Bipolar Cell Diversity in the Primate Retina: Morphologic and Immunocytochemical Analysis of a New World Monkey, the Marmoset Callithrix jacchus

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ABSTRACT
The aim of this study was to identify the bipolar cell types in the retina of a New World monkey, the common marmoset, and compare them with those found in the Old World macaque monkey. Retinal whole-mounts, sections, or both, were stained by using Dil labeling and immunohistochemical methods. Semithin sections were analyzed by using quantitative methods. We show that the same morphologic types of bipolar cell as described for the Old World macaque monkey by Boycott and Wässle (Boycott and Wässle [1991] Eur. J. Neurosci. 3:1069–1088) are present in marmoset retina: two types of midget bipolar cells, six type of diffuse bipolar cells, a blue cone bipolar cell, and one type of rod bipolar cell. The pattern of staining with different immunohistochemical markers (“fingerprint”) of each bipolar cell type in marmoset was also the same as described for macaque, with one exception: the flat midget bipolar cell (FMB) class is labeled by antibodies to recoverin in macaque but is labeled by antibodies to CD15 in marmoset. The labeled FMB cells in marmoset make contact with multiple cone photoreceptors throughout most of the extrafoveal retina. The spatial density of bipolar cells in marmoset is shown to be sufficient to support one-to-one connectivity of midget bipolar and ganglion cells in the fovea and to allow for parallel pathways to ganglion cells throughout the retina. Quantitative differences in the morphology and receptor connectivity between marmoset and macaque can be related to differences in cone and rod photoreceptor density between the species. We conclude that bipolar cell diversity is a preserved feature of the primate retina. J. Comp. Neurol. 437:219–239, 2001.

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Indexing terms: parallel pathways; CD15 immunohistochemistry; Dil labeling; midget bipolar cells; DB6 cells

In all mammalian retinae studied to date, including cat, rat, rabbit, and macaque monkey, multiple types of cone bipolar cell and one type of rod bipolar cell have been identified. Bipolar cells are the most numerous interneurons in these retinae (Boycott and Dowling, 1969; Martin and Grünert, 1992; Euler and Wässle, 1995; Strettoi and Masland, 1995; Jeon et al., 1998). The significance of bipolar cell diversity is proposed to lie in the existence of parallel pathways to different ganglion cell types (reviewed by Sterling et al., 1995; Wässle, 1999; Dacey, 2000). For example, midget bipolar cells make contact with a single cone pedicle in the fovea (Polyak, 1941; Kolb et al., 1969). They provide the dominant synaptic input to midget ganglion cells (Kolb and DeKorver, 1991; Calkins et al., 1994). By contrast, blue-cone bipolar cells are selective for short-wavelength-sensitive (SWS) cones (Mariani, 1984a) and transfer this chromatic signal to the small bistratified (blue-ON) ganglion cell (Dacey, 1993; Dacey and Lee, 1994; Ghosh et al., 1997; Calkins et al., 1998; Ghosh and Grünert, 1999). In macaque and human rei-
Here, we identified multiple bipolar cell classes in the marmoset retina by using DiI labeling and immunocytochemical markers. Bipolar cell classes homologous to major classes identified in macaque retina are demonstrated. Furthermore, we show that the spatial density of bipolar cells in marmoset is sufficient to support multiple, parallel pathways to ganglion cells, and to preserve the spatial acuity of the foveal cone mosaic. These features are common to dichromatic and trichromatic marmosets. Systematic differences in bipolar cell morphology between marmoset and macaque can be attributed to the high spatial density of cone photoreceptors in marmoset. The results give support for the hypothesis that parallel processing streams in the visual system have their origin at the first synapse in the retina, where photoreceptors make contact with bipolar cells.

MATERIALS AND METHODS

Tissue preparation

Twenty-two eyes from 19 adult male and female marmosets (Callithrix jacchus) were obtained either from the Australian CSIRO/NIH&MRC combined breeding facility in Adelaide, following experiments unrelated to this study or after electrophysiology experiments carried out in our laboratory. Marmosets were killed with an overdose of Saffan (14 mg/kg, i.m.) or pentobarbitone sodium (180 mg/kg, i.v.). All procedures used conform to the provisions of the Australian National Health and Medical Research Council (NH&MRC) code of practice for the care and use of animals.

The eyes were removed and opened by an encircling cut, lens and vitreous were dissected out, and the posterior eyecup was fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for approximately 3 to 4 hours. In some cases, the fixative also contained a small percentage of glutaraldehyde (0.1 or 0.25%). The retina was dissected, and the isolated retina was cut into quadrants and stored in PB with 0.01% sodium azide or immersed in 30% sucrose in PB and stored at −70°C until use.

DiI labeling and photoconversion of bipolar cells

Some of the retinal quadrants used in the present study were also used in our previous studies of marmoset horizontal cells and their connections with the SWS cones (Chan et al., 1997; Chan and Grünert, 1998). The method of DiI (1,1’, dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate) labeling and photoconversion is described in detail in these studies. Small crystals of DiI (up to five pieces per retinal quadrant) were inserted into the retina from the photoreceptor side. The retinal piece was then kept in a solution of 0.1% paraformaldehyde in PB at 37°C for 2 to 20 days. Subsequently, DiI-labeled cells were viewed under a Zeiss Axioskop microscope and photoconverted (Sandell and Masland, 1988) in the presence of 0.01% diaminobenzidine tetrahydrochloride (DAB) by using a 20× Neofluor objective (Zeiss filter set BP 546, FT 580, LP 590).

Antibodies

Most of the antibodies used in the present study have been used previously to study bipolar cell types in ma-
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A mouse monoclonal antibody to protein kinase Ca (Amersham) was used to stain rod bipolar cells (Greferath et al., 1990). The mouse monoclonal antibody MAB115A10 was kindly provided by Dr. S.C. Fujita (Mitsubishi Kasei Inst Life Sci Machida, Japan). It was raised against a homogenate of rabbit olfactory bulb (ROB) (Mori et al., 1985). The mouse monoclonal antibody against the calcium binding protein calbindin (CaBP D-28K) was obtained from Sigma (St. Louis, MO). It was used at a dilution of 1:20,000. The rabbit antiserum against recoverin was a gift from Dr. K.-W. Koch (Institut für Biologische Informationsverarbeitung, Jülich, Germany) (Lambrecht and Koch, 1992). The rabbit antiserum against the glutamate transporter protein (GLT-1) was provided by Dr. B.J. Kanner (The Hebrew University, Jerusalem, Israel) (Danbolt et al., 1992). The mouse monoclonal antibody against the G-protein Goα (mAb3073) was obtained from Chemicon (Temecula, CA). The mouse monoclonal antibody against the carbohydrate epitope CD15 was a gift from Prof. J.K. Mai (Universität Düsseldorf, Germany) (Andressen and Mai, 1997).

