

Stimulus chromatic properties affect period doubling in the human cone flicker ERG

Sowjanya Gowrisankaran · Kenneth R. Alexander

Received: 23 January 2012 / Accepted: 16 April 2012 / Published online: 13 May 2012
© Springer-Verlag 2012

Abstract Period doubling in the full-field cone flicker electroretinogram (ERG) refers to an alternation in waveform amplitude and/or shape from cycle to cycle, presumably owing to the operation of a nonlinear gain control mechanism. This study examined the influence of stimulus chromatic properties on the characteristics of period doubling in order to better understand the underlying mechanism. ERGs were acquired from 5 visually normal subjects in response to sinusoidally modulated flicker presented at frequencies from 25 to 100 Hz. The test stimuli and the pre-stimulus adaptation were either long wavelength (R), middle wavelength (G), or an equal combination of long and middle wavelengths (Y), all equated for photopic luminance. Fourier analysis was used to obtain the response amplitude at the stimulus frequency F and at a harmonic frequency of $3F/2$, which was used as the index of period doubling. The frequency–response function for $3F/2$ typically showed two peaks, occurring at approximately 33.3 and 50 Hz. However, the magnitude of period doubling within these frequency regions was dependent on the chromatic properties of both the test stimulus and the pre-stimulus adaptation. Period doubling was generally smallest when an R test was used, even

though the stimuli were luminance-equated and the amplitude of F did not differ between the various conditions. The pattern of results indicates that the mechanism that generates period doubling is influenced by chromatic signals from both the test stimulus and the pre-stimulus adaptation, even though the high stimulus frequencies presumably favor the achromatic luminance system.

Keywords Electroretinography · ERG · Chromatic · Flicker · Luminance · Period doubling

Introduction

The light-adapted flicker electroretinogram (ERG), typically measured at a stimulus frequency of approximately 30 Hz, is an important clinical tool for assessing the integrity of the cone system in ocular diseases. Because of its proven clinical value, the flicker ERG was incorporated into the protocol recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) [1]. Nevertheless, there are aspects of the light-adapted flicker ERG that remain incompletely understood. In particular, the phenomenon of period doubling in the flicker ERG has received relatively little attention.

Period doubling refers to a condition in which a system switches to a new behavior with a period twice that of the initial response. This phenomenon typically

S. Gowrisankaran · K. R. Alexander (✉)
Department of Ophthalmology and Visual Sciences,
University of Illinois at Chicago, 1855 W. Taylor St,
Chicago, IL 60612, USA
e-mail: kennalex@uic.edu

indicates that the system is approaching a transition from a stable periodic state to a chaotic state. The phenomenon of period doubling has been described in many nonlinear dynamical systems, including biological systems [2–4]. In particular, period doubling has been observed in electrophysiological recordings from the visual system, including the ERG of salamander [5], rat [6], rabbit [7], and human [5, 8]; in ganglion cell recordings from mouse retina [9]; and in the human visual-evoked potential [5]. It has been proposed that period doubling within the visual pathway may represent the operation of a nonlinear retinal feedback mechanism that alters response gain [5].

In the flicker ERG of the human cone system, period doubling can be observed as an alternation in response amplitude from cycle to cycle and/or a change in waveform shape that has a period twice that of the stimulus period. Period doubling is also evident in the Fourier spectrum of the ERG waveform as a subharmonic ($F/2$) that occurs at half the stimulus frequency (F) and at harmonics of $F/2$ that are generated by retinal nonlinearities [5, 8]. In the human flicker ERG, period doubling is typically observed at stimulus frequencies between approximately 30 and 70 Hz [5, 10, 11]. However, the magnitude of period doubling and the frequency region over which it occurs can vary somewhat among visually normal subjects [8, 10]. In addition, period doubling is affected by stimulus contrast, such that the amplitude of period doubling is reduced and the region of period doubling shifts to higher frequencies as stimulus contrast is decreased [10]. Moreover, within the frequency range of period doubling, two distinct regions with different contrast–response properties have been reported [10].

Period doubling is often not observed in clinical ERG recordings because multiple sweeps are usually averaged and these sweeps are typically not synchronized with the onset of the flicker train. The random averaging of even and odd cycles would tend to obscure any period doubling that might exist. Furthermore, the greatest amount of period doubling is typically found at frequencies higher than the standard clinical test frequency of approximately 30 Hz.

At the high frequencies at which period doubling occurs in humans, the flicker ERG is presumably driven by the luminance mechanism [12], so that luminance-equated stimuli with different chromatic properties should nevertheless result in the same magnitude of period doubling. However, there is both

psychophysical and electrophysiological evidence for a chromatic input to the luminance system. For example, psychophysical flicker sensitivity is affected more by a long-wavelength than by a luminance-equated middle-wavelength adapting field [13–15]. This finding has generally been interpreted as evidence that long-wavelength adapting fields reduce the relative weight of the long-wavelength (L) cone input to the luminance system compared to input from the middle-wavelength (M) cones. Long-wavelength adaptation can also inhibit the response of cells within the magnocellular (MC) pathway [16], which are presumed to form the physiological substrate of the luminance channel [17], and MC cells can respond, although weakly, to red–green chromatic modulation [18, 19]. The psychophysical and electrophysiological results have generally been modeled by assuming that there is a cone opponent input to the luminance system that involves both M and L cones [13, 14, 18].

