichromats was adapted to X-chromosome-linked color-defective observers. The modifications that we made were: (1) the peak wavelength of the LWS' pigment of average protan observers was set at 553 nm; (2) the peak wavelength of the MWS' pigment of average deutan observers was set at 560 nm; and (3) we assumed that the HFP spectral sensitivities of color-defective observers were mediated only by the normal Smith and Pokorny (1975) fundamentals (MWS in protan observers and LWS in deutan observers). Once we determined the values of the parameters using the TVR data, the modified model predicted the increment threshold sensitivity functions (Expt 1), HFP spectral sensitivity (Expt 2) and the purity discrimination thresholds (Expt 2). We allowed no free parameters in determining the increment spectral sensitivities or the colorimetric purity thresholds.

The model generally accounted for anomalous trichromacy as an alteration system and dichromacy as a reduction system. We did not make any distinction between anomalous trichromats and dichromats when we fit the model to the TVR data. We considered a particular observer did not demonstrate a functional (L – M) opponent system if the difference between the predicted sensitivity at 639 nm by the S – (L + M) opponent system and the measured increment sensitivity at 639 nm was < 0.2 log unit. According to this criterion, four observers (one EPA, one P, one of the five DAs, and the one D) were regarded as having no functional (L – M) opponent system under the current experimental conditions. The increment threshold spectral sensitivity functions and purity discriminations of these observers were well predicted without an (L – M) opponent system. Thus, some of the anomalous trichromats as well as the dichromats may be regarded as having no functional (L – M) opponent system.

Previous studies showed that dichromats (Smith & Pokorny, 1977; Nagy, 1980; Breton & Cowan, 1981; Nagy, 1982) and extreme anomalous trichromats (Nagy, 1982) had smaller matching ranges in the Rayleigh match with the use of a larger field. Nagy (1982) found that the matching ranges of dichromats and extreme anomalous trichromats converged to the matching ranges of the corresponding simple anomalous trichromats under large field conditions with rods suppressed. Thus, we expected that the use of a large test stimulus might enhance chromatic sensitivity mediated by the (L – M) opponent system in the dichromatic and the extreme anomalous trichromatic observers. However, the results of this experiment showed that the dichromats, the extreme anomalous trichromat, and the one of the simple anomalous trichromats did not have any functional (L – M) opponent system that could be revealed by the

current procedures even with the 10 deg test stimulus. There are two possible explanations for these observations: (1) these observers do lack a functional (L - M) opponent system; or (2) the sensitivity of the (L - M) opponent system remains lower than the sensitivity of the S - (L + M) opponent system at all the wavelengths measured for both field sizes. For the two anomalous trichromats and the deuteranope, the second explanation seems more plausible because their anomaloscope matching ranges were restricted with an 8 deg field. For the protanopic observer (RR), who accepted the full range in all the anomaloscope settings employed, the first explanation is possible.

In general, the concordance of the observed data with the molecular genetic analysis was good. Protanomalous observer JK appeared to show a greater loss in chromatic sensitivity than predicted by the proximity of the theoretical photopigments of DeMarco *et al.* (1992). As noted above, his genotype revealed two types of M opsin gene. The genotype predicts a separation of 2-4 nm, consistent with his reduced chromatic discrimination compared with the "standard" PA. The four deuteranomalous observers (JH, MM, PM, and TJ) showed loss in chromatic sensitivity consistent with the predicted 6 nm proximity of the "standard" DA. The genotypes revealed multiple L opsins conferring possible pigment separations of 5-7 nm. The extreme protanomalous (DK), protanope (RR) and deuteranope (MT) showed no evidence for a functional (L - M) opponent system. Two of these observers, however, showed large field Rayleigh matches narrowing from full range. All three observers showed only one opsin type according to the molecular analysis of exons 3-5. However exon 2 was not investigated and may possibly have revealed variation. The deuteranomalous trichromat (WZ) who showed narrow Rayleigh matches but no evidence for (L - M) spectral opponency remains a puzzling case. Unfortunately, he declined to participate in the molecular genetic analysis.

