Aging and human cone photopigments

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We used a noninvasive technique to investigate changes in photoreceptor function with aging in observers 13–69 years of age. This technique, steady-state color matching, provides estimates of the optical density of cone photopigments, the illuminance that bleaches half of the photopigment, and the ratio of the primaries required at moderate light levels. In normal human retinas, we found that the optical density for a 4-deg field is affected minimally by aging from the second through the seventh decades. The average optical density is 0.27 ± 0.054. The optical densities of older observers varied more than those of younger observers. The change in optical density with age is nonmonotonic, with slightly lower values for the youngest and oldest observers in our study. The retinal illuminance that bleaches half of the cone photopigment varied little across observers, averaging 4.37 log Td ± 0.119. The change with age, which averages 0.00018 log Td per year over all observers, also is nonmonotonic. Moderate illuminance color matches did change with age, as expected, with a monotonic decrease with age in the ratio of the long-wavelength primary to the short-wavelength primary required for the color match.

INTRODUCTION

We have two interests in investigating the age-related changes in vision: (1) understanding developmental changes in the visual system, which may affect performance of everyday tasks, and (2) understanding ocular disease processes. To understand the etiology of age-related changes in visual function, it is necessary to identify the loci of these changes. For example, the most common cause of untreatable loss of vision in the elderly is age-related maculopathy.[1] In this disease, the retinal pigment epithelium (RPE) is the most-cited locus of morphological change.[2] Because the RPE provides the metabolic support for the photoreceptors, tests that examine the health of the photoreceptor–RPE complex should provide the best information for diagnosis of its dysfunction. However, not all people develop age-related maculopathy or other visual disorders, and those who do develop disease do so at different ages, with a dramatic upswing in the seventh and eighth decades.[1] Thus any useful measure of visual function change should show differences among observers before the onset of clinical disease and should reflect the sudden upswing in disease that occurs in the seventh and eighth decades rather than a gradual trend over large age ranges.[3] A uniform trend would, however, indicate a developmental change that may have important consequences for visual function, including performance on visual function tests or on everyday tasks.

Morphological studies in humans[4–6] support the notion that there are changes in the photoreceptor–RPE complex with age, including loss of photoreceptor outer segments and changes in Bruch's membrane in older eyes. Whether such changes are either common or large enough to affect significantly the photoreceptor function of persons in the general population has seldom been tested. The number of retinas per photoreceptor study is often so small, sometimes only one per age group or per study, that a large variability among samples[7] or techniques could account for the apparent loss of cones. There are developmental changes in the eye with age, and the eyes of children, particularly those under the age of 45 months, which should not be taken as representative of adult eyes without pathology. The foveae are immature at birth, and the photoreceptors continue changing in length and distribution postnatally, reaching only half the adult length by 45 months.[6] In older adults, in addition to the loss of photoreceptors that follows damage to the RPE, other types of damage to the photoreceptor–RPE complex have been discussed in morphological or function studies, including diminished ability to regenerate pigment,[8,9] damage to cone structure,[10] and loss of cones without damage to the RPE and adjacent layers.[5] Thus, from infancy on, there could be changes in photoreceptor function that are not directly related to impairment of visual function in older adults.

Color Matching As a Technique for Studying Cone Function

To examine cone function, we have developed a noninvasive technique, steady-state color matching. This technique and similar ones were used previously to measure photoreceptor function in normal adults,[10–12] as well as in patients with diseases such as diabetes, central serous retinopathy, and retinitis pigmentosa.[12–16] In steady-state color matching, the observer matches a standard light with mixtures of two primary lights, one longer and one shorter in wavelength than the standard light. Since all primary lights are 546 nm or longer (within the Rayleigh region of color space), only two primary lights are required for making a color match, rather than the usual three primaries. With the use of these primaries, our technique, as is the Nagel anomaloscope, is sensitive only to changes in long-wavelength-sensitive (LWS) and middle-wavelength-sensitive (MWS) cones and does not test changes in short-wavelength-sensitive cones (SWS). The chief difference between steady-state color matching and that performed with the Nagel anomaloscope is that with the former several retinal illuminances are used and the difference between the moderate- and high-illuminance color matches is used to compute a change in photopigment concentration. The technique is based on the dependence of the absorption spectrum of a pigment on the

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pigment concentration.[17] When the concentration of photopigment is decreased by bleaching, the photoreceptors undergo a change in their spectral sensitivities, which causes a predictable change in the color match. We typically report results from three parameters: the baseline color match (moderate retinal illuminance color match, photopigments at full concentration), optical density (the difference between baseline and high-illuminance matches, photopigments at full versus bleached concentrations), and the half-bleach illuminance I_0 (the amount of light required to bleach half the photopigment). If only the baseline color matches are examined, rather than the difference between high- and low-illuminance color matches, the effects of optical density are difficult to separate from changes in media transmission, e.g., spectral changes in lens absorption, as is discussed below.