It has also been reported that chromatic adaptation can affect the properties of the high-frequency flicker ERG [20, 21]. Specifically, adaptation to a reddish background decreases ERG responses at long wavelengths compared to neutral adaptation, whereas adaptation to a greenish background has only a minimal effect on ERG amplitude. The fact that chromatic adaptation can affect the magnitude of the high-frequency flicker ERG raises the possibility that period doubling may also be affected. In fact, in a preliminary investigation, we observed that the magnitude of period doubling appeared to differ for stimuli of different wavelengths that were luminance-equated. The purpose of this study was to evaluate this effect more systematically. ERGs were acquired using temporally modulated test stimuli and steady pre-stimulus adaptation that had different excitation ratios for the L and M cones, ranging from approximately equal cone excitation to excitation that was strongly biased toward L cones. The intent was to provide new constraints regarding the possible physiological mechanism(s) underlying period doubling in the human cone flicker ERG.

Methods

Subjects

Five visually normal individuals—aged 22 (S_1 and S_2), 30 (S_3), 57 (S_4), and 59 (S_5) years—participated in

the study. Subject S_4 was a woman; the others were men. All subjects had best-corrected visual acuity of 20/20 or better in each eye and normal color vision. The study protocol was approved by an institutional review board of the University of Illinois at Chicago, and all subjects gave informed consent before participating in the study.

Stimuli and recording system

Stimuli were generated by arrays of light-emitting diodes (LEDs) and were presented in a Diagnosys ColorDome desktop Ganzfeld (Diagnosys LLC, Littleton, MA). Test stimuli consisted of sinusoidally modulated full-field luminance flicker that was either long-wavelength (peak wavelength: 640 nm [R_T]), middle-wavelength (peak wavelength: 516 nm [G_T]), or a combination of equal luminances of these long and middle wavelengths [Y_T]. In addition, three pre-stimulus adapting conditions were used (R_A , Y_A , and G_A) derived from the same LEDs, so that there were nine possible combinations of pre-stimulus adaptation and test stimulus ($G_A G_T$, $G_A Y_T$, $G_A R_T$, $Y_A G_T$, $Y_A Y_T$, $Y_A R_T$, $R_A G_T$, $R_A Y_T$, and $R_A R_T$). All stimuli were presented against a short-wavelength (peak wavelength: 464 nm), rod-saturating background with a luminance of 12.3 cd/m^2 (39.7 scot cd/m^2 or 3.3 log scot td , based on a dilated pupil diameter of 8 mm). Test stimuli were presented at frequencies ranging from 25 to 100 Hz, with a duration of approximately 1 s (the exact duration was dependent on the stimulus period).

The mean luminance of each of the test stimuli was 200 cd/m^2 and the nominal Michelson contrast was 100 %; although against the short-wavelength field, the effective contrast was 94.2 %. The luminance of each of the pre-stimulus adapting fields was also 200 cd/m^2 . The luminance and spectral characteristics of the stimuli were calibrated using a spectroradiometer (PR-650 SpectraScan colorimeter, Photo Research, Inc., CA). Photopic luminances were based on the 10-degree luminous efficiency function [$V_{10}(\lambda)$], given that the non-foveal retina is the major contributor to the full-field ERG. The $L/(L + M)$ cone excitation ratios for the G, Y, and R stimuli were 0.56, 0.72, and 0.89, respectively [22].

Electroretinograms were recorded using a DTL electrode referenced to the forehead, with an ipsilateral earlobe ground electrode. Signals were acquired

with a Diagnosys E^2 electrophysiology console, using a sampling frequency of 2 kHz and an amplifier band-pass setting of 0.3–500 Hz. The ERG recordings were synchronized with the onset of the flicker presentation.

Procedure

Electroretinogram testing was done monocularly with the non-tested eye occluded. Prior to the ERG recordings, the pupil of the tested eye was dilated using 2.5 % phenylephrine and 1 % tropicamide drops. The DTL electrode was then inserted under room illumination. The nine conditions were presented pseudorandomly across three sessions of approximately 1 h each. During each session, the same adapting condition (G_A , Y_A , or R_A) was used throughout. Before presenting the first test stimulus, subjects were exposed to the adapting field for 3 min. Then, a test stimulus (G_T , Y_T , or R_T) was chosen at random and the test stimuli were presented in order of increasing temporal frequency. The test stimulus presentation was repeated at each frequency until five reliable ERG responses were obtained. Between each presentation of the test stimulus, the appropriate adapting field was presented for 5 s. Following the acquisition of each set of recordings for a given chromatic test stimulus, the adapting field was presented for 1 min before acquiring a set of recordings across frequency for the next chromatic test stimulus.

