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Impact of aging and age-related maculopathy on inactivation of the *a*-wave of the rod-mediated electroretinogram ☆

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Abstract

This study examined the impact of aging and age-related maculopathy (ARM) on the inactivation of phototransduction in rod photoreceptors by measuring the recovery of the *a*-wave using a paired flash electroretinogram technique. Measurements were made on 32 older adults in normal retinal health, 25 with early ARM, 7 with late ARM, and 20 young adults for comparison purposes. ARM presence and severity were defined by the Wisconsin Age-Related Maculopathy Grading System based on grading of fundus photographs. The inactivation of rod phototransduction exhibited an aging-related slowing. Those with early ARM did not exhibit inactivation slowing over and above what would be expected based on normal retinal aging. Persons in the late stages of ARM exhibited dramatic slowing in inactivation kinetics.

Keywords: Electroretinogram; Rod photoreceptor; Phototransduction; Aging; Age-related maculopathy

1. Introduction

Age-related maculopathy (ARM) is the most common cause of new, irreversible vision impairment in older adults in many countries including the US (National Advisory Eye Council, 1999). ARM is a heterogeneous disorder affecting the retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris, and secondarily, the photoreceptors (Green & Enger, 1993; Sarks, 1976). Early ARM can be clinically characterized by large drusen and/or pigmentary changes in the macula. Late ARM is characterized by the presence of geographic atrophy and/or choroidal neovascularization (Bressler, Bressler, & Fine, 1988). Treatment options for ARM are for the most part directed at the late phases (exudative form) of the disease when vision loss is already severe (Bressler & Bressler, 2000). Proven treatments for early disease are limited to antioxidative nutritional supplements that slow progression to advanced disease for a subset of patients with early disease of intermediate severity (Age-related Eye Disease Study Research Group, 2001). The biological mechanisms causing ARM remain unknown although risk factors have been suggested by previous research including smoking (Delcourt, Diaz, Ponton-Sanchez, Papoz, & POLA Study Group, 1998; Eye Disease Case Control Study Group, 1991; Smith et al., 2001) and complement factor H (Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005). An improved understanding of ARM pathogenesis

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is clearly needed in order to facilitate the development of preventative measures and more effective treatments (Gottlieb, 2002; National Advisory Eye Council, 1999). One strategy for understanding early ARM pathogenesis is to focus research on photoreceptor dysfunction because it provides insight into the pathways and mechanisms before anatomical changes are visible in the fundus (Jackson, Curcio, Sloan, & Owsley, 2004; Jackson, Owsley, & Curcio, 2002).

Histopathologic studies on human donor retinas indicate a predilection for parafoveal loss of rod photoreceptors over cones in the early, nonexudative form of ARM (Curcio, 2001; Curcio, Jackson, & Owsley, 2000; Curcio, Medeiros, & Millican, 1996). Although both rods and cones in the parafoveal degenerate in ARM, rod loss precedes and is more severe than cone loss in most donor retinas evaluated (Medeiros & Curcio, 2001). During the normal course of retinal aging, rod density declines whereas cone density remains relatively unchanged throughout adulthood (Curcio, Millican, Allen, & Kalina, 1993; Gao & Hollyfield, 1992). Given the loss of rods early in ARM pathogenesis, a test probing rod function may reveal a functional counterpart to these histopathological changes, which could have the potential for being an earlier marker of the disease, or a method for monitoring progression following interventions. Previously we and others have shown that rod-mediated dark adaptation, as measured psychophysically, is dramatically delayed in those with early ARM, even for patients with good visual acuity (Owsley, Jackson, White, Feist, & Edwards, 2001; Steinmetz, Haimovici, Jubb, Fitzke, & Bird, 1993). Interestingly this impairment of dark adaptation was found adjacent to the macula, suggesting that some dysfunction associated with early ARM may extend beyond the macula. Older adults in good retinal health also exhibit rod-mediated dark adaptation delays, although not as severe as those with early ARM (Jackson, Owsley, & McGwin, 1999). This slowing in light sensitivity recovery appears to be mediated by a slowing in the rate of rhodopsin regeneration in these persons, as revealed by their exhibiting abnormal parameters for the second component of rod-mediated dark adaptation. This second component is dictated by the rate of rhodopsin regeneration as indicated by electrophysiologic work on animal models (Baylor, Matthews, & Yau, 1980; Dowling, 1960; Lamb, 1980, 1981) and retinal densitometry findings in human (Rushton, Campbell, Hagins, & Brindley, 1955).

