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Calcium-binding protein distribution in the retina of strepsirhine and haplorhine primates

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Abstract

Calcium-binding proteins are involved in numerous functional roles in the retina and are widely distributed in almost all retinal neurons. The present study aimed to characterize the distribution of the calcium-binding proteins calbindin, calretinin, parvalbumin and recoverin in relation to retinal cell types in a strepsirhine primate (mouse lemur, *Microcebus*) in comparison with primate species of the three main haplorhine lineages (marmoset, macaque and human), as well as a rodent (gerbil, *Taterillus*). The main findings show that whereas the recoverin antibody labels both rod and cone photoreceptors in all species, calbindin consistently labels cones, but not rods, in the haplorhine primates marmoset, macaque and human, but none of the photoreceptors in the mouse lemur. Marmoset and macaque also show a distinct label of cone outer segments with calretinin. Depending on the species, bipolar cells express calbindin and/or recoverin, while amacrine, horizontal and ganglion cells are labeled to varying degrees with calbindin, calretinin and parvalbumin. Haplorhine and strepsirhine primates clearly differ in the expression of calcium-binding protein expression in horizontal cells. In all haplorhine species, horizontal cells are densely labeled with parvalbumin whereas in mouse lemur horizontal cells express calbindin but not parvalbumin. Several characteristics of the calcium-binding immunostaining in the retina of the mouse lemur are similar to those observed in the rodent, and distinguish this species from the diurnal haphorhine primates. These differences may be related to adaptations of retinal structure and function to the nocturnal niche, since nocturnal strepsirhine and haphorhine (*Tarsius* and *Aotus*) primates share some features of calcium-binding expression.

Keywords: Photoreceptor; Calretinin; Parvalbumin; Calbindin; Recoverin; Prosimian

1. Introduction

Strepsirhines primates are of significant interest because they represent the most ancestral living primates and possess many anatomical and behavioral characteristics of ancestors that are also common to present-day monkeys, apes, and humans [28]. The retina of nocturnal and diurnal primates differs according to several features. Information on the structure of the retina in nocturnal primates mainly concerns ganglion cells and photoreceptors. Nocturnal strepsirhine (*Microcebus*, *Galago*) and haplorhine (*Tarsius*, *Aotus*) have rod-dominated retinas and are dichromates, characterized by a low number of MW cones and extremely sparse (or absent) SW cones [9,22,24,60]. Apart from opsin expression in photoreceptors, very little information is available on the characteristics of other retinal neurons especially in strepsirhine primates. In contrast, identification of the different cell types in the retina of haplorhine primates has been extensively studied using antibodies directed against specific cell or membrane proteins and other molecules. Several classes of anatomical markers have contributed to the classification of neuronal sub-types and to the understanding of neuronal networks in the retina. For example, photoreceptor organization, distribution, and development has been studied using antibodies raised against their opsin content [8,9,52,55]. Bipolar cells, which are the most numerous neurons in the inner nuclear layer (INL) can be distinguished by expression of glutamate [36], protein kinase C [14], calbindin [14,25,34,36], cholecystokinine [34] or recoverin [15,37,58]. Horizontal cells can be distinguished as

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either H1 cells, expressing only parvalbumin [56] or H2 cells, expressing both parvalbumin and calbindin [56]. Amacrine cells are recognized using a large spectrum of immunocytochemical markers, including calretinin [31,57], parvalbumin [4,11], choline acetyltransferase, cholecystokinine, glycine, neuropeptide Y, tyrosine hydroxylase or gamma-aminobutyric acid.

The family of calcium-binding proteins, which are present in almost all retinal neurons, is useful for studying neural pathways, synaptic connections, and specific retinal cell types [1]. The calcium-binding proteins most commonly used include calbindin, calretinin, calmodulin, parvalbumin, recoverin, caldendrin, hippocalcin, VILIP, the S100 protein, guanylate cyclase-activating proteins, and protein kinase C [1,17,21]. These calcium-binding proteins are expressed in both the retina and the brain and their distribution depends on cell type, species [42] and stage of development [39,61]. Calcium-binding proteins mediate or regulate the effects of Ca^{2+} ions on intracellular metabolism of neurons. Many aspects of neuronal activity, ranging from rapid modulations of channel functions to long-term switches in gene expression, are controlled by changes in the cytosolic concentration of Ca^{2+}.

The present study aims to characterize the distribution of calcium-binding proteins in the retina of the nocturnal strepsirhine gray mouse lemur (*Microcebus murinus*), in comparison to three haplorhine species: New World (*Callithrix jacchus*) and Old World (*Macaca fascicularis*) monkeys, and humans. For further comparison, the distribution of these markers is also studied in the retina of a nocturnal rodent (gerbil, *Taterillus petteri*). The study of nocturnal primates is of particular interest since the absence or reduced number of SW cones is associated with nocturnality in some primates [9,22,24,60], rodents, and carnivores [23,43]. Furthermore our recent study has shown that in contrast to other primate species, SW cones in humans and all cones in the nocturnal mouse lemur lack calbindin [6]. Thus, due to this variation within the primates, a comparison of the expression of calcium-binding proteins between different primate lineages is useful for understanding retinal organization.

2. Material and methods

2.1. Tissue samples

Three human eyes were obtained from donors and fixed within 16 h after death from the Department of Anatomy (University of Lyon, UCBL1), under approval of the Institutional Human Subjects Committee. Donors were of both sexes and varied on age from 65 to 80 years, and had no previous history of eye disease. The eyes were placed in Zamboni's fixative (4% paraformaldehyde with 15% saturated picric acid in phosphate buffer; 0.1 M, pH 7.4) overnight at 4 °C and rinsed in phosphate buffer (PBA; 0.1 M; sodium azide 0.1%, pH 7.4) the next day.

Retinae were obtained from four macaques (*M. fascicularis*), two marmosets (*C. jacchus*), three mouse lemurs (*M. murinus*) and four rodents (*T. petteri*). The mouse lemurs were obtained from the Laboratory of General Ecology in Brunoy, France (license approval No. A91.114.1). The other primates were part of the colony in INSERM (license approval No. B 69-685). All animals were maintained and treated according to current national and international standards. Animals were perfused with Zamboni's fixative. The cornea and lens were removed and after one night in fixative, the eyes were transferred to PBA at 4 °C.

Fixed eyes were cryoprotected by immersion in 30% sucrose overnight, embedded in a solution of agar (2.5%) with 30% sucrose and cut on a freezing microtome (Polycut, Reicher-Jung) at a thickness of 15 μm. Free-floating retinal sections were placed in titration wells containing PBA and stored at 4 °C until use.

