Electroretinographic responses that may reflect activity of parvo- and magnocellular post-receptoral visual pathways

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The electroretinogram (ERG) is a complex retinal response to visual stimuli that contains receptoral and post-receptoral components. Here, data are presented using stimuli that isolate the responses of L (long wavelength sensitive)- or M (middle wavelength sensitive)-cones or that stimulate the two simultaneously. The data show that at a temporal frequency of 12 Hz, ERG responses are L- to M-cone opponent with little inter-individual variability. Furthermore, the ratio of L- to M-cone-driven response strengths in the ERGs is about unity. These are also properties of the L- and M-cone opponent chromatic channel mediated by parvocellular activity. Similar to the parvocellular-mediated temporal sensitivity, the ERG response is robust to moderate changes in state of cone adaptation. Thus, the 12-Hz ERG shares distinct characteristics with the post-receptoral red-green sensitive parvocellular pathway. At higher temporal frequencies, the responses are not cone opponent, the inter-individual variability is larger, the mean L/M ratio is larger than unity, and the responses change more strongly when the state of cone adaptation is altered. These properties are reminiscent of the magnocellular non-opponent channel. The data suggest that under well-controlled conditions, the ERG can be used to study post-receptoral processes of the visual system.

Keywords: electroretinography, magnocellular, parvocellular, L-cones, M-cones, cone opponency, luminance


Introduction

The electroretinogram (ERG) is a mass retinal potential that is elicited by light. Although the retinal visual pathways projecting to the lateral geniculate nucleus and the ERG generating pathways are both initiated by photon absorption in the photopigments, different post-receptoral processes and cells are involved. But the ERG is an objective non-invasive electrical signal that may reflect the physiological integrity of the retina and therefore has enormous clinical and physiological value, especially for the diagnosis and screening of disorders affecting the photoreceptors (Heckenlively & Arden, 1991; Kremers, 2003).

Under certain circumstances, ERG signals correlate with perceptual phenomena related to photoreceptor activity. For instance, it was found that spectral sensitivities and the ratio between L- and M-cone-driven responses reflect the numbers and sensitivities of L- and M-cones in the retina (Brainard et al., 2000; Kremers et al., 2000).

Some components in the ERG were shown to be correlated with post-receptoral activity for instance of bipolar cells or of spiking retinal ganglion cells (Holder, 2001; Sieving, Frishman, & Steinberg, 1986; Viswanathan, Frishman, & Robson, 2000; Viswanathan, Frishman, Robson, Harwerth, & Smith, 1999; Viswanathan, Frishman, Robson, & Walters, 2001) although no correlation with the light evoked responses of retinal ganglion cells could be identified so far.

Hence, to date there is very little evidence for the existence of ERG signals that are directly driven by mechanisms of the retinal visual pathways that project to the lateral geniculate nucleus (LGN). There are a few reports that suggest that cone opponent signals can be identified in the ERG (Baron, 1980; Donovan & Baron, 1982). But, the effects were generally not very obvious and only small subcomponents of the ERG signal were involved.

The purpose of the experiments presented here was to identify visual processes, similar to those in the LGN projecting pathways, which are involved in generating the ERG waveform. The value of the ERG will increase if direct correlations with retinal visual pathways can be identified. Retinal visual processing in normal and diseased eyes could then be studied with objective and non-invasive electrophysiological methods. Furthermore,
the ERG could then be used to describe the perceptually relevant changes caused by a disorder mainly involving post-receptorial structures (such as glaucoma). New diagnostic methods could be developed that are more specific and sensitive to neuronal changes. Finally, the method could be a useful extension of already existing ERG methods to monitor progression and treatment of a disorder.

In the present paper, we present the results of ERG measurements that were performed using stimuli in which L (long wavelength sensitive)- and M (middle wavelength sensitive)-cones were selectively modulated or simultaneously modulated in different relative proportions. These measurements were performed at different temporal frequencies. When the two cone types are selectively stimulated or modulated in counter-phase at 12 Hz, the ERG displays distinct properties reminiscent of activity in the parvocellular pathway with L- and M-cone opponency. The data cannot be explained on the basis of receptorial activity. We therefore propose that, under these stimulus conditions, the ERG responses are directly and causally related to post-receptorial parvocellular activity and possibly originate in the midget bipolar cells. The data may also shed new light upon the interpretation of ERG data that can be correlated with activity of the non-opponent magnocellular channel (Kremers, Stepien, Scholl, & Saito, 2003).