Analysis

Harmonic components of the ERG responses were obtained using a fast Fourier transform (FFT) implemented in Matlab. In order to avoid onset and offset transients, approximately 200 ms of the initial part of the waveform and approximately 100 ms of the end of the waveform were omitted, so that the segment of the waveform that was Fourier-analyzed was approximately 700 ms in length (the exact duration was dependent on the stimulus period and was always an even number of cycles).

An example of this approach is illustrated in Figs. 1 and 2, which present a typical ERG waveform and its Fourier spectrum, respectively. The waveform shown in Fig. 1 represents the mean of 5 responses from S_1

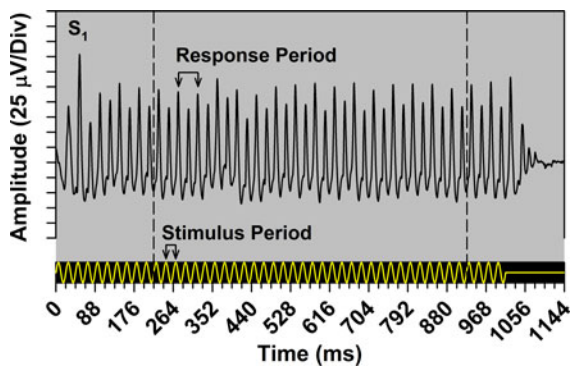


Fig. 1 Mean of 5 ERG responses of S_1 for the $Y_A Y_T$ condition at a stimulus frequency of 45.4 Hz, with the stimulus waveform represented along the x -axis, and the stimulus and response periods indicated by arrows. Vertical dashed lines indicate the portion of the waveform used in the Fourier analysis

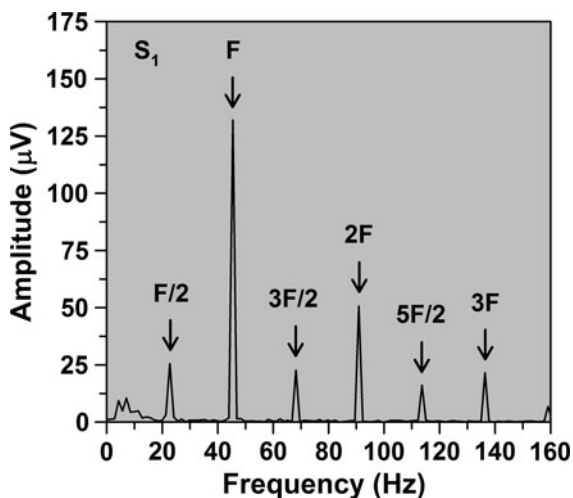


Fig. 2 Fourier spectrum of the portion of the ERG waveform in Fig. 1 demarcated by the vertical dashed lines. Arrows and labels indicate the various harmonic components. Peaks at $F/2$ and its odd multiples indicate the presence of period doubling

that were elicited by Y_T at a frequency of 45.4 Hz and that was preceded by Y_A . The test stimulus, which is indicated by the trace plotted along the x -axis, had a period of 22 ms, as indicated by the arrows. Following an initial onset transient, the ERG waveform began to show an alternation in amplitude, such that the response period (indicated by the arrows above the ERG trace) was twice the stimulus period. This amplitude alternation represents period doubling.

Figure 2 illustrates the amplitude spectrum obtained from the Fourier analysis of the waveform segment between the vertical dashed lines in Fig. 1.

The arrows in Fig. 2 indicate the various spectral components of the ERG. In addition to the fundamental (F) and its even and odd harmonics ($2F$ and $3F$), there were also components at a subharmonic of F ($F/2$) and its odd harmonics ($3F/2$ and $5F/2$; the even harmonics of $F/2$ are indistinguishable from the fundamental and its harmonics). The presence of components at $F/2$ and its odd harmonics is indicative of period doubling.

For each test condition, the derived amplitudes at $F/2$, F , and $3F/2$ were compared to a noise estimate defined as the mean of the amplitudes at the neighboring frequencies, which were approximately 2 Hz on either side of the frequency of interest. Amplitudes that were less than three times the noise estimate (corresponding to a signal-to-noise ratio of 2) were considered non-detectable and are plotted as 0 amplitude in Fig. 3 and as “ND” in Figs. 4, 5, and 6. Consistent with previous observations [5, 8], the amplitude of $F/2$ was generally low and typically did not exceed the noise level, even though it was readily apparent in the data shown in Fig. 2. This has been attributed previously to an attenuation of the $F/2$ harmonic at a site subsequent to the nonlinearity that generates the higher harmonics of the flicker ERG [8]. Therefore, as in previous studies [5, 10], the amplitude of $3F/2$ was selected as the most representative index of period doubling.

