Amplitude Increase of the Multifocal Electroretinogram during Light Adaptation

Mineo Kondo, Yozo Miyake, Chang-Hua Piao, Atsubiro Tanikawa, Masayuki Horiguchi, and Hiroko Terasaki

PURPOSE. To determine using the multifocal ERG technique whether there are any regional differences in the increase in the amplitude of cone electroretinograms (ERGs) during light adaptation.

METHODS. Multifocal ERGs were recorded with the Visual Evoked Response Imaging System from five normal subjects. Thirty-seven hexagonal stimulus elements and a recording time of 60 seconds were used. After 20 minutes of dark adaptation, multifocal ERGs were repeatedly recorded every 2 minutes over a period of 16 minutes. The amplitudes of the multifocal ERGs at different eccentricities were compared during the 16 minutes of light adaptation.

RESULTS. During the 16 minutes of light adaptation, the summed responses of the multifocal ERGs increased in amplitude an average of 36% and 47% for the negative and positive components, respectively. The magnitude of increase was minimal in the central retina at 22% and was significantly larger in the peripheral retina at 58%. The implicit time was slightly increased (<4%) with light adaptation, but there were no regional differences.

CONCLUSIONS. The results demonstrated that there are topographic variations in the amplitude increase of cone ERGs during light adaptation. This topographic variation indicates that the mechanism for the increase must be based on known regional differences in the retina. (Invest Ophthalmol Vis Sci. 1999;40:2633–2637)

If an eye is first dark adapted and then light adapted, the amplitude of the electroretinograms (ERGs) elicited by photopic stimuli increases progressively during the course of light adaptation, stabilizing in approximately 15 to 20 minutes.1–12 The mechanism for this unique phenomenon has not been fully determined, although several explanations have been proposed—for example, a change in the standing potential,3 interaction between rods and cones,1,5–7 and a repolarization of the cone photoreceptors.8 Most of the investigators who have studied this phenomenon used traditional full-field cone ERGs, and very little is known about how different areas of the retina contribute to this phenomenon. However, Weiner and Sandberg13 elicited focal ERGs and reported that the foveal cone ERGs elicited by a 4° fast-flickering stimulus also increase during light adaptation, as do the full-field cone ERGs. Unfortunately, they did not compare the magnitude of the increase at different retinal locations.

The multifocal ERG, recently developed by Sutter and Tran14 and Bearse and Sutter,15 allows the simultaneous recording of focal ERGs from multiple retinal locations in a single recording session of several minutes. Because it has been demonstrated that the multifocal ERG is a cone-mediated response and that the negative and positive components of the multifocal ERG behave as do the a- and positive peaks of the traditional full-field photopic ERGs,16,17 it also could be expected that the amplitude of the multifocal ERG increases during the course of light adaptation. The multifocal technique is valuable, because it allows us to explore regional variations.

In the present study, we first determined whether the multifocal ERGs increase in amplitude during light adaptation as do the full-field cone ERGs. We then explored whether there are any topographic variations in this phenomenon across the human retina.

MATERIALS AND METHODS

Subjects
Five normal subjects aged 26 to 35 years (mean age, 30.3 years) participated. Except for refractive errors of −1.00 to −5.25 D, no ophthalmologic or systemic abnormalities were present. Informed consent was obtained after a full explanation of the procedures. All studies were conducted in accordance with the principles embodied in the Declaration of Helsinki.

Methods
The method for recording the multifocal ERG with the Visual Evoked Response Imaging System (EDI, San Mateo, CA) has been described in detail previously.14,15 In a clinical setting, 61 or 103 stimulus elements are widely used for eliciting the
multifocal ERGs, with a total recording time of 4 to 8 minutes. However, such a long recording period is not suitable for studying the time course of this phenomenon, because it is known that the cone ERGs increase rapidly in amplitude during the first several minutes of light adaptation. Therefore, we used 37 stimulus elements with a total recording time of 60 seconds.

The stimulus matrix consisted of 37 hexagonal elements that were displayed on a color monitor (Model GDM 2038; Sony, Tokyo, Japan) driven at a 75-Hz frame rate. These hexagons were scaled with eccentricity to elicit approximately equal signal amplitudes at all locations. At a viewing distance of 27 cm, the diameter of stimulus array subtended approximately 60°. A small red fixation point was placed at the center of the stimulus matrix. Each hexagon alternated between white and black according to a pseudorandom binary m-sequence at a rate of 75 times/sec. The luminance of the white frame and black frame was 100.0 and 1.0 cd/m², respectively, resulting in a contrast of 98.0%. To suppress scattered light, the periphery of the TV monitor, the diameter of which subtended 78°, was set to a luminance of 50 cd/m².