An issue that has arisen in the literature concerns whether (L - M) spectral opponency is derived from labeled receptors which determine segregation of LWS and MWS cone types in centers and surround of cells in the parvocellular pathway (Reid & Shapley, 1992; Smith *et al.*, 1992). An alternative model (Lennie *et al.*, 1991) is that spectral opponency can arise if centers are cone selective but surrounds contain mixtures of LWS and MWS cones. Such cells could be frequent in the fovea where the centers are driven by a single cone. Our data do not address this issue directly. However, we note the finding of spectral opponency in protanomalous observer JK with two types of M opsin gene. It would appear that if labeled receptors do exist, the source of their identification is not in the opsin code.

REFERENCES

- Alpern, M., & Pugh, E. N. (1977). Variation in the action spectrum of erythrolabe among deuteranopes. *Journal of Physiology (London)*, 266, 613-646.
- Alpern, M., & Torii, S. (1968a). The luminosity curve of the deuteranomalous fovea. *Journal of General Physiology*, 52, 738-749.
- Alpern, M., & Torii, S. (1968b). The luminosity curve of the protanomalous fovea. *Journal of General Physiology*, 52, 717-737.
- Asenjo, A. B., Rim, J. & Oprian, D. D. (1994). Molecular determinants of human red/green color discrimination. *Neuron*, 12, 1131-1138.
- Boynton, R. M., Ikeda, M. & Stiles, W. S. (1964). Interactions among chromatic mechanisms as inferred from positive and negative increment thresholds. *Vision Research*, 4, 87.
- Boynton, R. M. & Kambe, N. (1980). Chromatic difference steps of moderate size measured along theoretically critical axes. *Color Research and Application*, 5, 13-23.
- Breton, M. & Cowan, W. (1981). Deuteranomalous color matching in the deuteranopic eye. *Journal of the Optical Society of America*, 71, 1220-1223.
- Calkins, D. J., Thornton, J. E. & Pugh, E. N. J. (1992). Monochromatism determined at a long-wavelength/middle-wavelength cone-antagonistic locus. *Vision Research*, 32, 2349-2367.
- Chan, T., Lee, M. & Sakmar, T. P. (1992). Introduction of hydroxyl-bearing amino acids causes bathochromic spectral shifts in rhodopsin. *Journal of Biological Chemistry*, 267, 9478-9480.
- Chapanis, A. (1944). Spectral saturation and its relation to color-vision defects. *Journal of Experimental Psychology*, 34, 24-44.
- CIE (1964). *Proceedings 1963 (Vienna Session)* (Vol. B., Committee Report E-1.4.1, pp. 209-220). Paris: Bureau Central de la CIE.
- Curcio, C. A., Allen, K. A., Sloan, K. R., Lerea, C. L., Hurley, J. B., Klock, I. B. & Milam, A. H. (1991). Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *Journal of Comparative Neurology*, 312, 610-624.
- Deeb, S. S., Lindsey, D. T., Hibiya, Y., Sanocki, E., Winderickx, J., Teller, D. Y. & Motulsky, A. G. (1992). Genotype-phenotype relationships in human red/green color-vision defects: Molecular and psychophysical studies. *American Journal of Human Genetics*, 51, 687-700.
- DeMarco, P., Pokorny, J. & Smith, V. C. (1992). Full-spectrum cone sensitivity functions for X-chromosome-linked anomalous trichromats. *Journal of the Optical Society of America A*, 9, 1465-1476.
- Guth, S. L. & Lodge, H. R. (1973). Heterochromatic additivity, foveal spectral sensitivity and a new color model. *Journal of the Optical Society of America*, 63, 450-462.
- Hood, D. C. & Greenstein, V. (1990). Models of the normal and abnormal rod system. *Vision Research*, 30, 51-68.
- Ingling, C. R. & Tsou, B. H. P. (1977). Orthogonal combination of the three visual channels. *Vision Research*, 17, 1075-1082.
- Jacobs, G. H. & Neitz, J. (1993). ERG flicker photometric evaluation of spectral sensitivity in protanopes and protanomalous trichromats. In Drum, B. (Ed.), *Color vision deficiencies XI. Documenta Ophthalmologica Proceedings Series*, 55, 25-31.
- Jameson, D. & Hurvich, L. M. (1956). Theoretical analysis of anomalous trichromatic color vision. *Journal of the Optical Society of America*, 46, 1075-1089.
- Judd, D. B. (1951). Colorimetry and artificial daylight. In Technical Committee No. 7 Report of Secretariat United States Commission, International Commission on Illumination, Twelfth Session, Stockholm. (pp. 1-60).
- Kaiser, P. K. (1988). Sensation luminance: A new name to distinguish CIE luminance from Luminance dependent on an individual's spectral sensitivity. *Vision Research*, 28, 455-456.
- Kalloniatis, M. & Harwerth, R. S. (1990). Spectral sensitivity and adaptation characteristics of cone mechanisms under white-light adaptation. *Journal of the Optical Society of America A*, 7, 1912-1928.
- King-Smith, P. E. & Carden, D. (1976). Luminance and opponent-color contributions to visual detection and adaptation and to temporal and spatial integration. *Journal of the Optical Society of America*, 66, 709.
- Kishto, B. N. (1970). Variation of the visual threshold with retinal location. *Vision Research*, 10, 745-767.

