Primate Retina: Cell Types, Circuits and Color Opponency

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Abstract—The link between morphology and physiology for some of the cell types of the macaque monkey retina is reviewed with emphasis on understanding the neural mechanism for spectral opponency in the light response of ganglion cells. An in vitro preparation of the retina is used in which morphologically identified cell types are selectively targeted for intracellular recording and staining under microscopic control. The goal is to trace the physiological signals from the long...
1. INTRODUCTION

1.1. Primate Retinal Cell Types

The mammalian retina displays a diversity of cell types and functionally distinct synaptic pathways (Masland, 1996). Much of this anatomical diversity has been recently summarized (Rodieck, 1988; Sterling, 1990; Vaney, 1990): at least 2 horizontal cell and 10 bipolar cell populations transmit signals in parallel from photoreceptors to ganglion cells. In turn, the ganglion cells further subdivide into an estimated 20–25 anatomically distinct populations that project in parallel to about a dozen target structures in the midbrain and thalamus. Still more complex, the link between ganglion cells and bipolar cells is modulated by the amacrine cell types. To a first approximation there appears to be a number of distinct amacrine cell types dedicated to a given bipolar–ganglion cell pathway, with the total number of amacrine cell populations currently estimated to be at least 40 (Vaney, 1990; Wässle and Boycott, 1991). Thus the “retinal circuit” is not a single circuit but many “microcircuits”, comprising on the order of 80 neural cell types. One purpose of these multiple microcircuits is to create the characteristic physiological properties of the parallel visual pathways that link the retina to a diverse set of target structures in the brainstem. A challenge then, is to systematically characterize retinal cell types with the goal of clarifying the structure and function of the circuitry dedicated to each of the central visual pathways.

1.2. Microcircuits for Color Opponency

The neural code for color begins in the retina. The voltage response of a photoreceptor initiated by the absorption of a photon is independent of the wavelength of that photon. The probability that a photon is absorbed is thus a function of both wavelength and the density of photons incident on the photoreceptor. In the first step toward the generation of chromatic signals the wavelength independent responses of the long, middle and short wavelength sensitive cone types are transformed into spectrally opponent responses of certain retinal ganglion cells (see review in Kaplan et al., 1990). The diversity of cell types of the trichromatic primate retina raises a question as to which cell types display spectral opponency or other physiological properties (such as input from only a single cone type) that would suggest a critical role in an opponent transformation. My goal in this chapter is to briefly review what is currently known of the physiology of morphologically identified cell types in the macaque monkey retina with reference to the generation of color-related signals. The classical approach to this problem has been to combine intracellular recording and cell staining methods to reveal the morphology of a cell whose response to light has been observed (e.g., Dacheux and Raviola, 1990). Recently we have applied this basic technique to an in vitro preparation of the macaque retina. In a modification of this method identified cell types are targeted for intracellular recording and staining under direct microscopic control (Yang and Masland, 1994). Before
reviewing the results some critical aspects of the method are outlined in the next section.

2. METHODS

2.1. In Vitro Retina–choroid Preparation of Macaque Retina

Intracellular techniques for directly relating morphology to physiology were first applied with limited success to the intact eye of the anesthetized macaque monkey (DeMonasterio, 1979; Zrenner et al., 1983) (see further discussion in Section 5; ganglion cells). A superfused eyecup preparation was later used to make the first intracellular recordings from the non-spiking, H1 horizontal cell type (Dacheux and Raviola, 1990). This approach was also of limited value because the in vitro eyecup was physiologically viable for only a few hours. More successfully, an isolated in vitro retina, has now been used to make whole cell patch electrode recordings of rod and cone photoreceptor light responses (Schneeweis and Schnapf, 1995). In a variant of this latter approach we have developed an in vitro preparation of the macaque retina in which the retina, retinal pigment epithelium (RPE) and choroid layers are dissected intact from the eyecup and mounted flat in a superfusion chamber (Dacey and Lee, 1994) (Fig. 1A). A significant advantage of this dissection is that the anatomical relationship of the photoreceptors to the RPE is undisturbed—since the reactions of the visual cycle that return all-trans retinol to the photosensitive 11-cis configuration take place in the RPE, the retina–RPE–choroid can be maintained even at high photopic levels and retains the ability to regenerate photopigment in vitro.

A second advantage of the retina–RPE–choroid preparation is that retinal cell types can be directly observed and targeted for intracellular recording under microscopic control. The recording chamber is mounted on the stage of a light microscope and retinal cells are selectively stained with a variety of vital fluorescent markers and observed with high resolution water immersion optics and episcopic illumination. Because photopigment is regenerated the retinal cells retain normal light responses after targeting. Figure 1B shows cell bodies in the ganglion cell layer of the macaque retina in vitro stained with the dye acridine orange (observed as they would be through the microscope) prior to selecting a particular cell for intracellular recording.
To deliver visual stimuli to the *in vitro* retina, a small optic bench is mounted above the microscope. Light passes down through the camera port and the optics of the microscope are used to bring the light into focus as a small spot on the retinal surface. Red, green and blue light emitting diodes (LEDs) with dominant wavelengths of 638, 554 and 445 nm respectively, serve as light sources. The output of each LED is an independently modulated waveform (square or sine) that can be adjusted as to radiance, temporal frequency, phase or modulation depth (Swanson *et al.*, 1987). The size and shape of a given stimulus can be adjusted by passing the light through slits, annuli or apertures of different sizes—the position of this stimulus relative to the receptive field center of a cell can be further controlled with a motorized *x*–*y* positioner mounted on the microscope camera port.

