Topical Review

Colour processing in the primate retina: recent progress

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Colour vision in the majority of humans is trichromatic, relying on a comparison of the quantal absorption in three different types of cone photoreceptors. The first steps in this comparison process take place at an early level of the visual system, in the retina. This topical review will highlight recent experiments which have advanced our understanding of how cone signals are compared to generate cone-opponent responses in the primate retina.

In trichromatic humans, and in Old World monkeys such as the macaque, the cone photoreceptors have peak spectral sensitivity in the short wavelength (S or 'blue'), medium wavelength (M or 'green') and long wavelength (L or 'red') range of the visible spectrum (Schnapf et al. 1988; Bowmaker et al. 1991; Jacobs, 1996). The majority of retinal ganglion cells and thalamocortical relay cells in the lateral geniculate nucleus (LGN) show cone-opponent behaviour: they are excited by some wavelengths of light and inhibited by others (De Valois, 1965; Wiesel & Hubel, 1966; De Monasterio & Gouras, 1975; Derrington et al. 1984; Lee et al. 1988). Red–green cells draw their opponent input from M versus L cones, and blue–yellow cells draw their opponent input from S versus some combination of the M and L cones. Three major lines of study have in recent years given new insights into the origin and nature of cone opponency. First, the development of in vitro preparations of primate retina (Cohen & Miller, 1994; Dacey & Lee, 1994; Dacey et al. 1996) has clarified the response properties and morphological identity of cone-opponent cells. Second, analyses of the microcircuitry of the primate retina with light microscopic (Boycott & Wassle, 1991; Ahnelt & Kolb, 1994), immunocytochemical (Kouyama & Marshak, 1992; Grünter et al. 1994; Ghosh et al. 1997) and electron microscope-based methods (Kolb & Dekorver, 1991; Grünter & Ghosh, 1997; Hopkins & Boycott, 1997; Calkins et al. 1998) have delineated the intra-retinal pathway devoted to processing cone signals. Third, analytic and computational advances have breathed new life into extracellular single cell recording studies in the primate retina and LGN (Reid & Shapley, 1992; Smith et al. 1992; Benardete & Kaplan, 1997; Lankheet et al. 1998b; Lee et al. 1998). Here I will describe some of the major discoveries and new questions that have arisen from these lines of inquiry.

Shedding new light on cone opponency: ganglion cells

The majority of ganglion cells which project to the parvocellular layers of the LGN (the 'midget' cells; Polyak, 1941) have small dendritic fields, whereas the main projection to the magnocellular layers is from large-field, 'parasol' morphology cells (Leventhal et al. 1981; Perry et al. 1984; Rodieck & Watanabe, 1993). It was long assumed that both blue–yellow and red–green opponent cells would have midget morphology. However, this view has changed. Dacey & Lee (1994) reported results obtained from an intact (whole-mount) in vitro preparation of the macaque retina, which allows long-lasting intracellular recordings from ganglion cells. In this preparation the choroid is kept intact, and the receptors remain attached to the pigment epithelium, which allows the process of regeneration of bleached photopigment to continue. The retina is stimulated by coloured lights delivered through the microscope objective lens; the wavelength and amplitude of the light is arranged to modulate the S, M or L cones, either alone or in combination.

