Multifocal Electroretinography as a Function of Age: The Importance of Normative Values for Older Adults

William Seiple,[1,2,3] *Thasarat S. Vajaranant,*[2] *Janet P. Szlyk,*[2,3] *Colleen Clemens,*[1] *Karen Holopigian,*[1] *Jennifer Paliga,*[2] *David Badawi,*[2] *and Ronald E. Carr*[1]

Purpose. To determine the influence of age on local electroretinographic responses in humans.

Methods. Multifocal electroretinograms (mfERGs) were obtained from 62 normally sighted subjects ranging in age from 21 to 81 years. A stimulus array of 103 scaled hexagons was used to measure electrical signals within a retinal area approximately 46° in diameter. Commonly reported mfERG methods were used to quantify the responses: peak-to-peak amplitudes and implicit times, scalar product amplitude, and amplitude and time scales derived from the algorithm of Hood and Li, published in 1997.

Results. Regression analysis showed significant linear relationships of amplitude and timing measures with age. The rates of losses were 10.5% per decade for peak-to-peak amplitude, 11.7% per decade for scalar product amplitude, and 9.5% per decade for a-scale. The rate of amplitude reduction was highest in the central 3°. Age had less influence on implicit time measures. The rates of timing losses were 1.4% per decade for the N1 component and 1.0% per decade for both the P1 component and the t-scale measure. Using predicted interval ranges, the age was calculated at which 50% of the expected values would fall below the lower 95% prediction interval band of younger subjects.

Conclusions. The age-associated mfERG alterations are presented to emphasize the importance of appropriate normative data in interpretation of mfERGs. (*Invest Ophthalmol Vis Sci.* 2003;44:1783–1792) DOI:10.1167/iovs.02-0518

Because the size of the elderly population is increasing, an understanding of senile changes in the visual system has become an important topic of interest. Pathologic findings in the senescent retina are numerous. For example, intracellular aberrancies associated with aging include refractile bodies in the inner segments of cone photoreceptors,[1] mitochondrial abnormalities in the foveal cones,[2] and reduction of foveal cone pigment density.[3–5] Declines in the number of retinal pigment epithelial cells, cone and rod photoreceptors, and ganglion cells have also been reported.[6–12] The influence of age on electroretinographic (ERG) results have been investigated. Investigators in studies involving conventional full-field ERGs[13–16] and focal (f)ERGs[17–19] have reported substantial age-related alterations in retinal function. More recently, the multifocal (mf)ERG has generated much interest in the field of retinal electrophysiology research.[20,21] This technique allows the simultaneous recording of local ERG responses from multiple sites. The mfERG topographical data have been used frequently to describe the retinotopic distribution of disease effects in the central retina.[22]

Our goal was to determine the retinotopic distribution of the effects of aging on electrophysiologic function, as measured with the mfERG. We investigated age-related changes on measures of amplitude and implicit time that are commonly used in quantifying the mfERG.[20,21,23] In addition, we calculated the confidence interval around the regression lines to allow assessment of the normality of future measurements. The relationships among commonly used mfERG parameters were also examined. We hoped that these investigations of the normal aging process would lead to a better knowledge of electrophysiological changes associated with aging and an increase in the accuracy in distinguishing pathologic states from normal age-related changes.

Materials and Methods

Subjects

We recruited 62 normally sighted subjects: 47 from the University of Illinois at Chicago (UIC) and 15 from New York University School of Medicine (NYU). The subjects' ages ranged from 21 to 81 years, with a mean age of 45.7 years. We excluded any individual with visual acuity worse than 20/25 or with clinically significant media opacities. All subjects had normal contrast sensitivity, as measured with the Pelli-Robson chart.[24] None of the subjects had any ocular or medical condition that might have affected retinal function or altered ERG responses. Only one eye of each subject was tested; the other eye was patched. All subjects were informed of the purpose of the study and signed a consent form. The research adhered to the tenets of the Declaration of Helsinki, and the institutional review boards at UIC and NYU approved the protocols.

Testing Technique

Multiple retinal areas were stimulated to record local retinal responses with the mfERG technique (VERIS; Electro-Diagnostic Imaging, San Mateo, CA). We used a stimulus array of 103 hexagons subtending a retinal area approximately 46° in diameter. The area of the hexagons was scaled with eccentricity to obtain mfERG responses of approximately equal amplitude. The luminance of the individual hexagons was modulated between 0.45 and 280 cd/m^2, according to a binary m-sequence. The stimulus luminances were equivalent for the UIC and the NYU sites. The stimulus was displayed on a black-and-white mon-

From the [1]Department of Ophthalmology, New York University School of Medicine, New York, New York; the [2]Department of Ophthalmology and Visual Science, University of Illinois at Chicago, Chicago, Illinois; and the [3]Research and Development Service, Chicago Veterans Administration Health Care System, West Side Division, Chicago, Illinois.

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Corresponding author: William Seiple, Department of Ophthalmology, BEL5N15, New York University School of Medicine, 550 First Avenue, New York, NY 10016; whs4@nyu.edu.

