The contribution of human cone photoreceptors to the photopic flicker electroretinogram

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We aimed to noninvasively estimate the relative contribution of the cone photoreceptors to the photopic flicker electroretinogram (ERG) in humans. Responses to ganzfeld square-wave flicker (15, 30, and 60 Hz) were recorded in the presence of a rod-saturating background. We used a modified paired flash technique in which a bright probe flash was presented at different times during a flicker cycle and the ERG response was recorded. The cone photoreceptor response was estimated by comparing the relative amplitude of the response during a flicker cycle to the response in the absence of flicker, at early times after the presentation of the probe flash. The normalized responses were then scaled to absolute values based on the assumption that the response to 15-Hz flicker is dominated by the photoreceptor response at early times in a cycle (<15 ms). The postreceptoral contribution was obtained by subtracting the absolute photoreceptor response from the flicker response. The derived cone response showed a peak around 25 ms, from the start of the flicker cycle for 15-Hz and 30-Hz flicker with no clear peak for 60-Hz flicker. In humans, the postreceptoral components dominate the photopic flicker ERG at 15, 30, and 60 Hz. The findings are comparable to those from pharmacological studies in monkeys.

Keywords: cone photoreceptors, flicker electroretinogram (ERG), paired flash technique


Introduction

In a clinical setting, the electroretinogram (ERG) provides an objective functional assessment of the retina. It is particularly useful in cases of unexplained vision loss and in the differential diagnosis of hereditary retinal diseases like retinitis pigmentosa and congenital stationary night blindness. To make clinical ERG testing consistent across the world, the International Society for Clinical Electrophysiology of Vision (ISCEV, Marmor, Holder, Seeliger, & Yamamoto, 2004) has proposed a standard that comprises five test protocols. These tests are designed to evaluate the rod and cone systems separately. Under dark-adapted conditions, dim and moderate luminance flashes are used to assess rod function; the cone system is evaluated by employing a moderate luminance flash under light-adapted conditions. The standard also recommends the use of a 30-Hz flickering stimulus for assessment of the cone system. Two indices, amplitude and implicit time, are typically used to evaluate the flicker ERG waveform (Berson, 1981; Sandlberg, Effron, & Berson, 1978). In disease conditions that affect the cone system, the responses typically show reduced amplitude and an altered implicit time (Alexander, Rajagopalan, Raghuram, & Fishman, 2006; Zeitz et al., 2005).

Electrophysiological studies on animals using pharmacological techniques to isolate receptor and postreceptoral responses have shown that the flicker ERG response is dominated by postreceptoral elements and have provided a better understanding of how postreceptoral elements, particularly the ON and OFF bipolar cells, interact to shape the steady-state flicker ERG response (Kondo & Sieving, 2001, 2002). Kondo and Sieving (2001) demonstrated that after blocking signal transmission from the photoreceptors to postreceptoral cells using 2-amino-4-phosphonobutyric acid (APB) and cis 2,3, piperidine dicarboxylic acid (PDA), there was an ∼80% decrease in amplitude of the ERG response for frequencies above 30 Hz. The relative contribution of the postreceptoral elements to the photopic flicker ERG has not been studied in humans. The aim of this study was to noninvasively measure the receptoral and postreceptoral contributions to the human photopic flicker ERG.

We present a technique that can isolate the cone photoreceptor component of the photopic flicker ERG and hence allows the determination of the postreceptoral contribution. The technique is based on the paired flash paradigm that has been used to isolate the photoreceptor component of the flash ERG response in vivo (Birch, Hood, Nusinowitz, & Pepperberg, 1995; Friedburg, Allen, Mason, & Lamb, 2004; Pepperberg, Birch, & Hood, 1997). We have modified this technique and applied it to the steady-state flicker ERG to isolate the cone photoreceptor component of the response to square-wave flicker at temporal frequencies of 15, 30, and 60 Hz. We then...
use the cone photoreceptor component to estimate the postreceptoral contribution at each frequency and demonstrate that postreceptoral components dominate the flicker ERG response in humans at all temporal frequencies studied, as they do in primates. Some results of this study have appeared previously as an abstract (Verma & Pianta, 2006).

