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Horizontal cells in the chameleon retina

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SUMMARY

Using the Golgi method, we have identified three morphological types of horizontal cell (HC) in the chameleon retina. Type I is located at the level of the retinal fovea and has a dense dentritic tree occupying a circular area of 4-5 μm. Type II is situated in the peripheral and central parts of the retina. Its dentritic tree ranges between 20 and 40 μm in diameter, increasing from the central retina to the periphery. Type III is found in the peripheral most zones of the retina and has a dendritic field of scarce, elongated prolongations covering an area of 40-70 μm. In all three types of HC described, we observed that some neurons show among their dendrites a longer prolongation ending in a pointed swelling that could be the cell axon. In addition, we used inmunohistochemistry to analize the expression of several neurotransmitters that may be used by horizontal cells. Our results suggest that horizontal cells have: GABAergic-Ir negative. Glycinergic-Ir negative, Glutamatergic-Ir negative, and Aspartatergic-Ir positive properties. This latter aspect is mainly seen in the peripheral parts of the chameleon retina.

Key Words: Retina - morphological types - horizontal cells - Golgi method-immunohistochemistry.

INTRODUCTION

Horizontal cells (HC) are second-order neurons that modulate the transfer of information between photoreceptors and bipolarcells in the outer plexiform layer of the retina (Kolb, 1974; Dacheux and Raviola, 1982; Naka, 1982; Boycott et al., 1987; Wässle et al., 1989). This cell type is known to contact cones through their dendrites and rods through their axon terminals (Kolb, 1974; Gallego, 1975; Boycott et al., 1987; Linberg and Fisher, 1988). Their structural and physiological properties varies considerably among different species. In retinas of non-mammalian species in which there is good trichromatic or even tetrachromatic vision, three or four different morphological types of HC can be recognized and these are known to have colour-specific connections to cones (Stell and Lightfoot, 1975; Leeper, 1978b; Kolb and Lipetz, 1991) or to cones and rods (Stell, 1975; Mariani and Leure du Pree, 1977; Leeper, 1978b; Van Haesendock and Missoten, 1979; Naka, 1982). In mammalian species with dichromatic colour vision, two types of HC have been described (Cajal, 1892; Kolb, 1974; Mariani, 1985; Gallego, 1975; Boycott, 1988), while in rabbit, monkey and human retinas three types can be found (Famiglietti, 1990; Kolb et al., 1994).

Some reptiles, such as members of the chameleon family, appear to have a visual system different to that of other vertebrates. Each eye has motor independence, leading to all-round spatial vision for each (Rochon-Duvigneaud, 1943; Harkness, 1977; Ambrosiani et al., 1979). Their retinal foveas are highly developed, comparable to those of birds of prey —known to have great visual acuity (Cajal, 1892). The chameleon retina is characterized by having an axon layer between the outer and inner nuclear layers. This axonal layer, which is known as the Henle layer, is similar to thatobserved in human retina (Cajal, 1892; Rochon-Duvigneaud, 1943). Furthermore, the chameleon retina has only cone photoreceptors (Cajal, 1892; Rochon-Duvigneaud, 1943; Armengol et al., 1988). The morphological characteristics

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of some chameleon retinal cells, such as bipolar cells (Quesada and Génis-Gálvez, 1981) or Müller cells (Cajal, 1892; Prada et al., 1979) are different from those seen in other vertebrate. Information about the morphological characteristics of the HCs of chameleons is very scanty and indeed Cajal (1892), studying the cytological organization of the chameleon retina, did not even mention them. Accordingly, to date this cell type remains practically unknown.

This paper details the morphological characteristics of different HC types recognized in Golgi preparations of chameleon retina together with their ultrastructural characteristics. In addition, to identify the amino acids used by HCs as neurotransmitters we tested the retina immunohistochemically, using antibodies against the neurotransmitters most frequently detected in most vertebrates: gamma-aminobutyric acid (GABA), glycine, glutamate and aspartate (Ehinger, 1982, Yazulla, 1986; Hendrickson et al., 1988; Ehinger, 1989; Marc, 1989; Massey, 1990; Davanger et al., 1991; Yaqub and Eldred, 1991). An antibody against calbindin was also used to verify the position and immunohistochemical expression of the HCs.

Materials and Methods

Tissue preparations

Five adult chameleons (Chamaleo chamaleo), provided by the Consejeria del Medio Ambiente of the Junta de Andalucía, were used in this study for electron microscopy and immunohistochemistry procedures. Animal care protocols used in our laboratory are in conformity with the appropiate national legislation (Decres 223/1988, BOE n.° 67) and guidelines from the European Communities Council Directive 86/609/EEC. Chameleons were anaesthetized with pentobarbitone (Nembutal, 150 mg/Kg) and perfused intracardially with the appropriate fixative for the technique to be followed.

Light microscopy procedures

Golgi illustrations were obtained from our histological collection of chameleons preparations stained by the Colonnier (1964) method. After enucleation, these had seen prepared by inmersing the eyes globes in fixative: 2.3 g potasium dichromate, 20 cc glutaraldehyde 40% in 80cc distilled water, for 5-7 days. After washing in tap water, the pieces were inmersed in silver nitrate solution for 3 days, dehydrated, and some of them were mounted "in toto" and others were embedded in low viscosity nitrocellulose. Serial sections, 80-90 µm thick, were cut in the transverse plane. Photomicrographs were obtained with a Zeiss microscope. We photographed cells in the section's thickness.

Electron microscopy procedures

After enucleation, one retina was dissected and fixed by immersion in 3% glutaraldehyde, 1% formaldehyde and 0.5% acrolein in 0.1 M sodium cacodylate buffer (pH 7.1) and postfixed in 2% osmium tetroxide, dehydrated in a graded series of acetone, and stained in the block (0.5% uranyl acetate in 70% acetone for 1 or 2h). The pieces of retina were embedded in Araldite CY 212 (Durcupan). Ultrathin sections (60 nm) were stained with uranyl acetate and lead citrate and examined under a Jeol 100C electron microscope.