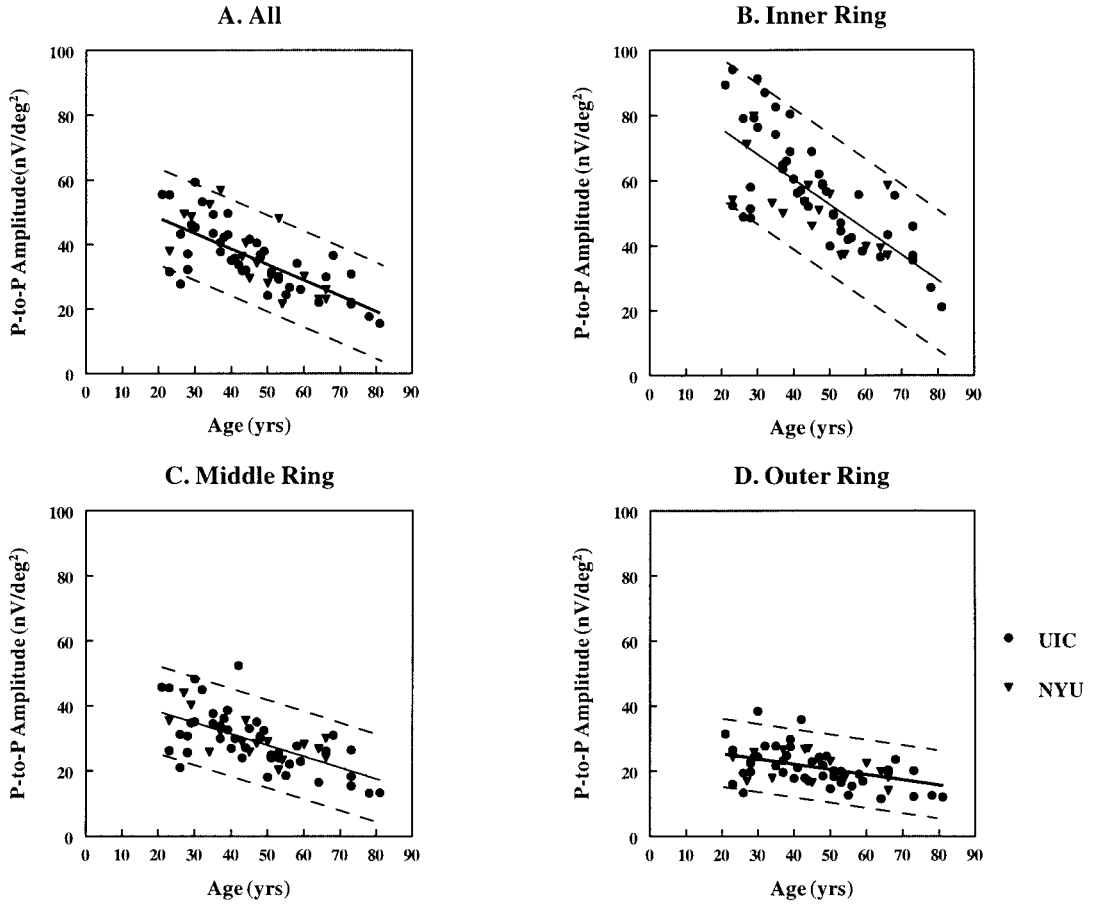

FIGURE 1. Averaged peak-to-peak amplitudes plotted as a function of age for all hexagons (**A**), the inner ring (**B**), the middle ring (**C**), and the outer ring (**D**). *Solid lines*: the fits of linear regression analyses; *dashed lines*: the 95% PI bands.

itor (Nortec, Plymouth, MN) driven at a frame rate of 75 Hz. Each subject's pupil was dilated with 2.5% phenylephrine and 1% tropicamide, and the pupil diameter was measured before testing. ERGs were recorded with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Laboratories, Iowa City, IA) that was grounded to the ipsilateral ear. Before insertion of the contact lens electrode, the subject's cornea was anesthetized with 0.5% proparacaine. The total recording time was 7 minutes and 17 seconds, divided into 32 segments. The subject was required to maintain fixation during each segment. Segments with large eye movements, losses of fixation, or blinks were discarded and rerecorded. The raw data were filtered at a band pass of 10 to 300 Hz, amplified at a gain of 100,000, and digitized at 1200 Hz. Each local ERG response was isolated according to the system's algorithm (VERIS; Electro-Diagnostic Imaging).[20,21]

Correction of Refractive Error

With the UIC system, each subject's vision was optimally corrected with the imaging system's refractor/camera system. To ensure equal magnification of the stimulus array, the distance between the subject's eye and the refractor/camera was adjusted for each subject by obtaining a sharp image on the control monitor. With the NYU system, a 60-mm diameter lens was placed in front of the subject's eye to obtain best correction of the subject's refraction at the viewing distance. The subject's eye position and eye movements were monitored with a charge-coupled device (CCD) camera.

Analysis of the mfERG

mfERG amplitude and timing were quantified for each hexagon. There are three methods commonly used for measuring mfERG responses:

1. Peak-to-peak amplitude was measured from the trough of the first negative wave (N1) to the peak of the following positive wave (P1). The implicit times of these two waves were also measured.
2. Scalar product amplitude was measured using the system's software. This represents the dot product between a normalized template for a group of hexagons and each local response within that group.[20,21]
3. Amplitude and implicit time scaling data were derived using the algorithm of Hood and Li.[23] For this analysis, template waveforms were constructed for each hexagon from the average of the younger control subjects. Two parameters were then calculated: amplitude scale (a-scale), which is derived by scaling the template amplitude at each point by a single value, and timing scale (t-scale), which is derived by scaling the time vector by a single value. The best-fit scaling values are obtained by using a least-squares fitting procedure.

In this study, we measured each subject's mfERGs by all three methods and reported the results as a function of age. We also compared the relationships among these measures. Linear regressions were fitted to each data set, and 95% prediction interval (PI) bands were calculated (Analyze-It software; Analyze-It Software, Ltd., Leeds, UK).

RESULTS

Peak-to-Peak Amplitude

Peak-to-peak amplitudes for waveforms grouped across all hexagons are plotted as a function of age in Figure 1A. UIC data

TABLE 1. Values of the Regression Fits

	Slope	r	r^2	$F_{(1,60)}$	P		Slope	r	r^2	$F_{(1,60)}$	P
Peak-to-peak amplitude						N1 implicit time					
All	−0.48	0.62	0.38	62.15	<0.001	All	0.02	0.46	0.21	15.80	<0.001
Inner ring	−0.77	0.73	0.53	68.34	<0.001	Inner ring	0.02	0.27	0.07	4.80	0.032
Middle ring	−0.35	0.63	0.40	40.52	<0.001	Middle ring	0.02	0.39	0.15	10.68	0.002
Outer ring	−0.16	0.41	0.17	11.83	0.001	Outer ring	0.03	0.56	0.31	25.11	<0.001
Scalar product amplitude						P1 implicit time					
All	−0.15	0.68	0.46	51.40	<0.001	All	0.03	0.31	0.10	6.30	0.015
Inner ring	−0.26	0.72	0.52	62.45	<0.001	Inner ring	0.04	0.39	0.15	10.69	0.002
Middle ring	−0.11	0.62	0.38	37.05	<0.001	Middle ring	0.02	0.22	0.05	3.04	0.086
Outer ring	−0.06	0.48	0.23	17.91	<0.001	Outer ring	0.03	0.30	0.09	5.30	0.025
A-scale						T-scale					
All	−0.011	0.69	0.48	54.17	<0.001	All	0.001	0.43	0.18	13.45	<0.001
Inner ring	−0.013	0.75	0.56	20.95	<0.001	Inner ring	0.001	0.43	0.18	13.50	<0.001
Middle ring	−0.011	0.67	0.45	48.43	<0.001	Middle ring	0.001	0.37	0.14	9.58	0.003
Outer ring	−0.011	0.60	0.36	16.12	<0.001	Outer ring	0.001	0.44	0.19	14.04	<0.001

are plotted as circles and NYU data as triangles. There were no statistically significant differences between the data from the two sites; therefore, the data were combined for analysis. As age increased, amplitude decreased. A linear regression was performed on these data (Table 1) and is shown as the solid line in Figure 1A. The slope of the regression line fitting these data was −0.48 nV/deg^2 per year. Because each of our measures had a different metric and because we wanted to compare the age-related changes among measures, we converted the slopes into percentage of change in predicted means from the predicted mean at 25 years. For peak-to-peak amplitude, the predicted mean at 25 years is 45.8 nV/deg^2, yielding predicted decreases of 10.5% per decade.

