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ORIGINAL RESEARCH ARTICLE

Age-related change in fast adaptation mechanisms measured with the scotopic full-field ERG

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Abstract
Purpose To quantify the response dynamics of fast adaptation mechanisms of the scotopic ERG in younger and older adults using full-field m-sequence flash stimulation.
Methods Scotopic ERGs were measured for a series of flashes separated by 65 ms over a range of 260 ms in 16 younger (20–26, 22.2 ± 2.1; range mean ±1 SD) and 16 older (65–85, 71.2 ± 7) observers without retinal pathology. A short-wavelength ($\lambda_{peak} = 442$ nm) LED was used for scotopic stimulation, and the flashes ranged from 0.0001 to 0.01 cd s m^{-2}. The complete binary kernel series was derived from the responses to the m-sequence flash stimulation, and the first- and second-order kernel responses were analyzed. The first-order kernel represented the response to a single, isolated flash, while the second-order kernels reflected the adapted flash responses that followed a single flash by one or more base intervals. B-wave amplitudes of the adapted flash responses were measured and plotted as a function of interstimulus interval to describe the recovery of the scotopic ERG. A linear function was fitted to the linear portion of the recovery curve, and the slope of the line was used to estimate the rate of fast adaptation recovery.
Results The amplitudes of the isolated flash responses and rates of scotopic fast adaptation recovery were compared between the younger and older participants using a two-way ANOVA. The isolated flash responses and rates of recovery were found to be significantly lower in the older adults. However, there was no difference between the two age groups in response amplitude or recovery rate after correcting for age-related changes in the density of the ocular media.
Conclusions These results demonstrated that the rate of scotopic fast adaptation recovery of normal younger and older adults is similar when stimuli are equated for retinal illuminance.

Keywords Aging · Electroretinogram · Scotopic adaptation · Ocular media

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Introduction

A common visual deficit among the elderly is the decreased ability to see under dim illumination and at night, especially following brief exposure to bright light [1]. Poor vision at low light levels in older adults has been associated with a higher risk of falls that

result in injury [2] and motor vehicle collisions [3]. While loss in rod photoreceptors might be thought to contribute to the age-related decline in light sensitivity in the dark, the relationship between photoreceptor number and sensitivity is not straightforward above the absolute threshold. The human retina of a healthy young adult contains about 120 million rods. However, approximately 37 % of rods are lost between the ages of 18–85 years [4], with an average loss of 570 rods/mm^2 per year [5]. There is also a non-uniform decline in rod density across the senescent retina as the total number of rods within 4 mm ($\sim$14°) of the fovea decreases by about 30 % between the fourth and ninth decades of life, while the number of rods 13 mm ($\sim$45°) from the fovea remains relatively stable throughout adulthood [6]. To investigate the functional consequences of the variable rod loss, Jackson et al. [7] measured the scotopic (i.e., dark-adapted) sensitivity at several retinal eccentricities from 4° to 38° of visual angle using psychophysical methods in normal younger and older adults. Overall, older adults had significantly diminished scotopic sensitivity compared to younger adults, but the age-related sensitivity losses were similar across retinal eccentricities and were not exacerbated at locations with heightened rod loss (i.e., 4° and 7°). Thus, rod photoreceptor death cannot completely explain the diminished scotopic sensitivity associated with advanced age.

A potential contributor to the decreased sensitivity under low illumination in older adults is an impaired ability to adapt to fluctuations in luminance due to changes in the rate of phototransduction inactivation. Rod phototransduction begins when rhodopsin is activated by light. Rhodopsin activation leads to the hydrolysis of cyclic guanosine monophosphate (cGMP), and the decrease in cGMP causes the cyclic nucleotide-gated cation channels to close and the cell membrane to hyperpolarize. While rhodopsin is active and the cation channels are closed, the rod is less sensitive and further light activations produce attenuated or adapted responses. In order to regain maximal sensitivity and reopen the cation channels, the phototransduction proteins need to be inactivated. The time between phototransduction activation and inactivation can be quantified in vivo using a double-flash electroretinogram (ERG) protocol [8]. In the full-field ERG signal the initial negative deflection, or a-wave, reflects a potential change in the photoreceptors due to a cessation of the rod circulating current following a flash of light [9]. The dark-adapted eye produces a sizeable ERG a-wave in response to a bright flash, but the amplitude of the a-wave in response to a second flash that immediately follows is notably smaller. If sufficient time is allotted between two flashes, however, the amplitude of the a-wave will be equal for both flash responses, indicating that the circulating current has been restored. In the double-flash ERG technique, the time needed to recover the dark-adapted ERG a-wave after a saturating flash is determined by varying the time interval between the first and second flash and creating an a-wave recovery curve [8]. The double-flash ERG has been used to probe the senescent human retina and investigate the age-related changes in the phototransduction inactivation kinetics. On average, adults 60 years of age and older with healthy eyes required 6 s longer to reach their half-maximal response during recovery compared to adults between the ages of 16 and 30 years [10]. This result suggested that fast adaptation mechanisms within the rod photoreceptors exhibit age-related delays that could contribute to impaired night vision in older adults.

