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Distribution of GABA and Glycine Receptors on Bipolar and Ganglion Cells in the Mammalian Retina

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ABSTRACT The amino acids GABA and glycine mediate synaptic transmission via specific neurotransmitter receptors. Molecular cloning studies have shown that there is a great diversity of GABA and glycine receptors. In the present article, the distribution of GABA and glycine receptors on identified bipolar and ganglion cell types in the mammalian retina is reviewed. Immunofluorescence obtained with antibodies against GABA and glycine receptors is punctate. Electron microscopy shows that the puncta represent a cluster of receptors at synaptic sites. Bipolar cell types were identified with immunohistochemical markers. Double immunofluorescence with subunit-specific antibodies was used to analyze the distribution of receptor clusters on bipolar axon terminals. The OFF cone bipolar cells seem to be dominated by glycinergic input, whereas the ON cone bipolar and rod bipolar cells are dominated by GABAergic input. Ganglion cells were intracellularly injected with Neurobiotin, visualized with Streptavidin coupled to FITC, and subsequently stained with subunit specific antibodies. The distribution and density of receptor clusters containing the α1 subunit of the $GABA_A$ receptor and the α1 subunit of the glycine receptor, respectively, were analyzed on midget and parasol cells in the marmoset (a New World monkey). Both $GABA_A$ and glycine receptors are distributed uniformly along the dendrites of ON and OFF types of parasol and midget ganglion cells, indicating that functional differences between these subtypes of ganglion cells are not determined by GABA or glycinergic input. *Microsc. Res. Tech. 50: 130–140, 2000.*

INTRODUCTION

The major inhibitory neurotransmitters in the mammalian retina are γ-aminobutyric acid (GABA) and glycine (reviewed by Marc, 1989; Pourcho and Goebel, 1990; Vaney, 1990; Wässle and Boycott, 1991; Freed, 1992). GABA is present in horizontal cells, in some bipolar cells, and in about half of the amacrine population. Glycine-containing amacrine cells make up the other half of the amacrine cell population.

Electrophysiological recordings from bipolar and ganglion cells show that they respond to GABA and/or glycine and their agonists (Bolz et al., 1985a,b; Tauck et al., 1988; Karschin and Wässle, 1990; Suzuki et al., 1990; Ishida, 1992; Müller et al., 1992; Feigenspan et al., 1993; Rörig and Grantyn, 1993; Zhou et al., 1994; Gillette and Dacheux, 1995; Hartveit, 1997; Protti et al., 1997; Euler and Wässle, 1998; Tian et al., 1998).

Consistently, electron microscopic studies revealed synaptic input from GABA and glycinergic amacrine cells to bipolar cell axons and ganglion cell dendrites (Marc and Liu, 1985; Freed et al., 1987; Hendrickson et al., 1988; Chun and Wässle, 1989; Pourcho and Owczarzak, 1989; Grünert and Wässle, 1990; Koontz and Hendrickson, 1990; Kim et al., 1998; Owczarzak and Pourcho, 1999). Thus, nearly all cell types in the retina receive GABAergic and/or glycinergic input.

Three pharmacologically and physiologically distinct types of GABA receptors (termed A, B, and C) have been described (Bormann, 1988; Bormann and Feigenspan, 1995; Johnston, 1996). The $GABA_A$ and $GABA_C$ receptors are ionotropic receptors that mediate fast synaptic transmission, whereas $GABA_B$ receptors are metabotropic receptors. $GABA_A$ receptors are composed of five subunits that form a chloride channel (Seeburg et al., 1990). To date, 14 $GABA_A$ receptor subunits have been cloned: six α, four β, three γ, and one ϵ subunit. Despite the enormous number of theoretically possible subunit combinations, it is thought that the number of major $GABA_A$ receptor subtypes is less than ten (McKernan and Whiting, 1996). In the brain, a heterogeneous distribution of different subunits has been demonstrated (Laurie et al., 1992; Wisden et al., 1992; Fritschy and Mohler, 1995). In the mammalian retina, $GABA_A$ receptors are localized on amacrine, bipolar, and ganglion cells (reviewed by Wässle et al., 1998).

$GABA_B$ receptors couple with G-proteins and activate second messenger systems (Bowery, 1989, 1993; Kaupmann et al., 1997, 1998). Presynaptic $GABA_B$ receptors regulate Ca^{2+} channels and thus affect neurotransmitter release, whereas postsynaptic $GABA_B$ receptors regulate K^+ channels, suggesting that there are two functional types (Bettler et al., 1998). In the mammalian retina, $GABA_B$ receptors were found presynaptically and/or postsynaptically on horizontal cells, amacrine, and ganglion cells by immunohistochemistry (Koulen et al., 1998b) and by in situ hybrid-

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ization (Zhang et al., 1998). $GABA_B$ receptors have not been found on mammalian bipolar cells, neither in electrophysiological recordings nor by immunohistochemical staining or in situ hybridization (Suzuki et al., 1990; Yeh et al., 1990; Gillette and Dacheux, 1995; Koulen et al., 1998b; Zhang et al., 1998).

$GABA_C$ receptors differ significantly from $GABA_A$ and $GABA_B$ receptors in their pharmacological and biophysical properties (reviewed by Bormann and Feigenspan, 1995; Johnston, 1996; Feigenspan and Bormann, 1998). $GABA_C$ receptors are composed of ρ subunits exclusively (ρ1, ρ2, ρ3 subunits, reviewed by Enz and Cutting, 1998) and are predominantly expressed in retina. In mammalian retina $GABA_C$ receptors are only found on bipolar cells (Wässle et al., 1998).

Glycine receptors are ionotropic receptors, composed of five subunits that form a chloride channel (reviewed by Langosch et al., 1990). To date at least three different α subunits (α1, α2, α3) and one β subunit have been identified. In situ hybridization and immunohistochemistry show that different glycine receptor subunits in rat retina have distinct distributions (Greferath et al., 1994a). Glycine receptors are expressed by bipolar, amacrine, and ganglion cells (Greferath et al., 1994a; Sassoè-Pognetto et al., 1994; Grünert and Wässle, 1996).

An extensive review of the distribution of GABA and glycine receptors in the mammalian retina was recently published (Wässle et al., 1998). The functional diversity of GABA receptors in the retina has also been reviewed recently (Lukasiewicz, 1996; Feigenspan and Bormann, 1998; Lukasiewicz and Shields, 1998). The present review focuses on the distribution of GABA and glycine receptors on identified bipolar and ganglion cell types.