Factors That Influence Color Matches

Several factors influence color matches. An increase in the optical density of the photopigment causes a shift in the moderate illuminance color matches,[12] so that more short-wavelength (green) primary light is required to match the standard. A decrease in photopigment concentration causes a shift in the moderate-illuminance color matches in the opposite direction: more long-wavelength primary light is required to match the standard, the so-called pseudoprotanomalous shift. Disorientation of the photoreceptors can cause a pseudoprotanomalous shift in color matches[18] as well as cause more light to be required for bleaching the photopigment.[13,15,16] With disoriented photoreceptors, two parameters are involved: optical density and half-bleach illuminance. Cataracts causes preretinal filtering and, typically, a shift in the moderate illuminance matches, so that more short-wavelength (green) primary light than normal is required in the match.[19] Since we use narrow-bandwidth primaries, spectral filtering from cataracts or other sources changes only the relative amounts of primary lights reaching the retina but not the spectral characteristics of each primary light. By measuring at both high and low retinal illuminances, and determining the difference, we minimize spectral filter effects. However, spectrally neutral filter effects must be considered. A decrease in retinal illuminance permits a rod contribution to at least the low-illuminance color matches, so that somewhat more longer-wavelength primary light is required to match the standard.[13] For an artifact from rod contribution to be measurable with our technique in 25–35-year-old observers, a 2-log-unit decrease below our lowest retinal illuminance is necessary. If so great a decrease in illuminance should occur, it would be impossible to bleach the photopigments, given our range of test illuminances. To avoid these two problems of greatly reduced retinal illuminance owing to increased preretinal filtering, we chose not to recruit observers with clinically observable cataracts. Also, we monitored pupil size and position to minimize artifacts in retinal illuminance caused by, for instance, decreased pupil sizes in older observers.

In previous studies of the possible change with age in photopigment optical density, not only color matching[10,19–21] but also retinal densitometry[9,22–24] was used. In retinal densitometry, the difference between the light entering and leaving the eye is used to calculate the optical density of the photopigment, under both bleached and unbleached conditions. There are two main differences between the measurements of optical density from densitometry and those from color matching. First, in retinal densitometry, any light absorbed contributes to the measured optical density, so that the number of cones, as well as the pigment concentrations, contributes to the measure. Second, scattered light, which increases with cataract and other media changes, causes an artifact in the amount of light reaching the detector. In contrast, for steady-state color matching, both of these factors are unimportant so long as two criteria are met.[13] First, vision must be good enough to permit the task to be performed. Second, enough light must pass through the media so that a good estimate of cone function, uninfluenced by rod contribution, is available for both the low- and high-illuminance matches. The number of photoreceptors will not influence color matches, so long as an equal number of quanta for each cone type are caught by functioning photoreceptors in the standard and mixture fields. However, as described above, color matching is greatly influenced by a change in the pigment within the photoreceptors or photoreceptor structure and alignment.

Previous Color-Matching Results for Effects of Aging

Previous studies in color matching offer little evidence that changes in cone function during or before the seventh decade are large or widespread or explained by a single biological mechanism. Results of color matching confined to low retinal illuminances showed that there are differences between younger and older observers.[10,19,20] In those previous studies, as well as in the present study, color matches were used that reflect only the contribution of LWS and MWS cones. Although changes in the SWS cone system are detected readily in older observers and in age-related eye disease, such as age-related maculopathy,[20,21] such changes do not necessarily reflect changes in pigment concentration, and they must be separated from lenticular changes.[21] One finding is that the matches of older observers require slightly more short-wavelength (green) primary light than do those of younger observers.[10,19] The typical explanation of this finding is that the increased absorption of the short-wavelength primary light in the lens of older observers leads to more of this primary light's being required to make the match, with no change in optical density.[19] An alternative explanation by Alpern is that the cone outer segment length increases in older observers, resulting in a higher optical density of the cone photopigment, which in turn causes more short-wavelength primary light to be required for the match.[10] The Alpern study included both high and low retinal illuminances; since the high-illuminance color matches showed no trend with age, preretinal filtering could not explain the data. The study included only six observers; and the test stimuli were foveal fields of only 0.7 deg, much smaller than is typical in either color matching or retinal densitometry.