While effective, the double-flash ERG technique can be time-consuming, for the technique requires the experimenter to collect responses to multiple, individual paired-flash sequences with a 2-min recovery period between each measurement [8, 10]. The full-field ERG with m-sequence flash stimulation, however, can be used to record responses to various flash sequences simultaneously and within a few minutes for a given flash strength [11]. The m-sequence is a pseudorandom binary sequence that allows for the analysis of systems with a large number of inputs. From the responses to m-sequence flash stimulation, the complete binary kernel series can be derived. A detailed account of how to derive the binary kernel series has been described elsewhere [12–15]. The first-order kernel response represents a response to a single flash without contributions from any preceding or succeeding flashes (i.e., isolated flash response). Second-order kernel responses represent the effect of adaptation on a single-flash response that follows the adapting flash by one or more base intervals (i.e., the minimal amount of time between two flashes). A related study using this approach, conducted by Gerth et al. [16], examined the effect of age on the second-order responses in the photopic multifocal ERG

(mfERG). It was found that the response density amplitude of the mfERG, which was measured from the first negative peak (N1) to the first positive peak (P1), showed a significant age-related decline for both the first-order response and the second-order response that followed a single flash by one base interval (13.3 ms). This suggested that the light-adapted fast adaptation mechanisms of the outer retina display senescent impairments. However, it remains to be determined whether the second-order responses measured at longer time intervals also undergo age-related reductions. Furthermore, it is unknown whether the second-order responses elicited by m-sequence flash stimulation exhibit age-related decreases under dark-adapted conditions.

In this study, the first- and second-order kernels derived from the scotopic full-field ERG responses to m-sequence flash stimulation were compared between normal younger and older adults. The amplitude of the first-order responses was predicted to be smaller for the older adults compared to the younger adults. The second-order responses at different time intervals were used to construct recovery curves of the scotopic full-field ERG and to estimate the rate at which the rod-mediated retina recovers from a brief flash of light. The recovery rate was predicted to be slower for the older adults compared to the younger adults. Furthermore, to determine whether optical or neural factors underlie the age-related change in the recovery rate of the scotopic ERG, the optical density of the ocular media for each subject was calculated and used to adjust the responses so that retinal illuminance was constant across subjects.

Materials and methods

Participants

Full-field scotopic ERGs were recorded from 16 younger adults between 20 and 26 years of age (22.2 ± 2.1 years; mean ± 1 SD) and 16 older adults between 65 and 85 years of age (71.2 ± 7 years). Nine younger adult females and eight older adult females participated in the experiment. All participants underwent a comprehensive ophthalmological examination, including an evaluation of their fundus photographs by a retinal specialist. Individuals with ocular disease or systemic diseases known to affect vision (e.g., diabetes) were excluded from the study. Participants had a best-corrected visual acuity of 20/25 or better and a refractive error between $+3.00$ and -5.00 diopters. All subjects had normal color vision as determined by the Cambridge Colour Test (Cambridge Research Systems Ltd, UK). Eyes with intraocular lens implants were not used for testing. All experimental procedures were explained to the participants prior to testing and have been approved by the Office of Human Research Protection of the University of California, Davis, School of Medicine. Written informed consent was obtained in compliance with the tenets of the Declaration of Helsinki.

Procedure

Only one eye per subject was tested, which was the eye with the best visual acuity. The participant's eye was dilated and cyclopleged with 1–2 drops of tropicamide (1 %) and phenylephrine hydrochloride (2.5 %). A standard ground electrode was placed on the center of the forehead, and a reference gold cup electrode was placed near the temporal canthus of the tested eye. An active Dawson–Trick–Litzkow electrode (DTL, Diagnosys LLC, MA, USA) was placed on the lower conjunctival sac of the eye. The impedance of the electrodes was measured and maintained below 5 kΩ. Following dilation and electrode placement, the participant was dark-adapted for 40 min. Electrode position was checked before and after the recording of the dark-adapted ERGs.

Scotopic full-field ERGs were recorded using the Electro-Diagnostic Imaging System (EDI, CA, USA). The stimuli were displayed on a LED-based Ganzfeld stimulator and delivered using the visual response imaging system software (VERIS Pro, ver. 6.3.2; EDI). Before testing commenced, participants were instructed to fixate on a red LED at the back of the Ganzfeld and to avoid blinking during the flash stimulation. Participants' fixation was monitored with a built-in infrared camera. Subjects were presented with a series of flashes controlled by an m-sequence. The time-averaged flash luminance ranged from 0.0001 to 0.01 photopic cd s m^{-2}. Each flash luminance level was tested independently and in ascending order. The blue LED ($\lambda_{peak} = 442$ nm) was used for scotopic stimulation in order to minimize the contribution of cones. The base interval was 65 ms, and the m-sequence exponent was 11, which resulted in about

4 min of recording per flash luminance step. Each recording segment lasted approximately 30 s, and breaks between segments were included to avoid the effects of long-term adaptation to the stimulus and fatigue. If the participant blinked during a single recording segment, the record was immediately discarded and re-recorded. Signals were sampled at 3000 Hz with a gain of 10 K. The low and high frequency filtering cutoffs were 3 and 300 Hz, respectively.

