Effects of Aging on the First and Second-order Kernels of Multifocal Electroretinogram

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Purpose: To determine the effects of aging on the first and second-order kernels of the multifocal electroretinogram (ERG).

Methods: Multifocal ERGs were recorded from 52 healthy subjects (52 eyes) ($0 \geq$ refractive error ≥ -3.0 diopter). The ages of the subjects ranged from 12–76 years with a mean (±SD) of 44.0 ± 20.2 years. The Visual Evoked Response Imaging System was used. The effects of aging on the response densities and on the implicit times of the first positive wave (P1) of the first-order kernel, and of the second and third positive waves (P2 and P3) of the second-order kernel were analyzed for the different age groups.

Results: The response densities of the first-order kernel P1 and second-order kernel P2 waves decreased significantly, and the implicit times of the second-order kernel P2, and P3 were significantly prolonged ($P < .05$) in subjects over 50 years of age.

Conclusion: The results suggest that the age of the subject should be considered when evaluating retinal function using multifocal ERGs in basic and clinical studies. **Jpn J Ophthalmol 2002;46:261–269** © 2002 Japanese Ophthalmological Society

Key Words: Aging, electroretinogram, multifocal electroretinogram, retina, visual evoked response imaging system.

Introduction

The recently developed technique of recording the multifocal electroretinogram (ERG) makes it possible to record local ERGs simultaneously from different regions of the retina.[1] In this technique, the visual field of about 50° is divided into a number of regions and each region is stimulated pseudorandomly. Because this recording technique is relatively simple, it is being widely used for research and clinical studies. In addition, special computational programs are provided with the recording system for multifocal ERG to extract the first and second-order kernels. Because there is some evidence that the second-order kernel is related to functions of the inner retina, analysis of the second-order kernel of the multifocal ERGs is being done to evaluate its inner retinal functions in diseases such as glaucoma[2] and diabetic retinopathy.[3,4]

It is well known that the conventional ERGs are influenced by age and myopia, and there is some evidence that age and myopia also affect the multifocal ERGs. Thus, Anzai et al[5] and Mohidin et al[6] have reported on the influence of aging while Kawabata et al[7] and our laboratory[8] have reported on the influence of myopia on the first-order kernel of the multifocal ERGs. We have shown that myopia decreases the second-order kernel. We also have reported the aging effect on the first and second-order kernels of the multifocal ERG.[9] In the present study, we reviewed our previous results by elucidating subjects and analyzing methods.

Materials and Methods

In compliance with the Declaration of Helsinki, the study content was explained to the subjects and informed consent was obtained from each one. Fifty-two healthy volunteers (52 eyes) with refractive errors

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between 0 and −3.0 diopter (D) (−0.76 ± 0.94 D; mean ± SD) were studied. The best-corrected visual acuity was 0.7 or better in all eyes. All subjects had normal ophthalmological findings except for incipient cataracts (cases with slight nuclear opacity were included while those with diagonal opacities were excluded).

The age of the subjects ranged between 12 and 76 years with a mean of 44.0 ± 20.2 years. The subjects were divided into decade age groups as shown in Table 1. There was no significant difference in refractive error between the age groups.

Each subject was examined by a refractometer (RM-6000; TOPCON, Tokyo Kagaku, Tokyo) after mydriasis by topical 0.5% tropicamide and 0.5% phenylephrine hydrochloride, and the uncorrected and corrected visual acuities were determined. The cornea and conjunctiva were anesthetized by topical oxybuprocaine chlorhydrate and 4% lidocaine hydrochloride, and a bipolar corneal contact lens electrode (Kyoto Contact Lens, Kyoto) of +3.0 D filled with 15% hydroxymethylcellulose was inserted. The refractive error was corrected by the spherical equivalent of the refractive error. The distance between the stimulus screen and the chin stand was adjusted by the method of Kondo et al.[10] A silver disc electrode was attached to the right earlobe to ground the subject. The opposite eye was occluded with a patch.

The Visual Evoked Response Imaging System III (VERIS III; Tomey, Nagoya) was used to record the multifocal ERGs. The stimulus consisted of 61 black and white hexagonal stimulus elements that were arranged concentrically around a fixation point. The overall stimulus array subtended an angle of about 40° vertically and 50° horizontally. The stimulus was displayed on a 17-inch CRT monitor (Sony, Tokyo) and each black and white element was reversed pseudorandomly in binary m-sequence[1,11] at 75 Hz. The luminance of the stimulus elements was set at approximately 5 cd/m^2 for the dark phase and 200 cd/m^2 for the bright phase. Thus, the mean luminance was about 100 cd/m^2 and the black and white phases were reversed with 1/2 probability. The background luminance was fixed at 100 cd/m^2. The illuminance of the testing room was 252 lux and the subjects were adapted to this room light for 15 minutes before the start of the recording.

