The multifocal visual evoked potential and cone-isolating stimuli: Implications for L- to M-cone ratios and normalization

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Multifocal visual evoked potentials (mfVEP) were recorded with a pattern-reversing display that modulated only the long wavelength-sensitive (L) cones or only the middle wavelength-sensitive (M) cones. Outside the central 5.8° (radius), the ratio of the amplitudes of the mfVEP responses to L- and M-cone modulation varied across the six subjects, ranging from 1.1 to 1.7. The responses from the central 1° (radius) showed a substantially lower ratio, ranging from 0.8 to 1.1 (average of 0.9). The variation among individuals outside the central fovea is probably due to differences in the ratio of the L/M cone input to both magno- and parvocellular pathways. The substantially lower ratios for the central responses is consistent with an L/M cone ratio closer to 1.0 in the central 1° and/or an adjustment in the gain of the L- versus M-cone contributions to the central parvocellular pathways. Taking into consideration evidence from other techniques, we believe it is unlikely that most individuals have a L/M cone ratio of 1.0 in the fovea. Instead, it appears that there is a change in gain before the mfVEP is generated in area 17.

Keywords: evoked potentials, multifocal, cones, ERG, color vision

Introduction

Based on evidence from a variety of techniques, it appears that the ratio of the number of long wavelength-sensitive (L) cones to middle wavelength-sensitive (M) cones varies widely among normal human trichromats (for recent reviews, see *Journal of the Optical Society of America A*, March 2000). Although it has been known for some time that, on average, a ratio of L/M cones of 1.5 to 2.0 is needed to describe typical spectral luminous efficiency functions obtained psychophysically, such as the 1924 CIE V(λ) function (e.g., Guth, Alexander, Chumbly, Gillman, & Patterson, 1968; Vos & Walraven, 1971; Smith & Pokorny, 1975; Stockman & Sharpe, 2000), it also has been long known that this value can range widely among individuals (e.g., de Vries, 1946, 1948; Rushton & Baker, 1964). For example, with neutral adapting conditions and a small (2° diameter) foveal target, the values of L/M ratios needed to describe heterochromatic flicker (25 Hz) photometric (HFP) sensitivities of normal trichromats range from 0.03 (very M-cone dominated) to 5.19 (very L-cone dominated), with a mean of about 1.4 (H.J., Jagla, Knau, & L.T.S., in preparation).

Similarly, other psychophysical paradigms (e.g., the point source detection technique) yield estimates of L/M ratios varying from 1.6:1 to greater than 7:1 (e.g., Wesner, Pokorny, Shevell, & Smith, 1991; Otake & Cicerone, 2000). With the electroretinogram (ERG) and a procedure

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akin to flicker photometry, the estimates of L/M ratios obtained ranged from 0.6:1 to 12:1 (e.g., Jacobs, Neitz, & Krogh, 1996; Carroll, McMahon, Neitz, & Neitz, 2000). More recently, ranges of L/M ratios at least as large as these have been obtained with two new ERG paradigms (Kremers, Usui, Scholl, & Sharpe, 1999; Albrecht, Jägle, De Luca, & Sharpe, 2002). In addition, two relatively new techniques, direct visualization with adaptive optics and mRNA measurements, attack the question of L/M cone ratios with entirely different approaches but come to similar conclusions. In the first, L- and M-cones are directly visualized and counted in the human retina employing a combination of high-resolution imaging and selective bleaching of M- and L-cones (Roorda & Williams, 1999; Brainard, Roorda, Yamauchi, Calderone, Metha, Neitz, Neitz, Williams, & Jacobs, 2000). With this technique, very different L/M ratios (about 1.1:1 and 3.8:1) were observed for two color normal individuals. With the second technique, when the mRNA of patches of retina from 23 human donor eyes were analyzed, L/M ratios from about 0.8:1 to over 4:1 were obtained for patches from the central 20° (Hagstrom, Neitz, & Neitz, 1997, 1998, 2000).

Of course, the ratios obtained with any of these techniques should be interpreted with caution. All of the techniques require assumptions to get from the parameters that are measured (e.g., ERG amplitude and intensity for minimum flicker) to an estimate of L/M cone ratios. In this context, it is reassuring that a number of studies have shown reasonably good correspondence among the estimates of L/M ratios obtained with different behavioral, anatomical and ERG measures (e.g., Pokorny, Smith, & Wesner, 1991; Wesner et al., 1991; Chang, Burns, & Kreitz, 1993; Brainard et al., 2000; Kremers, Scholl, Knau, Berendschot, Usui, & Sharpe, 2000; Albrecht et al., in press). Thus, it appears certain that, on average, there are more L- than M-cones and that L/M cone ratios of individuals differ from well under 1:1 to 10:1 or more. However, the degree to which L/M cone ratios vary with eccentricity is under debate.

Two separate techniques—one molecular genetic, the other electrophysiological—have been used to address this issue. Molecular biological analysis of opsin mRNA assayed from postmortem human eyes, in retinal patches of 20° diameter, suggested a central L/M cone ratio of 1.5:1, which increases to 3.1:1 in the mid periphery (about 41° eccentricity) (Hagstrom et al., 1998). Similarly, in the accompanying multifocal ERG (mfERG) study (Albrecht et al., in press), the results are consistent with a lower L/M cone ratio in the central fovea (5° diameter) than in the periphery (annular ring centered at 40°). However, the mfERG data are open to alternative explanations (Albrecht et al., in press), and their resolution is currently limited to about 5° (diameter), even in the important central foveal region.

Thus far, our estimates of the L/M ratio in the central 2° or so come entirely from behavioral techniques. In general, these techniques (i.e., spectral sensitivity functions, HFP, and two-point detection) agree and argue for L/M ratios greater than 1.0 in the central fovea of the average observer. However, at least three paradigms produce results consistent with something closer to a 1:1 weighting of L- and M-cone inputs: (1) the settings of unique yellow (e.g. Pokorny & Smith, 1987; Pokorny et al., 1991); (2) the appearance of brief, small lights (e.g., Krauskopf, 2000); and, (3) the detection of relatively large (e.g., 2°) foveal lights that are slowly modulated in time (e.g., < 8 Hz) (Krauskopf, 2000; Kremers et al., 2000). These results pose a challenge for models of foveal vision. To account for them, Krauskopf (2000) suggested that the ratio of cones in the fovea is close to 1:1 in all normal trichromats. If this is the case, then a gain adjustment must take place in the magnocellular (MC) pathway to produce the near 2:1 balance of L- and M-cone inputs observed in tasks such as HFP, which are believed to be mediated by that pathway. On the other hand, Pokorny, Smith, and colleagues pointed to the agreement between the data from point source detection and HFP experiments and argued that the L/M cone ratio in the fovea varies widely among individuals (e.g., Pokorny et al., 1991; Wesner et al., 1991). Again, a normalization was hypothesized to occur—but now in the parvocellular (PC) pathway—to produce the near 1:1 balance of L- and M-cone inputs suggested by the other paradigms, including the setting of the unique yellow wavelength (see also Miyahara, Pokorny, Smith, Baron, & Baron, 1998; Otake & Cicerone, 2000). This speculation has since been put forward by others (Kremers et al., 2000). In short, two questions remain concerning the central fovea: Is the ratio of L- to M-cones 1:1 or does it vary among individuals? If it does vary among individuals, where does the normalization, or change in gain, take place?

