Cone Signal Contributions to Electrograms in Dichromats and Trichromats

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PURPOSE. To find out how the different cone types contribute to the electroretinogram (ERG) by quantifying the contribution of the signal pathways originating in the long (L-) and the middle (M-) wavelength-sensitive cones to the total ERG response amplitude and phase.

METHODS. ERG response amplitudes and phases were measured to cone-isolating stimuli and to different combinations of L- and M-cone modulation. Conditions were chosen to exclude any contribution of the short wavelength-sensitive (S-) cones. The sensitivity of the ERG to the L and the M cones was defined as the cone contrast gain.

RESULTS. In the present paper, a model is provided that describes the ERG contrast gains and ERG thresholds in dichromats and color normal trichromats. For the X-chromosome-linked dichromats, the contrast gains of only one cone type (either the L or the M cones) sufficed to describe the ERG thresholds for all stimulus conditions. Data suggest that the M-cone contrast gains of protanopes are larger than the L-cone contrast gains of deuteranopes. The response thresholds of the trichromats are modeled by assuming a vector summation of signals originating in the L and the M cones. Their L- and M-cone contrast gains are close to a linear interpolation of the data obtained from the dichromats. Nearly all trichromats had larger L- than M-cone contrast gains. Data from a large population of trichromats were examined to study the individual variations in cone weightings and in the phases of the cone pathway responses.

CONCLUSIONS. The data strongly suggest that the missing cone type in dichromats is replaced by the remaining cone type. The mean L-cone to M-cone weighting ratio in trichromats was found to be approximately 4:1. But there is a substantial interindividual variability between trichromats. The response phases of the L- and the M-cone pathways can be reliably quantified using the response phases to the cone-isolating stimuli or using a vector addition of L- and M-cone signals. (Invest Ophthalmol Vis Sci. 1999;40:920–930)

The classical electroretinogram (ERG) is measured with stimuli that usually excite more than one photoreceptor type simultaneously. Thus, it is generally not possible to quantify the stimulus strength for each photoreceptor type or to know whether the long (L-) and the middle (M-) wavelength-sensitive cones and their respective postreceptoral pathways contribute in a similar manner to the ERG signal or how their signals interact when stimulated simultaneously. To investigate such questions, chromatic adaptation procedures have been used in ERG recordings.1,2 However, although the response of the adapted or desensitized cone type in such procedures may often be very small, it may not be negligible. Furthermore, these techniques have the disadvantage that the system may be pushed into a nonlinear range. Finally, it is difficult to come to a quantitative description of the interaction between photoreceptor signals when only cone-isolating conditions are used.

The present paper provides new data on ERG responses to cone-isolating stimuli and to stimuli in which the L- and M-cones are stimulated simultaneously with known contrasts without changing the overall state of adaptation.3-4 Our technique is reminiscent of the silent substitution paradigm (see review in Ref. 5) and of the heterochromatic flicker photometry ERG.6,7 We extend these stimulus paradigms to conditions in which the L- and M-cone types are modulated simultaneously and quantify the modulation of the individual cone excitations in terms of cone contrast. A similar method has been used by Brainard et al.8 and by Shapley and Brodie.9 Our chosen stimuli did not modulate the short-wavelength-sensitive (S-) cones. Moreover, the mean luminance (66 candela [cd]/m²) and the temporal frequency of the stimulus (30 Hz) were both high enough to desensitize the rods substantially.3

We introduce a model that describes the response amplitude data of both trichromats and X-chromosome-linked dichromats. This allows us to address several important issues. Because all stimuli were quantified in terms of cone contrast, the response data of deuteranopes, missing functional M cones, and protanopes, missing functional L cones, could be directly compared. We find that L-cone pathway signals in deuteranopes are significantly larger than M-cone pathway signals in protanopes.
Model fits to the trichromatic data allow the ratios of L-cone to M-cone weightings to be estimated and reveals phase differences between the responses of the L- and the M-cone pathways. Considerable interindividual variations of the cone weighting ratios and of the phase differences are implied. The response phase differences of L- and M-cone pathways obtained from the fits could be compared for each subject separately with directly measured response phase differences using the cone-isolating stimuli.

It is controversial whether the missing cone types in X-linked dichromats are completely or incompletely replaced by the remaining cone type. Our ERG measurements argue for a complete replacement.

A subset of the data was reported previously.

METHODS

Subjects

Thirty-six trichromats (9 to 57 years of age), 6 protanopes (20 to 46 years of age), and 9 deuteranopes (18 to 41 years of age) participated in this study. The classification of dichromacy was performed by the Nagel type I anomaloscope, or the panel D15, and had normal ophthalmologic findings with corrected visual acuity of 20/20. A genetical analysis was performed on blood samples of some of the male trichromats, and the presence of functional genes expressing for the L- and the M-cone photopigment was established.

In all subjects, one eye was dilated with a mydriatic (0.5% tropicamide) and kept light-adapted at least 10 minutes before ERG recording to reduce rod responses. Corneal ERG responses were measured with a DTL fiber electrode. The reference and ground skin electrodes were attached to the ipsilateral temple and the forehead, respectively. Informed consent was obtained from all subjects after explanation of the purpose and possible consequences of the study. This study was conducted in accordance with the tenets of the Declaration of Helsinki and with the approval of our institutional ethics committee in human experimentation.

