

# Effect of Age On Short-Wavelength Sensitive Cone Electroretinogram and Long- and Middle-Wavelength Sensitive Cone Electroretinogram

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**Abstract:** Using a light-emitting diode (LED) built-in contact lens electrode, we studied the effect of age on short-wavelength sensitive cone electroretinograms (S-cone ERG) and long- and middle-wavelength sensitive cone ERGs (LM-cone ERG). We recorded ERGs in 31 pseudophakic subjects to avoid the effect of yellowing in the human crystalline lens. The intensities of our stimuli were on the asymptote of the intensity-response curve. Linear regression analysis against age was carried out on the S-cone ERG b-wave, and the LM-cone ERG a-, b-, and d-waves. We found significant age-dependent reduction in the amplitude of the S-cone ERG b-wave, and the LM-cone ERG a- and d-waves, significant prolongation in the peak time of the S-cone b-wave and the LM-cone ERG b-wave, significant increase in the b/a ratio of the LM-cone ERG, but no significant age correlation between the LM-cone ERG a-wave and the amplitude of the b-wave. Our results provide evidence that age-related changes in S- and LM-cone systems begin in early adulthood in the human eye. A significant increase in the b/a ratio suggests that off-bipolar cells are more vulnerable to aging than on-bipolar cells. **Jpn J Ophthalmol 1998;42:424-430** © 1998 Japanese Ophthalmological Society

**Key Words:** Aging, LED, LM-cone, ERG, pseudophakia, S-cone ERG.

## Introduction

Although there have been many studies<sup>1,2</sup> of age-related change in cone electroretinograms (ERGs), there is no report in which cone ERG was analyzed by classification into short-wavelength sensitive cone (S-cone) ERG and long- and medium-wavelength sensitive cone (LM-cone) ERG, followed by separate analyses of the two systems. This is because it was difficult to record S-cone ERG and there was a problem of the lens becoming yellow with aging. The standard technique for separating S-cone ERG employs an intense blue stimulating light and yellow background light,<sup>3-5</sup> which are not available from an ordinary ERG stimulator. In the new method re-

cently reported by Gouras et al,<sup>6</sup> such intense lights are not required, but the amplitude was too small for our studies on aging.<sup>6-8</sup> The present study applied a new technique to separate S- and LM-cone ERG by the use of a light-emitting diode (LED) built-in contact lens electrode, which provides an intense light with wavelengths of 450 nm and 566 nm.<sup>9</sup> We recorded the ERGs in pseudophakic subjects to avoid the influence of lens yellowing. Only the b-wave was recorded in S-cone ERG and a-, b- and d-waves were recorded in LM-cone ERG. The nature of these components has been intensively studied by Sieving and others.<sup>4,10,11</sup> They suggested that the S-cone b-wave originates from depolarizing bipolar cells and the LM-cone a-wave comes from hyperpolarizing bipolar cells. The LM-cone b-wave results from interaction between depolarizing and hyperpolarizing bipolar cells.

The effects of age on these retinal neurons in human S- and LM-cone systems are discussed in this paper.

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## Methods and Subjects

### Recording of ERG

A contact lens electrode with built-in LED was used for stimulation and recording. The background illumination was delivered with a filter placed in the carousel of a slide projector (Kodak Ectagraphic 3-JE) and projected onto the electrode, which works as a diffuser to produce full-field background illumination (Figure 1). The electrode contains a white lens beneath the LED, which produces homogeneous stimuli and background illumination. The wavelength and intensity of background illumination can be adjusted by inserting various filters into the carousel of the slide projector. As a slide projector produces loud exchange noises, a metal cover connected to the ground was placed over the projector to reduce the noise. S-cone ERGs and LM-cone ERGs were recorded under the following conditions.

