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AGING AND HUMAN MACULAR PIGMENT DENSITY

APPENDED WITH TRANSLATIONS FROM THE WORK OF MAX SCHULTZE AND EWALD HERING

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Abstract—The optical density of human macular pigment was measured for 50 observers ranging in age from 10 to 90 years. The psychophysical method required adjusting the radiance of a 1°, monochromatic light (400–550 nm) to minimize flicker (15 Hz) when presented in counterphase with a 460 nm standard. This test stimulus was presented superimposed on a broad-band, short-wave background. Macular pigment density was determined by comparing sensitivity under these conditions for the fovea, where macular pigment is maximal, and 5° temporally. This difference spectrum, measured for 12 observers, matched Wyszecki and Stiles's standard density spectrum for macular pigment. To study variation in macular pigment density for a larger group of observers, measurements were made at only selected spectral points (460, 500 and 550 nm). The mean optical density at 460 nm for the complete sample of 50 subjects was 0.39. Substantial individual differences in density were found (*ca.* 0.10–0.80), but this variation was not systematically related to age.

Macular pigment Color vision Aging

INTRODUCTION

The central portion of the human retina contains a yellow pigment called the macular pigment. This pigment is most dense in the receptor fiber layer and inner plexiform layer (Snodderly *et al.*, 1984) and, as such, screens short-wave light enroute to the outer segments. Based on its absorption spectrum, the macular pigment is generally believed to be a carotenoid (3,3′-dihydroxy-α-carotene) similar to leaf xanthophyll (Wald, 1949) or perhaps a mixture of two carotinoids (Bone *et al.*, 1985). Although several other photostabile, yellow pigments have been identified in the primate retina (Snodderly *et al.*, 1984), they are visually less prominent in difference spectra because of their relatively flat spatial density distribution across the retina. In contrast, psychophysical and microspectrophotometric data show that the macular pigment density of most subjects declines in an exponential fashion from the fovea radially outward, so that by about 5° there is virtually no change in density with further eccentricity (Stabell and Stabell, 1980; Viénot, 1983; Snodderly *et al.*, 1984; Moreland and Bhatt, 1984; Wooten *et al.*, to be published; also see Hurvich, 1969).

The confinement of most of the macular pigment to the avascular zone of the retina suggests that it may have an important metabolic function, although clear evidence for this hypothesis is lacking (Dartnall and Thomson, 1949). Two facts that render this hypothesis less compelling are that the foveola is probably not completely avascular in some subjects (Bird and Weale, 1974; Medina *et al.*, 1986), and, as Ruddock (1963) pointed out, some observers have virtually none of this yellow pigment. The function most popularly accorded to the macular pigment is to reduce the effects of chromatic aberration and glare, a hypothesis suggested in 1866 by Max Schultze (see Appendix 1; Walls and Judd, 1933). By absorbing short wavelengths prior to the arrival of the stimulus at the outer segments of the photoreceptors, effects of chromatic aberration are reduced, particularly in the area centralis. Indeed, Reading and Weale (1974) calculated the ideal filter necessary to reduce the blur circle associated with chromatic aberration to below threshold and found that its absorption spectrum is similar to macular pigment.

The macular pigment, in concert with the crystalline lens, may also play a protective role in retinal function since their combined absorption is greatest for wavelengths that are most

effective in producing actinic damage to the retina (Ham *et al.*, 1982). Interestingly, Schultze (1866) also entertained this hypothesis. The crystalline lens is an excellent absorber of potentially damaging ultraviolet radiation while the macular pigment provides some protection against the hazards of short-wave visible light. In addition, by virtue of its concentration in the fovea, the macular pigment is well situated to protect the retinal area, and the class of photoreceptors (the short-wave cones) most vulnerable to actinic damage (Sykes *et al.*, 1981; Sperling *et al.*, 1980). One might speculate that this protective function is not only made possible by selective spectral absorption, but perhaps also by some involvement in scavenging for cytotoxic free radicals (see also Feeney and Berman, 1976; Kirschfeld, 1982). Krinsky (1979) has described several mechanisms by which carotenoid pigments can protect cells against photosensitized oxidations.

Historically there has been much debate about the macular pigment (Nussbaum *et al.*, 1981). Its very existence has been denied by several prominent investigators (e.g. Gullstrand, 1907) who considered it a post-mortem artifact. Others have assigned a primary role to the macular pigment in their explanations of entoptic phenomena such as Haidinger's brushes and Maxwell's spot, as well as for anomalies in color vision. Although the macular pigment is likely to be partially responsible for these phenomena, it has not been established as sufficient to entirely account for them.

These complications and debates about macular pigment function notwithstanding, it has generally been argued since the early investigations of Schultz (1866; Appendix 1) and Hering (1885; Appendix 2), and the more modern quantitative studies by Wright (1928–29), that individual variation in the macular pigment contributes significantly to individual differences in color vision. If macular pigment density changes with age, as do other pigments of the eye, it may also produce developmental variations in color vision. Indeed, changes in color vision, particularly color matching, have been used to infer the nature of age-correlated changes in macular pigment density. This approach has not, however, yielded consistent answers, with some color-matching data suggesting the possibility of age-related changes in macular pigmentation (Wright, 1947; Stiles and Burch, 1959; Lakowski, 1962) and others suggesting no change (Ruddock, 1965). Several more recent investigations indicate that there is no systematic change in macular pigment density with age (Bone and Sparrock, 1971; Yasuma *et al.*, 1981), but in these studies (1) the age range was limited or not specified, and/or (2) the methods involved psychophysical procedures that failed to control for inhomogeneity of the receptor mosaic (i.e. rods and short-wave cone distribution), and/or (3) it was not established that the methods yielded a density spectrum of the proper shape (i.e. xanthophyll-like).

