British Journal of Medicine & Medical Research
19(8): 1-25, 2017; Article no.BJMMR.31018
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Ultrastructure of Teleost Retina II: Interstitial Amacrines, Dislocated Amacrines, Ganglion Cells, Müller Fiber, Oligodendroglia, Adrenergic Terminals, Inner Plexiform Layer, Glycogen Cytochemistry

Jose Antonio O'Daly[1*]

[1]*Instituto Venezolano de Investigaciones Científicas (IVIC), Center of Microbiology and Cell Biology, Caracas, Venezuela.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/BJMMR/2017/31018
Editor(s):
(1) Ravi Kumar Chittoria, Department of Plastic Surgery & Advanced Centre for Microvascular, Maxillofacial & Craniofacial, Laser Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India.
Reviewers:
(1) Italo Giuffre, Catholic University of Roma, Rome, Italy.
(2) Tomás Ortiz Basso, Hospital Italiano de Buenos Aires, Argentina.
Complete Peer review History: http://www.sciencedomain.org/review-history/17735

Original Research Article

Received 15th December 2016
Accepted 30th January 2017
Published 6th February 2017

ABSTRACT

Aims: To analyze the ultrastructure of internal interstitial amacrine cell, dislocated amacrine cell, ganglion cells, oligodendroglia, Müller cells, inner plexiform layer, adrenergic terminals, as well as the connections found between the cells and the cytochemistry of glycogen distribution from *Mugil brasiliensis* teleost retina.
Methodology: The retina was fixed with glutaraldehyde and osmium tetroxide and radial and tangential sections stained with lead citrate and uranyl acetate to be observed in a Hitachi 11B electron microscope at 75Kv. To demonstrate glycogen fragments glutaraldehyde fixed osmium post-fixed were, sectioned in the MT2 ultramicrotome and processed with periodic acid thiosemicarbizide for oxidized molecules.
Results: Retinal morphology revealed external interstitial amacrine cells as the most voluminous cell above inner plexiform layer. Dislocated amacrine cells described by the first time, localized at the inner plexiform layer, had clear cytoplasm, few ribosomes, few vesicles of rough endoplasmic reticulum and elongated mitochondria with clear matrix. Dark and clear amacrine piriform cells

**Corresponding author: E-mail: jaocjesus@hotmail.com;*

were characterized by glycogen density, and their functional contacts described. External horizontal cells, dark piriform amacrines, stellate amacrines and Müller cells exhibited the highest glycogen concentration in teleost fish retina.

Conclusion: This paper is a comprehensive analysis of ultrastructure of five retinal cells, adrenergic terminals inner plexiform layer and glycogen distribution in the retina, finding one new cell, the dislocated amacrine cell and also describing the amacrine interstitial external cell the most voluminous cell in teleost retina.

Keywords: Adrenergic vesicles; inner plexiform layer; interstitial amacrine cells; retina glycogen; retina ultrastructure.

1. INTRODUCTION

Retinal cell morphology was organized in cell layers and their connections were described in detail with light microscopy by Cajal using the method of Golgi [1]. Neuronal and glial types of amacrine cells, the last one compared to astrocytes were described in fish retina [2]. In the cat and the pigeon retinas synaptic laminas were observed in association with multiple synaptic contacts in the inner plexiform layer. Four main kinds of synapse were identified (a) The conventional kind, as described in the CNS, (b) The ribbon type, similar to those described in the outer plexiform layer. (c) The spine type, consisting of a post-synaptic process invaginating a pre-synaptic process. (d) The serial type, one being similar to the inter-receptor synapses described previously in the guinea-pig, and a second type involving a process which is postsynaptic to one process and pre-synaptic to another, all the synaptic contacts involved being of the conventional kind [3]. Synaptic laminas were identified as structures belonging to bipolar cells (BC) axons, describing also one type of piriform amacrine cells of neuronal nature in monkey retina [4]. In mammals (*Ictidomys tridecemlineatus*) amacrine cells take a blue-on signal from a short wavelength blue cone to a BC and to glycinergic amacrine S-cone inhibitor cell, sending blue-off responses to ganglion cells [5]. In mammals, AII-amacrines built a field of stratified glycinergic neurons, collecting escotopic signals from rod BC to be distributed to cones OFF and ON pathways into a net of inhibitory synapses and cellular unions AII::ON cone bipolar-gap cells. AII-amacrines have the most complex repertoire of interactions that any other neuron in vertebrates, contacting 28 different cell classes including BC [6]. In electron microscopy, serial sections showed BC connecting to OFF-amacrine cells (CASs) close to the cell soma. Space-time wiring specificity supports direction selectivity in the retina [7]. There are several morphological types of retinal ganglion cells (RGC) codifying specific aspects of the visual scene. In a transgenic mouse expressing green fluorescent protein (GFP) in cells, it was found that Islet2 (IsL2)-GFP RGC have different morphologies and dendritic patterns in the inner plexiform layer. Islet2 is a transcription factor in LIM-home dominion in the developing retina in 40% RGC, it has dendrites in layer S3 of inner plexiform layer and innervates the nucleus geniculate and the superior colliculus when the eye opens [8]. RGC usually do not branch before exiting retina, thus do not provide synaptic feedback. However, small groups of RGC have collateral axons in humans, monkeys (macaque), mice, cats and turtles, their function remains unknown. Some RGC express melanopsine, irradiating a signal to brain visual nuclei, contributing to image formation, circadian rhythm and pupil reflex to light. Inmuno labeling with anti-melanopsine in monkeys revealed a group of ipRGC, with branching axons, that branch via the optic disc forming intraretinal axon collaterals, ending in the inner plexiform layer, probably building a synaptic feedback to modulate responses to light [9]. For night vision you require retinal microcircuits to send signals of one photon to RGC via rod bipolar (RB) cells and AII-amacrine cells. Tridimensional reconstruction of serial sections with the electron microscope, in C57BL/6J mouse revealed that each rod contacted by ribbon synapses two adjacent RB in 94% of cases and each RB was contacted by 25 rods. Each RB axon contacted 4 or 5 AII-amacrines via 53 synapses, thus one rod signal might be represented in 106 replicas in 2 RB axons [10]. Glycogen has an important role in brain energy, localized mainly in astrocytes and Müller cell fibers together with glycogen synthetase and glycogen synthetase kinase 3β (GSK3β) [11]. Glycogen granules have been found in cones but not in rods in the rat retina [12]. Adrenergic receptors alfa 1 and beta 2 have been identified in glial cells in rat anterior brain, rabbit brain and human optic nerve and beta 2 using histochemistry and autoradiography, in

astrocytes of the same animals [13]. Glycogen phosphorilase (GP) have been found by monoclonal antibodies in Müller cell fibers of rat and rabbit retina [14]. In this second paper we analyzed the ultrastructure of five additional cells in the teleost retina as well as their membrane connections together with the localization of adrenergic terminals, the structure of the inner and outer plexiform layers and the glycogen distribution in all retinal cells, as a continuation of first paper on Ultrastructure of Teleost Retina I [15].

2. MATERIALS AND METHODS

Teleost fish, family Mugilidae, genera Mugil, specie brasiliensis, was used. Retinas were extracted after equatorial section of the eye to eliminate the anterior part and crystalline, cutting the choroid and sclerotic around the optic nerve which was preserved, taking out the vitreous humor. Retinas were fixed in 2% glutaraldehyde in 0.1 molar Na phosphate buffer pH 7.2 for 3 hrs at 4°C [16]. Afterwards, retinas were washed in 0.18 molar sucrose in 0.1 molar sodium phosphate buffer pH 7.2, with six changes one every 10 minutes. Subsequently retinas were cut in pieces 1 mm wide 1-3 mm long selecting the bests under the inverted microscope and post fixed in equal parts of 2% osmium tetroxide and 0.18 molar sucrose in sodium phosphate buffer pH 7.2 at 4°C overnight, as published [17]. After dehydration half the retinas were processed in Araldita and the other half in Maraglas. Radial and tangential sections were obtained in a Porter Blum MT2 ultramicrotome and stained with lead citrate and uranyl acetate to be analyzed in a Hitachi Hu-11B at 75 Kv. To demonstrate glycogen, fragments glutaraldehyde fixed osmium post-fixed were, sectioned in the MT2 ultramicrotome and processed with periodic acid thiosemicarbizide for oxidized molecules technique [18]. Some sections were treated with saliva amylase for 1 hr. at 37°C [19]. Finally sections were treated with osmium acid vapors. All sections were stained with uranyl acetate to increase contrast.