**Methods**

**ERG recordings**

All research on human subjects followed the tenets of the Declaration of Helsinki and was approved by the Human Research Ethics Committee of the Department of Optometry and Vision Sciences, The University of Melbourne. Informed, written consent was obtained from all subjects. All participants had best corrected visual acuities of 6/6 with no color vision defects, ocular pathology, or history of hereditary retinal disease. Monocular mydriasis was achieved with two drops of 1% tropicamide (Mydriacyl, Alcon Laboratories, NSW, Australia) instilled at an interval of 5–10 min, followed by further instillation of drops, if necessary. Pupil diameter was measured at the start and end of the session. Luminances in photopic candela per square meter were converted to photopic trolands using the method of Friedburg et al. (2004), which takes into account the Stiles–Crawford effect. When this method is used with an effective area of the dilated pupil of 20 mm², 1 photopic cd m⁻² corresponds to 20 photopic trolands (phot Td or Td).

Subjects were prepared for skin electrode placement. The forehead and the temple were swabbed with alcohol (70% isopropyl alcohol, Smith & Nephew, Australia) and scrubbed with Nuprep abrasive skin preparation gel (D.O. Weaver, USA) to minimize the resistance of the skin. ERGs were recorded using custom conductive fiber electrodes (Card windings, Diagnosys LLC, Boston, MA). The conductive fiber electrode was attached to conductive nickel/copper fabric tape (LessEMF, NY, USA) at one end, and insulating tape on the other end. Electrodes with resistance of 100–200 Ω were used for recordings. The length was matched to the subject’s palpebral length. Electrodes were soaked in Renu Multi-Plus disinfecting contact lens solution (Bausch & Lomb, USA) prior to insertion. The end with the masking/insulating tape was fixed on the nasal side and the DTL fiber was placed loosely across the cornea, resting in the lower fornix. The conductive tape on the temporal end was attached to an alligator clip and fixed to the electrode on the subject’s temple using Velcro. The electrodes were placed with minimal exposure on both canthi to minimize photovoltaic effects and to avoid contact with the skin.

The reference and ground electrodes were disposable silver/silver chloride ECG electrodes (ConMed) or Skin-tact tab electrodes (CNS Medical, NSW, Australia). The reference electrode was placed on the temple close to the active electrode and the ground electrode was placed on the forehead. The impedance of the electrodes was tested prior to the start of the test. A resistance of less than 5000 Ω between any two electrodes suggested good contact with the skin (Hogg, 2006).

**Visual stimulation and light calibration**

All our stimuli were presented in a custom-built ganzfeld bowl, which was designed to accurately deliver controlled light stimuli to one eye. The ganzfeld bowl was constructed from two hemispherical bowls (radius of 33.3 cm). The front hemisphere was modified to taper to a cone with a 4-cm monocular viewing port at its apex (similar to Smith & Lamb, 1997). The top of the ganzfeld bowl had two square access ports (3.5 × 3.5 cm), one for the flash stimulus (Xenon flashgun, Mecablitz 60CT4, Metz) and the other for the flicker stimulus. The flicker stimulus was produced by mounting a light chopper (Frequency Programmable, 750, MKII) in front of an incandescent light source (Kodak Carousel, S-AV 2010, Projector). The base of the bowl had a square aperture for a set of short-wavelength light-emitting diodes (LEDs, peak wavelength 470 nm and width at half-height was 40 nm), which produced the rod saturating background. The subject position was maintained using an adjustable head and chin rest.

Luminance calibration was performed using an IL-1700 Photometer (International Light, Newburyport, MA, USA). The inner surface of the ganzfeld bowl was painted in flat white (Barloworlds Coatings, Australia) and all luminance measurements were performed at the monocular viewport. The maximum and minimum luminances of the flicker stimulus together with the background were 5.0 and 60.3 cd m⁻². The xenon flash was calibrated at seven different exposure levels and its luminance ranged from 1.99 to 295 cd m⁻² s⁻¹. The quantal catch for L and M cones was similar for both the flicker and flash stimuli.

**Amplification and data acquisition**

A CED 1902 (Cambridge Electronic Design, Cambridge, UK) single channel differential amplifier was used for ERG recordings. All recordings were performed in isolated EEG mode and with AC coupling enabled. Signals were amplified by 3000 times and band-pass filtered from 1 Hz to 500 Hz.