There are several reasons for suspecting that macular pigment density might change with age. Many of the 19th Century histological investigations (e.g. by Schultze, Hering, and others; see Appendices 1 and 2) indicated relatively little macular pigmentation in young retinas. It is well known that other ocular pigments accumulate or change during aging, including that of the crystalline lens (Said and Weale, 1959; Werner, 1982) and lipofuscin in cells of the retinal pigment epithelium (Feeney, 1978; Wing *et al.*, 1978). Finally, macular pigment is dependent on diet (Malinow *et al.*, 1980); *if* there are significant age-correlated changes in diet, a change in macular pigmentation—possibly a decrease—might be found in older individuals. In their review of the literature, Nussbaum *et al.* (1981) argue that the developmental course of macular pigment accumulation in early life, as well as in aging and senescence, is one of the major unresolved issues in our understanding of this pigment. For these reasons, we sought to measure the developmental variation in macular pigmentation.

METHODS

A variety of direct and indirect methods have been used to estimate the macular pigment density spectrum (Ruddock, 1972; Vos, 1972). The former consist of extracting the pigment directly or measuring the absorption spectrum by reflection densitometry or microspectrophotometry. The latter involve deriving an absorption spectrum from the difference between foveal and parafoveal sensitivity or color matches. The procedures used in this investigation were introduced, but not elaborated, in an earlier paper by Werner and Wooten (1979a; also see Knoblauch, 1981). The first task of the present study was to establish the reliability and validity of the method (see also Wooten *et al.*, to be published) and then to apply it to a large group of observers of varying age.

Subjects

Fifty trichromats, male and female Caucasians, participated in the experiments. Their ages ranged from 10 to 90 years and they had little or no previous experience as psychophysical observers. The participants were all in good general health, had normal color vision according to the Dvorine plates, and no evidence of retinal or ocular pathology according to self-reports and/or examinations by a local ophthalmologist. Corrected visual acuities exceeded 0.65 for all subjects. Seven other subjects were brought to the laboratory but their data are not included because they were not able to complete the task.

Stimulus

Figure 1 illustrates the stimulus that was viewed with the right eye. The central 1° test portion consisted of a 460 nm standard (Ditric interference filter with 8 nm bandpass at half power) presented in square-wave counterphase at 15 Hz with a variable wavelength. The observers fixated straight ahead for foveal presentations or at a fixation point (a dim red LED or a black arrow) located 5° to the right for parafoveal (5° temporal) presentations. This test stimulus was presented superimposed on a 10°, steady, broadband background (Corning 5–60; $\lambda_d = 445$ nm). The luminance of the test and background portions of the stimulus were -0.49 and -0.92 log cd/m^2, respectively.

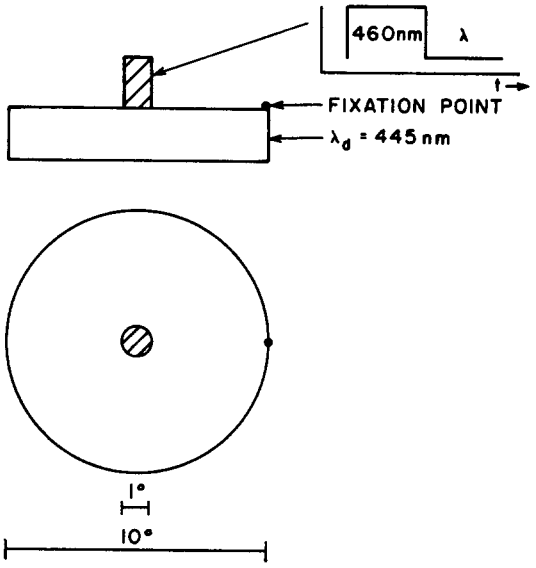

Fig. 1. Schematic of the stimulus; the 1° test portion consisted of a 460 nm standard presented in counterphase (usually at 15 Hz) with a variable wavelength. It was superimposed on a steady, 10°, short-wave background. The test stimulus was viewed both foveally and parafoveally (when fixation of the right eye was directed to a point at the edge of the background, the test stimulus was imaged 5° temporally).

Apparatus

The optical system was a three-channel projection system derived from a single source, a 1 kW xenon arc lamp that was regulated by a d.c. power supply at 980 W. Channels 1 and 2 were used to form the test stimulus, and channel 3 provided the broadband background.

Because the three channels were similar, only channel 1 will be described in detail. The light in channel 1 was collimated by a lens and passed through an 18 cm water bath to filter out infrared radiation, and then focused onto the entrance slit of a holographic grating monochromater (Instruments SA). Entrance and exit slits were 3 mm, resulting in a 12 nm bandpass at half-power. The emergent beam was collimated and refocused onto a circular neutral density wedge (Kodak, Inconel type), which was coupled to a potentiometer so that the position could be read on a digital voltmeter. An aperture was placed at a focal point to pass the arc image, but eliminate the electrode image. This beam was then recollimated and passed through a filter box which held the neutral density filters. The light was focused onto a rotating sectored mirror that was mounted to a regulated light chopper (Brower Engineering) where it was brought together at a right angle with the focussed beam from channel 2.

Channels 2 and 3 were spectrally shaped by narrow-band interference and broadband filters, respectively, rather than by a monochromater. The combined beam from channels 1 and 2 was then collimated and passed through a field stop, conjugate with the projection screen, which determined the subtense of the stimulus. A similar, but larger, field stop was used in channel 3. A pellicle combined the test field with the background field from channel 3. The final lens projected the three beams onto a rear-projection screen located in a light-shielded booth.

The observers were positioned in an adjustable dental chair approximately 50 cm from the projection screen. An adjustable chin rest was used to hold their heads steady and to center their right eye with respect to the stimulus.

