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The Effects of Gender and Age on the Range of the Normal Human Electro-Oculogram

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Abstract

Purpose—To define the normal ranges for the slow oscillations (SO) and fast oscillations (FO) of the electro-oculogram (EOG) recorded to International Society for Clinical Electrophysiology of Vision (ISCEV) standards. The effects of age and gender on the EOG ranges were examined.

Methods—ISCEV standard SOs and FOs were recorded from 121 subjects (51% male) aged from 7 to 72 years. Study variables for the SO were dark trough (DT) and light peak (LP) amplitudes (μ V), times to DT and LP (minutes), and the Arden ratio (LP/DT amplitude). The FO was fit by a sine wave and peak-to-peak amplitude (μ V), phase (degrees), and peak-to-trough (PT) ratios derived. The effects of age, gender and pupil size on EOG parameters were examined by multiple regression analysis.

Results—The average Arden ratio was 2.5. Arden ratio decreased with age at a rate of 0.13 per decade of age ($R^2=0.14$, $P<0.0001$). The 5th percentile of the Arden ratio decreased from 2.0 to 1.7 between 10 and 60 years of age. Median time to LP was 9 minutes (interquartile range 8-9 minutes). Time to LP was age-dependent and increased by 2 minutes for subjects over 55 years of age compared with those less than 25 years. EOG amplitudes were greater in women than in men ($P<0.005$). The average PT ratio was 1.18, which was not affected by age or gender. Time to reach the light trough of the FO was 40 s, which increased with age (1.1 s/decade). No correlation was observed between Arden ratio and PT ratio.

Conclusions—The major strength of this study is the definition of the normal range and associated lower limits of ISCEV standard EOGs based on recordings from 121 subjects balanced by gender and spanning the 1st through 8th decades of life. Decreased Arden ratio and increased time to LP are associated with aging, which is likely due to the intricate mechanisms involved in generation of the light rise. Differences between the FO and SO with respect to the effects of aging are consistent with separate generation of these two EOG signals.

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Keywords

EOG; electro-oculogram; fast oscillation; Arden ratio; clinical electrophysiology; retinal pigment epithelium

Introduction

The retinal pigment epithelium (RPE) regulates the extracellular environment and provides metabolic support to the photoreceptors and outer retina [1]. RPE dysfunction and death occurring in isolation or secondary to photoreceptor disease is the hallmark of several maculopathies including Best vitelliform dystrophy, Stargardt disease and age-related macular degeneration (AMD).

The structure of the RPE layer can be visualized with optical coherence tomography and fundus autofluorescence, but these imaging techniques do not provide insight into the functional integrity of RPE cells. The c-wave of the electroretinogram provides a measure of RPE function but also contains a significant contribution from the Muller cells [2]. The electro-oculogram (EOG) provides a non-invasive means by which to assess integrity of the photoreceptor-RPE complex by recording the changes in voltage across the RPE in response to changes in retinal illumination. The EOG recorded for two different patterns of light stimulation can be used to assess distinct aspects of RPE physiology.

The slow oscillation (SO) is generated by alternating 15-minute periods of dark and light and is characterized by a dark trough (DT) during the dark phase followed by a light peak (LP) during the light phase. The ratio of the amplitudes during the first dark-light cycle (LP/DT) defines the Arden ratio, which is used clinically in the differential diagnosis of several bestrophinopathies including Best vitelliform dystrophy [3,4]. Generation of a light rise requires input from the photoreceptors that ultimately results in RPE basement membrane depolarization [1].

The fast oscillation (FO) is generated by alternating 60-75-s periods of dark and light. In contrast to the SO, the FO is characterized by a dark *peak* and light *trough* whose amplitudes remain relatively constant throughout the dark-light cycles. Fast shifts in potassium and chloride concentrations leading to *hyperpolarization* of the basal side of the RPE membrane underlie generation of the FO. The FO is larger and slower in patients heterozygous for mutations in the cystic fibrosis gene, CFTR which encodes a chloride channel [5]. The contrasting changes in the SO and FO in Best disease and cystic fibrosis support the conclusion that the slow and fast oscillations of the EOG are derived from different RPE mechanisms and that both may be clinically useful [6].

The International Society for Clinical Electrophysiology of Vision (ISCEV) first established standards for recording and analyzing the fast and slow oscillations of the EOG in 1993 [7], with subsequent updates, most recently in 2010 [4]. Existing studies that established normal ranges for these tests in large numbers of subjects (>50) were completed prior to 1993 and used settings that were often outside the ranges subsequently adopted by ISCEV. Light intensities ranged from 250-8611 cd/m² and were usually delivered by light boxes that did

not provide full-field stimulation [8-14]. Other variations from the ISCEV standard included the use of undilated pupils and fixation excursions ranging from 30° to 55°. As a result, the lower limit of normal for the Arden ratio reported across studies varies, ranging from 1.5 to 2.0.

