Title

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THE RELATIVE NUMBERS OF LONG-WAVELENGTH-SENSITIVE TO MIDDLE-WAVELENGTH-SENSITIVE CONES IN THE HUMAN FOVEA CENTRALIS

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Abstract—The determination of the relative numbers of different cone types in the human retina is fundamental to our understanding of visual sensitivity and color vision; yet direct measurements which provide this basic information have not previously been made for all cone types. Here we present a model which links the detection of a test light of small dimension to the number of cones contributing to detection of the light. We selectively isolated either the long-wavelength-sensitive (L) or the middle-wavelength-sensitive (M) cones, by choosing combinations of wavelengths of adapting backgrounds and tests to favor detection by the cone class of interest. Our model was applied to the detection functions measured for six color normal observers to obtain estimates of the relative numbers of L to M cones. Our estimates ranged between 1.46 and 2.36 for our observers with a mean value near two L cones for every M cone in human fovea centralis.

Cones Human fovea centralis Relative numbers of L to M cones

INTRODUCTION

The determination of the relative numbers of different cone types in the retina is fundamental to our understanding of human visual sensitivity and color vision, and this information would be required for any quantitative models of human vision. Direct measurements which provide this basic information have not been previously made for all cone types.

There continues to be a gratifying convergence of psychophysically derived evidence from humans (Williams *et al.*, 1981) and anatomically derived evidence from baboon (Marc and Sperling, 1977), macaque (deMonasterio *et al.*, 1985), and human (Ahnelt *et al.*, 1987) on the numerosity and distribution of the short-wavelength-sensitive (S) cones in the primate retina.

In the cases of the long-wavelength-sensitive (L) and middle-wavelength-sensitive (M) cones, there are no previous direct psychophysical measurements from which the relative numbers of L and M cones can be derived, and estimates based on various indirect means vary widely. To our knowledge, DeVries (1946, 1948) was the first to suggest that the individual variability in luminosity functions could be related to individual variability in the relative numbers of different cone types. Rushton and Baker (1964) subsequently reported that retinal densitometric measurements yielding the density of M and L cone pigments could be correlated to the flicker photometric matches between red and green lights made by their observers. Rushton and Baker's estimates, based on densitometric measurements, of the relative numbers of L to M cones in normal trichromatic observers spanned a wide range of three times more L as compared to M cones to one third as many L as compared to M cones. Another approach has been based on estimates deriving from curve fits required to make various sets of psychophysical data consistent one to another. Examples of this kind of analysis include Walraven's (1974) and Smith and Pokorny's (1975) estimates based on fits of the cone primaries to the luminosity function; Vos and Walraven's (1971) estimate based on comparisons of Weber fractions for the different Stiles π mechanisms; and Walraven's (1974) estimate based on the relative heights of the spectral sensitivity functions of the cone primaries. These methods yield estimates of the relative numbers of L to M cones which vary between 1.6 and 2.0. There are as yet no morphological criteria whereby L and M cones can be distinguished. Marc and Sperling (1977) used a histochemical assay to estimate that there were fewer L cones than M cones (in a ratio of 1:2) in the baboon retina. This estimate falls near one end of Rushton and

Baker's range but is quite the reverse of the indirect estimates based on other human psychophysical results, as have been reviewed above.

In our study we attempted a more direct psychophysical approach. When a tiny light on the order of 1 min arc is viewed by the fovea, its color appearance cannot always be predicted from the color appearance of a large patch of the same wavelength. Krauskopf (1964) reported that a 580 nm light subtending 1 min of visual angle did not have a stable color appearance from flash to flash but could appear red, green, yellow or white. Indeed, a range of wavelengths when presented as tiny, brief flashes show this instability of color appearance from flash to flash (Walraven, 1962; Krauskopf, 1978). We presented wavelengths between 520 and 660 nm as stimuli of 1 min of visual angle and for 50 msec duration. All lights were set to a level so that they could be detected approx. 70% of the time. Figure 1 shows a plot of the proportion of time a light of a given wavelength was seen as red or green for two of our observers. (Lights seen as yellow or white have been excluded from this plot.) For most of these wavelengths, the color appearance of a light is sometimes judged to be red and at other times green. Only at the ends of this range is any light exclusively called either red and never green or green and never red.

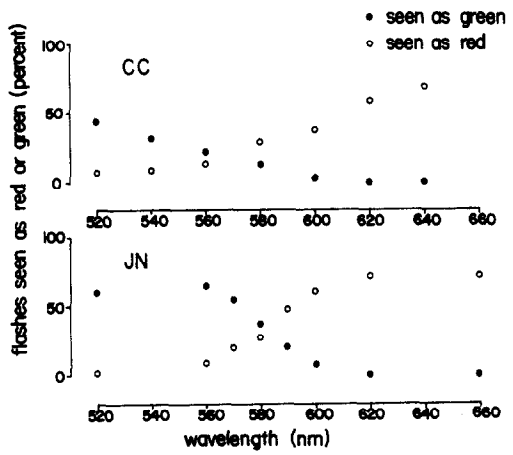

Fig. 1. Test lights subtending a visual angle of 1 min were presented in fovea centralis of observers CC and JN. All lights were set at an intensity to be seen approx. 70% of the time. The percentage of these lights seen as green (solid symbols) or as red (open symbols) is plotted as a function of wavelength of the light. For any given wavelength, these two percentages as plotted may not sum to 100, since those test flashes which were seen as yellow or white are not included. The test wavelengths ranged between 520 and 660 nm. Except for the ends of this range, most wavelengths appeared red on some trials and green on others.

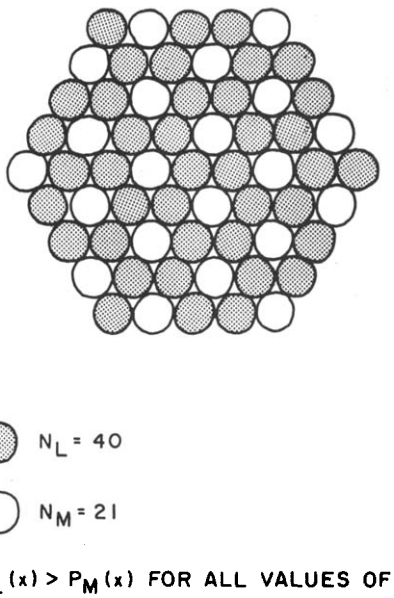

Fig. 2. This hypothetical patch of cones in human fovea centralis is shown to contain 40 *L* cones (stippled) and 21 *M* cones (open). For such a mosaic it is readily seen that the probability of detecting a test spot falling on this patch would be greater if detection is mediated solely by the *L* cones as compared to that mediated solely by the *M* cones.

