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Multifocal electroretinogram: age-related changes for different luminance levels

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Abstract

Background—Age-related changes in the first-order multifocal electroretinogram (mfERG) responses were measured for two different luminance levels (200 and 700 $\text{cd}\cdot\text{m}^{-2}$). The relative contribution of optical and neural factors to senescent change in response was evaluated.

Methods—Data were obtained from one eye of each of 71 normal phakic subjects, age 9–80 years. The mfERG responses were recorded with the 7" stimulus-refractor unit (EDI) and VERIS 4.3 using the following protocol: bipolar contact lens, 103 hexagons, consecutive stimulation with 200 and 700 $\text{cd}\cdot\text{m}^{-2}$, pupils ≥ 6 mm, amplification of 10^5 , filter cut-offs at 10 and 300 Hz.

Results—Age-correlated decreases in amplitude and response density and increases in P1 implicit time were found for both luminance levels. The mean response density ($\text{nV}\cdot\text{deg}^{-2}$) was higher for the 700 $\text{cd}\cdot\text{m}^{-2}$ stimulus, but the rate of change with age was not significantly different from that obtained with the 200 $\text{cd}\cdot\text{m}^{-2}$ stimulus. Implicit time was not significantly different for the two light levels, nor was the rate of change with age. The decrease in response density and the increase in implicit time with age were significant across all retinal regions, dividing the 50 deg stimulus into six concentric rings. Age-related change in response density was greatest for the central retina and decreased with increasing retinal eccentricity.

Conclusion—Log mfERG response changes linearly as a function of age. Analyses of the effects of reduced ocular media transmission and increased stray light, along with ancillary data obtained from pseudophakes, imply that age-related changes in the mfERG are due to both optical and neural factors.

Introduction

The multifocal technique introduced by Sutter and Tran [25] permits recordings of multiple, spatially localized electroretinograms (ERG). Hood et al. [8] showed that the biphasic response of the multifocal cone ERG (mfERG) appears to be generated by the same cells generating the a-wave and the positive peaks of the full-field ERG. The need for age-based norms for both fundamental and clinical studies is widely recognized from studies of the Ganzfeld ERG, which generally show a linear change in amplitude and latency over the adult years [2,22,27,30]. Several studies of age-related changes in the mfERG have also been reported [1,5,10,18,19, 21,24,26]). Many of these studies are based on limited sample sizes and/or limited age ranges, and they do not show consistent changes in relation to age.

The mfERG may provide useful tests of psychophysically based models of the aging visual system. It is known from a number of studies that the sensitivity of the pathways carrying

signals from the three classes of cone photoreceptor decreases with age, due to both optical and neural factors (see [14] for review). The magnitude of the age-related loss depends on the state of adaptation of sites that are early in the visual system [23]. For this reason, we obtained the mfERG at two different light levels for 71 subjects covering a broad age range. The results show significant age-related decreases in log amplitude, log response density and log implicit time at both light levels. The effects of ocular media senescence were modeled, and it is concluded that the reductions in mfERG responses with age are due to both optical and neural factors, with response density influenced more by optical factors and P1 implicit time changes influenced more by neural factors.

Materials and methods

Subjects

MfERGs were obtained from one eye of each of 80 normal phakic observers. The data for nine subjects were omitted due to excessive noise, so the analyses presented are based on 71 subjects, approximately evenly distributed by gender (5 females and 5 males in each decade) and across age (10 each decade, 10–80 years, plus a 9-year-old boy). The sample was ethnically diverse (44 Caucasian, 13 Asian, 10 African American, 10 Hispanic and 3 Native American).

The presence of retinal disease or abnormal ocular media in the tested eye was ruled out by ocular examination including visual acuity, slit-lamp examination, intraocular pressure, and direct and indirect ophthalmoscopy. Color stereo fundus photographs of the macula and optic disc (ETDRS fields 1 and 2) were evaluated by a retinal specialist using a stereo viewer. The retinæ of all but one subject were found to have no more than five small ($\leq 63 \mu\text{m}$) drusen and no vascular, retinal, choroidal or optic nerve findings known to disrupt visual function. One subject, whose mfERG data were consistent with his age cohort, had one drusen $> 63 \mu\text{m}$. Intraocular pressure was $\leq 22 \text{ mmHg}$. All subjects demonstrated a corrected Snellen acuity of $\geq 20/20$ in the tested eye, as well as normal color vision when tested with the Neitz anomaloscope, the HRR pseudo-isochromatic plates, and the Farnsworth F-2 plate. Subjects with refractive errors greater than $+4.00 \text{ D}$ or -6.00 D were excluded.