The intracellular recordings showed that the cone-opponent cells are of two morphological types. Red–green opponent cells had the expected midget morphology (Fig. 1A and B), but one subgroup of blue–yellow opponent cells ('blue-on') turned out to be the recently discovered small-field bistratified (SBS) cells (Fig. 1D and E). The SBS ganglion cells, like midget ganglion cells, project to the LGN but occur at a much lower density than midget cells (Dacey, 1993; Rodieck & Watanabe, 1993). The SBS cells are the only ganglion cells recorded so far which show significant input from S cones (Fig. 1E). Curiously, none of the ganglion cells had blue-off receptive fields, although this type has been consistently identified (albeit much less frequently than blue-on cells) in extracellular recording studies (De Monasterio & Gouras, 1975; Derrington et al. 1984; Valberg et al. 1986).
The intracellular recordings from retina, and extracellular studies from macaque LGN (Wiesel & Hubel, 1966; Lee et al. 1987; Lankheet et al. 1998a) agree that four different functional types of red–green opponent ganglion cells can be distinguished. They all show some degree of red–green opponency, and differ in their excitatory synaptic drive (either from M or L cones), and by their response phase to luminance modulation (either on- or off-type response; Fig. 1C). In accord with the basic buypian of the vertebrate retina, the dendrites of off-centre cells are located in the outer half of the inner plexiform layer (IPL), and the dendrites of on-centre cells are located in the inner half of the IPL. No morphological distinction between L or M centre cells can be made (Dacey & Lee, 1994). Parasol cells also come in on- and off- varieties (Fig. 1F). They respond best to achromatic stimuli presented at high temporal frequencies, but show little or no sign of M–L cone opponency, or of functional input from S cones (Fig. 1G and H). This basic anatomical and functional distinction of S cone pathways and M–L cone pathways is already present at the level of bipolar cells, as shown below.

**Bipolar inputs to opponent cells: specificity and evolution**

More than ten different morphological subtypes of bipolar cell have been distinguished in the primate retina (Polyak, 1941; Boycott & Dowling, 1969; Rodieck, 1988; Boycott & Wässle, 1991; Grünert et al. 1994). Midget bipolar cells connect individual L and M cones to midget ganglion cells (Polyak, 1941; Boycott & Dowling, 1969; Kolb & Dekorver, 1991; Calkins et al. 1994; Wässle et al. 1994). A different bipolar subtype, called the blue cone bipolar cell (Mariani, 1984) makes specific contact with S cones (Kouyama & Marshak, 1992; Wässle et al. 1994). The axon terminal system of the blue cone bipolar cell is stratified with, and makes synaptic contact with, the inner layer of dendritic terminals of the blue-on cells (Kouyama & Marshak, 1992; Dacey & Lee, 1994; Calkins et al. 1998). The same S cone pathway can also be defined in a New World primate, the marmoset (Ghosh et al. 1997; Grünert & Ghosh, 1997). This species shows a high proportion of dichromatic (red–green colour blind) individuals (Travis et al. 1988; Yeh et al. 1995). The S cone pathway is identical in dichromatic and trichromatic marmosets (Ghosh et al. 1997) suggesting that, in an evolutionary sense, it predated the development of trichromatic primate vision.

**Horizontal cell connectivity and opponent responses**

Horizontal cells are likely candidates to provide the inhibitory, opponent surround to bipolar cells and ganglion cells (Mangel, 1991; Wässle & Boycott, 1991), but their role in creating opponent responses has long been controversial. Horizontal cells in primate retina do show some chromatic selectivity, but this selectivity is different to that seen in lower vertebrates (Dacheux & Raviola, 1990; Kamermans & Spekreijse, 1995; Dacey et al. 1996). The two morphologically distinct horizontal cell classes, H1 and H2 (Kolb et al. 1980; Boycott et al. 1987; Wässle et al. 1989a) receive input from L and M cones, but have distinct patterns of output from S cones (Ahnelt & Kolb, 1994; Dacey et al. 1996; Goodchild et al. 1996). The H1 cells are very insensitive to S cone modulation and are only sparsely connected to S cones, but H2 cells always make substantial contact with, and receive depolarizing input from S cones as well as M and L cones (Fig. 2A, B and E). Furthermore, the same pattern of connectivity of the homologous H1 and H2 subtypes (Fig. 2E) is present in both dichromatic and trichromatic primates (Chan & Grünert, 1998) showing that the segregation of S cone signals to H2 horizontal cells is a basic feature of the organization of the primate retina.

