

Chromatic input to cells of the magnocellular pathway: Mean chromaticity and the relative phase of modulated lights

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Abstract

If the relative phase of red and green modulated lights is changed, at low temporal frequencies the response of cells of the magnocellular (MC) pathway has been found to be minimal not to counterphase, chromatic modulation (as expected of a luminance mechanism) but shifted to some phase intermediate between luminance and chromatic modulation. The results could only be modeled by assuming interaction between achromatic and chromatic inputs to MC cells. The ‘phase shift’ resembled that seen with psychophysical threshold measurements using the same stimuli. Psychophysical results also showed that the phase shift is dependent on the chromaticity of a background. The results reported here show that the direction of the phase shift in MC cells is reversed by changing the background from long to short wavelengths and is consistent with psychophysical observations. Cell behavior was again modeled by assuming vector summation of achromatic and chromatic inputs. The reversal of phase-shift direction requires a reversal in polarity of the chromatic input. The underlying physiological mechanism may involve summation of chromatic signals of opposite polarity; if the relative size of these signals depends on the background, this may determine the direction of phase shift.

Keywords: Ganglion cells, Macaque, Magnocellular, Luminance, Chromatic mechanism

Introduction

There is substantial evidence that cells of the magnocellular (MC) pathway form the physiological substrate of a psychophysical luminance channel (Lee et al., 1988; Kaiser et al., 1990), but these cells also show indications of chromatic input. This was noted in one of the first systematic studies of macaque lateral geniculate nucleus neurons (Wiesel & Hubel, 1966), in which it was reported that MC-cells’ firing was inhibited by “flooding the surround with red light.” A further manifestation of a chromatic response is a frequency-doubled response to red–green chromatic modulation (Lee et al., 1989a), which appears to be a rectification of a chromatic signal, rather than a nonlinearity of middle- (M) and long-wavelength (L) cone summation (Lee & Sun, 2003). Another indication of chromatic input is seen on changing the relative phase of red and green sinusoidally modulated lights. The minimum response of a MC cell to this stimulus is not to counterphase, chromatic modulation (± 180 deg) but shifted to some intermediate phase. This could only be modeled by assuming that there is a chromatic input to the cell (Smith et al., 1992). This “phase shift”

effect seen in MC cells closely mirrors psychophysical detection performance of such stimuli as a function of the relative phase of the lights. Both the frequency-doubled response and the phase effect become much smaller if small spot stimuli, restricted to the receptive-field center, are used. However, experiments with annuli show that both effects are restricted to a surround region only ~ 1.5 times the center diameter; there also exists a larger, achromatic surround (Lee & Sun, 2003). They thus appear linked.

The physiological origin of this chromatic input to MC cells remains uncertain. In an attempt to further define this input, we analyze here the effect of chromatic backgrounds on the phase shift. Earlier studies used a long-wavelength mean chromaticity. Use of a short-wavelength adapting background has been shown to reverse the direction of the psychophysical phase shift (Swanson et al., 1988; Stromeyer et al., 1997), that is, minimum sensitivity is found in the “green-leads-red” rather than the “red-leads-green” phase quadrant (see below). We test here if the same reversal is seen in MC cells, and if the chromatic input model can account for this result.

Materials and methods

Cells were recorded from the retinas of anesthetized macaques (*Macaca fascicularis*). The animals were initially sedated with an

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intramuscular injection of ketamine (10 mg/kg) followed by thiopental (10 mg/kg). Anesthesia was maintained with inhaled isoflurane (0.2–2%) in a 70:30 N₂O–O₂ mixture. Local anesthetic was applied to points of surgical intervention. Electroencephalogram (EEG) and electrocardiogram (ECG) were monitored continuously to ensure animal health and adequate depth of anesthesia. Muscle relaxation was maintained by a constant infusion of gallamine triethiodide (5 mg/kg/h i.v.) with accompanying dextrose Ringer solution (5 ml/h). Body temperature was kept close to 37.5°C. End tidal CO₂ was adjusted to close to 4% by adjusting the rate of respiration. All procedures were approved by the SUNY Animal Care Committee and conform to ARVO guidelines for ethical care of animals.

