Functional consequences of the relative numbers of L and M cones

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Direct imaging of the retina by adaptive optics allows assessment of the relative number of long-wavelength-sensitive (L) and middle-wavelength-sensitive (M) cones in living human eyes. We examine the functional consequences of variation in the relative numbers of L and M cones (L/M cone ratio) for two observers whose ratios were measured by direct imaging. The L/M cone ratio for the two observers varied considerably, taking on values of 1.15 and 3.79. Two sets of functional data were collected: spectral sensitivity measured with the flicker electroretinogram (ERG) and the wavelength of unique yellow. A genetic analysis was used to determine L and M cone spectra appropriate for each observer. Rayleigh matches confirmed the use of these spectra. We determined the relative strength of L and M cone contributions to ERG spectral sensitivity by fitting the data with a weighted sum of L and M cone spectra. The relative strengths so determined (1.06 and 3.38) were close to the cone ratios established by direct imaging. Thus variation in L/M cone ratio is preserved at the sites tapped by the flicker ERG. The wavelength of unique yellow varied only slightly between the two observers (576.8 and 574.7 nm). This small variation indicates that neural factors play an important role in stabilizing unique yellow against variation in the L/M cone ratio. © 2000 Optical Society of America [S0740-3232(00)01003-6]

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1. INTRODUCTION

It has long been clear that human color vision is mediated by three classes of light-sensitive cone, those that are sensitive to long (L), middle (M), and short (S) wavelengths. Anatomical[1–10] and behavioral[11–13] techniques have provided a good understanding of the number and packing arrangement of the S cone submosaic. But identifying the number and arrangement of the L and M cone submosaics has been a considerably less tractable problem.

Recently, new techniques have been developed to mea-

sure the relative numbers of L and M cones (L/M cone ratio) in individual human eyes. These techniques are direct imaging of the living human retina[10] and analysis of the messenger RNA expressed in retinas from male eye donors.[14–16] These methods indicate that there is considerable individual variation in the L/M cone ratio. Roorda and Williams[10] studied two observers and found L/M cone ratios of 1.15 and 3.79, respectively. Hagstrom *et al.*[16] studied 23 male eye donors and found that the L/M cone ratio varied between 0.82 and 3.00. That sample has been expanded recently to include more than 100 male eye donors, and the range of variation for the larger sample was from 0.82 to 9.71.[17] Individual variation in the ratio of L cone to M cone pigment is also revealed by retinal densitometry.[18,19]

Individual variation in the L/M cone ratio might be expected to have consequences for vision. For example, there is considerable individual variation in the relative strength of the L and M cone input (L/M signal ratio) to mechanisms that mediate both psychophysical flicker photometry[18,20–23] and the flicker electroretinogram (ERG).[24–28] This variation may be mediated by the relative numbers of L and M cones.

There is also individual variation in the wavelength of unique yellow. Cicerone has suggested that the wavelength of unique yellow depends strongly on the L/M cone ratio,[29] but this suggestion remains controversial.[30,31]

Other functional measures, such as relative efficiency of detection for lights of different spectral composition,[32–34] the appearance of brief, small flashes of light,[35,36] hyperacuity measurements for small spots of different spectral composition,[37] and the spectral response of individual neurons in the visual pathways,[38–40] may also reflect variation in the L/M cone ratio.

Conclusions about the relation between L/M cone ratio and functional measurements have generally been drawn somewhat indirectly. An important exception is a classic paper by Rushton and Baker.[18] They showed that individual variation revealed by heterochromatic flicker photometry correlated with estimates of the amount of L and M cone pigment derived from retinal densitometry (see also Ref. 23).

The direct imaging technique of Roorda and Williams[10] allows assessment of L/M cone ratios in living human eyes. In this paper we examine the functional consequences of variation in the L/M cone ratio for two individuals whose ratios were studied by direct imaging. The L/M cone ratio for the two observers was quite different. A genetic analysis was used to determine L and M cone spectra appropriate for each observer. Rayleigh matches confirmed the use of these cone spectra. For both observers, we measured spectral sensitivities by using ERG flicker photometry and the spectral locus of unique yellow.