Table 1. Age Distribution, Numbers of Eyes, and Mean Refractive Error of the Subjects

Age	Subjects	Eyes	Refractive Error (D)
12–19	9	9	−0.71 ± 0.84
20–29	7	7	−1.63 ± 1.42
30–39	7	7	−1.07 ± 0.94
40–49	3	3	−0.50 ± 0.66
50–59	8	8	−0.23 ± 0.37
60–69	15	15	−0.55 ± 0.79
70–76	3	3	−0.83 ± 1.13
Total	52	52	−0.76 ± 0.94

The subjects were instructed to maintain a steady fixation on the fixation point placed at the center of the stimulus screen. Each recording session was set for about 27.3 seconds, and 16 sessions were recorded for a total of 7.28 minutes of recording. During the recording, the subjects were instructed to try not to move their body or eyes, or to blink. If artifacts larger than ±50 μV were noted more than 3 times during the 27.3 seconds of recording, the record was discarded and another session was recorded. The retinal responses were amplified (Model 12 A5C; Astro-Med, West Warwick, RI, USA) with a bandpass from 10 to 300 Hz. The multifocal ERGs were analyzed by the VERIS analytical software, Veris Science, version 3.0.1 (Tomey). The responses analyzed were the "All traces" response, which is the sum of the retinal responses from the 61 different retinal regions. Also analyzed were the five different "Ring" responses that were the sum of the individual multifocal ERGs from within a ring of different sizes centered on the fixation point. The central multifocal ERG was elicited by the one 3.2° element at the center of the screen and was called the response from Ring 1. The responses of rings 2 to 5 represented the sums of the responses elicited by the elements in rings 2 to 5 (Figures 1A and 1B and Table 2).

For the analysis, the height from the baseline to the peak of P1 and P2 (Figures 1C, 1D), and from the trough of N2 to the peak of P3 (Figure 1D) were defined as the response density, and the time from the start of the stimulus to the peak of the wave was defined as the implicit time.

Spatial averaging to improve the signal-to-noise ratio was not performed in the response analysis but artifact removal[1] was performed once.

Analysis of variance was used for statistical analysis for the different age groups and $P < .05$ was considered significant.

Results

The first-order kernel waves and the second-order kernel waves of the multifocal ERGs recorded from the left eye of a 26-year-old man are shown in Figures 1A and 1B. The sum of all of the first-order kernels ("All traces") is shown in Figure 1C and the sum of all of the second-order kernels, in Figure 1D.