A newly developed technique based on the visual evoked potential (VEP) offers a different approach to this problem. The VEP is a gross electrical potential generated by the cells in the occipital cortex and easily recorded with scalp electrodes. With the traditional VEP technique, only a few field locations can be tested within a single session. The multifocal VEP (mfVEP) technique, by way of contrast, allows for the simultaneous measurement of 60 focal VEP responses from locations from the fovea out to 45° or more (Baseler, Sutter, Klein & Carney, 1994). Although the mfVEP recently has received considerable attention, relatively little has been done with cone-isolating stimuli (Klistorner, Crewther, & Crewther, 1998; Baseler, Schneck & Sutter, 1996). In this study, mfVEP potentials were recorded to focal L- or M-cone modulation. This allowed us to ask whether the relative responses to L-cone modulation compared with M-cone modulation changed with eccentricity. A preliminary report of these results was presented at the 2001 annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, FL (Yu, Hood, Zhang, Albrecht, Jägle, & Sharpe, 2001).

Methods

Subjects

The six subjects, four females and two males, in this study ranged in age from 16 to 58 years and had 20/20 corrected acuity and normal color vision as determined by pseudoisochromatic plates and Nagel Type I anomaloscope. None of the subjects had a history of color blindness in their pedigree. Molecular genetic analysis of the opsin gene array on the X-chromosome was performed in one male (D.H.) and two female (A.Y. and C.C.) observers (Jagla, private communication). This included the female observer (A.Y.) who had the lowest estimated L/M cone ratio (near 1.0, which is consistent with her previous mfERG measurements; Albrecht et al., in press). All three have a normal L-cone pigment gene (1st gene in the array) and one or more normal M-cone pigment genes (downstream) on their X-chromosomes, but no L/M or M/L hybrid genes. Given that both females have only normal L- and M-cone pigment genes on their two X-chromosomes, they are very unlikely to be carriers for any protan or deutan color-vision deficiencies.

Procedures followed the tenets of the Declaration of Helsinki, and the protocol was approved by the committee of the Institutional Board of Research Associates of Columbia University, New York, NY.

L- and M-Cone Isolation

A pattern-reversing display (**Figure 1A**), described below, alternated between red and green lights calibrated so that only the L-cones or only the M-cones were modulated. The logic here is akin to the silent substitution technique (for a review, see **Estevez & Spekreijse [1982]**). For example, the pattern of the L-cone-isolating stimuli (called "L-cone modulation" here) alternated between red and green lights that were equally effective for the S- and M-cones, and, thus, only modulated the L-cones. The red and green lights for the L- and M-cone stimuli were calibrated from the emission spectra of the three phosphors and the cone fundamentals for 10° and larger viewing conditions (Stockman & Sharpe, 1998, 1999, 2000) as described in detail in Albrecht et al. (2002, in press). These cone-isolation settings were adjusted slightly based on recordings from a protanope and a deuteranope to eliminate the possibility of any residual response from the unwanted cone type that would contaminate the recordings. In terms of cone contrast, these adjustments were extremely small (<1.6% or a change from 49.2% to 50.0% contrast), and insignificant, given that a factor of 2 change in cone contrast (50% vs. 25%) decreases the mfVEP amplitude by only a factor of about 1.3. The basic findings were confirmed for two subjects (D.H. and A.Y.) using the settings before these slight adjustments were made. In addition, the consequences of using cone fundamentals for 2° viewing conditions (Stockman & Sharpe, 2000), as opposed to 10° or larger, were considered. Adjusting our settings to conform to the 2° fundamentals would change the cone-contrast values by less than 1.5% and the linearized gun values by about 2% (the phosphor settings by about 1%), well within our error of measurement and insignificant in terms of the mfVEP response amplitudes and their variability.

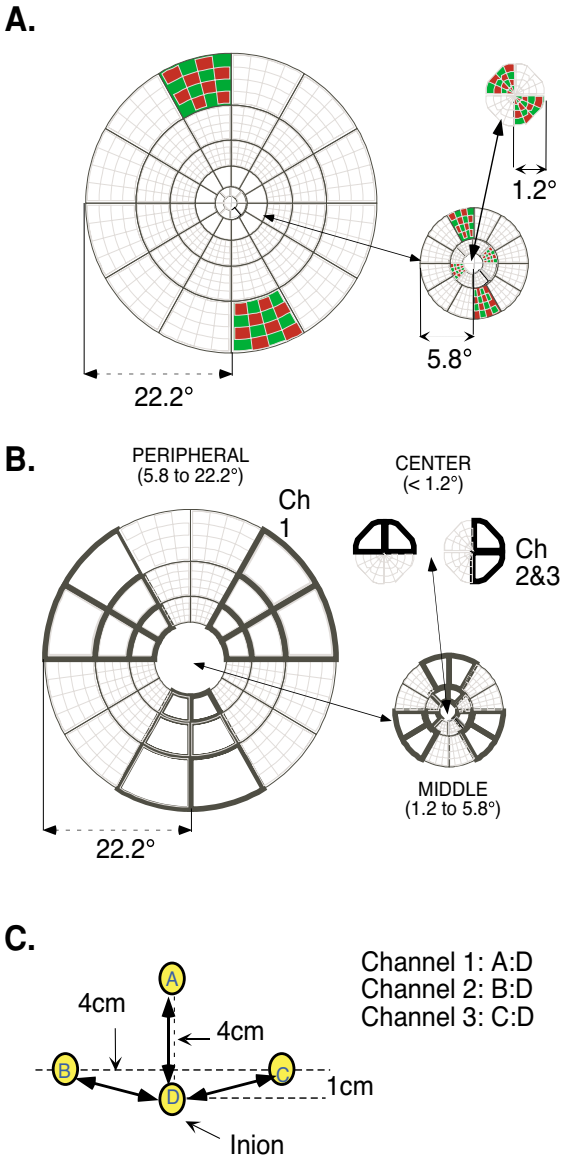

Figure 1. A. The dartboard array with 60 sectors. B. Responses were summed within the groups shown. C. Electrode positions and configurations for the three channels of recording.

