

Cone selective adaptation influences L- and M-cone driven signals in electroretinography and psychophysics

Jan Kremers

Department of Experimental Ophthalmology,
University of Tübingen Eye Hospital, Tübingen, Germany



Maciej W. Stepień

Department of Experimental Ophthalmology,
University of Tübingen Eye Hospital, Tübingen, Germany



Hendrik P. N. Scholl

Department of Experimental Ophthalmology,
University of Tübingen Eye Hospital, Tübingen, Germany



Cézar Saito

Departamento de Fisiologia, Universidade Federal do
Pará, Centro de Ciências Biológicas, Belém, Brazil



To assess the influence of selective adaptation of long (L) and middle (M) wavelength sensitive cones with electroretinography (ERG) and psychophysics, a novel adaptation procedure was developed, which comprises a selective and quantifiable change in the state of adaptation in the different cone types. One adaptation condition was used as a reference. In four additional conditions, the M-cones or the L-cones were selectively adapted, so that they absorbed either more or less photons. At each of these five states of adaptation, the ERG response amplitudes to 30Hz L- and to M-cone selective stimuli were measured. Furthermore, the psychophysical sensitivities to L- and M-cone selective stimuli were measured at different temporal frequencies. In subjects with normal color vision, adaptation can have a strong influence on the L- and M-cone driven response amplitudes in the ERG and on both the L- and the M-cone sensitivities in the psychophysical luminance channel. As a result, the L- to M-cone ERG and psychophysical ratios can change dramatically at the different states of adaptation. The cone sensitivity thresholds and the L- to M-cone sensitivity ratio in the psychophysical chromatic channel are about unity at all states of adaptation, suggesting the presence of a compensatory mechanism. In dichromats, the responses and sensitivities to stimulation of the absent cone type were generally small at all states of adaptation. But, with reddish backgrounds residual ERG responses and residual psychophysical sensitivities were observed, indicating the presence of either a robust rod driven signal or an additional adaptation mechanisms that are not cone driven and that have not been described before.

Keywords: cone specific ERG, selective adaptation, luminance channel, chromatic channel

Introduction

The signals in the various postreceptoral pathways of the visual system originate in the photoreceptors. The gains and distributions of the photoreceptor driven signals depend on the postreceptoral pathway the signals enter (Brainard, Calderone, Nugent, & Jacobs, 1999; Kremers, Usui, Scholl, & Sharpe, 1999; Yeh, Lee, & Kremers, 1995). Furthermore, there is strong evidence that the L- and M-cone driven signals in the electroretinogram (ERG) and their ratio can vary considerably among individual trichromatic subjects (Brainard et al. 1999; Carroll, McMahon, Neitz, & Neitz, 2000; Kremers et al. 1999). The L- to M-cone weighting ratio in the ERG is correlated with the sensitivity ratio to L- and M-cone isolating stimuli in the psychophysical luminance channel. These ratios are probably related to the number of L- and M-cones that are stimulated (Brainard et al., 2000; Kremers et al., 2000). However,

when the psychophysical sensitivities are mediated by the chromatic channel, the L-/M-cone sensitivity ratio is around unity for all subjects with normal color vision (Hood et al., 2002; Krauskopf, 2000; Kremers et al., 2000).

The close correspondence between the ERG and the luminance channel is reflected in the resemblance between the spectral sensitivities of the ERG and the psychophysical spectral luminosity function, V_λ (Jacobs, & Deegan, 1997; Jacobs, Deegan, & Moran, 1996; Jacobs, Neitz, & Krogh, 1996). But the human V_λ and other luminance related spectral sensitivities can be changed by the state of adaptation (Eisner & Macleod, 1981; Swanson, 1993). Further, the spectral sensitivity of the ERG can be altered in a very similar manner by using different chromatic adaptations (Padmos & van Norren, 1971). But, it is not known how well the changes in spectral sensitivities of ERG and of the psychophysical luminance channel correlate with each other. A change in

spectral sensitivity implies that the contributing cone signals and therefore the L- to M-cone weighting ratio change. Until now, no quantitative description of the changes in L-/M-cone ratio by adaptation is available. Furthermore, it is not known how the adaptation in one cone type affects the responses of the same cone type and of cone types that are not adapted.

We found that the L- to M-cone weighting ratio in the chromatic channel is about unity for all trichromatic observers irrespective of the ratios in the ERGs or the luminance channel, indicating the presence of a compensatory mechanism (Kremers et al., 2000).

However, it is not clear how the cone driven sensitivities in the chromatic channel are influenced by adaptation.

It is the purpose of the present paper to address the above mentioned questions by studying the influence of selective adaptation in the L- or the M-cone on the L- and the M-cone driven signals in the ERG and the psychophysical luminance and chromatic channels of color normals. This paradigm enables us not only to study the influence of adaptation on the L- to M-cone weighting ratios but also the effect of cone selective adaptation on the responses driven by the adapted and the non-adapted cones. Recently, it was found that adaptation in the L- or the M-cones influences only the responses of horizontal cells to modulation in the adapted cone, but not in the non-adapted cone (Lee, Dacey, Smith & Pokorny, 1999). But, this has not been studied in other postreceptoral mechanism with non-invasive methods.

Finally, we were interested in the influence of cone selective adaptation on the ERG responses and psychophysical sensitivities in color vision deficient subjects: protanopes, deuteranopes and S-cone monochromats. The smaller number of photoreceptor types in these observers allows the identification of some adaptation mechanisms, that may also be present in trichromats.

Methods

Subjects

This study was conducted in accordance with the tenets of the declaration of Helsinki and with the approval of the institutional ethical committee in human experimentation.

Eight trichromats (five males and three females), five male dichromats (three deuteranopes and two protanopes) and one S-cone monochromat participated in ERG recordings and psychophysical measurements. The classification of trichromacy or dichromacy of the subjects

was based upon Rayleigh matches in the Nagel type I anomaloscope. Blood samples of the dichromats and the S-cone monochromat were analyzed genetically (genetic data were kindly provided by Wolfgang Jagla and Christiane Wolf of the University of Tübingen Eye Hospital). All deuteranopes and one protanope had only one gene coding for a normal L- or M-cone respectively. The second protanope had multiple genes, that all coded for identical and normal M-cone photopigments. A genetic analysis was also performed on blood samples of three male trichromats and confirmed the presence of trichromacy. Informed consent was obtained from all subjects after explanation of the purpose of the study.

L- and M-Cone Selective Stimuli

The methods on the cone isolating stimuli, the ERG recording procedure and the psychophysical measurements are described in detail elsewhere (Kremers et al., 2000; Kremers et al. 1999; Usui, Kremers, Sharpe, & Zrenner, 1998a; Usui, Kremers, Sharpe, & Zrenner, 1998b). Briefly, the stimuli were presented on a computer-controlled color monitor (BARCO CCID 121) driven at 100 Hz by a VSG 2/3 graphics card (Cambridge Research System). The spectral characteristics of the monitor phosphors were measured with a spectroradiometer (CAS 140, Instrument Systems). The luminance output was calibrated using the internal luminance measuring device of the monitor. The VSG software automatically performed the gamma correction.

To stimulate and to adapt the photoreceptors, the outputs of the red, green and blue phosphors were changed. The appropriate calculations were based on the emission spectra of the monitor phosphors and the psychophysical cone fundamentals (Stockman, MacLeod & Johnson, 1993). The sensitivities of all cone types to all phosphors were calculated by multiplying the emission spectra with the cone fundamentals and by integrating over the wavelength. The sensitivities and mean luminances of each phosphor (Table 1) were used to calculate the modulation contrast in each phosphor, required to obtain the appropriate photoreceptor (rod or cone) contrast. Either the L- or the M-cones were selectively stimulated without a stimulation in the remaining two cone types. Thus, the short wavelength sensitive (S-) cones were not stimulated (S-cone contrast was 0%) in any of the measurements. By controlling the contrasts in each cone type it is not possible to additionally control the rod contrast. But the rod contrast could be calculated for each stimulus.

Table 1. Monitor Outputs and Receptor Excitations.