In addition to the rate of rhodopsin regeneration being essential for the recovery of light sensitivity by rods, the onset time of the inactivation phase of transduction, and the rate of that inactivation are also critically important (Birch, Hood, Nusinowitz, & Pepperberg, 1995; Lyubarsky & Pugh, 1996). After the eye's exposure to a very intense light, there is a time delay before the onset of photocurrent recovery, and then the response recovers at an exponential rate described by a time constant. This rapid inactivation phase is the earliest phase of light sensitivity recovery when the dark current is being re-established, and in the dark adaptation literature is referred to as the first component of dark adaptation (Lamb, 1980, 1981; Lamb & Pugh, 2004; Leibrock, Reuter, & Lamb, 1998). Traditional dark adaptometry techniques cannot be used to examine the first component of rod-mediated sensitivity recovery because it is obscured by the cone portion of the dark adaptation function (i.e., the cones are more sensitive than the rods and recover faster). In addition, using full-field, single-flash electroretinogram (ERG) recording techniques, the inactivation phase of the *a*-wave is typically obscured by the *b*-wave and other post-receptoral components. To estimate the parameters of inactivation of transduction in humans, Birch and colleagues (Birch et al., 1995) developed the "double flash" technique. Using this method, recovery of the *a*-wave response can be inferred by measuring the recovery of the *a*-wave to a probe flash presented after the saturating flash. By varying the interval between the saturating flash and the probe flash, a recovery function of the *a*-wave can be estimated which provides information for both the time delay for the onset of inactivation and the time constant of inactivation.

In the study reported here, the double-flash ERG technique allowed for the examination of whether the delays in rod-mediated dark-adaptation observed in older adults and in those with ARM may extend to the first-component of rod-mediated dark adaptation. That is, is there a deficit in the inactivation phase of phototransduction in rods relative to normal young eyes? If inactivation is delayed or the rate of recovery is slower relative to normal eyes, this would imply that at least some of the mechanistic basis of dark adaptation impairment in aging and early ARM is intrinsic to the rod itself, irrespective of dysfunction in Bruch's membrane or the RPE. This is because the first component of dark adaptation does not depend on the functioning of Bruch's membrane/RPE complex (Lamb & Pugh, 2004).

2. Method

2.1. Subjects

Participants were recruited from the comprehensive ophthalmology and the retina services of the Department of Ophthalmology, University of Alabama at Birmingham. Eligibility criteria for older adults were as follows: (1) at least 60 years of age; (2) best-corrected, distance visual acuity as listed in the medical record of 20/80 or better in at least one eye. Since the primary focus of the study was on the early phases of ARM, an acuity cut-off of 20/80 was used; (3) diagnosis of ARM or normal retinal health based on grading of stereoscopic color 30° fundus photographs taken on the day of ERG testing after dilation of the pupil to at least 6 mm. Photographs were taken with a FF4 Zeiss fundus camera on the eye selected for ERG testing, which was the eye with better visual acuity. Photographs were evaluated using the Wisconsin Age-related Maculopathy Grading System (WARMGS) (Klein et al.,

1991) at the University of Wisconsin Reading Center by graders masked to patient characteristics including previous diagnoses.

For the purposes of this study, retina were considered to not have signs of early ARM based on the following WARMGS grades: (1) maximum drusen type coded 0 (none), 1 (hard indistinct drusen), or 2 (hard distinct drusen); (2) maximum drusen size coded 0 (none), 1 (drusen indistinct or questionable), or 2 (distinct drusen with a diameter less than 63 μm); (3) increased retinal pigmentation coded 0 (none), or 1 (questionable); and (4) decreased RPE pigmentation coded 0 (none) or 1 (questionable). Early ARM was defined as WARMGS grade of: (1) maximum drusen type coded 3 (soft distinct drusen) or 4 (soft indistinct or reticular drusen); (2) maximum drusen size $\geqslant 3$ (drusen diameter greater than 63 μm); (3) increased retinal pigmentation $\geqslant 2$ (presence of increased pigmentation); and (4) decreased RPE pigmentation $\geqslant 2$ (presence of decreased pigmentation). Late ARM was defined as those with a grade of 2 on the late ARM variable, indicating the presence of geographic atrophy and/or choroidal neovascularization. Late ARM eyes could also have some of the other characteristics of early ARM patients as described above, but their having a late ARM grade of 2 automatically placed them in the Late ARM category.

Patients whose test eye had a grade of 8 (''cannot grade'') on any of the following WARMGS variables were excluded from the sample: maximum drusen type, maximum drusen size, decreased pigmentation, increased pigmentation, late ARM, geographic atrophy, retinal detachment, subretinal hemorrhage, subretinal scar, and ARM treatment. Patients having evidence of diabetic retinopathy and its associated lesions (i.e., any patient with grades other than 10 (absent), 12 (non-diabetic), or 13 (questionable) on diabetic retinopathy level) were also excluded.

Young adults were also enrolled in the study to have a reference group against which older participants without signs of early ARM could be compared on ERG parameters. They were recruited from the comprehensive ophthalmology service as described above. Inclusion criteria were (1) aged 16–30 years; (2) no ophthalmic conditions or signs of maculopathy noted in a dilated comprehensive eye exam performed within the previous six months; (3) best-corrected distance acuity of 20/20 or better in each eye according to this exam. Fundus photographs were not taken on young enrollees.

Patients, regardless of age, were excluded if their medical record or a general health interview indicated that they had any of the following: (1) glaucoma, optic neuropathy, or any ocular conditions other than ARM, refractive error or dry eye; (2) refractive error (spherical equivalent) whose absolute value was >6 diopters; (3) neurological diseases such as Alzheimer's disease, Parkinson's disease, and a history of stroke; (4) diabetes; (5) serious frailty or medical conditions expected to lead to mortality or disability within 12 months.