Spectral energy measurements and calibrations of the neutral density filters and wedges

were made with a *P-I-N*-10 silicon photodiode and linear readout system (United Detector Technology Corp.). Calibrations of this instrument are traceable to the National Bureau of Standards. Photometric measurements were obtained using an S.E.I. photometer and spectral transmission measurements of interference and broad band filters were made with a scanning spectrophotometer (Cary Model 219). Logarithms and optical densities are expressed in decadic units throughout this paper.

Procedure

Sessions began with 10 min dark adaptation followed by 4 min adaptation to the broadband background. The psychophysical procedure involved the method of adjustment. The subject's task was to turn a dial, moving the wedge in the path of the variable wavelength, to eliminate or minimize flicker. This task is different from standard heterochromatic flicker photometry only in that we use a 460 nm standard, the wavelength of maximal absorption by macular pigment. After several practice trials, subjects could reliably adjust the wedge for the fovea, where macular pigment density is greatest, and 5° in the parafovea, where macular pigment density is at a low, relatively constant level with further changes in eccentricity. For several observers one or two practice sessions were required.

Measurements were taken at 10 nm intervals over the region from 400 to 540 or 550 nm when generating the complete spectral density curves and at selected spectral points when determining only the peak density for the larger group of observers. The exact number of wavelengths and settings per wavelength varied depending on the difficulty experienced by the subjects with the task. The complete spectral curves are based on data from 12 observers who were tested on an average of 10 wavelengths (range: 5–16) with a mean of 19 settings per wavelength (range: 8–28). The mean number of sessions required was 2 (range: 1–4). The selected spectral measurements obtained with the other 38 subjects were taken at three wavelengths with a mean of 5 measures per wavelength (range: 4–12). The presentation order for the different wavelengths was pseudorandom.

The assumption underlying this method is that the difference spectrum between foveal and parafoveal sensitivity under these conditions is dependent only on the density of the macular pigment. (It is necessary to normalize the data relative to a wavelength, such as 540 or 550 nm, where macular pigment absorption is zero.) The broadband blue background and 15 Hz flicker are assumed to eliminate responses from rods and short-wave sensitive cones (Brindley *et al.*, 1966), leaving sensitivity mediated by middle- and long-wave cones. There is evidence that the middle- and long-wave color mechanisms mediating sensitivity in this task are represented in equal ratios for the fovea and parafovea (Wooten and Wald, 1973), but the ultimate justification for this assumption is in the similarity between the difference spectra and a standard curve representing macular pigment absorption.*

RESULTS AND DISCUSSION

Macular pigment absorption spectrum

Figure 2 illustrates the results from a young adult who had limited previous experience in psychophysical experiments. The top panel shows her foveal and parafoveal spectral sensitivity from 400 to 550 nm. Although the error bars are somewhat larger for parafoveal compared to foveal viewing, the data seem quite reliable for both conditions. In agreement with Wald (1945), who measured absolute thresholds during dark adaptation following a high bleach, the parafoveal sensitivity curve is elevated at short wavelengths, whereas the data are essentially superimposable for the two conditions from 530 to 550 nm. The elevation in Wald's data may be partially due to changes in short-wave cone density with eccentricity (Weale,

*A complication with our method arises because of the greater optical density of foveal relative to parafoveal cones and the resultant effects of self-screening (Pokorny and Smith, 1976). Fortunately, control experiments and theoretical considerations by Wooten *et al.* (to be published; also see Knoblauch, 1981) with normal trichromats and a protanope suggest that self-screening would, at best, have a small effect on absolute density of the macular pigment measured in this way. Since their calculations are based on data for young adult observers we are still faced with the possibility that differential self-screening for foveal and parafoveal cones would affect the data from older observers. This is likely only if foveal cone optical density *increases* with age, but the limited existing data suggest no change or the opposite (v. Norren and v. Meel, 1985; Kilbride *et al.*, 1986; Eisner, in press); a decrease in foveal and/or parafoveal optical density would negligibly affect our macular pigment density determinations. Finally, there is nothing in the data that would require the assumption of significant self-screening changes in foveal relative to parafoveal cones of the elderly.

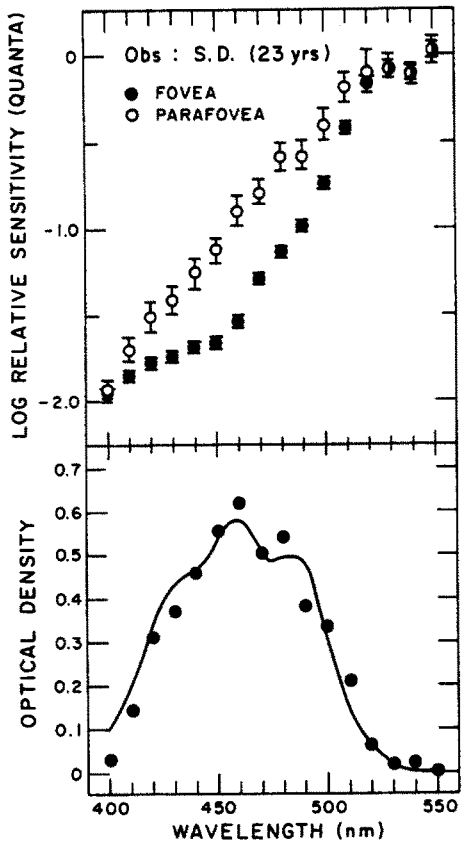

Fig. 2. The top panel shows log quantal sensitivity as a function of wavelength for foveal and parafoveal viewing. Error bars denote ±1 SEM. The difference spectrum, plotted in the lower panel, is fit by a scalar adjustment of the macular pigment density spectrum (smooth curve) of Wyszecki and Stiles (1982).

1953; Wooten *et al.*, 1975), but this seems unlikely for our conditions. Because it can be assumed that short-wave cones and rods are not sensitive under our conditions, we attribute this difference to macular pigment absorption. The lower panel shows this difference spectrum plotted against the standard curve of Wyszecki and Stiles (1982), with the latter adjusted by a scalar. To the extent that the data points fit the smooth curve, the difference spectrum under these conditions can be attributed to macular pigment absorption.