Our interpretation of these observations goes as follows: Since the lights were tiny, each flash illuminated only a few cones, presumably slightly different ones from flash to flash, leading to the differences in color appearance. These results are only suggestive. In order to exploit these observations so as to obtain estimates of the relative numbers of *L* to *M* cones, we developed a model which describes how the relative numerosity of a particular cone type affects the function measuring the probability of detection based on that cone type. In essence, the model relates the steepness of the detection function to the numbers of cones contributing to the detection of the test spot. The idea behind our model is illustrated in Fig. 2, which shows a hypothetical cone mosaic. For the purpose of the argument, this patch of cones is shown to contain roughly twice as many *L* as compared to *M* cones. For such a mosaic, it is easily seen that the probability of detection is greater if detection is mediated solely by *L* cones as compared to that mediated solely by *M* cones. We measured the probability of detection as a function of the intensity of such tiny lights upon either an *L* or *M* cone isolating background, according to the method of Stiles (1978). This

allowed us to estimate the relative numbers of L to M cones in fovea centralis of six trichromatic color normal observers using three estimation procedures, each requiring additional elaborations of our basic model. A brief report of the results for two of these six observers has been previously presented (Cicerone and Nerger, 1985).

METHODS

Observers

Three females (CC, JN and VV) and three males (EM, HA, and YP) served as observers. These observers were tested on anomaloscope matches and small field color matches, which confirmed that they were color normal trichromats. All observers, except one female (VV) and one male (HA), were emmetropic. Subjects VV and HA were each mildly myopic (less than 1.5 D correction) in the right eye which was used for these experiments. Optical corrections were applied for these two observers.

Apparatus

Two channels of a four-channel Maxwellian-view apparatus were employed for most of these experiments. One channel provided the background field which subtended 12 deg of visual angle. Interference filters (Ditric Optics) were used to change the wavelength of the background field. Placed in this channel was a glass plate with four small, opaque fixation dots arranged as the corners of a square whose diagonal extent spanned 3 deg of visual angle. The second channel provided the test field which appeared as a flash of 50 msec duration in the center of the array of fixation points. Precision pinhole apertures (Newport Corporation, PH series) of 25, 50 and 100 μm were used in this channel to provide test sizes of 0.5, 1.0 and 2.0 min arc in visual angle, respectively. The wavelength of the test was controlled by a monochromator (Instruments SA, H-20V). The radiance of light in the two channels was varied by means of neutral density filters and wedges. All optical components, as well as the bite bar, were firmly anchored to an optical table (Newport Corporation, MS series). The control of the experiment was aided by a computer (Apple IIE).

The two other channels of the Maxwellian-view apparatus were employed for measurements of heterochromatic flicker photometry. The broadband standard and the narrowband test, subtending 1 deg of visual angle, were modulated sinusoidally out of phase by means of a rotating polarizer spinning between two fixed out-of-phase polarizers and the light source. Neutral density filters and a wedge allowed the radiance of the test to be varied. The wavelength of the test was varied using interference filters (Ditric Optics).

A standard radiometer/photometer (EG & G, 450) was used for all calibrations.

Procedures

Detection of small colored tests upon colored backgrounds. The observer was dark adapted for 15 min. This was followed by a 5-min period of adaptation to the background light. The radiance of the background light was chosen to elevate the threshold for the test by 0.5 log unit above its dark-adapted value. The subject's task was to detect the tiny test light. The subject was instructed to initiate each trial by pressing a button when ready and confident of accurate fixation. After the test light had been presented, the subject indicated whether the light was seen or not. Stimuli were presented in blocks of 20 trials with the stimulus intensity chosen randomly for each block.

For the main experiments, two conditions were presented in each experimental session. In one condition the test wavelength was of 520 nm and the background wavelength was of 640 nm to provide the conditions favoring M cone detection. In the second condition, the test wavelength was 640 nm with a background of 520 nm, favoring detection by the L cones. The choices of wavelengths for tests and backgrounds were guided by the work of Stiles (1978) and by the results of the experiments of Fig. 1. The order of presentation of these conditions was randomized from session to session. Different combinations of test and background wavelengths, as noted below, were employed for the control conditions.

Heterochromatic flicker photometry. The observer was dark adapted for 15 min. The observer fixated a 1 deg test spot composed of a broadband standard and a light of variable wavelength flickered at 15 Hz in sinusoidal counterphase. Stimuli were presented according to a random staircase procedure with the radiance of the variable wavelength set by the experimenter. On any trial, the observer indicated whether flicker was present or absent.

Determinations of the best-fitting theoretical functions. Determinations of the best-fitting the-

oretical functions for our measurements were made by a least-squares method ("zxssq" subroutine from the IMSL Mathematical Library).

RESULTS

Isolation of L and M cones and measurement of the detection functions

Since our reasoning was directly dependent on comparisons of the slopes of the detection functions and required that our background lights had adequately isolated the L and M cones, we first checked that the shapes of the detection functions remained stable with changes in the adaptation level in a range about our standard value. Any changes in slope with small changes in the intensity level of the adapting light could result for at least two reasons. First, if a test and background combination we had chosen had not adequately isolated the cone mechanism of choice so that both L and M cones were contributing to the detection, then as the adaptation level was increased, we should have measured a decline in the slope of the detection function as greater isolation was achieved. Second, if our adapting backgrounds had significantly desensitized both L and M cones, and not only the intended cone class, then as the intensity of the background was increased, the numbers of cones contributing to detection should have declined, again producing a decline in the slope of the detection function with increasing intensity. Even if neither of these factors had played a role, if for any reason the slope of the detection function was affected by small changes in the adapting level, then our model linking the slope of the detection function to the number of cones contributing to detection would be less viable as a means for estimating the relative numbers of different cone types. We therefore measured the probability of detection as a function of the radiance of our test lights for three different levels, above and below our standard value, of the background light. These elevated thresholds 0.2, 0.5 and 0.8 log unit above the dark-adapted value. As Fig. 3 shows for observer CC, the slopes of the functions were virtually identical when measured under L or M cone isolation conditions. This set of experiments was also conducted for observer JN, whose results are also shown here.

