

Factors Affecting Mydriasis-Free Flicker ERGs Recorded With Real-Time Correction for Retinal Illuminance: Study of 150 Young Healthy Subjects

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PURPOSE. A small, full-field flicker electroretinogram (ERG) recording system was recently developed to record flicker ERGs without mydriasis (RETeval). The device delivers a stimulus with constant retinal illuminance by adjusting the retinal luminance to compensate for changes in the pupillary area. The purpose of this study was to determine what factors affect the fundamental components of the flicker ERGs recorded by RETeval in young healthy subjects.

METHODS. Flicker ERGs were recorded with the RETeval system from 150 eyes of 150 young healthy subjects (age, 20–29 years). Univariate and multivariate linear regression analyses were performed to identify the factors that affected the implicit times and amplitudes of the fundamental component of the flicker ERGs. The independent variables included age, sex, refractive error, axial length, and pupillary area.

RESULTS. Multivariate regression analyses indicated that a longer axial length ($P = 0.03$) and larger pupillary area ($P = 0.008$) were independent factors that were significantly associated with longer implicit times of the fundamental component of the flicker ERGs. Multivariate regression analyses also showed that the female sex ($P = 0.03$) was an independent factor, which was significantly associated with larger amplitude fundamental component of the flicker ERGs.

CONCLUSIONS. These results indicate that the fundamental components of the RETeval flicker ERGs are significantly affected by the axial length, pupillary area, and sex of young healthy subjects. The results also suggest that it would be better to compensate for the Stiles-Crawford effect when flicker ERGs are recorded with natural pupils.

Keywords: electroretinogram (ERG), flicker ERG, RETeval, axial length, pupil size, sex, implicit time, amplitude

Full-field electroretinography (ERG) is a basic clinical test that is used to evaluate the retinal function in healthy subjects and patients with various types of retinal diseases. Recording the flicker ERGs is one of the standard methods that is used to assess the physiology of the retina. The flicker ERGs are elicited by intermittent stimulation at a frequency of 30 Hz.¹ The rods do not respond to such high frequencies, thus, the flicker ERGs elicited by 30 Hz are cone-mediated responses.

Although it is known that flicker ERGs are useful for the assessment and diagnosis of retinal diseases,^{2–15} they have not been widely used for mass screening for retinal abnormalities because a large space is needed for the conventional ERG recording devices, the need of mydriasis and topical anesthesia, and the placement of the electrodes on the cornea or conjunctiva requires specific expertise.

A full-field flicker ERG recording system called the RETeval system was recently developed.^{16–21} This system is comprised of a small, hand-held Ganzfeld dome used with a special single-use, skin electrode array. This system can record flicker ERGs without mydriasis because the device delivers stimulus flashes with constant retinal illuminance

(Td-s) by adjusting the luminance (cd-s/m^2) to compensate for changes in the pupillary area (mm^2) in real time. Two recent studies^{22,23} reported that the implicit time of fundamental component of flicker ERG recorded by RETeval was a useful index of retinal function with high sensitivity of detecting diabetic retinopathy (DR) or DR requiring ophthalmic treatment. However, it has not been determined what factors can affect the implicit times or amplitudes of the fundamental component of the flicker ERGs obtained by RETeval in healthy subjects.

Thus, the purpose of this study was to determine the factors that can affect the implicit times and amplitudes of the fundamental component of the flicker ERGs recorded with the RETeval device in healthy subjects. To accomplish this, we recorded flicker ERGs with the RETeval device and collected biometric measurements from 150 young, healthy individuals whose age ranged between 20 to 29 years. Univariate and multivariate regression analyses were used to determine the relationships between the biometric values and the implicit times and amplitudes of the flicker ERGs.



METHODS

Study Design

This was a prospective, single center study conducted at the Mie University Hospital between March 2014 and February 2017. The Medical Ethics Committee of Mie University Hospital (No. 2680) approved the procedures used, and they conformed to the tenets of the Declaration of Helsinki of the World Medical Association. All patients signed a written informed consent form after they were provided with information on the procedures to be used.

