The effect of age on human cone and rod ganzfeld electroretinograms

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The ganzfeld-evoked electroretinogram (ERG) is the most effective measure of the mass electrical response of cones and rods; however, all variables affecting the various components of the ERG have not been identified. The scotopic b-wave amplitude has been appreciated to be age-dependent, but little or no information exists in the literature regarding age dependency for other components. Linear regression analysis against age and multiple regression analysis against age and log intensity of the stimulus were performed on ERG responses from 24 prospectively normal subjects. Significant age dependency was found for scotopic rod-mediated b-wave amplitude, scotopic mixed rod-and cone–mediated bx-wave amplitude, and for photopic cone-mediated b-wave amplitude. No significant age correlation was found for dark-adapted cone a-wave amplitude, scotopic cone-mediated x-wave amplitude, scotopic a-wave amplitude from mixed rod and cone responses to bright stimuli, or for implicit times for any scotopic or photopic responses. These findings indicate that for clinical patient evaluation, age-corrected normal ranges derived from linear or multiple regression coefficients should be used for rod- and cone-mediated b-wave response, whereas normal ranges derived from mean and standard deviation are more appropriate for scotopic a-wave amplitude, scotopic cone-mediated x-wave amplitude, and for all implicit times.

Key words: electoretinogram, human, ganzfeld stimulation, effects of aging

Ganzfeld stimulation has been found to be the most effective way of eliciting an electroretinogram (ERG) representative of the entire population of cones and rods in the retina. With ganzfeld electroretinography, repeatable peak amplitudes and implicit times can be obtained. The b-wave of the dark-adapted, nonganzfeld ERG decreases in amplitude with age.[1-3] Most laboratories do not have specific age-matched or corrected normal data in the ranges from 10 to 50 or 60 years of age for comparison with patients. The present experiment examines the amplitudes and implicit times of specific components of the ganzfeld-evoked ERG in 24 normal subjects. The b-wave amplitudes mediated by light-adapted cones and dark-adapted rods decreased with age. The dark-adapted cone a-wave amplitude, the x-wave amplitude (a dark-adapted cone-mediated corneal positive wave response), and the implicit times for all positive wave peaks were not age-dependent.

Patients

Twenty-four Caucasian individuals age 9 to 67 years (10 men, mean age 39.6; 14 women, mean age 32.7) were studied by ganzfeld electroretinography. All were asymptomatic with clear media, normal eye examinations, and low or in-

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Table I. Integrated light values for photostimulator settings

	Log ft-Lambert-sec		*Log μjoule/cm ²-steradian*	
Photostimulator setting	*With 2.4 neutral density filter*	*With 0.2 neutral density filter*	*With KW 26 (red)*	*With KW 47, 47A 47B, +0.6 N.D.*
1	−3.67	−1.22	−0.455	−1.008
2	−3.26	−0.85	−0.162	−0.741
4	−3.02	−0.61	+0.019	−0.580
8	−2.73	−0.32	+0.239	−0.372
16	−2.29	+0.11	+0.623	+0.003

significant refractive errors (none were myopic over −5.00 diopters). In order to avoid the influence of lenticular nuclear sclerosis, only patients with apparently clear lens on dilated biomicroscopy were studied.

