



Macular pigment densities derived from central and peripheral spectral sensitivity differences

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Abstract

Estimates of the density spectrum of the macular pigment (Wyszecki G, Stiles WS. *Color Science: Concepts and Methods, Quantitative Data and Formulas*. 1st ed. New York: Wiley, 1967); (Vos JJ. Literature review of human macular absorption in the visible and its consequences for the cone receptor primaries. Institute for Perception. Soesterberg, The Netherlands, 1972) are partially based on the difference between central and peripheral spectral sensitivities, measured under conditions chosen to isolate a single cone class (Stiles WS. *Madrid: Union Internationale de Physique Pure et Appliquée*. 1953:1:65–103). Such derivations assume that the isolated spectral sensitivity is the same at both retinal locations, save for the intervening macular pigment. If this is true, then the type of cone class mediating detection should not influence the calculated difference spectrum. To test this assumption, we measured central and peripheral spectral sensitivities in a deuteranope, a protanope and a normal trichromat observer: (a) for short-wave sensitive (S-) cone detection; and (b) for long-wave sensitive (L-) cone detection (deuteranope), for middle-wave sensitive (M-) cone detection (protanope) or for both L- and M-cone detection (normal trichromat). The difference spectra determined for L- or M-cone detection deviate significantly from those measured for S-cone detection, at wavelengths below 450 nm. A theoretical analysis suggests that the discrepancies are owing, in part, to regional variation in the optical density of the cone pigments; and that such receptor variation cannot be ignored when deriving the standard density spectrum of the macular pigment. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Recently, we have begun comparing centrally and peripherally measured spectral sensitivities in normal and dichromatic observers. We have undertaken this work for two reasons. The first is to estimate the macular pigment densities in a group of dichromatic observers, who have a single-gene in their X-chromosome opsin gene array. An estimate of each dichromat's macular pigment density, derived from the ratio of the peripheral to central sensitivities, is required to correct the centrally measured sensitivities for individual variation in pre-receptoral absorption. Only after applying such corrections can different genotypes be reliably compared to determine the influence of amino acid substitutions upon the short-wave spectral sensitivity of the expressed photopigment.

The other, more general, reason for comparing spectral sensitivities is to validate and extend the presently available estimates of the spectral distribution of the macular pigment [1,2,4,5] (Bone, personal communication; see Fig. 1 for a comparison of the spectra). The optical densities estimated by Bone are similar to those estimated by Wyszecki and Stiles and by Vos, except at very short wavelengths, where the Bone values, which extend to 380 nm, are higher. Higher optical densities for the macular pigment in this spectral region are consistent with other data [6].

Estimating the density spectrum of the macular pigment from ratios of spectral sensitivities relies upon the assumption that centrally and peripherally measured spectral sensitivities differ only in the presence of the macular pigment, which screens the central, but not the peripheral retina. Unfortunately this assumption is only approximately valid. Two other factors vary significantly with retinal region: the relative sensitivities of the photoreceptors, (i.e. the change in the receptor contri-

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butions to the overall spectral sensitivity) and the optical density of the photopigment. Both will influence the peripheral to central sensitivity ratios and, hence, the derived shape of the macular pigment spectrum.

The possible contaminating influence of changes in relative cone spectral sensitivity upon macular pigment estimates has been circumvented by choosing conditions favouring detection by a single cone class. For instance, in his measurements of the macular pigment optical density, Stiles [3] chose conditions to favour detection by the short-wave sensitive (S-) cones. His values were given the greatest weight in the template derived by Wyszecki and Stiles [1,4], from the results of several psychophysical studies, to describe the relative optical density of macular pigment with wavelength (Fig. 1).

If the isolated spectral sensitivity is the same at both retinal locations, save for the intervening macular pigment, then the type of cone class mediating detection should not influence the peripheral and central difference spectrum. That is, conditions chosen to favour detection by either the long-wave sensitive (L-) or middle-wave sensitive (M) cones should yield identical results to those chosen to favour detection by the S-cones. However, the limited information that exists in the literature suggests that this is not the case. Pease et al. [7], who attempted flicker isolation of the M-cones, obtained macular pigment spectra, which deviate from the values of Stiles [3] and from the values of the Wyszecki and Stiles template. Their spectra, however, are similar to those reported by Ruddock [8], who used the WDW colour-matching paradigm of Wright [9] to