Based on the amplitude-versus-age data, it might be useful to determine whether a new subject had significantly reduced amplitude for his or her age. The dashed lines in each figure demarcate the limits of the upper and lower 95% PIs. Future amplitude observations have a 95% probability of falling within this range. Amplitudes declining below the lower dashed line would be statistically ($P \leq 0.025$) smaller than the predicted range of normal subjects for that age.

In addition, it might be useful to determine the age at which there is a significant decrease in amplitude from younger subjects. In other words, when do the effects of aging cause a significant loss in peak-to-peak amplitude? Regressions state only whether there is a statistically significant relation between the variables and that the best-fit line accounts for a significant amount of the variance; slope values alone do not allow statements concerning the statistical significance of the rate of change. In prior studies, the question of significance has been addressed by comparing means from different age subgroups. For example, subjects aged 20 to 30 years might be compared with subjects aged 50 to 60 years. To make this comparison, mean and variance values would be estimated using each subset of the data. However, the validity of these estimates of sample statistics depends on the number of subjects and the age distribution of each subgroup. Typically, in studies that have used this method, samples sizes were small, and no details were given concerning age distributions. Therefore, to avoid this problem, we used the values of the regression parameters derived from the entire data set to compare predicted means to the PI, as described earlier. The predicted mean peak-to-peak amplitude fell below the PI for 25-year-old subjects at an age of 57 years (Fig. 1A). If a line beginning at the lower PI at 25 years is drawn horizontally, it intersects with the regression line (predicted mean value) at an age of 57 years. That is, at 57 years, approximately 50% of the amplitudes from a sample of normal subjects would be expected to fall outside the lower PI of the 25-year-old normal subjects (Table 2). By examining these data for the measures presented later in the article (Table 2), the rates of amplitude and implicit time changes can be compared with measures of variation to determine the relative effects of aging.

We next examined whether the age-related change in amplitude varied as a function of retinal eccentricity. To do this, the mfERG responses for each subject were grouped into three concentric rings centered at the fovea, as shown in Figure 2, and amplitudes were measured. For the inner ring (Fig. 1B),

TABLE 2. Comparison of Change as a Function of Age

Parameter	Slope	Change per Decade (%)	Age at Which Mean Falls Outside PI for 25-year-old Subjects
Peak-to-peak	−0.48	10.5	57
Scalar product	−0.15	11.7	64
A-scale	−0.011	9.5	58
N1 implicit time	0.02	1.4	87
P1 implicit time	0.03	1.0	123
T-scale	0.001	1.0	87

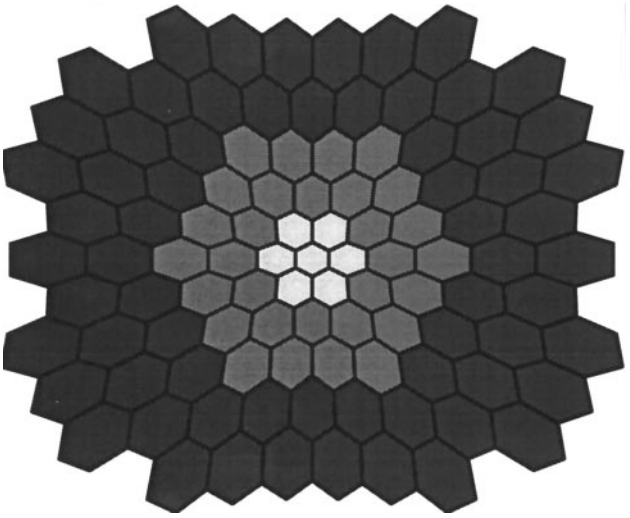

FIGURE 2. Pattern of grouping of the local mfERG responses into three concentric rings.