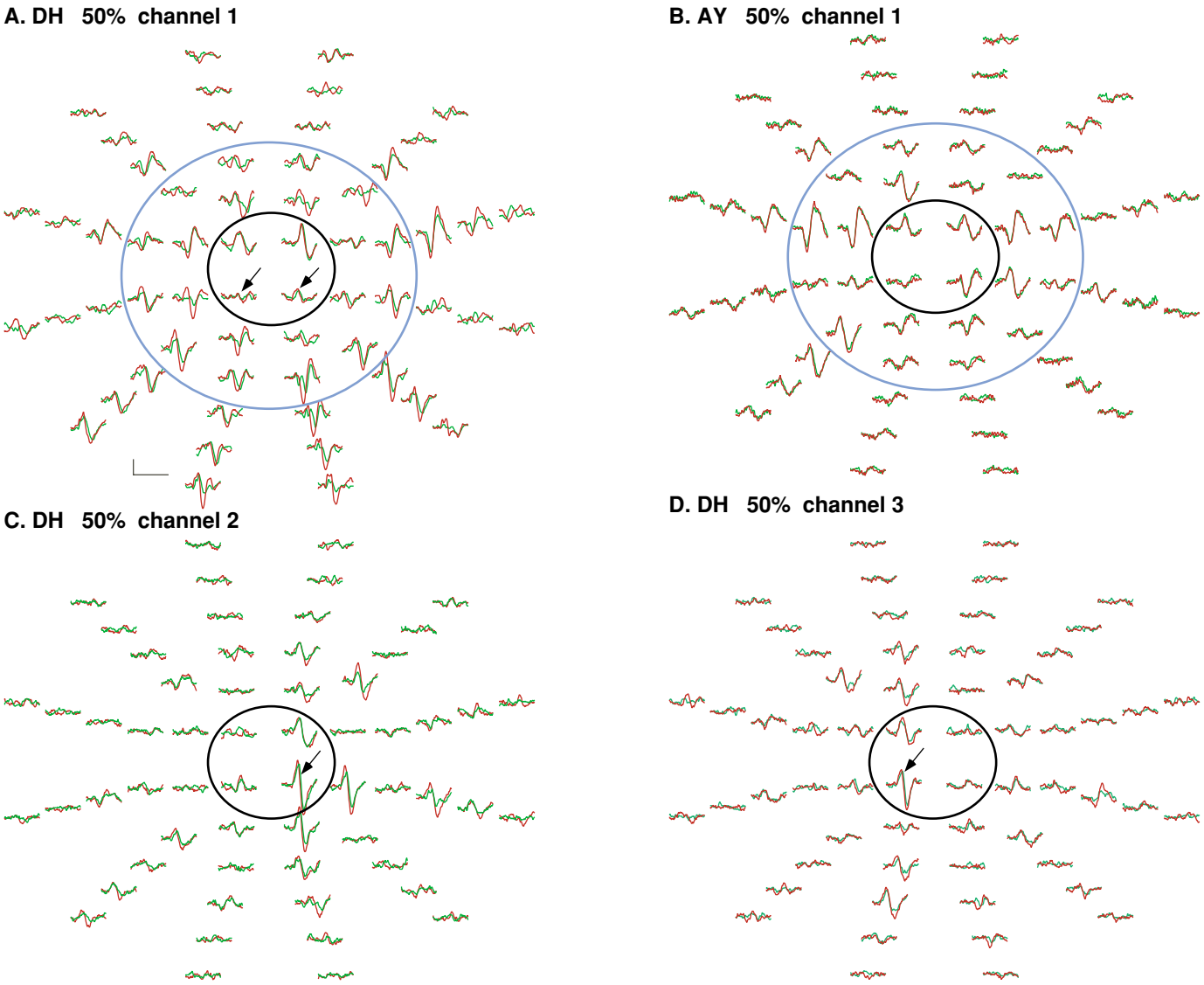

Figure 2. A. The 60 mfVEP responses from subject D.H. to the L-cone (red traces) and M-cone (green traces) modulated stimuli of 50% contrast. B. Same for subject A.Y. C and D. The recordings as in A but from channels 2 (C) and 3 (D). The calibration bars indicate 200 nV and 200 ms.

The mfvEP Display

The stimulus array was produced with VERIS software (Dart Board 60 with Pattern; Electro-Diagnostic Imaging, San Mateo, CA). The stimulus (Figure 1A) consisted of 60 sectors, each with 16 checks, 8 red and 8 green. The mfVEPs were recorded to M- and L-cone modulation of equal mean quantal catch and equal contrast (50%). The entire display had a radius of 22.2°. The sectors were scaled to take into consideration cortical magnification (Baseler et al., 1994). However, due to variations in the folding of the cortex, the orientation of the cells relative to the electrodes will differ for local regions of the cortex. Thus, the amplitude of the response, even to sectors scaled perfectly for cortical magnification, will vary both within and among subjects. In fact, there is a wide variation in amplitude even for the responses from sectors at the same eccentricities (see Figures 2A and 2B).

The central 4 sectors of the display fell within 1.2° (i.e., a diameter of 2.4°) of the foveal center (see insert, Figure 1A) and the 20 sectors of the next two rings within 5.8° (see insert, Figure 1A). The surround of the display was set at the time average mean luminance, which was 30.6 and 16.8 cd/m^2 for the M- and L-cone modulation. The stimulus array was displayed on a 21-inch Apple Studio Display monitor (Apple Computer, Inc., Cupertino, CA) driven at a frame rate of 75 Hz. The 16-element checkerboard of each sector had a probability of 0.5 of reversing on any pair of frame

changes, and the pattern of reversals for each sector followed a pseudorandom (m) sequence. For a more detailed description of the multifocal technique, see Sutter (1991), and for more information about the mfVEP, see Baseler et al., (1994); Hood, Zhang, Greenstein, Kangovi, Odel, Liebmann, & Ritch (2000); and Hood & Zhang (2000).

mfVEP Recordings

Multifocal VEPs were recorded on three channels with four gold electrodes placed as shown in Figure 1C. The electrodes, indicated as A, B, and C, were each referenced to electrode D placed at the inion and the associated differential signals recorded on three separate channels as indicated in Figure 1C. A forehead electrode served as the ground. All responses in the figures are displayed with the reference (inion) electrode as negative. The records of primary interest are from channel 1; this electrode configuration has been employed in previous work (Hood, Zhang, et al., 2000; Hood, Odel, & Zhang, 2000). Channels 2 and 3 were added because past experience indicated that the central responses can be very small in some individuals and that signals recorded with the laterally placed electrodes are often larger (Klistorner & Graham, 2000; Hood, Zhang, Hong, & Chen, in press).

The continuous VEP record was amplified, with the low- and high-frequency cutoffs set to 3 and 100 Hz (1/2 amplitude; Grass preamplifier P511J, Quincy, MA), and was sampled at 1200 Hz (every 0.83 ms). The m-sequence had 2^{15}-1 elements requiring about 7 minutes of recording. Unless otherwise specified, the records presented in the figures are the averages of three of these runs. To improve the subject's ability to maintain fixation, the run was broken into overlapping segments, each lasting about 27 s. Second-order local response components were extracted using VERIS 4.2 software from Electro-Diagnostic Imaging.

mfERG Recordings

For two subjects, A.Y. and D.H., mfERGs were recorded to M- and L-cone modulation of equal mean quantal catch and equal contrast (47%) in the Division of Experimental Ophthalmology, University Eye Hospital, of the University of Tübingen in Germany. The procedures for these recordings are described in detail in Albrecht et al. (2002, in press). Briefly, mfERGs were recorded with DTL electrodes to a display with 103 scaled hexagons that subtend a field of 84° by 75°. A.Y. was one of the subjects in the study by Albrecht et al., in press. For her, the ratio of summed mfERG amplitudes for the L- and M-cone modulation was near 1. Because D.H. and A.Y. represented the ends of a continuum of mfVEP amplitude ratios in this study (see below), D.H.'s mfERGs were recorded in Tübingen as well.

Displaying the Responses

The recordings of primary interest came from channel 1 (inion plus 4 cm [A] to inion [D] in Figure 1C). Figure 2 contains the response arrays for two subjects, D.H. (panel A) and A.Y. (panel B), recorded from this channel. The red and green records correspond to the responses for L-cone and M-cone modulation, respectively. As indicated above, channels 2 and 3 were added to help assure measurable responses to the central 4 sectors. The recordings from channels 2 and 3 in Figures 2C and 2D for D.H. illustrate this point. At least two of the central responses, indicated by the arrows in Figures 2C and 2D, are clearly larger than the corresponding responses from channel 1, indicated by the arrows in Figure 2A.