The results of three different experiments are presented. In Experiment 1, the influence of temporal frequency (12 and 30 Hz) and of stimulus contrast on the ERG signal using was studied. A relatively large number of cone stimulus combinations including selective stimulation, in-phase, and counter-phase modulation was used. Experiment 2 aimed at a more detailed study of the interaction of cone-driven ERG signals when stimulating the L- and M-cones selectively and simultaneously in counter-phase. In this experiment, intermediate temporal frequencies and more subjects were included. Finally, Experiment 3 tested whether a change in state of adaptation has a different influence on the recorded ERG at 12 and 30 Hz.

Parts of the data were presented before as an abstract (Kremers, Jüenemann, & Link, 2007).

Methods

Subjects

All subjects underwent an extensive ophthalmological investigation, and no signs of retinal and ocular disorders were found. They all had normal color vision as tested with a Nagel anomaloscope. In each subject, the pupil of one eye was dilated by a drop of Mydriaticum (Tropicamide; all pupil sizes were at least 7 mm during testing). If requested, topical anesthesia (Oxybuprocain) was administered.

Prior to the experiments, informed consent was obtained from the subjects. The experiments were conducted in accordance with the tenets of the Declaration of Helsinki.

ERG recordings

ERGs were recorded with a DTL electrode that was positioned on the lower canthus of the eye. The reference electrode was a gold cup electrode positioned at the ipsilateral temple. The ground electrode was a gold cup electrode on the forehead. The skin under the reference and ground electrodes was cleaned with abrasive gel. ERG signals were amplified (100,000×) and band-pass filtered (cut-off frequencies 3 and 300 Hz; Grass amplifier) and sampled at 1 KHz by a CED1401 on-line computer. The first 4 sec to a stimulus were discarded to avoid stimulus onset artifacts. The responses to each stimulus were recorded for 48 sec in 12 blocks of 4 sec each. The signals were Fourier analyzed and were found to be mainly determined by the first harmonic component at stimulus frequencies of 12 Hz and higher (Kremers & Scholl, 2001). Thus, the amplitude and phase of the first harmonic component was used to describe amplitude and phase of the complete ERG signal.

Visual stimuli

The visual stimuli were square wave modulations presented on a BARCO color monitor (CCID 7751 MKII; 100 Hz frame rate) driven by a VSG 2/2 card (Cambridge Research Systems). Three different experiments were performed. The stimuli of each experiment will be discussed separately below. In all experiments, spatially homogeneous stimuli, covering the whole monitor (124 by 108 deg at the 10-cm viewing distance), were used. The spectral output of the monitor phosphors was spectrally calibrated by a spectroradiometer (Instrument Systems CAS 1400) and the luminance output was calibrated with a Minolta luminance meter (LS-110). The modulation in phosphor output was used to modulate the excitation in the L- and M-cones and in the rods in a defined manner (Kremers, 2003). Modulation depth and phase were chosen to stimulate selectively the L- or the M-cones or the two simultaneously (either in-phase or in counter-phase). The rods were not stimulated in any of the measurements using the silent substitution paradigm (Estévez & Spekreijse, 1974, 1982; Kremers, Usui, Scholl, & Sharpe, 1999; Usui, Kremers, Sharpe, & Zrenner, 1998a). We chose to silence the rods rather than S (short wavelength sensitive)-cones because rods can alter flicker detection sensitivity for stimuli that modulate the L- and M-cones, whereas the S-cones do not (Cao, Zele, & Pokorny, 2006). Furthermore, rod-driven responses may also influence the ERG responses especially at low temporal frequencies (Kremers & Scholl,
With the three monitor phosphors, it is possible to control the modulation in only three photoreceptor types. Stimulation of the S-cones therefore could not be controlled. In a few additional experiments, the S-cones were silenced with uncontrolled modulation in the rods. The results of these experiments will be mentioned explicitly. Different stimuli were employed in three different experiments.

**Experiment 1**

As mentioned in the Introduction, it was the purpose of Experiment 1 to study the influence of temporal frequency and stimulus strength on the cone-driven signals and their interactions.

**Stimuli**

The stimuli used in Experiment 1 were similar to those described previously (Kremers et al., 1999). Briefly, the mean luminance and modulation in the output of the three monitor phosphors were set. The mean luminance of the monitor was 66 cd/m² (20, 40, and 6 cd/m² from the red, green, and blue phosphors, respectively). The CIE (1964) chromaticity coordinates were (0.32, 0.31). Eight different ratios of L- to M-cone modulation (including selective L- and M-cone modulation) were presented. For each of these ratios, ERGs were measured at four stimulus strengths (expressed in cone contrast as defined by Kremers et al., 1999). Unlike our previous studies, rod activity was silenced and S-cone contrast was uncontrolled. The measurements were performed at 12 and 30 Hz. These measurements were performed in three subjects (two females age 32 and 28 years and one 47-year-old male).