Difference spectra for four other observers are presented in Fig. 3. The data are normalized by a scalar to a $\lambda_{max} = 0.50$ for comparison with the shape of the macular pigment density spectrum of Wyszecki and Stiles (1982). It should be noted that these data were all obtained with naive, unpracticed observers who had little previous experience as psychophysical subjects. Yet, there is fairly good agreement between the data and smooth curve. Average data from twelve subjects are presented in Fig. 4; the number of subjects contributing data to each point ranged from 1 to 12. There is excellent agreement between the data and the standard curve of Wyszecki and Stiles. The average absolute deviation between the data and the curve is 0.02 optical density. Although these deviations from the smooth curve are small, they appear systematic at short wavelengths. A similar pattern was also observed by Wooten *et al.*, using highly practiced subjects and Vos (1972) has

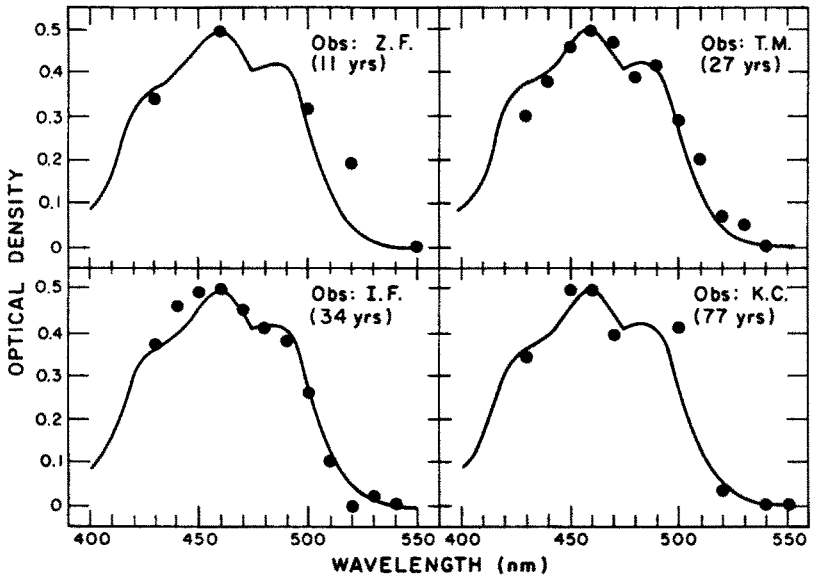

Fig. 3. Difference spectra for foveal and parafoveal viewing are plotted for individual observers with scalar adjustments to fit the macular pigment density spectrum (smooth curves) of Wyszecki and Stiles.

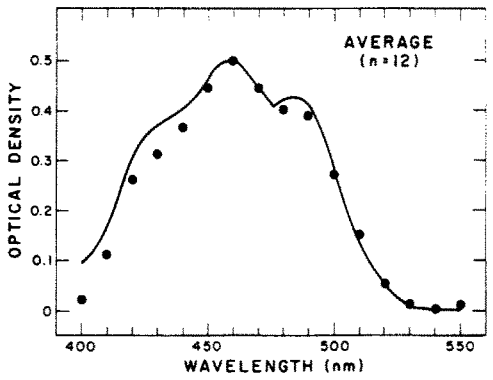

Fig. 4. Macular pigment density (normalized and averaged, for twelve observers) is plotted with the standard density spectrum (smooth function) of Wyszecki and Stiles. Observer ages were: 11, 19, 20, 22, 23, 23, 26, 27, 27, 34, 56 and 77.

proposed that minor modifications in the standard macular pigment spectrum may be necessary to account for the difference between 2 and 10° color matching functions. Thus, it is not clear whether our data are too low or the standard curve is too high at short wavelengths, but the differences are slight in any case. It may be concluded that our technique does allow for precise determinations of the macular pigment absorption spectrum over the region from 400 to 550 nm.

An additional validation of our method was obtained by measuring the macular pigment density of an observer with foveal test stimuli of different diameters. Because the macular pigment density declines with eccentricity, a decrease in density with increasing foveal field sizes should be observed. These results are shown in Fig. 5 with density plotted as a function of the radius of the foveal test spot. The smooth curve, which the data fit well ($r^2 = 0.96$, $P < 0.01$), was determined by Wooten *et al.* (to be published) using tiny test probes at various retinal eccentricities. The fact that their function fits the radius of the foveal test spots implies that sensitivity in this task is entirely dependent on retinal stimulation at the edge of the stimulus, an idea that has been proposed before in other contexts (Walraven, 1973; Ratliff, 1978). In terms of our macular pigment data this result strongly implies that the density measured for a 1° spot is not an average within the area stimulated, but rather the density at the edge of the area of stimulation. Thus, insofar as the density declines exponentially with eccentricity, our method will underestimate the average density within a 1° area. This may, of course, be appropriate for correcting psychophysical data that may also be dependent on sensitivity at the stimulus edge.

Methodological controls

It appears that our method is adequate to determine the macular pigment density spectrum from 400 to 550 nm. However, several problems in using this method with observers covering a wide age range were anticipated since it is well known that there are many age-correlated changes in vision (Weale, 1963). To rule out potential confounds from these latter variables, ancillary tests were conducted with young subjects to simulate visual deficits that might be present in some of the elderly.

Accommodation and refraction. Compared to young subjects, older observers tended to have lower visual acuity and they could not accommodate to the projection screen. We tested a young observer under normal conditions and with trial lenses to induce optical blur of the test stimulus. His visual acuity was reduced to 0.3, an acuity worse than any subject included in this study. This reduction in visual acuity was not, however, associated with a changed estimate of his macular pigment density. Thus, the lower visual acuity in older subjects does not seem to significantly affect our ability to measure their macular pigment density.