**Recording of S-cone ERG.** After pupil dilation using a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride, an LED built-in contact lens electrode (wavelength: 450 nm) was placed on the cornea. The steady background illumination was yellow in color (Kodak Wratten No. 12) and 4.8 log photopic trolands in intensity<sup>4</sup>. The maximum stimulus intensity was 3.3 log photopic trolands. In this condition, the rod and LM-cone system were sufficiently suppressed and only the S-cone ERG was elicited. This was proved in a preliminary experiment where the same waves as in normal subjects were recorded in a blue cone monochromat deficient of LM-cone or in a fundus albipunctatus with dysfunction of the rod.<sup>9</sup>

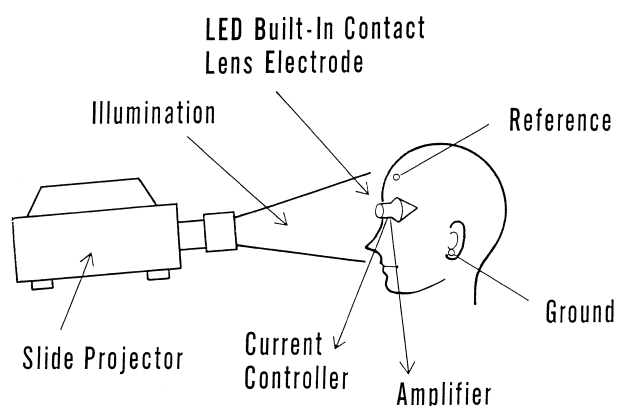
**Recording of LM-cone ERG.** To record LM-cone ERG, an LED built-in contact lens electrode (wavelength 566 nm) was placed on the cornea after mydriasis and a blue light (Kodak Wratten No. 43), 3.2 log photopic trolands in intensity, was irradiated as background illumination from a slide projector. The maximum stimulus intensity was 4.1 log photopic trolands and the frequency of stimulation was 3 Hz. The On:Off ratio was 1:1. This intensity was equal to that of the background illumination of photopic ERG in the protocol<sup>12</sup> proposed by the International Society of Clinical Electrophysiology and Vision. This background illumination was considered to suppress the rod and S-cone sufficiently.

The time constant of the amplifier was 0.1 second and high-cut was 100 Hz. Thirty-two responses were averaged, requiring about 20 seconds. During recording, one investigator confirmed alignment of the contact lens and the projector in a sealed room, whereas the other manipulated the stimulator and amplifier outside the room.

### Subjects

To obtain intensity-response curves of S-cone ERG and LM-cone ERG, both ERGs were recorded in 8 healthy subjects (25–29 years old) with clear media. Intensities of stimulus used for recording of S-cone ERG were 2.0, 2.5, 2.8, 3.1, and 3.3 log trolands; those used for recording of LM-cone ERG were 2.1, 2.5, 3.1, 3.8 and 4.1 log trolands.

The intensities of stimulus giving the maximum amplitudes were determined from the intensity-response curves obtained from the same 8 normal subjects and, using these stimuli, S-cone ERG and LM-cone ERG were recorded in 31 pseudophakic subjects (between 25 and 91 years) with no congenital dyschromatopsia. These subjects did not have high refractive error before cataract surgery, and had no chorioretinal diseases, lattice degeneration, or drusen. The visual field and visual acuity were normal in all subjects. Phacoemulsification and intraocular lens implantation were performed without complication within 20 minutes. In all cases, the diameter of dilated pupils was more than 8.0 mm at the time of recording ERGs, and no retinal damage from operating microscope was found in any case. The transplanted intraocular lens, a polymethylmethacrylate (PMMA) lens 6 mm in diameter (not transmitting ultraviolet rays), was fixed in the capsule. There was no case with severe secondary cataract in the study. The cataract in young patients (under 40 years) was caused by trauma in the anterior segment.



**Figure 1.** Recording system of ERG (see text for description).

The purpose and nature of the examination was fully explained to all subjects before the study and their informed consent was obtained.