**Recording and Data Analysis**

The subject's pupils were fully dilated with a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride drops. After 20 minutes of dark adaptation, the Burian Allen bipolar contact lens electrode was inserted under dim red illumination. A ground electrode was attached to the ipsilateral earlobe. The opposite eye was occluded. Optical lenses were used to correct the visual acuity that had been determined before testing. After 5 additional minutes of readaptation to darkness, multifocal ERGs were repeatedly recorded every 2 minutes over a period of 16 minutes. Between each recording, the subjects were asked to place their heads in a Ganzfeld dome with a constant illumination of 50 cd/m².

The signals were amplified by 100 K (Grass, Quincy, MA) with a band-pass of 10 to 300 Hz. The data sampling rate was 1200 Hz. An artifact-elimination technique was used once. The length of the m-sequence used for the present study was $2^{10} - 1$, resulting in a total recording time of approximately 60 seconds. Each local response (first-order kernel) was calculated (VERIS Science 3.01 software, EDI). The array of local responses was plotted in the same manner as the visual field.

**RESULTS**

**Time Course of Amplitude Increase**

The amplitude of summed multifocal ERGs gradually increased with time. Figure 1 shows the time course of the relative amplitudes for both the negative and positive components of summed multifocal ERGs. The amplitude of the initial negative component (N1) was measured from the baseline to maximum initial negativity and the following positive component (P1) was measured from the first trough to the positive peak. The means ± SEM of the relative amplitudes from the five subjects were plotted. The negative component increased by approximately 36%, and the positive component increased by approximately 47% during the 16 minutes of light adaptation. The increase of the positive component tended to be larger than that of the negative component, although there was no statistically significant difference between the two components. This process was rapid for the first several minutes, then continued to increase more slowly, and reached maximal values at approximately 16 minutes. The implicit times for the negative and positive components of the summed multifocal ERG increased slightly by approximately 3% (0.5 msec) and 4% (0.8 msec), respectively, during the 16 minutes of light adap-

![Figure 1](http://iovs.arvojournals.org/doi/abs/10.1167/iovs.98-4217)

**Figure 1.** Relative amplitude of both the negative and positive components of the summed multifocal ERGs during the course of light adaptation. The means ± SEM of the relative amplitude obtained from five normal subjects were plotted.

![Figure 2](http://iovs.arvojournals.org/doi/abs/10.1167/iovs.98-4217)

**Figure 2.** Multifocal ERGs recorded at different times after the onset of light adaptation obtained from the left eye of a normal subject. Note that the amplitude increase was more prominent in the peripheral retina than in the central retina.
Amplitude Increase of the Multifocal ERG during Light Adaptation

To examine the influence of the stimulus configuration on our results, we repeated the same experiment with 37 unscaled hexagonal stimuli in one subject and confirmed that the magnitude of the increase was consistently smallest in the central retina and became larger toward the peripheral retina (data not shown).

We also explored the amplitude increase of the second-order kernel (representing the temporal interaction between two consecutive focal flashes) and found that the time course, magnitude, and regional variations of the amplitude increase were similar between first-order and second-order kernels.

**DISCUSSION**

Our results clearly demonstrate that the multifocal ERGs using fast-flicker m-sequence stimulation increased in amplitude during light adaptation. This phenomenon had been previously explored mainly with full-field stimuli (30-Hz flicker, brief- or long-flash stimuli). We found that both the negative and positive components of the multifocal ERG increased during light adaptation and that its time course was similar to that of conventional full-field cone ERGs. These results, however, were not unexpected, according to the results of two previous reports. First, Gouras and MacKay reported that the traditional photopic ERG a-wave and b-wave increase during light adaptation. Second, it was recently shown that the negative and positive components of the multifocal ERG have the same origins as the a- and positive peaks of the traditional full-field photopic ERG.