To minimize the AC mains (50 Hz) interference, a notch filter is often used while recording the flicker ERG response. However, Bock, Gerth, and Lorenz (2000) have shown that a 50-Hz notch filter alters the phase of the response. The use of a notch filter has also been shown to affect the b-wave of the flash ERG signal (Lachapelle & Molotchkinoff, 1986). Even though we were primarily
concerned with the a-wave, a notch filter may also alter the a-wave response. Instead of using a notch filter, we used a line noise model to minimize the loss of any signal information from the flash and flicker ERG responses. Our model assumed that the line noise was sinusoidal with a frequency of 50 Hz. Amplitude and phase were adjusted to obtain a best fit to the first cycle of the flicker response at 30 Hz and 15 Hz and up to the fourth cycle at 60-Hz test frequency. The best fit model was then subtracted from the raw ERG signal to obtain a signal without mains noise. The first flicker cycle was useful to estimate the line noise because during this time the flicker response was small compared to the line noise. We compared the flicker response obtained with the notch filter on to the flicker response obtained using the line noise model. The responses were no different in amplitude but showed a slight phase difference (<5 ms).

Stimulus trigger timing and data acquisition were controlled by a digital acquisition board (PCI-6036E, National Instruments) with 12-bit resolution using an input sampling rate of 10 kHz and an output sampling rate of 1 kHz. The timing of the flash and the light chopper was synchronized by a hardware trigger. Acquisition and analysis were performed using custom software developed in MATLAB. Responses were averaged over 30 to 40 sweeps, depending on the quality of the signal.

Theory of the flicker-flash technique

Based on the theory of the paired flash technique, a bright probe flash delivered at different times during the flicker cycle should shut down the remaining circulating current during the photoreceptor response to the flicker. The flicker alone response (FA) when subtracted from the combined flicker-plus-probe response (F+P) gives the isolated probe response. The isolated probe response, normalized to the probe alone response (i.e., the response to the flash alone without the flicker), provides an estimate of the fractional probe response and this can be used to indirectly estimate the amount of circulating current shut down in cone photoreceptors by the flickering stimulus at any given time. The fractional probe response, when measured at early times, should not be affected by responses from postreceptoral elements and hence should estimate a pure cone response.

Designing the flicker-flash technique

Application of the paired flash technique to the flicker ERG required three critical issues to be addressed.

**Rod saturation**

To estimate an adapting background level at which the rods are saturated and cones are minimally adapted, flicker ERG responses were measured at different levels of background luminance. Subjects were dark adapted for 20 min prior to these recordings. Flicker ERG responses elicited by the same flicker stimulus were measured without a background present and in the presence of seven different intensities of the short-wavelength background (after at least 2 min of adaptation to each background).

**Stability of the flicker response**

It has been established that on exposure to bright light after dark adaptation the flicker ERG amplitude gradually increases over time (Peachey, Alexander, Derlacki, & Fishman, 1992a, 1992b). These studies dark adapted their subjects before recording the flicker ERG response, but we did not require prior dark adaptation, so we decided to determine the time required for the flicker response to stabilize after exposure to room lighting. This was critical, as the growth in the flicker ERG amplitude could lead to inconsistency between subjects, as well as differences between and within recording sessions. To measure the time required for the flicker response to stabilize, the responses were measured at various times after the subject moved from room lighting to flicker adaptation.

**Probe flash intensity**

The choice of probe flash was important, as it should be bright enough to shut down the remaining circulating current, with only short-term alterations to the steady-state flicker response. To select the ideal probe flash luminance, we first selected a probe flash that gave a saturating flicker response. To select the ideal probe flash luminance, we first selected a probe flash that gave a saturating response as early as 4 ms. Then, a series of lower probe flash intensities were delivered at a fixed time (12 ms) after the start of the flicker cycle, and the fractional probe response was estimated at this time.

Ideally, we wanted a probe flash that would give the same estimate of the fractional probe response as the bright probe and give a reliable estimate (i.e., good signal-to-noise ratio) but have minimal adaptation effect on the flicker response. A probe flash luminance of 79.4 cd m$^{-2}$ s$^{-1}$ achieved these goals and was close to intensities used in similar studies (Friedburg et al., 2004). The absence of an adaptation effect was confirmed by comparing the mean flicker alone responses for the first five consecutive probe flashes to those for the last five consecutive probe flashes in a series of 30 sweeps for three subjects. The responses from the start of the series were not different from the responses at the end of the series, confirming that there was no adaptation effect with our probe flash and inter-probe interval.