		Adaptation conditions				
		Baseline	Lmax	Lmin	Mmax	Mmin
Monitor phosphors luminance (Cd/m ²)	Red gun	20	28.1	11.9	13.6	26.4
	Green gun	40	36.1	43.9	49.1	30.9
	Blue gun	6	6	6	5.9	6.1
	Total	66	70.2	61.8	68.6	63.4
Total retinal illuminance (photopic td)	ERG	3318	3529	3106	3448	3187
	Psychophysics	467	496	437	485	448
CIE (1964) large field coordinates	X	0.32	0.35	0.29	0.30	0.35
	Y	0.31	0.305	0.31	0.325	0.29
Photoreceptor illuminance in ERG (cone or rod td)	L-cones	3648	4012	3283	3649	3647
	M-cones	2964	2964	2964	3261	2667
	S-cones	2197	2203	2190	2193	2201
	Rods	3246	3153	3340	3555	2937

The outputs of the red, green and blue phosphors of the monitor, the total output of the monitor, total retinal illuminance during electroretinography and the psychophysical experiments, the large field (10°) CIE coordinates and the excitations in the four photoreceptor types during the ERG measurements at all adaptation conditions. The total excitations in the photoreceptors during the psychophysical measurements were approximately 7 times smaller than those in the ERG recordings.

L- and M-Cone Selective Adaptation

ERG recordings and psychophysical measurements were performed at five different states of adaptation: one reference or baseline state of adaptation. In four additional states of adaptation, the mean excitation in only one cone type was altered by a fixed value relative to the baseline condition. The excitations in the two other cone types were not altered. The mean excitation in the rods could not be controlled. In Table 1 the luminances of the three phosphors, the total luminance of the monitor, the mean retinal illuminances during ERG and psychophysical measurements, the large field (10°) CIE coordinates and the mean photoreceptor excitations during the ERG measurements are given. In Figure 1, the mean excitations in the L- and the M-cones (expressed in cone td) during ERG recordings at each state of adaptation are displayed. Figure 1 clearly visualizes that, relative to the baseline condition, the excitation in either the L- or the M-cones were altered. Furthermore, the plot shows that in two conditions (Lmax and Mmax) the cone excitations were increased, whereas in the remaining two adaptation conditions (Lmin and Mmin) the cone excitations were decreased. The amount of increase and decrease of M-cone excitation in the Mmax and Mmin conditions respectively were identical. Similarly, the amount of increase and decrease of L-cone excitation were identical in the Lmax and Lmin conditions. The excitation changes in the L- and the M-cones were very similar.

ERG Measurements

The pupil of the observer was dilated with 0.5% tropicamide resulting in a pupil diameter of about 8 mm. On the basis of the differences in pupil size (and neglecting the Stiles-Crawford effect), the mean retinal illuminances and the mean excitations in the photoreceptors were a factor of approximately 7 smaller in the psychophysical experiments. The eyes were light adapted for at least 10 minutes prior to recording. Corneal ERG responses were measured with DTL fiber electrodes which were positioned on the conjunctiva directly beneath the cornea. Gold cup electrodes were attached to the ipsilateral temple and the forehead and served as reference and ground electrodes respectively. The monitor was positioned at 10 cm from the observer's eye resulting in a stimulus size of 124 by 108 degrees. The stimuli were 30 Hz square wave modulations of the red, green and blue phosphors with predefined Michelson contrast ($= \frac{I_{max} - I_{min}}{I_{max} + I_{min}} \times 100\%$ in which the I_{max} and I_{min} are the maximal and minimal luminant output in the phosphor) so that the appropriate excitation modulation of the cone or rod photopigments (expressed in cone or rod contrasts) was obtained. The excitations of the phosphors were modulated by the graphics card on the basis of a temporal envelope, which was independent of the refresh rate. In this manner a 30 Hz stimulus is possible even if the refresh rate of the monitor is not an integer multiple of the stimulus frequency.

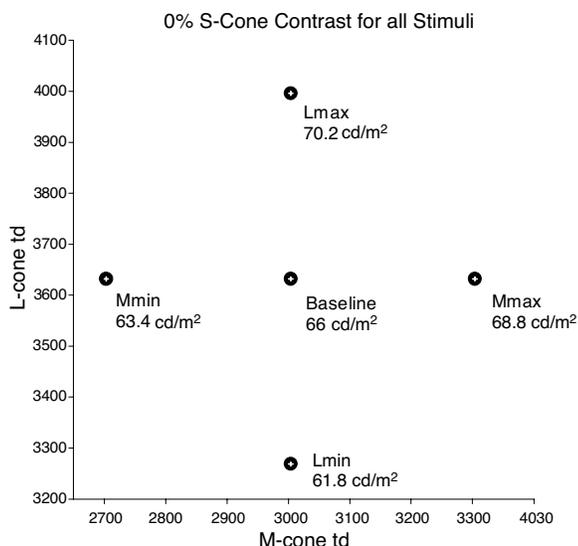


Figure 1. Schematic representation of the adaptation conditions during ERG recordings. The used states of cone adaptation, expressed as time averaged L- and M-cone trolands, given for the five states of adaptation. During the psychophysical measurements, the mean excitations were a factor of approximately 7 smaller. Observe that, in comparison with the baseline condition, only one cone type is adapted. All adaptations are approximately equidistant to the baseline conditions, indicating that the change in adaptation is about the same for all conditions. The state of S-cone adaptation was constant at all conditions (about 2200 S-cone td during ERG recordings). The mean luminances are also given. At each state of adaptation, selective L- and M-cone modulation is presented. In the ERG measurements, L- and M-cone contrast was always 15%. In none of the stimuli the S-cones were modulated. Rods were modulated by the stimuli. The rod contrasts were larger in the selective M-cone stimuli.

The ERG responses were obtained from the averages of 48 sweeps each lasting one second. At the 30 Hz stimulus frequency, the first harmonic component out of the Fourier analysis on the ERG signals dominated the total response. Therefore, the ERG amplitudes and phases were defined as the amplitudes and phases of the

first harmonic components. Data acquisition started about 4 sec after a change in background. This adaptation time was considered enough for these relatively subtle changes in background. Furthermore, we did not notice a systematic change in the ERG response over time.

For the baseline adaptation condition, and at the 30 Hz stimulus frequency, we found a linear relationship between cone contrast and response amplitude (Kremers & Scholl, 2001; Kremers et al. 1999; Usui et al. 1998a). We assumed that the linear relationship was also present at the other states of adaptation. We checked this for one subject. Owing to this linear relationship we were able to describe the complete changes in ERG amplitudes by measuring the response amplitudes at one cone contrast: either 15% L-cone contrast and 0% M-cone contrast or *vice versa*. As was stated above, S-cone contrast was 0% at all conditions. The rod contrasts at each stimulus are given in Table 2. The L-cone selective stimuli stimulated the rods with 3.9-5.1 % contrast (dependent on the state of adaptation) in counter-phase with the L-cones (indicated by the negative sign). For the M-cone selective stimuli, the rods were modulated in phase with the M-cones with 14.0-14.8 % contrast (both M-cone and rod contrasts have the negative sign indicating a counter-phase modulation with the modulation of the red phosphor. In previous measurements, we found that rod responses did not influence the ERGs at the baseline condition (Kremers & Scholl, 2001). The issue of possible intrusion of rod responses will be discussed in a later section.

Psychophysical Measurements

For the psychophysical measurements, the monitor was positioned at 114 cm from the observer’s eye. The stimuli were circular and had a diameter of 4 deg. The stimuli were viewed through a 3 mm artificial pupil positioned close to the subject’s eye. Owing to the artificial pupil the mean retinal illuminance and the mean photoreceptor excitations were about a factor of 7 lower than in the ERG measurements. The subjects were asked to fixate the outer edge of the circular stimulus. Thus the center of the stimulus was located at 2 deg.

Table 2. Rod Contrasts During ERG Recordings.

		Adaptation conditions				
		Baseline	Lmax	Lmin	Mmax	Mmin
Rod contrast in ERG (%)	Selective L-cone modulation (15% L-cone, 0% M-cone, 0% S-cone contrast)	-4.5	-5.1	-3.9	-4.1	-4.9
	Selective M-cone modulation (0% L-cone, -15% M-cone, 0% S-cone contrast)	-14.4	-14.8	-14.0	-14.4	-14.3

The rod contrasts during ERG recordings at L- and M-cone selective stimulation and at different states of adaptation. The rod contrast is substantially larger at M-cone selective stimulation than at L-cone selective stimulation. L-cones were modulated in phase and M-cones in counter phase (indicated by a negative sign of the M-cone contrast) relative to the modulation of the red phosphor. The rods were modulated in counter phase with L-cones and in phase with M-cones.

eccentricity. To avoid Troxler's fading, they were encouraged to make eye movements along the outer edge of the stimulus. The stimuli were sinusoidal modulations at 11 different temporal frequencies varying between 1 and 50 Hz (1, 2, 4, 5, 8, 10, 15, 20, 30, 40 and 50 Hz). Owing to the temporal characteristics of the phosphors' excitation and decay at the 100 Hz refresh rate, the stimuli are substantially distorted at temporal frequencies above about 20 Hz. This had only a minor influence on the results.