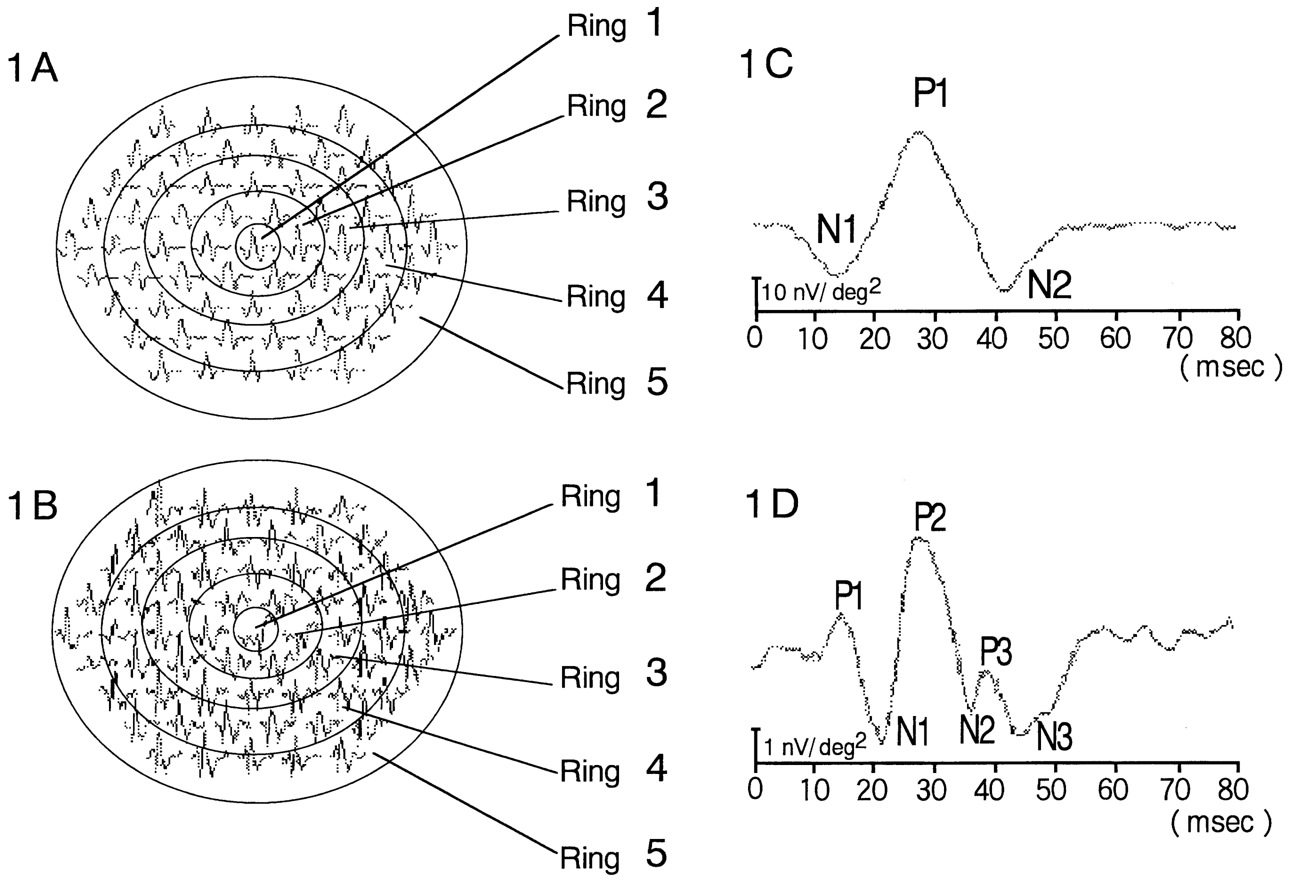

Figure 1. Multifocal electroretinograms from the left eye of a 26-year-old man. (**A**) First-order kernels. (**B**) Second-order kernels. (**C**) An "All trace" wave obtained by averaging the sum of the waves of the first-order kernel. (**D**) An "All trace" wave obtained by averaging the sum of the waves of the second-order kernel.

First-order Kernel

"All trace" waves. Figure 2 and Table 3 show the mean ± SD of the response densities for P1 of the first-order kernel and for the "All trace" wave for the respective age groups. The response densities did not change significantly up to the fourth decade but then showed a linear decrease with increasing age. The mean response densities in the 60- and 70-year age groups were significantly lower than those in the 10-, 20-, 30-, and 40-year age groups ($P < .05$).

Table 2. Visual Angle and Number of the Elements in Each Ring

Ring	Visual Angle (Degree)	Elements
1	3.2	1
2	3.2–6.8	6
3	6.8–11.2	12
4	11.2–16.4	18
5	16.4–25	24

The mean ± SD of the implicit times of P1 for the "All trace" waves are shown in Table 3. The mean implicit time did not differ for the different age groups.

Responses in the Different Rings

The mean ± SD of the P1 response densities for the different rings are shown in Figure 3 and Table 4 for the different age groups. The response densities in all "Rings" showed a linear decrease from the 50-year age group, as was seen in the "All traces" responses. The values were significantly lower in the 60s and 70s than in every age group younger than 40 ($P < .05$).

The mean ± SD of the P1 implicit times for rings 1 to 5 are shown in Table 4. The implicit times did not show a uniform tendency.

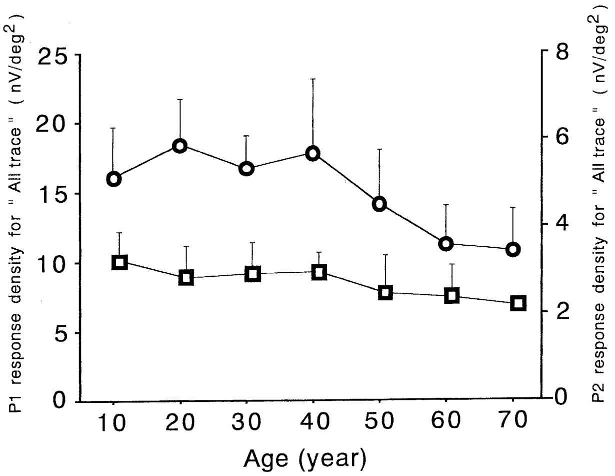

Figure 2. Response density of P1 (○) of the first-order kernel and P2 (□) of the second-order kernel of "All trace" waves as a function of age.