**Derived cone photoreceptor response at 15, 30, and 60 Hz**

Experiments were performed in separate sessions for 15, 30, and 60 Hz square-wave flicker using the following...
protocol: The probe alone (PA) response was recorded after adapting for 2 min to the rod saturating background. The subjects then adapted to the flicker stimulus for 10 min and the flicker alone (FA) response was recorded. The combined flicker-plus-probe (F+P) responses were obtained by delivering the probe flash at specific times during the flicker cycle. The PA response was recorded at the start and end of the session and the FA and F+P responses were recorded for each probe time.

For 15 Hz, the probe flash was triggered in 3-ms steps for the first half of the cycle and in 6-ms steps for the second half of the cycle. For 30 Hz, the probe flash was triggered in 3-ms steps, and for 60 Hz, the probe flash was delivered in 2-ms steps throughout the cycle. For each probe time, we used the flicker response from the cycle immediately prior to the combined flicker-plus-probe response as the FA response. This was subtracted from the F+P response to give the isolated probe response. The probe alone PA response was recorded again at the end of the session to ensure that the PA response did not change during the experiment due to pupil or electrode factors. The time intervals between consecutive flashes were around 5 s. The total recording time for one session, including the 10-min adaptation time was 40 to 70 min, depending on the stimulus frequency.

Results

Adapting luminance and flicker adaptation

Flicker ERG responses were measured at different levels of background luminance to determine the adapting background level at which rods are saturated but cones are.

Figure 1. The effect of adapting luminance and adapting duration on the flicker response. (A) The mean normalized amplitude (±SEM, n = 3) of the flicker ERG response decreases with increasing luminance of a short-wavelength background. The data have been fitted with an exponential saturation function (solid line, see text for details). (B) The mean normalized implicit time (±SEM, n = 3) decreases with background luminance. The data have been fitted empirically with a sigmoid function (solid line). The background luminance selected for use in this study is 2.04 log Td (dashed line). The mean luminance can be calculated by adding the (constant) mean luminance of the flicker stimulus to the background luminance. (C) The mean normalized flicker ERG amplitude (±SEM, n = 3) for 15 Hz (solid line) and 30 Hz (dotted line) grows during adaptation. The amplitude plateaus after ~10 min of flicker adaptation. (D) There is little change in the mean normalized implicit time for 15 Hz and 30 Hz (±SEM, n = 3) during adaptation.
minimally adapted. Figures 1A and 1B show a decrease in amplitude and implicit time of the flicker ERG response (to 30 Hz) recorded for the same flicker stimulus in the presence of short-wavelength backgrounds that differed in luminance. The amplitude and the implicit time of the flicker response have been normalized to the maximum for each subject and then averaged (explaining why none of the data points has a value of 1). The decrease in amplitude (Figure 1A, solid line) is well described by an exponential saturation function, similar to that used by Friedburg et al. (2004) and Lamb and Pugh (1992):

\[ R(I) = \exp\left(\frac{-I}{I_0}\right) \]

where \( R \) is the normalized response amplitude, \( I \) is the adapting luminance (time averaged flicker luminance plus the short-wavelength background luminance), and \( I_0 \) is the luminance required to reduce the normalized response amplitude by \( 1/e \). The decrease in implicit time can be described empirically by a sigmoid function (Figure 1B, solid line).

The decrease in amplitude and implicit time presumably represents a reduction in rod contribution with increasing background luminance. For the flicker-flash paradigm, a luminance of 2.04 log Td was selected as the rod saturating luminance. At this background luminance, there was only a small decrease in flicker ERG amplitude and implicit time was shorter. Previous studies have shown that the cone system begins adapting around 2.2 log Td (Hood & Birch, 1993), hence the selected luminance should minimally desensitize the cone photoreceptors.

To determine the time required for the flicker response to stabilize, flicker ERG responses were measured during adaptation to the flicker stimulus. Figures 1C and 1D show the change in amplitude and implicit time for the response to 15- and 30-Hz flicker after the subject is removed from room lighting and begins adapting to flicker. The average normalized amplitude and implicit time for three subjects have been plotted in Figures 1C and 1D (again, the amplitude and implicit time have been normalized for each subject and then averaged). For 30 Hz, the growth in amplitude was steepest from time zero to 5 min. The standard deviation (SD) was maximal (0.18) at the onset of the flicker adaptation, perhaps indicating different levels of adaptation early in the experiment. The growth in amplitude was \( \sim 30\% \) after 10 min of flicker adaptation. For the same responses, the implicit time (Figure 1D) did not show any change with adaptation time. The 15-Hz response showed a similar growth in amplitude to 30 Hz and no change in the implicit time with increase in flicker adaptation (Figures 1B and 1D). The growth in flicker ERG amplitude during adaptation to flicker asymptotes \( \sim 10 \) min after starting adaptation, so we used a minimum of 10 min of flicker adaptation to ensure a stable flicker response for all our experiments.