A second finding is that the color matches for a large-area target become somewhat more similar to those for a small-area target for older observers than for younger observers (i.e., the color-match area effect decreases slightly with age).[10,20] Even with the elimination of variable observers, the difference between older and younger observers in color-match area effect still exists, although it is small.[20] These results also cannot be explained by preretinal filtering.

Since the baseline color matches are not reported, it is not known whether the baseline color matches of these older observers are consistent with the hypothesis of increased optical density. Increased optical density of all the cones might lead to less difference among them. The area-effect data, but not Alpern's data, are consistent with a third hypothesis, the relative decrease in the optical density of the central cones.

A third finding is that there is an altered Stiles–Crawford effect in some older observers, accompanied by a change in the color-match area effect, such that more long-wavelength light is required for the match.[25] This is consistent with the results obtained in patients with diseases that cause retinal deformation, implying some disorientation of the photoreceptors.[18]

A fourth finding is that there is more variation across individuals for the color matches of older observers, even older observers with 20/20 visual acuity,[10] in comparison with those of younger observers.[19] In summary, although the variability of measuring color matches seems to increase with age, whether the optical density increases, decreases, or remains constant with age is not well established.

Cone-Function Results from Densitometry

Results of studies with retinal densitometry[22,23] disagree about the effects of aging on human cone optical densities. Using an imaging densitometer with a large area of stimulation, Kilbride *et al.* found an age-related decrease in optical density among their 19 subjects.[23] With a smaller field of illumination, van Norren and van Meel found no age-related density difference among the 77 eyes of their subjects under the age of 50 years.[24] However, with the same instrument, Keunen *et al.*[9,24] found an age-related decrease in optical density among 29 subjects, with the decrease measurable only for subjects over age 60 years. In addition, for subjects in their seventh and eighth decades, more time was required to regenerate photopigment after exposure to a 2-min 1,000,000-Td light. No correlation was found with moderate-illuminance color matches by both densitometry methods.

METHOD

Apparatus and Stimulus

Using the computer-controlled, four-channel Maxwellian-view apparatus and the procedure described previously,[12] the observers made color matches for 4-deg bipartite fields at nine retinal illuminances from 260 to 260,000 Td, with 10 settings at each illuminance. Each observer matched the standard half field (589.6 nm) with a mixture of green primary (546 nm) and red primary (650 nm) lights in the variable half field. A dim 480-nm light minimized the contribution to the match of the SWS cones and rods. The 480-nm light was 2 log units lower in luminance than the standard light. The settings do not vary over a wide range of proportions of the 480 nm and standard lights.[12] By turning the color knob, the observer adjusted the ratio of the red versus the green primaries in the mixture, at approximately constant luminance. By turning the brightness knob, the observer adjusted the luminance of the red–green mixture. A computer continuously monitored the mixture and stored the settings.

At the beginning of the session, the observer was aligned to the optical axis of the apparatus by using infrared light-emitting diodes, a television camera, a video monitor, and a bite-bar positioner. The observer's pupil was centered on the 2.1-mm exit pupil of the apparatus. The observer's pupil size and position were monitored throughout the experiment so that all the light from the stimulus entered the pupil. Thus changes with age in pupil size were not a factor in our measurements.

After each session, the contribution of each primary light to the matches was calibrated with a photometer (EG&G Model 550), and the mean log ratio of the red-to-green primary luminances, log (R/G), was computed. Using a microcomputer-based simplex routine, we fitted a cumulative normal distribution to these data to obtain (a) the baseline color match, (b) the optical density, and (c) the half-bleach illuminance I_0. In addition, the standard deviation of the 10 settings at each of the nine retinal illuminances was calculated.[26]

Subjects

We report data for 52 normal observers between 13 and 69 years of age.[27] Informed consent was obtained after a full explanation of our experimental procedure and purpose. An inclusion criterion was good health, with the exception of arthritis and respiratory illnesses. We excluded observers taking drugs known to be retinotoxic. Observers who were more than 50 years old had eye examinations to ensure that they had normal retinas and optic media. We excluded those with ocular abnormalities, including drusen. Although possibly not reflecting the population as a whole, our older observers represent a group of people in whom any changes in visual function must be due to the normal aging process rather than to the side effects of illness or medication. The above criteria did decrease our sample size, since many older observers with clinically normal retinas, particularly older males with high blood pressure or heart disease, were taking medications. The ratios of males to females were 3:7, 8:9, 2:5, 2:3, 0:7, and 1:5 for the second, third, fourth, fifth, sixth, and seventh decades, respectively. No age distributions were reported for the proportions of males versus females in the two recent studies[9,23] in which retinal densitometry was used with the 29 and 19 subjects, respectively. Clearly, separate analyses of our 16 males and 34 females provide sample sizes comparable with the densitometry reports of positive findings of age-related changes in cone function.