Written informed consent was obtained following the Tenets of Helsinki, and with approval of the Office of Human Research Protection of the University of California, Davis, School of Medicine.

Procedure

Pupils were dilated with 2.5% phenylephrine and 1% tropicamide to a pupil diameter of greater than 6 mm. Topical anesthetic (0.5% propacaine hydrochloride) was instilled prior to inserting a Burian-Allen electrode (Hansen Ophthalmic Development Laboratory, Iowa). To protect the cornea and to ensure electrode-cornea contact, 1.0% carboxymethylcellulose sodium (Celluvisc) was used on the inner contact lens surface. The untested eye was patched. A silver cup electrode positioned on the forehead was used as the ground electrode. The subjects were corrected for refractive errors through the refractive unit, whereby the subjects adjusted their correction to optimally focus the stimulus. Before and during recording, a correct and centered position of the contact lens in relation to the pupil and to the stimulus was ensured by monitoring a video camera image.

Stimulus

The recordings were performed with a VERIS (version 4.3) stimulus-refractor unit (frame rate 75 Hz) using a stimulus with 103 hexagons and a standard m-sequence length with $m=14$, resulting in a total recording time of 3.38 min. Signals were sampled at 1200 Hz (i.e. 0.83 ms between samples). The luminances of the stimuli were $200 \text{ cd}\cdot\text{m}^{-2}$ (test 1), $700 \text{ cd}\cdot\text{m}^{-2}$ (test

2) (white), and $<1 \text{ cd}\cdot\text{m}^{-2}$ (black). The resulting Michelson contrast was 99%. The surround was set to 50% of the mean luminance. The recordings were performed under room light conditions. A 4.8 deg (pen diameter 8%) black fixation cross was used. The data were acquired at a gain of 10^5 over a frequency range of 10–300 Hz (GRASS pre-amplifier CP 511). The amplifier was calibrated with an oscilloscope. Noise-contaminated segments were rejected and repeated. Recordings with more than two repeated segments or too much noise were not used for further analyses.

Stimulus luminance was calibrated with the EDI autocalibrator, while the spectral radiance of the monitor was measured with a Photo Research Model PR703-A spectroradiometer/photometer. The recording protocol was chosen according to the recommended ISCEV guidelines for basic mfERG [16], except for the high luminance condition.

Response analysis

One iteration of an artifact rejection procedure was applied to the raw data. No spatial smoothing was performed. First-order kernel responses for both luminance levels were analyzed for the P1 (first positive peak) implicit time, the response densities (density-scale average obtained from the first negative trough to the first positive peak) and the response amplitude (from the first negative trough to the first positive peak). Analyses were conducted using the overall response as well as for a series of six concentric rings: ring 1 = central hexagon, 1 deg in radius, ring 2 = 1–5 deg, ring 3 = 5–10 deg, ring 4 = 10–15 deg, ring 5 = 15–20 deg and ring 6 = 20–25 deg. In addition, the data were analyzed separately for superior/inferior and temporal/nasal hemifields.

Statistical analysis

The data were analyzed using regression statistics applied to the entire sample [11]. Only linear regressions are presented because no statistically significant improvement with a nonlinear regression (quadratic equation) was found in any analysis. An analysis of covariance (ANCOVA) was used to compare differences in responses across rings, following the methods of Judd et al. [12]. Because each subject's mfERG yields multiple measures, many statistical comparisons were possible. To reduce the probability of type I errors, an adjusted α -level (from $P \leq 0.05$ to $P \leq 0.001$) was chosen based on the number of tests conducted [13].

Results

Overall responses: age, luminance, and gender

Raw values for each subject were transformed to decadic logarithms to facilitate an analysis of the *proportional* change across age and conditions.

Figure 1 presents overall (mean of 103 hexagons) response density ($\text{nV}\cdot\text{deg}^{-2}$) as a function of age for the two luminance levels. The mean response density is approximately 0.157 log units higher for $700 \text{ cd}\cdot\text{m}^{-2}$ than for $200 \text{ cd}\cdot\text{m}^{-2}$. The slopes of the linear regression equations show a 0.03 log unit decrease in response density per decade of age. This indicates that the proportional change is not significantly different for the two light levels.