Another test of the adequacy of our chosen stimulus conditions was conducted in the following way. We measured detection thresholds using a 575 nm test presented upon either the

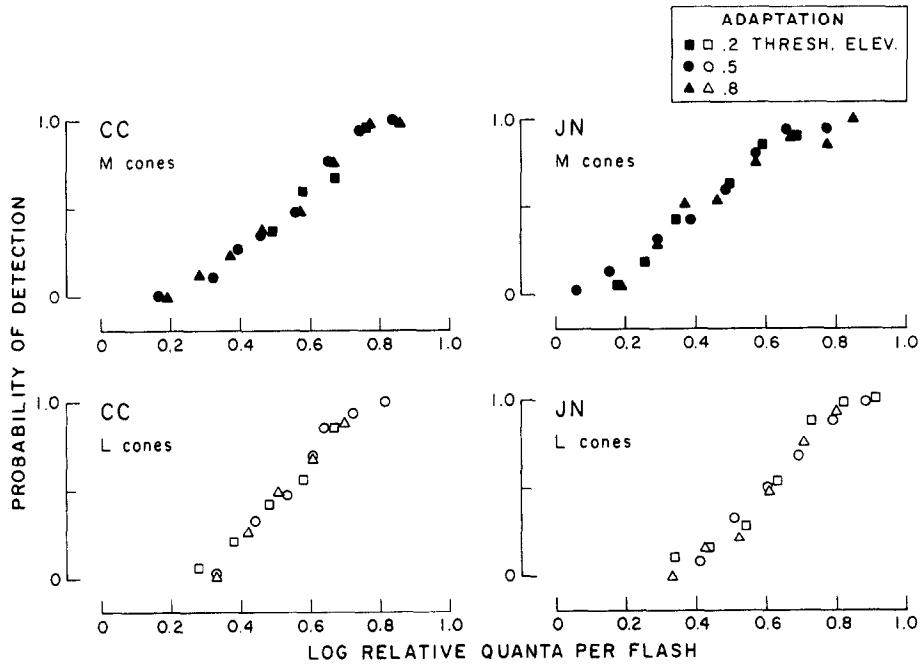

Fig. 3. Shown are measurements of the probability of detection as a function of the radiance of the test lights under M (solid symbols) and L (open symbols) cone isolation conditions. The adapting backgrounds were chosen to elevate thresholds for the test 0.2 (squares), 0.5 (circles), and 0.8 (triangles) log unit above the dark-adapted value. The slopes of the detection functions are seen not to change for this range of adaptation.

red background used for isolation of M cones or upon the green background used for isolation of L cones. If the choice of test wavelengths affected the detection functions significantly (perhaps due to chromatic aberration of the eye, for example) then changing the test wavelength would be expected to change the slope of the detection function. If, however, the chosen backgrounds provided adequate isolation of each cone type, and optical and other preretinal factors did not come into play in any significant way, then the detection functions should not depend on the specific wavelength of the test. Figure 4 shows that when a test was detected upon the red background chosen to isolate M cones, the detection function measured with a 575 nm test was nearly identical to that measured with a 520 nm test. Also, when detection was measured with a green background designed to isolate L cones, the 575 nm and the 640 nm tests provided closely similar results. A more quantitative comparison based on the method, described below, of finding the best-

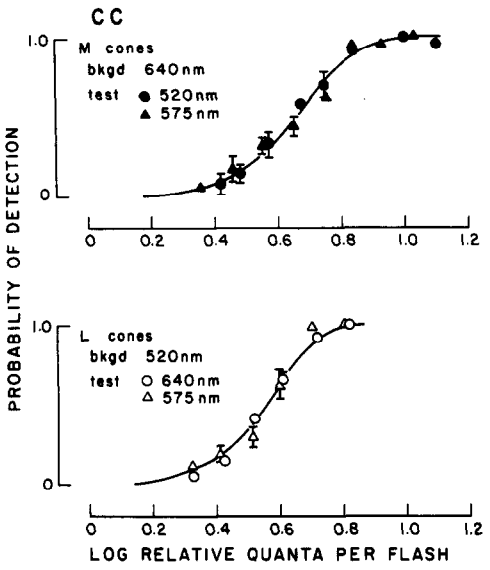

Fig. 4. Detection functions were measured using a 575 nm test presented upon either the red background used for isolation of M cones (solid triangles) or upon the green background used for isolation of the L cones (open triangles). These functions are compared to the results using our standard 520 nm test upon the red background (solid circles) and the 640 nm test upon the green background (open circles). The detection functions measured upon the red background are similar whether the test light was of wavelength 520 or 575 nm. The detection functions measured upon the green background are similar for test lights of wavelength 640 or 575 nm. The smooth curves are the theoretical functions chosen to best fit the measurements from the standard conditions (circles) and are identical to those shown in Fig. 7.

fitting theoretical functions for the measurements of Fig. 4 confirmed that the particular choices of the wavelengths of the test lights did not bias our results. Details of this comparison are deferred until after the description of the complete model.

A remaining concern was that the few S cones observed in central fovea by anatomical procedures (Ahnelt *et al.*, 1987) may contribute to detection under our M cone isolation conditions, for which the test light is of wavelength 520 nm. If this were the case, we would overestimate the number of M cones, but the expected error in any case is small, since no more than 1 in 20 cones in fovea centralis is an S cone. The involvement of S cones in the detection of the 520 nm test is unlikely since under the conditions of our experiment, S cone sensitivity to the 520 nm test is two orders of magnitude less than that of M cones. Furthermore, the experiments conducted with either a 575 nm or a 520 nm test upon a red background provide evidence against the involvement of S cones, since the shapes of the detection functions in these two conditions are nearly identical. S cones are almost surely not involved in detection of the 575 nm test, and the virtual identity of the detection functions lends credibility to the assumption that they are not involved in our standard M cone isolation conditions using a 520 nm test.