3. RESULTS

3.1 Amacrine Interstitial External Cells

In a general view of the retina below cones and rods photoreceptors, three layers of horizontal cells with lateral process with plasma membranes fused were seen, and also BC bodies with big nuclei, undulated amacrine cells, located in the layer of medium and internal horizontal cells close to the tubular cells layer. Amacrine interstitial external cells (Amle) were localized below tubular and stellate amacrine cells and constitute the most voluminous cell in the teleost retina (Fig. 1). Amle form a tangential net among pairs of clear and dark piriform amacrine cells and nuclei of the Müller cells (Fig. 2). Functional contacts with dense undulated plasma membranes were observed between Amle with numerous microtubules and dark piriform amacrine cells surrounded by the Müller cell fiber with glycogen granules and dilated vesicles in the cytoplasm. Piriform amacrine cells had the highest density of glycogen granules and many elongated dilated mitochondria, with hypertrophic crests. In the cytoplasm many dilated clear elongated vesicles as well as dark dense lamellar structures were observed (Fig. 3). Amle had processes with many parallel microtubules, surrounded by cell terminals with a high content of dense vesicles, probably adrenergic vesicles. BC terminals had membrane fusion to adrenergic terminals exhibiting two synaptic lamina-like structure parallel to the membranes at the contact site with very dense plasma membranes, one of them close to a clear piriform amacrine cell (Fig. 4). Amle exhibited oval nuclei with prominent nucleolus, perinuclear polyribosomes, vesicles of rough endoplasmic reticulum and Golgi apparatus with numerous mitochondria and fusion of plasma membrane to a neighbor Amle (Fig. 5). Amle cells with many microtubules have lateral expansions in the same layer forming functional contacts characterized by fusion of both high density membranes without synaptic vesicles, which may correspond to synapses of reciprocal flux or electrical synapses (Fig. 6), similar to horizontal cells functional contacts.

3.1.1 Amacrine interstitial internal cells

Amacrine Interstitial internal cells (Amli) structured another layer located in the inner plexiform layer, above the optical nerve fibers, exhibiting numerous microtubule , dense granules, elongated big mitochondria and fusion of very dense undulated plasma membranes in adjacent cells (Figs. 7, 8). All amacrine cell membrane contacts were similar to functional contacts between horizontal cells membranes. Some expansions terminals showed two laminar components surrounded by a halo, vesicles and granules (Fig. 9). There are also fusion of dense

plasma membrane contacts between the terminal BC process, with a synaptic lamina-like structure, and the body of the Amle as well as with clear piriform amacrine cells membranes (Fig. 10).

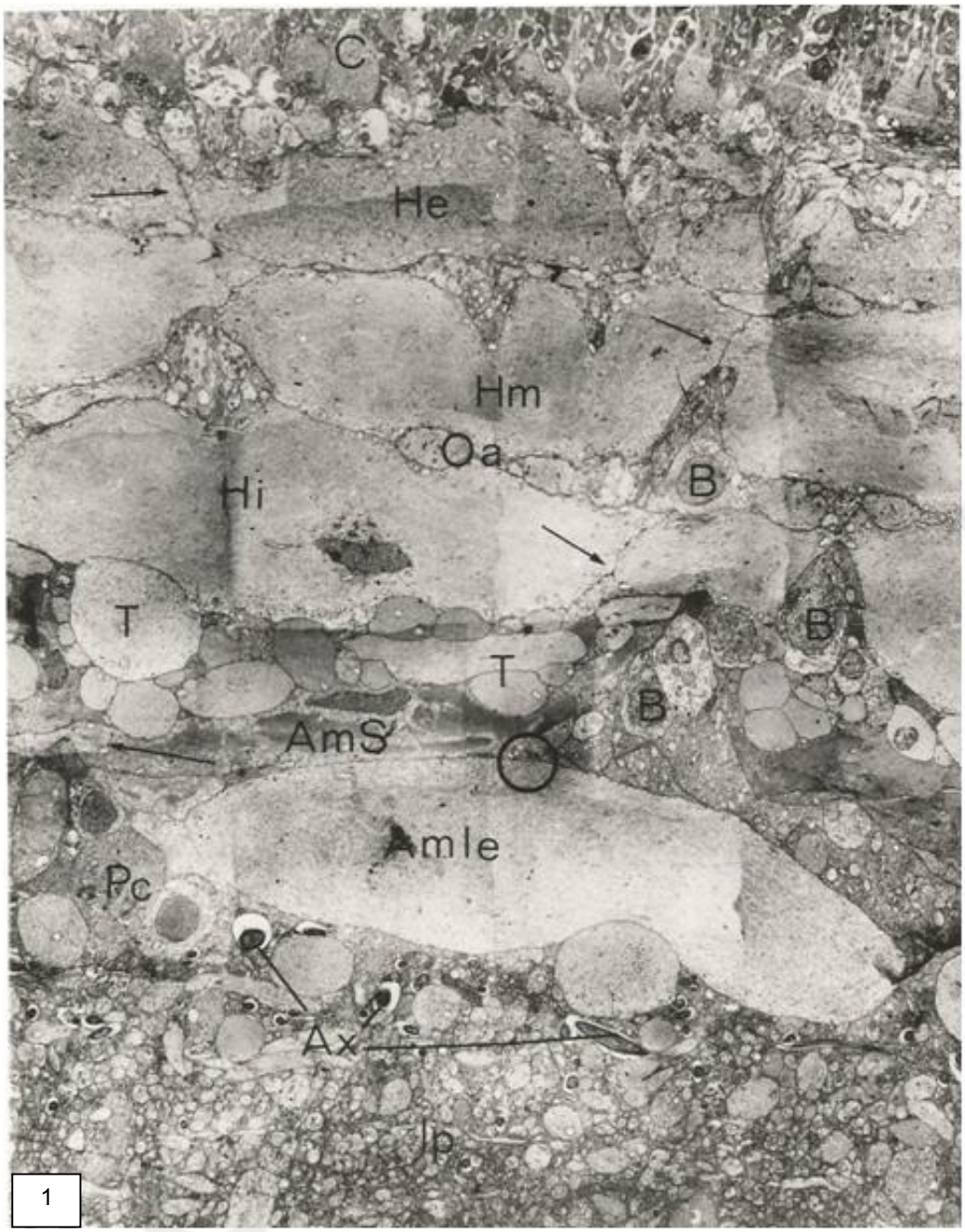


Fig. 1. Retina mosaic [around 150 photos]. Cone [C], external horizontal [He], medium horizontal [Hm], internal horizontal cell [Hi] with nucleus. Functional contacts with membrane fusion between horizontal cells [arrows]. Other cells: Tubular cell [T], Amacrine stellate [AmS] cell, amacrine interstitial external [Amle] cell, undulate amacrine [Oa] cell, bipolar cell [B], clear piriform cell [Pc], myelinated axons [Ax], inner plexiform layer [Ip]. Circle: fusion between Amle and AmS. Radial section. Each photo X 1800

