

# Red-green flicker photometry and nonlinearities in the flicker electroretinogram

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We investigated whether responses from different cone classes are combined before or after the nonlinearity that is responsible for generating nonlinear response components of the flicker electroretinogram (ERG). We measured the nonlinear response of the retina while systematically varying the modulation in the long-wavelength-sensitive and middle-wavelength-sensitive cones by changing the proportions of flickering 633- and 543-nm lights that compose a sum-of-sinusoids temporal waveform. We found that at high temporal frequencies the ERG responses are best accounted for by a model in which the principal retinal nonlinearity is located before the convergence of signals from the two cone classes. At low temporal frequencies the ERG signal is dominated by cone-antagonistic responses. At frequencies of 30 Hz and higher, the flicker ERG and psychophysical flicker photometry give similar estimates of the relative proportions of long- and middle-wavelength-sensitive cones. The ERG photometric null is frequency dependent, whereas the psychophysically determined ratio is much less sensitive to changes in frequency.

## INTRODUCTION

The electroretinogram (ERG) is a mass electrical response that is elicited when the retina is stimulated by light. This retinal response is complex, arising from multiple neural and nonneuronal sources.<sup>1-3</sup> The ERG is one of the few techniques available to the visual researcher that permit probing of human retinal physiology *in vivo*. For this reason there has been interest in devising ERG techniques that are capable of identifying components of the mass response from known retinal substrates. One approach has been to use flickering lights. Flicker has been assumed to bias the ERG toward cone responses by decreasing the relative response from glial and inner retinal generators of the ERG response, and it gives a good estimate of the activity of the late receptor potential.<sup>4-5</sup> Flicker ERG recordings have been used to give excellent estimates of the spectral sensitivity of cones<sup>6-8</sup> and to provide information on the recovery of cones from bleaching.<sup>9</sup> However, it is well known that retinal generators other than the photoreceptors respond with high temporal frequencies.<sup>10-11</sup> Thus, although the responses may be driven by the photoreceptors, there is no guarantee that they arise from the photoreceptors. It is, however, likely that the ERG response to high frequencies of flicker arises from neural elements, since the glial elements are fairly slow.<sup>12,13</sup>

In the current study we use both the spectral sensitivity of the ERG and the existence of nonlinear components in the retinal response to light to study the flicker ERG further. Our overall goal is to use retinal nonlinearities, as represented in the flicker ERG response, to permit us to probe the functioning of cone photoreceptors. The existence of retinal nonlinearities<sup>14-19</sup> provides us with a means to partition the retinal contributions to the ERG into those that occur before a nonlinearity and those that occur after a nonlinearity.<sup>17-26</sup> By additionally varying the spectral composition of the stimulating light, we

can also vary the contributions of different cone classes to the retinal response, which permits us to probe the location of the nonlinearity relative to the convergence of cone signals.

In a linear system it is not possible to understand the nature of the underlying components of a system from the system's behavior. However, in a nonlinear system it is sometimes possible to deduce the behavior of individual components of the system.<sup>19-33</sup> In recent years there has been increasing use of the concept of a sandwich model,<sup>23</sup> or a linear-nonlinear-linear model, in analyses of the visual system.<sup>20-32</sup> The name comes from the fact that the sandwich model partitions the system under study into three boxes. The first box represents the input and initial processing of the system, and it is assumed to be linear. Following this first linear stage is a nonlinear processing stage and then a final linear stage. The analysis uses as its basis the fact that a nonlinearity generates distortion in the system response. For instance, if a linear system is stimulated with the sum of two sine waves, the output will be the sum of two sine waves. The amplitude and the phase of the response sinusoids will change depending on the actual system tested, but the frequency content will not. However, if a nonlinear system is stimulated with the sum of two sine waves, the output will contain power not only at the stimulus frequencies but also at all sum and difference frequencies. For instance, if the eye is stimulated with the sum of a 26- and a 35-Hz sine wave, the ERG response will contain power at the difference frequency, in this case 9 Hz. By varying the input frequencies but keeping the beat frequency constant, we can measure the transfer function of the initial linear stage of processing. Since the beat frequency is kept constant across stimulus conditions, the third stage of the model, which is linear, does not differentially affect the response. The sandwich model assumes that the nonlinearity is not stimulus dependent; that is, it must be instantaneous, an assumption that may not be met by some

physiological systems.<sup>33</sup> However, it is important to note that this linear-nonlinear-linear or sandwich model can be generalized to more-complex systems.<sup>34</sup>

Using the two-sinusoid technique, we have found<sup>20</sup> that the early stages of the temporal response of the retina act as a low-pass filter, with a corner frequency of  $\sim 50$  Hz. This early temporal-frequency filtering occurs either before or at the site of the ERG nonlinearity.<sup>19,20</sup> This early, fast stage of the retinal response may well represent the response of the photoreceptor outer segments.

In the present study we further examine this possibility, asking whether the nonlinearity occurs before or after convergence of information from the long-wavelength-sensitive (LWS) and middle-wavelength-sensitive (MWS) cones. If the visual system is linear up to the convergence of cone signals, then by varying the relative response and phase of the two cone classes we should be able to null, or cancel, the response of the retina. If there is a nonlinearity before convergence, then we should not be able to null the nonlinear response components. Although we discuss the experiments by using the framework of the sandwich model, the results of this study can be generalized to some dynamic nonlinearities, since an early linear combination of cone signals will result in a null for these systems as well. In addition, to understand the relation of the ERG to psychophysical measurements more thoroughly, we directly compared the null ratio that was measured electrophysiologically and psychophysical measurements.

## METHODS

### Subjects

The authors served as subjects. All were color-normal males, and none had evidence of ocular disease.