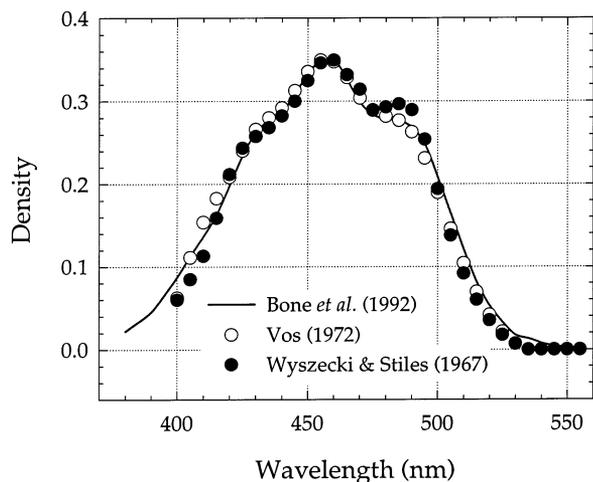


Fig. 1. The macular pigment density spectra of Wyszecki and Stiles [1] (filled circles), Vos [2] (open circles) and Bone et al. [5] (continuous line). The Bone et al. [5] optical density values were derived by mixing lutein and zeaxanthin in the same ratio as found in the foveal region and then incorporating the mixture into phospholipid membranes. This membrane environment is believed to more or less duplicate that of the carotenoids in the macula. Bone et al. [5] published only data at wavelengths greater than 420 nm. The values below 420 nm were obtained from Bone (private communication).

estimate the retinal variation in photoreceptor sensitivity. Both studies found higher density values of the macular pigment for wavelengths on the long-wavelength side of the absorption maximum and lower density values for wavelengths on the short-wavelength side. These discrepancies have never been satisfactorily explained. A possibility is that the M-cone isolation in the Pease et al. study was incomplete. However, it is also possible that the Wyszecki and Stiles template, as well as the Vos template, is incorrect. It is known, for example, that both templates yield implausible values when used to estimate the S-cone photo-pigment optical density from corneal S-cone spectral sensitivities [10].

Surprisingly, the other factor that can influence central and peripheral spectral sensitivity comparisons, the change in optical density of the photopigment with retinal eccentricity, has been almost completely neglected in deriving the macular pigment spectrum. The photo-pigment density is believed to be directly proportional to the length of the photoreceptor outer segment, which, in turn, depends upon retinal location [11–14]. In the very central fovea (foveola), cone outer segments have been estimated as being 36 [11], 38 [12], 35 [13], < 45 [14] and 41–63 [15] μm long; whereas those on the foveal slope have been estimated as 22 [12] and 25–45 [15] μm . These values probably apply to M- and L-cone outer segment lengths. Less is known about the regional differences in S-cone outer segment length. However, some preliminary evidence indicates that the pattern of change may be similar (Ahnelt, private communication). At the fovea, outer segments of S-cones may be 5% shorter than those of the M- and L-cones; whereas in the periphery, at retinal eccentricities greater than 5 mm (17.7° of visual angle), they may be shorter by 15–20%, for L- and M-cone outer segments of 15–20 μm . A conservative interpretation of these values, assuming a density per unit length or specific absorbance of $0.015 \mu\text{m}^{-1}$ [16], suggests a change in optical pigment density between central foveal cones (with an outer segment length of c. 40 μm) and peripheral cones (with an outer segment length of c. 25 μm) of about 0.20 for the L- and M-cones and perhaps a slightly smaller value for the S-cones.

Fundamental reflectometric measurements also support a decrease in the photo-pigment optical densities of the L- and M-cones with retinal eccentricity. Kilbride et al. [17] have reported an average change in density from 0.35 (foveola) to 0.15 (1° eccentricity) to 0.10 (2° eccentricity). Likewise, Elsner et al. [18] have reported a reduction from 0.33 (0.24–0.41) to 0.24 (0.12–0.35) going from a retinal location of $0.5\text{--}4^\circ$. Psychophysical estimates are quite variable and, at present, pertain only to the central fovea [19,25].

