Electron Microscopic Analysis of the Rod Pathway of the Rat Retina

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ABSTRACT

Two immunocytochemical markers were used to label the rod pathway of the rat retina. Rod bipolar cells were stained with antibodies against protein kinase C and AII-amacrine cells with antibodies against parvalbumin. The synaptic circuitry of rod bipolars in the inner plexiform layer (IPL) was studied. Rod bipolar cells make approximately 15 ribbon synapses (dyads) in the IPL. Both postsynaptic members of the dyads are amacrine cells; one is usually the process of an AII-amacrine cell and the other one frequently provides a reciprocal synapse. No direct output from rod bipolar cells into ganglion cells was found. AII-amacrine cells make chemical output synapses with cone bipolar cells and ganglion cells in sublamina a of the IPL. They make gap junctions with cone bipolar cells and other AII-amacrine cells in sublamina b of the IPL. The rod pathway of the rat retina is practically identical to that of the cat and of the rabbit retina. It is very likely that this circuitry is a general feature of mammalian retinal organization.

In the cone pathway of the mammalian retina, cones feed into cone bipolar cells, which comprise two basic physiological types: ON-cone bipolars that are depolarized and OFF-cone bipolars that are hyperpolarized by a light stimulus projected into their receptive field center. In the rod pathway, rods are connected to a single type of rod bipolar cell, which is depolarized by light (for review see Wässle et al., '91). In the inner plexiform layer (IPL) rod bipolar cells make no direct output synapses onto ganglion cells, but a distinctive type of narrowfield amacrine, the so-called AII-amacrine cell, is interposed (Kolb and Famiglietti, '74; Famiglietti and Kolb, '75; Kolb, '79; Sterling, '83; Strettoi et al., '92). AII-amacrine cells make conventional inhibitory chemical synapses with OFF-cone bipolar and OFF-ganglion cells and make electrical synapses with ON-cone bipolar cells. In this way they could produce signals of opposite polarity in ON- and OFF-ganglion cells. This circuitry of the dark adapted retina has mainly been established in cats (Kolb and Famiglietti, '74; Famiglietti and Kolb, '75; Freed et al., '87; Sterling, '83; McGuire et al., '84; Sterling et al., '87, '88) and in rabbits (Dacheux and Raviola, '86; Raviola and Dacheux, '87; Strettoi et al., '90, '92).

The morphology, the synaptic contacts, and the topographic distribution of rod bipolar cells in the cat retina were originally studied from electronmicroscopic serial reconstructions (Kolb, '79; McGuire et al., '84; Smith et al., '86; Freed et al., '87; Sterling et al., '88). However, the recent finding that rod bipolars of the mammalian retina can selectively be labelled with antibodies against a certain isoform of protein kinase C (PKC; Negishi et al., '88) made it possible to study rod bipolar cells more readily in a variety of mammals (cat, rat, and rabbit: Greferath et al., '90; primates: Grünert and Martin, '91; rabbit: Young and Vaney, '91). Rod bipolar cells express also the L-7 protein (Berrebi et al., '91; Grünert and Martin, '91) and the PEP 19-protein (Berrebi et al., '91), both of which have been isolated from cerebellar Purkinje cells (Oberdick et al., '88; Ziai et al., '88). Rod bipolar cells are also immunolabelled with antibodies MAb 115A10, directed against olfactory bulb (Onoda, '88; Greferath et al., '90; Grünert and Martin, '91).

