1040-5488/11/8807-0001/0 VOL. 88, NO. 7, PP. 1–●●●
OPTOMETRY AND VISION SCIENCE

ORIGINAL ARTICLE

Macular Pigment: Its Associations with Color Discrimination and Matching

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ABSTRACT

Purpose. Macular pigment (MP) acts as a prereceptoral filter which selectively absorbs short wavelengths. It has the potential to alter color vision but the literature is conflicting on whether it does and, if so, to what extent, possibly reflecting differences between color mechanisms and color tests. This study was designed to identify and investigate relationships, if any, between MP optical density (MPOD) and color sensitivity using a battery of techniques to quantify the color vision of color-normal observers.

Methods. Color vision was assessed with the Farnsworth-Munsell 100-Hue test (FM100), Moreland match on the HMC anomaloscope, and a customized short wavelength automated perimetry (SWAP) technique at the foveola and at 1, 2, 3, 4, and 5° eccentricity. MPOD spatial profile was measured using customized heterochromatic flicker photometry.

Results. Total error scores and % partial error scores on the FM100 were uncorrelated to MPOD. Moreland matches showed a significant long wavelength shift with MPOD at between 1 and 3° (at 1.75°, $r = 0.489$, $p < 0.001$). Sensitivities on customized SWAP (cSWAP) using foveal targets were significantly inversely correlated with MPOD at both 1.75° ($r = -0.461$, $p < 0.001$) and 3° ($r = -0.393$, $p < 0.001$). Partial correlation analysis suggests that none of these findings can be attributed to age effects within the range 18 to 40 years.

Conclusions. Our findings suggest that dietary supplementation to increase MPOD is unlikely to adversely affect hue discrimination. The association of MPOD with cSWAP may be a temporally limited effect to which the visual system normally adapts. We suggest that cSWAP may provide a clinical tool for assessing short-wavelength foveal sensitivity. (Optom Vis Sci 2011;88:1–●●●)

Key Words: hue discrimination, anomaloscope, SWAP, macular pigment

Macular pigment (MP), consisting of the carotenoids lutein, zeaxanthin, and meso-zeaxanthin, is concentrated at the macula and is not detectable optically beyond about 7° from the foveal center.[1] Of these carotenoids, the zeaxanthins predominate at the fovea whereas lutein dominates beyond the fovea.[2] The extent of macular pigmentation has recently been found to be related to the width of the foveal cup, as assessed by optical coherence tomography.[3] Because these pigments are located in the fibers of Henle at the foveola and in the inner nuclear layer beyond the foveola,[4] they act as a prereceptoral filter and are believed to contribute a variety of potentially beneficial properties for vision, including reduction of the effects of chromatic aberration[5] (though not supported by Engles et al.,[6]), improvement of spatial vision and contrast enhancement,[7] increased photopic increment sensitivity,[8] reduced glare sensitivity in some studies[9,10] but not others,[11] and increased critical flicker frequency.[12]

Hue discrimination and color vision in general are most acute at the fovea[13] corresponding to increased cone density, specialized anatomic relationships and minimal spatial summation in this region (although with appropriate stimulus size scaling, surprisingly good color vision is possible beyond the fovea[14]). It is plausible that color discrimination at a small angular subtense would be influenced by the optical density (OD) of MP at the fovea. Indeed, it has long been speculated that interobserver differences in color matching by color-normal observers are at least partly because of differences in macular pigmentation.[15,16] Also, it is known that even subjects with ophthalmoscopically normal fundi exhibit substantial variations in MPOD, contributing to a range of prerecep-

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toral light absorption at 460 nm from 3% to almost 100%.[17] Dietary supplementation with the macular carotenoids has been shown to increase MPOD[18] and may retard development of age-related macular degeneration because of its antioxidant and short wavelength light filtering properties. Such hypotheses are currently the subject of a major randomized controlled clinical study (AREDS 22)[19] and follows potentially significant results from the LAST II study.[20]