2. OBSERVERS

Two young adult male observers, AN and JW, were used in this study. These observers were chosen because the relative numbers of L and M cones in their retinas had been established previously.[10] The imaging procedure used to identify individual L, M, and S cones in the retinal mosaic combines high-resolution imaging and retinal densitometry and has been described previously.[10] For each observer, the eye's aberrations were measured with a Hartmann–Shack wave-front sensor and were compensated for with a deformable mirror.[41] This imaging procedure makes it possible to resolve the mosaic of cone photoreceptors. Images were taken with 4-ms flashes (550 nm; 20-nm bandwidth, 1-deg diameter). Images were acquired at 1 deg nasal retina for both AN and JW and at 1 deg temporal retina for JW. Identification of the type of each cone was done by comparison of the images acquired before and after selective bleaching of cone photopigment with 650- and 470-nm light.

Figure 1, reprinted from Roorda and Williams,[10] shows pseudocolor images of the cone mosaics for AN and JW. Both images show a 0.5-deg square patch of retina (1 deg eccentricity; nasal retina for AN, temporal retina for JW). A second image (not shown) was acquired for JW in nasal

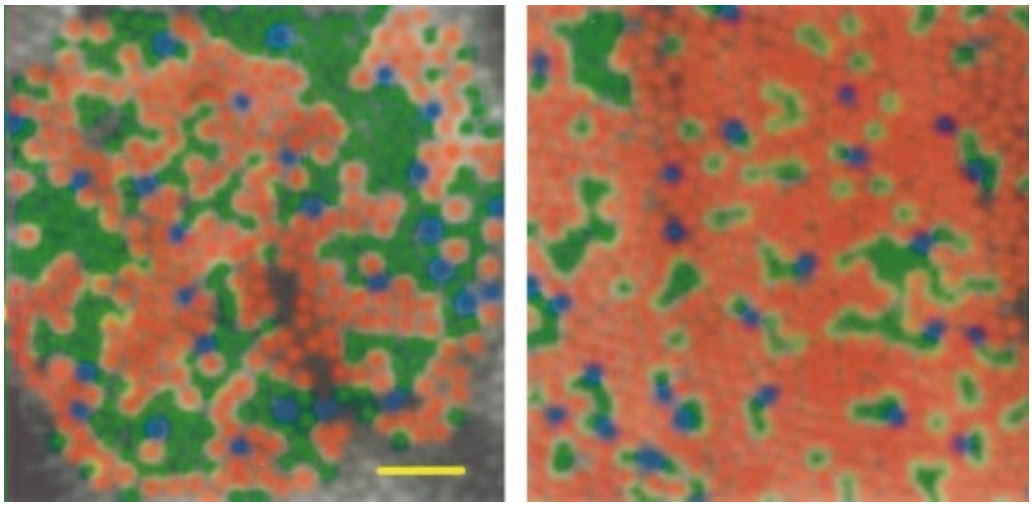

Fig. 1. Pseudocolor images of the trichromatic cone mosaics of JW and AN at 1 deg eccentricity. Red, green, and blue colors represent the L, M, and S cones, respectively. Left: AN's nasal retina; right: JW's temporal retina. The scale bar represents 5 min of visual angle. Images reprinted from Ref. 10 with permission from its authors and with copyright permission from its publisher.

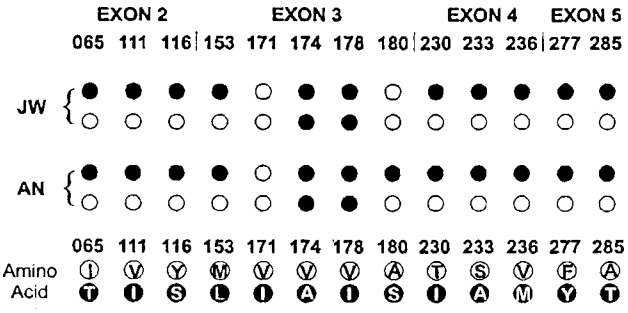

Fig. 2. Codon numbers and amino acid positions are given for the 11 polymorphic positions encoded by exons 2–4. For exon 5, only the two positions involved in determining the spectral difference between L (Y277 and T285) and M (F277 and A285) pigments are shown. For each observer, sequence information is provided in two rows. The first row is for the L cones; the second row is for the M cones. At each position, for each cone type, there is either a black or a white circle. A key translating circle color to amino acid identity at each position is provided in the bottom two rows of the figure. The single-letter amino acid code is used: T, threonine; I, isoleucine; V, valine; Y, tyrosine; S, serine; M, methionine; A, alanine; F, phenylalanine.