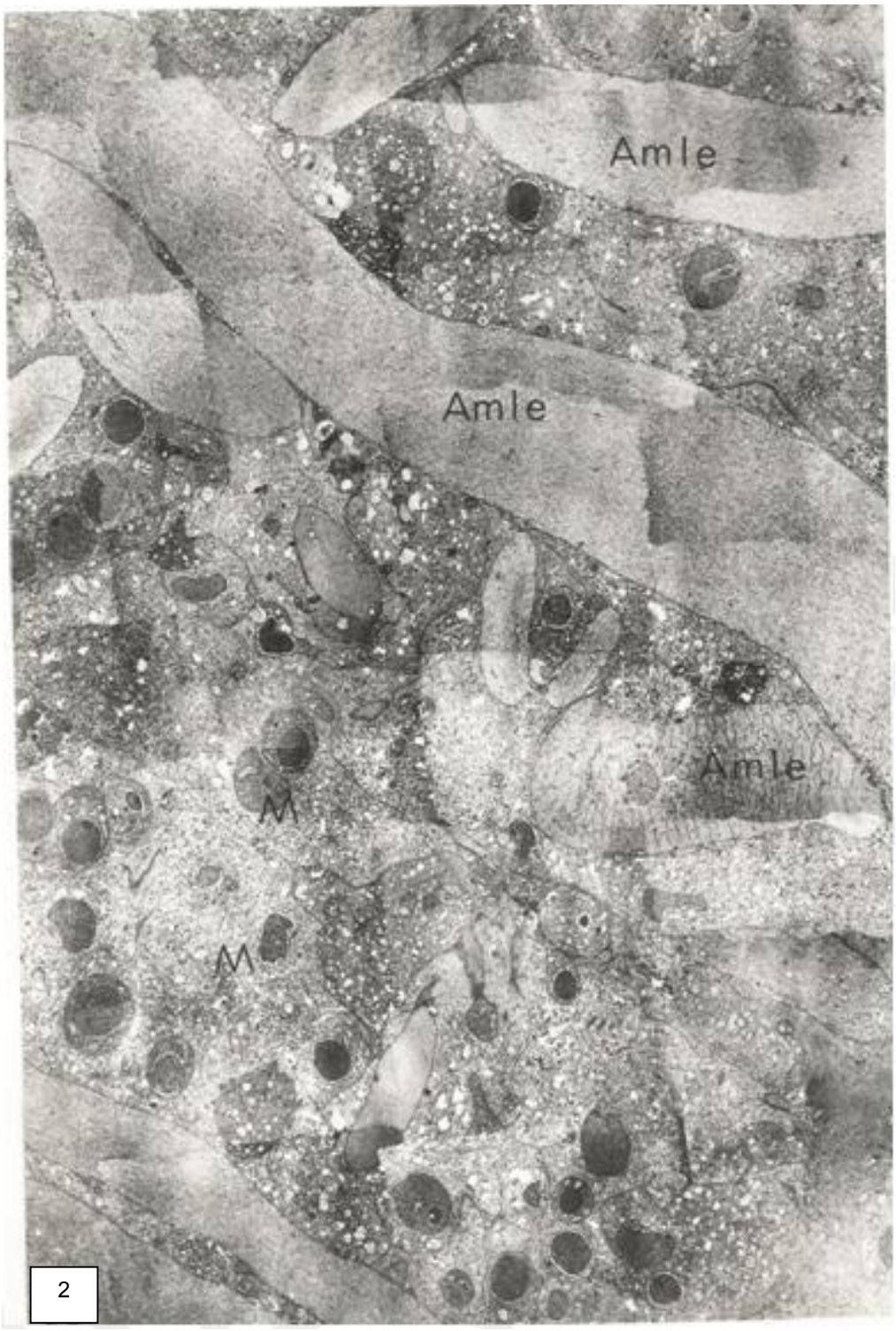


Fig. 2. Mosaic [around 150 photos] tangential section at level of Amle and piriform amacrine cells. Amacrine interstitial external [Amle] cell. Müller cell fiber [M]. Noteworthy abundant pairs of dark and clear piriform cells between Amle. Tangential section. Each photo X 1800

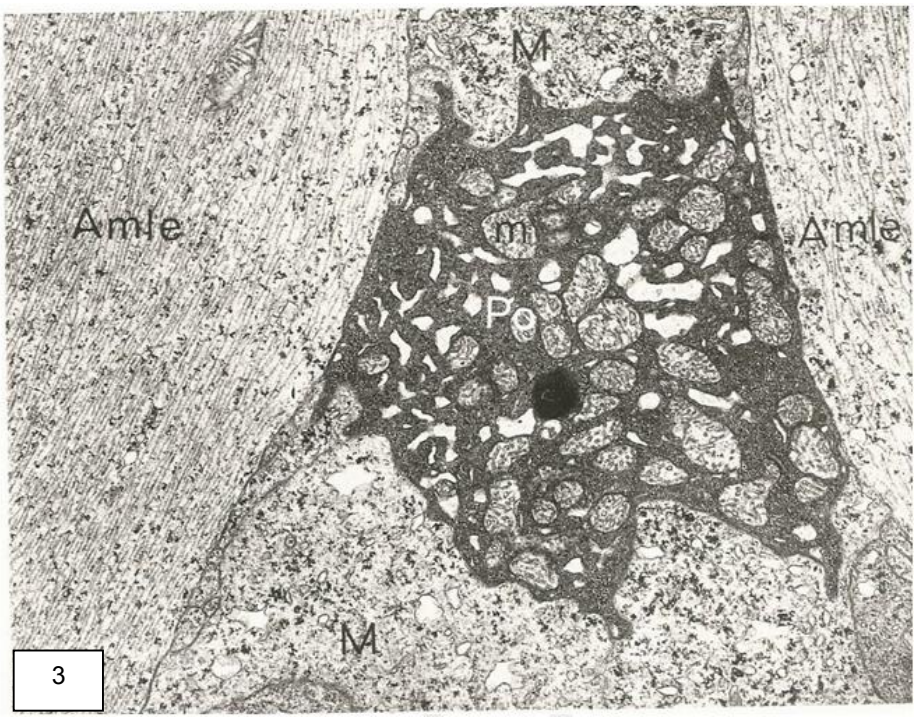


Fig. 3. Dark piriform amacrine cell [Po], amacrine interstitial external cell [Amle] with microtubules, Müller cell fiber [M]. Mitochondria [m]. Tangential section X 10000

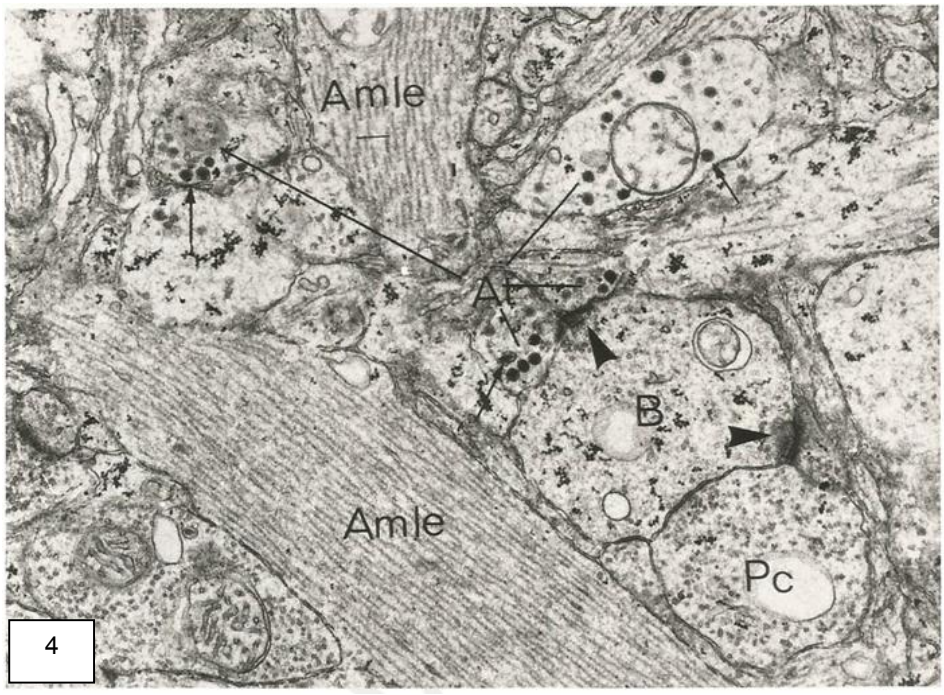


Fig. 4. Amacrine interstitial external cell [Amle], with numerous microtubules, bipolar cell terminal [B] with synaptic lamina [triangle] close to fused plasma membranes, adrenergic terminals with dense vesicles [At], [arrows], clear piriform amacrine cell [Pc]. Tangential section. X 11750