Methods

Ganzfeld electroretinography was performed with the principles established by Gouras,[4] Berson et al.,[5-7] and Brunette,[8] using a ganzfeld stimulator made according to guidelines described by Rabin and Berson[9] and Gunkel et al.[10] An electrical box (Pomona box No. 3311) was modified to house the xenon tube. Another important modification was the coating of the inside of the sphere with Eastman Kodak white reflectance coating (cat. no. 6080). The pupils were dilated maximally with phenylephrine (either 10% or 2½%) and tropicamide (1%), and the subjects were dark-adapted for 30 min. Under Dorsacaine (0.4% benoxinate HCl) topical anesthesia, Lawwill-Burian bipolar contact lens electrodes were inserted bilaterally, with Lyteers used as a coating agent. A battery-operated differential microelectrode preamplifier with bandpass of from 0.1 to 1000 Hz and a gain of 100 was used to amplify the ERG signal. Slide holders built into the ganzfeld stimulator allowed attenuation (or change of predominant wavelength) of the 10 μsec xenon flash with 75 mm square, glass-mounted gelatin filters. Flashes were presented to the patient at various intensities with each of the following filters: Kodak Wratten No. 2.4 neutral density filter to elicit a low-level rod b-wave response; Kodak Wratten No. 26 filter (red; λ >600 nm) to elicit a response which consists of a complex waveform with separable x-wave (dark-adapted cone-mediated response) and b-wave (rod-mediated response); Kodak Wratten No. 47, 47A, and 47B filters (blue; λ <470 nm) plus a 0.6 neutral density filter, a combination that elicits a pure b-wave or rod-mediated response and is scotopically balanced to give rod-mediated b-wave amplitude approximately equal to that obtained with Kodak Wratten filter 26; and No. 0.2 neutral density filter to elicit maximal scotopic a-wave and positive peak wave (termed bx-wave), which represents activity of both dark-adapted cones and rods to bright intensity stimuli. Stimulus flashes were separated by intervals of at least 10 sec to avoid adaptation of the rods. After this, the patient was light-adapted to 20 millilambert ganzfeld background illumination and presented with flashes of white light (with a 0.2 neutral density filter), red light (Kodak Wratten 26), and blue light (Kodak Wratten 47). With all flash-filter combinations and under both scotopic and photopic conditions, several responses from each eye were elicited at multiple intensities. For certain subjects the responses at a given intensity may not have been obtained or may have been contaminated by excessive blink artifact. In the latter cases the data were deleted from further analysis. With the 2.4 and 0.2 neutral-density filters, the integrated light value of the flashes produced with the capacitor-switched stimulator settings was measured in foot-Lambert-second (ft-Lambert-sec) with the use of a pulse photometer with a detector head calibrated to respond according to the Comité Internationale de l'Eclairage photopic photometric special sensitivity curve. For the colored filters the integrated light value was measured in microjoules per square centimeter per steradian by a pulse radiometer with a detector head filtered to level the average photodiode sensitivity to within ±7% between 460 and 975 nm (Table I).

The ERG responses were either photographed directly from a storage oscilloscope display, or for the last two subjects, the responses were digitized and stored on a disc. Measurements were made from the photographs by hand with calipers for a-wave amplitude and for both amplitude and implicit time for x-wave, bx-wave, and b-wave. The responses of both eyes for each stimulus condition were averaged. The average amplitudes and implicit times were analyzed statistically by a

Table II. Linear regression for ERG amplitude

	Photo-stimulator setting	*No.**	*Constant*	*Age coefficient*	*R^2 age*	*Significance (two tail)*	*S.D.*
Light-adapted cone-mediated response:							
White (0.2 N.D.)							
b-Wave	1	23	34.0	−0.197	0.227	0.0216	6.5
	4	22	101.7	−0.614	0.325	0.0056	16.2
	16	23	284.0	−2.112	0.374	0.002	49.6
Red (K.W. 26)							
b-Wave	16	17	104.9	−0.660	0.414	0.005	13.4
Blue (K.W. 47)							
b-Wave	16	17	70.9	−0.629	0.465	0.002	11.5
Dark-adapted rod-mediated b-wave amplitude:							
White (2.4 N.D.)							
	1	19	103.4	−0.600	0.146	0.106	25.7
	4	21	230.5	−1.020	0.149	0.042	44.3
	16	19	449.7	−2.515	0.378	0.0051	55.9
Red (K.W. 26)							
	1	14	148.5	−1.077	0.283	0.050	30.7
	4	17	278.7	−1.627	0.371	0.0094	38.3
	16	16	476.1	−2.861	0.433	0.003	64.8
Blue (K.W. 47, 47A, 47B + 0.6 N.D.)							
	1	20	174.2	−1.551	0.474	0.0008	28.3
	4	19	349.6	−2.749	0.462	0.0014	49.9
	16	22	489.2	−2.397	0.293	0.0093	64.0
Dark-adapted mixed cone and rod-mediated amplitude (0.2 N.D.)							
a-Wave	1	22	75.1	−0.008	0.0001	0.968	15.3
	4	8	214.8	−0.255	0.0213	0.730	27.7
	16	24	307.2	0.191	0.0043	0.762	52.2
bx-Wave	1	22	543.3	−2.818	0.436	0.0004	57.2
	4	8	660.9	−3.92	0.520	0.022	60.4
	16	24	677.8	−2.59	0.310	0.005	68.8

*Number of observations.

