Annu. Rev. Neurosci. 2000. 23:743–775

PARALLEL PATHWAYS FOR SPECTRAL CODING IN PRIMATE RETINA

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Key Words color vision, photoreceptors, bipolar cells, horizontal cells, ganglion cells

■ **Abstract** The primate retina is an exciting focus in neuroscience, where recent data from molecular genetics, adaptive optics, anatomy, and physiology, together with measures of human visual performance, are converging to provide new insights into the retinal origins of color vision. Trichromatic color vision begins when the image is sampled by short- (S), middle- (M) and long- (L) wavelength-sensitive cone photoreceptors. Diverse retinal cell types combine the cone signals to create separate luminance, red-green, and blue-yellow pathways. Each pathway is associated with distinctive retinal architectures. Thus a blue-yellow pathway originates in a bistratified ganglion cell type and associated interneurons that combine excitation from S cones and inhibition from L and M cones. By contrast, a red-green pathway, in which signals from L and M cones are opposed, is associated with the specialized anatomy of the primate fovea, in which the "midget" ganglion cells receive dominant excitatory input from a single L or M cone.

INTRODUCTION

Cell-Type Diversity Creates Parallel Pathways

The vertebrate retina is one of the most accessible parts of the central nervous system for clarifying the links between cellular morphology, physiology, and coding by neural circuits. The basic retinal cell classes and their interconnections were revealed over a century ago (Ramon y Cajal 1892). Modern application of intracellular recording and staining completed a neural architecture of beautiful simplicity (Werblin & Dowling 1969): a straight-through, three-neuron excitatory pathway composed of 1) photoreceptors that transduce the light stimulus and 2) bipolar interneurons that relay the photoreceptor signals to 3) ganglion cells, the output neurons of the retina. Added to this pathway are two sets of inhibitory interneurons: the horizontal cells that modify transfer at the photoreceptor-bipolar synapse and the amacrine cells that function at the bipolar-

ganglion cell synapse. These excitatory and inhibitory pathways are the origin of the fundamental center-surround receptive field characteristic of most neurons in the visual pathway.

This basic framework of retinal circuitry, however, belies a more fundamental architecture, fully appreciated only since the late 1980s, of diverse retinal cell types whose total number, approaching 80, rivals that of just about any other brain structure. Thus rod photoreceptors subserving scotopic vision, and multiple cone photoreceptor types subserving photopic vision, transmit to at least 10 bipolar cell types that in turn connect to at least 20 ganglion cell types and an even greater number of amacrine cell types, estimated at between 30 and 40 (Vaney 1990, Wässle & Boycott 1991, Masland 1996a, MacNeil & Masland 1998). These types are clearly defined as morphologically distinct cell populations with characteristic spatial densities, synaptic connections, and physiological properties (e.g. Rodieck 1998).

Why does the retina, at the earliest stages of visual coding, require a neural complexity comparable to the cerebral cortex? Much remains to be learned about retinal cell types, but it is now clear that parallel visual pathways emerge at the first synaptic steps in vision, where signals from each cone photoreceptor diverge to multiple bipolar cell types. Bipolar cell pathways diverge further onto ganglion cells such that each ganglion cell population establishes its own complex circuitry of associated interneurons (Boycott & Wässle 1999). Each of the many ganglion cell populations may be viewed, then, in analogy with the diverse mosaic of visual neocortical areas, as a separate, functionally distinct map of the visual field, relayed separately to one of an array of central brainstem targets.

The Primate Retina and Spectral Coding

The recognition of retinal cell-type diversity provides a key for understanding mechanisms of color coding in the primate visual system, and this topic has been the focus of several current reviews (e.g. Dacey 1996, Lee 1996, Martin 1998, Boycott & Wässle 1999, Calkins 1999, Dacey 1999, Lee 1999). Here we focus on recent advances and hypotheses about color coding circuitry. Our understanding of primate retinal organization derives mainly from studies of the macaque monkey. Macaques have photoreceptor types with the same spectral tuning as their human counterparts (Schnapf et al 1987, 1988) and similar overall visual capacities. Beyond the photoreceptors, as far as has been determined, the cell types and circuits of macaque and human retina are virtually indistinguishable (Kolb 1991; Kolb & Dekorver 1991; Dacey & Petersen 1992; Kolb et al 1992; Dacey 1993a,b; Peterson & Dacey 1997, 1998), establishing the macaque retina as an ideal model for discovering the neural mechanisms at the earliest stages of human trichromatic color vision.

The neural code for color depends on the trichromatic sampling of the visual image by cone photoreceptor types maximally sensitive to long (L cones), middle (M cones), or short (S cones) wavelengths. Cones signal the absorption of a

photon, but this signal is ambiguous with regard to the wavelength of the absorbed photon because the probability that a photon is absorbed is given by both wavelength and the density of photons incident on the photoreceptor. Thus a single cone is "colorblind," and it has long been appreciated that the first step toward the generation of signals that code for wavelength must involve a comparison of the relative activities of the three cones signals at some postreceptoral site (Young 1802, Helmholtz 1924).

The manner in which the cone signals are combined gives rise to three perceptual dimensions in normal human color vision: 1) an achromatic or luminance axis, 2) a red-green axis, and 3) a blue-yellow axis (Lennie & D'Zmura 1988). The two chromatic axes are defined by antagonistic, or opponent, color pairs that cannot coexist. Thus a balanced mixture of red and green light cancel to give a yellow percept that contains neither red nor green. The implication of opponent cancellation is that perception along, say, the red-green dimension must be determined by a single neural mechanism in which signals that mediate red versus green perception are antagonistic. Opponent process theory thus postulates that blue-yellow and red-green information is represented by two parallel channels in the visual system that combine cone signals differently. It is now generally accepted that at an early stage in the red-green opponent pathway, signals from L and M cones are opposed, and in the blue-yellow pathway signals from S cones oppose a combined signal from L and M cones (e.g. Krauskopf et al 1982).

The basic connection between the three cone types and spectral opponency in the macaque visual system has been clarified only recently. Spectral opponency was revealed in the macaque monkey in the light responses of certain retinal ganglion cells (Gouras 1968, de Monasterio & Gouras 1975, de Monasterio et al 1975a, de Monasterio 1978) and their targets in the lateral geniculate nucleus (LGN) (DeValois et al 1966, Derrington & Lennie 1984). Spectrally opponent neurons were excited by light from one portion of the spectrum and inhibited by light from another portion of the spectrum, typically showing, at some intermediate point, a response minimum where excitation and inhibition cancel. Initial recording experiments suggested a great diversity of opponent types, including, for example, trichromatic opponent cells that appeared to receive input from all three cone types (de Monasterio et al 1975b), so that the links between neural spectral opponency and the two perceptual opponent channels was not clear. More recent studies, employing stimulus techniques that isolate signals from each of the cone types, find instead that there are only two major classes of spectral-opponent light responses that relay chromatic information to visual cortex. One is red-green opponency, in which signals from L and M cones are differenced. The other is blue-yellow opponency, in which signals from S cones are opposed to an (L + M) cone signal (Derrington et al 1984; Lee et al 1987; Lee et al 1989; Lee et al 1990; Lankheet et al 1998a,b). From the perspective of retinal organization the significant remaining problems are to determine 1) the number of neural pathways that transmit opponent signals and 2) the underlying mechanisms that create the antagonistic cone interactions observed at the ganglion cell level.

Growing Complexity of the Retinogeniculate Pathway

How many ganglion cell populations transmit signals to visual cortex via the LGN? In an early attempt to directly link morphology to physiology using intracellular recording and staining methods in the intact primate eye, De Monasterio (1979) addressed the question of which ganglion cell types transmitted spectrally opponent signals. He suggested that two common types, the morphologically distinct "parasol" and "midget" ganglion cells (Polyak 1941), might transmit blue-yellow and red-green opponent signals respectively to the LGN. It is now well understood that the parasol ganglion cells project to the magnocellular layers of the LGN (Perry et al 1984) and are not spectrally opponent but show a broad spectral sensitivity created by additive input from L and M cones (e.g. Lee et al 1988). Midget ganglion cell axons project to the parvocellular layers of the LGN and were considered equivalent to the spectrally opponent P cells that have also been studied intensively by extracellular recording in both retina and LGN (for review, see Kaplan et al 1990). It has been suggested that the midget cells are a highly specialized cell type evolved uniquely for the purpose of color vision in trichromatic primates (Shapley & Perry 1986). However, ganglion cell types other than midget and parasol cells project to the LGN (Rodieck & Watanabe 1993), calling into question the simple one-to-one correspondence between these two ganglion cell types and the physiologically defined magnocellular and parvocellular pathways.

One such newly recognized ganglion cell type, the small bistratified, blue-ON cell, provides a signal for the blue-yellow axis of color vision (Dacey & Lee 1994, Calkins et al 1998, Cottaris & DeValois 1998). There is growing evidence that these small bistratified cells project to a recently identified LGN relay cell population that is intercalated between the main cellular layers (Martin et al 1997, White et al 1998). The intercalated cells in turn appear to provide a newly recognized parallel pathway to visual cortex that terminates in the "blob" region of supragranular visual cortex (Hendry & Yoshioka 1994, Hendry & Calkins 1998) where cortical color-opponent cells may be segregated (Ts'o & Gilbert 1988). It is therefore possible that the blue-ON signal pathway remains anatomically distinct from a red-green pathway or pathways through the first synapse in visual cortex. Other recent data have further modified the classical view of the color-coding midget pathway. Red-green spectral opponency appears to be restricted to the specialized wiring of midget pathway cells in the foveal region of the retina where central vision is mediated. Beyond the fovea, in the retinal periphery, midget cells receive additive input from L and M cones and show a nonopponent, luminance response indistinguishable from the parasol cells (Dacey & Lee 1997, Diller et al 1999). Figure 1 summarizes the current view of the ganglion cell populations that project to the LGN. The picture should be considered incomplete, however, because the list of ganglion cell types that project to the LGN and their physiological properties is incomplete.

The following sections focus on recent advances in clarifying the retinal circuits and mechanisms that create the spectral properties of the parasol, midget, and small bistratified cell classes. Major features of the intraretinal circuitry for all three classes have been identified anatomically. These data together with recent intracellular recordings from identified interneurons and ganglion cells suggest a strong dichotomy in the way S-cone versus L- and M-cone signal pathways are built, possibly reflecting the distinct evolutionary origins of the cone types themselves.

L- AND M- VERSUS S-CONE PATHWAYS

Beginning at the level of the cone photopigments the L- and M-cone pathways are closely linked and together differ significantly from the S-cone pathway. Molecular genetic analysis of the L-, M-, and S-cone photopigments (Nathans et al 1986a,b) established that the L- and M-cone photopigment gene loci are located in a tandem array on the X chromosome and that the amino acid sequences for these two proteins are nearly identical. Their spectral tuning, dictated by the amino acid sequence, is consequently highly overlapping, with a separation at the peak of only about 30 nm. The inference is that these two genes originated by duplication very recently in primate evolution. New World monkeys lack this duplication, with the key mutation occurring some 30 million years ago, after the divergence of New and Old World primate lineages, but before the separation of the Old World monkeys and the great apes (Dulai et al 1994). By contrast, the S-cone pigment gene appears to have had a long evolutionary history independent of the L- and M-cone pigments and predating the origin of the mammals. It is located on somatic chromosome 7, is divergent from the L- and M-cone pigments in sequence and spectral tuning, and is common to most mammals (for a review, see Jacobs 1993).

The distinct evolutionary histories of the cone photopigments are also reflected in their anatomy and postreceptoral pathways. S cones are morphologically distinct (Ahnelt et al 1990, Calkins et al 1998) and spatially form an independent and nonrandom arrangement across the retina (Curcio et al 1991). The S cones are clearly recognized by postreceptoral neurons, as is demonstrated by the circuitry for the blue-ON opponent pathway. By contrast, the L and M cones cannot be distinguished morphologically, do not form independent spatial arrays, and as we consider in more detail later in this chapter, do not appear to be recognized selectively by each other (Tsukamoto et al 1992) or by the bipolar and horizontal cell interneurons to which they connect. Both the luminance and red-green opponent pathways combine signals from L and M cones and lack a significant contribution from S cones. Finally, the circuitry for red-green opponency appears not to arise by selective circuits and cell types devoted to L- versus M-cone signals but by capitalizing on the unique circuitry of the central midget pathway.

