

# Rod-Driven Focal Macular Electroretinogram

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**Purpose:** To determine the stimulus conditions required to elicit rod-driven, focal macular electroretinograms (rod FMERGs).

**Methods:** A blue ( $\lambda_{\max} = 470$  nm) stimulus,  $5^\circ$  in size, was imaged at a luminance of  $1.5 \text{ cd/m}^2$  on different regions of the human retina. Electroretinograms (ERGs) elicited by this stimulus were recorded from the light- and dark-adapted retina of four subjects without any ophthalmological abnormalities. A subject with cone dystrophy was also tested by this method.

**Results:** Stimulus luminance  $\leq 1.5 \text{ cd/m}^2$  did not elicit a response when it was imaged on the optic disc, but higher intensities elicited a small b-wave from stray light. When this stimulus was imaged on the macular area or the  $15^\circ$  temporal retina, an ERG was elicited that had the shape of the full-field scotopic ERG. This stimulus with a luminance of  $1.5 \text{ cd/m}^2$  did not elicit a response from stimulation of the macular area of a light-adapted retina but elicited a slow-rising positive b-wave after 30 minutes of dark adaptation. In a subject with cone dystrophy, focal rod response was elicited from the macula, despite no response under photopic conditions.

**Conclusion:** We conclude that this stimulus will elicit a response that is derived exclusively from rods and is a focal response with no contribution from stray light. **Jpn J Ophthalmol 2003;47:356–361** © 2003 Japanese Ophthalmological Society

**Key Words:** Dark adaptation, macular focal electroretinogram, rod.

## Introduction

Standardized electroretinograms (ERGs) elicited by *Ganzfeld* stimuli are used to evaluate the physiological condition of the retina and to diagnose several retinal disorders. These ERGs originate from the activation of retinal cells over a large area of the retina,<sup>1–6</sup> and localized retinal diseases such as macular holes, epimacular membranes, and age-related macular degeneration, cannot be investigated by these full-field ERGs.<sup>1,7,8</sup>

To overcome this limitation, several researchers have developed techniques to elicit focal macular ERGs (FMERGs) to study macular and localized diseases.<sup>9–17</sup> Because cone photoreceptors are most concentrated in the macular area<sup>18–20</sup> and are important for visual functions,<sup>21</sup> most of these methods have focused on studying the responses originating from cones.<sup>9–17</sup> These focal responses are thus referred to as photopic or cone FMERGs.

The concentration of rods is maximal within the retinal vessel arcade and decreases gradually toward the fovea.<sup>18,20</sup> Because rods are not completely absent in the macula area, stimuli designed to be optimal for eliciting rod activity should elicit rod-driven responses.<sup>22</sup> Nevertheless, the properties of the rods within the macular area have not been studied in detail, and alterations of their function in retinal diseases have not been adequately determined.

Subjective tests, such as dark adaptometry and two-color perimetry,<sup>23</sup> are used to evaluate rod function in the macular area. Unfortunately, there are no well-described objective clinical tests to examine the rods although some researchers have studied rod FMERG.<sup>22,24–27</sup> In spite of the technical difficulties of eliminating stray light and of monitoring the stimulus in the macular region, some success has been obtained for recording rod-driven responses from the macular region.

We report on the stimulus and recording conditions necessary to obtain FMERGs elicited exclusively from rods, and on the effect of retinal adaptation on the rod FMERGs. The usefulness of this technique was tested by examining whether rod FMERGs could be elicited from a patient with cone dystrophy.

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## Materials and Methods

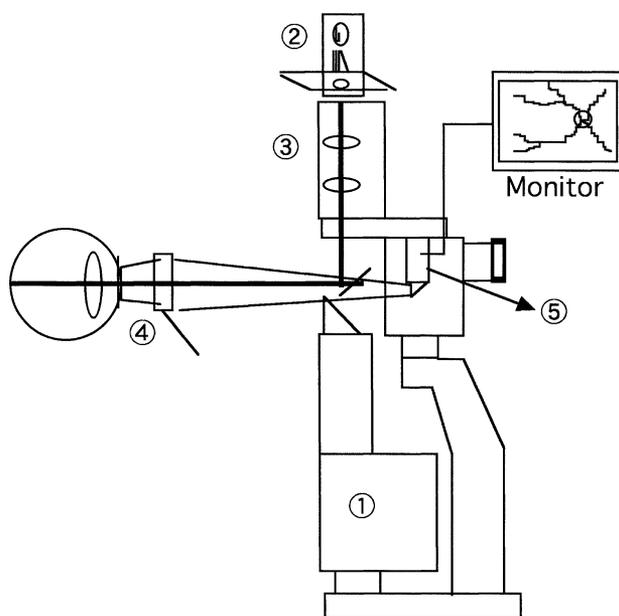
### *Stimulating and Monitoring Instrument*