The effects of age and gender on EOG parameters are also difficult to assess from previous studies due to heavy bias of one gender and/or narrow age ranges of test subjects. A notable exception is the study of Adams (1973), who recorded the EOG from 120 subjects between 10 and 69 years of age, evenly distributed by decade of life and gender [11]. Adams found that the Arden ratio decreased with increasing age in women only and that women had larger absolute EOG amplitudes.

Further characterization of the normal EOG variation using current ISCEV standards is still needed. The present study establishes the normal ranges and examines the effects of age and gender on the SO and FO in a large cohort of 121 subjects with an approximately equal number of male and female subjects spanning the 1st through 8th decades of life.

Materials and Methods

This study was approved by the National Institutes of Health Institutional Review Board. All procedures performed involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. EOGs were recorded from a total of 132 healthy volunteers recruited to the National Eye Institute in Bethesda, MD from 1995 to 2001. Informed consent was obtained from all individual participants included in the study. Inclusion criteria included best-corrected visual acuity 20/20 and normal ophthalmological exam with normal visual fields and color vision perception. Exclusion criteria included subjective or objective evidence of visual loss, abnormal night vision, or light sensitivity. Volunteers were also excluded if they had a personal history of significant ocular disease, family history of hereditary ocular disease, current systemic disease, or were currently taking neuropharmacological medication.

EOG recordings

A commercial Ganzfeld-based UTAS E-2000 electrophysiology system (LKC, Gaithersburg, MD) was used for controlling and recording all EOGs. Following dilation, EOGs were recorded binocularly from silver/silver chloride electrodes placed near each canthus with an additional electrode placed on the forehead serving as ground. EOG signals were band pass filtered prior to storage (-3 dB at 0.05 and 30 Hz for FO and 0.05 and 100Hz for SO respectively). A 60 Hz notch filter was used for FO recording. At the completion of EOG recording, pupil size was measured (mm) using a pupil diameter reference card. For the FO protocol, fixation light-emitting diodes (LEDs) at 30° separation were alternately illuminated at 0.5 Hz and subjects continuously alternated gaze between the LEDs for the entire test. The FO consisted of six cycles, each comprised of 75 s of light adaptation (100 cd/m²) followed by 75 s of dark adaptation. To determine the amplitude of each saccade, the UTAS E-2000 software averaged the amplitude data between 300 and 600 ms after the fixation light was switched

The SO was then recorded at the same visit. For the SO protocol, 5 minutes of pre-adaptation (20 cd/m²) were followed by 15 minutes of dark adaptation, then by a minimum of 20 minutes of light adaptation (100 cd/m²). Fixation LEDs alternated at 1.5 Hz and the subject was tested for 15 s once per minute during the 35 or more minutes of recording. For each 15-s interval, the average saccade value was calculated by UTAS E-2000 software using the following algorithm (Also see Appendix 1): 1) A 5-point median filter was applied to the waveform to eliminate spike artifacts, 2) the peak to trough amplitude was found for each saccade, 3) the largest and smallest saccade amplitudes recorded during the 15-s sweep were discarded, and 4) average saccade amplitude for each 15-s sweep was calculated from the remaining values.

EOG Analysis

Measurement of SO parameters—At the completion of the SO test, the UTAS E-2000 software automatically located the lowest point in the dark-adapted phase and highest point in the light-adapted phase. The location of the trough and peak could be moved by the user if he/she felt the software had not selected the correct locations. Study variables for the SO were absolute amplitudes at the dark trough (DT amplitude (μV)) and light peak (LP amplitude (μV)), time from onset of the dark phase to the DT (minutes), time from onset of the light phase to the LP (minutes), and the Arden ratio. The Arden ratio was calculated as LP amplitude/DT amplitude.

Measurement of FO parameters—A sine wave provides the best description of the shape of the FO data [6]. The 15 minutes of absolute amplitudes recorded during the FO was smoothed (5-point moving filter) then auto fit with a sinusoidal wave by UTAS E-2000 software (See Appendix 2). The following parameters were derived from the sinusoidal wave fit: FO amplitude (μV) calculated from the difference between the absolute amplitudes of the peak and trough of the sine wave fit (Appendix 2, Equation A2), the peak-to-trough (PT) ratio calculated from the ratio of absolute amplitudes of the peak and trough of the sine wave fit (Appendix 2, Equation A4), and the phase (degrees) of the sine wave measured relative to the time the light turned on for the light phase (Appendix 2, Equation A5). For analysis, FO phase was converted to time to light trough using the formula:

$$\text{Time to light trough (s)} = \text{FO phase} / 360 * 150\text{s/cyc} - 75\text{s} \quad \text{Equation (1)}$$

Eleven subjects were excluded from analysis due to poor technical quality of recordings. They were not significantly different in age or male/female ratio from the included subjects. Both eyes of the remaining 121 subjects for a total of 242 eyes were included in subsequent analyses. R statistical software (version 3.1.1, Vienna, Austria) was used for statistical analysis [15]. Plots were created using the R package ggplot2 [16]. Data were tested for normality using the Shapiro-Wilk test.