### Apparatus

Red (633-nm) and green (543-nm) He-Ne lasers were used as light sources.<sup>35</sup> The average intensity of each laser beam was independently modulated by a separate acousto-optic modulator with the use of pulse-frequency modulation.<sup>36</sup> The relative luminance, frequency, and phase of the red and green laser lights were varied with the use of an analog voltage produced by a programmable-function generator. The first-order beams from each acousto-optic modulator were used to illuminate the end of a rotating motor shaft that had been coated with MgO.<sup>35</sup> The diffused light was then used as the source for a standard Maxwellian-view system, producing an entrance pupil in the plane of the eye of  $\sim 1$  mm and a field size of 40 deg. A white surround was used to suppress stray light responses.<sup>37,38</sup> Light from the surround entered the eye through the natural pupil. The signal generators and data acquisition were driven by a single master clock, which was divided to generate the 512-Hz sample rate and the 1024-Hz stimulus update rate. The visual stimulus was generated with the use of 12-bit digital-to-analog converters, and the corneal ERG response was similarly digitized at 12 bits.

### Stimuli

The flicker ERG was elicited by temporally modulating the retinal illuminance of the 633- and 543-nm lights. The modulations for each wavelength were excursions

above and below the mean luminance, and thus the time-averaged luminance of the red and green lights was kept strictly constant across stimulus conditions, except for the control experiments. In experiment 1 the red and green modulation were varied inversely. The average retinal illuminance of the 633-nm light was 3.65 log Td, and the average retinal illuminance of the 543-nm light was 3.75 log Td. The temporal waveform of each light could be independently programmed.

### ERG Recording

ERG responses were recorded by using either a JET ERG lens (for subject YC) or a Burian-Allen ERG lens (for subjects SB and MK). The ERG response was amplified in two stages. The first-stage gain was 1000, and the second-stage gain was 100. The amplifier bandwidth corner frequencies were set at a high-pass value of 3 Hz and a low-pass value of 100 Hz. Occasionally a 60-Hz notch filter was used to reduce noise, although in general it was not used. The 60-Hz filter setting was never changed within a session, and data were not compared across conditions in which the filter settings differed. The output of the second amplifier was digitized by a 12-bit analog-to-digital converter (Data Translation) at a sampling rate of 512 Hz. A total of 16,384 samples were recorded without averaging and were stored on a hard disk for later analysis.

### Signal Analysis

We analyzed the ERG data off line by computing the discrete Fourier transform for each data block (16,384 samples). The magnitude, the phase, and an estimate of the signal-to-noise ratio were then saved to the disk for all stimulus frequencies as well as for the second- and third-order nonlinear product frequencies. We estimated the signal-to-noise ratio by comparing the magnitude of the ERG response at the frequency under consideration with the average magnitude at the ten adjacent lower-frequency bins (that is, averaged over the adjacent 0.3 Hz). This is equivalent to estimating the ratio of signal-plus-noise to noise assuming that the noise is additive.

## EXPERIMENT 1. MODELING THE ERG RESPONSE WITH VARIATION OF THE RED/GREEN RATIO

### Spectral Response of the Photoreceptors

In this study we varied the contributions of the LWS- and MWS-cone photoreceptors by varying the modulation of a red (632.8-nm) and a green (543-nm) flickering light such that the total modulation and the time-averaged chromaticity remained constant. For the majority of conditions reported in this paper, the red and the green lights were flickering out of phase. With this approach the responses of the MWS and LWS cones vary with the mixture ratio. That is, when the green light is at 100% modulation, both cones are stimulated in phase. As the relative modulation of the lights is varied from all green to all red, the response of each cone type varies systematically. Figure 1 below shows the relative quantal absorption for each cone type for the stimuli used in this study. The Smith-Pokorny<sup>39</sup> fundamentals were used to derive the cone absorptions. When the proportion of red modula-

tion is zero, both LWS and MWS cones are stimulated in phase. As the proportion of red modulation is increased (and the proportion of green modulation is decreased), there is a mixture ratio of which the response of the LWS cone is nulled, the so-called silent LWS ratio. Increasing the red modulation beyond this ratio results in increasing LWS modulation, but now the LWS and MWS stimuli are out of phase. At some ratio the LWS and MWS modulations are equal but of opposite phase, and at this ratio the stimulus for a system that linearly adds the cone quantal catches is zero. Increasing red modulation further decreases the MWS response and increases the LWS response until we reach a mixture for which there is no stimulus for the MWS cones, the silent MWS ratio. As the proportion of red approaches 1.0, the two cones again respond in phase. The possibility of phase differences between LWS and MWS cones<sup>40,41</sup> changes the quantitative predictions to the extent that the cones would not be completely silenced, but, unless the phase shifts were extreme, the general pattern of responses would be the same, except the degree of cancellation would vary.

This linear relation between the red/green (R/G) ratio and the cone stimulus permitted us to make distinct qualitative predictions for different classes of nonlinear models. To make these predictions we needed to make an assumption regarding the sources of the flicker ERG

response. We adopted a model that assumes that all stages of the neural retina can contribute to the total response. That is, there might be recordable contributions to the total current that arise from all three stages of the sandwich model. This assumption permitted us to incorporate possible ERG contributions from the photoreceptors that might precede any nonlinearity. This is different from the more familiar analysis in which we examine only the output of a mechanism, a method that constitutes a major limitation on formal modeling of the ERG. It is this limitation that has restricted us to comparing classes of models rather than examining a more specific quantitative model. We also assumed that the response of glial cells is minimized at high frequencies.<sup>12,13,20</sup> Finally, we modeled only static nonlinearities, that is, nonlinearities for which the input-output properties do not depend on the stimulus history. The major conclusions of this study also apply to many other classes of nonlinearities, since we tested for the presence of a null in the retinal response. For each model the response to a series of red-green mixtures was calculated. The resulting model response was Fourier transformed, and the responses at the frequencies of interest were plotted as a function of the red/(red + green) ratio (the proportion of red). We examined a number of parameter choices for each type of model, but, because of the number of unknowns relating