This study was approved by the Institutional Review Board of the University of Alabama at Birmingham. The research followed the Tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after the nature and possible consequences of the study were explained.

2.2. Procedures

Prior to ERG testing, best-corrected, distance visual acuity was measured for each eye on the day of ERG testing using the ETDRS chart (Ferris, Kassoff, Bresnick, & Bailey, 1982; Ferris & Sperduto, 1982) and expressed as logMAR. Contrast sensitivity was assessed for each eye using the Pelli–Robson chart and its standard administration protocol (Pelli, Robson, & Wilkins, 1988) and scored with the letter-by-letter method (Elliott, Bullimore, & Bailey, 1991).

The apparatus used to measure the rod *a*-wave was the UTAS-E 3000 visual electrodiagnostic system (LKC Technologies) a commonly used, clinical instrument also suitable for research purposes. Our general technique for measuring the rod *a*-wave is similar to that of previous studies (Birch, Hood, Locke, Hoffman, & Tzekov, 2002; Cideciyan & Jacobson, 1996; Hansen & Fulton, 2005; Hood & Birch, 1993; Smith & Lamb, 1997). Measurement of the inactivation of the *a*-wave is based on the double-flash method of Birch et al. (1995) and Hansen and Fulton (2005). The tested eye (the eye with better acuity) was dilated to at least 6 mm in diameter using 1% tropicamide and 2.5% phenylephrine hydrochloride. Pupil size was measured before and after ERG recording to ensure adequate dilation of the pupil was achieved throughout testing. Participants were dark-adapted for 45 min prior to ERG recording. The cornea was anesthetized with 0.5% proparacaine hydrochloride. Recordings were obtained through the use of a Burian Allen bipolar electrode placed on the anesthetized cornea. Responses were amplified (band-pass 0.5–8000 Hz; 4-pole) and digitized at a 7 kHz sampling rate for a duration of 60 ms. Subjects were instructed to look straight ahead and to keep their eye stable. A 10.9 ln scot T_d—second test flash (55,000 K) was used to saturate photoreceptor response. After an interval of time had elapsed (inter-flash interval), a second probe flash was presented to measure the photoreceptor response. The inter-flash intervals used were 2, 4, 8, 16, 32, 48, and 64 s. A blue probe flash (8.9 ln scot T_d—second; 450 nm; Kodak 47A) was used to predominantly stimulate the rods. For each inter-flash interval, a photopically matched red flash (605 nm; Kodak 26) was used to predominantly stimulate the cones in a separate trial. In addition to the double flash pairings, responses to each of the probe flashes were measured to estimate the maximum photoreceptor response for each individual. After each ERG measurement, a 2-min recovery period was used to allow full recovery of the photoreceptors. To isolate the rod photoreceptor response, the response to the red probe flash was computer subtracted from the response to the blue probe flash.

The recovery of the rod *a*-wave response (R/R_{max}) was expressed as a proportion of the *a*-wave amplitude of the paired flash response (R) divided by the *a*-wave amplitude (R_{max}) of the probe flash alone. To describe the response recovery kinetics, in the following equation was used as:

$$R/R_{max} = 1 - \exp[-(t - T_c)/(T_r)], \quad (1)$$

where t is the time in seconds, T_c is the delay before the start of recovery in seconds, and T_r is the time constant of the recovery (seconds). The equation was fit to the data with T_c constrained to >0 s. Because older adults and especially ARM patients may have recovery functions of a different shape compared with young normals, the time at which R/R_{max} reached its half maximal value, T_{50}, was calculated using linear interpolation. Calculating T_{50} using linear interpolation describes the speed of *a*-wave recovery independent of the shape of the recovery (Hansen & Fulton, 2005). Thus, the same parameter can be calculated and compared across groups that are each best described by a different model.

To compare demographic and visual function variables across groups of study subjects, analysis of variance (for continuous variables) and χ^2 tests (for categorical variables) were used. Rod-mediated ERG parameters were compared across the older adult groups with and without adjustment for age using analysis of covariance. The role of pseudophakia versus phakia in ERG parameters was assessed by analysis of variance. The association between ERG parameters and fundus features were assessed by analysis of variance. SAS Version 8.02 (SAS Institute, Cary, NC) was used for all statistical analyses.

3. Results

Table 1 presents descriptive characteristics of the sample for demographics and visual function. There were 32 persons 61–80 years of age meeting the definition of normal retinal health who were classified as "old normal", 26 persons (62–88 years of age) classified as early ARM, and 7 persons (67–95 years of age) as late ARM; of the latter, three had geographic atrophy and four had choroidal neovascularization. The fundus grading data, which dictated which group older adults were in, were not available until several months after ERG testing. Although the focus of the study is on early ARM (and we specifically tried to recruit persons whose most recent eye exam indicated that they had early ARM), we decided to include the late ARM patients as a separate group in the analyses because the data from the entire protocol including ERG data had already been collected.