The current ERG protocol differed from the ISCEV Standard ERG series [17] in two ways. First, several of the flash luminances used for testing were lower than the ISCEV dark-adapted 0.01 cd s m^{-2} ERG. This was to allow for the assessment of fast adaptation mechanisms over a range of flash intensities while keeping the flashes dim enough to only elicit rod-mediated responses. The second difference from the ISCEV standards was that instead of using a single flash, the current protocol used pseudorandom flicker controlled by an m-sequence. The purpose of using the pseudorandom flicker was to be able to rapidly record the responses to different flash combinations [14]. However, ISCEV recommendations for electrode placement, impedance levels, and pupillary dilation were followed accordingly.

Response analysis

Full-field isolated flash responses and subsequent flash interactions were analyzed using the VERIS Pro software. The responses to m-sequence flash stimulation were derived for each subject from the binary kernel series, as described by Sutter [12]. Response synthesis was completed for the first- and second-order kernels [14]. Higher-order kernels were not analyzed because their signal was not consistently visible above the noise. The first-order kernel response represented the isolated flash response and was synthesized by subtracting out the appropriate combinations of higher-order kernels shifted by integral multiples of the base interval. The isolated flash response is analogous to a single-flash response under similar adaptation conditions. The second-order kernel responses represented the subsequent flash responses, or the adapted flash responses, and were synthesized by subtracting the initial, adapting flash response (i.e., isolated flash response) from a double-flash response in which the two flashes were separated by one or more base intervals. An epoch length of 500 ms was chosen such that all of the induced components were included. No artifact rejection was applied prior to response synthesis.

The amplitude of the isolated and subsequent flash responses was analyzed and measured from the DC baseline to the peak of the corneal positive b-wave. The amplitude of the a-wave was not measured because the flash luminance settings were not high enough to generate an appreciable a-wave. Subsequent flash response amplitudes were measured at 65, 130, 195, and 260 ms following the initial, adapting flash. To assess the recovery of the scotopic ERG, the subsequent flash responses were plotted as a function of interstimulus interval and a linear function was fitted to the linear portion of the recovery curve. The slope of the line was used to estimate the rate of recovery of the scotopic response for the younger and older observers.

Statistical analysis

Using the open-source software R [18], a two-way analysis of variance (ANOVA) was computed to compare the scotopic isolated flash response amplitudes and recovery rates between the healthy younger and older subjects, with age and flash luminance as the independent variables.

Optical density of the ocular media

The optical density (OD) of the ocular media, primarily the crystalline lens, increases with age and reduces retinal illuminance in older adults [19]. The greatest difference in OD between younger and older adults occurs at short wavelengths. Using the bilinear model proposed by Pokorny et al. [20], the OD of the ocular media was estimated for each participant using their age. Figure 1a illustrates the estimated OD from 400 to 650 nm for a 20-year-old and 85-year-old standard observer.

The spectral radiance of the short-wavelength LED stimulus was measured using a spectroradiometer (PhotoResearch, PR-670 SpectraScan, CA, USA) from 380 to 780 nm in 2-nm step increments. The spectral power distribution measurement is displayed in Fig. 1b. The spectral power distribution was adjusted for each participant from his or her estimated OD values. Figure 1c shows the OD-adjusted spectral

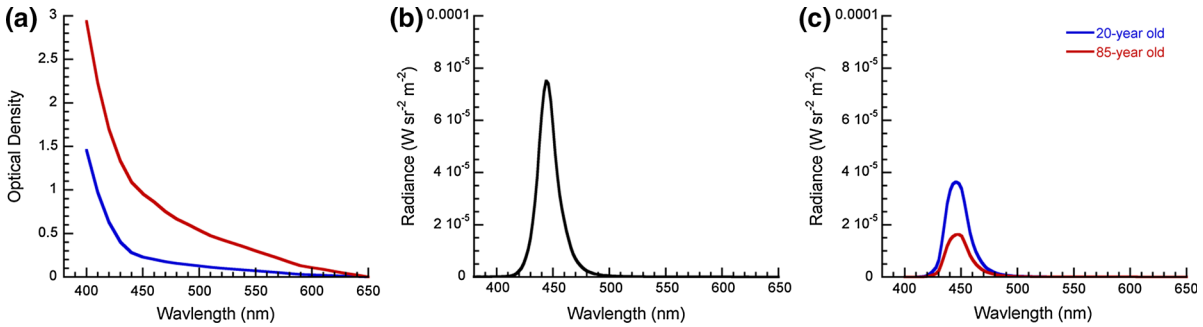

Fig. 1 Optical density (OD) estimates of the aging ocular media. **a** OD as a function of wavelength for a typical 20-year-old and 85-year-old based on the bilinear model of Pokorny et al. [20]. **b** Spectral distribution of the blue LED stimulus source measured in situ. **c** OD-adjusted spectral distribution of the stimulus source for the 20-year-old and 85-year-old calculated by multiplying the OD values in plot (**a**), by the radiance values in plot (**b**)

power distributions for the standard 20- and 85-year-old observers. The integral of the OD-adjusted spectral power distribution for each participant's age was used to estimate the amount of light absorbed from the stimulus and shift the responses to the appropriate flash luminance at the retina.