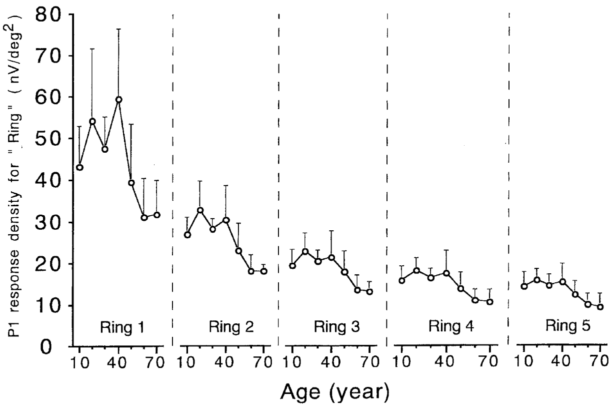

Figure 3. Mean and SD of the response density of "Ring" waves for P1 of the first-order kernel as a function of age.

Second-order Kernel

"All trace" waves. The mean ± SD of the P2 response densities of the second-order kernel for the "All trace" waves are shown in Figure 2 and Table 5 for the different age groups. The mean P2 values did not change significantly from the 10- to 40-year age groups but they showed a linear decrease from the 50s, as seen in the first-order kernels. The values for the 50-, 60-, and 70-year age groups were significantly lower than in the teens group ($P < .05$).

The mean ± SD of the P2 implicit times for the "All trace" waves are shown in Figure 4 and Table 5 for the different age groups. The values did not vary significantly between the 10- and 30-year age groups but lengthened from the 40s. The implicit times in the 40- and 60-year age groups were significantly longer than the values in the 20s group ($P < .05$).

Table 3. Relationship Between Response Density and Implicit Times of P1 of the First-order Kernel of "All Trace" Waves as a Function of Age

Age	Response Density (nV/deg^2)	Implicit Time (msec)
12–19	16.06 ± 3.59	26.47 ± 1.01
20–29	18.41 ± 3.20	26.57 ± 0.34
30–39	16.61 ± 2.38	26.19 ± 1.16
40–49	17.73 ± 5.27	27.50 ± 1.70
50–59	14.08 ± 3.89†	26.86 ± 1.16
60–69	11.13 ± 2.75$^{*\dagger\ddagger\S\|}$	27.01 ± 0.94
70–76	10.63 ± 3.04*†‡§	26.63 ± 1.44

*Significant difference ($P < .05$) between teens
†Significant difference ($P < .05$) between 20s.
‡Significant difference ($P < .05$) between 30s.
§Significant difference ($P < .05$) between 40s.
‖Significant difference ($P < .05$) between 50s.

The mean P3 response densities of the second-order kernel for "All trace" waves did not differ significantly among the age groups and were not correlated with age. The mean ± SD of P3 implicit times for "All trace" waves for the different age groups are shown in Figure 4 and Table 6. The values did not differ significantly between the teens and the 40s, but became significantly longer in the 50-, 60-, and 70-year age groups than the values in the age groups younger than 30 ($P < .05$).

Responses in the Different Rings

Because the P2 component of the "Ring" 1 response was very small, it was excluded from analysis and only the waves in "Rings" 2 to 5 were examined. The mean ± SD of the P2 response densities of the second-order kernel for the different rings as a function of age are shown in Figure 5 and Table 7. The response densities in "Rings" 3 to 5 were high in the teens, 20s, 30s, and 40s, and showed a linear decrease from the 50s. The values in "Ring" 3 were significantly lower in the 60- and 70-year age groups compared to those in the teens ($P < .05$), and those in "Rings" 4 and 5 were significantly lower in the 50-, 60-, and 70-year age groups compared to the teens ($P < .05$).

Table 7 shows the mean ± SD of the P2 implicit times for the different "Ring" waves for the different age groups; there was no tendency to correlate with age.

The mean (± SD) of the P3 response densities of the second-order kernel for the different "Rings" did not correlate with age.

Table 4. Means and Standard Deviations of the Response Density and Implicit Times of "Ring" Waves for P1 of the First-order Kernel as a Function of Age