Immunohistochemistry

The chameleons were perfused with Zamboni's fixing solution at 4°C (Zamboni and Martino, 1976). After fixing, the eyes were cryoprotected in 10%, 20% and 30% sucrose solution, and cryostat sections of 10-20 µm were incubated in 10% normal goat serum and 0.25% Triton X-100 for 30 min to block non-specific staining.

Primary antisera

Sections were then incubated for 24h at 4°C with primary antisera diluted in PBS containing 0.25% Triton X-100 and 1% normal goat serum. The antisera were aspartate polyclonal antiserum (Sigma A-9684) at a dilution of 1/250; GABA polyclonal antiserum (Sigma A-2052) diluted 1/500; glutamate polyclonal antiserum (Chemicon AB-133) at a dilution of 1/250; glycine polyclonal antiserum (Chemicon AB-139) at a dilution of 1/500, and calbindin D-28K monoclonal antiserum (Sigma C-8666) at a dilution of 1/500.

Other immunoreagents

After washing three times for 10 min in PBS with 0.25% Triton X-100, sections were incubated in the secondary antiserum. In this study we used anti-rabbit IgG and anti-mouse IgG, depending on the case, conjugated to fluorescein isothiocyanate (FITC) (Chemicon AP-132F) (Sigma F-6257) and tetramethyl rhodamine isothiocyanate (TRITC) (Sigma T-6778) (Sigma T-7782), diluted at 1/100 in PBS for 2h at room temperature in the dark.

Control sections were processed with each set of immunofluorescence preparations. The solution used to block non-specific staining replaced the primary or the secondary antibody. No significant fluorescence was detected in any of these sections.

Results

Light microscopy study

The HCs in the chameleon retina displayed the usual layered organization found in the retina of other vertebrates. HCs were seen lying at the outer margin of the inner nuclear layer (INL) forming a sheet which separated receptor and bipo-

lar cells in both the central (Fig. 3a, arrow head) and the peripheral retina (Fig. 3b, h). These cells comprised an irregular layer of perikarya of varying shape —spherical or ovoid-and normally with a single nucleolus. Their cytoplasm was clear and their perikarya large, in contrast to the scarce cytoplasm and smaller size of the bipolar cells. This was more noticeable in the peripheral retina (Fig. 3b, compare h with bi). The lateral surfaces of the HC perikarya (Fig. 3c, h) were separated from the outer prolongations of the bipolar cells (Fig. 3c, bi) by expansions of Müller cells (Fig. 3c, M). Using the Golgi method, three main types of HC were identified, depending on the morphological variations of the dendritic branches.

The type-I horizontal cells (I-HC) were found at the level of the retinal fovea (Fig. 1a) and of the parafoveal region (Fig. 1b, short arrow). Their cell bodies were normally piriform (Figs. 1a, short arrow; 1g) or round (Fig. 1a, long arrow; 1h). A dense mass of short, straight dendritic expansions emanated from the external pole of the perikaryon (Figs. 1f; 1g), ascending via the outer plexiform layer (OPL) to divide successively (Fig. 1i), and terminate forming between 15 and 20 synaptic boutons (Figs. 1h; 1i; 1v, arrowheads). Their dendritic field was circular and occupied a small area of approximately 4-5 μm in diameter (Figs. 1t; 1v). These cells did not show an axon with the typical axonal arborization observed in other animals. However, some of them displayed a branch or "accessory prolongation" that was longer than the rest. This prolongation was separated from the dendritic tree and its trajectory through the OPL followed a straight line (Figs. 1c, arrow; 1j, arrow) or forming knots and bows (Figs. 1a, arrowhead; 1u, arrowhead; 1w, arrowhead), terminating in a bouton-like swelling– possibly synaptic (Figs. 1c, arrowhead; 1j, arrowhead).

Type-II HC cells (II-HC) (Fig 1b, long arrow) were found in all regions of the retina except the fovea. The size of their perikarya was similar to that of the I-HC and its shape was round (Fig. 1k) or piriform (Figs. 1m; 1n). The dendritic tree coursed directly from the external pole of the cell body (Figs. 1k; 1l) or from a small trunk arising in the same region (Figs. 1n; 1o). The main dendrites were abundant and arose laterally from the perikaryon in opposite directions, giving the cell a double-crested aspect (Fig. 1p; 1x) which was more or less rectangular (Fig. 2a) or asymmetric (Fig. 2e) and covered a dendritic field between 20 and 40 μm in diameter (Figs. 1k; 1o). The area increased from the central to the peripheral retina. The main dendrites divided successively to form branches that became finer and shorter, ascending vertically through the OPL and terminating in synaptic spines that were isolated or in small accumulations of 3 or 4 synaptic boutons (Figs. 1x, arrowhead; 2a, arrowhead; 2c, arrowhead). Overall, there were between 20 and 30 boutons per field. Independently of the size of the dendritic field, some II-HCs showed an "accessory prolongation" that was varicose or had swellings at intervals (Figs. 1y, arrowhead; 2b, arrowhead; 2d, arrowhead), similar to what was observed for I-HCs.

Type-III horizontal cells (III-HC) were mainly found in the more peripheral zones of the retina (Figs. 1d; 1e; 1p). Their perikarya were normally round (Fig. 1r) or oval (Fig. 1s). From the scleral part of the cell body emerged 6 or 7 main dendrites (Figs. 2h; 2j) that coursed horizontally through the innermost part of the OPL. Throughout their trajectory their divided successively into scarce branches that ascended through the OPL (Figs. 1q; 1r; 2e) to terminate in synaptic boutons (Figs. 1q, arrowhead; 1r, arrowhead; 2e, arrowhead; 2f, arrowhead; 2i, arrowhead; 2j, arrowhead; 2k, arrowhead). The dendritic field was circular (Fig. 2h) or rectangular (Fig. 2i), with a diameter between 40 and 70 μm, and contained between 25 and 50 synaptic boutons. As in the case of the I-HCs and II-HCs, among the III-HCs cells an isolated, longer expansion could also be observed (Figs. 1r, long arrow; 2g, arrow).