Results

Isolated flash responses and recovery of the scotopic ERG

Synthesized scotopic ERG responses from a representative younger (23-year-old) and older (67-year-old) subject are shown in Fig. 2. The responses were generated at a flash luminance of 0.001 cd s m^{-2}. The left, uppermost ERG trace for each subject is an isolated flash response, and the trace directly below the isolated flash response is a double-flash response with one base interval separating the two flashes. The remaining ERG traces are the subsequent flash responses at different time intervals, with the initial flash response subtracted out. The double-headed arrows on each trace designate how the b-wave was measured, and scale bars are provided for reference at the lower, left-hand corner. The white boxes represent the addition or subtraction of a flash, and the black boxes mean no flashes were added or subtracted at that time point.

The b-wave amplitudes of the responses from the representative younger and older subjects are plotted in Fig. 3. Absolute amplitudes of the isolated and subsequent flash responses for the two subjects are shown in Fig. 3a, and the normalized amplitudes are shown in Fig. 3b. From these plots, it is apparent that the b-wave of the subsequent flash responses increased as the time between the adapting and test flashes increased. Also, the amplitudes of the subsequent flash response at 195 and 260 ms were equal to the amplitude of isolated flash response, demonstrating a recovery of the response under the current condition. A line was fitted to the data points at 65, 130, and 195 ms for both plots in Fig. 3 to characterize the recovery of the scotopic ERG. This linear function was used to fit the data for each individual subject at each flash luminance. On average, the linear function provided a good fit for both the younger ($R^2 = 0.96 \pm 0.08$, mean $\pm$ SD) and older ($R^2 = 0.95 \pm 0.08$) adult data. There was no significant difference in the fits between the younger and older adults [$F(1, 0.85)$, $p = 0.36$].

Figure 4 shows the average b-wave amplitude of the isolated flash response (a) and rate of recovery of the scotopic ERG (b) for the younger and older observers as a function of flash luminance. The relationship between response amplitude and luminance and between recovery rate and luminance could be described with the following exponential function:

$$y = a + b \times [1 - \exp(-cx)]$$

In the equation, a represents the smallest response that could be recorded, b represents the magnitude of the range of responses, and c represents the rate at which the response changes with flash luminance. The function was fitted to the averaged data using the curve fitting function in KaleidaGraph (ver. 4.5.0; Synergy Software, Inc, PA, USA) and provided good fits for both the younger and older subjects. At the

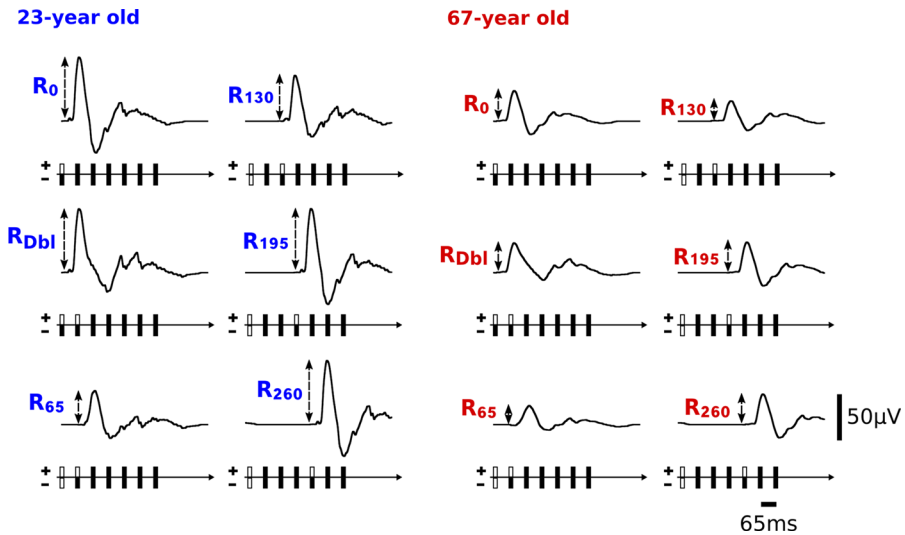

Fig. 2 Representative scotopic ERG responses derived from the m-sequence flash stimulation from a younger, 23-year-old (*left/blue traces*) and older, 67-year-old (*right/red traces*) observer. The *left* uppermost ERG trace for each participant is the isolated flash response, which does not contain any contributions from preceding or succeeding flashes. Directly below the isolated flash response is the double-flash response, which is elicited by two flashes separated by one base interval. The first subsequent flash response, which is located beneath the double-flash response, is the response to a single flash that follows an adapting flash by one base interval. The first subsequent flash response is calculated by subtracting the isolated flash response from the double-flash response. The remaining subsequent flash responses on the right for each participant are the responses to a single flash that follows an adapting flash by two, three, and four base intervals, with the response from the initial flash subtracted out. The amplitude of the scotopic ERG responses was measured from the DC baseline to the *peak* of the b-wave, as symbolized by the *double-headed arrows*. R_x denotes the time between the adapting and test flashes. The *white boxes* underneath the ERG traces denoted by the (+) represent the presence of a flash, and the *white boxes* denoted by the (−) represent the flash responses that are subtracted out. The *black boxes* mean a flash response was neither added nor subtracted. Each *box* is separated by one base interval, or 65 ms

highest flash luminances, the degree to which the isolated flash response amplitudes and rates of recovery increase in magnitude is smaller relative to the lower flash luminances, which may reflect a plateau and the start of the transition between rod-mediated and cone-mediated vision. In comparing the results from the younger and older subjects, the amplitudes of the isolated flash responses were found to be significantly smaller for the older subjects compared to the younger subjects [$F(1, 15.13)$, $p < 0.001$]. Moreover, the recovery rates of the scotopic ERG were also significantly different between the younger and older subjects, with the older subjects having slower rates of recovery [$F(1, 20.85)$, $p < 0.001$].