Results

Figure 3 plots the amplitude of $3F/2$ vs. stimulus frequency for S_1 . The top graph shows the results for the homochromatic conditions, in which the adaptation and test stimuli had the same chromatic properties. The bottom graph shows the results for the conditions in which R_T was used and the adapting stimulus was altered (the condition $R_A R_T$ was included in both graphs, so that its properties could be seen more clearly). The results for the other four conditions ($G_A Y_T$, $Y_A G_T$, $R_A G_T$, and $R_A Y_T$) did not differ systematically from those for $G_A G_T$ and $Y_A Y_T$ for this subject or any of the other subjects and are not illustrated. The implications of this result are considered in the “Discussion.”

In agreement with a previous report [10], the frequency–response function for the $Y_A Y_T$ condition (Fig. 3, top) showed two distinct regions of period

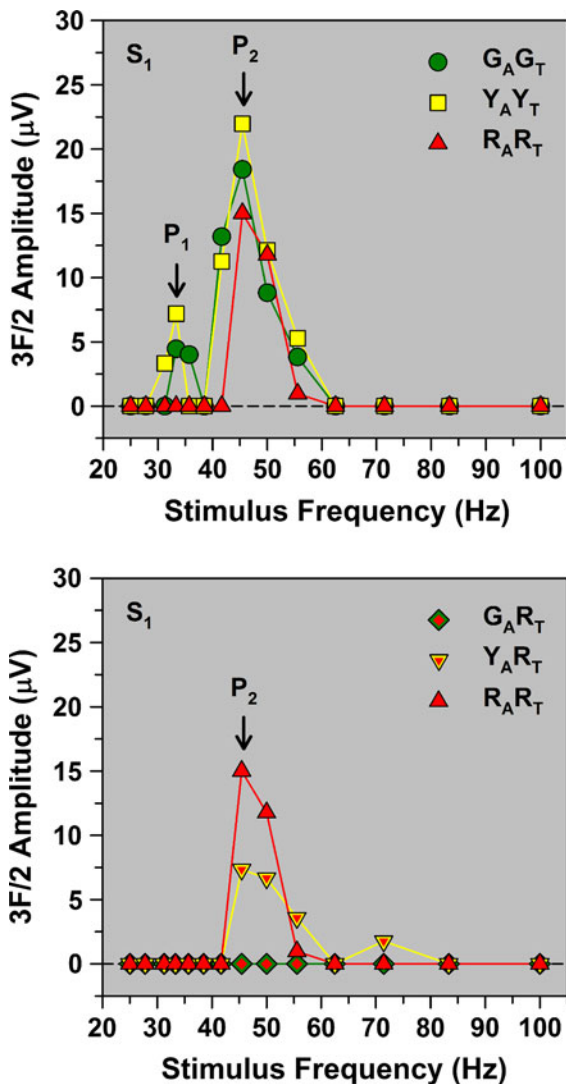


Fig. 3 Amplitude of 3F/2 for S_1 as a function of stimulus frequency for the homochromatic conditions (top) and for conditions in which the test stimulus was long wavelength (bottom). Arrows indicate the lower-frequency peak (P_1) and higher-frequency peak (P_2) of the response functions. The $R_A R_T$ condition is included in both plots for comparison

doubling, with a peak at 33.3 Hz (labeled P_1) and a second larger peak at 45.4 Hz (labeled P_2), and with an amplitude minimum between the two peaks. This was also the case for the $G_A G_T$ condition (Fig. 3, top), although the amplitude of 3F/2 was slightly lower for this condition. However, this subject showed no peak at P_1 in the frequency–response function for R_T (Fig. 3, top), regardless of the pre-stimulus adaptation (Fig. 3, bottom).