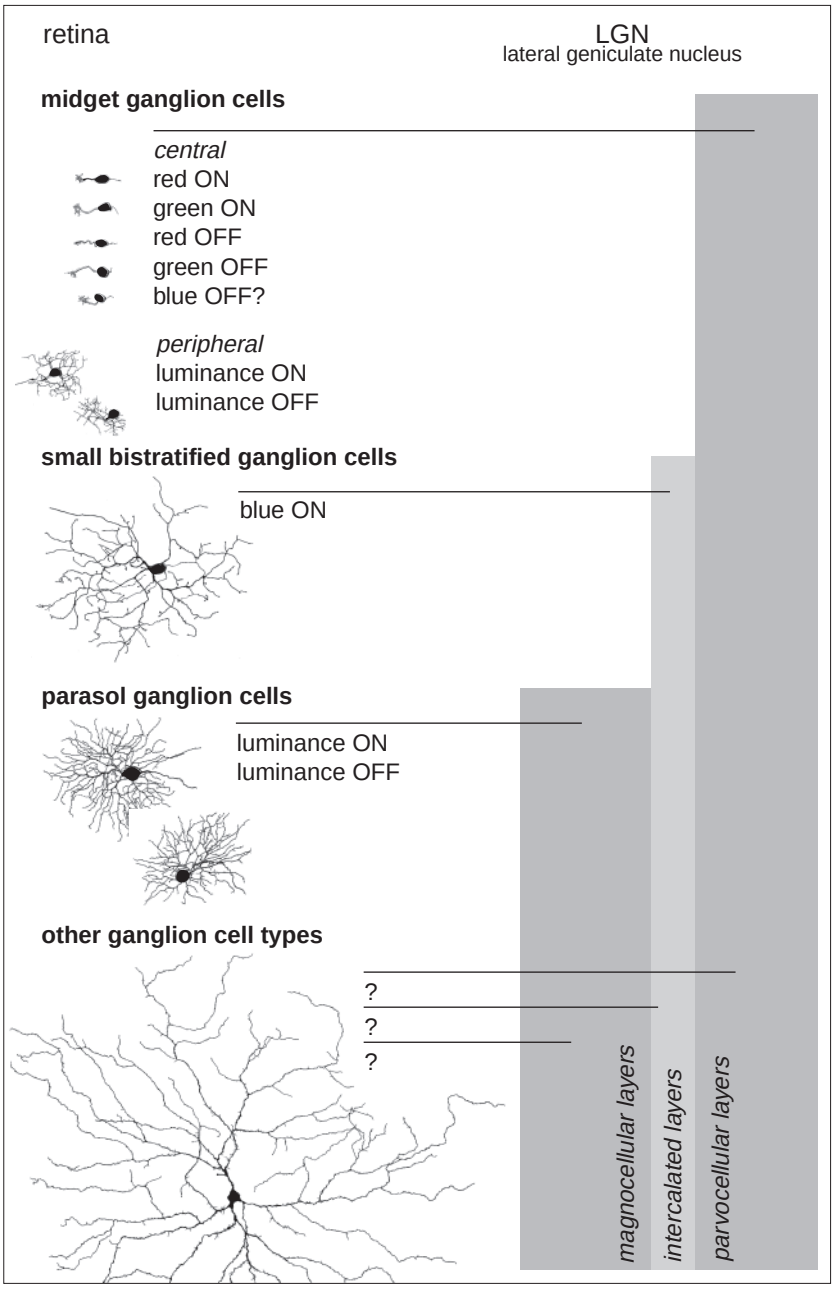

Figure 1 The retinal projection to the lateral geniculate nucleus (LGN) of the dorsal thalamus originates from a growing diversity of ganglion cell types. Midget ganglion cells project to the parvocellular layers; those subserving central vision (~0–7 degrees

(*continued*)

← **Figure 1** (*continued*) visual angle) have private-line connections to L, M, and S cones and transmit ON and OFF red-green and possibly blue-OFF opponent signals. In the retinal periphery both ON and OFF midget ganglion cells increase in dendritic field size, receive additive input from L and M cones, and transmit a luminance signal. The small bistratified cells project to the intercalated layers, receive excitatory input from S cones, and transmit a blue-ON/yellow-OFF opponent signal. ON and OFF parasol ganglion cells receive additive input from L and M cones and transmit a luminance signal via the magnocellular layers. Other LGN-projecting ganglion cell types exist (Rodieck & Watanabe 1993), but the total number of types, their site of termination in the LGN, and their functional roles remain to be clarified. Cell drawings $\sim \times 100$.

S-CONE PATHWAYS

S cones contribute little to achromatic spatial vision, and the anatomy and physiology of the S-cone pathway also reveals a design for chromatic but not spatial vision. The S cones make up only about 5–10% of the total number of cones and therefore do not exist at a fine enough spatial grain to preserve the high spatial frequencies in an image (Curcio et al 1991). Also the S-cone spectral sensitivity does not contribute to the overall spectral sensitivity of the eye, which is well modeled by combining the L- and M- cone spectra only (for review, see Lennie et al 1993). Finally, the receptive field structure of ganglion cells that receive S-cone input transmit a chromatic signal without the spatial tuning conferred by classical center-surround receptive field structure. Thus the earliest recordings of blue-ON/yellow-OFF cells describe a receptive field of spatially coextensive excitation and inhibition (Wiesel & Hubel 1966) with S cones providing the ON input and L and M cones combining to provide the OFF input (Derrington & Lennie 1984). Beyond the photoreceptor, however, the degree to which the S-cone pathway is a specialized retinal module for color coding has become clear only recently.

Small Bistratified Cells and the Blue-ON Pathway

Initial descriptions of color opponent neurons in the retina and the LGN included cells with excitatory S-cone input: the blue-ON/yellow-OFF cells (e.g. DeValois et al 1966, Wiesel & Hubel 1966, de Monasterio & Gouras 1975). A number of early studies pointed out large differences in the physiology of blue-yellow versus red-green opponent cells (e.g. Gouras & Zrenner 1981). With current knowledge of ganglion cell diversity, it is not surprising that cells with S-cone input correspond to an anatomical type distinct from the parasol and midget cells. The small bistratified cell was first anatomically recognized as a single, distinctive ganglion cell population by intracellular staining in both macaque and human retina (Dacey 1993a). The bistratified dendritic tree typically showed a sparsely branched outer tier, stratified near the outer border of the inner plexiform layer, and a somewhat larger inner tree stratified near the inner border of the inner plexiform layer. The

inner dendritic tier costratifies with the axon terminals of a cone bipolar cell type that makes exclusive contact with S cones—the blue-cone bipolar cell (Kouyama & Marshak 1992)—suggesting the transmission of an excitatory S-cone signal. This cell type was also shown to project to the parvocellular layers of the LGN (Rodieck & Watanabe 1993), suggesting a role in color coding (Rodieck 1991). Intracellular recordings from the small bistratified ganglion cell, identified in the in vitro retina, subsequently revealed the spectral-opponent light response with a major ON input from S cones opposed to an OFF input from L and M cones (Dacey & Lee 1994) (Figure 2).

Beginning with the identification of the blue-ON bistratified ganglion cell type, the major elements in this S-cone opponent pathway were assembled rapidly and are summarized in the schema shown in Figure 3. The bistratified dendritic tree of the blue-ON cell suggested a simple but unexpected mechanism for the opponent light response (Dacey & Lee 1994), termed the ON-OFF pathway hypothesis (Dacey 1999). This hypothesis proposes that S-ON/L + M-OFF opponency originates at the level of the excitatory bipolar-ganglion cell connection by converging an ON S-cone bipolar input and an OFF L + M cone bipolar input to the inner and outer dendritic tiers of the bistratified dendritic tree, respectively. Thus the small bistratified cell would correspond to an ON-OFF cell type, excited in parallel by both an ON- and an OFF-bipolar population.

What type of receptive field structure would be predicted from such an ON-OFF anatomical circuit for the small bistratified blue-ON cell? Wiesel and Hubel (1966) were the first to attempt to link red-green and blue-yellow spectral opponency to the receptive field structure of parvocellular LGN relay cells. They described two types of cells: Type 1 cells had center-surround receptive field organization, and Type 2 cells lacked spatial antagonism but showed two spatially coextensive fields that differed in their wavelength sensitivities. The ON-OFF pathway hypothesis predicts that the bistratified blue-ON cell should have two spatially overlapping receptive field center mechanisms of opposite polarity derived from S cones and L + M cones. Previous measurements of the receptive fields of blue-ON cells at the level of the LGN indicate this type of receptive field structure (e.g. Wiesel & Hubel 1966, Derrington & Lennie 1984).

Testing the ON-OFF Hypothesis for Blue-Yellow Opponency

The ON-OFF hypothesis provides a novel and efficient mechanism for generating spatially coextensive receptive fields. If this is the basis for the blue-yellow receptive field, then there should be significant synaptic input from blue-cone bipolar cells to the inner dendritic tier as well as L- and M-cone input from other bipolar cell types to the outer dendritic tier. However, electron microscopic reconstructions of cone bipolar synapses to bistratified cells presumed to be blue-ON cells support this idea only partially. In the parafovea, bistratified cells receive synaptic input to inner stratifying dendrites from a cone bipolar cell that makes

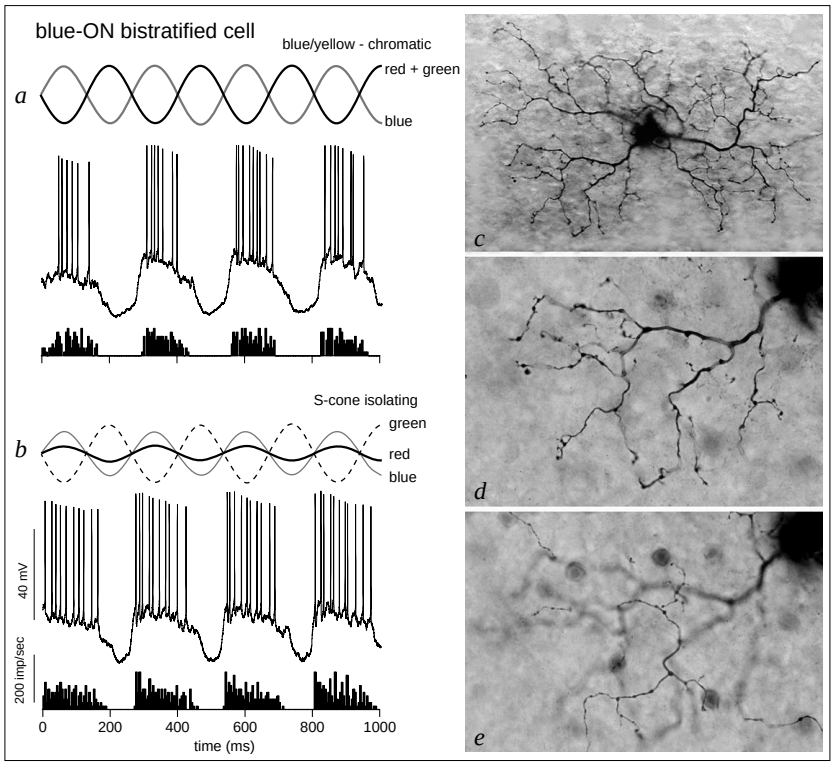

Figure 2 Light response and morphology of the small bistratified blue-ON ganglion cell type. (*a*) Blue-ON response to a blue-yellow chromatic stimulus. *Top:* the stimulus waveform (output from a blue light is modulated in counterphase to equiluminant red and green lights). *Middle:* intracellular voltage response shows strong blue-ON depolarization and spike discharge. *Bottom:* poststimulus time spike histogram. (*b*) S-cone-mediated ON response. *Top:* amplitude and phase of red, green, and blue lights adjusted to silence L and M cones while modulating only the S cones. S cones are modulated in phase with the blue light. *Middle:* voltage response shows strong depolarization and spike discharge in phase with S-cone modulation. *Bottom:* poststimulus time histogram as in (*a*). (*c*) Intracellular injection of Neurobiotin (Vector Labs) and subsequent horseradish peroxidase histochemistry demonstrate the dendritic morphology of a cell whose light response is shown in (*a).* (*d*) Higher magnification of a small portion of the dendritic tree shows the inner tier of dendrites. (*e*) Same field as in (*d*), but plane of focus is shifted to the more sparsely branched outer dendritic tier.

exclusive contact with S cones as well as input from L and M cones to the outer stratifying dendrites via two diffuse cone bipolar cell types (DB2 and DB3) (Calkins et al 1998). By contrast, in the peripheral retina, bipolar input to the outer dendritic tier is extremely rare (Ghosh et al 1997), although blue-yellow oppo-