Effects of senescent changes in ocular media density on adaptation recovery

Although the scotopic isolated flash response amplitudes and rates of recovery of the scotopic ERG were significantly different between the younger and older observers, correction for the ocular media density difference is required to separate contributions from optical and neural mechanisms. The OD of the ocular media was calculated based on the spectral radiance of the stimulus and each subject's age using the bilinear model [20]. Figure 5a, b are plots of the isolated flash response amplitudes and rates of recovery, respectively, for each individual subject versus retinal flash luminance. The blue, upright triangles and the red, upside-down triangles denote the younger and older subjects, respectively. From the plots in Fig. 5, it is evident that both the response amplitudes and recovery rates were all shifted toward lower flash luminances, but the older participants had a larger shift due to more dense ocular media.

Isolated flash response amplitudes and rates of recovery of the scotopic ERG were fitted with the same exponential function used in Fig. 4 for each subject using data corrected for ocular media density.

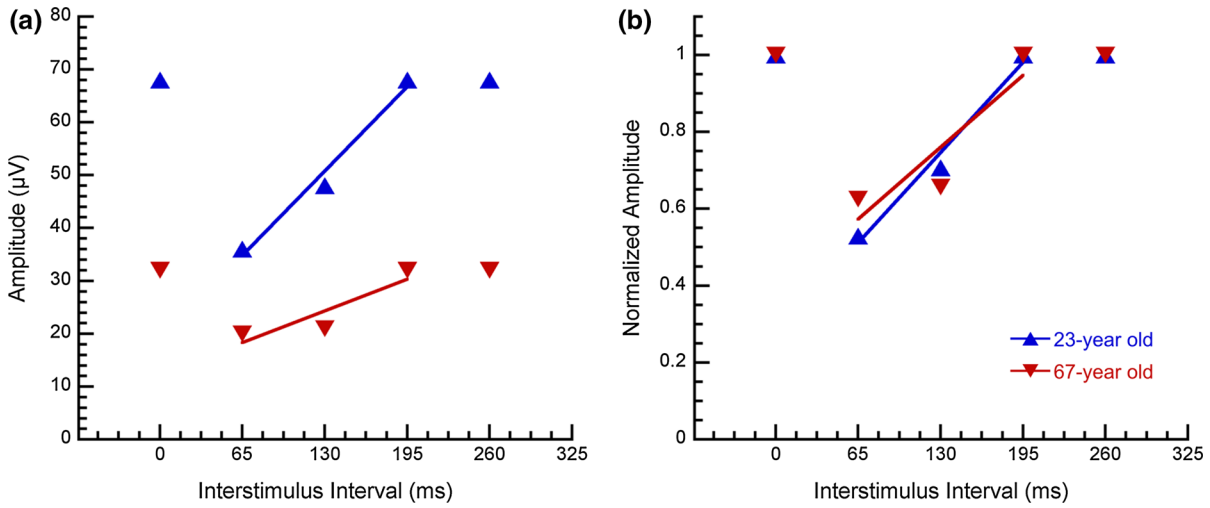

Fig. 3 Recovery of the scotopic ERG. **a** Absolute response amplitudes measured from the ERG traces shown in Fig. 1 plotted as a function of interstimulus interval for a 23-year-old and 67-year-old. **b** Relative response amplitudes that were normalized to the amplitude of the isolated flash response plotted as a function of interstimulus interval for the 23-year-old and 67-year-old. For both plots, the point at 0 ms is the isolated flash response. The remaining points are the subsequent flash responses plotted as a function of the time that occurred between the initial flash and subsequent flash. The first three subsequent flash responses at 65, 130, and 195 ms were fitted with a linear function

