The spatial structure of cone-opponent receptive fields in macaque retina

Barry B. Lee a,b,⇑, Bonnie Cooper a, Dingcai Cao c

a State University of New York, College of Optometry, NY 10036, NY, USA
b Max Planck Institute for Biophysical Chemistry, D-37077 Göttingen, Germany
c Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL 60612, USA

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The receptive field structure of long (L) to middle (M) wavelength (L/M) cone-opponent ganglion cells of the parafoveal macaque retina was investigated using drifting gratings. Gratings were luminance, chromatic or selective for the L- or M-cones. Based on these spatial tuning curves, receptive field profiles for the individual cones were derived. Receptive field profiles were coarse compared to single cones, and often could not be described by a simple Gaussian, having shallower flanks. There was a continuum of spatial properties, which blurred any systematic distinction between Type I and Type II receptive fields. Opponent center-surround organization within a single cone was rare. Usually, responses to all four grating types could be described based on the cone receptive field profiles. An exception was a few cells that showed irregularities of amplitude and phase at high spatial frequencies for one or other of the cone isolating conditions. The data are related to standard models of M/L opponent receptive fields and implications for central processing are considered.

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1. Introduction

It is well established that retinal ganglion cells, and cells of the lateral geniculate nucleus (LGN), show a center-surround receptive field structure. In the primate however, Wiesel and Hubel (1966) noted variability of structure of color-opponent cells in the macaque LGN. Some cells in the parvocellular (PC) layers showed the usual center-surround organization (Type I) while in other cells antagonistic cone mechanisms seemed to be co-extensive (Type II), seen in Fig. A,C. It is now well established that cells with short-wavelength (S) cone input generally fall toward this latter category; differences in diameter of antagonistic cone mechanisms, if present, are small (reviewed in Martin and Lee (2014)). Cells with opponent middle (M) and long (L) wavelength cone input may also show Type II structure. Quantitative measurements have suggested a continuum from Type I to Type II structure, rather than discrete cell classes (Derrington & Lennie, 1984; Lee, Shapley, Hawken, & Sun, 2012). The results presented here describe the receptive field structure of these cells in more detail.

An additional issue concerns specificity of cone inputs to center and surround of M/L opponent cells; such specificity was assumed in early studies (de Monasterio & Gouras, 1975; Wiesel & Hubel, 1966). The canonical midget morphology (Boycott & Dowling, 1969; Poljak, 1941) provides one-to-one cone-selective input to receptive field centers in central retina. This alone could provide some degree of opponency even with mixed cone input to the receptive field surround (Lennie, Haake, & Williams, 1991; Paulus & Kröger-Paulus, 1983). This issue has been much debated (Calkins & Sterling, 1996). However, all direct measurements suggest at least some degree of cone specificity to the receptive field surround, at least in central retina (Buzas, Blessing, Szmajda, & Martin, 2006; Lee, Kremers, & Yeh, 1998; Lee et al., 2012; Reid & Sharpley, 1992, 2002).

Receptive field structure of midget ganglion cells has also been studied in vitro, using single-cell recording (Crook, Manookin, Packer, & Dacey, 2011) or electrode arrays (Field et al., 2010; Freeman et al., 2015). Mixed cone input to both center and surround was found. These recordings were from much higher eccentricities than in the in vivo experiments. At high eccentricities substantial convergence from midget bipolar to midget ganglion cell occurs; this would account for mixed cone input to the center. Further comparison of results from central and peripheral retina is taken up in the discussion section.
Here we investigate parafoveal receptive field structure, in terms of spatial extent and cone connectivity. This is of functional relevance to luminance and chromatic visual processing. Models of visual processing often assume marked center surround structure in midget, PC cells, to provide luminance contrast enhancement or to enable extraction of an achromatic signal (Ingling & Martinez-Uriegas, 1983). If center-surround structure is poorly expressed in PC cells (i.e., Type II structure were common), this would argue against such models. We do not attempt to here to provide a detailed model of spike generation and response structure, as we have done elsewhere (van Hateren, Ruttiger, Sun, & Lee, 2002); our concern is spatial receptive field structure with stimuli of restricted contrast, to which responses are likely to remain in the linear range.