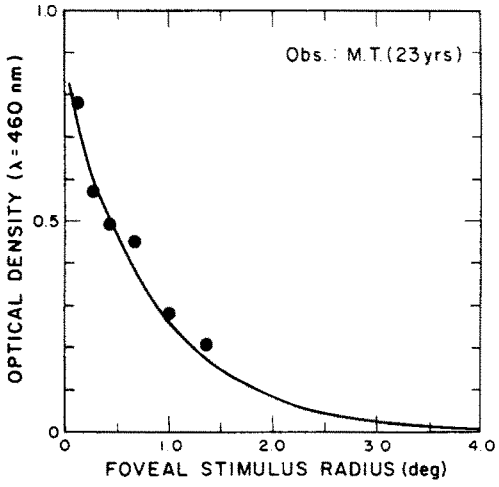

Fig. 5. Macular pigment density at 460 nm is plotted as a function of foveal test stimulus radius. The smooth function, optical density = $0.83\,e^{-1.15d}$ (where d is the visual angle in degrees), was derived by Wooten *et al.* to fit macular pigment density as a function of retinal eccentricity. The agreement between the data and the smooth function implies that our density estimates are based on the macular pigment absorption only at the edge of the test stimulus, rather than an average for the entire foveal stimulus.

Retinal illuminance. The retinal illuminance of the stimulus decreases for older observers as a function of age-correlated decreases in pupillary diameter (Kadlecová *et al.*, 1958). For one young subject, measurements were made under normal conditions and after topical application of a 0.5% solution of Mydriacyl which increased his pupillary diameter from 3.5 to 9.0 mm. This did not alter our measurements of his macular pigment density. Conversely, the results of reducing the luminance of the adapting background or the test stimulus are shown for one subject in the top two panels of Fig. 6. Similar results were obtained for two other observers. Arrows indicate our standard conditions. Note that the reduction in luminance of the background and test probe, well beyond what might be expected from senile miosis, has no effect on the macular pigment density. Surprisingly, no effect was observed when the background was entirely eliminated; either the luminance of the background is too low to influence sensitivity or short-wave cone and rod contributions were already eliminated by virtue of the 15 Hz flicker. This obviates a potential criticism raised by Pease and Adams (1983) that the use of a short-wave background will adapt parafoveal mechanisms more than foveal mechanisms because of prereceptoral absorption by the macular pigment in the foveal region.

Flicker sensitivity. Nine of the older subjects reported difficulty in seeing flicker at 15 Hz so they were tested at 12 Hz. This problem is not surprising in view of the evidence that the senescent visual system of some individuals undergoes a loss in temporal resolution (McFarland *et al.*, 1958). To be certain that changing the flicker rate did not influence the difference spectrum, we repeated the measurements over a frequency range from 6 to 18 Hz using an observer who was sensitive to all temporal rates. As can be seen in Fig. 6, the difference spectrum was only trivially affected by the temporal frequency.

Parafoveal stimulus diameter. Flicker sensitivity is lower at 5° than in the fovea. Many observers, particularly the elderly, found the task possible only when the parafoveal stimulus was increased to 2°. To test the influence of a large parafoveal stimulus, while keeping the foveal stimulus at 1°, we repeated the measurements with larger stimuli, again going beyond the largest parafoveal stimulus used by any observer. These results, shown in the bottom panel of Fig. 6, establish that larger parafoveal stimulus diameters do not influence our density estimates. This is to be expected given the data in Fig. 5 and much other data (Ruddock, 1963; Stabell and Stabell, 1980; Wooten *et al.*, to be published) establishing a nearly exponential decline in macular pigment density from the fovea to about 3° in the parafovea.

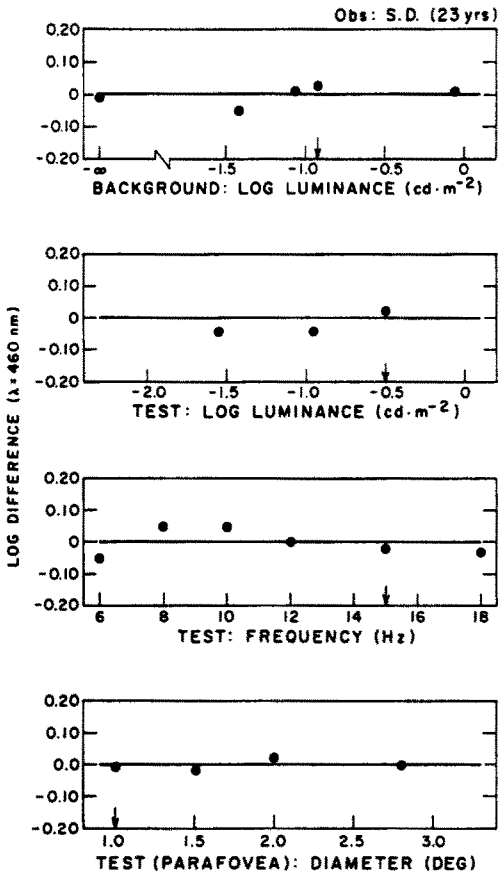

Fig. 6. Four control conditions varying background and test luminance (top two panels), as well as the flicker rate and parafoveal diameter of the test stimulus (bottom two panels), are presented for the same observer. Data points show deviations relative to data obtained in a different session under our standard conditions. Arrows denote the standard conditions.