At each state of adaptation, flicker detection thresholds were determined using a PEST procedure (Taylor & Creelman, 1967). Briefly, a stimulus was presented until the subject indicated, by pressing a button, whether or not he/she perceived the presence of a temporal modulation. After the subject had pressed a button, the next stimulus was immediately presented. If the subject perceived flicker at a certain stimulus strength, the contrast in the next presentation was decreased. Conversely the contrast was increased when the subject did not perceive flicker. After a direction reversal, the changes in contrasts were halved. Thresholds were assumed to be reached when the changes in contrast were less than 0.14 times the actual contrast. Two randomly interleaved staircases were used: one starting at zero modulation, the other starting at maximal modulation.

Results

Cone Specific Adaptation in Trichromats

We measured ERG responses in normal trichromats to 15% L-cone and 15% M-cone modulation at the five different states of adaptation (Lmax and Lmin in which the L-cones are more and less adapted relative to the baseline condition respectively; and the Mmax and the Mmin conditions in which the M-cones are more and less adapted relative to the baseline condition respectively; see methods). The response amplitudes of six subjects (MS, CS, JM, US, VK, BK) are shown in Figure 2(a) and Figure 3(a). The response amplitudes are similar in the baseline, Lmin and the Mmax conditions. However, the responses in the Lmax and the Mmin conditions differed from the others: the L-cone driven responses were much smaller than in the other conditions whereas the M-cone driven responses were larger. The data show that selective adaptation in one cone type can affect the ERG responses driven by the non-adapted cone. The differences in state of adaptation in the different cones are relatively small. Nevertheless, the changes in the responses can be dramatic, indicating that adaptation can have a very large influence on the cone driven ERG responses.

In the Lmax and Mmin conditions, the output of the red phosphor was about 40 cd/m² during a short phase of the modulation, approaching the maximal possible output of this phosphor. To exclude the possibility that the described results were caused by stimulus artifacts owing to distortions at high outputs of the red phosphor, which were not captured by the calibration of the monitor, we repeated the measurements in one subject with 10% L- and 10% M-cone modulation. Apart from an overall decrease in response amplitude, the results were identical, indicating that stimulus distortion is not the cause of the described effects.

Similar to previous experiments (Kremers et al., 2000), we measured the flicker detection thresholds to L- and M-cone isolating stimuli at different temporal frequencies. As an extension, we did not measure the detection thresholds only at the baseline condition but also at the other adaptation conditions. In Figure 2(b)-2(e) and Figure 3(b)-3(e), the psychophysical L- and M-cone sensitivities (expressed as the inverse of the L- or M-cone contrasts at threshold) of the same subjects, whose ERG data are shown in Figure 2(a) and Figure 3(a) are displayed for the different states of adaptation. Clearly, for all adaptation conditions, the subjects had similar L- and M-cone sensitivities at low temporal frequencies (below 5 Hz). But at high temporal frequencies (8 Hz and higher), the L- and M-cone sensitivities were quite different. In the baseline, Lmin and Mmax conditions, most subjects were more sensitive to L-cone than to M-cone modulation, whereas in the Lmax and Mmin conditions all subjects were more sensitive to M-cone than to L-cone modulation. We calculated the L-/M-cone sensitivity ratios from the psychophysical data for each state of adaptation and plotted these as a function of temporal frequency. These plots are shown in Figure 2(f) and Figure 3(f). At the right side of the plots, the L-/M-cone amplitude ratios derived from the ERG data are displayed. The psychophysically measured L-/M-cone sensitivity ratios at high but not at low temporal frequencies are similar to the L-/M-cone ratios estimated from the ERG data. Cone selective stimuli, as used in the present study, contain both luminance and chromatic modulation. For such combined luminance and chromatic stimuli it has been proposed that the chromatic channel mediates detection at low temporal frequencies, whereas the luminance channel is responsible for detection at high temporal frequencies (Kremers, Lee, & Kaiser, 1992; Kelly & Norren, 1977). Thus, the data indicate that when the luminance channel mediates detection (high temporal frequencies), the ratio of L- to M-cone sensitivities depends on the state of adaptation. When the detection is caused by the chromatic contents in the stimuli (low temporal frequencies), the sensitivity ratios are around unity for all adaptation conditions.

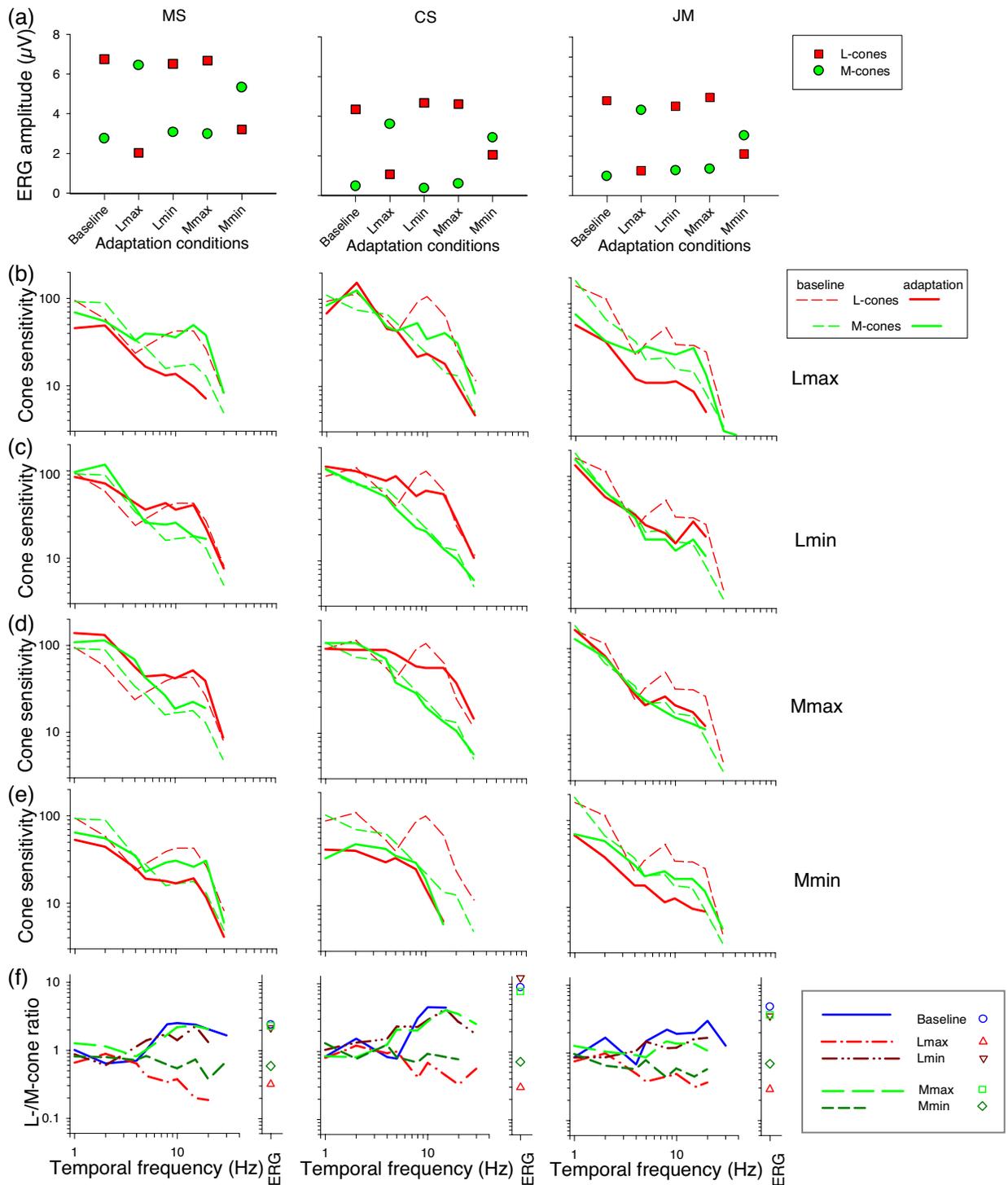


Figure 2. ERG response amplitudes and psychophysical sensitivities in three trichromatic subject. **(a)** The ERG response amplitudes to L-cone (red squares) and to M-cone (green circles) selective stimuli at different states of adaptation. In comparison with the baseline condition the ERG amplitudes are quite different when adapted to a reddish background (Lmax and Mmin). An adaptation in one cone also affects the responses in the non-adapted cone. **(b- e)** Psychophysical L- and M-cone sensitivities [defined as 100/(L- or M-cone contrast at detection threshold)] as a function of temporal frequency, plotted separately for the different states of adaptation. For comparison the sensitivities at baseline condition are shown in each plot. At all adaptations, the sensitivities to L- and M-cone selective stimuli are very similar at low temporal frequencies. At high temporal frequencies, the sensitivities to L- and M-cone selective stimuli differ. **(f)** The psychophysical L-/M-cone ratio as a function of temporal frequency. Clearly the ratio is about unity at low temporal frequencies. At high temporal frequencies the ratios are either larger (baseline, Lmin, Mmax) or smaller (Lmax, Mmin) than one and correspond to the L-/M-cone ratios derived from the ERGs (given on the right side of the plot).