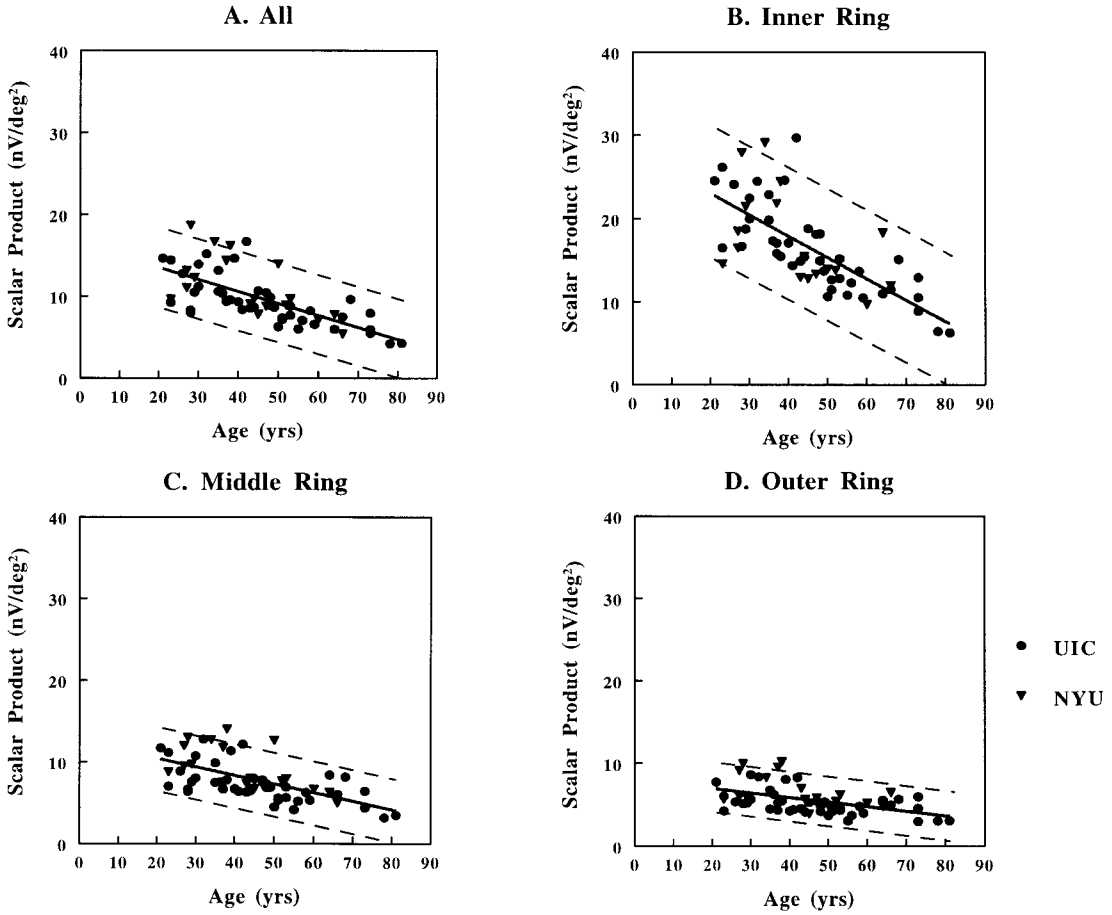

FIGURE 3. Averaged scalar product amplitudes plotted as a function of age for all hexagons (**A**), for the inner ring (**B**), for the middle ring (**C**), and for the outer ring (**D**). *Solid lines*: the fits of linear regression analyses; *dashed lines*: the 95% PI bands.

the slope of amplitude loss as a function of age (-0.77 nV/deg^2 per year) was steeper than that for the summed data. For the middle and outer rings (Figs. 1C, 1D), the slopes were shallower than the slopes of the summed data and of the inner ring (Table 1).

Scalar Product Amplitude

The template for the scalar product amplitude data presented in Figure 3A was derived from all the hexagons. Each waveform was compared with this template, and scalar product amplitudes for all the hexagons were averaged for each subject. Figure 3A shows a plot of scalar product amplitudes as a function of age. Scalar product amplitude decreased with increasing age (-0.15 nV/deg^2 per year, Table 1). Based on the regression, it is predicted that, at 25 years of age, mean scalar product amplitude is 12.8 nV/deg^2, yielding a calculated scalar product loss of 11.7% per decade (Table 2). The predicted mean scalar product amplitude falls below the PI for 25-year-old subjects at 64 years.

To examine variations with eccentricity, we also calculated scalar product amplitudes using templates based on concentric ring groupings (Fig. 2). For the innermost hexagons, the slope of amplitude loss (-0.26 nV/deg^2 per year) was steeper than for the grouped data, whereas for the middle and outer rings, the slopes were shallower (Table 1).

A-scale

To derive a-scale data, the individual waveform for each hexagon was compared with a template waveform calculated for that hexagon. The templates were constructed with the data from all subjects between the ages of 20 and 30 years. In Figure 4A, a-scale values averaged across all hexagons are plotted as a function of age. These data can be viewed as amplitude loss relative to the younger subgroup due to increasing age. A-scale values averaged across all hexagons decreased at a rate of -0.011 per year (Table 1). Based on the regression, the predicted mean a-scale value for age 25 is 1.16 yielding a calculated a-scale loss of 9.5% per decade (Table 2). The predicted mean a-scale at 58 years falls below the PI for 25-year-old subjects. Similar to peak-to-peak and scalar product amplitudes, a-scale data for the inner ring had a steeper loss with age than for the summed data and for the other rings (Table 1).

Implicit Time

Implicit times for N1 (open symbols) and P1 (filled symbols) response components for waveforms averaged across all hexagons are plotted as a function of age in Figure 5A. For both components, there was a statistically significant relationship between implicit time and age. The slope of the regression for the N1 component was 0.02 ms per year (Table 1) or 1.4% per decade (Table 2). The predicted mean N1 implicit time at 87 years would fall above the PI (upper dashed line) for 25-year-old subjects. That is, at 87 years, 50% of negative peak (N)1 implicit times would be longer than the upper limit values for 25-year-old subjects ($P \leq 0.025$). P1 implicit time increased at a rate of 0.03 ms per year (Table 1), or 1.0% per decade (Table 2). The predicted mean P1 implicit time would fall above the