**Results**

In the upper plots of Figure 1, averaged responses to L- and M-cone isolating stimuli are displayed for the three subjects. The middle and lower plots display the amplitudes and the phases of the responses to L- and M-cone isolating stimuli respectively as a function of cone contrast for the same subjects. The ERG amplitudes increase in an approximately linear manner with stimulus contrast (Figure 1 middle plots). The slopes of the linear regression are therefore proportional to the sensitivity of the ERG signal to each condition and inversely proportional to an arbitrary threshold. In agreement with previous data (Kremers & Scholl, 2001; Kremers et al., 1999; Usui, Kremers, Sharpe, & Zrenner, 1998b), it was found that, within this contrast range, the response phase (Figure 1 lower plots) is constant or slightly positively correlated with stimulus contrast. From these phases, the difference between L- and M-cone-driven responses can be estimated. For the three subjects, the phase differences at 12 Hz are larger than those at 30 Hz and have values between 150 and 180 deg, indicating near cone opponency when stimulated simultaneously. The L- and M-cones were stimulated with equal phases. The larger phase difference in the signal is therefore the result of processing in the pathways leading to the ERG signal.

In Figure 2, the thresholds (i.e., the inverse of the slopes of the linear regression through the amplitudes vs. contrast data) at the eight different L/M stimulus ratios are displayed for the same three subjects and for the measurements at 12 and 30 Hz. Data points on the x- and y-axes indicate threshold contrasts for M- and L-cone-selective stimuli, respectively. Other points indicate thresholds for combined stimulation of the two (1st and 3rd quadrants represent in-phase modulation; 2nd and 4th quadrants represent counter-phase modulation; the plot is rotation symmetric indicating that data points in the 1st and 3rd quadrants and in the 2nd and 4th quadrants represent identical stimuli of opposite polarity). The ellipses are fits of a vector addition model to the data. The vector addition model (Kremers, 2003; Kremers et al., 1999) is a simple linear model that assumes that L- and M-cone-driven signals are added at every instant. The form and orientation of the ellipses is determined by three parameters: the amplitudes of the L- and of the M-cone-driven ERG signals and by the phase difference between the two. The fits were further constrained by using estimates of the phase differences obtained from the responses to L- and M-cone isolating stimuli (derived from the data shown in Figure 1 lower plots). Thus, two free parameters, the amplitudes of the L- and M-cone-driven signals, remained. In agreement with previous data (Kremers et al., 1999), the 30-Hz data could be described satisfactorily by the fits. Large phase differences between L- and M-cone-driven signals (suggesting cone opponent in the ERG signal) could be confirmed for the 12-Hz stimuli for all combinations of L- and M-cone modulation and all subjects because the fits were satisfactory for all stimulus conditions with the exception of one. The exception is the condition in which the L- and M-cones were modulated in-phase and with equal strength. These stimuli have a strong luminance component but elicit no L–M-cone opponent signal (see arrows Figure 2). For this condition, large phase differences in the responses were expected to result in response cancellation of L- and M-cone-driven responses, leading to small response amplitudes and large thresholds. This was not the case, indicating that in this condition the responses were probably determined by a different mechanism. These data points were therefore excluded from the fits. From the fits, the amplitudes of the L- and of the M-cone-driven ERG signals and their ratio were estimated. This ratio is about unity for the three subjects despite the different L/M ratios at 30 Hz (see...
This can be derived from the fact that thresholds are about equal for L- and M-cone isolating stimuli. At 30 Hz, the thresholds for L-cone isolating stimuli are considerably smaller than those for M-cone isolating stimuli for subjects BL and JK. Thus, the responses to L-cone isolating stimuli are larger than those to M-cone isolating stimuli at equal contrast and hence the L/M ratio is larger than one.