Non-normally distributed continuous data (Arden ratio, PT ratio, and FO phase) were well-described by a Gamma distribution with the corresponding probability density function:

$$\rho(y|\alpha\beta) = \frac{\beta^\alpha}{\Gamma(\alpha)} y^{\alpha-1} e^{-\beta y} \text{ where } \Gamma \text{ is the gamma function Equation (2)}$$

Equation 2 describes the probability of y given α and β where y is the independent count variable (e.g. number of subjects with Arden ratio >2.0 and <2.1) and derived parameters are α , which describes the “shape” of the distribution and β , which describes the “rate” of change of the distribution. The mean of the Gamma distribution is α/β . The limits of normal were determined as the 5th and 95th percentiles calculated from Equation 2.

Standard Gaussian functions were fit to normally-distributed log-transformed amplitude data (SO DT and LP amplitudes and FO amplitudes). Gamma distribution and Gaussian fits along with derived parameters are reported in Supplemental Figure 1. The times to DT or LP could not be fit by probability density functions because these times (measured in 1-minute integers) were distributed over a small range.

Statistical Analysis

In order to identify the variables affecting each EOG parameter, an exploratory multiple regression analysis was performed with main variables of age, gender, and pupil size. Generalized estimating equations (GEEs) from the R package *geepack* were applied in all regression analyses to account for the correlation between eyes of each subject [17,18]. An exchangeable correlation structure was used for GEE models, which assumes that the correlation between eyes for each subject is the same for all subjects.

To answer the primary question of how age affects EOG parameters, linear regressions with respect to age were then calculated for parameters that were found to be statistically significant for age in the exploratory analysis. In order to account for the possibility that certain age-related changes might be gender-specific, linear regressions were also calculated by gender subgroup for the parameters that showed statistically significant gender differences in the exploratory analysis. To reduce the increased risk of type I (family-wise) errors associated with multiple comparisons, a Holm-Bonferroni adjustment for the 10 comparisons (4 parameters including all subjects vs. age and 3 parameters that were divided into gender subgroups vs. age) was applied to each P Value and presented in the results. A family-wise adjusted P Value <0.05 was considered statistically significant.

Results

The results from 121 subjects ranging in age from 7 to 72 years with a mean age of 36.2 ± 16.3 years (mean $\pm$ standard deviation) were included in the final analysis (Table 1). Male and female subjects were equally represented and not significantly different in age. Right and left eyes within individual subjects were highly positively correlated for the Arden ratio ($R^2=0.79$; $P <0.0001$) and PT ratio ($R^2=0.79$; $P <0.0001$).

Effect of Age on EOG Parameters

Table 2 summarizes the results of the multiple regression analysis performed for each EOG parameter for the main effects of age, gender and pupil size (See Methods). The only

parameters that changed as functions of age were the Arden ratio and time to LP for the SO and phase of the sine wave fit to the FO data. None of these three parameters were affected by gender when corrected for age and pupil size.

SO: Arden Ratio—Figure 1a shows the fit of the gamma distribution to the Arden ratio data for all subjects. Mean Arden ratio was 2.5, slightly higher than the median value of 2.4. Ratios of 1.72 and 3.44 demarcate the 5th and 95th percentiles respectively (Figure 1a, Table 3).

Figure 2 illustrates that the Arden ratio is inversely correlated with age ($R^2=0.14$; $P<0.0001$; Table 2). Each decade increase in age resulted in a 0.13 decrease in Arden ratio. Because the Arden ratio varied with age, quantile regression analysis (R package quantreg [19]) was used to calculate the 5th and 95th percentiles of the Arden ratio as a function of age. As seen in Figure 2, the 5th percentile line declined at a slow rate of 0.06/decade of life ($y = -0.00597x + 2.05$), while the 95th percentile line decreased at a much greater rate of 0.31/decade of life ($y = -0.0314x + 4.66$). The 5th and 95th percentiles of the Arden ratio for the midpoint of each decade are listed in Table 1. When calculated from this regression, the 5th percentile lies at 2.0 for 10 year old people and 1.7 for 60 year old people. Over the same age range, the 95th percentile falls from 4.3 to 2.8.