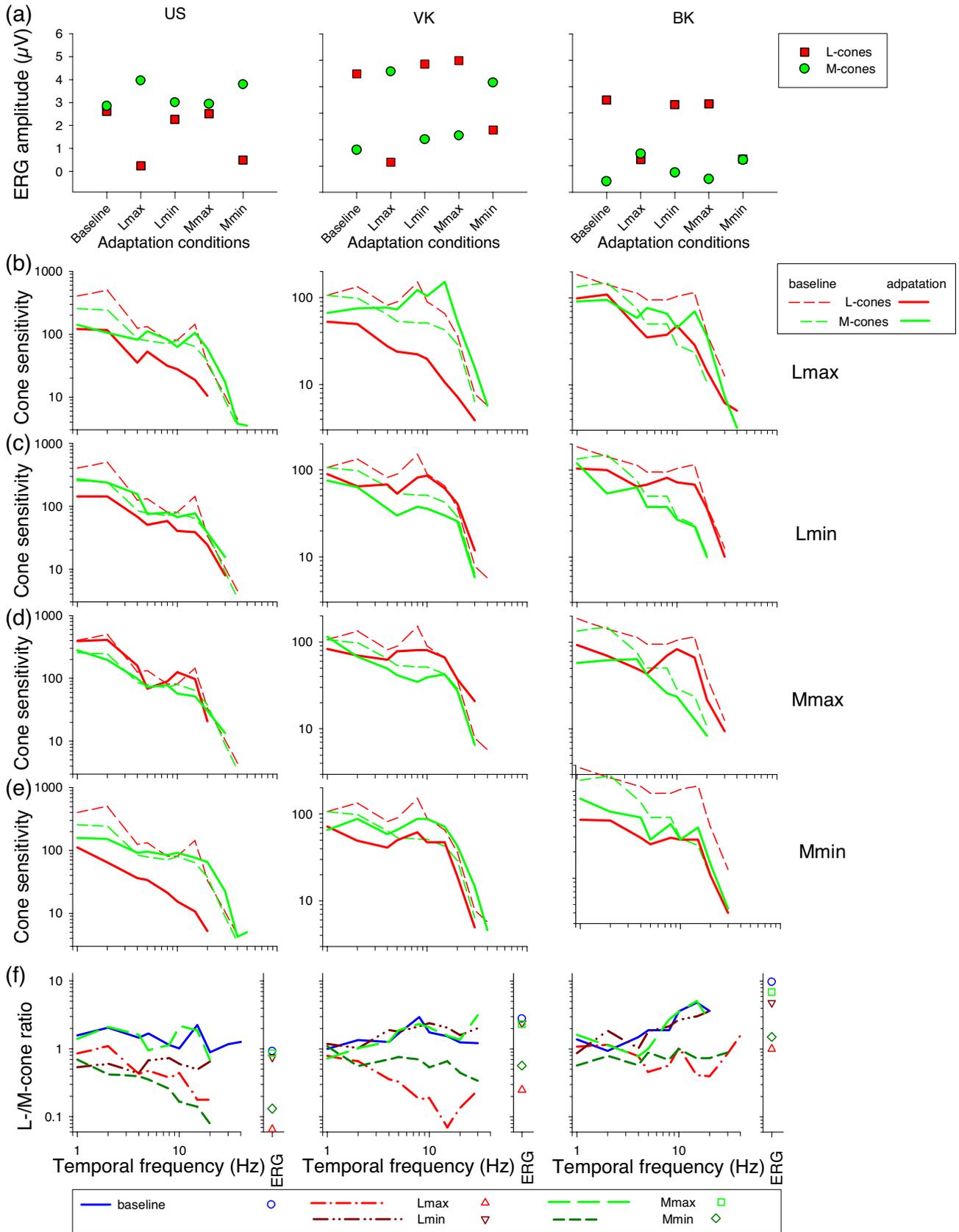


Figure 3. Data of three additional subjects (US, VK, BK) presented in the same format as Figure 2.

To pursue this issue, we calculated the ratio of L- to M-cone sensitivity estimated from the psychophysical measurements for eight trichromatic subjects at each of the five adaptation conditions. At each temporal frequency and each adaptation condition, we calculated the mean ratio by first converting the individual ratios into their logarithm (to give the data a normal distribution) averaging them and converting them back into the linear range. In Figure 4(a), the mean ratios are plotted as function of temporal frequency separately for the five different adaptation conditions. It can be seen that the L-/M-cone sensitivity ratio for detection mediated by the chromatic channel (low temporal frequencies) is

about unity for all adaptation conditions, whereas the ratio for detection mediated by the luminance channel (high temporal frequencies) depends on the state of adaptation. The mean L-/M-cone ratios estimated from the ERG recordings on the same subjects are given on the right side of the plot. Again, there is a correspondence between the L-/M-ratios in the ERGs with those derived from the psychophysical data in which detection is mediated by the luminance channel (high temporal frequencies) but not with the sensitivity ratios when the chromatic channel mediates detection (low temporal frequencies).