retina (1 deg eccentricity). As is clear from the figure, the relative numbers of L and M cones are quite different for the two observers. Observer AN had a ratio of 1.15, while JW had a ratio of 3.79 (3.66 nasal, 3.90 temporal). Roorda and Williams also report the percentages of L and M cones likely to have been misidentified for each observer. These percentages can be used to generate confidence intervals for the L/M ratios by recomputation of the ratios under the assumptions that (a) all misidentified cones are L cones and that (b) all misidentified cones are M cones. The resulting intervals are [0.91, 1.44] for AN and [3.35, 4.33] for JW. Since it seems highly unlikely that all misidentified cones are of one type, these intervals provide upper bounds on the uncertainty.

To interpret the ERG and psychophysical data as precisely as possible, we estimated each observer's individual L and M cone spectra. These estimates were derived from a genetic analysis of DNA isolated from blood obtained from each observer in conjunction with spectral sensitivity measurements made from dichromats. Fragments from L and M genes were amplified in the polymerase chain reaction by use of primers and conditions that have been described in detail elsewhere.[42,43] The polymerase chain reaction products were used directly in automated fluorescent DNA sequence analysis as described previously.[42]

Exons 2–5 of the L and M genes encode amino acid positions involved in tuning the spectral absorption properties of the pigments.[44–46] Exon 5 encodes amino acids that produce the spectral difference between L and M pigments, and the corresponding gene sequence differences were utilized in the selective amplification of L or M genes. Exons 2–4 encode amino acid positions that produce relatively small spectral shifts; these exons are responsible for producing spectral subtypes of L and M pigments.

Exons 2–4 of the M genes from both AN and JW encode identical amino acid sequences and thus would encode pigments with identical spectral peaks. Exons 2–4 of the L genes for AN and JW encode pigments that differ at a single amino acid position, 180. For each subject, only one M gene sequence was found and only one L gene sequence was found, indicating that each subject had genes encoding one spectral type of M pigment and one spectral type of L pigment. Figure 2 summarizes the results of the genetic analysis.

Substitution of serine for alanine at position 180 in the L pigment is common among people with normal color vision and produces a spectral shift of 3–7 nm, with the pigment containing serine absorbing at the longer wavelength.[42,44,46–48] Although the results reported in Refs. 42, 44, and 46–48 are in good agreement that the L pigment of AN should be shifted long relative to that of JW, they agree less well about the exact location of their respective peaks. This undoubtedly reflects small differences imposed by the various measurement techniques that have been employed to establish the peaks. We elected to use peak values derived from ERG flicker photometry obtained in the same apparatus and with the same methods used to measure AN and JW's spectral sensitivities.

AN and JW have spectrally identical M pigments. Direct measurement of the M pigment was made in a large sample of protanopes by the same ERG flicker-photometric technique described above.[49] The spectra obtained from these subjects were corrected for lens density[20] so that they were expressed at the retina and fitted by the Dawis photopigment absorption nomogram, computed to allow variation in the wavelength of its peak value according to the Mansfield–MacNichol transform.[50–52] No adjustment for pigment self-screening was made to the nomogram. The wavelength of peak quantal sensitivity was varied in 1-nm steps along the wavelength axis to obtain the best least-squares fit (computed in log sensitivity coordinates) between the data and the nomogram. The average peak value obtained was 531 nm, and this value was used as an estimate of the M cone λ-max value for both AN and JW. Similar analysis of the distribution of L pigment peaks in a sample of deuteranopes[53] suggests that the two common versions of the L pigment have λ-max values of approximately 563 and 559 nm, and those values were assumed appropriate for AN and JW, respectively.

The genetic analysis was confirmed by Rayleigh matches obtained for each observer by use of an apparatus and procedures that have been described previously.[54] L cone λ-max values necessary to explain the measured matches (assuming cone spectral sensitivities derived from the Dawis nomogram and an M cone λ-max value of 531 nm) were 563 and 558 nm for AN and JW, respectively.