The average age of the 20 young-normal participants was 24 years (range 17–29 years) while each of the three older groups had average ages in the late sixties and seventies (Table 1). The old normal group was younger than the early and late ARM groups ($p = 0.002$ and $p = 0.002$, respectively); the ages of the early and late ARM groups were not significantly different. There were a higher proportion of African Americans in the young normal group and early ARM group compared with the old-normal and late ARM groups. However, results from analyses comparing ERG parameters in young and old-normal adults described below are unchanged if analyses are limited to white participants (data not shown). Visual acuity was best in young subjects, and was decreased in the older groups, from better to worse as follows: old-normal, early ARM, late ARM (all p values <0.01, Table 1). A similar pattern of results was also observed for contrast sensitivity with the exception of contrast sensitivity in the tested eye for the old normal and early ARM groups; this difference was not significant. Otherwise all group comparisons on contrast sensitivity were significant with p values of <0.01. These findings were true for both the tested and fellow eyes.

Fig. 1 shows examples of the *a*-wave families for a normal young adult, a normal old adult, an early ARM patient, and a late ARM patient. Blue-flash responses are

Table 1
Demographics and visual function for the patient groups

	Young normal ($n = 20$)	Old normal ($n = 32$)	Early ARM ($n = 25$)	Late ARM ($n = 7$)
Age, years mean (SD)	23.9 (3.2)	68.7 (5.2)	73.5 (6.3)	77.4 (9.3)
Gender, *n* (%)				
Female	15 (75.0)	17 (53.1)	13 (52.0)	2 (28.6)
Male	5 (25.0)	15 (46.9)	12 (48.0)	5 (71.4)
Race/ethnicity, *n* (%)				
White, non-Hispanic	16 (80)	32 (100)	19 (76)	7 (100)
African American	4 (20)	0	5 (20)	0
Hispanic	0	0	1 (4)	0
Visual acuity (logMAR), mean (SD)				
Tested eye	−0.06 (0.07)	0.02 (0.09)	0.13 (0.17)	0.25 (0.14)
Fellow eye	−0.03 (0.07)	0.14 (0.23)	0.38 (0.39)	0.94 (0.21)
Contrast sensitivity (log), mean (SD)				
Tested eye	1.65 (0.11)	1.48 (0.13)	1.34 (0.35)	1.21 (0.26)
Fellow eye	1.60 (0.12)	1.47 (0.13)	1.24 (0.38)	0.83 (0.51)

