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The biocytin wide-field bipolar cell in the rabbit retina selectively contacts blue cones

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Abstract

The biocytin wide-field bipolar cell in rabbit retina is a sparsely populated ON cone bipolar cell with a broad dendritic arbor that does not contact all cones in its dendritic field. The purpose of our study was to identify the cone types that this cell contacts. We identified the bipolar cells by selective uptake of biocytin, labeled the cones with peanut agglutinin and then used antibodies against blue cone opsin and red-green cone opsin to identify the individual cone types. The biocytin-labeled cells selectively contacted cones whose outer segments stained for blue cone opsin and avoided cones that did not. We conclude that the biocytin wide-field bipolar cell is an ON blue cone bipolar cell in the rabbit retina and is homologous to the blue cone bipolar cells that have been previously described in primate, mouse, and ground squirrel retinas.

Keywords

biocytin; bipolar cells; blue cones; fluorescence

Introduction

Bipolar cells are the essential link between the light sensitive photoreceptors in the outer retina and the centrally projecting ganglion cells in the inner retina. In rabbits, 13 bipolar types have been identified: a single rod bipolar cell that conveys rod signals to AII amacrine cells in layer 5 of the inner plexiform layer (Raviola and Raviola, 1967; Dacheux and Raviola, 1986; Vaney et al., 1991), and 12 cone bipolar cell types that transfer cone signals from red-green (L-type) and blue (S-type) cones to ganglion and amacrine cells (Famiglietti, 1981; McGillem and Dacheux, 2001; MacNeil et al., 2004). Most bipolar cell dendrites do not discriminate between the two cone types and contact all cones within their dendritic fields (Boycott and Wässle, 1991; MacNeil et al., 2004). However, some wide-field bipolar cells are exceptions and make few contacts with overlying cones. Single examples of wide-field bipolar cells have been observed in Golgi-stained tissue (Famiglietti, 1981). The cells had wide axonal arbors that stratified in sublamina *a* (type wa) or sublamina *b* (type wb) of the inner plexiform layer and contacted a fraction of the overlying cones, suggesting that the cells are capable of carrying a single chromatic signal. Later, Jeon and Masland (1995) identified a single population of wide-field bipolar cells by selective uptake of biocytin. The labeled cells had broad axonal arbors that extended up to 300 μ m in diameter in layer 5 of the inner plexiform layer and had wide, sparsely branched dendrites that contacted a fraction of the available cones in their dendritic fields. Their morphology was similar to the description of wb bipolar cells (Famiglietti, 1981) as well as those of blue cone bipolar cells in primate (Mariani, 1984; Kouyama and

Marshak, 1992), mouse (Haverkamp et al., 2005), and ground squirrel retinas (Li and DeVries, 2006), suggesting that the biocytin wide-field cell type may be a homologous cell type.

The substrate for a blue signal could arise from the orderly arrangement of cones in the outer plexiform layer. In rabbit retinas, the green and blue cones form two regular mosaics and express either red-green opsin or blue cone opsin in their outer segments (Szél et al., 1988; Famiglietti and Sharpe, 1995; Ahnelt and Kolb, 2000; Haverkamp et al., 2005; Li and DeVries, 2006). Of these two cone types, the blue cones have a lower density and account for approximately 10% of all cones. Spectral information collected by either of these cone types could be preserved by a bipolar cell making specific contacts with a single type of cones. Wide-field bipolar cells in rabbit retina do not appear to contact all cones in their dendritic fields (Famiglietti, 1981; Jeon and Masland, 1995), making them prime candidates to selectively transfer chromatic signals through the retina.

The purpose of our study was to test whether the biocytin wide-field bipolar cell selectively contacts blue cones in rabbit retina. The cells were labeled by selective uptake of biocytin injected into the vitreous body of each eye and then the retinas were subsequently stained with peanut agglutinin and antisera against blue cone opsin to identify the cones they contacted. Our results show that the biocytin wide-field bipolar cell selectively contacts blue cones in the outer plexiform layer and is homologous to the blue cone bipolar cells in primate, mouse and ground squirrel retinas. A preliminary account of our findings has been given in abstract form (MacNeil and Gaul, 2006).