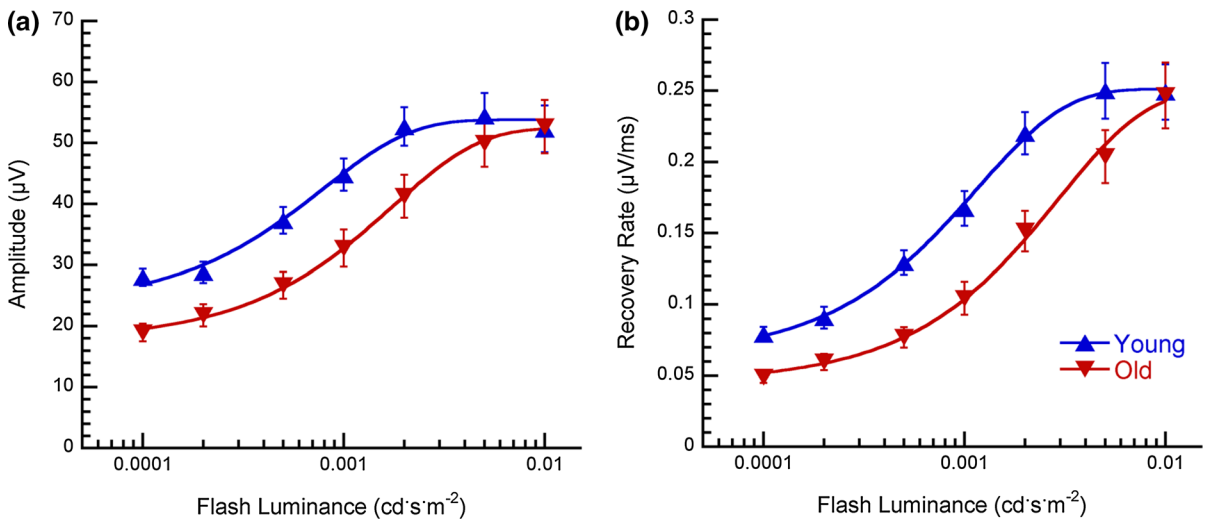

Fig. 4 Averaged isolated flash response amplitudes and rates of recovery for the younger and older observers. Response amplitude and recovery rate increase as a function of flash luminance. The younger subjects have larger isolated flash responses ($p < 0.001$) and faster rates of recovery ($p < 0.001$) compared to the older subjects. Error *bars* are ±1 standard error of the mean. The average response amplitudes and recovery rates for the younger and older participants were fitted with the exponential function $y = a + b \times [1 - \exp(-cx)]$. The R^2 values for all *curve* fits were >0.99

The exponential equations used to fit each individual's adjusted data were then used to determine what the amplitude of the isolated flash responses and rates of recovery would be if the retinal flash luminance equaled the input flash luminance settings. This allowed for a direct comparison of the results between the younger and older subjects. It is of note that the responses between 0.0001 and 0.005 cd s m^{-2} could be interpolated from the exponential function, while the responses at 0.01 cd s m^{-2} needed to be

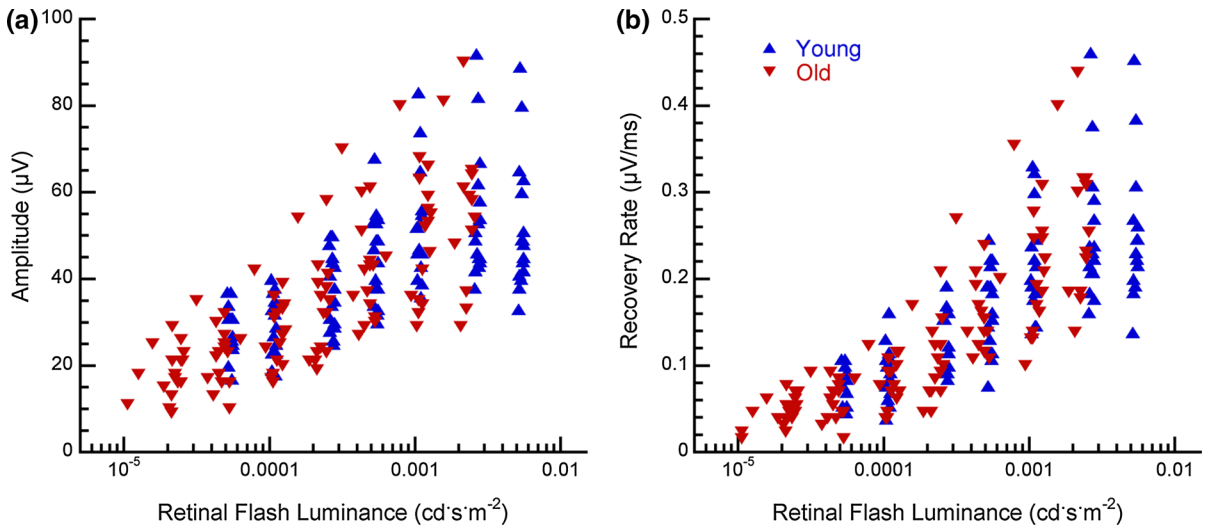

Fig. 5 OD-adjusted flash luminance. **a** Individual isolated flash response amplitudes for each subject as a function of flash luminance at the retina estimated using the OD of the ocular media. **b** Individual recovery rates for each subject as a function of flash luminance at the retina

extrapolated because that setting fell outside the range of the flash luminances at the retina (see Fig. 5). The results of these calculations are plotted in Fig. 6, which shows the average isolated flash response amplitude (a) and rate of recovery (b) as a function of retinal flash luminance for the younger and older observers. After adjusting the responses according to the age-related changes in ocular media, there was no significant difference between the two age groups for either the isolated flash responses [$F(1, 0.36)$, $p = 0.55$] or the rates of recovery [$F(1, 0.28)$, $p = 0.59$].

Discussion

The objective of this study was to quantify the rapid response dynamics of the dark-adapted retina in normal younger and older adults using the scotopic full-field ERG with m-sequence flash stimulation. Over a range of flash luminances, the isolated flash response amplitudes and rates of recovery of the scotopic ERGs were lower in the older adults compared to the younger adults. However, after adjusting the responses for age-related changes in optical density (OD) of the ocular media, the isolated flash response amplitudes and rates of recovery were approximately equal and not statistically different between the two age groups. Thus, scotopic fast adaptation recovery is similar between healthy younger and older adults when the stimuli are equated for retinal illuminance.