The apparatus used to record photopic FMERGs, described in detail earlier, was modified for these studies.<sup>2</sup> In brief, a microscope (30 SL-M, Carl Zeiss-Humphrey, Dublin, CA, USA) with a built-in light-emitting diode (LED) formed the basic unit. The fundus observation system consisted of a near-infrared camera, an infrared light source (wavelength peak 800 nm), and a removable infrared light scope (Figure 1). The infrared light irradiated the fundus through a panfundus contact lens with built-in bipolar electrodes.

A round 5° spot of blue light (light emitting diode with  $\lambda_{\max} = 470$  nm,  $\Delta\lambda_{1/2} = 35$  nm, Nichia Corporation, Tokushima) was used as the stimulus. The stimulus duration was 10 msec and the interval between stimuli was 210 msec. The ERGs were processed and averaged by a Neuropack  $\Sigma$  (MEB-5500, Nihon Kohden, Tokyo), and the noise level with the electrodes immersed in saline was less than 0.05  $\mu$ V for a recording with 50 summations.

### *Subjects*

Three women (age 21, 23, and 26) and one man (age 29) with normal visual acuity and no ophthalmological diseases and one patient (age 26) with cone dystrophy were studied. An informed consent was signed by the



**Figure 1.** Schematic drawing of apparatus for recording focal electroretinograms. (1) Microscope (30 SL-M, Carl Zeiss), (2) Blue LED, (3) Telescope, (4) Panfundus contact lens with built-in bipolar electrodes (Kyo-Con), (5) Near-infrared camera.

four volunteers and one patient following an explanation of the purpose and procedures of the experiment. The experiments were conducted to conform to the tenets of the Declaration of Helsinki.

### *Procedures*

The pupils were fully dilated with 0.5% tropicamide, and the ERGs were recorded with a contact lens bipolar electrode placed on the anesthetized cornea. A silver disc ground electrode was placed on the ear.

To improve the signal-to-noise level, 200 to 400 responses were averaged. The amplitudes and implicit times of the a- and b-waves were measured.

### *ERGs Elicited from Stimuli Imaged on Optic Disc*

To determine the optimal stimulus to elicit only rod activity, the stimulus intensity had to be balanced between one that would elicit a relative large and reliable b-wave and one that did not elicit a stray light response. To determine the optimal stimulus intensity, a 5° blue stimulus was imaged on the optic disc, and ERGs were recorded to increasing stimulus intensities.

### *ERGs from Different Areas of the Retina*

In addition to the responses from the optic disc, the same stimulus was used to elicit focal MERGs from two other retinal areas that have different proportions of rods. After 30 minutes of dark adaptation, the weak blue light stimulus (luminance, 1.5  $\text{cd}/\text{m}^2$ ) was projected onto the macula area and onto a 15° temporal retinal area where the density of rods is highest.