Materials and Methods

Wide-field bipolar cells were selectively labeled in rabbit retinas by vitreal injections of biocytin (Sigma, St. Louis, MO). Adult New Zealand White rabbits were first anesthetized with an intramuscular injection of ketamine (30 – 60 mg/kg) and xylazine (10 mg/kg) and then 20 μ l of 5% biocytin in ddH₂O and 0.5% DMSO were injected into each eye. After 40 – 50 hour incubations, the rabbits were reanesthetized using the same agents and the eyes were enucleated, hemisected and the retinas removed from the eye cup while immersed in oxygenated Ames Medium (Sigma, St. Louis, MO). Rabbits were euthanized with Euthosol (100 mg/kg, iv). All procedures using animals were carried out in accordance with NIH Guidelines and were approved by the Institutional Animal Care and Use Committee at York College, CUNY. Free floating retinas were fixed for 60 – 90 minutes in 4% formaldehyde (Ted Pella, Redding, CA) and rinsed thoroughly in three changes of sodium phosphate buffer (0.1M, pH 7.4) over 30 minutes. For retinal wholemounts, tissue was stained immediately with antibodies. For retinal sections, tissue was embedded in 4% agarose (low melting point; Sigma, St Louis, MO) and cut into 60 μ m sections using a vibratome.

Antibodies

Polyclonal antibodies were used to identify the two types of cones in rabbit retina. Blue opsin expressing cones were detected with a goat polyclonal antibody derived from a 20 amino acid synthetic peptide mapping within amino acids 1-50 of human blue-sensitive opsin (EFYLFKNISSVGPWDGPQYH, catalog #sc-14363, Santa Cruz Biotechnology, Santa Cruz, CA). Red-green cones were labeled with a rabbit polyclonal antibody against recombinant human red-green opsin (MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWVYHLT SVWMIFVVIASVFTNGLVLAATMKFKLRHPLNWILVNLAVADLAETVIASISVV NQVYGYFVLGHPMCVLEGYTVSLCGITGLWSLAIISWERWMVVCCKPFGNVRFDAK LAIVGIAFSWIWAAVWTAPPIFGWSRYWPHGLKTSCGPDVFSGSSYPGVQSYMIVL MVTCCITPLSIIVLCYLQVWLAIKAVAKQKQKESESTQKAEKEVTRMVVVMVLAFCF CWGPYAFFACFAAANPGYPFHPLMAALPAFFAKSATIYNPVIYVFMNRQFRNCILQ

LFGKKVDDGSELSSASKTEVSSVSSVSPA; catalog #AB5405, Chemicon, Carlsbad, CA). In each case, the specificity of the antibody was tested by the manufacturer with a Western blot analysis using retinal extract containing the antibody. Each analysis revealed a band of ~40kD, which corresponded to the respective sizes of the opsin proteins.

Immunocytochemistry and PNA labeling

Free-floating tissue was processed using a combination of bench and microwave staining techniques. Extended incubations were carried out at 4° C with gentle agitation and all microwave steps were carried out in a PELCO Biowave microwave oven with variable wattage control (Ted Pella, Redding, CA) set at 150 watts (Muñoz et al., 2004). In each case, the tissue was permeabilized in 0.1M sodium phosphate, pH 7.4 with 0.5% Triton-X 100 (Sigma, St. Louis, MO; immunobuffer) for 60 seconds, blocked in immunobuffer with 4% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) for 60 seconds and then rinsed in immunobuffer (3 × 60 sec). Permeabilized retinas were incubated with goat antiserum against blue-sensitive opsin, n-20 (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, catalog #sc-14363) and 2% normal donkey serum in immunobuffer while irradiating the tissue in the microwave for short intervals over a period of 13 minutes (3 min ON, 2 min OFF, 3 min ON, 2 min OFF, 3 min ON). After rinsing, the retina was incubated in the secondary antibody (1:100 Alexa Fluor 647 donkey anti-goat IgG; Molecular Probes, Eugene, OR, catalog #A21447) for 10 minutes (2 min ON, 2 min OFF, 2 min ON, 2 min OFF, 2 min ON) followed by three changes of immunobuffer. In one experiment, red-green cones were also labeled using the same procedure described above. The rabbit polyclonal antibody against recombinant human red-green opsin (1:50; Chemicon, Carlsbad, CA, catalog #AB5405) was applied to label red-green cones and was subsequently revealed with Alexa Fluor 488 donkey anti-rabbit IgG secondary antibody (1:200; Molecular Probes, Eugene, OR, catalog #A21206).