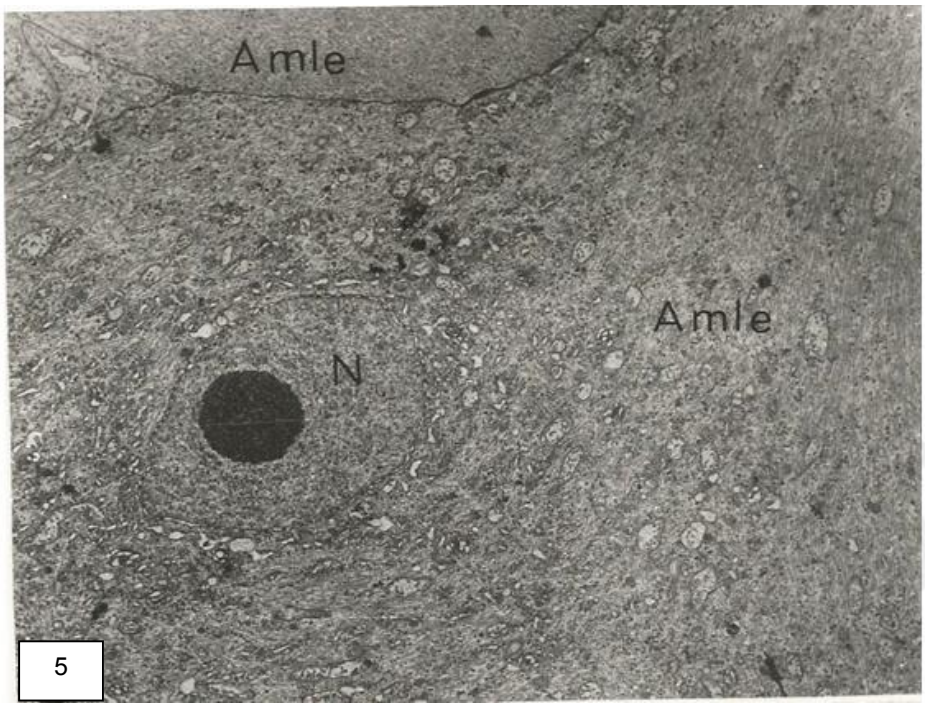


Fig. 5. Amacrine interstitial external [Amle] cell; nucleus [N] with prominent nucleolus surrounded by mitochondria, rough endoplasmic reticulum and Golgi apparatus. Tangential section X 5500

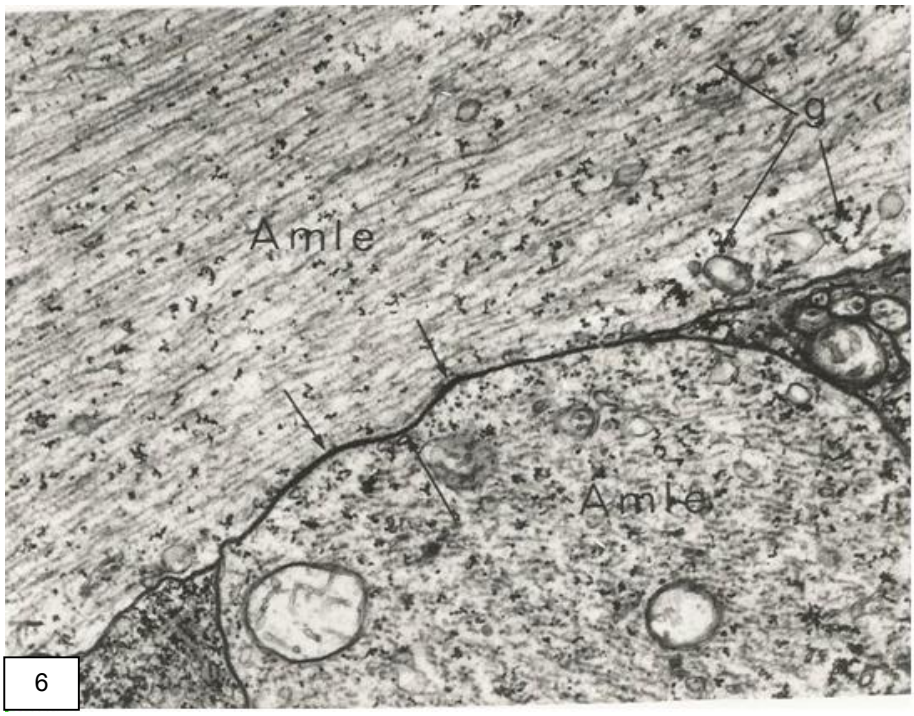


Fig. 6. Amacrine interstitial external [Amle] cell with numerous microtubule and glycogen granules (g) in cytoplasm, with fussed plasma membranes at functional contact [arrows]. Radial section X 37200

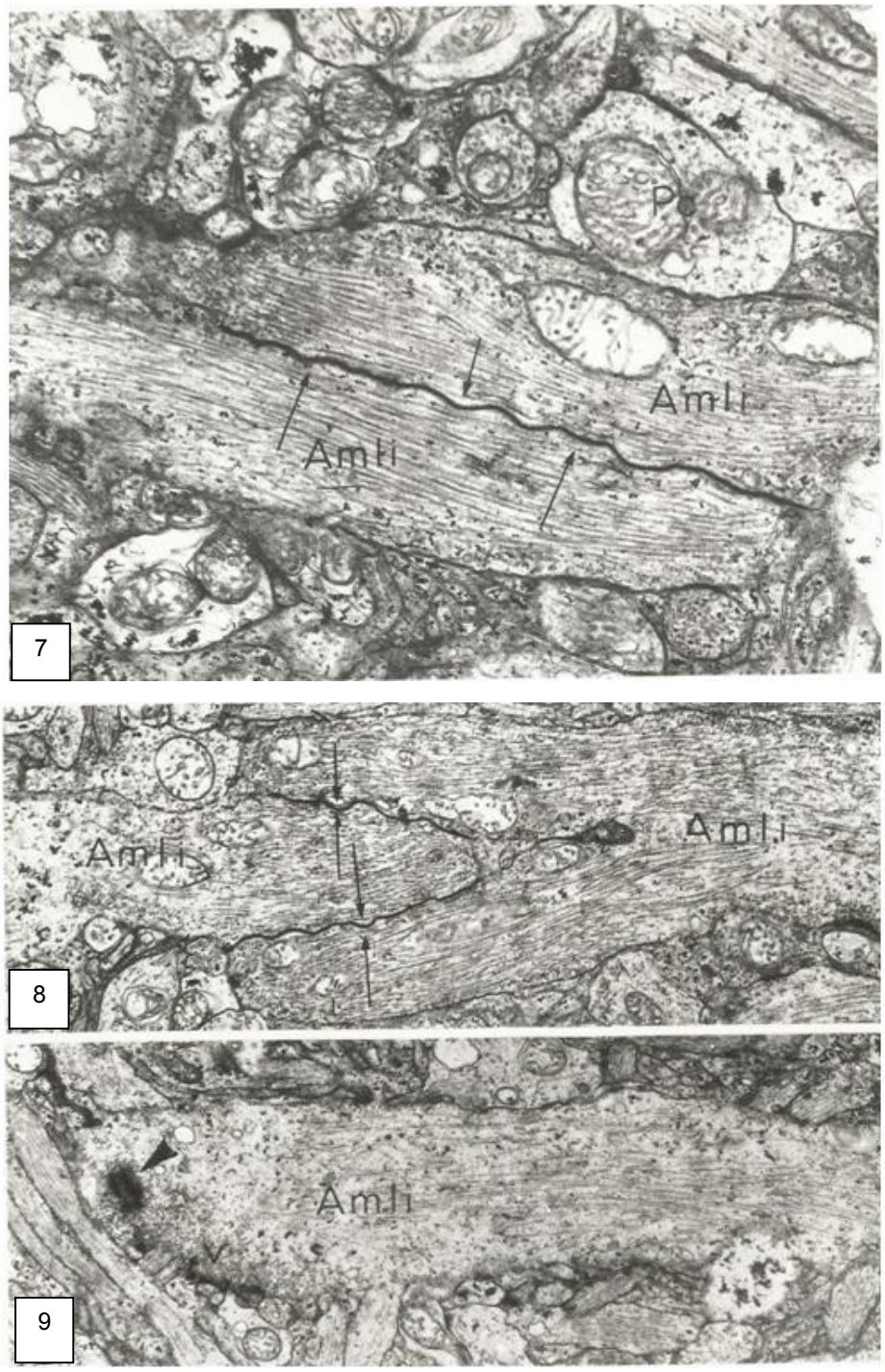
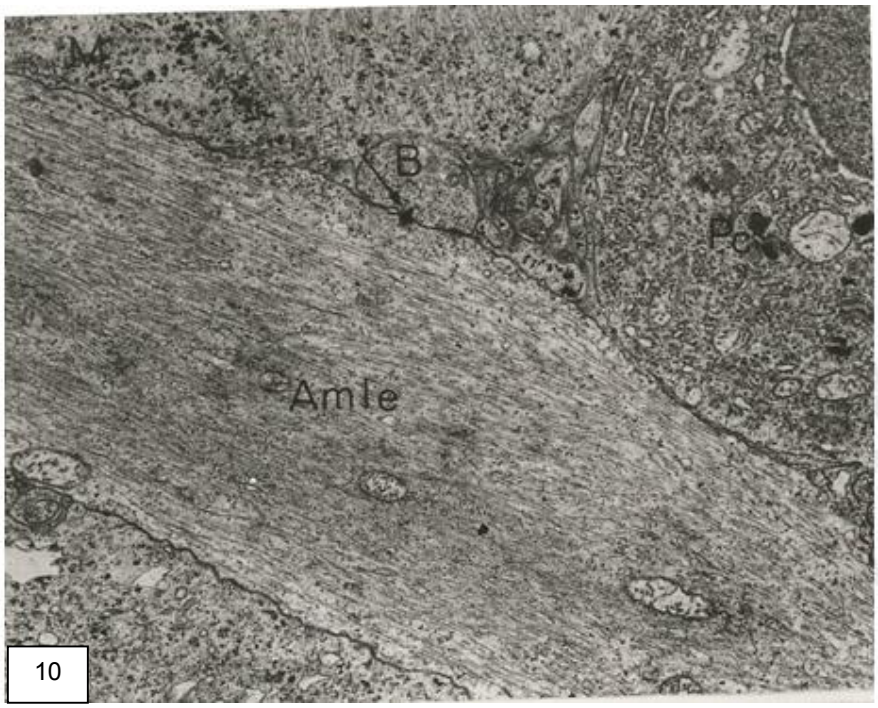