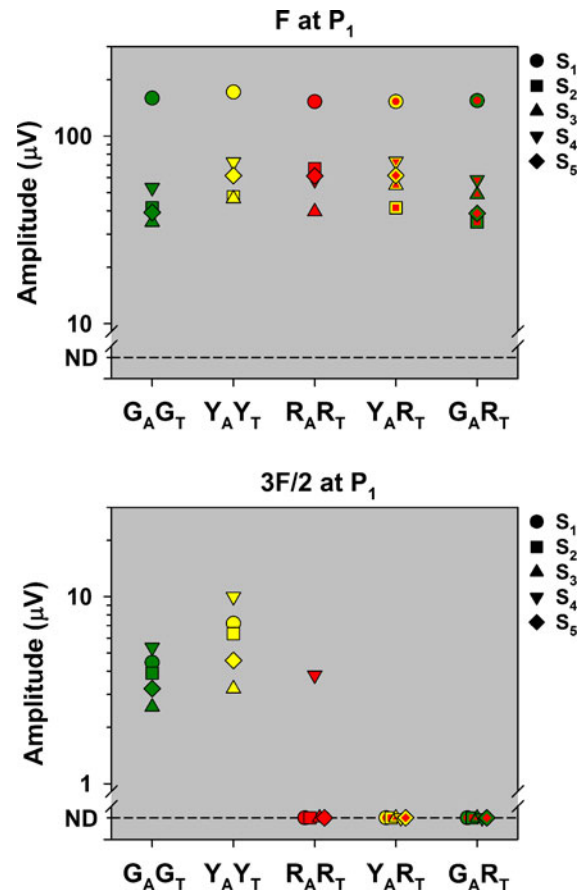


Fig. 4 Amplitude of F (top) and 3F/2 (bottom) at P_1 for the individual subjects (indicated by the key) for the 5 chromatic conditions indicated along the x-axis. Data points along the dashed line labeled “ND” have been jittered horizontally for clarity

At P_2 , in comparison, the magnitude of period doubling using R_T was strongly influenced by the state of chromatic adaptation (Fig. 3, bottom). That is, the greatest amplitude of 3F/2 at P_2 occurred for the $R_A R_T$ condition, and the amplitude of 3F/2 decreased with an increasing difference between the relative cone excitations for the adaptation and test conditions. In fact, this particular subject showed no period doubling at any stimulus frequency when R_T was preceded by G_A , which entailed a change in the ratio of cone excitations from 0.56 (adaptation) to 0.89 (test).

In order to determine whether a similar pattern of results held true for the other subjects, we examined the amplitude of 3F/2 at P_1 and P_2 , as well as the amplitude of F at those same stimulus frequencies. A complication is that the stimulus frequencies at which

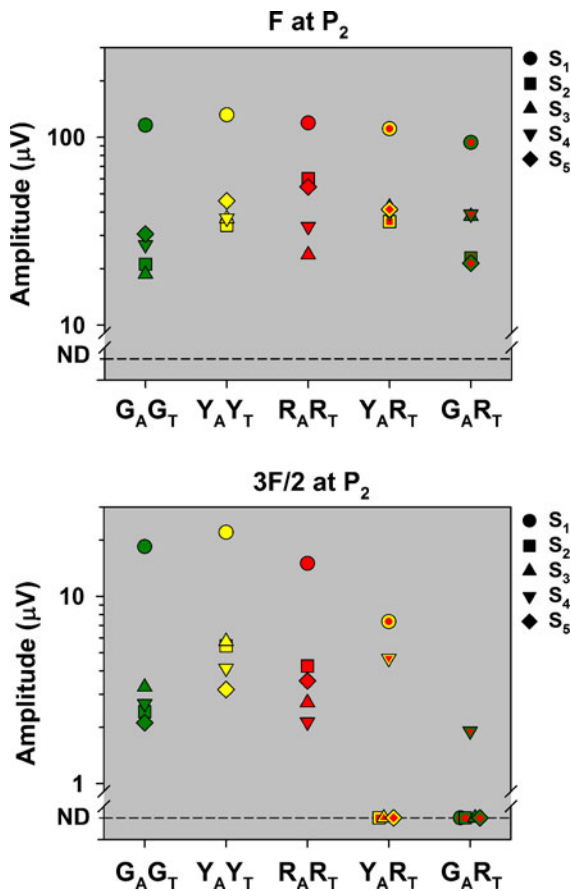


Fig. 5 Amplitude of F (*top*) and 3F/2 (*bottom*) at P₂ for the individual subjects (indicated by the key) for the 5 chromatic conditions indicated along the x-axis. Data points along the dashed line labeled “ND” have been jittered horizontally for clarity

the maximum 3F/2 response occurred varied among the subjects, and the frequency of maximum response also differed slightly between chromatic conditions for a given subject. The lower-frequency peak occurred most frequently at 33.3 Hz (range, 33.3–41.6 Hz), and the higher-frequency peak occurred most frequently at 50 Hz (range, 45.4–55 Hz). For a given subject and a given condition, P₁ was defined as the stimulus frequency at which the maximum 3F/2 amplitude occurred across the frequency region of 30–45 Hz, and P₂ was defined as the stimulus frequency at which the maximum 3F/2 amplitude occurred at stimulus frequencies between 45 and 60 Hz. For some conditions, the 3F/2 amplitude was not significantly different from noise at stimulus frequencies between 30 and 45 Hz or between 45 and 60 Hz. In those cases, the