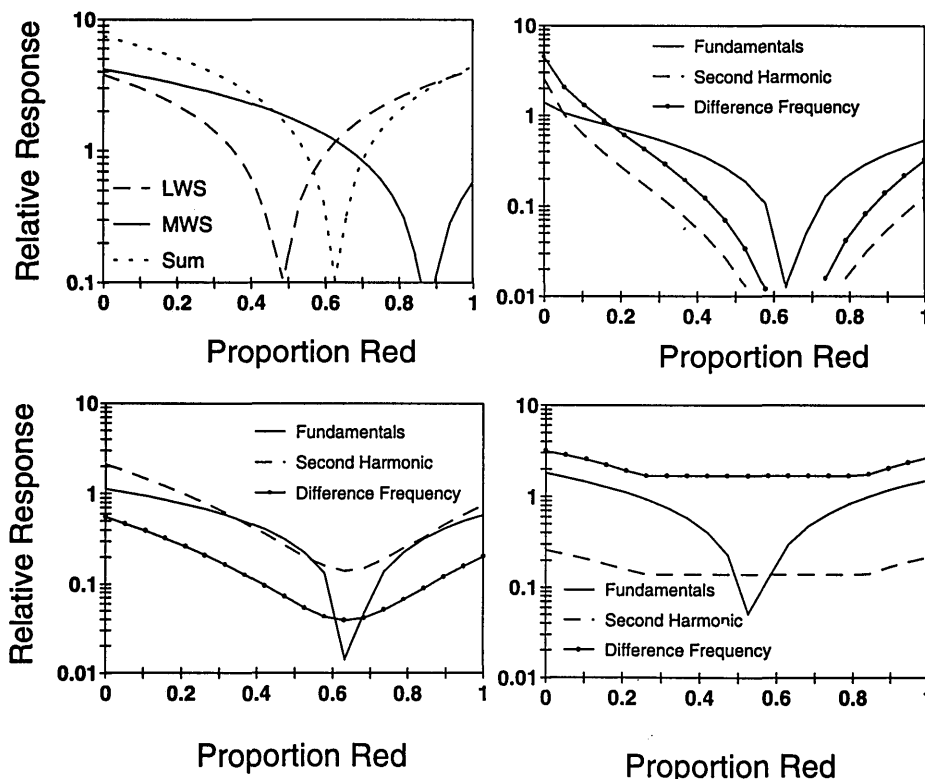


Fig. 1. Predictions of ERG modeling. Top left: Relation between the proportion of red in the flickering stimuli and the modulation of the cone absorptions. Going from left to right, the modulation of the green stimulus is linearly decreasing and that of the red stimulus is linearly increasing. Also shown is the response of a system that linearly sums the cone absorptions. Top right: predictions for a model incorporating linear combinations of cone signals and a later nonlinearity. A compressive nonlinearity was used. Both fundamental responses and nonlinear-response components drop to zero at the location of the photometric null. Bottom left: predictions for a model in which the output of each cone passes through a compressive nonlinearity before combination. Whereas responses at the stimulus frequencies drop to zero, nonlinear-response components show only a shallow minimum. Bottom right: predictions for a model in which the output responses of each cone are rectified and are then summed. The nonlinear responses in the regions where both cones are in phase have a shallow slope, but there is no minimum at the photometric null. All models treat the frequencies uniformly and do not incorporate phase shifts between LWS and MWS cones. Models incorporating frequency-dependent phase shifts and phase shifts between LWS and MWS cones produce qualitatively similar results but with quantitative differences related to the degree of cancellation.

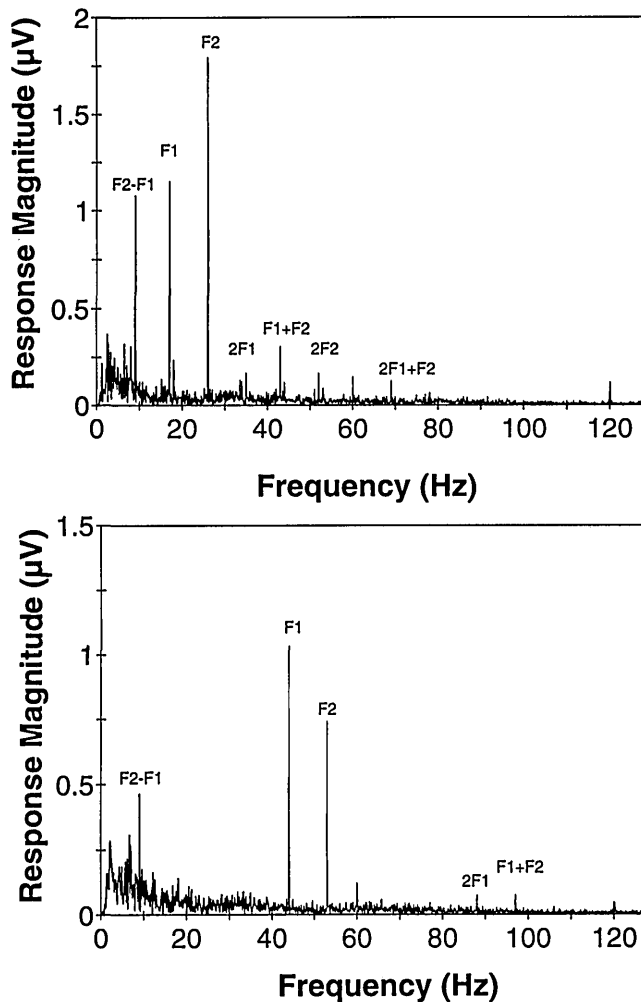


Fig. 2. Amplitude spectrum for an ERG response to a sum of a 17- and a 26-Hz sine wave (top) and a sum of a 44- and a 53-Hz sine wave (bottom). The response components are labeled. Note that nonlinear-response components are easily recorded.

the corneal response to the underlying retinal components, we restrict our analysis to the general behavior of the models.

#### Model 1. Static Nonlinearity After Convergence of Cone Signals

The first class of model we discuss assumes that the nonlinearity occurs after cones linearly combine their responses. That is, the response of the cones is linear, the cone responses are linearly combined regardless of their spectral type, and the resulting signal goes through a nonlinear stage. Predicted response functions for this type of model are shown in the top-right-hand panel of Fig. 1. The linear combination of cone signals means that at some R/G ratio the system will not respond to the stimulus. That is, the MWS response and the LWS response will cancel each other in the early linear stage. If we measure the response of such a system to a sum of sinusoids, then the responses at the fundamental frequencies, at the second-harmonic frequency, and at the beat frequency will disappear when the LWS and MWS responses are equal but at a phase of 180 deg relative to one another. This provides us with our first question: Is there a R/G ratio

for which the nonlinear response of the retina is nulled? Note that if the cones interact negatively then the response minimum can occur at a ratio that is not between the silent LWS and the silent MWS points, but there will still be a distinct minimum. Such an antagonistic process can be revealed to a greater extent by varying the relative phases of the red and green stimuli (see experiment 2 below).