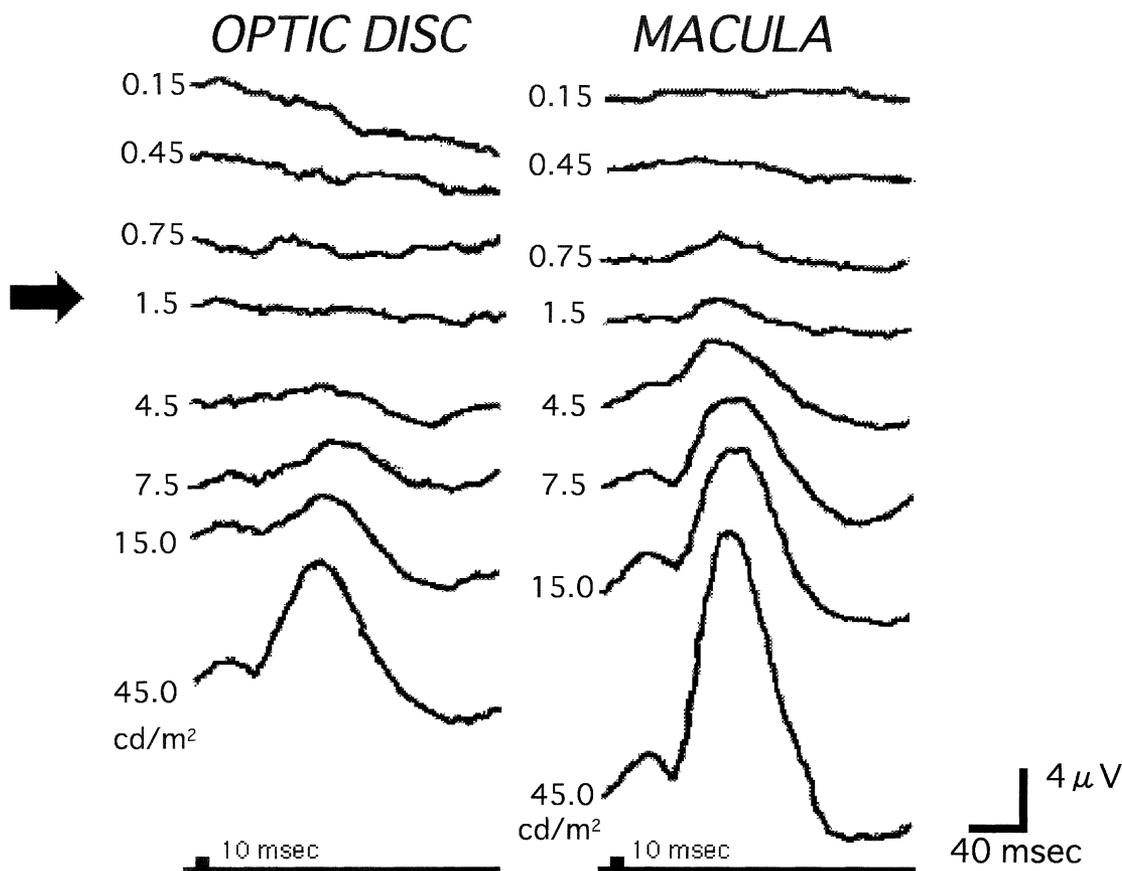
### *Effect of Retinal Adaptation*

Because the rods are more sensitive after dark-adaptation, we tested the effectiveness of our 5° blue stimulus of 1.5  $\text{cd}/\text{m}^2$  in eliciting rod FMERGs after different periods in the dark. We first light-adapted the eye with 600  $\text{cd}/\text{m}^2$  for 5 minutes, then recorded an ERG immediately after turning the light-adapting light off. ERGs were also recorded after 30 and 60 minutes of dark adaptation.

## Results

### *FMERGs from Stimulating Optic Disc*

The ERGs elicited by the 5° blue flash of different intensities imaged on the optic disc or on the macula are shown in Figure 2. With the stimulus spot on the optic disc, no response was elicited by stimulus intensities of 1.5  $\text{cd}/\text{m}^2$  and weaker. However, these stimulus intensities



**Figure 2.** Focal electroretinograms elicited by different stimulus intensities from a normal volunteer. With a stimulus luminance [dbltequ] 1.5 cd/m<sup>2</sup>, no response was recorded when the stimulus was imaged on the optic disc (left), but the same stimuli imaged on the macula elicited responses. At higher stimulus intensities, a response was elicited when the stimulus was imaged on the optic disc. Similar findings were obtained for the other normal volunteers.

elicited a small but definite ERG when stimulating the macular area. With light stimuli  $\geq 4.5$  cd/m<sup>2</sup> imaged on the optic disc, a small b-wave was elicited probably by the stray light. The slow-rising positive b-wave wave resembled the scotopic b-wave elicited by a weak full-field stimulus in shape and latency. Similar findings were made on all four volunteers (Figure 2, arrow).

The response elicited by a 1.5 cd/m<sup>2</sup> blue flash imaged on the macular seemed to be a focal rod response because the same stimulus imaged on the optic disc did not elicit a response.