Because the MP absorption spectrum ranges from about 400 to 520 nm and peaks at 460 nm,[21] it would appear likely that these pigments influence color vision through selective absorption of short wavelengths, thereby influencing the short-wave sensitive (SWS) cones and the blue-yellow opponent-color channel. Moreland and Dain[22] reported that hue discrimination, measured using the Farnsworth-Munsell 100-Hue test (FM100), is indeed adversely affected primarily for short wavelengths by simulation of high MPOD using liquid filters containing carotene in a benzene solution. Comparing the results with those obtained with a neutral filter, they concluded that this effect was not simply the result of reduced retinal illuminance. However, to our knowledge, there are no published studies on the effects of actual (rather than simulated) MPOD on conventional measurements of hue discrimination thresholds. Further evidence supporting an effect of MPOD on short wavelength vision has been obtained from studies of SWS cone sensitivity.[8,23] Finally, it has been shown that color discrimination measured by a color matching technique is influenced by MPOD.[24,25]

However, two recent studies using alternative methods, produced conclusions differing from those of the above mentioned studies. First, a study of the effects of dietary supplementation with macular carotenoids on MP found no correlation between the level of MP [measured by heterochromatic flicker photometry (HFP)] and red-green (RG) or yellow-blue (YB) color discrimination thresholds, although it was reported that RG vision tends to improve with augmentation of MP.[26] Second, RG cancellation profiles have been reported to be highly correlated with MPOD, whereas profiles for YB were independent of both eccentricity and MPOD.[17] However, changes in spectral sensitivity across the fovea, macula, and paramacula are accompanied by relatively little change in color appearance, depending on whether corrections are made for MP absorption.[27,28]

Thus, there is no consensus in the literature on the relationships, if any, between MPOD and color vision parameters on the one hand, and mechanisms on the other hand. This may or may not simply reflect the innate differences between, for example, spectral sensitivity measurements of the isolated SWS cone mechanism and the overarching hue discrimination function at short wavelengths. It is also necessary to distinguish between the effects on color vision (mechanisms, sensitivity, or appearance) of (1) distribution of MP across the retina and (2) variation of MPOD between subjects at a given retinal locus.

The objective of this study was to evaluate, in a cross-sectional manner, the associations between color variables and MPOD, using a much larger sample of subjects than in most previous studies and a battery of color assessments rather than relying on a single method of quantification. This study was part of a larger study of the association between MPOD and a wide range of vision parameters.[11]

The color vision tests used in this study were (a) hue discrimination using the FM100 test, (b) hue matching using the Moreland match on an anomaloscope, and (c) short wavelength automated perimetry (SWAP) increment thresholds using a customized procedure (cSWAP) to provide optimal foveal and parafoveal stimuli. This study has clinical implications for the visual effects of dietary supplementation of patients with age-related macular degeneration and at-risk patients.

METHODS

Identical instrumentation and test protocols were used in the Macular Pigment Research Group laboratories in Dublin and Waterford, Ireland.

Subjects

One hundred two healthy subjects aged 18 to 40 years and resident in either Dublin or Waterford, Ireland, were recruited to participate in this dual-center study, which was approved by Research Ethics Committees of Waterford Institute of Technology and of Dublin Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Potential subjects underwent a full eye examination. The exclusion criteria comprised: any ocular pathology (including abnormal macula appearance or cataract); corrected visual acuity $<6/9$ in the better eye; refractive error outside -6 to $+6$ diopters; and defective color vision. One eye only of each subject was tested, that with better corrected acuity. Full color vision data were available for 84 subjects.

Color Threshold/Sensitivity Techniques

The FM100 test (X-Rite UK, Poynton)

This test was administered under color-corrected fluorescent lighting supplied by a pair of 15W 46 cm lamps (The Daylight Co., London, UK) providing minimum luminance of 94 cd.m^{-2} reflected from each color sample as measured with a spot telephotometer. Maximum background luminance reflected from the supplied black sample trays was 12 cd.m^{-2}. Color temperature is rated at 6400° K. Subjects were allowed to review the arrangement in each tray if they so requested.

Individual error scores and total error scores (TES), summed across the visible spectrum and purple hues, were determined using the software supplied by the manufacturer. Partial error scores (PES) were used to assess hue discrimination specifically among blue and cyan hues using samples 50 to 68 and 36 to 54, respectively, and were divided by TES to obtain percentage values (%PES).