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Figure 2. The flicker-plus-probe response for (A) 15, (B) 30, and (C) 60 Hz (subject RV). Vertical markers represent the probe trigger times and are plotted using the same color as the response to that probe flash. For clarity, the individual flicker-plus-probe responses have been truncated after the peak. Dashed lines show the flicker stimulus trace. Arrows in (A) and (B) highlight low-amplitude responses that occur \( \sim 25 \) ms after the start of the flicker cycle.
Combined flicker-plus-probe response

Figure 2 shows the combined flicker-plus-probe responses to temporal frequencies of 15, 30, and 60 Hz for subject RV. The combined flicker-plus-probe response was obtained by delivering the probe flash at different times during the flicker cycle. The flicker-plus-probe responses are shown for one complete cycle at each temporal frequency. In Figure 2, for each temporal frequency, the flicker-plus-probe response for the probe delivered at the latest time is shown as a thick solid line to highlight the flicker cycle preceding the probe response. The vertical markers in Figure 2 represent the probe onset times and the corresponding responses have been plotted using the same color. The time is measured from the onset of the flicker cycle and the stimulus is indicated by the dashed line. The flicker-plus-probe response varied with probe onset time and showed a maximal response early in the cycle and a minimal response for probes triggered at ~25 ms for both 15 and 30 Hz (arrows in Figures 2A and 2B). The flicker-plus-probe response for 60 Hz did not vary greatly for the entire cycle.

Isolated probe response

Figure 3 shows the isolated probe response obtained by subtracting the flicker alone response from the combined flicker-plus-probe response. The left panel (A, B, C) of Figure 3 shows these responses after shifting them in time to align the probe trigger times at zero. This highlights the relative change in the response amplitude to the probe at different times during the flicker cycle. The right panel of Figure 3 shows the same responses on a larger time scale.

According to the theory of the paired flash technique, the isolated probe response can be used to estimate the amount of circulating current shut down by the test (flicker) stimulus at different times during the flicker cycle. To demonstrate the difference in the isolated probe response derived at 0 ms and at 33, 16, and 8 ms (for 15, 30, and 60 Hz, respectively), these responses have been plotted as a dashed line. The larger amplitude response at the start of the cycle indicates that the flicker stimulus minimally reduced the circulating current at this time, leaving most of the remaining current to be shut down by the probe flash. Similarly, near the middle of the cycle, the isolated probe response was relatively small since, at this time, a larger amount of circulating current had been shut down by the flicker stimulus, with less current left to be shut down by the probe flash.

Deriving the fractional cone response

Figure 4 shows the derived fractional cone current for 15, 30, and 60 Hz from three subjects. The average data from three subjects is plotted with the fractional cone response measured at 5, 6, 7, and 8 ms shown using different symbols. The amplitude of the isolated probe responses measured at these times (Figure 3) was normalized to the probe alone response at the same times to derive the fractional cone response for each time. The data are from an average of two sets of recordings for MP and RV and a single set of recordings for subject JK. The time of measurement is from the onset of the flicker cycle, so the fractional probe response for a probe triggered at the onset of the flicker cycle and measured at 5 ms after the probe flash gives the derived fractional probe response at 5 ms into the flicker cycle. Applying this for various probe trigger times gives a series of fractional probe responses spanning a full flicker cycle. As the flicker stimulus is cyclic, data measured at times beyond one cycle have been shifted to times near the start of the cycle by subtracting the stimulus period.

A value of one for the fractional cone response in Figure 4 represents the maximum amount of circulating current remaining (i.e., the flicker stimulus has not reduced the circulating current). The derived fractional cone response for the 15- and 30-Hz flicker stimuli varied from a maximum of 1 to a minimum of ~0.6 in all three subjects. Hence, it represented a peak reduction in the circulating current of ~40% in the cone photoreceptors in response to the flicker stimulus. The response showed complete recovery near the end of the cycle. The fractional cone response for the 60-Hz stimulus varied from 0.9 to 0.7 indicating a smaller change in circulating current compared to the 15- and 30-Hz stimuli. The maximum value of 0.9 at 60 Hz indicates that there is at most 90% of the circulating current remaining, suggesting the cone photoreceptor response never fully recovers at this high temporal frequency. Also for the 15-Hz and 30-Hz stimuli, the fractional cone response showed a peak ~25 ms after the onset of the flicker cycle, but for 60 Hz, there was no clear peak.