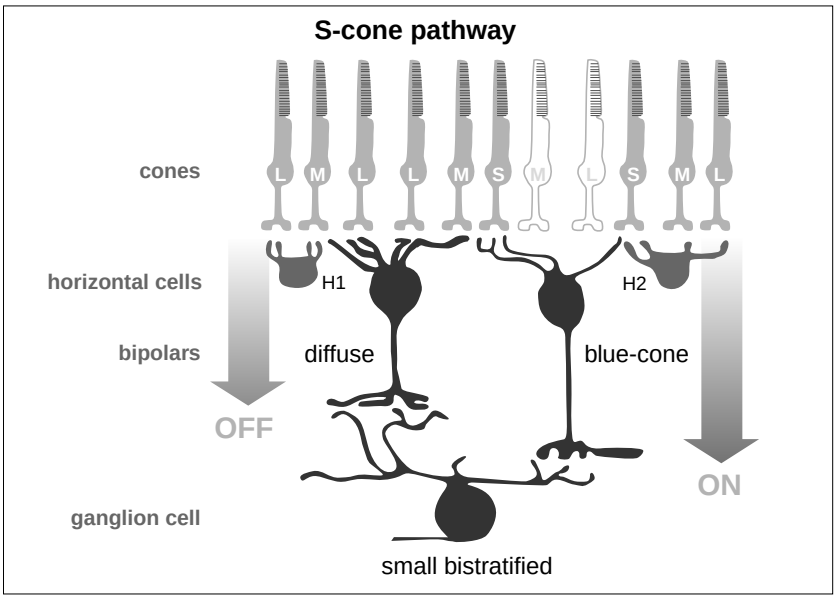

Figure 3 Identified cell types and circuitry of the S-cone, blue-ON pathway. The small bistratified ganglion cell receives synaptic input to the inner stratifying dendrites from a distinct S-cone-contacting bipolar cell, the blue-cone bipolar cell. H2 horizontal cells contact L, M, and S cones and are the probable basis for a surround in the blue-cone bipolar cell and the origin of S- versus (L + M)-cone spectral opponency. The sparse outer stratifying dendrites of the small bistratified ganglion cell receive input from diffuse cone bipolar types that contact L and M cones nonselectively. H1 horizontal cells contact L and M cones and are the probable basis for an inhibitory surround in the diffuse bipolar cell. Combined input from these ON and OFF bipolar cell pathways creates a spatially coextensive S-ON, OFF (L + M)-opponent receptive field (see Figure 4).

nency is strong, calling into question the role that the outer dendrites play in the opponent mechanism.

Recent measurements of the receptive field structure of blue-ON bistratified cells in vitro (Dacey 1996) have permitted a more careful examination of the ON-OFF hypothesis using pharmacological methods. We first determined the spatial profile of the S- and (L + M)-cone contribution to the receptive field by measuring a blue-ON cell's spatial frequency sensitivity to drifting sinusoidal stimuli that modulated either the S cones or the L + M cones in isolation (Figure 4). The responses to both S- and (L + M)-cone modulation are both low pass, and the data are best fit by single Gaussian functions with similar amplitudes and diameters. These results alone do not reveal the basis for the spatially coextensive receptive field structure. We reasoned that if the S-cone-mediated ON response and the (L + M)-cone-mediated OFF response were derived from dual S-ON and

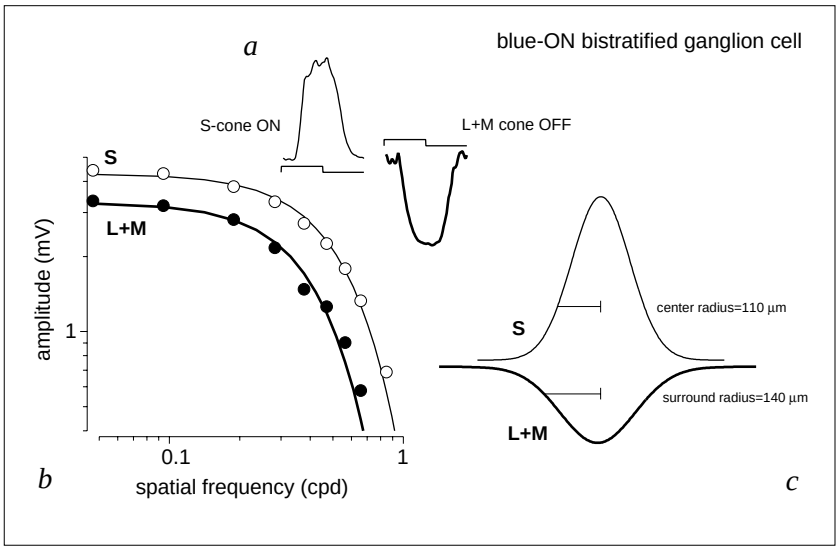

Figure 4 Spatially coextensive receptive field structure of the blue-ON bistratified ganglion cell in the retinal periphery. (*a*) Averaged synaptic potentials (spike discharge removed by filtering) in response to S- and (L + M)-cone square wave modulation. Stimulus time course shown in relation to each waveform. (*b*) Plot of response amplitude (*first harmonic*) as a function of the spatial frequency (cpd, cycles per degree of visual angle) of a drifting sine wave grating for both S- and (L + M)-cone modulations. Both S- and (L + M)-cone-mediated responses are low pass in shape and are best fit by single Gaussian functions (*solid curves*). (*c*) The radii and relative amplitude of the S- and (L + M)-cone receptive field components derived from the fits shown in (*b*).

OFF (L + M)-cone bipolar input, then it should be possible to selectively abolish the S-cone input and leave the (L + M)-cone input intact by using the well-documented ON pathway blocker 2-amino-4-phosphonobutyric acid (AP-4) (Slaughter & Miller 1981). However, bath application of 200 μM AP-4 to the retina in vitro abolished not only the S-cone component but also much of the OFF (L + M)-cone input. This result does not completely support the ON-OFF hypothesis but suggests rather that the S-(L + M) opponency must already be present in the light response of the blue-cone bipolar cell.

Recordings have not yet been made from identified blue-cone bipolar cells, but it is likely that these cells will show center-surround receptive field organization in which a depolarizing S-cone-mediated receptive field center combines with a hyperpolarizing (L + M)-cone-mediated surround. In the first study of bipolar cell physiology in primate retina, all recorded bipolar cells showed strong center-surround receptive fields (Packer et al 1999), indicating that, as in non-mammalian retinas, spatial antagonism is a fundamental property of primate bipolar-cell receptive fields.

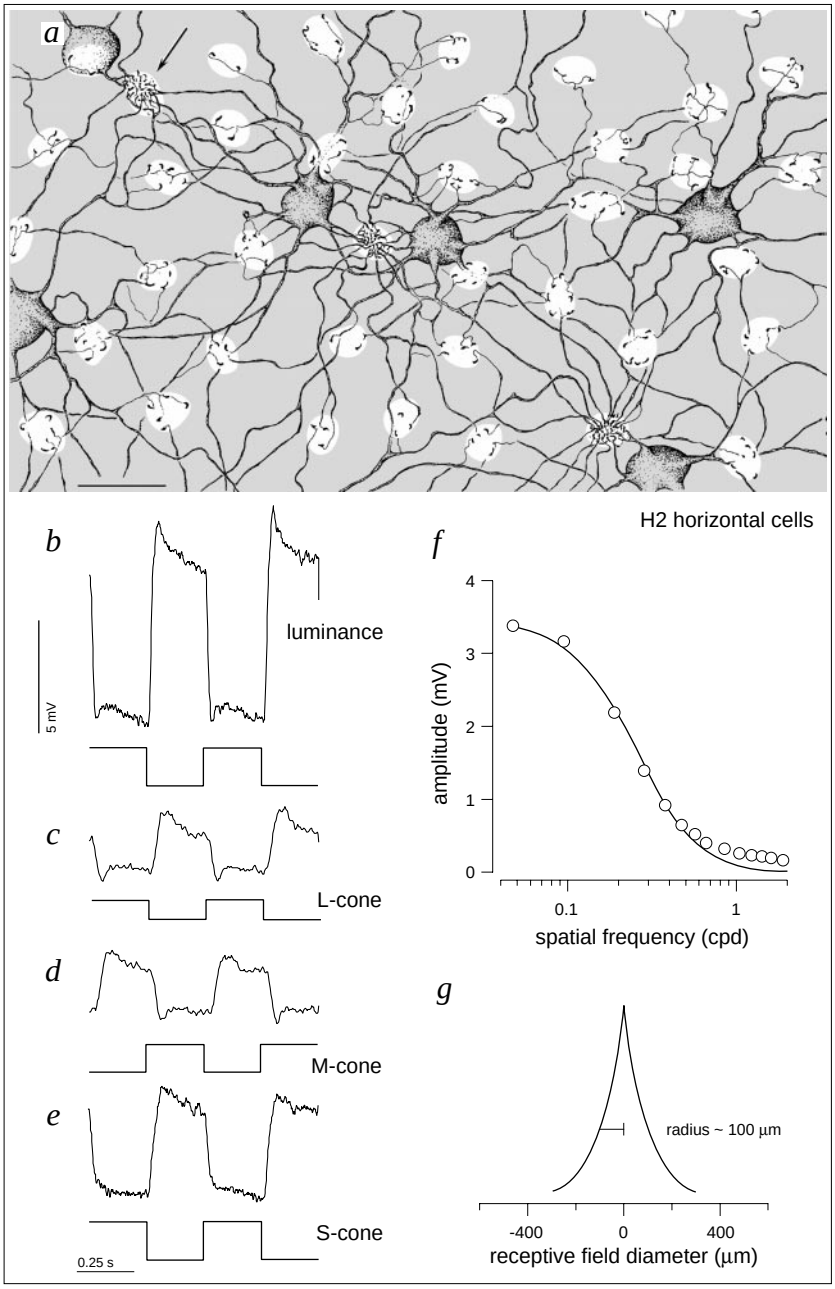

Figure 5 Morphology and physiology of the H2 horizontal cell. (*a*) Camera lucida tracing of H2 cell dendritic network revealed by injection of Neurobiotin during intracellular recording. H2 cell bodies and dendrites are shown by stippling, and white holes in

(*continued*)

← **Figure 5** (*continued*) the gray background indicate position and size of cone axon terminals. The majority of cones are sparsely innervated and correspond to L and M cones. Three cone pedicles (one pedicle indicated by arrow) are densely innervated and have the spacing and density of S cones. (*b*) Large hyperpolarizing voltage response to luminance modulation (all three cone types modulated; stimulus time course shown below voltage trace). (*c–e*) Hyperpolarizing voltage responses to L-, M-, and S-cone-isolating conditions as indicated. (*f*) Plot of response amplitude as a function of the spatial frequency of a luminance modulated drifting grating (protocols as in Figure 4). Data were fit with a single exponential function (*solid curve*). (*g*) Exponential fit shown in (*f*) gives a receptive field radius of 100 μm.

The probable basis for an (L + M)-cone-mediated surround in the blue-cone bipolar cell would be via negative feedback from a distinct horizontal cell type—the H2 horizontal cell—recently shown to be a major component of the S-cone pathway (Dacey et al 1996). The H2 horizontal cell makes contact with all three cone types, but like the blue-cone bipolar cell, many dendrites preferentially seek out and make contact with the sparse S cones (Anhelt & Kolb 1994, Dacey et al 1996, Goodchild et al 1996). The result is that the light response of the H2 horizontal cell is driven with relatively equal strength by L-, M-, and S-cone input (Figure 5). The H2 horizontal cells could therefore contribute an (L + M)-cone opponent surround to the S cone via a negative feedback that would then be conveyed to the blue-cone bipolar cell.

Would an H2 horizontal cell–mediated bipolar surround be of the appropriate size to provide for the spatially coextensive (L + M)-cone inhibitory field observed in the blue-ON ganglion cell? The answer appears to be yes. In general, horizontal cells form an electrically coupled syncytium that generates large receptive fields extending far beyond the extent of the photoreceptor contacts of a single cell. H2 horizontal cells also form a coupled network; however, the spatial extent of the H2 horizontal cell receptive field is relatively small and comparable in size to the blue-ON ganglion cell receptive field (Figure 5*g*).

If blue-yellow opponency originates in the segregation of S- and (L + M)-cone signals to the center and surround, respectively, of the blue-cone bipolar cell, then what is the role of the bistratified ganglion cell dendritic tree and input from an OFF cone bipolar cell? The experiments described earlier in this chapter, in which AP-4 was used to eliminate the blue-cone bipolar pathway, showed that a small component of the OFF (L + M)-cone input is unaffected by AP-4 and corresponds to the OFF cone bipolar input to the outer dendritic tier. Analysis of the spatial properties of this AP-4-resistant input suggests that it functions importantly to enhance the spectral opponency in the normal blue-ON receptive field (Figure 4). The OFF cone bipolar input itself has a center-surround organization that would affect the ganglion cell receptive field in two ways. First, the OFF-center component, although small, would add significant gain such that, in the blue-ON ganglion cell, the (L + M)- and S-cone-mediated fields would become approximately equal in strength. Second, the ON-surround component is large and would

spatially antagonize the OFF-surround of the blue-cone bipolar pathway, making the S- and (L + M)-cone-mediated fields more spatially coextensive.