**Results**

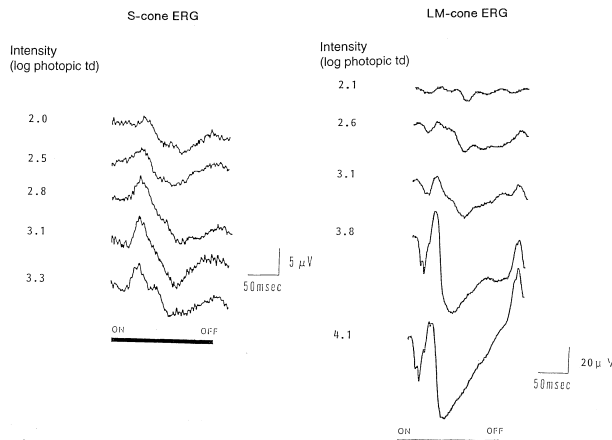
*Intensity-Response Curves for S-Cone ERG and LM-Cone ERG*

Figure 2 shows an example of the intensity series of S-cone ERG and LM-cone ERG recorded in normal subjects. Only the b-wave was recorded at any intensity for S-cone ERG, whereas a-, b-, and d-waves were recorded for LM-cone ERG. In both S- and LM-cone ERG, the implicit times were almost constant at all intensities. Figures 3 and 4 show the intensity-amplitude curves (mean  $\pm$ SD) of the b-wave in the S-cone ERG and the LM-cone ERG.

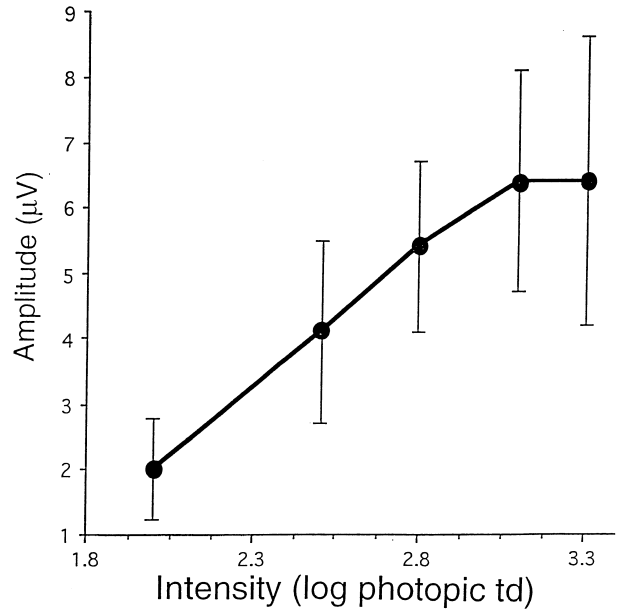
In the present study, we employed the minimum intensity to give the maximum amplitude of the b-wave for the study of aging. The stimulus intensity was 3.1 log trolands for S-cone ERG and 3.8 log trolands for LM-cone ERG. The mean amplitude at these intensities of stimulus was 6.4  $\mu$ V for the b-wave of S-cone ERG and 17.8  $\mu$ V for the a-wave, 36.1  $\mu$ V for the b-wave and 27.5  $\mu$ V for the d-wave of LM-cone ERG. The mean implicit times were 54.7 msec in the b-wave of S-cone ERG, and 25.7 msec, 38.2 msec and 190.5 msec, respectively, in the a-, b- and d-waves of LM-cone ERG.