**Immunocytochemistry**

Immunoreactivity was demonstrated by using the indirect immunofluorescence method or the avidin-biotin peroxidase complex (ABC) method, as described previously (Grüner et al., 1994; Luo et al., 1999). Vertical cryostat sections were incubated in the primary antibodies for approximately 16 hours, retinal quadrants were incubated for up to 20 days. For immunofluorescence, the following secondary antibodies were used: goat anti-mouse Alexa 488, goat anti-mouse Alexa 594, goat anti-rabbit 488, goat anti-rabbit 594 (all from Molecular Probes). For the ABC method, horse anti-mouse biotin and the ABC elite kit (Vector) were used. Sections and whole-mounts were cover-slipped with Mowiol (Hoechst, Sydney, Australia) (Harlow and Lane, 1988).

**Semithin sections**

Semithin vertical (radial) sections were taken from three retinae of three animals with known color vision phenotype: MG4, a dichromat male; MG7, a dichromat female; and MG23, a trichromat female. These retinae were previously used for analysis of ganglion cell and photoreceptor density (Wilder et al., 1996). Full details of the tissue preparation are given in that study. Briefly, semithin sections (0.5 μm thickness) were cut from blocks taken at defined eccentricities along an axis passing through the fovea and the center of the optic disk. Sections were stained with toluidine blue or processed for GABA-like immunoreactivity by using a polyclonal antiserum (made by I. Wulle; see Wässle and Chun, 1989) or parvalbumin-like immunoreactivity by using a polyclonal antiserum (Stichel et al., 1986).

**Analysis**

*Whole-mounts.* Maps of whole-mounts were drawn at a final magnification of 12.9× by using a camera lucida attached to an Olympus microscope. Dil-stained and CD15-immunolabeled bipolar cells were drawn at a final magnification of 1,875×. The eccentricity of each labeled cell was measured from the whole-map portrait. In some retinal pieces, the mosaic of the overlying cones could also be seen and the outlines of the cone inner segments could be drawn together with the labeled bipolar cells. Thus, taking into account the displacement due to the Henle fibers, the number of cone contacts of some of the bipolar cells could be estimated.

The level of stratification in the inner plexiform layer was measured for the axon terminals of Dil-labeled bipolar cells with the 100× oil-immersion objective and Zeiss Axioplan microscope. By using Nomarski optics, the outer and inner borders of the inner plexiform layer were determined and the position of these borders and of the axon terminals was measured from the fine-focus scale of the microscope. The area of axon terminals was measured from scanned images of the camera lucida drawings (MatLab image processing toolbox, Mathworks, Natick, MA). A polygon connecting the outermost tips of the axon terminals was drawn, and the diameter of a circle with equivalent area was calculated (Boycott and Wässle, 1991; Goodchild et al., 1996b). Spatial density of CD15 labeled cells, somata, and/or axon terminals was measured by using a computer-assisted camera lucida system (Halasz and Martin, 1984; Wilder et al., 1996).

**Semithin sections.** The spatial density of inner nuclear layer cells was calculated from the semithin sections by using stereologic methods as described in full by Martin and Grüner (1992) and Wilder et al. (1996). Briefly, cell profiles cut through amacrine cells, Müller cells, bipolar cells, and horizontal cells were distinguished by their size, position within the inner nuclear layer, and parvalbumin-like immunoreactivity. Bipolar and horizontal cells were counted together in toluidine blue sections in all retinae. Horizontal cell density was measured from parvalbumin-stained sections in one retina (MG7). Spatial density was calculated from the size and number of labeled cell profiles by using the recursive reconstruction method (Rose and Rohlich, 1988). Our previous studies (Martin and Grüner, 1992; Wilder et al., 1996) showed that the recursive reconstruction method gives results that are consistent with other unbiased stereologic measures (see also Sterio, 1984; de Groot and Bierman, 1986). Values were corrected for shrinkage (linear factor 0.87; Wilder et al., 1996).

A three-stage combination of exponentials function was fit to the density estimates for each population. The function has the form

\[ f(x) = a_1e^{-bx_1} + a_2e^{-bx_2} - a_3e^{-bx_3} \]  

(1)

where \( x \) is eccentricity (mm), \( f(x) \) is spatial density (cells/mm² × 1,000), \( e \) is the natural exponent, \( a_{1-3} \) are multiplicative coefficients, and \( b_{1-3} \) are exponential coefficients. This function can account for the “bimodal” density functions of inner nuclear layer cells, which fall close to zero at the fovea, rise to a peak, then fall again with increasing distance from the fovea (Azzopardi and Cowey, 1996). Optimal fit parameters were found by least-squares error minimization using a Levenberg-Marquardt algorithm (MATLAB Optimisation toolbox, Mathworks, Natick, MA.). These functions were used for estimates of cell density and calculation of numerical convergence as shown in Fig. 13.
Fig. 1. Photomicrographs of Di-labeled bipolar cells in a whole-mounted marmoset retina. The top panel of each montage shows the dendrites of a given cell, the bottom panel shows the axon terminal. Some unrelated passing processes are also labeled. The circle indicates the presumed position of the SWS cone pedicle contacted by the blue cone bipolar cell (BB). FMB, flat midget bipolar cell; IMB, invaginating midget bipolar cell; DB, diffuse bipolar cell; RB, rod bipolar cell. Scale bar = 20 μm.
RESULTS

Identification of bipolar cell types by DiI labeling

A total of 68 DiI-stained bipolar cells was analyzed at eccentricities from 0.25 to 9.24 mm. Seventeen cells were from six male marmosets and, thus, from presumed dichromats. Fifty-one cells were from nine female marmosets. Four were dichromats (MY53, MY54, MY58, and MY62) and two were trichromats (MY52, MY60) as determined by electrophysiology (White et al., 1998). The chromatic phenotype of the other females (MY31, MY57, and MY66) was unknown. Examples conforming to the morphology of each of the bipolar cell types described in the Old World macaque monkey by Boycott and Wässle (1991) were identified in the DiI-stained marmoset retinae. Figure 1 shows examples of these cells. Passing processes from other cells are frequently seen, often making it difficult to photograph individual bipolar cells. It is probably through these processes that bipolar cells take up the dye. The panels on the top show two types of midget bipolar cells in the fovea (a flat midget bipolar cell [FMB] and an invaginating midget bipolar cell [IMB]) and a blue cone bipolar cell (BB). The panels on the bottom show two diffuse bipolar cells (DB) and a rod bipolar cell (RB). No systematic differences in the morphology of bipolar cell types were detected between the retinae of male and female marmosets.

