Eur J Anat, 5 (3): 113-117 (2001)

Immunoreactivity for GABA in the retina of the chameleon *(Chamaeleo chamaeleon)*

Mohamed Bennis[1] and José A. Armengol[2]

1- Unite Neuroscience du Comportement, Faculté des Sciences, Marrakech, Morocco
2- División of Neuroscience, LAB, CSIC-UPO, Seville, Spain

SUMMARY

Antibodies directed against gamma-aminobutyric (GABA) and L-glutamic acid decarboxylase (GAD) were used to study the GABAergic cell populations of the chameleon retina. GABA immunoreactivity was found in the two main retinal interneurons: amacrine and horizontal cells. Amacrine, displaced amacrine, intra– and interplexiform cells displayed the strongest GABA-immunoreactivity of the retinal cell types. Horizontal cells formed a continuous GABA-immunoreactive cell layer lying in the outermost portion of the inner nuclear layer. In contrast with previous studies, describing the absence of horizontal GABAergic cells in the chameleon retina, the present results demonstrated that the vast majority of horizontal cells of the chameleon retina possess GABA, and agree with the key role that GABAergic synaptic transmission plays in the outer plexiform layer of the vertebrate retina.

Key Words: Amacrine cells – Chameleon – Gamma-aminobutyric acid – Horizontal cells – Retina

INTRODUCTION

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate central nervous system, and is present in the retina of all species analyzed. Retinal interneurons, such as horizontal cells and several amacrine cell subtypes, possess GABA as a neurotransmitter (for a review, see Yazulla, 1986). Further, some cones, and displaced amacrine and ganglion cells are also GABA-immunoreactive (GABA-Ir) (Pourcho and Goebel, 1983; Agardh et al., 1986; Pessac et al., 1987; Sherry and Ulshafer, 1992; Hurd and Eldred, 1994; Versaux-Botteri et al., 1994; Haverkamp and Wässle, 2000). Among the vertebrates, the chameleon is unique in exhibiting a motor independence of its eyes, leading to all-round spatial vision for each eye (Harkness, 1977; Bennis et al., 1994). This characteristic has been related to certain peculiarities of its retina. However, all cell types of the vertebrate retina are present in the chameleon retina, whose organization is very similar to that of the rest of vertebrate retinas (Ramón y Cajal, 1893; Rochon-Duvigneaud, 1943). In fact, as in other diumal lizards (Crescitelli, 1972), the chameleon retina is considered as an all-cone retina (Ramón y Cajal, 1893; Rochon-Duvigneaud, 1943; Armengol et al., 1988). Further, like other diurnal lizards (Sherry and Ulsahfer, 1992), GABA-Ir retinal interneurons are also found in the chameleon retina (Bennis and Versaux-Botteri, 1995). Despite this, two recent reports by Quesada et al. (1996, 1999) describe the absence of GABA-Ir horizontal cells in the chameleon retina. Indeed, these reports raise the possibility that the chameleon retina might be an exception to the general pattern of the outer plexiform layer inhibitory pathways of the vertebrate retina. Therefore, with a view to solving this question, here we have re-examined the distribution of GABA-Ir cells in the chameleon retina.

MATERIALS AND METHODS

Three adult chameleons were used in this study. Animal handling and tissue acquisition protocols

Submitted: August 17, 2001
Accepted: October 25, 2001

Correspondence to:
José A. Armengol. División de Neurociencias. LAB, CSIC-UPO. Ctra. Utrera, km 1, E-41013 Sevilla, Spain. Telephone: +34-954-349-373. E-mail: jarmengo@cica.es