*Effect of age on S-cone ERG and LM-cone ERG*

We measured the amplitude and implicit times of the b-wave in S-cone ERG and those of the a-, b- and

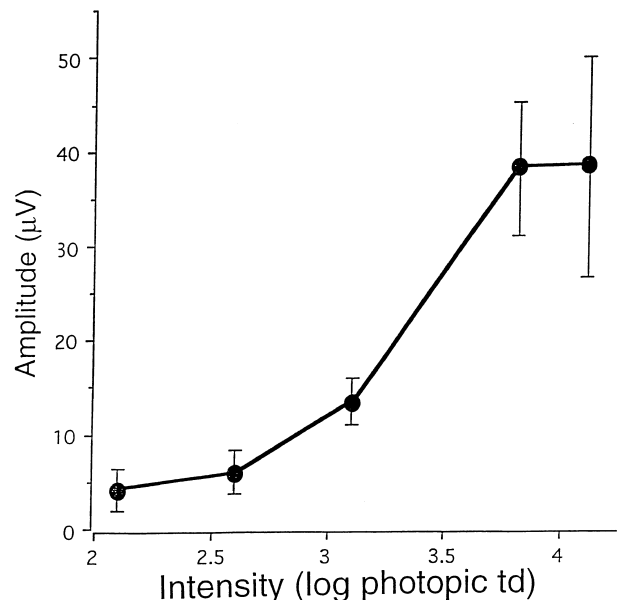


**Figure 2.** Example of S-cone ERG and LM-cone ERG. td:troland



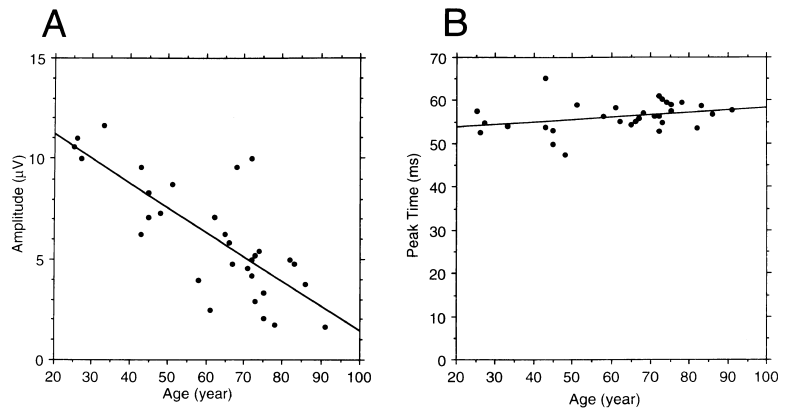
**Figure 3.** Intensity-response curve of S-cone ERG b-wave (mean  $\pm$  SD, n = 8).

d-waves of LM-cone ERG in the pseudophakic eyes and performed linear regression analysis. Figure 5 shows the effects of age on the amplitude and implicit times of b-wave in S-cone ERG. The amplitude of b-wave in S-cone ERG showed a statistically sig-



**Figure 4.** Intensity-Response curve of LM-cone ERG b-wave (mean  $\pm$  SD, n = 8).

**Figure 5.** S-cone ERG b-wave. **(A)** Correlation between age and amplitude of b-wave.  $Y = -0.12 X + 13.7$ ;  $r = -0.75$  ( $P < 0.01$ ). **(B)** Correlation between age and peak time of b-wave.  $Y = 0.056 X + 52.9$ ;  $r = 0.40$  ( $P < 0.05$ ).