Midget bipolar and blue cone bipolar cells. The morphology of midget bipolar cells has been described in detail for Old World primates (Polyak, 1941; Kolb et al., 1969; Wässle et al., 1994). Midget bipolar cells can be distinguished by their cone contacts and the level of stratification of their axons in the inner plexiform layer (IPL). “Single-headed” midget bipolar cells have a single apical dendrite, which forms a “bouquet” close to the cone pedicle and contacts a single cone pedicle. In macaque retina, single-headed midget bipolar cells have been found throughout central and mid peripheral retina up to an eccentricity of 10 mm (Wässle et al., 1994). Two-headed midget bipolar cells (contacting two cones) have also been described throughout the retina of macaque, whereas three-headed or four-headed midget bipolar cells have mostly been observed in far peripheral retina (Wässle et al., 1994).

Figure 2 shows camera lucida drawings of DiI-labeled midget bipolar cells from different eccentricities in marmoset retina. Only two single-headed midget bipolar cells were found: one IMB cell (from 0.12 mm eccentricity) and one FMB cell (from 0.34 mm eccentricity) both were from female marmosets. The IMB cell was from an identified trichromat; the FMB cell was from an animal whose color vision phenotype was not determined. In addition, seven midget bipolar cells that contacted more than one cone were found in peripheral retina of female and male animals. Three cells (one IMB and two FMB) were from mid-peripheral retina (between 1.6 and 4 mm eccentricity). The IMB cell contacted three cones, the two FMB cells contacted four cones. In far peripheral retina (5.76 to 7.4 mm), four IMB cells but no FMB cells were found. One cell was double-headed, two cells were triple-headed, and one cell contacted five cones.

The axons of the three FMB cells stratified in the outer half of the IPL at approximately 15 to 41% of the IPL, the axons of the six IMB cells stratified at approximately 76 to 92% of the IPL. The axon terminals of two FMB cells in mid-peripheral retina occupied an area of 68 and 87 μm², respectively. The axonal diameter was between 6 and 11 μm.

At 6 mm eccentricity, a labeled IMB cell was located in close vicinity (approximately 30 μm) to a labeled OFF midget ganglion cell (Fig. 3). This finding enables the relative area of these two cell types at the same eccentricity to be compared. The area occupied by the axon terminal of this IMB cell is 101 μm² (ellipse axes 9.7, 13.2 μm), and the area occupied by the dendritic tree of this midget ganglion cell is 2101 μm². Thus, the dendritic terminal of this midget ganglion cell is large enough to be contacted by approximately 21 midget bipolar cells.

Blue cone bipolar cells are easily identified by their dendrites, which are horizontally oriented in the outer plexiform layer, and their distinct axon terminal stratifying close to the ganglion cell layer (Mariani, 1984a). The morphology and connections of CCK-labeled blue cone bipolar cells with SWS cones has been described in macaque and marmoset retinae (Kouyama and Marshak, 1992, Kouyama and Marshak, 1997; Wässle et al., 1994; Ghosh et al., 1997, Luo et al., 1999). DiI-labeled blue cone bipolar cells of marmoset retina are shown in Figures 1 and 2. Their dendrites reach toward one or more presumed SWS cones, their axon terminals are relatively large and stratify close to the ganglion cell layer (88 to 112% depth of the IPL). The axon terminal area of the blue cone bipolar cell on the right in Figure 2 is 155 μm², and the axonal diameter is 15 μm.

Diffuse cone bipolar cells. Boycott and Wässle (1991) distinguished six types of diffuse bipolar cell (DB1 to DB6) in Golgi-impregnated macaque retina. These types differ with respect to their cone contacts and in the shape and branching level of their axons. The DB1-type stratifies closest to the inner nuclear layer; the DB6-type stratifies closest to the ganglion cell layer.

A total of 46 DiI-stained diffuse bipolar cells was analyzed in marmoset retina. They were from eccentricities between 0.25 and 9.24 mm. The dendritic tree and the axon terminals of the cells were drawn, and the stratification level of their axon terminals was determined. Because the cells were found at a relatively wide range of eccentricities, it was difficult to compare them directly with the cell types defined for macaque retina at 7 mm eccentricity by Boycott and Wässle (1991). However, by using the shape and stratification of the axon terminal as main criterion, allowed a classification of marmoset bipolar cells, which is comparable to that established in macaque (Boycott and Wässle, 1991). Thus, we use the same terminology (DB1 to DB6) to describe marmoset diffuse bipolar cell types. In the following descriptions of cone connectivity, we use the term “nonselectively” to mean that the spacing of dendritic terminals matched the spacing and distribution of the cone mosaic. The specific question of connectivity with SWS cones is addressed in a separate section below. The morphology and axonal stratification of 28 presumed OFF-bipolar cells (8 DB1, 8 DB2, 12 DB3 cells), and 18 presumed ON-bipolar cells (5 DB4, 10 DB5, 3 DB6 cells) was analyzed (Table 1).

Figure 4 shows drawings of six diffuse bipolar cells from far peripheral marmoset retina in whole-mount view. The cells in the top panel stratify in the OFF-sublamina and correspond to the cell types DB1, DB2, and DB3. The cells
in the bottom panel stratify in the ON-sublamina and correspond to the cell types DB4, DB5, and DB6.

The cell on the left of the top panel in Figure 4 is a presumed DB1 cell from 7.3 mm eccentricity. Its axon terminal is relatively large with a diameter of 13 μm (axon terminal area: 128 μm²). It stratifies close to the inner nuclear layer, at between 0 to 12.5% depth of the IPL. The dendritic tree of this cell is quite large; the dendrites are thin and seem to make only a few contacts per cone pedicle. This cell contacts approximately 14 cones nonselectively.

The cell in the middle of the top panel in Figure 4 is a presumed DB2 cell from 5.6 mm eccentricity. Its axon terminal is relatively compact and broadly stratified (between 17 and 58% depth of the IPL). The dendrites of this cell are relatively thick and contact approximately 17 cones nonselectively.

The cell on the right of the top panel in Figure 4 is a presumed DB3 cell from 7.4 mm eccentricity. The axon of this cell is characterized by many varicosities, and its narrow stratification at approximately 30% of the IPL. The dendrites of this cell are relatively thick and contact approximately 17 cones nonselectively.

The cell on the left of the bottom panel in Figure 4 is a presumed DB4 cell from 5.7 mm eccentricity. The axon terminal of this cell is relatively compact and broadly stratified (between 20 and 60% depth of the IPL). As for macaque DB4 cells (Boycott and Wässle, 1991), a star-like shape of the axon terminal was frequently observed. The dendrites of this cell are relatively fine and terminate in dendritic terminals and maybe another cone with some terminals. The other DB4 cells contact three, three, and two cones (from left to right). The DB2 cell on the left is a single-header, the one on the right is a four-header. The numbers indicate the eccentricities. Scale bar = 20 μm.