### 2.2. Heterochromatic Modulation Photometry

Stimulus protocols were generated to address two related questions: (1) what are the cone inputs to the recorded cell, and (2) which cells show red–green or blue–yellow spectral opponency? The psychophysical paradigm of heterochromatic modulation photometry (HMP) was used to identify red–green spectral opponency in macaque retinal cells (Pokorny *et al.*, 1989). In this variant of heterochromatic flicker photometry (HFP) a pair of chromatic lights are presented in temporal alternation. In HFP, the relative luminances of the two lights are varied until the perception of flicker is minimized or eliminated—at this point the two lights are considered equal in luminance. The spectral sensitivity function (*V*<sub>L</sub>) that derives from such a method is consistent with a postreceptoral process that sums input from L- and M-cones and receives little or no S-cone contribution. In HMP the mean luminance of the two lights is held constant but the modulation amplitude around the mean is varied to acquire a threshold for flicker detection. An advantage of HMP over conventional flicker photometry is that mean luminance and chromaticity (and presumably the relative state of adaptation of the L- and M-cones) remain constant. To study the spectral sensitivity of retinal neurons using HMP, equiluminance for the red and green LEDs was first determined by obtaining a perceived flicker minimum for human subjects observing the LEDs as they are projected onto the retinal surface. The dominant wavelengths of the red and green LEDs are such that excitation of the S-cone is negligible. A human observer adjusts the luminances of the LEDs until the perception of flicker is minimized and this setting is taken as the human equiluminant point. The equiluminant setting used in these experiments were also checked radiometrically and corresponded closely to that of the standard observer (Lennie *et al.*, 1993). The relative modulation depth of the LEDs is then adjusted to produce a series of stimuli that vary in the degree to which the L- and M-cones are modulated (Fig. 2). The relative stimulation of the L- and M-cones is calculated from the Smith–Pokorny cone fundamentals (Smith and Pokorny, 1975) given a mean retinal illuminance estimated at 2000 trolands and the spectral luminous-efficiency function of the Judd standard observer. To illustrate the HMP protocol Fig. 2 shows the voltage responses of a recorded H1 horizontal cell to one stimulus series (More detail on the light response and cone input to macaque horizontal cells is given in section. (3)). Note that the neuron gives a hyperpolarizing light response to stimuli which modulate the L- and M-cones in isolation, and that there is a response null in between these two points. These cells thus receive additive input from both L- and M-cones. The response minimum is interpreted as the point at which the strength of the L-cone + M-cone signals are equal for both lights, so as to cancel with counterphase modulation. By contrast, a neuron that receives antagonistic input from L- and M-cones will show a large response to red–green chromatic modulation since L- and M-cones will be modulated in phase and combine additively.

### 2.3. Selective Modulation of S-Cone Signals

To determine whether a given retinal neuron receives S-cone input the method of silent substitution was also used to generate an S-cone isolat-
Fig. 2. Characterization of spectral sensitivity using the paradigm of heterochromatic modulation photometry. (A) Red (dominant wavelength: 638 nm) and green (dominant wavelength: 553 nm) are modulated in counterphase; relative modulation depths and of LEDs are varied. 100% R, 100% G point corresponds to equiluminance for the nominal human observer with the sensitivity of the spectral-luminosity function, V(λ). (B and C) relative L- and M-cone modulations in response to LEDs; L-cone is modulated in phase with the red LED, M-cone modulates in phase with the green LED; silent-L and silent-M conditions are indicated. (D) Predicted response of an L–M opponent neuron with equally weighted L- and M-cone inputs. Note that silent-L and silent-M conditions elicit responses of the same phase (i.e., ON-response to M-cone modulation and OFF-response to L-cone modulation). (E) Predicted response of an L + M non-opponent neuron. Response null is expected at equiluminance; silent-L and silent-M conditions elicit responses in counterphase (i.e., ON-response to M-cone modulation and ON-response to L-cone modulation). (F) Voltage response of an H1 horizontal cell to this set of stimuli; cell responds in phase to both L and M cone modulation with a response minimum near the equiluminant point.
ing stimulus. To silence both the L- and M-cones the blue LED is first adjusted to be equal in luminance to the red and green LEDs by flicker photometry as described above. The blue LED is then modulated in phase with the red diode and counterphase to the green diode. Relative modulation depths for the three diodes are then adjusted to silence L- and M-cones leaving a remaining S-cone contrast of ~85% (Lee and Yeh, 1995).

The blue LED and, to a lesser extent, the green LED will also strongly modulate rod photoreceptors and have been used to study rod input to certain retinal cell types under dark adapted conditions (Verweij et al., 1997). For the results discussed in this paper, rod signals were saturated by exposures to high photopic levels and did not normally contribute to the light responses recorded with our photopic stimuli, though rod signals could be easily recorded after periods of dark adaption.

3. HORIZONTAL CELLS

3.1. History: a Role in Color Coding?

Horizontal cells are the lateral interneurons of the outer retina; their dendritic processes innervate the axon terminals of the photoreceptors and form the lateral elements of the synaptic triad. Horizontal cells of a given type are electrically coupled to one another by gap junctions and form a widespreading electrical syncytium; they provide a negative feedback signal to photoreceptors and play an important role in the generation of receptive field surrounds in bipolar cells and ganglion cells either via the feedback pathway and/or by a direct feedforward connection to bipolar cell dendrites.

Intracellular recordings from horizontal cell types in teleost retina (Svaetichin and MacNichol, 1958) provided initial evidence that there could be a simple physiological basis for the opponent process stage in human color vision (Hurvich and Jameson, 1957). The underlying mechanisms for the spectral opponent responses found in fish horizontal cells, and their functions in the vision of fishes is still not fully understood (Burkhardt, 1993; Kamermans and Spekreijse, 1995), although results in fish pointed to the horizontal cell networks in the trichromatic primate retina as a possible locus for an opponent transformation.

In primates, including man, the anatomy of horizontal cells and speculation about their function has been controversial. Boycott and Wässle concluded that in macaque monkey, the dendritic terminals of two distinctive horizontal cell types, the H1 and H2 cells, made non-selective contact with all cone types (Wässle et al., 1989). It was suggested that in primates the horizontal cells might sum inputs from all cones and play no role in cone type-specific spectral opponency. The first intracellular recordings of the H1 cell light response supported this interpretation of the anatomy (Dacheux and Raviola, 1990). By contrast, other anatomical data, principally from human retina, indicated that the primate horizontal cells could make preferential connections with cones. Golgi stained H2 cells made increased contact with S-cones, H1 cells connected to all three cone types indiscriminately, and a third horizontal cell type (H3) was identified that was nearly identical in morphology to the H1 cell but avoided contacting S-cones (Ahnelt and Kolb, 1994b). From this data the possibility was considered that spectral opponency, like that present in fish horizontal cells, might be found in primate if one looked carefully enough.

3.2. Cone Type-Specific Connections Without Color Opponency for H1 and H2 Cells

The in vitro preparation permitted a fresh look at the cone connections and light responses of macaque horizontal cells (Dacey et al., 1996). To selectively target H1 and H2 cells for recording in vitro we labeled them with the nuclear stain, diaminophenylindole (DAPI), which had been used previously to stain various retinal cell types in other mammals (e.g. Tauchi and Masland, 1984; Mills and Massey, 1992). Two populations could be distinguished based on differences in nuclear size and staining intensity. An orderly mosaic of large, brightly stained cells corresponded to the H1 population and a less regular, lower density
array of smaller, less brightly labelled nuclei belonged to the H2 cell population. When the low molecular weight tracer Neurobiotin was intracellularly injected into the horizontal cells it passed through the gap junctions that couple cells of the same type so that a local patch of either the H1 or H2 mosaic was revealed (Figs 3F and 4F).