SO: Time to Light Peak and Dark Trough—The median time to LP from onset of the SO light phase was 9 minutes with a narrow interquartile range of 8 to 9 minutes (Table 3). Figure 3 graphically highlights the progressive increase in the time needed to reach the SO LP with advancing age. For the youngest age group (<15 years), a median of 8 minutes was required to reach the LP and a time to LP >10 minutes was not observed for any subject <25 years old (Figure 3). For the two oldest age groups (>55 years), the median time to LP was 10 minutes and no subject over 55 years had a time to LP of <8 minutes (Figure 3).

The time to LP was negatively correlated with Arden ratio ($y = -0.1266x + 3.6059$; $R^2=0.11$; $P<0.05$; Supplemental Figure 2). An increase in time to LP from 8 to 10 minutes would be associated with a decrease in AR from 2.59 to 2.34.

The median time to DT from the onset of the dark phase was 13 minutes with an interquartile range of 12 to 13 minutes (Table 3). Time to DT was not dependent on age, gender or pupil size (Table 2).

FO: Phase—FO phase was converted to the time to reach the light trough from onset of the light phase (see Methods: Equation 1). A Gamma distribution fit to the time to reach the light trough of FO produced a mean of 40 s with 5th and 95th percentiles of 26 and 53 s, respectively (Table 3, Supplemental Figure 1d). The time to reach the FO light trough was positively correlated with age ($R^2=0.048$; $P<0.01$) and increased at a rate of 1.1 s/decade ($y = -0.1118x + 35.77$). The practical implication of this increase is that time to FO light trough would increase ~5.5 s from 10 to 60 years of age.

Effect of Gender on EOG Parameters

Figure 4 highlights that women had significantly larger SO and FO absolute amplitudes compared with men when corrected for age (also see Table 2). The difference in amplitude was 28% and 15% for slow oscillation DT and LP amplitudes, respectively, and 21% for FO amplitude (Table 3). The amplitude of the slow oscillation LP was correlated with both DT amplitude ($R^2=0.62$; $P<0.0001$) and FO amplitude ($R^2=0.17$; $P<0.0001$).

Effect of Pupil Size on EOG Parameters

FO: Peak-to-Trough Ratio—Figure 2b shows the fit of the Gamma distribution to the PT ratio data for all subjects. Mean PT ratio was 1.18, almost identical to the median value of 1.17. The PT ratio was negatively correlated with pupil size (Table 2; $R^2=0.04$; $P<0.05$; $y = -0.0393x + 1.506$). An increase in pupil size from 7 to 9 mm would be associated with a decrease in PT ratio from 1.23 to 1.15. The 5th and 95th percentiles were demarcated by PT ratios of 1.06 and 1.32, respectively (Figure 2b, Table 3).

Absolute amplitude of the SO LP and time to light trough of the FO (FO phase) were also positively correlated with pupil size (Table 2). Although statistically significant, the correlation coefficients for these variables with respect to pupil size were small (For LP amplitude, $R^2=0.02$, $P<0.05$, $y = -119.5x + 241.6$; For time to FO light trough, $R^2=0.02$, $P<0.05$, $y = 5.33x + 231.9$). The small effect of pupil size likely reflects the narrow range of pupil sizes (7 to 9 mm) observed in our dilated patients.

SO-FO Correlations

The Arden ratio was not correlated with any of the FO parameters (Supplemental Table 1). FO PT ratio was statistically significantly negatively correlated with LP and DT amplitude of the SO ($P<0.05$), but the slopes of the linear regressions were very small (-6.17×10^{-5} and -1.131×10^{-4} , respectively).

Discussion

The major strength of this study is the recording of the EOG to ISCEV standards in 121 subjects with equal representation of male and female subjects across a wide age range. Subjects received SO and FO testing during the same session, allowing us to correlate normal findings between these two tests. The use of GEEs enabled data recorded from both eyes to be included in the final analysis.

Tables 5 and 4 detail previous studies that have established normal ranges from at least 50 eyes for the slow and fast oscillations of the EOG, respectively. The majority of these studies were completed prior to establishment of the ISCEV standards for EOG recording and differ from our study in the recording conditions used, including stimulus size, stimulus intensity and the use of undilated pupils. To our knowledge, the only previous study to record slow and fast EOG oscillations in the same subjects was that of De Rouck and Kayembe (1981) [14].