Electron microscopy study

Ultrastructurally, the basic characteristics of the HCs in the chameleon appeared to be similar to those already described for the retina of other vertebrates. Apart from the shape of the cells there were no essential ultrastructural differences between peripheral (Fig. 3b, h) and central HCs (Fig. 3a, arrowhead). The perikarya of the peripheral neurons had a predominantly horizontal shape (Fig. 3b) while that of the central cells was more rounded (Fig. 3a). The nucleus was usually round or oval, but in the peripheral cells it was sometimes irregular or deeply indented. The chromatin content of the nucleus was finely and uniformly– granulated and the nucleolus was not centrally located. Their cytoplasm was clear in both the perikaryon and prolongations, greatly helping their identification (Fig. 3c, h). The mitochondria were numerous, variable in size and shape, and polarized in the scleral portion of the perikaryon (Fig. 3c, m). The Golgi complex had a similar location. Rough endoplasmic reticulum and free ribosomes were very scarce. The main horizontal processes had a fine structure, similar to that observed for the soma, and usually contained numerous mitochondria and frequently one or two centrioles. These expansions formed a dendritic plexus in the inner zone of the OPL, where the finest HC dendrites synapsed with the pedicles of photoreceptors (Fig. 3c, p). At the base of the cone pedicles, it was clearly seen that the HCs formed the lateral element of triads in the typical ribbon synapse (Fig. 3c, arrowhead). Adjacent triads and diads sometimes shared a