In summary, identification of the small bistratified blue-ON, yellow-OFF cell has led to a number of detailed anatomical and physiological studies probing the mechanism for spectral opponency in the S-cone pathway. Experiments designed to test the hypothesis that the origin of the opponency occurs in the bistratified dendritic tree via dual S-cone ON and (L + M)-cone OFF bipolar input suggest instead a more complex picture: Spectral opponency originates in the blue-ON bipolar cells, by segregating S-cone versus (L + M)-cone signals to the receptive field center and surround, respectively. This opponency is modified by the OFF bipolar pathway at the level of the blue-ON ganglion cell to enhance spectral antagonism and reduce spatial antagonism.

Midget Cells and the Blue-OFF Pathway

Most S-cone opponent cells identified in extracellular recordings from the retina or the LGN were blue-ON cells, receiving excitatory input from S cones, but the existence of a more rarely recorded S-OFF opponent cell is also well documented (Valberg et al 1986). The correspondence of the blue-ON cell with a novel bistratified ganglion cell type raises the question of whether some other non-midget ganglion cell type or types project to the LGN and transmit a blue-OFF signal. No such novel type has been observed, and the morphology of an identified blue-OFF cell has not been identified. However, each S cone, in addition to its output to the blue-cone bipolar cell, is also connected to a single midget bipolar cell (Klug et al 1992, 1993), suggesting that a subset of midget cells in the central retina could transmit a blue-OFF signal.

Electron microscopic reconstruction of these S-cone-connected midget bipolar cells indicates that they correspond to OFF-bipolar cells. The dendritic terminals make flat contacts with the cone pedicle and the axon terminal stratifies in the outer portion of the inner plexiform layer—features strongly correlated with OFF-center light responses (for review, see Boycott & Wässle 1999). With surround responses derived from L and M cones via the H2 horizontal cell, like that suggested for the blue-cone bipolar cell, these S-cone-connected midget cells should show blue-OFF/yellow-ON spectral opponency. That a small proportion of parafoveal midget cells transmits a blue-OFF signal could explain why these cells are rarely encountered by the recording electrode. Blue-OFF midget cells would make up only a very small proportion (about 2–3%) of the total midget cell population.

In addition, linking the blue-OFF pathway to the midget system suggests a second anatomical limitation. Beyond the parafovea, all midget cells begin to receive input from multiple cones (Dacey 1993b). Thus, any blue-OFF midget cell should become nonopponent because S-, L-, and M-cone-connected midget bipolar cells would increasingly converge on a single OFF midget ganglion cell. Thus only the blue-ON pathway would have a representation beyond central vision. This kind of eccentricity-related asymmetry appears also to be present

when comparing the blue-ON pathway with the red-green pathway, as discussed in the next section.

L- AND M-CONE PATHWAYS

A basic property of the cone array critical for ultimately understanding the pathways for luminance and red-green spectral opponency is the relative number and spatial arrangement of L and M cones across the retina. A variety of techniques applied over many years have indirectly suggested a random arrangement and great variability in the ratio of L to M cones across individuals. This variability is the most likely explanation for individual variation in photopic spectral sensitivity (for review, see Lennie et al 1993).

Direct observations of the two cone types in situ have confirmed that the cones are arranged randomly and vary greatly in relative numbers across individuals (Mollon & Bowmaker 1992, Roorda & Williams 1999). Adaptive optics was used to attain the sharpest images ever of the retina and directly view individual cones (Liang 1997). These images were then combined with retinal densitometry to identify the photopigment of each cone. Roorda & Williams (1999) found L- to M-cone ratios of about 1:1 and 4:1 for two human subjects (Figure 6). The consequence of random arrangement is an apparent large-scale clumping of cones of like type. How are the postreceptoral pathways that derive from the L and M cones affected by this tremendous anatomical variability, and what consequence, if any, does this variability have for the circuits that code luminance and color?

In the next two sections we discuss evidence that the red-green and luminance pathways are not as clearly related to distinct ganglion cell populations, as was found for the link between the bistratified ganglion cell and the blue-ON pathway. In retinal periphery, both the midget and parasol pathways make nonselective connections with all of the L and M cones in their receptive fields and lack spectral opponency. The variability in the relative numbers of L and M cones both locally in the retina and across individual retinae is reflected in the spectral sensitivities of both cell populations. Red-green opponency appears to be a property restricted to midget ganglion cells of the central retina, where a single cell is dominated by excitatory input from a single cone.

Midget Pathways: Red-Green Opponency and Luminance Coding

Red-green opponency derives from antagonistic interaction between L- and M-cone signals and is conveyed to primary visual cortex via the parvocellular layers of the LGN (e.g. Merigan 1989, Schiller et al 1990). The morphologically distinct midget ganglion cell population (e.g. Watanabe & Rodieck 1989, Dacey 1993b) provides a major projection to the parvocellular LGN (Leventhal et al 1981, Perry et al 1984) and reaches a very high density in the parafoveal retina, where red-

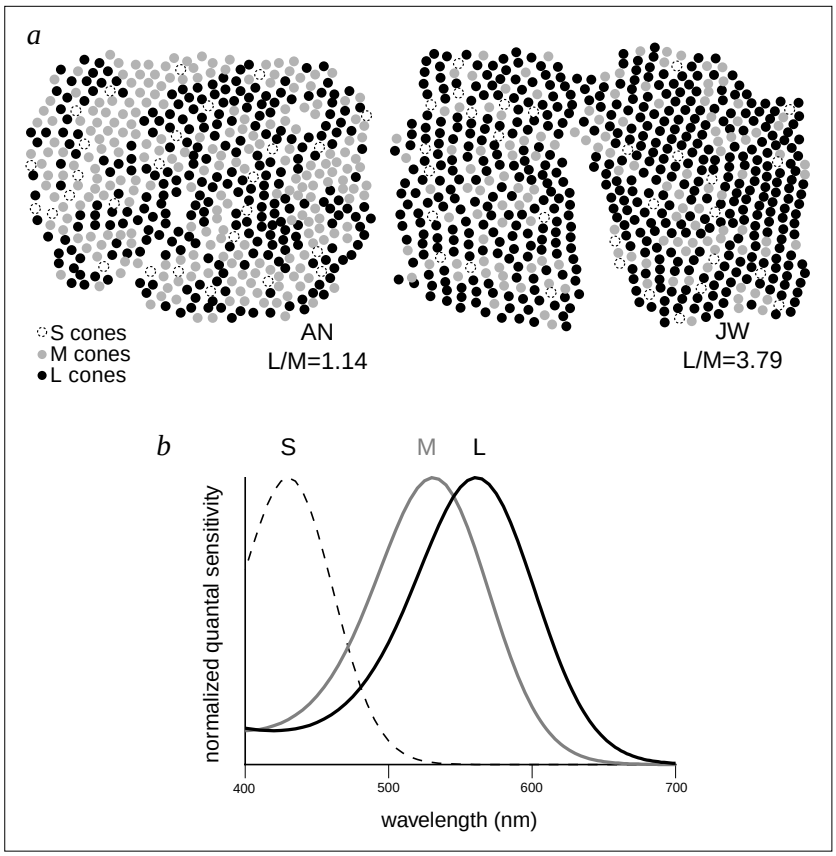

Figure 6 Mosaic and spectral sensitivities of S, M, and L cones. (*a*) Direct imaging of cone mosaic and assignment of photopigments in two living human eyes (modified from Roorda & Williams 1999). One individual's retina (*left patch*, AN) showed about equal numbers of L and M cones (dotted, gray, and black dots indicate S, M, and L cones, respectively), whereas a second subject (*right patch*, JW) demonstrated an L- to M-cone ratio of nearly 4:1. Variation in the relative numbers of L and M cones accounts for variation across individuals in the overall spectral sensitivity of the eye (Brainard et al 2000) and for variation in the spectral tuning of retinal neurons that receive L- and M-cone input (Dacey 2000a). (*b*) Spectral sensitivity functions for S, M, and L cones of macaque retina (data taken from Baylor et al 1987).

green cells are recorded reliably. Therefore, the evidence is strong that the midget ganglion cells are the major if not sole source of an opponent signal that compares the output of L and M cones. Wiesel and Hubel (1966) proposed a simple circuit for opponency based on center-surround receptive field antagonism in which the receptive field center was excited by only one of the two cone types and input to

the inhibitory receptive field surround was derived from the other. Physiological maps of cone inputs to the receptive fields of red-green opponent ganglion cells (Lee et al 1998) and LGN relay cells (Reid & Shapley 1992), using stimuli designed to modulate either L or M cones in isolation, have provided evidence that both center and surround receive such cone type–selective input, in agreement with the original Wiesel and Hubel hypothesis.

The cell types and circuits that could underlie red-green opponency constitute a major issue, however, because segregation of L- and M-cone inputs to the receptive field of a midget ganglion cell poses a formidable problem for the retinal circuitry. Any postreceptoral cone type–specific connections must be able to recognize and seek out either L or M cones and ultimately segregate their signals to the receptive field center and surround of the red-green opponent ganglion cell.

The cone type–specific hypothesis appears to be supported at least partially by the remarkable microcircuitry of the midget system in the parafovea (Figure 7). A midget bipolar cell receives all of its photoreceptor input from a single cone and transmits virtually all of its output to a single midget ganglion cell (Kolb & Dekorver 1991, Calkins et al 1994) (Figure 7). This "private-line" synaptic arrangement in the parafovea could provide for a pure L- or M-cone input to the receptive field center of a midget ganglion cell. Recent measurements of responses to very high spatial frequencies for parafoveal midget pathway cells demonstrate that the dominant input must derive from a single cone (McMahon et al 2000). However, evidence indicates that more than one cone can contribute to the receptive field center (for review, see Lee 1996), arguing against a strict private-line interpretation of the midget circuit. It is possible that electrical coupling among cones (Tsukamoto et al 1992), and perhaps among midget bipolar cells, may enlarge the receptive field center size beyond that of a single cone.

There is more uncertainty about a possible cone type–specific surround mechanism. Attempts to determine the cone pathways that drive the receptive field surround of midget ganglion cells, and consequently the underlying opponent mechanism, have failed to support the cone type–specific hypothesis. Measurements of the strength and chromatic signature of surround inhibition led to the conclusion that, with extracellular recording techniques, it was not possible to distinguish between selective or indiscriminate cone input to the surround (Smith et al 1992, Lankheet et al 1998b). Horizontal cells, known to contribute to the formation of the receptive field surround (Mangel & Miller 1987), receive a combined input of the same sign from both L and M cones in macaque (Dacey et al 1996). Horizontal cells are thus excluded from a role in generating a cone type–selective surround in the midget pathway; indeed it appears that they would contribute mixed L- and M-cone input to the surround (Masland 1996b). Amacrine cells, the other candidates for cone type–selective lateral inhibitory connections, contact multiple midget bipolar cells with no selectivity, also arguing against a role for amacrine cell circuitry in the formation of a cone-pure surround (Calkins & Sterling 1996).