Anomaloscope

This test was administered using the Moreland match on an HMC MR anomaloscope (type 7700: Oculus, Wetzlar, Germany). This provides a 2° field within which 436 and 490 nm sources are matched to a mixture of 480 and 589 nm, the latter mixture providing a brightness match. Control of stimuli and calculation of blue/green mixture were achieved with the anomaloscope under computer control using the manufacturer's software. Neutral preadaption was not used as this

was found to produce transient adaptation effects on stimulus saturation. Stimuli were presented under continuous viewing mode. After practice, subjects toggled the mixture to obtain four matches, two each with the mixture preset to blue bias and green bias. The mean of six blue/green matches was calculated for each subject to obtain the midpoint.

Customized Short-Wavelength Automated Perimetry

Foveal and parafoveal increment sensitivities were measured using an adaptation of the standard SWAP routine on a Humphrey Field Analyzer 2i (Carl Zeiss Medetec, Jena, Germany). Yellow (530 nm) background luminance was 100 cd.m^2. Size V targets of 440 nm and 200 ms duration subtending 1.7° at the eye were presented at 0, 1, 2, 3, 4, and 5° eccentricity from a fixation target. The number of targets at each eccentricity beyond the foveal center varied from 4 to 20. On each presentation, a single target was presented. Increment thresholds were obtained using the SWAP adaptive staircase full thresholding technique. Subjects were given 3 min to adapt to the background before testing began. Sensitivity for each eccentricity was the mean of values for all targets in the group at that eccentricity.

Macular Pigment Optical Density

MPOD was measured by customized HFP (cHFP) using a densitometer (Macular Metrics Corp., Providence, RI), which alternates 460 and 550 nm stimuli, the former being maximally absorbed by MP whereas the latter is not absorbed by MP. A spatial profile of MPOD was obtained by performing five measurements at each eccentricity (0.25, 0.5, 1, 1.75, and 3°), and at 7°, to provide a reference point at which MP is optically undetectable. Further details have been published elsewhere.[29] This instrument and technique have been shown to be valid and have high reproducibility.[30]

Statistical Methods

Data were analyzed using PASW Statistics 17 (SPSS, Chicago, IL). Correlation coefficients and first-order partial correlation coefficients were calculated using the Pearson product-moment method because scatter-plots showed no evidence of non-linearity. Statistical analysis was based on two-tailed tests and interpreted with reference to 0.05 significance levels and Bonferroni correction.

RESULTS

Fig. 1 shows the MPOD spatial profile. These data compare well with previously published data using the same cHFP method.[3] Mean (±SD) MPOD for the 0.25° stimulus was 0.45 (±0.18), range 0.16 to 0.93.

Mean (±SD) hue discrimination TES for our subjects was 55 (±23), comparable with Kinnear and Sahraie's data for the 30 to 39 age group.[31] TES was found not to correlate significantly ($p > 0.001$ after Bonferroni correction). Possible associations between MPOD and (1) short wavelength hue discrimination in the region of peak absorption by MP and (2) discrimination at the short wavelength end of the expected axis of a type III acquired color vision defect were investigated by calculating %PES for color samples 50 to 68 and 36 to 54, respectively, i.e., %(PES/TES). An example of this analysis is provided in Fig. 2, which is a scattergram of % PES for FM100 samples 36 to 54 against MPOD at 1.75° eccentricity. Despite an apparent trend of increased %PES with higher MPOD, both (1) and (2) were found to be non-significantly

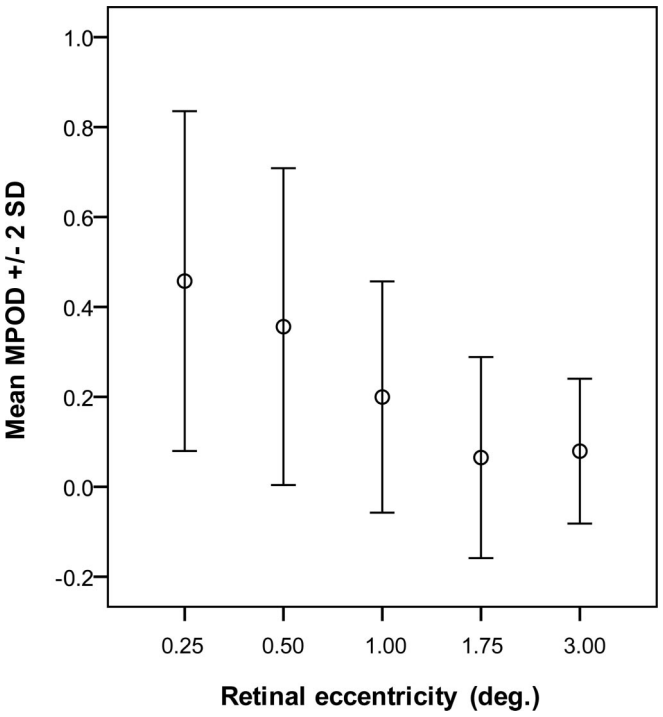

FIGURE 1.
Spatial profile of MPOD. Abscissa: eccentricity in degrees. Ordinate: mean MPOD across subjects ± 2 SDs.