We used sinewave gratings with four variants: luminance gratings, chromatic gratings, and gratings modulating either the L- or M-cones alone using silent substitution (Estévez & Spekreijse, 1974), so as to derive the receptive field structure of a single cone. We attempt to capture responses to all variants with a single model. Receptive field structure has usually been modeled as a difference between two Gaussian distributions (DoG) (Rodieck, 1965). In Fig. 1A is sketched a receptive field for a Type I cell with an L-cone center and either a surround derived from the M-cone or from both L- and M-cones. With achromatic gratings (black curve) there will be an attenuation of responsivity at low spatial frequencies (Fig. 1B, black curve). With a grating only modulating the excitation of the L-cone, if there were input to the surround just from the M-cone (i.e., no L cone), the spatial frequency response will be low-pass (‘Pure surround’; solid grey curve). If there were mixed cone input to the surround (i.e., the surround had L-cone input) there should also be low spatial frequency attenuation with L-cone isolating gratings (mixed surround; dashed grey curve). For a Type II cell (Fig. 1C), little low spatial frequency attenuation is to be expected for either achromatic or center cone-isolating gratings (Fig. 1B). The degree of low spatial frequency attenuation can be quantified comparing responsivity at peak ($R_{\max}$) with that at low spatial frequency ($R_0$). In a previous study (Lee et al., 2012), we reported on measures of this attenuation in retinal and LGN PC cells, and found most cells showed little or no indication of mixed cone input to the surround. This paper is an attempt to model the retinal data from this paper in more detail. It should be noted that the mixed surround model (Lennie et al., 1991; Paulus & Kröger-Paulus, 1983) is predicated on a center input from a single cone, with a larger surround drawing from a random cone array. The existence of Type II cells does not seem a priori compatible with this model approach; the complexities of this issue are taken up in the discussion.

Here we first attempted to describe cells’ spatial frequency tuning curves to the four grating stimuli with a DoG model. Firstly, slopes of tuning curves were often shallower than expected from a Gaussian profile. In a few cells, response phase reversals occurred in some cells to cone isolating gratings at high spatial frequencies, a phenomenon previously described by Crook et al. (2011) in peripheral retina. We consider possible receptive field structures that might account for this behavior.

**Fig. 1.** Sketch of hypothetical receptive field structure of opponent cells. A. Type I cell has center-surround structure in which the center is provided by one cone, and the surround by the other cone, or a mixture. B. Spatial frequency tuning expected of Type I cell. For achromatic, luminance gratings a band-pass tuning is expected. For a grating selectively modulating the center cone, a low-pass is tuning is expected if the surround is cone-specific, but some degree of band-pass character is anticipated if the surround is mixed. This effect can be quantified by taking the ratio $R_0/R_{\max}$. C. For a Type II cell, center and surround have similar extents. D. This leads to a spatial tuning low-pass in shape. A mixed surround leads to a decrease in chromatic responsivity relative to a cone-selective surround.
2. Methods

The technical details listed below largely duplicate those described in previous publications. In particular, descriptions have been drawn from Cooper, Lee, and Cao (2016).

Ganglion cell responses were recorded in vivo from the retinas of macaque monkeys (M. fascicularis). The animals were initially sedated with an intramuscular injection of ketamine (10 mg/kg). Anesthesia was induced with sodium thiopental (10 mg/kg) and maintained with inhaled isoflurane (0.2–2%) in a 70:30 N2O-O2 mixture. Local anesthetic was applied to points of surgical intervention. EEG and 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/hr i.v.) with accompanying dextrose Ringer solution (5 ml/kg/hr). Body temperature was kept close to 37.5°C. End tidal CO₂ was adjusted to close to 4% by adjusting the rate of respiration. Procedures were in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved by the SUNY State College of Optometry Institutional Animal Care and Use Committee.

Neuronal activity was recorded directly from retinal ganglion cells by an electrode inserted through a cannula entering the eye behind the limbus. Gas-permeable contact lens of the appropriate power was used to bring stimuli into focus on the retina. Responses of macaque retinal ganglion cells were recorded between 4 and 20 deg eccentricity. Cell identification was achieved through standard tests (Crook, Lange-Malecki, Lee, & Valberg, 1988). These included achromatic contrast sensitivity and responses to lights modulated in different directions of cone space. For each cell, the locus of the receptive field center was determined and the stimulus field centered on this point.

Times of spike occurrence were recorded to an accuracy of 0.1 ms and averaged histograms were accumulated. Fourier analysis of the histograms was carried out and 1st harmonic response amplitude and phase calculated. For response phase estimates, response phase was plotted against spatial frequency. Any error in receptive field centering is reflected in a phase displacement linearly related to spatial frequency (Lee, Virsu, & Elepfandt, 1981). Major centering errors were corrected to yield phase values described in the results.

Visual stimuli were generated via a VSG series 2/3 graphic controller (Cambridge Research Systems, Rochester, UK) and