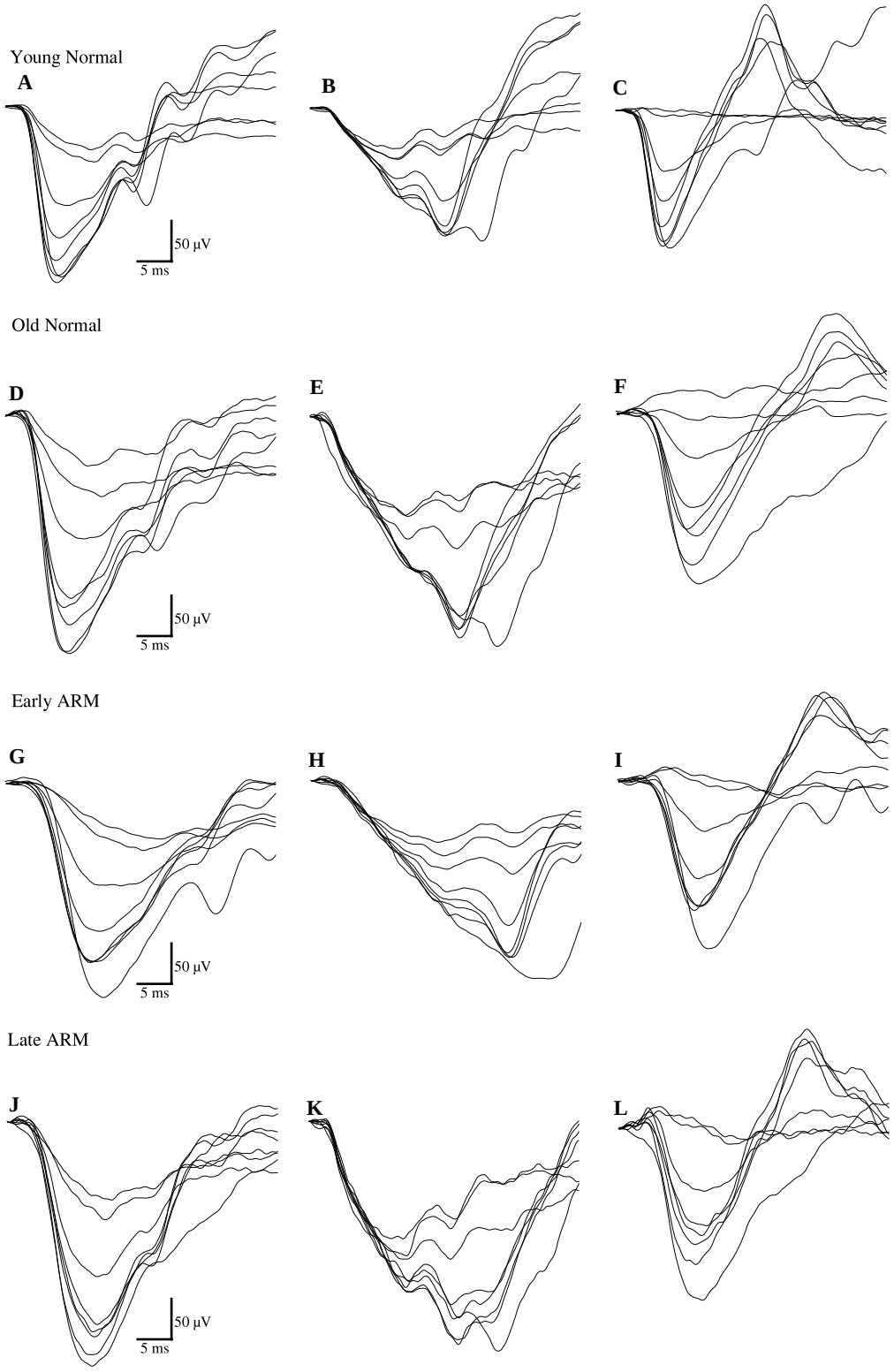

Fig. 1. *a*-Wave families for a normal young adult (A–C), normal old adult (D–F), early ARM patient (G–I), and late ARM patient (J–L). The first column is the response to the blue flash. The second column is the response to the red flash and the third column is the computer subtracted rod response. In each *a*-wave family, the responses from smallest *a*-wave amplitude to largest amplitude are the inter-flash intervals (2, 4, 8, 16, 32, 48, and 64 s) and probe flash alone.

in the first column, and red-flash responses are in the second column. Computer-subtracted rod responses are in the third column. The R/R_{max} function of a normal young adult is shown in Fig. 2. This figure illustrates the fit of both Eq. (1), from which T_c and T_r are estimated (stippled curve), and the half maximal response, T_{50}, by linear

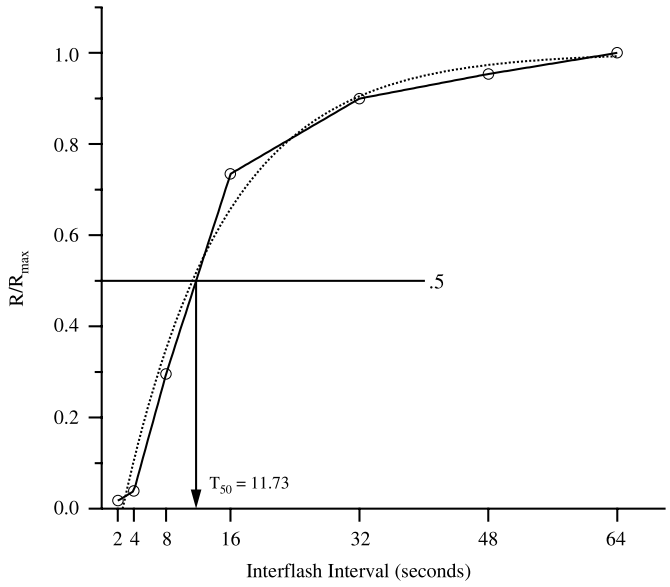

Fig. 2. R/R_{max} (circles) plotted as a function of inter-flash interval (seconds) for a normal young adult. The stippled curve is the fit of the recovery model from which T_c and T_r are estimated. The half maximal response, T_{50}, is estimated by linear interpolation (solid line).

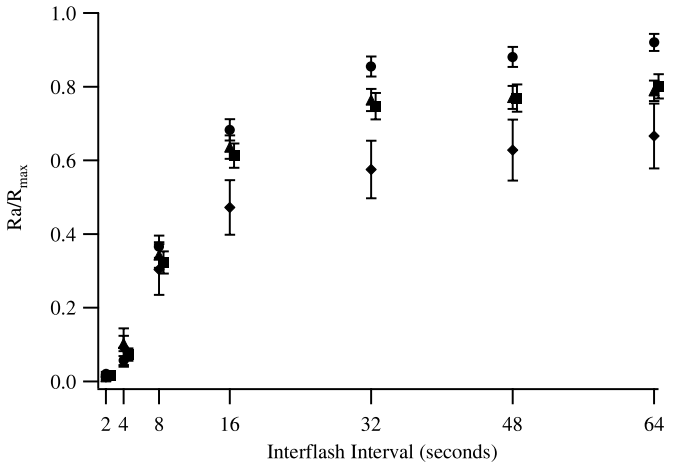

Fig. 3. Ra/R_{max} plotted as a function of inter-flash interval for young normal (circles), old normal (squares), early ARM (triangles), and late ARM (diamonds) groups. Old normal group's Ra/R_{max} values were offset 0.5 s for visibility. Error bars are ±1 standard error of the mean.

interpolation (solid line). Mean ERG parameters and standard deviations for each group are listed in Table 2.