Age	Ring 1	Ring 2	Ring 3	Ring 4	Ring 5
	Response Density (nV/deg^2)				
10	43.10 ± 9.71	26.81 ± 4.12	19.38 ± 3.81	15.62 ± 3.52	14.04 ± 3.62
20	54.17 ± 17.31	32.81 ± 6.87*	22.87 ± 4.25	17.94 ± 3.10	15.63 ± 2.53
30	47.44 ± 7.60	28.16 ± 2.45	20.40 ± 2.55	16.17 ± 25.25	14.36 ± 2.55
40	59.40 ± 16.87*	30.40 ± 8.20	21.33 ± 6.21	17.33 ± 5.54	15.17 ± 4.28
50	39.46 ± 13.81†§	22.88 ± 6.66†‡§	17.73 ± 5.01†	13.63 ± 3.85†	12.09 ± 3.25†
60	31.08 ± 9.30*†‡§	18.05 ± 3.82*†‡§‖	13.55 ± 3.31*†‡§‖	10.86 ± 2.69*†‡§	9.76 ± 2.66*†‡§
70	31.67 ± 8.28†§	18.00 ± 1.56*†‡§	13.07 ± 2.42*†‡§	10.33 ± 3.18*†‡§	9.20 ± 3.22*†‡§
	Implicit Time (msec)				
10	27.31 ± 0.69	26.77 ± 0.88	26.20 ± 0.47	26.11 ± 0.85	26.57 ± 0.98
20	27.84 ± 1.58	27.39 ± 0.72	26.67 ± 0.70	26.31 ± 0.18	26.80 ± 0.58
30	27.27 ± 1.35	26.41 ± 0.93	26.19 ± 1.16	25.73 ± 1.22	26.29 ± 1.16
40	28.90 ± 1.28	28.07 ± 0.98‡	27.50 ± 1.70	27.50 ± 1.70‡	27.77 ± 1.76
50	26.85 ± 1.51§	27.08 ± 1.17	26.76 ± 1.13	26.26 ± 0.90	27.08 ± 1.20
60	28.11 ± 1.37	27.45 ± 1.12	26.96 ± 0.87	26.72 ± 1.21	27.29 ± 1.16
70	25.00 ± 2.98*†‡§¶	27.77 ± 2.54	26.63 ± 1.44	26.63 ± 1.44	26.37 ± 1.72

*Significant difference ($P < .05$) between teens
†Significant difference ($P < .05$) between 20s.
‡Significant difference ($P < .05$) between 30s.
§Significant difference ($P < .05$) between 40s.
‖Significant difference ($P < .05$) between 50s.
¶Significant difference ($P < .05$) between 60s.

The implicit times of P3 of the second-order kernel for "Rings" 2 and 3 tended to be prolonged in the age groups over the 40s, and for "Rings" 4 and 5, to be significantly prolonged in the 70s compared to the values in the age groups younger than 30 ($P < .05$) (Figure 6 and Table 8).

Discussion

Because multifocal ERGs are being widely used in research and clinical laboratories, it is necessary to standardize the recording conditions and to determine the normative values for the response density and implicit times. As part of this process, the influence of age on the first and second-order kernels of the multifocal ERGs was examined in the present study.

The second-order kernel of the multifocal ERG is very sensitive to noise in the recordings. Under the usual stimulus condition of 103 elements and a 4-minute

Table 5. Means and Standard Deviations of the Response Density and Implicit Times of "All Trace" Waves for P2 of the Second-order Kernel as a Function of Age

Age (y)	Response Density (nV/deg^2)	Implicit Time (msec)
12–19	3.23 ± 0.65	26.84 ± 0.92
20–29	2.81 ± 0.74	26.33 ± 2.08
30–39	2.93 ± 0.69	26.79 ± 1.24
40–49	2.93 ± 0.45	28.37 ± 1.44†
50–59	2.44 ± 0.88*	27.40 ± 1.04
60–69	2.35 ± 0.73*	27.55 ± 1.02†
70–76	2.17 ± 0.12*	27.80 ± 1.91

*Significant difference ($P < .05$) between teens.
†Significant difference ($P < .05$) between 20s.

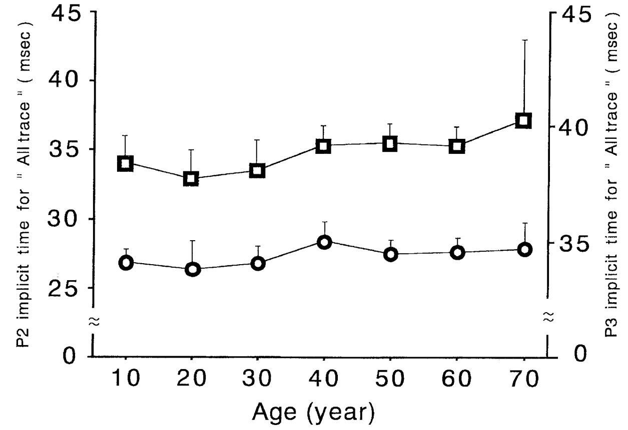

Figure 4. Mean and SD of the implicit times of "All trace" waves for P2 (○) and P3 (□) of the second-order kernel as a function of age.

