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# The Focal Cone Electroretinogram

S. YAMAMOTO,* P. GOURAS,* R. LOPEZ*

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**The focal cone electroretinogram (ERG) in monkey retina has been examined with a 3 deg pulse of laser light (544 and 633 nm) centered on a 25 deg steady white rod saturating field. The stimuli were viewed simultaneously through a slit lamp and corneal contact lens. Cone ERGs were studied at different eccentricities from the fovea and compared with full-field corneal and intraretinal ERGs. The cone ERG is maximum at the fovea. There are two components to the on- (*b*-wave) and off- (*d*-wave) response, one slower, more long wavelength sensitive and more foveally oriented than the faster response. This makes the foveal cone ERG slower and more longer wavelength sensitive than the perifoveal ERG. This difference disappears at high rates (> 20 Hz) of stimulation. The foveal cone ERG is larger and slower than that of more peripheral retina. The slowness appears to be due to a subcomponent of the response which is especially prominent in the fovea and has a slightly greater long wavelength sensitivity than the more peripherally generated ERG. It may depend on a unique difference in L–M cone bipolar systems or in L–M cone interactions that are more prominent near the fovea.**

Cone ERG  Laser ERG  Focal ERG  Monkey retina

Using the electroretinogram (ERG) non-invasively to obtain information about local retinal function is difficult because the corneal signal produced by a focal light stimulus is small and there is an additional problem of scattered light eliciting non-focal responses. Although there have been many earlier descriptions of the focal ERG in human subjects (Armington, Tepas, Kropel & Hengst, 1961; Gouras, Gunkel & Jones, 1962; Brindley & Westheimer, 1965; Arden & Banks, 1966; Aiba, Alpern & Maaseidvaag, 1967; Biersdorf & Diller, 1969) it has only been recently that a practical clinical application of this technique has succeeded, facilitated by allowing direct observation of the test stimuli on the retina (Hirose, Miyake & Hara, 1977; Sandberg, Effron & Berson, 1978; Miyake, Yanagida, Kondo, Yagasaki & Ohta, 1981). We have modified the original idea of Hirose *et al.* (1977) which employs a slit lamp to view the fundus during stimulation by introducing paired lasers to produce focal retinal stimulation (Lopez, Yamamoto, Gouras & Rosskothen, 1992; Yamamoto, Gouras, MacKay & Lopez, 1992). In order to determine the effectiveness of this instrument we examined rhesus monkeys under general anesthesia. The rhesus monkey's retina is extremely similar to that of man and the use of anesthesia facilitates stable recording of the corneal ERG over relatively long periods of time (1–2 hr). This method has revealed an unusual waveform to the foveally centered focal cone ERG in monkey retina that has not been detected before. This provides a new parameter to examine local retinal function experimentally and clinically.

*Department of Ophthalmology, Columbia University, 630 West 168 Street, New York, NY 10032, U.S.A. [*Fax* 1 212 305 9087].

## MATERIALS AND METHODS

Adult male rhesus monkeys (*Macaca mulatta*) were examined over the course of a year, each monkey was tested at monthly intervals. Monkeys were anesthetized by ketamine hydrochloride (10 mg/kg/hr, i.m.) in conjunction with sodium pentobarbital (5–10 mg/kg/hr, i.p.). The head was held by supports in the external auditory canals and under the hard palate. The pupils were dilated fully with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride. All experiments conformed to the ARVO Resolution of the Use of Animals in Research.

Figure 1 shows a diagram of our experimental system. Using a fiber optic cable to introduce a laser beam into a slit lamp to elicit local ERGs was previously reported by Hirose *et al.* (1977). We have modified this method to include two interchangeable helium-neon lasers, 544 and 633 nm to provide a means of testing response univariance. The laser beam was chopped by accurate rotation of a sector disk driven by an electronic motor that also provided a synchronization signal for a computer (CA-1000, Nicolet) averaging ERG responses from the cornea. This produced a square-wave on- and off-stimulus with equal duty cycles, which elicited discrete on and off ERG responses at frequencies of 10 Hz

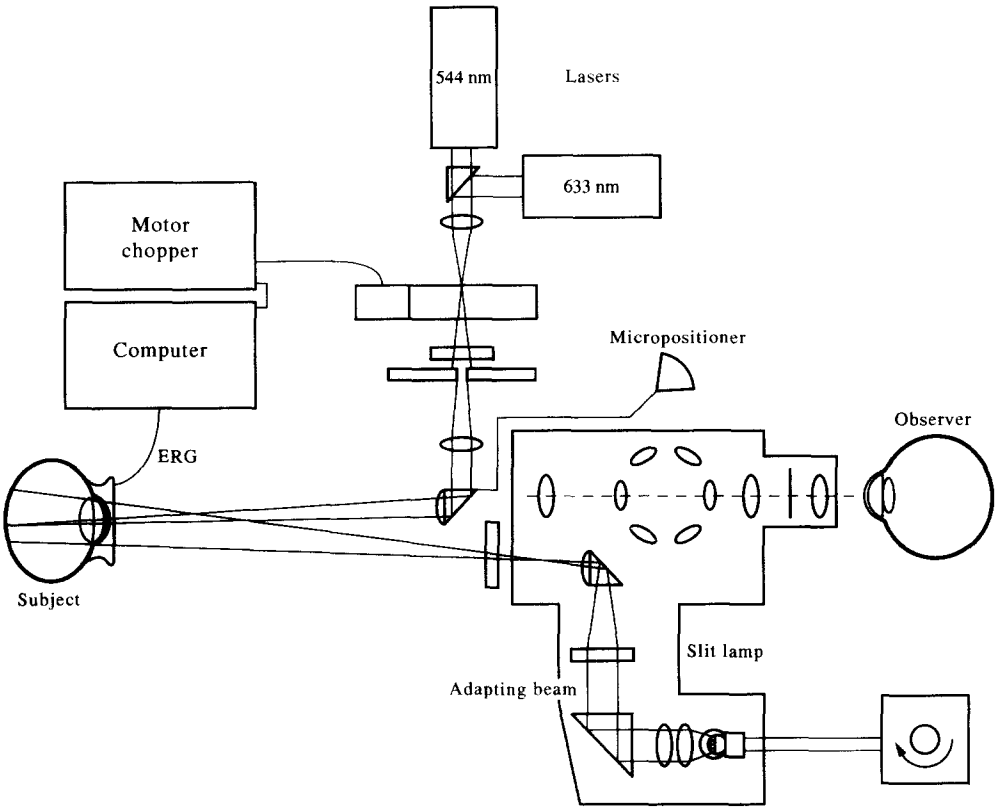


FIGURE 1. Diagram of the laser slit lamp testing system.