followed European Union Guidelines (86/609/ECC) for the use of animals in experiments. Under deep pentobarbital anesthesia (Nembutal 10-2 mg/kg), the chameleons were perfused transcardially with a solution of 4% paraformaldehyde in phosphate buffer (PB) 0.12 M pH 7.2. The eyes were enucleated, partially opened behind the cornea and immersed in fresh fixative overnight at 4º C. The eyecup was cryoprotected in 10% sucrose in the same buffer and quickly frozen in isopentane cooled with liquid nitrogen. Vertical cryostat sections (25 µm thick) were processed by immunohistochemistry. Tissue sections were preincubated in PB and 0.9% NaCl (PBS) with 0.5% gelatin, 0.25% Triton X-100 (PBS-G-T) and 0.1 M lysine for 1 hour at room temperature, and then incubated with a mouse monoclonal antibody against GAD65 diluted in PBS-G-T (1:1000). This GAD6 antibody, developed by D.I. Gottlieb (Washington University School of Medicine, St. Louis, MO), was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences (Iowa City, IA). After several rinses in PB-T, sections were incubated in Cy3-goat anti-mouse IgG (Jackson Immunoresearch) in PBS-G-T (1:200) for 1 hour. Then, sections were washed in PB-T and incubated overnight in a rabbit polyclonal antibody against GABA (1:2000; Sigma) diluted in PBS-G-T. Thereafter, sections were washed in PB-T and incubated in FITC-sheep anti-rabbit IgG (AMRAD Biotech) (1:100) for 1 hour, rinsed several times in PB, and mounted in Mowiol (Calbiochem). Observations were made under a Leica DMR microscope. Images were viewed in the Meta Imaging Series 4.5 program and with the Adobe Photoshop on a Macintosh computer.

RESULTS

Our chameleons showed a similar retinal organization to that of all other vertebrates. In the central retina, the outer nuclear layer, formed by several rows of photoreceptor cell nuclei, was separated from the outer plexiform layer by a thick Henle's layer composed by densely packed photoreceptor axons (Fig. 1A). The outermost part of the inner nuclear layer was occupied by strong GABA-Ir horizontal cell somata (Fig. 1 B-G). The horizontal cells of the central retina possessed small and rounded cell bodies, whose size increased progressively towards the peripheral retina (Fig. 1B, D, E, G). GABA immunoreactivity delineated the delicate endings of the horizontal cell dendrites (Fig. 1C, D, E). These dendrites formed dendritic fields of several sizes that, like the horizontal cell somata, progressively increased from the central to the peripheral zones of the retina (Fig. 1C, E, G). At this level, straight and isolated neurites, resembling an axon, arose from a lateral edge of the dendritic field (Fig. 1D, G). Most of these long prolongations ended in a sharp GABA-Ir tip (Fig. 1D, G), similar to that described on using the Golgi method (Quesada et al., 1999).

GABA-Ir amacrine cells were located throughout the deep tier of the inner nuclear layer (Fig. 1), and distributed their plexuses throughout the sublayer of the inner nuclear layer, the strongest immunoreactivity being observed within sublayers 1, 3 and 5 sublayers (Fig. 1B-C). GABA-Ir amacrine cells resembled those described in the lizard retina by Ramón y Cajal (1893). Two main types of amacrine cells were observed, according to the distribution of their dendritic ramifications: (i) diffuse or non-stratified amacrine cells, whose plexuses end in several inner plexiform layer strata (Fig. 1H), and (ii) stratified amacrine cells, which distribute their dendritic branches in one of the sublayers of the inner plexiform layer (Fig. 1E). Scattered intraplexiform GABA-Ir cell bodies were observed throughout all the retinal regions (not shown). Displaced GABA-Ir cells formed a discontinuous band located at the external limit of the ganglion cells layer. Their dendritic trees endings were distributed through the innermost sublayer of the inner plexiform layer, mimicking in a mirror fashion the distribution of radial amacrine plexuses described by Ramón y Cajal (1893).

In the peripheral retina, interplexiform cells projecting to both retinal plexiform layers were found (Fig. 1F). The outer prolongation branched in few small dendrites behind the horizontal cell somata (Fig. 1F), which were distributed within the outer plexiform layer. After a short and irregular trajectory through the inner nuclear layer, the inner prolongation divided into smooth branches, which resembled the plexus of non-stratified amacrine cells (Fig. 1F).