Fig. 3. Physiology and cone connectivity of the H1 horizontal cells. (A) Light response of H1 cell network to square wave luminance modulation (red and green LEDs in phase). (B) Response minimum to heterochromatic modulation (red and green LEDs in counterphase modulation—green: 0.7, red: 1.0). (C) Response to silent-L-cone condition (M-cone modulation in phase with green LED). (D) Response to silent-M-cone condition (L-cone modulated in phase with red LED). (E) Lack of response to selective modulation of S-cone. Blue LED is added (thicker gray trace) in phase with red LED (see Section 2.3). (F) Camera lucida tracing of the H1 cell network revealed by intracellular injection of Neurobiotin. H1 cell bodies and dendritic processes are shown by stippling—white “holes” in the gray background indicate approximate position of cone axon terminals (pedicles). Majority of cones are densely innervated by dendritic terminals. Three cones in this field (~10%) lack innervation and show the spacing and density of S-cones. (G) Plot of responsivity as a function of modulation depth of red and green LEDs. Silent-M- and silent-L-cone conditions are indicated by arrows. Equiluminance for the nominal observer (indicated by the asterisk) corresponds to a red/red + green ratio of 0.5. Temporal modulation 2.44 Hz—luminance estimated at 2000 trolands.
H1 cells hyperpolarize to light across the spectrum and show a spectral sensitivity like that of the photopic luminosity function, strongly dominated by L- and M-cone input (Dacheux and Raviola, 1990). Two major questions remained unanswered. Firstly, does a second population of...
H1-like cells exist that show selectivity in the degree to which they contacted the three cone types, and if so, do these cells show spectral opponency? Secondly, what is the nature of the light response of H2 cells? Do these cells receive a major physiological input from S-cones as some anatomical data had suggested? In recordings from now over 200 cells with the characteristic morphology of H1 cells, I have found a single characteristic response to cone isolating stimuli: H1 cells hyperpolarize to light that modulates either the L- or M-cone in isolation, but do not respond to S-cone isolating modulation (Fig. 3). The spectral sensitivity of the H1 cells as measured with the HMP protocol (Figs 2 and 3G) reflected additive input from L- and M-cones and showed a response minimum near the equiluminance. The spectral sensitivity as shown by the HMP data reinforces the anatomical observation that H1 cells draw input indiscriminately from L- and M-cones and show a spectral sensitivity like the photopic luminosity function, \(V_l\). Since \(V_l\) is well characterized by a 1.6 to 1 ratio in the relative number of the L- and M-cones (or the relative synaptic gain of the two cone types) the HMP data provides indirect evidence that, at least in the retinal periphery, the L- and M-cone mosaic is similarly organized in macaque and human retina. This is consistent with the same conclusion derived from analysis of the spectral sensitivity of M-ganglion cells (Lee et al., 1988), and is discussed further below.

An anatomical basis for the lack of response to S-cone stimulation was revealed in the anatomy of the connections of the H1 cell dendrites with cone axon terminals. The majority of cone pedicles were densely innervated by the H1 cells as previously observed but a small percentage of cones were “skipped” over. The density and spacing of these skipped cones indicated that they corresponded to the S-cones. This conclusion has recently been confirmed by directly labelling both the H1 cells and the S-cones with an S-cone opsin-specific antibody (Goodchild et al., 1996).

What of the anatomical distinction made between H1 cells, which were suggested to contact all cone types indiscriminately, and the “H3” cells which were suggested to avoid contact with S-cones (Ahnelt and Kolb, 1994a)? The large bodied cells that we have termed H1 show properties of both of the previously described H1 and H3 cells in that they have a non-opponent light response and a broad spectral sensitivity derived from additive L- and M-cone input (H1 cells) but they make little or no contact with S-cones (“H3” cells). There is now direct anatomical evidence that some H1 cells do make a reduced contact with a small percentage of S-cones (Goodchild et al., 1996). Thus we conclude that the previous distinction made between H1 and H3 cells does not reflect the existence of two anatomically and functionally distinct horizontal cell mosaics. Instead there is a single, electrically coupled mosaic of large bodied horizontal cells—H1—that show a greatly reduced connection to the S-cone mosaic, and this is reflected in the response of the overall network to our cone isolating stimuli.

As with the H1 cells, the H2 horizontal cells also receive hyperpolarizing input from both L- and M-cone—the null point for these cells in the HMP protocol was similar to that of the H1 cells (Fig. 4G) suggesting that the H2 cell network does not show any preference for selecting L- or M-cone input. Unlike the H1 cells however, the H2 cells also give a large response to the S-cone stimulus (Fig. 4A–E). The response to S-cone modulation however is also hyperpolarizing so that no spectral opponency is conferred by this additional cone input.

A particularly striking anatomical basis for the light response of the H2 network was discovered in its anatomical connections with the cones. The majority L- and M-cone population was innervated only sparsely—with a few dendritic terminals entering each pedicle. However the H2 cell dendrites also converged upon and densely innervated a sparse array of cones, with the expected spacing and density (~7% of the total) of the S-cones, the result being a near reverse image of the H1 network pattern of cone contacts. The identity of these densely innervated pedicles as S-cones has also been directly confirmed (Goodchild et al., 1996).

Even though the S-cones make up less than 10% of the cone population they appear to have a stronger anatomical, and correspondingly physiological, input to the H2 cells than either the L- or M-cone population. The significance of this S-
cone dominated horizontal cell pathway is not clear but it raises a problem for understanding the nature of the presumed feedback signal from horizontal cells to cones. Given that the H2 cell contacts L- and M-cones it would be expected that strong S-cone stimulation should have a depolarizing effect on the H1 cell via a feedback pathway, but this is not observed. The two horizontal cell types therefore may function in parallel with their output primarily feedforward to bipolar cell dendrites.

In sum, evidence is now strong that the primate, like other mammals, has two horizontal cell types; these types are capable of selectively avoiding or seeking out S-cone axon terminals. However, no selectivity is found for L- or M-cones and all cone inputs to both cell types are hyperpolarizing. Thus unlike the horizontal cells in certain non-mammalian retinas, primate horizontal cells do not show spectral opponency and cannot provide a simple mechanism for cone-type specific signals to the receptive field surround of inner retinal neurons—the bipolar, amacrine or ganglion cells.