2.2. Immunohistochemical procedure

2.2.1. Antibodies

Anti-calbindin (Sigma C-8666, 1/500, monoclonal antibody, Ab), anti-calretinin (Chemicon, AB149, 1/8000, polyclonal Ab), anti-parvalbumin (Sigma P-3088, 1/2000, mAb) and anti-recoverin (from A. Polans, University of Wisconsin-Madison, Medical School, Madison, USA, 1/1000, polyclonal Ab) immunochemistry were performed on free-floating sections. Peanut agglutinin lectin (PNA) was used as a general cone marker, since it binds specifically to the extracellular matrix of all cones (biotinylated PNA, Vector ref. # B-1075, 1/20).

2.2.2. General procedure

For all processing, free-floating retinal sections were placed in filtered wells (72 μm mesh Costar®). All rinses and incubations were carried out under gentle agitation. Immunostaining with each antibody alone was carried out. Positive immunoreactivity was revealed using avidin–biotin complex and diaminobenzidine (DAB) reaction. Free-floating sections were first incubated in a solution of alcohol–saline–H_2O_2 (30 min, absolute alcohol 50%–saline solution 50%–H_2O_2 0.05%) and then rinsed twice in phosphate buffered saline (PBS 0.01 M, 0.9% NaCl, pH 7.4, 10 mn). Retinal sections were incubated in normal horse serum (Vector ref. # S-2000, 1/100, 1 h) or normal goat serum (Vector ref. # S-1000, 1/100, 1 h), according to the source of the antibody. Then they were incubated in an anti-calcium-binding protein antibody solution (calbindin, calretinin, parvalbumin or recoverin) at 4 °C for 48 h. Sections were then rinsed twice in PBST (PBS with 0.3% triton) and incubated in the secondary biotinylated antibody (anti-mouse IgG, Vector ref. # BA-2000, or anti-goat IgG, Vector ref. # BA-9500, dilution 1/100, 2 h) followed by two rinses in PBST, and an incubation in avidin–biotin complex (Vectastain ABC Rabbit IgG; ref. # PK-6100) for 2 h. Retinal sections were rinsed once in PBST and twice in TRIS (0.05 M, pH 7.6) solution. The sections were pre-incubated 10 min in a mixture of DAB (Sigma, ref. # D5637) and nickel ammonium sulfate (0.5%) with 0.001% H_2O_2 added for 5–10 min under visual control. The sections were mounted on gelatinized slides and coverslipped. Negative controls were performed using the same technique but omitting each primary antibody.

Digitized images were captured using a Spot II camera (Diagnostic Instruments™) with 40, 63 and 100× immersion objectives. Image processing was carried out with Adobe Photoshop™ software.

3. Results

3.1. Calbindin-immunopositive neurons

In mouse lemur the photoreceptor layer is immunonegative for calbindin (Fig. 1B). Some vertically oriented nerve processes probably corresponding to Müller cells are seen at the base of the outer nuclear layer (ONL). Calbindin immunopositive cells also include a scattered population of neurons in the INL and a few sparse neurons in the ganglion cell layer (GCL). Horizontal cells are immunopositive with evident dendritic processes in the outer plexiform layer (OPL). Judging from the morphology, the cells in the inner part of the INL appear to be mainly amacrine cells although dendritic morphology is not very evident.

Unlike the mouse lemur, calbindin is present in the photoreceptor layer of all haplorhine primates studied. All calbindin positive photoreceptors have a cone-like morphology in humans, macaque, and marmoset (Fig. 2A–C). The calbindin positive