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Fig. 3. A similar analysis for a Type II cell variant; +M-L cell. The luminance grating data show a low-pass shape. For the cone-selective conditions, slopes are shallower than could be described by a single Gaussian. Over all conditions, 99.4% of data variance was accounted for by the dual Gaussian model and 96.0% with the single Gaussian model. However, it should be stressed that the shape of the curve for the M cone condition is more satisfactorily captured by the dual model.
provided on a CRT monitor (SONY Trinitron GDM-F500, 150 Hz frame rate) 2.28 m away from the monkey. The spectrum of each phosphor was measured using a PhotoResearch Spectroradiometer. The chromaticity, relative luminance ($10^4 \Phi_v$), and cone excitation for each phosphor was calculated for each by multiplying each spectrum with cone fundamentals (Smith & Pokorny, 1975) modified to the CIE 1964 $10^4$ color matching and luminosity functions (Smith & Pokorny, 1972, 1975). The mean luminance of the red and green phosphors were set equal to give a mean luminance of $31.34 \text{ cd/m}^2$ and chromaticity of $(0.436, 0.476)$ in CIE x, y $10^4$ coordinates. Stimuli were horizontal or vertical gratings presented in a $5^\circ \times 5^\circ$ window: luminance gratings (60$\%$ contrast), equiluminant red-green gratings (15$\%$ contrast for the L-cone, 33$\%$ for the M-cone), and L- or M-cone isolating gratings (36, 42$\%$ contrast respectively), drifted at 2.5 Hz. For the chromatic and silent substitution conditions, we used the maximum contrast achievable to improve the response signal-to-noise ratio. For luminance gratings, we used a higher contrast since PC-cell achromatic contrast sensitivity is low. About 8$\,$s of activity were accumulated per spatial frequency per condition. For some cells, responses to both horizontal and vertical gratings were measured.

In the model fitting procedures, contrast values of the individual cones were inserted as necessary. Fits were performed in the complex plane, to incorporate both response amplitude and phase. Fits were based on a least-squares criterion using a nonlinear generalized reduced-gradient routine imported from Microsoft Excel. We found that the optimal procedure was to fit first the curves for the cone-isolating conditions individually for each cell. The parameters so derived were then used as initial values for fitting the whole data set for a given cell. Mean variance of the data was calculated and the mean percentages accounted for by the fits are given in the results section.

3. Results

3.1. Qualitative description of cell responses

We first describe some examples of cell responses and then attempt to develop a model of receptive fields. We would stress that the cell sample ($n=63$) showed a continuum of properties rather than falling into distinct groups.

Amplitude and phase of the first harmonic were derived by fourier analysis of response histograms; most energy (>75$\%$) was in the first harmonic (Cooper et al., 2016). There was also no indication of an increase in mean firing rate with spatial frequency, as occurs with Y cells of the cat (Enroth-Cugell & Robson, 1966), and is thought to be associated with the kind of subunit structure proposed by Freeman et al. (2015). To illustrate this, we show in Figs. 2A and 3A the response histograms for different conditions; the solid curves are fits for the model described in the next section. For each condition, amplitudes have been normalized to the total cone contrast and expressed as contrast gain (imp/s/% contrast). Apart from minor clipping due to response rectification, the responses are sinusoidal in shape, and higher harmonic distortions are not apparent. Fig. 2C shows data from a + M-L cell that can be described as Type I, with an on L-cone center. The curves show model fits to be described later; dashed curves are fits with a single Gaussian mechanism for each cone, and the solid curves using the dual-Gaussian model. The luminance response is weak, as expected from the low contrast gain of PC cells to luminance modulation (Kaplan & Shapley, 1986, 1982; Lee, Hicks, & Vidyasagar, 1983) but shows bandpass spatial tuning. The chromatic response is more vigorous and low-pass in shape. The lower panels show responses to the cone isolating conditions. Responses are vigorous and both curves are low-pass in shape with two notable features. Firstly, the L-cone curve shows no low spatial frequency attenuation, as might be expected if there were input from the L-cone to the surround. This is consistent with the earlier study (Lee et al., 2012). In this case, the L-cone data could be described by a single Gaussian. For the M-cone data there is some discrepancy to a single Gaussian fit, which was improved using the sum of two Gaussians. Residual errors are given in the figure legend Fig. 3C shows a cell for which the luminance response function is low-pass in shape, indicating center-surround organization is poorly expressed; such a cell could thus be classified Type II. The other conditions evoked more vigorous responses and the curves are low-pass. Phase behavior appears orderly, except where responses were weak. The single-Gaussian curves are too steep to describe the data, but the dual Gaussian curves improved the fit. Residual errors are given in the figure legend. The cells in Figs. 2 and 3 were representative of most members (ca. 2/3) of the cell sample. Goodness-of-fit estimates are given in the next section.

Fig. 4 shows examples of cells in which other features were observed. Some cells (11 of 63) showed indications of mixed cone input to the cone mechanism providing the surround. One example is shown in Fig. 3A. The luminance tuning curve shows a marked bandpass character, and there was a phase reversal at the lowest spatial frequency. In the cone-isolating conditions, the M-cone tuning shows a band-pass character with low frequency attenuation but the L-cone tuning is low-pass, with the shallow slope seen in cells of Fig. 2. There are some drifts in phase at high spatial frequencies that are likely to be associated with inaccurate centering. These data are consistent with an M-cone center with some M-cone input to the surround. Some low spatial frequency attenuation is also visible in the chromatic curve. In this example the low spatial frequency rolloff for the M-cone was obvious, but usually it was less apparent. A quantitative analysis is found in Lee et al. (2012).