4. BIPOLAR CELLS

4.1. History: Midget and Diffuse Bipolar Classes

The retinal bipolar cells convey photoreceptor signals to the amacrine and ganglion cells, yet despite this central position in the retinal circuitry, very little is known about their responses to light. In non-mammalian retina, bipolar cells show a distinct center-surround receptive field organization (e.g., Kaneko, 1973). Some non-mammalian bipolar cells also show spectral opponency (Kaneko and Tachibana, 1983). In mammalian retina, bipolar cell physiology has been studied mainly at the biophysical level via recordings made from dissociated cells or slice preparations (e.g., Yamashita and Wässle, 1991; Euler et al., 1996)—light responses from the intact retina have been rarely recorded, and then only briefly, probably due to the small size of these interneurons. There is little evidence that mammalian cone bipolar cells show a center-surround receptive field organization (Nelson and Kolb, 1983; Dacheux and Raviola, 1986).

In primates we now have a detailed picture of the morphology and synaptic connections of the diverse bipolar cell types. Briefly, primate cone bipolar cells can be divided into two main classes: diffuse bipolar cells, distinguished by connections to multiple cones; and midget bipolar cells, distinguished, over much of the retinal area, by a “private line” connection to a single cone axon terminal. The diffuse bipolar cells have been classified at the light microscopic level into six distinct types according to the depth of stratification of the axon terminal in the inner plexiform layer (Boycott and Wässle, 1991)—dB1–3 stratify in the outer portion of the IPL (presumed OFF or hyperpolarizing cells) cells, dB4–6 stratify in the inner portion of the IPL (presumed ON or depolarizing cells). The midget bipolar cells also comprise inner and outer stratifying types. In addition, a bipolar cell which selectively connects to S-cones, called the blue cone bipolar, has recently been characterized (Kouyama and Marshak, 1992).

The richness of detail now available for the morphology of macaque bipolar cells is a necessary starting point for a detailed analysis of the bipolar cell light response. A first question, relevant to this review, is whether primate cone bipolar cells show strong center-surround receptive field organization and if so, does this provide for a red–green or blue–yellow opponency in one or more types. In the in vitro preparation cone bipolar cells can be seen in DAPI stained retinas. Study of the physiology of these macaque bipolar cells is just beginning. However, results thus far—17 diffuse bipolar cells and a single, presumed midget bipolar cell have been recorded—show clearly that both diffuse and midget bipolar cells have strong center-surround organization.

4.2. Diffuse Bipolar Cells: Center-Surround Organization

Center-surround structure could be demonstrated by changing the size of the stimulus field. Figures 5 and 6 illustrate center-surround interaction for an OFF and ON diffuse bipolar cell re-
Fig. 5. Light response of an OFF-center diffuse cone bipolar cell. This cell was tentatively identified as a dB3 cell based on the depth of stratification of the axon terminals in the inner plexiform layer. (A) Spectral sensitivity determined from HMP paradigm shows additive input from L- and M-cones for both center- and surround-dominated responses. Response minimum was located near equiluminance (*) for both center and surround responses. (B) Response to square wave luminance modulation shows a depolarizing OFF-center response to a 1.8 stimulus field (top trace) and surround-dominated response to a 10.8 stimulus field (bottom trace). (C) Center and surround responses to an M-cone isolating stimulus. (D) Center and surround responses to an L-cone isolating stimulus. (E–F) Photomicrograph of Neurobiotin-filled cell from whole mount with focus on (E) axonal tree in the IPL and (F) dendritic arbor in OPL.
Fig. 6. Light response of an ON-center diffuse cone bipolar cell. This cell was tentatively identified as a dB5 cell based on the depth of stratification of the dendrites in the inner plexiform layer. (A) Spectral sensitivity determined from HMP paradigm shows additive input from L- and M-cones for both center- (1° stimulus field) and surround-dominated (10° stimulus field) light responses. Response minimum was located near equiluminance (*) for both center and surround responses. (B) Response to square wave luminance modulation shows a depolarizing ON-center response to a 1° stimulus field (top trace) and a hyperpolarizing surround-dominated response to a 10° stimulus field (bottom trace). (C) Center- (top trace) and surround-dominated (bottom trace) responses to an M-cone isolating stimulus—M-cone input to center and surround respectively depolarize and hyperpolarize the cell. (D) Center- and surround-dominated responses to an L-cone isolating stimulus—L-cone input to center and surround also respectively depolarize and hyperpolarize the cell. (E–F) Morphology of recorded cell after intracellular injection of Neurobiotin and HRP histochemistry, wholemount preparation. (E) Plane of focus on the axonal tree in the inner portion of the IPL. (F) Focus shifted to cell body, note coupling to mosaic of neighboring cells. (G) Focus shifted to level of dendritic tree in OPL.
Fig. 7. Light response of a presumed OFF-center midget bipolar cell shows red–green spectral opponency. This cell was tentatively identified as a midget cone bipolar cell based on the morphology of the cell as observed in vitro after dye staining. However, the cell was not recovered for later analysis with Neurobiotin staining so its identification is considered tentative. (A) Spectral sensitivity determined from HMP paradigm for a 5° field shows strong input from M-cones and a strong response to equiluminant chromatic modulation—response minimum was located near silent-M condition. (B) Response to square wave luminance modulation shows a hyperpolarizing OFF-center response to a 1° stimulus field (top trace) but only small transient responses at stimulus ON and OFF for a 5° stimulus field (bottom trace). (C) Center- (top trace) and surround-dominated (bottom trace) responses to an M-cone isolating stimulus—hyperpolarizing M-cone input dominates both responses. (D) Center- and surround-dominated responses to equiluminant red/green chromatic modulation—cell hyperpolarizes to both stimulus fields. (E) Center- and surround-dominated responses to L-cone isolating stimulus. 1° field elicits small hyperpolarization; 5° field elicits a larger depolarizing response—reversal of response phase (compare with response to M-cone stimulus for large field). Indicates that this cone bipolar is an M-cone dominated OFF-center and L-cone dominated ON-surround opponent cell.
spectively. Small fields 1° in diameter or less evoked a sustained, center-dominated response. By contrast, larger stimulus fields (10° in diameter) almost completely inverted the polarity of the light response, though a transient component of the center response remained. Diffuse bipolar cells received additive input from both L- and M-cones to both the center and surround of the receptive field. This was shown by obtaining the HMP null point for both center-dominated and surround-dominated responses (Fig. 5A, Fig. 6A). The location of the response minimum was similar to that found for the H1 cells and indicates that diffuse bipolar cells draw input indiscriminately from the L- and M-cones. This is in agreement with the pattern of cone connections for diffuse bipolar cells determined anatomically (Boycott and Wässle, 1991). Thus far no significant S-cone input has been found for diffuse bipolar cells, though an anatomical connection with S-cones has been suggested (Boycott and Wässle, 1991). Physiological recording of diffuse bipolar cell light responses is just beginning and no attempt has yet been made to reach conclusions about the specific type (dB1–6) of diffuse bipolar cell recorded. No recordings have yet been made from an identified blue-cone bipolar cell.