#### Model 2. Nonlinearity before Convergence of Cone Signals

The second class of model assumes that there is a nonlinearity at a site in the visual system where the signals of the two cone types are relatively separate. Model responses for a soft or compressive, type of nonlinearity are shown in the bottom-left-hand panel of Fig. 1. Model responses for a hard nonlinearity, in this case a full-wave rectification, are shown in the bottom-right-hand panel of Fig. 1. Note that in these cases there is no true null ratio. Depending on the nature of the nonlinearity, we might find a R/G ratio at which the fundamental response goes to zero. However, the beat response does not go to zero. The actual shape of the amplitude-versus-R/G-ratio curve depends on the nature of the nonlinearity. A compressive nonlinearity produces a shallow minimum in the vicinity of the ratio, which produces equal LWS- and MWS-generated responses (lower-left-hand panel of Fig. 1). A hard nonlinearity, such as full-wave rectification, produces no minimum (lower-right-hand panel of Fig. 1).

#### Methods

Beat responses with a frequency of 9 Hz were generated from sums of two sine waves. The frequency combinations used were 5 + 14 Hz (5,14), 17 + 26 Hz (17,26), 29 + 38 Hz (29,38), and 44 + 53 Hz (44,53). The average luminance of each stimulus was kept constant while the R/G ratio was varied at 15 levels from all green to all red.

#### Results

Figure 2 shows the response amplitude for subject MK in the 17,26 (top) and 44,53 (bottom) conditions. Note that there are strong response components at sum and difference frequencies as well as at the frequency of stimulation. Second- and third-order nonlinear responses are easily recorded. Also note that the signal-to-noise ratio is quite high for this condition (proportion of red equal to zero in Fig. 3). The low-frequency noise that is evident in these plots was the reason for using a difference frequency of 9 Hz as opposed to, for instance, 4 Hz in these studies.

Figure 3 shows the ERG-response magnitudes for both the fundamental and the beat frequencies as a function of the R/G ratio in a single run of the 17,26 condition for MK. The beat response is almost independent of the mixture ratio, whereas the 17- and 26-Hz fundamental responses have nulls between 0.4 and 0.6 of the R/G ratio. Figure 4 shows 17,26 condition data obtained from subject SB in two separate sessions approximately 3 weeks apart. The top panel shows amplitudes for the 17-Hz, the 34-Hz (the second harmonic of 17 Hz), and the difference frequency (9 Hz). The bottom panel shows results for the 26- and 52-Hz response components. Data points with a ratio of signal-plus-noise to noise of <3.0 have been omitted from the plot.

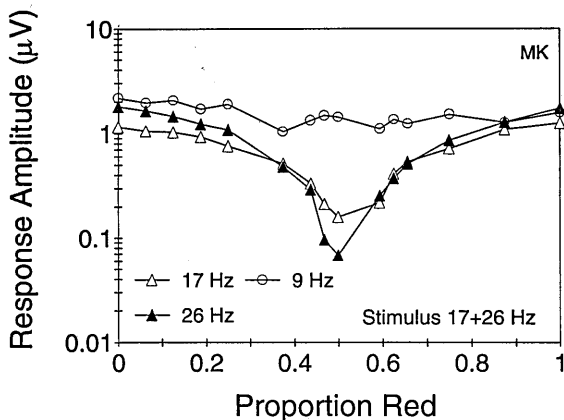


Fig. 3. Amplitude of the fundamental and beat responses as a function of the proportion of red for a single session in which the 17,26 sum-of-sinusoids stimulus was used on subject MK. The proportion of red is the modulation of the red stimulus. In all cases the modulation of the green is 1 minus the modulation of the red stimulus.

Figure 5 shows average responses for all three subjects in all frequency conditions as a function of the R/G ratio. The average amplitude (top) and phase (bottom) are shown for both the fundamental-response components (left-hand panels) and the beat frequency (right-hand panels). The fundamental-response data have been shifted vertically for clarity.

At moderate frequencies ( $\sim 30$  Hz) the fundamental-response component showed cancellation ( $>8:1$ ), but the beat-response component did not show cancellation. It seems that two response domains are evident: a low-frequency region ( $<17$  Hz) and a high-frequency region. In the low-frequency region the fundamental-response components were variable across subjects and showed no systematic null ratio (Figs. 6 and 7). At 17 Hz and above there was a reliable null ratio. The null ratio shifted progressively toward red with increasing frequency for all three subjects. At 44 Hz the null is near the luminance match for each subject (see below). Partial data sets obtained with sinusoidal stimulation revealed no major differences between the fundamental response to single sinusoids and the fundamental response to the sum-of-sinusoids conditions.

#### Beat Responses

For the 5, 14 and 17, 26 conditions the beat responses show no null ratio. There is a shallow null for the beats resulting from higher-frequency stimulation, but the maximum trough represents a decrease in response amplitude by a factor of only  $\sim 4$ , whereas the fundamental response decreases in amplitude by a factor of 10.

#### Response Phase

The response phase at the fundamental frequencies depended systematically on the frequency of stimulation.<sup>19,20</sup> The first-order response phase underwent the expected 180-deg phase shift in the vicinity of the response null. This reversal in phase matches the reversal in phase that is expected from a simple summation of cone responses. The phase of the beat response (see Fig. 5, bottom right) did not change with either the stimulus frequency or the R/G ratio. Variations in the phase of the beat response

observed at high frequencies of stimulation correspond to records in which the signal-to-noise ratio was small.