RESULTS

Color matches changed as a function of retinal illuminance in a manner similar for all observers, irrespective of age (Fig. 1). Thus the same parameters adequately describe the data for all observers.

Optical Density

Optical density, as determined from the difference between high- and low-illuminance color matches, changed insignificantly with age; the slope was 0.0081 per year ($r = 0.250$, $p > 0.05$, two-tail test). Optical density was nonmonotonic with age: younger and older observers had slightly less optical density than did middle-aged observers (Figs. 2 and 3). The

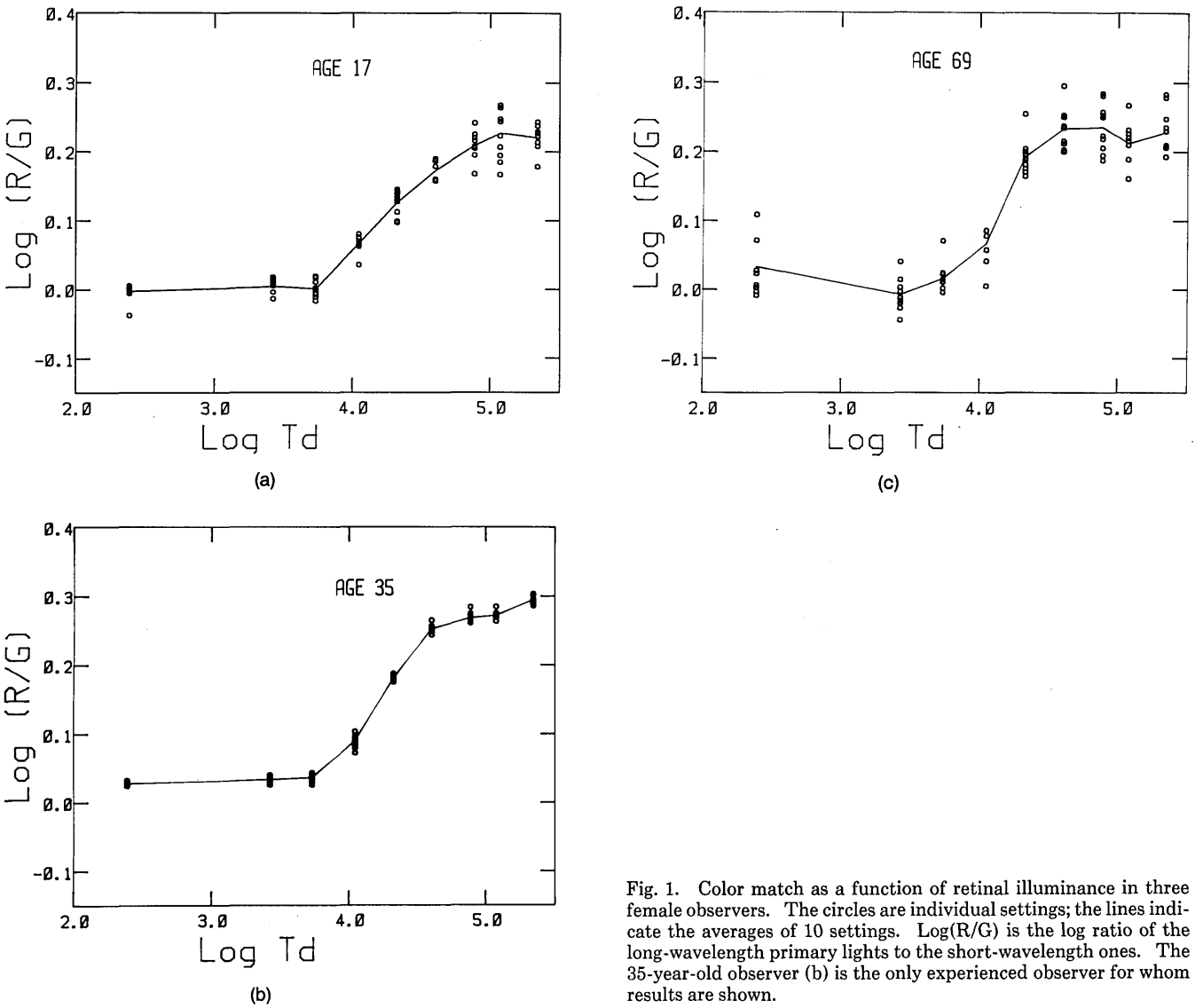
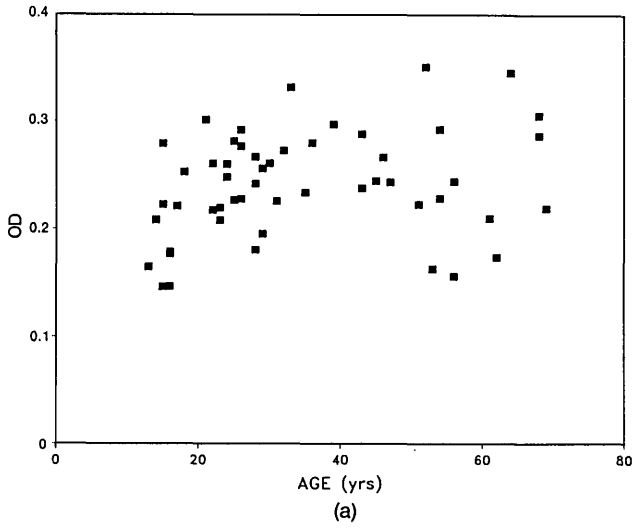