4.3. Midget Bipolar Cells: Red–green Spectral Opponency?

The physiology of the midget bipolar cell may hold the key to understanding the mechanisms of red/green opponency. Individual midget bipolar cells receive synaptic connections from a single cone over much of the retinal area (from fovea to ~50° eccentricity) (Milam et al., 1993; Wässle et al., 1994). This private line connection has the potential of delivering a cone type specific input to the receptive field center of a midget ganglion cell. Does the midget bipolar cell show spectral opponency, and if so, what is the nature of the cone input to the receptive field that generates the opponent response? Thus far only one midget bipolar cell has been recorded but the response showed clear red–green spectral opponency (Fig. 7). This cell was a hyperpolarizing, OFF-center cell. The light response was dominated by a hyperpolarizing M-cone input when a 1° diameter stimulus field was used (Fig. 7A–C). When a 5° stimulus field was used a depolarizing L-cone input was added to the response (Fig. 7E). These two inputs act synergistically in response to red–green chromatic modulation, since the L- and M-cones are modulated in counterphase (see Fig. 2) resulting in a large red-ON, green-OFF response (Fig. 7D). The response to a luminance modulated square wave was also characteristic of a spatially as well as chromatically opponent cell. The cell showed a strong OFF response to stimulation of the receptive field center; when the surround was added the L- and M-cone inputs (now modulated in phase) acted agonistically to greatly diminish the light response (Fig. 7B). It was not possible in this cell to determine whether the cone input to the surround was derived entirely from L-cones or from both L- and M-cones.

In sum, the first recordings from primate cone bipolar cells show evidence for strong center-surround receptive field organization. Diffuse bipolar cells studied thus far sum L- and M-cone inputs to both center and surround and show, like the H1 cells, a spectral sensitivity, that derives from L- and M-cone input with a weighting like that of the photopic luminosity function. Much more will need to be done on a large number of morphologically identified diffuse types before any conclusions can be reached about their overall functional role. A single, midget bipolar cell has been recorded and showed a spectral opponent M-cone OFF, L-cone ON light center surround light response.

5. GANGLION CELLS

5.1. History: Parasol, Midget and Small Bistratified Types

In order to understand the circuitry that gives rise to spectral opponency it is necessary to clearly identify the ganglion cell types that transmit wavelength-selective signals. In an early attempt to directly link morphology to physiology using intracellular recording and staining methods in the intact primate eye, DeMonasterio
addressed the question of which ganglion cell types transmitted spectrally opponent signals (DeMonasterio, 1979). He suggested that two common types, the parasol and midget ganglion cells, first described and named by Polyak (1941), might transmit blue–yellow and red–green opponent signals respectively to the lateral geniculate nucleus. It is now well understood that the parasol ganglion cells project to the magnocellular layers of the LGN, where cells with non-opponent light responses are recorded. The parasol cells have for this reason been considered the anatomical counterpart of the “M” cells which have been well characterized in extracellular recordings of primate ganglion cells and show a broad spectral sensitivity that matches the photopic luminosity function (Lee et al., 1988). Midget ganglion cells, whose axons project to the parvocellular layers are considered equivalent to the spectrally opponent “P” cells which have also been studied intensively by extracellular recording in both retina and LGN (see review in Kaplan et al., 1990). However, ganglion cell types other than midget and parasol cells project to the LGN (Rodieck and Watanabe, 1993), calling to question the simple one-to-one correspondence between these two ganglion cell types and the physiologically defined magnocellular and parvocellular pathways. To determine which of the many ganglion cell types (Rodieck, 1988; Kolb et al., 1992) project to the LGN (and contribute to color vision) it is necessary to directly link ganglion cell dendritic morphology, physiology and central connection. As a first step towards this goal we have recorded the light responses of parasol cells, midget cells and the small bistratified cell, a recently characterized ganglion cell type (Dacey, 1993) that is retrogradely labelled after tracer injections made into the parvocellular LGN (Rodieck, 1991).

5.2. Parasol Cells: Non-Opponent Cells of the Magnocellular Pathway

Parasol ganglion cells have large cell bodies easily identified in the in vitro retina after staining with acridine orange (Fig. 1B, arrowheads). Intracellular staining of recorded cells shows the distinctive dendritic morphology of parasol cells observed previously (Fig. 8A) (Perry et al., 1984; Watanabe and Rodieck, 1989; Dacey and Petersen, 1992). Consistent with their projection to the magnocellular LGN parasol cells show non-opponent physiology with a response minimum near the equiluminant condition as expected (Fig. 8B, D–F). Using a small spot or an annulus it is possible to isolate a relatively pure center and pure surround response (Fig. 8C); both components show a similar spectral sensitivity (Fig. 8B). Interestingly it is the surround that shows a frequency doubled, response minimum (Fig. 8E) suggesting that this property is transmitted to the parasol cell surround via the H1 horizontal cell network (Fig. 3). The recordings from identified parasol cells confirm their identity as a type of M-cell, but does not determine whether all magnocellular projecting ganglion cells are parasol cells. To address this question it will be necessary to use the intracellular approach in combination with retrograde labelling (e.g. Pu et al., 1994; Yang and Masland, 1994).