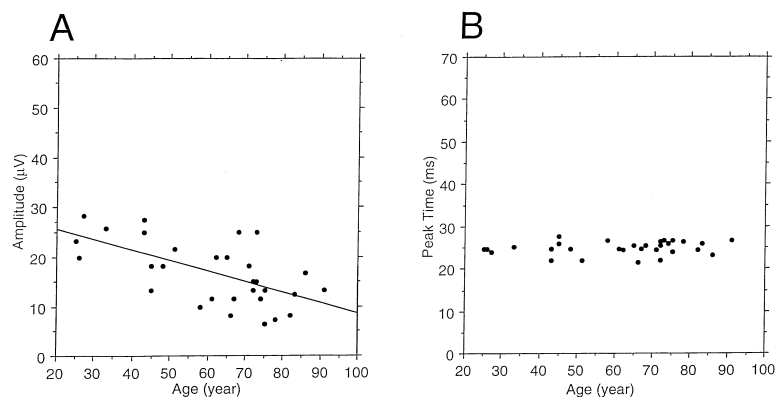
nificant decrease ( $r = -0.75$ ,  $P < 0.01$ , [Spearman's rank correlation]). The implicit time showed a significant increase ( $r = 0.40$ ,  $P < 0.05$ ). Figure 6 shows the effects of age on the amplitude and implicit time of the a-wave in LM-cone ERG. The amplitude of the a-wave in LM-cone ERG decreased significantly ( $r = -0.59$ ,  $P < 0.01$ ). The implicit time revealed no significant difference. Figure 7 shows the effects of age on the amplitude and implicit time of the b-wave of LM-cone ERG. The amplitude showed no significant difference, whereas the implicit time was prolonged significantly ( $r = 0.49$ ,  $P < 0.01$ ). Figure 8 shows the effects of age on the amplitude and implicit time of the d-wave in LM-cone ERG. The amplitude showed a statistically significant decrease ( $r = -0.36$ ,  $P < 0.05$ ) and the implicit time showed no significant difference. Figure 9 shows the effects of age on the b/a ratio of LM-cone ERG. The ratio showed a statistically significant increase ( $r = 0.44$ ,  $P < 0.05$ ).

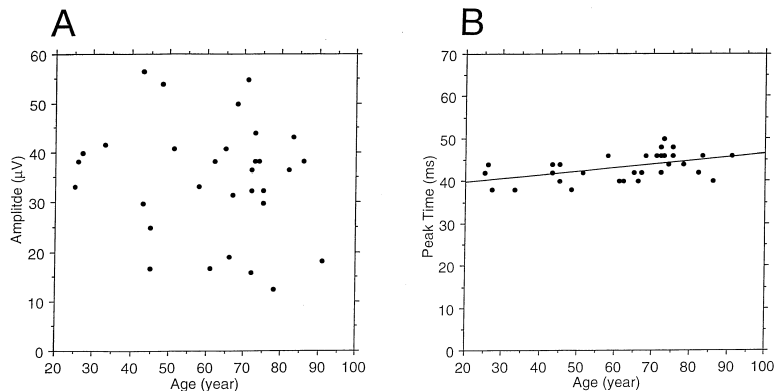
### Discussion

In the present study, age-related changes were observed in both S-cone ERG and LM-cone ERG. We

found a decrease in amplitude of the b-wave in S-cone ERG, decrease in amplitude of the a- and d-waves, and prolongation of the implicit time of the b-wave in both S- and LM-cone ERGs. In the literature, there are numerous reports<sup>12-16</sup> on pathologic examination of the effects of aging on photoreceptors. In qualitative examination, the outer segment was damaged in both rods and cones, and accumulation of lipofuscin,<sup>14</sup> refractile particles,<sup>13</sup> and refractile bodies<sup>16</sup> in the inner segment of cones have been reported. Quantitatively, the rods were more affected by age than the cones.<sup>15</sup> At 90 years of age, 30% of the rods were lost at the central area of 28°, but the inner segments of the remaining rods were enlarged for compensation. Changes in the cones were negligible at the central area and 23% of peripheral cones was lost. Reduction of S-cones is believed to start in early adulthood.<sup>13</sup> There is no doubt that such age-related changes in the photoreceptors played a great role in the aging phenomena we observed in S-cone and LM-cone ERGs. However, prolongation of the implicit time of the b-wave and increase in the b/a ratio cannot be explained by the reduced number of photoreceptors alone. Age-

**Figure 6.** LM-cone ERG a-wave. **(A)** Correlation between age and amplitude of a-wave.  $Y = -0.21 X + 29.8$ ;  $r = -0.59$  ( $P < 0.01$ ). **(B)** Correlation between age and peak time of a-wave.





**Figure 7.** LM-cone ERG b-wave. (A) Correlation between age and amplitude of b-wave. (B) Correlation between age and peak time of b-wave.  $Y = 0.082 X + 38.2$ ;  $r = 0.49$  ( $P < 0.01$ ).