**The provenance of red–green opponency: could the midget system do the job?**

A potential substrate for red–green opponent responses can be identified in the precise one-to-one connectivity of the fovea—a retinal specialization which (like red–green colour vision) is unique to primates among the mammals (Schein, 1988; Wässle et al. 1989; Martin & Grünert, 1992; Calkins et al. 1994). This idea is encapsulated as the 'random wiring' hypothesis, where each midget ganglion cell in the fovea gets excitatory input (either on- or off-) to its receptive field centre from a single cone via a midget bipolar cell, and antagonistic input from a small number of neighbouring cones via H1 horizontal cells (Paulus & Krüger-Paulus, 1983; Shapley & Perry, 1986; Young & Marrocco, 1989; Lennie et al. 1991; De Valois & De Valois, 1993). Since the H1 cells get input from both L and M cones, their wavelength of peak spectral sensitivity (Fig. 3A) lies between that of M and L cones alone (Dacheux & Raviola, 1990; Lennie et al. 1991; Dacey et al. 1996). It should not really matter if the H1 cell-mediated inhibition is ‘mixed’ from M and L cones, because the opponent cone still contributes to the inhibitory mixture. But the attractiveness of an hypothesized state of affairs should not be confused with evidence for its existence, and recent electrophysiological studies have asked whether the behaviour of red–green opponent ganglion cells and horizontal cells really is consistent with the random wiring scheme.

The nature of the cone inputs to red–green opponent cells can be explored quantitatively with linear systems analysis. The pioneering studies of Gielens et al. (1982) and Derrington et al. (1984) first showed that opponent cell responses can be well predicted by linear combination of the responses of L and M cones. If the inhibitory surround of red–green opponent cells is combined from M and L cones (Fig. 3A), then the action of both cone types should be measurable for stimuli which activate the surround, or generate centre–surround interaction. One way to produce centre–surround interaction is to stimulate with spatially uniform, rapidly modulated (flickering) light. The distinct temporal properties of the centre and surround (the surround is ‘slower’) mean that centre and surround become synergistic at high temporal frequencies (Gouras & Zrenner, 1979; Frishman et al. 1987; Lee et al. 1989). A second way to search for the action of the surround is to use spatially
discrete stimuli (such as gratings, bars or edges) to elicit centre–surround spatial antagonism (Lee et al. 1998).

Smith et al. (1992) and Lankheet et al. (1998) recorded from ganglion cells in macaque retina and relay cells in macaque LGN, using flickering stimuli to modulate specifically the M and L cones. Both studies found that the behaviour of many of the red–green opponent cells was consistent with input from both M and L cones to the receptive field surround (random wiring), but Smith et al. (1992) also found some cells which were better accounted for by a selective surround model where, say, an L centre cell has only M cones in the surround. More recently, Lee et al.

Figure 1. Anatomy and physiology of opponent and non-opponent pathways in the primate retina
A, midget pathway. Schematic view of a vertical section through the parafoveal retina. Midget bipolar cells contact a single cone photoreceptor and provide excitatory input to midget ganglion cells. Each L and M cone makes contact with both an on-centre and an off-centre midget bipolar cell; only one example of each is drawn for clarity. B, response of a ‘green-on’ midget cell. The intracellular response and averaged spike discharge rate are shown below a schematic representation of the cone modulation due to the stimulus (red–green modulation). The cell responds to increases in M cone activation but is inhibited by L cones. C, 4 subclasses of red–green opponent cells can be distinguished on the basis of their response to achromatic (LUM) or red–green chromatic (RG) modulation at 4 Hz. Open red circles, red-on; filled red circles, red-off; open green squares, green-on; filled green squares, green-off. The phase of cell response is shown relative to the phase of the stimulus; negative phase representing increasing response lag relative to the stimulus. D and E, the SBS (blue-on) pathway. SBS cells receive excitatory (on) input from the blue cone-contacting bipolar cell, and excitatory (off) input from an off-centre diffuse bipolar cell. They respond vigorously to S cone modulation. F, parasol cells receive excitatory input from diffuse (on–off) bipolar cells, which contact both L and M cones and hence are non-opponent. The response of an off–parasol cell is shown in G. This cell responds to combined modulation of L and M cones, but is unaffected by a stimulus (shown in H) which modulates selectively the S cones. Panels B, E, G and H are modified from Dacey & Lee (1994). Panel C is modified from Lankheet et al. (1998). Calibration values for B, E, G and H are 50 mV and 400 impulses s$^{-1}$. 
(1998) used a discrete spatial stimulus (a bipartite field with a straight edge) and likewise found many red-green opponent ganglion cells where the inhibition was opponent and specific, again supporting a ‘selective surround’ model. This result was similar to one obtained previously using the reverse correlation technique (Reid & Shapley, 1992), but the receptive field sizes reported by Lee et al. (1998) are much closer to other reports from the literature (De Monasterio & Gouras, 1975; Derrington & Lennie, 1984; Crook et al. 1988).