3. METHODS

A. Electroretinogram Flicker Photometry

ERG flicker photometry was used to measure spectral sensitivity. ERG responses were recorded to an interleaved train of pulses from test and reference lights. Each pulse was 8 ms in duration, and there was an 8-ms interstimulus interval between pulses. Thus one period of the overall test–reference pulse train was 32 ms, yielding a frequency of 31.25 Hz. The effectiveness of the test

and reference lights was equated by iterative adjustment of the intensity of the test light until the ERG response that it produced was equivalent to that produced by the reference light. General features of the apparatus and the procedures have been fully described elsewhere.[55]

Briefly, light pulses were derived from a three-beam optical system and were presented to the eye in Maxwellian view (in the form of a 59-deg circular spot). Head position was stabilized with the aid of a dental-impression bite bar. The test light of the photometer was drawn from a high-intensity grating monochromator (half-energy passband, 10 nm). Intensity control of this light was accomplished by adjustment of the position of a 3.0-log-unit neutral-density wedge placed in the optical pathway. The resulting wedge settings were read to an accuracy of 0.01 log unit. The reference light of the photometer originated from a tungsten halide lamp; neutral-density step filters controlled its intensity. For the measurements reported here, the reference light was achromatic (2850 K) and had a retinal illuminance of 2.37 log trolands (td). Both test and reference lamps were underrun at 11 V from regulated dc power supplies. High-speed mechanical shutters were used to control the timing of test and reference lights.

The pupil of the test eye was dilated by topical application of 0.5% mydriacyl. ERG's were differentially recorded from DTL electrodes referenced to a location on the forehead.[56] Recordings were made in an illuminated room. To complete the photometric equations we examined the averaged response to the last 50 of a total of 70 stimulus cycles, repeating this procedure for a total of 22 test lights that were located at 10-nm intervals from 450 to 660 nm. During the course of the experiment photometric equations were obtained twice[57] for each of the test lights, and these values were subsequently averaged.

B. Unique Yellow

The wavelength perceived as unique yellow by AN and JW was measured in a Maxwellian-view apparatus. Light from a 200-W 120-V quartz halogen tungsten bulb was filtered by a monochromator (Instruments SA, Inc.) to produce a narrow-band (1-nm full-width at half-height) stimulus. A field-stop conjugate with the pupil set the entrance pupil size to 1.62 mm, while a field-stop conjugate with the retina set the spatial structure of the retinal stimulus.

Observers viewed a 0.52-deg circular spot with their right eye. The left eye was uncovered but was not exposed to the stimulus. The stimulus spot was located at 1 deg nasal retina, corresponding to the location where the L/M cone ratio had been established for both observers. The retinal illuminance was approximately 50 td. A small, dim broadband spot (luminance approximately 1.6 log units above detection threshold; color temperature, 3240 K) served as a fixation point. The fixation point was turned off during dark adaptation and was turned on continuously during the stimulus presentation sequence. The stimulus was viewed in an otherwise dark surround. During the experiment the stimulus was presented repeatedly for 500 ms with an interstimulus interval of 3.5 s.

At the start of the experiment the observer dark adapted for 1 min. Control measurements made by use of one of the authors (YY) as an observer indicated that the locus of unique yellow was not affected by 30 min of dark adaptation and did not differ between 1 deg eccentricity and the rod-free fovea. This control experiment indicates that results in the main experiment were not affected by rod activity. After dark adaptation the observer adjusted the monochromator so that the test flash appeared neither reddish nor greenish. Six adjustments were made: three from starting wavelengths that clearly appeared greenish, and three from wavelengths that clearly appeared reddish.

The mean wavelength set with the method of adjustment was used to guide the selection of wavelengths for a forced-choice experiment by the method of constant stimuli. On each trial of the forced-choice experiment, the experimenter set one of five wavelengths, and the observer indicated whether it appeared reddish or greenish. During a session 20 trials of each of five wavelengths were presented in random order, for a total of 100 trials. The five wavelengths used in the forced choice experiment were equally spaced at 1-nm intervals around a center frequency. In the first session the center frequency was taken as the mean wavelength of the adjustment experiment. In subsequent sessions the experimenter adjusted the center frequency on the basis of the results of the previous session so as to ensure that the observer viewed wavelengths seen both as greenish and reddish during the forced-choice trials.

For each session, the wavelength of unique yellow was determined as the 50% point of a psychometric function fitted by eye to the forced-choice data. This value was averaged over sessions to determine a mean value for each observer. There were four sessions for observer AN and two sessions for observer JW.