Estimates based on test sizes yielding matching detection functions

Our first estimation procedure followed the logic illustrated in Fig. 2. If a test light is increased in size, then the number of cones contributing to the detection of the test light should increase accordingly. When the detection function obtained under M cone isolation conditions matches that obtained under L cone isolation conditions, then the numbers of cones contributing to the detection of the test lights must be equal. If the match is attained when the test lights are of equal size, this must imply that the relative numbers of L and M cones are equal. If the match in detection functions is attained for unequal sizes, however, then the smaller test size must be associated with the more numerous population. Since the cones are of finite size, a simple comparison of the areas of the matching test lights will not yield an accurate estimate of the relative numbers. Instead, the dimensions of the cones need to be considered in the estimation. For this purpose,

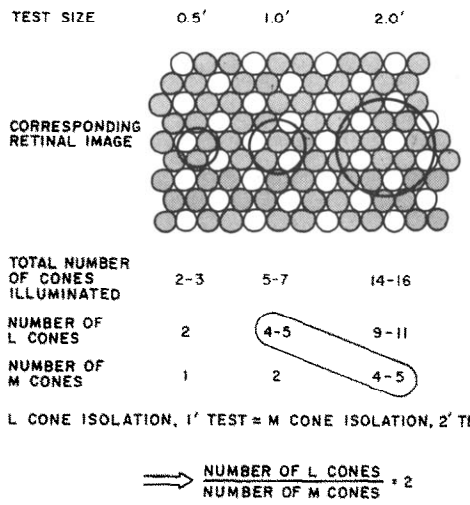

Fig. 5. Illustrated here is the logic behind the experiments described in this section. In our idealized cone mosaic, the separation between cone centers is uniformly 0.6 min arc, the cones are hexagonally packed, and there are roughly twice as many L cones (stippled) as M cones (open). The retinal image of the variously sized tests are transformed by an optical spread function to produce the retinal images as shown. If the detection function measured with a test 1 min in diameter under L cone isolation conditions matches that measured with a 2 min test under M cone isolation conditions, then the same number of cones must be contributing to detection of each of these tests. An estimate of the relative numbers of L to M cones is obtained as the ratio of the total numbers of cones illuminated by these tests.

we used anatomical results on the total numbers of cones in the human fovea centralis (Osterberg, 1935; Miller, 1979; Curcio *et al.*, 1987). Using a mosaic with 0.6 min arc separation between cone centers, we can estimate the total number of cones illuminated by a test of any given size. As illustrated in Fig. 5, the corresponding retinal image, obtained by convolution of the test aperture with the optical spread function of Campbell and Gubisch (1966), when placed on the mosaic allows one to estimate the total number of cones illuminated. The relative numbers of L cones as compared to M cones can be obtained as the ratio of total numbers of cones illuminated under L and M cone isolation conditions for which the detection functions are identical. In the example of Fig. 5 detection under L cone isolation with a test size of 1 min arc matches that under M cone isolation measured with a test of 2 min arc. Associated with these test sizes are cone counts of 5–7 and 14–16, respectively, yielding a ratio near 2.

We conducted this experiment for all six of our observers (Fig. 6). The estimate for observers CC and JN was 2.0–2.3 since the detection functions match when L cones are detecting a 1-min test and M cones are detecting a 2-min test. Here we can only specify a range of possible values, since there is uncertainty in the exact numbers of cones illuminated by the test lights as noted above. For observers EM and VV the detection functions for L cones measured with a 1 min test is steeper than that for M cones measured with a 2 min test; therefore, the ratio of L to M cones for these observers must be greater than for CC or JN. The results for observer HA indicate a ratio less than or possibly equal to two. Observer YP's results are consistent with the lowest proportion of L to M cones (less than two), since the difference between the detection functions measured under M and L cone isolation with the same size test of 1 min in diameter is smallest for this observer.

Estimates based on comparisons of the slopes of the detection functions

In order to refine our estimation procedure we extended our model to require that a test flash which delivers an average number of quanta (x) will be detected if any one of the number (N) of illuminated cones attains a specified quantum catch. Then, the probability of detection (P) can be expressed in terms of Q, the probability that any one cone has not caught the required number of quanta, as follows:

$$P(x) = 1 - Q(x)^N. \tag{1}$$

What this equation expresses is that a test will be detected if any one of the N cones illuminated by the flash catches the required number of quanta. The slope of the function can be expressed as:

$$\mathrm{d}P(x) = -NQ(x)^{N-1}\,\mathrm{d}Q(x).$$

These equations can be separately written for L and M cone detection under our isolation procedures. Then, under the assumption that the value of Q is the same for L and M cones for a fixed value of the variable x, we can express the ratio of the number of L cones (N_L) to the number of M cones (N_M) as follows:

$$N_L/N_M = (\mathrm{d}P_L/\mathrm{d}P_M)(1 - P_M)/(1 - P_L). \tag{2}$$

We have achieved an expression for the relative numbers of L to M cones which is dependent upon the slopes of the detection functions and the probabilities of detection. The probabilities of detection are exactly what we measured, and the slopes of the detection functions can be obtained as two-point estimates for a number of