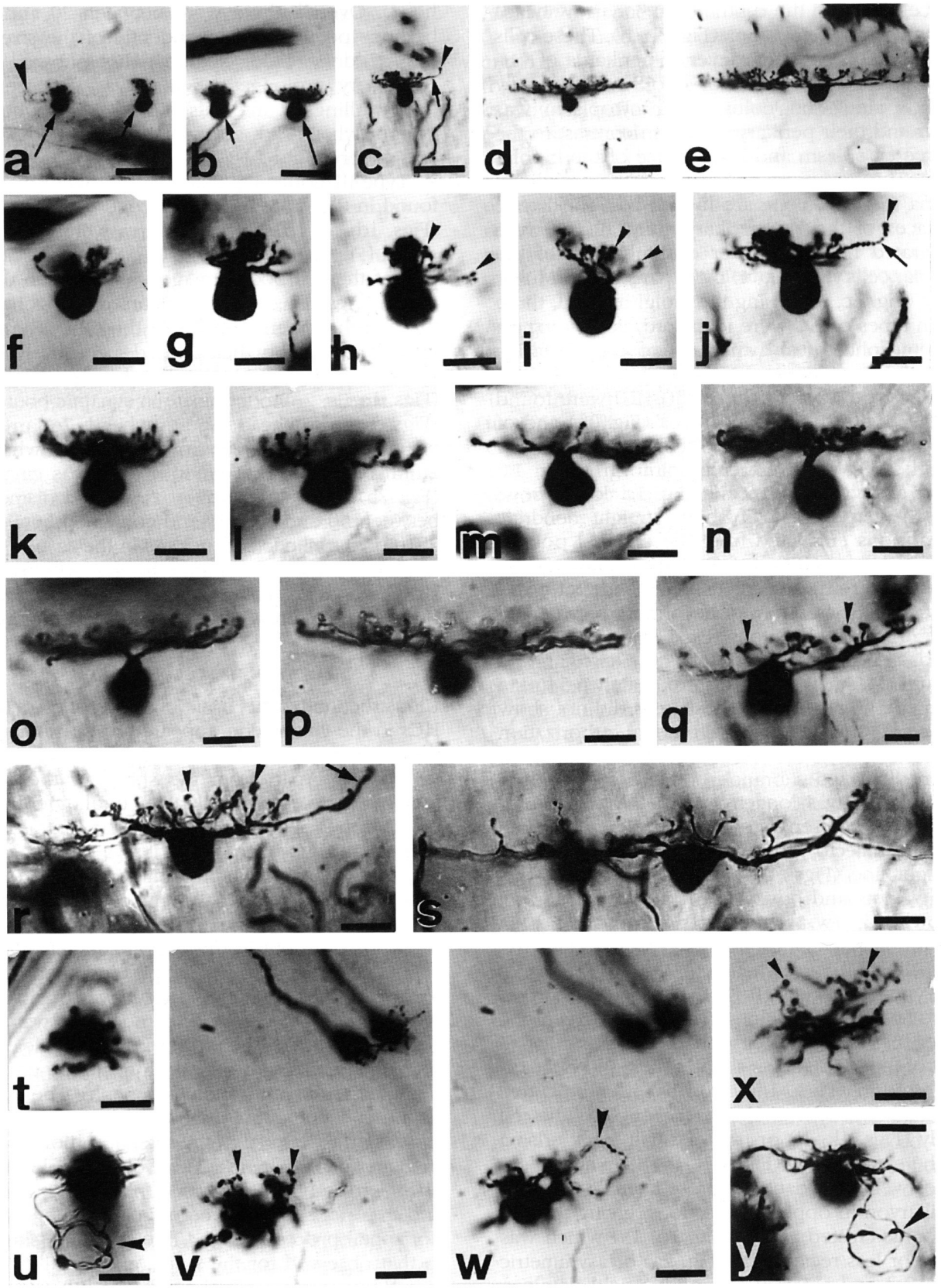

Fig. 1.– Vertical sections (**a-s**) and whole mounts (**t-y**) of chameleon retina stained by the Golgi method. **a)** Region of the fovea, showing type-I HCs (short and long arrows); arrowhead indicates an "accessory prolongation"; **b)** Parafoveal region, type-I HC (short arrow) and type-II HC (long arrow). **c)** and **j)** Type-I HC with "accessory prolongation" (arrow) terminating in a possibe synaptic swelling. **d)** Type-II HC. **e)** Type-III HC. **f, g, h, i)** Type-I Hcs; arrowheads indicate the terminal synaptic boutons. **k, l, m, n, o)** Type-II HCs localized from the central retina to the peripheral, and in which the dendritic field becomes progressively wider. **p, q, r, s)** Type-III HCs; arrowheads indicate the terminal synaptic boutons and the arrow shows an "accessory prolongation". **t, u)** Dendritic fields of type-I HCs; arrowhead indicates an "accessory prolongation". **v, w)** Different focal planes of the same type-I HC; small arrowheads indicate the dendritic terminal synaptic boutons, and the large arrowhead shows the "accessory prolongation". **x, y)** Type-II HCs with small dendritic fields showing the terminal synaptic boutons (small arrowheads) and "accessory prolongation" (large arrowhead). Bar = 25 μm (**a-e**). Bar = 8 μm (**f-y**).

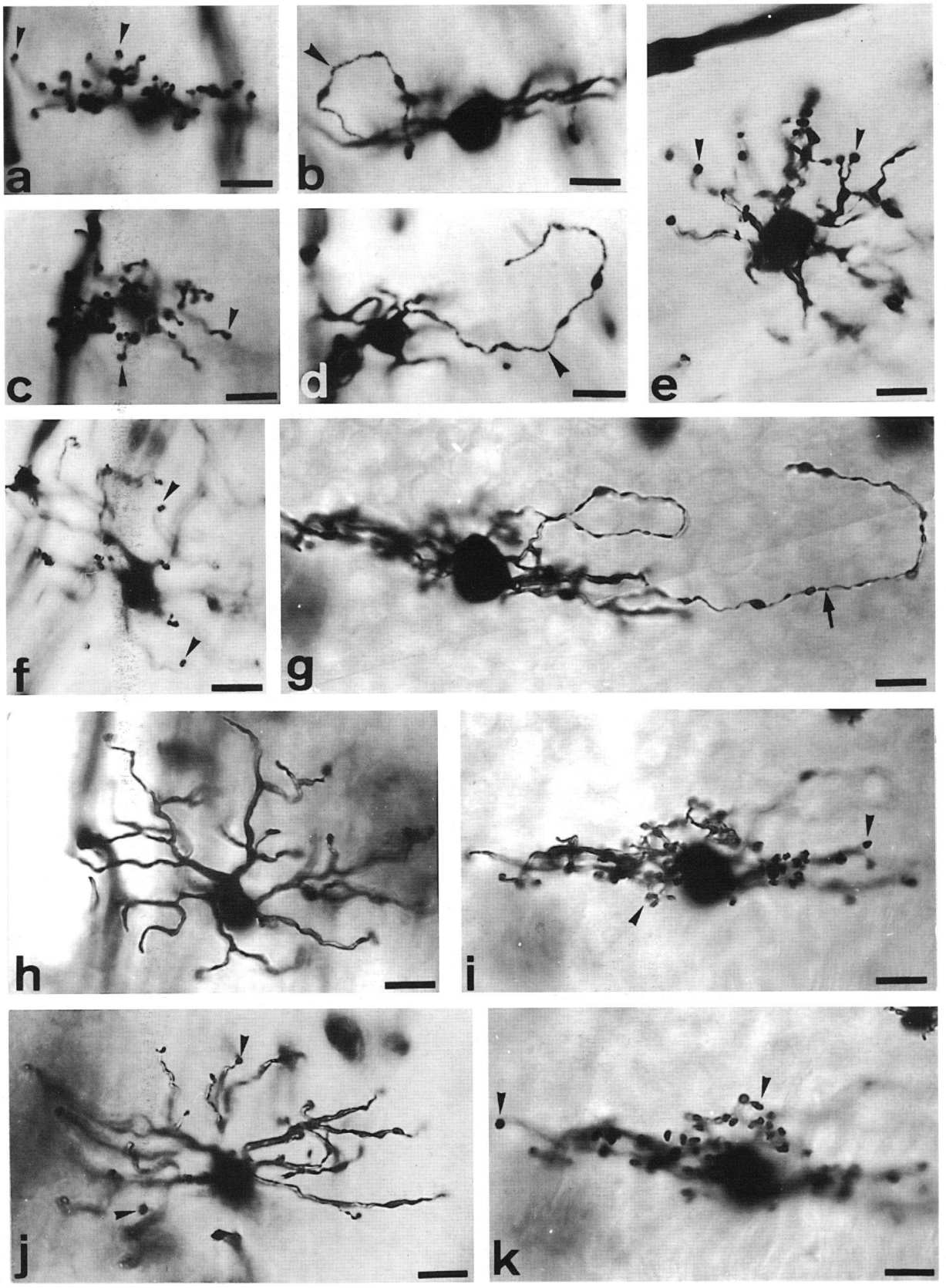

Fig. 2.– Retinal whole stained by the Golgi method. **a, b)** Different focal planes of the same type-II HC with medium-sized dendritic field; small and large arrowheads indicate the terminal synaptic boutons and "accessory prolongation", respectively. **e, d)** Different focal planes of the same type-II HC; samall and large arrowheads indicate the terminal synaptic boutons and "accessory prolongation", respectively. **e, k)** Type-I HCs with different-shaped dendritic fields and an "accessory prolongation" (arrow); arrowheads indicate the dendritic terminal synaptic boutons. **f, h)** and **g, i, k)** Different focal planes of the same cell. Bar = 8 μm.