It has been found that the responses to luminance stimuli have a large 2nd harmonic component at temporal frequencies between 10 and 20 Hz (Kondo & Sieving, 2001; Odom, Reits, Burgers, & Riemslag, 1992; Viswanathan, Frishman, & Robson, 2002). We studied whether this was also the case for the stimuli used in Experiment 1. For all stimulus conditions, the ratio of 1st to 2nd harmonic component at 12 Hz was calculated. For all combinations of L- and M-cone stimuli, this ratio was only minimally influenced by cone contrast. For each L/M cone stimulus combination, the mean amplitude ratio for all contrasts was calculated. The results are shown in Figure 3. The distance to the origin represents the mean ratio. Points on the red circle would indicate responses with equal mean amplitudes of the 1st and 2nd harmonic components. For all combinations of L- and M-cone stimulation, the data lie outside the circle indicating that the 1st harmonic components dominate all responses. This is in contrast with previous data (Kondo & Sieving, 2001; Odom et al., 1992; Viswanathan et al., 2002). Possibly, the difference

Figure 1. ERG responses to cone isolating stimuli. Original tracings (200 ms excerpts; averages of 48 sweeps) to L-cone isolating (22.6% cone contrast; dark red traces) and to M-cone isolating (25.6% cone contrast; green dashed traces) stimuli; upper traces at 12 Hz; lower traces at 30 Hz. Amplitudes (upper plots) and phases (lower plots) of the fundamental component in the response for L (dark red symbols) and M-cone (green symbols) isolating stimuli, plotted separately for the three subjects. The data are shown for 12 Hz (closed circles) and 30 Hz (inverted triangles) stimuli. The relationship between amplitude and cone contrast is approximately linear (upper plots). The linear regressions are displayed (drawn lines: 12 Hz; dashed lines: 30 Hz). The L- and M-cone-driven responses have similar amplitudes at 12 Hz. At 30 Hz, the L-cone-driven ERGs have larger amplitudes than the M-cone-driven amplitudes for subjects BL and JK (see also the original traces). The response phases (lower plots) are relatively stable within this contrast range. Therefore, the phase difference between L- and M-cone-driven ERGs can be estimated at each temporal frequency.
is caused by the absence of rod stimulation in the experiments described here. But Figure 3 also shows that the 1st to 2nd harmonic ratio is relatively small (and thus 2nd harmonic components are relatively large) for M-cone isolating conditions and for those conditions in which the L- and M-cones are modulated in phase.

In conclusion, at 12 Hz, the responses are dominated by the 1st harmonic components, the L/M cone ratio is about unity and there are indications for L- and M-cone opponent processing in the ERG provided there is a chromatic component in the stimulus. This is especially seen when using cone isolating stimuli and when the two cones are modulated simultaneously in counter-phase. In Experiment 2 this issue was pursued in more detail by analyzing the 1st harmonic components in ERG recordings in more subjects and using more temporal frequencies but restricting the stimuli to cone-selective and counter-phase modulations and by using only one contrast for each condition.

**Experiment 2**

Experiment 1 showed that phase difference between L- and M-cone-driven ERG signals increases to about 180 deg when the temporal frequency is decreased to 12 Hz. Furthermore, the amplitude gain ratio is about 1 at 12 Hz. To study the influence of temporal frequency in more detail ERG responses were measured in a larger subject group and more temporal frequencies were employed.

**Stimuli**

Thirteen subjects (4 females and 9 males aged between 29 and 51 years) participated in this experiment. The stimuli had the same mean luminance and chromaticity as in Experiment 1. The stimulus conditions used in Experiment 2 are displayed in Figure 4. In this experiment, the
L- and M-cones were either modulated selectively or the two were modulated simultaneously in counter-phase at different ratios. To be able to present the ERG data more clearly, the L- to M-cone modulation ratios are also expressed in terms of L-cone modulation fraction \((L/(L + M))\). Because the results of Experiment 1 showed that the relationship between stimulus strength and ERG response amplitude was approximately linear for all ratios of L- to M-cone stimulus strengths (Figure 1 middle plots), the measurements were performed at one stimulus strength for each ratio. Stimulus conditions with L/M cone stimulus ratios smaller than 1 (L-cone stimulus fractions \(<0.5\)) were measured more densely because it was expected from previous data (Kremers et al., 2000) that ERG signal amplitudes and phases would change more strongly for these conditions at 30 Hz. This is caused by the fact that most subjects display larger sensitivities to L-cone cone stimuli than to M-cone stimuli (probably related to the larger number of L-cones). As a result, the minimal response in conjunction with large ERG phase changes can be found for conditions in which M-cone contrast exceeds L-cone contrast (Brainard et al., 2000; Kremers et al., 2000; Pokorny & Smith, 1987; Pokorny, Smith, & Wesner, 1991). At 12 Hz, all responses have similar amplitudes and phases, indicating, in accordance with the data from Experiment 1, that the L/M ratio is about unity at this temporal frequency. Furthermore, the responses to L- and M-cone isolating stimuli have similar phases at 12 Hz. Taking into account the fact that excitation modulation in the L- and M-cones were 180 deg phase shifted relative to each other, this means that the L- and M-cone-driven ERG phases differ by about 180 deg. The data at 18 and 24 Hz are intermediate. The curve fits are based upon the same vector addition model as described for Experiment 1. The model was simultaneously fitted to the amplitude and phase data by converting the data into