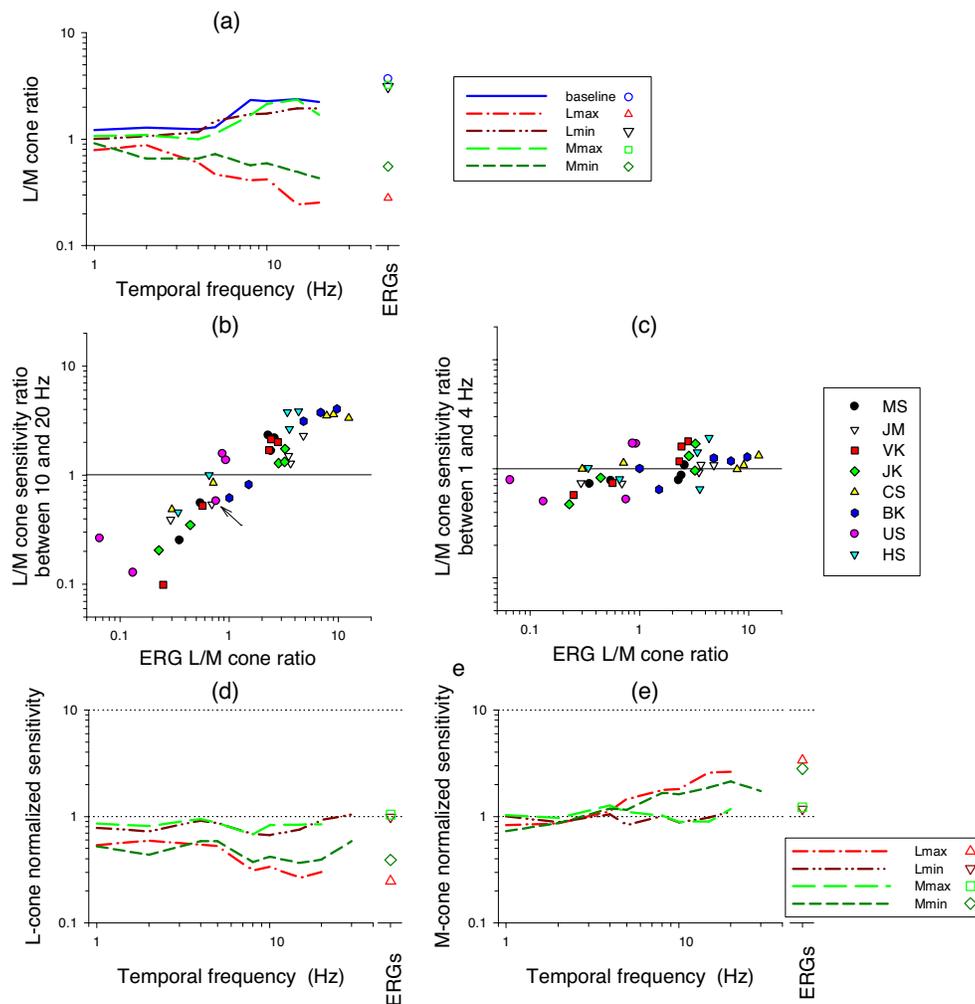


Figure 4. Summary of the data obtained in trichromats. (a) Averaged psychophysical L-/M-cone ratio plotted versus temporal frequency. The mean ratio is about unity at low temporal frequency. At high temporal frequencies, the mean ratio is larger than one in the baseline, Lmin and Mmax conditions and smaller than one in the Lmax and Mmin conditions. The ratios at high temporal frequencies correspond to the mean L-/M-cone ratios estimated from the ERG data. (b) Psychophysical L-/M-cone ratios, estimated from high temporal frequency data as a function of the ERG derived L-/M-ratios for each individual subject and each state of adaptation. There is a close correlation between the two. (c) Psychophysical L-/M-ratios, estimated from low temporal frequency data plotted versus ERG derived L-/M-cone ratio. The psychophysical ratios are all around unity, and are therefore not strongly correlated with the ERG data. Mean L-cone (d) and M-cone (e) sensitivities for each observer normalized to the sensitivities at the baseline condition as a function of temporal frequencies. At the right side normalized ERG response amplitudes are displayed. The normalization eliminates individual differences in the data and isolates effects of adaptation. The normalized ERG responses correspond with the normalized psychophysical data at high temporal frequencies.

To extract a more reliable estimate of the L-/M-cone sensitivity ratio in the luminance channel, we averaged for each individual and each state of adaptation the L-/M-cone sensitivity ratios obtained at 10, 15 and 20 Hz. Similarly, the individual ratios in the chromatic channel were calculated by averaging the ratios estimated from the 1, 2 and 4 Hz data. [Figures 4\(b\) and 4\(c\)](#) show the psychophysical ratios mediated by the luminance and the chromatic channels respectively as a function of the ERG derived L-/M-cone ratios for each individual and each adaptation condition. Each symbol displays the data of one subject. It can be seen that the L-/M-cone ratios in the ERG signals correlate closely with the sensitivity ratios in the luminance channel ($r^2 = 0.85$), but much less with the ratios in the chromatic channel ($r^2 = 0.32$), which all cluster around unity.

The psychophysical ratios based upon action in the luminance channel obtained in the baseline, Lmin and Mmax conditions were all larger than unity (with the exception of data point of subject US marked with an arrow), whereas those measured in the Lmax and Mmin conditions were all below unity. Furthermore, the L-/M-cone ratios in the ERGs and in the psychophysical luminance channel obtained in the baseline, Lmin and Mmax conditions varied between different individual observers, confirming previous results ([Kremers et al., 2000](#)).

To study the effects of adaptation on the signals driven by the individual cones without confounding the data with the above mentioned individual differences, we normalized the L- and M-cone mediated sensitivities (for the psychophysical data) and response amplitudes (for the ERG data) at the different adaptation conditions to those obtained in the baseline conditions. The mean normalized L- and M-cone sensitivities at the different states of adaptation are shown as a function of temporal frequency in [Figures 4\(d\) and 4\(e\)](#) respectively. The data indicate, that the effects of adaptation in the luminance channel correlate closely with the changes in the ERG responses and that they are larger than the changes in the chromatic channel.

Cone Specific Adaptation in Dichromats

To check that the adaptation conditions and stimuli indeed selectively adapted and stimulated only the L- or the M-cones, we repeated the measurements in two male protanopes and three male deuteranopes. Because all deuteranopes and one protanope had only a single gene on their X-chromosomes, coding for a normal L- or M-cone pigment respectively and because the remaining dichromat was a multigene protanope with M-cone genes, coding for identical and normal M-cone photopigments, we were confident that these subjects were true dichromats. The mean ERG amplitudes and the mean psychophysical L- and M-cone sensitivities of the

protanopes are shown in [Figures 5\(a\)-5\(c\)](#) respectively. The mean data of the deuteranopes are displayed in [Figures 5\(d\)-5\(f\)](#). The data are quite different in comparison with those obtained in the trichromats. Generally, the protanopes' L-cone driven and the deuteranopes' M-cone driven ERG responses and psychophysical sensitivities are small at all adaptation conditions, indicating that the response amplitude changes in the trichromats are not caused by stimulus artifacts. Furthermore, in contrast with the trichromatic data (*cf.* [Figure 2](#)), the psychophysical sensitivity curves all have similar shapes, indicating that the thresholds are mediated by one postreceptoral pathway: the luminance channel. A chromatic channel is absent.

However, the dichromats displayed residual but significant responses and sensitivities to stimulation of the non-present cone type when adapted to the reddish backgrounds (Lmax and Mmin). Furthermore, Lmax and Mmin adaptation, seemed to have some influence on the response amplitudes of the present cone type in protanopes and deuteranopes respectively. These effects cannot be explained on the basis of simple mechanisms located in the L- and M-cone driven pathways. The possible causes will be discussed in a later section. But these effects are generally smaller than those found in trichromats, indicating that they can only partially explain the trichromatic data so that the conclusions about cone adaptation in the trichromats are still valid.

Measurements in the S-Cone Monochromat

We also performed the same ERG and psychophysical measurements with an S-cone monochromat, lacking both L- and M-cones. The data are displayed in [Figures 5\(g\)-5\(i\)](#). At all adaptation conditions and for both L- and M-cone selective stimuli, the ERG amplitudes and the psychophysical sensitivities were extremely small or not measurable.

The Role of Rod Driven Signals

In the measurements described above, rods were adapted and also stimulated. Previously, we found that the rod driven ERG signals in the baseline adaptation condition is very small when the stimulus frequency is 30 Hz ([Kremers & Scholl, 2001](#)). However, we do not know what role rod signals can play at the other states of adaptation. To address this issue, additional ERG measurements on four trichromats, the single gene protanope and one single gene deuteranope were conducted at the same adaptation conditions for the L- and the M-cones as before. Furthermore, the L- and M-cone contrasts at the different adaptation conditions were identical with those used before. In contrast with the previous conditions the state of adaptation of rods was