To improve the signal to noise and to make it easier for the reader to see the differences among subjects, the individual mfVEP responses in Figure 2 were grouped as shown in Figure 1B and summed. In particular, the 36 sectors of the three most peripheral rings, falling between 5.8° and 22.2°, were divided into six groups of six sectors and their responses summed. These are the responses falling outside of the blue circles in Figures 2A and 2B. The central 4 sectors, falling within 1.2° of the foveal center and within the black circles in Figures 2A and 2B, were divided into two groups as shown in Figure 1B. (Notice the grouping is different for channel 1 as opposed to channels 2 and 3.) Finally, the remaining 20 sectors of the middle 2 rings, falling between 5.8° and 1.2° and between the black and blue circles in Figures 2A and 2B, were divided into two groups of 4 sectors and four groups of 3 sectors and their responses summed. For all groups, the responses were summed within regions producing responses of similar waveforms in most individuals (Klistorner & Graham, 1999; Hood, Odel, et al., 2000, Hood, Zhang, et al., 2000). Further, in the case of channel 1, the responses from the upper and lower fields are, in general, reversed in polarity as expected from the topography of V1 in the calcarine fissure (Baseler et al., 1994; and see Figure 8 in Hood & Zhang, 2000). Although the precise relationship between the mfVEP and the traditional pattern-reversal VEP (e.g., Harding, Odom, Spileers, & Spekreijse, 1996) has yet to be determined, the first prominent peak of the mfVEP appears to correspond to N75 and the second to P100. In channel 1, the polarity of N75 tends to be positive in the lower field and negative in the upper field, although variations exist across the hemi-fields due to variations in the folding of local regions of the cortex (see Figures 3, 5, and 8 in Hood & Zhang, 2000). Similarly, the responses for channels 2 and 3 typically are reversed in polarity as the vertical midline is crossed. Thus, for the central responses, the upper two and lower two were grouped together in the case of channel 1 and the left and right two in the case of channels 2 and 3.

Results

In Figure 3, the mfVEPs from D.H. and A.Y. from Figures 2A and 2B are shown for the groups indicated in Figure 1B and described in the section above. The **red** records were elicited by the L-cone modulation and the **green** records by the M-cone modulation. There are three key findings illustrated in Figure 3. First, for the central responses, the M- and L- cone modulations produce responses of approximately similar amplitude and similar waveform for both subjects. Second, D.H.'s responses from the middle and periphery of the field were larger to the L-cone modulation than to the M-cone modulation, whereas A.Y.'s responses were approximately the same amplitude. Third, the responses to L- and M-cone modulation from the middle and periphery of the field differ in waveform for D.H. but not for A.Y. The difference in waveform is particularly conspicuous in the case of D.H.'s peripheral responses. The significance of this difference in waveform will be considered below.

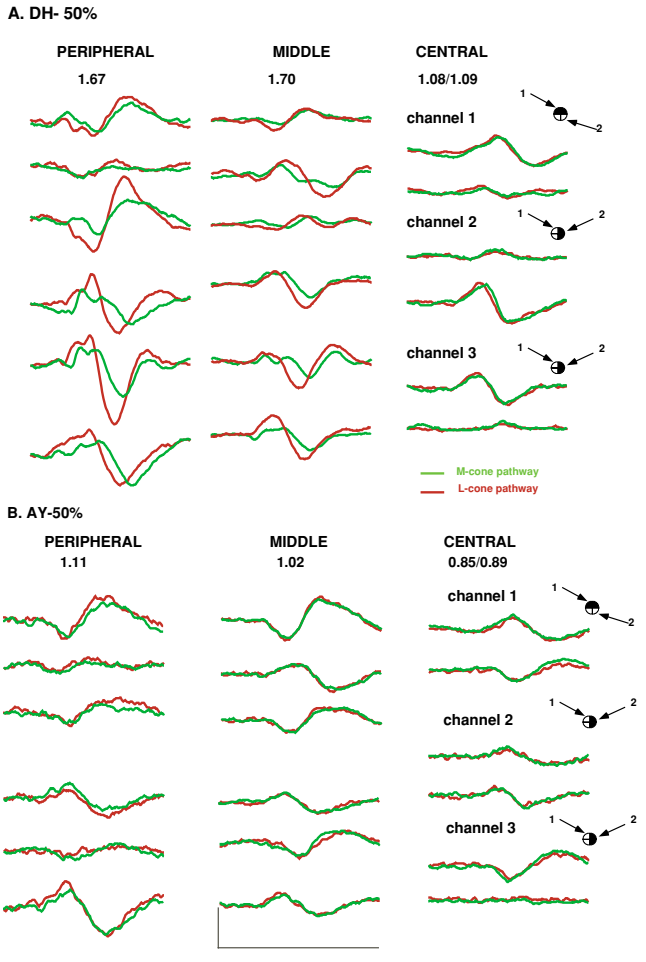

Figure 3. The mfVEPs summed as in Figure 1B for subjects D.H. (A) and A.Y. (B). The numbers are the ratios of the root mean square (RMS) amplitudes to the L- versus M-cone modulation. The calibration bars indicate 1 mV and 200 ms.

Figures 4 and 5 show the responses for the central and peripheral regions for the other four subjects. These are displayed as in Figure 3 with one minor exception. For conciseness of presentation, in Figure 4, responses for channels 2 and 3 are shown only for the half of the field with the larger responses. To a first approximation, the central responses are similar in waveform and amplitude for these four additional subjects (Figure 4) as for subjects D.H. and A.Y. (Figure 3). In general, unlike the central responses, the peripheral responses differ in amplitude and waveform for the two modes of stimulation (Figure 5), although there is a wide range of variation among individuals. Among the six subjects, the two sets of responses (central and peripheral) are most similar for A.Y. and most dissimilar for D.H. (Figure 3).

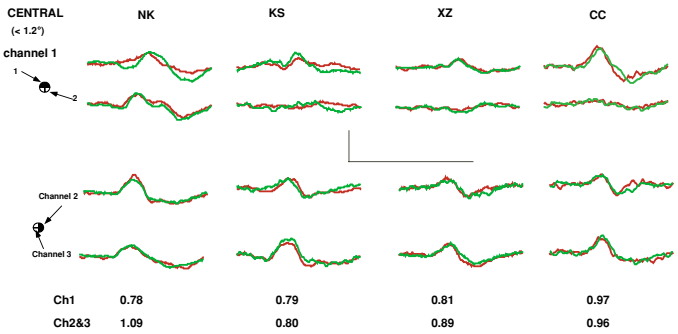

Figure 4. The summed mfVEPs for the central groups (see Figure 1B) from four subjects. The numbers are the ratios of the root mean square (RMS) amplitudes to the L- versus M-cone modulation. The calibration bars indicate 1 mV and 200 ms.

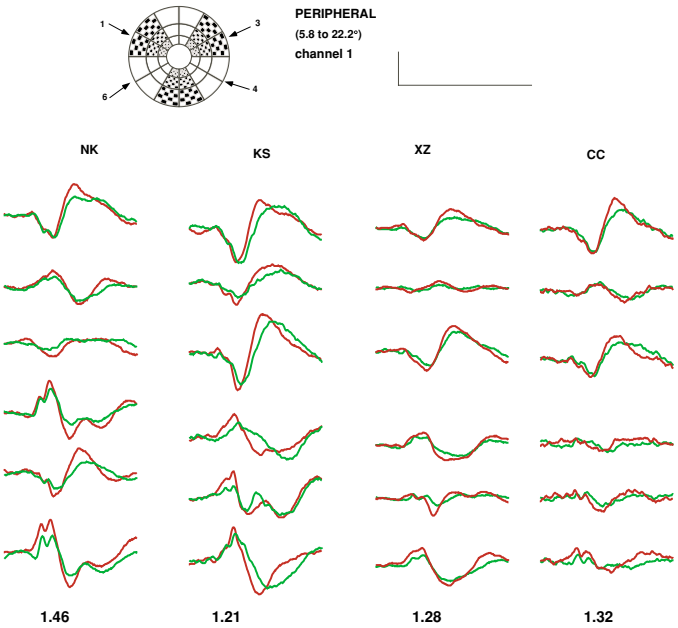

Figure 5. The summed mfVEPs for the peripheral groups (see Figure 1B) from four subjects. The numbers are the ratios of the root mean square (RMS) amplitudes to the L- versus M-cone modulation. The calibration bars indicate 500 nV and 200 ms.