The tissue was processed to reveal biocytin next. Retinas were incubated overnight at 4°C in 0.25M Tris buffer with 0.5% Triton-X 100, rinsed in the same buffer the next day and then incubated for two days in avidin DN (1:50; Vector Laboratories, Burlingame, CA, catalog #A-3100). After rinsing, the retinas were incubated overnight in fluorescein anti-avidin D (1:200; Vector Laboratories, Burlingame, CA, catalog #SP-2040) followed by thorough rinsing in buffer.

The final step was to label all cones with peanut agglutinin, which labels certain disaccharides on the surface of outer and inner segments of all cones and in punctuate regions at the cone pedicles (Blanks and Johnson, 1984). Endogenous avidin and biotin in the retina was blocked by preincubating the tissue in an avidin/biotin blocking solution for 30 minutes at room temperature (Vector Laboratories, Burlingame, CA, catalog #SP-2001), rinsed in tris buffer and then incubated with biotinylated peanut agglutinin (1:50; Vector Laboratories, Burlingame, CA, catalog #B-1075) in the microwave at 150 watts for 13 minutes. The tissue was rinsed in sodium phosphate buffer (3 × 60 sec) and the lectin revealed with Texas Red conjugated streptavidin (1:100; Vector Laboratories, Burlingame, CA, catalog #SA-5006) for 10 minutes, rinsed thoroughly in phosphate buffer and coverslipped in Vectashield (Vector Laboratories, Burlingame, CA) shortly before viewing in the microscope.

Retinal sections were processed using the same procedures except for the biocytin step; incubation in avidin DN was shortened to overnight (rather than several days) and to two hours in fluorescein anti-avidin.

Cone densities

To verify the topographical distribution of cone types in retinal wholemounts, we sampled the distribution of PNA labeled cones that were also labeled with antibodies against red-green cone

opsin and blue cone opsin. Cell counts were made from a series of dorsal (d) and ventral (v) locations along the retinal midline with the origin located in the middle of the optic disk.

Imaging

Labeled retinas were imaged with an Olympus Fluoview 300 Confocal microscope equipped with helium, argon and neon lasers and an Olympus 60 X water immersion objective (NA 1.2) at resolutions of 1024×1024 or 2048×2048 . A series of optical sections in a single channel were collected every $0.5 \mu\text{m}$ in the inner plexiform layer to show the wide-field bipolar cell axons, in the outer plexiform layer to show the contacts made by the bipolar cell dendrites with overlying cones, and through the outer nuclear layer to link individual cone pedicles with corresponding outer segments. Each channel was scanned separately to reduce spectral cross-talk between channels. Comparisons of the cones, opsins and the biocytin labeled cells were made by merging images of like focal planes using MetaVue (v 6.1) or Adobe PhotoShop and corrected for brightness and contrast using Adobe Photoshop.

Results

Biocytin injected into the vitreous body of the rabbit eye labeled an assortment of palely-stained ganglion, amacrine and bipolar cell somas and one population of well-stained wide-field bipolar cells (Jeon and Masland, 1995). The wide-field bipolar cells have large, irregular axonal arbors that stratify narrowly in layer 5 of the inner plexiform layer and form a regular mosaic of cells with a low density of $40 - 120 \text{ cells/mm}^2$ (Figure 1). The axonal arbors ranged in size from about $100 \mu\text{m}$ in diameter in central retina to $300 \mu\text{m}$ in diameter in the periphery, and had numerous swellings along both the length of the processes and at their terminal endings. They were variable in shape, sometimes radially symmetric while at other times having one axis significantly longer than the other. The axons tiled the retina without overlap, but extended thinner processes that made apparent contacts with axons from nearby wide-field cells, sometimes making it difficult to judge when one cell ended and another began.

The dendrites of the wide-field cells were relatively thinner in diameter than the axons and arose from 2 - 5 thick, proximal segments (Figure 1B). Characteristic features of the dendrites included clusters of terminals occurring at branch points or at transitions from thick to thin segments along the length of the dendrite. Occasionally, the transition points included a fine process emerging from the cluster that eventually merged with a similar process from an adjacent wide-field cell (Figure 2A, arrow). The diameter of the dendritic arbor ranged from $75 - 200 \mu\text{m}$ depending on retinal eccentricity, but it could be significantly wider if the fine dendritic processes were included. These findings are consistent with those of Famiglietti (1981) and Jeon and Masland (1995).