A theoretical treatment of how optical density differences can influence estimates of the macular pigment

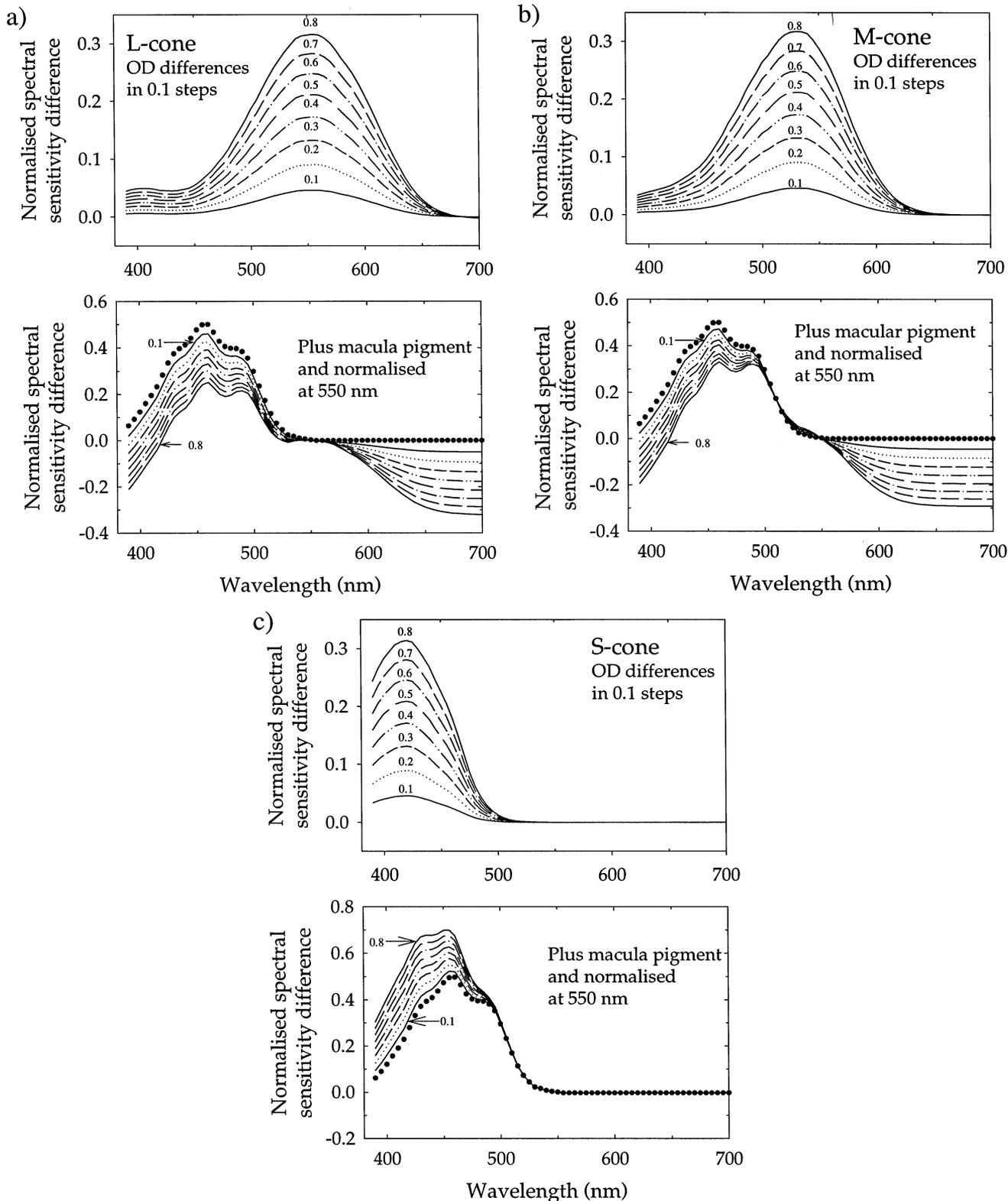


Fig. 2. Influence of optical density differences in photopigment upon the difference spectrum for central and peripheral viewing, obtained for the L- (a), M- (b) and S-cones (c). The upper panels show, as a function of wavelength, the assumed optical density difference between central and peripheral L-, M- and S-cones in increasing steps of 0.1. The lower panels show the same differences after adding in the macular pigment and normalising the curves at 550 nm. The filled circles indicate the macular density values of Bone et al. [5].