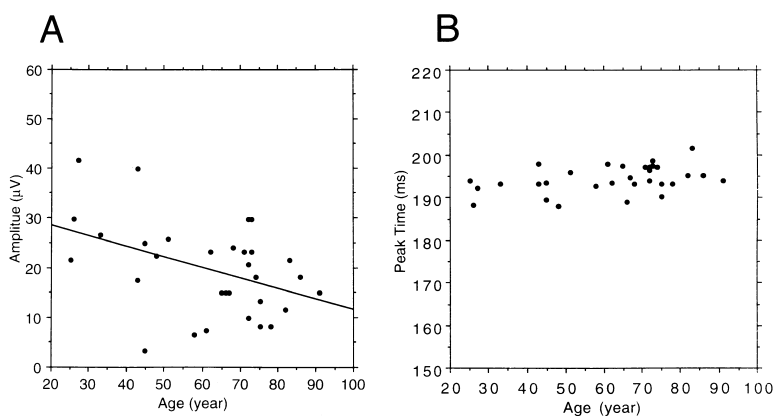
related changes in secondary neurons should have some contribution to the phenomenon we observed in this study.

Although the S-cone ERG b-wave is believed to originate from on-bipolar cells (depolarizing bipolar cells<sup>10</sup>, Sieving et al<sup>11</sup> recently reported that LM-cone ERG involves on- and off- (hyperpolarizing) bipolar cell components. According to their hypothesis, both bipolar cells receive glutamate from the cones by photostimulation and on-bipolar cells release  $K^+$  and off-bipolar cells take in  $K^+$  simultaneously. The Müller cells respond to the change in extracellular  $K^+$ , and the input from the on-bipolar cells is cornea-positive, whereas that from the off-bipolar cells is cornea-negative on ERG. The onset of the negative component induced by off-bipolar cells is earlier than that of the positive component induced by on-bipolar cells, resulting in a cornea-negative component, the a-wave. Then, the positive component from on-bipolar cells grows and produces the b-wave. Therefore, LM-cone ERG a-wave is produced by off-bipolar cells and b-wave is produced by the interaction between on- and off-bipolar

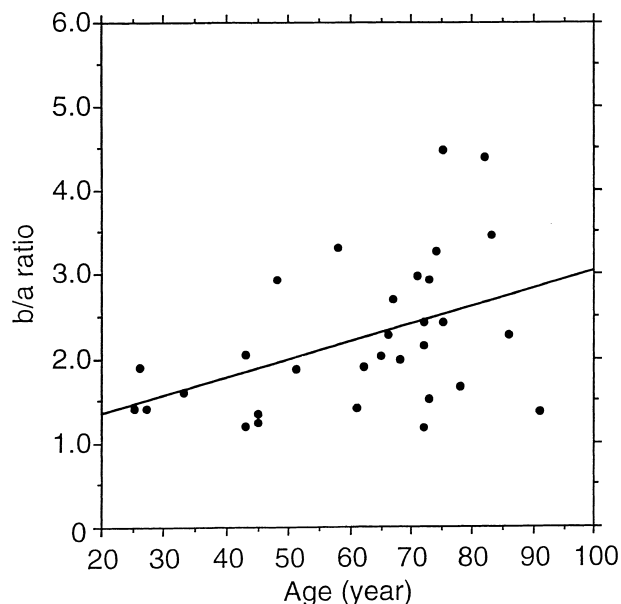
cells. They suggest that cone receptor potential contributes to the a-wave when the stimulus is very bright, but that in clinical studies, the photopic a-wave is mostly from off-bipolar cells.