The clusters of synaptic terminals found on the wide-field bipolar cell dendrites were not randomly positioned on the dendrites but instead coincident with a subset of cone pedicles. Wherever terminal clusters were present on the wide-field dendrites, the varicosities were observed in through focus series entering the PNA-labeled cone pedicles. Four such clusters can be seen at higher magnification in Figure 2B. The wide-field dendrite, labeled in green, extended tiny grape-like clusters sclerally into the overlying cone pedicle. There was a clear separation between the main dendrites (which appear out of focus in Figure 2B) and the varicosities as they extended into the base of the cone. Points of possible contact between the terminal clusters and the PNA labeled cones appear white in the image and suggest a synaptic relationship (Haverkamp et al., 2001). In contrast, dendrites traveling below a pedicle without extending terminal varicosities into it are not likely to be making synaptic contacts (2B, arrow). Many cones in the dendritic field of the wide-field bipolar cell are not contacted, making this cell unlike other cone bipolar types in mammals that contact all available cones within their dendritic fields (Boycott and Wässle, 1991; MacNeil et al., 2004).

Cone distribution

To assess the topographical distribution of cone types in wholemounts, we labeled all cones using PNA and the cone subtypes with antibodies against red-green cone opsin and blue cone opsin. In rabbit retina, PNA binds to the membrane of the cone inner and outer segments as well as the cells bodies and axonal extensions of the cones (Blanks and Johnson, 1984). However, the staining is brightest at the cone pedicles and outer segments. In triple labeled preparations, the distribution of cones resembled the patterns and distributions reported by others (Blanks and Johnson, 1984; Szél and Röhlich, 1988; Famiglietti and Sharpe, 1995; Peichl, 2005). The PNA labeled cone photoreceptors were most dense in the visual streak, numbering $\sim 12,000$ cells/mm², and declined slightly to a plateau of $\sim 10,000$ cells/mm² from mid-peripheral retina to the ventral margin (Figure 3B, black line). In dorsal retina, the densities of cones ranged from $\sim 6,000$ - $9,000$ cones/mm². The distribution pattern of red-green cones closely mirrored the distribution of the PNA labeled cones; the peak density of red-green cones was in the visual streak and then gradually declined with increasing eccentricity (Figure 3B, green line). In contrast, cones with outer segments that stained with blue cone opsin maintained a relatively low, but constant density across the retinal surface ($\sim 1,000$ cells/mm²) that increased sharply in number at the extreme ventral margin (v14, 14 mm ventral to the optic disk) to form the “blue streak” (Juliussen et al., 1994; Famiglietti and Sharpe, 1995; Ahnelt and Kolb, 2000) where nearly every cone stained for blue cone opsin (Figure 3A, lower right).

In peripheral retina, we also observed the presence of cones expressing more than one cone opsin. Mixed cones were not seen in dorsal (d) or ventral (v) retina superior to v8 (8 mm inferior to the optic disk) where PNA labeled cones stained for either blue cone opsin or red-green cone opsin, but not both (Figure 3A, upper panels). The numbers of mixed-opsin cones were more numerous toward the ventral rim and accounted for about half of all cells in the “blue streak” (Figure 3A, lower panels). Since it was not possible to differentiate the function of blue cones from cones expressing dual cone opsins, we restricted our study of cone-to-bipolar cell contacts to retinal eccentricities superior to v8.

Type-specific cone contacts of wide-field bipolar cells

Cone type-specific contacts of the wide-field bipolar cells were studied in sections and wholemounts that had been double-labeled with PNA and blue cone opsin. In sections, it was possible to see both the dendrites of a wide-field cell enter a cone pedicle and its corresponding blue-opsin stained outer segment perfectly in register (Figure 4). In some instances however, the sections were not oriented perfectly vertical, causing the outer segment to be slightly offset from its pedicle. Confocal stacks taken from wholemounted retina confirmed that pedicles contacted by the wide-field bipolar dendrites corresponded to blue cone opsin stained outer segments. Figure 5A shows the dendrites of a single wide-field bipolar cell with its four terminal clusters indicated by arrows. Each one of these clusters was coincident with a cone pedicle (magenta) whose outer segment expressed blue cone opsin (Figure 5B). This correspondence was confirmed by tracing each of these cones in a stack of optical planes taken every 1 μ m throughout the outer nuclear layer (ONL). Five of these images are shown in Figure 6 with each cone indicated by an arrow as it traveled through the ONL. The base of each outer segment corresponds to each of the four blue cones in Figure 5B.