Bipolar cells are the most numerous interneurones in the retina (Missotten, 1974; Martin and Grünert, 1992; Grünert et al., 1994; Euler and Wässle, 1995; Strettoi and Masland, 1995; Jeon et al., 1998). They transfer the signal from the photoreceptors to the inner retina. A single type of rod bipolar cell, which connects to rods exclusively (Cajal, 1893; Wässle et al., 1991), and several types of cone bipolar cell (reviewed by Wässle, 1999) have been found in all mammalian species studied to date. Cone bipolar cells differ with respect to the number of cones they contact, the location of their soma within the inner nuclear layer, and the stratification of their axon in the inner plexiform layer.

Ganglion cells can be subdivided into many physiological and morphological classes (reviewed by Wässle and Boycott, 1991). One crucial morphological identification criterion is the stratification level of their dendrites. OFF-center ganglion cells stratify in the outer half of the inner plexiform layer (IPL), whereas ON-center ganglion cells stratify in the inner half of the IPL (Nelson et al., 1978). Thus, it is likely that different ganglion cell types are involved with different types of amacrine and bipolar cells.

$GABA_A$ RECEPTOR SUBUNITS IN THE INNER RETINA

Figure 1A,B shows a vertical cryostat section through a retina of a New World monkey, the common marmoset Callithrix jacchus, which was stained with antibodies against the α1 subunit of the $GABA_A$ receptor. Strong punctate immunoreactivity is present in the entire IPL. Similar staining patterns have been observed in other mammalian retinae, including, rat, rabbit, and macaque monkey (Brecha, 1992; Grünert et al., 1993; Greferath et al., 1994b, 1995).

In addition to the α1 subunit, nearly all $GABA_A$ receptor subunits described so far have been localized in retina (reviewed by Wässle et al., 1998). Early immunohistochemical studies found the α1 and/or β2/3 subunits to be expressed by ganglion, bipolar, and amacrine cells (Mariani et al., 1987; Richards et al., 1987; Hughes et al., 1989, 1991; Brecha, 1992; Vardi et al., 1992; Greferath et al., 1993b, 1994b; Grünert et al., 1993; Grünert and Hughes, 1993; Vardi and Sterling, 1994). More recently, immunocytochemical and in situ hybridization studies using antibodies and probes against various $GABA_A$ receptor subunits, respectively, showed that each subunit shows a distinct expression pattern (Greferath et al., 1995; Gutiérrez et al., 1996; Khan et al., 1996). For example, the γ2 subunit is distributed most abundantly, whereas the δ subunit has the most restricted distribution (Fig. 2).

In order to find out which subunit compositions are present in the rat retina, double-labeling experiments were carried out. The combination of α1/β2,3/γ2 subunits was most common (Greferath et al., 1995). Different subunit combinations could be attributed to distinct cell types, e.g., cholinergic amacrine cells are thought to express α2, β1, β2/3, δ, and γ2 subunits (Greferath et al., 1993a, 1995; Brandstätter et al., 1995). Similarly, it was found in rabbit retina that cholinergic amacrine cells express the β2/3 subunits but do not express the α1 subunit (Zucker and Ehinger, 1998), whereas bistratified DAPI-3 amacrine cells (Vaney, 1990; Wright et al., 1997) show high concentrations of the α1 and β2/3 subunits (Zucker and Ehinger, 1998). Thus, there is a great diversity among $GABA_A$ receptors in the retina, suggesting that different receptor subunits participate in different functional synaptic circuits.

Glycine Receptor Subunits in the Inner Retina

Figure 1C,D shows a vertical cryostat section through marmoset retina stained with an antibody against the α1 subunit of the glycine receptor. Immunoreactivity is concentrated in two broad bands of the IPL (see also Grünert and Ghosh, 1999). The outer band contains more puncta than the inner band. The staining pattern for the α1 subunit of the glycine receptor is comparable in all mammalian species studied, including rat, cat, rabbit, mouse, and macaque monkey (Grünert and Wässle, 1993, 1996; Greferath et al., 1994a; Pinto et al., 1994; Sassoè-Pognetto et al., 1994).

Immunostaining with an antibody against all subunits of the glycine receptor is more abundant than immunostaining for the α1 subunit alone (Grünert and Wässle, 1993; Greferath et al., 1994a), indicating that not all glycine receptors in the retina contain the α1 subunit. Results from in situ hybridization (Greferath et al., 1994a) and single cell polymerase chain reaction (PCR) (Enz and Bormann, 1995) support the idea that glycine receptor subunits are differentially distributed. The α1 subunit seems to be expressed predominantly by bipolar cells and some, but not all, ganglion cells. The α2 subunit in the inner nuclear layer is probably