**Results**

In Figure 5, the ERG response amplitudes and phases measured in one subject (SR) are displayed as a function of L-cone stimulus fraction plotted separately for four different frequencies. An L-cone stimulus fraction of one indicates an L-cone isolating stimulus whereas a fraction of zero indicates an M-cone isolating stimulus (see Figure 4). At 30 Hz, the ERG response to L-cone isolation is larger than the response to M-cone isolation. This is in accordance with previous data and is probably related the larger numbers of L- than M-cones in the retina (Brainard et al., 2000; Kremers et al., 2000; Pokorny & Smith, 1987; Pokorny, Smith, & Wesner, 1991). At 12 Hz, all responses have similar amplitudes and phases, indicating, in accordance with the data from Experiment 1, that the L/M ratio is about unity at this temporal frequency. Furthermore, the responses to L- and M-cone isolating stimuli have similar phases at 12 Hz. Taking into account the fact that excitation modulation in the L- and M-cones were 180 deg phase shifted relative to each other, this means that the L- and M-cone-driven ERG phases differ by about 180 deg. The data at 18 and 24 Hz are intermediate. The curve fits are based upon the same vector addition model as described for Experiment 1. The model was simultaneously fitted to the amplitude and phase data by converting the data into

![Figure 3](image-url)  
**Figure 3.** Mean ratio of 1st to 2nd harmonic response components at 12 Hz for different combinations of L- and M-cone stimulation. Points on the red circle indicate responses in which the 1st and 2nd harmonic components are equal.

![Figure 4](image-url)  
**Figure 4.** Stimulus conditions for the Experiment 2. Every point in this plot represents a particular stimulus. Points are plotted on the axes and in the 2nd quadrant indicating that the stimuli selectively modulated the L- or M-cones or the two in counter-phase. The ratio of L/M cone stimulus strength and the L-cone stimulus fraction \((L / (L + M))\) are given for each stimulus.
Figure 5. ERG amplitudes and phases at different temporal frequencies. Response amplitudes (upper four left plots) and phases (upper four right plots) as a function of L-cone stimulus fraction. The closed circles are the measured data. The curves are fits of the vector addition model to the data. At 30 Hz the response to the L-cone isolating stimuli (L-cone stimulus fraction 1) is larger than the response to the M-cone isolating stimulus (L-cone stimulus fraction 0). This difference decreases with decreasing temporal frequencies and the responses have similar amplitudes at 12 Hz. The response phase changes strongly at 30 Hz. This phase change decreases with decreasing temporal frequency. At 12 Hz, the phase is nearly constant, indicating that M- and L-cone responses are nearly 180 deg apart (taking into account that the L- and M-cones are modulated in counter-phase). From the fits, the L/M ratio and the phase difference between L- and M-cone-driven ERGs can be estimated. These are shown in the lower plots. The L/M ratio decreases with decreasing temporal frequency and is close to unity at 12 Hz whereas the phase difference increases with decreasing temporal frequency and is close to 180 deg at 12 Hz.
vectors (the lengths of which are determined by the response amplitudes and the angles with the positive x-axis are set by phase) and fitting the model in the vector space. The model data were then converted back into amplitude and phase data.

The lower plots in Figure 5 show the amplitude ratio (left plot) and the phase difference (right plot) of the L- and M-cone-driven ERGs as a function of temporal frequency. It can be seen that with decreasing temporal frequency the L/M ratio decreases and is about unity at 12 Hz and the phase difference increases and is close to 180 deg at 12 Hz. This was found for all subjects.

Figure 6 (left plots) shows the averaged L/M ratio and phase differences for the 13 subjects that participated in Experiment 2, confirming that the data for subject SR (Figure 5) are representative for all subjects. Moreover, the phase differences at 12 Hz display less inter-individual variation than those at other temporal frequencies (Figure 6, lower plot). In eight subjects, the experiments were repeated with stimuli that silenced S- (short wavelength sensitive) cone responses and that stimulated rods in an uncontrolled manner. The data obtained from these experiments were similar to those describe above for the silent rod conditions.