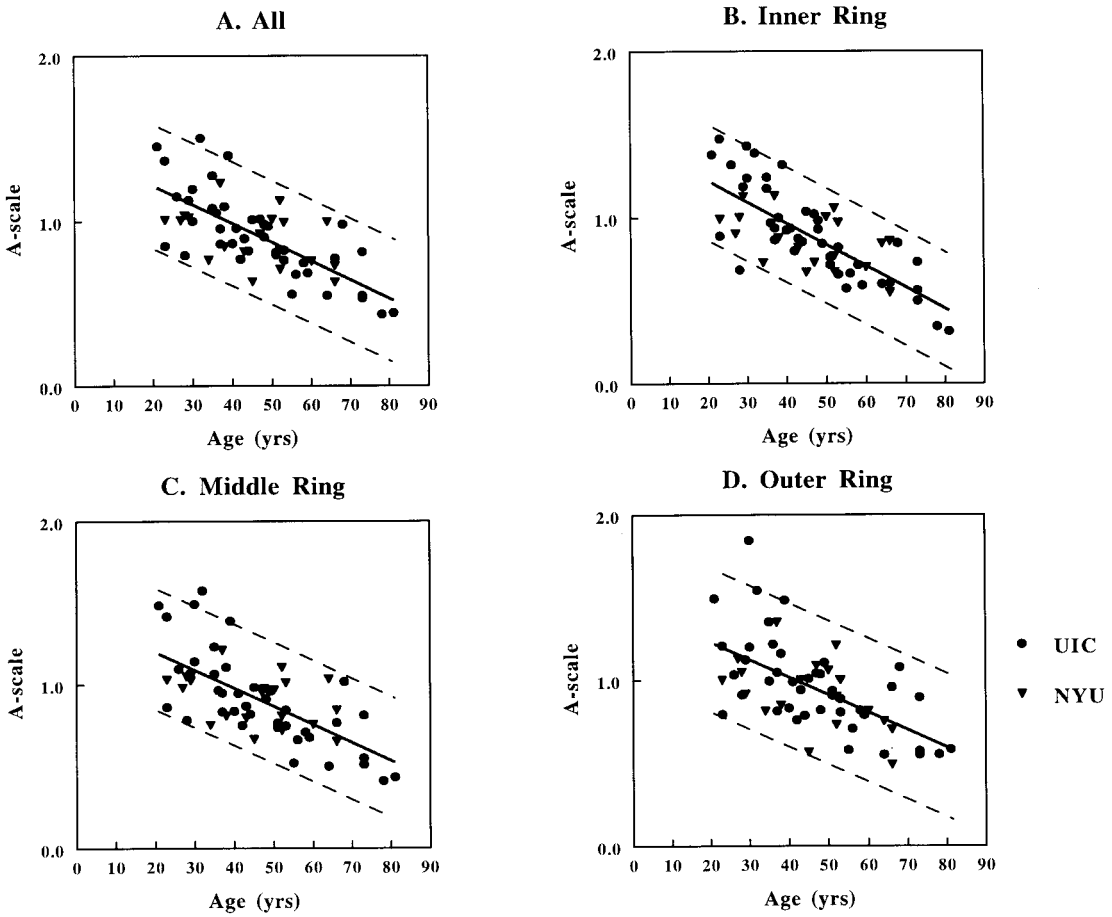

FIGURE 4. Averaged a-scale values plotted as a function of age for all hexagons (**A**), for the inner ring (**B**), for the middle ring (**C**), and for the outer ring (**D**). *Solid lines*: the fits of linear regression analyses; *dashed lines*: the 95% PI bands.

PI for 25-year-old subjects at 123 years. Although absurd in terms of life span, this simply means that P1 implicit times increased very slowly with age. Similar data for implicit times calculated for waveforms averaged into the three concentric rings are shown in Figures 5B–D, and the fit parameters of the linear regressions are presented in Table 1.

T-scale

T-scale was derived by scaling the time vector by a single value.[23] In other words, the time for each point was multiplied by a constant. This constant is calculated as the value that yields the least-squared error between the stretched template and the original waveform. The derived constant is the t-scale value. T-scales, averaged over all hexagons, increased at a rate of 0.001 per year (Fig. 6A), or 1.0% per decade. The predicted mean t-scale at 87 years would fall above the PI for 25-year-old subjects. T-scale for the three concentric rings are plotted in Figures 6B–D, and the regression fit parameters are presented in Table 1.

Correlation among Measures

Peak-to-peak amplitudes are plotted against scalar product amplitudes in Figure 7A and a-scale values for each subject are plotted against his/her corresponding peak-to-peak amplitudes and scalar product amplitudes in Figures 7B and 7C. Values of the linear regression to each set of data are shown in Table 3. There were statistically significant relationships among all amplitude measures. The correlation between peak-to-peak and scalar product amplitudes was 0.63, and between a-scale and peak-to-peak amplitudes, it was 0.71, and between a-scale and scalar product amplitude, it was 0.66. This deviation from unity in Figure 7C probably reflects the differences caused by the differences in the choice of the reference template in the two methods.

The correlation between N1 and P1 implicit times was 0.76 (Fig. 7D). The correlations between t-scale and implicit times (Figs. 7E, 7F) were lower ($r = 0.33$ for N1 and $r = 0.40$ for P1). This was anticipated, because t-scales were derived by scaling the entire time vector (80 ms), not just by matching the peak times of the early response components.

Effects of Pupil Diameter

One confounding factor in examining age-related changes in ERG amplitude is the inability to fully dilate the pupils of some older patients (senile miosis). In Figure 8A, we have plotted dilated pupil diameter as a function of age of our subjects. All younger subjects had dilated pupil diameters of 8 mm or more, whereas some of the older subjects' pupils dilated only to 6 mm. The question is whether the reduced amplitudes that we observed in older subjects were attributable to smaller pupils. Reduced pupil area affects the illuminance of all light impinging on the retina. Illuminance is measured as pupil area times luminance (reported in trolands [td]). With reductions in pupil diameters, not only is the illuminance of the light increments