A second group of cells (13 of 63 cells, 5 of which showed indications of surround mixing) displayed a further feature. This was a secondary peak in the spatial frequency tuning curves for one of the cones. An example is shown in Fig. 3B, for a + M-L cell with an M cone center. The luminance response curve shows a band-pass shape and the chromatic response is low pass. The M-cone tuning is low-pass in shape with some indication of M-cone input to the surround. However, for the L-cone tuning the inhibitory input is dominant at low spatial frequencies but near 4 cpd there is a response null associated with a reversal of response phase. This second peak was of variable size in different cells. It is suggestive of mixed cone input to the center, but alternative explanations are discussed below. Such an effect has been described in peripheral macaque retina in vitro (Crook et al., 2011; Field et al., 2010; Freeman et al., 2015) and in PC cells of the marmoset LGN (Buza et al., 2006). In our cell sample, such cells were only found at greater than 10 deg eccentricity (Lee et al., 2012). A major problem in interpreting these data is possible artifacts due to chromatic aberration (Forte, Blessing, Buzas, & Martin, 2006; Thibos, 1987). However, such effects were always found just in one of the cone response curves, but not in responses of the other cone or responses to chromatic gratings. This would argue against a sole source of this effect in aberration.

3.2. Modeling of cell responses

To generate a spatial tuning for single cones with shallow slope as in Fig. 3, a narrow peak region (to generate a response to high spatial frequencies) surrounded by flanks to provide a shallow slope. We attempted to model this with a sum of two Gaussians for each cone. Thus for each cone, the response ($F_{M}(f)$) as function of spatial frequency ($f$) is given by

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with $C_{ML}$ representing the M or L cone contrast, and each receptor's response function $(R(f))$ is given by the usual Gaussian equation

$$R(f) = k\pi r^2 e^{-r^2 f^2}$$

where $k$ is an amplitude constant and $r$ the radius of that particular receptive field mechanism. This is a sum of Gaussians rather than a difference as in common receptive field models. An additional factor is the possibility of phase differences between center and surround field components. One of these is of spatial origin, the other temporal.

Spatially, when measuring responses to drifting gratings, any error in centering of the receptive field over the display will cause a linear phase shift as a function of spatial frequency (Lee et al., 1981). In addition, there is the possibility that center and surround are not concentric. This might occur in cone-opponent neurons, if, due to the random distribution of M and L cones, there were differences in the loci of the two mechanisms. This would cause a spatial frequency dependent phase difference between the cone responses. We included a spatial phase parameter for each cone to see if we could detect such displacements.

Temporally, response latency will affect response phase. In addition, in cat ganglion cells, center-surround latency difference can cause a response phase advance at low spatial frequency (Enroth-Cugell, Robson, Schwitzer-Tong, & Watson, 1983), which was also noted here for luminance gratings (e.g., in Fig. 2A). This is because center-surround balance changes as spatial frequency increases, and the vector sum of the two signals changes in phase as well as amplitude. We have also noted such effects in temporal responses of macaque PC ganglion cells (Smith, Lee, Pokorny, Martin, & Valberg, 1992). We therefore included a temporal phase parameter for each cone to try and capture these effects.

Fig. 4. Two further cell variants. A. Example of cell with indication of mixed surround. Details as in Fig. 2. Luminance tuning shows marked band-pass shape, so extreme that there is a response phase reversal at the lowest spatial frequency, which is also captured by the model. The M-cone and chromatic curves also show an indication of band-pass character. The L-cone curve is too shallow in slope to be fit with a single Gaussian. B. Example of cell in which there is an amplitude irregularity at high spatial frequencies associated with a phase reversal for the L-cone isolating grating. For this cell the model described in the text provided an adequate fit to the data, but this was not always the case.
Incorporating these phase parameters, Eq. (1) becomes

\[
\begin{align*}
F_{M1}(f) &= C_{M1}(R_{1M1}(f) + R_{2M2}(f))\cos(\phi_{M1} + \phi_{M2}) \\
F_{L1}(f) &= C_{L1}(R_{1L1}(f) + R_{2L2}(f))\sin(\phi_{L1} + \phi_{L2})
\end{align*}
\]

(3)

and

\[
\begin{align*}
F_{M2}(f) &= C_{M2}(R_{1M2}(f) + R_{2M2}(f))\cos(\phi_{M1} + \phi_{M2}) \\
F_{L2}(f) &= C_{L2}(R_{1L2}(f) + R_{2L2}(f))\sin(\phi_{L1} + \phi_{L2})
\end{align*}
\]

(4)

for the real and imaginary response components, where \(\phi_{M2}\) and \(\phi_{L2}\) are spatial and temporal phase parameters respectively, for each cone.

Response amplitude and phase data allowed data to be fitted in the complex plane. Each single cone response curve was first fitted alone using a least-squares criterion (see Methods). These parameters were used to initiate a fit of the entire data set for each cell.

Cells such as those in Figs. 2 and 3 were almost always fitted satisfactorily, as can be seen from the curves in the figure, which capture most features of the data. As an example, for luminance modulation, a phase advance at low spatial frequencies associated with center-surround latency difference is captured. Fig. 5A shows the spatial profiles of the cone mechanisms derived from these fits.