density spectrum, derived from ratios of central and peripheral spectral sensitivities, is given in Fig. 2. The top panels in each group show, as a function of wavelength, the changes in cone spectral sensitivity (normalised at long-wavelengths) that are caused by photo-pigment optical density differences between peripheral and central L-, M- and S-cones of 0.1–0.8 in steps of 0.1 (i.e. the optical density for central is higher than for peripheral cones by the amounts shown). These curves have been calculated from corneal cone spectral sensitivities [6,10] by correcting the values to the retinal level by removing the effects of the lens pigment, adjusting them to infinitely dilute photopigment spectra, and then adding the appropriate photopigment densities for the periphery and centre and finding the difference. (Note: in order to make these calculations, it is necessary to adopt a value for the optical density of the photopigment in the periphery, where we made the extra-foveal measurements. Within limits, this choice has a minimal effect on the estimate of the change in density. We assumed a value of 0.20 for the peripheral cones).

The bottom panels in each group show the same differences after adding in a fixed amount of macular pigment for a central 2° field and normalising the curves at 550 nm, where the effect of the macular pigment is assumed to be negligible (Fig. 1). The filled points show the macular density values of Bone et al. [5]. Clearly, the influence of photo-pigment optical density differences on macular pigment density estimates is not the same for all three cone classes. For the L- and M-cones, photo-pigment optical density differences effectively produce lower erroneous estimates of macular absorption values (and even negative values) at wavelengths below the absorption peak (460 nm); whereas for the S-cones, they produce higher values.

How exactly do such theoretical predictions about the influence of changes in optical pigment density correspond to actual differences between central and peripheral spectral sensitivities? To find out, we have made measurements of the density spectrum of the macular pigment in observers, under S-, M- and L-cone isolation conditions. The measurements were made in dichromat observers, as well as in a normal observer, to control for the possible variation in M- and L-cone receptor contribution with retinal location.

2. Methods

2.1. Subjects

A deuteranope (VM), a protanope (MH) and a normal trichromat co-author (LTS) participated in the experiments. VM and MH were diagnosed by their Rayleigh matches and by heterochromatic flicker pho-

tometry. This psychophysical diagnosis is consistent with results provided by quantitative southern blotting and PCR amplification and sequencing of exons 2–5 of their X-chromosome-linked opsin genes [26]. VM is an emmetrope; MH and LTS are slightly myopic, but required no optical correction.

2.2. Stimuli and procedure

Two channels of a Maxwellian-view system produced the flickering test stimulus and the steady adapting field [10]. The target was 1° of visual angle in diameter. It was square-wave flickered at 3 Hz for S-cone isolation and at 15 Hz for L- and/or M-cone isolation. Flicker was accomplished by the imposition of mechanical shutters driven by a computer-controlled square-wave generator. The target wavelength was shaped by a grating monochromator (Jobin-Yvon H-10 Vis), with 0.5 mm entrance and exit slits, into a triangular profile having a full-bandwidth at half-maximum (FWHM) of c. 4.0 nm. The wavelength was varied from 390 to 600 nm. The adapting field was 18° in diameter. Its wavelength was shaped by a grating monochromator (Jobin-Yvon H-10 Vis), with 2 mm entrance and exit slits, into a triangular profile, peaking at 430 nm (FWHM = 17 nm) for the L- and M-cone isolation experiments and at 580 nm (FWHM = 16.5 nm) for the S-cone isolation experiments. The intensity of the 430 nm adapting field was 11 log quanta s⁻¹ degree⁻² or (3.19 log photopic trolands); that of the 580 nm adapting field for S-cone isolation was 11 log quanta s⁻¹ degree⁻² or (4.8 log photopic trolands). The 580 nm adapting field condition provides S-cone isolation in normal observers at wavelengths below c. 520 nm. The target and adapting field were either presented centrally or 12° peripherally in the nasal field of view. (At a peripheral location of 12°, the effect of macular pigment is assumed to be essentially negligible [5]). For the peripheral experiments, fixation was aided by a dimly illuminated, centrally viewed red cross.

The position of the observer's head was maintained by a dental wax impression. During an experimental session, the spectral sensitivities at 20–26 wavelengths, depending upon the isolation condition (central or peripheral viewing, S- or M- or L-cone isolation), were measured in a random order, two to three times each, by the method of adjustment. The values at each wavelength were then averaged. The final spectral sensitivities for a particular condition represent the averages of data from 20 to 30 separate experimental sessions.