The upper right plot of Figure 6 shows psychophysically measured ratios of flicker detection sensitivities to L- and M-cone-selective stimuli (redrawn from Kremers et al., 2000). The psychophysical task at low temporal frequencies is mediated by the L- and M-cone opponent chromatic channel, the ratios of which are similar to those of the 12-Hz ERG signal (Kremers, Lee, & Kaiser, 1992). The psychophysical task at high temporal frequencies is...
mediated by the luminance channel (Kremers et al., 1992; Lennie, Pokorny, & Smith, 1993). The sensitivity ratios correspond to the 30-Hz ERG ratios, although it should be noted that two different subject populations participated in the ERG and the psychophysical experiments.

The data provide evidence that the ERGs measured at 12 Hz may reflect L/M opponency and have other properties that are shared with the parvocellular channel. As can be seen in the upper right plot of Figure 6, we previously found that the ratio of detection sensitivities for selective L- and M-cone isolating stimuli are about unity at low temporal frequencies (<5–6 Hz), at which the chromatic channel determines detection. To be able to establish a more firm correlation, we have considered another property that distinguishes the L/M cone opponent channel from the luminance channel. A possible distinction can be found in the different behavior of the two channels when the cones are selectively adapted: the psychophysical ratio does not change at different states of adaptation when the chromatic channel determines flicker detection (Kremers et al., 2003). In contrast, the ratio can strongly vary with the state of adaptation when luminance mediated detection sensitivities are measured and the psychophysical ratios correlates with the 30-Hz ERG ratios. In Experiment 3 we therefore studied the possibility that cone-selective adaptation has distinct influences on the 12- and 30-Hz ERGs.

**Experiment 3**

Experiments 1 and 2 show that the ERG signal may display different characteristics at 12 and 30 Hz. The properties of the ERG signals at 12 Hz resemble those of the parvocellular retinal pathway. It was found previously (Kremers et al., 2003) that adaptation may have different influences on the magno- and parvocellular pathways. The purpose of Experiment 3 was to study whether adaptation may also have a different influence on cone-driven ERG signals at 30 and at 12 Hz.

**Stimuli**

Twelve subjects (7 females and 5 males; aged between 29 and 58 years) participated in this experiment. The subject population partially overlaps with the one that participated in Experiment 2. In Experiment 3, the conditions were similar to those described by Kremers et al. (2003). Briefly, either L- or M-cones were stimulated with 15% cone contrast. These measurements were repeated for five states of adaptation. The so-called baseline condition was identical to those used Experiments 1 and 2. In comparison with this condition, in the other adaptation conditions the number of photon catches in the L- or the M-cones was selectively changed. In two conditions, the cones absorbed more photons (“L\text{max}” and “M\text{max}” conditions); in the remaining two conditions they absorbed fewer quanta (“L\text{min}” and “M\text{min}” conditions). The L\text{max} and M\text{min} conditions were a change into the reddish direction in comparison with the baseline condition whereas the L\text{min} and M\text{max} conditions represented a change into the greenish direction. The changes in quantal catches in comparison with the baseline condition were similar in all adaptation conditions. The rods were neither stimulated nor were they adapted by any of the adaptation conditions. As in Experiments 1 and 2, S-cones were stimulated and state of S-cone adaptation was different in the different adaptation conditions. Table 1 shows the cone td and scot td in the five adaptation conditions together with the luminance in the three phosphors. To calculate the cone td, we have normalized the cone fundamentals to a maximal value of one.

**Results**

The rationale for Experiment 3 is that we wanted to study how far adaptation can differently influence the ERG amplitudes to 15% cone contrast L- and M-cone at 12 Hz and thus see whether the ERG displays the same characteristics as previously published psychophysical data.

The upper panels in Figure 7 display the 12- and 30-Hz L/M ratios measured at the different adaptation conditions for three different subjects the data of whom represent the range of results we obtained at 12 Hz. The

<table>
<thead>
<tr>
<th>Troland</th>
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<th>M</th>
<th>L</th>
<th>Scot</th>
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<tr>
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<td>2193</td>
<td>3004</td>
<td>3632</td>
<td>8177</td>
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<tr>
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<td>2388</td>
<td>3004</td>
<td>3996</td>
<td>8177</td>
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<td>L\text{min}</td>
<td>1997</td>
<td>3004</td>
<td>3269</td>
<td>8177</td>
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<tr>
<td>M\text{max}</td>
<td>1558</td>
<td>3304</td>
<td>3632</td>
<td>8177</td>
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<tr>
<td>M\text{min}</td>
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<td>2703</td>
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<tr>
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<tbody>
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<td>M\text{min}</td>
<td>27.8</td>
<td>28.8</td>
<td>8.3</td>
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Table 1. S-, M-, and L-cone troland and the scotopic troland values for the five adaptation conditions; the luminances in the red, green, and blue phosphors of the monitor at each condition are given in the lower table. The conditions are not identical to those published before (Kremers et al., 2003) because here rods are not stimulated and their state of adaptation was held constant, whereas previously the S-cones were not simulated and not adapted in the different conditions.
ratios change dramatically at 30 Hz whereas the change at 12 Hz is either smaller (subjects JK and EM) or in an opposite direction (subject SR). The phase differences between L- and M-cone-driven responses are displayed in the lower plots. The phase difference at 30 Hz strongly depends on the state of adaptation whereas at 12 Hz all phase differences are relatively constant and close to 180 deg.