Because response density can be affected by an interaction between amplitude and timing, separate analyses of amplitude were conducted. Statistical analyses of the amplitude data indicate that there is no significant difference between the two light levels in age-related decreases in response amplitude. With respect to timing, Fig. 2 shows log implicit time (P1) as a function of age for the two light levels. As expected, the mean implicit time is lower for the higher light level, 0.7 ms on average. Note, however, that the rate of change with age is

similar for the two light levels, with increases in implicit time of 0.28 and 0.26 ms per decade for the 200 and 700 $\text{cd}\cdot\text{m}^{-2}$ conditions, respectively. These values do not differ significantly.

In all of these analyses, the slopes of the regression equations were not significantly different for the two light levels. Therefore, subsequent data analyses are presented only for the 200 $\text{cd}\cdot\text{m}^{-2}$ condition.

Full-field ERG studies have demonstrated that the mean age-adjusted b-wave amplitude is higher in females than in males (reviewed in [18]). An analysis of covariance (ANCOVA) indicated that for response density and implicit time in this mfERG sample, there was no significant effect of gender and no significant age $\times$ gender interaction.

Age-related changes related to retinal topography

To ascertain whether age-related changes in mfERG are variable across the retina, the responses were grouped in six concentric rings as defined in Materials and methods. Figure 3 shows response density plotted as a function of age for the different rings. To reduce clutter, rings 3 and 4 are averaged (geometric mean) as are rings 5 and 6. Statistical analyses were, however, conducted with all individual rings.

Regression analyses demonstrated that for each ring, the response density decreased significantly with age. The slopes, however, were steepest for the central areas and decreased with increasing retinal eccentricity, as can be seen in Fig. 3. An ANCOVA demonstrated that the slope relating response density to age for ring 1 was not significantly steeper than for ring 2 ($F_{1,69}=1.273$, $P>0.05$) and for ring 3 ($F_{1,69}=6.612$, $P=0.0123$)¹, but the slope for ring 1 was significantly steeper than for ring 4 ($F_{1,69}=12.608$, $P=0.0007$), ring 5 ($F_{1,69}=13.452$, $P=0.0005$), and ring 6 ($F_{1,69}=15.786$, $P=0.0002$). It should be noted that ring 1 consists of only one hexagon and the data from this ring may be noisier than the data from the others, which have more hexagons. For this reason, the ANCOVA was repeated with ring 2 to make comparisons with rings 3–6. In each case, the results paralleled those expected from the ANCOVA using ring 1 as the base for comparison. Ring 2 was not significantly different from ring 3 ($F_{1,69}=8.457$, $P=0.0049$)¹, but there was a statistically significant difference in slope relating response density to age between ring 2 and rings 4–6.

For each of the six rings, log implicit time increased significantly with age ($P<0.0001$). The slopes were: ring 1 = 0.0004, ring 2 = 0.0003, ring 3 = 0.0005, ring 4 = 0.0004, ring 5 = 0.0003, and ring 6 = 0.0004. An ANCOVA did not reveal any significant differences in slopes between ring 1 and rings 2–6 or between ring 2 and rings 3–6.

We also compared mfERG data partitioned along the horizontal and vertical meridians. As expected, there was a significant decrease in response density with age, together with a significant increase in implicit time with age for all hemifields. The slopes of the regression equations relating response density to age and implicit time to age were identical for superior and inferior retina and for nasal and temporal retina: -0.003 and 0.0004 in each case, respectively.

Discussion

Age-related changes in psychophysical thresholds depend upon light level; however, these variations occur primarily at lower light levels [23]. In this study, both the response density and P1 implicit time varied with light level, but there was no interaction between age and light

¹Note that this would be considered statistically significant had we not chosen a P value corrected for the number of statistical tests.

level. In other words, the rate of age-related change in the mfERG was similar for 200 $\text{cd}\cdot\text{m}^{-2}$ and 700 $\text{cd}\cdot\text{m}^{-2}$ stimuli.