#### Discussion

These data most clearly resemble the predictions of models that place the nonlinearity before convergence of the cone signals. It is important to note in this context that with the ERG we were sampling the whole 40-deg extent of the central retina. Thus the majority of the responses are presumed to arise from retinal areas in which there is considerable convergence of cone signals. The low- to moderate-frequency data are best described by models incorporating a hard nonlinearity before the site of convergence of cone signals. In fact, we did not generate a model that produced a response as invariant with the proportion of red as was evident in the 17, 26 responses (see Figs. 3 and 4). It is notable that even if the nonlinear responses arise after cone-cone inhibitory processes, the models all agree that there should be a mixture ratio that produces no response. At higher frequencies there is evidence of a dip in the beat response in the vicinity of the photometric match. We conclude that the data are most consistent with a model of the generation of the ERG that includes an early, cone-specific nonlinearity. This conclusion is in accord with recent psychophysical investigations.<sup>26</sup>

There are several other notable features of this set of data. First, the relative insensitivity of the phase of the beat to the stimulus condition is consistent with the same nonlinearity acting under all conditions. That is, the phase of the beat component of the response is a measure

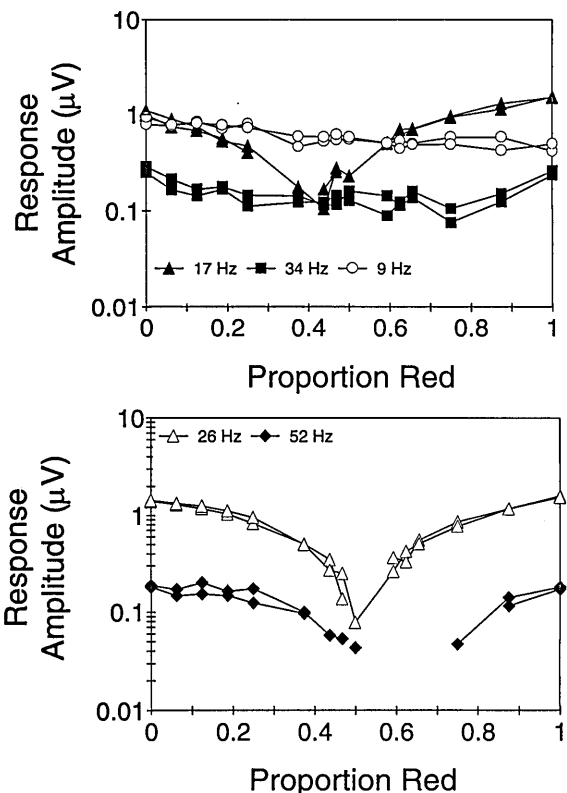


Fig. 4. Response amplitudes for two separate sessions, obtained 3 weeks apart, in which the 17, 26 sum-of-sinusoids stimulus was used on subject SB. Symbols are as in Fig. 3, with the addition of the second-harmonic-response components. Points with estimated signal-to-noise ratios of  $<3.0$  are not plotted.

of the phase with which the two fundamental components interact at the nonlinearity. This is because the phase of the beat follows the envelope of the composite-stimulus waveform. If one of the signals changes in phase, the envelope changes correspondingly. Suppose that there were two nonlinear sites, for instance, one within the cones<sup>26</sup> and a later nonlinearity arising from the amacrine-cell interaction or the construction of nonlinear subunits.<sup>28</sup> In this case it is likely that the phase of the beat generated at these two locations would differ, since the transmission characteristics of signals are liable to change through the retina. Thus if the beat signal recorded in the ERG were a composite signal, with the contributions of the components varying with changes in the proportion of red modulation, then we might expect to see changes in the phase of the beat with changes in stimulus conditions. We do not. The average beat response for the 5, 14 stimulus does deviate from this phase stability, but we have independent evidence (see below) that at low frequencies responses are arising from cone-cone interactions. In addition, the majority of the variation in the phase with the R/G ratio for the 5, 14 stimulus occurred as a result of the responses of subject SB. The other two subjects showed no major

interaction between the phase of the beat response and the R/G ratio at 5 Hz.

Second, the nonlinear components behave differently from ganglion-cell data.<sup>41</sup> For stimulus conditions that were near the R/G null ratio the response was dominated by nonlinear responses. However, the actual amplitude of the nonlinear components did not increase at these ratios; rather, the linear-response components decreased. For the phasic ganglion cells the nonlinear responses increase near isoluminance.<sup>41</sup> There is no pronounced null in the 5- or the 14-Hz fundamental responses (see Figs. 6 and 7), and the fundamental-response phase does not show the 180-deg phase shift that is expected if the response is dominated by either cone responses or signals derived from summed cone responses (not shown). In experiment 2 we investigated possible sources of these response differences to low- and high-frequency stimuli.

## EXPERIMENT 2. ELECTRORETINOGRAM AT LOW FREQUENCIES

Traditionally it is assumed that the low-frequency flicker ERG is dominated by responses from glial cells and photo-