DISCUSSION

The present results demonstrate the presence of two populations of GABA-Ir cells in the chameleon retina: horizontal and amacrine cells. GABA-Ir horizontal cells are distributed throughout the entire chameleon retina. According to their size and dendritic ramification, the horizontal cell types described by Quesada et al. (1999) as type I for the central retina, type II or transitory dendritic tree cells from the central to the peripheral retina, and type III –or peripheral horizontal cells– display an important GABA immunoreactivity. This immunoreactivity expands from the soma to the delicate dendrite endings

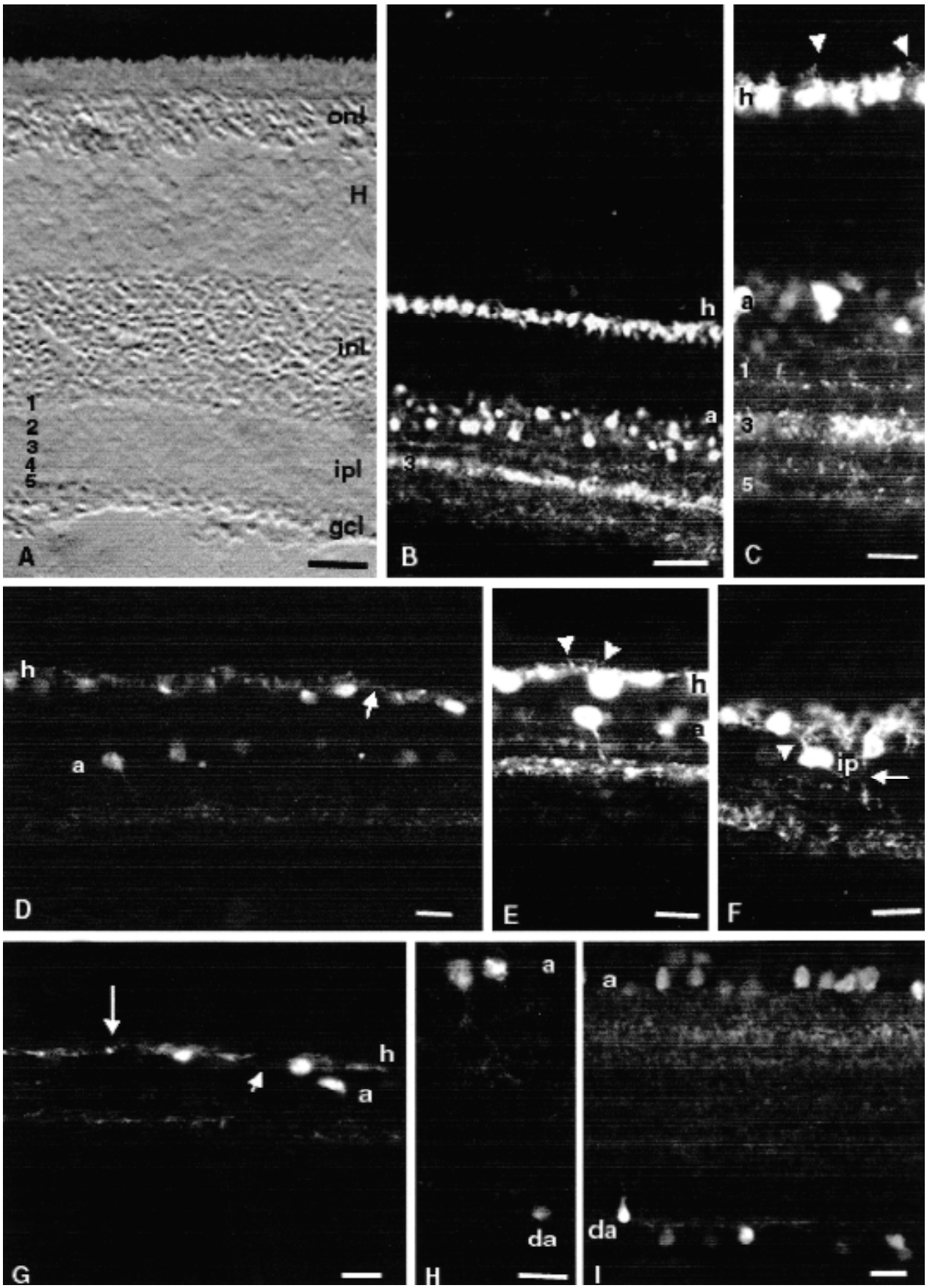

Fig. 1.- Vertical sections through the central and peripheral retina of the chameleon. Nomarski's interferential contrast shows the different layers of the retina (**A**). Horizontal (h) and amacrine cells (a) are strongly GABA-Ir in both the central (**B**, **C**, **H** and **I**) and peripheral retina (**D**, **E**, **G**). GABA immunoreactivity delineates the dendritic endings of the horizontal cells within the outer plexiform layer (C and E, arrowheads), and the varicose tip (G, arrow) of their axons (D and G, short arrows). Displaced amacrine (da) cells occupy the outermost portion of the ganglion cells layer (H-I). In the peripheral zones of the retina, GABA-Ir interplexiform cells (**F**, ip) send their prolongations to the outer (arrowhead) and the inner (arrow) plexiform layers. onl: outer nuclear layer; H: Henle's layer; inl: inner nuclear layer; ipl: inner plexiform layer; gcl: ganglion cell layer; 1-5: 1-5 sublayers of the inner plexiform layer. Scale bars mark 100 µm (A, B), and 30 µm (C-I).

and the putative axonal prolongation. Thus, the present results extend the first description of GABAergic horizontal cells (Bennis and Versaux-Botteri, 1995) to almost all horizontal cell types of the chameleon retina, and strongly disagree with the absence of GABA-Ir horizontal cells, twice reported by Quesada et al. (1996; 1999). This discrepancy is quite surprising because these authors reported the presence of GABA-Ir amacrine cell types similar to those found in the lizard (Sherry and Ulshafer, 1992) and chameleon retina (Bennis and Versaux-Botteri, 1995; present results). A possible explanation for this discrepancy might be based on the quality of the antibody utilized in each experiment. Indeed, Haverkamp and Wässle (2000), using three different antibodies, did not observe the presence of GABA-Ir horizontal cells in the mouse retina, and in our hands, the GAD-antibody failed to label any synaptic ending of GABA-Ir cells in the chameleon retina. Further, the fixation method used has an essential role in the preservation of retinal antigenic epitopes (Haverkamp and Wässle, 2000). Thus, the fact that Quesada et al. (1996, 1999) utilized Zamboni's fluid instead of 4% paraformaldehyde may also explain this loss of immunoreactivity in their preparations (for a review, see Landis, 1985).