Previous studies of age-related changes in the mfERG have produced varying results. Palmowski et al. [21] reported no significant change in amplitude for groups with mean ages of 34 ($n=9$) and 47 ($n=8$) years. No age-related response density changes were found from 2 deg to 8 deg by Anzai et al. [1] (33 subjects, age groups 10–20 years and 60–70 years) or within the central 5 deg by Mohidin et al. [18] (90 subjects, age 18–52 years). The small number of subjects and/or limited age range in these studies may have limited the statistical power to detect the aging effects found in our data. Jackson et al. [10] (46 subjects, age groups 19–30 years and 60–74 years) found an age-related reduction in amplitude density in the central 36 deg diameter field. Seeliger et al. [24] (recording with dilated pupils) and Fortune and Johnson [5] (recording with undilated pupils and not under room-light conditions; Fortune, personal communication) reported that implicit time increases by 0.4 ms and 0.7 ms per decade respectively, values that are somewhat higher than those found in this study. The different study designs and the stimulus luminances may have contributed to different age-related changes in response density and implicit time.

Are the age-related changes in the mfERG found in this study due to optical and/or neural factors? To address this question, we measured the spectral radiance from 400 nm to 700 nm (2-nm steps) and converted to relative luminance using the CIE luminosity function (V_λ). These spectral values were filtered by theoretical ocular media density spectra for subjects aged 25 years and 75 years. The latter were obtained from a quadratic equation fitted to measured ocular media density values for observers between 12 and 88 years of age [28]. Values at other wavelengths were obtained from the standard density function of van Norren and Vos [20]. Based upon this analysis, we conclude that the difference in light reaching the retina for 25- and 75-year-old observers in our dilated-pupil sample was only 0.12 log units. The same value was obtained (between the ages of 21 and 69 years) from analysis of measurements reported by Fortune and Johnson [5] for their mfERG sample.

The effect of a 0.12 log unit reduction in luminance on the mfERG was evaluated based on ancillary data obtained with four subjects tested for a series of nine stimulus intensities (0.15 log steps) ascending from 50 $\text{cd}\cdot\text{m}^{-2}$ to 700 $\text{cd}\cdot\text{m}^{-2}$. Three phakic observers (ages 16, 32, and 73 years) and one pseudophakic observer (age 72 years) were tested in a single session using the protocol described in Materials and methods. The data are shown in Fig. 4, with best-fitting functions to the mean data. Reduced stimulus luminance results in lower response density and higher implicit time. Using the regression equations fitted to the normal data (Figs. 1, 2), the predicted change between 25 and 75 years would be a reduction in response density of 0.15 log units and an increase in log implicit time of 0.0215. The results in Fig. 4 can be used to predict the response changes due to age-related decreases in ocular media transmission between the ages of 25 and 75 years. These predicted changes in mfERG response are substantially less (0.0511 decrease for log response density and 0.00492 increase for log implicit time) than the actual findings. Thus, most of the change in mfERG over our age range cannot be ascribed to senescent changes in light transmission by the eye's optics.

Intraocular scatter is another important optical factor that might contribute to age-related changes in the mfERG. Age-related increases in intraocular scatter have been well documented [7,9,29], and this will reduce the contrast of the retinal image in elderly observers. Fortune and Johnson [5] estimate that scatter reduces image contrast by ~20% between the ages of 20 and 70 years. The relation between contrast and mfERG response, however, can be complex. While Brown and Yap [3] report a linear reduction in response amplitude with decreasing contrast, Fortune and Johnson [5], found that the first-order kernel of the response in the central 5–10 deg of retina increases for their middle contrast level and that the rise time to the peak is actually

shorter at the lower contrast levels tested. Those results were interpreted as indicating that implicit time changes with age are affected more by changes in ocular media density than by changes in contrast.

Figure 5 shows ancillary data for three subjects (29, 31, and 69 years of age) obtained using the protocol described in Material and methods. Six stimulus contrasts (48%–99%) were tested in ascending order with the same mean luminance as in the 200 $\text{cd}\cdot\text{m}^{-2}$ condition. The results are presented in Fig. 5 for individual subjects with polynomials fitted to the mean log response density and the mean log implicit time. Log response density increased with log contrast. Assuming a 20% reduction in contrast between the ages of 25 and 75 years, one would expect a reduction in log response density of 0.009. This decrease, combined with the decrease due to retinal illuminance (0.05), is insufficient to explain all of the age-related reduction in response density. A decrease of approximately 0.09 units in log response density over this age range remains and may be ascribed to retinal neural senescence. The results for implicit time, shown in the right-hand panel of Fig. 5, demonstrate that log implicit time is actually *less* with a reduction in contrast. Thus, the reduction in retinal contrast in the elderly eye resulting from large particle scatter may compensate for the effects of reduced retinal illuminance under these conditions. A 20% reduction in contrast due to age-related increases in intraocular scatter would be expected to decrease log implicit time by 0.024 between the ages of 25 and 75 years. This change, combined with the increase in log implicit time due to reduced retinal illuminance (0.005), implies that essentially all of the implicit time change with age may be ascribed to neural factors. An additional correction for age-related changes in retinal illuminance might be considered necessary due to reductions in undilated pupil diameter with increasing age. No such correction was applied, however, because all subjects' pupils were dilated with phenylephrine, and Korczyn et al. [15] have reported that there is no significant difference between younger and older groups of probands (spanning the approximate age range of our sample) in the diameter of the phenylephrine-dilated pupil. Recently, Jackson et al. [10] reported a small (0.7 mm) difference in dilated pupil diameter between younger and older subjects. An additional correction of our data based on this value would not alter our conclusions. Overall, the results imply that age-related reductions in retinal illuminance and increases in intraocular scatter do not explain all of the age-related loss in the mfERG. These optical factors have a somewhat greater effect on response density than on P1 implicit time. Both optical and neural factors mediate senescent changes in the mfERG.