4. RESULTS

A. Electroretinogram Flicker Photometry

Figure 3 shows the spectral sensitivities obtained for JW and AN by the ERG flicker-photometric procedure. These sensitivities are corrected for lens density[20] so that they express sensitivity at the retina rather than at the cornea. Each spectral sensitivity was fitted by a weighted sum of the individual observer's L and M cone spectra:

$$S(\lambda) = \log 10[(N_l/N_m)L(\lambda) + M(\lambda)] + c. \qquad (1)$$

In Eq. (1), $S(\lambda)$ is the predicted log spectral sensitivity (expressed at the retina), $L(\lambda)$ and $M(\lambda)$ are the individual L and M cone spectra derived from the Dawis nomogram as described above, N_l/N_m is the L/M signal ratio, and c is a normalizing constant. For each observer, numerical search was used to find the L/M signal ratio (parameter N_l/N_m) that yielded the minimum mean-squared error between predicted and measured log spectral sensitivity. On each iteration of the search the constant c was computed analytically to provide the best fit, given the current value of N_l/N_m. No parameter was allowed for individual variation in optical or lens density. Error was evaluated over the wavelength range of 460–

660 nm. The 450-nm data were excluded to minimize distortions introduced by uncertainty about preretinal absorption at shorter wavelengths.

The fits are shown as the solid curves in Fig. 3 and provide a reasonable description of the data. The derived L/M signal ratio is 1.06 for AN and 3.38 for JW. These estimates are close to the values for L/M cone ratios obtained from direct imaging.

Although the agreement between derived L/M signal ratio and L/M cone ratio is good, a number of cautionary points should be made. First, to yield a reliable signal the ERG spectral sensitivities were obtained with a 59-deg field, while estimates of L/M cone ratio were obtained from 1-deg parafoveal images. If there is substantial variation in L/M cone ratio across the retina, this would distort the relation between L/M signal ratio and L/M cone ratio. The L/M cone ratio as assessed by analysis of messenger RNA increases in the far periphery.[15,16] Even though the ERG field size is large, it is nonetheless an area within which the messenger RNA measurements indicate that the cone ratio is fairly constant.

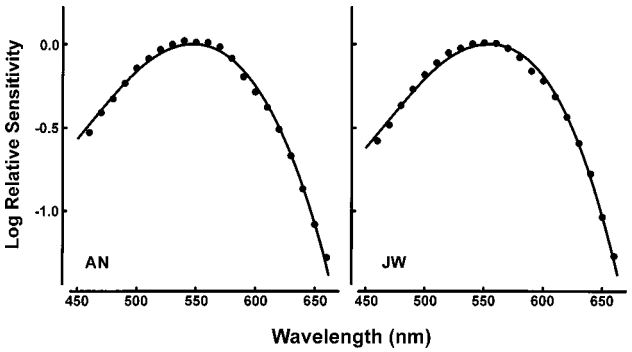

Fig. 3. ERG spectral sensitivity functions. Each panel shows data for one observer. The solid curves show the best fit to the measured spectra obtained with a weighted sum of L and M cone photopigment absorption spectra. Left (AN): fit with M cone λ-max value of 531 nm; L cone λ-max value of 563 nm; L/M cone ratio, 1.06. Right (JW): fit with M cone λ-max value of 531 nm; L cone λ-max value 559 nm; L/M cone ratio, 3.38. The maximum standard error of measurement for the measured sensitivities was 0.02 log unit, comparable in size with the plotted data points. The data shown are normalized so that each observer has a peak sensitivity of 0 log unit. Tabulated spectral sensitivity data may be obtained from the World Wide Web at http://color.psych.ucsb.edu/ERG/JOSA2000.txt.

Table 1. Estimates of L/M Signal Ratio Derived from Flicker ERG[a]

	Observer	
	AN	JW
L Cone λ-max Value		
559	1.73	3.38
561	1.32	2.31
563	1.06	1.72
Spectral Range (nm)		
460–660	1.06	3.38
450–660	1.09	3.72
460–620	0.94	2.70
500–660	0.98	2.93

[a] The top section shows the effect of varying L cone λ-max. L and M cone spectra were derived from the Dawis nomogram as described in the text. The M cone λ-max value was 531 nm, and the L cone λ-max value is given in the table. The bottom section provides L/M signal ratios derived from the data restricted to various spectral ranges with the same assumptions about λ-max as were used in the main analysis.