When this same stimulus was projected onto the 15° temporal retina, larger amplitude focal ERGs were recorded (Figure 3, lower).

#### *Effect of Dark Adaptation*

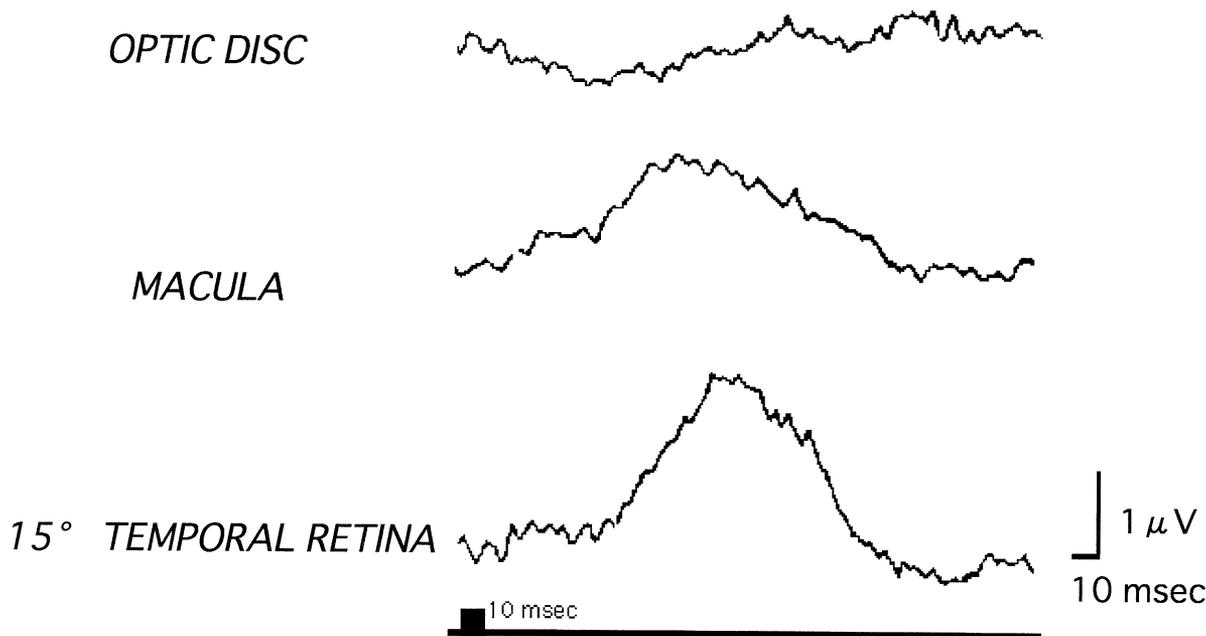
The rod FMERGs elicited by the 5° blue stimulus of 1.5 cd/m<sup>2</sup> after different times in the dark are shown in Figure

4. This stimulus did not elicit a response when stimulating the macula area immediately after turning off the light-adapting light (Figure 4, upper trace). However, after 30 and 60 minutes dark adaptation, a small b-wave was recorded (Figure 4, middle and lower trace). Similar findings were obtained from the other volunteers.

#### *Amplitude and Implicit Times of Rod FMERGs*

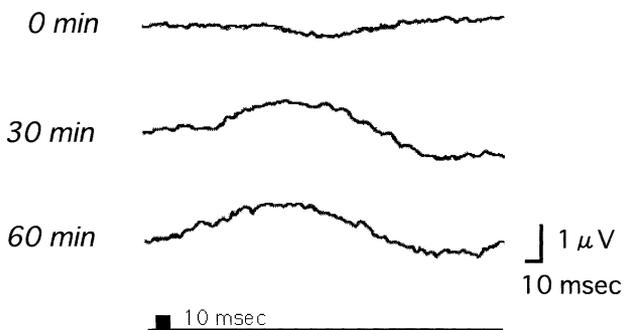
The mean amplitude of the b-wave of the rod FMERGs for the four volunteers was  $1.04 \pm 0.14$   $\mu$ V (mean  $\pm$  SD) after 30 minutes and  $1.12 \pm 0.08$   $\mu$ V after 60 minutes of dark-adaptation when the stimulus spot was imaged on the macula. The difference in the mean ERG amplitudes at 30 and 60 minutes was not significantly different ( $P > .05$ ), suggesting that 30 minutes of dark-adaptation is sufficient to record rod FMERGs.