Subjects

Individuals whose age ranged from 20 to 29 years were recruited from the students of Mie University. Those who had any known ocular or systemic diseases or myopia of -6.0 diopters (D) or more were excluded. A total of 150 healthy Japanese subjects were studied. We selected this age range because we wanted to minimize the effect of age on the flicker ERGs because all ERG components are strongly influenced by the age.²⁴⁻²⁸ In addition, it was recently reported that the RETeval flicker ERGs recorded with a stimulus intensity of 8 Td-s can be affected by cataracts.²⁰

Protocols of Ocular Examinations

The examinations of the right eye consisted of measurements of the best-corrected visual acuity, refractive error (spherical equivalent) by autorefractometry (KR-800; Topcon, Tokyo, Japan), anterior segment examinations by slit-lamp biomicroscopy, fundus examination by indirect ophthalmoscopy, and nonmydriatic color fundus photography (AFC-330; Nidek, Gamagori, Japan). The axial length was measured by partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Inc., Dublin, CA, USA).

Flicker ERG Recordings by RETeval

Full-field flicker ERGs were recorded with the RETeval system (LKC Technologies, Gaithersburg, MD, USA). The components of the RETeval system have been described in detail.¹⁶⁻²³ In brief, full-field stimuli are presented with a 60-mm diameter dome, and the white stimuli are created by a combination of three colored light emitting diodes. The frequency of the flicker stimulus was 28.306 Hz, and the pulse duration was less than 1 msec. A small red fixation spot was present at the center of the dome.

During the flicker stimulation, the pupil size (mm^2) was automatically measured in real-time, and the stimulus flash luminance ($\text{cd}\cdot\text{s}/\text{m}^2$) was continuously adjusted to maintain a constant flash retinal illuminance (Td-s) by the following equation:

$$\begin{aligned} \text{Photopic flash retinal illuminance (Td - s)} \\ = \text{photopic flash luminance (cd - s}/\text{m}^2) \\ \times \text{pupillary area (mm}^2). \end{aligned} \quad (1)$$

The ERGs were recorded with a special skin electrode array (Sensor Strip; LKC Technologies, Inc.) placed 2 mm from the margin of the lower eyelid. This electrode array contained an active, a reference, and a ground electrode in a single adhesive tape. The electrical potentials are direct current (DC)-amplified and digitized with a sampling rate of 2 kHz. The data resolution was 24 bits for ± 0.6 V, which is equal to approximately 0.07 μV .

We selected a fixed stimulus flash retinal illuminance of 8 photopic Td-s, which is the recommended default stimulus setting for flicker ERGs for non-dilated eyes in the RETeval system. This stimulus is different from the recommended International Society for Clinical Electrophysiology of Vision (ISCEV) standard light-adapted flicker ERGs used to assess the cone system in retinal diseases. Specifically, this flicker stimulus of 8 Td-s was approximately 1.1 log units lower than the ISCEV standard, which is approximately 150 Td-s with a typical dilated pupil. No background illumination was used.

The recording time of the flicker ERGs ranged from 5 to 15 seconds depending on the reliability of the results as assessed by estimating the standard error of the means estimate of the implicit time from all of the recordings.

The amplitudes and implicit times of the fundamental component were automatically measured and displayed by the RETeval system using a special algorithm with discrete Fourier transformation (DFT) and cross-correlation analysis.²⁹ We also measured the conventional peak implicit times and peak-to-peak amplitudes of the "raw" flicker ERGs (e.g., reconstructed flicker ERG waveform using the first 8 harmonic components).

To reduce common-mode interference (e.g., from 50/60 Hz power lines), the RETeval device used the following methods. To prevent common-mode signal from being recorded by the device, the RETeval used an active ground (right leg drive) and a shielded electrode cable. To prevent common-mode signals from turning into difference-mode signals measurable by the data acquisition system, all of the electrodes in the Sensor Strip had the same area to match their impedances, and the data acquisition system had a high-impedance analog input at the power line frequencies. To prevent measured power line interference from contaminating the processed ERG waveforms, the RETeval device used a stimulus frequency of 28.3 Hz and used Fourier analysis that attenuates the contribution of 50/60 Hz interference in the second harmonic of the ERG by $\times 1000$.