Second, it has been argued that estimates of L/M signal ratio derived from fits of luminancelike spectral sensitivities with weighted sums of L and M cone spectra should be assigned rather large confidence intervals.[58] We therefore used a resampling procedure to determine the uncertainty in the estimated L/M signal ratios. The maximum standard error for individual photometric equations obtained from both observers was 0.02 log unit. We synthesized 500 spectral sensitivity functions for each observer by perturbing their measured log spectral sensitivities with noise that was distributed normally with a standard deviation of 0.02. We then fitted each of the 500 synthesized sensitivities to generate the distribution of estimated L/M cone ratios to be expected from repeated experimentation. For AN, the 95% confidence interval obtained from this distribution was [0.96, 1.16]; for JW, it was [2.81, 4.12].

Third, as has been pointed out previously, the derivation of relative L/M signal ratio depends on the λ-max value chosen for L and M cone spectra.[59] Table 1 provides L/M signal ratios obtained with cone spectra derived from the Dawis nomogram by use of a variety of values for L cone λ-max for each observer. The L/M signal ratios vary considerably with L cone λ-max, especially for JW. Thus, to estimate L/M cone ratios from measurements of luminance mechanisms, it is important to have accurate information about the photopigment complement of the individual observers.

Other fitting choices might influence the exact L/M signal ratio derived from the fit. The effect of varying the wavelength range used in the fit is also shown in Table 1. The effect is very small for AN and modest for JW. The choice of pigment nomogram could also have an effect. We fitted the data with a second nomogram.[60] This nomogram provides a narrower template than does the Dawis nomogram, and the fit quality obtained by direct use of Eq. (1) is poor. Allowing a parameter for photopigment optical density (OD) in the numerical search yields fits comparable with those shown in Fig. 3 as well as very similar L/M signal ratios (AN: 1.12, OD 0.23; JW: 3.42, OD 0.31.)

Finally, the L/M signal ratio derived from flicker ERG might vary with the observer's state of chromatic adaptation.

B. Unique Yellow

The wavelength of unique yellow was 576.8 nm for AN (standard error, 0.6 nm) and 574.7 nm for JW (standard error, 0.7 nm). Cicerone presented a simple additive model of how the wavelength of unique yellow could be expected to vary with the relative numbers of L and M cones.[29] This model is based on the ideas that (a) a stimulus appears neither red nor green when the output of a linear red–green mechanism is zero and that (b) the contribution of L and M cones to the red–green mechanism varies in proportion to their relative numbers. This model may be expressed as

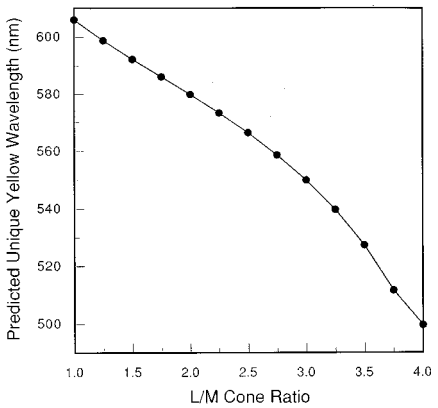

Fig. 4. Predictions for unique yellow based on model described in the text. The figure shows predicted wavelength for unique yellow as a function of L/M cone ratio. To make the predictions we used the Smith–Pokorny estimates of the cone spectral sensitivities, and we set the constant k by requiring that the prediction for an L/M cone ratio of 2 be 580 nm.

$$(N_l/N_m)L(\lambda_y) - kM(\lambda_y) = 0, \tag{2}$$

where λ_y is the wavelength of unique yellow, N_l/N_m is the L/M cone ratio, k is a constant that describes any neural factors that govern the relative contribution of L and M cones to the red–green mechanism, and the functions $L(\lambda)$ and $M(\lambda)$ represent the M and L cone spectral sensitivities. The constant k is assumed to be fixed across individuals for any given stimulus conditions. A similar model has been used by Pokorny and Smith.[30]

This model suggests that the variation in L/M cone ratio measured between AN and JW should have a large effect on the wavelength of unique yellow. Figure 4 plots the wavelength of unique yellow predicted by Eq. (2) against cone ratio. To make this plot we used the Smith–Pokorny estimates[61,62] of cone spectral sensitivity and we determined the constant k by requiring that the predicted unique yellow wavelength for an L/M cone ratio of 2 be 580 nm. When implemented in this way, the model predicts that AN will perceive 602-nm light as unique yellow, while for JN the wavelength of unique yellow will be 518 nm. Although this predicted difference of 84 nm is in the right direction, it is a factor of 40 greater than the difference that we obtain (2.1 nm) between the two observers.