To obtain a quantitative measure of these differences, the root mean square (RMS) amplitude of the responses was measured with a period of analysis from 45 to 200 ms (all responses in all figures are 200 ms in length). The RMS amplitude was calculated for each of the peripheral records shown in Figures 3 and 5, and then summed. The numbers in Figures 3 and 5 are the ratio of these summed RMS values for L-cone compared with M-cone modulation. For example, the RMS amplitude of D.H.'s peripheral responses was 1.67 larger for the L-cone modulation than for the M-cone modulation. On average, for all six subjects, this ratio was 1.34, with D.H. and A.Y. having the largest (1.67) and smallest (1.11) values. A similar analysis was performed for the central records and the results are shown in Figures 3 and 4. Here two numbers are shown, one for channel 1 and one for the combination of channels 2 and 3. On average for all 6 subjects, these ratios were 0.88 (channel 1) and 0.95 (channels 2 and 3). The responses from the central 1° clearly had a significantly lower ratio than the peripheral responses. The ranges of ratios were nonoverlapping, and the ratio for the center was lower than the ratio for the periphery for each of the six subjects.

To ensure that our choice of groups did not affect our conclusions, the RMS amplitudes were also obtained for each of the 60 individual responses, and then these RMS amplitudes were summed for sectors of equal distance from the central fovea. Figure 6 shows the ratios of these summed RMS amplitudes versus the distance of the center of the sectors from the fovea. For example, the point at zero represents the ratio for the four central sectors, the next point for the eight sectors in the second ring, and so on. It appears that most of the difference with eccentricity is seen within 2° of the foveal center.

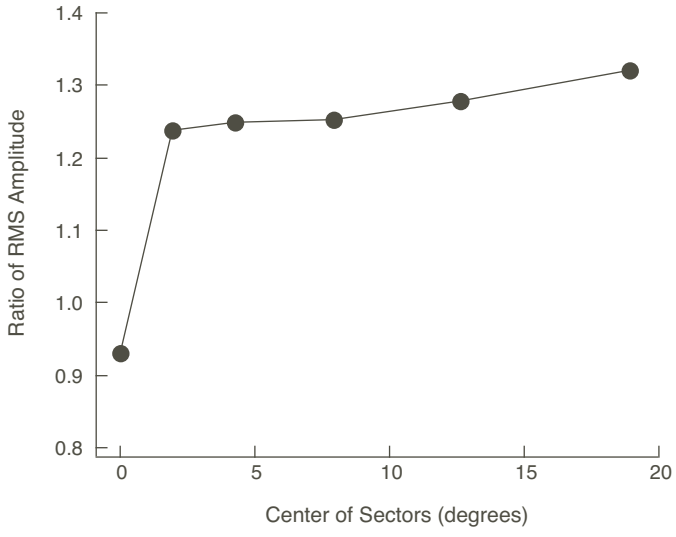

Figure 6. The mean (n = 6) ratio of root mean square (RMS) amplitude to the L- versus M-cone modulation as a function of the eccentricity of the center of the sectors.

The Effects of Contrast

The responses in Figures 3-5 were for the 50% contrast condition, the maximum cone-specific contrast available. Figure 7 shows the results for the 25% condition for the two subjects with the most extreme ratios of RMS amplitudes to L- versus M-cone modulation. The results are, in general, the same as for the 50% condition in Figure 3. For both subjects, the responses inside the center tend to be similar in amplitude for the M- and L-cone modulation, whereas in the periphery, D.H.'s responses to the L-cone modulation tend to be larger.

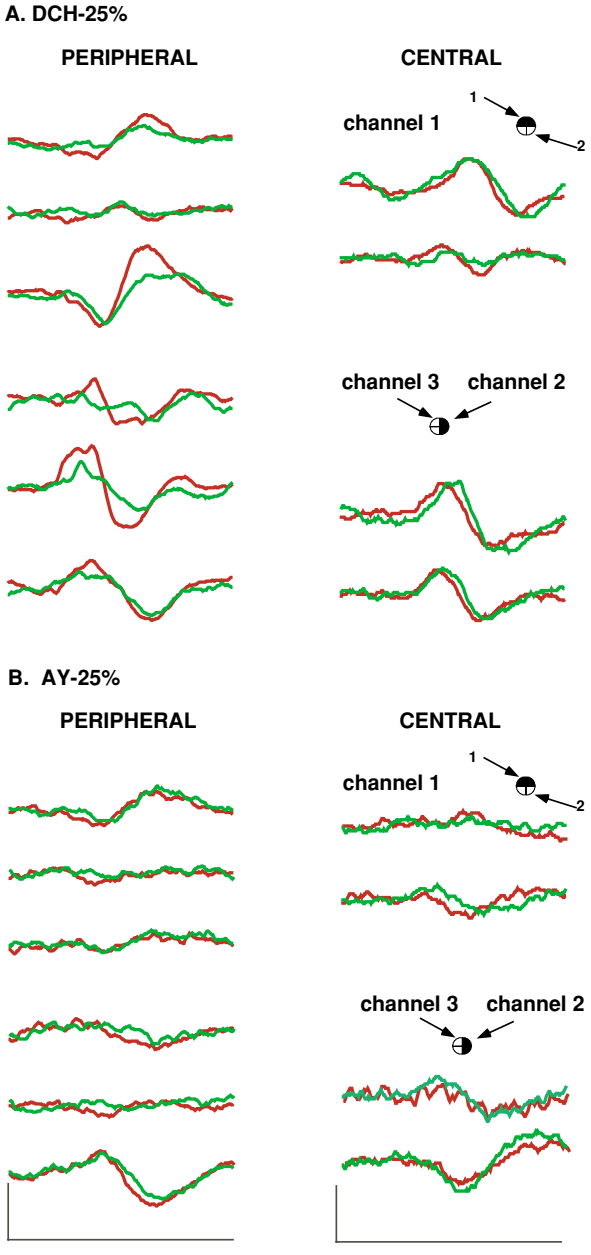

Figure 7. The mfVEPs to the 25% contrast displays summed as in Figure 1B for subjects D.H. (A) and A.Y. (B). The vertical calibration bars indicate 1 μV (left column) and 500 nV (right column) and the horizontal bars 200 ms.