CYBER computer with scattergram, condescriptive, linear regression, and multiple regression subprograms (Statistical Package for the Social Sciences, or SPSS, Version 7). The ERG parameters of each class of stimulus condition (dim white, bright white, red, and blue light) were analyzed by multiple regression against the variables, age and log intensity of the flash (log ft-Lambert-sec for white light, and log of μjoules/cm^2-steradian for red and blue light).

Results

The results of linear regression analysis of amplitudes vs. age and for multiple regression analysis of the amplitudes and implicit times by age and log intensity are shown in Tables II and III. Fig. 1 depicts averaged waveforms for the various stimuli. At the higher intensities for scotopically presented white light stimuli, the response is composed of a bx-wave mediated by both dark-adapted cones and dark-adapted rods. Note that an a-wave was obtainable only at the higher intensities. A small corneal positive wave, termed the *i-wave* by Nagata,[11] occurred following the b-wave of the light-adapted ERG. Under scotopic conditions and with red stimuli of bright intensity, this wave was only variably present. Figs. 2 to 4 represent scattergrams for cone-mediated responses, rod-mediated b-wave responses, and responses mediated by mixed cones and rods. For none of the implicit times was the linear regression

Table III. Multiple regression for ERG amplitude and implicit time

	No.[A]	Constant	Age coefficient (sig.[B])	Age R^2 (sig.)	Intensity coefficient (sig.)	Int. R^2 (sig.)	Mult. R^2 (sig.)	S.D.
Dark-adapted cone-mediated response (K.W. 26):								
x-wave amp.	62	157.1	−0.457 (0.135)	0.0005 (0.866)	253.8 (0)[C]	0.881 (0)	0.886 (0.000)[D]	39.2
x-wave imp.	51	48.2	−0.002 (0.945)	0.00004 (0.965)	3.39 (0.017)	0.114 (0.016)	0.114 (0.055)	3.9
Dark-adapted rod-mediated response:								
Red KW 26								
b-wave amp.	49	287.6	−1.96 (0.000)	0.106 (0.022)	243.8 (0)	0.769 (0)	0.846 (0.000)	48.9
b-wave imp.	49	102.1	0.069 (0.331)	0.012 (0.455)	−43.04 (0)	0.834 (0)	0.838 (0.000)	8.4
Blue KW 47, 47A, 47B +0.6 N.D.								
b-wave amp.	63	484.7	−2.22 (0.000)	0.057 (0.059)	282.2 (0)	0.787 (0)	0.861 (0.000)	49.7
b-wave imp.	63	74.1	0.116 (0.023)	0.0067 (0.524)	−35.4 (0)	0.838 (0)	0.852 (0.000)	6.3
White 2.4 N.D.								
b-wave amp.	68	869.9	−1.23 (0.000)	0.017 (0.285)	206.6 (0)	0.821 (0)	0.852 (0.000)	46.4
b-wave imp.	68	29.7	0.063 (0.251)	0.0012 (0.778)	−23.0 (0)	0.716 (0)	0.722 (0.000)	7.6
Dark-adapted mixed cone and rod–mediated response (0.2 N.D.):								
a-wave amp.	54	293.1	0.120 (0.696)	0.0027 (0.711)	180.2 (0)	0.901 (0)	0.901 (0.000)	37.5
bx-wave amp.	54	674.9	−2.80 (0)	0.202 (0.001)	107.8 (0)	0.414 (0.000)	0.638 (0.000)	61.7
bx-wave imp.	54	50.2	−0.024 (0.587)	0.007 (0.550)	−2.45 (0.050)	0.074 (0.046)	0.080 (0.120)	5.5
Dark-adapted white light response (2.4 and 0.2 N.D.):								
b- or bx-wave amp.	122	661.9	−1.892 (0)	0.010 (0.264)	131.71 (0)	0.861 (0)	0.890 (0.000)	63.4
b- or bx-wave imp.	122	41.4	0.0249 (0.637)	0.003 (0.572)	−18.98 (0)	0.874 (0)	0.874 (0.000)	9.8
Light-adapted cone-mediated response (0.2 N.D.):								
b-wave amp.	75	214.9	−0.886 (0.000)	0.031 (0.130)	139.0 (0)	0.787 (0)	0.822 (0.000)	35.3
b-wave imp.	75	26.8	0.0147 (0.171)	0.016 (0.285)	2.91 (0.000)	0.481 (0)	0.494 (0.000)	1.6