The details of the preparation and cell-identification techniques can be found elsewhere (Lee et al., 1989b). Visual stimuli were generated using a light emitting diode (LED) based Maxwellian-view system (Lee et al., 1990). Three LED sources with dominant wavelengths of 470 nm, 554 nm, and 638 nm were used. The 554- and 638-nm LEDs were each modulated with an amplitude of 500 td, at either 1.22, 4.88, or 19.52 Hz. This modulated stimulus was superimposed on 1000-td backgrounds of 470, 554, and 638 nm. Relative phase of the LEDs was varied in 22.5-deg steps. A 4.7-deg diameter stimulus was used, which encompassed both center and surround. Approximately 6 s of activity was recorded in each condition. First-harmonic amplitudes and phases were extracted by Fourier analysis.

Results

MC-cell responses

Data sets were obtained from 14 MC cells (10 on- & 4 off-center). The effects of mean chromaticity on response amplitude and phase are shown in Fig. 1 for examples of on- and off-center cells, with three different chromatic backgrounds and at three different temporal frequencies. The two cells were chosen to show the range of intercell variability. The curves in the plots were derived from a model described in a later section. For the on-center cell with the 638-nm background, at 1.22 Hz (◆) the response minimum is not at ± 180 deg (as expected of an achromatic mechanism) but near ~ 45 deg, that is, it is shifted toward 0-deg phase shift. This phase shift can be described as being in the “red-leads-green” quadrant since the 638-nm LED leads the 554-nm LED. The shift becomes less at the higher temporal frequencies, moving rightward in the plot toward ± 180 deg phase shift (4.88 Hz—▲; 19.5 Hz—□). On the shorter wavelength backgrounds there is much less phase shift. The off-center cell showed a small phase shift on the 638-nm background (although still in the “red-leads-green” quadrant), but on the shorter wavelength backgrounds the phase shift is reversed, that is, to the “green-leads-red” quadrant. It is ≈ 10 deg at 1.22 Hz and decreases with increasing temporal frequency, moving leftward toward ± 180 deg (≈ 75 deg at 4.88 Hz and ≈ 170 deg at 19.5 Hz). In summary, under all conditions, the response minimum can be seen to move toward ± 180 deg as temporal frequency increases and to be dependent on mean chromaticity, tending to move through ± 180 deg from the “red-leads-green” to the “green-leads-red” quadrant with short-wavelength backgrounds. Twelve of 14 cells showed a change in this direction, the other two cells not showing a change in phase of least response as a function of background chromaticity. Both these cells showed response minima near ± 180 deg.

Response phase plots usually show a rapid change close to the response amplitude minimum. The shape of the phase curves are dependent on background, typically becoming shallower with short-wavelength backgrounds. This reflects an increase in the relative L/M cone weighting, and is analyzed in more detail in a later section.

The stimulus phase of minimum response was determined for each condition by fitting each set of amplitude data points with a cosine template (Swanson et al., 1987). Mean-MC cell data for the three chromatic conditions are shown in Fig. 2A; error bars indicate 95% confidence limits of the mean and are large, reflecting intercell variability. The direction of the phase shift reverses on the short-wavelength backgrounds. Also shown are mean data from Smith et al. (1992). These data show a somewhat larger phase shift than in the current experiments. This may be due to differences in adaptation conditions but may also reflect the considerable degree of intercell variability. The two examples in Fig. 1 represent extremes of behavior in the cell sample, which appeared to form a continuous distribution. In our previous work (Smith et al., 1992) there was no difference, on average, between on- and off-center cells in such plots as in Fig. 2. The two examples in Fig. 1 are of on- and off-center cells, but there was no obvious difference in phase-shift effects over these two groups and their data are treated together.

Comparison 2000-td psychophysical data replotted from Swanson et al. (1988) are shown in Fig. 2B. Thresholds for the detection of sinusoidal modulation were measured as a function of the relative phase of red and green LEDs and the relative phase of lowest sensitivity (highest threshold) determined at different temporal frequencies. The details of the adaptation conditions in the two sets of experiments were not identical, but nevertheless the two sets of data resemble one another. Similar results to those of Swanson et al. were reported by Stromeyer et al. (1997), who used a different psychophysical protocol involving minimum motion. We suggest that the comparison in Fig. 2 supports the idea that the MC pathway underlies the phase-shift effects seen psychophysically.