Pupillary Area Measurements

In the RETeval system, the pupil area (mm^2) was automatically measured in real time by a built-in infrared pupillometer during the flicker ERG recordings. The pupil size at each time point of 28.306 Hz was obtained with software provided by LKC Technologies (in the public domain, <http://www.lkc.com/products/RETeval/index.html>). Thus, we estimated the pupillary areas at each time point during the ERG recordings using the equation:

$$\text{pupillary area (mm}^2) = (\text{radius of pupil (mm)})^2 \times \pi. \quad (2)$$

Using this software and equation, we calculated the average pupillary area during the flicker ERG recordings for all 150 subjects.

To determine the accuracy of the pupillary tracking system, the pupil measurements using RETeval software were plotted against hand-measured values of more than 50,000 pupil images. The correlation coefficient (r^2) was over 0.99, showing an excellent correlation between two measurements.

Statistical Analyses

Univariate and multivariate linear regression analyses were used to determine the factors, which affected the implicit times and amplitudes of the fundamental component of the flicker ERGs recorded by the RETeval system. The implicit times and amplitudes of the fundamental component of the flicker ERGs were used as the dependent variables. The independent variables included age, sex, axial length, refrac-

TABLE 1. Demographic Data of 150 Eyes of 150 Healthy Subjects

Number of Subjects/Eyes	150/150
Age (y), mean \pm SD (range)	22.8 \pm 1.8 (20–29)
Sex (male/female)	100/50
Axial length, mm, mean \pm SD (range)	24.9 \pm 1.0 (22.5–27.1)
Spherical equivalent refractive error, D, mean \pm SD (range)	−2.7 \pm 1.7 (+0.6 to −5.5)
Pupillary area during ERG, mm ² , mean \pm SD (range)	7.8 \pm 3.2 (3.2–20.7)
Implicit time of fundamental component, ms, mean \pm SD (range)	33.3 \pm 1.3 (30.0–36.8)
Amplitude of fundamental component, μ V, mean \pm SD (range)	14.1 \pm 4.1 (5.1–31.6)

tive error (spherical equivalent), and pupillary area. The coefficients of correlation (r) and P values were calculated for the univariate linear regression analysis, and standardized partial regression coefficient (β) and P values were calculated for the multivariate linear regression analyses for the five independent variables. After confirming that the data were approximately normally distributed, the differences in the amplitudes of the fundamental component between the male and female subjects were compared with unpaired t -tests. The results were considered statistically significant when P less than 0.05. Analyses were performed with SPSS software (IBM SPSS Statistics 20; IBM Corp., Armonk, NY, USA).

RESULTS

Demographic information of the 150 eyes of 150 young healthy subjects is shown in Table 1. The mean \pm SD age of the subjects was 22.8 \pm 1.8 years with a range of 20 to 29 years. The ratio of males:females was 2:1. The mean refractive error (spherical equivalent) was −2.7 \pm 1.7 D (range, +0.6 to −5.5 D). This male predominance and myopic tendency reflects the population recruited primarily from young medical students at our university.

We performed univariate and multivariate linear regression analyses to determine the factors that affected the implicit times of the fundamental component of the flicker ERGs recorded by the RETeval system (Table 2). Univariate linear regression analyses showed that an older age ($P = 0.03$), longer axial length ($P < 0.001$), higher myopic refractive error ($P = 0.004$), and larger pupillary area during the flicker ERG recordings ($P = 0.003$) were significantly associated with longer implicit times of the fundamental component of the RETeval flicker ERGs (middle panel of Table 2). Consecutive multivariate linear regression analyses identified two independent factors; the axial length ($P = 0.03$) and pupillary area ($P =$

0.008) as independent factors, which can affect the implicit times of the fundamental component of the RETeval flicker ERGs (right panel of Table 2).

The axial lengths are plotted against the implicit times of the fundamental component of RETeval flicker ERGs in Figure 1A. A linear regression fit to the data indicates that a 1-mm increase of axial length was accompanied by an approximately 0.39-msec delay in the implicit time of the fundamental component of the RETeval flicker ERG (Fig. 1A). The refractive error was not analyzed because of strong collinearity with the axial length.