Accuracy of fixation. Ancillary tests were conducted to check whether fixation accuracy might vary with age. An important concern was whether naive subjects could accurately fixate a point while attending to a parafoveal stimulus. Four observers, ranging in age from 15 to 71 years, were tested with a task that simulated our macular pigment tasks. They observed a video display and pressed a button to indicate the presence of a 0.33° alphanumeric character that flickered aperiodically at a rate of 15 Hz. The dependent variable was the percentage of time during ten 33-sec intervals that the observers

Table 1. Fixation accuracy for foveal (F) and parafoveal (PF) stimuli

Subject	Age (yr)	±0.50° F (%)	PF (%)	±0.67° F (%)	PF (%)	±1.00° F (%)	PF (%)
L.M.	15	98	81	99	86	99	99
M.T.	22	97	83	100	97	100	100
J.R.	42	98	91	100	95	100	99
S.C.	71	94	92	97	96	100	99

were able to accurately maintain fixation for foveal (directly viewing the test stimulus) and parafoveal (viewing a fixation point 5° from the test stimulus to which they were to attend) conditions of viewing. Attention to the flicker was evident from nearly 100% detection rates under both conditions. Fixations were measured with an Applied Sciences eye view monitor (Model 1996) that is based on the pupil-corneal reflection method and provided 60 Hz samples of eye position. Data reduction and calibration programs have been described by Kliegl and Olson (1981).

Table 1 presents the results on fixation accuracy for our four observers. Clearly, all observers could accurately fixate the foveal stimulus. In the worst case, 94% of the fixation time was within ±0.50° of the stimulus. Fixation accuracy was lower for all observers when attending to a parafoveal stimulus, but this reduced accuracy does not seem to depend on the age of the observer. Further, data already presented in Fig. 4 show that the parafoveal stimulus can be enlarged to almost 3° without altering our estimates of macular pigment density. Since all subjects tested could accurately fixate to within ±1.00° while attending to the parafoveal stimulus, it seems unlikely that our data are confounded by developmental changes in fixational accuracy.

Developmental variation

Measurements of macular pigment density in a large group of observers were made feasible by obtaining difference spectra only at selected points, 460, 500 and 550 nm. Since it is safe to assume a common extinction spectrum for all subjects based on our complete curves, the density at 460 nm is, in principle, sufficient for specifying the entire density spectrum. However, density measurements at 500 nm were also made as a reliability check. The rationale was that if we measure 0.50 optical density at 460 nm, we would expect the density at 500 nm to be approximately 0.28, based upon the standard density spectrum tabled by Wyszecki and Stiles (1982). To the extent that an observer with 0.50 density at 460 nm deviates from 0.28 at 500 nm, doubt is cast on the reliability of that observer's data. By this criterion the data seem reliable, as the mean value at 500 nm (after normalizing to 0.50 at 460 nm) was 0.27, with a standard deviation of 0.05; the average absolute deviation from the density expected at 500 nm for the 50 observers included in the study was 0.04.

The results for our 50 observers are shown in Fig. 7; the density at 460 nm ranges from 0.09 to 0.81. Inspection of the figure suggests—as proposed by Schultze (1866)—a slight decrease in macular pigment density with age, but this relation is not statistically significant. The Pearson product-moment correlation coefficient is −0.21, implying that age accounts for only about 4% of the variation in macular pigment density. Additional checks of the most variant points indicate that there are no statistical outliers (using Grubb's test; see Dunn and Clark, 1974). Nevertheless, the correlation between age and density was calculated without the two most extreme points, but the relation was still not statistically significant.

Wyszecki and Stiles's (1982) density for an average observer is 0.50, but based on calculations from color matching data, Vos (1972) suggested that 0.35 might be a more appropriate value. Our average density value, 0.39, is closer to that suggested by Vos. It should be emphasized, however, that our average value is specific to the central 1° of the retina. It is common to use average values taken from standard sources

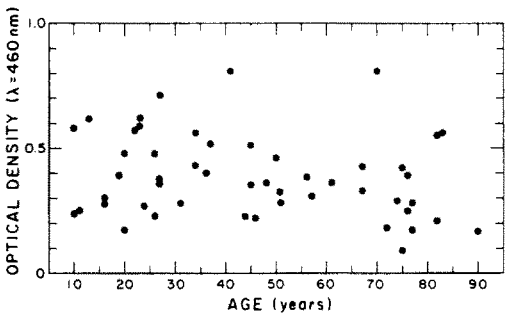

Fig. 7. Macular pigment density at 460 nm is plotted as a function of age (years) for 50 observers.

to refer psychophysical data to the retinal level. The data in Fig. 5 highlight the importance of considering field size in making such corrections; an appropriate adjustment would require that the tabled values themselves be corrected for the appropriate field size.

It may be concluded that there is a great deal of individual variation in macular pigmentation, but (at least after age 10) this variation and the average density are not correlated with age. It should be emphasized that these data obviously cannot be generalized to the younger ages that were not included in this study; alternative methods are required to measure their macular pigment. Both Schultze (1866) and Hering (1885) observed less macular pigment in infants than in young children or adults. Variation in macular pigment density will have profound consequences for the color vision of these young subjects (Werner and Wooten, 1979b; Kliegl *et al.*, 1984) and, as with subjects of any age, this variation must be taken into account for quantitative predictions and models.

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APPENDIX 1

Ueber den gelben Fleck der Retina, seinen Einfluss auf normales Sehen und auf Farbenblindheit. [On the Yellow Spot of the Retina: Its Influence on Normal Vision and on Color Blindness.] by Max Schultze, von Max Cohen & Sohn, Bonn, pp. 1–5 and 15–16 in the original (1866).

[pp. 1–5]

On April 4, this year, in a presentation to this society on "the anatomy and physiology of the retina,"[1] I have, I believe as the first person, drawn attention to the point that the pigment of the macula lutea and fovea centralis of our retina must have an influence on the length and intensity of the spectrum as we see it. Since the yellow spot must be penetrated by the rays of light on their way to the perceiving elements, the cones, it will absorb a certain amount of blue. How much blue passes through the yellow of the macula lutea and whether rays other than blue ones are absorbed as well, must depend on the nature and density of the pigment. Lately I have cut out the part of the retina containing the yellow spot and the fovea centralis from a number of corpses which were as fresh as I could get them. These pieces were placed in serum on the stage of a microscope and exposed to colored light. As far as I could observe, the yellow color of the macula lutea and fovea centralis (the latter was thought to be colorless, but is as yellow as the former) did not change noticeably within the first days after death.