The mean Arden ratio of 2.5 in our population is consistent with average values ranging from 2.2 to 2.7 reported in five other large studies, despite wide variation in recording

conditions and differences in the age and gender distributions of the study populations (Table 5) [8-14]. In the current study, Arden ratio declined with age and was independent of gender, which contrasts with the decline in age only seen in women reported by Adams (1973) and Krogh (1975) [11,13]. While recording conditions were different between our study and those of Adams and Krogh, it is not clear that such methodological differences between studies would account for the differences in the effects of gender on the age-related decline in Arden ratio. Regardless, the results from three large studies with subjects stratified by age and gender highlight that the Arden ratio declines with age. The direct implication from the current study is that the lower limit of normal for the Arden ratio may be defined for each decade as outlined in Table 1.

Time to LP is an important parameter that may be delayed in patients with retinal degeneration and AMD [20]. In contrast, patients with cystic fibrosis exhibit a normal Arden ratio, but have a *shorter* time to LP compared to normal subjects [5]. Based on the data from the present study, the range of normal for the time to LP lies between 6 and 10 minutes for subjects <25 years of age. This range shifts higher by approximately 2 minutes to 8 to 11 minutes for subjects >55 years of age, which is consistent with Krogh's findings that the time to LP of the normal EOG increases with age [12]. Time to LP was negatively correlated with Arden ratio in our population, confirming the link between these two parameters first noted by Arden (1962) [8].

Current ISCEV EOG guidelines recommend reporting of the DT amplitude. Our mean DT amplitude of 469 μ V is consistent with previously reported DT values ranging from 416 to 553 μ V (Table 5). Krogh reported somewhat lower absolute DT values, which may be attributed to the use of lead electrodes in that study [12]. Absolute values of both SO and FO amplitudes have been observed to vary enormously in all studies describing the normal range of the EOG (Tables 5 and 4). In our study, DT amplitude varied over a 6-fold range from 211 to 1314 μ V. FO amplitude showed even greater variation ranging from 15 to 423 μ V. Variations in electrode positioning, facial structure, and subcutaneous fat content, which are associated with changes in electrical impedance, may all contribute to high variability in EOG amplitudes [8]. Based on the present study and that of De Rouck and Kayembe [14], a DT amplitude of 200 μ V for the SO could be considered the lower limit of normal. DT values below 200 μ V may indicate retinal dysfunction or poor electrical contact between electrodes and skin, which may in turn produce an unreliable Arden ratio.

The larger absolute EOG amplitudes in women compared with men reported in the current study are consistent with previous reports (Tables 5 and 4). The larger amplitudes in women are unlikely to be explained by hormones given the wide age ranges (pre-pubertal to postmenopausal) tested in each of these EOG studies. Indexes of subcutaneous fat, such as triceps and subscapular skin fold measurements are higher in females than males across all age groups [21]. Higher average subcutaneous fat content associated with higher electrical impedance in women compared with men may be a contributing factor to the higher mean absolute EOG voltage values in women observed in EOG studies, as could variation in facial features and subsequent electrode placement.

For the FO, our average PT ratio of 1.2 is consistent with previous reports ranging from 1.13 to 1.54 (Table 4) [14,22,23]. Commonly-used normal limits of 1.05 and 1.30 are appropriate, as they correspond in this study to the 4th and 92nd percentiles, respectively [4]. The implication of a lower limit for the PT ratio of 1.05 would mean that almost any FO that was not completely flat would lie within the normal range. The FO is larger and slower in patients heterozygous for mutations in the cystic fibrosis gene (CFTR) and the upper limits for the FO provide a basis for investigating FO changes in diseases such as cystic fibrosis. The current study used alternating 75-s periods of dark and light adaptation to elicit the FO, whereas only 60 s is recommended in the most recent ISCEV EOG standard [4]. Because the FO produces a light trough within 30-40 s of light onset, little variation would be expected between 75- and 60-s dark/light cycles. The four large FO studies listed in Table 4 used between 6 and 10 alternating dark and light periods to derive FO parameters. Varying the numbers of dark/light cycles between four and six does not alter FO parameter values [23], indicating that the current recommendation of four cycles is sufficient to describe the FO.

The age-related changes observed for the SO (Arden ratio and time to LP) suggest that the mechanisms involved in generation of the light peak of SO are vulnerable to the effects of aging. The mechanisms underlying the light rise remain to be elucidated but generation of the light rise requires input from the photoreceptors that ultimately results in RPE basement membrane depolarization [1]. The decrease in rod (0.2-0.4%/year) and RPE (0.3%/year) densities with age may contribute to age related changes in the Arden ratio and time to LP [24-28]. Although mechanisms involved in generation of the SO light rise remain unknown, one essential step in this pathway is known to involve an increase in RPE intracellular calcium which activates the calcium-activated chloride channel (CaCC) on the RPE basal membrane [1].