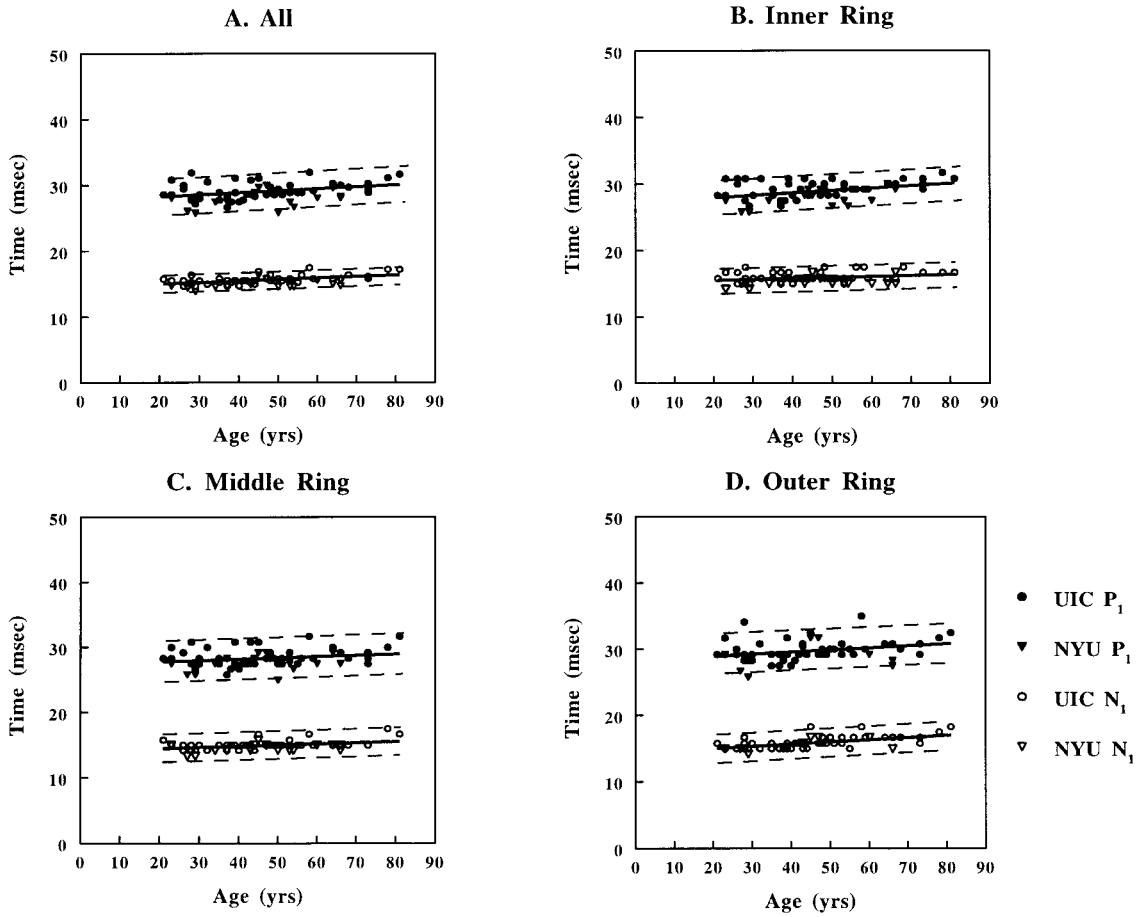

FIGURE 5. Averaged implicit times for N1 and P1 plotted as a function of age for all hexagons (**A**), for the inner ring (**B**), for the middle ring (**C**), and for the outer ring (**D**). *Solid lines*: the fits of linear regression analyses; *dashed lines*: the 95% PI bands.

for the hexagons (the stimulus) reduced, but the level of adaptation of the retina is also reduced. For example, with an 8-mm pupil diameter and a time-averaged mean luminance of the mfERG display of approximately 140 cd/m^2 (hexagons alternate between 0.45 and 280 cd/m^2), the level of retinal illuminance would be 3.84 log td. With a 6-mm pupil diameter, the retina would be adapted to a lower illuminance of 3.60 log td. With a 2-mm smaller pupil diameter, the reduction in stimulus illuminance would also be 0.24 log units. In a previously published study, we measured VlogI functions for peak-to-peak amplitudes measured at different levels of retinal adaptation by using the mfERG array.[25] The Naka-Rushton fits for adaptation levels of 3.8 and 3.5 log td are plotted in Figure 8B. The vertical dashed line is at the level of luminance increment for the mfERG hexagon flashes, assuming an 8-mm pupil diameter. From this, a response of 11.4 μV is predicted (horizontal dashed line). Logically, a reduction in only stimulus illuminance at this level of adaptation would produce smaller ERG amplitudes. A reduction in adaptation level due to, for example, a reduced pupil area, would shift the entire VlogI function to the left. At this lower adaptation level, larger ERGs would be produced by the same flash illuminance. However, smaller pupil diameters (areas) would reduce both the level of adaptation and the stimulus level equally, and nearly identical amplitudes would be predicted (vertical and horizontal solid lines in Fig. 8B).

We also examined our current data for supporting evidence. One younger subject had larger-than-average pupil diameter (9 mm vs. 8 mm). This subject did not have larger amplitudes or shorter implicit times relative to subjects of similar age who had smaller pupil diameters. A 59-year-old subject had a 7.5-mm pupil diameter. This subject did not have lower amplitudes or increased implicit times relative to subjects of equivalent age. Three older subjects had the smallest pupil diameters; however, we do not have age-equivalent subjects with 8-mm pupils with whom to compare their amplitudes and timing. Therefore, we reanalyzed the data omitting these subjects. We found that the values of the regressions were essentially the same with and without these older subjects. For example, the slope of the function of peak-to-peak amplitude versus age decreased from -0.48 nV/deg^2 per year for the entire sample to -0.45 nV/deg^2 per year without the three oldest subjects. That is, the data for these older subjects fell close to the predicted mean data for subjects their age based on the fit to the entire data set.

Our conclusion is that pupil diameter decreases of the magnitude we observed cannot account for the age-related reductions in mfERG amplitude observed in our data.

DISCUSSION

We have confirmed that mfERG amplitude decreased with increasing age. We also observed that the responses of the

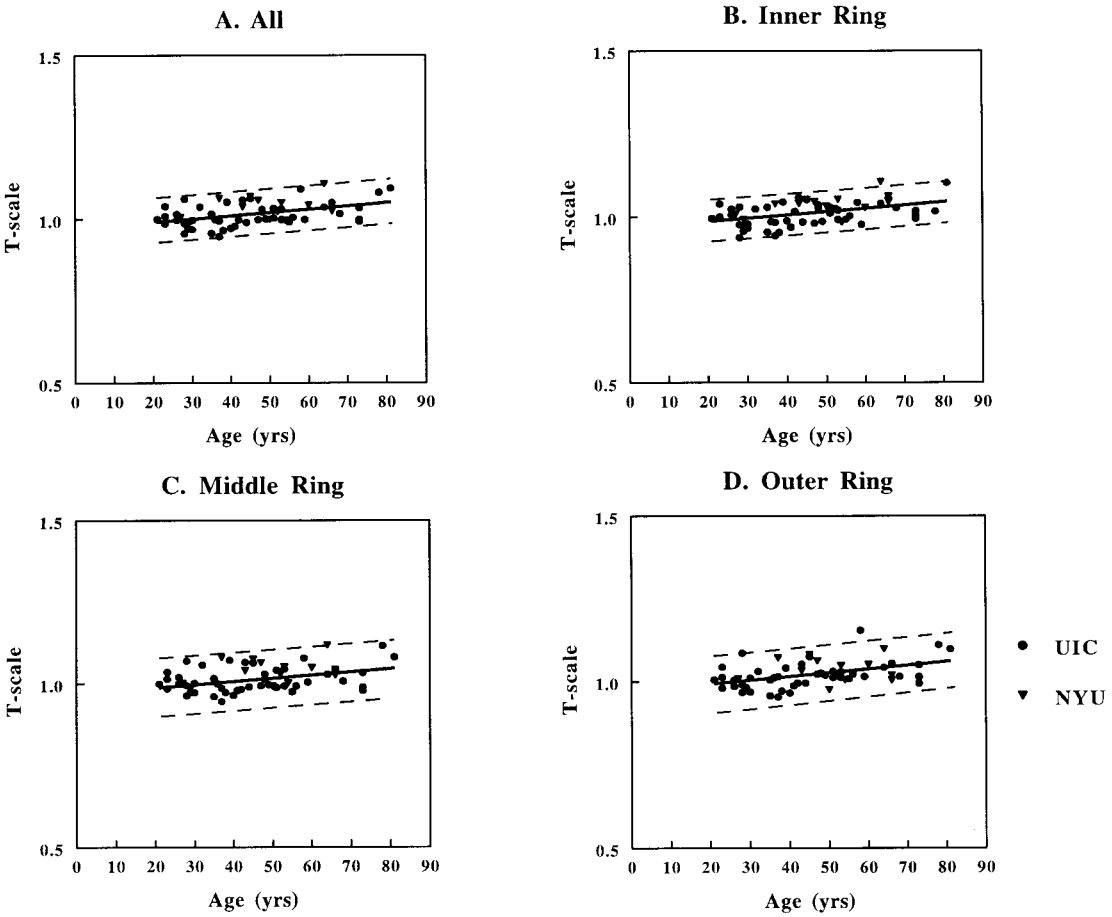

FIGURE 6. Averaged t-scale values plotted as a function of age for all hexagons (**A**), for the inner ring (**B**), for the middle ring (**C**), and for the outer ring (**D**). *Solid lines*: the fits of linear regression analyses; *dashed lines*: the 95% PI bands.