The current study was the first to characterize the age-related changes in the fast adaptation mechanisms of rod-mediated responses using the full-field ERG with m-sequence flash stimulation. Compared to the double-flash ERG method, the m-sequence flash stimulation can be achieved with shorter recording times and permits adaptation mechanisms to be probed on a rapid timescale (i.e., milliseconds). The analysis of the ERG responses, however, is similar between the m-sequence flash stimulation and double-flash techniques as both methods track the recovery of the signal amplitude. Jackson et al. [10] used the double-flash technique to examine the phototransduction inactivation kinetics of rods in elderly adults and found that the recovery of the a-wave in older adults was slower relative to the younger adults, even after taking into account the age-related variations in ocular media absorbance. They also found that the difference in a-wave amplitude between the younger and older adults was not significantly different until 16 s, which could account for why no differences in the fast adaptation recovery over the course of milliseconds were observed in the present study. Furthermore, Jackson et al. [10] measured the amplitude of the

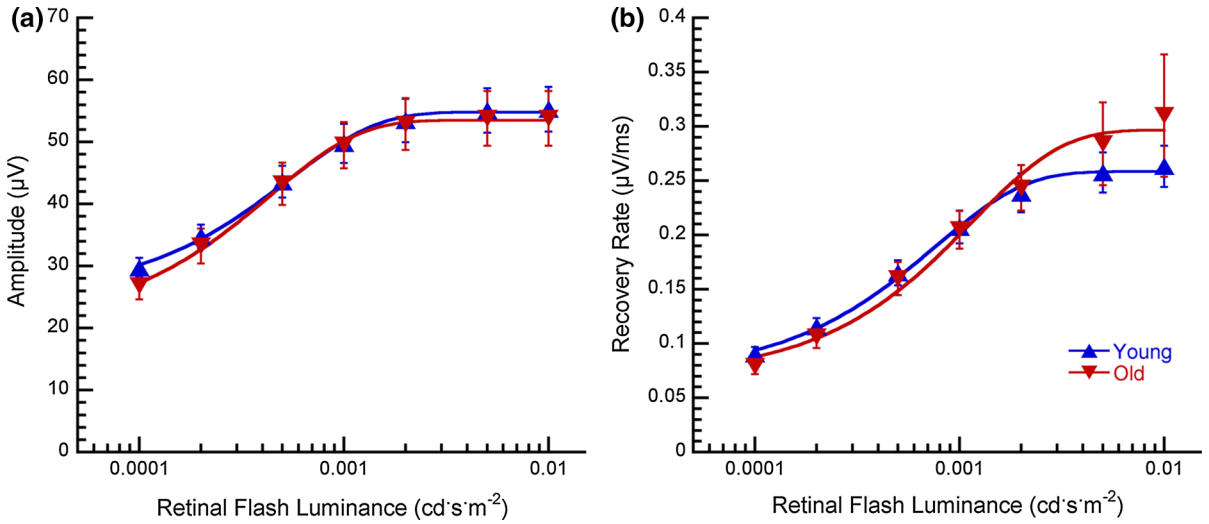

Fig. 6 OD-adjusted response amplitudes and rates of recovery. The estimated isolated flash response amplitudes and slopes were calculated with the exponential functions used to fit the individual data seen in Fig. 4 and averaged for the younger and older subjects. The average isolated flash responses (**a**) and rates of recovery (**b**) were plotted versus the flash luminance at the retina equal to the input flash luminance settings used for testing. Error *bars* are ±1 standard error of the mean. The average response amplitudes and recovery rates for the younger and older participants were fitted with the exponential function $y = a + b \times [1 - \exp(-cx)]$. The R^2 values for all *curve* fits were >0.99. The isolated flash responses and rates of recovery were not significantly different between the younger and older participants

a-wave, while in the current study the amplitude of the b-wave was measured because lower scotopic light levels were used and they were not high enough to elicit an a-wave. The flash luminances selected for this study were lower because the flashes occurred so closely in time that the signal would be saturated if the strength of the flashes were high enough to generate an a-wave.

Potentially, the recovery of the rod photoreceptor response (i.e., a-wave) could exhibit an age-related slowing, while the recovery of the rod ON-bipolar cell response (i.e., b-wave) remains stable during adulthood. For example, the convergence between rod photoreceptors and rod ON-bipolar cells is high with a 15-to-1 ratio [21]. Due to this redundancy, it is possible that the age-related decline in rod density would not significantly affect the response properties of the rod bipolar cell at some light levels. A second, perhaps complementary, hypothesis would be related to the plasticity of the retina. In mice and humans, investigators have noted that the dendritic fibers of the rod ON-bipolar cells in aged, postmortem retinas extended beyond their normal boundary of the outer plexiform layer into the outer nuclear layer [22, 23]. Moreover, in postmortem human eyes with retinitis pigmentosa, which is an inherited disease that leads to the degeneration of the photoreceptors, Li et al. [24] observed rods with neurites that extended far into the inner retina. Although untested, this irregular growth of rod neurites may also exist in the older adult. Combined, the abnormal growth of the rod and bipolar cell processes may be able to compensate for rod photoreceptor death. This remains to be tested.