These results lend support to a long-standing alternative hypothesis: that red-green opponency arises not from a cone type–selective circuitry but from ran-

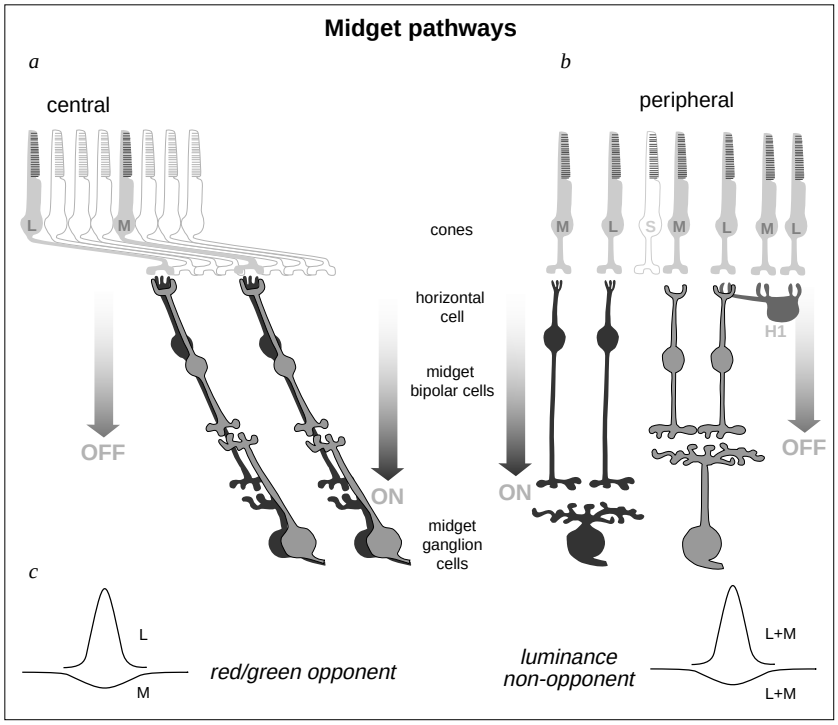

Figure 7 Cell types and circuits for the L- and M-cone midget pathways in central and peripheral retina. (*a*) In the central retina (~0–7 degrees eccentricity) a private line exists in which a single midget ganglion cell receives input from a single midget bipolar cell, which in turn contacts a single cone pedicle (either L or M cone); each cone terminal links to both an ON and an OFF pathway cell. (*b*) In the peripheral retina, midget ganglion cell dendritic trees enlarge greatly and receive convergent input from a number of midget bipolar cells. (*c*) In the central retina, receptive fields show strong L- and M- (red-green) cone opponency. In the peripheral retina, larger receptive fields show additive input from L and M cones to receptive field center and surround, lack spectral opponency, and show the spectral sensitivity of cells in the luminance pathway (see Figure 8).

dom connections of both L and M cones to the midget receptive field (Paulus & Kröger-Paulus 1983, Lennie et al 1991, DeValois & DeValois 1993, Mullen & Kingdom 1996) (Figure 7). The basis for the cone type–mixed hypothesis is that the relative strength of L- versus M-cone input to the receptive field center and surround determines the strength of a red-green opponent signal. In the parafovea, given a greater synaptic strength and input to the receptive field center dominated by a single cone, indiscriminate mixed-cone input to a large weak surround will result in strong red-green opponency (Lennie et al 1991). The key to this hypothesis is the great reduction in cone inputs to the receptive field center of the midget

ganglion cell. Given the private-line midget circuit, input to the receptive field center will be dominated strongly by a single L or M cone. By contrast, the receptive field surround will sample from several cones and, in most cases, include input from the opposing cone type.

The mixed-cone hypothesis also predicts that red-green opponency will be degraded in the retinal periphery because at eccentricities greater than approximately 7 degrees, midget ganglion cells increase steadily in dendritic field size (e.g. Watanabe & Rodieck 1989, Dacey 1993b), presumably gather input from multiple midget bipolar cells (Milam et al 1993) (see Figure 7), and are probably driven by input from 30–40 cones. Because the mixed-cone hypothesis is based on a lack of cone type–selective connections, the peripheral midget cells are predicted to receive a similar combined (L + M)-cone input to both receptive field center and surround and therefore show a nonopponent light response.

Using an in vitro preparation of macaque retina, it has been possible to address the mixed-cone hypothesis by mapping the cone inputs to both the receptive field center and surround of identified midget ganglion cells in the retinal periphery (Dacey & Lee 1997, Dacey 1999). In the far periphery, midget ganglion cell dendritic fields can be as large as 150–200 μm in diameter (Figure 8*a*). These peripheral midget ganglion cells receive input from both L and M cones to the receptive field center and surround (Figure 8*c–d*). Not only do these midget ganglion cells show a complete lack of red-green opponency, but they show a spectral sensitivity (Figure 8*b*), like that shown previously for ganglion cells that project via the magnocellular layers of the LGN (e.g. Lee et al 1988).

The lack of opponency in peripheral midget ganglion cells is also present in the physiology of the cone bipolar cells that generate the midget receptive field. The first intracellular recordings of identified midget bipolar cells showed that the loss of spectral opponency in the peripheral midget pathway is already present at the bipolar cell level (Dacey et al 2000b) (Figure 9). Unlike midget ganglion cells, midget bipolar cells maintain the private-line single-cone connection over most of the retina (Milam et al 1993). However, receptive field center size is large relative to that expected from a single cone input, suggesting that neighboring cones must contribute, perhaps via electrical coupling among bipolar cells. The consequence is that the receptive field center can show additive input from both L and M cones. Midget bipolar cells also possess a large and strong receptive field surround that receives combined input from L and M cones. The result is a nonopponent receptive field like that observed for midget ganglion cells.

The L- and M-cone input to the midget bipolar cell surround is likely derived from negative feedback to L and M cones via the H1 horizontal cell. Unlike H2 horizontal cells of the S-cone pathway, H1 cells receive strong input from L and M cones but do not transmit an S-cone signal (Figure 10*b–e*). The lack of S-cone input is reflected in the anatomy of the H1 cell mosaic, in which L and M cones are nonselectively and densely innervated but S cones are avoided (Figure 10*a*). The relatively large diameter of the H1 receptive field (Figure 10*g*) is also consistent with the surround diameters of midget bipolar cells in the retinal periphery.

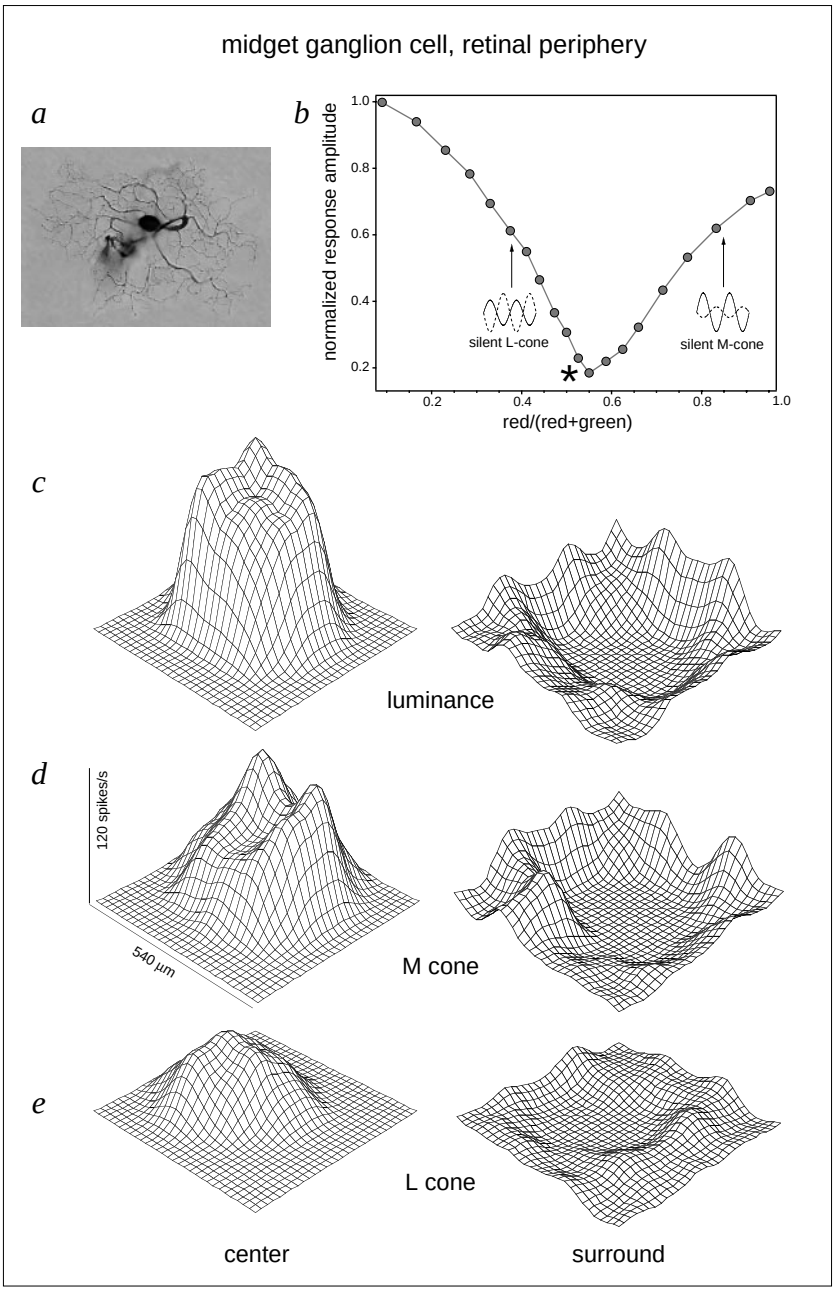

Figure 8 Midget ganglion cells lack spectral opponency in the retinal periphery. (*a*) Dendritic morphology of an intracellularly recorded and stained macaque midget ganglion cell from the far temporal periphery (~11 mm from the fovea). The dendritic

(*continued*)

← **Figure 8** (*continued*) field diameter is approximately 150 μm. With increasing eccentricity midget ganglion cells increase in dendritic field size and receive convergent input from many cones (Watanabe & Rodieck 1989, Dacey 1993b). (*b*) Cone inputs and spectral sensitivity assessed using the paradigm of heterochromatic modulation photometry (Dacey & Lee 1999; see also text). Plot of mean response amplitude as a function of the ratio of red-green modulation depth for 15 identified midget cells. Insets show stimulus waveform for L- and M-cone-isolating points (*solid sine wave,* red light; *dotted sine wave,* green light). Stimulus ranges through selective L-cone, red-green pure color, and selective M-cone modulation. These cells received additive same-sign input from both L and M cones and showed a response minimum near red-green equiluminant modulation (*). (*c–e*) Cone inputs were mapped using luminance-modulated M- and L-cone-isolating spot stimuli, 40 μm in diameter and modulated at 2.44 Hz temporal frequency. The spot was moved to successive locations in a 13 × 13 grid covering a 540-μm square field. Center and surround responses were identified clearly by an approximately 180-degree shift in response phase. The left-hand column shows three-dimensional mesh plots of the location and amplitude of center-OFF responses to each of the three stimulus conditions (surround response locations were given zero values). The receptive field center received additive input from M and L cones. The right-hand column shows mesh plots of the surround-mediated ON response to the same stimuli (center response locations were given zero values); these responses were strongest around the edges of the center. As for the center, the surround received additive input from M and L cones; the M-cone input was the stronger of the two.

These results support the mixed-cone hypothesis in the retinal periphery for both midget ganglion cells and midget bipolar cells and are also compatible with psychophysical evidence for a gradual decline in the sensitivity of red-green color vision with increasing distance from the fovea (Mullen 1991; Mullen & Kingdom 1996, 1999). For the central retina, where the private-line midget pathway is present, the degree to which random and mixed-cone connectivity can be discerned remains a major unanswered question (e.g. Lee 1996, McMahon et al 2000). If connectivity is truly random, then given the variable patchwork of L- and M-cone submosaics as illustrated in Figure 6, a lack of opponency should occur in those midget cells that are located in a large patch of cones of like type.

Making the distinction between the cone type–mixed and cone type–specific hypotheses for red-green opponent circuitry is not simply a fine point but is central for understanding the functional and anatomical organization of the primate retina. According to the cone type–specific hypothesis, a tremendous amount of connectional specificity is required that must be characterized both in terms of the adult and developing retina and in terms of the evolution of primate color vision. According to the cone type–mixed hypothesis, no specific circuitry is required, and red-green opponency would be considered a byproduct of the evolution of single cone–connecting midget ganglion cells in the parafovea for the purpose of increased spatial resolution (Mollon et al 1990, Boycott & Wässle 1999).