Fig. 7. Amacrine interstitial internal [Amli] cells, with mitochondria, many fibrils and glycogen granules, with functional contacts [arrows]. Tangential section X 17500

Fig. 8. Amacrine interstitial internal [Amli]; with functional contact [arrows]. Tangential section X 9800

Fig. 9. Expansion of amacrine interstitial internal [Amli] cell with 2 dense laminar components [triangle] and also horizontal synaptic lamina in membrane both surrounded by vesicles. Tangential section X 8750



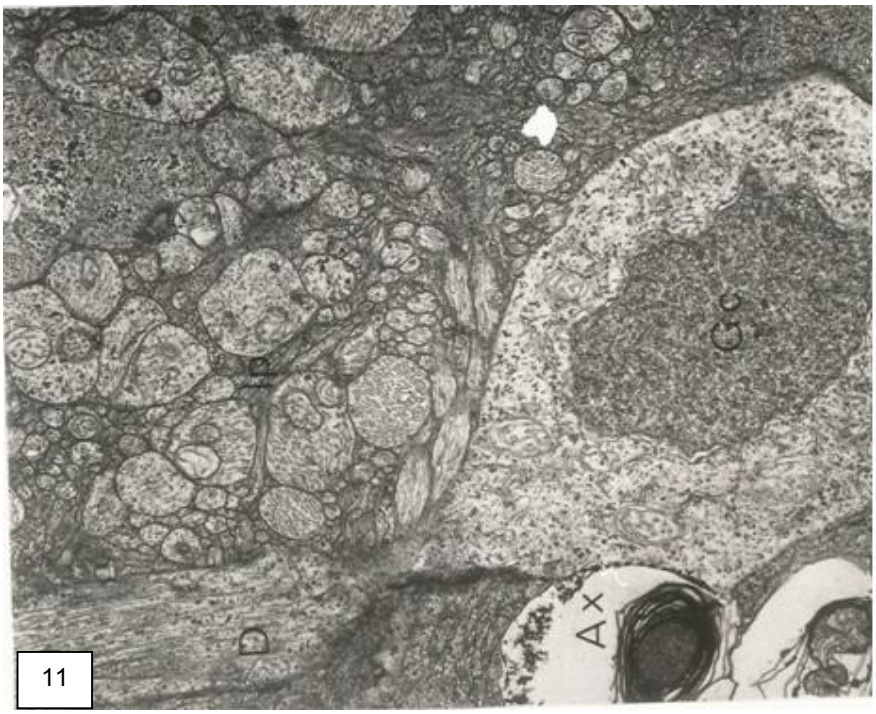


Fig. 10. Amacrine interstitial external cell [Amle] with many microtubules and functional contact with bipolar cell terminal [B] with synaptic lamina [arrow]. Clear piriform cell [Pc] in functional contact with Amle, Müller cell fiber [M]. Tangential section X 8750
Fig. 11. Ganglion cell [Gc] with main dendrite [D], inner plexiform layer [Ip], myelinated axons [Ax] from optical fibers layer. Radial section. X 9600

3.1.2 Ganglion cells

Ganglion cells of variable size are located between optical nerve fibers and the inner plexiform layer sometimes as pairs in contact. Ganglion cell dendrites penetrate the inner plexiform layer, have many microtubules and elongated mitochondria with clear matrix around the dense nucleus (Fig. 11). The Cytoplasm exhibits abundant ribosomes and rough endoplasmic reticulum vesicles, many mitochondria with hypertrophic crests, as well as dense bodies encircled by membranes. Ganglion cells exhibit plasma membrane fusion among ganglion cells pairs (Figs. 12, 13). Amacrine interstitial internal cells (Amli), with many microtubules also present fusion to ganglion cell membranes, which had mitochondria with hypertrophic cristae, abundant glycogen granules dense bodies and many dilated vesicles of rough endoplasmic reticulum in the cytoplasm and close to the membranes fused (Figs. 14, 15).