2.3. Calibration

The radiant fluxes of the test and adapting fields were measured at the plane of the observer's pupil with a silicon photo-detector (United Detector Technology)

combined with a picoammeter (Keithley, model 486). The silicon photo-detector had been calibrated by the manufacturers against standards traceable to the National Bureau of Standards, Washington. The optical waveforms produced by the mechanical shutters were monitored periodically using a silicon detector (United Detector Technology, operational amplifier and oscilloscope).

3. Results

Fig. 3 shows the difference spectrum (open circles) obtained for the deuteranope (a) protanope (b) and normal trichromat (c) under S-cone isolation conditions. The curves were derived from the logarithmic differences between the central and the 12° peripheral measurements. The filled circles indicate the macular density values of Bone et al. [5]; while the dotted lines indicate the influence of adding in assumed optical density differences between the peripheral and central S-cones in steps of 0.1. A base optical density value of 0.2 for the peripheral S-cones is assumed (i.e. a difference value of 0.1 corresponds to a central optical density value of 0.3). The best fits (solid line) for all three observers lie between the Bone et al. [5] template with no optical density difference between the centre and periphery (filled circles) and the Bone et al. template with an added optical density difference of 0.1. This implies that spectral sensitivity differences between the central and peripheral retina, measured under S-cone isolation conditions are well described by the Bone et al. macular density values (a slightly poorer fit is found with the density spectrum of Wyszecki and Stiles) [1]. However, it cannot be ruled out that a small optical density difference between the central and peripheral cones (< 0.1) is influencing the difference spectrum (i.e. adding to the macular pigment differences to increase the optical density values at short wavelengths).

Fig. 4 shows the difference spectrum obtained for the deuteranope (a) protanope (b) and normal trichromat (c) under conditions chosen to favour L-cone detection in the deuteranope, M-cone detection in the protanope and L- and/or M-cone detection in the normal observer. (Because both M- and L-cones may be mediating detection in the normal trichromat, the possible influence of regional variation in photoreceptor sensitivities upon the difference spectrum cannot be ruled out. However, independent evidence suggests that both the central and peripheral flicker sensitivities in normal trichromat observer LTS are L-cone dominated.) Here, the difference spectra are quite different from the Bone et al. template and from the observers' own corresponding S-cone difference spectra, at wavelengths below 455 nm. For the deuteranope VM, the best fit is closest to the Bone et al. template with an added optical

density difference of 0.2; for the protanope MH, it is closest to the Bone et al. template with an added optical density difference of 0.3; and for the normal observer LTS, it is closest to the Bone et al. template with an