We tested if a smaller phase shift on the 638-nm background (i.e. closer to ± 180 deg) was correlated with a larger reverse phase shift on a 470-nm background. This appeared to be the case ($r = 0.565$; $t = 2.36$, $n = 14$, $P < 0.05$). However, intercell variability was considerable.

Modelling of cell responses

In Smith et al. (1992), data were modeled based on vector summation of achromatic and chromatic inputs to the cell. The chromatic input became insignificant above 10 Hz and cell responses resembled those expected of an achromatic, luminance mechanism. Simpler models, which were based, for example, on cone-weighting differences, were unsatisfactory. The results presented here with a 638-nm background resemble those of Smith et al. We now consider if this model can account for our data with short-wavelength backgrounds. In the following, it is important to distinguish *stimulus phase*, which refers to the relative phase of the LEDs, and *response phase* of the cell, which is expressed relative to modulation of the 638-nm LED.

The first step in this approach is to calculate M- and L-cone excitation amplitude and phase as a function of stimulus phase. These relationships were shown in Fig. 11 of Smith et al. (1992). Chromatic backgrounds do not change these curves' amplitude or shape but shift the amplitude curves vertically. An achromatic mechanism sums, and the chromatic mechanism subtracts, M- and

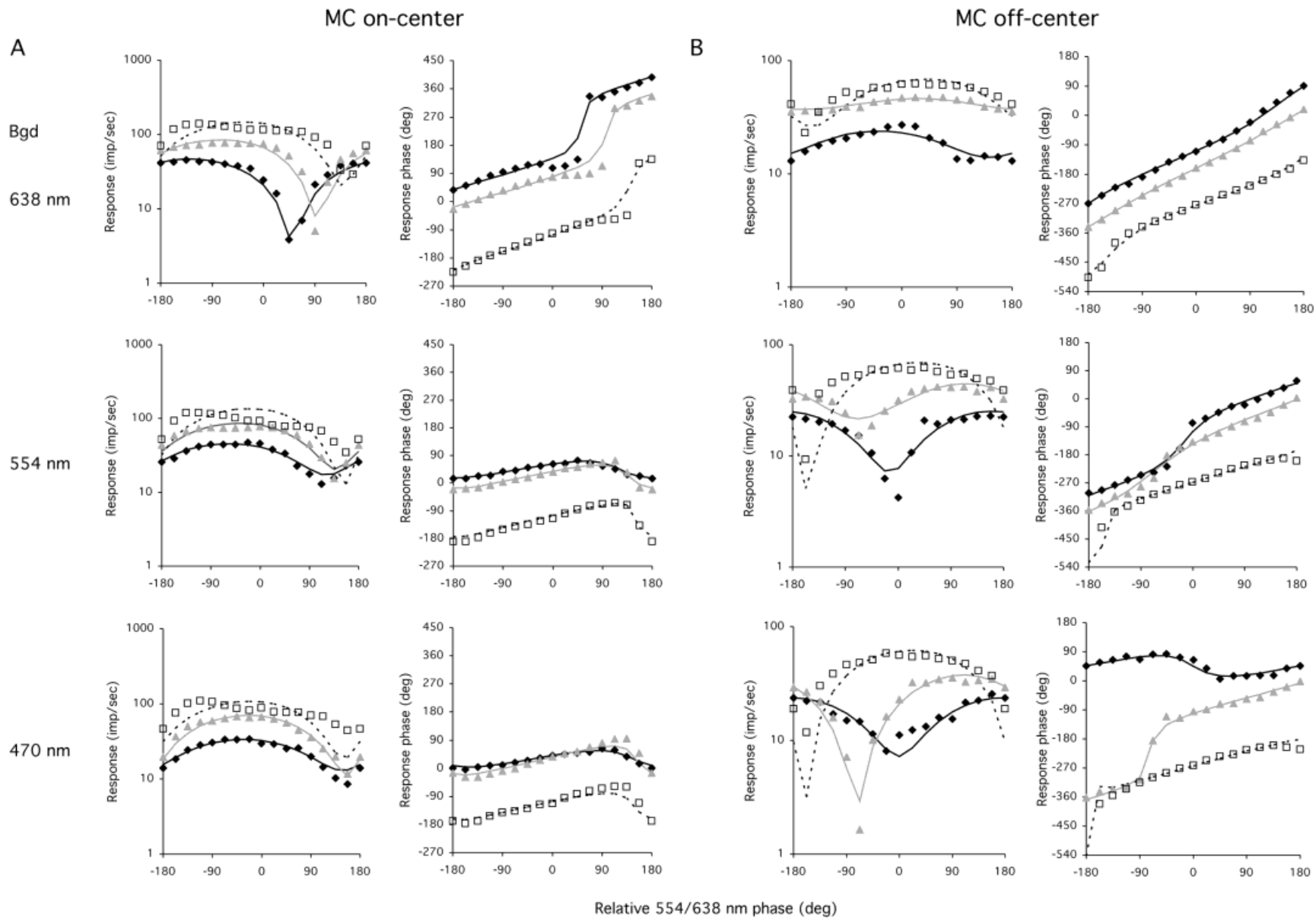


Fig. 1. Response amplitude and phase of an MC on-center (A) and off-center cell as a function of the relative phase of 554/638-nm modulated lights, for three chromatic background conditions. Data for three frequencies are shown (1.22 Hz— \blacklozenge ; 4.88 Hz— \blacktriangle ; 19.5 Hz— \square). The abscissa represents the phase of the 638-nm LED relative to the 554-nm LED; positive values are termed the “red-leads-green” quadrant and negative values the “green-leads-red” quadrant. The curves near the data points indicate model fits. Note that the relative LED phase of minimum response depends on the chromatic background. Mean illuminance 2000 td, 4.7-deg field, response amplitude, and phase was derived from Fourier analysis of averaged histograms derived from ~ 6 -s activity.