5.3. Midget Ganglion Cells and the Circuitry for Red–green Opponency

It is generally accepted that the midget ganglion cells form the substrate for the red–green opponent pathway (e.g. Kaplan et al., 1990; Lee, 1996). The issue that remains controversial is the nature of the circuitry that accomplishes the L/M cone opponent transformation. The recording from a single midget bipolar cell illustrated in Fig. 7 suggests that opponency is already present at the bipolar cell level. If a midget ganglion cell receives input from a single midget bipolar cell, as occurs in the central 7–10° eccentricity, then the opponent response of the midget bipolar cell can be simply transmitted to the midget ganglion cell. This is consistent with the large number of red–green opponent cells that are recorded in the parafoveal retina where the “private-line” midget system dominates. However, with increasing distance from the fovea midget ganglion cells increase greatly in dendritic field diameter relative to the midget bipolar cells. Do these peripheral midget ganglion cells have the ability to make
Fig. 8. Light response of a parasol ganglion cell. (A) Intracellular stain of an inner parasol cell from retinal periphery (M. nemestrina). (B) Plot of responsivity (1st harmonic amplitude of averaged synaptic potential) of the cell shown in A as a function of red and green LED modulation depth. Separate plots are shown for 1° stimulus field (open symbols), 10° stimulus field (gray symbols), and an annulus with a central occluding disc ~1.5° diameter which elicited a pure surround response (solid symbols). Synaptic potentials for the silent-L, response null, and silent-M points in this plot are shown in D–E. Other conventions as described in Fig. 3. (C) Response to 9.77 Hz square wave luminance modulation. The top trace shows a sustained-ON response (peak discharge rate 280 spikes/sec) to a 1° stimulus field; the lower trace shows a sustained-OFF response to an annulus. (D) Synaptic potential, averaged over ~5 sec stimulus modulation, in response to M-cone isolating stimulus (silent-L condition indicated in B) for both ON-center and OFF-surround response. (E) Averaged synaptic potential at response minimum (red, 1.0, green, 0.6) for both ON-center and OFF-surround response. (F) Averaged synaptic potential, in response to L-cone isolating stimulus (silent-M condition indicated in B) for both ON-center and OFF-surround response.
selective connections with either the L- or M-cone connecting bipolar cells? Or is there a lack of cone-type selectivity with the consequent loss of opponency in the periphery? In the in vitro preparation it has been possible to answer this question by recording from midget cells in the periphery of the retina and mapping the cone inputs to both the receptive field center and surround.

In the retinal periphery midget ganglion cells can be as large as 150–200 μm in diameter (Fig. 9A) and must receive convergent input from

![Image of a midget ganglion cell](image)

**Fig. 9.** Light response of a midget ganglion cell in the retinal periphery. (A) Intracellular stain of an inner midget ganglion cell at 11 mm eccentricity in the temporal retina (M. nemestrina). (B) Plot of responsivity (1st harmonic amplitude of averaged synaptic potential) of the cell shown in A as a function of red and green LED modulation depth. Separate plots are shown for 0.5° stimulus field (open symbols) and a 10° stimulus field (filled symbols). Cell receives additive input from both L- and M-cones but the response to M-cone modulation is stronger, shifting the response null toward the silent-M condition. Synaptic potentials for the silent-L, response null, and silent-M points in this plot are shown in D–F. Other conventions as described in Fig. 8. (C) Response to 13 Hz sine wave luminance modulation. The top trace shows a sustained-ON response (peak discharge rate, 100 spikes/sec to a 1° stimulus field—the lower trace shows an OFF surround-dominated response to a 10° stimulus field. (D) Synaptic potential, averaged over ~5 sec stimulus modulation, in response to M-cone isolating stimulus (silent-L condition indicated in B) for both center-dominated and surround dominated responses. (E) Averaged synaptic potential at response minimum (red: 1.0, green: 0.3) for center and surround-dominated responses. (F) Averaged synaptic potential, in response to L-cone isolating stimulus (silent-M condition indicated in B) for center and surround-dominated responses.
a large number of midget cone-bipolar cells. We have found that these peripheral midget ganglion cells receive input from both L- and M-cones to the receptive field center as well as the receptive field surround (Fig. 9C–F). This results in a non-opponent light response similar to that found for all other cells, including the parasol ganglion cell, that receive additive L- and M-cone input and lack significant S-cone input (Fig. 9B). Spatial maps of the cone inputs to the receptive field also very clearly reveal L- and M-cone input to both the center and the surround components (Fig. 10). These results suggest that the surround of the midget ganglion cell, whether it derives exclusively from the midget bipolar or via amacrine cell input or both is not cone type-selective and that opponency is manifest when the number of cone inputs to the center is reduced to one, as occurs in the parafovea. This is consistent with the class of non-selective, “mixed-surround” and “hit or miss” models (Buchsbaum and Gottschalk, 1983; Lennie et al., 1991; DeValois and DeValois, 1993) and with psychophysical evidence for a gradual decline in the sensitivity of red–green color vision with increasing distance from the fovea (Mullen and Kingdom, 1996).

5.4. Small Bistratified Cells: Blue-ON/Yellow-OFF Cells of the Parvocellular Pathway

A number of early recording studies pointed out large differences in the physiology of S-cone opponent cells (e.g. Zrenner and Gouras, 1981), so with hindsight and current knowledge of the great diversity of ganglion cell types, it is not surprising that cells with S-cone input correspond to a distinctive anatomical type that projects to the LGN in parallel with the parasol and midget ganglion cells. The small bistratified cells were recently recognized by intracellular staining in both macaque and human retina (Dacey, 1993). These cells were also shown to project to the parvocellular layers of the LGN (Rodieck and Watanabe, 1993; Rodieck, 1991) suggesting a role in color coding. The distinctive bistratification of the dendritic tree (Fig. 11C–E) suggested that these cells might convey S-cone signals: the inner dendritic tree appeared to costratify with the axon terminals of a cone bipolar cell type, the blue cone bipolar, whose dendrites connect exclusively to S-cones (Kouyama and Marshak, 1992). The relatively large and distinctive cell body of the small bistratified cell was identified in the in vitro macaque retina stained with acridine orange and recordings showed clearly a blue-ON/yellow-OFF opponent light response (Dacey and Lee, 1994) (Fig. 11A). The S-cone isolating stimulus elicits a strong ON depolarization and spike discharge in phase with the S-cone modulation, in striking contrast to either the parasol or midget ganglion cells that show no response to the same stimulus.

The receptive field structure of the Blue-ON cell taken together with its bistratified morphology suggests a simple underlying mechanism for the cone opponent response. Firstly, the S-cone ON and L + M-cone OFF responses do not represent a classical center-surround organization but instead appear as two fields that are spatially similar in size and nearly coextensive (Fig. 12). Secondly, the inner dendritic tier (Fig. 11D), as mentioned above is well suited to receive direct depolarizing input from the blue-cone bipolar cell and in addition the more sparsely branching outer dendritic tree (Fig. 11E) is stratified close to the amacrine cell layer, in the outer, presumed OFF portion of the inner plexiform layer. Recent electron microscopic reconstruction indicates that these outer dendrites are the sites of synaptic input from a diffuse cone bipolar that is connected non-selectively to L- and M-cones (Calkins et al., 1995). Thus the spatially coextensive blue-ON and yellow-OFF fields appear to derive from separate ON and OFF cone-bipolar pathways.