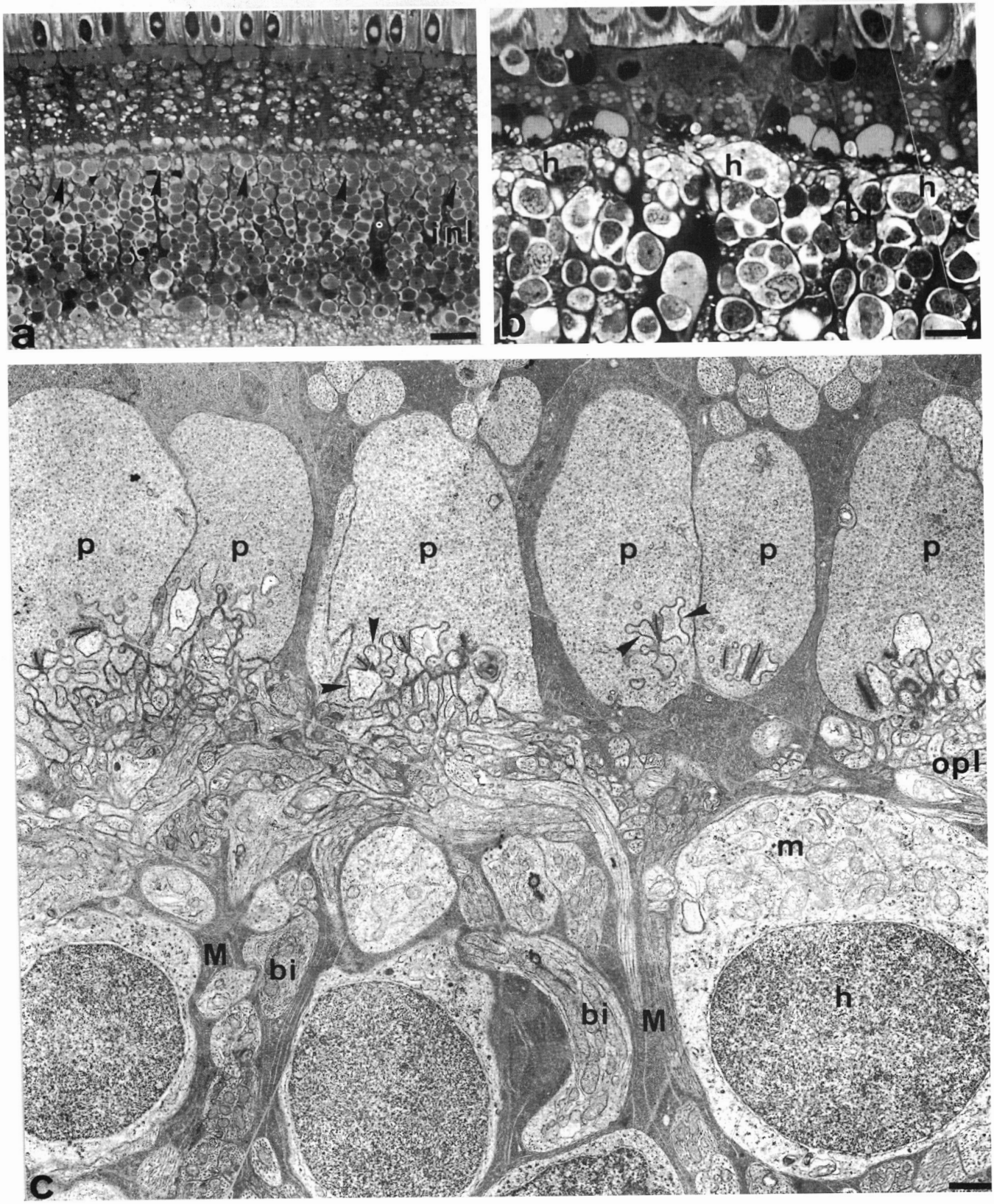

Fig. 3.– Semithin (**a,b**) and ultrathin **c)** sections of the central (**a,c**) and peripheral (**b**) retina of the chameleon. In **a)** arrowheads indicate the perikarya of the HCs, while in **c)** they indicate the dendritic processes of the HCs that form part of the lateral elements of the synaptic bar. p: Photoreceptor; h: horizontal cell. bi: bipolar cell. M: Müller cell. m: mitochondria. opl: outer plexiform layer. inl: inner nuclear layer. Bar = 20 μm (**a**). Bar =10 μm (**b**). Bar = 800 nm (**c**).