The morphology and topographic distribution of AII amacrine cells have been elaborated in the cat (Vaney, '85), in the rabbit (Mills and Massey, '91; Vaney et al., '91a,b), and in the rat retina (accompanying paper, Wässle et al., '93). AII-amacrine cells are small, distinctly bistratified cells, with so-called lobular appendages in sublamina a of the IPL and bushy dendritic fields in sublamina b. Cells of this characteristic shape have been recognized in practically all mammalian retinae studied, including those of marsupials (Wong et al., '86; Young and Vaney, '90). In the rat

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Fig. 1. A: Vertical frozen section (15 μm thick) of a rat retina processed for protein kinase C (PKC) immunoreactivity. Rod bipolar cells and a few amacrine cells are labelled. B: Vertical frozen section of a rat retina stained for parvalbumin (PV) immunoreactivity. At the inner border of the IPL a single row of AII-amacrine cells is labelled. Ganglion cells and optic nerve fibres are also lightly labelled. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NF, optic nerve fibre layer. Scale bar = 50 μm.

retina AII-amacrine cells were revealed by Golgi-staining (Perry and Walker, '80) and by intracellular injection with Lucifer Yellow (LY) (Voigt and Wässle, '87).

In the present paper rod bipolar cells of the rat retina were immunolabelled with antibodies against PKC and their output and input synapses were studied. AII-amacrine cells were labelled with antibodies against parvalbumin (PV) and the distribution of their synapses within the IPL was studied. Finally both markers (PKC and PV antibodies) were applied together to reveal synapses between rod bipolars and AII-amacrines, and to study the general features of the scotopic circuitry of the rat retina.

MATERIALS AND METHODS

Tissue preparation for electronmicroscopy

Four rat retinas were used. The rats were deeply anaesthetized by intraperitoneal injection of 4% chloral hydrate (1 ml/100 g body weight). The eyes were enucleated and the animals were killed immediately afterwards by an overdose of 4% chloral hydrate. The eyes were then opened by an encircling cut and the posterior eyecup was immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 30 minutes, and then the retinas were dissected from the choroid and further immersion fixed in the same fixative for 2 hours at room temperature. After several washes in PB small pieces of the retinas were cut out from the region close to the optic nerve head and immersed in 30% sucrose in PB for 6 hours at 4°C, and then the retinal pieces were rapidly frozen in liquid nitrogen and thawed at room temperature in order to enhance the antibody penetration. The retinal pieces were then embedded in 4% Agar (Merck, Germany) and vertically sectioned with a vibratome at a thickness of 40 μm. The vibratome sections were transferred into 0.1 M phosphate-buffered saline (PBS, pH 7.4) and then processed for free-floating immunostaining.

Immunostaining

Immunostaining was performed by using the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., '81). The vibratome sections were incubated in 10% normal goat serum (NGS) in PBS for 1 hour at room temperature in order to block non-specific binding sites and were then incubated in a mixture of mouse monoclonal antibody against protein kinase C (PKC antibody) and anti-cat parvalbumin antibody in PBS containing 3% NGS for 12 to 18 hours at 4°C. The PKC antibody was purchased from Amersham (clone MC 5; RPN. 536) and the dilution ratio was 1:50. The anti-cat parvalbumin antibody was raised in rabbit (Stichel et al., '86) and diluted 1:2,000. The antibody was kindly provided by Dr. C.C. Stichel. The following immunocytochemical procedures were carried out at room temperature. After washing in PBS for 45 minutes (3 x 15 minutes), sections were incubated in a mixture of biotin-
labelled goat anti-mouse IgG and biotin-labelled goat anti-rabbit IgG for 2 hours. Both secondary antisera were purchased from Sigma and diluted 1:50 in PBS containing 3% NGS. After washing in PBS for 45 minutes (3 x 15 minutes), they were incubated in avidin peroxidase (Sigma) diluted 1:400 in PBS for 1 hour, washed in 0.05 M Tris-HCl buffer (TB), and then incubated in 0.05% 3',3'-diaminobenzidine (DAB) in TB containing 0.01% H₂O₂ to visualize the peroxidase. The reaction was monitored using a low-power microscope and terminated by replacing the DAB and H₂O₂ solution with PB.