The first question addressed was what impact normal aging had on the inactivation of the rod-mediated ERG *a*-wave. The time at which the young-normals' *a*-wave reached its half maximal value, T_{50}, was about 6 s faster than old normals ($p < 0.04$). The time at which recovery commenced (T_c) was similarly distributed for young and old-normal adults ($p = 0.12$). The time constant of the *a*-wave amplitude recovery (T_r) was on average 13.17 s higher for old-normal adults compared with young adults ($p < 0.004$). Although the model's goodness of fit (r^2) was high for both young adults and old-normals, the goodness of fit was significantly lower for the old-adults ($r^2 = 0.93$, range: 0.76–0.99) compared with the young normals ($r^2 = 0.97$, range: 0.92–1.00) ($p < 0.02$).

To determine whether poorer model fits in the old normal group biased the analysis, four old normals with an r^2 value less than 0.85 were excluded in a subsequent analysis. With these four individuals excluded the old adults' mean R^2 increases to 0.95, similar to the young adults ($p = 0.09$). T_c values between the two groups were comparable ($p = 0.38$). The mean T_r of the old adults was elevated about 9 s compared with young adults ($p < 0.02$).

The slowing of *a*-wave recovery in the older age groups is best illustrated in Fig. 3 where mean R/R_{max} is plotted as a function of inter-flash interval for each group. For the first three inter-flash intervals (2, 4, and 8 s), there is no difference R/R_{max} between the four groups ($p = 0.88$). For the remaining inter-flash intervals ($\geqslant$16 s), R/R_{max} values are significantly higher for young adults compared with all the older groups ($p < 0.03$). Old normals and early ARM patients exhibit similar *a*-wave recovery ($p = 0.86$). Late ARM patients exhibit slowed recovery (i.e., lower R/R_{max} values) compared with the old normals ($p < 0.03$) and early ARM patients ($p < 0.03$).

To examine the ERG parameters of the ARM patients, an analysis of covariance was performed on each ERG parameter (T_c, T_r, and T_{50}) as the dependent variable, examining the main effect of group. Because the age distributions of the three older groups were significantly different ($p < 0.001$), age was used as a covariate. After age adjustment, the mean T_{50} value was delayed about 16 s for late ARM patients compared with normal old adults

Table 2
Rod-mediated ERG parameters for each group estimated from Eq. (1)

	Young normal ($n = 20$)	Old normal ($n = 32$)	Early ARM ($n = 25$)	Late ARM ($n = 7$)	p Value young vs. old-normal	p Value older groups
T_c, mean (SD)	2.58 (0.78)	1.98 (1.58)	1.29 (1.39)	1.28 (1.21)	.12	
Age-adjusted[a]		1.99	1.29	1.26		.5
T_r, mean (SD)	11.03 (4.32)	24.21 (18.85)	32.76 (20.42)	37.62 (27.36)	.004	
Age-adjusted[a]		24.60	32.49	36.81		.13
T_{50}, mean (SD)	11.82 (4.20)	17.72 (11.69)	17.31 (12.44)	33.67 (24.05)	.04	
Age-adjusted[a]		18.42	16.83	32.23		.004

[a] Age-adjustments were not applied to the young and old-normal group comparisons. In comparing parameters among the three older adult groups, age-adjustments were made in evaluating statistical significance since age was significantly different in these three older age groups.

and early ARM patients ($p < 0.004$). The T_c, and T_r, parameters were similar for all three groups ($p = 0.50$; $p = 0.13$, respectively). The model's goodness of fit (r^2) was statistically similar for both early and late ARM participants, averaging 0.91 (range: 0.75–1.00) and 0.88 (range: 0.67–0.97), respectively ($p = 0.46$).

Because the model did not fit some of the ARM patients as well as the old normal adults, we attempted to fit a nested exponential model (Kang Derwent et al., 2004) to each individual's recovery function. R/R_{max} was normalized and fitted with the following equation:

$$[1 - (R/R_{max})] = 1 - \exp\{-A_1[\exp(-t/T_1) + A_2 \times \exp(-t/T_2)]\}, \quad (2)$$

where A_1 and A_2 are scaling factors and T_1 and T_2 are exponential time constants. The double exponential equation was selected because the recovery during the first 8 s was similar between groups and group differences arise after 8 s. Thus, the expectation is the first time constant (T_1) would be similar between the groups, where as, the second time constant (T_2) would be slower in the older groups compared to the young groups.

Estimated time constants from Eq. (2) for each group are listed in Table 3. The first time constant (T_1) was similar between the old normal group compared with the young group ($p = 0.94$) and similar between all three old groups ($p = 0.86$). The second time constant of the old normal group was almost twice as large in magnitude compared with the young group, but did not reach statistical significance ($p = 0.10$). The second time constant was similar among the three old groups ($p = 0.49$). The nested exponential equation (Kang Derwent et al., 2004) appeared to fit better than the equation originally applied to the data (Birch et al., 1995) in that the mean R^2 was higher for nested exponential equation (mean = 0.99; range 0.90–1.00) compared with the single exponential (mean = 0.93; range = 0.67–1.00) ($p < 0.001$). However the variability of the fits were much higher for nested exponential compared to single exponential. The average standard deviation for an individual's estimate of T_1 is 13.56, thus the 95% confidence interval was −23.66 to 30.03. The average standard deviation for an individual's T_2 parameter estimate was 360.47 yielding a confidence interval of −601.78 to 811.26. Because the confidence in the parameters estimated using Eq. (2) was low, and the equation failed to converge for four normal old adults and four early ARM patients, parameters estimated using Eq. (2) were considered unreliable for this application. The T_{50} parameter may be a more reliable estimate of recovery speed across all of the groups in this study.