The number of cones that each bipolar cell contacted depended on retina eccentricity, but usually ranged between 2 and 7. In mid-peripheral retina, each wide-field bipolar cell contacted a unique set of cones and all blue cones were contacted by only one wide-field bipolar cell (Figure 7). As the size of the dendritic fields increased, the number of cones contacted increased as well. This pattern varied in dorsal retina and in the “blue streak” of the ventral retina. In dorsal retina, where the density of blue cones is lower and the diameter of the wide-field cell dendrites is large, each cone was routinely contacted by more than one wide-field cell, while

in the far ventral retina, which contains a combination of blue cones and dual opsin cones, only a subset of cones expressing blue cone opsin was contacted by each wide-field bipolar cell (not shown). This may be because the cones residing in the “blue streak” in rabbit retina contain only some “true blue” cones (Ahnelt and Kolb, 2000).

Discussion

The biocytin stained wide-field bipolar cell is the rabbit's equivalent of the mammalian ON blue cone bipolar cell that has been described in monkey, mouse and ground squirrel retinas (Kouyama and Marshak, 1992; Haverkamp et al., 2005; Li and DeVries, 2006). Like its mammalian counterparts, the rabbit's wide-field cell is more than twice as large as any other bipolar cell type (MacNeil et al., 2004). Its axon resides in layer 5 of the inner plexiform layer and is thus presumed to be an ON bipolar cell. Moreover, the dendrites of the wide-field cell extend throughout the inner plexiform layer and make selective contacts with cones that express blue cone opsin in their outer segments and avoid cones that do not. We assume that these points of contact represent synaptic junctions because we were able to follow the dendrites in through focus series into blue cone pedicles and see them terminate adjacent to patches of PNA labeling. Invaginating bipolar processes are a hallmark of ON cone bipolar cells and mark the locations where synapses between bipolar cells and cones occur (Hopkins and Boycott, 1995). A confocal study of the cone pedicle has shown that PNA labeling overlaps that of bassoon-immunoreactive ribbons and is in register with Go α , a marker for ON-bipolar cells (Haverkamp et al., 2001). This suggests that the proximity of the PNA label with the biocytin stained processes in the blue cone pedicles reflects a synaptic relationship. Synaptic communication between blue cone bipolar cells and blue cones has been clearly shown by Li and DeVries (2006) in ground squirrel retina. They demonstrated that only one type of bipolar cell made exclusive contacts with blue cones and these contacts were functional; depolarization in a presynaptic cone generated a prominent outward current in a coupled bipolar cell.

In addition to the contacts that the wide-field cell dendrites made with cones, the dendrites also included thin processes (Famiglietti, 1981) that merged with the thin dendrites of adjacent cells (Jeon and Masland, 1995). These processes usually arose from a terminal cluster aligned with a blue cone and were smooth and not associated with any cone pedicles that might suggest a synaptic junction. It is likely that gap junctions exist between the thin dendrites of adjacent cells, but their existence could not be established in the confocal images.

One significant difference observed in the cone contacts of the rabbit wide-field bipolar cell was the degree of divergence of the photoreceptor input. In primates and mice, the density ratio of blue cone bipolar cells to blue cones is approximately 1:2 and each blue cone is contacted by more than one blue cone bipolar cell (Kouyama and Marshak, 1992; Kouyama and Marshak, 1997; Schein et al., 2004; Haverkamp et al., 2005). In rabbits, the divergence of blue cone input onto each wide-field bipolar cell depends on retinal eccentricity. In dorsal retina, where the density of blue cones is low relative to the numbers of wide-field bipolar cells, blue cones were contacted by dendrites from more than one wide-field cell. In ventral retina, multiple cones were more likely to converge onto each wide-field bipolar cell with none of them contacting more than one (Figure 7). The advantage of this arrangement could be to preserve spatial representation of the blue signal in ventral retina while allowing for better detection of the blue-cone signal in dorsal retina where the number of blue cones is lowest.