Figure 8 shows the means for all 12 subjects. The mean L/M ratio was obtained by calculating the mean of the logarithms of the individual ratios and then converting them back into linear terms. Generally, the data confirm what has been found for the individual data: The changes in the 12-Hz L/M ratios are opposite to those at 30 Hz. It is difficult to provide a satisfactory explanation for this. But it shows that different adaptation mechanisms may be involved at 12 Hz and at 30 Hz. The mean phase difference between L- and M-cone-driven responses changes dramatically at 30 Hz. Furthermore, the large standard deviations indicate large inter-individual variability. At 12 Hz, the phase differences are close to 180 deg for all adaptation conditions and there is a less inter-individual variability.

Discussion

The purpose of the present study was to investigate the processing of L- and M-cone-driven signals at different temporal frequencies. The data show that different post-receptoral processes are involved at 30 and at 12 Hz. In agreement with previous data (Kremers & Scholl, 2001), the phase difference between L- and M-cone-driven ERGs increases with decreasing temporal frequency. A decrease in phase difference with decreasing temporal frequencies would be expected if a single simple mechanism (with, e.g., delay and filter properties) would have determined the responses. The data therefore suggest a change of mechanisms that underlie the ERG responses as temporal frequency is altered. A transition between two mechanisms that drive the ERGs when changing the temporal frequency was also suggested by others (Odom et al., 1992; Viswanathan et al., 2002). In Experiment 1, we found a further indication for the presence of two different ERG driving mechanisms: at 12 Hz, the ERG thresholds can be described by a simple vector addition model for the majority of L/M cone stimulus ratios except for the
condition in which the stimulus does not contain an L- and M-cone opponent chromatic signal (arrows in Figure 2), suggesting that the ERG responses are driven by another mechanism in this particular condition.

We previously postulated that the transition between ERG mechanisms, as temporal frequency is varied, may be caused by intrusion of rod-driven signals at lower temporal frequencies (Kremers & Scholl, 2001). In our laboratory, we have confirmed that rod-driven signals may be large at low temporal frequencies (Kremers, Czop and Link, unpublished data). In the present experiments, we have used stimuli that did not modulate rod excitation. Thus, intrusion of rod-driven signals cannot explain the transition of mechanisms that drive the ERGs. From the data presented here, we propose that the ERG responses at 12 Hz reflect parvocellular activity. This proposal is based upon several observations: First, at 12 Hz the phase differences are close to $180^\circ$, suggesting that a cone opponent mechanism determines the responses (Smith, Lee, Pokorny, Martin, & Valberg, 1992). Cone responses at higher temporal frequencies interact less antagonistically. Second, the amplitude ratio decreases with decreasing temporal frequency, so that the ratio is about unity at 12 Hz. In psychophysical measurements, it was found that the red-green chromatic system (of which the parvocellular channel is the physiological basis) also displays an L/M ratio of about unity (Brainard et al., 2000; Krauskopf, 2000; Kremers et al., 2000) (see Figure 5 right plot). Third, the inter-individual variability in the phase differences is larger at 30 Hz than at 12 Hz. It was found previously that the red-green chromatic channel indeed shows substantially less inter-individual variability than the luminance channel (Brainard et al., 2000; Krauskopf, 2000; Kremers et al., 2000, 2003; Neitz, Carroll, Yamauchi, Neitz, & Williams, 2002). Finally, we found that the phase difference and, in some individuals, the L/M ratio at 12 Hz is relatively robust for changing adaptation levels, whereas the two vary strongly at 30 Hz. Again this resembles some properties of the L- and M-cone opponent chromatic channel, which was found to be stable at different adaptation conditions (Krauskopf, 2000; Kremers et al., 2003; Neitz et al., 2002). This was, however, not true for the L/M ratios of all individuals in the ERG experiments.