Fig. 2. Camera lucida drawings of DiI-labeled bipolar cells in marmoset retinal whole-mounts. The top panel shows invaginating midget bipolar (IMB), the bottom panel shows flat midget bipolar (FMB) and blue cone (BB) bipolar cells. Numbers indicate the distance from the fovea. The IMB cell contacts one cone with an aggregate of dendritic terminals and maybe another cone with some terminals. The other IMB cells contact three, three, and two cones (from left to right). The FMB cell on the left is a single-header, the one on the right is a four-header. The numbers indicate the eccentricities. Scale bar = 20 μm.
distinct knobs. Several dendritic terminals seem to contact the same pedicle. This cell contacts approximately 10 cones nonselectively.

The cell in the middle of the bottom panel in Figure 4 is a presumed DB5 cell from 6.7 mm eccentricity. Its axon terminal stratifies narrowly at approximately 70% depth of the IPL. The dendrites are relatively fine and end in distinct terminals with several terminals contacting the same pedicle. Approximately 11 cones are contacted nonselectively.

TABLE 1. Quantitative Data for DiI-Labeled Cone Bipolar Cells in Marmoset Retina

<table>
<thead>
<tr>
<th></th>
<th>DB1</th>
<th>DB2</th>
<th>DB3</th>
<th>DB4</th>
<th>DB5</th>
<th>DB6</th>
<th>FMB</th>
<th>IMB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eccentricity range (mm)</td>
<td>2.3 to 7.3</td>
<td>1 to 6.7</td>
<td>2.3 to 9.2</td>
<td>2.7 to 6.9</td>
<td>0.25 to 7.4</td>
<td>5 to 7</td>
<td>0.3 to 3.8</td>
<td>0.1 to 7.4</td>
<td>3.5 to 4.6</td>
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<tr>
<td>No. of cells</td>
<td>6</td>
<td>8</td>
<td>11</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No. of cones contacted</td>
<td>5 to 14</td>
<td>5 to 15</td>
<td>7 to 19</td>
<td>9 to 13</td>
<td>4 to 13</td>
<td>14 to 15</td>
<td>1 to 4</td>
<td>1 to 5</td>
<td>1 to 2</td>
</tr>
<tr>
<td>Average no. of cones contacted</td>
<td>11</td>
<td>9.6</td>
<td>13.5</td>
<td>10.6</td>
<td>7.5</td>
<td>14.5</td>
<td>3</td>
<td>2.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Depth of axon terminal (% of IPL)</td>
<td>5–21</td>
<td>12–52</td>
<td>27–38</td>
<td>42–74</td>
<td>58–72</td>
<td>84–110</td>
<td>14–41</td>
<td>76–92</td>
<td>88–113</td>
</tr>
<tr>
<td>SWS cone contacts</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>—</td>
<td>2</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Average no. of SWS cones in dendritic field</td>
<td>0</td>
<td>0</td>
<td>0.29</td>
<td>0</td>
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<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>Average no. of SWS cones contacted</td>
<td>—</td>
<td>—</td>
<td>0.14</td>
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1DB, diffuse bipolar; FMB, flat midget bipolar cell; IMB, invaginating midget bipolar cell; BB, blue cone bipolar cell; IPL, inner plexiform layer; SWS, short-wavelength sensitive.
The cell on the right of the bottom panel in Figure 4 is a presumed DB6 cell from 5 mm eccentricity. It is characterized by a large axon terminal system that terminates close to the ganglion cell layer (91 to 127% of the IPL). The dendritic tree is also large and contacts approximately 15 cone pedicles nonselectively. Each pedicle seems to receive only a few dendritic terminals.

In summary, the morphology of the bipolar cell types identified in Dil-labeled marmoset retina resembles closely that of macaque bipolar cells. We did not see examples of “giant” type bipolar cells (Mariani, 1983; Rodieck, 1988).

Cone contacts of Dil-labeled bipolar cells. For a total of 42 diffuse bipolar cells, the number of cones con-
tacted could be estimated (see Materials and Methods), and for 14 diffuse bipolar cells and three midget bipolar cells, the number of SWS cones in the dendritic field could be determined (Table 1).

SWS cones make up less than 10% of the cones in marmoset retina (Martin and Grünert, 1999); thus, it is still possible that diffuse cone bipolar cells are “biased” in their SWS cone connectivity. The antiserum against the SWS cone opsin labels the entire cone, including the pedicle, and, thus, can be used to analyze synaptic connections of SWS cones directly (Goodchild et al., 1996a; Chan and Grünert, 1998). Most bipolar cells analyzed did not have any SWS cone in their dendritic field (Table 1). Figure 5 shows the cone contacts of a DiI-labeled DB3 cell from 9 mm eccentricity. This cell contacts 19 cones, including the immunolabeled SWS cone pedicle, suggesting that diffuse bipolar cells might receive SWS cone input. This finding is consistent with EM reconstructions of DB2 and DB3 cells in macaque fovea that found SWS cone input to these cell types (Calkins, 1999). We did not see examples of presumed OFF-type (outer stratifying) SWS cone specific bipolar cells.

Rod bipolar cells. The dendrites of rod bipolar cells contact rods exclusively, and their axons stratify close to the ganglion cell layer. They do not contact ganglion cells directly but provide synaptic contact to AII amacrine cells, which convey the scotopic signal into the cone pathway (Daw et al., 1990; Wässsel et al., 1991; Sharpe and Stockman, 1999). A total of 11 DiI-labeled rod bipolar cells was analyzed in marmoset retina, they were from an eccentricity range of 2.64 to 7.6 mm. Examples of rod bipolar cells are shown in Figures 1 and 6. They have fine dendrites that pass the cone pedicles to reach rod spherules. Approximately 40 rod spherules are contacted by the cell on the left, and approximately 60 rod spherules are contacted by the cell on the right (Fig. 6). The axon terminals are characterized by large varicosities, and the axon field is relatively compact. In summary, the rod bipolar cells found in marmoset retina show the typical characteristics of rod bipolar cells described in other mammals.

**Immunocytochemical staining of marmoset bipolar cells**

It has been demonstrated for macaque retina, that different antibodies for bipolar cells show different patterns of selectivity (Grünert et al., 1994). Some label only one type of bipolar cell, others label several types of bipolar cell as well as other neuronal populations in the retina.

Figure 7 compares the staining pattern of five antibodies that label different groups of bipolar cells in marmoset retina. Figure 7A shows a vertical section that was processed with the antibody to GLT-1. A heterogeneous population of bipolar cells is labeled. They have their cell bodies in the middle of the INL, and their axons form a broad band located mainly in the outer region of the IPL. Figure 7B shows a higher resolution photomicrograph of a GLT-1–labeled section from central retina. Many labeled bipolar cell dendrites terminate in distinct bouquets opposite to individual cone pedicles, suggesting that antibodies to GLT-1 stain FMB cells. However, the broad labeled band in the IPL suggests that GLT-1 antibodies also stain other OFF bipolar cell types. Consistently, in macaque retina, comparison of sections labeled for GLT-1 with sections labeled for recoverin—a selective marker for FMB cells (Milam et al., 1993; Wässsel et al., 1994)—revealed that GLT-1 labels FMB, DB2, and possibly DB1 cells (Grünert et al., 1994; Jacoby et al., 2000). In marmoset, antibodies to recoverin labeled only photoreceptors but not bipolar cells (result not shown).