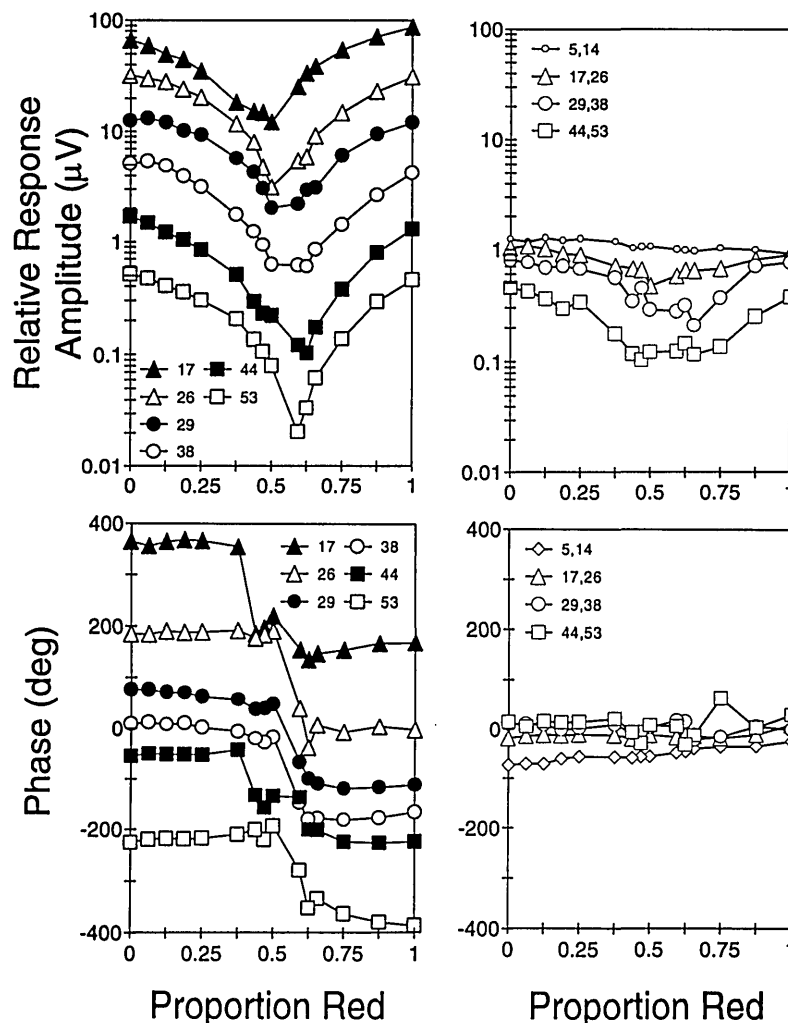


Fig. 5. Average responses for all subjects for all conditions in experiment 1. The top panels show the amplitudes for the fundamental (left) and beat (right) responses. The bottom panels show the response phase. The amplitude and the phase of the fundamental-response components (bottom left) have been shifted vertically for clarity. Fundamental responses have been plotted for stimulus frequencies of 17 Hz and greater. At lower frequencies there was no consistent null, and individual differences precluded averaging.

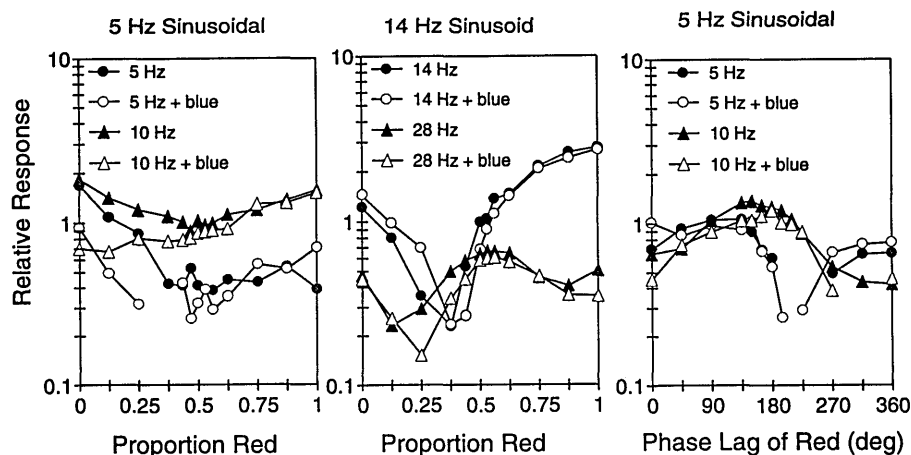


Fig. 6. Effect of the added blue field on the amplitude of the ERG response to sinusoidal stimuli. The left-hand panel shows that the added blue decreased the amplitude of both the fundamental and the second-harmonic responses to 5-Hz flicker. The middle panel shows that the response to 14-Hz flicker is minimally affected by the blue field. The right-hand panel shows the responses to changing the phase of the red light relative to the green light at a proportion of red of 0.5. Data points with a ratio of signal-plus-noise to noise of  $<3.0$  are not plotted.

receptor cells, the primary contributors to the *b* wave and the *a* wave seen in the flash electroretinogram. Since data from experiment 1, as well as data of other investigators,<sup>19</sup> suggest that there are qualitative differences between responses to frequencies higher and lower than 17 Hz, we investigated whether either rod intrusion or some form of cone-originated neural interaction could give rise to the low-frequency results of experiment 1. To test for possible rod intrusion we added a steady rod background that doubled the scotopic retinal illuminance of the field while only slightly changing the modulation for the LWS and MWS cones. Since the potential rod modulation arises mainly from modulation of the green light, the rod contribution should decrease linearly with increases in the proportion of red modulation. Since rod pathways are slower than cone pathways, a rod contribution should produce an effect of the phase of the stimuli on the ERG responses. To examine potential cone-cone antagonism, we varied the phase difference between the red and the green stimuli at a constant modulation.

### Methods

The conditions used in experiment 1 had a scotopic retinal illuminance of 3.9 log scotopic Td, an intensity that is expected to saturate the rod responses.<sup>42</sup> To increase further the likelihood of rod saturation we added a steady, 3.9-log-scotopic-Td, 488-nm light to the test field. The added blue field reduced rod modulation by 50% but reduced MWS modulation by only ~12%. We used 100%-modulated temporal sine waves to improve the signal-to-noise ratio, which tends to be poor at low frequencies. To test further for rod contributions we also decreased the retinal illuminance of the original field by 1.0 log unit and replicated some of the conditions from experiment 1. In a second set of conditions we fixed the proportion of red modulation at 0.5 and varied the relative phases of the red and green stimuli. This was done with 5- and 14-Hz sinusoidal stimulation with and without the blue field, and it was also done for a series of sinusoidal frequencies and sum-of-sinusoid conditions without the blue field. For sinusoidal stimulation the modulation of each frequency was twice the modulation of that frequency used in the

sum of two sinusoids conditions. Portions of experiment 2 were performed on all three subjects; however, we did not perform every condition on all subjects.