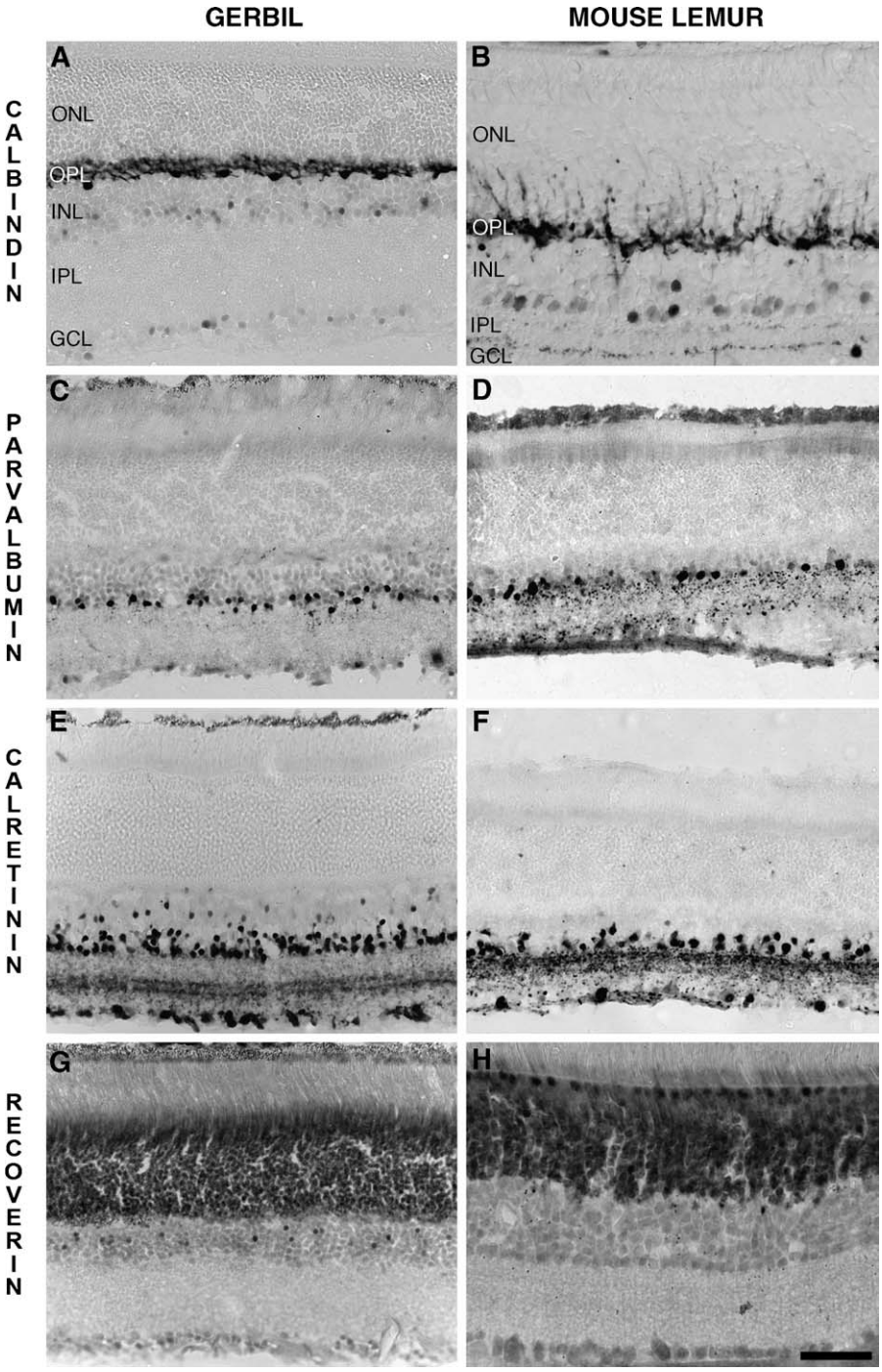

Fig. 1. Distribution of retinal immunostaining using antibodies against calcium-binding proteins in a rodent (gerbil, *Taterillus*) and the strepsirhine mouse lemur (*Microcebus*). In both species, calbindin (A and B) is absent from photoreceptors and bipolar cells, but present in horizontal, amacrine and ganglion cells. Parvalbumin (C and D) immunoreactivity is observed in amacrine cells and sparse ganglion cells. Calretinin (E and F) expression is absent from photoreceptors but evident in amacrine cells and cells located in the ganglion cell layer. Recoverin (G and H): immunoreactivity is only expressed in photoreceptors and bipolar cells (scale bar in $H = 50\ \mu m$). ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

cones are strongly stained from the inner segment to the cone pedicle in humans and macaque whereas the distribution of label within individual cone cells is not homogenous in marmoset (Fig. 2A–C). In the latter species, staining is densest in the distal part of the inner segment corresponding to the connecting cilium forming a ring-like band. The outer segment of cones in the marmoset are also unstained. No rod-like photoreceptors are calbindin positive in any primate species, including the mouse lemur.

In the INL, all primate species show similar distribution of calbindin labeling of a scattered population of amacrine cells but several differences in the label of horizontal cells. In macaque

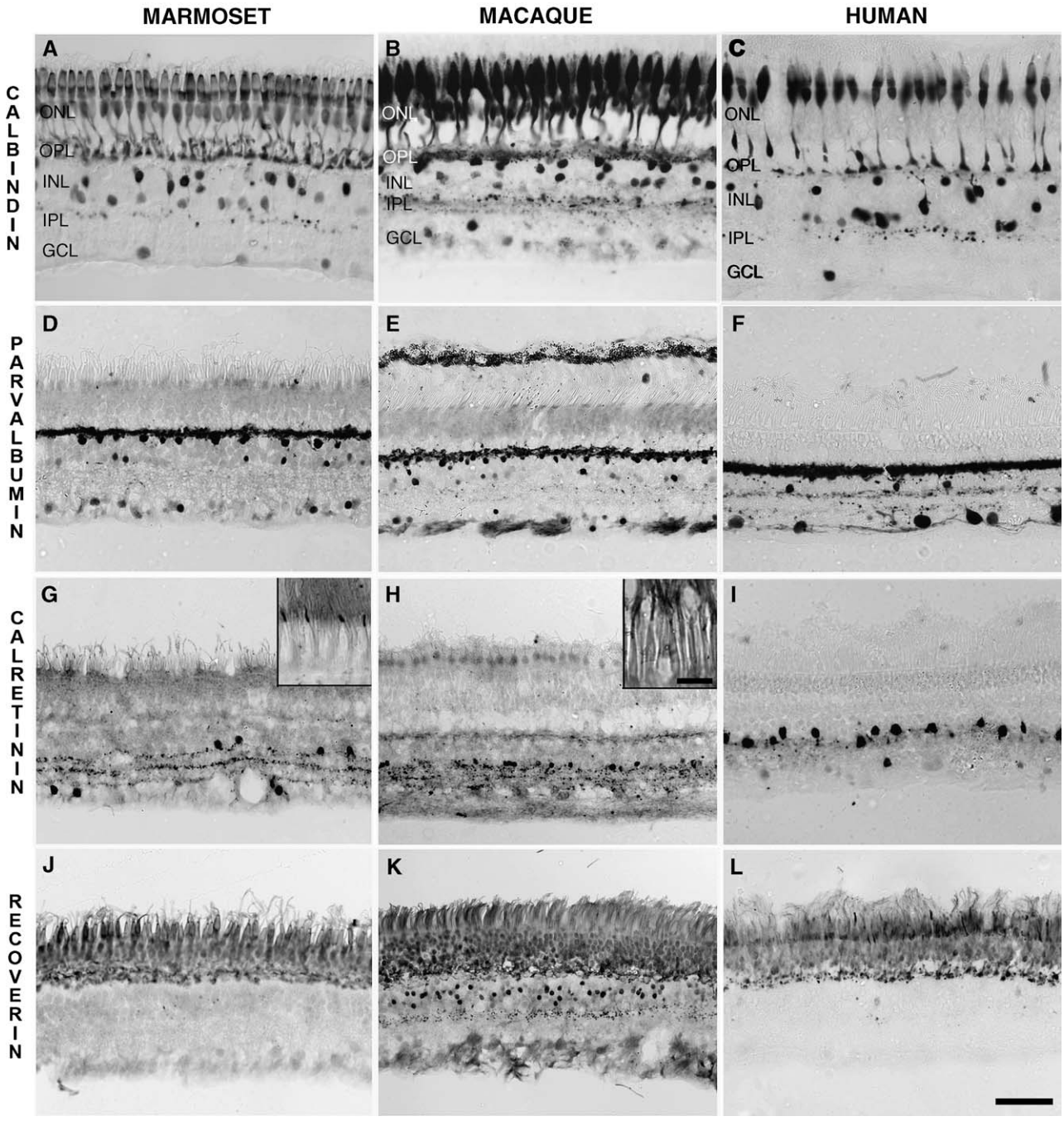

Fig. 2. Distribution of immunoreactivity using antibodies against calcium-binding proteins in the retinae of three haplorhine primates (marmoset, macaque and human). In all haplorhines, calbindin (A–C) labels cones, as well as amacrine, bipolar and a few scattered cell bodies in the ganglion cell layer. Horizontal cells are clearly evident in the macaque, but sparse or difficult to distinguish in human and marmoset. Parvalbumin (D–F) stains horizontal cells (soma and cells processes), a few amacrine cells and sparse neurons in the ganglion cell layer in all species. Calretinin (G–I) in all primates mainly stains cone photoreceptors and neurons in the INL with an amacrine-like morphology. The enlargements in (G and H) show that outer cone segments are distinctly labeled in both marmoset (G) and macaque (H) but not in humans. Recoverin (J–L) is present in the photoreceptor layer (rods and cones) of all primates, with immunopositive label of the outer segments and the cell bodies. In macaque and humans, cone outer segments are distinctly labeled whereas rod outer segments are faintly stained. Recoverin immunopositive cells also included a scattered population of bipolar cells in the macaque (scale bar in $L = 50$ μm; in insert in $H = 10$ μm). ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

and mouse lemur, the horizontal cell somas and dendritic processes in the OPL are clearly evident (Figs. 1B and 2B). In humans only a few horizontal cells, with little or no dendritic processes are labeled, whereas in the marmoset it is difficult to identify calbindin positive horizontal cells (Fig. 2A and C). A few scattered bipolar cells are observed in the outer part of the INL in the haplorhine primates.