The stained sections were fixed in 2.5% glutaraldehyde in PB for 1 hour, and after washing in PB containing 4.5% sucrose for 15 min (3 x 5 minutes), they were then post-fixed in 1% osmium tetroxide in PB for 1 hour. Afterwards they were washed again in PB containing 4.5% sucrose, dehydrated in a graded series of alcohol, during the dehydration procedure they were en bloc stained with 1% uranyl acetate in 70% alcohol for 1 hour, infiltrated in propylene oxide, and embedded in Epon 812 (Serva, Germany). After polymerization at 60°C for 3 days, well-immunostained areas were cut out and attached to Epon support for further sectioning with an ultramicrotome (Reichert-Jung, Germany). Consecutive ultrathin sections (about 90 nm thick) were obtained and mounted on one-hole grids coated with Formvar films. After being contrasted with lead citrate, they were observed with a Zeiss EM 10 electron microscope.

In order to define the kinds and numbers of synapses made by an individual rod bipolar axon, five PKC-labelled rod bipolar axons and their terminals were reconstructed. For reconstruction of one rod bipolar axon, about 40 consecutive sections were needed, and mosaic microphotographs of each axon and axon terminal in the IPL were made with an electron microscope at a final magnification of x20,000. Afterwards the outline of the axon and axon terminal was drawn on tracing paper, and then individual drawings were assembled.

To establish the output synapses of AII amacrine cells, parvalbumin-labelled amacrine cell processes, presumably belonging to AII amacrine cells, were observed in the IPL and the types and relative frequency of their postsynaptic processes were counted with respect to the sublaminae of the IPL.

**Tissue preparation for light microscopy**

Details of staining frozen sections of the rat retina for the light microscopical analysis of PKC-labelled rod bipolar cells are given in Greferath et al. ('90) and Grünert and Martin ('91). The staining of AII-amacrine cells with antibodies against parvalbumin is described in detail in the accompanying paper (Wässle et al., '93).

**RESULTS**

**Immunostaining of the rod pathway**

Two markers were used in the present study to stain rod bipolar cells and AII-amacrine cells: antibodies against...
protein kinase C (PKC) and antibodies against parvalbumin (PV) respectively. Figure 1A shows a vertical section of the rat retina immunostained with antibodies against PKC. Rod bipolar cells and occasionally amacrine cells were found to be labelled (Negishi et al., '88; Greferath and Wässle, '90; Karschin and Wässle, '90; Zhang et al., '91). The section in Fig. 1B was stained for PV-immunoreactivity. A distinctive type of amacrine cell is strongly labelled and based on the results presented in the accompanying paper these are very likely to be AII-amacines. In whole mounted retinae a sparsely distributed second type of amacrine cell is also immunoreactive for PV (Wässle et al., '93). Weak label is also present in ganglion cells.

Semithin and ultrathin sections were cut from vibratome sections, which were double stained for both PKC- and PV-immunoreactivity. Figure 2 shows a low power electronmicrograph of three PKC-labelled rod bipolar cells and one AII-amacine cell. PKC-immunoreactivity is confined to the cytoplasm of the rod bipolars, while PV-immunoreactivity is also found in the nucleus of the AII-amacine cell. AII cell labelling appears denser. In agreement with descriptions from cat and rabbit AII-cells, many large mitochondria can be found in AII-cell processes (see descending dendrite of AII-amacine cell in Fig. 2).

**Rod bipolar cells**

It has been shown for the cat and monkey retina by electronmicroscopy that PKC labelled bipolars have dendritic processes in rod spherules (Greferath et al., '90; Grünert and Martin, '91). Figure 3A,B shows dendrites of labelled bipolar cells invaginating into rod spherules. Horizontal cell processes, the synaptic ribbons, and the labelled (H) and a labelled central element, a putative rod bipolar cell dendrite (RB), form a triad. B: Another rod spherule containing a triad with a labelled rod bipolar dendrite (RB) forming the central element. Scale bar = 2 μm.
ROD PATHWAY OF THE RAT RETINA

Fig. 4. Vertical section through the inner part of a rat retina stained for PKC- and PV-immunoreactivity. The section passes through a PKC-labelled rod bipolar axon (RBA) and its terminal (RBT). In the upper half of the micrograph the rod bipolar axon makes a ribbon synapse (large arrow) onto a PV-labelled AII-amacrine cell process (AII) and an unlabelled amacrine cell process (A*). This in turn makes a reciprocal synapse onto the rod bipolar cell (open arrow). In the lower half two other such postsynaptic pairs (AII, A) are marked by circles. Small arrows show input synapses of the rod bipolar axon from unlabelled amacrine cells. The arrowhead indicates another synaptic ribbon. Scale bar = 1 μm.