Although, in previous experiments, there were signs that certain components in the ERG could be correlated with red-green opponent processing (Baron, 1980; Donovan & Baron, 1982), the present report is to our knowledge the first in which the complete ERG signal under particular conditions (12 Hz temporal frequency and the L- and M-cones selectively stimulated or the two simultaneously in counter-phase, adaptation to white or slightly greenish backgrounds) can be correlated with known activity properties of the parvocellular pathway.

It is difficult to speculate upon which cell types directly cause the ERG responses. The cone opponency in the responses, the L/M ratio of about unity and the presence of a mechanism that compensates for inter-individual differences strongly suggests a post-receptoral origin of the ERG signal. It has been proposed that the flicker ERG originates in the bipolar cells (Bush & Sieving, 1996). In accordance with our data, others found a transition between two mechanisms that mediate the ERG response at responses between 6 and 20 Hz (Odom et al., 1992; Viswanathan et al., 2002). The transition was still present when ganglion cell activity was abolished by tetrodotoxin (TTX; which blocks the spiking activity) and NMDA (suppressing light-driven activity in the inner retina). However, the transition was removed after blockade of signals transmission of the photoreceptors to the bipolar
cells (Viswanathan et al., 2002). This suggests that the transition in the ERG data is caused by a change in bipolar cells that drive the responses. We therefore postulate that at the lower temporal frequencies, the ERG signals to stimuli with a large chromatic content are generated by the midget bipolar cells. Possibly, properties of the retinal ganglion cells and the amacrine cells are also reflected in the ERG through feedback loops onto the midget bipolar cells because TTX and NMDA did change the characteristics of the transition between the two mechanisms (Viswanathan et al., 2002). Our proposal assumes that midget bipolar cells display cone opponency. This needs to be confirmed. Furthermore, our proposal implicitly assumes that ERG activity driven by midget bipolar cells that are depolarized by L-cone excitation and by bipolar cells that are depolarized by M-cone excitation are not completely balanced. Otherwise the two ERG signals would have canceled each other out.

Another question is why the 12-Hz ERG is able to reflect parvocellular processes. Partially, the low-pass characteristics of cells belonging to parvocellular pathway (Lee, Pokorny, Smith, Martin, & Valberg, 1990) may result in a larger participation of these cells in mediating the ERG response at low and intermediate temporal frequencies. Furthermore, our data suggest that a red-green chromatic component should be present in the stimuli because the stimuli that lack this component (Figure 2 arrows) evoke a response that is mediated by another mechanism. The ERG response to luminance stimuli shows a conspicuous dip at a frequency between 10 and 20 Hz (Odom et al., 1992; Viswanathan et al., 2002). Possibly, this dip in the luminance response introduces a possibility to detect red-green opponent responses that are otherwise overruled by the luminance responses.

These data may also shed new light upon the interpretation of other ERG data. It was found that the ERG measured at high temporal frequencies (ca. 30 Hz) shares several features with the magnocellular based luminance system such as a V_L-like spectral sensitivity (Jacobs & Neitz, 1993; Jacobs, Neitz, & Krogh, 1996), very similar inter-individual variability in L/M ratios (Kremers et al., 2000) and similar changes in L/M ratios at different states of adaptation (Kremers et al., 2003). Previously, these data were interpreted in such a way that there is no causal relationship between the ERGs and the luminance channels but rather reflect similar cone signal processing in which L- and M-cone signals interact synergistically and are drawn randomly from the available cones. The present data may indicate that the ERGs at high temporal frequencies may indeed reflect activity of the magnocellular system and are possibly driven by diffuse bipolar cells. This interpretation would also explain why cone-selective adaptation has similar effects upon L- and M-cone-driven response amplitudes and L/M ratios in the ERG and in luminance mediated flicker detection sensitivities (Kremers et al., 2003), which cannot be understood solely on the basis of cone numbers.

If ERG responses under well-chosen stimulus conditions reflect post-receptoral parvocellular or magnocellular activity, then new electrophysiological studies of the retinal cells (e.g., bipolar cells) subserving human chromatic and luminance channels are possible. For instance, the involvement of the parvocellular pathway in diseases of the inner retina can be studied. Preliminary data show that in glaucoma patients, the phase difference between L- and M-cone-driven responses are changed when the ERGs reflect parvocellular activity (Link, Jüinemann, & Kremers, 2007). More basic questions such as the dependency of the response of the L- and M-cone opponent chromatic channel upon spatial frequency and/or upon retinal eccentricity are then open for non-invasive electrophysiological investigations in human subjects.

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