The effects of lens density must be taken into account when interpreting these results, since the aging lens preferentially absorbs the short-wavelengths (Pokorny, Smith, & Lutze, 1987; van Norren & Vos, 1974) used to isolate the rod response, thus effectively reducing the flash's retinal illuminance more so for older adults than young. Paired flash ERG techniques like that used here are more robust to the effect of aging-related increases in lens density compared with single flash techniques because probe responses are normalized to an individual's maximum response. The concern, however, is that the saturating flash may be less effective and allows the older individual to recovery faster compared with an individual with a more transparent lens. To examine whether older adults' increased lens density impacts their ERG parameters, we compared the ERG parameters in older adults having an intraocular lens (IOL) of the conventional type (Alcon Acrysof which has an ultra-violet radiation filter) in the test eye ($n = 13$) to those older adults who were phakic ($n = 44$). Normal old adults and early ARM patients groups were combined because the ERG parameters between the two old groups were similar. Because the pseudophakic patients were about 6 years older on average than the phakic patients ($p < 0.0006$), analyses were adjusted for age. There were no IOL versus phakic differences in the combined old-normal and early ARM groups with respect to either T_c, T_r, or T_{50}. ($p = 0.59$; $p = 0.62$; $p = 0.40$) Late ARM patients were not included in the analysis because their recovery was significantly slower than old normals and early ARM patients; however, if they are included, there remains no difference in the ERG parameters between IOL and phakic patients. T_c, T_r, or T_{50}. ($p = 0.88$; $p = 0.50$; $p = 0.18$).

Are rod-mediated inactivation ERG parameters related to fundus characteristics in the older participants as characterized by the WARMGS? The WARMGS characteristics of interest were maximum drusen type, maximum drusen size, drusen area, increased RPE pigmentation, and RPE depigmentation. To insure adequate power, the levels of each characteristic were concatenated as follows. For maximum drusen type 2 levels were created: (1) none or hard drusen and (2) soft drusen. For maximum drusen size, 3 levels were created: (1) none or questionable drusen, (2) <63 μm diameter, and (3) ⩾63 μm diameter. Three levels were created for maximum drusen area: (1) <63 μm diameter, (2) <250 μm diameter, and (3) <0.5 disc area. Two levels representing the absence or presence of pigmentary lesions were created for increased RPE pigmentation

Table 3
Rod-mediated ERG parameters for each group estimated from Eq. (2)

	Young normal ($n = 20$)	Old normal ($n = 27$)	Early ARM ($n = 21$)	Late ARM ($n = 7$)	p Value young vs. old-normal	p Value older groups
T_1, mean (SD)	2.85 (1.49)	2.82 (1.22)	3.02 (1.45)	3.03 (1.87)	.94	.86
T_2, mean (SD)	55.29 (77.93)	109.32 (128.87)	120.62 (151.24)	180.67 (157.01)	.10	.49

Table 4
Rod-mediated ERG parameters as a function of fundus features as defined by the WARMGS

ARM lesion	F value	p Value
Maximum drusen size		
T_c	1.21	0.31
T_r	0.80	0.50
T_{50}	0.39	0.76
Maximum drusen type		
T_c	0.46	0.63
T_r	0.30	0.74
T_{50}	0.34	0.72
Drusen area		
T_c	1.42	0.25
T_r	1.32	0.28
T_{50}	0.36	0.78
Increased RPE pigmentation		
T_c	0.97	0.39
T_r	2.24	0.12
T_{50}	0.68	0.51
RPE Depigmentation		
T_c	0.92	0.41
T_r	2.65	0.08
T_{50}	1.44	0.25

and RPE depigmentation. Late ARM characteristics (geographic atrophy and choroidal neovascularization) were not specifically evaluated as part of this analysis since only a relatively small percentage of the sample had geographic atrophy ($n = 3$) or choroidal neovascularization ($n = 4$). As displayed in Table 4, there were no significant associations between the ERG parameters and any of the anatomic fundus characteristics as defined by the WARMGS (all $p > 0.05$).

4. Discussion

The speed of phototransduction inactivation as estimated by T_r is slowed in older adults with normal retinal health compared with young normals. For inter-flash intervals up to 8 s there is no difference between young and old adults. The slowing of phototransduction becomes apparent for inter-flash intervals 16 s and longer For the longest inter-flash interval (64 s) old adults exhibited 12% less recovery compared with young adults even though they were exposed to a dimmer saturating flash because of increased optical density. Adults with normal appearing retinas required on average 6 s longer to reach their half maximal response during recovery, as compared to young adults. These results provide evidence for the first time that the first component of rod-mediated dark adaptation, namely the inactivation of phototransduction, exhibits aging-related slowing.