It is also possible to employ the individual L and M cone spectra derived from the genetic analysis to examine the unique yellow data. We set the constant k from the data for JW by using his L and M cone spectra and by requiring that his wavelength of unique yellow correspond to his L/M cone ratio measured for nasal retina. This value of k was then used along with AN's cone spectra to predict that AN's wavelength of unique yellow would be 617 nm, again in striking disagreement with the data. For this analysis, cone spectral sensitivities derived from the nomogram were corrected for lens and macular pigment density.[20]

5. DISCUSSION

The L/M signal ratios derived from flicker ERG were in good agreement with the L/M cone ratios measured for the same observers. Although it is not necessarily the case that the flicker ERG taps the same sites as psychophysical heterochromatic flicker photometry, studies that have compared ERG and psychophysical flicker photometry indicate that the two techniques yield similar estimates of L/M signal ratio.[23,63] Recently, Verweij *et al.*[39] and Diller *et al.*[40] have shown that L/M signal ratios are very similar for horizontal, bipolar, and ganglion cells of primate retina. This preservation of L/M signal ratio at multiple retinal sites may underlie the agreement between the techniques.

Our conclusion that individual variation in the flicker ERG is mediated by individual variation in L/M cone ratio is in accord with that of Rushton and Baker,[18] who compared results from retinal densitometry and psychophysical flicker photometry (see also Ref. 23). This conclusion supports the hypothesis that individual variation in L/M cone ratio mediates much of the individual variation in photopic luminous efficiency, however measured.[18,20–28]

The striking discrepancies between the predictions of Eq. (2) and the measurements of unique yellow for AN and JW indicate that individual variation in unique yellow is dominated by factors other than L/M cone ratio. Although more complicated models of the dependence of unique yellow on cone ratio could be developed, it seems unlikely that use of these would change this basic conclusion. Our conclusion that unique yellow does not vary substantially with L/M cone ratio is in agreement with that reached by Pokorny and colleagues on the basis of comparisons of variation in unique yellow with variation in L and M cone contributions to luminance for individual observers.[30,31] Moreover, the evidence reviewed in Section 1 suggests that there is large individual variation in the L/M cone ratio among male color-normal observers. Even if we take the conservative estimate of the range as being 1–4, Fig. 4 indicates that individual variation in unique yellow should span the range of 500–600 nm. This is much larger than the range actually observed, which might reasonably be taken to be 568–592 nm.[29,30] This point has been made previously.[30]

Our finding that unique yellow is not influenced by L/M cone ratio is consistent with the observation that L/M signal ratios derived from threshold experiments thought to be mediated by a red–green chromatic mechanism show considerably less individual variability than do detection thresholds thought to be mediated by a luminance mechanism.[23,64]

Why does the L/M cone ratio affect the luminance mechanism (as measured by ERG) but not the red–green mechanism (as measured by unique yellow)? An intriguing suggestion, offered by Pokorny *et al.*,[22] is that the relative contribution of L and M cones to the red–green mechanism is set by experience with the environment. Because the red–green mechanism is opponent, a mechanism response of zero provides a natural reference level for calibration. Experience with the environment, either during development or continuing throughout life, could be used to adjust the relative strength of L and M inputs so that the time-averaged response magnitude of the red–green mechanism is minimized.

Although the variation in unique yellow that we measure between AN and JW is not consistent with a strong

influence of their L/M cone ratios, it remains true that the spectral position of unique yellow varies across the population. It is not at present clear what mediates these differences, but the idea outlined above suggests that environmental factors could play an important role in this process. It would be interesting both to test this idea and to try to uncover the nature of the key factors involved.

In contrast to the red–green mechanism, the luminance mechanism is additive and thus does not have an easily identifiable reference level for calibration. It may be that luminance preserves individual differences in the L/M cone ratio because the visual system is unable to compensate for these differences. Alternatively, it may be that, for natural scenes, luminance contrast is not much affected by differences in luminance spectral sensitivity, so that little or no functional benefit would be conferred by precise calibration of the luminance mechanism.

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