- Kranda, K. & King-Smith, P. E. (1979). Detection of coloured stimuli by independent linear systems. *Vision Research*, *19*, 733–745.
- LeGrand, Y. (1968). *Light, colour and vision*. London: Chapman & Hall.
- Lennie, P., Haake, P. W. & Williams, D. R. (1991). The design of chromatically opponent receptive fields. In Landy, M. S. & Movshon, J. A. (Eds), *Computational models of visual processing*, (pp. 71–82). Cambridge, Mass.: MIT Press.
- Lindsey, D. T., Pokorny, J. & Smith, V. C. (1986). Phase-dependent sensitivity to heterochromatic flicker. *Journal of the Optical Society of America A*, *3*, 921–927.
- MacAdam, D. L. (1949). Colour discrimination and the influence of colour contrast on acuity. *Documenta Ophthalmologica*, *3*, 214–233.
- Merbs, S. L. & Nathans, J. (1993). Role of hydroxyl-bearing amino acids in differentially tuning the absorption spectra of the human red and green cone pigments. *Photochemistry & Photobiology*, *58*, 706–710.
- Miyahara, E., Pokorny, J. & Smith, V. C. (1995). The red–green chromatic system in anomalous trichromats. *Documenta Ophthalmologica Proceedings Series*, *58*, 149–157.
- Miyahara, E., Smith, V. C. & Pokorny, J. (1993). How surrounds affect chromaticity discrimination. *Journal of the Optical Society of America A*, *10*, 545–553.
- Miyahara, E., Smith, V. C. & Pokorny, J. (1994). Increment on white and purity discrimination thresholds in X-chromosome-linked color defectives. *Investigative Ophthalmology and Visual Science*, *35*, 1556.
- Mollon, J. (1991). Discussion: Biophysics and psychophysics of photoreceptors. In Valberg, A. & Lee, B. B. (Eds), *From pigments to perception* (pp. 35–40). New York: Plenum Press.
- Moreland, J. D. & Bhatt, P. (1984). Retinal distribution of macular pigment. *Documenta Ophthalmologica Proceedings Series*, *39*, 127–132.
- Nagel, W. A. (1907). Zwei apparate für die augenärztliche Funktionsprüfung: Adaptometer und kleines Spektralphotometer (Anomaloskop). *Zeitschrift für Augenheilkunde*, *17*, 201.
- Nagy, A. L. (1980). Large-field substitution Rayleigh matches of dichromats. *Journal of the Optical Society of America*, *70*, 778–784.
- Nagy, A. L. (1982). Homogeneity of large-field color matches in congenital red–green color defectives. *Journal of the Optical Society of America*, *72*, 571–577.
- Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B. & Hogness, D. S. (1986b). Molecular genetics of inherited variation in human color vision. *Science*, *232*, 203–210.
- Nathans, J., Thomas, D. & Hogness, D. S. (1986a). Molecular genetics of human color vision: The genes encoding blue, green and red pigments. *Science*, *232*, 193–202.
- Neitz, J., Neitz, M. & Jacobs, G. H. (1993). More than three different cone pigments among people with normal color vision. *Vision Research*, *33*, 117–122.
- Neitz, M., Neitz, J. & Grishok, A. (1995b). Polymorphism in the number of genes encoding long-wavelength sensitive cone pigments among males with normal color vision. *Vision Research*, *35*, 2395–2407.