It is likely that the response to the probe at 5 ms represents a pure cone response, but it is possible that the response at later times is contaminated by responses from postreceptoral cells (Friedburg et al., 2004). Since most probe times were separated by 3 ms, we were able to investigate the impact of postreceptoral contamination by comparing the fractional cone response measured at 8 ms for a probe triggered at time t to the fractional cone response measured at 5 ms for a probe that was triggered at time t + 3 ms. These two measures represent different assays of the fractional cone response at the same time during the flicker cycle (t + 8 ms and (t + 3) + 5 ms = t + 8 ms). Figure 4D shows the agreement between these two measures using a mean-difference plot (Altman & Bland, 1983; Bland & Altman, 1986). The difference between the estimates of the fractional cone response measured at 5 and 8 ms (response at 5 ms minus the response at 8 ms) is plotted against the mean of the two estimates. The average differences for the responses to
15, 30, and 60 Hz were $-0.07$, $-0.10$, and $-0.02$, respectively. The average difference for all three frequencies was $-0.07$, which represents a change of $\sim 10\%$, suggesting that the impact of estimating the fractional cone response at times as late as 8 ms is small.

Figure 3. Isolated probe responses for (A) 15, (B) 30, and (C) 60 Hz for subject RV obtained by subtracting the flicker alone responses from the flicker-plus-probe response (see Figure 2). The responses have been shifted in time to align the probe trigger times at zero. Vertical lines are drawn at measurement times of 5, 6, 7, and 8 ms. The right panels replot the responses from the left panels on a larger time scale. Waveforms plotted as dashed lines are the responses to probe flashes triggered at the start and near the middle of the cycle (timings as marked) and highlight the difference in amplitude at different times in the cycle.

The postreceptoral component of the flicker ERG

To estimate the contribution of postreceptoral components of the flicker ERG, we needed to convert the
normalized cone response (fractional cone current) into absolute values that could be subtracted from the raw flicker ERG waveform to give the postreceptoral component of the response. We converted the derived fractional cone current to absolute values using a similar method to Friedburg et al. (2004). Since the shape of the derived cone response was similar to the 15-Hz flicker response at early times (at 15 ms after the start of the cycle, Figures 5A and 5B), and the photoreceptor response to 15-Hz flicker recovers within a cycle, we assumed that at these early times the raw flicker ERG response was dominated by the cone photoreceptor response, with minimal postreceptoral influence. The conversion between the fractional cone response ($F_c$) and the cone ERG response ($V_c$) is

$$V_c = K(1 - F_c),$$

where $K$ is a scaling constant (in $\mu V$) and a scaling constant of $-10 \mu V$ gave the best match to the raw flicker ERG waveform recorded with the 15-Hz flicker stimulus at times earlier than 15 ms (Figure 5B).

We used Equation 2 and $K = -10 \mu V$ to estimate the cone ERG responses at each frequency and these were then subtracted from the raw flicker ERG waveform to estimate the postreceptoral ERG responses (Figure 5). The relative cone contribution was calculated by taking the ratio of the amplitude of the cone ERG response and the amplitude of the raw flicker ERG response. The data are summarized in Table 1.

As shown in Figure 5, and the “Average” column of Table 1, the postreceptoral component dominates the flicker ERG response at all three temporal frequencies studied. In Figure 5, the estimated postreceptoral component follows the raw flicker ERG response very closely. The dominance of the postreceptoral component is
Table 1. Estimated cone and postreceptoral response amplitudes for 15, 30, and 60 Hz. The data in the column titled “Monkey” are from Figure 3A of Kondo and Sieving (2001).
maximal at 30 Hz (79%), followed by 15 Hz (63%) and 60 Hz (36%). The estimated postreceptoral contribution from our study is similar to that reported by Kondo and Sieving (2001), where they showed that, at 30 Hz, 83% of the response is due to the postreceptoral component.