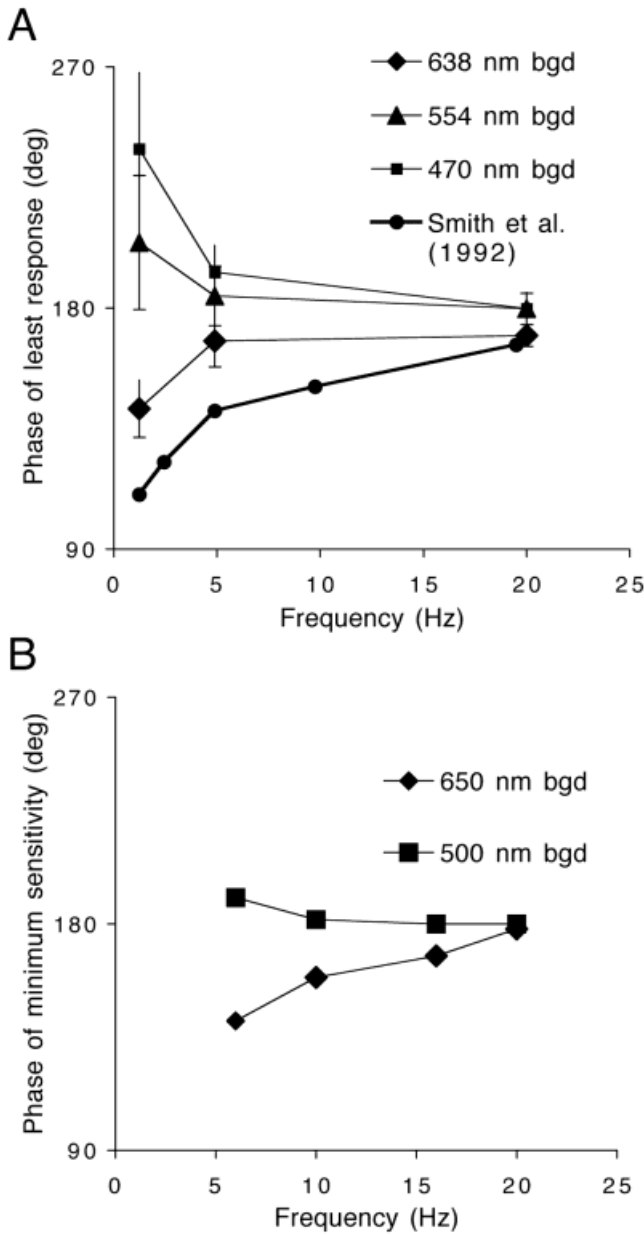


Fig. 2. A: The phase of minimum response was determined by fitting each MC-cell's response amplitude with a cosine template. Mean values ($n = 14$) are plotted as a function of temporal frequency for the three chromatic background conditions. Data from on- and off-center cells have been combined. Error bars are 95% confidence limits of the mean. Also shown are data obtained with a long-wavelength mean chromaticity replotted from Smith et al. (1992). B: Psychophysical data (phase of minimum psychophysical sensitivity) replotted from Swanson et al. (1988) for two chromatic-adaptation conditions.

L-cone signals. Examples of achromatic and chromatic amplitude templates are shown in Fig. 3A. The achromatic template shows a maximum at 0 deg and a minimum at ± 180 deg, and *vice versa* for the chromatic template. The exact shape of achromatic and chromatic amplitude and phase templates is dependent on the calculated M- and L-cone phases, on the cone weighting (see below) and any phase lag intrinsic to a given mechanism.

The next step is to combine the achromatic and chromatic signals by vector summation to predict cell response amplitude and

phase, as sketched in Fig. 3B. This represents a response phase space in which the amplitude and phase of the achromatic and chromatic mechanisms are drawn. Amplitude is represented by the length of the vector (normalized to unity in this sketch) and phase of the vector angle relative to modulation of the 638-nm LED, which is the abscissa. The cell response is the sum of the two vectors, as shown. The minimum cell response can then show a phase shift in terms of stimulus phase, as sketched in Fig. 3C. The equations describing the model are set out in Smith et al. (1992). For fitting the model to cell data, there are four free parameters: the weights of the achromatic relative to the chromatic mechanisms, achromatic phase (the intrinsic phase of the achromatic input), chromatic phase (the intrinsic phase of the chromatic input), and an amplitude scalar.