central retina decreased at a greater rate with age than the responses of more peripheral locations. Measures of response timing demonstrated much less change with age than the amplitude measures.

Prior reports of age-associated changes in cone full-field ERGs found significant amplitude reductions[13–16] and implicit time delays.[14,15] Similarly, fERGs consistently showed reduction of amplitudes as a function of age within a central retinal area of 3° to 15° in diameter. However, other studies reported no changes in fERG implicit times with age.[17–19]

In the present study, we examined the changes in mfERG amplitude and implicit time measures as a function of age. For all amplitude measures, there were statistically significant relationships with age. Palmowski et al.[26] reported no significant differences in the scalar product amplitudes when they divided their 17 subjects into two age groups (mean of 34 years versus mean of 47 years). Our current data also indicate that comparing the predicted means of subjects aged 34 and 47 years would not yield statistically significant differences. Anzai et al.[27] reported a significant correlation between mfERG amplitude density and age in 33 subjects, but only for the hexagons in the central 8°. They found smaller amplitudes (no statistics given) in this retinal region when they compared responses for older subjects (60–70 years) to those of younger subjects (10–20 years). Mohidin et al.[28] examined age-related changes in 90 normally sighted, relatively young subjects (18–52 years). These authors reported no statistically significant differences in amplitude density among three age groups (18–22, 33–37, and 48–52 years). Analysis of the responses from the center hexagon and first surrounding ring found significant differences among the groups, and post hoc analysis showed that the older subgroup's mean amplitude was significantly lower than those of the other two subgroups.[28] Jackson et al.[29] examined 46 subjects in two age groups (19–30 years and 60–74 years). They found that both scalar product and peak-to-peak amplitudes showed the largest differences between age groups for the inner hexagons, with less difference between the groups as a function of eccentricity. Significantly reduced peak-to-peak amplitudes were also reported by Nabeshima.[30] in subjects more than 50 years of age. In none of these studies were scatter plots of the data presented. Therefore, it is impossible to determine the age distribution within the subgroups and, thus, the validity of the statistical comparisons. In a recent publication, Gerth et al.[31] reported a statistically significant relationship between peak-to-peak amplitude and age (0.03 log units per decade), based on a linear regression performed on their entire dataset of 71 subjects. This agrees with our peak-to peak amplitude data, for which we found a loss of 0.06 log units per decade.

We also found a significant relationship between age and implicit time, but the slopes of the regression lines were very shallow. Anzai et al.[27] reported no significant differences in implicit time between their younger and older groups. Jackson et al.[29] reported that the mean latency of the P1 component

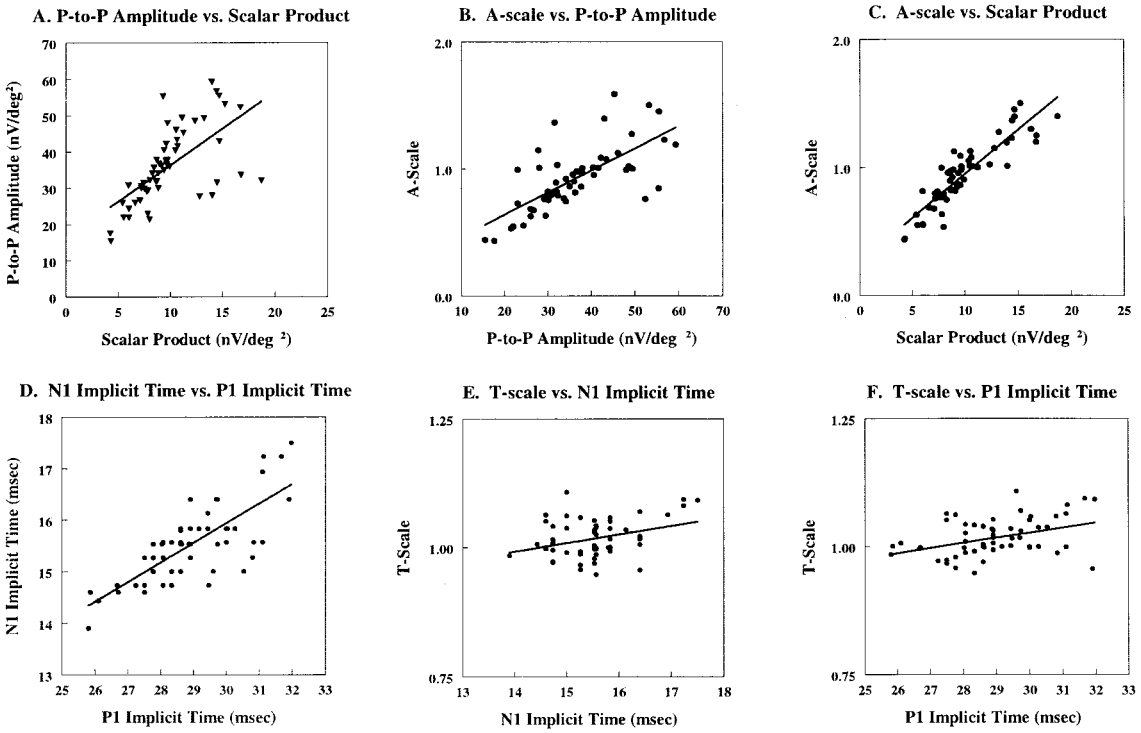

FIGURE 7. (**A–C**) Scatterplots of peak-to-peak amplitudes (P-to-P), scalar product amplitudes, and a-scale. (**D–F**) Scatterplots of N1 implicit times, P1 implicit times, and t-scale.

was 1.31 ms greater in their older group (60–74 years) than in their younger group (19–30 years). For comparison, the difference in predicted mean P1 implicit times in our subjects aged 25 versus those aged 67 was 1.19 ms. Gerth et al.[31] reported an increase in P1 implicit time of 0.28 ms per decade (1.12 ms over 40 years) for their lowest luminance condition. This is also in agreement with our present data and with the findings of Jackson et al.[29] In conclusion, implicit time increases as a function of age, but the rate of change is very slow. In fact, we calculated that predicted mean timing of N1 and P1 would not fall outside the PI of our 25-year-old subjects until very advanced ages.