Reducing the contrast decreases the amplitude in all regions. However, the waveform differences within D.H.'s records for M- versus L-cone modulation could not be mimicked with a change in contrast. Figure 8 provides a direct comparison between D.H.'s responses to the 25% L-cone modulation and the 50% M- cone modulation. In the center, the responses to the 50% M-cone modulation are larger, whereas in the middle and peripheral regions, they are more similar in amplitude, although, on average, the responses to the L-cone modulation are still slightly larger. The important point here is that although decreasing contrast brings the amplitudes of the peripheral responses closer, clear differences in waveform exist between the M- and L-cone modulation. The waveforms to the left in Figure 8 were amplified by a factor of 3 to illustrate this point. A particularly obvious difference is the relative amplitudes and latencies of the local positive peaks indicated with the dashed vertical lines. These differences are a very consistent and reproducible feature of many of the records from these regions from this subject. As discussed below, these features of the mfVEP waveform have been attributed to MC- and PC-pathway activity (Baseler & Sutter, 1997). In contrast, in the fovea where the PC pathway is expected to predominate, the waveforms for the L- and M-cone modulation are more similar. For the sets of records from channels 2 and 3, the responses to the L-cone modulation were amplified by a factor of 1.5 and displayed to the right. Compared with the peripheral responses, the central responses are more similar in waveform.

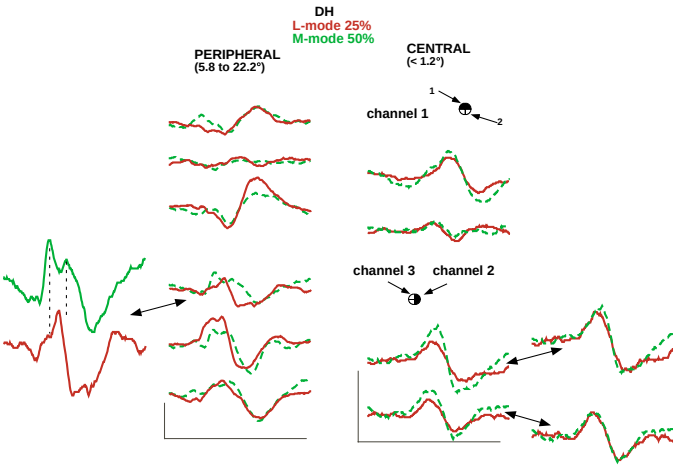

Figure 8. A comparison of D.H.'s mfVEPs elicited by the L-cone modulated stimulus of 25% and the M-cone modulated stimulus of 50%. The vertical calibration bars indicate 1 mV and the horizontal bars 200 ms.

For the six subjects, the RMS amplitude for the L-cone modulation was 1.30 times larger for the 50% contrast stimuli compared with the 25% contrast stimuli. Recall that for the same six subjects, the ratio of the RMS amplitudes to L- as opposed to M-cone modulations of 50% contrast was 1.34. Thus, the relative effectiveness of the M- and L-cone modulations is approximately equivalent to halving the contrast of the L-cone modulation.

The mfERG

The mfERGs recorded in Tübingen (see "Methods") from subjects D.H. and A.Y. are shown in Figure 9. These records are the summed mfERG responses from the entire field (Figures 9A and 9B) or from annuli (Figure 9C). A.Y.'s responses to M- and L-cone modulation are nearly the same (panel A). The peak-to-trough amplitude to the L-cone modulation was 10% smaller than to the M-cone stimulus. For D.H., on the other hand, the peak-to-trough amplitude to the L-cone stimulus was 225% greater (panel B). There is a qualitative agreement between these results and the mfVEP responses from the periphery of these subjects. The implications will be considered below.

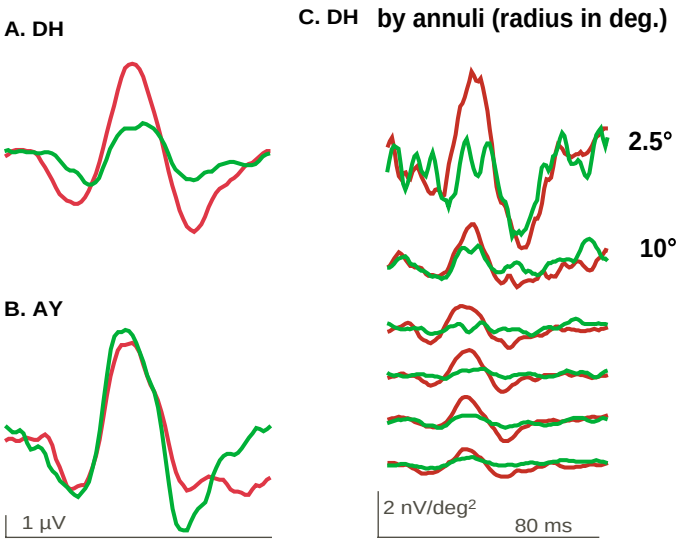

Figure 9. The mfERGs recorded in Tübingen are shown summed over the entire 103 hexagons and the 84° by 75° field for D.H. (A) and A.Y. (B) and summed by annuli for D.H. (C).

Discussion

The mfVEPs to cone-isolating stimuli were recorded from individuals with normal color vision. We were particularly interested in the relative amplitudes of the mfVEP responses to L- and M-cone modulation in the fovea compared with the periphery. Outside the central 5.8° (radius), the ratio of the amplitudes (RMS) of the responses to L- and M-cone modulation of equal cone contrast varied across individuals ranging from about 1.1 to 1.7 with an average ratio of 1.34. Inside the central 1.2°, the range was smaller and the mean ratio of RMS amplitudes was about 0.9. The waveforms of the responses are of interest as well. Within each subject, the central responses to M- versus L-cone modulation were

similar in waveform. On the other hand, in the periphery, most subjects showed different waveforms for the two modes of stimulation. The clear exception was A.Y., the subject whose mfERG records suggested an approximately equal number of L- and M-cones. Her mfVEP responses to M- and L-cone modulation were essentially identical in waveform both in the periphery and in the fovea. To consider the implications for estimates of L/M cone ratios, the mfVEP responses should be placed in the context of what is known about the PC and MC pathways.

PC and MC Pathways and L- and M-Cone Inputs

The foveal mfVEP

The mfVEP, as recorded here, is largely generated in striate cortex (Slotnick, Klein, Carney, Sutter, & Dastmalchi, 1999) presumably in response to inputs from both PC and MC pathways. In an anatomical study of the human retina, Dacey (1993) estimated that the midget ganglion cells (PC pathway) make up about 95% of the total ganglion cells in the central retina. Thus, it is likely that the mfVEP responses generated by foveal stimulation will be dominated overwhelmingly by the PC pathway. The high spatial frequency of the central segments will also act in favor of the PC pathway. Further, the similarity of the mfVEP response waveform to L- and M-cone modulation is consistent with a single pathway controlling the mfVEP from the central 1.2°. Recall that all the subjects in this study had nearly equal mfVEP amplitudes to the M- and L-cone modulations in this region. Therefore, the PC pathway of the central retina appears to produce approximately equal amplitude mfVEP responses to the L-cone and M-cone modulations. This suggests that either the ratio of L/M cones in the fovea is close to 1.0, as proposed by Krauskopf (2000), or that there is a cone-type specific adjustment in the gain of the input to the PC pathway at or before the generation of the mfVEP in striate cortex (e.g., Pokorny et al., 1991; Kremers et al., 2000; Otake & Cicerone, 2000). The bulk of the evidence favors the latter.