Representative flicker ERGs recorded from three subjects who had approximately the same pupillary area during the ERG recordings of 6.9, 6.4, and 6.8 mm² but had different axial lengths of 22.5, 25.3, and 26.2 mm, respectively, are shown in Figure 1B. The implicit time of fundamental component became longer with longer axial lengths.

Similarly, the pupillary areas during flicker ERG are plotted against the implicit times of the fundamental component in Figure 2A. A linear regression analyses of the data indicated that a 1-mm² increase in the pupillary area was accompanied by an approximately 0.10-msec delay in the implicit time of the fundamental component of the RETeval flicker ERGs (Fig. 2A).

Representative flicker ERGs recorded from three subjects who had approximately the same axial length of 24.6, 24.3, and 24.6 mm but different pupillary areas during ERG recording of 5.3, 9.9, and 16.2 mm², respectively, are shown in Figure 2B. The implicit time of the fundamental component became longer with larger pupillary areas during the flicker ERG recordings.

Next, we performed univariate and multivariate linear regression analyses to determine the factors, which affected the amplitudes of the fundamental component of the flicker ERGs (Table 3). Univariate linear regression analysis showed that the sex and pupillary area during the flicker ERG recordings were marginally associated with larger amplitudes of the fundamental component of the RETeval flicker ERGs but they were not statistically significant ($P = 0.053$ and $P = 0.067$, respectively, middle panel of Table 3). Subsequent multivariate linear regression analyses showed that sex was an independent factor, which influenced the amplitudes of the fundamental component of RETeval flicker ERGs ($P = 0.01$, right panel of Table 3). The mean (\pm SD) of the amplitude of the fundamental component was 15.0 \pm 4.7 μ V in women and 13.6 \pm 3.7 μ V in men ($P = 0.053$, unpaired t -test, Fig. 3). This indicated that the female sex was associated with larger amplitude fundamental component of RETeval flicker ERGs although the difference was not significant.

We also measured the conventional peak implicit times and peak-to-peak amplitudes of the “raw” flicker ERG waveform (e.g., reconstructed flicker ERG waveform using the first eight

TABLE 2. Results of Univariate and Multivariate Regression Analyses

Independent Variables	Univariate Regression Analysis		Multivariate Regression Analysis	
	r	P Value	β	P Value
Age, y	0.175	0.032*	0.087	0.276
Sex	−0.135	0.101	−0.027	0.755
Axial length, mm	0.317	<0.001*	0.266	0.030*
Refractive error, D	−0.231	0.004*	−0.019	0.867
Pupillary area during ERG, mm ²	0.241	0.003*	0.208	0.008*

Correlation coefficient (r), standardized partial regression coefficient (β) and P value are shown for five independent variables, which can affect the implicit time of fundamental component of flicker ERG obtained by RETeval. Refractive error is shown in the value of spherical equivalent refractive error (D). Correlation coefficient (r) in univariate linear regression analysis. Standardized partial regression coefficient (β) in multivariate regression analysis.

* $P < 0.05$ was considered significant.