Where does this leave the random wiring model? On one hand, the above studies show that some red–green opponent...
cells have pure cone inputs to the surround, as proposed over 30 years ago (De Valois et al. 1966; Wiesel & Hubel, 1966). On the other hand, in the studies of both Lankheet et al. (1998) and Lee et al. (1998), the responses of most cells were always compatible with the presence of at least a weak input from the ‘wrong’ cone type in the surround. In other words, even if some cells do have specific surrounds, they need not be specific in order to produce a perfectly respectable degree of red–green opponency. So the critical issue returns to the question of whether horizontal cells could provide a surround which is sometimes specific, and sometimes not.

**Chromatic processing in horizontal cells: a beautiful day in the neighbourhood?**

Horizontal cells are coupled together by gap junctions to form a functional network. When they have been measured in non-primate mammals, the receptive fields of horizontal cells have been much larger than their anatomically measured dendritic fields (Nelson, 1977; Dacheux & Raviola, 1982; Bloomfield et al. 1995). If a similar state of affairs holds in the primate retina, then the surround of foveal midget cells would be at least 1 deg in diameter, which is over 20 times larger than contemporary estimates of the surround diameter of red–green opponent cells (Derrington & Lennie, 1984; Croner & Kaplan, 1995; Lee et al. 1998). However, there may be a way out of this dilemma. From the point of view of a midget bipolar cell, the important feature of the receptive field of the horizontal cell is not its total diameter as measured at the soma for high contrast stimuli, but the efficacy with which changes in the horizontal cell membrane potential are translated to changes in bipolar cell membrane potential. This will determine the extent to which any one cone will influence bipolar cells in its neighbourhood, and may well be subject to non-linear effects such as thresholds and/or shunting inhibition (Smith, 1995).

If this ‘effective space constant’ in horizontal cells is small (Fig. 3B), then the inhibitory input to a bipolar cell will be dominated by the cones within its immediate neighbourhood. By contrast, if the effective space constant of the cell is large (Fig. 3C), then the inhibitory effect of the horizontal cell will reflect the average of a larger number of cones, both in the spatial and in the chromatic domains. Now, the

![Figure 3](image-url)