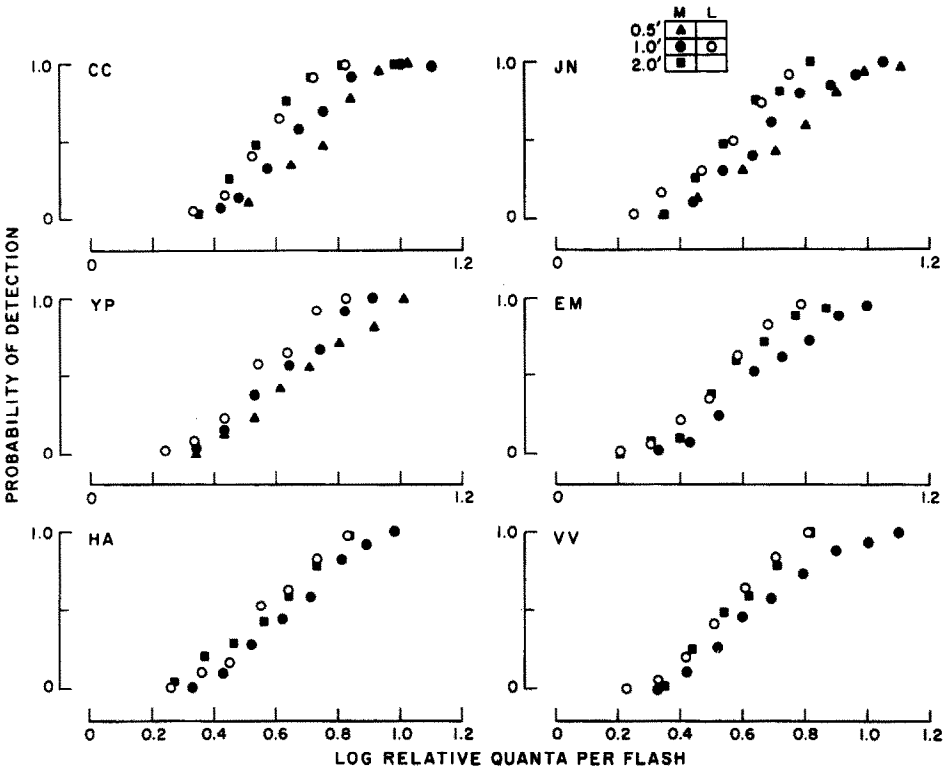

Fig. 6. Shown here are results for experiments designed to determine the test sizes yielding matching detection functions under M and L cone isolation conditions. The test size used under L cone isolation conditions was 1 min in diameter (open circles). Under M cone isolation conditions, measurements were made with tests of diameter of 0.5 min (solid triangles), 1 min (solid circles) or 2 min (solid squares). A match between the detection functions measured with a 2 min test under M cone isolation and a 1 min test under L cone isolation is produced for observers CC and JN, indicating an equal number of cones contributing to detection of the tests in these two conditions. As explained in the text, this result is consistent with a foveal mosaic containing between 2.0 and 2.3 L cones for each M cone. Results for observers EM and VV indicate that more L cones contribute to the detection of a 1 min test as compared to the number of M cones contributing to a 2 min test, by comparison of the respective detection functions, indicating an L to M ratio greater than 2. Observer HA's results indicate a ratio less than or equal to 2. Observer YP's results are consistent with the lowest proportion of L to M cones (less than 2) since the difference between the detection functions measured under M and L cone isolation with the same size test of 1 min diameter is smallest for this observer.

values of the variable x. These two-point estimates are reasonable estimates of the slope of the function in its nearly linear portion. The values for the L to M cone ratio obtained by means of equation (2) for a number of values of the variable x (in the nearly linear portion of the detection function) were averaged to obtain our second estimate. The values obtained for each of our observers were as follows: 2.06 for CC; 2.01 for JN; 1.41 for YP; 2.24 for EM; 2.16 for VV; and 2.08 for HA.

Estimates based on the best-fitting theoretical functions

We added further definition to our model by assuming that the absorption of quanta in any cone follows a Poisson process (Hecht *et al.*, 1942; Brindley, 1963; Marriott, 1963). This allowed us to specify that:

$$Q(x) = \Sigma(e^{-x}x^k/k!), \qquad (3)$$

where the summation runs from $k = 0$ to $k = (m - 1)$ and m is the required number of quanta to activate a cone. Our choice of the value of m was guided by the results of Marriott (1963) which set a lower bound of 5 for this number. Our measurements were best fit by a choice of m equal to 6. Equation (3) in combination with the basic model expressed in equation (1) allowed us to find best-fitting theoretical functions for our measured detection functions. The best-fitting functions as compared to our measurements are shown in Fig. 7 for our six observers. The values of N_L and N_M

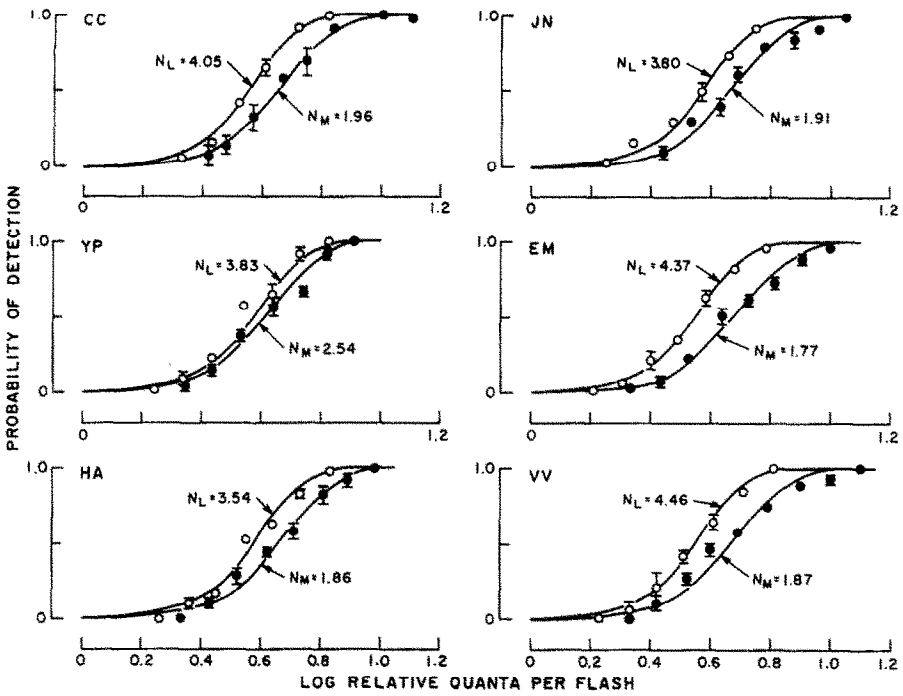

Fig. 7. The best-fitting theoretical functions (smooth curves) derived from equation (3) allowed us to obtain estimates of the numbers of cones contributing to detection of a test of 1 min in diameter under L and M cone isolation conditions. The results for M cone isolation conditions are plotted as closed symbols, for L cone isolation as open symbols. The error bars indicate the between-day SEM. The numbers of estimated L cones (N_L) and M cones (N_M) are shown next to the associated detection function.

associated with these best-fitting functions gives us another way to estimate the relative numbers of L and M cones. The values we obtained for each of our observers were as follows: 2.07 for CC; 1.99 for JN; 1.51 for YP; 2.47 for EM; 2.39 for VV; and 1.90 for HA.

The values obtained by means of each of the estimation procedures for each of our observers are listed in Table 1.

Estimation of total cone density

We estimated the total number of cones illuminated by our test of 1 min of visual angle by summing our estimates of the numbers of L and M cones. These values are shown in Table 2 for each of our six color normal observers. By comparison, anatomical results provide an estimated range of five to seven cones, likely to be illuminated by the retinal image of a test spot of 1 min in diameter. The retinal image corresponding to this 1 min test was estimated as before by assuming the optical spread function of Campbell and Gubisch (1966). The density of cones in fovea centralis for each of our six observers was then calculated. Figure 8 shows the mean cone density obtained for our six color normal observers as compared to the anatomically derived estimates of Osterberg (1935), Miller (1979), and Curcio *et al.* (1987). We have also applied these methods to obtain cone density in fovea centralis of dichromatic observers (Cicerone and Nerger, 1986). The mean values obtained for these dichromatic observers are also plotted in Fig. 8. In addition, for CC, estimates of total numbers of L and M cones in locations at 0.5, 1.0, 2.0 and 4.0 deg eccentricity