### Results

There was a clear effect of the blue stimulus on the ERG for a 5-Hz stimulation frequency (Fig. 6, left-hand panel)

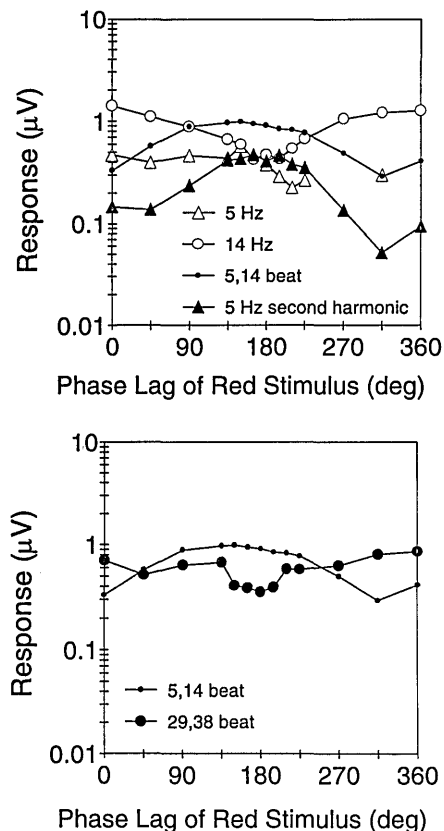


Fig. 7. Effect of phase on the flicker ERG. The top panel shows that the nonlinear-response components elicited by a sum of 5- and 14-Hz sine waves increase sharply when the red and green stimuli are out of phase. The bottom panel compares the amplitude of the 9-Hz beat elicited by a 5,14 beat and a 29,38 beat as a function of the phases of the red and green stimuli. Data points with a ratio of signal-plus-noise to noise of  $<3.0$  are not plotted.

but not for 14-Hz (Fig. 6, middle panel) or higher (not shown). The effect was consistently one of lower response to the green stimulus. This asymmetric effect was seen in both the fundamental response and the second-harmonic response to 5-Hz sine-wave stimulation (Fig. 6, left-hand panel). When we decreased the retinal illuminance of the ERG stimulus we did not find a major change in the shape of the response versus proportion-red curves or in the fact that the beat response was independent of proportion (not shown).

There was a large effect of the relative phases of the red and green stimuli on both the linear and the nonlinear response components (Fig. 6, right-hand panel, and Fig. 7) at low frequencies. The blue field did not appreciably affect the response with changes in the relative phases of the red and green stimuli (Fig. 6, right-hand panel). For the 5, 14 stimulus condition the nonlinear response component was larger when red and green stimuli were out of phase than when they were in phase. This result was measured in all three subjects. The fundamental-response components varied with the frequency of stimulation in their response to changes in phase (not shown). There was a slight decrease in the beat response when the red and green stimuli were out of phase in the 29, 38 condition (Fig. 7, bottom panel).

### Discussion

The results with the blue adapting field show that at high frequencies there is no evidence for rod contributions to the ERG responses. Thus the majority of behavior seen in Figs. 3–5 cannot be attributed to rod responses. This is to be expected since at best the rod response is only minimally modulated at these high frequencies. We are left with the fact that the added blue light caused large changes in the low-frequency ERG response, but the results were not affected significantly by decreasing the retinal illuminance. In addition, the ERG response to different phases of the red and green light at equal modulation was not affected by the presence of the steady blue field. These results cannot rule out a rod contribution to the ERG results from experiment 1 that include a 5-Hz stimulus, but they lead us to suspect that a cone–cone interaction, potentially involving short-wavelength-sensitive-cone stimulation, is a more likely cause of the 5-Hz results. This possibility is supported by the strong dependence of the response amplitude on the relative phases of the red and green lights (Figs. 6 and 7). The amplitudes of the nonlinear components of the ERG are large when the red and green lights are flickering in counterphase (see also Donovan and Baron<sup>43</sup>). The finding of a nonlinear response to color implies that a large proportion of the low-frequency ERG response is contributed after a stage of cone–cone interaction. Note that the data still require that there be a nonlinearity before, or at, the site of combination of cone signals, since otherwise we would find evidence for a null in the mixture experiments. There is no evidence that this cone–cone interaction contributes significantly to the response at higher frequencies, since there is a slight dip in the amplitude of the beat when the red and green stimuli are 180 deg out of phase in the 29, 38 condition (Fig. 7, bottom panel). This dip for higher stimulus frequencies may be due to the decreased cone modulation for this condition. Thus we believe that the results obtained at fre-

quencies above 14 Hz in experiment 1 are not affected by rods. Results from the use of stimulus frequencies of 5 and 14 Hz are best explained by the existence of an early nonlinearity followed by a stage of cone–cone interaction. This stage does not seem to correspond to a typical psychophysically determined color-opponent stage, since it is quite nonlinear and may represent a nonlinear gain control involving all three cone classes.

### EXPERIMENT 3. COMPARISON OF THE ELECTRORETINOGRAM AND PSYCHOPHYSICS

It is often assumed that flicker-photometry samples the average retinal contribution of the LWS and MWS cones; that is, it is thought to be sensitive to the relative number of each cone type in the stimulus field. Similarly, the flicker ERG represents a mass summation (in this case at the cornea) of currents generated by stimulation of all the cones in the stimulus field. If the two techniques give similar estimates of the relative contributions of LWS and MWS signals, then we have additional evidence for the summation by flicker photometry. To examine this ques-

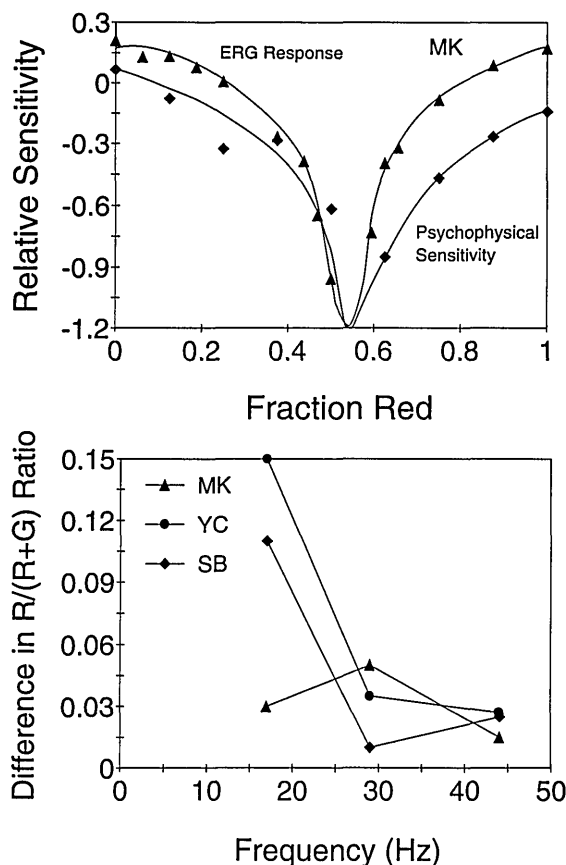


Fig. 8. Comparison of psychophysical-modulation sensitivity and ERG-fundamental-response amplitude. The top panel compares data obtained with each technique as a function of the proportion of red in a 29-Hz stimulus for subject MK. The symbols represent either the amplitude of the ERG (triangles) or the psychophysical sensitivity (diamonds). The solid curves are drawn to go through the data, and the minimum is taken as the photometric match as determined by that technique. The bottom panel shows the difference between the psychophysical and the ERG-determined matches at 17, 29, and 44 Hz for all three subjects.