[A]Number of observations.
[B]Significance (two-tailed p value) of regression coefficient or R^2 occurring at given value vs. zero.
[C](0) indicates $p < 0.00005$.
[D](0.000) indicates $p < 0.0005$.

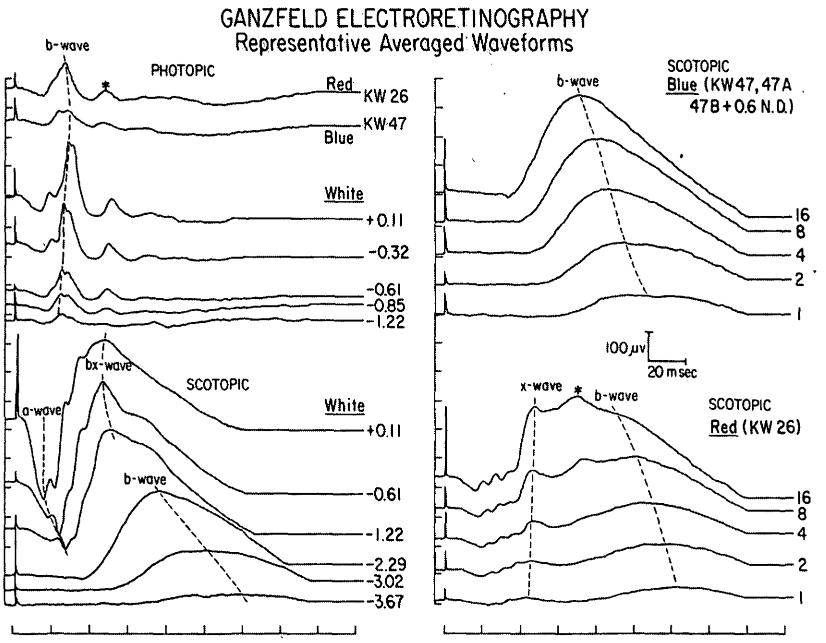

Fig. 1. Representative averaged waveforms of ganzfeld-evoked electroretinograms. The wave noted with the asterisk has been termed the *i-wave* by Nagata.[11] The intensity of the stimulus is indicated to the right of the waveform, as either log ft-Lambert-sec (for white light) or the photostimulator setting (for the red and blue light).

by age significant, although with greater data a small correlation (greater implicit time with older age) may exist. The x-wave amplitude and the a-wave amplitude for dark-adapted cones and for mixed responses of dark-adapted cones and rods to bright light stimuli also showed no significant age correlation. The light-adapted cone a-wave (measured only at a single flash intensity of 1.28 ft-Lambert-sec) showed significant correlation with age, but the age coefficient was quite small. The linear regression of dark-adapted rod-mediated and light-adapted cone-mediated b-wave amplitudes, whether elicited by white, red, or blue light, in general showed significant age correlation. Indeed, the linear regression age coefficient itself appeared to increase with increased intensity of stimulus (Table II). The amplitude response of mixed dark-adapted cones and rods (the bx-wave amplitude) showed significant age dependency; however, the age dependency seen with the higher bright light intensity stimuli was similar to that with the lower intensity bright light stimuli. The coefficients of determination, R^2, for the linear regression of b- and bx-wave amplitudes by age ranged from 0.146 to 0.520, indicating that for the data analyzed, 15% to 52% of the variation in responses can be attributed to age.

With the use of multiple regression with the data analyzed against age and log intensity, significant age correlation was again found for the amplitude of rod-mediated b-wave, scotopic bx-wave response mediated by mixed cones and rods, and the b-wave amplitude mediated by light-adapted cones. The rod-mediated b-wave implicit time elicited by the blue light showed a small but significant positive age coefficient; however, the age R^2 was very small with low significance.