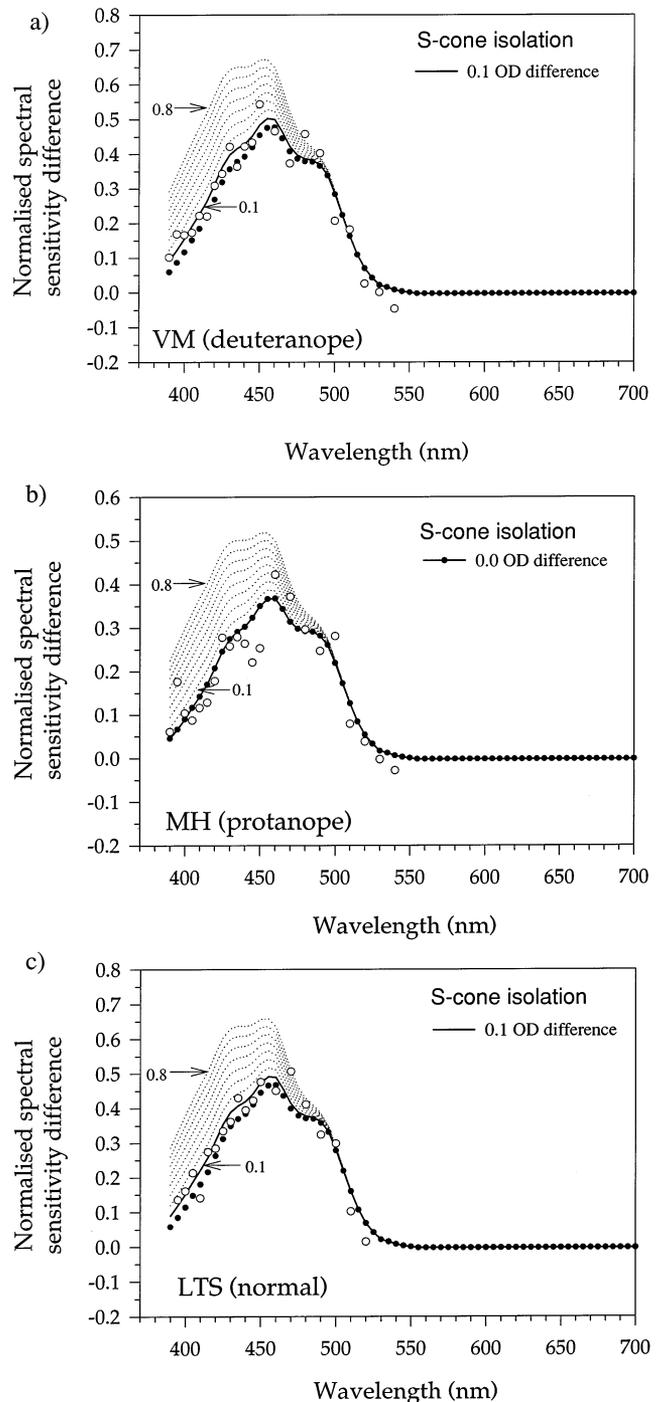


Fig. 3. The difference spectrum (open circles) for central and 12° peripheral viewing, measured under S-cone isolation conditions for a deuteranope VM (a) a protanope MH (b) and a normal trichromat LTS (c). The filled circles indicate the macular density values of Bone et al. [5]; the dotted lines indicate the influence of adding in optical density differences between peripheral and central S-cones in steps of 0.1 (Fig. 2c, lower panel). The closest fitting function for each observer is indicated by the solid line.