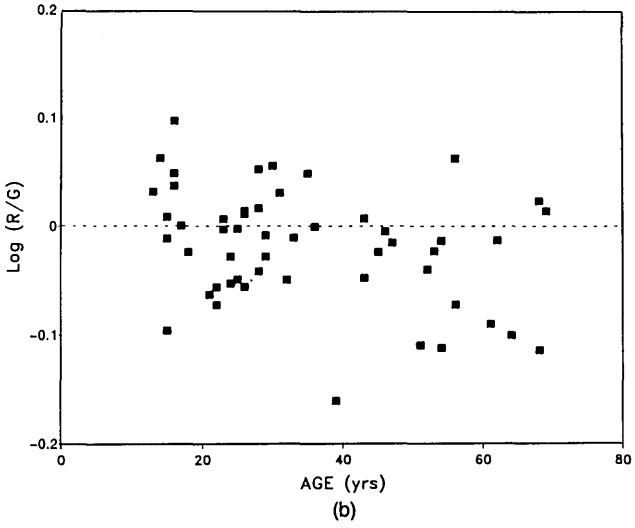
Fig. 1. Color match as a function of retinal illuminance in three female observers. The circles are individual settings; the lines indicate the averages of 10 settings. Log(R/G) is the log ratio of the long-wavelength primary lights to the short-wavelength ones. The 35-year-old observer (b) is the only experienced observer for whom results are shown.

data were divided into two groups, for observers below the age of 30 (n = 27) and for observers of age 30 or more (n = 25). Optical density significantly increased for observers aged 13 to 30, with a slope of 0.014 per year (r = 0.566, $p <$ 0.01, two-tail test). For observers of ages 30 through 69, optical density decreased only slightly, with a slope of −0.00057 per year. This trend was not significant (r = −0.123, $p > 0.2$, two-tail test), nor is there a significant change if only the observers of age 40 or more or those of age 50 or more were considered ($p > 0.20$). There was no significant trend for either the entire sample of males or females [log(R/G) increases per year of 0.000312 (r = 0.0797) and 0.000755 (r = 0.275), respectively]. Thus we could not reject the hypothesis that there is no uniform change with age in optical density for observers of age 30 and older. The average optical density for the entire sample was 0.27 ± 0.054, a combined value for both the MWS and LWS cone pigments.[28]