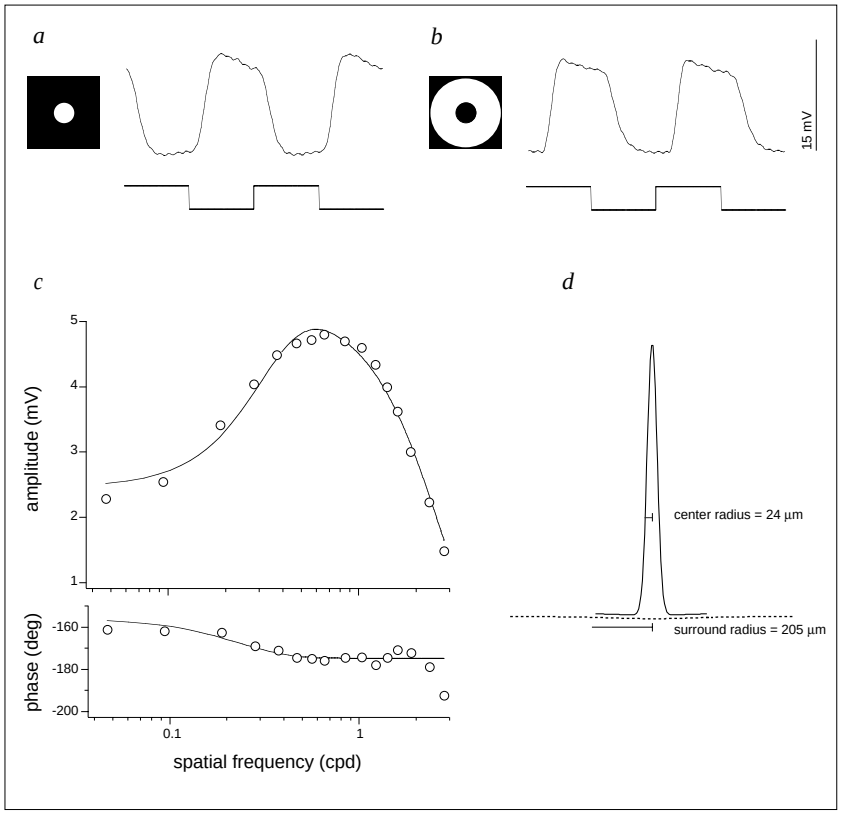

Figure 9 Center-surround receptive field organization of a midget bipolar cell. (*a*) Hyperpolarizing OFF-center voltage response to a square wave–modulated spot (*inset*) centered on the receptive field. (*b*) Depolarizing surround response to an annular stimulus placed over the receptive field. Time course of stimulus waveforms are shown under voltage traces in (*a*) and (*b*). (*c*) Plot of first harmonic response amplitude and phase as a function of spatial frequency (drifting, full field sine wave grating; 100% luminance contrast; cpd, cycles per degree). Bandpass spatial frequency tuning is best fit by a difference of Gaussian receptive field model (*solid line*) (Dacey et al 2000b). (*d*) Receptive field center and surround amplitudes and Gaussian radii derived from the fit shown in (*c*) are indicated.

Luminance Coding and the Variable L-and M-Cone Mosaic

A basic question about the retinal basis for a luminance signal is whether variability in the L- and M-cone mosaic (e.g. Figure 6) has a neural representation beyond the cones or whether neural processing can adjust the relative strengths of L- and M-cone signals (e.g. Calkins et al 1994, Shapley 1995). In the retinal periphery the midget ganglion cells are similar to the parasol ganglion cells—both cell populations receive additive L- and M-cone input to the receptive field

center and surround and demonstrate a spectral sensitivity similar to that of the psychophysical luminance channel (Dacey & Lee 1997, Lee et al 1988). Like midget cells, parasol cells demonstrate a receptive field surround that is probably derived from H1 horizontal cell feedback to L and M cones. H1 horizontal cells also demonstrate a spectral sensitivity expected for the luminance channel (Dacey 1999). Unlike midget ganglion cells the bipolar cell input to parasol cells derives from the class of diffuse bipolar cells (Jacoby et al 1996) (Figure 11). These bipolar cells contact multiple cones and, like the H1 horizontal cells, also sum L- and M-cone input (Dacey et al 2000b).

If the midget and parasol pathway cells draw randomly from all the L and M cones in their receptive fields, would the physiological balance of the two cone inputs to the receptive field reflect local variability in the L- and M-cone ratio? Would the physiological weights of the two cone inputs be similar or different for midget and parasol cells that draw input from the same or nearly the same patch of cones? In a recent series of experiments we addressed these questions directly by measuring the relative strengths of L- and M-cone input to H1 horizontal cells, midget ganglion cells, and parasol ganglion cells across many different retinas and retinal locations (Diller et al 1999, Dacey et al 2000a). To make these measurements, we designed stimuli that maintained all three cone types at the same level of adaptation while systematically varying the amount of L- and M-cone contrast in the stimulus. The relative L- and M-cone contrast gains were best fit with a linear model in which cone contrast was simply proportional to cone input strength.

For a large sample of H1 cells we found a striking variability in L- to M-cone contrast gain ratio with a mean of 1.5:1 (L/L + M) (Figure 12*a*). This mean value is close to the relative L- and M-cone weights that characterize the photopic luminosity function (Lennie et al 1993). The large variability from cell to cell was accounted for by systematic variation in L- and M-cone gain at different retinal locations and overall variation across different retinas. Both of these sources of variability are now well-documented properties of the cone mosaic itself (Hagstrom 1998, Roorda & Williams 1999; see also Figure 6) and suggest that the gain ratios that we measured directly reflect the anatomical cone ratios. Recent molecular genetic analysis of the L- to M-cone ratios, in pieces of macaque retina in which the physiological gain ratios were measured, strongly supports this conclusion (Deeb et al 2000).

The physiological L:M variability was also present at the next synaptic step at the level of the midget and parasol ganglion cells (Diller et al 1999) (Figure 12*a*). In addition, measures of the L- to M-cone contrast gain ratio for single H1, midget, and parasol ganglion cells at the same retinal locations recorded in sequence revealed highly correlated L:M gain ratios for all three cell types (Figure 12*b–c*), indicating that the physiological gain set by the anatomical cone ratio is preserved from outer to inner retina and is identical in both the nonopponent midget and parasol pathways.

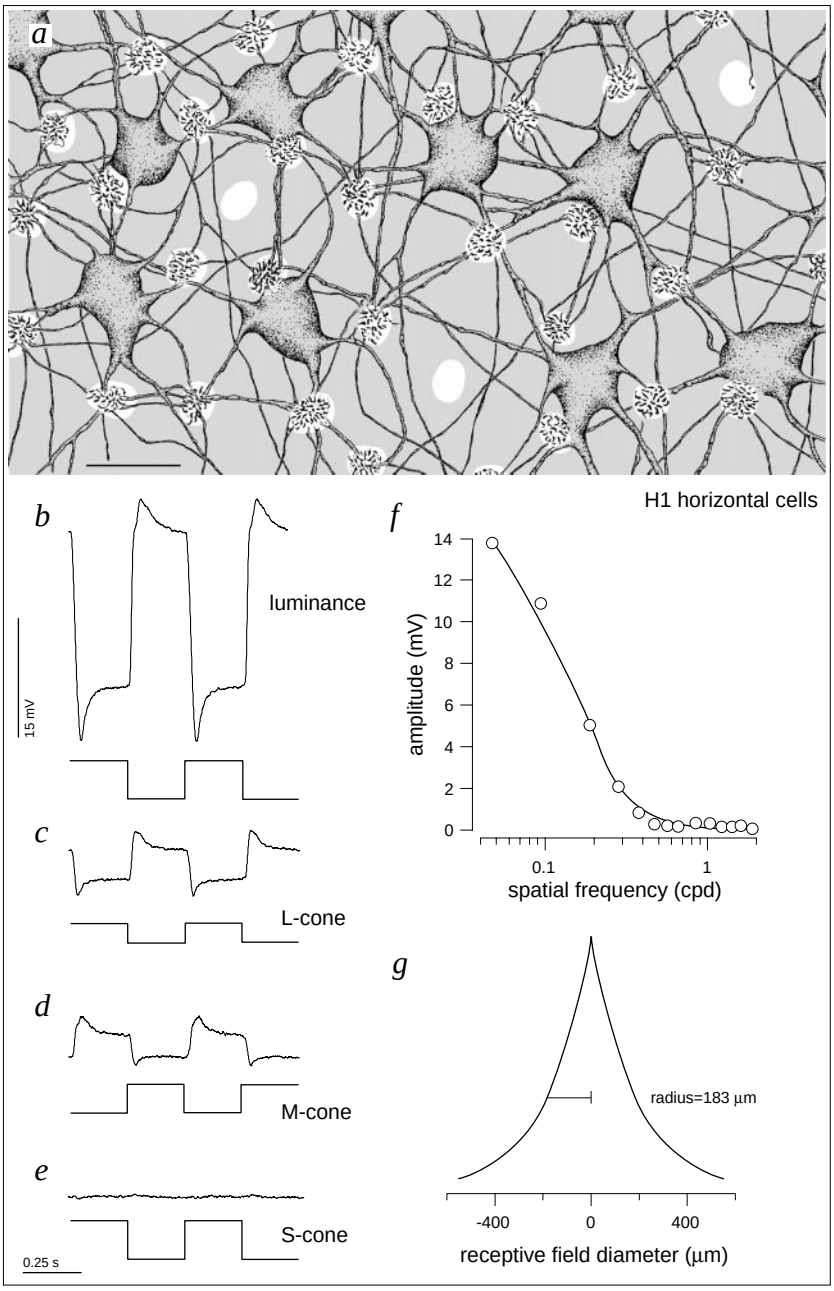

Figure 10 Morphology and physiology of the H1 horizontal cell. (*a*) Camera lucida tracing of the H1 cell network; conventions as shown for H2 cell in Figure 5. Note that in contrast to the H2 cell the H1 cell network densely innervates all L- and M-cone axon

(*continued*)

← **Figure 10** (*continued*) terminals but skips over three terminals in the field with the spacing and density expected of S cones. (*b*) Large hyperpolarizing voltage response to luminance modulation (all three cone types modulated in phase, stimulus time course shown below voltage trace). (*c–d*) Hyperpolarizing voltage responses to L- and M-cone-isolating stimuli (note that L- and M-cone stimuli are in counterphase). (*e*) Lack of response to S-cone modulation. (*f*) Plot of response amplitude as a function of the spatial frequency of a luminance modulated drifting grating (protocols as in Figure 4). Data were fit with a single exponential function (*solid curve*). (*g*) Exponential fit shown in (*f*) gives a receptive field radius of 185 μm.

What are the implications of these results? First, both midget and parasol pathways can serve as a neural basis for the photopic luminosity function, at least in the retinal periphery. Second, no special circuitry is devoted to the L- and M-cone signals that adjusts their relative gain to produce a neural code for fixed photopic spectral sensitivity (e.g. Shapley 1995). This conclusion agrees with psychophysical measurements that show similar variability in spectral sensitivity across individuals that can also be directly correlated with the L- to M-cone ratio (Brainard et al 2000). Finally, the lack of cone-type specificity in setting the gain

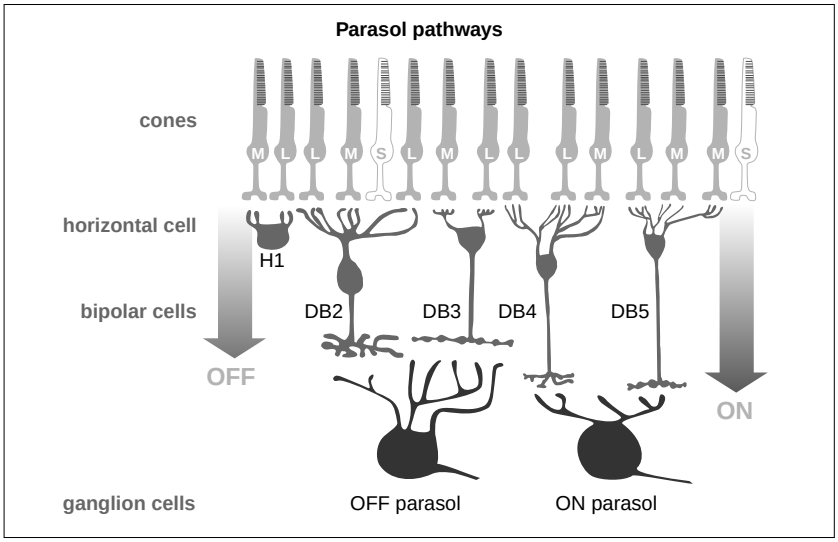

Figure 11 Schematic representation of identified cell types and circuitry of the L- and M-cone parasol pathways. ON and OFF pathway parasol populations are represented. Diffuse cone bipolar cells (DB2, DB3, DB4, DB5) contact L and M cones nonselectively and, like H1 cells, do not receive significant S-cone input. Diffuse cone bipolar cells show a nonopponent center-surround receptive field structure that is transmitted to the parasol cells. H1 horizontal cells contact L and M cones and are the probable basis for an inhibitory surround in diffuse cone bipolar cells.