Table 1. Relative numbers of L to M cones in fovea centralis

Estimates based on:	Observers CC	JN	YP	EM	VV	HA
Test sizes giving matching detection functions	2.0–2.3	2.0–2.3	<2.0	>2.0	>2.0	≤2.0
Ratio of slopes of detection functions	2.06	2.01	1.41	2.24	2.16	2.08
Best-fitting theoretical functions	2.07	1.99	1.51	2.47	2.39	1.90

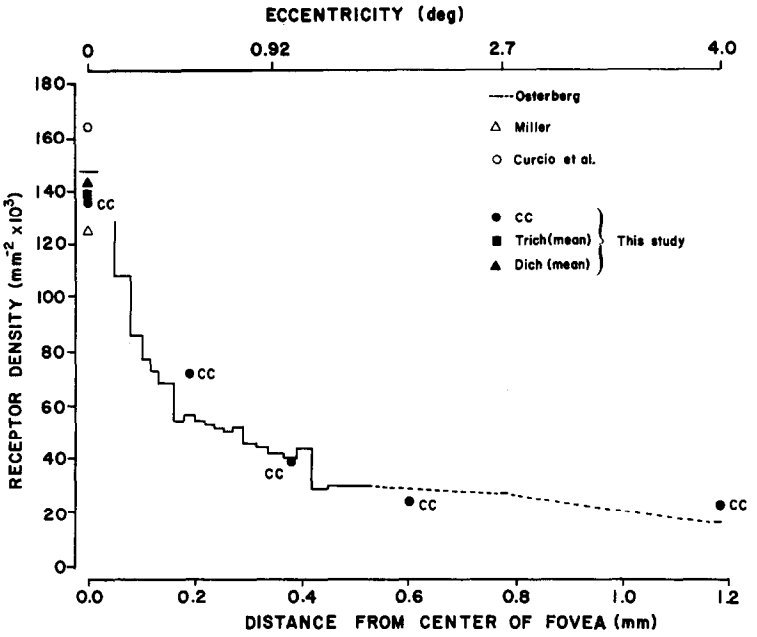

Fig. 8. The total number of cones illuminated by our test of 1 min diameter can be estimated as the sum of the numbers of L and M cones obtained by our previous estimates. The density of cones in fovea centralis can then be easily calculated. Plotted here are the anatomical results of Osterberg (straight and dashed lines), Miller (open triangle) and Curcio *et al.* (open circle) as compared to the results of our study. Shown here are the mean values obtained from our sample of six trichromatic observers (solid square) and from a sample of six dichromats (solid triangle), as well as the values for trichromat CC (solid circle) for whom we obtained estimates in fovea centralis and at eccentricities of 0.5, 1.0, 2.0 and 4.0 deg.

were obtained. Measurements were made as before but with a 580 nm test upon a blue–green background, chosen to favor detection by L as well as M cones, and to eliminate the rods and S cones from contributing to detection. Estimates of total numbers of L and M cones were obtained by means of the best-fitting theoretical function as detailed above. Results are plotted in Fig. 8 without correcting for S cones. Overall the comparisons to the anatomical results are reassuring, and lead us to be confident of our estimation procedures.

Predicted spectral efficiency functions

Shown in Fig. 9 are comparisons of the spectral efficiency functions measured by heterochromatic flicker photometry for three observers. Deuteranope LH's results are compared to the Smith and Pokorny (1972) fundamental L function, protanope KG's results to the M function, and trichromat CC's results to the sum of the L and M functions, in the proportion 2.07 to 1.00, as dictated by our estimates of the relative numbers of these cone types in the fovea for this observer. The generally accepted model is that the flicker photometric value of a light is given by the weighted sum of the signals from the L and M cones (e.g. Eisner and MacLeod, 1981). Here we have applied weighting factors which solely reflect our estimate of the relative numbers of L to M cones, ignoring such possible factors as differences in the gain of the neural pathways of the different cone types. The fits of the measurements to the predicted functions are shown to be satisfactory for these observers.

Table 2. Number of cones in fovea centralis contributing to detection of a 1 min test spot

Observers	Test λ/background λ 520/640	640/520	Total cones $(L+M)$
CC	1.96	4.05	6.01
JN	1.91	3.80	5.71
YP	2.54	3.83	6.37
EM	1.77	4.37	6.14
VV	1.87	4.46	6.33
HA	1.86	3.54	5.40

DISCUSSION

We have obtained estimates of the numerosity of L as compared to M cones in six color normal trichromatic observers. These estimates ranged

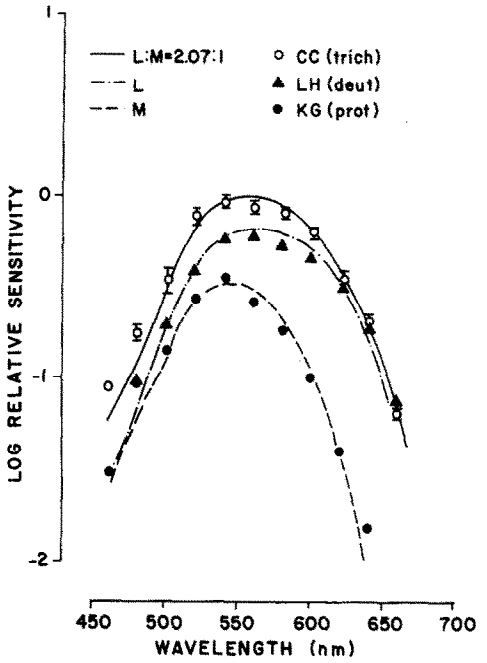

Fig. 9. The spectral efficiency functions for trichromat CC (open circles), deuteranope LH (solid triangles), and protanope KG (solid circles) were measured by the method of heterochromatic flicker photometry. The dashed line is the Smith and Pokorny *M* fundamental, the dot–dashed line is the *L* fundamental, and the smooth curve is the linear sum of *L* and *M* fundamentals in proportion 2.07 to 1.00, reflecting our estimate of the relative numbers of *L* to *M* cones for observer CC. Error bars indicate the between-day SEM.

between a factor of 1.46 to 2.36 more *L* as compared to *M* cones in human fovea centralis. The range of values we obtained overlaps the range of earlier estimates based on modeling procedures designed to make various psychophysical data sets consistent one to another (Vos and Walraven, 1971; Walraven, 1974; Smith and Pokorny, 1975), and our mean value is virtually identical to the ratio proposed by Vos and Walraven (1971) and Walraven (1974). We have sought to verify our estimates by comparing the total numbers of *L* and *M* cones we obtained to anatomical estimates of the total numbers and densities of the cones in human fovea (Fig. 8). This comparison in the fovea, as well as at a number of eccentric locations, shows a close correspondence between our psychophysical results and the anatomical results of others (Osterberg, 1935; Miller, 1979; Curcio *et al.*, 1987).