Discussion

Although previous investigators[1-3, 12] have appreciated the age dependency of the b-

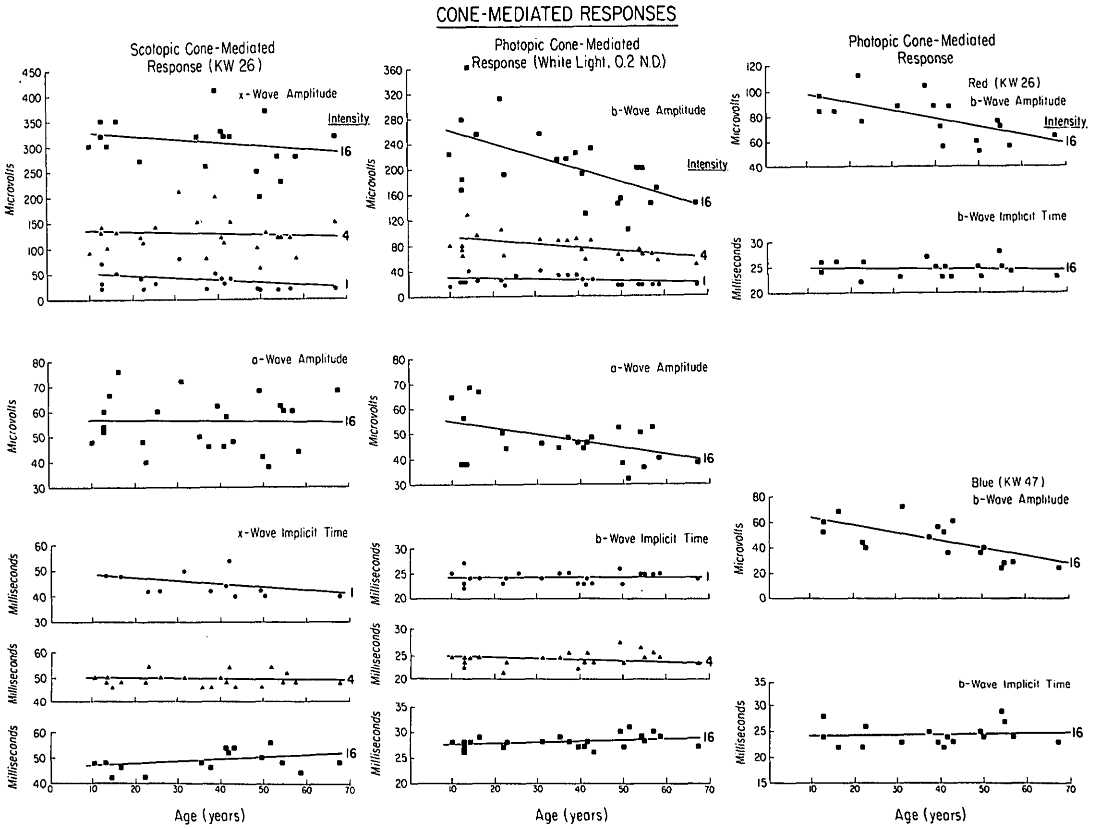

Fig. 2. Scattergrams for cone-mediated responses under dark-adapted (scotopic) and light-adapted (photopic) conditions. The linear regression slopes were significant for light-adapted a-and b-wave amplitudes only.