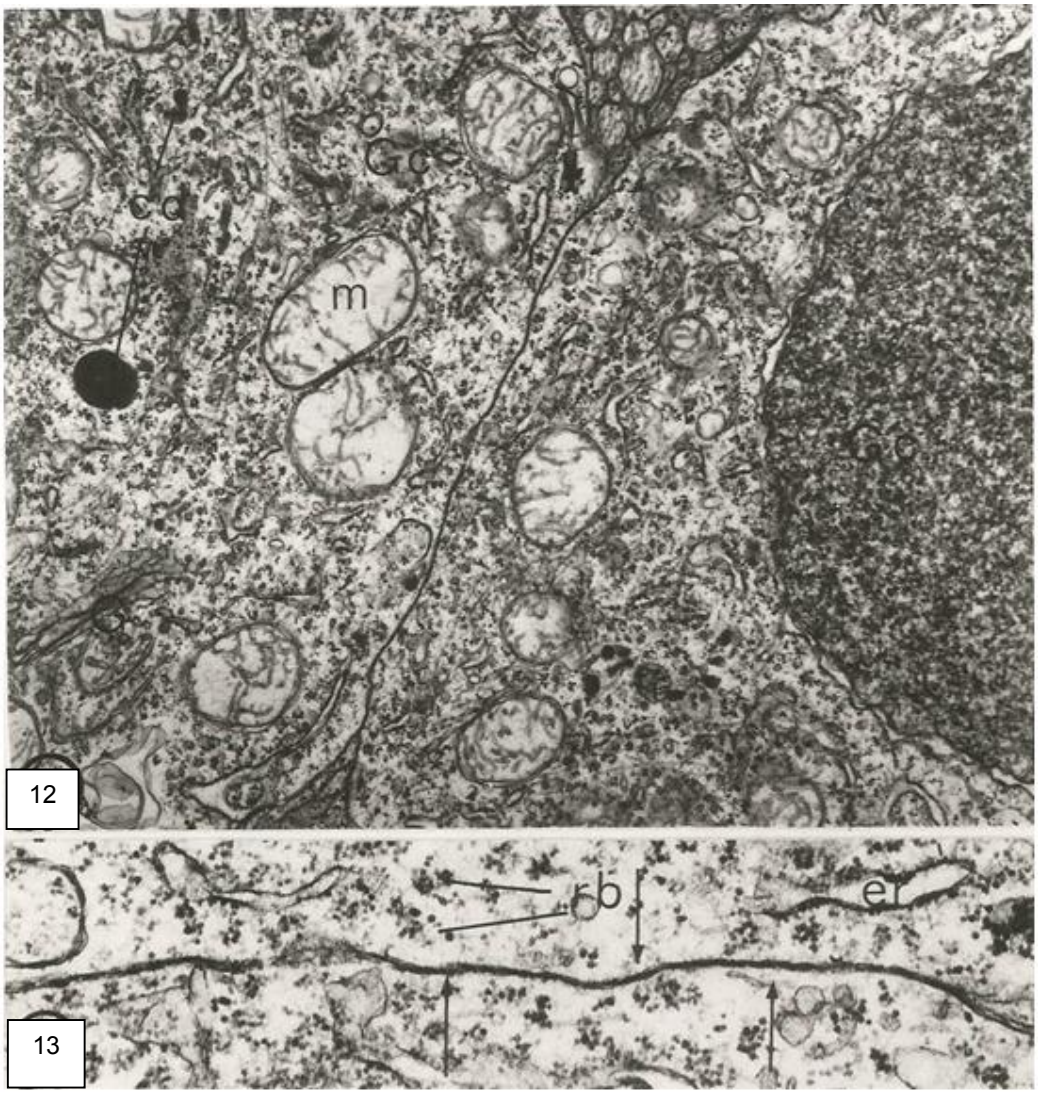


Fig. 12. Ganglion cell [Gc] pairs, elongated mitochondria [m], dense corpuscles [cd], abundant rough endoplasmic reticulum and Golgi apparatus. Radial section X 15200

Fig. 13. Functional contact with dense membranes between adjacent ganglion cells [arrows] from Fig. 12, ribosomes [rb], rough endoplasmic reticulum [er]. X 40000

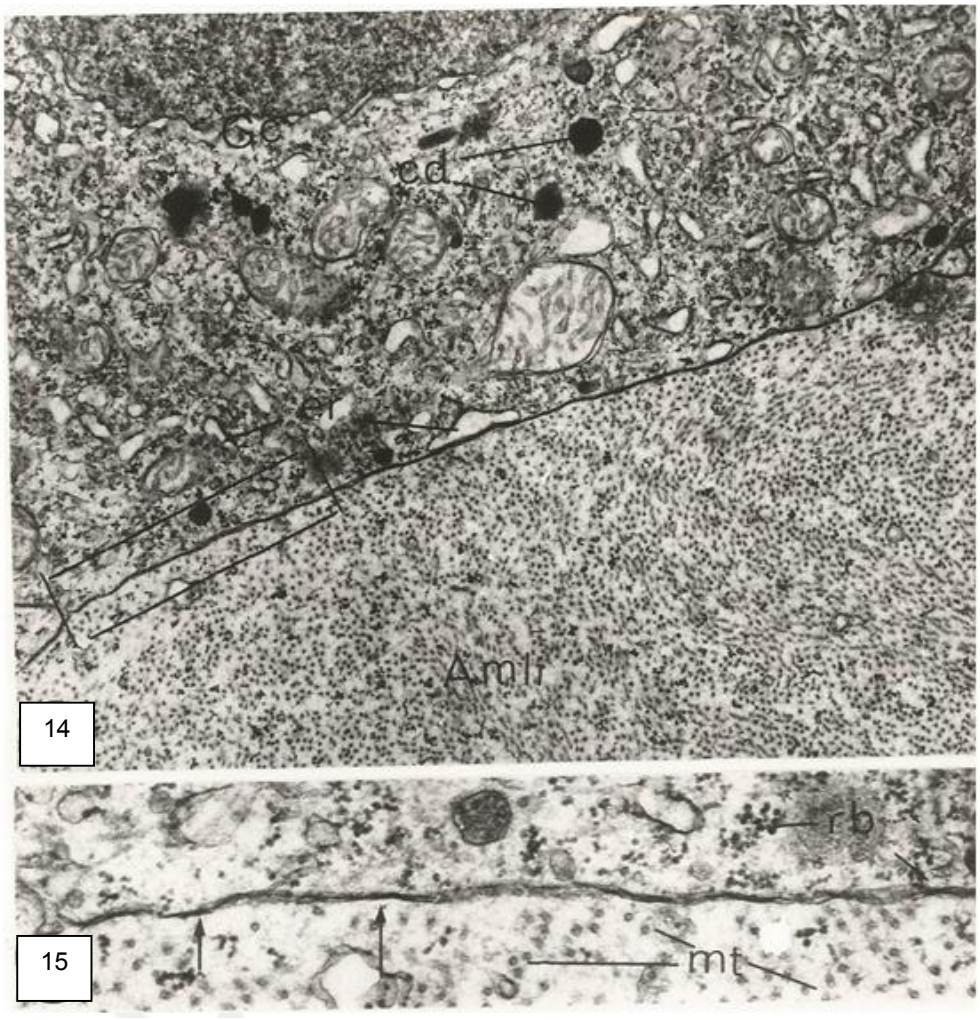


Fig. 14. Ganglion cell [Gc] in contact with amacrine interstitial internal cell [Amli], dense corpuscles [cd], rough endoplasmic reticulum [er]. Radial section X 2500
Fig. 15. Rectangle from fig 14 with fusion of plasma membranes [arrows], microtubules [mt]: ribosomes [rb]. X 38500

3.1.3 Amacrine interstitial internal cells

Amli structured another layer located in the inner plexiform layer, above the optical nerve fibers, exhibiting numerous microtubules, dense granules, elongated big mitochondria and fusion of membranes in adjacent cells (Figs. 7, 8). All amacrine membrane contacts are similar to functional contacts between horizontal cells. Some expansions terminals showed two laminar components surrounded by a halo and granules (Fig. 9). There are also membrane contacts between the terminal bipolar cell (BC) process, with a synaptic lamina, and the body of the Amle as well as with clear piriform amacrine cells (Fig. 10). Amli were located in the inner section of the inner plexiform layer, were seen in contact with BC terminal expansions with synaptic lamina (Fig. 16) and also with terminals full of dense vesicles, probably adrenergic vesicles (Figs. 19, 20, 21). Close to the BC terminal prolongations of clear piriform cells and ganglion cell dendrites with fused dense plasma membranes in the contact area and synaptic lamina in a triad configuration are seen, with synaptic vesicles surrounding the lamina absent from the GC dendrite (Fig. 17). Internal amacrine cells in contact to BC terminals with synaptic lamina and fused plasma membranes (Fig. 18) and several adrenergic terminals (Figs. 19, 20, 21).