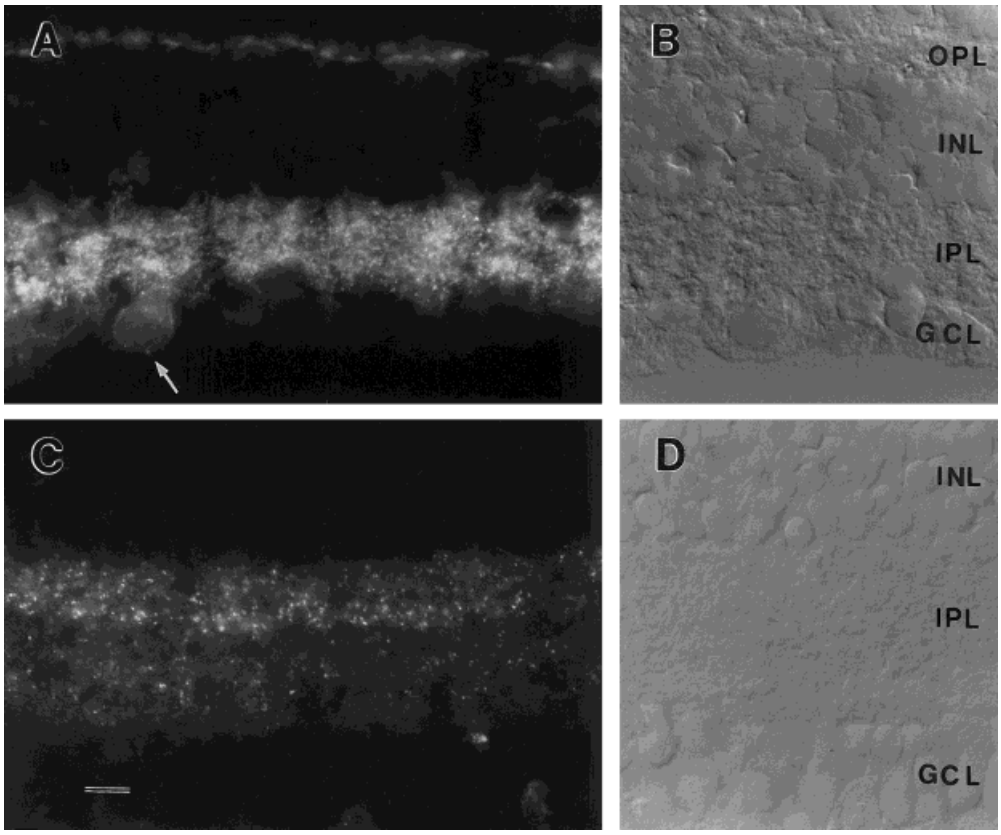

Fig. 1. Micrographs of vertical cryostat sections through marmoset retina immunolabeled with antibodies against the α1 subunit of the $GABA_A$ receptor (**A**,**B**) and the α1 subunit of the glycine receptor (**C**,**D**). **A**,**C**: Fluorescence micrographs. **B**,**D**: Nomarski micrographs of the sections revealing the retinal layers. Strong punctate immunoreactivity is present in the inner plexiform layer (IPL) with both antibodies. The antibody against the α1 subunit of the $GABA_A$ receptor also shows a more diffuse staining in the outer plexiform layer (OPL), inner nuclear layer (INL), and the ganglion cell layer (GCL). Some ganglion cell somata show hot spots of immunoreactive puncta (arrow). Scale bar = 10 μm.

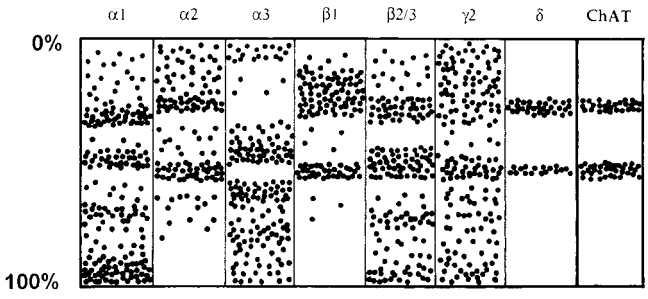

Fig. 2. Schematic diagram summarizing the expression of $GABA_A$ receptor subunits in the IPL of the rat retina. The border with the INL corresponds to 0%; the border with the ganglion cell layer corresponds to 100%. Each subunit shows a characteristic staining pattern. Modified from Greferath et al. (1995).

expressed mainly by amacrine cells and by nearly all cells in the ganglion cell layer. The α3 subunit is expressed in the entire inner nuclear layer and to a lesser extent in the ganglion cell layer. It was difficult to attribute this subunit to certain cell types. The β subunit showed a widespread distribution, consistent with the idea that is required to form functional glycine receptors (Bormann et al., 1993).

Gephyrin in the Inner Retina

Gephyrin is a peripheral membrane protein that copurifies with the postsynaptic glycine receptor in spinal cord (Betz et al., 1994). Gephyrin binds to subsynaptic tubulin and is essential for the clustering and anchoring of glycine receptors (Kirsch et al., 1991, 1993b; Prior et al., 1992). In the retina as well as in other regions of the brain gephyrin is expressed abundantly and is present in regions where no glycine receptors are found (Grünert and Wässle, 1993; Kirsch and Betz, 1993; Greferath et al., 1994a; Zucker, 1998). Electron microscopic studies showed that in the IPL gephyrin is localized postsynaptically on amacrine and ganglion cell processes but not on bipolar cells (Pourcho and Owczarzak, 1991; Grünert and Wässle, 1993; Sassoè-Pognetto et al., 1994). Double immunofluorescence experiments in rat retina showed that gephyrin clusters are extensively colocalized with α2 and γ2 subunits of the $GABA_A$ receptor (Sassoè-Pognetto et al., 1995). Since $GABA_A$ and glycine receptors are not colocalized (Sassoè-Pognetto et al., 1995), the colocalization of $GABA_A$ receptor subunits and gephyrin indicated that gephyrin is also involved with the clustering of $GABA_A$ receptors. This hypothesis has recently been supported by experiments with γ2 subunit-deficient

mice, showing that the loss of the γ2 subunit is accompanied with the loss of gephyrin and synaptic GABAergic function (Essrich et al., 1998). It thus has been suggested that both the γ2 subunit of the $GABA_A$ receptor and gephyrin are essential for $GABA_A$ receptor clustering.

GABA AND GLYCINE RECEPTORS ARE CLUSTERED AT POSTSYNAPTIC SITES

Electron microscopy demonstrated that the punctate immunoreactivity seen in the light microscope (Fig. 1) represents a cluster of receptors at postsynaptic sites. Figure 3 shows an example of a labeled synapse in macaque retina. Immunoreactivity for the α1 subunit of the glycine receptor is concentrated in the synaptic cleft (see also Sassoè-Pognetto et al., 1994; Grünert and Wässle, 1996) consistent with the finding that the antibody recognizes an extracellular epitope of the receptor (Schröder et al., 1991). Similarly, it has been shown that immunoreactivity for $GABA_A$ (Nusser et al., 1995a,b; Sassoè-Pognetto et al., 1995) and $GABA_C$ (Fletcher et al., 1998; Koulen et al., 1998a) receptor subunits is concentrated at synapses. Thus, double-labeling experiments make it possible to study the distribution GABA and glycinergic synapses on specific neurons by light microscopy (Sassoè-Pognetto et al., 1994; Grünert and Wässle, 1996; Koulen et al., 1996; Wässle et al., 1998). In the following the distribution of $GABA_A$, $GABA_c$, and glycine receptors on identified bipolar cell types is summarized. As outlined above, $GABA_B$ receptors have not been found on mammalian bipolar cells.

Localization of $GABA_A$ and $GABA_C$ Receptor Subunits on Bipolar Axons

$GABA_A$ receptor subunits on bipolar cells have been analyzed by a number of methods. Immunohistochemical staining at the light and electron microscopic level demonstrated that α1, β2/3, and γ2 subunits are expressed by rod and cone bipolar cells (Brecha, 1992; Greferath et al., 1993b, 1994b; Grünert and Hughes, 1993; Vardi and Sterling, 1994). In situ hybridization confirmed these findings (Greferath et al., 1993b, 1995). Single cell PCR detected β1 and β3 subunits on the majority of bipolar cells investigated and the β2 subunit on only a few bipolar cells (Grigorenko and Yeh, 1994), suggesting that different bipolar cell types express different β subunits.