The rodent retina shows a pattern of labeling similar to that of the mouse lemur with a absence of calbindin in photoreceptors and bipolar cells, a dense staining of horizontal cell bodies and

processes, and a sparse population of lightly stained amacrine and ganglion cells (Fig. 1A).

3.2. Parvalbumin-immunopositive neurons

In mouse lemur, parvalbumin is present in some amacrine cells and a few sparse cells in the GCL (Fig. 1D). In all primates, including the mouse lemur, the photoreceptors and bipolar cells are devoid of parvalbumin.

In contrast to the mouse lemur, all haplorhine primates show a dense label in horizontal cell bodies and dendrites (Fig. 2D and F). In all species, amacrine like immunopositive cells are present in the inner part of the INL. The characterization of these amacrine cells was not possible since the dendritic morphology is not very evident. In addition, a population of cells with particularly large soma diameters is observed in the GCL in humans (Fig. 2F). Parvalbumin immunopositive cells also include a few sparse neurons in the GCL in all species.

The immunolabeling in the rodent retina (Fig. 1C) is similar to that of the mouse lemur with presence of parvalbumin restricted to amacrine and sparse ganglion cells.

3.3. Calretinin-immunopositive neurons

Retina of the mouse lemur is characterized by the presence of calretinin in a subset of amacrine cells, very few bipolar cells and ganglion cells but the absence from photoreceptors and horizontal cells (Fig. 1F).

Although calretinin is also absent from photoreceptors in humans (Fig. 2I), this calcium-binding protein is present in the outer segments of a subset of photoreceptors in macaque and marmoset (enlargements in Fig. 2G and H). In both these species, double labeling using PNA (data not shown) showed that all these photoreceptors are cones.

In all primate species, a sub-population of amacrine cells is calretinin-immunopositive, with somas located in the inner part of the INL (Fig. 2G–I). Amacrine cells are densely labeled, with large, round or mitral-shaped cell bodies and dendrites that pass down through the IPL. Some calretinin immunoreactive amacrine cells appear to have the typical morphology of AII amacrine cells. The dendrites are not fully evident in the human retina (Fig. 2I). In macaque, marmoset and mouse lemur labeled dendrites are distributed in several strata of the IPL suggesting that different sub-populations of amacrine cells are labeled (Figs. 1F, 2G and H). Calretinin is not expressed in horizontal cells of any primates. Furthermore, no cells with a clear bipolar like morphology are immunopositive, although a few weakly stained cell bodies (at the outer margin of the INL) and dendritic processes (in the OPL) are seen in the macaque. A few sparse immunopositive cells are seen in the GCL in the human, the marmoset (Fig. 2G and I), the mouse lemur and the rodent (Fig. 1E and F).

In rodent, more numerous calretinin positive cells are seen in the INL (amacrine-like cells) and the GCL (Fig. 1E). As in macaque and marmoset, two strata of dendritic processes are evident in the IPL (Figs. 1E, 2G and H). Similar to human and mouse lemur, no photoreceptors are calretinin positive.

3.4. Recoverin-immunopositive neurons

In the mouse lemur, rod and cone photoreceptor inner segments and cell bodies are immunopositive (Fig. 1H). The position of a row of large more densely stained cell somas close to the outer limiting membrane suggests that these correspond to cone cell bodies. Recoverin immunopositive cells also include a scattered population of neurons in the inner part of the INL that, judging from the morphology, appear to be bipolar cells.

In humans, macaque, and marmoset cone photoreceptors are recoverin immunopositive and the label is evident in the inner segment, the cell body, and cone pedicles (Fig. 2J–L). In addition, the outer segment of cones is labeled in humans and macaque. In the marmoset the staining is densest in the distal part of the inner segment corresponding to the connecting cilium, forming a ring-like band, with the same distribution as seen for calbindin immunoreactivity. In all primates, rod outer segments and cell bodies are lightly stained. Only the macaque exhibits a labeling of bipolar cells and dendrites in the IPL (Fig. 2K). No immunopositive cells are detected in the GCL of any primate species.

The pattern of recoverin immunoreactivity in the rodent is very similar to that observed in the mouse lemur, with a widespread labeling of the photoreceptors and a small subset of bipolar cells (Fig. 1G).

4. Discussion

As in other mammals, calcium-binding proteins are widely expressed in various neuronal cell types of the primate retina from photoreceptors to ganglion cells. Calcium is known to regulate several biochemical functions such as axonal transport, neurotransmitter release, and transduction processes. In the retina, Ca^{2+} is involved in light transduction (activation of transducin), in the regulation of the switch-off of the visual cascade at the level of rhodopsin, the regulation of cGMP synthesis and cGMP gated ion channels, and in the synaptic release of the neurotransmitter glutamate [49].

There is, however, substantial inter-specific variability in the expression and distribution of calcium-binding proteins among primates (see summary in Fig. 3). Although the expression of some calcium-binding proteins has been studied in several primates, no results are currently available concerning any of the strepsirhine species.

4.1. Calcium-binding proteins as neuroanatomical markers of retinal neurons in primates and rodents

4.1.1. Photoreceptors

In primates, the presence of recoverin has been previously described in humans and macaque [27,32,37,38], including the fovea [27]. We report the expression of recoverin in photoreceptors of all primate species, in contrast to that of other calcium-binding proteins such as calbindin, parvalbumin and calretinin which are absent from the photoreceptors of the mouse lemur. Calbindin has been reported to be present in cone photoreceptors of all diurnal haplorhine species [13,18,19,45], with however an