'90). However, it is also possible that one of the labelled postsynaptic processes belongs to the type of widefield amacrine which also expresses PV-immunoreactivity, as described in the preceding paper.

Table 1 and Figure 6 show synapses observed at five rod bipolar cell terminals. They were completely reconstructed from serial sections. An individual rod bipolar terminal makes on the average 15 ribbon synapses. In nearly all of these one postsynaptic member is an AII-amacrine process and the other one is an unstained amacrine cell process, which provides a feedback synapse onto the rod bipolar cell in about half of the dyads. No ganglion cell dendrites were contacted directly by rod bipolar cells. Rod bipolar cell axons receive on the average 16 synapses from amacrine cells, including conventional as well as reciprocal synapses.

As can be seen from the reconstructions (Fig. 6), ribbon synapses are mainly found at the axon terminal of rod bipolars in the inner third of the IPL, while some conventional input synapses are also present along the shaft of the axon in the outer part of the IPL (Sterling and Lampson, '86). The axon terminals have a bulbous appearance and are made up of one to three branches.

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AII-amacrine cells

The following description concentrates on output synapses made by AII-amacrine cells. AII-amacrines as well as a type of widefield amacrine cell are immunoreactive for PV, and an isolated PV-labelled process could belong to either class (Wässle et al., '93). By following a process through several sections, by defining the stratum where it is found and by ultrastructural criteria, we could in many instances positively identify AII-cell processes. The ultrastructural preservation of the antibody treated sections was not ideal, however, with some experience it was possible to reliably identify the synapses made by AII-cells. Figure 7A,B shows part of a lobular appendage of an AII-cell in stratum S2. The retina was immunolabelled with antibodies against PV. A cone bipolar process (CB) is inserted into the stained lobular appendage. The cone bipolar cell makes a ribbon synapse (arrowhead) and receives a reciprocal synapse (open arrow) from an unidentified amacrine cell (A*). At the process inserted into the stained AII-cell lobular appendage the cone bipolar receives an input synapse both in Figure 7A,B (arrows). Figure 7C,D shows two other consecutive sections through an AII-amacrine cell body and the primary dendrite (PD). The cone bipolar cell makes a ribbon synapses (arrowhead) in Figure 7C, and the AII-cell primary dendrite (PD) is postsynaptic at this ribbon synapse. The AII-cells makes also an output synapse (Fig. 7D, arrow) onto the bipolar cell.

This example shows that output synapses into cone bipolars are not restricted to lobular appendages; however, as shown in Table 2, such synapses are restricted to the outer third of the IPL, where OFF cone bipolar axons and OFF-ganglion cell dendrites are found. We therefore conclude that most of the chemical output synapses of AII-amacrines cells are made onto OFF-cone bipolar cells. Occasionally we also observed lobular appendages of AII-amacrine cells penetrating the INL. In one case we followed such a process through a series of sections and found an output synapse onto a bipolar cell perikaryon (not shown).