or lower. At higher frequencies, the on–off components merged (see Results). The laser beam subtended 3 deg and could be moved along the horizontal and vertical meridians by a micropositioner. The photopic luminance of laser beams are 30 ft-L at 633 nm and 33 ft-L at 544 nm; these could be reduced by neutral density filters. The light source for constant background illumination was a tungsten viewing light which subtended 25 deg and was adjusted to produce a brightness (230 cd/m$^2$) sufficient to saturate rod responses. The steady adapting field was centered on the fovea for all experiments except those using the mirror contact lens (see below). The focal laser spot could be positioned anywhere within this background field. When the focal laser spot was also centered on the fovea, the test and background beam were concentric. When the laser spot was moved eccentrically from the fovea it was closer to the edge of the adapting beam. The ERG was detected by a platinum electrode embedded in a corneal contact lens which allowed the examiner to observe the fundus through the slit lamp's microscope. The reference electrode was placed subcutaneously at the lateral canthus and a ground electrode on the scalp. Between 500 and 1000 ERG responses to the same stimulus were averaged to obtain an adequate signal-to-noise ratio. In several experiments we aimed the laser stimuli on the front surface of a bipolar Burian–Allen contact lens electrode in order to compare ERGs to focal stimuli with those to quasi-full-field stimuli. In order to examine the focal ERG from more peripheral retina we used a mirror contact lens with a platinum corneal electrode, which delivered the focal laser stimuli and the adapting field to areas 30–50 deg from the fovea.

In order to record the intraretinal ERG (IERG) we introduced an insulated tungsten microelectrode through a pars plana port and guided it to specific retinal loci. The test stimuli for the intraretinal recordings were obtained from a full-field stroboscope flash superimposed on a bright white (tungsten) adapting field of 17,000 phot td.

## RESULTS

Figure 2 shows ERGs to 3 deg stimulus flickering at 5 Hz centered at the fovea and at various eccentricities along the horizontal [Fig. 2(top)] and vertical [Fig. 2(bottom)] meridian. The ERG exhibits corneal positive on- (*b*-wave) and off- (*d*-wave) responses which are similar to each other. The response at the fovea is larger with a later peak latency to both 633 nm (red) and 544 nm (green). There are two distinct components to both the on- and off-responses. One component is faster and more phasic than the other. The fast component (arrow) is more responsive to green and the slow component (asterisk) is more responsive to red light. With more peripheral stimulation along both the horizontal and vertical meridians, the slow component of the *b*-wave decreases in amplitude more than the fast one does; i.e. the slow component is more prominent than the fast component in the fovea.

Figure 3 shows the decrease in amplitude of the focal ERG with eccentricity. At 10 deg from the fovea the response is about 50% of the foveal response. The fall