6. AMACRINE CELLS

6.1. History: Anatomical Diversity

The lack of spectral opponency in the H1 and H2 horizontal cells focuses attention on the amacrine cells, the laterally connecting interneurons of the inner retina, as a basis for cone-opponent circuitry. One possibility, for example, is that a “midget” amacrine cell exists. That is, an ama-
Fig. 10. Map of cone inputs to receptive field center and surround of an OFF-center midget ganglion cell in the retinal periphery. Cone inputs were mapped using luminance modulated, M-cone and L-cone isolating spot stimuli, 40 \( \mu \text{m} \) in diameter—stimuli were modulated at 2.44 Hz temporal frequency. The spot was moved to successive locations in a 13 \( \times \) 13 grid covering a 540 \( \mu \text{m} \) square field. Center and surround responses were clearly identified by a 180° shift in response phase. The left hand column shows 2D 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 righthand 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—with the M-cone input the stronger of the two.
Fig. 11. (Caption on next page)
Fig. 12. Spatial map of S-cone ON and L + M cone OFF field for a blue-ON bistratified ganglion cell. Map was made as described in Fig. 10 using an S-cone selective stimulus and a luminance modulated stimulus (red and green LEDs run in phase—S-cone modulation is negligible). (A) S-cone ON response is shown in the surface plot and the image plot. (B) L + M-cone OFF field is shown as both a surface plot and image plot. The two fields are similar in size but, surprisingly, do not overlap completely but are adjacent to one another. As a result, one edge of the OFF field was not mapped with the grid shown here.

Fig. 11. Light response and morphology of the bistratified blue-ON ganglion cell type. (A) “Blue-ON” response to equiluminant blue–yellow modulation. Top: stimulus waveform, blue LED output is run in counterphase to red and green LED output. Middle trace: intracellular voltage response shows strong blue-ON depolarization and spike discharge. Post stimulus time histogram of spike discharge, shown under voltage record, is averaged over six seconds stimulus modulation. (B) S-cone mediated ON-response. Top trace, stimulus waveform, blue LED is modulated in phase with red LED and counterphase to green LED. Modulation depths were set to silence L- and M-cones (see Section 2.3)—S-cones are modulated in phase with the blue LED. Middle trace, voltage response shows strong depolarization and spike discharge in phase with S-cone modulation. Post stimulus time histogram as in A. (C) Dendritic morphology of cell whose light response is shown in A. Note that overall dendritic tree is more sparsely branching than the parasol cell shown in Fig. 8. Morphology demonstrated by intracellular injection of Neurobiotin and subsequent HRP histochemistry. (D) Higher magnification of a small portion of the dendritic tree—plane of focus on the inner tier of dendrites. (E) Same field as in D but plane of focus shifted to the outer tier of dendrites.
crine cell type that receives input from a single midget bipolar connected to, say, an L-cone, and directs inhibitory output exclusively to a midget ganglion cell that receives excitatory M-cone input from another midget bipolar cell—this circuitry, would require a great deal of connectional specificity in the midget system. This question has recently been addressed by reconstructing amacrine cell processes that synapse onto the private line connection of a midget bipolar cell and midget ganglion cell (Calkins and Sterling, 1996). No anatomical evidence was found to suggest that an amacrine cell could receive input from only one cone type so as to provide a simple basis for a

Fig. 13. Light response and morphology of the A1 amacrine cell. (A) Morphology of the A1 cell in the retinal periphery. The dendritic tree (shown in white) is ~400 μm in diameter and moderately branched—a distinctive axon-like arbor arises near the cell body and gives rise to a second, more sparsely branched arbor (shown in black) that extends for long distances (4–5 mm) in the inner plexiform layer; scale bars = 300 μm. (B) Plot of responsivity (1st harmonic amplitude of averaged synaptic potential) of the cell shown in A as a function of red and green LED modulation depth. This amacrine cell type receives additive input from both L- and M-cones with a response minimum near equiluminance. (C) ON–OFF spiking light response to squarewave luminance modulation. Responses for the silent-L, response null, and silent-M points taken from the plot in B are shown in D–F.
cone type-specific inhibitory pathway into the midget system. However, the physiology of mammalian amacrine cells is complex (Masland, 1988), and little understood. Given the presence of up to 40 distinct amacrine cell populations, most of which have yet to be characterized physiologically, the specific role of identified amacrine cell types in an opponent transformation remains an unsettled and difficult question.

In macaque retina use of the in vitro retina-choroid preparation, has permitted the first physiological studies of identified amacrine cell types (Stafford and Dacey, 1996; Dacey, 1996). Thus far, it has been possible to determine the cone inputs and light responses of two distinctive amacrine types in macaque and these results are briefly reviewed below.

6.2. The A1 Cell: a Spiking, Axon-Bearing Amacrine Type

It is possible to divide the amacrine cells physiologically into two broad groups, those cell types that spike and those, like the other retinal interneurons, that show only graded changes in potential in response to a synaptic input. For the spiking amacrinics the nature of the spiking behavior varies greatly and the exact origins of the spike trigger zones have not been defined for any type. Several mammalian amacrine types show both dendritic trees and distinctive axon-like arbors which project for long distances within the inner retina (reviewed in Vaney, 1990; Wässle and Boycott, 1991). In general these “axon-bearing” types are good candidates for long-range, spiking inhibitory connections that could contribute to the properties of the receptive field surrounds of bipolar and ganglion cells. One of these types in the primate retina, the A1 cell, is a particularly striking example of an axon-bearing type (Dacey, 1989) (Fig. 13). In addition to a distinctive, long range, axon-like arbor, the dendritic tree of this cell type stratified broadly across the ON–OFF subdivision of the IPL suggesting that this type might show a spiking, ON–OFF light response.