GABA-Ir amacrine cell types belong those described previously in the lizard (Sherry and Ulshafer, 1992) and chameleon retina (Bennis and Versaux-Botteri, 1995). Some small GABA-Ir cell somata were found within the outermost part of the ganglion cells layer. According to their size and location, these cells are considered here to be similar to the GABA-Ir displaced amacrine cells described in several species (for a review, see Yazulla, 1986; Vigh et al., 2000). Few GABA-Ir neurons locate their somata within the inner plexiform layer. Intraplexiform cells, of unknown function, earlier described in the chameleon retina by Genis-Galvez et al. (1978), may represent misplaced inverted amacrine cells that have arrested their migration within the inner plexiform layer during development (Hinds, 1979). However, in contrast to the first description of GABA immunoreactivity in the chameleon retina (Bennis and Versaux-Botteri, 1995), neither GABA-Ir ganglion cells nor fibers coursing through the optic nerve fiber layer were found in present immunohistochemical experiments.

Interplexiform cells are a special type of amacrine cell that project to both plexiform layers (Dowling and Ehinger, 1975; 1978). These cells have been described as dopaminergic cells that interconnect and modulate the information between the inner and the outer plexiform layers (Djamgoz and Wagner, 1992) in the retina of several vertebrates [fishes (Van Haesendonck et al., 1993; Dalil-Thiney et al., 1996), amphibians (Vigh et al., 2000), lizards (Lanuza et al., 1996), birds (Dkhissi et al., 1993), and mammals (Gustincich et al., 1999)]. Interplexiform cells possess GABA receptors (Gustincich et al., 1999), indicating that they might act under GABA modulation. However, their ability to synthesize GABA in amniotes is discussed (Mosinger et al., 1986; Wulle and Wagner, 1990; Brecha et al., 1991). In the chameleon retina, interplexiform cells have been identified in Golgi-stained retina (Quesada and Genis-Galvez, 1983), but they lack GABA immunoreactivity (Quesada et al., 1996). In the present work, some interplexiform cells located in the peripheral retina were GABA-Ir, reinforcing the developmental idea, earlier proposed by Ramón y Cajal (1893) and Rochon-Duvigneaud (1943), concerning the similarities between the chameleon and the avian retina.