Table 6. Mean and Standard Deviation of the Implicit Times of P3 of the Second-order Kernel Component for the "All Trace" Waves as a Function of Age

Age (y)	Implicit time (msec)
12–19	38.43 ± 1.14
20–29	37.71 ± 1.23
30–39	38.09 ± 1.32
40–49	39.17 ± 0.85
50–59	39.28 ± 0.83[†]
60–69	39.11 ± 0.87[†]
70–76	40.30 ± 3.48[*†‡]

[*] Significant difference ($P < .05$) between teens.
[†] Significant difference ($P < .05$) between 20s.
[‡] Significant difference ($P < .05$) between 30s.

recording time, the signal-to-noise ratio for the second-order kernel is relatively low. In the present study, the noise of the second-order kernel was reduced by decreasing the number of stimulus elements to 61 and by extending the recording time to 7.28 minutes. Under these conditions, reliable responses were obtained for the second-order kernel of the multifocal ERGs.

Anzai et al[5] reported that the response density of P1 of the first-order kernel for the "All traces" response in the group under 30 years of age did not differ significantly from the response density in the age group over 60 years of age. In the present study, however, the response densities in the 50s, 60s, and 70s were significantly lower than those in the younger age groups. We cannot explain this discrepancy at present.

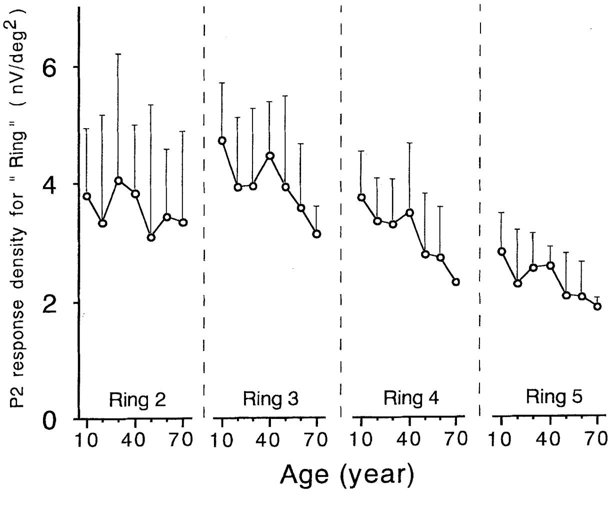

Figure 5. Mean and SD of the response density of P2 of the second-order kernel component for the "Ring" waves as a function of age.

Anzai et al[5] also compared the P1 response densities of the first-order kernel from rings of 2 and 8° for the young and the old age groups, and concluded that the old group showed lower response densities. The central angle of 2° corresponds to "Ring" 2 and that of 8° to "Ring" 3 in the present study. Our results showed that the P1 response densities of the first-order kernel also decreased significantly in all rings from 1 to 5 in the 60- and 70-year age groups compared to the response density in age groups younger than the 40s or 50s.

Mohidin et al[6] also reported the influence of age on the first-order kernel of multifocal ERG. They reported that the response density of only Rings 1 and 2 were significantly lower in a group older than 48 years of age. In the present study, the response densities of all of the "Rings" for subjects over 50 years old were significantly lower than for younger subjects. Although the reason for this difference is unknown, the fact that photoreceptors decrease with age evenly in the whole retina[12] supports our result described above.

Hayashi et al[13] examined the relationship between aging and the focal macular ERGs obtained by stimulating areas of 5, 10, and 15° squared in the macular area. They reported that amplitudes of the a-waves in the 10 and 15° areas, amplitudes of the b-waves in the 15° area, and amplitudes of the oscillatory potentials in the 5, 10, and 15° areas decreased significantly with age. Hood et al[14] mentioned that the first negative wave of the first-order kernel of multifocal ERG (N1) comprised the same components as the a-wave of the full-field ERG, and that the positive wave of the multifocal ERG (P1) had the same combination of positive components (b-wave and oscillatory potentials) as the full field ERG. If the N1 and P1 of the multifocal ERG correspond to the a- and b-wave (with oscillatory potentials) of the full-field ERG, respectively, their results are similar to the findings in the present study.

On the other hand, Birch and his colleagues[15] reported that the focal macular ERG elicited by a 42-Hz stimulus of 3° decreased with increasing age in the fovea but did not change in the parafovea. Although our results were similar to those of Birch et al in the fovea, they were different in the parafovea. This difference is probably due to the stimulus: They used a 42-Hz flicker stimulation while we used pseudorandom stimulation at 75-Hz binary m-series.[1,11] Because the retinal sensitivity to flicker stimulation differs in the central and peripheral retina,[16] this might account for the difference in these two studies.