Discussion

This study aimed to isolate the cone photoreceptor response underlying the photopic flicker ERG in humans using a noninvasive technique. To our knowledge, this is the first attempt to derive the human cone photoreceptor responses from ERG responses to flickering stimuli.

Derived cone photoreceptor response

With our flicker stimulus, which was of moderate luminance, the time to peak of the isolated cone response was around 25 ms. While not directly comparable because of difference in stimulus duration, the overall shape of the response is comparable to that found by Friedburg et al. (2004), where they isolated the human cone response to a brief flash and found the time to peak for a moderate luminance flash to be around 20 ms.

Comparison with other ERG studies

We have compared our derived cone response to the isolated cone responses from the Kondo and Sieving (2001, 2002) studies on monkeys, where the cone component was isolated using pharmacological agents (APB and PDA). Figure 6 shows the postdrug data from Figure 4 of Kondo and Sieving (2002), for 32-Hz square-wave flicker, along with the 30 Hz derived cone response from our study. We have normalized the responses from all four monkeys to range from 0.6 to 1 and shifted the responses to align the timing of the peak response without altering the shape of the response. The kinetics of the response from monkey closely matches our derived waveform, particularly during the deactivation phase.

The study by Kondo and Sieving (2002) highlighted the dominance of the postreceptoral contribution to the flicker ERG. The relative postreceptoral contributions found here are comparable to their findings and the findings of an earlier study using sine-wave stimuli (Kondo & Sieving, 2001). In our study, with 30-Hz and 15-Hz square-wave stimuli, the amplitude of the postreceptoral component was ~85% and ~75% of the flicker ERG response amplitude, which is similar to the 80% and 65% contribution found in monkeys. The relative postreceptoral contribution at 60 Hz was lower in our human subjects: ~60% compared to ~85% in monkeys.

Our results are also consistent with the derived cone response of the photopic flicker ERG found in monkeys by Viswanathan, Frishman, and Robson (2002). This study used pharmacological agents to block the postreceptoral component and found that the time to peak for cone photoreceptors was ~25 ms for flicker stimuli of moderate intensities. The similar shape of the derived waveform for 15 and 30 Hz suggests that the differences in the raw flicker ERG response as a function of temporal frequency are mainly due the differences in the postreceptoral responses.

Comparison with isolated cone photoreceptor recordings

We have also compared our data to results from in vitro studies. We selected waveforms that showed a fractional cone response of 0.6 (Kraft, 1988; Schneeweis & Schnapf, 1999). Figure 7 shows the data from Figure 1A (trace 3) of Schneeweis and Schnapf (1999), where photovoltage measurements were made using a patch electrode on long-wavelength sensitive cones from monkey retina.

The data from Kraft (1988) are from photocurrent recordings obtained by suction electrode from ground squirrel cones. The photovoltage and photocurrent recordings have been converted to fractional cone response. The average derived cone response obtained from our flicker-flash technique closely follows the in vitro recordings,
especially for the activation phase of the response. The deactivation phase appears to be faster in humans compared to the isolated cone response from ground squirrel and monkey. The similarity of the responses is striking, despite the difference in stimulus durations of 10 ms used by Schneeweis and Schnapf (1999) and 11 ms used by Kraft (1988). Our derived cone response is, however, not consistent with cone photocurrent recordings by Schnapf et al. (1990), where the time to peak was around 50 ms. It has been suggested (Schneeweis & Schnapf, 1999) that photocurrent and photovoltage recordings can show differences in the measured kinetics, with the time to peak from photovoltage measurement (in vitro) occurring at ~20 ms compared to the photocurrent recording (in vitro), which occurs at ~50 ms. As photovoltage recordings are influenced by postreceptoral mechanisms, they do not directly measure the circulating current within the outer segment. The derived cone response presented here is more like a photovoltage measurement (in vivo) and shows a time to peak ~25 ms, which is comparable to the in vitro photovoltage recordings.

**Conclusion**

The flicker-flash technique can be used to noninvasively isolate the cone photoreceptor response to flickering stimuli in humans. The results show that the photopic flicker ERG is dominated by responses from postreceptoral cells at all temporal frequencies studied (15, 30, and 60 Hz). The relative contribution of receptoral and postreceptoral components to the human flicker ERG is similar to that demonstrated previously in monkeys. This study provides new insight into the relative contribution of cone photoreceptors and postreceptoral cells to human flicker ERG waveform, which may inform the interpretation of changes in the flicker response in retinal disease.

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