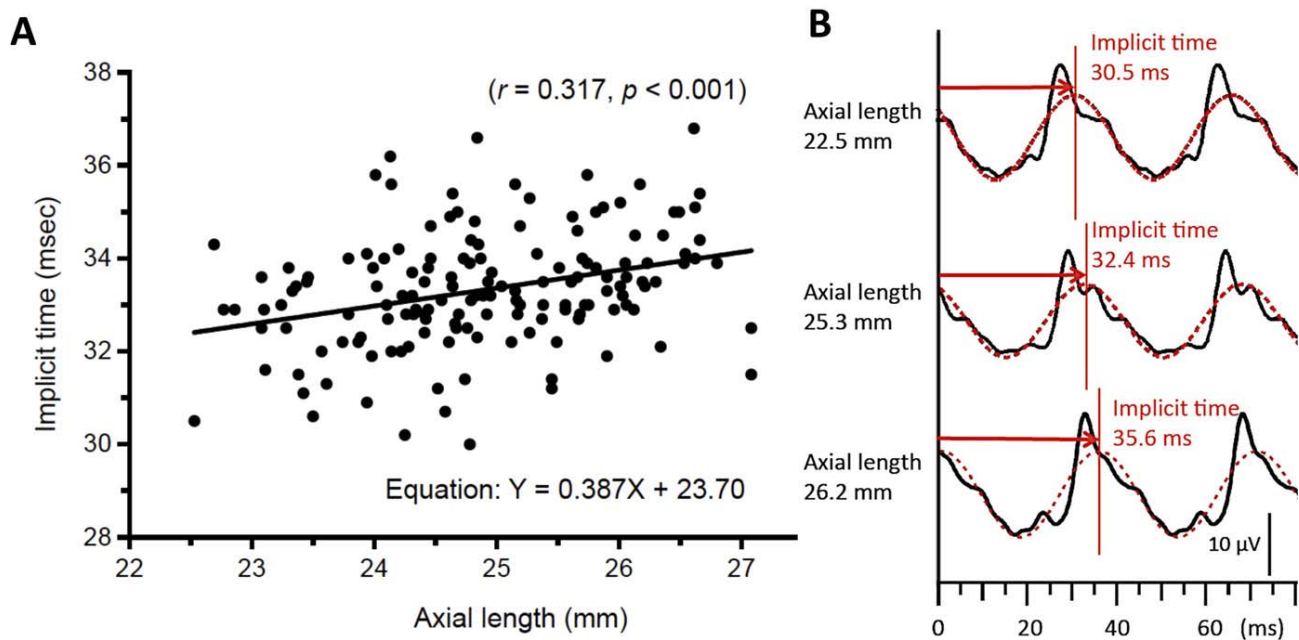


FIGURE 1. Relationship between implicit times and axial length of healthy eyes. **(A)** Implicit time of the fundamental component as a function of axial length. There is a significant correlation between the axial length and the implicit times of the fundamental component ($r = 0.317$, $P < 0.001$). The best-fit linear regression line is also shown. **(B)** Representative flicker ERGs recorded from three subjects who had approximately the same pupillary area of 6.9, 6.4, and 6.8 mm² during the ERG recordings but different axial lengths of 22.5, 25.3, and 26.2 mm respectively. The fundamental component (dotted red line) is superimposed on the reconstructed flicker ERG waveform using the first eight harmonics (solid black line). Red vertical lines are drawn at the implicit times of the fundamental component. The implicit time of fundamental component is longer with longer axial lengths.

harmonic components and performed similar regression analyses). The results were approximately the same as those for the analyses of the fundamental component. Multivariable regression analyses showed that longer axial lengths were

significantly associated with longer peak implicit times of the “raw” flicker ERGs ($P = 0.008$), and larger pupillary area was marginally associated with a longer peak implicit time of the “raw” flicker ERGs ($P = 0.07$, [Supplementary Table S1](#)) The