The generally accepted model for spectral efficiency which linearly combines the quantum catches in *L* and *M* cones was applied in its simplest form, using only the proportion of *L* to *M* cones as weighting factors, to predict the function as measured by heterochromatic flicker photometry for one color normal observer (Fig. 9). We showed a reasonable match between the predicted functions and measurements, thus lending credibility to this model for flicker photometric sensitivity, as well as to our estimates of the relative numbers of *L* and *M* cones.

Experimental conditions

Our conclusions are dependent on the extent to which we have succeeded in adequately isolating the *L* or *M* cones in each of our conditions so that detection is not contaminated by contributions from quanta caught in other than the intended cone type. The experiments of Fig. 3 provide experimental evidence that this was achieved, inasmuch as the slopes of the detection functions were stable for a range of adapting intensities around the value we used. On the contrary, if proper isolation had not been attained, then as the intensity of, say, the red background was increased, more *L* cones should have been eliminated from contributing to detection, and the slopes of the detection functions would be expected to decline.

We also conducted the following control experiment (Fig. 4) to buttress this argument. We measured detection functions using a 575 nm test light upon either the green background used for isolation of *L* cones or upon the red background used for isolation of *M* cones. If the backgrounds we had chosen adequately isolated *L* and *M* cones, then the slopes of the detection functions measured with a 575 nm test should be determined by the wavelength of the background and would be expected to match those measured with the red and green tests, respectively. Indeed, this was our result. When the method using best-fitting theoretical functions was applied to the detection functions of Fig. 4, the following values were obtained as estimates of the number of cones contributing to detection: upon an *M* cone isolating background of 640 nm, the estimated number of cones contributing to the detection of a test of 1 min diameter was 1.96 if its wavelength was 520 nm and 1.80 if its wavelength was 575 nm. Upon an *L* cone isolating background of 520 nm, the estimated number of cones contributing to detection was 4.05 if the test wavelength was 640 nm and 3.81 if the test wavelength was 575 nm. These values yield relative numbers of

L to M cones estimated at 2.07 and 2.12, respectively.

The experiments of Figs 3 and 4 were also of importance in making the converse point, namely, that the backgrounds we used were not likely to have significantly desensitized the cone class of interest. This is an important point to consider for the following reason: if this had occurred, we might have underestimated the numbers of L or M cones, since all the cones of interest might not have contributed to the detection of the test. In our favor, the results show no change of slope for a reasonable range of intensity levels of the background (Fig. 3) and when the wavelength of the test is not optimal for the cone class of interest (Fig. 4). Hence, it is unlikely that the cone class of interest was significantly desensitized.

Thus, these two sets of experiments allow us to conclude that we have not erred significantly in either overestimating or underestimating the relative numbers of L to M cones in the human fovea centralis.

The results of the experiments with a 575 nm test detected upon either an M or L cone isolating background also give us assurance that preretinal factors, such as chromatic aberration, for example, did not significantly distort our results. If these factors had entered into the measurements in any significant way, then the stability of the slopes of the detection functions which we obtained would not be predicted.

Specific aspects of the model

The core version of our model, illustrated in Figs 2 and 5, is satisfying in that it is independent of a number of specifications which were used in our subsequent elaborations of this basic model. These include the specific detection rule, the extent of pooling among the stimulated receptors, and the number of quanta required to excite a single cone, each of which we will subsequently discuss in detail. We emphasize here, that the conclusions based on this model are nearly agnostic as to these kinds of assumptions. The estimate, based on the results shown in Fig. 6, that the relative numbers of L to M cones present in human fovea centralis are approximately in the ratio of 2:1, depends on two rather mild assumptions. First is that the quantum efficiency of the L cones (and M cones) does not change for the small range of test radiances we explored. Second is that any neural factors involved in the detection of near threshold lights of the tiny sizes we used are the same for L and M cones, provided, as was the case in our experiments, the colored backgrounds have comparable effects upon the L and M cones.

The second version of our model allowed us to obtain numerical estimates of the relative numbers of L and M cones based on the slopes of the detection functions, in exchange for the assumption that if any one of the illuminated cones absorbed the required number of quanta, then the test was detected. According to this assumption, under the conditions of our experiments each cone in fovea centralis is an independent detector, and the usual rule of probability summation applies to the small number of cones illuminated by our test light. These model assumptions are similar to those made by Brindley (1963) and Marriott (1963) to estimate the number of quanta required for detection by cones and by Krauskopf and Srebo (1965) and Krauskopf (1978) to estimate the spectral sensitivities of the cone mechanisms and to specify the nature of the detection process.

The third version of our model allowed us to obtain estimates, not only of the relative numbers of L to M cones, but also of the numbers of cones contributing to detection of the test under L as well as M cone isolation conditions. We obtained these estimates by assuming that quantal absorption follows a Poisson process. The parameters of the best-fitting theoretical functions then yielded our estimates of the numbers of cones contributing to detection of the tests.

The three methods produced estimates of the relative numbers of L to M cones which were tightly clustered for any given observer (Table 1). The distinct advantage of the estimates based on the third version of the model was that the sum of the estimates of the numbers of L and M cones derived from this version of our model can be compared to the anatomical estimates of the total number of cones which are contained in the retinal image of our test spot, in human fovea centralis. As shown in Fig. 8, this comparison is satisfactory, and therefore validates our model and our estimates. It should be noted that although the second and third versions of the model would not be expected to yield differences in the estimated relative numbers of L and M cones, our estimates of the total numbers of cones obtained by our third procedure need not necessarily have matched the anatomical results so closely. An underestimate of the total numbers of cones could have oc-

curred, for example, if our assumption of cones as independent detectors was seriously flawed. If detection thresholds were determined by a pool of cones rather than by independent pathways stemming from single cones, then the third procedure is likely to underestimate the number of cones contributing to detection of the test. Provided that the number of cones contributing to a pool is the same for L and M cones, this would not affect our estimates of the relative numbers of cones, only our estimates of the total numbers of cones contributing to detection of the test. Additionally, if our assumptions on the extent of optical spread (Campbell and Gubisch, 1966) had been incorrect, then our estimates might have deviated significantly from the anatomical estimates of total cone density.