Patch clamp recordings from rod and cone bipolar cells showed that they have both $GABA_A$ and $GABA_C$ receptors (reviewed by Feigenspan et al., 1993; Euler and Wässle, 1998). The proportion of $GABA_A$ and $GABA_C$ mediated responses was different for different bipolar cell types and the $GABA_C$ response dominated in rod bipolar cells (Euler and Wässle, 1998). Single cell PCR detected ρ1 and ρ2 subunits of the $GABA_c$ receptor in rod bipolar cells (Enz et al., 1995; Yeh et al., 1996) and immunocytochemistry showed the presence of these subunits on rod and cone bipolar axon terminals (Enz et al., 1996; Koulen et al., 1997, 1998a).

Localization of $GABA_A$ and $GABA_C$ Receptor Subunits on Rod Bipolar Axons

GABAergic synapses provide the majority of inhibitory input to rod bipolar axon terminals (Freed et al., 1987; Kim et al., 1998). Recently, the distribution of α1, α2, α3, and γ2 subunits of the $GABA_A$ receptor and the subunits of the $GABA_c$ receptor has been analyzed quantitatively on rod bipolar axons in rat and rabbit retinae (Fletcher et al., 1998). Double-labeling experiments were carried out with antibodies against protein kinase C to label rod bipolar cells (Greferath et al., 1990) and antibodies against $GABA_A$ and $GABA_C$ receptor subunits. Fletcher et al. (1998) found on average 30 α3-positive and 60 ρ-positive puncta on rod bipolar axons in peripheral rabbit retina. Thus, rod bipolar axons in rabbit retina express at least three $GABA_A$ receptor subunits (α1, α3, and γ2), and ρ subunits of the $GABA_C$ receptor. A single rod bipolar varicosity in rat retina had on average three ρ subunit clusters, two clusters of each α1, α3, and γ2 subunits but usually no α2 subunit cluster. Thus, a single rod bipolar axon terminal in rat retina has between 14 and 21 GABA receptor clusters. Previous studies showed that α1, α2, α3, and ρ subunits do not colocalize (Greferath et al., 1995; Koulen et al., 1996, 1998a) and thus are present at different synaptic sites. These findings taken together suggest that rod bipolar axons express at least three different types of GABA receptors: $GABA_A$ receptors, which contain the α1 subunit, $GABA_A$ receptors, which contain the α3 subunit, and $GABA_C$ receptors. The question whether different amacrine cell types are presynaptic to each of these receptors was addressed in a recent study by Fletcher and Wässle (1999). They found that indoleamine-accumulating amacrine cells are presynaptic to rod bipolar axons through $GABA_C$ receptors and possibly through $GABA_A$ receptors containing the α3 subunit.

Localization of the α1 Subunit of the Glycine Receptor on Cone Bipolar Axons

Using electron microscopy we found that in rat (Sassoè-Pognetto et al., 1994) and in macaque retina (Grünert and Wässle, 1996) the majority of synapses involving the α1 subunit of the glycine receptor in the outer half of the IPL were made by amacrine cells onto cone bipolar axons (Fig. 4). In order to find out which bipolar and amacrine cell types are involved with these synapses we carried out double-labeling experiments. In rat retina, we used antibodies against the calcium-binding protein recoverin to label two populations of bipolar cells (Milam et al., 1993), termed Cb2 and Cb8 (Euler and Wässle, 1995). The axons of Cb2 cells terminate in the outer half (OFF-sublamina) of the IPL and the axons of Cb8 cells terminate in the inner half (ON-sublamina) of the IPL. Consistently, patch clamp recordings have shown that they are OFF- and ON-bipolar cells, respectively (Euler et al., 1996; Hartveit, 1997). As shown in Figure 4, the majority of subunit immunoreactive puncta in the outer half of the IPL colocalized with the axon terminals of Cb2 cells (Sassoè-Pognetto et al., 1994). In macaque retina, recoverin labels OFF-midget bipolar cells (Milam et al., 1993; Grünert et al., 1994; Wässle et al., 1994) and similar double-labeling experiments showed that the majority of α1 subunit immunoreactive puncta were colocalized with the axon terminals of OFF-midget bipolar cells (Grünert and Wässle, 1996). Other double-labeling experiments in macaque retina indicated that the axons of calbindin-labeled diffuse bipolar cells (DB3 cells)

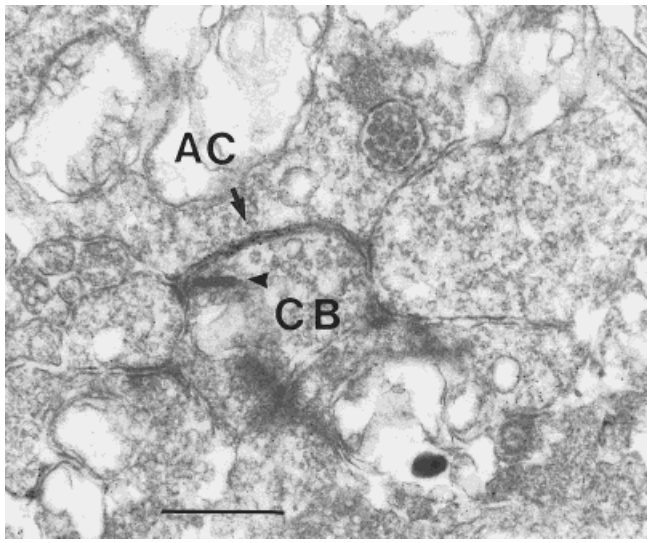

Fig. 3. Electron micrograph showing the ultrastructural localization of the α1 subunit of the glycine receptor in macaque monkey retina. An amacrine cell (AC) makes a conventional synapse (arrow) onto a cone bipolar axon (CB). The arrowhead points to the synaptic ribbon of the cone bipolar terminal. The peroxidase reaction product was silver-intensified and gold-toned. Immunoreactivity is concentrated in the synaptic cleft. Scale bar = 0.4 μm.