The mean of the multifocal ERG amplitude increase was 36% for the negative component and 47% for the positive component. Both values are smaller than the previously reported 60% to 75% increase with full-field cone ERGs. The reason for the lower increase in the multifocal ERGs can be explained partly by two differences in the methods used. One difference is the longer recording time of the multifocal ERG in our study (60 seconds) compared with those of traditional full-field cone ERGs (less than 10 seconds). The amplitude of the multifocal ERG in the first 60 seconds of recording just after dark adaptation may increase considerably, even during the 60 seconds of recording, because of the light-adapting effect of the stimulus. Because the increase of the amplitude is calculated relative to this first response, a larger initial response makes subsequent values smaller. Another difference is the area of the retina stimulated. The stimulus of the multifocal ERG falls within the

**TABLE 1.** Amplitudes for the Multifocal ERG Positive Component at Various Retinal Eccentricities with Light Adaptation

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central area</td>
<td>1.03 ± 0.11</td>
<td>1.09 ± 0.10</td>
<td>1.18 ± 0.17</td>
<td>1.22 ± 0.16</td>
<td>1.25 ± 0.16</td>
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<tr>
<td></td>
<td>(100.0)</td>
<td>(106.3 ± 0.1)</td>
<td>(114.3 ± 8.7)</td>
<td>(118.0 ± 12.2)</td>
<td>(121.7 ± 8.6)</td>
</tr>
<tr>
<td>Second ring</td>
<td>2.95 ± 0.82</td>
<td>3.65 ± 1.19</td>
<td>3.94 ± 1.26</td>
<td>4.24 ± 1.70</td>
<td>4.29 ± 1.44</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(121.8 ± 8.1)</td>
<td>(132.5 ± 18.2)</td>
<td>(139.5 ± 24.6)</td>
<td>(143.7 ± 17.2)</td>
</tr>
<tr>
<td>Third ring</td>
<td>5.55 ± 1.16</td>
<td>6.56 ± 0.84</td>
<td>7.36 ± 1.01</td>
<td>7.79 ± 1.56</td>
<td>8.57 ± 1.52</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(119.8 ± 10.8)</td>
<td>(134.8 ± 18.5)</td>
<td>(141.8 ± 19.8)</td>
<td>(156.4 ± 20.4)</td>
</tr>
<tr>
<td>Fourth ring</td>
<td>8.95 ± 1.40</td>
<td>11.20 ± 0.95</td>
<td>12.04 ± 1.11</td>
<td>12.71 ± 1.88</td>
<td>14.04 ± 1.76</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(126.5 ± 8.89)</td>
<td>(136.3 ± 14.4)</td>
<td>(142.8 ± 7.5)</td>
<td>(158.0 ± 8.8)</td>
</tr>
</tbody>
</table>

Amplitude data are expressed as mean microvolts ± SD recorded every two minutes up to 16 minutes, with percentage amplitude relative to the first response shown in parentheses.
Adaptation phenomenon. There is evidence in the frog and carp retina that not only cone but also rod light adaptation contributes to the magnitude of redepolarization of the cone photoreceptors during light adaptation. In the dark-adapted condition, rods may normally inhibit cone response, and in the light-adapted condition, this inhibition may be released, resulting in the cone ERG growth. Although this idea still remains controversial, our present results support this hypothesis. It is well known that the activity of the rod system is lowest in the central retina and increases toward the periphery. This lower rod activity in the central retina may result in the smaller amplitude increase at the center. It can be expected that, if the amplitude increase is caused by suppression of the rod system, the magnitude of increase would be maximal at a retinal eccentricity of 15° to 25° where the rod density is maximal. We could not confirm this point because of the limitation of the equipment. Further, chromatic preadaptation periods may provide a good test for the rod-suppression hypothesis.

Tables and figures are included to support the text. Table 2 presents Implicit Time for the Multifocal ERG Positive Component at Various Retinal Eccentricities with Light Adaptation.

**Table 2. Implicit Time for the Multifocal ERG Positive Component at Various Retinal Eccentricities with Light Adaptation**