In conclusion, the present findings demonstrate, as in the rest of vertebrate retinas, that GABA may play a relevant role in the horizontal cell inhibitory feedback to cones to account for color discrimination and surround responses in cones and bipolar cells (Baylor et al., 1971; Marc et al., 1978; Watt et al., 2000) in the chameleon retina.

ACKNOWLEDGEMENTS

The authors thank Dr de la Villa for critical reading of the manuscript. This work was supported by grants to J.A.A. from the D.G.I.C.Y.T. (PB 95-0154), the Junta de Andalucía (Ayuda para la consolidación de grupos de investigación científica y tecnológica), Spain.

REFERENCES

AGARDH E, BRUUN A, EHINGER B and STORM-MATHISEN J (1986). GABA immunoreactivity in the retina. *Invest Ophthalmol*, 27: 674-678.

ARMENGOL JA, PRADA F, AMBROSIANI J and GENIS-GALVEZ JM (1988). The photoreceptors of the chameleon retina (*Chamaleo chamaleo*). A Golgi study. *J Hirnsforsch*, 29: 403-409.

BAYLOR DA, FUORTES MGF and O'BRYAN PM (1971). Receptive fields of the cones in the retina of the turtle. *J Physiol (Lond)*, 214: 265-294.

BENNIS M, REPÉRANT J, RIO JP and WARD R (1994). An experimental reevaluation of the primary visual system of the chameleon, *Chamaeleo chameleon*. *Brain Behav Evol*, 43: 173-188.

BENNIS M and VERSAUX-BOTTERI C (1995). Cathecolamine–, indoleamine–, and GABA-containing cells in the chameleon retina. *Vis Neurosci*, 12: 785-792.

BRECHA NC, STERNINI C and HUMPHREY MF (1991). Cellular distribution of L-glutamate decarboxylase (GAD) and gamma-aminobutyric acid receptor mRNAs in the retina. *Cell Mol Neurobiol*, 11: 497-509.

CRESCITELLI F (1972). The visual cells and visual pigments of the vertebrate eye. In: Dartnall HJA (ed). *Handbook of Sensory Physiology*. Springer-Verlag, New York, Vol. VII/1, pp 245-363.

DALIL-THINEY N, VERSAUX-BOTTERI C and NGUYEN-LEGROS J (1996). Electron microscopic demonstration of tyrosine

hydroxylase-immunoreactive interplexiform cells in the lamprey retina. *Neurosci Lett*, 207: 159-162.

DJAMGOZ MB and WAGNER HJ (1992). Localization and function of dopamine in the adult vertebrate retina. *Neurochem Int*, 20: 139-191.

DKHISSI O, DALIL-THINEY N, VERSAUX-BOTTERI C, CHANUT E, REPÉRANT J and NGUYEN-LEGROS J (1993). Dopaminergic interplexiform cells in the retina of pigmented and hypopigmented quails (*Coturnix coturnix japonica*). *Ophthalmic Res*, 25: 280-288.

DOWLING JE and EHINGER B (1975). Synaptic organization of the amine-containing interplexiform cells of the goldfish and Cebus monkey retinas. *Science*, 18: 270-273.

DOWLING JE and EHINGER B (1978). The interplexiform cell system. I. Synapses of the dopaminergic neurons of the goldfish retina. *Proc R Soc Lond B Biol Sci*, 201: 7-26.

GENIS-GALVEZ JM, PRADA FA and ARMENGOL JA (1978). The intraplexiform cells of the retina of the chameleon. *An Desarr*, 22: 21-25.

GUSTINCICH S, FEIGENSPAN A, SIEGHART W and RAVIOLA E (1999). Composition of the GABA (A) receptors of retinal dopaminergic neurons. *J Neurosci*, 19: 7812-7822.

HARKNESS L (1977). Chameleons use accommodation cues to judge distance. *Nature*, 267: 346-349.