While our data and analyses reveal a neural basis for some of the age-related changes in mfERG, there are insufficient anatomical and physiological data to localize the specific sites of loss. Age-related losses in cone density have been reported [4,6], but these losses are greatest outside the central retina, the opposite of our mfERG findings. Psychophysical data and modeling indicate that much of the sensitivity loss in cone pathways is due to the loss in ability of photoreceptors to capture quanta [23]. It is not clear why this occurs, although there is some evidence of morphological changes in cone outer segments with age [17] that would be expected to decrease receptor sensitivity.

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Fig. 1.

Overall log response density (nV · deg⁻²) is plotted as a function of age for two light levels, 200 cd · m⁻² (*open symbols*) and 700 cd · m⁻² (*filled symbols*). Least-squares linear regression lines are shown for each data set. The regression equations are: $y(200) = -0.003 \text{ age} + 1.43$ ($r = -0.60$, $P < 0.0001$) and $y(700) = -0.003 \text{ age} + 1.59$ ($r = 0.55$, $P < 0.0001$)

Fig. 2.

Overall log implicit time (ms) is plotted as a function of age for two light levels, 200 $\text{cd}\cdot\text{m}^{-2}$ (*open symbols*) and 700 $\text{cd}\cdot\text{m}^{-2}$ (*filled symbols*). Least-squares linear regression lines are shown for each data set. The regression equations are: $y(200)=0.00043 \text{ age}+1.435$ ($r=0.52$, $P<0.0001$) and $y(700)=0.00041 \text{ age}+1.426$ ($r=0.51$, $P<0.0001$)

Fig. 3.

Log response density (nV · deg⁻²) is plotted as a function of age for various concentric rings. Least-squares linear regression lines are shown for each data set. The regression equations are: $y(\text{ring 1}) = -0.005 \text{ age} + 2.114$ ($r = -0.70$, $P < 0.0001$); $y(\text{ring 2}) = -0.005 \text{ age} + 1.824$ ($r = -0.72$, $P < 0.0001$); $y(\text{rings 3 \& 4}) = -0.004 \text{ age} + 1.555$ ($r = -0.643$, $P < 0.0001$); and, $y(\text{rings 5 \& 6}) = -0.003 \text{ age} + 1.372$ ($r = -0.55$, $P < 0.0001$)

Fig. 4.

Normalized log response density (nV·deg⁻²) and log implicit time (ms) are plotted as a function of log stimulus luminance (cd·m⁻²) in the left and right panels, respectively. Data points represent individual subjects. The smooth curve in the left-hand panel represents the best-fitting polynomial to the mean data: $y(\text{response density}) = -0.18x^2 + 1.125x - 0.326$ ($r=0.99$, $P<0.0001$). The regression line in the right-hand panel is: $y(\text{implicit time}) = -0.041x + 1.552$ ($r=0.97$, $P<0.0001$).

Fig. 5.

Normalized log response density ($\text{nV} \cdot \text{deg}^{-2}$) and log implicit time (ms) are plotted as a function of log stimulus contrast (%) in the left and right panels, respectively. Data points represent individual subjects. The smooth curves represent best-fitting polynomials to the mean data: $y(\text{response density}) = -0.894x^2 + 0.621x + 1.497$ ($r=0.99$, $P=0.0004$), and $y(\text{implicit time}) = 0.369x^2 + 0.287x + 1.431$ ($r=0.99$, $P=0.0015$)