For the Type I cell of Fig. 2, the center mechanism could be well fit using a single Gaussian, as represented in the profile. The surround mechanism is spatially broader and looks Gaussian in shape, but relative to the peak amplitude, the flanks are wider than for a single Gaussian distribution. The Type II profiles of the cell of Fig. 3 for the single cones were also described by a combination of two Gaussians, so that a sharp peak with broad flanks. The Type II panel in Fig. 5 shows these profiles. Both these cell variants could be well fitted; on average, 96.6% (S.D. 1.7%, n = 44) of response variance was captured.

For the cells such as those in Fig. 4A, with indications of mixed cone input to the surround, one of the response functions in Eq. (2) became of opposite sign, so as to provide standard center-surround structure for a single cone. This usually described cell responses satisfactorily. This is demonstrated for the cell of Fig. 4A in Fig. 5B, where the M cone can be seen to have weak center-surround structure. For the cells of this group, on average 95.0% (s.d. 2.8%, n = 13) of variance was accounted for be the model. For the ‘notch’ cell in Fig. 4B we added a third Gaussian of opposite sign to the other two to capture the peak with reversed phase. This results in the unusual receptive field profile shown in Fig. 5C. Even so some of these cells could not be fit adequately; 89.1% (S.D. 3.7%, n = 11) of variance could be accounted. Although for each cone alone a satisfactory fit could be obtained, as in Crook et al. (2011), responses to luminance and chromatic gratings were sometimes poorly described by combining responses from the individual cones.

Fig. 5D-F shows distributions of some parameters associated with the fits. The distribution of L/M cone weighting is shown in Fig. 5D. These ratios are distributed around one, i.e., an equal cone balance of the opponent mechanisms. This is as in previous studies (Derrington, Krauskopf, & Lennie, 1984; Lee, Valberg, Tigwell, & Tryti, 1987); in most ganglion cell types, the center weight dominates, so midget ganglion cells are unusual in this respect. This equal balance of cone mechanisms suggests that midget ganglion
cells are primarily concerned with transmitting chromatic information. We also assessed the distribution of receptive field dimensions between center and surround by calculating the ratio of the standard deviations of center to surround such as those in Fig. 5A. A ratio close to zero indicates that the surround is much bigger than the center, and a ratio of one that they are coextensive. This distribution is shown in Fig. 5E. It can be seen that the distribution forms a continuum, i.e., that there is a continuum between Type I and Type II. The cells in Figs. 2 and 3 had ratios of 0.13 and 0.65 respectively. We also assessed in how far the single-cone tuning curves could be described by a single Gaussian function. We calculated the relative weights (large radius to small radius) of these mechanisms, and this distribution is plotted in Fig. 5F; each cell is represented in this distribution twice for the two opponent mechanisms. A ratio near zero indicates a single Gaussian was adequate to fit the data. This was the case in ca. 25% of cases (ratios 0.0–0.2). In most cases, two mechanisms were required, i.e., spatial frequency tuning curves were too shallow in slope to be described by a single Gaussian. There were also 15 instances in which some indication of spatial opponency was seen in one cone mechanism; these are the negative ratios (Lee et al., 2012). The mean Gaussian radii from the fits can be found in Table 1. The values (ca. 0.06 deg, 4 arc min, for smaller mechanism, ca. 0.4 deg, 25 arc min) for the larger mechanism, were similar for both cone types.

Temporal and spatial phase parameters are also included in Table 1. Temporal phase shows a slight lag for both cone mechanisms, consistent with other data (Lee, Pokorny, Smith, Martin, & Valberg, 1990) for the temporal frequency used here (2.5 Hz). The spatial phase parameter was primarily a measure of the accuracy of centering the display on the receptive field. A phase shift proportional to spatial frequency is due to this factor (Lee et al., 1981). If the phase shift parameter differed for the two cones, this would be evidence that the centers of the receptive fields for the two cone mechanisms were not concentric. There were two cells where such displacement was apparent, but usually the centers were closely aligned (mean displacement 0.013 deg, 0.8 arc min; Table 1). We also tested if variability of cone weighting was associated with receptive field structure, but no correlation was observed and we tested if spatial structure (Fig. 5E) was associated with eccentricity but this was not the case (both not shown).

There are multiple parameters in Eqs. (3) and (4). Each Gaussian function necessary contains an amplitude and standard deviation term. In addition, there are two phase parameters associated with each cone mechanism, one temporal and one spatial parameter.