HAVERKAMP S and WÄSSLE H (2000). Immunocytochemical analysis of the mouse retina. *J Comp Neurol*, 424: 1-23.

HINDS JW (1979). Early neuron differentiation in the CNS: a comparison of several regions studied with serial thin sections. In: Meisami E, Brazier MA (eds). *Neural Growth and Differentiation*. Raven Press, New York, pp. 133-151.

HURD LB and ELDRED WD (1989). Localization of GABA and GAD-like immunoreactivity in the turtle retina. *Vis Neurosci*, 3: 9-20.

LANDIS DMD (1985). Promises and pitfalls in immunocytochemistry. *Trends Neurosci*, 8: 312-317.

LANUZA E, MARTÍNEZ-MARCOS A, FONT C and MARTÍNEZ-GARCÍA F (1996). Catecholaminergic interplexiform cells in the retina of lizards. *Vision Res*, 36: 1349-1355.

MARC RE, STELL WK, BOK D and LAM DM (1978). GABA-ergic pathways in the goldfish retina. *J Comp Neurol*, 182: 221-244.

MOSINGER JL, YAZULLA S and STUDHOLME KM (1986). GABA-like immunoreactivity in the vertebrate retina: a species comparison. *Exp Eye Res*, 42: 631-644.

PESSAC B, TOWLE AC, GEFFARD M and WU JY(1987). Presence of glutamic acid decarboxylase and gamma-aminobutyric acid immunoreactivity in photoreceptors of hatching quail retina. *Dev Brain Res*, 1: 156-159.

POURCHO RG and GOEBEL DJ (1983). Neuronal subpopulation in cat retina which accumulates the GABA agonist 3H-muscimol: A combined Golgi and autoradiographic study. *J Comp Neurol*, 219: 25-35.

QUESADA A and GENIS-GALVEZ JM (1983). The interplexiform cell in the chameleon retina. *Anat Histol Embryol*, 12: 70-76.

QUESADA A, CHMIELEWSKI CE, GENIS-GALVEZ JM and PRADA F (1996) Immunohistochemical localization of GABA in chameleon retina (*Chamaleo chamaleo*). *Cell Biol Internat*, 20: 395-400.

QUESADA A, GARCÍA-LOMAS V, ESPINAR A, GENIS-GALVEZ JM and PRADA F (1999). Horizontal cells in the chameleon retina. *Eur J Anat*, 3: 13-25.

RAMÓN y CAJAL S (1893). La retine des vertébrés. *La Cellule*, 9: 119-257.

ROCHON-DUVIGNEAUD A (1943). *Les yeux et la vision des vertébrés*. Masson Ed., Paris.

SHERRY DM and ULSHAFER RJ (1992). Neurotransmitter-specific identification and characterization of neurons in the all-cone retina of *Anolis carolinensis*, I: Gamma-aminobutyric acid. *Vis Neurosci*, 8: 515-529.

VERSAUX-BOTTERI C, HERGUETA S, PIEAU C, WASOWICZ M, DALIL-THINEY N and NGUYEN-LEGROS J (1994). Early development of GABA-like immunoreactive cells in the retina of turtle embryos. *Dev Brain Res*, 83: 125-131.

VAN HAESENDONCK E, MARC RE and MISSOTTEN L (1993). New aspects of dopaminergic interplexiform cell organization in the goldfish retina. *J Comp Neurol*, 333: 503-518.

VIGH J, BANVOLGYI T and WILHELM M (2000). Amacrine cells of the anuran retina: morphology, chemical, neuroanatomy, and physiology. *Microsc Res Tech*, 50: 373-383.

WATT CB, KALLONIATIS M, JONES BW and MARC RE (2000). Studies examining the neurotransmitter properties of horizontal cell populations in the goldfish retina. *Invest Ophthalmol Vis Sci*, 41: S943.

WULLE I and WAGNER HJ (1990). GABA and tyrosine hydroxylase immunocytochemistry reveal different patterns of colocalization in retinal neurons of various vertebrates. *J Comp Neurol*, 296: 173-178.

YAZULLA S (1986). GABAergic mechanisms in the retina. In: Osborne NN, Chader GJ (eds). *Progress in Retinal Research*. Pergamon Press, Oxford, vol. 5, pp 1-52.