Fig. 4. Drawing of a section through rat retina double-labeled for recoverin immunoreactivity and the α1 subunit of the glycine receptor. Only the INL and the IPL are shown. The axon terminals of Cb2 bipolar cells terminate form a broad band in the outer half of the IPL, the axon terminals of Cb8 bipolar cells form a more narrow band in the inner half of the IPL. Most of the immunoreactive puncta (indicated by black dots) are colocalized with the axon terminals of Cb2 cells. Modified from Sassoè-Pognetto et al. (1995).

(Boycott and Wässle, 1991; Grünert et al., 1994), which stratify in the OFF sublamina, colocalize with some of the α1 positive puncta (Grünert and Wässle, 1996). These findings suggested that OFF cone bipolar cells receive glycinergic synapses involving the α1 subunit.

In order to identify the presynaptic process at this synapse we performed similar double-labeling experiments using antibodies to stain AII amacrine cells (Sassoè-Pognetto et al., 1994; Grünert and Wässle, 1996). The AII amacrine cell is the best-characterized glycinergic amacrine cell; it is known to play a crucial role in the scotopic pathway (reviewed by Daw et al., 1990; Wässle et al., 1991). In rat retina, AII amacrine cells can be stained with antibodies against parvalbumin (Wässle et al., 1993) and in macaque retina with antibodies against calretinin (Wässle et al., 1995). In both rat and macaque retinae, double immunofluorescence showed that the majority of α1 immunoreactive puncta colocalized with labeled AII amacrine cell processes in the outer half of the IPL. Thus, the α1 subunit of the glycine receptor is present at the chemical synapses made by AII amacrine cells with OFF-cone bipolar cells.

In the inner part (the ON-sublamina) of the IPL of rat retina, only a small number of α1 positive puncta were colocalized with recoverin-labeled Cb8 axon terminals (Fig. 4, see also Sassoè-Pognetto et al., 1994). Thus, Cb2 cone bipolar cells receive significantly more glycinergic synapses involving the α1 subunit than Cb8 bipolar cells. Chun et al. (1999) found, in an electron microscopic study, that GABAergic input to recoverin-labeled Cb2 cells made up around 50% of the total amacrine input, whereas GABAergic input to recoverin-labeled Cb8 cells made up around 70%. These findings suggest that GABAergic amacrine input predominates for Cb8 (ON bipolar) cells, but glycinergic input predominates for Cb2 (OFF bipolar) cells.

Localization of the α1 Subunit of the Glycine Receptor on Rod Bipolar Axons

Evidence from electron microscopy and electrophysiology has shown that rod bipolar terminals receive input form glycinergic amacrine cells (Freed et al., 1987; Suzuki et al., 1990; Kim et al., 1998). However, as can be seen in Figures 1C and 4 the vast majority of immunoreactive puncta are localized in the outer half of the IPL and only very few puncta are located close to the ganglion cell layer, where rod bipolar axons terminate (Greferath et al., 1990). Two electron microscopic studies analyzed the pre- and postsynaptic elements at α1-positive synapses (Sassoè-Pognetto et al., 1994; Grünert and Wässle, 1996). Both studies concentrated on the outer half of the IPL and thus could not determine whether rod bipolar cells express the α1 subunit. However, results from in situ hybridization showed a strong signal for the α1 subunit in the outer half of the inner nuclear layer, where the somata of both rod and cone bipolar cells are located (Greferath et al., 1994). Furthermore, there is evidence from PCR and patch clamp recordings that the glycine receptor in rod bipolar cells is a heteromeric protein composed of α1 and β subunits (Enz and Bormann, 1995). In conclusion, it seems likely that rod bipolar axons receive some glycinergic input through glycine receptors containing α1 and β subunits. Whether other glycine receptor subunits are present at rod bipolar terminals remains to be determined.

LOCALIZATION OF GEPHYRIN ON BIPOLAR CELLS

Despite the fact that gephyrin is thought to be essential for clustering glycine receptors (Kirsch et al., 1993b) and the known presence of glycine receptors on bipolar cells, electron microscopy failed to detect gephyrin immunoreactivity on bipolar axon terminals (Pourcho and Owczarzak, 1991; Grünert and Wässle,

1993; Sassoè-Pognetto et al., 1994). Furthermore, gephyrin mRNA was not detected using single cell PCR on rod bipolar cells (Enz and Bormann, 1995). These results suggest that gephyrin is not required by glycine receptors in bipolar cells. Alternatively, bipolar cells could express a different gephyrin splice variant (Kirsch et al., 1993a).

GABA AND GLYCINE RECEPTORS ON GANGLION CELLS

Electrophysiological recordings from mammalian ganglion cells in vivo, in slice preparations, and in isolated cell preparations have suggested that all ganglion cells have $GABA_A$ and glycine receptors (reviewed by Tian et al., 1998). However, recent patch clamp studies have raised some doubts that all ganglion cell types express both inhibitory receptors. Tian et al. (1998) found in mouse retina that all ganglion cells had $GABA_A$ receptor-mediated synaptic responses, but only half of the ganglion cells had glycine receptor-mediated responses. Protti et al. (1997) found in rat retina that some ganglion cells had both $GABA_A$ and glycine receptors, but most cells had only $GABA_A$ receptors and some had only glycine receptors. One potential problem with both of these studies is that recordings were made in slice preparations and thus the dendritic tree of the recorded ganglion cell is most likely not fully intact (Tian et al., 1998). The other problem is that the ganglion cell types were not identified morphologically and thus it is still unclear whether the physiological differences have a morphological correlate. Despite these reservations these studies suggest that there is specificity in responses of distinct ganglion cell types for GABA or glycine.

The presence of $GABA_A$ receptors on ganglion cells is supported by a large number of immunohistochemical studies. The presence of α1 and β2/3 subunits of the $GABA_A$ receptor on some but not all ganglion cells has been demonstrated for rat, cat, rabbit, and monkey retinae (Hughes et al., 1989, 1991; Brecha, 1992; Greferath et al., 1993a, 1994b, 1995; Grünert et al., 1993). In macaque monkey retina it was found that in the fovea only parasol cells express α1 and β2/3 subunits, but in peripheral retina the majority of ganglion cells was labeled (Grünert et al., 1993).

In rat and rabbit retinae the γ2 subunit was found on many ganglion cells (Greferath et al., 1994, 1995), suggesting that many ganglion cells express a combination of α1, β2/3, γ2 subunits. In rat retina some ganglion cells, including giant ganglion cells, expressed the α4 subunit (Khan et al., 1996). These giant cells were not labeled with other $GABA_A$ receptor antibodies (Khan et al., 1996). Gutierrez et al. (1996) found the α6 subunit on rat ganglion cells.