Diminished retinal illuminance due to aging optics or decreased pupil diameter remains a major concern in any study of ERG changes in older adults. According to Fortune and Johnson,[32] the influence of age on the mfERG is primarily due to preoptical factors. They tested a group of 32 normally sighted subjects (16–69 years) with the mfERG using natural pupils (pupil size ranging from 2.5 to 4.5 mm) and also obtained psychophysical measures of lens density in their older subjects. After adjustment for the effect of aged lens and senile miosis in the older group, significant effects of age on the mfERG were limited to the central 5° area. However, these authors accounted only for decreases in stimulus luminance as a function of optical density and failed to account for the concomitant decreases in the level of retinal illuminance. Gerth et al.[31] also examined the effects of optical media density on mfERG responses. They calculated a 0.12-log-unit reduction in light reaching the retina of a 75-year-old subject compared with a 25-year-old subject. The effect of reduced luminance was tested in an experiment in which stimulus luminance was decreased. Their conclusion was that reduced optical density did not account for their findings of decreasing amplitude with age. However, similar to Fortune and Johnson,[32] Gerth et al.[31] accounted for changes in stimulus luminance only, failing to account for the equivalent reduction in adaptation level that would be caused by increased optical density. In Figure 8B, we demonstrated that changes in both stimulus luminance and adaptation level would predict no change in mfERG amplitudes.

Other studies have examined the effects of lens opacities. Arai et al.[33] simulated moderate cataracts, which decreased visual acuity to a level of 20/70, and reported only small changes in mfERG responses. Jackson et al.[29] also found no differences in mfERG responses between older subjects with aged lenses and those with artificial lenses. Our study did not include individuals with clinically significant lens changes (as determined by clinical examination, visual acuity, and contrast sensitivity). As a result, we conclude that preretinal optical density factors and small pupils in older adults do not account for the mfERG changes observed in our study.

TABLE 3. Relationship among Measures

	r	r^2	$F_{(1,60)}$	*P*
Peak-to-peak amplitude versus scalar product	0.63	0.40	35.66	<0.001
A-scale versus peak-to-peak amplitude	0.71	0.50	54.24	<0.001
A-scale versus scalar product	0.66	0.43	42.68	<0.001
N1 implicit time versus P1 implicit time	0.76	0.57	80.30	<0.001
T-scale versus N1 implicit time	0.33	0.11	6.94	0.011
T-scale versus P1 implicit time	0.40	0.16	10.80	0.002

Possible Pathophysiology of Age-Related mfERG Alterations

The findings of reduced mfERG amplitude density with age are consistent with losses of photoreceptors in older retinas.[34]

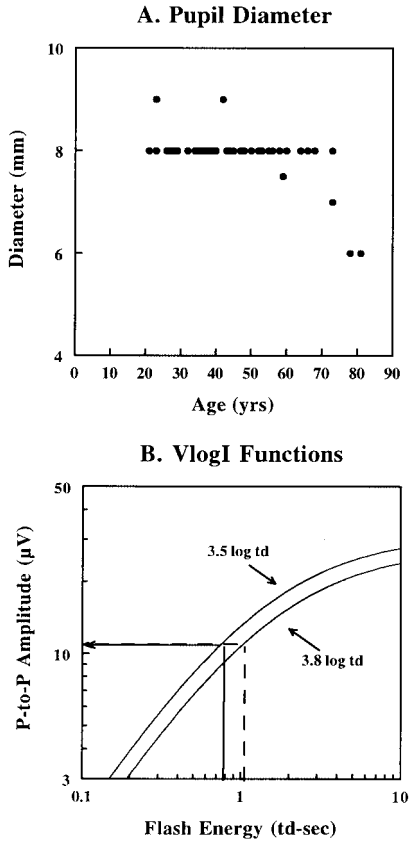

FIGURE 8. (**A**). Dilated pupil diameters plotted as a function of age. (**B**) VlogI functions fit with the Naka-Rushton equation for adaptation levels of 3.8 and 3.5 log td. *Vertical dashed line*: flash energy associated with the mfERG hexagon flashes, assuming an 8-mm pupil diameter; *horizontal solid line*: the amplitude predicted in response to this stimulus intensity. With a 0.3-log unit reduction in the stimulus intensity (*solid vertical line*) and a level of adaptation such as caused by reduced pupil diameter, nearly the same response amplitude is predicted.

Histopathologic studies have reported a decline in the number of cone photoreceptors with age.[6–12] However, findings of cone loss in the area tested with the mfERG were inconsistent.[6,8,9] Age-associated cone loss in the macula has been demonstrated by Gartner and Henkind,[6] whereas Gao and Hollyfield[8] and Curcio et al.[9] reported no statistically significant loss of foveal cones in senile retinas. Gao and Hollyfield[8] noted that a high variability of cone density observed in each age group could have limited their ability to assess age-related cone reductions accurately in the fovea.

Jackson et al.[29] concluded that age-related mfERG reductions were due to functional impairment of the aged retina rather than to anatomic alteration. They postulated that their findings of decreased second-order mfERG amplitudes associated with increased first-order mfERG implicit times were consistent with slowed temporal adaptation in aged retinas.

Other abnormalities previously proposed to account for functional impairment of the fovea included misalignment of photoreceptors and inefficient synaptic transmission.[34] These deficits could also explain changes in ERG amplitudes and possibly implicit times observed in aged retinas. In addition, age-associated mfERG alterations could well be caused by structural and/or functional impairments of the central cones.[1–5] Our findings showed a significant amplitude reduction beginning around the age of 60. Age, however, had less effect on timing measures. A significant implicit time delay was found only at advanced age (sometimes beyond the normal life span). These findings emphasize the importance of appropriate age-matched normative data for accurate mfERG amplitude interpretation in older adults.

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