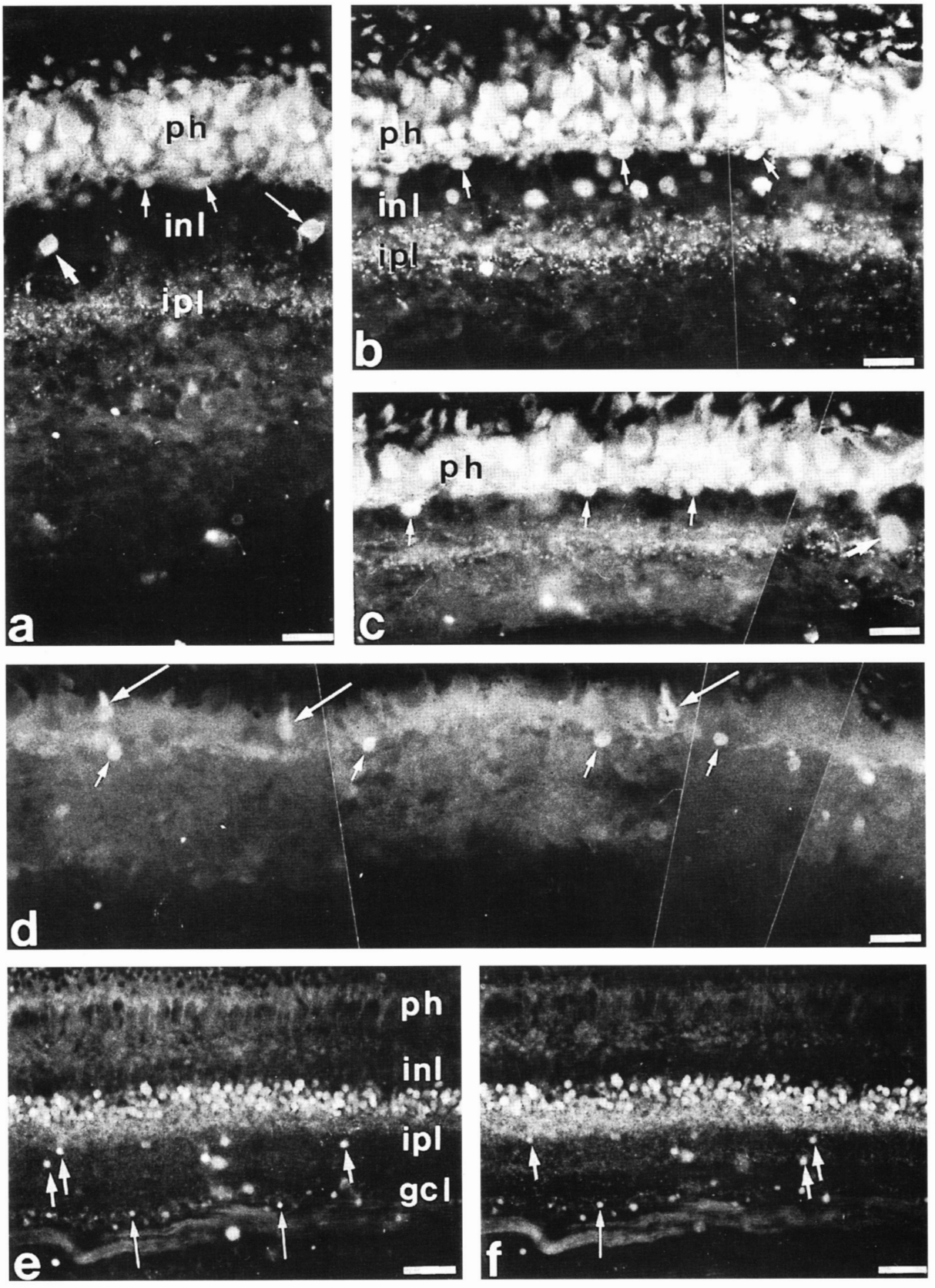

Fig. 4.– Vertical sections of chameleon retina. **a)** Calbindin-Ir in central retina; horizontal cells (short arrows); bipolar cell (long arrow); amacrine cell (thick arrow); photereceptor layer (**ph**); inner nuclear layer (**inl**); inner plexiform layer (**ipl**). **b)** Calbindin-Ir in equatorial retina; horizontal cells (short arrows); photoreceptor layer (**ph**); inner nuclear layer (**inl**); inner plexiform layer (**ipl**). **c)** Calbindin-Ir in peripheral retina; horizontal cells (short arrows); amacrine cell (thick arrow) photoreceptor layer (**ph**). **d)** Aspartate-Ir in peripheral retina; photoreceptors (long arrow); horizontal cells (short arrow). **e)** GABA-Ir in central retina; intraplexiform cells (short arrows); displaced amacrine cells (long arrows); photoreceptor layer (**ph**); inner nuclear layer (**inl**); inner plexiform layer (**ipl**); ganglion cell layer (**gcl**). **f)** Colocalization of labelling for GABA-Ir and glycine-Ir; intraplexiform cells (short arrows); displaced amacrine cells (long arrows). Bar = 15 μm (**a-c**); Bar = 10 μm (**d**); Bar = 30 μm (**e, f**).

lateral process. The terminal synaptic knobs of HCs were of considerable size, rounded, slightly swollen and occasionally collapsed. They showed branching in the form of evaginations 500-700Å in diameter. The OPL of the chameleon retina has a single synaptic stratum as can be observed in Fig. 3c (opl).

Immunocytochemical study

We attempted to identify the neurotransmitter content of the Hcs using conventional immunocytochemical techniques and we studied the patterns of immunoreactivity shown by the retina against GABA, glycine, aspartate and glutamate antisera. In order to identify and localize the HCs, we incubated vertical sections of retina with an anticalbindin monoclonal antibody.

Calbindin-Ir localization

All regions of the chameleon retina contained neurons that expressed calbindin-Ir. The general pattern of calbindin-Ir expression was mainly localized in the photoreceptor layer (Fig. 4a, ph) and in the inner nuclear layer (Fig. 4a, inl). The central retina (Fig. 4a) showed staining of most of the photoreceptors (ph), many of the horizontal cells (short arrows), and some amacrine (thick arrow) and bipolar (long arrow) cells. The inner plexiform layer of the central retina expressed weak calbindin-Ir (Fig. 4a, ipl). The equatorial retina (Fig. 4b) showed a pattern of calbindin-Ir expression similar to that of the central retina, for both the photoreceptors (ph) and the horizontal cells (short arrows). However, the number of amacrine and bipolar cells expressing calbindin-Ir was higher (Fig. 4b, inl). Similarly, the inner plexiform layer showed a more intense calbindin-Ir (Fig. 4b, ipl). The peripheral retina (Fig. 4c) expressed calbindin-Ir in all the photoreceptors (ph) and in most of the horizontal cells (short arrows). The horizontal cells in this region were less numerous than in the central retina (compare Fig. 3b with 3a). Only a few large amacrine cells expressed calbindin-Ir (Fig. 4c, thick arrow).

Aspartate-Ir localization

In the central retina, no aspartate positive immunoreactivity was observed. However, in the peripheral retina aspartate-Ir was distinguished in some randomly distributed photoreceptors (Fig. 4d, long arrow) and in some randomly distributed horizontal cells (Fig. 4d, short arrow).

GABA-Ir localization

The chameleon retina showed GABA-Ir only at the level of the INL, inner plexiform layer (IPL) and more sparsely in the ganglion cell layer (GCL) (Fig. 4e). The HCs, which in non-mammalian retinas do contain GABA, do not show GABA-Ir in the chameleon retina (Quesada et al., 1996). The highest GABA staining intensity was localized in the cell bodies of the inner third of the INL in vertical sections of central retina where the bodies of amacrine cells, displaced ganglion cells, and a type of interplexiform cell (Cajal, 1892; Prada et al., 1989; Quesada and Génis-Gálvez, 1983). At the level of the IPL, it was possible to observe GABA-Ir in the cell bodies of some the intraplexiform cells (Génis-Gálvez et al., 1978) (Fig. 4e, short arrows). More rarely, we also observed labelled cell bodies in the GCL. These were small and round, suggesting that they might be displaced amacrine cells (Fig. 4e, long arrows).