Because the A1 cell body is one of the largest in the amacrine cell layer it was easily targeted for intracellular recording in the in vitro retina stained with the non-selective vital dye acridine orange. We found the A1 to truly be a spiking cell, with a phasic ON–OFF discharge. The diameter of the A1 receptive field showed a close correspondence with the size of the dendritic tree (shown in white in Fig. 13A), suggesting that the spikes originate at the axonal origins near the soma and propagate distally for long distances. The A1 cells received additive input from both L- and M-cones to both ON and OFF components of the receptive field and lacked any significant response to an S-cone stimulus (Fig. 13C–F). The HMP protocol showed a null response near the equiluminant point (Fig. 13B), as for the other cell types described thus far that sum L- and M-cone inputs. Thus this cell type has a spectral sensitivity like the photopic luminosity function and is excluded from a role in transmitting a cone-type specific inhibitory signal to bipolar cells or ganglion cells.

6.3. The AII Cell: Cone Inputs to a Rod-Aamacrine Type

The AII amacrine is one of the best characterized amacrine types of the mammalian retina. It reaches the highest spatial density of any amacrine population and is considered the pivotal cell type for the transmission of rod photoreceptor signals to ganglion cells. The circuitry that accomplishes this has been well studied and reviewed in detail (e.g., Wässle et al., 1991). In brief, depolarizing (ON) rod bipolar cells synapse not upon ganglion cell dendrites but on the inner dendrites of the AII amacrine. The AII is a bistratified cell (Fig. 14A, B), that makes sign-inverting inhibitory synapses with cone bipolar cells in the OFF part of the IPL and sign-conserving gap junctions with cone bipolar cells in the ON portion of the IPL—the AII cell thus “piggy-backs” on the cone bipolar cells to transmit both ON and OFF rod signals to ganglion cells. Why then consider the possibility that the AII rod amacrine plays a role in photopically driven cone signal pathways and color coding? Surprisingly the AII cell also receives significant synaptic input from OFF-cone bipolar cells (Dacheux and Raviola, 1986). In primate retina it has recently been shown that presumed OFF-midget bipolar cells...
Fig. 14. Light response and morphology of the AII amacrine cell. (A) Intracellular Neurobiotin injection reveals characteristic network of AII cells—plane of focus on inner, "arboreal" dendrites. (B) Plane of focus shifted to level of AII cell bodies and outer, "lobular" dendrites. (C) Plot of responsivity (1st harmonic amplitude of averaged synaptic potential of the cell shown in A as a function of red and green LED modulation depth. Center (1° field) and surround-dominated (10° field) responses are shown by the open and filled symbols respectively. This cell type receives additive input from L- and M-cones and shows a response minimum near equiluminance (asterisk). (D) Light response to luminance modulated square wave for both 1° (top trace) and 10° (lower trace) diameter fields. Note the strong hyperpolarizing component elicited by the 10° stimulus. (E–G) Center- and surround-dominated responses taken from the plot in C, for response null (red = 1.0, green, 0.7), and for M-cone and L-cone isolating conditions.
synapse upon the AII cell (Grünter and Wässle, 1996). In addition it might be expected that under photopic conditions that bidirectional gap junctions with ON-cone bipolar cell might function to transmit photopic signals to the AII. It was possible then that the AII could introduce cone-type selective inhibition into the midget pathway.

To address these questions the AII cell was identified in vitro by selective staining with DAPI applied under somewhat different conditions than used to label the horizontal cell nuclei (Dacey, 1996). In the in vitro prep high intensity light is used to observe the fluorescent dyes that label retinal cells and this produces a strong bleach of the rods. We found that a threshold rod response in an AII cell required ~30 min dark adaptation. However, after the rod saturation, AII cells show a strong cone driven light response at light levels 3–4 log units above cone threshold. This response is similar in amplitude to the light response of cone bipolar cells and, like the cone bipolar cells shows a strong center-surround receptive field organization that is easily observed by varying the diameter of the stimulus field (Fig. 14D). The waveform of the light response to a large field stimulus that engages both center and surround is complex and multiphasic, showing a transient depolarization at light ON, followed by a more sustained surround-mediated hyperpolarization. A second more phasic hyperpolarization appears at light OFF followed by a weaker, sustained depolarization. Responses to cone-isolating stimuli show that, like the A1 amacrine, the AII cell receives additive input from L- and M-cones, but lacks a significant S-cone input to both the center and the surround of the receptive field (Fig. 14E–G). Characterizing the spectral sensitivity with the HMP paradigm gave response minimums for both center and surround near the equiluminant condition, similar to all other cell types recorded thus far that combine L- and M-cone input.

7. SUMMARY AND FUTURE DIRECTIONS

One major problem for fully characterizing the retinal origins of spectral opponency is the great diversity of cell types in the primate retina whose physiology and morphology remain largely uncharacterized. A fresh approach to this problem is the use of an in vitro preparation of the macaque monkey retina that permits a systematic study of the cone inputs to morphologically identified ganglion cells and retinal interneurons. Horizontal cell types which have long been suggested as key components in opponent circuitry are shown not to play such a role in macaque retina. The first recordings from cone bipolar cell types demonstrate a strong center-surround receptive field organization, but the specific roles of the distinct types of cone bipolar cell in the transmission of spectral opponent signals remains to be established. Cone inputs to three morphologically identified ganglion cell types whose axons project via the retino–geniculate pathway, the magnocellular projecting parasol cells, and the parvocellular projecting small bistratified and midget ganglion cells, have been determined. Parasol ganglion cells, as anticipated from previous extracellular recordings, receive additive input from L- and M-cones, lack significant S-cone input to both center and surround of the receptive field and thus show non-opponent characteristic of cells in the magnocellular visual pathway. The small bistratified cells are ON–OFF cells that receive a major excitatory input from S-cones and transmit a blue-ON/yellow-OFF opponent signal. The mechanism for the cone opponency appears to be the segregation of S-cone and L + M-cone signals respectively into ON- and OFF-bipolar cell channels. Midget ganglion cells of the retinal periphery, expected to show red–green opponency, surprisingly receive additive input from L- and M-cones to both center and surround of the receptive field and give non-opponent light responses. The precise circuitry for red–green opponency thus remains elusive. Finally, the first recordings from two identified amacrine cell types have been made in the macaque: the spiking, axon-bearing A1 cell; and the AII amacrine cell, a key amacrine type in the rod signal pathway. Both amacrine types receive additive input from L- and M-cones to all parts of the receptive field and can be excluded from any role in a cone-opponent transformation in the inner retina. The great majority of identified amacrine cell types remain to be studied so no conclusions can yet be reached about their specific roles in the
generation of opponent circuitry. It is likely though that the list of physiologically and anatomically identified retinal cell types will continue to grow and to provide new insights into the basic structure and function of the primate retina and its role in color vision.

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