In contrast to the Arden ratio of the SO, no study has observed an effect of age on the PT ratio of the FO (Table 4). In the present study, we observed an age-related increase in the time to light trough. The lack of an effect of age on the PT ratio may provide insight into the RPE mechanisms selectively affected in generating the SO LP. Absent from FO generation is modulation of RPE intracellular calcium concentration, which raises the possibility that mechanisms regulating RPE intracellular calcium concentration involved in generation of the SO light rise may be altered by the aging process. Calcium dysregulation through reduced ER calcium buffering is a well-established feature of aging cells in-vitro [29]. Such disturbances in intracellular calcium with aging of the RPE might also alter the light rise in-vivo.

The SO data for this study was extracted from the raw recordings and future work might apply curve-fitting to determine the physiologic peaks and troughs as recommended by the current ISCEV standards [4]. Given the large number of subjects in this study, however, the physiologic data is likely still captured in our results. By presenting the raw data, our results are generalizable to clinicians using similar software seeking to apply EOG results directly into clinical practice.

This large study of 121 subjects with ages spanning the 1st through 8th decades of life shows changes in the Arden ratio and time to LP associated with age that give insight into the mechanism of the SO. We propose the time to LP as a diagnostic parameter different from the Arden ratio and provide a range of normal values for each age group. We also provide a set of reference values for measured parameters in SO and FO of normal subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix 1

This appendix outlines the algorithm used by the UTAS E-2000 software (as detailed in the Manufacturer's Operation Manual) for calculating average saccade amplitude during recording of the SO. For each 15 sec interval: 1) A 5-point median filter was applied to the waveform to eliminate spike artefacts. With the median filter, a group of 5 points centered on the point in question were sorted and the value of the point in question replaced by the middle value in the group; 2) the peak to trough amplitude was found for each saccade using the following algorithm. A local maximum (minimum) was defined as a point that was higher (lower) than its 24 nearest neighbors (12 on either side) and that exceeds a predefined threshold. Saccade amplitude was determined from the difference between the maximum and minimum amplitudes; 3) Saccade amplitudes for a 15 s sweep was sorted and the largest and smallest amplitudes discarded; 4) the average saccade for each 15 s sweep was calculated from the remaining values.

Appendix 2

This appendix details the analysis performed by the UTAS E-2000 software (as detailed in the Manufacturer's Operation Manual) for deriving FO parameters from the fit of a sine wave to the absolute amplitude data recorded during the FO protocol.

The amplitude of the sine and cosine components of the FO were determined using a discrete Fourier transform:

$$A_{\sin} = \frac{2}{N} \sum_{n=0}^{N-1} X_n \sin(2\pi fT) \quad A_{\cos} = \frac{2}{N} \sum_{n=0}^{N-1} X_n \cos(2\pi fT) \quad \text{Equation (A1)}$$

Where N = total number of samples, T is the time for a full saccade, X_N is the amplitude of the FO at time nT and f is the frequency of the FO. For our FO recording conditions, N = 450 points, T = 2 s for a full saccade and f=0.00666Hz

Peak to trough amplitude (μV), of the sine wave was estimated from:

$$A_{p-t} = 2 \sqrt{(A_{\cos}^2 + A_{\sin}^2)} \quad \text{Equation (A2)}$$

The mean amplitude of the standing FO was estimated as

$$\bar{X} = \frac{1}{N} \sum_{n=0}^{N-1} X_n \quad \text{Equation (A3)}$$

The results from Equations 2 and 3 are used to calculate the peak-to-trough ratio:

$$R_{p-t} = \frac{\bar{X} + \frac{A_{p-t}}{2}}{\bar{X} - \frac{A_{p-t}}{2}} \quad \text{Equation (A4)}$$

The phase of the sine wave (θ , deg) measured relative to the time the light turns on in the light phase) is estimated from:

$$\theta = \frac{180}{\pi} \tan^{-1} \left(\frac{A_{\sin}}{A_{\cos}} \right) \quad \text{Equation (A5)}$$

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Fig. 1.

Fit of the Gamma distributions (red curves) to the Arden ratio (a) and fast oscillation peak-to-trough (PT) ratio (b) data. Vertical dashed lines indicate the 5th and 95th percentile lines calculated from the fits. Frequency was determined from bins of 0.2 and 0.02 for Arden and PT ratios respectively. The parameters derived from the fit of the Gamma distribution to the Arden ratio data were α (shape) = 22.958 and β (rate) = 9.128 with resulting 5th percentile at 1.72 and 95th percentile at 3.44. Similarly, the fit of the Gamma distribution to fast oscillation PT ratio showed α (shape) = 220.0 and β (rate) = 185.7 with the 5th percentile at 1.06 and 95th percentile at 1.32

Fig. 2.