Because the amplitude of the first-order kernel response density is low in the periphery, Sutter et al[1]

Table 7. Means and Standard Deviations of the Response Density and Implicit Times of P2 of the Second-order Kernel Component for the "Ring" Waves as a Function of Age

	Response Density (nV/deg^2)			
Age (y)	Ring 2	Ring 3	Ring 4	Ring 5
10	3.79 ± 1.14	4.72 ± 0.97	3.74 ± 0.77	2.81 ± 0.63
20	3.34 ± 1.82	3.93 ± 1.17	3.34 ± 0.71	2.26 ± 0.91
30	4.06 ± 2.15	3.94 ± 1.30	3.29 ± 0.75	2.53 ± 0.59
40	3.83 ± 1.16	4.47 ± 0.90	3.47 ± 1.17	2.57 ± 0.32
50	3.10 ± 2.23	3.93 ± 1.54	2.77 ± 1.03*	2.06 ± 0.70*
60	3.44 ± 1.13	3.57 ± 1.09*	2.72 ± 0.85*	2.03 ± 0.58*
70	3.33 ± 1.55	3.13 ± 0.45*	2.30 ± 0*	1.87 ± 0.15*
	Implicit Time (msec)			
10	29.27 ± 0.66	26.94 ± 0.93	26.30 ± 0.95	26.84 ± 1.09
20	30.13 ± 1.48	27.27 ± 0.61	26.43 ± 0.65	26.93 ± 0.39
30	27.96 ± 1.58†	26.79 ± 1.24	26.07 ± 1.52	27.03 ± 1.18
40	30.83 ± 2.23‡	29.17 ± 1.65*†‡	27.77 ± 0.92*‡	28.33 ± 0.85
50	29.41 ± 2.03	27.74 ± 0.92	27.27 ± 1.15‡	27.86 ± 1.15
60	29.98 ± 1.49‡	27.55 ± 0.91§	26.95 ± 0.92	27.78 ± 1.48
70	29.43 ± 1.27	27.80 ± 1.91	27.80 ± 1.28*‡	27.50 ± 2.21

*Significant difference ($P < .05$) between teens.
†Significant difference ($P < .05$) between 20s.
‡Significant difference ($P < .05$) between 30s.
§Significant difference ($P < .05$) between 40s.

concluded that the response density corresponded to the cone cell density. Kondo et al[17] stated that the 75-Hz stimulation frequency of multifocal ERG would produce a light adaptation effect in the retina and, thus, that the multifocal ERGs obtained under light adaptation were cone-driven ERG.

Horiguchi et al[18] injected 2-amino-4-phosphonobutyric acid intravitreally in rabbits to block neural transmission between photoreceptors and bipolar cells, and then recorded full-field ERGs and multifocal ERGs. They compared the wave shapes and reported a strong association of the on- and off-bipolar cells to the first-order kernel of multifocal ERG, similar to photopic short flash ERGs. Suzuki et al[19] demonstrated that the off-bipolar cells were more sensitive to aging than the on-bipolar cells. There is histological evidence that the density of cone and bipolar cells decreases with aging.[12,20,21] As the P1 response density of the first-order kernel showed a significant decrease with increasing age, the first-order kernel of multifocal ERG was considered to demonstrate a decrease in the densities of cone and bipolar cells with age.

Anzai et al[5] stated that the implicit times of the "All trace" waves and the P1 waves for the "Ring" of 8° correlated with age while correlation was absent in the "Ring" of 2°. The implicit times of "All trace" and "Ring" waves except "Ring" 1 did not correlate with age in the present study; the reason for the difference from the findings of Anzai et al[5] is not known.

The influence of aging on the second-order kernel waves of the multifocal ERG is reported here for the first time. The P2 and P3 waves of the second-order kernel waves were examined and aging influenced

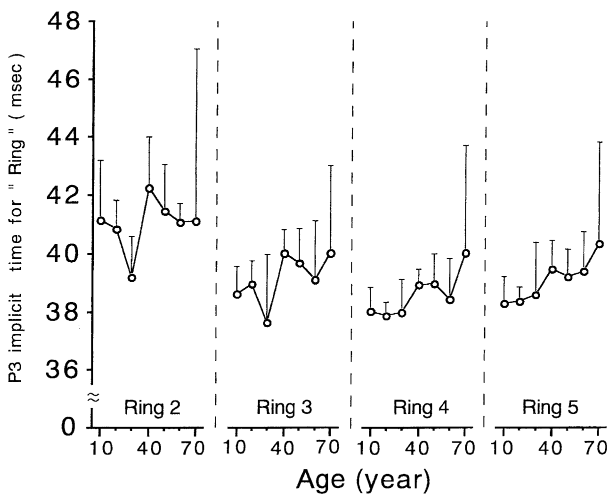

Figure 6. Mean and SD of the implicit times of P3 of the second-order kernel component for the "Ring" waves as a function of age.