Visual Stimuli and ERG Recording

The method of ERG recording has been described previously. The stimuli were presented on a computer controlled monitor. The monitor subtended 124 by 108° at the 10-cm viewing distance. We used a 30-Hz square wave modulation of the red, green, and blue phosphors, with predefined Michelson contrast. The emission spectra of the three phosphors were measured with an Instruments Systems spectroradiometer. The red phosphor had multiple narrow peaks at approximately 594, 616, 626 (the largest peak), and 704 nm. The blue and green phosphors had relatively broad emission spectra with peak emissions at 448 nm (bandwidth at half-maximum 62 nm) and 530 nm (bandwidth at half-maximum 76 nm), respectively.

The time-averaged luminance of the monitor was 66 cd/m² (40 cd/m² for the green phosphor, 20 cd/m² for the red phosphor, and 6 cd/m² for the blue phosphor). The excitation in one cone type by the monitor phosphors was calculated by multiplying the phosphor emission spectra with psychophysically based cone fundamentals integrated over the wavelength range. For example, the excitation of the L cones by the red phosphor (E_L(R)) was calculated as follows:

\[ E_L(R) = F_L \cdot L(t) \cdot \int_{380}^{780} I_d(\lambda) \cdot A_L(\lambda) \cdot d\lambda \]

where \( E_L(R) \) is the luminance of the red phosphor, \( L(t) \) changes as a function of time, \( I_d(\lambda) \) is the emission spectrum of the red phosphor at 1 cd/m² luminance. We have checked that the emission spectrum did not change with the luminance output of the phosphors. \( A_L(\lambda) \) is the L-cone fundamental. \( F_L \) is a conversion factor for the red phosphor relating the photometric measurements to the cone fundamentals, which are expressed in radiometric terms. The total excitation of the L cones is the sum of the excitations caused by each phosphor. Similarly, the excitation in the S and M cones could be calculated.

Recalculations of the cone excitations using another set of cone fundamentals resulted in only minor differences in stimulus conditions. The modulation of cone excitation was quantified by the cone contrast. The L-cone contrast was defined separately. The S cones were not modulated (S-cone contrast was 0%) in this study.

The ERGs were measured with two different but similar setups. With the first setup, we measured all the dichromats and 12 trichromats. The monitor was a BARCO CCID 7751 MKII (100-Hz frame rate) driven by a VSG 2/2 graphics card (Cambridge Research Systems). The time-averaged chromaticity in CIE (1964) large field coordinates were as follows: \( x = 0.3329, y = 0.3181 \). We calculated that the average foveal quantal catches were approximately 4.42 log quanta ° cone⁻¹ in the L cones and approximately 4.31 log quanta ° cone⁻¹ in the M cones. ERG responses to 32 different stimuli were measured: eight conditions with different L cone to M cone contrast ratios (1:0, 2:1, 1:1, 1:2, 0:1, −1:2, −1:1, −2:1; negative ratios and negative cone contrasts indicate counter-phase modulation) with four contrasts within each condition (100%, 40%, 20%, and 10% of the maximally possible cone contrast). A threshold contrast increase for a 1 μV ERG response increase was determined at each condition. The spaces of possible L- and M-cone contrasts that can be generated by the monitor are displayed in Figure 1, in our previous publication.

The largest possible cone contrast was 28.6% in the M-cone-isolating condition and 22.7% in the L-cone-isolating condition. Signals were amplified and band-pass filtered be-
between 10- and 100-Hz corner frequencies (half amplitude at -6 dB; Grass Instruments). The signal was sampled at 1000 Hz with a CED 1401 on-line computer. ERG responses to 12 runs, each lasting 4 seconds, were averaged in each measurement. The stimulus and the data acquisition were synchronized by a trigger pulse generated by the VSG graphics card.

With the second setup, 25 trichromats were measured. It was designed to be implemented in the clinical routine, requiring only minor hardware and software differences. The monitor was a BARCO CCID 121 driven at 100 Hz by a VSG 2/5 graphics card. The same mean values of luminance of the three phosphors were used as in the first setup, resulting in nearly identical mean quantal catches in the L and M cones, and in a very similar space of feasible cone contrasts. The largest possible cone contrast was 31.2% in the M-cone-isolating condition and 24.7% in the L-cone-isolating condition. The responses were measured at 100%, 75%, 50%, and 25% of the maximally possible cone contrasts at each L-cone to M-cone contrast ratio. The signals, recorded with the second setup, were amplified and filtered between 1 Hz and 300 Hz (half amplitude at -6 dB; Grass Instruments) and sampled at 1000 Hz with a National Instruments AT-MIO-16DE-10 data acquisition card. The gain set by the analysis software was different. As a result, the response data were qualitatively very similar to those obtained with the first series, but the absolute amplitudes differed. Therefore, these data cannot be compared directly with the dichromatic data. One subject (LTS) was measured with the two setups. The results for the ratio of cone weightings as well.

An estimation of the magnitude of stimulus artifacts, caused by electromagnetic radiation of the monitor, was obtained using a signal recording when the monitor displayed a stimulus but was covered by black cardboard. In all cases, the stimulus artifacts were negligibly small.