Table 1
Summary of fit parameters for the cell sample. Numbers in parentheses are standard deviations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>+L-M (n = 33)</th>
<th>+M-L (n = 30)</th>
</tr>
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<tbody>
<tr>
<td>Eccentricity (deg arc)</td>
<td>9.51 (4.1)</td>
<td>8.51 (4.36)</td>
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<tr>
<td>Cone radius 1 (deg arc)</td>
<td>0.058 (0.04)</td>
<td>0.077 (0.05)</td>
</tr>
<tr>
<td>Cone radius 2 (deg arc)</td>
<td>0.42 (0.21)</td>
<td>0.53 (0.20)</td>
</tr>
<tr>
<td>Temporal phase (deg)</td>
<td>-4.92 (7.93)</td>
<td>-3.92 (12.4)</td>
</tr>
<tr>
<td>Spatial disp. (deg arc)</td>
<td>0.012 (0.013)</td>
<td>0.016 (0.012)</td>
</tr>
</tbody>
</table>

Fig. 6. A. Sketch of hypothetical cone matrix of macaque at ca. 10 deg eccentricity. An L:M proportion of 1:1 has been assumed, and each cone assigned as L or M at random. The circle shows the size of the peak mechanism (+/- 2SD.) and the profile the average profile of a cone mechanism. B. Photomicrograph of midget ganglion cells stained by retrograde transport of horseradish peroxidase injected into the optic tract, eccentricity ca. 18 degrees. The lower ganglion cell has two dendritic tufts. C. A receptive field of a single cone is isotropic, and gratings drifted horizontally or vertically will give the same spatial tuning and phase. D. A receptive field made up of two cones (or two patches of cones) is anisotropic. Spatial tuning is different at different orientations, and when drifted parallel to the two cones will give spatial tuning with a null followed by a secondary peak associated by a phase reversal.
Temporal phase parameters were very similar to those derived from other measurements (Lee et al., 1990). Spatial parameters played little role in the fits. Thus, overfitting of the data was unlikely to have occurred. Some cells were also tested with gratings drifted horizontally as well as vertically. The responses to the two directions were similar in pattern, although sometimes differing in detail. Factors such as cone balance and spatial parameters of the receptive field were similar.

These analyses suggest that the receptive field structure of midget ganglion cells varies between Type I and Type II and the center-surround structure is often weak. In this sense, there is more similarity to the Type II S-cone cells of the koniocellular pathway than is commonly held to be the case. In the spatial frequency tuning of single cone mechanisms, tuning curves often had shallow slopes. These could not be fit well with a single Gaussian (e.g., Fig. 3, M cone) but a pair of Gaussians provided more satisfactory fits. Although the decrease in residual error was often modest, the shape of the spatial tuning curves is more satisfactorily captured. These shallow spatial tuning curves require a cone receptive field profile with a central peak (to give a high spatial frequency response) with shallow flanks (to provide the shallow tuning curve slope). We describe this profile as a sum of two Gaussians, which we will term a peak mechanism and a shallow mechanism, but would not assume that these mechanisms are physiologically separate. We now briefly consider such profiles in relation to the underlying cone matrices and ganglion cell morphology.

3.3. Anatomical considerations

In this section we attempt to frame our response modeling into an anatomical context. The mean eccentricity of the cells in our sample was ca. 10 degrees. Based on the cone density estimates of macaque retina (Packer, Hendrickson, & Curcio, 1989), this would give a cone density of ca. 800 per square degree, and an array with the density shown in Fig. 6A would be expected if arranged in a hexagonal matrix. The different circles represent a random distribution of M and L cones, assuming a 1:1 L- to M-cone ratio in the macaque (Dobkins, Thiele, & Albright, 2000; Mollon & Bowmaker, 1992). The circle drawn on this matrix represents the mean center size (+/- two Gaussian radii) from our data and the profile is seen to cover numerous cones. The center size is somewhat larger than dendritic tree diameters estimates from, for example, Goodchild, Ghosh, and Martin (1996). This is the case in the cat (Wässle, Boycott, & Illing, 1981). However, in the primate parafovea there is an additional complication.

Midget ganglion cell morphology, with a ganglion cell receiving input from a single midget bipolar, is maintained up to 10 degrees eccentricity and beyond (Boycott & Dowling, 1969); although there is some electron microscopic evidence for some convergence from more than one midget bipolar onto a midget ganglion cell near the fovea (Kolb & Marshak, 2003), it appears restricted. One-to-one connectivity from cones to midget bipolars is largely maintained right to the edge of the retina (Wässle, Grünter, Martin, & Boycott, 1994) but convergence of midget bipolar cells onto midget ganglion cells begins to occur beyond 10 degrees eccentricity (Boycott & Dowling, 1969). This is illustrated in a flat-mount photomicrograph in Fig. 5B, obtained by retrograde labeling of macaque retina by injections of horseradish peroxidase in the optic tract (P.R. Martin and B. B. Lee, unpublished observations). A fraction of midget ganglion cells have been stained; eccentricity is ca. 18 deg. Some midget ganglion cells have a single dendritic tuft, but others show two, as in the lower cell in the photomicrograph. With increasing eccentricity, convergence increases until at 30–40 degrees eccentricity convergence is substantial, as described by Goodchild et al. (1986). However, in the intermediate range of 10–20 degrees, there is some patchiness in dendritic connectivity.