Arden ratio plotted as a function of age. Scatterplot shows a negative correlation between Arden ratio and age following the linear regression $y = -0.013x + 3$. Dotted lines indicate quantile regressions of the 5th and 95th percentile lines following $y = -0.00597x + 2.05$ and $y = -0.0314x + 4.66$, respectively. Vertical lines connect eyes within the same subject

Fig. 3.

Boxplot of the time to reach the light peak of the slow oscillation by age group. The time to reach the SO light peak increases with age. Thick horizontal lines represent median values, horizontal lines bordering each box represent first and third quartiles, and ends of whiskers represent minimum and maximum values excluding outliers. Dots within boxes represent means and dots beyond whiskers represent outliers

Fig. 4.

Effect of gender on SO and FO absolute amplitudes. Dark trough (DT) and light peak (LP) absolute amplitudes of the slow oscillation were significantly larger in women ($P < 0.05$ for both). Similarly, FO absolute amplitudes were significantly larger in women compared to men ($P < 0.01$)

Table 1**Number of Volunteers and Limits of Arden Ratio by Age**

Age (years)	Male – No. (%) (n=62)	Female – No. (%) (n=59)	All – No. (%) (N=121)	Arden ratio 5 th percentile	Arden ratio 95 th percentile
<15	8 (12.9)	10 (16.9)	18 (14.9)	1.99	4.35
15-24	11 (17.8)	8 (13.6)	19 (15.7)	1.93	4.03
25-34	10 (16.1)	14 (23.7)	24 (19.8)	1.87	3.72
35-44	10 (16.1)	9 (15.3)	19 (15.7)	1.81	3.40
45-54	12 (19.4)	11 (18.6)	23 (19.0)	1.75	3.09
55-64	9 (14.5)	6 (10.2)	15 (12.4)	1.69	2.78
65-75	2 (3.2)	1 (1.7)	3 (2.5)	1.63	2.46
Mean Age (years)	37.7 ± 15.9	34.6 ± 15.9	36.2 ± 16.3		
Mean Pupil Diameter (mm)	8.3 ± 0.5	8.1 ± 0.5	8.2 ± 0.5		

Table 2
Multiple Regression Analyses for Age, Gender, and Pupil size on EOG Parameters^a

Parameter	<i>P</i> Value of Age	<i>P</i> Value of Gender	<i>P</i> Value of Pupil size
Slow Oscillation			
Arden Ratio ^b	<0.0001***	0.39	0.16
Time to DT (minutes)	0.71	0.41	0.96
Time to LP (minutes)	<0.0001***	0.47	0.15
DT Amplitude (μV)	0.13	<0.05*	0.35
LP Amplitude (μV)	0.21	<0.05*	<0.05*
Fast Oscillation			
Peak-to-Trough Ratio	0.06	0.74	<0.05*
Amplitude (μV)	0.19	<0.01**	0.23
Phase (degrees)	<0.01*	0.61	<0.05*

^a Abbreviations: DT = Dark Trough; LP = Light Peak; M = Male; F = Female

^b Arden ratio is defined as Light Peak Amplitude / Dark Trough Amplitude

* indicates *P* Value < 0.05;

** indicates *P* Value < 0.01;

*** indicates *P* Value < 0.0001

Multiple regression analyses of the effects of age, gender and pupil size on each parameter with *P* Values reflecting the effect of each independent variable (age, gender, or pupil size) on the specified parameter accounting for the other two independent variables.

Table 3

Descriptive Statistics of Measured EOG Parameters^a

Parameter	Patients	Median	Interquartile Range	5th Percentile ^{d-g}	95th Percentile ^{d-g}
Slow Oscillation					
Arden Ratio	All	2.41	2.13-2.78	1.72 ^b	3.44 ^b
Time to DT (minutes)	All	13	12-13	NA ^c	NA ^c
Time to LP (minutes)	All	9	8-9	NA ^c	NA ^c
DT Amplitude (µV)	All	469	388-586	288 ^d	787 ^e
	M	419	360-538	263	752
	F	540	424-622	325	803
LP Amplitude (µV)	All	1160	958-1440	735 ^d	1867 ^d
	M	1090	921-1320	703	1742
	F	1250	1050-1520	784	1974
Fast Oscillation					
Peak-to-Trough Ratio	All	1.17	1.13-1.23	1.06 ^b	1.32 ^b
Amplitude (µV)	All	134	103-179	63 ^d	281 ^d
	M	122	97-165	57	260
	F	148	112-200	71	298
Phase (degrees)	All	275	265-285	244 ^b	309 ^b
(seconds) ^e		39.6	35.4-43.8	26.3 ^b	53.3 ^b

^a Abbreviations: DT = Dark Trough; LP = Light Peak; M = Male; F = Female; NA = not applicable

^b Percentile estimations calculated by fitting gamma

^c Times could not be fit by a probability density function.