There are inherent limitations when a monitor is used as a stimulator, which in principle, can interfere with our measurements, including the different excitation and decay times of the different phosphors and the sequential excitation of the pixels, which could influence the response phase. As described previously, the first potentially confounding factor could be excluded, because we found that in dichromats the ERG response amplitudes and phases were very similar in stimulus conditions that were physically different but that resulted in equal cone contrasts. Furthermore, measurement of the phosphor excitations with a photodiode revealed that the differences in peak excitation times between the three phosphors are much less than 1 ms, which is smaller than the phase effects measured here (see the Results section). The sequential pixel excitation might influence the phase data when there is an asymmetry in L- and M-cone distributions between the upper and the lower parts of the retina. However, we found that the response phases were not influenced by rotating the stimulus monitor by 180°, excluding a possible artifact of the sequential pixel excitation.

**Data Analysis**

The thresholds for a 1-µV ERG response increase were determined at each ratio of L-cone to M-cone contrast (see the Results section). These ERG thresholds were fitted with a model that is based on the assumption that the ERG responses are the result of a vector summation of the ERG signals originating in the two cone types. The response to pure L-cone modulation has an amplitude \( A_L \times C_L \) (which depends linearly on L-cone contrast \( C_L \)) with a gain of \( A_L \); as described in the Results section and as is displayed in Fig. 3 of a report published by our group previously, this linearity was found in all conditions and phase \( \alpha_L \). Similarly, pure M-cone modulation results in a response amplitude \( A_M \times C_M \) and phase \( \alpha_M \). For each condition at which a threshold is measured, the ratio of the L-cone to M-cone contrast is constant and predefined \((C_L/C_M = K, \text{ when } C_M \neq 0)\). The response vectors can be described as \( R_L = A_L \times C_L \times (\cos \alpha_L) \) responses originating in the L-cones and \( R_M = A_M \times C_M \times (\cos \alpha_M) \) responses originating in the M cones. We assume that the threshold will be reached when the sum of these vectors has a certain length \((i.e., \text{the sum of the signals has a threshold amplitude})\):

\[
\sqrt{A_L^2 \cdot C_L^2 + A_M^2 \cdot C_M^2 + 2 \cdot A_L \cdot A_M \cdot C_L \cdot C_M \cdot \cos (\alpha_L - \alpha_M)} = A_{\text{Thresh}} \quad (2)
\]

This model is identical to the model used by Lee et al. to explain the contribution of chromatic and luminance signals to the responses of retinal ganglion cells.

With \( A_L/A_{\text{Thresh}} = A_L^* \) (which is the L-cone weighting or L-cone contrast gain) and \( A_M/A_{\text{Thresh}} = A_M^* \) (the M-cone weighting or M-cone contrast gain) Equation 2 becomes

\[
A_L^* \cdot (K \cdot C_M)^2 + A_M^* \cdot C_M^2 + 2 \cdot A_L^* \cdot A_M^* \cdot K \cdot C_M \cdot \cos (\alpha_L - \alpha_M) = 1 \quad (3)
\]

and thus the contrasts \( C_M \) and \( C_L \) at threshold are

\[
C_M = \frac{1}{\sqrt{A_L^2 \cdot K^2 + 2 \cdot A_L^* \cdot A_M^* \cdot K \cdot \cos (\alpha_L - \alpha_M) + A_M^2}} \quad (4)
\]

When \( C_M = 0 \), the contrast \( C_L \) at threshold, defined by Equation 2 is

\[
C_L = \frac{1}{A_L^*} \quad (5)
\]

The model predicts elliptical threshold curves. For the fits, there were three free parameters: \( A_L^* \) and \( A_M^* \), which quantify the L- and M-cone contrast gains or weightings, respectively (which by definition are the inverse of the threshold contrasts for the cone-isolating stimuli) and \( (\alpha_L - \alpha_M) \), which is the phase difference between the L- and M-cone responses.

The cellular origins of the flicker ERG are not unequivocally established yet, but postreceptoral processes are almost certainly involved. However, this is not crucial for the model. The model only assumes that the responses originate in the photoreceptors. In the rest of the present article, the term “cone response” is used to refer to the ERG response that originates in a particular cone class including the subsequent postreceptoral stages. Therefore, the cone weightings we find should not be confused with cone numbers, because postreceptoral mechanisms can modify the cone weightings. However, the cone density will almost certainly influence the cone weightings as well.
RESULTS

Amplitude Data

Figure 1 shows the ERG responses to pure M-cone modulation (L:M cone contrast ratio 0:1; M-cone contrast: −28.6% M-cone contrast, the negative contrast indicates a counter-phase modulation relative to the trigger pulse), to pure L-cone modulation (L:M cone contrast ratio 1:0; 22.7% L-cone contrast), and to phase modulation of the L and the M cones (L:M cone contrast ratio 1:1; 76.8% L-cone contrast and 76.8% M-cone contrast) for a protanope (A), a deuteranope (B), and a trichromat (C). As expected, the responses to pure L-cone modulation were very small or absent in the protanope, whereas the deuteranope showed small responses to the M-cone-isolating stimulus. Substantial responses were measured in the trichromat in both conditions. For this trichromat, the response to the L-cone-isolating stimulus is larger than the response to the M-cone-isolating stimulus, despite the slightly larger M-cone contrast. Furthermore, the two dichromats display larger ERG responses to the stimuli isolating their remaining cone type than does the trichromat to the same stimuli. The responses to the condition in which the L and M cones are modulated in phase are very similar in the three subjects.