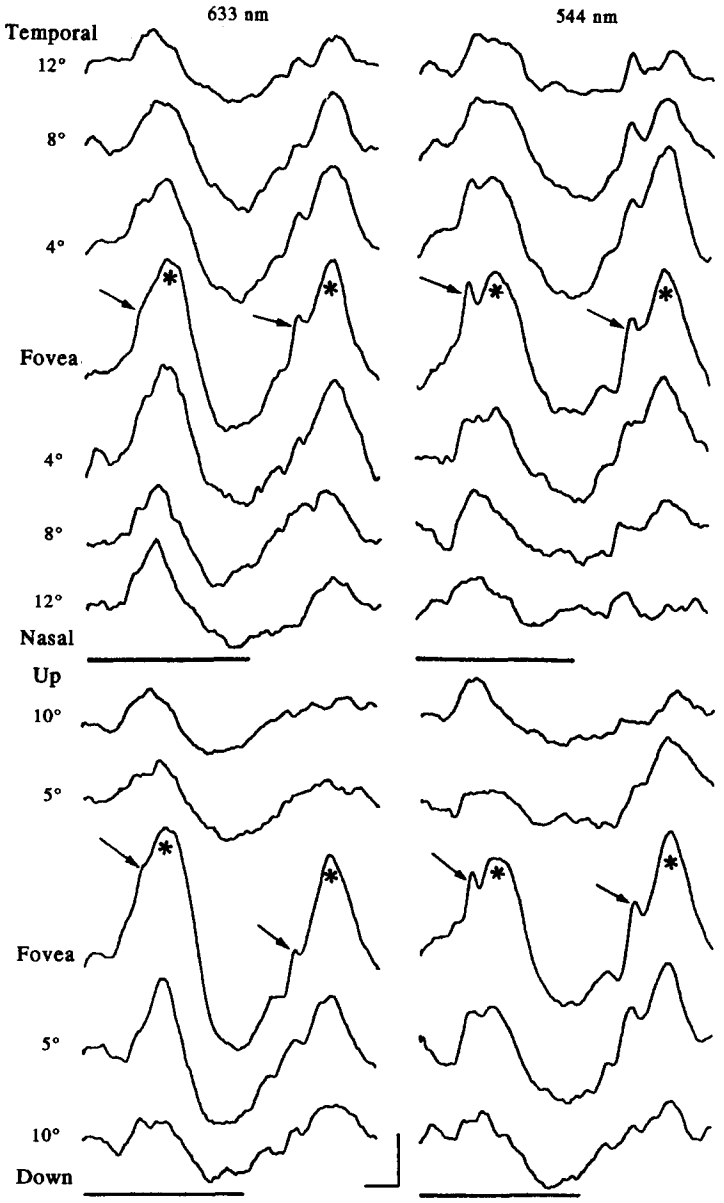


FIGURE 2. Focal ERG to a 3 deg stimulus at 5 Hz centered at the fovea and at various eccentricities along the horizontal (top) and vertical (bottom) meridian. There are two positive responses, one at the on-phase, the other at the off-phase of the stimulus. Each response has a quick (arrow) and a larger slow (asterisk) component. The horizontal line (below) indicates the duration of the light stimulus. The calibration indicates 1 $\mu$V vertically and 20 msec horizontally. Positivity is upward in this and all other figures.

off is similar for the on- and off-responses and to the two different spectral stimuli. The implicit time of the on-response is shorter than that of the off-response at all eccentricities (Fig. 4). At the fovea the implicit time of the on-response, either to green or red stimuli, is later and becomes progressively earlier at more peripheral loci. The implicit time to the 633 nm stimulus is later than it is to the 544 nm stimulus at the fovea, but this difference disappears at 10 deg from the fovea. This difference in the responses to red and green light is independent of response amplitude. Figure 5 shows the foveal response to these two lasers at different intensities. With weaker stimuli the responses diminish in amplitude but the response to green remains faster than that to red light.

In order to study the focal ERG from more peripheral retina, we used a mirror contact lens, to deliver the laser beam and the adapting light at 30–50 deg from the fovea. Figure 6 shows the focal cone response to a 633 nm stimulus at the fovea (top), at 30 deg temporal to the fovea (middle), as well as the cone ERG to a full-field stimulus using the same laser stimulus delivered to the surface of a Burian–Allen contact lens electrode (bottom). With foveal stimulation the peak latency of the on-response is later than it is to the other stimuli.

Figure 7 shows the relationship between the amplitude (ordinate) vs the peak latency (abscissa) of the on- and off-response to foveal, focal peripheral and the full-field stimuli. The full-field stimulus produces a quicker

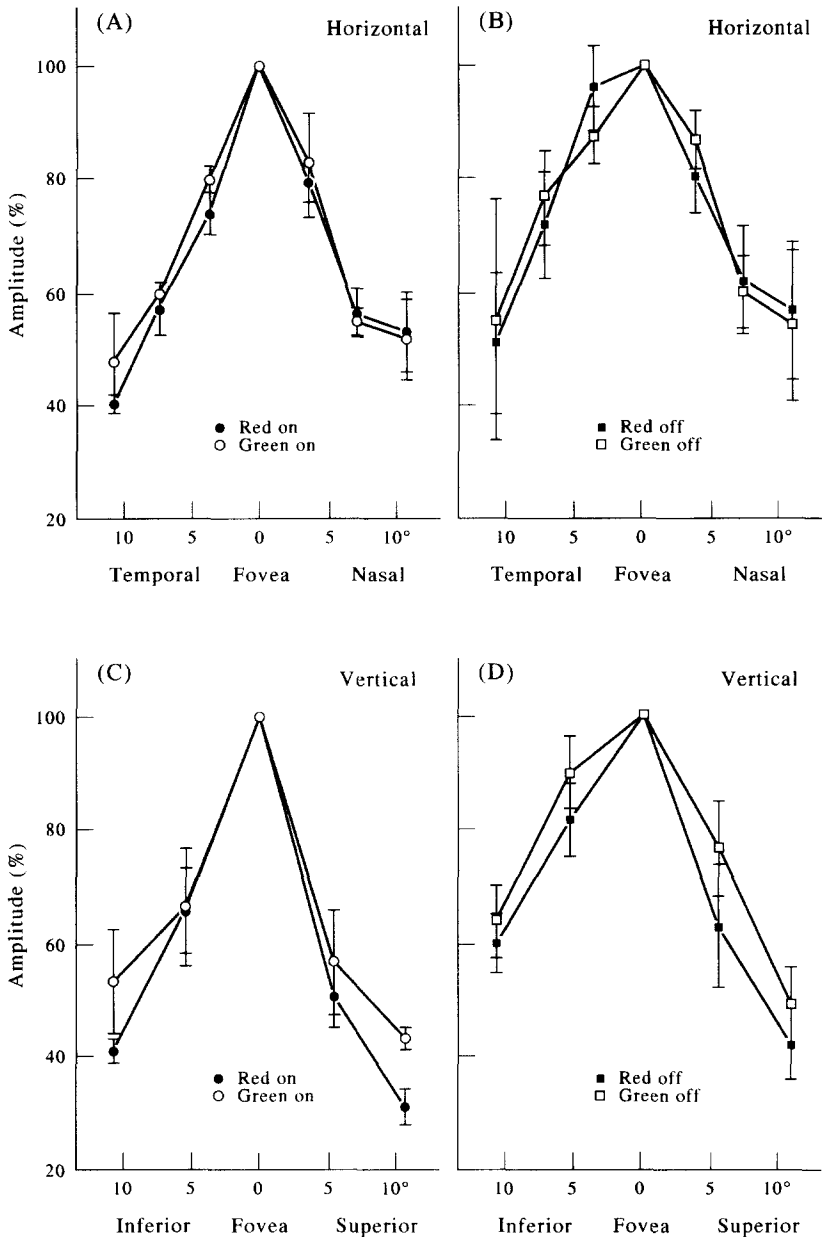


FIGURE 3. The relative amplitude of the focal ERG with eccentricity. The fall off of amplitude is similar along the horizontal and vertical meridian. The vertical bars indicate the SDs. Results from four separate experiments have been averaged in this and the other figures.

response than the foveal ERG for the off- as well as the on-response. The on-response to the focal stimuli at 30–50 deg from the fovea has a shorter peak latency than the foveal response, for the on- but not for the off-response. With a full-field stimulus the 544 nm stimulus also produces a quicker response than the 633 nm stimulus and this difference is independent of the amplitude.