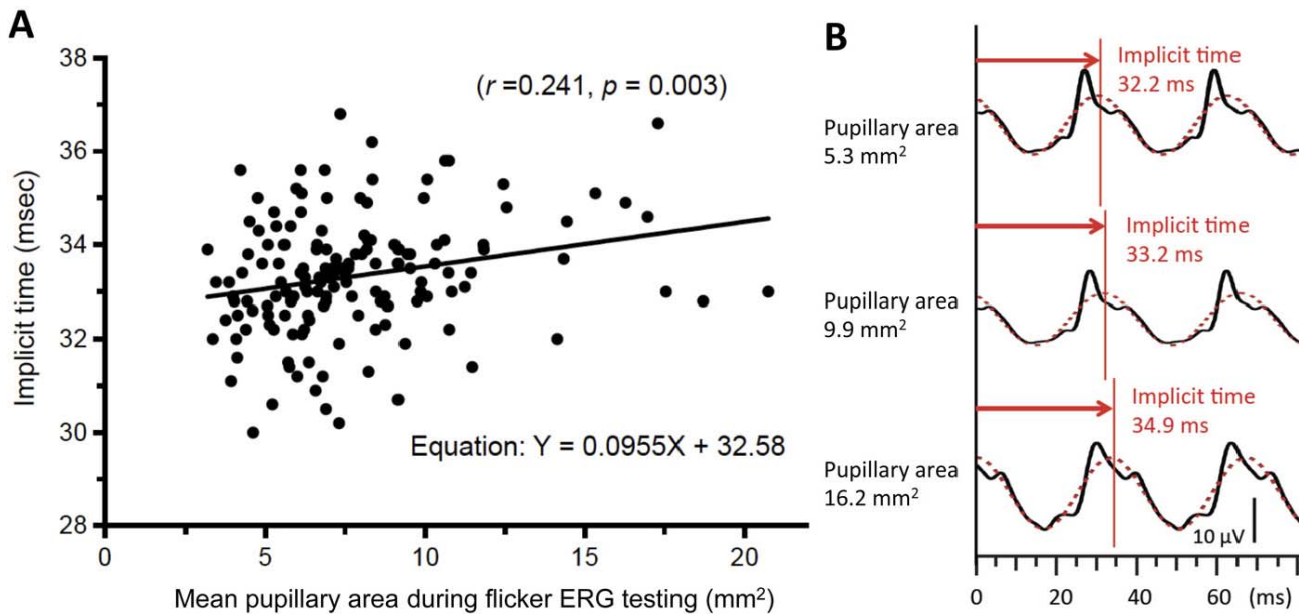


FIGURE 2. Effect of pupillary area on the implicit times of the fundamental component of the flicker ERGs recorded with the RETeval system. **(A)** Implicit time of the fundamental component as a function of pupillary area during RETeval flicker ERG. There is a significant correlation between the pupillary area and the implicit time of the fundamental component ($r = 0.241$, $P = 0.003$). The best-fit linear regression line is also shown. **(B)** Representative flicker ERGs recorded from three subjects who had approximately the same axial length of 24.6, 24.3, and 24.6 mm but different pupillary areas during ERG recording of 5.3, 9.9, and 16.2 mm², respectively. The fundamental component (dotted red line) is superimposed on the reconstructed flicker ERG waveforms using the first eight harmonics (solid black line). Red vertical lines are drawn at the implicit times of fundamental component. The implicit time of fundamental component is longer with larger pupillary area during the flicker ERG recordings.

TABLE 3. Results of Univariate and Multivariate Regression Analyses

Independent Variables	Univariate Regression Analysis		Multivariate Regression Analysis	
	<i>r</i>	<i>P</i> Value	β	<i>P</i> Value
Age, y	0.096	0.242	0.089	0.285
Sex	0.158	0.053	0.234	0.010*
Axial length, mm	0.015	0.852	0.167	0.191
Refractive error, D	0.009	0.904	0.150	0.215
Pupillary area during ERG, mm ²	0.150	0.067	0.152	0.064

Correlation coefficient (*r*), standardized partial regression coefficient (β), and *P* value are shown for five independent variables, which can affect the amplitude of fundamental component of flicker ERG obtained by RETeval. Refractive error is shown in the value of spherical equivalent refractive error (D). Correlation coefficient (*r*) in univariate linear regression analysis. Standardized partial regression coefficient (β) in multivariate regression analysis.

* *P* < 0.05 was considered significant.

female sex was significantly associated with a larger peak-to-peak amplitude of the “raw” flicker ERGs (*P* = 0.008, Supplementary Table S2).

DISCUSSION

The results showed that a longer axial length and a larger pupillary area were independently associated with longer implicit times of the fundamental component of the RETeval flicker ERGs. In addition, the female sex was also independently associated with larger amplitudes of the fundamental component of the RETeval flicker ERGs.

There is good evidence that the axial length affects the amplitudes and implicit times of full-field ERGs. Thus, Westall et al.³⁰ reported that the amplitude of the flicker ERGs became smaller with longer axial lengths as had been reported.^{31–34} They also reported that the implicit times of the flicker ERG became longer with longer axial lengths although the difference was not statistically significant. Two studies^{35,36}

using the multifocal ERG also showed that the amplitudes became smaller and implicit times became longer in eyes with higher myopia (i.e., longer axial lengths). Our results are in general agreement with these earlier reports. The reason why the axial length was not identified as an independent factor, which affected the amplitude of the fundamental component is unclear, but it may be because we excluded eyes with myopia of –6.0 D and higher.

The exact cause of the longer implicit times in eyes with longer axial lengths has not been determined, but three possible factors have been suggested.^{30–36} First, there is a decrease in the retinal illuminance in eyes with longer axial lengths. Second, there is an increase in the distance between the electrical signals in the retina and the electrodes. And third, there is a change in the retinal function caused by the stretching and thinning of the retina associated with the axial length elongation.