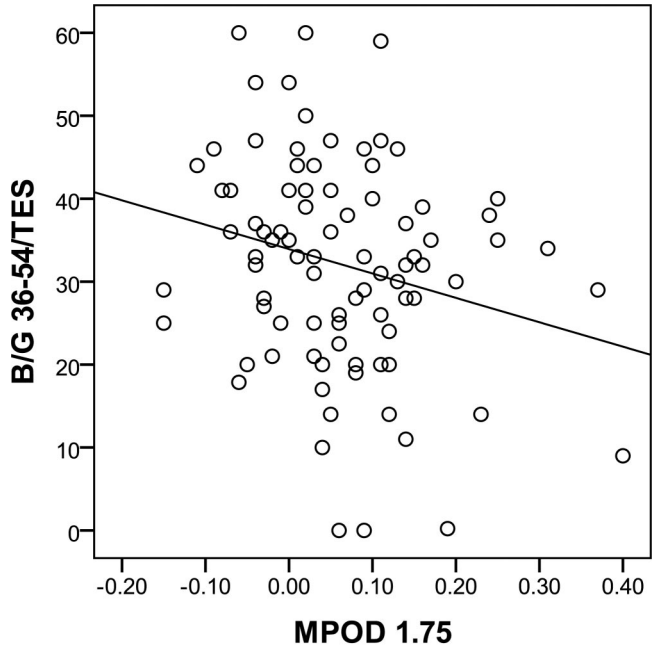

FIGURE 2.
Scattergram of %PES for FM100 caps 36 to 54 against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (%PES = −0.239 × MPOD + 33.92).

TABLE 1.
Correlations between color vision variables and MPOD

	%PES		Moreland	cSWAP					
MPOD	B/G 36–54	B 50–68	midpoint	Fovea	1	2	3	4	5
0.25°									
r_0	−0.188	0.114	0.343	−0.331	−0.189	−0.110	−0.003	−0.097	−0.032
r_1	−0.183	0.121	0.343	−0.328	−0.186	−0.106	0.005	−0.089	−0.025
p_0	0.084	0.301	0.001[a]	0.002[b]	0.083	0.314	0.982	0.378	0.769
df_0	83	83	91	83	83	83	83	83	83
0.5°									
r_0	−0.142	0.094	0.298	−0.267	−0.191	−0.116	−0.047	−0.134	−0.063
r_1	−0.138	0.099	0.295	−0.264	−0.189	−0.112	−0.042	−0.128	−0.057
p_0	0.195	0.393	0.004[b]	0.014[b]	0.079	0.292	0.667	0.223	0.567
df_0	83	83	91	83	83	83	83	83	83
1°									
r_0	−0.219	0.026	0.329	−0.285	−0.180	−0.200	−0.132	−0.165	−0.125
r_1	−0.218	0.028	0.331	−0.285	−0.178	−0.198	−0.130	−0.163	−0.123
p_0	0.044[b]	0.816	0.001[a]	0.008[b]	0.100	0.067	0.229	0.132	0.256
df_0	83	83	90	83	83	83	83	83	83
1.75°									
r_0	−0.224	0.113	0.489	−0.461	−0.288	−0.295	−0.215	−0.267	−0.203
r_1	−0.217	0.121	0.484	−0.458	−0.284	−0.291	−0.209	−0.261	−0.196
p_0	0.040[b]	0.304	0.000[a]	0.000[a]	0.008[b]	0.006[b]	0.048[b]	0.013[b]	0.063
df_0	83	83	90	83	83	83	83	83	83
3°									
r_0	−0.177	0.230	0.387	−0.393	−0.288	−0.317	−0.249	−0.307	−0.283
r_1	−0.154	0.258	0.371	−0.386	−0.278	−0.306	−0.229	−0.284	−0.263
p_0	0.105	0.034[b]	0.000[a]	0.000[a]	0.008[b]	0.003[b]	0.021[b]	0.004[b]	0.009[b]
df_0	83	83	90	83	83	83	83	83	83