Figure 8 shows the differences between the corneal, vitreal and intraretinal cone ERGs to ganzfeld flashes. The intraretinal ERG is about 2–3 times the amplitude of the corneal or vitreal ERG and reversed in polarity. Figure 9 shows that the cone *b*-wave implicit time is virtually independent of the amplitude of the responses. The intraretinal cone ERG in the para-macular area has a longer implicit time than the intraretinal ERG in the mid-peripheral retina. The para-macular intraretinal ERG obtained with a ganzfeld flash is also faster than the focal cone ERG obtained with a 3 deg stimulus at 30–50 deg from the fovea (Fig. 7).

In order to determine whether these differences in both the speed and the spectral behavior of these cone ERGs are due to the rate of stimulation, we examined the response over a range of frequencies. Figure 10 shows foveal cone ERGs to the 633 and 544 nm flicker at rates ranging from 5 to 40 Hz. At 5 and 7 Hz the responses to 633 nm are as large, or larger, than those to 544 nm with a relatively similar response to the on and off of the light pulse. Above 15 Hz the response begins to lose its double harmonic as on- and off-responses merge. At about 20 cps, the response to 633 nm becomes

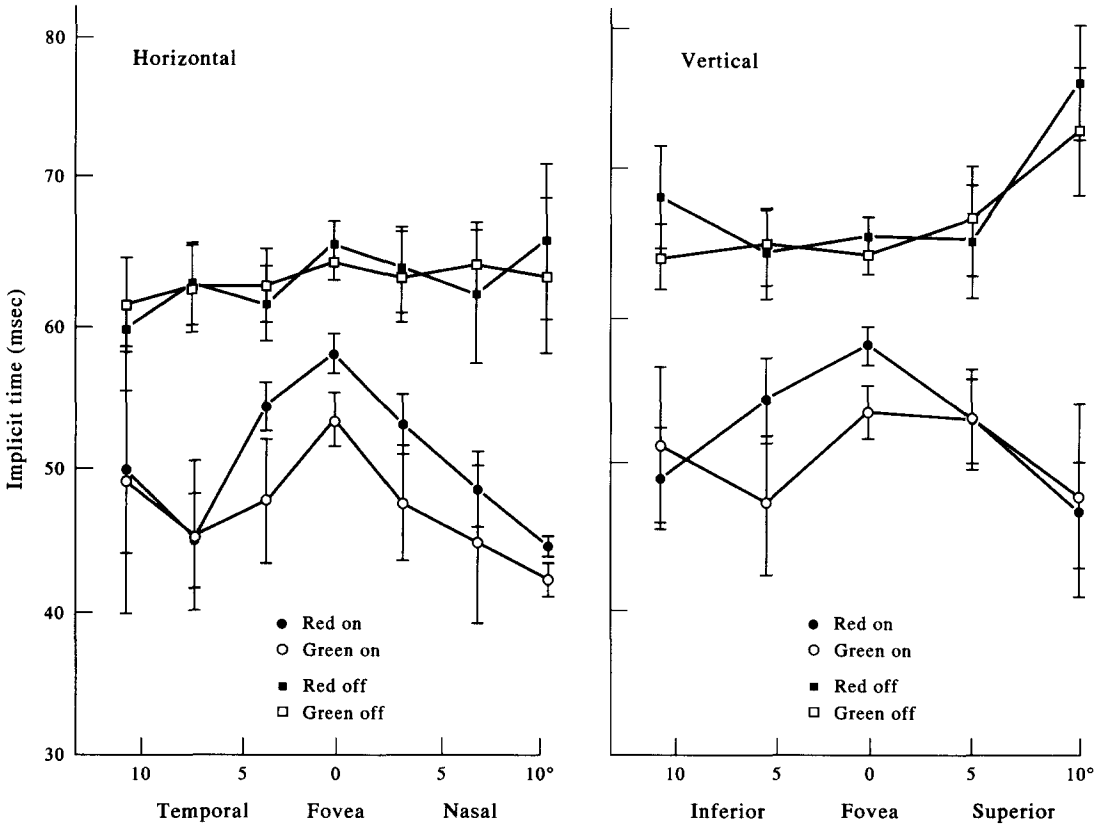


FIGURE 4. The relationship of the implicit time of the on- (*b*-wave) and the off-response (*d*-wave) with eccentricity from the fovea. The vertical bars indicate the SD.

smaller than that to 544 nm and this is maintained to the highest frequencies (30 and 40 cps). Figure 11(A) shows the relationship between the amplitude of these focal cone responses and flicker frequency. There is a minimum in amplitude at about 15 Hz where the double harmonic shifts to a more fundamental response. At low frequencies the on-response to the 633 nm is larger than that to the 544 nm, but at frequencies >15 Hz where the on- and off-responses have merged, the responses to 633 nm are smaller than they are to 544 nm. At low frequencies (<20 Hz) implicit times are late [Fig. 11(B)]. At very low frequencies (<10 Hz) later