There is an additional complication in the current experiments due to changes in cone weighting depending on the chromatic background, which requires a modification of the Smith et al. (1992) model. The templates of the achromatic and chromatic mechanisms depend on the relative weights of their L- and M-cone inputs. In the previous description (Smith et al., 1992), the different mechanisms were assigned specific cone weights. The weights for the achromatic mechanism were set at (L 0.62: M 0.38; i.e. $L+M = 1$) as expected of a Judd observer. The weights for the chromatic mechanism were set at (L 0.35: M 0.65), which were the mean weights derived for M and L opponent parvocellular (PC) cell measurements under identical adaptation conditions. In the current experiments, chromatic backgrounds are likely to change these weightings. The response amplitude and phase of the achromatic and chromatic mechanisms as a function of stimulus phase depend on these weightings, as sketched in Figs. 3D and 3E. The shape of the response amplitude and phase curves is seen to be dependent on weighting, especially in the case of the chromatic mechanism.

We estimated M- and L-cone weighting for the different adaptation conditions as follows. For the achromatic mechanism, it can be seen in Fig. 3D that the slope of the relation between response phase and stimulus phase is dependent on relative L- and M-cone weighting. At 19.5 Hz, the chromatic mechanism provides little input, and each cell's 19.5-Hz data were used to estimate the weighting. For each cell and each background, we fitted the five 19.5-Hz data points from -45 deg to $+45$ deg with a straight line and used this to estimate cone weight for the achromatic mechanism for that particular cell and condition.

There was a highly significant increase in relative L- to M-cone weighting as background wavelength decreased (650-nm, L 0.48: M 0.52M; 554-nm, L 0.67: M 0.33; 470-nm, L 0.79: M 0.21; ANOVA: $F = 18.6$, $n = 14$, $P < 0.001$). There was also significant intercell variability ($F = 3.55$, $P < 0.01$). The achromatic weights for each cell and condition were inserted into the equations at all three temporal frequencies for the full fitting procedure.