Figure 7C,D shows two sections that were processed with antibodies to Goc (Fig. 7C) and to ROB (Fig. 7D), respectively. Labeled bipolar cells have their somata mostly in the outer part of the INL. In the OPL, diffuse labeling can be seen, including many small processes reaching to the level of rod spherules. Axon terminals are most intensely labeled in the inner region of the IPL. We conclude that the antibodies to ROB and Goc stain ON-cone bipolar and rod bipolar cells in marmoset retina. This finding is consistent with findings in macaque retina (Grünert and Martin, 1991; Grünert et al., 1994; Vardi, 1998).

The staining pattern produced by the antibodies to calbindin (CaBP) and protein kinase C (PKCα) is more selec-
Fig. 7. Fluorescence photomicrographs of immunolabeled vertical cryostat sections through central marmoset retina (eccentricity 1 to 2 mm). A: Glutamate transporter (GLT-1) immunoreactivity is preferentially localized in the membrane of bipolar cells that have axon terminals in the outer half of the IPL. B: High-power photomicrograph of GLT-1 immunoreactive bipolar cells. They have smooth dendritic tops. C: The antibody against Goα stains bipolar cells that have their somata in the outer region of the INL. D: The antibody against rabbit olfactory bulb (ROB) stains bipolar cells that have their axon terminals in the inner half of the IPL. E: The antibody against calbindin (CaBP) labels cones including their pedicles, some bipolar and amacrine cells, and some displaced amacrine cells. In the inner plexiform layer two bands of immunoreactivity can be seen (arrows). F: The antibody against PKCa strongly labels rod bipolar cells whose axons form large terminals close to the ganglion cell layer (lower arrow). In addition a weakly labeled band can be seen located more sclerad in the IPL (upper arrow). ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar in A = 50 μm in A–F, 10 μm in B.
BIPOLAR CELLS IN MARMOSET RETINA

tive (Fig. 7E,F). Calbindin immunoreactivity is found in cones, some horizontal, bipolar, amacrine, and displaced amacrine cells (Fig. 7E). The somata of labeled bipolar cells are located relatively high in the inner nuclear layer, and their axon terminals stratify narrowly in the outer half of the IPL. Thus, consistent with previous studies, calbindin-labeled bipolar cells are classified as DB3 cells (Martin and Grünert, 1992; Grünert et al., 1994; Ghosh et al., 1997; Luo et al., 1999; Jacoby and Marshak, 2000). A second calbindin-labeled band is found in the inner IPL (Fig. 7E), but the origin of this band is still unknown.

Figure 7F shows a section that was processed with antibodies to PKCa. In macaque retina, antibodies against PKCa have been demonstrated to label rod bipolar as well as DB4 cells (Grünert et al., 1994). The staining pattern seen in marmoset resembles that of macaque retina. Bipolar cells that are strongly labeled for PKCa have axon terminals that stratify close to the ganglion cell layer. Their dendrites pass cone pedicles and reach deep into the outer plexiform layer, indicating that they contact rod spherules. In addition to the strongly labeled presumed rod bipolar cells, some evidence for a weakly labeled population of bipolar cells forming a second plexus of narrowly stratified axon terminals can be seen (arrows in Fig. 7F). Thus, we conclude that antibodies to PKCa label rod bipolar cells. In addition, a population of diffuse ON-cone bipolar cells may be labeled by PKCa in marmoset retina.

**CD15-labeled bipolar cells**

Figure 8A,B shows a vertical section through central marmoset retina processed with antibodies to CD15. Many bipolar cells are stained. The somata are mostly located in the middle of the inner nuclear layer. The axon terminals stratify in two bands in the IPL. A broad outer band borders the inner nuclear layer; a narrower inner band is located very close to the ganglion cell layer. Their dendrites pass cone pedicles and reach deep into the outer plexiform layer, indicating that they contact rod spherules. In addition to the strongly labeled presumed rod bipolar cells, some evidence for a weakly labeled population of bipolar cells forming a second plexus of narrowly stratified axon terminals can be seen (arrows in Fig. 7F). Thus, we conclude that antibodies to PKCa label rod bipolar cells. In addition, a population of diffuse ON-cone bipolar cells may be labeled by PKCa in marmoset retina.

**CD15-labeled FMB cells**

In central marmoset retina, CD15-labeled OFF-bipolar cells resemble recoverin-labeled FMB cells in central macaque retina (compare Fig. 8A in this study with Fig. 8A in Wässle et al., 1994), suggesting that the CD15-labeled OFF bipolar cells in marmoset retina are FMB cells. This hypothesis is further supported by double-labeling experiments. Figure 8C,D shows a vertical section through central marmoset retina that was double labeled with antibodies to CD15 (Fig. 8C) and antibodies to GLT-1 (Fig. 8D). The arrowhead indicates a dendritic terminal forming a bouquet opposite a cone pedicle. Although the CD15 label does not extend all the way to the dendritic tip it is clear that this presumed FMB cell is double labeled.

The GLT-1–labeled cells clearly include single-headed midget bipolar cells in the central retina. This finding can be seen by comparing Figure 8E, from marmoset, with GLT-1–labeled midget cells in baboon peripheral retina (Fig. 8F) (see also Grünert et al., 1994). In both species, the position of each cone pedicle is marked by an aggregate of labeled dendritic terminals.

The morphology and spatial distribution of CD15-labeled bipolar cells were further investigated in retinal whole-mounts. Figure 9 shows a focus series through a CD15-labeled whole-mount of marmoset retina from 5.5 mm eccentricity. The somata and dendrites of labeled bipolar cells are shown in Figure 9A,B. The photomicrographs in Figure 9C,D show the axon terminals of FMB cells and those of labeled DB6 cells, respectively. The outer and inner stratifying axon terminal systems show significant differences in size and morphology. The outer (FMB) axon terminals are small (diameter between 11 and 20 μm) and broadly stratified. The inner (DB6) axon terminals are large (diameter between 25 and 60 μm) and narrowly stratified. The outer terminals are present at a higher density than the inner terminals.