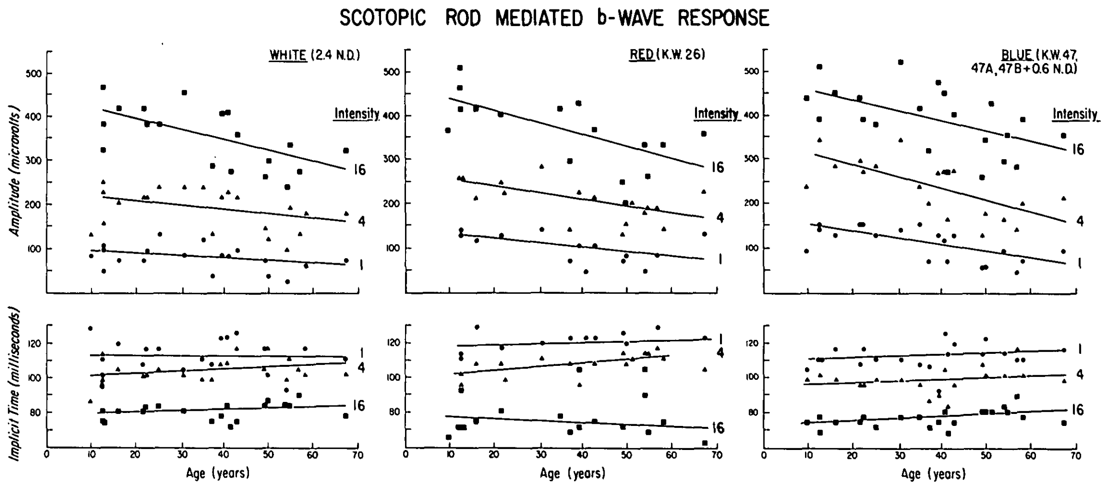

Fig. 3. Scattergrams for scotopic rod-mediated responses to white, red, and blue stimuli. Only amplitudes showed significant linear regression slopes. Note that slope for amplitude vs. age tends to steepen with greater intensity of stimulus.