For the chromatic mechanism, we estimated L- and M-cone weighting in five PC cells measured under identical conditions, using the model described in Smith et al. (1992). There was an increase in relative L- to M-cone weight with decreasing wavelength of the background (650-nm, L 0.38: M 0.62; 554-nm, L 0.62: M 0.38; 470-nm, L 0.69: M 0.31; $n = 5$). These mean values were used in the model fits.

Data for each frequency for each background were fitted separately in the complex plane using a least-squares criterion. As can be seen from the solid curves in Fig. 1, the model provided a satisfactory fit to the data. Model parameters behaved in an orderly manner. Of the four free parameters, under all adaptation condi-

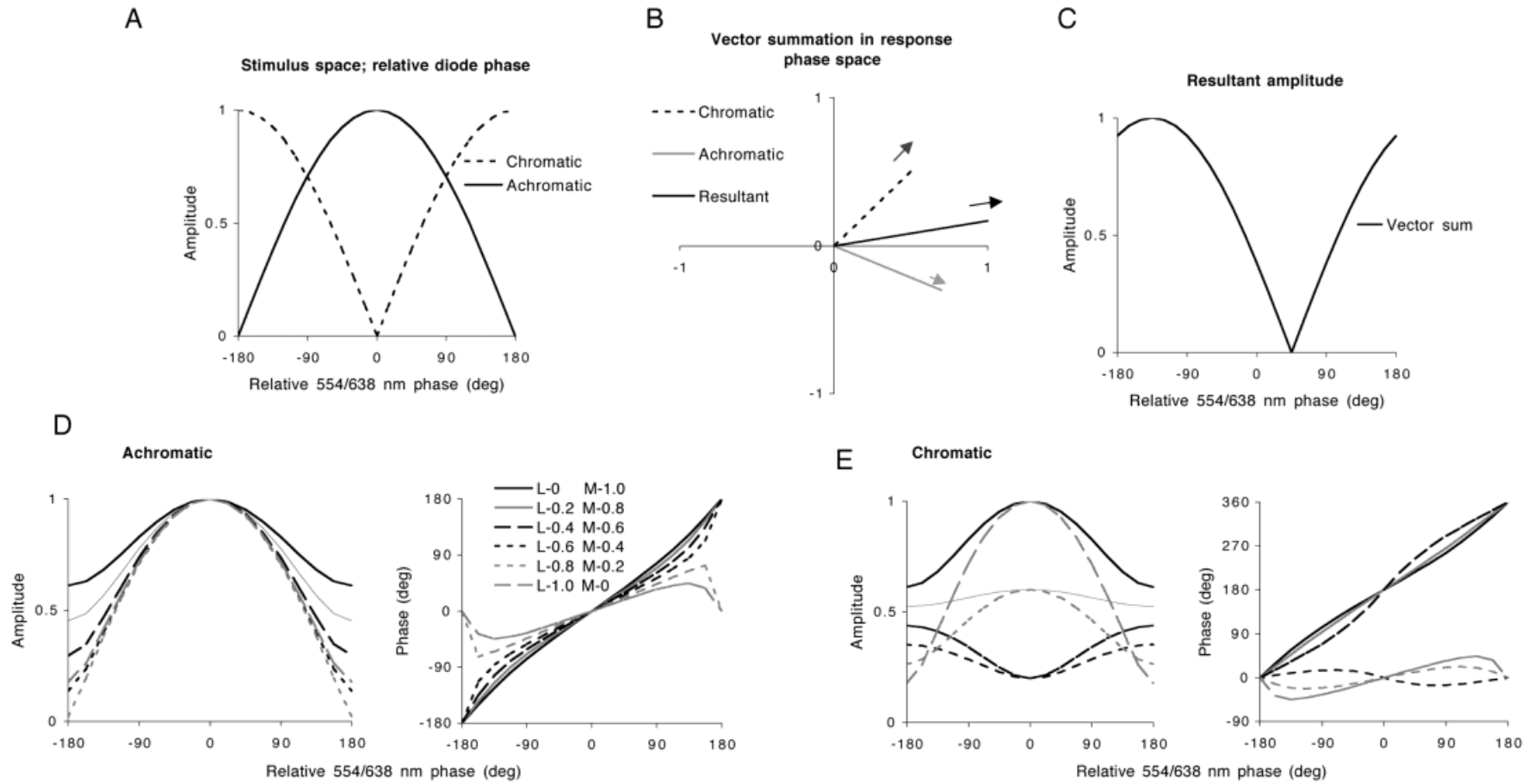


Fig. 3. A–C: Steps in modelling cell responses. Achromatic- and chromatic-mechanism have amplitude templates separated in the LED (stimulus) space by 180 deg. Achromatic- and chromatic-response components sum in a response phase space to give a resultant vector; phase of achromatic and chromatic components is determined by cone excitations and cone balance, as well as by a phase term intrinsic to the mechanism. The resultant output template can show a minimum at intermediate phase values. D–E: The amplitude and phase templates of achromatic and chromatic mechanisms are influenced by the M- and L-cone balance of the inputs. Chromatic backgrounds will affect cone balance, and this must be incorporated in the model.

tions achromatic phase showed a similar increase in phase lag with frequency. Values were consistent with earlier measurements of MC-cell response phase with luminance modulation (Lee et al., 1990). For a given chromatic background, the chromatic phase parameter also showed an increase in phase lag with frequency. The weighting of the chromatic relative to the achromatic mechanisms decreased with temporal frequency, that is, there was less evidence of chromatic input, as expected since the phase shift decreases as frequency increases. The most interesting aspect of the analysis was the effect on these parameters of different adaptation conditions. We compared parameters for 1.22 Hz, where the phase shifts and chromatic inputs were most marked. There was no significant effect of the different chromatic backgrounds on the achromatic phase parameter ($F = 2.85$, $P > 0.05$) or on the achromatic/chromatic weighting parameter ($F = 3.1$, $P > 0.05$). However, there was a highly significant effect of background on the chromatic phase parameter ($F = 56.1$, $P < 0.001$). The chromatic phase parameter frequently changed by more than 90 deg (11 of 14 cells), that is, effectively a phase reversal. There was significant intercell variability ($F = 3.68$, $P < 0.01$). It appeared that cells in which there was a reverse in direction of the phase shift showed the greatest change in this parameter.