Variability in Optical Density

There was more variability in the optical density measurements across individuals in an older group (ages 50–69 years) than in a younger group (20–39 years) [$F(12, 23) = 6.42$, $p <$ 0.0001] (Ref. 29) (Fig. 3). This difference was unlikely to be due only to poor performance for the older observers for three reasons. First, although the variances were larger for older than younger observers for the 10 settings at the lowest retinal illuminance, $F(10, 18) = 2.7636$, $p < 0.05$,[30] there was no difference at higher retinal illuminances. Second, the variances for the settings were uncorrelated with the optical density for the older observers; i.e., more-variable individuals had neither higher nor lower optical density than less-variable ones, so that elimination of the variable individuals would not change the results.[31] Third, the parameter estimation from all nine retinal illuminances was not worse for the older observers as a group [$t(35) > 0.10$] (Ref. 32); the mean rms errors on the curve fitting were 0.00205 ± 0.00274 and 0.00339 ± 0.00353 for younger and older observers, respectively. Similarly, the half-bleach illuminance estimates were uncorrelated with the optical density measurements for the whole group, as well as for older or younger observers separately, indicating that the optical density estimates were not confounded by changes in bleaching across observers.





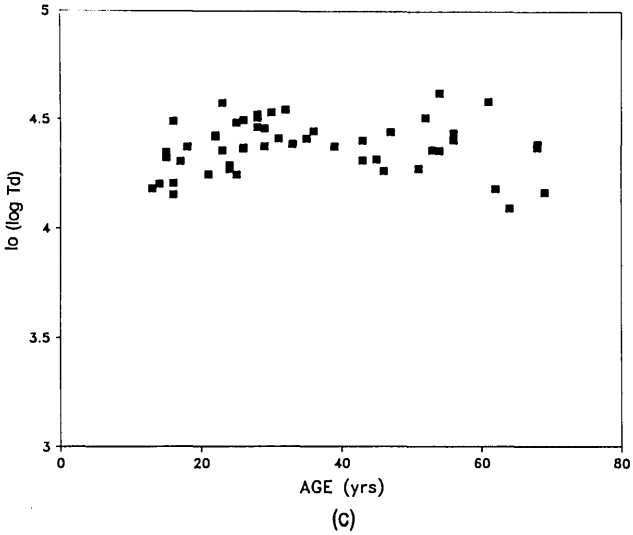
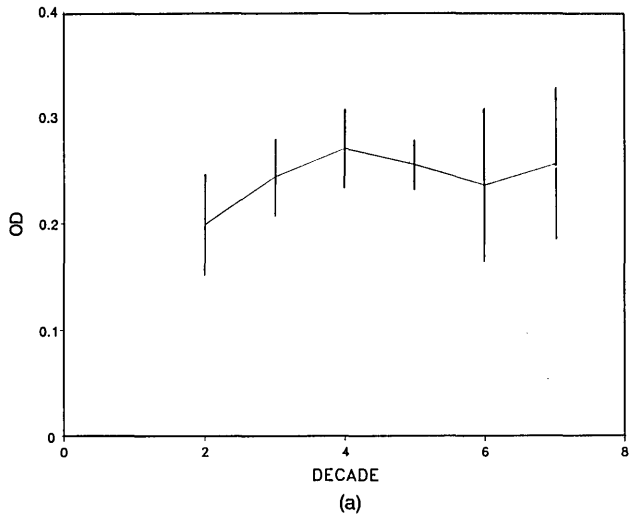
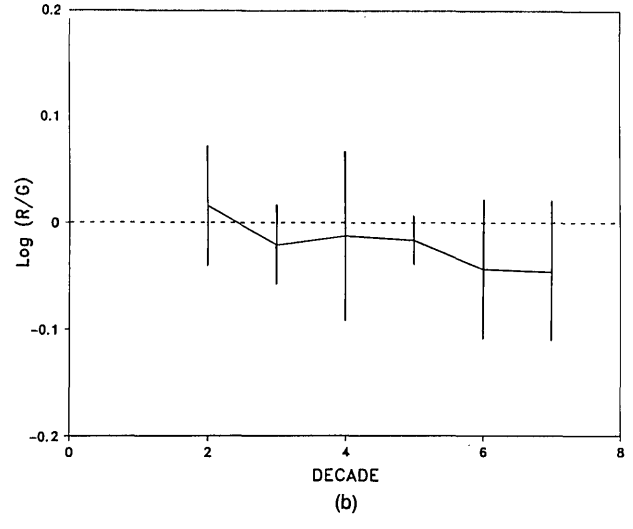


Fig. 2. Estimates for individual observers for each parameter. (a) Optical density as a function of age. (b) Baseline color match as a function of age. (c) Half-bleach illuminance as a function of age.