In some well-labeled patches in far peripheral retina, we were able to distinguish between the dendrites of CD15-labeled FMB cells and those of CD15-labeled DB6 cells. Figure 10 shows camera lucida drawings of FMB cells at 7.8 mm eccentricity. Figure 10B shows the somata and dendrites, Figure 10D shows somata and axon terminals of the cells. They have a compact dendritic field (diameter between 15 and 20 μm) and a compact axon terminal system (diameter between 17 and 30 μm). The dendrites end in terminal aggregates at the position of cone pedicles. They contact between five and nine cones. Comparison of the bipolar cell and cone mosaics (Fig. 10A,B) suggests that, in some areas, there is not complete coverage of the cone mosaic by the FMB cells. We do not know whether these “gaps” are an artefact of the immunostaining. The axon terminals are densely packed but show little overlap. In Figure 10A, the somata of the FMB cells are drawn together with the overlying cone mosaic. At this eccentricity (7.8 mm), the density of FMB cells was 1,821 cells/mm² and the cone density was 17,082 cells/mm². Thus, there are 0.12 bipolar cells per cone and 9.4 cones per bipolar cell. Thus, the dendritic morphology and cone convergence data both indicate that the FMB cells contact multiple cones in peripheral marmoset retina (Fig. 4, see also Boycott and Wässle, 1991). By contrast, in macaque retina, the FMB cells maintain single-cone contacting dendritic fields at least to 10 mm eccentricity (Boycott and Wässle, 1991; Wässle et al., 1994). This species difference is discussed further below (see Discussion section).

**CD15-labeled DB6 cells**

Figure 11 shows camera lucida drawings of CD15-labeled DB6 cells in a retinal whole-mount in the same patch of retina as shown in Figure 10. The dendritic tree of DB6 cells measures between 40 and 80 μm in diameter, and the large axon terminal is between 30 and 40 μm in diameter. In Figure 11A, labeled DB6 somata are drawn together with the overlying cone mosaic. The cell bodies of DB6 cells are regularly spaced across the retina, and their dendrites and axon terminals occupy distinct territories with little or no overlap. At this eccentricity (7.8 mm), the density of DB6 cells was 440 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell. At an eccentricity of 4.3 mm in nasal retina, the density of DB6 cells was 1,120 cells/mm² and the cone density was 17,082 cells/mm², resulting in a bipolar/cone ratio of 0.03 DB6 cells and a ratio (numerical convergence) of 39 cones per DB6 cell.
Fig. 8. Photomicrographs of vertical cryostat sections stained for CD15 and/or glutamate transporter (GLT-1) immunoreactivity. 

**A:** CD15-labeled section through central marmoset retina. Two populations of bipolar cells are labeled, one stratifying in the outer sublamina of the inner plexiform layer, one stratifying close to the ganglion cell layer. 

**B:** Same section as in A shown with Nomarski optics to reveal the retinal layers. 

**C,D:** Section through central marmoset retina that was double labeled for CD15 and GLT-1. GLT-1–labeled dendritic tops form distinct aggregates and end opposite to cone pedicles. CD15-labeled cells are not stained all the way to the top, but it is obvious that they double label with GLT-1 antibodies (arrowheads). 

**E:** Low-power photomicrograph of a GLT-1–labeled section through central marmoset retina. 

**F:** Low-power photomicrograph of a GLT-1–labeled section through peripheral baboon retina. 

Abbreviations as in Figure 7. Scale bars = 20 μm in A (applies to A,B), 10 μm in D (applies to C,D), 20 μm in F (applies to E,F).
conclude that the incomplete coverage is unlikely to be due to understaining of the labeled cells. This finding suggests that some cones are not contacted by any DB6 cell, giving a coverage factor of less than unity. However, there is no clear sign of cone selectivity in the labeled population analogous to that seen in the blue-cone bipolar cell population, where the dendritic terminals form aggregates at the sites of SWS cone pedicles (Kouyama and Marshak, 1992; Wässle et al., 1994; Luo et al., 1999).

Spatial density of bipolar cells

Figure 12 shows radial semithin sections through the foveal retina of one marmoset. As previously described (Ordy and Samorajski, 1968; Wilder et al., 1996), the foveal architecture is typical for a diurnal primate, with a massive accumulation of cone photoreceptor somata at the foveola and substantial displacement of the postreceptorial layers. Our previous quantitative analysis of ganglion
cells and photoreceptors included measurement of the displacements due to Henle’s fibers and the oblique orientation of the inner nuclear layer (Wilder et al., 1996). This analysis showed that the ganglion cells devoted to the centralmost cones are located up to 0.4 mm from the center of the fovea (Fig. 12A). A high-power view of the inner nuclear layer at 0.8 mm from the center of the fovea (Fig. 12B) shows the same distinct sublamination as described for macaque and human retina (Schein, 1988; Davanger et al., 1991; Martin and Grünert, 1992) with a single layer of Müller cell somata separating amacrine cells, in the proximal part of the inner nuclear layer, from bipolar and horizontal cells in the distal part. These criteria were consistent with observations on sections processed for GABA-like immunoreactivity (see Wilder et al., 1996, Fig. 4), which confirmed that amacrine cells were almost exclusively located proximal to the layer of Müller cells.

Figure 13A shows spatial density of inner nuclear layer cells in one marmoset (MG7). Density of all populations is maximal between 0.8 and 1 mm from the fovea. Peak density for the combined count of bipolar and horizontal cells (120,000–150,000 cells/mm²) is 3–6 times greater than that of Müller or amacrine cells. Combined (bipolar+horizontal) cell density falls rapidly with increasing eccentricity, whereas the Müller and amacrine cell density remains more constant. These features are the same as described for macaque retina (Martin and Grünert, 1992), suggesting that the overall architecture of the inner nuclear layer is preserved between these species. Comparison of cell density in retinae from two dichromat animals and one trichromat animal (Fig. 13B) showed individual variability that was not clearly related to the animals’ chromatic phenotype. Such variation is also present in the ganglion cell population in marmoset (Wilder et al., 1996) and macaque retinae (Perry and Cowey, 1985; Schein, 1988; Wässle et al., 1990). Least-square error minimization parameters for the density functions are given in Table 2.