The most important finding in this study was that the pupillary area was an independent factor, which significantly affected the implicit times of the fundamental component of the RETeval flicker ERGs. Earlier, we recorded RETeval flicker ERGs before and after mydriasis drops in 10 healthy subjects and found that the implicit times of the fundamental component of the RETeval flicker ERG were significantly affected by the pupillary area even after the compensation for the pupillary area.¹⁷ We hypothesized that this could have been due to the Stiles-Crawford effect of the cone system.^{37–39} We also suggested that the RETeval system delivers a stimulus with constant retinal illuminance if the pupil diameter was less than approximately 6.5 mm (pupil area, 33.2 mm²) because the implicit time remained relatively constant when the pupillary area was smaller than 33.2 mm².¹⁷ Because the majority of patients are likely to have a natural pupillary diameter of less than 6.5 mm, our previous conclusion was that the manufacturer’s claim that the RETeval system delivers constant retinal illuminance will be valid when testing is performed without mydriasis. At that time, we also stated that this conclusion must be verified after an analysis of the ERG data from many healthy subjects.¹⁷

Our results of both univariate and multivariate regression analyses indicated that the implicit times of the fundamental component of flicker ERG was significantly affected by the pupillary area during the recordings of the flicker ERGs (Table 2), even though the pupil diameter was less than 6.5 mm for all 150 subjects (Table 1). These results suggested that it would be better to compensate for the Stiles-Crawford effect when the flicker ERG is recorded if the ERG recordings are performed without mydriasis by the RETeval system. However, there is very little information about how to compensate the Stiles-Crawford effects across the entire pupil in order to compare the effectiveness of light through a small versus

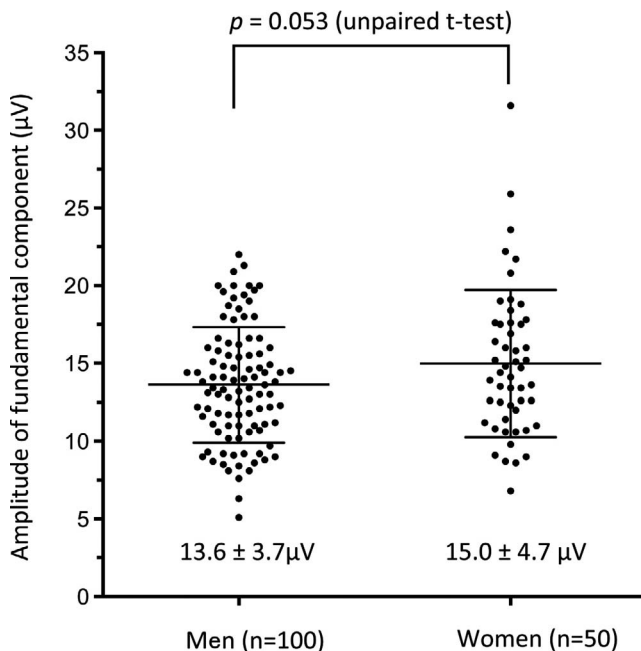


FIGURE 3. Scatter dot plots of the amplitude of fundamental component in 100 male subjects and 50 female subjects. Bars show the means \pm SDs. The difference in the amplitude of the fundamental component between the male and female subjects was marginally significant (*P* = 0.053, unpaired *t*-test).

large pupil. This modeling is an important topic for further studies.