Table 8. Mean and Standard Deviation of the Implicit Times of P3 of the Second-order Kernel Component for the "Ring" Waves as a Function of Age

	Implicit time (msec)			
Age (y)	Ring 2	Ring 3	Ring 4	Ring 5
10	41.14 ± 2.04	38.60 ± 0.94	37.97 ± 0.85	38.26 ± 0.90
20	40.84 ± 0.97	38.91 ± 0.81	37.84 ± 0.43	38.31 ± 0.49
30	39.17 ± 1.38	37.61 ± 2.33	37.93 ± 1.15	38.56 ± 1.78
40	42.23 ± 1.76[‡]	40.00 ± 0.80[‡]	38.90 ± 0.52	39.43 ± 0.98
50	41.43 ± 1.58[‡]	39.64 ± 1.18[‡]	38.93 ± 1.05	39.16 ± 0.97
60	41.05 ± 0.67[‡]	39.07 ± 2.02	38.39 ± 1.43	39.35 ± 1.38
70	41.10 ± 5.92	40.00 ± 2.98[‡]	40.00 ± 3.66[*†‡]	40.30 ± 3.48[*†]

[*] Significant difference ($P < .05$) between teens.
[†] Significant difference ($P < .05$) between 20s.
[‡] Significant difference ($P < .05$) between 30s.

these waves differently. The P2 response density of "All trace" waves decreased significantly with age while there was no change in P3. In addition, the P2 response density of the "Ring" waves decreased significantly with age in the periphery to "Ring" 3 whereas there was no influence of aging on P3 waves in any "Ring." These findings suggest that P2 and P3 of the second-order kernel showed different sensitivity to aging and, thus, possible difference in their origins.

In diabetic retinopathy, it is known that the inner retina is injured earlier than the outer layer, and the oscillatory potentials of the conventional ERGs are disturbed earlier than the a- or b- waves. Palmowski et al[3] reported that the second-order kernel contained more components from the inner retina because the response density of the second-order kernel in multifocal ERG recorded from diabetic patients without retinopathy decreased remarkably, even when the first-order kernel was intact. Wu and Sutter[22] reported that the second-order kernel recorded by reducing the stimulation frequency corresponded to the oscillatory potentials of full-field ERG.

Horiguchi et al[18] suggested that the second-order kernel and oscillatory potentials were strongly influenced by the state of the inner retina based on the experiment in which a neurotransmission inhibitor was used. Sutter and Bearse[23] pointed out that the second-order kernel included waves with fixed implicit times irrespective of the distance from the optic nerve head, and included other waves which had prolonged implicit times in proportion to the distance from the optic nerve head, ie, the length of the axons in ganglion cells. They called the former the retinal component[23] and the latter, the optic nerve head component, and suggested that the retinal component originated from the outer retina and the optic nerve head component from the inner retina. Hasegawa et al[24] recorded multifocal ERGs from eyes with branch retinal artery occlusion, and the second-order kernel reflected the inner retinal function.

Histologically, Gao and Hollyfield[21] reported that the density of the pigment epithelial cells and ganglion cells in the human retina decreased with aging. Therefore, the age-related changes in the response density and implicit times of P2 and implicit times of P3 of the second-order kernel observed in this study indicated that the second-order kernel of the multifocal ERG demonstrated changes in the inner retina from aging.

Sarks[25] stated that age-related histological change in the posterior pole of the retina would start with thickening of Bruch's membrane. Ichikawa[26] showed that Bruch's membrane thickened with aging and the choriocapillaries were partly replaced by collagenous fibers, resulting in thinning of the choroid. Therefore, the decrease in retinal function by aging is assumed to start from the choroid-dependent outer layer. Yagasaki[27] examined the influence of aging on the function of the retinal pigment epithelium using an electro-oculogram and reported a decrease in the light peak/dark trough ratio with aging. He considered that the change in the retinal pigment epithelium influenced the visual cell layer, showing the influence of aging in the multifocal ERG, and these findings support the results of the present study.

We suggest that the differences in the response densities of P1 of the first-order kernel and P2 and P3 of the second-order kernel demonstrated different influences of aging on the outer and inner retina. Furthermore, it seemed that the response densities of P2 and P3 reflected different influences of aging on the outer and inner retina. Our results showed a tendency for increasing response density and prolonged implicit times in the 40-year age group, but these findings were considered to derive from the smaller number of subjects in this age group. Thus, it will be necessary to repeat this study with a greater number of subjects in this and other groups. Our subjects were all phakic and the influence of lens opacity due to aging was likely in the multifocal ERG of the aged subjects. However, as the purpose of the present study was to obtain normative values with respect to age groups in normal subjects, only phakic eyes were used and lens opacity was limited to nuclear opacities. In the future, it will be necessary to examine the influence of aging on multifocal ERG waves in pseudophakic eyes.

In conclusion, our findings demonstrated that the age of a patient must be considered in research and clinical studies of the multifocal ERG.

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