The numerical convergence of bipolar to ganglion cells was calculated in two steps. First, bipolar cell density was estimated by subtracting horizontal cell density (estimated from parvalbumin-stained semi-thin sections), from the combined (bipolar+horizontal) cell density calculated from toluidine blue stained sections (Fig. 13C). Second, the number of cells in a semi-annulus (centered on the fovea) at different eccentricity ranges in the nasal or
temporal hemiretina was estimated by numerical integration:

\[ N = \frac{\pi}{2} (x_1^2 - x_2^2) \int_{x_2}^{x_1} f(x) \, dx \]  

(2)

where \( N \) is the number of cells, \( f(x) \) is the combinations of exponentials function (equation (1)), and \( x_1, x_2 \) are the outer and inner bounds of the desired eccentricity range. The numbers of ganglion cells in the same retinas were estimated by re-analyzing published data (Wilder et al., 1996) by using the curve-fitting method given above. Allowance was made for postreceptoral displacements around the fovea (Wilder et al., 1996) by adjusting the limits of the integral. Integration was carried out with a recursive Newton method (MATLAB, MathWorks, Natick, NJ). This integration gives a total of 42,000 ganglion cells and 122,000 bipolar cells within 1 mm of the fovea for temporal retina and 55,000 ganglion cells and 118,000 bipolar cells for nasal retina. These numbers are in broad agreement with data from macaque (Schein, 1988; Wässle et al., 1990; Martin and Grünert, 1992) and human (Curcio and Allen, 1990) retinas. We conclude that in marmoset retina, as for these other primates, the spatial density of bipolar cells is high enough to support one-to-one connectivity of midget bipolar cells with midget ganglion cells. The numerical convergence of bipolar to ganglion cells increases further to over 12:1 in peripheral nasal retina (Fig. 13D). Thus, throughout the marmoset retina, the spatial density of bipolar cells is, in principle, high enough to support multiple, independent inputs to parallel ganglion cell pathways.

The spatial density of DB6 cells was estimated along the horizontal meridian by using one nasal and one temporal quadrant of two retinas from two male animals, by counting the CD15-labeled axon terminals (Fig. 14). The maximum density was 1,285 DB6 cells/mm² at 1.12 mm eccentricity in temporal retina, falling to 551 DB6 cells/mm² at 7.4 mm eccentricity in nasal retina. Therefore, these cells represent only a small fraction of the total bipolar cell complement throughout the retina.

The density of FMB cells was estimated for three positions in temporal retina of one well-labeled whole-mount preparation, by counting the total number of CD15-labeled somata, then identifying the labeled DB6 axon terminals, and subtracting this density from the total. This calculation gave a FMB cell density of 15,400 cells/
mm$^2$ (SD 2,560) at 0.99 mm, 4,680 cells/mm$^2$ (SD 846) at 2.9 mm, and 4,767 cells/mm$^2$ (SD 426) at 5.9 mm. The average total bipolar cell density was 120,000 cells/mm$^2$ at 1.0 mm, 6,800 cells/mm$^2$ at 2.9 mm, and 41,500 cells/mm$^2$ at 6 mm. Thus, the FMB cells represent less than 15% of all bipolar cells at the positions measured. However, this conclusion is limited in respect of central retina because of the difficulties in achieving adequate antibody penetration where the retinal layers are thicker.

**DISCUSSION**

The main findings of the present study are as follows. First, the multiple bipolar cell classes described in Old World primates (Polyak, 1941; Boycott and Wässle, 1991;
Fig. 13. Spatial density of inner nuclear layer cells in marmoset. 

**A:** Marmoset MG7 (dichromat). Density of three populations measured along an axis passing through the fovea and optic disk is shown. Am, amacrine cells; M, Müller cells; B+H, bipolar and horizontal cells. Error bars show standard deviation. Lines show best-fit to the combination of exponentials function described in the text. 

**B:** Comparison of combined (bipolar+horizontal) cell spatial density on the fovea-optic disk axis in three animals (MG23 [open circles], trichromat; MG7 and MG4 [squares and filled circles] dichromats). 

**C:** Comparison of bipolar+horizontal cell (B+H), horizontal cell (H), and ganglion cell (G) density on the temporal axis. Bipolar+horizontal values averaged from animals MG7, MG4, MG23. Ganglion cell density recalculated from Wilder et al. (1996). Horizontal cell density from animal MG7. Peak density of all populations is close to 1 mm eccentricity. Bipolar and horizontal cell density falls less rapidly with increasing eccentricity than does ganglion cell density, indicating increasing convergence of bipolar to ganglion cells. 

**D:** Numerical convergence of bipolar to ganglion cells, calculated by numerical integration of the density functions as described in the text. n, nasal; t, temporal.
cell populations obtained in marmoset retina was mostly (single-headed) midget bipolar cells (see below).

Kolb et al., 1992) can be identified in marmoset retina. Second, the spatial density of bipolar cells is sufficient to allow parallel sampling of the cone array by multiple bipolar cell classes. Third, there is substantial homology in the immunocytochemical properties of bipolar cells in macaque and marmoset retina. We discuss these findings and their implication for our understanding of primate retinal circuitry.

**Morphology and immunoreactivity of bipolar cells**

Diverse morphologic types of cone bipolar cells, including midget, diffuse, and blue cone bipolar cells, and one type of rod bipolar cell, were identified in marmoset retina by DiI labeling. This finding suggests that the bipolar cell types in New World primates are comparable to those in Old World primates (Polyak, 1941; Mariani, 1984b; Boycott and Wässle, 1991; Kolb et al., 1992). Two previous studies on New World monkeys are consistent with this idea. Ogden (1974) found midget, diffuse, and rod bipolar cells in the retina of the nocturnal owl monkey Aotes by using Golgi-impregnation. Silveira et al. (1998) described midget bipolar cells in the retina of male capuchin monkeys and showed that these shared most morphologic features of the midget bipolar cells in macaque. However, differences between New World and Old World primates might exist in the distribution and occurrence of “true” (single-headed) midget bipolar cells (see below).

The immunohistochemical staining pattern for bipolar cell populations obtained in marmoset retina was mostly identical to that described for macaque retina. This finding is consistent with previous studies showing the blue cone bipolar cells in marmoset can be labeled with antibodies to cholecystokinin and DB3 cells with antibodies to calbindin, respectively (Ghosh et al., 1997; Luo et al., 1999). In contrast, in retinas of nonprimate species the staining pattern produced by the same antibodies is often quite different. Calbindin antibodies stain a population of ON-bipolar cells in rabbit (Massey and Mills, 1996) and no bipolar cell types in rat and mouse (Euler and Wässle, 1995; Haverkamp and Wässle, 2000). Recoverin antibodies label an ON- and OFF- bipolar population in rat (Euler and Wässle, 1995) and three types of bipolar cell in mouse (Haverkamp and Wässle, 2000). In rabbit retina, CD15 antibodies label selectively one population of bipolar cells that costratify with ON-starburst cells (Brown and Masland, 1999), whereas in other mammalian species, they label several neuronal types (Andressen and Mai, 1997).

Our results indicate that the immunohistochemical properties of most bipolar cell types are similar in primates. However, two differences were found between marmoset and macaque: (1) antibodies to recoverin stain FMB cells in macaque (Milam et al., 1993; Wässle et al., 1994) but do not stain any bipolar cells in marmoset retina; (2) antibodies to CD15 stain two populations of bipolar cells in marmoset (Andressen and Mai, 1997, this study) but only DB6 cells in macaque retina (Chan et al., 2001).