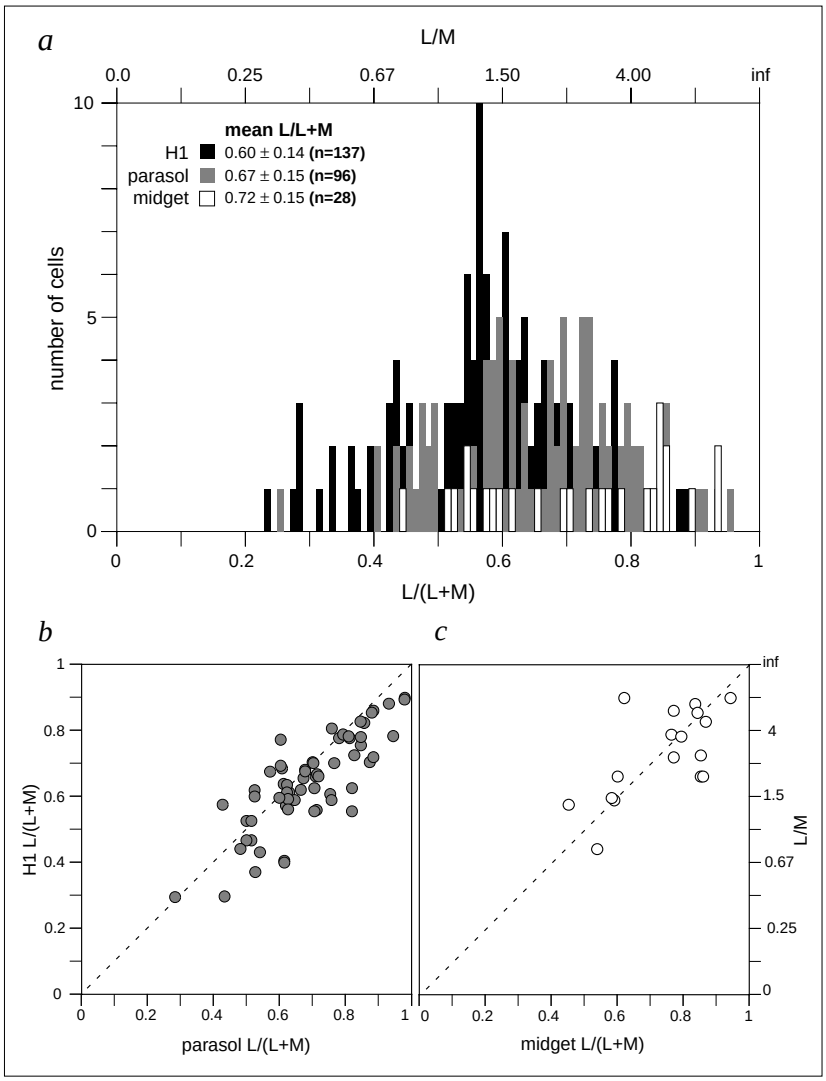

Figure 12 Relative strength of L- and M-cone input to H1 horizontal, parasol ganglion, and midget ganglion cells of the retinal periphery. Contrast gains for L- and M-cone signals were measured for stimuli that systematically varied L- and M-cone contrast. (*a*) Histogram of L- to M-cone gain ratio for H1 horizontal cells, parasol cells, and midget ganglion cells. L:M ratio is variable for all three cell populations. (*b–c*) For a subset of cells samples L:M ratio was measured for all three cell types in the same retina at the same retinal location. Scatter plots show that L:M gains are closely matched for overlapping H1 horizontal cells and parasol or midget ganglion cells. These and other data discussed in text indicate that variability in the relative numbers of L and M cones can explain the physiological L:M variability.

of the L- and M-cone signals in the achromatic midget and parasol cells reinforces the conclusion that L- and M-cone opponency in central vision is not based on any circuitry in which L or M cones make selective connections with bipolar or horizontal cell interneurons.

EVOLUTION OF PARALLEL SPECTRAL CODING PATHWAYS

The strong dichotomy between the S-cone and the L- and M-cone signal pathways fits well with the unified view that primate color vision is a composite of two distinct and well-separated adaptations during vertebrate evolution (Mollon 1989, Mollon et al 1990). All nonprimate mammals possess only two cone types—the S cone and a single long-wavelength-sensitive cone type—and consequently would be expected to show dichromatic color vision based on a comparison of the signals from these two types (for a review, see Jacobs 1993). A blue-ON ganglion cell light response has been identified in both cat (Daw & Pearlman 1969) and rabbit retina (Caldwell & Daw 1978). The distinctive bistratified morphology and circuitry of the blue-ON cell has also been observed in some dichromatic New World primate species (Yamada et al 1996, Ghosh et al 1997). It therefore seems likely that S-cone-pathway cell types represent a phylogenetically older pathway for color vision present in a mammalian ancestor and conserved in the primate lineage. By contrast, the presence of red-green opponency in the midget ganglion cell pathway must have arisen recently along with the appearance of L- and M-cone photopigments in Old World primates. In this scenario the appearance of distinct L- and M-cone photopigments, coincident with the presence of a fovea and a private-line midget circuit that evolved for the purpose of increased spatial resolution, are the necessary ingredients for L- and M-cone opponency. Thus trichromacy appears to be a very recent acquisition among the mammals and is mostly restricted to the Old World monkeys, although exceptions exist in foveate New World species (Jacobs et al 1996), great apes, and humans.

SUMMARY AND CONCLUSION

The mammalian retina contains on the order of 80 distinct cell types—each type recognized by a distinctive morphology, physiology, spatial distribution, and synaptic organization. The diverse cell types are used to build parallel pathways that originate at the first synaptic step in vision between the photoreceptors and bipolar cell interneurons. In trichromatic primates, signals from L-, M-, and S-cone types are combined to create red-green and blue-yellow spectrally opponent pathways. One blue-yellow pathway is built around a distinctive bistratified ganglion cell type and associated interneurons that selectively connect to S cones and generate

a spatially coextensive S-cone excitatory, (L + M)-cone inhibitory receptive field. This pathway is present in both central and peripheral retina, is probably characteristic of most mammals, and can be viewed as a primitive mammalian color-coding circuit. By contrast, the red-green pathway is restricted to central retina and arises by the union of two primate specializations: the unique midget cell architecture of the fovea, evolved first to permit high spatial resolution, and the more recent evolution of separate L- and M-cone photopigments in Old World species. The private-line pathway from a single cone to a midget ganglion cell in the fovea permits the segregation of L- and M-cone signals required for red-green opponency. Outside the fovea, midget ganglion cells increase in dendritic field size, combining inputs from multiple L and M cones, and show an achromatic spectral sensitivity. Cell types of the midget and parasol pathways that combine L- and M-cone inputs additively show a variability in the relative strengths of L- and M-cone input to the receptive field that is given directly by underlying variability in the cone ratios themselves.

ACKNOWLEDGMENTS

The author's work is supported by grants from the National Eye Institute (R01-EY06678, R01-EY09625) and by grants to the Regional Primate Research Center from the National Institutes of Health (RR00166). Technical assistance was provided by Toni Haun, Keith Boro, and Judy Johnson. The author would like to thank his collaborators—Barry Lee, Joel Pokorny, Vivianne Smith, David Williams, David Brainard, Jan Verweij, Lisa Diller, Beth Peterson, and Orin Packer—for their many critical contributions to studies on the primate retina in vitro described in this review.

LITERATURE CITED

Ahnelt P, Keri C, Kolb H. 1990. Identification of pedicles of putative blue-sensitive cones in the human retina. *J. Comp. Neurol.* 293: 39–53

Ahnelt P, Kolb H. 1994. Horizontal cells and cone photoreceptors in primate retina: a golgi-light microscopic study of spectral connectivity. *J. Comp. Neurol.* 343:387–405

Baylor DA, Nunn BJ, Schnapf JL. 1987. Spectral sensitivity of cones of the monkey *Macaca fascicularis. J. Physiol.* 390:145–60

Boycott BB, Wässle H. 1999. Parallel processing in the mammalian retina. *Invest. Ophthalmol. Vis. Sci.* 40:1313–27

Brainard DH, Roorda A, Yamauchi Y, Calderone JB, Metha A, et al. 2000. Functional consequences of the variation in relative numbers of L and M cones. *J. Opt. Soc. Am. A.* In press

Caldwell JH, Daw NW. 1978. New properties of rabbit retinal ganglion cells. *J. Physiol.* 276:257–76

Calkins DJ. 1999. Synaptic organization of cone pathways in the primate retina. See Gegenfurtner & Sharpe 1999, pp. 163–80

Calkins DJ, Schein SJ, Tsukamoto Y, Sterling P. 1994. M and L cones in macaque fovea connect to midget ganglion cells by different numbers of excitatory synapses. *Nature* 371:70–2

Calkins DJ, Sterling P. 1996. Absence of spectrally specific lateral inputs to midget ganglion cells in primate retina. *Nature* 381: 613–15

Calkins DJ, Tsukamoto Y, Sterling P. 1998. Microcircuitry and mosaic of a blue-yellow ganglion cell in the primate retina. *J. Neurosci.* 18:3373–85

Cottaris N, DeValois R. 1998. Temporal dynamics of chromatic tuning in macaque primary visual cortex. *Nature* 395:896–900

Curcio CA, Allen KA, Sloan KR, Lerea CL, Hurley JB, et al. 1991. Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *J. Comp. Neurol.* 312:610–24

Dacey DM. 1993a. Morphology of a small-field bistratified ganglion cell type in the macaque and human retina. *Vis. Neurosci.* 10:1081–98

Dacey DM. 1993b. The mosaic of midget ganglion cells in the human retina. *J. Neurosci.* 13:5334–55

Dacey DM. 1996. Circuitry for color coding in the primate retina. *Proc. Natl. Acad. Sci. USA* 93:582–88

Dacey DM. 1999. Primate retina: cell types, circuits and color opponency. *Prog. Retin. Eye Res.* 18(6):737–63

Dacey DM, Diller LC, Verweij J, Williams DR. 2000a. Physiology of L- and M-cone inputs to H1 horizontal cells in macaque monkey retina. *J. Opt. Soc. Am. A.* In press

Dacey DM, Packer OS, Diller LC, Brainard DH, Peterson BB, Lee BB. 2000b. Center surround receptive field structure of cone bipolar cells in primate retina. *Vis. Res.* In press

Dacey DM, Lee BB. 1994. The blue-ON opponent pathway in primate retina originates from a distinct bistratified ganglion cell type. *Nature* 367:731–35

Dacey DM, Lee BB. 1997. Cone inputs to the receptive field of midget ganglion cells in the periphery of macaque retina. *Invest. Ophthal. Vis. Sci. Suppl.* 38:S708 (Abstr.)

Dacey DM, Lee BB. 1999. Functional architecture of cone signal pathways in primate retina. See Gegenfurtner & Sharpe 1999, pp. 181–202

Dacey DM, Lee BB, Stafford DK, Pokorny J, Smith VC. 1996. Horizontal cells of the primate retina: cone specificity without spectral opponency. *Science* 271:656–59

Dacey DM, Petersen MR. 1992. Dendritic field size and morphology of midget and parasol ganglion cells of the human retina. *Proc. Natl. Acad. Sci. USA* 89:9666–70

Daw NW, Pearlman AL. 1969. Cat colour vision one cone process or several. *J. Physiol.* 201:745–64

Deeb SS, Diller LC, Williams DR, Dacey DM. 2000. Interindividual and topographical variations in red and green cone ratios in monkey retinae. *J. Opt. Soc. Am. A.* In press

de Monasterio FM. 1978. Center and surround mechanisms of opponent-color X and Y ganglion cells of retina of macaques. *J. Neurophysiol.* 41(6):1418–34

de Monasterio FM. 1979. Asymmetry of ON- and OFF-pathways of blue-sensitive cones of the retina of macaques. *Brain Res.* 166: 39–48

de Monasterio FM, Gouras P. 1975. Functional properties of ganglion cells of the rhesus monkey retina. *J. Physiol.* 251: 167–95

de Monasterio FM, Gouras P, Tolhurst DJ. 1975a. Concealed colour opponency in ganglion cells of the rhesus monkey retina. *J. Physiol.* 251:217–29

de Monasterio FM, Gouras P, Tolhurst DJ. 1975b. Trichromatic color opponency in ganglion cells of the rhesus monkey retina. *J. Physiol.* 251:197–216

Derrington AM, Krauskopf J, Lennie P. 1984. Chromatic mechanisms in lateral geniculate nucleus of macaque. *J. Physiol.* 357:241–65

Derrington AM, Lennie P. 1984. Spatial and

temporal contrast sensitivities of neurones in lateral geniculate nucleus of macaque. *J. Physiol.* 357:219–40

DeValois RL, Abramov I, Jacobs GH. 1966. Analysis of response patterns of LGN cells. *J. Opt. Soc. Am.* 56(7):966–77

DeValois RL, DeValois KK. 1993. A multi-stage color model. *Vision Res.* 33:1053–65

Diller LC, Verweij J, Williams DR, Dacey DM. 1999. L and M cone inputs to peripheral parasol and midget ganglion cells in primate retina. *Invest. Ophthalmol. Vis. Sci. Suppl.* 40:S817 (Abstr.)

Dulai KS, Bowmaker JK, Mollon JD, Hunt DM. 1994. Sequence divergence, polymorphism and evolution of the middle-wave and long-wave visual pigment genes of great apes and Old World monkeys. *Vis. Res.* 34(19):2483–91

Gegenfurtner KR, Sharpe LT, eds. 1999. *Color Vision: From Genes to Perception.* Cambridge, UK: Cambridge Univ. Press.