This change in anatomical convergence might be expected to have physiological consequences. We considered the spatial frequency tuning curves that might result. With a single cone input (Fig. 5C) to the center, the receptive field is isotropic, as in the horizontal and vertical profiles. The spatial frequency tuning for drifting gratings is low-pass and phase stable, and similar whether horizontal or vertical gratings are used. With two cones providing input, as in the example in Fig. 5B, the receptive field is obviously anisotropic, as in the profiles. The spatial frequency tuning for horizontal gratings remains the same as in Fig. 5A but that for vertical gratings shows a null followed by a secondary peak and phase reversal. The exact pattern of response amplitude and phase is dependent on the cone separation, the size of the cone sampling aperture and the angle a drifting grating makes with the cone pair. The phenomenon is effectively aliasing of the grating by the cones within the receptive field center; this is possible at this eccentricity since the cone separation has become comparable to the diameter of the point spread function. As the number of cones providing input to the center increase, we found that such effects persist until the receptive field size is ca. 6 cones across, when the cone receptive field becomes effectively isotropic once more. The example in Fig. 5D is used to demonstrate the possibility of such effects; further analysis of this issue is not pursued here.

The degree to which the surrounds of midget ganglion cells receive mixed cone input has been debated (Lennie et al., 1991; Paulus & Kröger-Paulus, 1983). As stated earlier, direct measurements of midget ganglion cell receptive field structure have been against mixed surrounds (Lee et al., 1998, 2012; Reid & Shapley, 1992, 2002). To put this result into context, we considered the variability of M/L cone weighting expected based on the receptive field profiles in Fig. 5A, on the assumption that these profiles draw cones at random from the underlying array. Different random cone arrays were generated; current evidence suggests that human cone arrays show little deviation from a random pattern of L and M cones (Hofer, Carroll, Neitz, Neitz, & Williams, 2005). With a 1:1 L:M cone ratio, weights typically ranged from 0.4 to 0.6, mean close to 0.5 and a standard deviation ca. 0.06. Thus random sampling from an array of these dimensions would be unlikely to generate any kind of cone-specific receptive field, whether for center or surround.

We have presented this analysis since the secondary peak and phase shifts resemble the data in Fig. 3B. Such effects in Crook et al. (2011) were attributed to mixed cone input to the center. This is plausible but possible aliasing effects might also occur. The vertical, dual-peaked receptive field in Fig. 5D is similar to the L-cone field in Fig. 4C. One way of testing between these alternatives would be measurements with gratings of different orientations, but we tested few such cells with orthogonal gratings.

4. Discussion

Receptive fields in the visual system are often described using Gaussian profiles. When fitting responses to luminance gratings of cells with opponent center-surround receptive fields, deviations from Gaussian profiles might be difficult to detect. With cone-opponent receptive fields in the primate, it is possible to define receptive fields of individual cone mechanisms in isolation. The receptive field profiles for individual cone mechanisms shown here might appear Gaussian-like at first glance (Fig. 5) but are distinguished by sharper peaks and broader flanks than can be fit by a single Gaussian. This shape further blurs the distinction between Type I and Type II cells, which are in any case extremes of a continuum. With large stimuli, the L:M opponent weighting of midget, PC cells is close to 1:1, as discussed in relation to Fig. 5. This indicates their signals are primarily contain red-green chromatic
information. It has been suggested that center-surround organization of midget, PC cells permits them to transmit luminance information at high spatial frequencies (Inglis & Martinez-Urijagas, 1983; Lennie & D’Zmura, 1988). The analysis here suggests center-surround organization is poorly expressed in midget, PC cells, due to the continuum between Type I and Type II cells. This argues against the double-duty interpretation.

Receptive profiles of single cone mechanisms have been described in the in vitro preparation (Crook et al., 2011; Freeman et al., 2015). These recordings were at eccentricities substantially greater than those reported here; for example, Freeman et al. (2015) recorded between ca. 30 and 70 degrees eccentricity. At these eccentricities, there is substantial convergence from midget bipolar cells onto midget ganglion cells. All these authors report some mixing of cone inputs to center and surround, although Field et al. (2007) nevertheless found evidence of selectivity in cone connectivity. These authors also noted some deviations of receptive field centers from a Gaussian profile, although this seems to have been associated with the punctate nature of the center structure at these eccentricities; individual cones are quite far apart, separated by rods. Freeman et al. (2015) found evidence of subunit structure, likely associated with individual cones, which caused a non-linearity of spatial summation. This differs from in vivo studies, in which no indication of such nonlinearities have been found (Blakemore & Vital-Durand, 1986; Derrington & Lennie, 1984; Kaplan & Shapley, 1982; Lee et al., 1983), when using the usual counterphase-reversed grating test (Emroth-Cugell & Robson, 1966). One reason might be the high contrasts used in the in vitro study. In any event, the linear model used here, and in numerous other studies in the in vivo preparation (Cooper et al., 2016; Kremers, Lee, Pokorny, & Smith, 1991; Lee, Pokorny, Smith, & Kremers, 1994), has provided an adequate description. One further difficulty in comparing in vivo and in vitro studies is that any functionally imposed cone selectivity (over and above that imposed by midget anatomy) may depend on eccentricity. In a in vivo study of midget ganglion cells at 20–50 degrees eccentricity (Martin, Lee, White, Solomon, & Rüttiger, 2001; Solomon, Lee, White, Rüttiger, & Martin, 2005), some cells were found with pronounced opponency but in others it was lacking. Until in vitro data at lower eccentricities are available, it is difficult to compare all these sets of data.