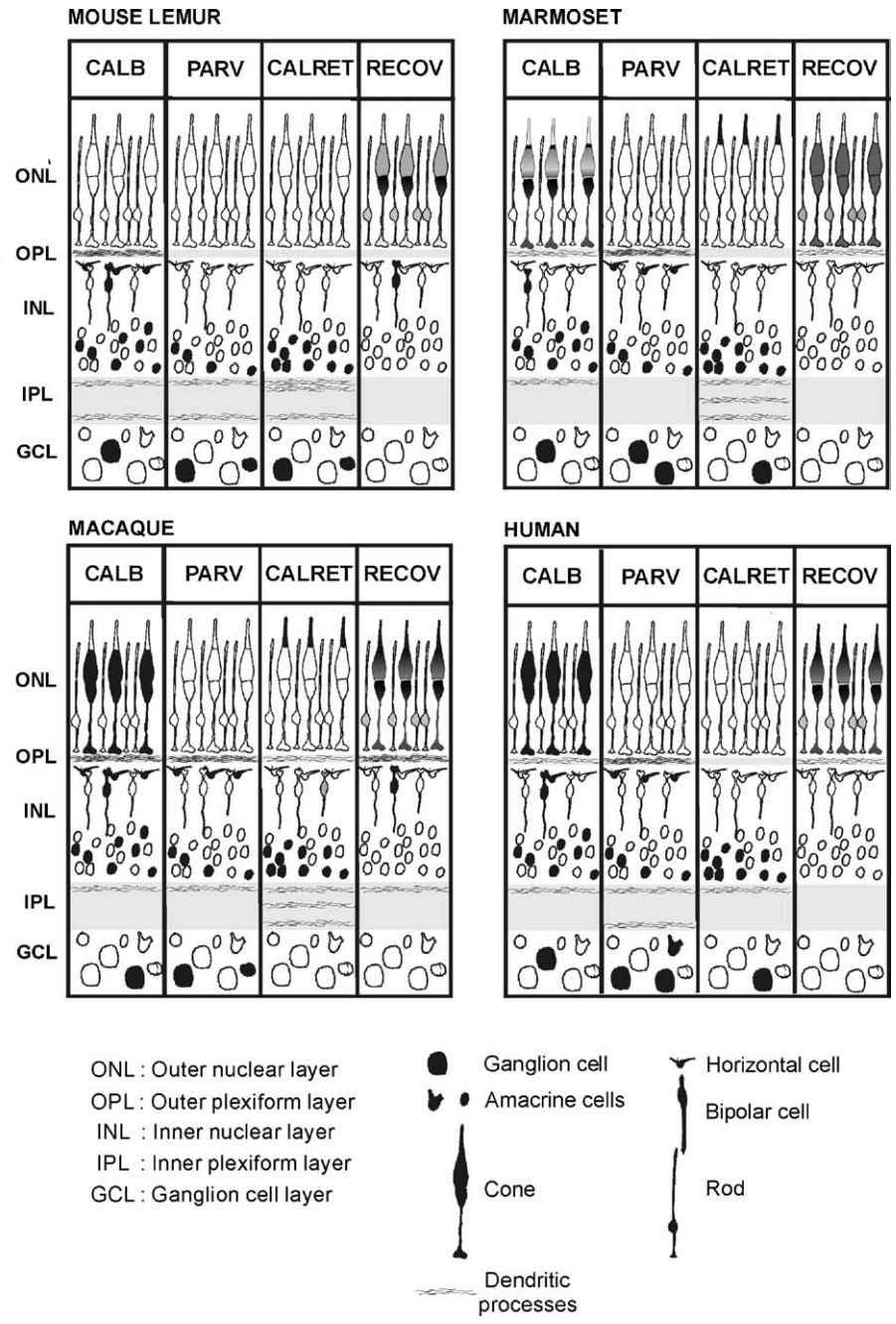

Fig. 3. A schematic diagram illustrating the distribution of calcium-binding proteins in haplorhine and strepsirhine primates (the distribution in the gerbil, which is similar to that of the mouse lemur, is not shown). Depending on the species, all calcium-binding proteins except parvalbumin are expressed in cone photoreceptors. Parvalbumin is a reliable marker for horizontal cells in all primates with the exception of the strepsirhine mouse lemur in which horizontal cells only express calbindin. Various types of amacrine cells are labeled with calbindin, calretinin, and parvalbumin but not with recoverin. Bipolar cells are immunopositive for calbindin and recoverin and to a lesser extent for calretinin (in the macaque).

absence in the foveal region [18,34]. Calbindin staining of cones in the human is similar to that seen in macaque. The marmoset retina exhibits a particular pattern of anti-calbindin staining with two areas of denser ring-like labeling of the inner segment [6]. In contrast, calbindin is absent in the photoreceptors of nocturnal species including strepsirhine (*Microcebus*) and haplorhine primates (*Tarsius* and *Aotus*) [22] as well as in the nocturnal gerbil and other rodents [7,21,37,42].

All PNA positive photoreceptors were also calretinin positive, suggesting that calretinin labels both SWS and MW/LW cones. Furthermore, we report a specific localization of calretinin in cone outer segments of marmoset and macaque only, which is complementary to that of calbindin staining of the inner segment and cell body [6]. Although calretinin staining has not been previously described in marmoset, Pasteels et al. reported labeling of the entire cone in the macaque [41], including the

fovea region. This difference between Pasteels' and our study could be related to the type of antibody used (antisera against chick calretinin raised against β-galactosidase calretinin fusion proteins [41]) or the technique of fixation and immunolabeling.

In summary, all nocturnal strepsirhine and haplorhine primates (*Microcebus*, *Tarsius*, *Aotus*) lack calbindin expression in photoreceptors and share this feature with rodents. Since calcium is thought to act as a modulator of light adaptation [47] the absence of calbindin in cones of nocturnal species may be related to different adaptational properties of the retina [18].

4.1.2. Bipolar cells

In all primate species our results show that calbindin is a reliable marker of a sub-population of bipolar cells. Previous studies have shown that these mainly correspond to diffuse bipolar cells (DB3 [5,15,25,34,56]) and a putative ON bipolar cell (DB5 [56]).

In the present study, the only other calcium-binding protein expressed in bipolar cells is recoverin which is present only in mouse lemur, macaque and gerbil. Previous studies have shown that recoverin is a reliable marker of bipolar cells in primates [20,37] and rodents [7,21,37].

In macaque and mouse lemur, recoverin positive bipolar cells could be clearly identified but the dendritic morphology was not distinctive enough to allow classification of bipolar cell types. Previous studies have shown that two populations of cone bipolar cells are recoverin positive in macaque: the flat midget cone bipolar cells [15,20,37] with a distinct pattern of narrow bistratification at the outer border (OFF-sublamina) of the IPL and diffuse bipolar cells with axonal stratification in the inner half (the ON-sublamina) of the IPL. In the macaque, recoverin and calbindin positive bipolar cells may correspond to two different bipolar cell types. Recoverin positive flat-midget cone bipolar cells are also immunoreactive for antibodies against glutamate transporters [25] but are immunonegative for protein kinase C (marker of rod bipolar cells), cholecystokinine (marker of invaginating midget bipolar cells and blue cone bipolar cells) or calbindin [37]. In the mouse lemur, the fact that bipolar cells are found in both the outer and the inner parts of the INL suggests that two sub-populations are labeled.

4.1.3. Amacrine cells

All strepsirhine and haplorhine primates (as well as rodents) show a similar pattern of expression of calcium-binding proteins in the amacrine cell population. In all species studied, amacrine cells are immunopositive for calbindin, parvalbumin and calretinin but are immunonegative for recoverin.

Amacrine cells labeled by calbindin antibody have also been previously described in macaque [31,41], marmoset [5] and humans [19,27]. In the macaque retina, a sub-population of calbindin amacrine cells, also calretinin immunopositive, are most commonly AII amacrine cells or wide field amacrine cells (A19 cells [31]).