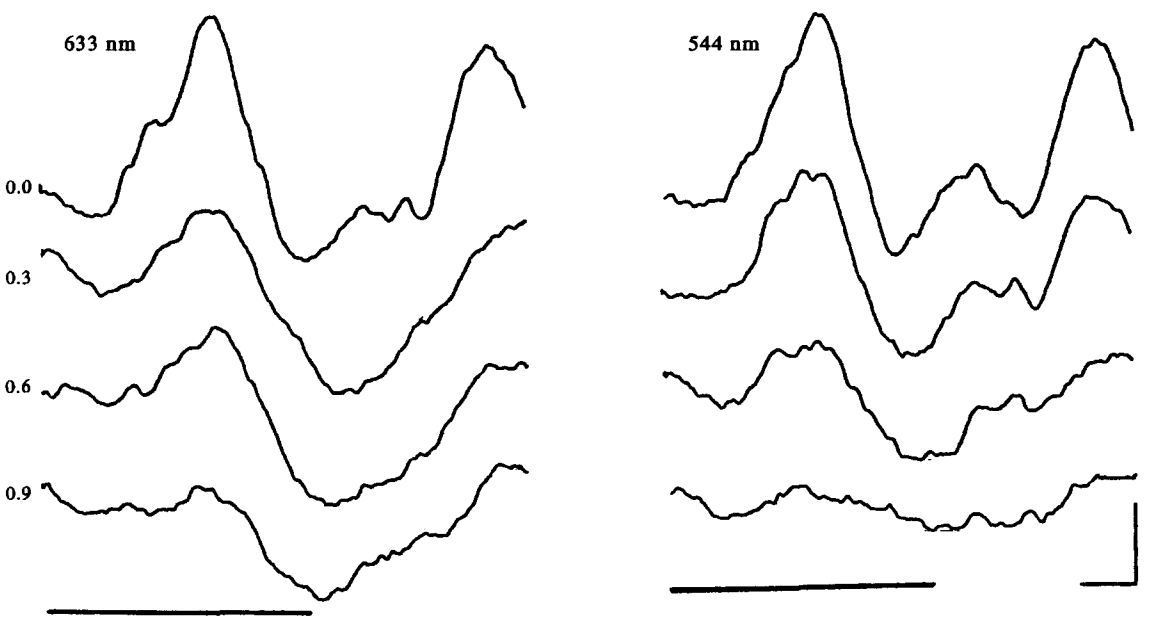


FIGURE 5. The foveally centered cone ERG to the red (633 nm) and green (544 nm) laser stimuli at different relative intensities indicated on the left by the amount of neutral density filtering interposed in the laser beam. The horizontal line below shows the duration of the light pulse. The calibration (lower right) indicates 1 $\mu$V vertically and 11 msec horizontally.

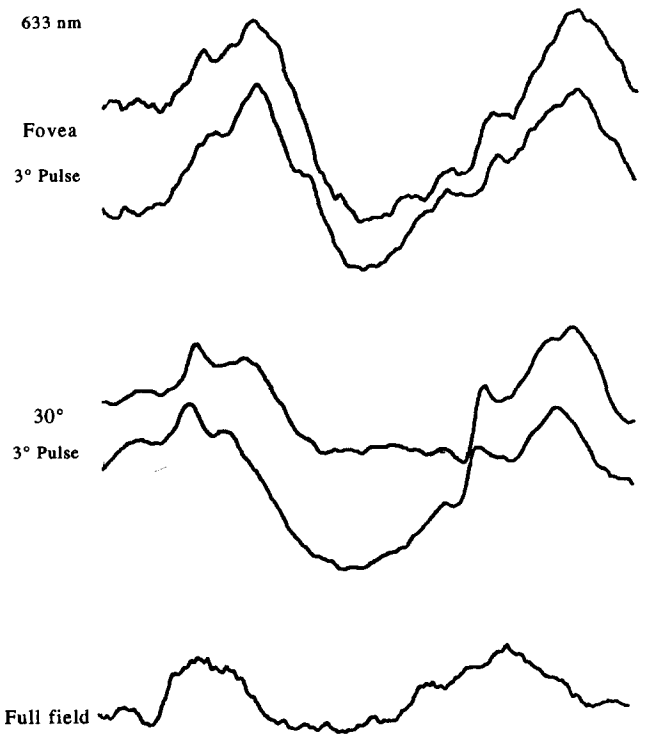


FIGURE 6. Focal cone ERG to the 633 nm stimulus at the fovea (top), at 30 deg temporal to the fovea (middle), and the cone ERG to a full-field stimulus using the same laser pulse and recorded with a bipolar Burian–Allen contact lens electrode (bottom). Each trace is 100 msec in duration.

on-responses are later to 633 nm than to 544 nm. At high frequencies (>30 Hz) the implicit time becomes very short and is similar for the red (633 nm) and green (544 nm) stimuli.

## DISCUSSION

The results reveal two new aspects to the primate focal cone ERG. One is its unique waveform in the vicinity of the fovea; the second is the speeding up of the cone *b*-wave with distance from the fovea. These two phenomena may be interrelated.

The foveal cone ERG has two distinct components to both its *b*- (on) and *d*- (off) wave responses. One component is fast and phasic; the second component is slower, more prolonged and more long wavelength sensitive. Both components decrease progressively with distance from the fovea but the slower component decreases more rapidly than the fast one. This makes the peripheral cone ERG faster than the foveal cone ERG.

One explanation of this phenomenon could be that it reflects differences between the physiology of the L and M cones and/or their postsynaptic neural responses. The red laser produces a relatively stronger stimulation of L than M cones; the green laser tends to do the converse. If the L cone response were slower or if there were appropriate antagonistic interactions between L and M cone signals, such a difference in waveform could occur. This hypothesis could be tested by examining the foveal cone ERG of human protanopes or deuteranopes presuming the human foveally centered cone ERG resembles that of the monkey. With only L or only M cones responding to the light there should be no difference in the waveform of the foveal cone ERG, if this unique waveform depends on differences in the physiology of L and M cone systems.