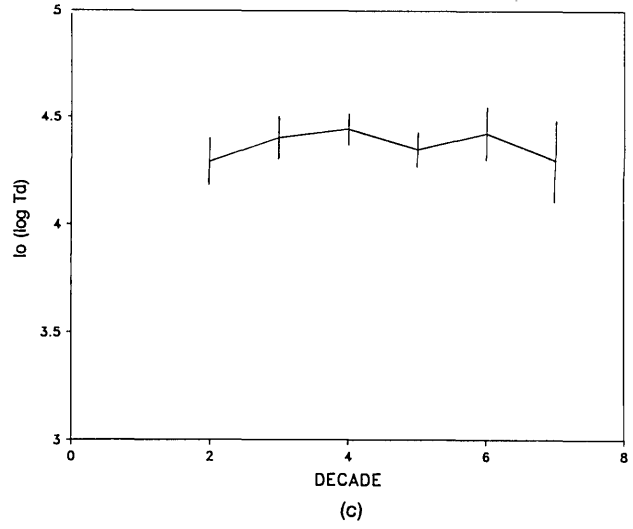


Fig. 3. Average of parameter estimates in each decade; vertical lines represent ±1 standard deviation. (a) Optical density as a function of age decade. (b) Baseline color match as a function of age decade. (c) Half-bleach illuminance as a function of age decade.

Changes with Age in the Color Match

In agreement with the results of previous studies, the moderate-illuminance (baseline) color matches changed with age; we measured changes of −0.00100 log(R/G) per year [$t(50) = -2.35$, $p < 0.025$, two-tail test]; that is, the shift was in the deuteranomalous direction, and there was a decrease with increasing age in the ratio of the long-wavelength primary lights to the short-wavelength ones required to match the standard.[30] If the change with increasing age in the baseline color matches could be explained by preretinal filtering, the high-illuminance color matches also should have changed in a similar manner. We found a change in the expected direction for the log(R/G) of the high-illuminance color matches of −0.0032 per year, but this trend did not reach significance. A possible explanation is that the variability of the high-illuminance color matches was significantly higher for all age groups [$F(51, 51) = 11.66$, $p < 0.0001$, worst case].

Half-Bleach Illuminance

The half-bleach illuminance, I_0, for all observers was 4.37 log Td ± 0.119. As shown in Figs. 2 and 3, the trends with age for the half-bleach illuminance were nonmonotonic. When the entire range of ages was analyzed, the change with age was insignificant, 0.00016 log Td per year, $t(51) = 0.157$. However, when the data were divided into groups, the half-bleach illuminance increased significantly between the ages of 10 and 30 years, 0.0123 per year [$t(24) = 3.43$, $p < 0.001$, one-tail test]. For subjects of ages 30 to 69 years the half-bleach illuminance decreased, −0.00385 log Td per year [$t(23) = -1.93$, $p < 0.05$].

DISCUSSION

We have shown that, as a group, older normal observers do not have measurably higher or lower optical densities than do younger normal observers. The technique that we used is insensitive to many optical factors, such as scattered light, preretinal filtering, and blur, that are expected to increase with age. In addition, the optical density measurements are insensitive to diseases, such as diabetes, in which no optical density change is found,[14] and glaucoma, that are more prevalent with increasing age. Since glaucoma affects primarily the inner retina, it is unlikely that the optical density measurements in the outer retina would be affected by glaucoma that is so early that it is undetected or subclinical. The screening of the older observers included a retinal examination and measurement of intraocular pressure. Our observers were healthy and took few, if any, medications. Since the risk factors for many diseases that are more common in older people are not well understood, we tried to eliminate as many risk factors as possible. Thus our observers cannot be considered to represent the population as a whole. They spanned the age range in which we would have measured a trend in optical density that reflects either (a) a single biological mechanism that contributes to visual deficits or age-related maculopathy or (b) a developmental change. We measured no such trend. Since our technique is sensitive enough to detect subtle disease-related changes in photoreceptor–RPE function,[13–16] we may conclude that the photoreceptor–RPE complex in healthy older individuals functions in a manner similar to that in younger observers.

The variability of the optical densities was higher for older than for younger observers. A useful clinical test of visual function should have a sufficient range of results across individuals to identify candidates with possible beginnings of eye disease. A high correlation of any measure with age gives no additional predictive power over age alone, since it provides redundant information. Some of the older individuals in our study approached the limits of what would be considered normal optical density for younger observers (Fig. 2); perhaps they are in the preclinical stages of retinal disease.