As mentioned in the "Introduction," the estimates of L/M ratios for the central 2° or so come largely from behavioral data. But, other techniques that approach the central 2° provide little support for a 1:1 ratio in the very central fovea. The mRNA results for the central 20° (Hagstrom et al., 1997, 1998, 2000), the direct visualization in one individual within 1° of the center (Roorda & Williams, 1999; Brainard et al., 2000), and the mfERG data within 5° (Figure 9 and Albrecht et al., in press) all show ratios greater than 1:1 in some individuals. In view of all the evidence, therefore, it is unlikely that the central 2° has a ratio close to 1:1 in all individuals. As an example, consider the mfERG results from D.H. in Figure 9C. The mfVEP responses from the central 1.2° of this individual were nearly equal in amplitude (Figure 3A). In contrast, the mfERG from the central 2.5° (radius) is clearly larger for the L-cone modulation. Although we cannot rule out the possibility that the retinal ratio changes abruptly, it seems unlikely given the other evidence. If this reasoning is correct, then these data argue that the gain adjustment is taking place in the PC pathway after the mfERG is generated but before the cells in area 17 respond. Because the mfERG, like the photopic full-field ERG, is dominated by the bipolar response (e.g., Sieving, Murayama, & Naarendorp, 1994; Hood, Frishman, Saszik, & Viswanathan, 2002; and see Figure 24 in Hood, 2000), the gain change implied by the mfVEP results must, at least in part, be beyond the bipolar cell, most likely in the inner plexiform layer before the ganglion cell responds.

The peripheral mfVEP

Presumably, the responses from the periphery are generated by a combination of MC- and PC-pathway activity. Consider the case where the receptive fields in the periphery are large enough so that all magno- and parvo-ganglion cells are receiving the same L/M ratio of cones. In the monkey, the relative number of L- to M-cones, which differs among individuals, appears to be preserved in the postreceptoral signal recorded in horizontal cells (Dacey, Diller, Verweij, & Williams, 2000). Thus, it seems safe to assume that these proportions continue to be preserved in the peripheral retina in the MC and PC pathways in which activity is contributing to the mfVEP recordings. To explain the qualitative differences in waveforms seen in most of our observers, we need to make the usual assumption that the MC pathway is more nonlinear and/or saturates at lower contrasts than does the PC pathway. For an individual with more L- than M-cones, the M- and L-cone modulations will produce similar MC-pathway activity because the MC pathway will have reached its maximum response to both modes of stimulation. On the other hand, the L-cone modulation will produce larger PC pathway activity than M-cone modulation because this pathway does not saturate as contrast is increased. If we assume further that the waveforms of the MC and PC contributions differ as suggested by the work of Baseler and Sutter (1997), then it becomes clear why the responses to the L-cone modulation will be both larger and of a different waveform. The response is larger because of the larger PC pathway contribution, and it has a different waveform because of a different proportion of MC to PC activity (with the proportion being smaller for the L-cone modulation). By the same line of reasoning, an individual with an approximately equal number of L- and M-cones should, like A.Y., have responses to the two modes of stimuli that are about equal in amplitude and of similar waveforms. The responses from A.Y. are consistent with this explanation.

By this line of argument, the ratio of the amplitudes of mfVEP is not a particularly good way to estimate the variation in L/M ratios across the retina. (This limitation does not apply to the mfERG, especially in the case of the peripheral mfERG traces.) In the center, a "gain change" may have already taken place. In the periphery, the responses will be indirectly related to the ratio of L/M cone input for two reasons. First, they are a sum of the responses from both MC and PC pathways. Hence, the combined response may not be a good representation of either. The positive and negative portions of these MC and PC responses could sum in ways to reinforce or cancel parts of the waveform. And, second, the amplitude of the MC-pathway's response is a nonlinear function of cone contrast. (This particular limitation does not apply to the mfERG because it is linear with contrast [Albrecht et al., 2002].) In the light of these caveats, it is surprising that, on average, the RMS amplitude ratio for the L- and M-cone modulations is about the same as a decrease in contrast of the L-cone modulation by a factor of 2. That is, the average results are consistent with a linear summation of cone-receptor signals, as is typically assumed, and a L/M ratio of about 2.

Bridging Between Single-Cell Physiology and Behavioral Data

For the reasons given, the mfVEP is not a particularly good way to estimate the variation in L/M ratios across the retina. It should be useful, however, in bridging the gap between models and hypotheses about PC and MC pathways derived from the physiology and anatomy of primates, including humans, on the one hand, and the wealth of behavioral data from humans, on the other. In this context, we intend to compare the mfVEP results obtained here to behavioral measures of the relative inputs of L- and M-cone inputs to PC and MC pathways.

Conclusion

Taken by themselves, the mfVEPs recorded here are consistent with an L/M cone ratio closer to 1.0 in the central 1° and/or an adjustment in the gain of the L- versus M-cone contributions to the central PC pathways before the mfVEP is generated. But taking into consideration the mfERG results in Albrecht et al., in press and in Figure 9, as well as evidence from other techniques, we conclude that it is unlikely that most individuals have a L/M cone ratio of 1.0 in the fovea. Instead, it appears that there is a change in gain in the PC pathway before the mfVEP is generated in area 17. This change may be required to optimize, or standardize among observers, foveal hue discrimination in the red-green region of the spectrum.

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References

Albrecht, J., Jägle, H., De Luca, E., & Sharpe, L. T. (2002). Long- and middle-wavelength-sensitive cone-isolating multifocal electroretinograms. Manuscript submitted for publication.

Albrecht, J., Jägle, H., Hood, D. C., & Sharpe, L. T. (in press). The multifocal electroretinogram (mfERG) and cone isolating stimuli: variation in L- & M-cone driven signals across the retina. *Journal of Vision.*

Baseler, H. A., Sutter, E. E., Klein, S. A., & Carney, T. (1994). The topography of visual evoked response properties across the visual field. *Electroencephalography and Clinical Neurophysiology*, *90*, 65–81. [PubMed]

Baseler, H., Schneck, M., & Sutter, E. E. (1996). Contributions of different receptor populations to mulitfocal ERGs and VEPs [Abstract]. *Investigative Ophthalmology and Visual Science*, *37*, S1061. Abstract No. 4873.

Baseler, H. A., & Sutter, E. E. (1997). M and P components of the VEP and their visual field distribution. *Vision Research*, *37*, 675–690. [PubMed]

Brainard, D. H., Roorda, A., Yamauchi, Y., Calderone, J. B., Metha, A., Neitz, M., Neitz, J., Williams, D. R., & Jacobs, G.H. (2000). Functional consequences of the relative numbers of L and M cones. *Journal of the Optical Society of America A*, *17*, 607–614. [PubMed]

Carroll, J., McMahon, C., Neitz, M., & Neitz, J. (2000). Flicker-photometric electroretinogram estimates of L:M cone photoreceptor ratio in men with photopigment spectra derived from genetics. *Journal of the Optical Society of America A*, *17*, 499–509. [PubMed]

Chang, Y., Burns, S. A., & Kreitz, M. R. (1993). Red-green flicker photometry and nonlinearities in the flicker electroretinogram. *Journal of the Optical Society of America A, 10*, 1413–1422. [PubMed]

Dacey, D. M. (1993). The mosaic of midget ganglion cells in the human retina. *Journal of Neuroscience, 13*, 5334–5355. [PubMed]

Dacey, D. M., Diller, L. C., Verweij, J., & Williams, D. R. (2000). Physiology of L- and M-cone inputs to H1 horizontal cells in the primate retina. *Journal of the Optical Society of America A, 17*, 607–614. [PubMed]

de Vries, H. L. (1946). Luminosity curve of trichromats. *Nature*, **157**, 736–737.

de Vries, H. L. (1948). The heredity of the relative numbers of red and green receptors in the human eye. *Genetica*, *24*, 199–212.