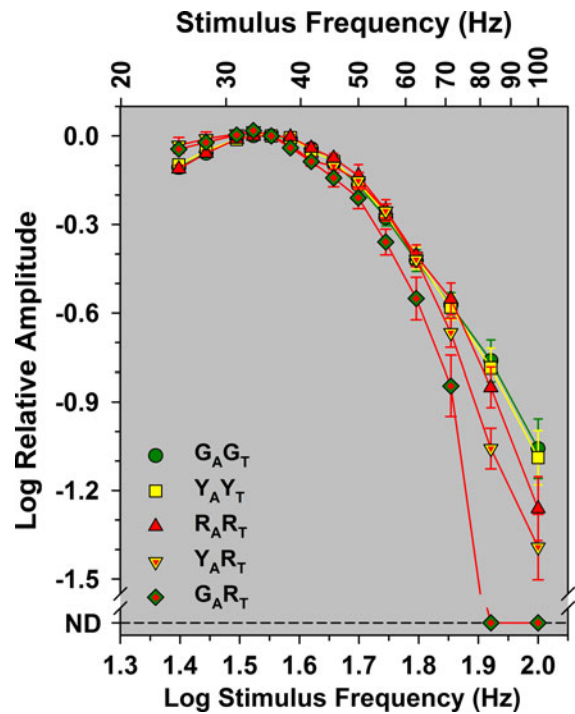


Fig. 6 Mean log relative amplitude of F as a function of log stimulus frequency for the chromatic conditions indicated in the key. Amplitudes have been normalized at 35.7 Hz. Error bars represent standard errors of the means. Linear values of the stimulus frequencies are indicated along the top x-axis

corresponding amplitude of F was identified based on the frequencies at which P₁ and P₂ occurred most commonly for the other test conditions for that subject.

The results for the individual subjects are plotted in Figs. 4 and 5. In these two figures, the top graphs plot the amplitudes of F and the bottom graphs plot the amplitudes of 3F/2. Figure 4 shows the results at P₁ and Fig. 5 shows the results at P₂. The amplitude of F at P₁ (Fig. 4, top) was similar across all these chromatic conditions for all of the subjects. In comparison, the amplitude of 3F/2 at P₁ was markedly different across conditions, in agreement with the results shown for S₁ in Fig. 3. All subjects showed appreciable period doubling at P₁ for the homochromatic conditions of G_AG_T and Y_AY_T. However, period doubling was essentially absent when R_T was used, regardless of the nature of the pre-stimulus adaptation. That is, the amplitude of 3F/2 at P₁ (Fig. 4, bottom) was non-detectable for the R_T stimulus for all subjects when it was preceded by G_A or Y_A, and only one subject showed detectable period doubling for R_T preceded by R_A.

Figure 5 presents the amplitudes of F (top) and $3F/2$ (bottom) at P_2 for all subjects. Similar to the results at P_1 , the chromatic properties of the pre-stimulus adaptation and test stimuli did not have a systematic effect on the amplitude of F at P_2 (Fig. 5, top). However, the amplitude of $3F/2$ at P_2 for the R_T stimulus was strongly influenced by the chromatic characteristics of the pre-stimulus adaptation for all subjects. That is, when R_T was used, adaptation to R_A resulted in period doubling at P_2 similar to that obtained in response to the homochromatic conditions of $G_A G_T$ and $Y_A Y_T$. However, period doubling at P_2 was non-detectable for 3 of the 5 subjects for the $Y_A R_T$ condition and was non-detectable for 4 of the 5 subjects for the $G_A R_T$ condition.

Although the chromatic properties of the pre-stimulus adaptation and the test stimulus appeared to have little effect on the amplitude of F in Figs. 4 and 5, previous studies have reported that the amplitude of the flicker ERG is reduced preferentially by long-wavelength adaptation [20, 21]. To examine this further under the present test conditions, we plotted the amplitude of F across stimulus frequency for the various adaptation/test combinations represented in Figs. 4 and 5. The mean results are shown in Fig. 6. In order to evaluate the shapes of the frequency–response functions, the functions were normalized at the peak (35.7 Hz).

The functions for the homochromatic conditions of $G_A G_T$ and $Y_A Y_T$ had similar shapes and corresponded to previous results for an achromatic test stimulus [23]. That is, the function was band-pass, with a peak at 35.7 Hz and a systematic decline in amplitude at higher temporal frequencies. When R_T was used, the amplitude of F at the highest stimulus frequencies was lower than for these other two conditions. The amplitude loss at high frequencies became greater as the spectral difference between the pre-stimulus adaptation and the test stimulus increased. In fact, when the long-wavelength test stimulus was preceded by middle-wavelength adaptation ($G_A R_T$ condition), there was no detectable ERG response at the two highest temporal frequencies.

Discussion

The aim of this study was to characterize the effect of stimulus chromatic properties on period doubling in

the human cone flicker ERG. At the high temporal frequencies at which period doubling occurs (i.e., approximately 30–70 Hz), it might be expected that luminance-equated stimuli with different wavelength composition would nevertheless yield the same magnitude of period doubling, because the luminance system would govern performance. On the other hand, there is evidence that long-wavelength adaptation can reduce preferentially the amplitude of the flicker ERG response within this frequency region [20, 21]. Therefore, the magnitude of period doubling might also be affected. In agreement with this latter possibility, we observed that the characteristics of period doubling were affected by the chromatic properties of a temporally modulated test stimulus as well as by the nature of the pre-stimulus adaptation. Moreover, the effect of chromatic adaptation varied systematically with stimulus temporal frequency.