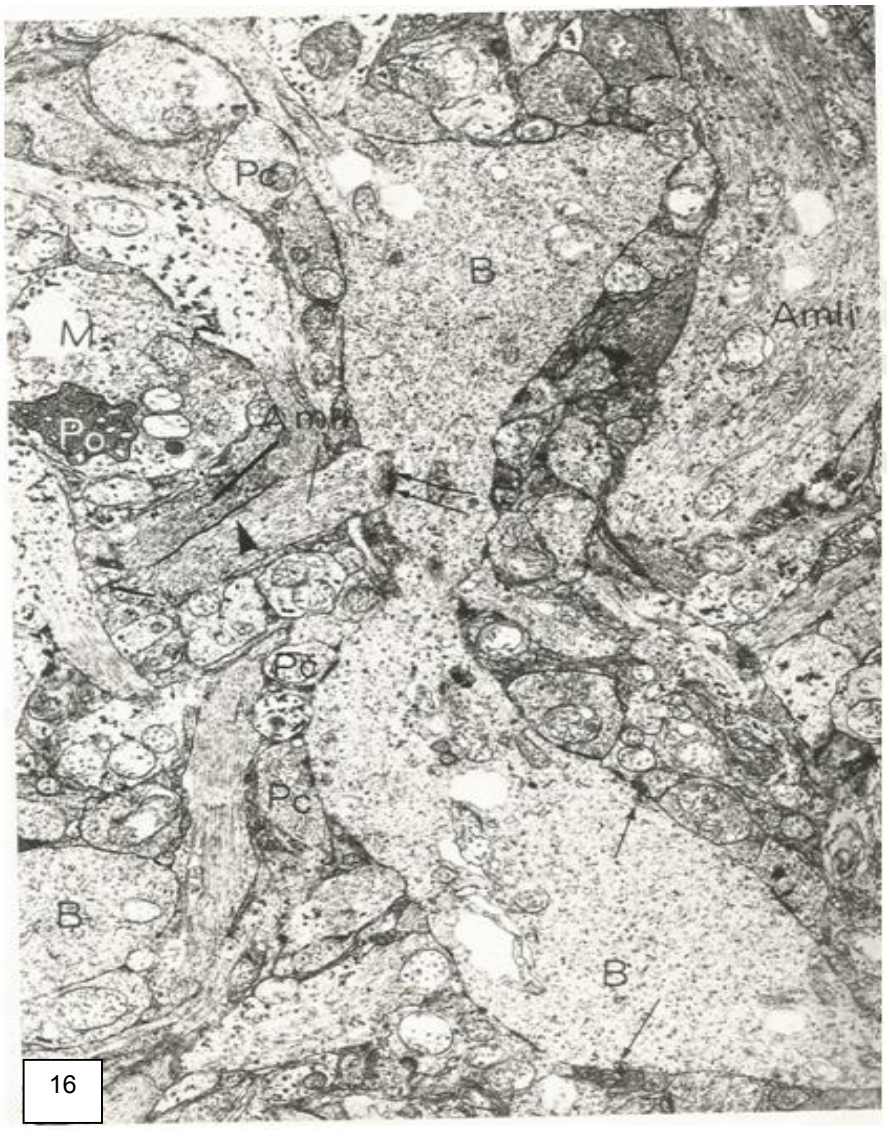


Fig. 16. Terminal ending of bipolar cell [B]. Terminal expansions from: Amacrine interstitial internal cell [Amli], clear piriform amacrine [Pc], dark piriform amacrine [Po] at inner plexiform layer. Müller cell fiber [M]. Dense triangle shows contacts between Amli with plasma membrane fusion, and double arrow synapses between Amli and bipolar cell [B]. Single arrow synaptic laminas in B. Tangential section X 5700

3.1.4 Inner plexiform layer

Terminal expansions of BC, ganglion cells dendrites, clear and dark piriform amacrine cells, Müller cell fiber and internal interstitial amacrines cells were identified in this layer (Figs. 1, 16). In the contact between clear piriform amacrine with ganglion cell dendrite and BC there are membrane densifications with numerous vesicles but not in the dendritic termination of ganglion cells forming a triad around a synaptic lamina. It should be noted that triads were also seen in BC terminals and are not exclusive of cones and rod photoreceptors (Fig. 16).

3.1.5 Cell terminals with dense vesicles

Cellular terminals with dense vesicles probably adrenergic, were observed in contact with internal interstitial amacrines, clear piriform amacrines and bipolar cell terminals and ganglion cell dendrites (Figs. 19, 20, 21). Several

plasma membrane fusion were observed between dark amacrine piriform cell and ganglion cell dendrites with elongated dense mitochondria, without synaptic vesicles close to the membranes (Figs. 22, 23), also between BC terminal with synaptic lamina and Amli cell (Fig. 24) and BC terminal with ganglion cell dendrites with mitochondria of dense matrix (Fig. 25).

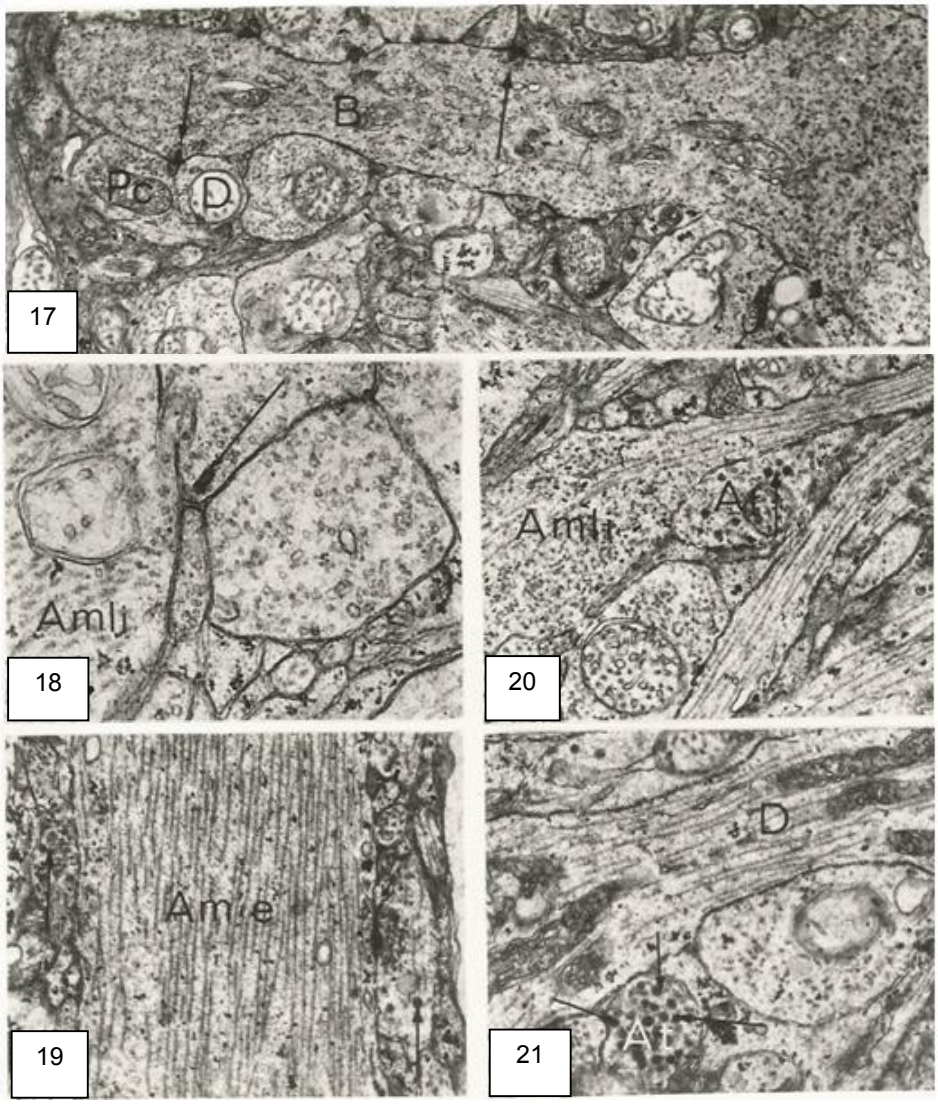


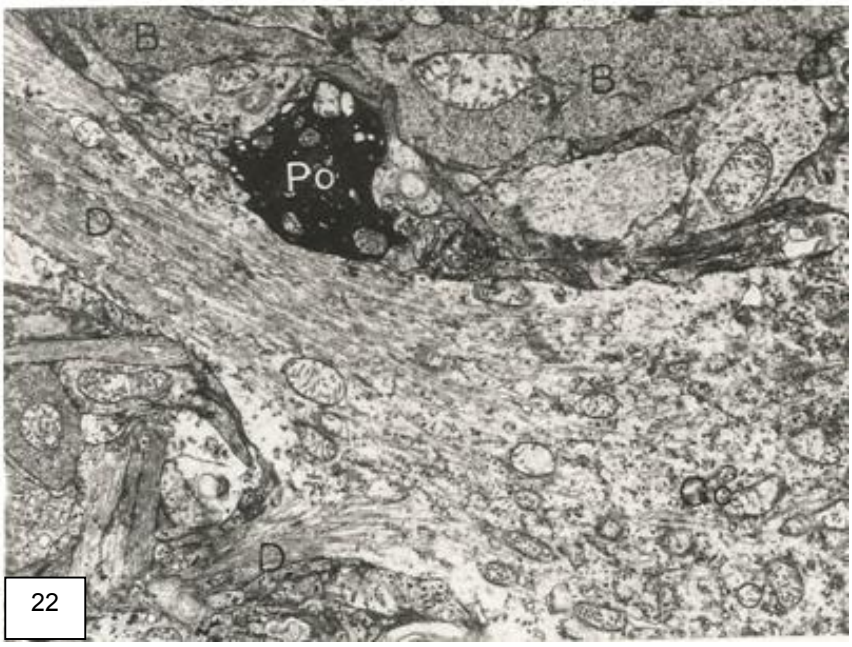
Fig. 17. Bipolar cell terminal [B], clear piriform amacrine [Pc] expansion, ganglion cell dendrite [D]. Synaptic lamina in bipolar cell cytoplasm [arrows] with triad formation between B, Pc and D. Tangential section X 10000