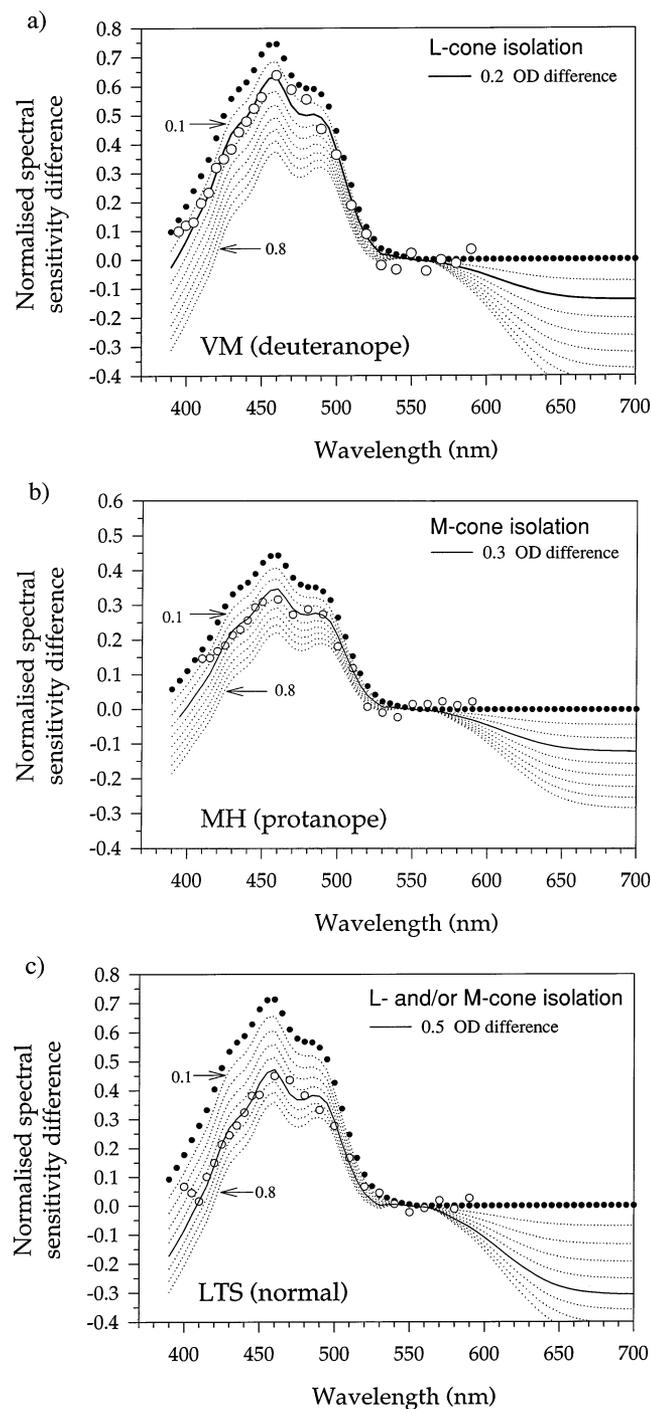


Fig. 4. The difference spectrum (open circles) for central and 12° peripheral viewing, measured under L-cone isolation conditions for a deuteranope VM (a) under M-cone isolation conditions for a protanope MH (b) and under M- and/or L-cone isolation conditions for a normal trichromat LTS (c). The filled circles indicate the macular density values of Bone et al. [5]; the dotted lines indicate the influence of adding in optical density differences between peripheral and central L-cones for the deuteranope VM and for the normal trichromat LTS (Fig. 2a, lower panel) and between peripheral and central M-cones for the protanope MH (Fig. 2b, lower panel) in steps of 0.1. The closest fitting function for each observer is indicated by the solid line.

added optical density difference of 0.5 (how these fits are made will be explained in detail in a longer manuscript).

4. Discussion

We have measured central and peripheral spectral sensitivities in a deuteranope, a protanope and a normal trichromat under S-cone and L- and/or M-cone isolation. These measurements were made to test the standard assumption invoked to derive the optical densities of the macular pigment from psychophysical measurements; that is, that isolated spectral sensitivities are the same at both retinal locations, save for the intervening macular pigment. As expected, we find that the difference spectrum calculated from the central and peripheral sensitivities for the various types of isolation conditions differ. The L- and/or M-cone isolation data also do not agree with the optical densities of the macular pigment of Wyszecki and Stiles [1], which is largely based on results obtained under S-cone detection conditions [3]; nor with those of Bone et al. [5]. The discrepancies seem to occur at short wavelengths.

Systematic discrepancies at short-wavelengths can also be found in the data of other psychophysical studies, such as those of Pease et al. [7] and Werner et al. [20], who used foveal and peripheral spectral sensitivities to derive the difference spectrum for the macular pigment. Both Pease et al. [7] and Werner et al. [20] used procedures that favoured M-cone isolation or allowed detection by other cone classes besides the S-cones. Pease et al. [7] used a fast, flickering (25 Hz), small (40 min) target, to disfavour S-cone detection and a red background to disfavour L-cone contributions. Werner et al. [20] used a 1° target square-wave flickering in counter-phase with a 460 nm standard null, superimposed on a broad-band, short-wave (445 nm) background.

If M-cone isolation was achieved in these studies, their results, along with ours, imply that other factors that vary across the retina must be considered when calculating the densities of the macular pigment from regional sensitivity differences. We are presently testing for the influence of such factors in a larger group of dichromat and normal trichromat observers.

One important factor is certainly the regional variation in receptor photo-pigment optical density. Its influence appears to be larger for the L- and M-cones than for the S-cones. But other factors may also have to be considered. One of these is possible waveguide modal patterns, which may vary in retinal receptors as a function of wavelength and receptor size [21,22]. However, such factors might be expected to be more pronounced at long-wavelengths than at the short-wavelengths where we find the significant deviations.

Another factor may be other short-wave absorbing photostable retinal pigments, the spatial density distribution of which changes with eccentricity. For instance, Snodderly et al. [23] have identified in the primate fovea by microspectrophotometry two short-wavelength absorbing photostable pigments, in addition to the macular pigment, with absorbance maxima at 410 and 435 nm. And, Bowmaker et al. [24] have identified a photostable pigment in the inner segments of rods and cones of old world monkeys, which has a peak sensitivity close to 420 nm and an absorbance spectrum that is narrower than that of a photosensitive visual pigment. For such pigments to explain our data, however, their distribution must be opposite to that of the macular; i.e. missing in the fovea and present in the extrafovea, which is very possible since the retinal layers are thinner in the central foveal area.

Until these factors are fully investigated, it may be impossible to reach a consensus about the optical density spectrum of the macular pigment. Additional work, involving comparisons of central and peripheral S-cone spectral sensitivities [10], however, suggests that the Bone et al. [5] optical density values are to be preferred over those of Wyszecki and Stiles [1] and Vos [2] at very short wavelengths.

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