[a]Significant with correction for a 5 by 9 correlation matrix.
[b]$p \leq 0.05$ without Bonferroni correction.
r_0, Pearson correlation coefficient; r_1, first-order partial correlation coefficient controlling for age; p_0, 2-tailed significance for r_0; df_0, degrees of freedom for r_0; B/G 36–54, blue/green caps (36–54); B 50–68, blue caps (50–68).

correlated ($p > 0.001$ with Bonferroni correction) to MPOD at all eccentricities.

The anomaloscope Moreland match midpoints were found to be negatively correlated to MPOD at all eccentricities (Table 1 and Fig. 3), indicating a shift toward green mixtures to match cyan. The coefficient was maximal for MPOD at 1.75°, corresponding to the anomaloscope stimulus diameter of 2°. MPOD at 1.75° accounted for 23.9% of variability (r^2) in Moreland match data. Coefficients were still significant after Bonferroni correction at all eccentricities except at 0.5°.

cSWAP data (sensitivity in dB) at all eccentricities measured were negatively correlated at high significance levels, with MPOD at both 1.75 and 3° of retinal eccentricity (Table 1). Fig. 4 is a scattergram of the data for cSWAP at 2° and MPOD at 1.75°. Furthermore, cSWAP at the fovea correlated negatively and significantly with MPOD at all eccentricities. Thus, high cSWAP sensitivities were associated with low MPOD. However, after Bonferroni correction, only foveal cSWAP correlated significantly with MPOD at 1.75 and 3°. The maximal proportion of variability in cSWAP attributable to MPOD (r^2) is 21.2% (for foveolar cSWAP and MPOD at 1.75°).

DISCUSSION

Our hue discrimination data do not support the findings of Moreland and Dain,[22] who found a significant increase in both TES and PES in the blue-green region with their MP1 carotene filter of 1.0 maximum absorbance. We found no statistically significant association between MPOD at any retinal eccentricity and TES or PES after application of Bonferroni correction. This discrepancy may be a reflection of the nature of Moreland and Dain's filter, which was considerably denser than typical MPOD values; it exceeded the MPOD of all our subjects at and between 1.75° and the foveola) and did not provide an exact fit to the spectral absorbance of MP. It may also reflect a difference between a physiological filter, to which the visual system has adapted, and a filter placed before the eye.

It is possible that an artificial filter creates short-term changes in color vision and that an autoregulatory process adjusts retinal and/or cortical color mechanisms on a long-term basis in response to their naturally occurring MPOD. This hypothesis is supported by data showing a consistent shift in achromatic locus over a 3 months period for cataract patients postsurgery,[32] by color con-

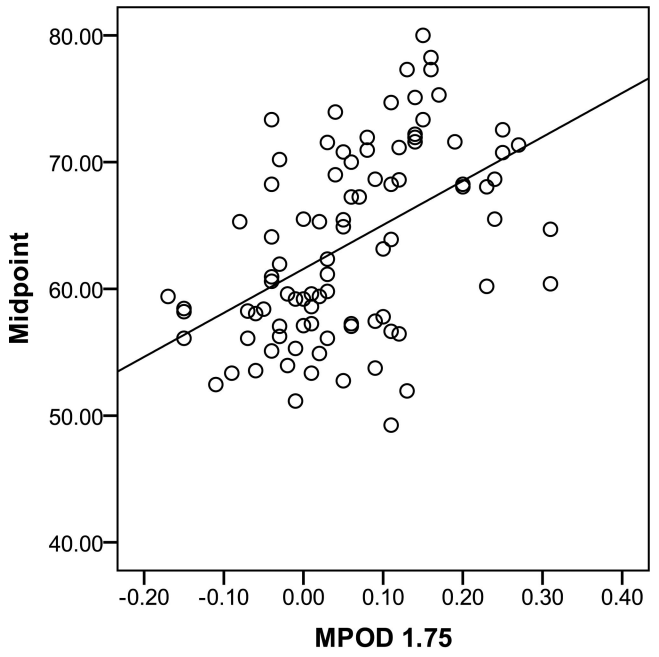

FIGURE 3.