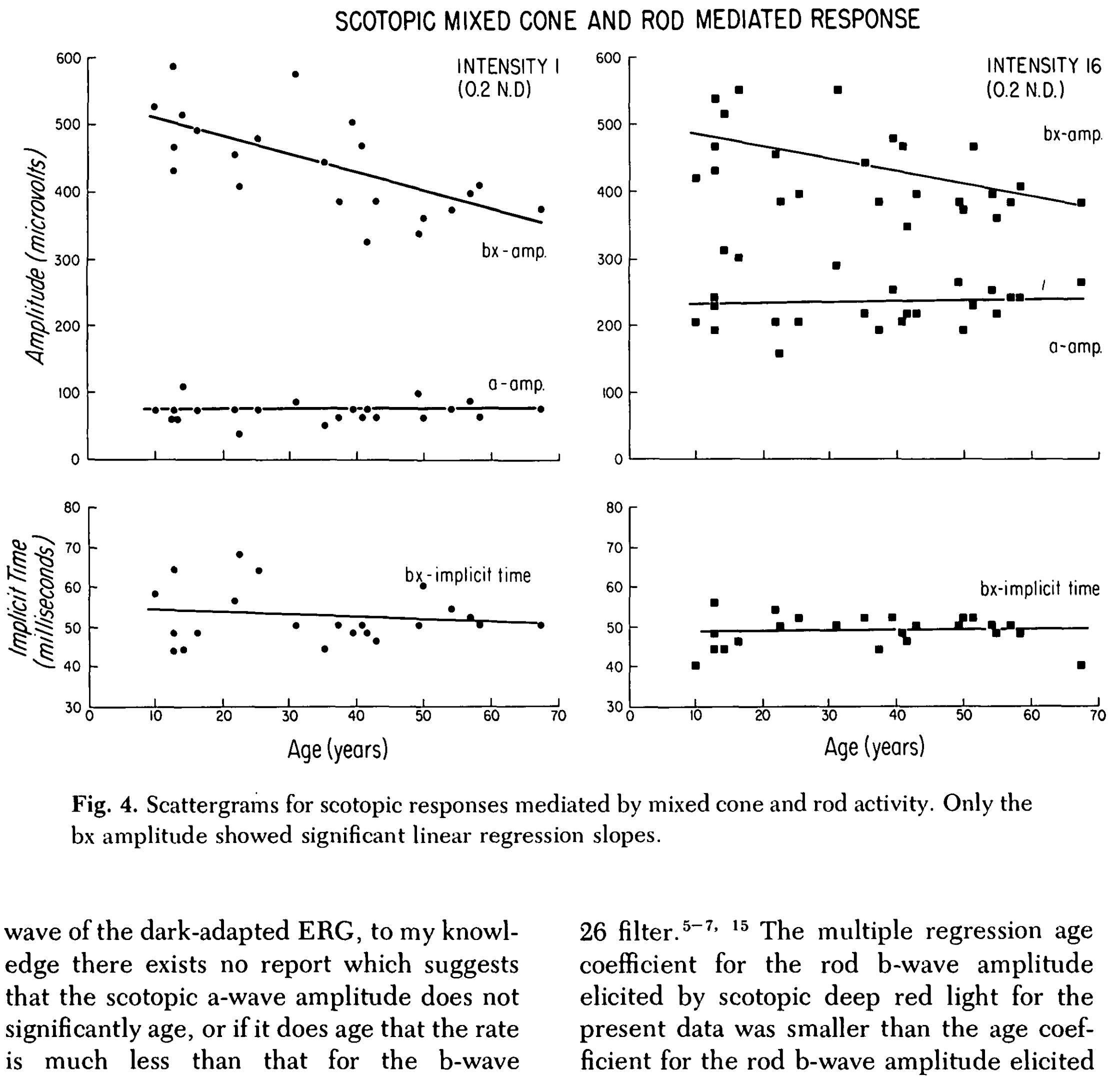

Fig. 4. Scattergrams for scotopic responses mediated by mixed cone and rod activity. Only the bx amplitude showed significant linear regression slopes.

wave of the dark-adapted ERG, to my knowledge there exists no report which suggests that the scotopic a-wave amplitude does not significantly age, or if it does age that the rate is much less than that for the b-wave amplitude.

Rod-mediated implicit times shorten significantly with higher intensities.[13, 14] The significant positive intensity coefficient for cone-mediated implicit times (x-wave and light-adapted b-wave implicit times) indicates that these cone-mediated implicit times lengthen significantly with increased intensity. The significant negative coefficient for age for the multiple regression of combined b-wave and bx-wave amplitudes with age and log intensity indicates that the slope itself for the stimulus-response curve decreases with age. (Table III).

Various authors have reported different deep blue filter combination as being scotopically matched with the Kodak Wratten 26 filter.[5-7, 15] The multiple regression age coefficient for the rod b-wave amplitude elicited by scotopic deep red light for the present data was smaller than the age coefficient for the rod b-wave amplitude elicited by the scotopic deep blue stimulus. Some of this difference at the higher intensity, such as intensity 16, may be related to uncertain identification of the peak of the b-wave or influence of the adjacent i-wave, especially when differentiation of these peaks is not clear as in Fig. 1. This difference in age coefficients may also be related to relatively greater absorbance of shorter wavelength light by normal aging lens, even when one attempts to study only clear lens. Thus the scotopic balancing of the responses elicited by Kodak Wratten 26 filter and 47, 47A, and 47B plus 0.6 neutral-density with regard to b-wave amplitudes may be valid only within a certain age range. It is important that the age of the normals be specified when discussing the balancing of scotopic or photopic long- and short-wavelength filters for equal b-wave responses. Indeed, a lesser neutral density filter in combination with the blue filter may be needed for scotopically balanced stimuli for older individuals.

Since all patients were specifically free of known eye disease and had clear media and normal complete ophthalmological examinations, the significant linear regression slope for the b-wave is most probably an effect of aging. Because women have greater ERG amplitudes than men,[1, 3] a division of normals into separate groups of males and females would be appropriate for evaluation of patients with suspected disease. Although this sex-related difference in ERG amplitudes might have slightly altered the individual slope or values for the age dependency curves, in the present experiment it could not have caused the marked age-dependent reduction of the b-wave amplitudes. Different mechanisms of membrane renewal between photoreceptors[16, 17] and middle retinal neurons may be responsible for the marked difference in aging between a-wave amplitude and b- or bx-wave amplitudes.

An important consequence of the aging effect on the human ERG is that normal 95% and 99% confidence ranges for clinical ERG laboratories should be calculated with mean and standard deviation only for those ERG waveform components which do not show significant aging. For rod- and cone-mediated b-wave amplitude the normal 95% and 99% confidence ranges should be calculated for each stimulus intensity with the use of either linear or multiple regression coefficients and the estimate of the standard deviation of y on x (i.e., the estimate of normal deviation around the linear regression line or multiple regression plane). The use of age-adjusted normal ranges will result in the ERG responses of certain patients falling outside of the normal range, whereas utilizing a non–age-corrected normal range will in these cases fail to allow significant discrimination from normal.

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