<table>
<thead>
<tr>
<th>Eccentricity</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central area</td>
<td>27.8 ± 0.4 (100.0)</td>
<td>28.2 ± 0.7 (101.2 ± 1.7)</td>
<td>28.3 ± 1.5 (101.7 ± 4.5)</td>
<td>28.8 ± 1.8 (103.6 ± 5.8)</td>
<td>28.7 ± 1.0 (103.6 ± 3.9)</td>
</tr>
<tr>
<td>Second ring</td>
<td>27.3 ± 0.9 (100.0)</td>
<td>27.5 ± 1.2 (100.6 ± 1.4)</td>
<td>27.8 ± 1.4 (101.8 ± 2.7)</td>
<td>27.8 ± 2.3 (101.7 ± 5.9)</td>
<td>27.7 ± 1.5 (101.2 ± 4.1)</td>
</tr>
<tr>
<td>Third ring</td>
<td>27.2 ± 1.3 (100.0)</td>
<td>27.5 ± 0.8 (101.3 ± 2.8)</td>
<td>28.2 ± 0.7 (103.7 ± 4.1)</td>
<td>27.8 ± 1.4 (102.5 ± 4.5)</td>
<td>27.8 ± 0.9 (102.5 ± 4.1)</td>
</tr>
<tr>
<td>Fourth ring</td>
<td>27.5 ± 1.0 (100.0)</td>
<td>27.8 ± 0.9 (101.2 ± 1.6)</td>
<td>28.2 ± 0.7 (102.6 ± 4.2)</td>
<td>28.2 ± 0.7 (102.6 ± 4.2)</td>
<td>28.2 ± 0.7 (102.6 ± 4.2)</td>
</tr>
</tbody>
</table>

Implicit time data are expressed as mean microseconds ± SD recorded every two minutes up to 16 minutes, with percentage implicit time relative to the first response shown in parentheses.

Central 60° of the retina, whereas traditional full-field ERGs are elicited from the entire retina. As noted in our results, the amplitude of amplitude increase was smaller in the central retina and was larger toward the peripheral retina, which may account for the smaller degree of increase with multifocal ERGs than with full-field stimuli.

Our results showing that there are regional variations in the amplitude increase across the retina are interesting in light of past work. Weiner and Sandberg have also examined the amplitude increase of foveal cone ERGs during light adaptation with a 4° flickering stimulus on the steady background illumination and found that the foveal cone ERG amplitude increase was smaller (27%) than that of reported full-field ERGs. Although they mentioned the possibility of regional variation of this phenomenon, unfortunately, they did not compare the magnitude of increase in different retinal locations. Instead, they concluded that several differences in method between foveal and full-field cone ERGs were the reasons for the differences in the increase. Earlier, we showed that patients with the incomplete type of congenital stationary night blindness had abnormally large amplitude growth of the full-field 30-Hz flicker ERG during light adaptation, but there was little amplitude growth of focal macular 30-Hz flicker ERGs.

The exact mechanism for the amplitude increase of the cone ERGs during light adaptation has not been determined. Armington and Biersdorf previously suggested that the standing potential of the eye may be involved. However, this possibility was ruled out, because this phenomenon is observed even when the retina is isolated from the retinal pigment epithelium, which is the origin of the standing potentials. Gouras and MacKay proposed that a redepolarization of the cone photoreceptors with light adaptation may cause the cone ERG amplitude growth, because the a-wave showed an increase. Findings in studies of the cone membrane potential during light adaptation are consistent with their suggestion. If the increase originates from the photoreceptors as they proposed, the present results would suggest that there are regional variations in the magnitude of redepolarization of the cone photoreceptors with light adaptation.

It has been also proposed that there may be factors other than the cone photoreceptors contributing to the increase phenomenon. There is evidence in the frog and carp retina that not only cone but also rod light adaptation contributes to the amplitude growth of the fast-flicker ERG during light adaptation. In the dark-adapted condition, rods may normally inhibit cone response, and in the light-adapted condition, this inhibition may be released, resulting in the cone ERG growth. Although this idea still remains controversial, our present results support this hypothesis. It is well known that the activity of the rod system is lowest in the central retina and increases toward the periphery. This lower rod activity in the central retina may result in the smaller amplitude increase at the center. It can be expected that, if the amplitude increase is caused by suppression of the rod system, the magnitude of increase would be maximal at a retinal eccentricity of 15° to 25° where the rod density is maximal. We could not confirm this point because of the limitation of the equipment. Further, chromatic preadaptation periods may provide a good test for the rod-suppression hypothesis.

Finally, it is important to emphasize that our results suggest that considerable intra- and interindividual variations may result when evaluating the multifocal ERG results in patients. We found that such variations can be higher in the peripheral retina than in the central retina. Therefore, to minimize variability, subjects should wait at least 10 to 15 minutes under the light-adapted condition before the test, if they have been tested previously in the dark.

**References**