The ERG responses were Fourier analyzed, and the ERG response amplitude was defined as the amplitude of the fundamental component. As reported previously, we found a linear relationship between ERG response amplitude and cone contrast in all conditions. The slope of this line was defined as the cone contrast gain and quantifies the increase of the ERG amplitude caused by a 1% increase of cone contrast. A linear relationship between response amplitude and stimulus contrast has also been reported by Wu et al. 20 at a very similar temporal frequency (28 Hz) but not at lower and higher temporal frequencies. This is potentially troublesome because a linear relationship between contrast and response amplitude is assumed for our model. However, for the model it is only necessary that linearity approximately holds near threshold.

The inverse of the contrast gain is the ERG threshold contrast needed for a 1-μV increase in response. These thresholds were obtained for all ratios of L-cone to M-cone contrasts. Figure 2 shows the measured thresholds for a protanope and a deuteranope. For the dichromats, the response thresholds are determined by a single cone type. When plotted as in Figure 2, the threshold data should lie on a straight line either parallel to the L-cone threshold axis (for the protanopes) or to the M-cone threshold axis (for the deuteranopes). This indeed seems to be the case, confirming the notion that the cone space of dichromats in the L/M-cone contrast coordinate system is one-dimensional. The ERG responses in protanopes to L-cone-isolating stimuli were extremely small. In the deuteranopes, we measured a small but substantial response in the M-cone-isolating condition. This is possibly caused by small imperfections in the calculations of the phosphor contrasts or by intrusion of small responses originating in the rods (we calculated that the maximal rod contrast is approximately 28% in this condition). In psychophysical control experiments, deuteranopes were able to detect this stimulus, although their sensitivity was about a factor of eight smaller than that for the L-cone-isolating stimulus.

Figure 3 shows the measured ERG thresholds for three different dichromats. The data of most dichromats can easily be distinguished from those of dichromats (A and B), although the thresholds of some subjects (an example is shown in Fig. 3C) are similar to those of deuteranopes. This has been observed previously. Furthermore, the displayed data indicate a substantial variability between trichromats.

We estimated the cone contrast gains from the model fits to the data when all conditions were measured or from the direct measurements for the dichromats, for whom only the cone-isolating stimuli were used. In Tables 1 and 2, the M- and L-cone contrast gains are shown for the dichromatic and trichromatic subjects measured with the first setup. The contrast gains differed between the groups: the M-cone contrast gains for the protanopes were 2.9 ± 0.95 (mean ± SD) and 1.90 ± 1.06 for the trichromats. The L-cone contrast gains was 2.66 ± 0.57 for the deuteranopes and 0.57 ± 0.45 for the trichromats.

Nearly all trichromats had larger L- than M-cone contrast gains, although a considerable interindividual variability is present. One female subject (JB; data are displayed in Fig. 3A) exhibited a ratio of L-cone to M-cone contrast gains that was considerably smaller than unity. This subject is possibly a carrier for protanopia (which is presently being investigated) because her mother's father was a protanope. The mean L-cone to M-cone contrast gain ratio was 4.24 ± 2.31.

Table 3 shows the results from measurement of 25 trichromats with the second setup in which different filter and gain settings were used. The mean ratio of the L-cone to M-cone contrast gains is 4.51 ± 5.57. This value is very similar to the one obtained with the first setup. The large SD again reflects a large interindividual variability of the ratio.

Phase Data

So far we have shown only the amplitude data of the ERG responses. We now consider the response phases. Extensive data on dichromats have been published previously. Here, we show extended data from trichromats. In Figure 4, the relationship between response phase and cone contrast is shown for the M- and L-cone-isolating stimuli. Only data obtained with cone-isolating stimuli at which all four cone contrasts were larger than 6% are shown. For these conditions, the response phases at all contrasts were large enough to get reliable response phases. The response phase increases with increasing cone contrast. A similar relationship between response phase and cone contrast was found for the conditions in which the two cone types were modulated simultaneously. In 10 subjects included in this analysis, the M-cone response led the L-cone response, whereas in 7 subjects the L-cone response led the M-cone response.

To compare the response phases of L and M cones shown in Figure 4, we assumed that for the displayed cone-contrast range the relationship between response phase and cone contrast was linear. Previous data have shown that an exponential function might describe these data more adequately when smaller cone contrasts are included. Using the linear regression through all data points we normalized the response phases to equal cone contrasts. With a paired t-test we found that the M-cone responses were significantly advanced relative to the L-cone responses (P = 0.035). The mean phase difference between M- and L-cone responses was approximately 15°. This would correspond to a 1.4-msec time difference, assuming that a delay difference is the cause of the phase difference.