^d log-normal distributions to continuous variables. See supplemental figure 1 for more details.

Converted to light trough time at seconds after onset of light phase using Equation 1 (see Methods)

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Table 4

Summary of Slow Oscillation Studies^a

Study	Subject Characteristics	Stimulus and Recording Conditions			Stimulus Order and Duration (min)	SO Characteristics (mean ± SD [range])			Effects of Age and Gender on SO	
	# Eyes	Intensity (cd/m ²)	Size/Generator	Bandpass Filter (Hz)		Arden Ratio	Time to Light Peak (min)	Dark trough amplitude (µV)	Age	Gender
	Age range (years)									
	% Men									
Arden (1962)	52	425			1. Room 2. 12 3. 10	2.5 ± 0.4 [1.9-3.8]			AR: ↓	NR
	NR	Box				8.6 ± 1				
	NR	0.5-50				553 ± 376				
Geijer-Mannerfelt (1966)	80	100			1. Room 2. 12 3. 15	2.65 ± 0.4 [1.6-3.9]			NR	NR
	12-59	Perimeter				NR				
	28	NR				NR				
Reeser (1970)	100	861.1			1. Room 2. 15 3. 15	2.48 [1.75-3.6]			None	NR
	20-62	Panel				“usually 6-8”				
	20	DC-NR				NR				
Adams (1973)	240	535			1. NR 2. 12 3. 12	2.2 ± 0.4			AR: ↓ F	LP amp: F>M
	10-69	45×56 °				NR				
	50	NR				NR				
Krogh (1975-1977)	142	2500-4000			1. 10 at 2500-4000 cd/m ² 2. Until DT seen 3. Until LP seen	2.41 [1.5-4.5]			AR: ↓F LP time: ↑ Pupil size: ↓	DT amp: F>M
	13-81	Box				8[5-12]				
	50	DC-15				238 [71-460]				
De Rouck & Kayembe (1981)	132	5000			1. Room for 20 → Fast oscillation testing for 20 2. 12 3. 10	2.0 ± 0.25 [1.5-3.2]			None	DT amp: F>M LP amp: F>M
	12-67	NR				NR				
	62	0.03-30				416 [196-857]				
Current Study	242	100			1. 5 at 20 cd/m ² 2. 15 3. 20	2.5 [1.6-5.3]			AR: ↓ LP time: ↑	DT amp: F>M LP amp: F>M
	7-72	Ganzfeld				9 [6-13]				
	51	0.05-100				469 [211-1314]				

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Abbreviations: SO=slow oscillation; SD=standard deviation; NR=not reported; AR=Arden ratio; LP=light peak; DT=dark trough; F=female; M=male; amp=amplitude; cd/m² = candela per square meter; Hz = hertz

Table 5

Summary of Fast Oscillation Studies

Study	Subject Characteristics	Stimulus and Recording Conditions	Cycles	FO Characteristics (mean $\pm$ SD [range])			Effects of Age and Gender on FO	
				Time per cycle (sec)	PT Ratio	FO Amplitude (μ V)	Age	Gender
De Rouck & Kayembe (1981)	#Subjects (# Eyes)	Intensity (cd/m ²)						
	Age range (years)	Size/Generator	# Cycles					
	% Men	Bandpass Filter (Hz)						
	75 (132)	5000	60			1.32 $\pm$ 0.06 [1.07-1.38]		
	12-67	NR	10			85 $\pm$ 43 [21-255]	None	None
	62	0.03-30				45		
Nakao (1995)	60 (120)	1270	60			1.54 $\pm$ 0.32 [1.06-2.43]		
	19-67	Ganzfeld	10			190 $\pm$ 104 [47-647]	None	PT Ratio: F>M FO amp: F>M
	42	0.05-20				45		
Mergaerts (2001)	51 (51)	250	60			1.13 $\pm$ 0.09		
	14-62	Ganzfeld	6			70 $\pm$ 38	None	None
	35	NR				NR		
Current Study	121 (242)	100	75			1.18 [1.01-1.51]		
	7-72	Ganzfeld	6			134 [14.6-423]	Time: $\uparrow$	FO amp: F>M
	51	0.05-30				40 [9-75]		

Abbreviations: FO=fast oscillation; SD=standard deviation; NR=not reported; F=female; M=male; amp=amplitude; cd/m² = candela per square meter; Hz = hertz