The results of the present study can be understood well by the following explanation taking the theory of Sieving et al<sup>11</sup> into consideration. The age-related changes occur in bipolar cells; off-bipolar cells are more vulnerable than on-bipolar cells. Therefore, the amplitude of the a-wave is reduced greatly but reduction in the amplitude of the b-wave is small. Thus, the a-wave decreased significantly, but the b-wave did not show a significant change and the resultant b/a ratio was increased. The implicit time of the b-wave was prolonged because the on-bipolar cell-induced depolarization reached a peak more slowly than the off-bipolar cell-induced hyperpolarization. In contrast, it is difficult to explain these changes by the conventional theory that the ERG a-wave is from photoreceptors and the b-wave is from bipolar cells.

There are numerous reports on age-related phenomena in the cone-ERG. Weleber<sup>2</sup> stated that the



**Figure 8.** LM-cone ERG d-wave. (A) Correlation between age and amplitude of d-wave.  $Y = -0.21 X + 33.0$ ;  $r = -0.36$  ( $P < 0.05$ ). (B) Correlation between age and peak time of d-wave.



**Figure 9.** LM-cone ERG b/a ratio. Correlation between age and b/a ratio.  $Y = 0.021 X + 0.94$ ;  $r = 0.44$  ( $P < 0.05$ ).

amplitude of the LM-cone ERG b-wave decreased, whereas the implicit time was shortened. Birch and Anderson<sup>1</sup> stated that the amplitude of the LM-cone ERG b-wave decreased with aging while its implicit time was shortened. Gouras et al<sup>6</sup> separated LM-cone ERG and S-cone ERG and examined them comparatively. However, because the stimulus in these reports was a short flash and most cases had phakic eyes, it is inappropriate to compare their results with the results of the present study. The LM-cone ERG b-wave produced and recorded by the short flash stimulation is probably a mixture of b- and d-waves produced by the long flash stimulation. Consequently, interpretation of the results is complicated. Furthermore, examination of age-related changes in phakic eyes is difficult because the amplitude of S-cone ERG is reduced by the yellowing of the lens. There are many reports on this point. According to Uji,<sup>17</sup> transmittancy of the lens decreased markedly in the short wavelength region even in the juvenile. Moreover, it has been reported by Dotz and Walther,<sup>18</sup> van Norren,<sup>19</sup> Uji,<sup>20</sup> Yoshida and Uji<sup>21</sup> and Machida et al<sup>22</sup> that the yellowed lens absorbs short wavelength light affecting ERG and the short wavelength light produces greater changes than the long and medium wavelength lights. Uji<sup>20</sup> compared ERG and b-wave amplitude at various wavelengths (400–680 nm, 16 colors) in cataractous eyes and aphakic eyes, under the white light-adapted

condition, and concluded that the b-wave increased markedly in the region below 500 nm in postoperative aphakic eyes in comparison to the preoperative state. Yoshida and Uji<sup>21</sup> recorded the S-cone ERG b-wave using yellow light for adaptation and reported that the b-wave in postoperative aphakic eyes increased greatly. Thereafter, Machida et al<sup>22</sup> compared changes in pseudophakic eyes at various wavelengths (400–600 nm, 14 colors) and concluded that the amplitude of the b-wave in the region below 520 nm changed markedly before and after surgery. It is probably best to employ pseudophakic eyes at present to avoid the problem of lens yellowing.

Vulnerability of the S-cone system has been reported psychophysically in many studies<sup>23</sup> and the S-cone ERG b-wave actually decreased markedly with aging in the present study using pseudophakic eyes, while the LM-cone ERG b-wave did not show a decrease. However, as mentioned above, the S-cone ERG b-wave derives from on-bipolar cells, whereas the LM-cone ERG b-wave is produced by the interaction of on- and off-bipolar cell response. In the present study, the b/a ratio and the LM-cone ERG increased, indicating that the balance between the on- and off-bipolar cells changed. Therefore, direct comparison of the S-cone b-wave and the LM-cone b-wave is difficult. Further study seems to be necessary to compare age-related changes in the S-cone system and the LM-cone system using ERGs.

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