Some of these assumptions may fail to hold for extrafoveal cones, for which psychophysical results indicate a fair degree of pooling of signals for detection and for which there is ample anatomical evidence for many-to-one connections of cones to flat bipolars. Thus, the assumption of cones as independent detectors is especially vulnerable for modeling detection by cones outside the fovea. In this regard, some support for the notion that cones in the fovea centralis act as independent detectors in the sense required by our model is given by our results showing a good match of our estimates of total cone density to that estimated in anatomical studies (Fig. 8).

Any differences in the direction of gaze from trial to trial would result in a different set of cones being illuminated by our test spot and thus the sample of L or M cones contributing to detection of the test would not be the same from trial to trial (Fig. 1). In this sense, the estimates we obtain from our model must be assumed to be mean values over all the possible slightly different placements of the image of the test spot due to any variability in the direction of gaze from trial to trial. To our knowledge there are no direct measurements which bear on our experimental situation. The r.m.s. deviation in the direction of gaze as measured over sustained (half a second or longer) attempts at fixation is small, estimated to be between 2 and 4 min arc (e.g. Steinman, 1965; Ditchburn, 1973). Snodderly and Kurtz (1985) estimated between-trial variability in the direction of gaze by measuring a final eye position as the mean of samples taken every 5 msec in the last 200 msec of trials lasting many seconds. Assessed in this way, their estimate is that the standard deviation of the direction of gaze in horizontal and vertical directions is between 2 and 6 min arc. In our experiments, the observer initiated a trial only when sure of accurate fixation, so that between-trial variability in direction of gaze might be smaller than these estimates. An indirect estimate of 2.5 min arc r.m.s. deviation in direction of gaze in an experimental situation close to ours (self-presentation of test flashes, 50 msec test flash durations) was obtained by modeling the spatial variations in sensitivity of S cones which are sparsely distributed in the retina (Williams *et al.*, 1981). This range of variability in the direction of gaze from trial to trial roughly spans a distance of four cones, assuming a center-to-center spacing among the cones of 0.6 min arc (Miller, 1979). Under our experimental conditions, with a test of 1 min arc in diameter, our expectation is that roughly six out of this pool of about 15 cones in fovea centralis are sampled on any one trial. Of course, our selective adaptation procedure raises the threshold of selected classes of cones, so that not all illuminated cones contribute to detection of the test. It should be noted that although we have taken advantage of this difference in direction of gaze from trial to trial, so that we can assume a random sample of the cones over trials, we have not specifically incorporated this source of variability into our expression for the psychometric functions.

It is perhaps worth noting here the basic differences in our approach and model to that employed by Williams *et al.* (1981) to estimate the distribution of the S cones in the human retina. The psychophysical estimation of the distribution of S cones in the human fovea presented by Williams *et al.* (1981) used to advantage the scarcity of this cone type. Using tiny, brief, violet test lights spaced 4 or 5 min arc apart and presented on a long-wavelength background, they measured large spatial variations in S cone sensitivity, with discrete peaks spaced roughly 10 min arc apart in peripheral regions of the fovea. They argued convincingly that these peaks represented individual S cones. We approached our task in estimating the density of L and M cones in fovea centralis in a different way. Far from being sparsely distributed, the L and M cones are densely packed, and reliably and consistently picking out individual ones of these would be nearly impossible due to optical considerations and the expected trial-to-trial variability in direction of gaze. We therefore used a model which would take into account the

contributions of several cones of a single class to the determination of threshold and tailored experimental conditions according to this model in order to obtain our results.

Individual variability

The results from our six observers yielded mean values for the relative numbers of L to M cones of 1.99 (SEM 0.13) by the ratio of slopes method and 2.05 (SEM 0.16) by the best-fitting function method. The different methods gave consistent estimates for each individual subject (Table 1). Our results are consistent with the generalization that in color normal trichromatic observers the number of L cones exceeds the number of M cones by a mean value near 2:1, with a range of individual variability of 1.46–2.36.

This range is modest as compared to that of DeVries (1948) who estimated the relative numbers of L to M cones by the ratio required to fit an individual's flicker photometric function. DeVries estimated a range of 0.59–4.00 of individual variability. This enormous range can be questioned on the following grounds. Although an individual's L to M cone ratio may reasonably be used to predict the luminous efficiency function in order to assess the consistency and predictive capability of the estimated ratio (Fig. 9), it is not straightforward to achieve the reverse. Reliable estimates of the relative numbers of L to M cones are not easily derived by analysis of an individual's photopic luminous efficiency function. This can be appreciated if one considers calculations of luminosity functions obtained as the sum of the contributions of L and M cones, with weighting factors representing the L to M cone ratio and ranging, for example, between 1.5 and 2.5. The resulting functions vary little; and when normalized, the long-wavelength portions (up to 660 nm) differ by no more than 0.07 log unit. At best, this is barely enough to span the usual variability in the psychophysical measurements made by heterochromatic flicker photometry. The uncertainty attached to this method of attaining the proportion of L to M cones was probably compounded in DeVries' (1948) experiments by the fact that he estimated flicker photometric functions with measurements made at only two wavelengths, 550 and 660 nm.

Our range of individual variability in the relative numbers of L to M cones can also be compared to that obtained by Rushton and Baker (1964) who reported estimates for their 21 male observers with a range of 0.33–3.00 for the ratio of L to M cones. Rushton and Baker arrived at this conclusion by assuming that the ratio of the numbers of L to M cones was proportional to the ratio of the density of erythrolabe as compared to chlorolabe measured at 535 nm, the erythrolabe measurement having been made after a bleach with a green light and the chlorolabe after a bleach with a red light. As these researchers themselves were careful to point out, their estimate establishes only the lower and upper bounds consistent with their measurements of pigment densities. This is because the proportionality constant which must be applied to the densitometric ratio to obtain the numerical ratio of the cones is not easily determined, since under their experimental conditions the red and green bleaching lights almost surely produced quantal absorptions in both L and M cones. Thus, the density of erythrolabe, after the bleach with the green light is not available in full strength, and neither is the density of chlorolabe after bleaching with the red light. The large range obtained by Rushton and Baker (1964) for the numbers of L to M cones cannot be safely assigned entirely to individual variability among color normal observers, but may, at least in part, be due to the large range in the boundaries of estimation that their methodology allows.

For a number of reasons, as discussed above, we have experimental evidence giving us a measure of assurance that our methods do not cause us to significantly overestimate or underestimate the relative numbers of L and M cones. We find a mean value of near two L cones for each M cone in fovea centralis of six color normal human observers, who show individual variability but in each case the results are consistent with more L than M cones composing the retinal mosaic of central fovea.

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