We also found that the sex was an independent factor, which affected the amplitude of the fundamental component of RETeval flicker ERGs ($P = 0.010$, right panel of Table 3). The results of unpaired t -tests on the difference in the amplitudes between males and females were not quite significant ($P = 0.053$), but the mean amplitude of the females was 9.3% larger than that of males in our cohort (Fig. 3). We also measured the peak-to-peak amplitude of the “raw” flicker ERGs and had similar results (Supplementary Table S2). This male-female difference in the amplitude of the flicker ERGs was unexpected and interesting. In 1992, Birch and Anderson²⁷ reported that the amplitudes of rod b-wave and cone b-wave were slightly larger in females than in age-matched males. They stated, “The cause of slightly smaller amplitudes in men than in women may be due to a longer axial length in men.” However, in this study the sex was identified as an independent factor, which affected the amplitudes of the flicker ERGs in the multivariate analyses. Ver Hoeve et al. also reported that the b-wave amplitude of the scotopic and photopic ERGs were 7% to 9% larger in female monkeys than male monkeys (Ver Hoeve JN, et al. *IOVS* 2014;55:ARVO E-Abstract 5130). In addition, larger multifocal ERG components with shorter implicit times in female humans⁴⁰ and monkeys⁴¹ are not explained by axial length or photoreceptor differences. Thavikulwat et al.⁴² also reported larger electro-oculogram (EOG) amplitudes in females.

The exact reason why the flicker ERGs is slightly larger in females than males is not known. Recently, Chaychi et al.⁴³ reported that the ERGs were larger in premenopausal than menopausal female rats, and also larger than those from age-matched male rats. They suggested that these sex differences might be due to the effect of the estrogen hormone on retinal function. It is known that estrogen receptors are expressed in the neurosensory retina and retinal pigment epithelium, and they play an essential role in normal retina function.⁴⁴ If that is the case, the fact that we used only young subjects might be the reasons why our analysis identified the male-female differences as a significant factor. To confirm this hypothesis, the ERG recordings at different days within the estrus cycle in healthy young females may be interesting.

Although the changes in the implicit times with axial length, mean pupillary area, and sex reached statistical significance, one question arises as to whether the magnitude of these influences is biologically meaningful. Calculating from the univariate slopes, the difference in mean implicit time between a short eye of 23 mm and a long eye of 27 mm would be 1.5 ms ($0.387 \text{ ms} \times 4 \text{ mm}$), and the difference in the means between a small pupil area of 3 mm² and a large pupil area of 20 mm² is 1.6 ms ($0.0955 \text{ ms} \times 17 \text{ mm}^2$). For 30-Hz flicker ERGs, a 1.5 ms difference in the implicit time is equal to a phase difference of 16.2°. The normative range for implicit time is approximately ± 2 ms (phase range $\pm 22^\circ$). Therefore, we believe that the differences of 1.5 ms associated with pupil size or axial length are substantial when considering the classification of patients for flicker ERGs.²³

There are three limitations in this study. The first limitation is that we measured mainly the fundamental component of flicker ERGs while the measurements of the peak implicit times or peak-to-peak amplitudes are more generally used in clinical situations. This was simply because the implicit times and amplitudes of the fundamental component are automatically displayed in the current RETeval system. To overcome this limitation, we also measured the conventional peak implicit times and peak-to-peak amplitudes of the “raw” flicker ERGs (Supplementary Tables S1, S2) and confirmed

that the results were approximately the same to those of the fundamental component.

Second limitation is that the stimulus illuminance used in this study (8 Td-s) was lower than that of conventional standard flicker ERGs (150 Td-s). This was because 8 Td-s was the default stimulus setting when this device was introduced in Japan. It is important to confirm that the similar correlations with axial length, pupillary area, and sex are obtained using the stronger flicker stimulus protocol. We believe that stronger stimuli (32 Td-s or more) may be more suitable for routine clinical examinations with the RETeval because larger flicker ERGs are obtained with smaller effect from cataracts. The relative sensitivity of ERGs to stronger versus weaker flicker stimuli for detection retinal dysfunction, such as high-risk DR, is unclear and requires further study.

The third limitation was that the ratio of the male and female subjects was 2:1. We found that the amplitude of the fundamental flicker ERGs tended to be higher in females, but this result would have greater statistical power if the sample of male:female was 1:1. This high proportion of males was because we recruited the subjects mainly from the medical students of our university.

In conclusion, regression analyses of the results of the RETeval flicker ERGs of 150 young healthy subjects showed that the axial length and pupillary area during the ERG recordings are independent factors, which affect the implicit time of the fundamental component of RETeval flicker ERG. In addition, the sex differences were an independent factor, which influence the amplitude of RETeval flicker ERG. We suggest that it would be better to compensate for the Stiles-Crawford effect when the RETeval flicker ERG is used with natural pupil settings.

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