The distribution of glycine receptor subunits in the ganglion cell layer was analyzed using in situ hybridization (Greferath et al., 1994a). Nearly all ganglion cells express the α2 subunit of the glycine receptor, whereas the α1 subunit is only expressed by a subpopulation of ganglion cells. Gephyrin is likely to be expressed by many ganglion cells, including α ganglion cells (Grünert and Wässle, 1993; Greferath et al., 1994a).

There is also evidence for the presence of $GABA_B$ receptors on ganglion cells from electrophysiology (re-

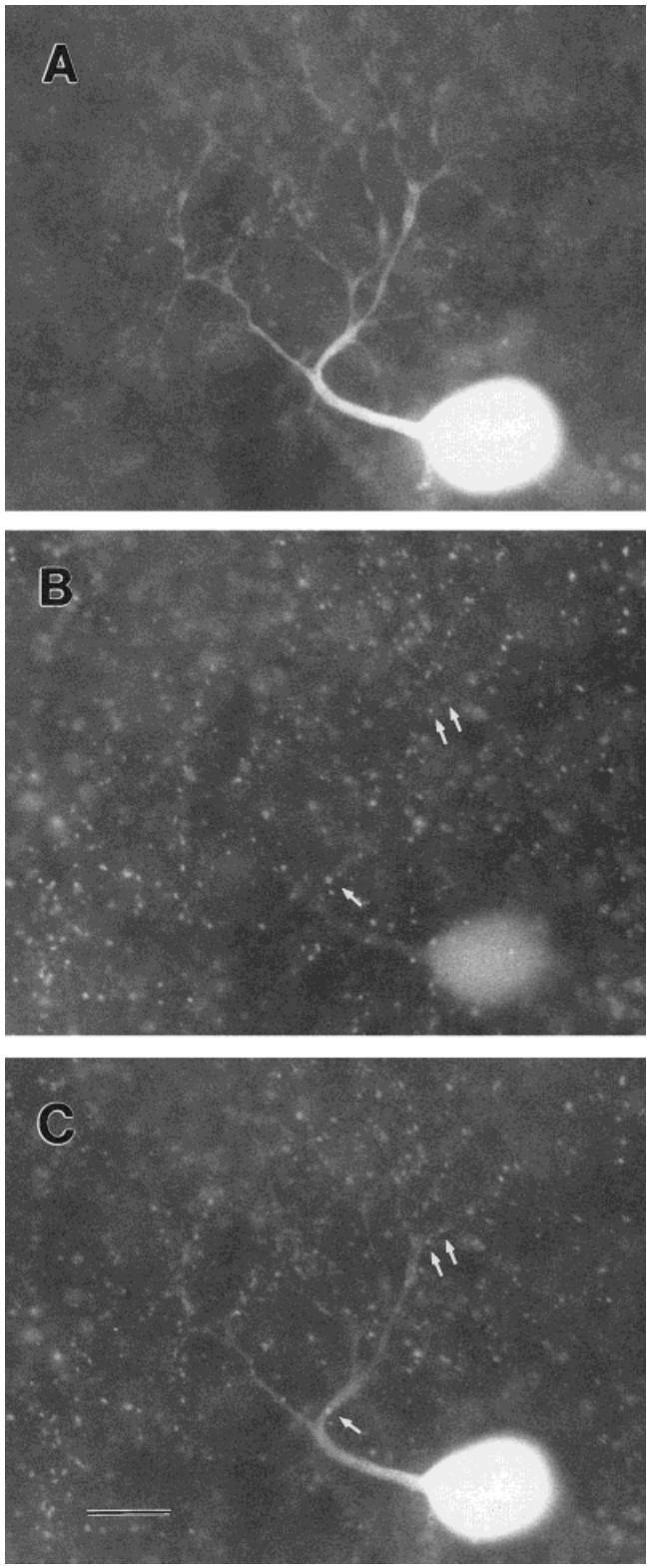

Fig. 5. Light micrographs of an OFF-midget ganglion cell of marmoset retina. **A**: The cell was injected with Neurobiotin and processed with Streptavidin coupled to FITC to show a green fluorescence. **B**: Immunoreactivity for the α1 subunit of the glycine receptor was made visible with secondary antibodies to Cy3 (red fluorescence). **C**: The dual band filter set shows that some immunoreactive puncta are colocalized with the dendrites of this cell (white arrows). Scale bar = 10 μm. Modified from Grünert and Ghosh (1999).

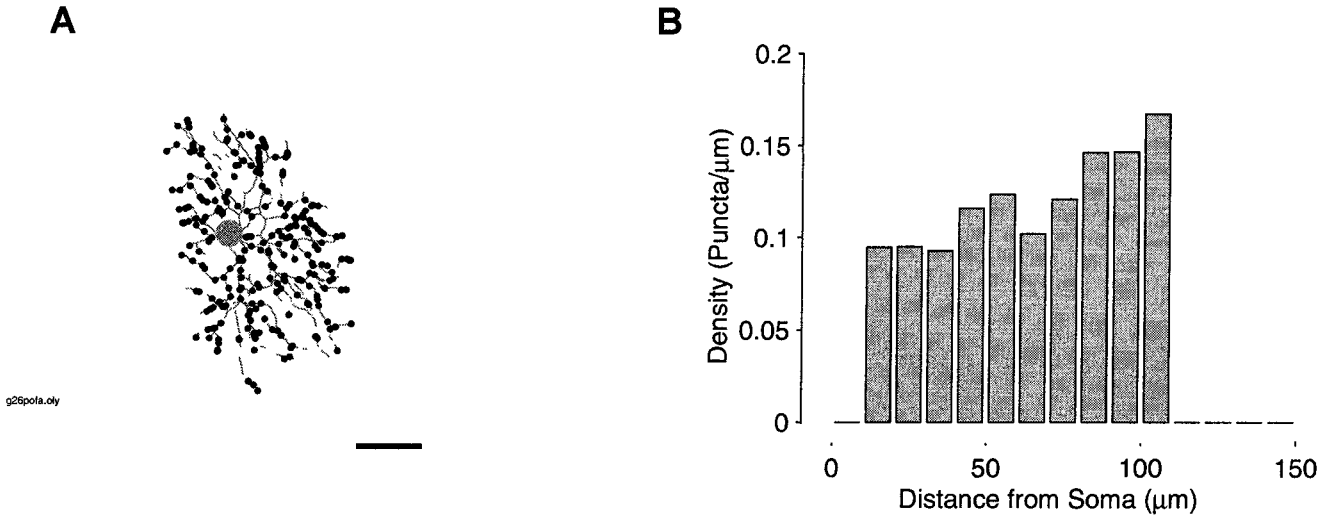

Fig. 6. Localization of the α1 subunit of the $GABA_A$ receptor on the dendrites of a Neurobiotin injected OFF-parasol ganglion cell in marmoset retina. **A**: Computer reconstruction of the dendritic tree of the cell and colocalized immunoreactive puncta (black dots). Scale bar = 50 μm. **B**: Histogram showing density of immunoreactive puncta on the dendrites of the cell shown in **A** as a function of the radial distance from the center of the soma. Modified from Macri et al. (2000).