Another explanation could be that there are separate sets of cone bipolar cells subserving both L and M cones. The L and M cones have at least one set of midget

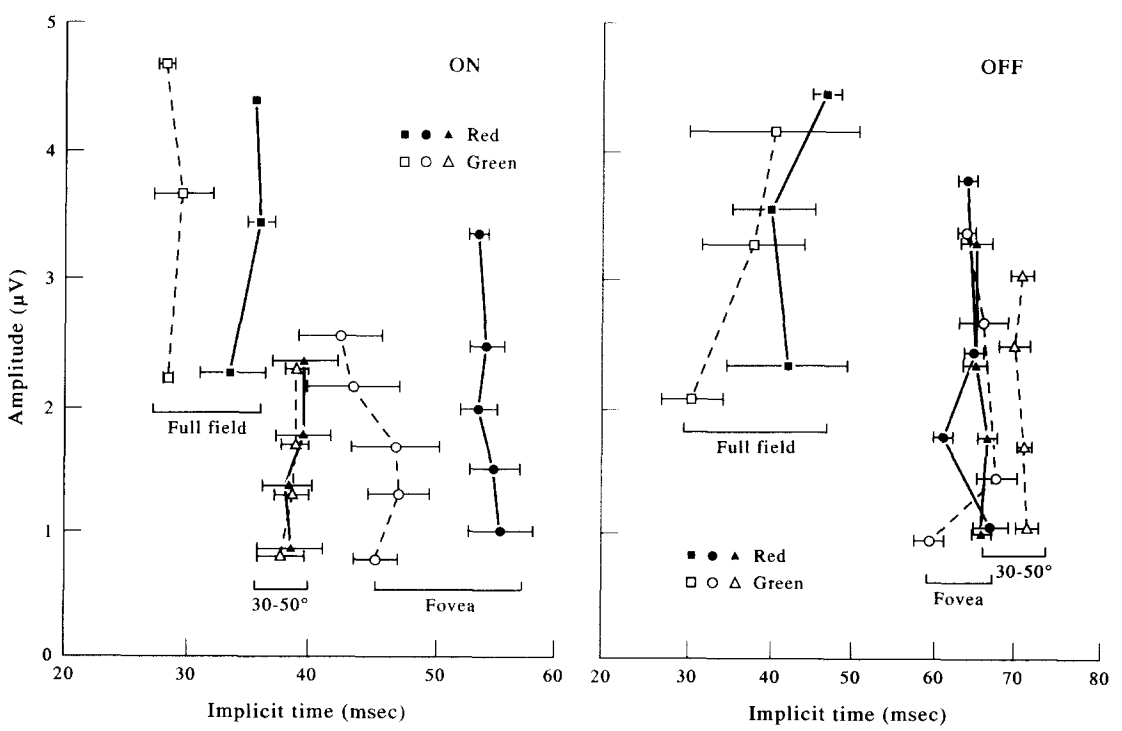


FIGURE 7. The relationship between the implicit time (abscissa) and the amplitude (ordinate) of the on- (ON) and off-response (OFF) to foveal, mid-peripheral, and full-field stimuli. Horizontal bars indicate the SD.

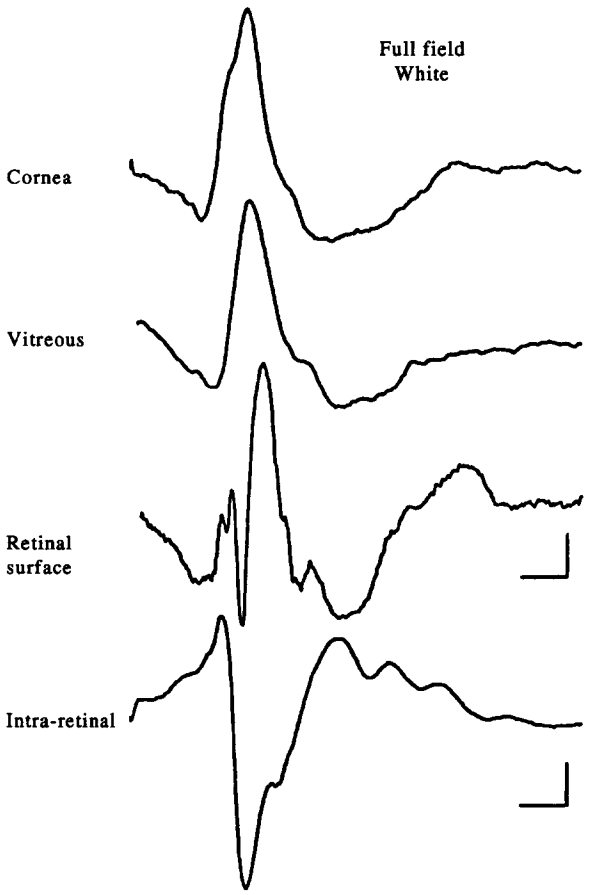


FIGURE 8. Cone ERG recorded with a corneal contact lens to a full-field ganzfeld flash (above), and the intraocular ERG from the same stimulus recorded in the vitreous, at the retinal surface, and intraretinally in the para-macular area (bottom) in the presence of a background light of 10 cd/m$^2$. Calibration indicates 5 $\mu$V vertically and 10 msec horizontally for the upper two traces; 10 $\mu$V vertically and 10 msec horizontally for the bottom trace.

or midget-like bipolar cells, both on- and off-variety (Kolb, Luberg & Fisher, 1992). These cone bipolars transmit signals to the small tonic ganglion cell system (Dacey & Lee, 1994) which synapses in the parvocellular layers of the lateral geniculate nucleus (Gouras, 1992). There may be a parallel set of cone bipolars which transmit signals to the large, phasic ganglion cell system which synapse in the magnocellular layers of the lateral geniculate nucleus (Kaplan, Lee & Shapley, 1992). The latter might generate the fast, and the former the slow, component of the *b*-wave of the foveal cone ERG. Such a response difference would be detectable even if there were only one set of L or M cones in the retina, such as in protanopes and deuteranopes.