The pigment is a homogeneous lemon to light orange-yellow mass located *between* the retinal fibers and cells. It does not mix with, and is not soluble in, water and appears to be of a lipoid nature. The color of these masses is still very intense if viewed 3 to 400 times enlarged and if one takes care to remove cells, fibers and other elements of the retina that cloud after death. This can easily be accomplished by rinsing the retina in serum and by slight piecing apart of the macula lutea. At a few spots the yellow-colored substance (located in the inner retinal layers, though absent in the cones) is so clearly visible that exposure to colored light provides completely reliable results. I chose colored glasses which could be inserted easily between the mirror of the

microscope and the stage. Care had to be taken to screen off all ambient light as completely as possible from the stage. As expected, it was possible to demonstrate the absorption of a certain amount of blue by the pigment of the yellow spot. The absorption was quite pronounced because insertion of a dark blue cobalt glass caused all spots of intense yellow color to appear as *black* on a blue background. Thus, the dark blue that I see through *one* yellow spot, that is my own, becomes black if a second yellow spot is held in front of my eye. Therefore, under an otherwise equal organization, a retina *without* a yellow spot would see more blue than one *with* such a spot. Consequently it is obvious that the yellow spot exerts an influence on the subjective brightness of the blue end of the spectrum, thus also on the so-called ultraviolet. Its influence will be much larger than that due to the fluorescence of the ocular media for the simple reason that the yellow spot lies at the place of the clearest retinal image. (Decisive experiments about the fluorescence of the ocular media we owe to Helmholtz[2] and later Sechenov.[3]) Thus, its influence must be taken into account along with the absorption of ultraviolet rays in the transparent ocular media as shown by Brücke. Donders's experiments with fluorescent fluids showed that the low subjective brightness of the ultraviolet could not be solely explained on the basis of the latter (see Helmholtz, Physiologische Optik, p. 233).

An absorption of other than blue rays in the yellow pigment of the macula lutea could not be demonstrated conclusively. Since the yellow showed a slight tinge of green, one also had to consider red absorption. That this color is absorbed somewhat, I would like to infer from the fact that the yellow spot becomes black when the dark blue cobalt glass is inserted. Since it is known that this glass transmits red to a small degree, the yellow spot had to appear red if it did not absorb red. But by means of red glasses it is difficult to prove absorption, since it is necessary to choose them in such a dark color that a confident decision about a hint of absorption is no longer possible. The same is true for illumination with spectral colors, which in addition have the disadvantage that, because of a rapidly decreasing intensity at the red end of the spectrum, it is impossible to achieve a visual field uniformly illuminated wtih dark red, as would be required to detect small differences of light and dark in the object.

It was noticed repeatedly that people with otherwise normal vision show different sensitivities for violet and ultraviolet light. Edm. Rose presents, among others, the example of an exquisitely ultraviolet-sighted man.[4] Such differences can be explained simply by a different density of the yellow color of the macula lutea. The yellow pigment develops in the human during the second year of life according to Ammon, Michaelis and Arnold. At least it becomes more dense around this time. To establish whether it is completely absent prior to this would require further research. In the fresh eyes of a mature infant that died during birth, a microscopic examination of the place of direct vision revealed a slight yellow tinge. This locus was well-preserved although slightly elevated in a crease. In advanced age the spot is assumed to bleach again. According to Huschke[5] the spot is *lighter* in blue-eyed than in brown-eyed people. More precise investigations on variations in the color of this spot are lacking, but all pigments of human and animal bodies are subject to individual differences. Therefore, the assumption that the macula lutea also varies between individuals should not meet any resistance....

[pp. 15–16]

In my earlier presentation, I hinted at the potential utility of the yellow color of the retina for the locus of direct vision. There are two points to which I thought it necessary to draw attention. Undoubtedly absorption of the most refractible violet and, to a small degree, of red diminishes the chromatic aberration of rays that are perceived at the most sensitive part of the retina. This correction will be all the more effective since it is located directly in front of the place where the clearest image is generated. The more complete the absorption of the extreme spectral rays, the more complete the correction. Thus, a dark yellow spot serves its purpose better than a light one. Indeed, I believe I have clearer vision with a yellow glass in front of my eye than without one. I and others to whom I pointed this out noticed this especially when we looked at the landscape with many trees that I see from my window. The perspective of this view appears much more precise, since under appropriate accommodation I obtain a more precise image of every single object out there. In experiments with writing samples this difference was not as pronounced. Also, my visual acuity did not increase when I looked at them while intoxicated with santonin. In any case, it seems advisable to extend the experiments in this direction and to test congenital color blind persons more carefully for their acuity.

Another advantage of the color of the yellow spot might be that yellow screens those rays from the perceiving elements that are usually described as the ones that are primarily chemically active in the spectrum. I will not speculate on the disadvantageous effects these rays might have on the most sensitive location if its yellow filter were missing....

1. *Archiv für mikroskopische Anatomie*, Vol. 2, p. 165.
2. *Poggendorff Annalen*, Vol. 104.
3. *Graefe Archiv*, etc., Vol. 5, part 2, p. 205.
4. *Virchow Archiv*, etc., Vol. 30, p. 442.
5. *Sömmering's Anatomie*, p. 727.

APPENDIX 2

Ueber individuelle Verschiedenheiten des Farbensinnes. [On Individual Differences in Color Vision.] by Ewald Hering. *Lotos* **6**, 142–198 (1885).