Consistent with a previous report [10], the frequency–response function for $3F/2$, which represents period doubling, typically showed two distinct regions: a lower-frequency region between approximately 30 and 45 Hz with a peak (P_1) near 33.3 Hz and a higher-frequency region between approximately 45 and 60 Hz with a peak (P_2) near 50 Hz. At P_1 , period doubling was robust for all of the G_T and Y_T conditions, regardless of the nature of the pre-stimulus adaptation. However, period doubling was essentially absent at P_1 when a long-wavelength test stimulus was used. That is, no subject showed period doubling for the $Y_A R_T$ and $G_A R_T$ conditions, and only one subject showed measurable period doubling for the $R_A R_T$ condition (Fig. 4). Thus, the chromatic properties of the test stimulus but not of the pre-stimulus adaptation determined the magnitude of period doubling at P_1 . At P_2 , in comparison, the amplitude of $3F/2$ was equivalent for all three test stimuli under the homochromatic conditions of $G_A G_T$, $Y_A Y_T$, and $R_A R_T$. However, the magnitude of period doubling at P_2 was strongly influenced by the nature of the pre-stimulus adaptation when a long-wavelength test stimulus was used. Specifically, period doubling at P_2 was reduced maximally when the excitation ratio for the L and M cones generated by the pre-stimulus adaptation diverged from that produced by the test stimulus (i.e., $G_A R_T$ condition, Fig. 5).

A plausible model for some of these chromatic effects on period doubling is that of Eisner and MacLeod [13], who hypothesized, based on

psychophysical data, that the relative contribution of signals from *L* and *M* cones to the luminance system is influenced by a chromatically opponent mechanism. According to their model, the signal within each cone pathway is subject to nonlinear feedback from both classes of cones before summing into the luminance system. It is the balance of excitation between the two cone types rather than the magnitude of the mean cone excitation that determines the level of neural activity within the luminance system. This model has been employed to account for the apparent reduction in the *L* cone input to the luminance system in the presence of long-wavelength adapting fields [13, 15], and it can also potentially explain the reduced period doubling observed at P_1 using a long-wavelength test stimulus.

The model also predicts that, when an adapting field changes color so that one cone type becomes more strongly stimulated (e.g., a change from a middle wavelength to a long wavelength), the signal from the cone type that is more greatly stimulated will be too large to be canceled by the feedback, and the summing mechanism will be overloaded, thereby reducing the response amplitude. Such a model could explain the severe reduction in the amplitude of period doubling for the $G_A R_T$ condition (Figs. 3, 5). In this case, there was a change from approximately equal excitation of the *M* and *L* cones provided by the middle-wavelength pre-stimulus adaptation to a substantially greater excitation of the *L* cones by the long-wavelength test stimulus.

This model would also account for the observation that other combinations of pre-stimulus and test stimulus conditions (i.e., $G_A Y_T$, $Y_A G_T$, $R_A G_T$, and $R_A Y_T$) did not entail a similar reduction of period doubling. In those cases, the change in relative cone excitation was insufficient to alter the magnitude of the putative feedback signal responsible for period doubling. However, this model does not account for all aspects of the data, including the differences observed between P_1 and P_2 and the lack of relationship between the amplitudes of $3F/2$ and F . Therefore, further work needs to be done in order to understand exactly how chromatic signals affect the characteristics of period doubling.

In addition to its effect on period doubling, there was also an effect of chromatic adaptation on the amplitude of F at high temporal frequencies, beyond the range at which period doubling occurred (Fig. 6). Specifically, the amplitude of F at high frequencies

was relatively lower for a long-wavelength than for a middle-wavelength test stimulus, and the amplitude was lowest when the long-wavelength test stimulus was preceded by middle-wavelength adaptation ($G_A R_T$ condition). These results are generally consistent with previous reports of the effect of chromatic adaptation on ERG amplitude [20, 21], which found that long wavelengths preferentially reduced the ERG response. However, the amplitude reduction for long-wavelength stimuli in the present study occurred at substantially higher temporal frequencies than in the previous reports. This may be related to the fact that high-contrast test stimuli were used in the present study, whereas stimuli were of relatively low contrast in the previous studies.

A nonlinear feedback model has been proposed to account for the period doubling that has been observed electrophysiologically in the visual system [5]. According to this model, the amplitude of the response to a periodic stimulus is determined by a gain control mechanism with input from a negative feedback signal that decays exponentially following stimulus presentation. Based on a pharmacological study of period doubling in the salamander retina, the retinal site for such a feedback mechanism has been proposed to include the cone photoreceptors and OFF bipolar cells [5]. Nevertheless, period doubling can also be observed in the flicker ERG when the ON pathway is isolated pharmacologically in the ON-dominant retina of rat [6]. This finding indicates that a pathway involving cone photoreceptors and ON bipolar cells can also form a substrate for period doubling. As noted previously [10], the nonlinear feedback model of period doubling proposed by Crevier and Meister [5] does not easily predict the two regions of period doubling that are observed in the frequency–response function for $3F/2$. The present results indicate that this model needs further modification in order to include a consideration of the effect of the chromatic properties of both the test stimulus and the pre-stimulus adaptation.