The analysis suggests that the model summing achromatic and chromatic signals can account for our results. The basis of the change in phase of the chromatic input is considered in the next section.

Discussion

The change in direction of the phase shift seen psychophysically with different backgrounds is also found in MC cells. Swanson et al. (1988) did not discuss in detail the reason for this reversal, although they noted it was inconsistent with adaptation causing changes in cone dynamics. Stromeyer et al. (1997) proposed a model to account for their results which assumed a chromatic input to MC cells (as assumed in our model) and this input changed polarity when the background shifted from long to short wavelengths. This would be equivalent to the large shift in the chromatic phase parameter which we observed in our results when changing background from 638 nm to 554 nm or 470 nm.

The origin of this chromatic input remains uncertain. The amplitude of the frequency-doubled response to chromatic modulation (which may derive from a chromatic signal) is also dependent on background chromaticity, typically becoming more pronounced on long-wavelength backgrounds (J. Pokorny, V.C. Smith, B.B. Lee, unpublished observations). The frequency-doubled response and the phase shift are both localized to a surround region not much larger than the center (Lee & Sun, 2003), which suggests a common physiological substrate. An input from the L- and M-cone opponent midgen pathway in inner retina appears plausible, perhaps through an amacrine cell of unknown

type. We suggest that the frequency-doubled response arises through nonlinear summation of +L–M and +M–L signals. Such summation (for example, through a leaky rectifier) could give rise to both first- and second-harmonic response components. The inversion of the polarity of the chromatic input responsible for the phase shift may be due to a change in balance of +L–M and +M–L signal components on different backgrounds.

There was considerable intercell variability in phase shift with different backgrounds, but shifts were consistent in that for 12 of 14 cells they were in the same direction. The reason for this intercell variability is unknown, but it suggests that this facet of MC-cell behavior might vary in prominence from observer to observer, as is the case for psychophysical performance (W. Swanson, J. Pokorny, V.C. Smith, personal communication).

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