Scattergram of anomaloscope Moreland match midpoints against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (midpoint = 35.91 × MPOD + 61.46).

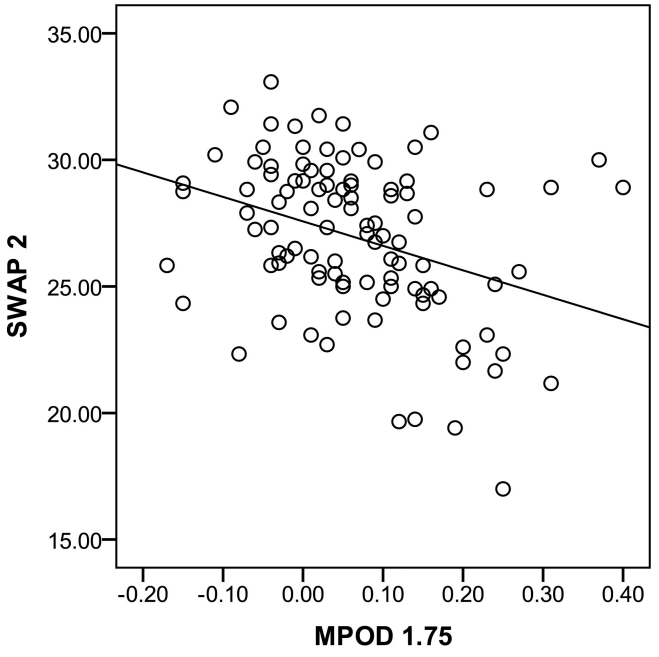

FIGURE 4.

Scattergram of sensitivity data on customized shortwave automated perimetry (cSWAP) at 2° eccentricity against MPOD at 1.75° eccentricity. Solid line = linear model least squares regression (cSWAP = −9.67 × MPOD + 27.57).

stancy effects for blue and green targets despite crystalline lens brunescence,[33] and by evidence of plasticity of adult neural color mechanisms.[34] Rodriguez-Carmona et al.[26] found no correlation between YB thresholds and MPOD using a technique in which threshold color differences were measured for detection of movement of a stimulus within a checkered array.

We did not assess the association, if any, of MPOD across subjects with color appearance other than by using the HMC anomaloscope Moreland match. Using this technique, we found that midpoint data were surprising in that subjects with high MPOD required less blue to match cyan; this finding was consistent for MPOD at all eccentrities. No directly comparable data exist in the literature, although Stringham and Hammond[17] found that YB cancellation thresholds were constant across the retina despite significant MPOD variability across the retinal region tested. It is of interest that in one study of Moreland match midpoint data, no difference was reported between postcataract patients with short wavelength-absorbing intraocular lenses and those with clear intraocular lenses.[35]

The cSWAP data show relatively constant sensitivity across the retina beyond the foveola (Fig. 5) despite substantial differences in MPOD across the retina (Fig. 1). This finding is consistent with that of Stringham et al.[36] who used Maxwellian-view multichannel optics except that they found slightly lower sensitivity at the foveola compared with parafovea using 16 subjects of similar age to those in this study. This suggests that parafoveal (but not foveolar) cSWAP may provide a valid clinical test of SWS cone function. The fact that we found statistically significant inverse correlations between short-wave sensitivity for the foveal stimulus and MPOD at two eccentricities does not in fact contradict Stringham et al.'s conclusions; our correlations relate to differences between subjects rather than to averaged measures across the retina which would not take into account the effects of intersubject variance in both SWS cone sensitivity and MPOD at any single retinal locus.

We hypothesize that the fact that SWS cone sensitivity exhibited significant inverse associations with MPOD, whereas hue discrimination thresholds showed no significant associations with