[From Section II, pp. 164–166 in the original]

I took the retina out of human eyes which were extirpated a few hours after death. I cut out the area of the macula, the coloration of which showed very large individual differences. I carefully dried it on a small piece of glass and especially avoided cracking which happens easily when drying is too rapid and too strong. Onto this fragment of dried retina a drop of concentrated glycerin was applied and after some time a cover glass. I store a series of yellow spots prepared this way in the dark, and only take them into the light for experimental purposes. If I hold such a macula in front of my eye such that it lies exactly in my line of vision I perceive white paper as yellow, the blue sky as gray and relatively dark, etc....

[From Section III, pp. 181–185 in the original]

If one examines a macula lutea prepared in the manner previously described in the microspectrum under slight

enlargement, one becomes easily convinced that it absorbs not only the violet and blue, but also the green rays, even though they are absorbed less than the blue. Only in the yellow and red [part of the spectrum] did I notice not the slightest trace of absorption.

Much more sensitive than the microspectrum for the examination of absorption phenomena are the approximate color equations, or hue equations, between one monochromatic light and a mixture of two monochromatic lights. If one mixes a yellow from a spectral red and a spectral green and adjusts next to it the spectral yellow to be as close as possible in hue and brightness, the yellow mixture viewed through a macula looks reddish; the stronger the coloration of the macula, the more reddish, whereas due to contrast the homogeneous yellow develops a tinge of green. This, then, proves the absorption of green light by the macula. The more yellowish the green light chosen for the mixture, the weaker is the absorption, as far as I could see, but in greenish yellow it still is very pronounced. Indeed, I was able to demonstrate with this method a weak absorption of greenish yellow of wavelength 565 nm by means of two weakly colored maculae put behind each other. The homogeneous yellow was of wavelength 578 nm, the mixed yellow was composed of the longest wavelength that under these conditions was still a sufficiently intense red and the greenish yellow mentioned above. I also made hue matches between a mixture of violet and green on the one hand and a homogeneous pure blue on the other, and saw both sides of the equation become much darker and the mixed blue turn greenish. Overall, I had the impression that all rays from violet to greenish yellow are absorbed by the macular pigment, and to a stronger degree the shorter the wavelength of the light in question.

But I will not discuss whether the absorption curve has a regular or an irregular shape. I am planning further more precise studies with an improved and refined method....

The macula, however, is not the only part of the eye that is relevant with respect to color absorption: in addition, the lens plays a very important role....

With respect to the coloration of the lens, Becker[1] notes:

> In the newborn child the lens is completely water clear.... As a rule one can notice a trace of yellow color of the nucleus in lenses that were taken from the corpses of 25- to 30-year old individuals. Upon palpation of the middle of the lens one feels a greater resistance. In exceptional cases both [phenomena] are still missing in 40-year-old persons.... While the lens may still be completely colorless in people of the most advanced age, from 80 to 90,... in individual cases at a much earlier age (50 years) the entire lens proper may already... have been baked into a homogeneous dark yellow, even reddish brown body.

I myself found the lenses of newborns in all ten cases which I happened to observe to be quite clear but still colored. This was the case even when I was able to examine them only a few hours after death. Even the lenses of an asphyxiated newborn infant which I examined 30 minutes after the heartbeat stopped were colored. The coloration is yellow-green, although only weak, still quite pronounced if the lens is put under water in a white porcelain dish. Outside the water the coloration might be overlooked very easily because of the ring of light which comes about by the strong refraction of the light.[2] Of the macula of infants (whose age was never more than four weeks) I noticed only an occasional hint of color. Perhaps it was masked by a clouding of the retina, because Max Schultze[3] believed he had seen under the microscope a tinge of yellow in the retina of a mature baby that died during birth.

A much more pronounced greenish yellow, but also absolutely clear were the lenses of a $1\frac{1}{4}$-year-old boy who was examined 11 hours after his death. In this case the stronger coloration could have been due to the much larger lens size. Similar observations were made on the lenses of a 3-year-old boy 10 hours after his death. The macula was quite pronounced and in its center (5 mm in the largest diameter) already pronouncedly yellow. In an 8-year-old boy I found a lens that was as weakly colored as the one of a newborn. It is known that after surgical removal of the pigmented lens (due to cataract), patients report that everything looks blue.

I have examined the absorption phenomena in the strongly colored lenses of adult men using methods identical to the ones used for the macula and obtained quite analogous results. The green light is also still strongly absorbed and with green corresponding to the wavelength of the thallium line I obtained very noticeable results with a lens verging into orange in color.

The coloration of the lens, then, is a quite common and constant appearance. In the lenses of newborns first only the absorption of violet rays is noticeable—consequently the greenish-yellow color. The more the pigmentation increases, and yellowish appearance of the lens, the greater the loss of blue and greenish-blue rays. Finally, one also notices the absorption of green rays and the color of the lens turns red-yellow. This sequence of colors is identical in the macula. Thus, macula and lens act in the same way. Whether the pigment of both is the same or at least behaves optically quite identically, and whether besides the great quantitative there are also qualitative differences in the macular pigment, I cannot decide at this point.

Max Schultze[3] previously emphasized the large individual differences in the macula and attempted to derive and explain differences in the color sense from that. Similarly, he attempted to trace back the "color blindness" resulting from the intake of santonin to stronger coloration of the macula generated by the santonin. Generally, he claimed that a more intensive coloration of the yellow spot may suffice to explain many cases of "violet blindness." Of course this would be quite a different kind of violet blindness than the one assumed by the followers of the three-color theory.

1. *Handbuch der Ophthalmologie*, v. Graefe und v. Saemisch, p. 170.
2. I have completely convinced myself that this coloration of the lens is not analogous to the sometimes quite pronounced icteric coloration of the cornea.
3. *Ueber den gelben Fleck der Retina*, Bonn, 1866.