In Tables 2 and 3 (fifth data column), the measured phase differences are given. Only phases at cone contrasts between 20% and 30% are shown, to avoid complications owing to the
Flicker ERGs in different subjects

A: Subject MH (protanope)

B: Subject MM (deuteranope)

C: Subject JK (trichromat)

D: Stimulus

FIGURE 1. Averaged ERG responses to pure M-cone modulation (left panels: M-cone contrast: −28.6%), pure L-cone modulation (middle panels: L-cone contrast: 22.7%), and in-phase modulation of the L and the M cones with equal cone contrasts (right panels: L-cone contrast: 76.8%; M-cone contrast: 76.8%) for a protanope (A; subject MH), a deuteranope (B; subject MM), and a trichromat (C; subject JK). The ERG signal is an average of 12 runs, and 150 msec of the responses are displayed. Note the different response scale for the in-phase modulation of the L and M cones. The small response in the deuteranope to the M-cone-isolating stimulus (B, left) might be caused by small errors in the calculation of the stimulus conditions or by rod response intrusion. The mean DC level is nonzero, owing to a small DC offset in the output of the amplifier. This DC offset had no influence on the data analysis, because only the first harmonic component of the Fourier transform was used. The ERG response in the M-cone-isolating condition is approximately 180° phase-shifted relative to the two other ERG measurements (also indicated by the negative cone contrast). This is because the M-cone excitation modulated in counter-phase with the trigger pulse generated by the graphics card; whereas in the two other conditions (L cone and L+M cone) the cone excitations and the trigger pulse modulated in phase. This phase shift has no influence on the amplitude data and was corrected for when analyzing the phase data. (D) Stimulus waveforms for the three conditions, separately displayed for the three phosphors. The values for mean luminance were 6 cd/m² for the blue phosphor, 40 cd/m² for the green phosphor, and 20 cd/m² for the red phosphor. The phosphor contrasts (red, green, blue) were 100%, −69.4%, and 4.3%, respectively, for the −28.6% pure M-cone modulation; 100%, −25.2%, and −2.8% for the 22.7% pure L-cone modulation; and 64%, 100%, and −20.5% for the 76.8% L- and 76.8% in phase M-cone condition. (Negative contrasts indicate counter-phase modulation.)
ERG-thresholds and model fits in dichromats

**RB: deuteranope**

**PH: protanope**

![Graphs showing threshold contrasts for dichromats](image)

**Figure 2.** Threshold contrasts (inverse of the contrast gains) in dichromats for the eight different ratios of L-cone to M-cone modulation. Data are shown for a deuteranope (A) and a protanope (B). A straight line is fitted to the data for each subject. The fits are based on the assumption that thresholds are determined by only one cone type in all conditions. The threshold data for the stimulus that isolated the nonpresent cone type were excluded from the fits. There was a very small response to the L-cone-isolating stimulus in protanopes even at the highest contrast. In the deuteranopes, a small but substantial response to the M-cone-isolating stimulus was measurable, possibly caused by small imperfections of our calculations or by rod response intrusion.

The interrelation between the phase and cone contrast. Sometimes, it was not possible to determine the phase differences because the response amplitude in the M-cone-isolating condition was too small to obtain a reliable measure for the response phase of the M-cone pathway. In seven subjects, the responses to L-cone modulation were found to lead the responses to M-cone-isolating stimuli. This is indicated by the negative values of the differences. In all other subjects the M cones were found to be

ERG thresholds and model fits in trichromats

**JB**

**JK**

**HK**

![Graphs showing threshold contrasts for trichromats](image)

**Figure 3.** Threshold contrasts in three different trichromats for the different ratios of L-cone to M-cone contrast. The ellipses are best fits of a vector addition model to the data points. The orientations of the ellipses span a large range of orientations found in our population of subjects. The ratio of L-cone to M-cone weighting was 0.28 for JB (A), 5.44 for subject HK (C), and 1.84 for JK (B).
TABLE 1. M- and L-Cone Contrast Gains Measured in Protanopes and in Deuteranopes

<table>
<thead>
<tr>
<th>Protanopes</th>
<th>M-Cone Contrast Gain</th>
<th>Deuteranopes</th>
<th>L-Cone Contrast Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>1.823</td>
<td>DS</td>
<td>2.992</td>
</tr>
<tr>
<td>AO</td>
<td>1.925</td>
<td>GE</td>
<td>2.768</td>
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<tr>
<td>MP</td>
<td>1.870</td>
<td>JH</td>
<td>2.466</td>
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<tr>
<td>MH*</td>
<td>2.729</td>
<td>SJ</td>
<td>2.387</td>
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<td>PH*</td>
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<tr>
<td>ML</td>
<td>1.409</td>
<td>AZ</td>
<td>2.743</td>
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<tr>
<td></td>
<td></td>
<td>MM*</td>
<td>2.602</td>
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<td></td>
<td></td>
<td>MB</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>RB*</td>
<td>2.846</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.01 ± 0.45</td>
<td>2.66 ± 0.57</td>
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</table>

In most subjects, the contrast gains were exclusively measured with cone-isolating stimuli. In four subjects, responses were obtained for all combinations of cone modulation. The given contrast gain for these subjects is the mean contrast gain of the remaining cone type in all these measurements, excluding only the condition in which the missing cone type was isolated.