- Neitz, M., Neitz, J. & Jacobs, G. H. (1991a). Relationship between cone pigments and genes in deuteranomalous subjects. *Documenta Ophthalmologica Proceedings Series*, *54*, 397–403.
- Neitz, M., Neitz, J. & Jacobs, G. H. (1991b). Spectral tuning of pigments underlying red–green color vision. *Science*, *252*, 971–973.
- Neitz, M., Neitz, J. & Jacobs, G. H. (1995a). Genetic basis of photopic variations in human dichromats. *Vision Research*, *35*, 2095–2103.
- Nelson, J. H. (1938). Anomalous trichromatism and its relation to normal trichromatism. *Physical Society of London*, *50*, 661–702.
- Noorlander, C., Heuts, M. J. G. & Koenderink, J. J. (1980). Influence of the target size on the detection threshold for luminance and chromaticity contrast. *Journal of the Optical Society of America*, *70*, 1116–1121.
- Pickford, R. W. (1967). Variability and consistency in the manifestation of red–green colour vision defects. *Vision Research*, *7*, 65–77.
- Pokorny, J., Jin, Q. & Smith, V. C. (1993). Spectral-luminosity functions, scalar linearity, and chromatic adaptation. *Journal of the Optical Society of America A*, *10*, 1304–1313.
- Pokorny, J. & Smith, V. C. (1977). Evaluation of single pigment shift model of anomalous trichromacy. *Journal of the Optical Society of America*, *67*, 1196–1209.
- Pokorny, J. & Smith, V. C. (1982). New observations concerning red–green color defects. *Color Research and Application*, *7*, 159–164.
- Quick, R. F. (1974). A vector-magnitude model of contrast detection. *Kybernetik*, *16*, 65–67.
- Regan, B. C., Reffin, J. & Mollon, J. D. (1994). Luminance noise and the rapid determination of discrimination ellipses in colour deficiency. *Vision Research*, *34*, 1279–1299.
- Reid, R. C. & Shapley, R. M. (1992). Spatial structure of cone inputs to receptive fields in primate lateral geniculate nucleus. *Nature*, *356*, 716–718.
- Sanocki, E., Shevell, S. K. & Winderickx, J. (1994). Serine/Alanine amino acid polymorphism of the L-cone photopigment assessed by dual Rayleigh-type color matches. *Vision Research*, *34*, 377–382.
- Shapiro, A., Zaidi, Q. & Hood, D. (1992). The effect of adaptation on the differential sensitivity of the S-cone color system. *Vision Research*, *32*, 1297–1318.
- Sloan, L. L. (1928). The effect of intensity of light, state of adaptation of the eye, and size of photometric field on the visibility curve. *Psychological Monographs*, *38*, 1–87.
- Smith, V. C., Bowen, R. W. & Pokorny, J. (1984). Threshold temporal integration of chromatic stimuli. *Vision Research*, *24*, 653–660.
- Smith, V. C., Lee, B. B., Pokorny, J., Martin, P. R. & Valberg, A. (1992). Responses of macaque ganglion cells to the relative phase of heterochromatically modulated lights. *Journal of Physiology (London)*, *458*, 191–221.
- Smith, V. C. & Pokorny, J. (1975). Spectral sensitivity of the foveal cone photopigments between 400 and 500 nm. *Vision Research*, *15*, 161–171.