We also observed output synapses from PV-labelled processes onto ganglion cell dendrites. Figure 8A shows such a synapse from stratum S2 and Figure 8B from stratum S4. Ganglion cell dendrites are also lightly labelled by PV-immunocytochemistry (Fig. 1B); hence the putative
Fig. 5. Consecutive sections through the axon terminal (RBT) of a PKC-labelled rod bipolar cell from stratum 5 of the IPL. A: Two synaptic ribbons which are presynaptic at dyads are marked by arrows. The postsynaptic elements at the upper dyad are a PV-labelled AII-amacrine cell (AII) and an unlabelled amacrine cell (A*). An AII-amacrine cell (AII) and an unlabelled amacrine cell (A) are also postsynaptic at the lower dyad. B: This consecutive section shows that process A* provides a reciprocal synapse (open arrow) back onto the rod bipolar cell, axon terminal (RBT). Another amacrine cell (marked by 'a' in A and B) also makes a synapse with the rod bipolar cell axon terminal (arrowhead). Scale bar = 1 μm.
rod amacrine cell makes a large gap junction (arrowhead) (Vaney, '91). It is interesting in this context that rod bipolar widefield amacrines and not to the AII-amacrine. Similar selective staining has been reported for most of their input synapses in strata S4 and S5, they make gap junctions in stratum S5, gap junctions between two labelled processes. These are probably AII-cell dendrites (Fig. 9B).

As summarized in Table 2, AII-amacrine cells have a clear stratification of their contacts in the IPL. They receive most of their input synapses in strata S4 and S5; they make their chemical output synapses preferentially in S1 and S2, and they make gap junctions in S3 and S4. They thus distribute signals within a narrow column within the IPL, which is the reason why Masland ('86, '88) considered AII-cells to be “through-conducting” neurons in contrast to other amacrine cells, which have horizontally extended functions.

**DISCUSSION**

**Rod bipolar cells**

Rod bipolar cells of the rat retina could be stained with antibodies against the α and β subunits of PKC (Negishi et al., '88; Greferath et al., '90; Karschin and Wässle, 1990). By showing their dendritic tips to be connected to rod spherules we proved that these cells are actually rod bipolar. Similar selective staining has been reported for the primate retina (Grünert and Martin, '91), the cat retina (Greferath et al., '90), and the rabbit retina (Young and Vaney, '91). It is interesting in this context that rod bipolar cells of the tree shrew (Tupaia glis) are also selectively labelled by the same antibody: the three shrew is clearly cone dominated and rods form only 5% of its photoreceptors (Müller and Peichl, '91). This shows that independent of the actual rod/cone ratio, rod bipolar cells are a well-defined homogeneous cell type in different mammalian retinæ.

It is also shown in the present paper by electron microscopy that rod bipolar cells make no direct output synapses onto ganglion cells. Approximately 15 ribbon synapses were found per rod bipolar axon and at practically all of the dyads one postsynaptic element was an AII-amacrine cell process. The detailed analysis and recognition of processes were greatly simplified by the use of two markers: PKC for the rod bipolar cells and PV for the AII-amacrinæ. The number of dyads made by rat rod bipolar cells is about 15 (range 11–18). This value is comparable to the 14–32 of rabbit rod bipolar cells (Strettoi et al., '90), and to the 7–23 of monkey rod bipolar cells (Grünert and Martin, '91). Cat rod bipolar cells make between 27 and 43 ribbon synapses (Kolb, '79; McGuire et al., '84). In all four mammals the second postsynaptic member of the dyad was an amacrine cell, which often made a reciprocal synapse back onto the bipolar axons. In the rabbit and in the cat retina, A17 amacrines quite frequently are the second member at the dyad (Kolb and Nelson, '83; Raviola and Dacheux, '87). Although A17-like amacrines have been described for the rat retina (Perry and Walker, '80), their involvement at the rod bipolar cell dyad still has to be demonstrated. A-17 cells have been shown to accumulate 3H-muscimol (Pourcho and Goebel, '83) and to contain endogeneous GABA (Chun and Wässle, '89; Wässle and Chun, '89; Sandell et al., '89); hence it is likely that the feedback of the reciprocal synapse involves GABAergic inhibition. Rod bipolar cells of all mammals, including rat, also have substantial input from other amacrine cells at conventional synapses along the axon (Sterling and Lampont, '96; Freed et al., '87). GABA has also been localized in some of these synapses (Chun and Wässle, '89; Freed et al., '87; Pourcho and Owczarzak, '89; Koontz and Hendrickson, '90). In the rat retina, rod bipolar cell axons have been shown to receive input synapses from glutamic acid decarboxylase (GAD) immunoreactive profiles (Vaughn et al., '81). All this suggests that a substantial proportion of the synapses, which rod bipolar axons receive in the IPL are from GABAergic amacrine cells.