Ghosh KK, Martin PR, Grünert U. 1997. Morphological analysis of the blue cone pathway in the retina of a new world monkey, the marmoset *Callithrix Jacchus. J. Comp. Neurol.* 379:211–25

Goodchild AK, Chan TL, Grünert U. 1996. Horizontal cell connections with short wavelength sensitive cones in macaque monkey retina. *Vis. Neurosci.* 13:833–45

Gouras P. 1968. Identification of cone mechanisms in monkey ganglion cells. *J. Physiol.* 199:533–47

Gouras P, Zrenner E. 1981. Colour vision: a review from a neurophysiological perspective. In *Progress in Sensory Physiology*, ed. D Ottoson, 1:139–79. Berlin/New York: Springer

Hagstrom SA, Neitz J, Neitz M. 1998. Variations in cone populations for red-green color vision examined by analysis of mRNA. *NeuroReport* 9:1963–67

Helmholtz H. 1924. *Physiological Optics.* Rochester, NY: The Optical Society of America

Hendry SHC, Calkins DJ. 1998. Neuronal chemistry and functional organization in the primate visual system. *Trends Neurosci.* 21:344–49

Hendry SHC, Yoshioka T. 1994. A neurochemically distinct third channel in the macaque dorsal lateral geniculate nucleus. *Science* 264:575–77

Jacobs GH. 1993. The distribution and nature of colour vision among the mammals. *Biol. Rev. Cambridge Philos. Soc.* 68:413–71

Jacobs GH, Neitz M, Deegan JFI, Neitz J. 1996. Trichromatic colour vision in New World monkeys. *Nature* 382:156–58

Jacoby R, Stafford D, Kouyama N, Marshak D. 1996. Synaptic inputs to ON parasol ganglion cells in the primate retina. *J. Neurosci.* 16:8041–56

Kaplan E, Lee BB, Shapley RM. 1990. New views of primate retinal function. *Prog. Retin. Eye Res.* 9:273–336

Klug K, Tiv N, Tsukamoto Y, Sterling P, Schein S. 1992. Blue cones contact OFF-midget bipolar cells. *Soc. Neurosci. Abstr.* 18:838

Klug K, Tsukamoto Y, Sterling P, Schein S. 1993a. Blue cone OFF-midget ganglion cells in Macaque. *Invest. Ophthalmol. Vis. Sci. Suppl.* 34:986 (Abstr.)

Kolb H. 1991. Anatomical pathways for color vision in the human retina. *Vis. Neurosci.* 7:61–74

Kolb H, Dekorver L. 1991. Midget ganglion cells of the parafovea of the human retina: a study by electron microscopy and serial section reconstructions. *J. Comp. Neurol.* 303:617–36

Kolb H, Linberg KA, Fisher SK. 1992. Neurons of the human retina: a Golgi study. *J. Comp. Neurol.* 318:147–87

Kouyama N, Marshak DW. 1992. Bipolar cells specific for blue cones in the macaque retina. *J. Neurosci.* 12:1233–52

Krauskopf J, Williams DR, Heeley DW. 1982. Cardinal directions of color space. *Vis. Res.* 22:1123–31

Lankheet MJM, Lennie P, Krauskopf J. 1998a. Distinctive characteristics of subclasses of red-green P-cells in LGN of macaque. *Vis. Neurosci.* 15:37–46

Lankheet MJM, Lennie P, Krauskopf J. 1998b. Temporal-chromatic interactions in LGN P-cells. *Vis. Neurosci.* 15:47–54

Lee BB. 1996. Receptive field structure in the primate retina. *Vis. Res.* 36:631–44

Lee BB. 1999. Receptor inputs to primate ganglion cells. See Gegenfurtner & Sharpe 1999, pp. 203–17

Lee BB, Kremers J, Yeh T. 1998. Receptive fields of primate retinal ganglion cells studied with a novel technique. *Vis. Neurosci.* 15:161–75

Lee BB, Martin PR, Valberg A. 1988. The physiological basis of heterochromatic flicker photometry demonstrated in the ganglion cells of the macaque retina. *J. Physiol.* 404:323–47

Lee BB, Martin PR, Valberg A. 1989. Sensitivity of macaque retinal ganglion cells to chromatic and luminance flicker. *J. Physiol.* 414:223–43

Lee BB, Pokorny J, Smith VC, Martin PR, Valberg A. 1990. Luminance and chromatic modulation sensitivity of macaque ganglion cells and human observers. *J. Opt. Soc. Am. A* 7(12):2223–36

Lee BB, Valberg A, Tigwell DA, Tryti J. 1987. An account of responses of spectrally opponent neurons in macaque lateral geniculate nucleus to successive contrast. *Proc. R. Soc. London B Biol. Sci.* 230:293–314

Lennie P, D'Zmura M. 1988. Mechanisms of color vision. *Crit. Rev. Neurobiol.* 3: 333–400

Lennie P, Haake PW, Williams DR. 1991. The design of chromatically opponent receptive fields. In *Computational Models of Visual Processing*, ed. MS Landy, JA Movshon, pp. 71–82. Cambridge, MA: MIT Press

Lennie P, Pokorny J, Smith VC. 1993. Luminance. *J. Opt. Soc. Am. A* 10(6):1283–93

Leventhal AG, Rodieck RW, Dreher B. 1981. Retinal ganglion cell classes in the old world monkey: morphology and central projections. *Science* 213:1139–42

Liang J, Williams DR, Miller DT. 1997. Supernormal vision and high resolution retinal imaging through adaptive optics. *J. Opt. Soc. Am. A* 14:2884–92

MacNeil MA, Masland RH. 1998. Extreme diversity among amacrine cells: implications for function. *Neuron* 20:971–82

Mangel SC, Miller RF. 1987. Horizontal cells contribute to the receptive field surround of ganglion cells in the rabbit retina. *Brain Res.* 414:182–86

Martin PR. 1998. Colour processing in the primate retina: recent progress. *J. Physiol.* 513:631–38

Martin PR, White AJR, Goodchild AK, Wilder HD, Sefton AE. 1997. Evidence that blue-ON cells are part of the third geniculocortical pathway in primates. *Eur. J. Neurosci.* 9:1536–41

Masland RH. 1996a. Processing and encoding of visual information in the retina. *Curr. Opin. Neurobiol.* 6:467–74

Masland RH. 1996b. Unscrambling color vision. *Science* 271:616–17

McMahon M, Lankheet MJM, Lennie P, Williams DR. 2000. Fine structure of primate parvocellular receptive fields in the fovea revealed by laser interferometry. *J. Neurosci.* In press

Merigan WH. 1989. Chromatic and achromatic vision of macaques: role of the P pathway. *J. Neurosci.* 9:776–83

Milam AH, Dacey DM, Dizhoor AM. 1993. Recoverin immunoreactivity in mammalian cone bipolar cells. *Vis. Neurosci.* 10:1–12

Mollon JD. 1989. The uses and origins of primate colour vision. *J. Exp. Biol.* 146:21–38

Mollon JD, Bowmaker JK. 1992. The spatial arrangement of cones in the primate fovea. *Nature* 360:677–79

Mollon JD, Estévez O, Cavonius CR. 1990. The two subsystems of colour vision and their roles in wavelength discrimination. In *Vision: Coding and Efficiency*, ed. C Blakemore, pp. 119–31. Cambridge, UK: Cambridge Univ. Press

Mullen KT. 1991. Colour vision as a post-receptoral specialization of the central visual field. *Vis. Res.* 31:119–30

Mullen KT, Kingdom FAA. 1996. Losses in peripheral colour sensitivity predicted from "hit and miss" post-receptoral cone connections. *Vis. Res.* 36:1995–2000

Mullen KT, Kingdom FAA. 1999. Different postreceptoral limits on red-green and blue-yellow contrast sensitivity. *Invest. Ophthalmol. Vis. Sci. Suppl.* 40:S176 (Abstr.)

Nathans J, Piantanida TP, Eddy RL, Shows TB. 1986a. Molecular genetics of inherited variation in human color vision. *Science* 232:203–10

Nathans J, Thomas D, Hogness DS. 1986b. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science* 232:193–232

Paulus W, Kröger-Paulus A. 1983. A new concept of retinal colour coding. *Vis. Res.* 23: 529–40

Perry VH, Oehler R, Cowey A. 1984. Retinal ganglion cells that project to the dorsal lateral geniculate nucleus in the macaque monkey. *Neuroscience* 12(4):1101–23

Peterson BB, Dacey DM. 1997. Morphology of human retinal ganglion cells with intraretinal axon collaterals. *Soc. Neurosci. Abstr.* 23:728

Peterson BB, Dacey DM. 1998. Morphology of human retinal ganglion cells with intraretinal axon collaterals. *Vis. Neurosci.* 15: 377–87

Polyak S. 1941. *The Retina.* Chicago: Univ. Chicago Press

Ramon y Cajal S. 1892. La rètine des vertèbrès. *La Cellule* 9:119–257

Reid RC, Shapley RM. 1992. Spatial structure of cone inputs to receptive fields in primate lateral geniculate nucleus. *Nature* 356:716–18

Rodieck RW. 1991. Which cells code for color? In *From Pigments to Perception: Advances in Understanding Visual Processes,* ed. A Valberg, BB Lee, pp. 83–93. London: Plenum

Rodieck RW. 1998. *The First Steps in Seeing.* Sunderland, MA: Sinauer

Rodieck RW, Watanabe M. 1993. Survey of the morphology of macaque retinal ganglion cells that project to the pretectum, superior colliculus, and parvicellular laminae of the lateral geniculate nucleus. *J. Comp. Neurol.* 338:289–303

Roorda A, Williams DR. 1999. The arrangement of the three cone classes in the living human eye. *Nature* 397:520–22

Schiller PH, Logothetis NK, Charles ER. 1990. Role of the color-opponent and broad-band channels in vision. *Vis. Neurosci.* 5:321–46

Schnapf JL, Kraft TW, Baylor DA. 1987. Spectral sensitivity of human cone photoreceptors. *Nature* 325:439–41

Schnapf JL, Kraft TW, Nunn BJ, Baylor DA. 1988. Spectral sensitivity of primate photoreceptors. *Vis. Neurosci.* 1:255–61

Shapley R. 1995. Parallel neural pathways and visual function. In *The Cognitive Neurosciences,* ed. MS Gazzaniga, E Bizzi, IB Black, C Blakemore, L Cosmides, et al, pp. 315–24. Cambridge, MA: MIT Press

Shapley R, Perry VH. 1986. Cat and monkey retinal ganglion cells and their visual functional roles. *Trends Neurosci.* 9:229–35

Slaughter M, Miller RF. 1981. 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retinal research. *Science* 211:182–85

Smith VC, Lee BB, Pokorny J, Martin PR, Valberg A. 1992. Responses of macaque ganglion cells to the relative phase of heterochromatically modulated lights. *J. Physiol.* 458:191–221

Ts'o DY, Gilbert CD. 1988. The organization of chromatic and spatial interactions in the primate striate cortex. *J. Neurosci.* 8(5): 1712–27

Tsukamoto Y, Masarachia P, Schein SJ, Sterling P. 1992. Gap junctions between the pedicles of macaque foveal cones. *Vis. Res.* 32:1809–15

Valberg A, Lee BB, Tigwell DA. 1986. Neurones with strong inhibitory S-cone inputs in the macaque lateral geniculate nucleus. *Vis. Res.* 26(7):1061–64

Vaney DI. 1990. The mosaic of amacrine cells in the mammalian retina. *Prog. Retin. Eye Res.* 9:49–100

Wässle H, Boycott BB. 1991. Functional architecture of the mammalian retina. *Physiol. Rev.* 71:447–80

Watanabe M, Rodieck RW. 1989. Parasol and midget ganglion cells of the primate retina. *J. Comp. Neurol.* 289:434–54

Werblin FS, Dowling JE. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus.* II. Intracellular recording. *J. Neurophysiol.* 32:339–55

White AJR, Wilder HD, Goodchild AK, Sefton AJ, Martin PR. 1998. Segregation of receptive field properties in the lateral geniculate nucleus of a new-world monkey, the marmoset *Callithrix jacchus. J. Neurophysiol.* 80:2063–76

Wiesel TN, Hubel DH. 1966. Spatial and chromatic interactions in the lateral geniculate body of the rhesus monkey. *J. Neurophysiol.* 29:1115–56

Yamada ES, Silveira LCL, Perry VH. 1996. Morphology, dendritic field size, somal size, density, and coverage of M and P retinal ganglion cells of dichromatic *Cebus* monkeys. *Vis. Neurosci.* 13:1011–29

Young T. 1802. On the theory of light and colours. *Philos. Trans. R. Soc.* 92:12–48

Annual Review of Neuroscience
Volume 23, 2000

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