Our data agree with those of previous reports in which only moderate-illuminance color matches were used, in that we found the expected shift in the deuteranomalous direction; i.e., relatively more short-wavelength primary light was required for making the match. We did not expect to measure a pseudoprotanomalous shift, such as that found either when the retinal illuminance of the test field is reduced, as previously reported,[12] or when the photoreceptors are misaligned with respect to the incoming light,[18] for two reasons. First, our observers were screened for lens changes and thus did not have dense lenses. Second, we controlled for the amount and direction of light entering the pupil through the use of a Maxwellian-view apparatus with a bite-bar positioner and a pupil-monitoring system. According to a recent report,[33] we calculated that there are changes in lens density for the 546-nm primary light that should produce about a −0.0035 change per year in log(R/G) for observers of ages 13–69 years. For the moderate-illuminance color matches, we found a significant change of −0.00100 log(R/G) per year, which is in reasonably good agreement.

For younger observers, the half-bleach illuminance may increase with age owing to decreases in clarity of the ocular media. Increases in lens density with age[33] predict that the half-bleach illuminance should increase ~0.0008 log Td per year for younger observers, while we found an increase of ~0.0123 per year. There may be other factors, such as changes in photoreceptor structure as the photoreceptors mature. For older observers, the media become denser, as indicated by the slight shift in the baseline color match. Although the effects of increasing lens density should cause the half-bleach illuminance to increase further, by about 0.00168 per year, the reverse was the case: the half-bleach illuminance decreased. This decrease is consistent with the hypothesis that the photopigments may regenerate more slowly, requiring less light to bleach half the photopigment. There is support for this hypothesis in the most recent retinal densitometry report.[9] The densitometry data are modeled by no increase in scattered light for older observers but rather by an increase in regeneration time and a decrease in optical density.[9] Although we found no obvious changes in optical density, our data are consistent with a change in regeneration time. We cannot rule out the possibility that the retinal densitometry data describe the effects of aging on the number of cones, while we are measuring the photopigment only in functioning cones. To resolve the differences between our findings and those obtained by retinal densitometry, it is necessary to test the same population with both techniques, using an adequate sample size and healthy observers with clear media.

To summarize, in healthy observers we found little age-

related change in optical density of cone pigments. There was greater variability in the optical densities of older observers in comparison with those of younger observers. There was a small, nonmonotonic change in the half-bleach illuminance. There was also a change in the baseline color matches that is consistent with increasing lens density with increasing age.

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26. Sandard deviations were not available for some observers, chiefly those who were tested in a previous report with several field sizes and fewer points.
27. Two observers were omitted from data analysis because their variability and I_0 values were so extreme that an accurate measure of optical density could not be obtained.
28. The reported density represents the densities for both the LWS and MWS cones, which are likely to have a density ratio of 1.33, as described in V. C. Smith, J. Pokorny, and S. S. Starr, "Variability of color matching data. I. Interobserver variability in the unit coordinates," Vision Res. **16,** 1087–1094 (1976). Thus the MWS density is slightly lower, and the LWS is higher, for computations based on the fundamentals of Smith *et al.* Our reported density is in close agreement with that predicted for a 4-deg field by the equation on p. 1098 of J. Pokorny, V. C. Smith, and S. S. Starr, "Variability of color matching data. II. The effect of viewing field size on the unit coordinates," Vision Res. **16,** 1095–1098 (1976).
29. It would be desirable to perform a planned comparison of the mean optical density of the two groups. However, the variances of the two means are unequal; thus one of the assumptions necessary to perform a t test to compare the two group means has been violated. The variances of observers in the two age ranges 20–39 and 50–69 years were compared with F ratios for optical density, baseline color match, and half-bleach illuminance. Only the F ratio for optical density was statistically significant.
30. The variances for all observers who had 10 settings per retinal illuminance were averaged for each group, the mean-square error was computed, and an F ratio was formed. Two observers (A.E. and S.B.) were omitted from the 20–40-year-old group, because they were highly practiced and could be expected to have smaller variances.
31. One low retinal illuminance (2600 Td) and one high one (130,000 Td) were selected as sample standard deviations. The correlation coefficients of each set of the standard deviations and optical density were computed for observers of ages 40–69 years and were not significantly different from 0 ($p > 0.1$).
32. The change with age in the results of the color match does not reach significance if the data are divided into two groups. Also, the variability across observers does not increase with age. The variability across older observers was not significantly greater than across younger observers ($p > 0.05$).
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