In conclusion, the magnitude of period doubling in the cone flicker ERG is dependent on the wavelength properties of luminance-equated stimuli. Long-wavelength stimuli, which stimulate *L* cones preferentially, generally demonstrated the least amount of period doubling. Period doubling was more robust when *L* and *M* cone stimulation was more nearly equal. These results provide further evidence that cone-

selective adaptation can influence cone-driven signals within the luminance channel, as described previously for the flicker ERG [20, 21]. The neural pathway by which chromatic signals influence the nonlinear feedback mechanism that is presumed to underlie period doubling [5] remains to be resolved.

Acknowledgments This research was supported by NIH research grant 5R01EY008301 and ARRA grant 3R01EY008301-18S1 (KRA), NIH Core Grant EY001792, and an unrestricted departmental grant from Research to Prevent Blindness.

References

- Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M (2009) ISCEV standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* 118(1):69–77
- Teresa RC (1984) Abnormal discharges and chaos in a neuronal model system. *Biol Cybern* 50(4):301–311
- Canavier CC, Clark JW, Byrne JH (1990) Routes to chaos in a model of a bursting neuron. *Biophys J* 57(6):1245–1251
- Stone L (1993) Period-doubling reversals and chaos in simple ecological models. *Nature* 365(6447):617–620
- Crevier DW, Meister M (1998) Synchronous period-doubling in flicker vision of salamander and man. *J Neurophysiol* 79(4):1869–1878
- Shah MR, Alexander KR, Ripps H, Qian H (2010) Characteristics of period doubling in the rat cone flicker ERG. *Exp Eye Res* 90(2):196–202
- Qian H, Alexander KR, Ripps H (2010) Harmonic analysis of the cone flicker ERG of rabbit. *Exp Eye Res* 91(6):811–817
- Alexander KR, Levine MW, Super BJ (2005) Characteristics of period doubling in the human cone flicker electroretinogram. *Visual Neurosci* 22(6):817–824
- Schwartz G, Berry MJ 2nd (2008) Sophisticated temporal pattern recognition in retinal ganglion cells. *J Neurophysiol* 99(4):1787–1798
- Alexander KR, Raghuram A (2007) Effect of contrast on the frequency response of synchronous period doubling. *Vision Res* 47(4):555–563
- Alexander KR, Raghuram A, McAnany JJ (2008) Comparison of spectral measures of period doubling in the cone flicker electroretinogram. *Doc Ophthalmol* 117(3):197–203
- Kremers J, Rodrigues AR, Silveira LC, da Silva Filho M (2010) Flicker ERGs representing chromaticity and luminance signals. *Invest Ophthalmol Vis Sci* 51(1):577–587
- Eisner A, MacLeod DIA (1981) Flicker photometric study of chromatic adaptation: selective suppression of cone inputs by colored backgrounds. *J Opt Soc Am* 71(6):705–716
- Stromeyer CF, Cole GR, Kronauer RE (1987) Chromatic suppression of cone inputs to the luminance flicker mechanism. *Vision Res* 27(7):1113–1137
- Swanson WH (1993) Chromatic adaptation alters spectral sensitivity at high temporal frequencies. *J Opt Soc Am A* 10(6):1294–1303
- Wiesel TN, Hubel DH (1966) Spatial and chromatic interactions in the lateral geniculate body of the rhesus monkey. *J Neurophysiol* 29(6):1115–1156
- Lee BB, Martin PR, Valberg A (1988) The physiological basis of heterochromatic flicker photometry demonstrated in the ganglion cells of the macaque retina. *J Physiol* 404:323–347
- Smith VC, Lee BB, Pokorny J, Martin PR, Valberg A (1992) Responses of macaque ganglion cells to the relative phase of heterochromatically modulated lights. *J Physiol* 458:191–221
- Lee BB, Sun H (2009) The chromatic input to cells of the magnocellular pathway of primates. *J Vis* 9(2):15, 1–18
- Padmos P, Van Norren D (1971) Cone spectral sensitivity and chromatic adaptation as revealed by human flicker electroretinography. *Vision Res* 11(1):27–42
- Kremers J, Stepien MW, Scholl HPN, Saito C (2003) Cone selective adaptation influences L- and M-cone driven signals in electroretinography and psychophysics. *J Vis* 3(2):146–160
- Cao D, Pokorny J, Smith VC (2005) Associating color appearance with the cone chromaticity space. *Vision Res* 45(15):1929–1934
- Alexander KR, Barnes CS, Fishman GA (2003) ON-pathway dysfunction and timing properties of the flicker ERG in carriers of X-linked retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 44(9):4017–4025