viewed by Slaughter, 1995), immunohistochemistry (Koulen et al., 1998b) and in situ hybridization (Zhang et al., 1998). In contrast, $GABA_C$ receptors are absent from mammalian ganglion cells (Lukasiewicz, 1996; Lukasiewicz and Shields, 1998).

Localization of $GABA_A$ and Glycine Receptors on α Ganglion Cells

A number of EM studies found that the vast majority of synaptic input to ON- and OFF-α ganglion cells in cat retina derives from amacrine cells (Freed and Sterling, 1988; Kolb and Nelson, 1993; Weber and Stanford, 1994). Amacrine synapses were uniformly distributed across the dendritic field of α cells. More recently, the distribution of GABAergic and glycinergic synapses on ganglion cell dendrites has been analyzed. Owczarzak and Pourcho (1999) reconstructed two OFF-α ganglion cells in cat retina using electron microscopy. Postembedding immunogold labeling for GABA and glycine was used to analyze the amacrine input to these cells. The results showed equal numbers of GABAergic and glycinergic synapses on the dendrites of the cells. These were relatively evenly distributed along the dendritic tree, with an increase in density with distance from the soma. Only very few synapses were located on the soma or primary dendrites. The ratio of GABA: glycine input did not change across the dendritic field. These electron microscopic findings are consistent with a light microscopic study of α ganglion cells in rat and rabbit retinae (Koulen et al., 1996). ON- and OFF-α ganglion cells were intracellularly injected with Lucifer Yellow and subsequently stained with antibodies against one of the subunits of the $GABA_A$ receptor, α1, α2, α3, β1, β2/3, γ2, and antibodies against glycine and gephyrin receptor subunits. All α ganglion cells injected expressed one of these subunits on the dendrites.

In order to find out whether α ganglion cells are a homogenous cell class expressing all these receptor subunits or whether there are subtypes of α cells expressing specific $GABA_A$ receptors the following experiments were carried out (Koulen et al., 1996). Lucifer Yellow-injected ganglion cells were cut into two sections. One section was immunostained for one receptor subunit, the other section was immunostained for another subunit. In this way all possible combinations of α-subunits of the $GABA_A$ receptor were tested, α1/α2, α1/α3, α2/α3. Receptor clusters of all three α-subunits were found on the dendrites of each α cell analyzed, indicating that α cells can express different combinations of $GABA_A$ receptor subunits. Double immunofluorescence also showed that the three α subunits of the $GABA_A$ receptor are not colocalized and thus probably are aggregated at different synapses (Koulen et al., 1996).

Both $GABA_A$ and glycine receptor clusters were distributed uniformly along the dendrites of on- and off-cell types. These findings suggest that the response characteristics of α cells are not related to a differential distribution of GABA and glycine receptors. However, it is possible that different types of amacrine cells are presynaptic to specific $GABA_A$ receptor clusters, thus producing different synaptic circuits (Koulen et al., 1996; Wässle et al., 1998).

Localization of $GABA_A$ and Glycine Receptors on Midget and Parasol Cells

Midget and parasol cells are the main ganglion cell classes in the primate retina. They differ with respect to their morphologies as well as in their functional roles. Midget ganglion cells have small dendritic fields, project to the parvocellular layers of the lateral geniculate nucleus, and give sustained responses to light stimuli. Parasol ganglion cells have large dendritic fields, project to the magnocellular layers of the lateral geniculate nucleus, and give transient responses to light stimuli (reviewed by Dacey, 1994; Lee, 1996).

Both midget and parasol cells in baboon retina respond similarly to GABA and glycine and thus are

TABLE 1. Localization of GABA and Glycine receptor subunits on bipolar and ganglion cells in the mammalian retina

Receptor types and subunits	Bipolar cells			Ganglion cells					
	OFF-Cb	ON-Cb	Rb	OFF-A (Nonprimate)	ON-A (Nonprimate)	OFF-P	ON-P (Primate)	OFF-M (Primate)	ON-M
$GABA_AR$									
α1	$+^{1,12,11,30}$	$+^{1,12,11,30}$	$+^{1,12,11,30}$	$+^{24}$	$+^{24}$	$+^{15,26}$	$+^{15,26}$	$+^{26}$	$+^{26}$
α2	$-^{10}$	$-^{10}$	$-^{10}$	$+^{10,24}$	$+^{10,24}$	n.d.	n.d.	n.d.	n.d.
α3	n.d.	n.d.	$+^{7}$	$+^{24}$	$+^{24}$	n.d.	n.d.	n.d.	n.d.
α4	$-^{21}$	$-^{21}$	$-^{21}$	$+^{21}$	$+^{21}$	n.d.	n.d.	n.d.	n.d.
α5	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$
α6	$-^{18}$	$-^{18}$	$-^{18}$	$+^{18}$	$+^{18}$	n.d.	n.d.	n.d.	n.d.
β1	most but not all bipolar cells[13]			$+^{24}$	$+^{24}$	n.d.	n.d.	n.d.	n.d.
β2/3	$+^{1,12,11,30}$	$+^{1,12,11,30}$	$+^{1,12,11,30}$	$+^{20,24}$	$+^{20,24}$	$+^{19}$	$+^{19}$	$+ ?^{19}$	$+ ?^{19}$
γ2	$+^{10}$	$+^{10}$	$+^{10}$	$+^{10,24}$	$+^{10,24}$	n.d.	n.d.	n.d.	n.d.
δ	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	$-^{10}$	n.d.	n.d.	n.d.	n.d.
$GABA_BR$	$-^{31}$	$-^{31}$	$-^{31}$	$+^{27}$	$+^{27}$	n.d.	n.d.	n.d.	n.d.
$GABA_CR$	$+^{4,23,5,}$	$+^{4,23,5,}$	$+^{3–7,22,23}$	$-^{31}$	$-^{31}$	$-^{31}$	$-^{31}$	$-^{31}$	$-^{31}$
GlyR									
α1	$+^{29,17}$	?	$+^{2}$	$+^{24}$	$+^{24}$	$+^{14}$	$+^{14}$	$+^{14}$	$+^{14}$
α2	$-^{9}$	$-^{9}$	$-^{9}$	$+^{9}$	$+^{9}$	n.d.	n.d.	n.d.	n.d.
α3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β	$+^{9}$	$+^{9}$	$+^{9}$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
All subunits	n.d.	n.d.	n.d.	$+^{24}$	$+^{24}$	$+^{25}$	$+^{25}$	$+^{25}$	$+^{25}$
Gephyrin	$-^{28,16,29}$	$-^{28,16,29}$	$-^{28,16,29}$	$+^{16,24}$	$+^{16,24}$	$+^{25}$	$+^{25}$	n.d.	n.d.