For the same stimulus intensity and location, the mean implicit time was  $99.3 \pm 11.0$  msec after 30 minutes of



**Figure 3.** Focal electroretinograms elicited by the standard stimulus with a luminance of  $1.5 \text{ cd/m}^2$  from a normal volunteer. An ERG was not recorded with the stimulus imaged on the optic disk (upper trace). An ERG that resembled the scotopic full-field ERG was recorded from stimulating the macula (middle trace), and a larger ERG was recorded when the stimulus was imaged in the  $15^\circ$  temporal retina (lower trace).

dark adaptation and  $98.8 \pm 13.5$  msec after 60 minutes. This difference in the implicit times was not significant, and also not significantly different from the standard rod ERGs using full field stimuli in our laboratory ( $95.6 \pm 5.1$  msec).



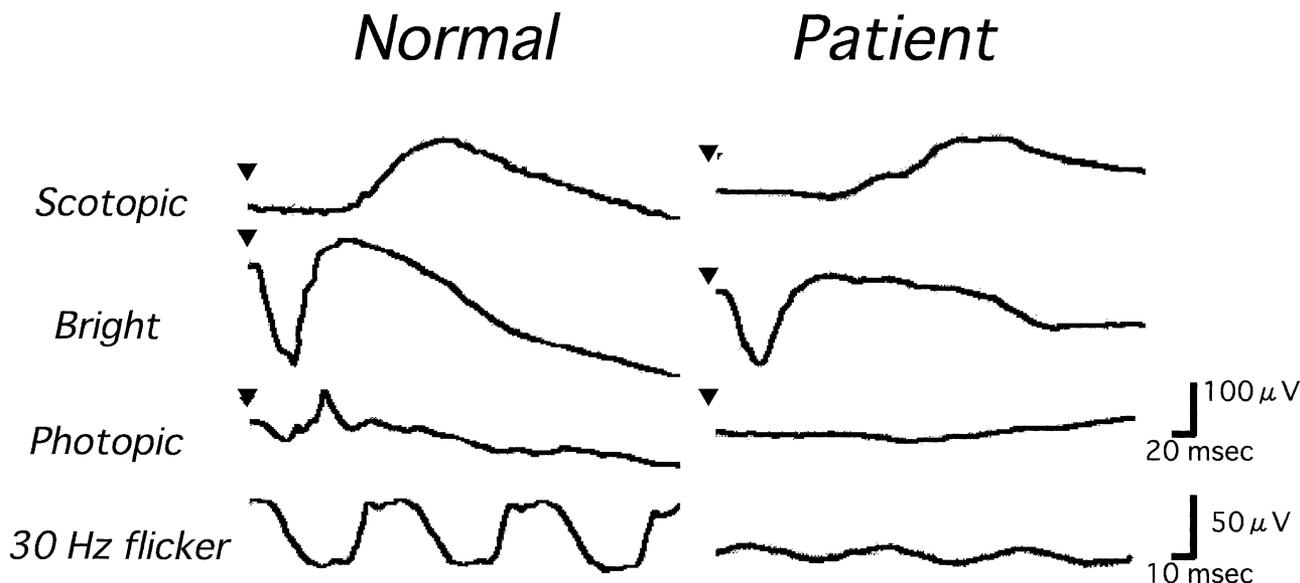
**Figure 4.** Macular focal electroretinograms from a normal volunteer. In the light-adapted condition (0 minute dark adaptation: upper trace), an ERG was not elicited by the standard stimulus. After 30 and 60 minutes dark adaptation, the same stimulus intensity elicited a b-wave that resembled a scotopic b-wave of the full-field ERG (middle and lower traces). (30 minutes =  $0.87 \mu\text{V}$ , 60 minutes =  $1.05 \mu\text{V}$ ).