There is earlier evidence that the primate foveal intraretinal ERG is different than the peripheral intraretinal response (Brown & Watanabe, 1962). The foveal response had a smaller *b*-wave and a relatively larger *a*-wave, a phenomenon thought to reflect the paucity of inner nuclear layer cells in the center of the fovea. In those experiments cone and rod responses were not distinguished from one another. We did not detect such a difference in our experiments, presumably because we used a 3 deg stimulus which is too large to distinguish activity that depends on the fovea, alone.

Both the focal peripheral and the full-field cone ERG *b*-waves are faster than those of the foveal cone ERG. This difference could be due to the possibility suggested above, that a faster cone bipolar system dominates the responses of the more peripheral retina. It is also possible that the peripheral cones respond more rapidly than foveal cones. Some of our experimental results, however, imply that these explanations may be inadequate. For example, the intraretinal cone *b*-wave to a full-field stimulus is faster than the focal response from the same or similar areas of the retina. This indicates that the size of the stimulus influences cone *b*-wave speed. This type of complex spatial interaction in the generation of the *b*-wave is reminiscent of results in cat retina, where small spot stimuli generate little to no *b*-wave compared to large spots subtending identical amounts of retinal area (Nelson, Zrenner & Gouras, 1978).

Cone *b*-wave speed is relevant to an interesting phenomenon that has long been observed in patients with retinal degenerations, such as retinitis pigmentosa (RP). In general these patients have reduced and delayed cone *b*-waves (Berson *et al.*, 1969; Gouras, 1970). There have been several hypotheses offered to explain why there is a delay. Sandberg, Effron and Berson (1983) suggested that this may be due to rod–cone interactions, which speed up the light-adapted cone *b*-wave in normal

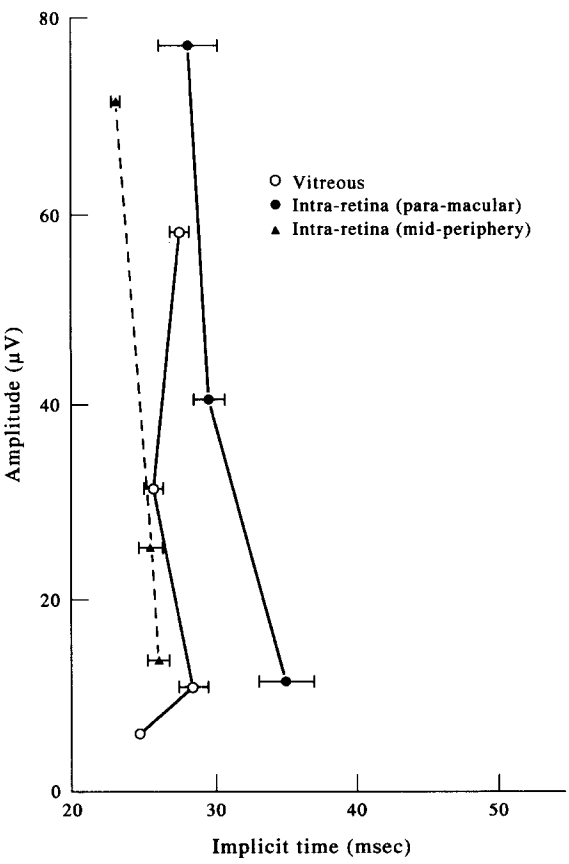


FIGURE 9. The relationship between the implicit time (abscissa) and the amplitude (ordinate) of the cone ERG *b*-wave recorded in the vitreous, in the para-macular retina and in the mid-peripheral retina. Horizontal bars indicate the SD.

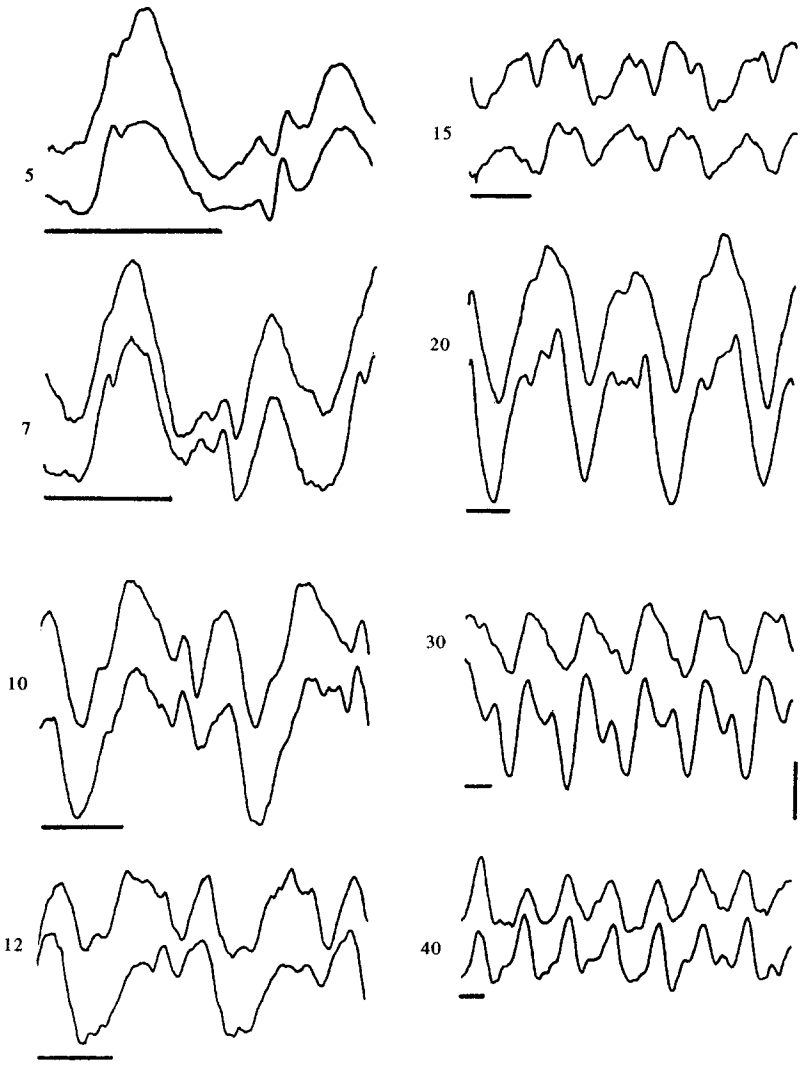


FIGURE 10. Foveal cone ERGs to the 633 and 544 nm stimuli at different flickering rates. The upper trace of each set shows the response to the 633 nm stimulus and the lower to 544 nm stimulus. Numbers on the left side of each set indicate flickering rates (Hz). Horizontal bars indicate duration of stimulus pulse and the vertical calibration indicates 1 $\mu$v. Each trace is 100 msec in duration.