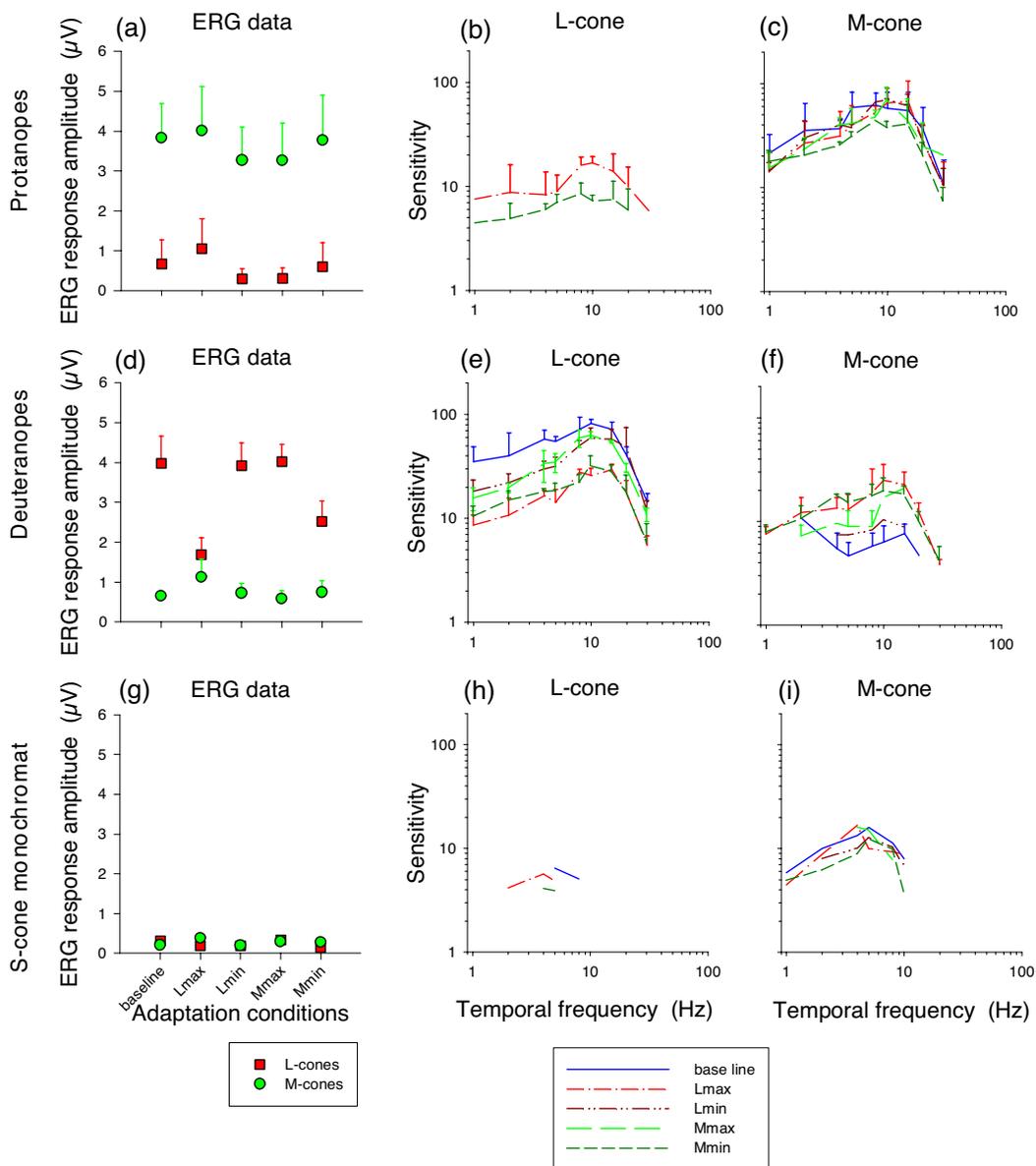


Figure 5: Summary of the data obtained in the dichromats and in an S-cone monochromat. (a-c) Mean data obtained from measurements with the protanopes. (a) Mean ERG response amplitudes to L- (red squares) and M-cone (green circles) selective stimuli plotted versus adaptation. (b) Mean psychophysical sensitivities to L-cone selective modulation at the different adaptations as a function of temporal frequency. (c) Mean psychophysical sensitivities to M-cone selective modulation at the different adaptations as a function of temporal frequency. If the sensitivity curve is not shown, then no threshold could be obtained. (d-f) and (g-i) Similar data as in (a-c) for the deuteranopes and the S-cone monochromat respectively.

kept constant. In addition, the rods were not stimulated by any of the stimuli (i.e. rod contrast was 0%). But, the S-cones were adapted and stimulated in the different conditions. It is known from previous studies (Usui et al. 1998a) that the S-cone driven response amplitude in 30Hz flicker ERG is very small and can be neglected. However, the S-cone modulation would result in a strong signal in the psychophysical task so that psychophysical measurements are not useful under these conditions. We therefore only performed ERG measurements with these stimuli. The results are depicted in Figure 6 together with

the results of the previous measurements (0% S-cone contrast; no S-cone adaptation) in the same subjects. In Figure 6(a) the mean data of the four trichromats are displayed. The squares show the ERG amplitudes for L-cone selective stimuli. The circular symbols show the M-cone driven ERG amplitudes. The basic results are similar in the two series of measurements. This strongly suggests that the adaptation effects are mainly mediated in the cones and their postreceptoral pathways. But, the ERG amplitudes are smaller in the conditions in which the rods are not stimulated and adapted, and the amplitude

decrease is generally larger for M-cone selective stimuli. This can be expected when rod responses do influence the data because the rod contrasts were larger in the original measurements for selective M-cone stimuli (about 14% see Table 1) than for the selective L-cone stimuli (about 4% see Table 1). As a result, the L-/M-cone ERG amplitude ratios (Figure 6(b)) are increased in most conditions with silent rod stimuli.

Figure 6(c) shows the ERG amplitudes in the single gene protanope. Again the response amplitudes are smaller, especially for selective M-cone stimuli suggesting that rod responses might be involved in the data with silent S-cone stimuli. The data on the single gene deuteranope, Figure 6(d), are less conclusive but indicate that rods driven signals might influence the results.

Discussion

Adaptation in Trichromats

The results show that cone selective adaptation can strongly influence the cone driven signals in the luminance channel and in the pathway leading to an ERG response, after adaptation to reddish backgrounds

as is the case in the Lmax and Mmin conditions; Figures 2(a), 2(b), and 2(e), also Figures 3(a), 3(b), and 3(e). When adapted to greenish backgrounds, as in the Lmin and Mmax conditions, L- and M-cone driven responses in the ERGs were similar to those measured in the baseline condition (Figure 2(a) and Figure 3(a)). Furthermore, the psychophysical sensitivities to L- and M-cone selective stimuli are similar in the Lmin, Mmax and the baseline conditions (Figures 2(c) & 2(d) and Figures 3(c) & 3(d)). Padmos and van Norren (1971) already described the influences of adaptation on the spectral sensitivities measured with a 40 Hz flicker ERG and with the psychophysical heterochromatic flicker photometry paradigm. In agreement with our data, they found that adaptation influences the two spectral sensitivities in a similar manner. Moreover, they showed that blue and red adapting backgrounds influenced the spectral sensitivities, but that an effect of a green background was absent.

Our data strongly suggest that L- and M-cone driven signals in the luminance channel and the ERG pathways are extremely sensitive to changes in adaptation. There is evidence that the L-/M-cone ERG ratio and luminance based psychophysical L-/M-ratio in individuals are correlated with the ratio of L- to M-cone numbers in the retina (Brainard et al., 2000; Kremers et al., 2000). The

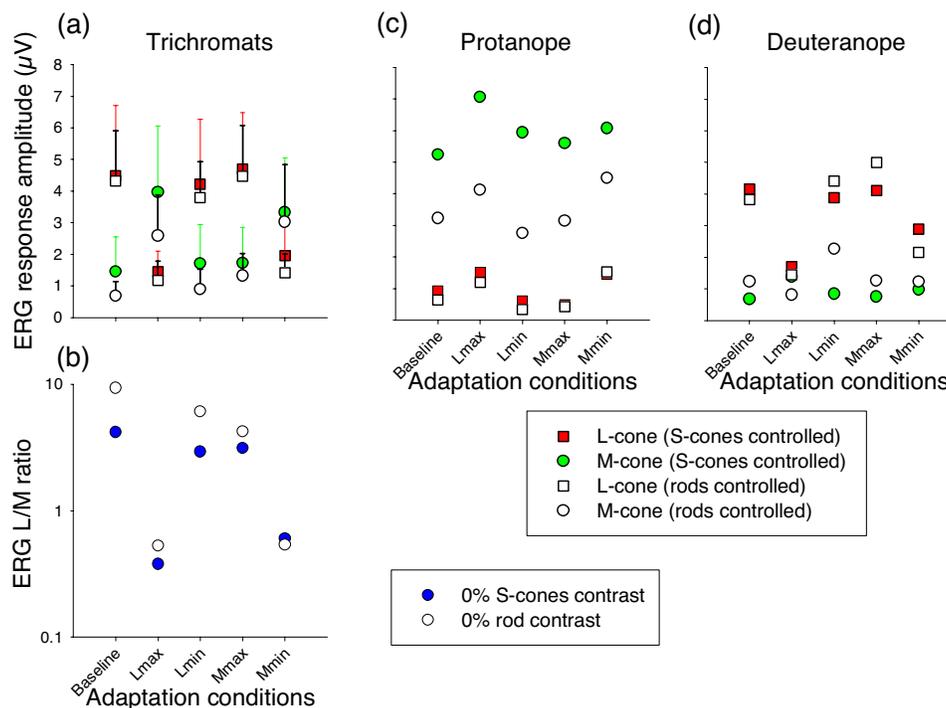


Figure 6. Results of additional ERG measurements (rods controlled; with 0% rod contrast and rod adaptation constant) on 4 trichromats, one protanope and one deuteranope presented together with those of the previously described measurements (S-cone controlled; with 0% S-cone contrast and contrast S-cone adaptation). The L- and M-cone response amplitudes of trichromats (a), the protanope (c), the deuteranope (d) and the trichromats' mean L-/M-cone ERG amplitude ratios (b) at different adaptation states are displayed. In trichromats silencing the rods instead of S-cones results in an amplitude decrease, which is generally larger for M-cone driven signals. As a result, the L-/M-cone ratio increases (except at the Mmin condition). The protanope M-cone driven amplitudes are also influenced; they are smaller at all conditions. The changes in the deuteranope are less conclusive.

results in the present study confirm that there are individual differences in L-/M-cone ERG and luminance based sensitivity ratios, but they also show that the ratios depend upon the state of adaptation and therefore the correlation with the number of cones can not be a one to one relationship.