Glycine-Ir localization

Double-labelling of the chameleon retina with an anti-GABA monoclonal antibody and an anti-glycine polyclonal antibody revealed a pattern of glycine-Ir distribution very similar to that observed for GABA immunoreactivity. However, it was found that at the level of the IPL (Fig. 4f), not all the intraplexiform cells expressing GABA expressed glycine, and vice versa (compare Fig. 4e, long and short arrows with Fig. 4f, long and short arrows).

Glutamate-Ir localization

The pattern of glutamate-Ir staining was negative for all the retinal neurons at both central and peripheral levels. An exception was at the optic disc, where glutamate-Ir fibres were observed; these were probably fibres located at the head of the optic nerve leaving the retina (data not shown).

Discussion

Morphological differences between HCs types

The morphology of HCs has been well described from Golgi staining, intracellular injections and neurofibrillar methods in many animals (Gallego, 1975; Bloomfield and Miller, 1982; Dacheux and Raviola, 1982; Drager, 1983; Boycott, 1988; Wässle et al., 1989). Studies in different species have concluded that two or three types of HC are observed in most retinas. The mammalian retina generally contains two types of HC, which are commonly termed A-type, or axonless HC, and B-type, or short axon HC (Cajal, 1892; Dowling et al., 1966; Kolb, 1974; Gallego 1975; Boycott, 1988; Linberg and Fisher, 1988; Mills and Massey, 1994). The dendrites of A-type HC contact exclusively with cones. The dendritic tree of the B-type HC establishes synaptic contact with cones, and its axon terminal with rods (Kolb, 1974; Dacheux and Raviola, 1982; Mariani, 1985; Boycott, 1988; Linberg and Fisher, 1988; Wässle et

al., 1989, Raviola and Dacheux, 1990; Müller and Peichl, 1993, Harman and Ferguson, 1994; Mills and Massey, 1994; Peichl and Gonzalez-Soriano, 1994). In the rabbit, primate and human retina, three types of HC have been described (Famiglietti, 1990; Kolb et al., 1994). Using the Golgi method in birds, Cajal (1892) differentiated two HC types: "brush-shaped" and "stellate", both with axonal prolongations. Gallego (1975) questioned that study and considered that there is only one type– the "brush-shape"– whose axon had been mistakenly described by Cajal (1892) as the "stellate" type. Later, Mariani and Leure-du Prée (1977) described the two types of HC seen by Cajal (1892), although the "stellate" type was reported to lack an axon. Later still, Génis-Gálvez et al., (1979) definitively established three types of HC in the chick retina: I-HC or "brush-shaped", II-HC or "stellate", and a new type, III-HC, which was named after the form of its dendrites as "candelabrum-shaped". In fish, there are two main types of HC: those that receive input from the cones and others that receive it from rods (Kaneko, 1970; Naka and Nye, 1971; Stell, 1975). In the reptilian retina, using Golgi-staining methods, Lasansky (1971) described two types of HC in the turtle retina resembling the "brush" and "stellate" cells that Cajal (1892) identified in the lizard. Later, also in Golgi preparations of turtle retina, four types of HC were observed (Leeper, 1978a). We report that in the chameleon retina three types of HC can be distinguished using morphological criteria: the dendritic tree size, the length and shape of dendrites, and the number of contacts with cones. The I-HC is similar to the "brush-shaped" cell originally described by Cajal (1892). The II-HC of the chameleon is similar to that described as "stellate" cells in the avian retina (Mariani and Leure-du Prée, 1977; Gallego, 1975). The dendritic field of this HC type is larger in the peripheral retina than in central retinal zones. This could be related to the area of the cone pedicles, which in the chameleon show a gradient in pedicle size from the central to the peripheral retina (Armengol et al., 1988). The morphology observed in the III-HCs of the chameleon cannot be correlated with the "candelabrum" HCs described in the chick (Génis-Gálvez et al., 1979).

The possible axon of the chameleon HC in relation to axonbearing HCs in the retina of other vertebrates

Horizontal cells axons with prominent terminal expansions are seen in urodele amphibians (Werblin and Skrzypek, 1982), reptiles (Leeper, 1978a), birds (Gallego, 1975; Génis-Gálvez et al., 1979) and some mammals (Kolb, 1994). By contrast, axons lacking a prominent terminal but emitting long branches that make contact with photoreceptor terminals are found in anuran amphibians (Stephan and Weiler, 1981; Tarres and Baron, 1983; Stone and Witkovsky, 1987), and in some mammals (West, 1978). The monkey retina appears to possess HC subtypes having both classes of axonal form: with (Boycott, 1988) and without terminal expansions (Kolb et al., 1994). In the human retina, many HCs emit a prolongation that descends through the external most zone of the INL; these HCs also have a normal axon that persists in the OPL (Mariani, 1985; Silveira et al., 1989; Wässle et al., 1989). The terminal swellings of HC axons similar to those described by us in the chameleon retina are a prominent feature of many teleost retinas (Stell, 1975; Weiler and Zettler, 1979; Teranishi, 1983; Raymond, 1990). Therefore, the posible axon prolongation displayed by some HC of the chameleon retina is not an excepcion.