* For these subjects, the responses were measured at all L-cone to M-cone contrast ratios.

The cone contrast gains (first two data columns), the ratio of the L-cone to M-cone contrast gains (third data column), and the phase difference between the L- and M-cone responses obtained from the model fits to the threshold data (fourth data column) were obtained with the first setup (see the Methods section). The directly measured phase differences (fifth data column) for each subject were obtained from the measurements with the cone isolating stimuli.

* M-cone contrast: 28.6%; L-cone contrast: 22.7%

TABLE 2. L- and M-Cone Contrast Gains in Trichromats

<table>
<thead>
<tr>
<th>Trichromats</th>
<th>L-Cone Contrast Gain (=A_L)</th>
<th>M-Cone Contrast Gain (=A_M)</th>
<th>L/M-Cone Contrast Gain Ratio</th>
<th>Vector Angle (α_L - α_M, deg)</th>
<th>Measured M-/L-Cone Phase Difference (deg)</th>
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<tr>
<td>HK</td>
<td>2.99</td>
<td>0.55</td>
<td>5.44</td>
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<td>LTS</td>
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<td>0.38</td>
<td>8.66</td>
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<td>—</td>
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<td>0.89</td>
<td>0.48</td>
<td>1.84</td>
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<td>4.51</td>
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<tr>
<td>CF</td>
<td>1.95</td>
<td>0.30</td>
<td>6.45</td>
<td>—</td>
<td>114.22</td>
</tr>
<tr>
<td>SW</td>
<td>3.68</td>
<td>0.79</td>
<td>4.68</td>
<td>0.11</td>
<td>2.08</td>
</tr>
<tr>
<td>HM</td>
<td>0.73</td>
<td>0.37</td>
<td>1.97</td>
<td>10.08</td>
<td>21.70</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.90 ± 1.06</td>
<td>0.57 ± 0.45</td>
<td>4.24 ± 2.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The cone contrast gains (first two data columns), the ratio of the L-cone to M-cone contrast gains (third data column), and the phase difference between the L- and M-cone responses obtained from the model fits to the threshold data (fourth data column) were obtained with the first setup (see the Methods section). The directly measured phase differences (fifth data column) for each subject were obtained from the measurements with the cone isolating stimuli.

* M-cone contrast: 28.6%; L-cone contrast: 22.7%
TABLE 3. Data as for Table 2 for a Different Population of Trichromats

<table>
<thead>
<tr>
<th>Trichromats</th>
<th>L-Cone Contrast Gain (=(A^*_L))</th>
<th>M-Cone Contrast Gain (=(A^*_M))</th>
<th>L-/M-Cone Contrast Gain Ratio ((\alpha_L / \alpha_M), deg)</th>
<th>Vector Angle ((\alpha_L - \alpha_M), deg)</th>
<th>Measured M-/L-Cone Phase Difference (deg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB</td>
<td>0.14</td>
<td>0.20</td>
<td>0.69</td>
<td>1.43</td>
<td>14.212</td>
</tr>
<tr>
<td>HI</td>
<td>0.34</td>
<td>0.06</td>
<td>6.08</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SK</td>
<td>0.23</td>
<td>0.17</td>
<td>1.37</td>
<td>19.77</td>
<td>17.115</td>
</tr>
<tr>
<td>MW</td>
<td>0.24</td>
<td>0.20</td>
<td>1.23</td>
<td>26.25</td>
<td>62.822</td>
</tr>
<tr>
<td>EB</td>
<td>0.22</td>
<td>0.05</td>
<td>4.48</td>
<td>27.10</td>
<td>−3.232</td>
</tr>
<tr>
<td>HL</td>
<td>0.23</td>
<td>0.12</td>
<td>1.86</td>
<td>43.10</td>
<td>—</td>
</tr>
<tr>
<td>HS</td>
<td>0.40</td>
<td>0.09</td>
<td>4.28</td>
<td>20.30</td>
<td>4.908</td>
</tr>
<tr>
<td>JR</td>
<td>0.23</td>
<td>0.17</td>
<td>1.40</td>
<td>9.59</td>
<td>—</td>
</tr>
<tr>
<td>US</td>
<td>0.15</td>
<td>0.24</td>
<td>0.65</td>
<td>31.98</td>
<td>26.041</td>
</tr>
<tr>
<td>SW†</td>
<td>0.27</td>
<td>0.06</td>
<td>4.13</td>
<td>−4.25</td>
<td>−21.139</td>
</tr>
<tr>
<td>AW†</td>
<td>0.31</td>
<td>0.13</td>
<td>2.36</td>
<td>36.08</td>
<td>24.534</td>
</tr>
<tr>
<td>RK†</td>
<td>0.46</td>
<td>0.03</td>
<td>13.32</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BK†</td>
<td>0.36</td>
<td>0.09</td>
<td>4.29</td>
<td>44.28</td>
<td>17.282</td>
</tr>
<tr>
<td>LS</td>
<td>0.31</td>
<td>0.16</td>
<td>1.95</td>
<td>−8.69</td>
<td>−10.894</td>
</tr>
<tr>
<td>BS</td>
<td>0.24</td>
<td>0.09</td>
<td>2.55</td>
<td>54.60</td>
<td>—</td>
</tr>
<tr>
<td>CJ</td>
<td>0.28</td>
<td>0.12</td>
<td>2.34</td>
<td>34.38</td>
<td>18.488</td>
</tr>
<tr>
<td>MC</td>
<td>0.20</td>
<td>0.15</td>
<td>1.38</td>
<td>−8.60</td>
<td>−8.337</td>
</tr>
<tr>
<td>WF</td>
<td>0.40</td>
<td>0.16</td>
<td>2.48</td>
<td>−25.13</td>
<td>−31.796</td>
</tr>
<tr>
<td>FH</td>
<td>0.47</td>
<td>0.02</td>
<td>28.16</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DB†</td>
<td>0.22</td>
<td>0.04</td>
<td>5.29</td>
<td>—</td>
<td>−42.879</td>
</tr>
<tr>
<td>FG†</td>
<td>0.41</td>
<td>0.10</td>
<td>4.12</td>
<td>69.56</td>
<td>—</td>
</tr>
<tr>
<td>RB†</td>
<td>0.29</td>
<td>0.24</td>
<td>1.22</td>
<td>11.07</td>
<td>1.917</td>
</tr>
<tr>
<td>JT†</td>
<td>0.30</td>
<td>0.06</td>
<td>5.27</td>
<td>—</td>
<td>−35.954</td>
</tr>
<tr>
<td>PM</td>
<td>0.42</td>
<td>0.12</td>
<td>3.56</td>
<td>83.88</td>
<td>83.438</td>
</tr>
<tr>
<td>LTS†</td>
<td>0.28</td>
<td>0.03</td>
<td>9.13</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean ± SD: 0.30 ± 0.09  0.12 ± 0.06  4.51 ± 5.57