**Figure 3**

A, schematic view of the retina showing the key features of the ‘random wiring’ hypothesis for generation of spectral opponency. The small graphs show spectral sensitivity for the cone photoreceptors (L and M), an H1 horizontal cell and a midget ganglion cell (G). The H cell spectral sensitivity is a combination of M and L cones, and hence is broader than that of either cone alone. The G cell spectral response is the difference between the single cone response and the horizontal cell response. B and C, schematic representations of horizontal cell inputs to bipolar cell receptive fields. The hypothetical curves represent the relative efficacy of stimulation at progressively greater distances from reference points at different positions in the dendritic field of the horizontal cell. The curves in B and C have different space constants. If the space constant is small (B), the strongest effect at a given point of the horizontal cell will be due to activity in a small number of cones. This would lead to variation in spectral weighting of the inhibition for each local domain within the H cell field, according to the local distribution of M and L cones. If the space constant is larger (C) the spectral weighting would be closer to the average of the M and L cones.
chromatic effect will obviously depend on the local distribution of M and L cones. If, as can be assumed from psychophysical and neurochemical evidence, the M and L cones are randomly arranged (Marc & Sperling, 1977; Roorda & Williams, 1998), then some of the surrounds will be predominantly due to M cones, some due to L cones, and most surrounds will have a mixture of the two cone types (Paulus & Krüger-Paulus, 1983; Lennie et al. 1991). This is substantially compatible with the results from ganglion cell recordings. In fact, a small space constant for horizontal cells is compatible with other data from the primate literature. Firstly, it would account for the variability in spectral weighting for the surrounds of both midget and ganglion cells is consistent with other data from the primate recordings. In fact, a small space constant for horizontal cells is compatible with the results from ganglion cell recordings. In fact, a small space constant for horizontal cells is compatible with other data from the primate literature. Secondly, it is consistent with surround space constants reported from the literature for red-green opponent cells (Derrington & Lennie, 1984; Lennie et al., 1991; Croner & Kaplan, 1995; Lee et al. 1998). Thirdly, it is consistent with the recent finding that there is relative independence of adaptation of M and L cone mechanisms in horizontal cells (Lee et al. 1997), under the assumption that each cone would have only a small effect (via feedback) on its neighbours. Fourthly, it is consistent with the fact that the anatomically demonstrated sparse S cone input to H1 cells (Goodchild et al. 1996; Chan & Grünt, 1998) is not functionally detectable at the horizontal cell soma (Dacey et al. 1996). The receptive field diameter of primate bipolar cells has not been measured, but such measurements could be made using the in vitro retinal preparation, which is bound to become the method of choice for addressing these kinds of functional questions.

A unified view of subcortical chromatic mechanisms, and some unanswered questions

A scheme for the basis of chromatic signal transfer in the primate subcortical visual pathway can be summarized as follows. The midget ganglion cells, whose general function is to carry high spatial resolution signals at high contrast levels in the photopic range, also carry a red-green opponent signal for central vision in trichromatic primates. Since both excitatory and inhibitory inputs to midget cells draw from a larger number of cones outside the fovea, the quality of the red-green opponent signal would be expected to decline with increasing visual angle. The small bistriated cells carry a blue-yellow opponent signal. The midget ganglion cell and small bistriated (blue-on) ganglion cell types have been identified in all diurnal primates studied so far (Ghosh et al. 1996, 1997; Yamada et al. 1996), so these pathways are presumed to be common to (red-green colour blind) dichromatic and trichromatic primates. The red-green and blue-yellow opponent signals travel through distinct subdivisions of the lateral geniculate nucleus to the primary visual cortex (Martin et al. 1997). The main attraction of this scheme is that it is compatible with known anatomical and physiological properties of the subcortical visual system in both dichromatic and trichromatic primates, but there remain unanswered questions, some of which are summarized below.

The random wiring hypothesis predicts that all the red-green opponent cells should have type I centre/surround receptive field structure, rather than type II overlapping centre and surround. In retrospect, it is obvious from the literature that the clear majority of type II cells is blue-on (De Valois et al. 1966; Wiesel & Hubel, 1966; Dreher et al. 1976; Derrington et al. 1984) and the existence of red-green opponent type II cells was questioned from the beginning (Wiesel & Hubel, 1966, p. 1128; Derrington et al. 1984). That amacrine cells could be mediators of specific red-green opponent interactions is now also thought to be unlikely (Calkins & Sterling, 1996), but there is still, quite reasonably, reluctance to give up any search for potential type II red-green circuitry in the retina (Rodieck, 1991; Calkins & Sterling, 1996). The random wiring hypothesis also predicts that some foveal midget cells would have surrounds with the same cone type as the centre cone. Such cells would be only a small proportion of midget cells and, because their action spectrum would be the same as that of either the M or L cone, would fall into one of the clusters shown in Fig. 1C rather than performing like parasol cells (which sum M and L cones). A parametric study of a large number of midget cells might reveal such a population, and give a better understanding of how the responses underlying red-green opponency change with retinal eccentricity. Finally, it could be argued that the largest gap in our knowledge of colour processing mechanisms in the primate retina is the morphological correlate of the elusive blue-off cell. It is impressive to look back at the progress which has been made since 1966, and encouraging to realise that this will remain an active field of study into the next millennium.


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