It is somewhat puzzling that the OD-adjusted scotopic ERG isolated flash response amplitudes were not smaller in the older adults where previous studies have demonstrated that rod responses to single flash decline with age [25–27]. Birch and Anderson [27], for example, have shown that the rod response to a -3.4 log cd s m^{-2} flash declines rapidly after the age of 55 years. While Birch and Anderson did not correct for density changes in the ocular media that occur during adulthood, this cannot explain the difference between the two studies. That is, the difference in optical density between the younger (15- to 24-year olds) and older (65- to 79-year olds) adults in Birch and Anderson's study using the bilinear model [20] and the peak wavelength of their stimulus (440 nm) would be approximately 0.49 log units, which is considerably smaller than the difference between the younger and older adult scotopic b-wave amplitudes

(1.86 log units). Though the results of the current study are different from those of Birch and Anderson, it is important to note that the isolated flash responses analyzed in the current study are derived from responses to rapid flicker stimulation. One limitation of using m-sequence flash stimulation to investigate the recovery of the scotopic ERG is that there is never sufficient time between flash sequences to allow the outer retina to reach a full recovery. Because the isolated and subsequent flash responses are extracted from a kind of flicker stimulation, the responses are adapted to the temporal modulations of the stimulus and a maximal, dark-adapted ERG response for a particular flash strength cannot be determined.

In the photopic mfERG and aging literature [28–32], controversy exists as to whether the age-related variations in the first-order or isolated flash responses are due to neural or optical factors. Comparing the scotopic full-field ERG results of the present study with those studies that utilized the photopic mfERG is relevant because they all used m-sequence flash stimulation to elicit retinal responses, and it is important to evaluate whether the m-sequence is a reliable technique for understanding the response dynamics of the outer retina. Several photopic mfERG studies have described age-related reductions in the response density amplitude of the P1 component of the mfERG waveform [16, 28–34], which is analogous to the b-wave in the full-field ERG waveform [35]. A number of investigations of the photopic mfERG and aging [16, 28, 30, 34] have calculated the expected differences in OD of the ocular media between their younger and older subjects using models of the aging ocular media and found that the difference in OD was smaller than the difference in the average response amplitudes across the retina of the younger and older subjects. These studies suggested that senescent changes in light absorbance by the ocular media could not entirely explain the losses in mfERG response density. Conversely, Fortune and Johnson [29] reported that the mfERG response density amplitude of both the first- and second-order response kernels was only significantly affected by age in the central 5° of the retina after correcting for pre-retinal optical factors. In their study, Fortune and Johnson obtained direct measurements of the OD of the ocular media for each subject using a psychophysical brightness-matching task to determine the relative sensitivity of the eye at 550 nm and compare the sensitivity to the relative sensitivity of rod photoreceptors in vitro at 550 nm. The difference between the sensitivities was used as an estimate of OD in order to adjust the mfERG responses for the corresponding subjects. Fortune and Johnson discovered that the significant age-related losses in mfERG response amplitudes observed before the adjustments were largely absent after correcting for the variations in OD between the subjects. Later studies by Tam et al. [31, 32] confirmed the results of Fortune and Johnson's study when they compared the mfERG responses between younger and older adults with intraocular lens implants and found that age had no significant effect on the mfERG response amplitude except in the central retina.

By assessing the second-order responses elicited during m-sequence flash stimulation, Gerth et al. [16] demonstrated an age-related decline in the response density of the second-order responses that could not be accounted for by changes in the density of the ocular media. This result suggested that fast adaptation mechanisms are compromised in the aged retina. The difference in the OD-corrected results between the current study and Gerth et al.'s could stem from the investigation of two different photoreceptor types. The present study examined the response dynamics of the rods, while Gerth et al. examined the cones. Multiple histological investigations, however, have revealed greater losses in rod density than cone density, which would suggest that rods should present with greater dysfunction. Another difference between the studies is that this study measured full-field responses, while Gerth et al. measured focal responses. In general, photopic mfERG studies show that the central 5° of the retina exhibits the largest changes between older and younger adults [16, 28–34]. Since the central retina only occupies a small percentage of the retinal surface, dysfunction limited to this region may not be resolved with the full-field ERG. Therefore, in the aged retina, the normal functioning of the rods in the periphery could mask the dysfunction of the rods in the central retina. Future studies should evaluate scotopic mfERG recovery as well as photopic full-field ERG recovery to determine whether impaired outer retinal activity is concentrated in the central retina or only in the cone photoreceptors.

In conclusion, the results of this study have demonstrated that the fast adaptation recovery of the scotopic ERG b-wave is delayed with age, but that the delay can be accounted for by the decreased light

transmission through the aging ocular media. A majority of the ocular media light loss in older adults is due to age-related increases in lens absorption [36], which may ultimately lead to cataract. Most age-related cataracts cannot be prevented, but surgery to remove the cataractous lens can rapidly improve visual function and quality of life [37]. Vision gains after cataract surgery are associated with increased mobility and independence as well as improved ability on every day tasks such as reading, recognizing people, and driving at night [38, 39]. The current study suggests that removal of cataracts may also enhance vision under dim illumination by allowing more photons to be absorbed by the rods, which would augment the responses of downstream neurons and potentially improve night vision in older adults.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standard of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statements of human rights All procedures performed in this study were in accordance with the ethical standard of the Office of Human Research Protection of the University of California, Davis, School of Medicine and with the 1964 Helsinki Declaration and its later amendments.

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