The presence of calretinin in amacrine cells is a conserved feature seen in primates, rodents [21,41], cat [26,41], rabbit [26], sheep [41], dog [26] and pig [41]. In all primate species studied here, amacrine cell processes form a dense stratum of varicosities in the inner part of the IPL. In the marmoset and the macaque, several calretinin-immunoreactive amacrine cells show a distinct morphology such as the rod bipolar-driven, AII amacrine cells [30,35,57]. Other sub-populations of amacrine cells have been defined based on colocalization of two calcium-binding proteins (calbindin and calretinin, or calretinin and parvalbumin) or colocalization with the neurotransmitters gamma-aminobutyric acid (wide-field A19 amacrine cells), or glycine (majority of AII amacrine cells [31]).

Parvalbumin is also a ubiquitous marker of amacrine cells in primates [15,31] and other non-primate mammals including rodents [7,51,59], rabbit [4,51] and cat [51].

4.1.4. Horizontal cells

Haplorhine and strepsirhine primates clearly differ in the expression of calcium-binding protein expression in horizontal cells. In all haplorhine species, horizontal cells are densely labeled with parvalbumin [31,50,51], whereas in the strepsirhine primate mouse lemur no horizontal cells are immunopositives. In contrast, a dense plexus of horizontal cell bodies and fibers labels with calbindin in the mouse lemur. The above features seen in mouse lemur are also observed in the gerbil, in other rodents [7,42], and non-primate mammals [50,51]. The absence of parvalbumin immunoreactivity in a primate has not been previously reported and may be related to the quasi absence of SW cones in the mouse lemur [9] (less than 0.2% of the total cone population) or the adaptation to the nocturnal environment.

Within the haplorhine primates, although parvalbumin is expressed in a high number of horizontal cells, differences are observed in the pattern of calbindin expression. The macaque shows distinct calbindin label of a population of large sized horizontal cells and processes, whereas few or no labeled cells can be distinguished in the marmoset and human retina. In the macaque, parvalbumin has been shown to label both the large diameter H1 and small diameter H2 horizontal cells, whereas calbindin only labels the H2 type [56]. In the marmoset we find it difficult to identify calbindin positive horizontal cells, in agreement with previous studies [5,34]. In humans, a few cell bodies, with little or no label of dendritic processes, can be seen adjacent to the OPL [19,27].

In our study no species displays horizontal cells labeled with anti-calretinin or anti-recoverin antibodies. This absence is consistent with descriptions in the rabbit [26] and rat [19] but contrasts with findings in other non-primate species such as pig [41], cat [26], dog [26] and sheep [41].

4.1.5. Ganglion cell layer

Immunopositive neurons were seen in the GCL of all primates using antibodies against calbindin, parvalbumin, calretinin but not recoverin. However, it is difficult to determine whether these neurons are ganglion cells or displaced amacrine cells. Most of the calbindin positive neurons in all species studied appeared to correspond to displaced amacrine cells, similar to observations in humans [19,27], marmoset [5] and macaque [14]. In our study a few calretinin immunopositive cells are seen in the GCL of both marmosets and humans. These have been shown to be displaced amacrine cells in the marmoset [57]. Calretinin neurons in the

GCL have also been observed in rodents, including gerbil (as seen in this study), rat [3], ground squirrel [7], and in other non-primate species, such as cat [26], dog [26], rabbit [26,54] and sheep [41]. A recent study has also described displaced calretinin bipolar cells in the GCL of the rat retina [16].

In summary, the calcium-binding protein signature of retinal neurons in the mouse lemur shares many features with that of rodents. This similarity is particularly evident concerning the absence of calbindin and calretinin label in photoreceptors, and in horizontal cells a calbindin-positive coupled with a parvalbumin-negative immunoreactivity. These similarities may be related to the high proportion of rods in these species associated with adaptations of retinal structure and function to the nocturnal niche. The above immunostaining features also distinguish mouse lemur from the diurnal haplorhine primates (Fig. 3). The nocturnal strepsirhine mouse lemur, however, shares an absence of calbindin expression in photoreceptors with the nocturnal haplorhines *Tarsius* and *Aotus* [22].

4.2. Neuroanatomical markers of neurons in animal models and in human disease

The distribution and alteration of calcium-binding protein immunoreactivity in the mammalian retina, including that of primates, is useful for the study of several animal models of human diseases. Calbindin has been used as a marker to study retinal development in the coneless transgenic mouse model [48], in RCS rats, and after transplantation of embryonic retinal tissue [62]. Calretinin has also been employed in the study of abnormalities of retinal function in ApoE-deficient mice [40] and the effects of metabolic stress in the rat following retinal ischaemia [2]. Calbindin antibodies also reveal alterations in synaptic terminals, cone, horizontal and amacrine cells after experimental retinal detachment in the cat due to the consistency of this marker to reveal morphological details [33]. Calbindin and parvalbumin are markers for the identification of different sub-populations of neurons in a model of monkey glaucoma [53], in human retinal disease such as retinitis pigmentosa [12,27] or retinoblastoma [29], and in human retinal cell cultures [44].

In some patients with retinitis pigmentosa, calbindin expression in cones is down regulated [12,27] whereas labeling of horizontal, bipolar and amacrine cells is unaltered. Anti-calbindin immunochemistry also revealed horizontal cells with abnormal processes extending apically near the external limiting membrane [12]. Mutations in the calcium-binding protein GCAP1 have been found to be associated with autosomal dominant cone dystrophy [10]. Retinitis pigmentosa further leads to a loss of immunoreactivity for recoverin [27]. Recoverin has also been reported to be inappropriately expressed in some tumor cells from non-retinal tissue of patients with cancer-associated retinopathy and acts as an autoantigen in this rare retinal degenerative disease [46].

In conclusion, definition of the distribution of calcium-binding proteins in the retina is a necessary prerequisite for understanding the patterns of evolution of retinal neurons and retinal function in strepsirhine and haplorhine primates, in particular concerning adaptation to the nocturnal and diurnal niche. This information is also relevant to studies of ocular pathologies in animal models and humans.