- Smith, V. C. & Pokorny, J. (1977). Large-field trichromacy in protanopes and deutanopes. *Journal of the Optical Society of America*, *67*, 213–220.
- Smith, V. C., Pokorny, J., Davis, M., & Yeh, T. (1995). Mechanisms subserving temporal modulation sensitivity in silent cone substitution. *Journal of the Optical Society of America*, *12*, 241–249.
- Smith, V. C., Pokorny, J. & Yeh, T. (1993). Pigment tests evaluated by a model of chromatic discrimination. *Journal of the Optical Society of America A*, *10*, 1773–1784.
- Sperling, H. G. & Harwerth, R. S. (1971). Red–green cone interaction in the increment-threshold spectral sensitivity of primates. *Science*, *172*, 180–184.
- Stiles, W. S. (1949). Increment thresholds and the mechanisms of colour vision. *Documenta Ophthalmologica*, *3*, 138–163.
- Stiles, W. S. & Crawford, B. H. (1933). The luminal brightness increment as a function of wave-length for different conditions of the foveal and parafoveal retina. *Proceedings of the Royal Society (London) B*, *113*, 496–530.
- Verriest, G. (1971). Les courbes spectrales photopiques d'efficacité lumineuse relative dans les déficiences congénitales de la vision des couleurs. *Vision Research*, *11*, 1407–1434.
- Verriest, G., Laethem, J. v. & Uvijls, A. (1982). A new assessment of the normal ranges of the Farnsworth–Munsell 100-hue test scores. *American Journal of Ophthalmology*, *93*, 635–642.
- Vollrath, D., Nathans, J. & Davis, R. W. (1988). Tandem array of human visual pigment genes at Xq28. *Science*, *240*, 1669–1672.
- von Kries, J. (1897). Über Farbensysteme. *Zeitschrift für Psychologie Physiologie Sinnesorg*, *13*, 241–324.
- von Kries, J. (1924). Normal and anomalous color systems. In von Helmholtz, H. L. F. (Ed.), *Treatise on physiological optics* (3rd edn, Vol. 2, pp. 395–425). Rochester, N.Y.: Optical Society of America.
- Went, L. N. & deVries-deMol, E. C. (1976). Genetics of colour vision. *Modern Problems in Ophthalmology*, *17*, 96–107.
- Winderickx, J., Lindsey, D. T., Sanocki, E., Teller, D. Y., Motulsky, A. G. & Deeb, S. S. (1992a). Polymorphism in red photopigment underlies variation in colour matching. *Nature*, *356*, 431–433.
- Winderickx, J., Sanocki, E., Lindsey, D. T., Teller, D. Y., Motulsky, A. G. & Deeb, S. S. (1992b). Defective colour vision associated with a

- missense mutation in the human green visual pigment gene. *Nature Genetics*, 1, 251–256.
- Wright, W. D. (1946). *Researches on normal and defective colour vision*. London: Henry Kimpton.
- Yeh, T. (1991). Colorimetric purity discrimination: Theory and data. Dissertation, University of Chicago, Chicago, IL.
- Yeh, T., Pokorny, J. & Smith, V. C. (1993a). Chromatic discrimination with variation in chromaticity and luminance: Data and theory. *Vision Research*, 33, 1835–1845.
- Yeh, T., Smith, V. C. & Pokorny, J. (1993b). Colorimetric purity discrimination: Data and theory. *Vision Research*, 33, 1847–1857.
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