Estevez, O., & Spekreijse, H. (1982). The "silent substitution" method in visual research. *Vision Research*, *22*, 681–691. [PubMed]

Guth, S. L., Alexander, J. V., Chumbly, J. I., Gillman, C. B., & Patterson, M. M. (1968). Factors affecting luminance additivity at threshold among normal and color-blind subjects and elaborations of a trichromatic-opponent colors theory. *Vision Research, 8*, 913–928. [PubMed]

Hagstrom, S. A., Neitz, J., & Neitz, M. (1997). Ratio of M/L pigment gene expression decreases with retinal eccentricity. In C. R. Cavonius (Ed.), *Color vision deficiencies XIII* (pp. 59–65). Dordrecht, The Netherlands: Kluwer.

Hagstrom, S. A., Neitz, J., & Neitz, M. (1998). Variation in cone populations for red-green color vision examined by analysis of mRNA. *Neuroreport, 9*, 1963–1967. [PubMed]

Hagstrom, S. A., Neitz, M., & Neitz, J. (2000). Cone pigment gene expression in individual photoreceptors and the chromatic topography of the retina. *Journal of the Optical Society of America A, 17*, 527–537. [PubMed]

Harding, G. F. A., Odom, J. V., Spileers, W., & Spekreijse, H. (1996). Standard for visual evoked potentials. *Vision Research*, *36*, 3567–3572. [PubMed]

Hood, D. C. (2000). Assessing retinal function with the multifocal technique. *Progress in Retinal and Eye Research*, *19*, 607–646. [PubMed]

Hood, D. C., Frishman, L. J., Saszik, S., & Viswanathan, S. (2002). The cellular basis of the primate multifocal ERG. Manuscript in preparation.

Hood, D. C., Odel, J. G., & Zhang, X. (2000). Tracking the recovery of local optic nerve function after optic neuritis: A multifocal VEP study. *Investigative Ophthalmology and Visual Science, 41*, 4032–4038.

Hood, D. C., & Zhang, X. (2000). Multifocal ERG and VEP responses and visual fields: Comparing disease-related changes. *Documenta Ophthalmologica*, *100*, 115–137. [PubMed]

Hood, D. C., Zhang, X., Greenstein, V. C., Kangovi, S., Odel, J. G., Liebmann, J. M., & Ritch, R. (2000). An interocular comparison of the multifocal VEP: A possible technique for detecting local damage to the optic nerve. *Investigative Ophthalmology and Visual Science, 41*, 1580–1587.

Hood, D. C., Zhang, X., Hong, J. E., & Chen, C. S. (in press). Quantifying the benefits of additional channels of multifocal VEP recording. *Documenta Ophthalmologica*.

Jacobs, G. H., Neitz, J., & Krogh, K. (1996). Electroretinogram flicker photometry and its applications. *Journal of the Optical Society of America A, 13*, 641–648. [PubMed]

Klistorner, A., Crewther, D. P., & Crewther, S. G. (1998). Temporal analysis of the chromatic flash VEP: Separate colour and luminance contrast components. *Vision Research, 38*, 3979–4000. [PubMed]

Klistorner, A. I., & Graham, S. L. (1999). Multifocal pattern VEP perimetry: Analysis of sectoral waveforms. *Documenta Ophthalmologica*, *98*, 183–196. [PubMed]

Klistorner, A., & Graham, S. L. (2000). Objective perimetry in glaucoma. *Ophthalmology*, *107*, 2283–2299. [PubMed]

Krauskopf, J. (2000). Relative number of long- and middle-wavelength-sensitive cones in the human fovea. *Journal of the Optical Society of America A, 17*, 510–516. [PubMed]

Kremers, J., Usui, T., Scholl, H. P., & Sharpe, L. T. (1999). Cone signal contributions to electroretinograms [correction of electrograms] in dichromats and trichromats. *Investigative Ophthalmology and Visual Science, 40*, 920–930.

Kremers, J., Scholl, H. P. N., Knau, H., Berendschot, T. T., Usui, T., & Sharpe, L. T. (2000). L/M cone ratios in human trichromats assessed by psychophysics, electroretinography, and retinal densitometry. *Journal of the Optical Society of America A*, *17*, 517–526. [PubMed]

Miyahara, E., Pokorny, J., Smith, V. C., Baron, R., & Baron, E. (1998). Color vision in two observers with highly biased LWS/MWS cone ratios. *Vision Research*, *38*, 601–612. [PubMed]

Otake, S., & Cicerone, C. M. (2000). L and M cone relative numerosity and red-green opponency from fovea to midperiphery in the human retina. *Journal of the Optical Society of America A*, *17*, 615–627. [PubMed]

Pokorny, J., & Smith, V. C. (1987). L/M cone ratios and the null point of the perceptual red/green opponent system. *Die Farbe*, *34*, 53–57.

Pokorny, J., Smith, V. C., & Wesner, M. F. (1991). Variability in cone populations and implications. In A. Valberg & B.B. Lee (Eds.), *From pigments to perception: Advances in understanding visual processes* (pp. 1–9). New York: Plenum Press.

Rushton, W. A. H., & Baker, H. D. (1964). Red/green sensitivity in normal vision. *Vision Research*, *4*, 75–85. [PubMed]

Roorda, A., & Williams, D. R. (1999). The arrangement of the three cone classes in the human eye. *Nature*, *397*, 520–522. [PubMed]

Sieving, P. A., Murayama, K., & Naarendorp, F. (1994). Push-pull model of the primate photopic electroretinogram: A role for hyperpolarizing neurons in shaping the b-wave. *Visual Neuroscience*, *11*, 519–532. [PubMed]

Slotnick, S. D., Klein, S. A., Carney, T., Sutter E. E., & Dastmalchi, S. (1999). Using multi-stimulus VEP source localization to obtain a retinotopic map of human primary visual cortex. *Clinical Neurophysiology*, *110*, 1793–1800. [PubMed]

Smith, V.C., & Pokorny, J. (1975). Spectral sensitivity of the foveal cone photopigments between 400 and 500 nm. *Vision Research*, *15*, 161–171. [PubMed]

Sutter, E. E. (1991). The fast m-transform: A fast computation of cross-correlations with binary m-sequences. *Society for Industrial and Applied Mathematics*, *2*, 686–694.

Stockman, A., & Sharpe, L. T. (1998). Human cone spectral sensitivities: a progress report. *Vision Research*, *38*, 3193–3206. [PubMed]

Stockman, A., & Sharpe, L. T. (1999). Cone spectral sensitivities and color matching. In K. Gegenfurtner, & L. T. Sharpe (Eds.), *Color vision: From genes to perception*. (pp. 53–88). Cambridge, UK: Cambridge University Press.

Stockman, A., & Sharpe, L. T. (2000). The spectral sensitivities of the middle- and long-wavelength sensitive cones derived from measurements in observers of known genotype. *Vision Research*, *40*, 1711–1737. [PubMed]

Vos, J. J., & Walraven, P. L. (1971). On the derivation of the foveal receptor primaries. *Vision Research*, *11*, 799–818. [PubMed]

Wesner, M., Pokorny, J., Shevell, S. K., & Smith, V. C. (1991). Foveal cone detection statistics in color-normals and dichromats. *Vision Research*, *31*, 1021–1037. [PubMed]

Yu, A., Hood, D. C., Zhang, X., Albrecht, J., Jägle, H., & Sharpe, L. T. (2001). M-cone to L-cone ratios and the multifocal VEP [Abstract]. *Investigative Ophthalmology and Visual Science*, *42*, S49.