Patch clamp recordings from isolated dissociated rod bipolar cells of the rat retina (Karschin and Wässle, '90; Suzuki et al., '90; Yeh et al., '90) have shown that GABA gates a Cl−-conductance in rod bipolar cells. Rod bipolar cells are therefore very likely under GABAergic control. Recent evidence suggests an unusual pharmacology of the GABA receptor Cl−-channels in rat retinal bipolar cells (Feigenspan et al., '93).

Rod bipolar cells have also glycine gated Cl−-channels (for review, see Wässle et al., '91), but less evidence is available about input from gicnicergic synapses (Pourcho and Owczarzak, '91).

**Allamacrine cells**

Allamacrine cells are rod bipolar cells, have been found to be a constant component of mammalian retinal circuitry (for review, see Vaney, '90; Vaney et al., '91a,b). In the rat retina they were first stained with the Golgi-method by Perry and Walker ('80). Later they were intracellularly injected with Lucifer Yellow (Voigt and Wässle, '87; Wässle et al., '93). They were immunostained in the preceding and in the present paper with PV-immunocytochemistry. However, since another amacrine cell turned out to be also PV-immunoreactive, additional criteria had to be used for the positive identification of Allamacrine cell processes. These were the characteristic lobular appendages, the bistratified organization of the dendrite tree with the long descending primary dendrite (Fig. 2), and finally ultrastruc-

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**TABLE 1. Kinds and Numbers of Synapses Found on Five Rod Bipolar Cells Which Were Completely Reconstructed From Ultrathin Serial Sections**

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Fig. 6. Reconstructions of five rod bipolar axons from electronmicrographs of serial ultrathin sections. Ribbon synapses (stars) and non-reciprocal conventional synapses (squares) are mainly found at the axons in the inner third of the IPL. Scale bar = 2 μm.

...tural criteria, such as their large mitochondria (Famiglietti and Kolb, '75; Kolb '79). All-amacrine cells of the rat retina have the same functional segmentation of their dendritic tree, which has already been described for cat and rabbit AII-cells (Kolb, '79; Kolb and Famiglietti, '74; Famiglietti and Kolb, '75; Sterling, '83; Sterling et al., '88; Strettoi et al., '92). They enter with chemical (presumed inhibitory) synapses into the OFF-cone pathway and make gap junctions into the ON-cone pathway. Their preferential target in the OFF-cone pathway is the OFF-cone bipolar cell; these cells are mainly postsynaptic at the lobular appendages. An interest-
Fig. 7. Electron micrographs of vertical sections through a rat retina stained for PV-immunoreactivity. A and B show two consecutive sections from stratum 2 of the IPL. A: The labelled lobular appendage (AII) of an AII-amacrine cell surrounds the axon of a cone bipolar cell (CB) and makes a conventional synapse (closed arrow). The cone bipolar cell contains a synaptic ribbon (arrowhead). Two amacrine cells (A*, A) are the postsynaptic targets at the ribbon. One of the amacrine cells (A*) makes a reciprocal synapse (open arrow). B: The labelled lobular appendage (AII) makes a second conventional synapse (arrow) onto the cone bipolar cell (CB). C and D are two sections apart and show the nucleus (N), the lower part of the cell body, and the primary dendrite (PD) of a PV-immunoreactive AII-amacrine cell. C: A cone bipolar cell (CB) makes a ribbon synapse (arrowhead) at which the primary dendrite (PD) is postsynaptic. D: The same cone bipolar cell (CB) receives a conventional synapse (arrow) from the labelled AII cell primary dendrite (PD). Scale bars = 0.5 μm.
ing detail of the rat retina is that such lobular appendages are found not only in the outer part of the IPL, but also in between cell bodies at the inner border of the INL. As demonstrated in Table 2, we also observed many output synapses from PV-labelled dendrites onto ganglion cell processes. Even if not all the labelled processes belong to AII-cells, it seems that rat AII-cells make more direct output into ganglion cell dendrites than those of the cat or rabbit retina (Strettoi et al., '92).