+, Subunit is expressed; −, subunit is not expressed; n.d., not determined; ?, not definite; Cb, cone bipolar; Rb, rod bipolar; A, Alpha ganglion cell; P, parasol ganglion cell; M, midget ganglion cell.

[1]Brecha (1992)
[2]Enz & Bormann (1995)
[3]Enz et al. (1995)
[4]Enz et al. (1996)
[5]Euler & Wässle (1998)
[6]Feigenspan et al. (1993)
[7]Fletcher et al. (1998)
[8]Fletcher & Wässle (1999)
[9]Greferath et al. (1994a)
[10]Greferath et al. (1995)
[11]Greferath et al. (1994b)
[12]Greferath et al. (1993)
[13]Grigorenko & Yeh (1994)
[14]Grünert & Ghosh (1999)
[15]Grünert et al. (1993)
[16]Grünert & Wässle (1993)
[17]Grünert & Wässle (1996)
[18]Gutiérrez et al. (1996)
[19]Hughes et al. (1989)
[20]Hughes et al. (1991)
[21]Khan et al. (1996)
[22]Koulen et al. (1998a)
[23]Koulen et al. (1997)
[24]Koulen et al. (1996)
[25]Lin et al. (2000)
[26]Macri et al. (2000)
[27]Müller et al. (1992)
[28]Pourcho & Owczarzak (1991)
[29]Sassoè-Pognetto et al. (1994)
[30]Vardi & Sterling (1994)
[31]Wässle et al. (1998)

thought to express similar types of GABA and glycine receptors (Zhou et al., 1994). In order to find out whether midget and parasol cells differ with respect to the distribution of $GABA_A$ and glycine receptors, we recently studied these cell types in primate retina. Ganglion cells were injected intracellularly with Neurobiotin in a live in vitro whole mount preparation (Vaney, 1991; Dacey and Brace, 1992). Retinal pieces were then processed with antibodies against receptor subunits. Streptavidin coupled to fluorescein isothiocyanate (FITC) was used to reveal the cells; goat anti-mouse IgG coupled to Cy3 was used as secondary antibody.

Figure 5 shows an OFF-midget ganglion cell from marmoset retina that was processed with the antibody against the α1 subunit of the glycine receptor. In Figure 5A the cell is viewed through the green (FITC) filter. In Figure 5B the cell is viewed through the red (Cy3) filter. The immunoreactive puncta of the glycine receptor staining is visible. Due to the strong green immunofluorescence the soma of the cell is also visible. In Figure 5C the cell is photographed with a dual-band filter set and it becomes clear that some of the immunoreactive puncta coincide with the dendritic membrane of the injected cell (arrows). Similar results were obtained for ON-midget ganglion cells and ON- and OFF-parasol ganglion cells (Grünert and Ghosh, 1999) suggesting that both ON- and OFF-midget and parasol cells express the α1 subunit of the glycine receptor. At first sight, the immunoreactive receptor clusters appear to be evenly distributed along the dendrites of the cells, but our more recent quantitative study suggests that the density of receptor clusters increases with distance from the soma (Lin et al., 2000).

Using the same technique, i.e., intracellular injection in combination with immunostaining, we also analyzed the distribution of the α1 subunit of the $GABA_A$ receptor in midget and parasol cells of marmoset retina. We found many of the immunoreactive puncta to coincide with the dendrites of both ganglion cell types. The distribution of the α1 subunit of the $GABA_A$ receptor was analyzed quantitatively for 13 parasol (three OFF- and nine ON-cells) and three midget cells (Macri et al., 2000).

Figure 6 shows an example of an OFF-parasol cell. In Figure 6A a computer reconstruction of the cell together with the colocalized immunoreactive puncta (representing receptor clusters) is shown. Receptor clusters were found along the entire dendritic tree. Figure 6B shows the density of colocalized immunoreactive puncta as a function of distance from the soma. Average densities of colocalized puncta were calculated at 10 μm intervals from the center of the soma to the outermost dendrites. Receptor cluster density increases slightly with distance from the soma. The total number of immunoreactive puncta varied substantially between the cells, but the average density of immunoreactive puncta (about 0.1 puncta/μm dendritic length)

stayed relatively constant for ON- and OFF-parasol and midget ganglion cells. Parasol and midget cells also did not differ in the distribution of receptor clusters containing the α1 subunit of the $GABA_A$ receptor.

Since $GABA_A$ and glycine receptor clusters are expressed at different synapses (Sassoè-Pognetto et al., 1995), our findings taken together suggest that GABAergic and glycinergic synapses are distributed throughout the dendritic tree of parasol and midget ganglion cells in the primate retina. Overall, these results are compatible with previous evidence that the main functional distinctions between parasol and midget cell populations (for example, sustained vs. transient properties) are already determined at the level of the bipolar cell input (Wässle and Boycott, 1991; Lee, 1996; Wässle, 1999).

SUMMARY

Table 1 summarizes the GABA and glycine receptor subunits found on identified bipolar and ganglion cell types in the mammalian retina. Most of these results were obtained using immunocytochemistry. Since subunit specific agonists or antagonists were rarely used in electrophysiological recordings to date there is still little known about the physiological significance of this diversity in the expression of inhibitory receptors.

Most of the inhibitory receptors expressed by the best-characterized ganglion cell classes, i.e., α ganglion cells and parasol and midget ganglion cells, are now known, but whether other ganglion cell types show a similar diversity is an important question for future studies.

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