Dual pigment cones

A small fraction of cones in peripheral ventral rabbit retina were labeled with antibodies against both blue and red-green cone opsins. Mixed opsin cones are common in many mammalian retinas (Szél et al., 2000; Lukáts et al., 2005), but their specific function is not known. In mouse retina, the majority of cones express dual cone opsins (Applebury et al., 2000), but blue cone

bipolar cells still make appropriate connections with genuine blue cones (Haverkamp et al., 2005) suggesting there exists a chromatically segregated blue channel in mouse retina. In the rabbit retina, the biocytin wide-field cells contacted all blue cones in their dendritic field except in the ventral rim where mixed pigment cones were observed. At this eccentricity, the cell dendrites contacted a subset of blue cones suggesting that cones expressing more than one cone opsin are functionally different from genuine blue cones. The advantage of having such mixed cones is not yet understood, but may be an adaptation of diurnal mammals for better detection of predators against a blue sky background (Ahnelt and Kolb, 2000; Peichl, 2005).

Function of wide-field bipolar cells

The blue cone selectivity of the biocytin wide-field bipolar cell and its structural similarity to blue cone bipolar cells in monkey, mouse and ground squirrel retinas suggests that it is involved with color processing in rabbit retina. The idea that wide-field cells would be selective for blue cones was first proposed by Famiglietti (1981). From counts of cells and measurements taken between terminal clusters on the wide-field bipolar dendrites, Famiglietti predicted that if the wide-field cells were selective for blue cones, one should see an increase in blue cone density toward the periphery. This prediction was born out by cone labeling studies showing a general increase in blue cone density toward the ventral periphery (Juliussen et al., 1994; Famiglietti and Sharpe, 1995) and a tendency for wide-field cells in ventral retina to contact more blue cones with increased distance from the visual streak. The ability of wide-field cells to make selective contacts with blue cones would allow them to deliver a spectrally segregated signal to ganglion cells that stratify in layer 5 of the inner plexiform layer.

There is still some question concerning the identity of OFF-type blue cone bipolar cells in the rabbit retina. Famiglietti (1981) described two populations of wide-field bipolar cells in Golgi-stained tissue: one that stratified in sublamina *b* (type wb), which is most likely the biocytin wide-field bipolar cell and a similar type that stratified in sublamina *a* (type wa). Wa cells possessed “extensive” axons of unspecified length and a narrow dendritic arbor that comprised between 1 and 4 primary dendrites. Each dendrite contacted a cone, including some cones that were shared with wb cells. So far, other studies of rabbit bipolar cells have not found an equivalent type (Mills and Massey, 1992; McGillem and Dacheux, 2001; MacNeil et al., 2004). The largest bipolar cell identified that stratified in sublamina *a* was type DAPI-Ba3 (Mills and Massey, 1992), but the dendrites were much more extensive than those of wa cells, and contacted an average of 16 cones per cell. Chiao and Liu (2006) injected a pair of OFF bipolar cells in dorsal retina that appeared to contact blue cones exclusively. The cells had narrow dendritic fields with four primary dendrites (like those of wa cells) and each dendritic terminal was aligned with blue cone opsin staining. However, the axon field diameters of these cells were relatively small compared with the axons of biocytin wide-field bipolar cells (73 μm versus $>200 \mu\text{m}$ in diameter), so it is unlikely that Chiao and Liu's cells are equivalent to type wa either. A more thorough examination of OFF bipolar cell types will be needed to clarify the morphology of the OFF blue cone bipolar cell population.

Circuitry of the biocytin wide-field bipolar cell

Recordings of ganglion cells in rabbit retina have revealed the presence of blue ON/green OFF, color opponent ganglion cells (Caldwell and Daw, 1978; De Monasterio, 1978; Vaney et al., 1981). The circuitry underlying these light responses is not yet known but could be formed by the selective wiring of ON and OFF cone bipolar cells onto a bistratified ganglion cell, as has been shown in primates (Dacey and Lee, 1994; Calkins et al., 1998). A good candidate for such wiring is the G3 ganglion cell in rabbit retina (Rockhill et al., 2002). It has a bistratified dendritic arbor with the inner dendrites located at the border between strata 4 and 5, in the vicinity of the biocytin wide-field cell axons, and an outer arbor located in stratum 2. We have shown that the dendrites of the wide-field bipolar cell selectively contact blue cones in the outer plexiform