These measurements were obtained with the second setup (see the Methods section).
* M-cone contrast: 23.4%; L-cone contrast: 24.7%.
† For these subjects, the responses were measured at only four conditions, with L- to M-cone contrast ratios: 0:1, 1:0, 1:1, and −1:1.

**Figure 4.** Response phase as a function of cone contrast in trichromats for the cone-isolating stimuli. A phase increase indicates a decrease of response lag. Clearly, the phase lags decrease with increasing cone contrast. A similar decrease in phase lag as a function of contrast is observed when more than one cone type is involved (not shown). There is a considerable interindividual variability in the phase data. But for the majority of subjects, the M-cone response leads the L-cone response.

**Figure 5.** Phase differences between responses originating in the L and the M cones in trichromats obtained from the model fits to threshold data plotted against the directly measured phase differences. There is a clearly positive correlation between the two values. The linear regression through the data (drawn line) lies close to the expected diagonal.
because the mean quantal catches in the L cones (4.42 log quanta $\cdot s^{-1} \cdot$ cone$^{-1}$; see the Methods section) are larger than in the M cones (4.31 log quanta $\cdot s^{-1} \cdot$ cone$^{-1}$). But, a similar difference was also found with other methods. Based on color-matching data on normal subjects, the ratio of peak optical density of the L-cone to M-cone photopigments has been estimated to be approximately 1.33.22-24 Fundus reflectometric measurement has revealed that the double density of L-cone photopigments in deuteranopes is approximately 1.13 times larger than the M-cone photopigment double density in protanopes.12 Thus, the difference between the L- and M-cone contrast gains might at least partially be caused by differences between the optical densities of the L- and M-cone photopigments. Assuming that deuteranopes and protanopes have the same number of cones, there might be several possible causes for this difference. According to Berendschot et al.,12 absorption by photopigment products in the short wavelength range or more reflection of long wavelength light from deeper layers might increase the relative absorption in the L cones. Alternatively, the L cones might possess more photopigment than the M cones. This would result in a stronger cone signal at the same cone contrast. Possibly, the gain of the L-cone signal transmission in the pathway leading to an ERG response is larger than that of the M-cone signal. However, this would not explain the ratios found with fundus reflectometry.

**Variability of L-/M-Cone Contrast Gains in Trichromats**

The ratios of L- and M-cone contrast gains obtained from the fits with the vector addition model differ substantially between different color normal subjects. One possible explanation might be that the relative amount of L and M cones differs between subjects. Alternatively, there might be gain differences in the postreceptoral pathways leading to the ERG response. Individual differences in cone weightings have been inferred from previous studies, in which different techniques have been used.10,11,25-31 Recently, we found that the cone weightings in the ERGs can be correlated with cone weightings obtained psychophysically, provided that the luminance channel (and not the chromatic channel) is isolated in the psychophysical experiments.32 This suggests that the individual differences originate in parts of the receptor and postreceptoral pathways that are shared by the ERG signal and the signal leading to a visual percept in the luminance channel.

Most trichromatic subjects display a larger L-cone weighting than M-cone weighting. The average weighting was approximately 4:1, measured with the two setups. However, cone weighting ratios exceeding a value of approximately 10:1 cannot be resolved by the fitting procedure so that excessively large values occasionally might occur (e.g., subjects RK and FH in Table 3). This might lead to a small overestimation of the mean values.