We believe that the absence of a typical axon terminal might be due to the fact that the retina of this animal lacks rod photoreceptors, which are found connected synaptically to HC axon terminals in other vertebrates (Kolb, 1974; Gallego, 1975). Although present day reptiles have a very high percentage of cones– around 93-97% compared with rods (Kouyama and Ohtsuka, 1985; Goede and Kolb, 1994; Reichenbach and Robinson, 1995)– the chameleon is an exception. Possibly, the lack of photoreceptor rods in the primitive reptile such as chameleon is because cones are phylogenetically 500 million years ago than rods (Raymond et al., 1993; Goldsmith, 1994; Reichenbach and Robinson, 1995).Other evidence supporting this phylogenetic antiquity of the cones over the rods is that the retinas of most larval fishes only have cones, while rods appear in the retina after metamorphosis (Zaunreiter and Kotrschal, 1989; Evans and Fernald, 1990; Johns, 1982). Similarly, cones predominate at the undifferentiated edges of the mammalian retina (Fine and Yanoff, 1972; Williams, 1991). Earlier studies in mammalian retinas suggested that type-A HCs (which synapse only with cones) differentiate prior to type-B cells (which synapse with cones and rods) (Polley et al., 1989), although more-recent studies indicate that they may be generated at the same time but that the appearance of an axon in type-B cells may be somewhat delayed (Redburn et al., 1992; Lyser et al., 1994). In growing goldfish, Raymond (1990) reported that the HC axon terminal is a very late developmental event. In chick embryos, the HCs that have still not differentiated their axon terminal have fibres with a full trajectory and swellings at intervals (Génis-Gálvez et al., 1981) similar to those described by us in the adult chameleon.

Neurotransmitter patterns in the chameleon retina

Pattern recognition of amino acid signals in the retinal cells of most species shows that 99% of the cell types are glutamatergic, GABAergic, glycinergic and aspartatergic neurons (Marc et al., 1995). The localization of the amino acid neurotransmitters shows that in the retina the photoreceptor-bipolar-ganglion cell pathway uses glutamate as a neurotransmitter, while the interneurons (horizontal and amacrine cells) use GABA and glycine (Ehinger, 1982; Yazulla, 1986; Ehinger, 1989; Marc, 1989; Massey, 1990; Davanger et al., 1991; Croks and Kolb, 1992; Barnstable, 1993). Immunohistochemical techniques have also been used as markers of certain neurons. We used an antibody against calbindin to localize the horizontal cells and to study the expression of different neurotransmitters in the retina of the chameleon.

Calbindinergic cells

The calcium-binding protein calbindin is important for the transport of intracellular calcium and is found throughout the central nervous system. Immunoreactivity for calbindin has proved to be an excellent marker for HCs in virtually all species examined (Rabie et al., 1985; Röhrenbeck et al., 1989; Pasteels et al., 1990; Pochet et al., 1991; Lyser et al., 1994; Peichl and Gonzalez-Soriano, 1994; Mitchell et al., 1995). However, despite its common presence in neuronal cells, its exact function remains unclear (Baimbridge et al., 1992). In some systems, calbindin has been shown to buffer calcium levels (Chard et al., 1993). It is used mostly as a cell marker, not only of HCs but also of bipolar cells (Pasteels et al., 1990; Pochet et al., 1991; Massey and Mills, 1996). In the chameleon retina, our results show that the distribution pattern of calbindin-Ir is expressed in all photoreceptors, in most horizontal cells, and in some amacrine cells. Bipolar cells do not seem to contain calbindin.

GABAergic cells and Glycinergic cells

The localization of the GABAergic neurons has been documented in the retina of vertebrates (Mossinger et al., 1986; Pourcho and Owczarzak, 1989; Grünert and Wässle, 1996; Müller and Marc, 1990; Davanger et al., 1991; Kalloniatis and Fletcher, 1993; Marc et al., 1995). All non-mammalian retinas examined before to this study have shown that HCs contain GABA-Ir (Mossinger et al., 1986; Kalloniatis and Fletcher, 1993). The chameleon retina is an exception because it does not contain GABAergic horizontal cells (Quesada et al., 1996; and this study). Since the photoreceptors and bipolar cells of the chameleon retina do not express GABA-Ir either we assume that GABA is not involved in the synaptic processes of the OPL. At the level of the inner third of the INL, GABA-Ir is intense and hence most of the amacrine cells, and possibly the type-II interplexiform cells (Quesada and Génis-Gálvez, 1983), are GABA-positive. Interplexiform cells with GABA-Ir have been identified in the mammalian retina (Hendrickson et al., 1985; Mossinger et al., 1986). The somal size of the GABA-positive cells observed at the level of the GL indicates that they are displaced amacrine cells. These cells may comprise up to 80% of all cells in the GL, depending on the species (Reichenbach and Robinson, 1995). The existence of glycinergic amacrine cells and interplexiform cells is not a novel finding since they have been previously reported in the retina of vertebrates (Müller and Marc, 1990; Yazulla, 1991; Kalloniatis and Fletcher, 1993; Zucker, 1993; Sassoè-Pognetto et al., 1994; Marc et al., 1995; Grünert and Wässle, 1996). In the present study, GABA-glycine co-localization indicates a similar immunostaining pattern similar between GABAergic and glycinergic cells, the only difference being that not all the GABAergic intraplexiform cells are glycinergic.

Glutamatergic cells and Aspartatergic cells

Observing that HC are not GABAergic and glycinergic in the chameleon retina, we studied the possible expression of others neurotransmitters such as glutamate and aspartate in these interneurons. Our results indicate the absence of glutamate-Ir in HC, although some photoreceptors and some HCs showed Aspartate-Ir, mosly at the level of the pheripheral retina. This is similar to the case of the turtle (Yaqub and Eldred, 1991) and the chicken (Ulshafer et al., 1990; Kalloniatis and Fletcher, 1993), which show higher immunoreactivity in the peripheral retina than in the central one. The diffuse or scarce staining of aspartate in the chameleon central retina probably reflects metabolic pools rather than neurotransmitter pools, while the high aspartate levels found in some horizontal cells and photoreceptors in the peripheral retina may reflect a neurotransmitter pool.

From this study it is difficult to speculate about the functional consequences of the neurotransmitters used or not used by HCs, since no detailed information about chameleon HCs in retinal information processing is available.

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