layer and its axon stratifies in layer 5 of the inner plexiform layer. With combined input from red-green cones carried by an OFF bipolar cell that stratifies in sublamina A of the inner plexiform layer, the G3 ganglion cell could be homologous to the blue-yellow cell described in primates (Calkins et al., 1998; Dacey, 2000). Alternatively, the wide-field bipolar cell could synapse with a monostратified ganglion cell that stratifies in layer 5, such as the Ib2 (Famiglietti, 2004) or G10 ganglion cell types (Rockhill et al., 2002). In recent recordings of blue cones in primate retina, Packer and colleagues have shown that blue-yellow spectral opponency originates within the blue cones themselves (Packer et al., 2007). Therefore, a bistratified ganglion cell would not be a prerequisite for generating the types of color opponent responses that have been recorded in rabbit retinal ganglion cells and the responses could be carried to the brain by a monostратified ganglion cell instead.

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Figure 1.

Vertical (A) and whole mount views (B and C) of biocytin labeled wide-field bipolar cells. B. The cell dendrites were made up of thin processes that possessed clusters of terminal swellings. C. The asymmetric axons ranged between 100 and 300 μm in diameter, contained numerous varicosities and in some instances, contacted axons of adjacent wide-field cells. They were narrowly stratified in layer 5 of the inner plexiform layer. Scale A = 50 μm , Scale B and C = 100 μm .

Figure 2.

A. The dendrites of three wide-field bipolar cells (green) in a patch of mid-peripheral retina together with the overlying cone pedicles (magenta); areas of closely opposed labeling appear white. The dendrites covered the retinal surface without overlap and had discrete clusters of varicosities that were coincident with a subset of cone pedicles. The arrow indicates a thin process between two cells that did not appear to contact any cone pedicles while traveling in the outer plexiform layer. The box indicates a region of interest shown at higher magnification below. Scale = 50 μ m. B. Higher resolution image of the terminal varicosities imaged 3 μ m scleral to the plane of the focused dendrites. Note that the terminal varicosities (green) extend quite a distance from the main dendrites and are opposite the lobes of the PNA labeled cone

pedicles (magenta). Processes in the vicinity of a cone pedicle that do not extend varicosities into it, are not likely to be making synaptic contacts (arrow). Scale = 50 μ m.

Figure 3.

A. Wholemounted retina triple-labeled with PNA, red-green cone opsin (green) and blue cone opsin (turquoise). In dorsal (d) and ventral (v) retina, 2 – 10 mm ventral to the middle of the optic disk (v2 - v10), PNA labeled cones stained for red-green opsin (upper left) or blue opsin (upper right) but not both. In the ventral periphery where the “blue streak” is located, nearly all cones were positive for blue cone opsin (v14, lower right) and about half of the cones were also labeled with red-green cone opsin (lower left). These dual opsin cones were first identified at ~v10 and then the numbers progressively increased toward the ventral periphery. Scale = 25 μ m. B. The density of blue cones (blue), red-green cones (green), and all cones (black), were sampled along the dorsal-ventral midline in a retina wholemount. Outside the “blue streak,” the ratio of blue cones to red-green cones was approximately 1:9. For simplicity, the number of dual opsin cones was included with the numbers of red-green cones.

Figure 4.

A. Vertical section showing a wide-field bipolar cell dendrite entering the base of a blue cone pedicle (boxed). Note that the pedicle is in register with its blue opsin stained outer segment (turquoise). B. Higher magnification view of the cone pedicle to show that the dendritic terminal cluster (green) is within the PNA labeled cone pedicle (magenta). Scale = 25 μ m.

Figure 5.

A. A wide-field bipolar cell (green) and the overlying PNA labeled cones (magenta). B. Cones contacted by the wide-field bipolar cell are identified by arrows and are all positive for blue cone opsin (turquoise). Scale = 25 μ m.

Figure 6.

Through-focus images of the cones in Figure 5 to show their path through the outer nuclear layer. The arrows identify the blue cones as they extend from the cone pedicles (bottom) to the cone outer segments (top). Scale = 50 μm .

Figure 7.

A composite image in mid-peripheral retina that shows the terminal varicosities on wide-field bipolar dendrites (circles) and the corresponding blue cone opsin staining (turquoise). In mid-peripheral retina, wide-field cells contact more than one blue cone and each blue cone is contacted by only one wide-field bipolar cell. Scale = 50 μ m