subjects but fail to do so in RP subjects where rods are preferentially lost. We have suggested that this delay may also be due to degenerate cones failing to absorb light effectively making RP subjects less light-adapted than normals. The results of the present paper suggest an alternate hypothesis that a loss of peripheral retinal function, typical of RP may expose a later response of the more central retina.

Although there have been numerous earlier publications that the focal cone ERG is maximal at the fovea (Armington *et al.*, 1961; Gouras *et al.*, 1962; Brindley & Wertheimer, 1965; Arden & Banks, 1966; Aiba *et al.*, 1967; Biersdorf & Diller, 1969; Sandberg *et al.*, 1978; Miyake & Awaya, 1989) only a few commented on any unique waveform difference to the foveally centered response. Brindley and Westheimer (1965) found that the foveal ERG had a different waveform than that of the more peripheral retina but their signal-to-noise ratio prevented examining this in any detail and there was a question whether rod intrusion occurred with the white light stimuli they had used. Using relatively large (15 deg) hemifield stimuli, Miyake *et al.* (1989a, b) found larger oscillations in the focal ERG response in the temporal compared to the nasal macular retina but did not report any uniqueness to the foveally centered focal ERG. Biersdorf (1981) noted that the foveal cone ERG was slower than that obtained with full-field stimuli and also suggested that this might be due to foveal cones being slower than peripheral cones. This idea receives support from the fact that the cone flicker fusion frequency is higher in the peripheral than in the central retina (Lythgoe & Tansley, 1929). Subsequent findings of Sandberg *et al.* (1983) that the foveal cone flicker ERG was slightly faster at the fovea than at the parafovea seemed inconsistent with this idea. Our results clarify this incongruity by revealing a unique slow subcomponent that is especially prominent in the foveal cone ERG but which is lost at high rates of flicker.

Previous research with the focal cone ERG has demonstrated its clinical usefulness using amplitude alone as the criterion of function (Sandberg *et al.*, 1978; Miyake *et al.*, 1989a, b; Matthews, Sandberg & Berson,

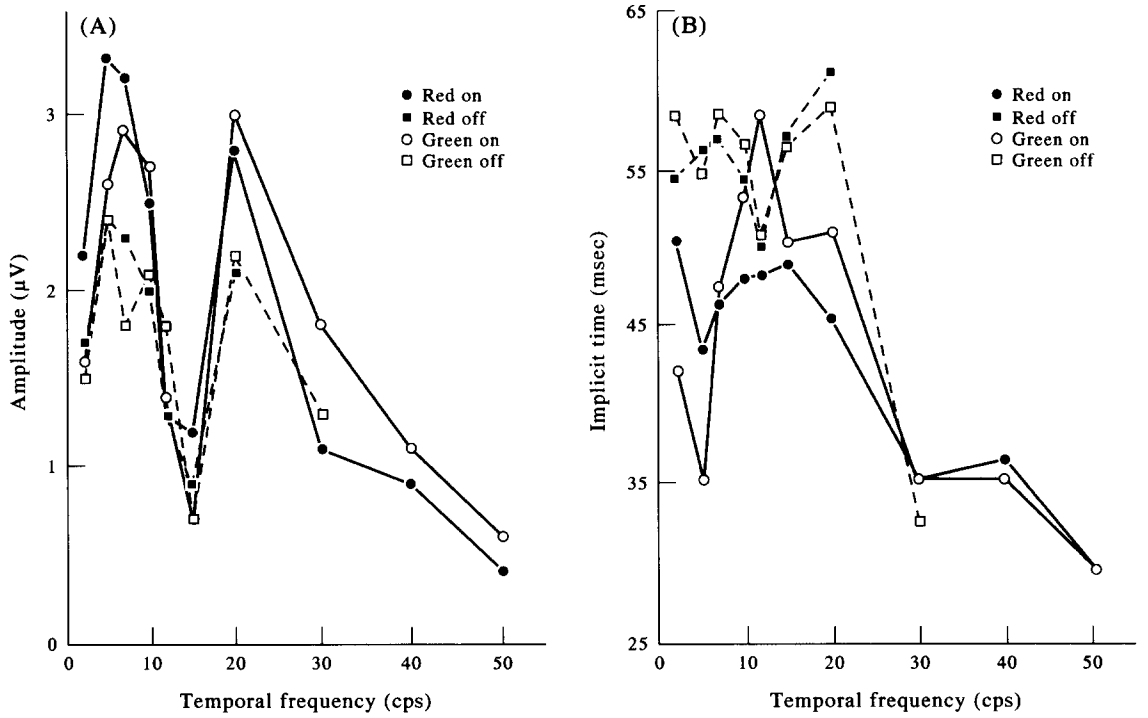


FIGURE 11. The relationship between the amplitude of the foveal cone ERG (ordinate) and the flicker frequency (abscissa) (A), and between the implicit time (ordinate) and the flicker frequency (abscissa) (B).

1992). The fact that there is also a unique waveform that defines the foveally centered cone ERG provides a new parameter to investigate both the monkey and human retina experimentally and clinically.

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