**Spatial density of bipolar cells**

We previously showed (Wilder et al., 1996) that the marmoset fovea, like the macaque and human fovea (Mitsotten, 1974; Schein, 1988; Wässle et al., 1990; Calkins et al., 1994) is characterized by divergence of cone photoreceptors to ganglion cells. This anatomic arrangement gives the potential for the (ON- and OFF-) midget ganglion cell arrays to transmit the full spatial resolving potential of the foveal cone array. We show here that the sampling density of bipolar cells in marmoset is likewise high enough to give a one-to-one “link” between the cone array and the ganglion cell array (Figs. 12, 13) in the fovea.

A quantitative comparison of cone convergence to bipolar cells in macaque and marmoset retinas is given in Table 3. The eccentricities are chosen to be close to equivalent visual angle in the two species. In macaque retina, the densities of cones and ganglion cells were measured by light and electron microscopy in the fovea (Calkins et al., 1994; Wässle et al., 1994). The total bipolar cell density was measured from toluidine blue–stained sections by Martin and Grünert (1992). In marmoset, the cone and ganglion cell densities were measured by Troilo et al. (1993) and Wilder et al. (1996). In macaque retina the density of FMB cells was measured by recoverin-like immunoreactivity in peripheral retina (Wässle et al., 1994),

![Fig. 14. Points show spatial density of diffuse bipolar 6 (DB6) cells measured close to the horizontal axis in one marmoset. Peak density (1,000–1,500 cells/mm²) is at 1- to 2 mm eccentricity. Continuous density function (solid line) drawn by hand.](Image)
and by EM serial reconstruction in the fovea (Calkins et al., 1994). There are clear similarities between the species, including the peak cone density and divergence to bipolar and ganglion cells in the fovea, as well as the convergence of cones to ganglion cells outside the fovea. These results are consistent with other evidence that, in both species, the neural machinery of the fovea is designed to transmit the highest spatial frequencies that the eye’s optics can deliver (for discussion, see Troilo et al., 1993). The relatively high cone density in peripheral retina in marmoset is only partially accompanied by a greater density of postreceptoral neurons, so the convergence of cones to bipolar and ganglion cells in peripheral retina is greater in the marmoset (Table 3; see also Goodchild et al., 1996b).

### TABLE 3. Convergence of Cone Photoreceptors to Bipolar and Ganglion Cells

<table>
<thead>
<tr>
<th>Retina</th>
<th>Cone density (cells/mm²)</th>
<th>Cone/bipolar ratio (Total bipolar cells)</th>
<th>Cone/FMB ratio (Recoverin labelled)</th>
<th>Cone/ganglion cell ratio (CD15-labeled)</th>
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<tbody>
<tr>
<td>Macaque</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fovea</td>
<td>250,000</td>
<td>0.2</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
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<td>0.4</td>
<td>1.1</td>
<td>6.7</td>
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<tr>
<td>10 mm (46 deg)</td>
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<td>0.5</td>
<td>1.1</td>
<td>12.7</td>
</tr>
<tr>
<td>Marmoset</td>
<td></td>
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<td></td>
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<tr>
<td>Fovea</td>
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<td>0.9</td>
<td>0.3</td>
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<tr>
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</tr>
<tr>
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<td>13.6</td>
</tr>
<tr>
<td>7.8 mm (65 deg)</td>
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<td></td>
</tr>
</tbody>
</table>

1Values show numerical convergence (cones/cell) at each eccentricity. Spatial density (cells/mm²) for cones is shown in parentheses.
2Data from Wässle et al. (1994).
3Data from Martin and Grünert (1992).
4Data from Wilder et al. (1996).
5Nasal retina. All other values are temporal, inferior, and superior retina.

Fig. 15. Comparison of spatial density of cones and bipolar cells in macaque (A) and marmoset (B). Density is normalized to peak foveal cone density for each species. Note the logarithmic scale. There is divergence of cones to bipolar cells throughout the retina. Flat midget bipolar cell (FMB) density in macaque is close to cone density throughout the retina. The FMB density in marmoset is close to that of cones near the fovea but is lower than cones in peripheral retina.
Does the marmoset have “true” midget bipolar cells?

Only two examples of single cone-contacting (“true”) midget bipolar cells were seen in the DiI-labeled material. These cells were restricted to the fovea. Likewise, in the CD15- and GLT-1-labeled material, single-headed FMB cells were seen in central retina. These data, together with the high spatial density of bipolar cells in the fovea (see above), are our strongest evidence that the one-to-one connectivity of cones, midget bipolar cells, and midget ganglion cells is attained in marmoset fovea, as in the fovea of diurnal Old World primates.

By contrast with the situation in the fovea, the midget bipolar cells that were identified in the DiI material at eccentricities above 3.5 mm (28 deg) and the peripheral CD15-labeled FMB cells all make contact with multiple cones. Thus, the peripheral marmoset retina is quite different to that described for macaque, human, and Cebus retina, where single cone-contacting midget bipolar cells predominate at 40-degree eccentricity, and the proportion of multiple-cone contacting midget bipolar cells rises only gradually with increasing eccentricity (Wässle et al., 1994; Silveira et al., 1998).

Table 3 and Figure 15 show a comparison of FMB cell density in macaque and marmoset. In macaque, at least as far as 50 degrees of eccentricity, the density of FMB cells is very close to that of cone photoreceptors, and the FMB cells form a large proportion of all bipolar cells. In marmoset retina, the spatial density of FMB cells (15,400 cells/mm²) represents 12.7% of all bipolar cells at 1 mm. Allowing for Henle’s fiber and postreceptoral dispersions (see Materials and Methods section), this proportion corresponds to a sampling density of 205,000 cells/mm², or 93% of peak foveal cone density. This high spatial density is consistent with FMB cells. The fact that FMB cells in peripheral retina contact multiple cone photoreceptors (Fig. 10) is likewise consistent with their lower spatial density and greater cone convergence (Fig. 15). In summary, both the numerical convergence data and the morphology of midget and diffuse bipolar populations (Figs. 2, 4, 10, 11) suggest that all bipolar populations in marmoset peripheral retina contact more than twice as many cones as their morphologic homologues in the macaque retina (Boycott and Wässle, 1991).

This species difference might be expected to have consequences for the color vision capacity of marmosets, as follows. In macaque retina, the one-to-one connectivity of cones to midget bipolar cells is proposed to underlie chromatic opponent responses of red-green opponent ganglion cells at least as far as 10 mm eccentricity in the retina (Lennie et al., 1991; Wässle et al., 1994; Martin et al., 2001) but see also Calkins and Sterling (1999). Our results show that in marmoset, such one-to-one connectivity is much more limited to the foveal region. It would follow that red-green opponent responses should also be limited to the fovea in trichromatic (female) marmosets. Electrophysiological data obtained so far are consistent with this prediction (Yeh et al., 1995; White et al., 1998), but the question has not been investigated systematically.

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