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References

[1] K.G. Baimbridge, M.R. Celio, J.H. Rogers, Calcium-binding proteins in the nervous system, Trends Neurosci. 15 (1992) 303–308.
[2] N.L. Barnett, N.N. Osborne, Prolonged bilateral carotid artery occlusion induces electrophysiological and immunohistochemical changes to the rat retina without causing histological damage, Exp. Eye Res. 61 (1995) 83–90.
[3] E. Bastianelli, K. Takamatsu, K. Okazaki, H. Hidaka, R. Pochet, Hippocalcin in rat retina. Comparison with calbindin-D28k, calretinin and neurocalcin, Exp. Eye Res. 60 (1995) 257–266.
[4] G. Casini, D.W. Rickman, N.C. Brecha, AII amacrine cell population in the rabbit retina: identification by parvalbumin immunoreactivity, J. Comp. Neurol. 356 (1995) 132–142.
[5] T.L. Chan, P.R. Martin, N. Clunas, U. Grunert, Bipolar cell diversity in the primate retina: morphologic and immunocytochemical analysis of a new world monkey, the marmoset *Callithrix jacchus*, J. Comp. Neurol. 437 (2001) 219–239.
[6] C. Chiquet, O. Dkhissi-Benyahya, N. Chounlamountri, A. Szel, W.J. Degrip, H.M. Cooper, Characterization of calbindin-positive cones in primates, Neuroscience 115 (2002) 1323–1333.
[7] N. Cuenca, P. Deng, K.A. Linberg, G.P. Lewis, S.K. Fisher, H. Kolb, The neurons of the ground squirrel retina as revealed by immunostains for calcium binding proteins and neurotransmitters, J. Neurocytol. 31 (2002) 649–666.
[8] C.A. Curcio, K.A. Allen, K.R. Sloan, C.L. Lerea, J.B. Hurley, I.B. Klock, A.H. Milam, Distribution and morphology of human cone photoreceptors stained with anti-blue opsin, J. Comp. Neurol. 312 (1991) 610–624.
[9] O. Dkhissi-Benyahya, A. Szel, W.J. Degrip, H.M. Cooper, Short and mid-wavelength cone distribution in a nocturnal strepsirhine primate (*Microcebus murinus*), J. Comp. Neurol. 438 (2001) 490–504.
[10] S.M. Downes, G.E. Holder, F.W. Fitzke, A.M. Payne, M.J. Warren, S.S. Bhattacharya, A.C. Bird, Autosomal dominant cone and cone-rod dystrophy with mutations in the guanylate cyclase activator 1A gene-encoding guanylate cyclase activating protein-1, Arch. Ophthalmol. 119 (2001) 96–105.
[11] T. Endo, M. Kobayashi, S. Kobayashi, T. Onaya, Immunocytochemical and biochemical localization of parvalbumin in the retina, Cell Tissue Res. 243 (1986) 213–217.
[12] R.N. Fariss, Z.Y. Li, A.H. Milam, Abnormalities in rod photoreceptors, amacrine cells, and horizontal cells in human retinas with retinitis pigmentosa, Am. J. Ophthalmol. 129 (2000) 215–223.
[13] K.K. Ghosh, P.R. Martin, U. Grunert, Morphological analysis of the blue cone pathway in the retina of a New World monkey, the marmoset *Callithrix jacchus*, J. Comp. Neurol. 379 (1997) 211–225.
[14] U. Grunert, P.R. Martin, H. Wassle, Immunocytochemical analysis of bipolar cells in the macaque monkey retina, J. Comp. Neurol. 348 (1994) 607–627.
[15] U. Grunert, H. Wassle, Glycine receptors in the rod pathway of the macaque monkey retina, Vis. Neurosci. 13 (1996) 101–115.