The data described here include as a subsample the ganglion cells from a previous paper (Lee et al., 2012), in which the possibility of cone-selective surrounds was addressed. In that paper, we concluded that, based on the shape of the cone spatial frequency tuning curves, there was little evidence for mixed surrounds, although there were some exceptions. Ganglion and LGN cells were compared in that paper. In the current analysis, the single-cone receptive fields showed more complexity than anticipated.

Spatial frequency tuning curves for individual cones were variable in shape, but in only ca. 12% of cases was there any indication of center-surround antagonism within a single cone mechanism, as in the earlier analysis (Lee et al., 2012). A further proportion (ca. 25%) of mechanisms could be described by a single Gaussian, but for most measurements spatial frequency tuning curves were shallower in shape, and could not be described by a single Gaussian. We chose to try and model these curves as the sum of two Gaussians. A similar structure has been used to model a two-part receptive field structure of H1 horizontal cells (Packer & Dacey, 2002, 2005); for H1 cells, a center is derived from direct input, while a second input through gap junctions provides a spatially broader input of the same sign. Field et al. (2010) also used a sum of Gaussians.

Generally, spatial frequency tuning to luminance, chromatic and cone-isolating gratings could all be captured with the same model parameters, except for cells with a second peak at high spatial frequencies. Different cone contrasts (generally the maximum achievable) were used for the different gratings, to evoke robust response for the modeling. Midget, PC cells show linear contrast-response curves (Purpura, Kaplan, & Shapley, 1988; Yeh, Lee, & Kremers, 1995), and do not show saturating non-linearities such as contrast gain control (Shapley & Victor, 1978). A linear analysis was adequate to capture most aspects of the data.

Some cells (ca. 18%) showed a further feature at high spatial frequencies. After a response minimum, a second, minor peak was present associated with a phase reversal. A similar effect has been observed in the in vitro preparation (Crook et al., 2011), in which recordings were made at eccentricities greater than in our sample. In our data, the response minimum occurred at spatial frequencies between 2 and 5 cpd. One difficulty with these measurements is possible artifacts due to chromatic aberration, which might become apparent in this frequency range (Thibos, 1987). The presence of such effects has been documented physiologically in cells of the dichromatic marmoset LGN (Forte et al., 2006). The microscope optics of the in vitro preparation in the Crook et al. (2011) study would be less liable to such artifacts. Also, in our data, such secondary peaks were only found for one of the cone conditions for a given cell, and were much less apparent or absent for chromatic gratings. This would argue against chromatic aberration as the sole origin for this effect. Crook et al. (2011), interpreted their data as indicating mixed cone input to the receptive field center, as might occur at high eccentricities due to convergence of midget bipolars onto midget ganglion cells. However, another possibility, as indicated in Fig. 6, might be an aliasing effect of the grating due to spatial anisotropy in the sample of cones within the cone mechanism. A test of these alternatives might involve using gratings at different orientations, or small punctate stimuli to map receptive fields of individual cone mechanisms. This issue remains unresolved. All these factors might contribute to these discontinuities in response amplitude and phase.

In our data, receptive field profiles of opponent mechanisms showed a continuum; from Type I to Type II, consistent with results of Derrington and Lennie (1984). The fact that the fields of individual mechanisms could often not be well described by a single Gaussian, but required a sum of two, further blurred the distinction between Type I and Type II profiles. Also, the size of cone receptive fields were always large in comparison to the cone mosaic. This would imply that the standard model, with its emphasis on single cone centers, is an oversimplification. There have been earlier suggestions that this is the case. For instance, measurements of spatial tuning of near-foveal LGN PC cells using interference gratings (which bypass the optics) showed more complex receptive fields than expected from the standard model (McMahon, Lankheet, Lennie, & Williams, 2000). Also, using adaptive optics, Sinich, Zhang, Tiruvedhula, Horton, and Roorda (2009) mapped L-cone receptive fields of LGN PC cells and found L-cone centers with multiple cone inputs. In terms of mechanism, Buzas et al. (2006) suggested a combination of inner and outer retinal inhibitory inputs to account for their data; random wiring with functional specificity. However, it is unclear how this might work, since pharmacological agents blocking inhibition do not seem to substantially affect receptive field organization (Crook et al., 2011). We suggest that, taken together, all these results indicate that current models of opponent receptive field structure are incomplete.

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