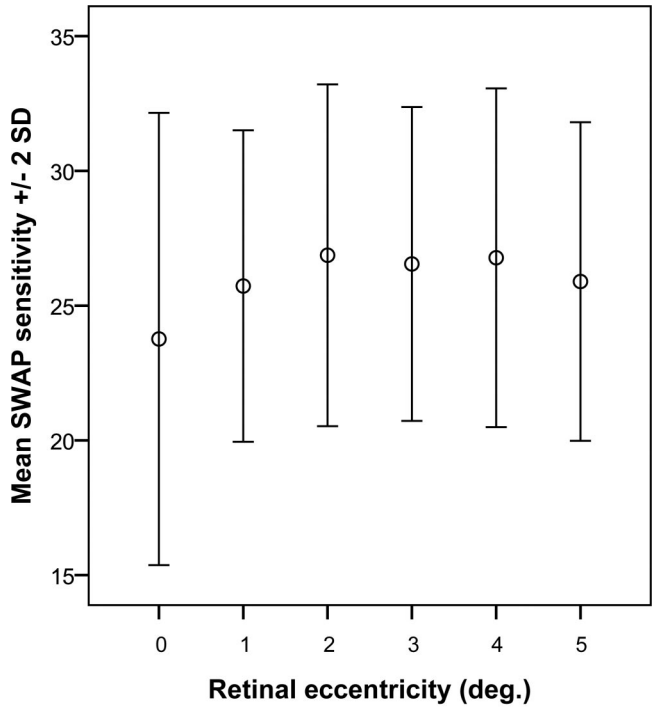

FIGURE 5.

cSWAP spatial profile. Abscissa: eccentricity in degrees. Ordinate: mean cSWAP sensitivity in decibels across subjects ± 2 SDs.

MPOD, may be related to temporal differences between the two measures. It is possible that, by using short stimulus presentations, the cSWAP technique (200 ms) produces transient effects quite different to those found with much longer presentations such as those of the FM100 test.

Confounding variables which might influence the relationship between MPOD and color vision include iris and choroidal pigmentation, age, stimulus size, and pupil diameter. The effect of iris pigment density has been studied by Woo and Lee,[37] who found that Asians have poorer PES in the blue quadrant, and by Hammond and Caruso-Avery,[38] who reported that subjects with darker irides had higher MPOD. Because all subjects in this study were white, the density range of both iris pigment and choroidal pigment was limited, and yet MPOD was found to correlate significantly with color sensitivity across a variety of measures. We suggest that our findings are independent of iris pigmentation, although such pigmentation is a factor in a less racially homogenous group of subjects.[39]

The effect of age on hue discrimination, in the blue-green spectral region in particular, is well known[40] and is partly because of wavelength-selective loss of light transmission by the aging crystalline lens.[41] An age effect on MPOD has also been reported, some studies having shown a statistically significant age-related decline in MPOD.[38,42] It is therefore possible that age is a confounding factor influencing our findings on MPOD and hue discrimination in the blue-green spectral region. A similar age effect is possible in relation to SWS cone function as measured by cSWAP.[43,44] Although our subjects were restricted to the age range 18 to 40 years, and our exclusion criteria included any evidence of cataract, potentially confounding contributions attributable to age cannot be dismissed. However, inspection of Table 1 shows that first-order partial correlation coefficients with age as the control variable are very similar to 0-order coefficients. In no case did a significance level change from significant to non-significant by controlling for age. We therefore suggest that our observed associations between MPOD and both Moreland midpoint and cSWAP are independent of age within the age range of this study (18 to 40 years, mean age $\pm$SD = 29 $\pm$ 6 years). However, the age factor may be important in older subjects.

Stimulus size and location are known to affect both color vision[45] and measures of MPOD.[3] In this study, MPOD was measured using targets subtending between 30 min and 3.5° at eccentricities between 0 and 3°. Color thresholds were measured using centrally fixated targets subtending ~1.5° (FM100), 2° (anomaloscope), and 1.7° at between 0 and 5° eccentricity (cSWAP). A clear pattern is evident from our data: MPOD correlated consistently across size and eccentricity parameters with cSWAP and Moreland midpoint. MPOD values were reported in this study at a range of eccentricities to assess the consistency of correlations, and because retinal images extend beyond their geometric optical limits as a result of aberrations, diffraction, and scatter. Furthermore eye movements produce translational shift of retinal images in a natural viewing environment.

The practical implications of this study are two-fold. First, dietary supplementation to increase MPOD is not likely to adversely affect hue discrimination. However, a longitudinal study of the effects of supplementation on color vision is needed to support this. Second, we have shown that appropriate customization of a standard clinical automated perimetry test (cSWAP) provides a potential clinical test for foveal SWS-cone sensitivity, although this awaits confirmation by a concordance study using Maxwellian view instrumentation.

ACKNOWLEDGMENTS

This work was supported by Enterprise Ireland and the Innovation Partnership of Bausch & Lomb Inc.

Received May 18, 2010; accepted January 27, 2011.

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