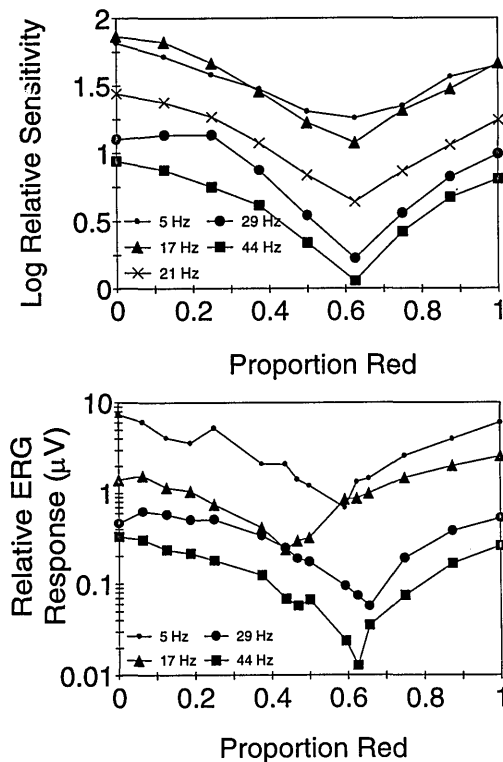


Fig. 9. Comparison of psychophysical-modulation thresholds (top) and ERG-fundamental-response amplitudes (bottom) for subject YC. Responses have been shifted vertically for clarity.

tion we measured flicker-photometric sensitivity by using the same stimuli as were used for the ERG recordings.

### Methods

Psychophysical estimates of the isoluminance ratio were determined by measurement of the modulation threshold for different ratios of red and green modulation. In a single session nine R/G ratios were tested at a single frequency with a yes-no staircase technique. Staircases for each ratio were randomly interleaved within a session. A stimulus period was 3 s. Modulation was linearly ramped to the maximum for that trial for the first 0.5 s, remained constant for 2 s, and then was ramped back down to zero during the remaining 0.5 s. The long trial durations were adopted to permit detection of low-frequency beat components that were generated in companion experiments. Each stimulus period was preceded by a warning tone. Subjects estimated the null proportions for both methods by drawing a smooth curve through the data that went to zero at some proportion of red (see below). Subjects SB, MK, and YC made measurements at 17, 29, and 44 Hz. MK and YC also made measurements at 5 and 21 Hz.

### Results

Figure 8 compares the psychophysical sensitivity and the ERG-response amplitude for subject MK at 29 Hz. The response amplitude was chosen since the amplitude-versus-modulation function of the ERG is well behaved,<sup>18</sup> and the same minima should be found regardless of whether a criterion-response measure or an amplitude measure is used. The R/G-ratio differences (indicated by the separation of the minima for the two curves) are

plotted as a function of stimulus frequency in the bottom panel of Fig. 8. At frequencies of 29 Hz and above, the two techniques give similar estimates of the relative proportions of red and green. The change between the photometric estimates at lower frequencies arose primarily from the ERG measurements. Figure 9 compares full psychophysical-modulation-sensitivity curves (top) with ERG-response curves (bottom) for subject YC. The psychophysically determined minima for all subjects were essentially invariant with stimulus frequency; both beat stimuli (not shown) and sinusoidal stimuli changed an average of only 0.007 in R/(R + G) ratio from 20 to 40 Hz. The ERG minima, however, were frequency dependent, moving to higher proportions of red with increasing frequency (see Fig. 5) for all three subjects, with an average change of ratio of 0.04 from 20 to 40 Hz. The variations with frequency measured by using the ERG were not an indication of noisy data, since they were generally replicable (see Fig. 4). At low frequencies the ERG null proportions differed across subjects, but these individual differences decreased with increasing stimulus frequency.

### Discussion

The agreement of psychophysical and electrophysiological measures of the relative luminance of the red and green stimuli supports the idea that the two are sampling the relative proportions of cones in the retina. Since the proportions of LWS and MWS cones do not change with retinal eccentricity, at least near the fovea,<sup>44</sup> use of the ERG may be a good way to assess the relative numbers of the two cone types quickly. However, it is not clear why the ERG photometric match changes with frequency. It is possible that the fundamental responses include multiple responding neural units, even at frequencies of 17 Hz and above, and that the LWS and MWS input to these different mechanisms varies. It is clear that there is a great deal more going on in the fundamental components of the ERG response than simply a summation of photoreceptor currents, even for our 17-Hz stimulation. In this respect it is interesting to note that when the subjects set modulation thresholds for the beat stimuli, thresholds behaved differently than the ERG results would lead one to predict. There was no evidence for an increased sensitivity to the beat stimuli, as opposed to the sinusoidal stimulus, at isoluminance. In all cases the subjects did not detect the beat until they were above threshold for the sinusoidal components of the stimuli. Thus we are left with the observation that the ERG results reflect retinal activity that cannot be simply assessed by performing this type of psychophysical experiment.

### CONCLUSIONS

These experiments are consistent with the following conclusions:

1. The nonlinear response of the retina, measured at stimulation frequencies of 17 Hz and above, is generated before the convergence of cone signals and thus is consistent with a locus in the cones or the cone pedicles.
2. At low frequencies of stimulation, the ERG response has a large response component that arises from cone-cone interactions.

3. At high frequencies the ERG and heterochromatic-modulation photometry give similar estimates of the proportions of LWS and MWS cones in the retina.

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