The ERG is a complex signal that arises in the action of many cell types, including the bipolar cells (Bush & Sieving, 1996). It has been shown that the ERG is not only the effect of a vector addition between L- and M-cone driven signals (Kremers et al. 1999) but also between signals originating in the on- and off-bipolar cells (Kondo & Sieving, 2002). The effects of cone selective adaptation might therefore not necessarily be present in the cones themselves by might also be present in a change of balance in the on- and off-bipolar cell contributions to the ERGs.

The effects of adapting one cone type leads also to a change in the response driven by the non-adapted cone. Interestingly, the changes in the ratios can be very large although the changes in state of adaptation for the different cones are relatively subtle. Responses of horizontal cells to cone isolating stimuli were not affected by adaptation in the other cone (Dacey, Lee, Stafford, Smith & Pokorny, 1996). H1 horizontal cells receive additive inputs from L- and M-cones and have spectral sensitivities similar to the luminosity function (Dacey & Lee, 1999; Dacey, Lee, Stafford, Smith & Pokorny, 1996). These data would suggest that H1 horizontal cells might play a role in the psychophysical luminance channel. The different influence of adaptation on the cone signals in the luminance channel and in the responses of the horizontal cells, however, indicates that there is not necessarily a causal relationship between the two.

Our data show that, when the chromatic channel mediates flicker detection, the L-/M-cone sensitivity ratios are around unity for all trichromatic observers and for all adaptation conditions used in the present study. This confirms previous proposals (Hood et al., 2002; Kremers et al., 2000; Pokorny, Smith & Wesner, 1991) that a mechanism is present in the chromatic channel that compensates for individual differences in L- and M-cone densities. From the present data, we can extend the presence of the compensatory mechanism to different adaptation conditions. We cannot exclude the possibility that the compensatory mechanism fails when stronger adaptation backgrounds are used. We suggest that the compensatory mechanism in the chromatic channel has a retinal origin. Possibly, the ontogenetic development of the compensatory mechanism is experience based, resulting in a continuous tuning of the L- and M-cone driven signals, so that the output of PC-cells, which are probably the physiological basis of the chromatic channel (Kremers et al. 1992; Lee, Martin, & Valberg, 1989; Lee, Pokorny, Smith, Martin, & Valberg, 1990, is set to nearly zero (indicating optimal cone opponency) when luminance modulation is presented and MC-cells are

responding strongly. To do so, the system must be able to distinguish between the L- and M-cone driven signals. The compensatory mechanism is not present in the pathway leading to a 30 Hz ERG response, which includes the activity of bipolar cells (Bush & Sieving, 1996). The most probable site of the compensatory mechanism is therefore at the stage of the amacrin cells or retinal ganglion cells.

Adaptation in Dichromats and in the S-cone Monochromat

Generally, the ERG responses and the psychophysical sensitivities of the dichromats to the stimulation of the non-present cones were small, indicating that the adaptation processes described for the trichromats have a physiological origin.

However, we measured residual responses and sensitivities to stimulation of the non-present cone in the Lmax and Mmin conditions, especially in the deuteranopes. Furthermore, selective adaptation of the L- and M-cones had an effect on a measured responses in protanopes and deuteranopes respectively.

There are three possibilities that can explain the above described data in the dichromats: (1) stimulus artifacts and miscalculations in the derivation of the stimulus conditions, (2) spatial differences in photoreceptors absorption spectra as result from variability in the cones or in pre-receptor absorption (caused by the macular pigment or by chromatic aberration) at different retinal eccentricities and (3) the intrusion of rod driven signal.

Stimulus artifacts and miscalculations in the derivation of the stimulus conditions

The ERG responses and the psychophysical sensitivities in the S-cone monochromat are very small, indicating that stimulus artifacts, resulting in substantial S-cone stimulation, are not present. Thus, these data confirm that stimulus artifacts and miscalculations do not have a large influence. An additional indication that stimulus artifacts can be excluded comes from the trichromatic data: stimulus artifacts would have identical effects at all temporal frequencies. But, the psychophysical data in the trichromats show that the change in sensitivity is different for low and high temporal frequencies (see Figures 2, 3, and 4).

Spatial differences in pre-retinal absorption

One of the causes of the residual responses and sensitivities to stimulation of the non-present cone in the Lmax and Mmin conditions might be the variability in pre-retinal absorption at different retinal locations, so that a cone response is never completely silenced. To study a possible influence of the spatial variability on the data, we repeated the psychophysical measurements in a deuteranope with the center of the stimulus presented at 4 deg. eccentricity. Furthermore, we repeated the

measurements with a centrally fixated stimulus, which was also smaller than in the other measurements (1 deg. in diameter). Apart from an absolute change in sensitivity, we did not observe any major changes in the relative sensitivities to L- and M-cone isolating stimuli, indicating that the residual sensitivities in the Lmax and Mmin conditions were still present. These data show that the spatial variability in the pre-retinal absorption cannot be the cause for the residual sensitivity.

Rod intrusion

Intrusion of rod driven signals could be another explanation for the residual responses and sensitivities in the dichromats. Indeed, the residual responses and sensitivities to selective M-cone stimuli in the deuteranopes are larger than those to selective L-cone stimuli in the protanopes. The rod contrasts are larger in the selective M-cone stimuli than in the selective L-cone stimuli (see Table 2). Thus, a possible rod intrusion could be expected to be larger in selective M-cone stimuli. Furthermore, we found that the residual ERG responses and psychophysical sensitivities were only found with reddish backgrounds (Lmax and Mmin) at which the rod adaptation (in terms of rod td; see Table 1) was smaller and thus at which rods can be expected to be more sensitive. Finally, the results of the ERG measurements in the conditions at which the rods were neither stimulated nor adapted, show that rod signals might indeed influence the data.

However, there are also some arguments against rod intrusion as explanation of the residual responses and sensitivities. Purely rod driven signals are measured in the S-cone monochromat because this subject lacks M- and L-cones and S-cones were not stimulated. Repeating the psychophysical measurements on the S-cone monochromat with stimuli in which the rods and S-cones were not modulated, so that only L- and M-cones were stimulated, completely abolished the sensitivities, indicating that the very small sensitivities of the S-cone monochromat shown in Figure 5h and 5i, are rod driven. These sensitivities are much smaller than the residual responses and sensitivities in the dichromats. Furthermore, in comparison with the data on the dichromats, the rod driven psychophysical sensitivities have a different dependency on temporal frequency. It therefore seems that the rod driven signals in the S-cone monochromat are very small and cannot explain the residual responses in the dichromats. But, there is strong evidence that two rod pathways exist (Kolb & Nelson, 1983; Nelson, 1977; Stockman, Sharpe, R  ther, & Nordby, 1995), a slower pathway (more sensitive at very low retinal illuminances) using the rod bipolar cells and the AII amacrin cells and a faster pathway (more sensitive at high scotopic or mesopic luminance levels) that uses the gap junctions between the rods and the cones (Kolb, 1977). The S-cone monochromat has no or only limited access to this latter pathway. Thus, although a rod driven

signal *via* the rod bipolar cells can be excluded as explanation for the residual responses and sensitivities in dichromats, the data from the S-cone monochromat cannot exclude the possibility of rod intrusion *via* gap junctions with the L- and/or the M-cones.

The repetitions of the psychophysical measurements in the deuteranope with stimuli at larger eccentricity and with the small foveal stimulus is a further argument against rod intrusion because in the different measurements the number of stimulated rods were quite different. Nevertheless the residual responses and sensitivities were still present. Finally, we repeated the psychophysical measurements on a deuteranope with a dilated pupil and without the use of the artificial pupil thereby increasing the retinal illuminance by about a factor of 7 to approximately 3300 td. Again the residual responses in the Lmax and Mmin conditions were present. These results suggest that either the rod intrusion is quite robust or that additional mechanisms play a role. These mechanisms would induce changes in the cone driven signals under reddish illuminations both in protanopes and deuteranopes. A conventional adaptation mechanism would not be able to explain these effects because they can be expected to be absent in protanopes and deuteranopes.