### Case Report

A 26-year-old man presented with a complaint of decreased vision. His corrected visual acuity was 0.1 in both eyes. The fundus appeared normal by indirect ophthalmoscopy and fluorescein angiography. Full-field ERGs, recorded with the ISCEV protocol (Figure 5),<sup>28</sup> showed a selective cone dysfunction or cone dystrophy. The blue  $5^\circ$  stimulus of  $1.5 \text{ cd/m}^2$  was used to elicit test rod and cone function in the macular area. A  $5^\circ$  stimulus of luminance  $38 \text{ cd/m}^2$  and the duration of 100 msec on a background of  $3.8 \text{ cd/m}^2$  was used to elicit photopic responses (Figure 6). The photopic FMERG was non-recordable, but a rod FMERG was recorded with amplitude and implicit time comparable to those of normal subjects.

### Discussion

We have shown that a  $5^\circ$  blue stimulus of luminance  $\leq 1.5 \text{ cd/m}^2$  does not elicit an ERG when imaged on the optic disc. This suggests that the intensity of stray light reflected from the highly reflective optic disc was not sufficient to elicit a stray light response. However, when this same stimulus was imaged on the retina, an ERG was elicited that can be considered to arise from



**Figure 5.** Full-field electroretinograms from a normal subject (30-year-old man) and the patient. The patient's scotopic (rod) ERG was nearly normal. His bright (mixed rod and cone) ERG was subnormal, photopic (cone) and 30 Hz flicker ERG were subnormal, photopic (cone) and 30 Hz flicker ERGs were nearly non-recordable. The arrowheads indicate stimulus onset.

the retinal neurons immediately beneath the spot, and the photoreceptors surrounding the spot stimulus were not activated. These responses can then be considered to be focal ERGs, and when imaged in the macular area, the responses are focal macular ERGs.

Our results also indicate that the responses elicited by this stimulus are derived exclusively from rods. First, a response was not elicited by this stimulus from a light-adapted retina. Second, the stimulus intensity is lower than the cone threshold. Third, the short wavelength blue stimulus and low stimulus intensity would favor the rods. Fourth, the slow-rising positive b-wave elicited by this stimulus closely resembles the scotopic b-waves elicited from the dark-adapted eye by low intensity, full-field stimuli. And fifth, this stimulus imaged in the macular area elicited a good response from a subject with cone dystrophy but did not elicit a response under photopic

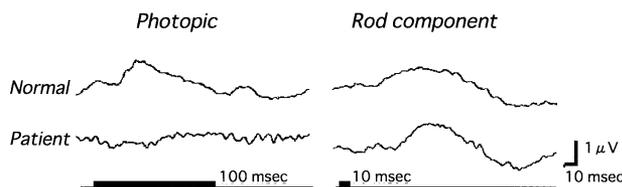
conditions. Taken together, we conclude that a 5° blue ( $\lambda_{max} = 470 \text{ nm}$ ) stimulus of luminance  $1.5 \text{ cd/m}^2$  will elicit a rod FMERG when placed in the macular area.

There are only a few reports about rod focal ERGs in the literature.<sup>22,24-27</sup> In one study,<sup>24</sup> a 40° diameter stimulus was used and the properties of the ERGs indicated that both rods and cones contributed to the response. The authors extracted the rod component from the mixed rod-cone response by wave subtraction techniques. In another report, a spot of 10° to 30° of 0.6-2.1 log scot td-sec blue light was imaged on the macula but the ERGs had two components; a faster and smaller component considered to be the focal response, and a second slower and larger positive component considered to arise from stray light.<sup>25</sup>

In a third study, the multifocal ERGs<sup>26</sup> elicited by 61 hexagonal stimuli on a background that should suppress stray light responses were reported to originate from rods. The other studies<sup>22,27</sup> used techniques similar to ours to record rod focal ERGs from only the targeted areas.

One advantage of our system was the ability to monitor the position of the stimulus on the retina by using an infrared camera. This was especially important in patients with macular disease and poor fixation. Using these stimulus conditions, we were able to record rod FMERGs but not cone FMERGs in a case of cone dystrophy (diagnosed by full-field ERGs).

In conclusion, we believe that examining the rod FMERGs is a useful method to study alterations of rod function in eyes with retinal diseases, particularly those with macular disease and night blindness.



**Figure 6.** Macular focal electroretinograms from a normal subject (30-year-old man) and the patient. Photopic response and rod component are shown.

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