[16] E. Gunhan, D. van der List, L.M. Chalupa, Ectopic photoreceptors and cone bipolar cells in the developing and mature retina, J. Neurosci. 23 (2003) 1383–1389.
[17] F. Haeseleer, I. Sokal, C.L. Verlinde, H. Erdjument-Bromage, P. Tempst, A.N. Pronin, J.L. Benovic, R.N. Fariss, K. Palczewski, Five members of a novel Ca(2+)-binding protein (CABP) subfamily with similarity to calmodulin, J. Biol. Chem. 275 (2000) 1247–1260.
[18] T.L. Haley, R. Pochet, L. Baizer, M.D. Burton, J.W. Crabb, M. Parmentier, A.S. Polans, Calbindin D-28K immunoreactivity of human cone cells varies with retinal position, Vis. Neurosci. 12 (1995) 301–307.
[19] K. Hamano, H. Kiyama, P.C. Emson, R. Manabe, M. Nakauchi, M. Tohyama, Localization of two calcium binding proteins, calbindin (28 kDa) and parvalbumin (12 kDa), in the vertebrate retina, J. Comp. Neurol. 302 (1990) 417–424.
[20] S. Haverkamp, F. Haeseleer, A. Hendrickson, A comparison of immunocytochemical markers to identify bipolar cell types in human and monkey retina, Vis. Neurosci. 20 (2003) 589–600.
[21] S. Haverkamp, H. Wassle, Immunocytochemical analysis of the mouse retina, J. Comp. Neurol. 424 (2000) 1–23.
[22] A. Hendrickson, H.R. Djajadi, L. Nakamura, D.E. Possin, D. Sajuthi, Nocturnal tarsier retina has both short and long/medium-wavelength cones in an unusual topography, J. Comp. Neurol. 424 (2000) 718–730.
[23] G.H. Jacobs, J.F.D. Deegan, Cone photopigments in nocturnal and diurnal procyonids, J. Comp. Physiol. [A] 171 (1992) 351–358.
[24] G.H. Jacobs, M. Neitz, J. Neitz, Mutations in S-cone pigment genes and the absence of colour vision in two species of nocturnal primates, Proc. Roy. Soc. London, Ser. B: Biol. Sci. 22 (1996) 705–710.
[25] R.A. Jacoby, A.F. Wiechmann, S.G. Amara, B.H. Leighton, D.W. Marshak, Diffuse bipolar cells provide input to OFF parasol ganglion cells in the macaque retina, J. Comp. Neurol. 416 (2000) 6–18.
[26] M.H. Jeon, C.J. Jeon, Immunocytochemical localization of calretinin containing neurons in retina from rabbit, cat, and dog, Neurosci. Res. 32 (1998) 75–84.
[27] S.K. John, J.E. Smith, G.D. Aguirre, A.H. Milam, Loss of cone molecular markers in rhodopsin-mutant human retinas with retinitis pigmentosa, Mol. Vis. 6 (2000) 204–215.
[28] R.F. Kay, C. Ross, B.A. Williams, Anthropoid origins, Science 275 (1997) 797–804.
[29] T. Kivela, Parvalbumin, a horizontal cell-associated calcium-binding protein in retinoblastoma eyes, Invest. Ophthalmol. Vis. Sci. 39 (1998) 1044–1048.
[30] H. Kolb, K.A. Linberg, S.K. Fisher, Neurons of the human retina: a Golgi study, J. Comp. Neurol. 318 (1992) 147–187.
[31] H. Kolb, L. Zhang, L. Dekorver, N. Cuenca, A new look at calretinin-immunoreactive amacrine cell types in the monkey retina, J. Comp. Neurol. 453 (2002) 168–184.
[32] H.W. Korf, B.H. White, N.C. Schaad, D.C. Klein, Recoverin in pineal organs and retinae of various vertebrate species including man, Brain Res. 595 (1992) 57–66.
[33] K.A. Linberg, G.P. Lewis, C. Shaaw, T.S. Rex, S.K. Fisher, Distribution of S- and M-cones in normal and experimentally detached cat retina, J. Comp. Neurol. 430 (2001) 343–356.
[34] X. Luo, K.K. Ghosh, P.R. Martin, U. Grunert, Analysis of two types of cone bipolar cells in the retina of a New World monkey, the marmoset, *Callithrix jacchus*, Vis. Neurosci. 16 (1999) 707–719.
[35] A.P. Mariani, Amacrine cells of the rhesus monkey retina, J. Comp. Neurol. 301 (1990) 382–400.
[36] P.R. Martin, U. Grunert, Spatial density and immunoreactivity of bipolar cells in the macaque monkey retina, J. Comp. Neurol. 323 (1992) 269–287.
[37] A.H. Milam, D.M. Dacey, A.M. Dizhoor, Recoverin immunoreactivity in mammalian cone bipolar cells, Vis. Neurosci. 10 (1993) 1–12.
[38] A.H. Milam, A.E. Hendrickson, M. Xiao, J.E. Smith, D.E. Possin, S.K. John, P.M. Nishina, Localization of tubby-like protein 1 in developing and adult human retinas, Invest. Ophthalmol. Vis. Sci. 41 (2000) 2352–2356.
[39] T.C. Nag, S. Wadhwa, Calbindin and parvalbumin immunoreactivity in the developing and adult human retina, Brain Res. Dev. Brain Res. 93 (1996) 23–32.
[40] J.M. Ong, N.C. Zorapapel, K.A. Rich, R.E. Wagstaff, R.W. Lambert, S.E. Rosenberg, F. Moghaddas, A. Pirouzmanesh, A.M. Aoki, M.C. Kenney, Effects of cholesterol and apolipoprotein E on retinal abnormalities in ApoE-deficient mice, Invest. Ophthalmol. Vis. Sci. 42 (2001) 1891–1900.
[41] B. Pasteels, J. Rogers, F. Blachier, R. Pochet, Calbindin and calretinin localization in retina from different species, Vis. Neurosci. 5 (1990) 1–16.
[42] L. Peichl, J. Gonzalez-Soriano, Morphological types of horizontal cell in rodent retinae: a comparison of rat, mouse, gerbil, and guinea pig, Vis. Neurosci. 11 (1994) 501–517.
[43] L. Peichl, K. Moutairou, Absence of short-wavelength sensitive cones in the retinae of seals (Carnivora) and African giant rats (Rodentia), Eur. J. Neurosci. 10 (1998) 2586–2594.
[44] S. Picaud, D. Hicks, V. Forster, J. Sahel, H. Dreyfus, Adult human retinal neurons in culture: physiology of horizontal cells, Invest. Ophthalmol. Vis. Sci. 39 (1998) 2637–2648.
[45] R. Pochet, B. Pasteels, A. Seto-Ohshima, E. Bastianelli, S. Kitajima, L.J. Van Eldik, Calmodulin and calbindin localization in retina from six vertebrate species, J. Comp. Neurol. 314 (1991) 750–762.
[46] A.S. Polans, J. Buczylko, J. Crabb, K. Palczewski, A photoreceptor calcium binding protein is recognized by autoantibodies obtained from patients with cancer-associated retinopathy, J. Cell Biol. 112 (1991) 981–989.
[47] E.E.J. Pugh, T.D. Lamb, CyclicGMP and calcium: the internal messengers of excitation and adaptation in vertebrate photoreceptors, Vis. Res. 30 (1990) 1923–1948.
[48] M.A. Raven, B.E. Reese, Mosaic regularity of horizontal cells in the mouse retina is independent of cone photoreceptor innervation, Invest. Ophthalmol. Vis. Sci. 44 (2003) 965–973.
[49] J.H. Rogers, Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons, J. Cell Biol. 105 (1987) 1343–1353.
[50] J. Rohrenbeck, H. Wassle, C.W. Heizmann, Immunocytochemical labelling of horizontal cells in mammalian retina using antibodies against calcium-binding proteins, Neurosci. Lett. 77 (1987) 255–260.
[51] P.P. Sanna, K.T. Keyser, M.R. Celio, H.J. Karten, F.E. Bloom, Distribution of parvalbumin immunoreactivity in the vertebrate retina, Brain Res. 600 (1993) 141–150.
[52] A. Szel, T. Diamantstein, P. Rohlich, Identification of the blue-sensitive cones in the mammalian retina by anti-visual pigment antibody, J. Comp. Neurol. 273 (1988) 593–602.
[53] J.C. Vickers, R.A. Schumer, S.M. Podos, R.F. Wang, B.M. Riederer, J.H. Morrison, Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma, Brain Res. 680 (1995) 23–35.
[54] B. Volgyi, E. Pollak, P. Buzas, R. Gabriel, Calretinin in neurochemically well-defined cell populations of rabbit retina, Brain Res. 763 (1997) 79–86.
[55] Y. Wang, J.P. Macke, S.L. Merbs, D.J. Zack, B. Klaunberg, J. Bennett, J. Gearhart, J. Nathans, A locus control region adjacent to the human red and green visual pigment genes, Neuron 9 (1992) 429–440.
[56] H. Wassle, D.M. Dacey, T. Haun, S. Haverkamp, U. Grunert, B.B. Boycott, The mosaic of horizontal cells in the macaque monkey retina: with a comment on biplexiform ganglion cells, Vis. Neurosci. 17 (2000) 591–608.
[57] H. Wassle, U. Grunert, M.H. Chun, B.B. Boycott, The rod pathway of the macaque monkey retina: identification of AII-amacrine cells with antibodies against calretinin, J. Comp. Neurol. 361 (1995) 537–551.
[58] H. Wassle, U. Grunert, P.R. Martin, B.B. Boycott, Immunocytochemical characterization and spatial distribution of midget bipolar cells in the macaque monkey retina, Vis. Res. 34 (1994) 561–579.

[59] H. Wassle, U. Grunert, J. Rohrenbeck, Immunocytochemical staining of AII-amacrine cells in the rat retina with antibodies against parvalbumin, J. Comp. Neurol. 332 (1993) 407–420.
[60] K.C. Wikler, R.W. Williams, P. Rakic, Photoreceptor mosaic: number and distribution of rods and cones in the rhesus monkey retina, J. Comp. Neurol. 297 (1990) 499–508.
[61] X.X. Yan, Prenatal development of calbindin D-28K and parvalbumin immunoreactivities in the human retina, J. Comp. Neurol. 377 (1997) 565–576.
[62] Y. Zhang, K. Arner, B. Ehinger, M.T. Perez, Limitation of anatomical integration between subretinal transplants and the host retina, Invest. Ophthalmol. Vis. Sci. 44 (2003) 324–331.