Summary

The present work shows that, in trichromats, the effects of cone selective adaptation originates in the cone driven pathways themselves. Cone selective adaptation influences the L-/M-cone ratios in the ERG signals and psychophysical sensitivities tapping the luminance channel. These two L-/M-cone ratios correlate closely with each other. The L-/M-cone sensitivity ratio in the chromatic channel is not influenced by cone selective adaptation providing further evidence for the presence of a compensatory mechanism.

Acknowledgments

This research was supported by Fortune Grant707-0-1 T  bingen to JK and HPNS; by the European Commission under contract QLGA-1999-50423 to MWS, Fellow of the Marie Curie Training site; JK was supported by a Heisenberg Fellowship of the German Research Council (DFG KR 131715-2); JK and CS were supported by a grant of the German Academic Exchange Council (DAAD) and CAPES (Brazil). The authors thank Bill Swanson for comments on an earlier version of the manuscript.

Commercial relationships: none.

References

- Brainard, D. H., Calderone J. B., Nugent A. K., & Jacobs G. H. (1999). Flicker ERG responses to stimuli parametrically modulated in color space. *Investigative Ophthalmology & Visual Science*, *40*, 2840-2847. [PubMed]
- Brainard, D. H., Roorda A., Yamauchi Y., Calderone J. B., Metha A. B., Neitz M., Neitz J., Williams D. R., & Jacobs G. H. (2000). Functional consequences of the relative numbers of L and M cones. *Journal of the Optical Society of America A*, *17*, 607-614. [PubMed]
- Bush, R. A. & Sieving P. A. (1996). Inner retinal contributions to the primate photopic fast flicker electroretinogram. *Journal of the Optical Society of America A*, *13*, 557-565. [PubMed]
- Carroll, J., McMahon C., Neitz M., & Neitz J. (2000). Flicker-photometric electroretinogram estimates of L:M cone photoreceptor ratio in men with photopigment spectra derived from genetics. *Journal of the Optical Society of America A*, *17*, 499-509. [PubMed]
- Dacey, D. M. & Lee, B. B. (1999). Functional architecture of cone signal pathways in the primate retina. In Gegenfurtner, K. R. & Sharpe, L. T. (eds), *Color Vision; From genes to perception*. (pp. 181-202). Cambridge: Cambridge University Press. [PubMed]
- Dacey, D. M., Lee B. B., Stafford D. M., Smith V. C., & Pokorny J. (1996). Horizontal cells of the primate retina: cone specificity without cone opponency. *Science*, *271*, 656-658. [PubMed]
- Eisner, A. & Macleod D. I. (1981). Flicker photometric study of chromatic adaptation: selective suppression of cone inputs by colored backgrounds. *Journal of the Optical Society of America*, *71*, 705-717. [PubMed]
- Hood, D. C., Yu A. L., Zhang X., Albrecht J., Jägle H., & Sharpe L. T. (2002). The multifocal visual evoked potential and cone-isolating stimuli: Implications for L- to M-cone ratios and normalization. *Journal of Vision*, *2*, 178-189, <http://journalofvision.org/2/2/4/>, DOI 10.1167/2.2.4. [Article]
- Jacobs, G. H. & Deegan II, J. F. (1997). Spectral sensitivity of macaque monkeys measured with ERG flicker photometry. *Visual Neuroscience*, *14*, 921-928. [PubMed]
- Jacobs, G. H., Deegan II, J. F., & Moran J. L. (1996). ERG measurements of the spectral sensitivity of common chimpanzee (*Pan troglodytes*). *Vision Research*, *36*, 2587-2594. [PubMed]
- Jacobs, G. H., Neitz J., & Krogh K. (1996). Electroretinogram flicker photometry and its applications. *Journal of the Optical Society of America A*, *13*, 641-648. [PubMed]
- Kelly, D. H. & Norren D. v. (1977). Two-band model of heterochromatic flicker. *Journal of the Optical Society of America*, *67*, 1081-1091. [PubMed]
- Kolb, H. (1977). The organization of the outer plexiform layer in the retina of the cat: electron microscopy observations. *Journal of Neurocytology*, *6*, 131-153. [PubMed]
- Kolb, H. & Nelson R. (1983). Rod pathways in the retina of the cat. *Vision Research*, *23*, 301-312. [PubMed]
- Kondo, M. & Sieving P. A. (2002). Post-photoreceptor activity dominates primate photopic 32-Hz ERG for sine-,square- and pulsed stimuli. *Investigative Ophthalmology & Visual Science*, *43*, 2500-2507. [PubMed]
- Krauskopf, J. (2000). Relative number of long- and middle-wavelength-sensitive cones in the human fovea. *Journal of the Optical Society of America A*, *17*, 510-516. [PubMed]
- Kremers, J., Lee B. B., & Kaiser P. K. (1992). Sensitivity of macaque retinal ganglion cells and human observers to combined luminance and chromatic modulation. *Journal of the Optical Society of America A*, *9*, 1477-1485. [PubMed]
- Kremers, J. & Scholl H. P. N. (2001). Rod-/L-cone and rod-/M-cone interactions in electroretinograms at different temporal frequencies. *Visual Neuroscience*, *18*, 339-351. [PubMed]
- Kremers, J., Scholl H. P. N., Knau H., Berendschot T. T. J. M., Usui T., & Sharpe L. T. (2000). L- and M-cone ratios in human trichromats assessed by psychophysics, electroretinography and retinal densitometry. *Journal of the Optical Society of America A*, *17*, 517-526. [PubMed]
- Kremers, J., Usui T., Scholl H. P. N., & Sharpe L. T. (1999). Cone signal contributions to electroretinograms in dichromats and trichromats. *Investigative Ophthalmology & Visual Science*, *40*, 920-930. [PubMed]
- Lee, B. B., Dacey D. M., Smith V. C., & Pokorny J. (1999). Horizontal cells reveal cone type-specific adaptation in primate retina. *Proceedings National Academy of Science*, *96*, 14611-14616. [PubMed] [Article]
- Lee, B. B., Martin P. R., & Valberg A. (1989). Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. *Journal of Physiology*, *414*, 223-243. [PubMed]

- Lee, B. B., Pokorny J., Smith V. C., Martin P. R., & Valberg A. (1990). Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. *Journal of the Optical Society of America A*, 7, 2223-2236. [\[PubMed\]](#)
- Nelson, R. (1977). Cat cones have rod input: a comparison of the response properties of cone-horizontal cell bodies in the retina of the cat. *Journal of Comparative Neurology*, 172, 109-136. [\[PubMed\]](#)
- Padmos, P. & van Norren D. (1971). Cone spectral sensitivity and chromatic adaptation as revealed by human flicker electroretinography. *Vision Research*, 11, 27-42. [\[PubMed\]](#)
- Pokorny, J., Smith, V. C., & Wesner, M. F. (1991). Variability in cone populations and implications. In A. Valberg & B. B. Lee (Eds.), *From pigments to perception: Advances in understanding visual process*. (pp. 1-9). New York: Plenum Press.
- Stockman, A., MacLeod D. I. A., & Johnson N. E. (1993). Spectral sensitivities of the human cones. *Journal of the Optical Society of America A*, 10, 2491-2521. [\[PubMed\]](#)
- Stockman, A., Sharpe L. T., Rütger K., & Nordby K. (1995). Two signals in the human rod visual system: a model based on electrophysiological data. *Visual Neuroscience*, 12, 951-970. [\[PubMed\]](#)
- Swanson, W. H. (1993). Chromatic adaptation alters spectral sensitivity at high temporal frequencies. *Journal of the Optical Society of America A*, 10, 1294-1303. [\[PubMed\]](#)
- Taylor, M. M. & Creelman C. D. (1967). PEST: efficient estimates on probability functions. *Journal of the Acoustical society of America*, 41, 782-787.
- Usui, T., Kremers J., Sharpe L. T., & Zrenner E. (1998a). Flicker cone electroretinogram in dichromats and trichromats. *Vision Research*, 38, 3391-3396. [\[PubMed\]](#)
- Usui, T., Kremers J., Sharpe L. T., & Zrenner E. (1998b). Response phase of the flicker electroretinogram (ERG) is influenced by cone excitation strength. *Vision Research*, 38, 3247-3251. [\[PubMed\]](#)
- Yeh, T., Lee B. B., & Kremers J. (1995). Temporal response of ganglion cells of the macaque retina to cone-specific modulation. *Journal of the Optical Society of America A*, 12, 456-464. [\[PubMed\]](#)