Because the inactivation phase does not depend on the functioning of the Bruch's membrane/RPE complex (Lamb & Pugh, 2004), these findings imply that at least some of the contributing cause(s) to this deficit are intrinsic to the aged rod photoreceptor itself. One candidate mechanism is reduced rhodopsin concentration which has been shown to delay the kinetics of recovery both in rats and humans (Fulton & Hansen, 2003; Hansen & Fulton, 2005). However, rhodopsin concentration measured in whole retinas of donor eyes appears to be relatively stable throughout adulthood (Fulton, Dodge, Hansen, & Williams, 1999). A potential contributor to reduced rhodopsin concentration, if in fact it does exists in the aged retina, is that 30% of rods in the macula die during the retinal aging process (Curcio et al., 1993). However, surviving rods could conceivably compensate by expressing more rhodopsin, so rod loss itself may not signify reduced rhodopsin concentration.

Another possible mechanism for slowing in inactivation is a prolonged lifetime of activated rhodopsin (Birch et al., 1995). Prolonged lifetime of activated rhodopsin by itself or in combination with changes in the expression of downstream proteins may explain the increased inactivation time (Pepperberg, Birch, Hofmann, & Hood, 1996). However, we do not know of any evidence that these protein concentrations change in the aged retina. To address this question, future work could compare young and old-normal adults with respect to the slope of T_c in response to a wide range of saturating flash intensities, which in turn be used to estimate the lifetime of activated rhodopsin in young versus old-normal adults (Birch et al., 1995). Other possible mechanisms exist such as abnormal deactivation of transduction which would prolong deactivation kinetics (Chen et al., 2000). Unfortunately, clinical electrophysiology has limited ability to differentiate among these more subtle changes that are readily apparent using single cell recording.

Early ARM patients exhibited similar rod-mediated ERG parameters compared with old-normal adults. Thus, these data, which were generated by full-flash ERG techniques, imply that early ARM does not cause slowing in rod phototransduction inactivation, above what is observed by aging alone. An important issue to consider, though, is that ARM is thought to be a largely focal disease of the macula, and thus the full-field flash stimulation and the recording of the full-field ensemble response (as done here) may be masking macular abnormalities (see also Jackson, McGwin, Phillips, Klein, & Owsley, 2004). However, despite the presumed focal nature of ARM, there is evidence that dark adaptation outside the macula in patients with early ARM is impaired. This issue of whether there is slowing in rod phototransduction inactivation in ARM could be clarified by using a focal (macular), paired ERG paradigm. This type of dysfunction is theoretically feasible because, in early ARM, rhodopsin expression (Ethen, Feng, Olsen, & Ferrington, 2005) and the number of rod photoreceptors in the macula (Curcio et al., 1996) are significantly reduced as compared with normal adults. For the moment though this issue will remain unresolved.

Late ARM patients did exhibit dramatically reduced inactivation kinetics. Compared with early ARM patients and normal old patients, late ARM patients required on average about 16 s longer to reach their half maximal a-wave amplitude. The mechanisms responsible for this

impairment in late ARM are most likely the dramatic rod loss in the macula (rods are almost non-existent in late disease) (Curcio et al., 1996) and severely reduced rhodopsin expression in the macula in late ARM (Ethen et al., 2005).

Using the inactivation model to fit the paired flash data in older adults raised some methodological challenges. In many instances, fitting the model triggered the constraint that T_c must be greater than 0 because of large increases in T_r, relative to young normals, causing T_c to move towards zero. Uniformly, subjects with the greatest T_r values had T_c constrained. The nested exponential was unsatisfactory because it produced very wide confidence intervals around the estimates of the recovery time constants and it failed for some participants. The recovery function appeared to be better fit by alternative models, such as a sigmoid function, but these models do not estimate the time delay before recovery. New ERG models need to be developed based on data collected from animal models of retinal aging in order to better characterize the recovery observed in this study. Measuring the time at which a criterion amount of recovery occurred (Hansen & Fulton, 2005) avoided the difficulties associated with applying the exponential model. The T_{50} parameter exhibits less variability in our data than the T_c and T_r estimates with fewer outliers. In patient populations that exhibit large elevations in T_r, it may be beneficial to measure T_{50} to compensate for difficulties in fitting the exponential model. Using either method, one is left with the conclusion that the speed of recovery is slower in old-normal adults compared with young adults.

In summary, the inactivation of rod phototransduction exhibits an aging-related slowing. At least some of the mechanistic basis of this deficit is intrinsic to the aged rod photoreceptor itself since the inactivation phase does not depend on the functioning of the Bruch's membrane/RPE complex. It is unlikely that reduced rhodopsin expression explains the effect since rhodopsin concentration measured in whole retinas appears to be relatively stable throughout adulthood. It remains to be determined to what extent a prolonged lifetime of activated rhodopsin is contributory. Persons with early ARM do not exhibit inactivation slowing over and above what one would expect from normal retinal aging; however, since ARM is a macular disease, focal ERG techniques will be needed to clarify this issue. Persons in the late stages of ARM exhibit dramatic slowing in inactivation kinetics, most likely due to widespread rod photoreceptor death and degeneration and severely reduced rhodopsin expression in the macula.

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