The gap junctions made by AII-amacrines were exclusively with putative ON-cone bipolars or with other AII-amacrines. No gap junctions with ganglion cell dendrites were observed. The chemical output synapses of AII-amacrines are probably sign inverting, since there is evidence from the cat retina (Pourcho and Goebel, '85; Pourcho and Owczarzak, '91) that AII cells release the inhibitory transmitter glycine. The gap junctions probably represent electrical synapses, which are usually sign conserving. Whether they are rectifying (Hille, '91) still has to be established. Recent work by Vaney ('91) has shown that they provide dye coupling under certain physiological conditions.

AII-cells receive many different kinds of input synapses from several different sources. At practically all rod bipolar cell ribbon synapses, AII-amacrines are one member of the dyad; hence in dark adapted retina they receive a major input from rod bipolar cells. However, AII-amacrine cells are also postsynaptic at ribbon synapses made by putative OFF-cone bipolars in the outer half of the IPL (Fig. 7C,D). Such synapses are very likely to be excitatory. This input is difficult to understand because AII-cells provide inhibitory input to the same bipolars, and their light responses have opposite polarity: AII-cells are ON-cells, while the bipolars are OFF-cells. It has been proposed by Strettoi et al. ('92) that the function of this reciprocal connection would be negative feedback from AII-cells onto OFF-cone bipolars. Along the same line of reasoning we would like to add a further speculation about this OFF-cone bipolar input. In complete darkness OFF-cone bipolars should be depolarized and would in turn depolarize AII-cells. However because depolarized AII-cells release glycine, which will inhibit those OFF-cone bipolars, the membrane potentials of OFF-cone bipolars and AII-cells might keep each other in a moderate depolarization. This depolarization of AII-cells in darkness might be important for their light sensitivity. AII-cells generate action potentials (Dacheux and Ravila, '86; Boos et al., '93), and the slight depolarization could keep the membrane close to spike threshold. Any input from rod bipolars would thus generate action potentials in AII-cells. Hence, the input from OFF-cone bipolars could sensitize AII-cells.

Another major source of input into AII-amacrine cells are other amacrine cells. We have not systematically investigated such synapses in the present paper. However, in a previous study of rat AII-amacrine cells from our laboratory (Voigt and Wässle, '87) it was shown that dopaminergic amacrine cells provide a prominent input into AII-cells. Like those of other mammals, rat AII-cell bodies are surrounded by dopaminergic “rings,” where they receive many large synapses (Törk and Stone, '79; Pourcho, '82; Kolb et al., '90, '91). In the fish retina dopamine has been shown to regulate electrical coupling between horizontal
Fig. 9. Vertical sections through the IPL stained for PV-immunoreactivity. A: The labelled vertical running process is an AII-amacrine cell dendrite. It makes a gap junction (arrowhead) in stratum S3 with a bipolar cell axon (CB). The bipolar cell makes a ribbon synapse (arrow) onto a ganglion cell (G) and an unidentified (U) process. B: Two labelled processes in stratum S5 make a gap junction (arrowheads). Scale bar = 0.5 μm.

### Physiology of the rod pathway of the rat retina

The rod bipolar cell of the rat retina has been shown to express the APB-type of glutamate receptor (Yamashita and Wässle, '91). It is therefore very likely that the light responses of rat rod bipolar cells are depolarizing. AII-amacrine cells of the rat retina express a conventional glutamate receptor of the kainate or AMPA-type (Boos et al., '93) and therefore will also give depolarizing light responses. If rat AII-amacrine cells, like those of the cat and of the primate retina, were glycinergic they could produce signals of opposite polarity in ON- and OFF-cone bipolar and ganglion cells. Through the chemical synapses at the lobular appendages they could inhibit the OFF-cone pathway and through the gap junctions they could excite the ON-cone pathway.

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### LITERATURE CITED


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