In one female subject (JB, Table 2) with normal color vision as established by the Nagel anomaloscope, we found that the L-cone to M-cone weighting ratio was substantially smaller than unity, implying that her retina has more M than L cones or larger M-cone than L-cone signal gains. She is possibly a carrier of protanopia, because her grandfather on her mother’s side was a protanope. Similar cone weightings have been found psychophysically in other female carriers of protanopia.33 It therefore seems that a defective gene for a cone photopigment on the X-chromosome in a female carrier may lead to a change in the cone signal weighting. The simplest mechanism to explain such a change would be an alteration in the relative numbers of L and M cones, rather than a change in the gains in the postreceptoral signal pathways.

**L- and M-Cone Phase Differences**

The results presented here confirm previous results4,20 that in normal subjects the response phase of the ERG increases with increasing cone contrast. But Wu et al.20 found that the relationship may differ at other temporal frequencies.

The data further confirm our previous observation that ERG responses to pure M-cone modulation are slightly phase advanced relative to the response phase to pure L-cone modulation (Fig. 4). In the previous paper,4 we noted that the phase differences between the responses to M- and L-cone-isolating stimuli vary between subjects but were relatively stable within subjects when repeatedly measured. Here, in a larger population of color normal subjects, we indeed find that the phase differences can vary between approximately $-30^\circ$ and $80^\circ$. Independently from the direct measurements, we obtained absolute values of phase differences from the model fits to the threshold data. The strong correlation between both values favors the conclusion that there is a consistent interindividual variability that has a physiological basis.

The smaller phase lags of the responses to M-cone-selective stimuli in the majority of subjects suggests that the M-cone signals are most often faster than the L-cone signals. Similar conclusions have been drawn in other studies in which several different techniques were used, including ERG recordings,34 psychophysics,35 and extracellular recording from retinal ganglion cells.36 Recordings of currents in single cones using the suction electrode method also indicate a tendency for M cones to be slightly faster than L cones, although this difference is not significant.37 Our data also show that there is a substantial interindividual variability in the phase of the cone ERGs that originates in response dynamics differences of the two cone types or their postreceptoral pathways.

**Cone Substitution in Dichromats**

Another aspect that can be addressed with our results is the question of whether missing cones are replaced in dichromats. A comparison between the ERGs of trichromats and dichromats is only valid when the postreceptoral processing in the ERG signals is identical in trichromats and protanopes (for signals originating in the M cones) and in trichromats and deuteranopes (for signals originating in the L cones), so that the postreceptoral processes have identical influence on the ERG signals in dichromats and trichromats. The fact that the mean M-cone contrast gain of protanopes is larger than that of trichromats, and that the mean L-cone contrast gain in deuteranopes is larger than in trichromats (cf. Tables 1 and 2), strongly suggests at least a partial replacement of the photopigment in dichromats.

The mean contrast gains for the dichromats and the trichromats are displayed in Figure 6. The drawn line connects the mean dichromatic mean data (2.66 and 2.01; open symbols) when only the contrast gains of the remaining cone types are considered. The connecting line can be described as follows: $A^*_A = 2.66 - (2.66/2.01) \times A^*_M$, in which $A^*_A$ and $A^*_M$ are the mean L- and M-cone contrast gains, respectively. Despite the large standard deviations in the trichromats, caused by the
the remaining cone type in dichromats. This interpretation is confirmed by the equal response amplitudes to the in-phase modulation of the L and M cones with equal cone contrasts (Fig. 1, right). A complete replacement of the missing cones might also explain why dichromats and trichromats in psychophysical experiments are about equally sensitive to luminance temporal modulation. The responses in the dichromats are driven solely by the L cones (in deuteranopes) or by the M cones (in protanopes). These responses are very similar to the responses found in the trichromats, which are driven by both the L and M cones. In contrast, the data of Meigen et al. suggest a much larger difference between the trichromatic data and the connecting line, probably caused by a large variability in the responses of dichromats. However, we found only a limited variation in the dichromatic contrast gains.

From fundus reflectometric measurements, Berendschot et al. concluded that there is a replacement of cones in dichromats but that the pigment density is lower in dichromats than in trichromats. This is only partially in agreement with our data, unless there are compensatory mechanisms in the ERG pathways of dichromats.

Acknowledgments

The authors thank Sabine Meierkord and Eva Burkhardt for technical assistance, Jeremy Nathans for providing the genotypes, and Eberhart Zrenner for general support.

References


Figure 6. Mean L- and M-cone contrast gains obtained in dichromats and trichromats. The data of the dichromats are displayed in two different ways. The open symbols, connected by the continuous line, represent the contrast gains when only the L cone (deuteranopes; open triangles) or the M cones (protanopes, open circles) are considered. The contrast gain values of the trichromats (open square) lie close to this line, indicating a complete photopigment replacement in the dichromats. The closed symbols connected by the dashed line represent the contrast gains of the dichromats when the responses to the stimuli that isolate the nonpresent cone types are also considered. Error bars, SD.

The authors thank Sabine Meierkord and Eva Burkhardt for technical assistance, Jeremy Nathans for providing the genotypes, and Eberhart Zrenner for general support.


26. de Vries HL. The heredity of the relative numbers of red and green receptors in the human eye. Genetica. 1948;24:199–212.