Fig. 18. Internal interstitial amacrine cell [Amli] in contact with B showing basilar lamina surrounded by vesicles [arrow]. Radial section X 23500

Fig. 19. Internal interstitial amacrine cell [Amli], adrenergic terminal [At] with dense vesicles [arrow]. Tangential section X 24000

Fig. 20. Internal interstitial amacrine cell [Amli] with parallel microtubules, adrenergic terminal [At] with dense vesicles [arrow]. Tangential section X 10250

Fig. 21. Ganglion cell dendrite [D], adrenergic terminal [At] with dense vesicles [arrows]. Tangential section. X 20750



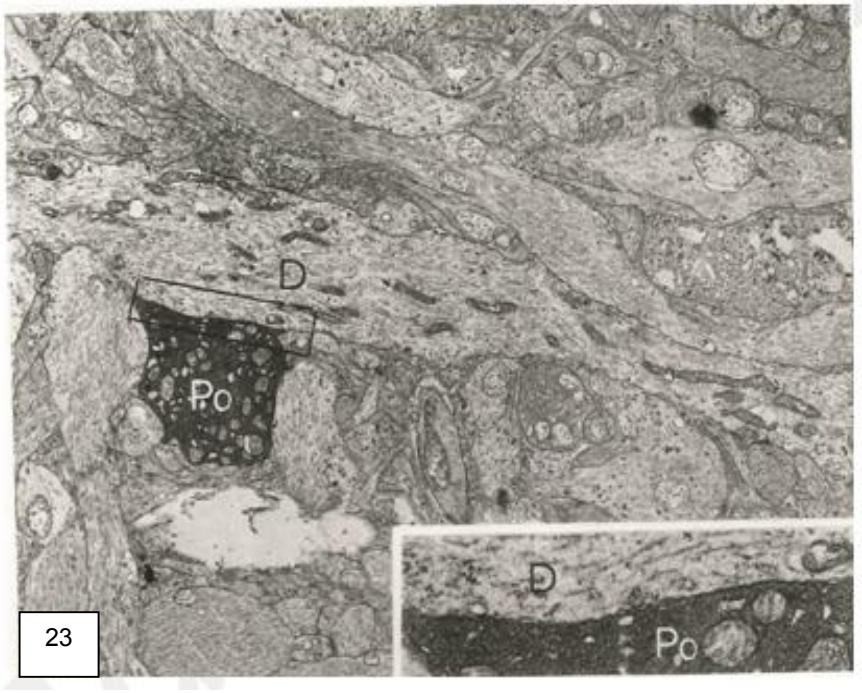
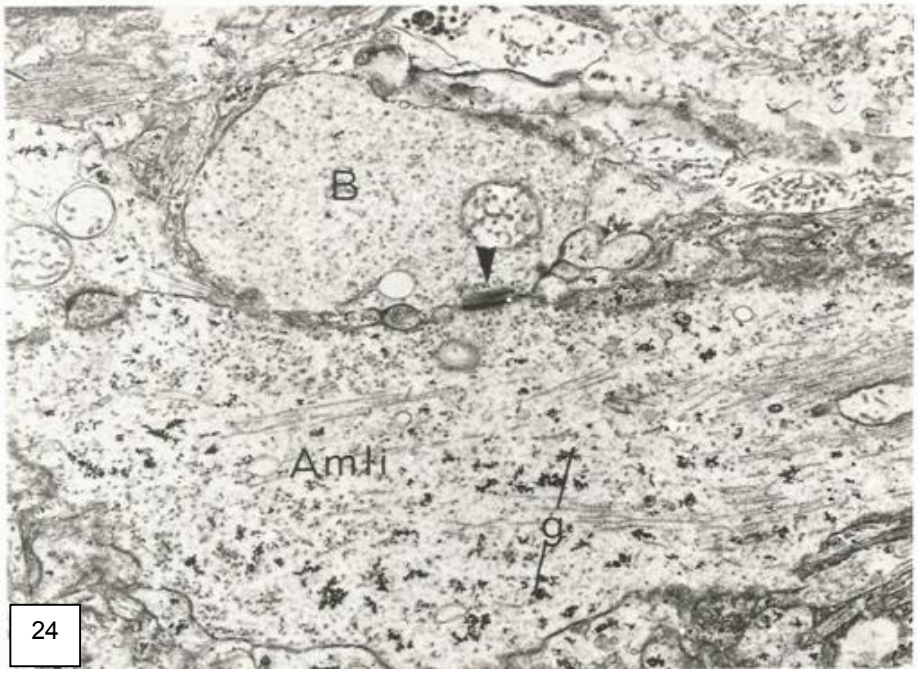


Fig. 22. Ganglion cell [Gc], dendrites [D], dark piriform amacrine cell [Po],: bipolar cell terminal [B]. Membrane contacts between Po, Gc and B. X 7500
Fig. 23. Ganglion cell dendrite [D], dark piriform amacrine cell [Po]. Tangential section X 7500. Insert lower right, D and Po contact with fussed undulated membranes X 22500

3.1.6 Dislocated amacrine cell

Localized at the inner plexiform layer, this novel cell of stellate configuration had big expansions terminals tangentially oriented and is considered as dislocated amacrine cell due to its cytoplasmic characteristics. The nucleus is oval with abundant granular chromatin, the cytoplasm has

a clear matrix similar to clear piriform amacrine cells, few ribosomes, and few vesicles of rough endoplasmic reticulum with glycogen granules and elongated mitochondria of clear matrix with hypertrophic crest (Fig. 26).



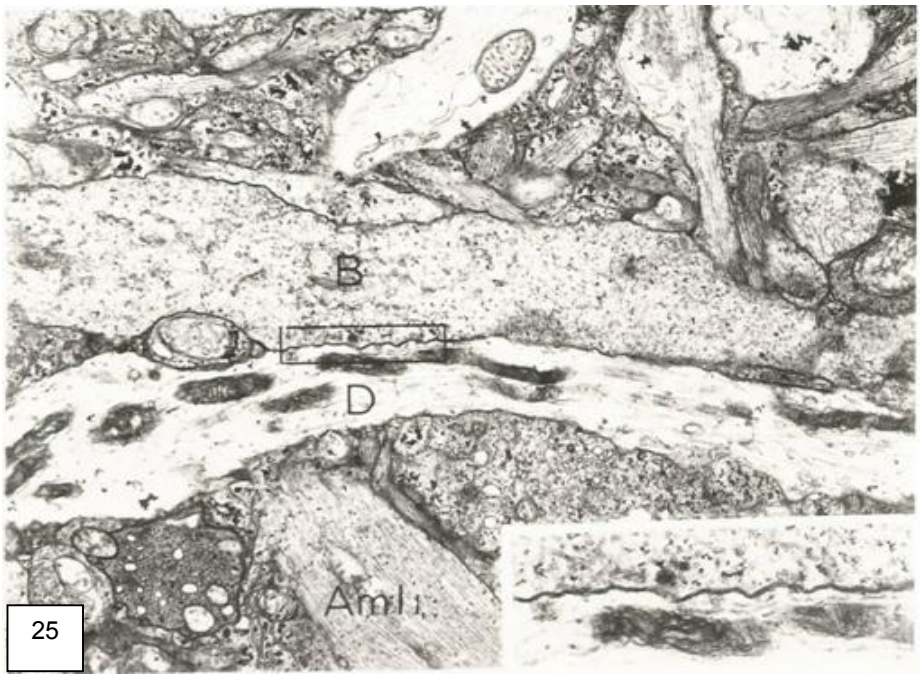


Fig. 24. Amacrine interstitial internal cell [Amli] with glycogen granules [g]. bipolar cell [B], mitochondria [m]. Synaptic lamina between both cells [triangle]. Tangential section. X 6250
Fig. 25. Ganglion cell dendrite [D], bipolar cell terminal [B], amacrine interstitial internal cell [Amli]. Tangential section X 10000. Insert: Plasma membrane fusion between B and D. X 20000

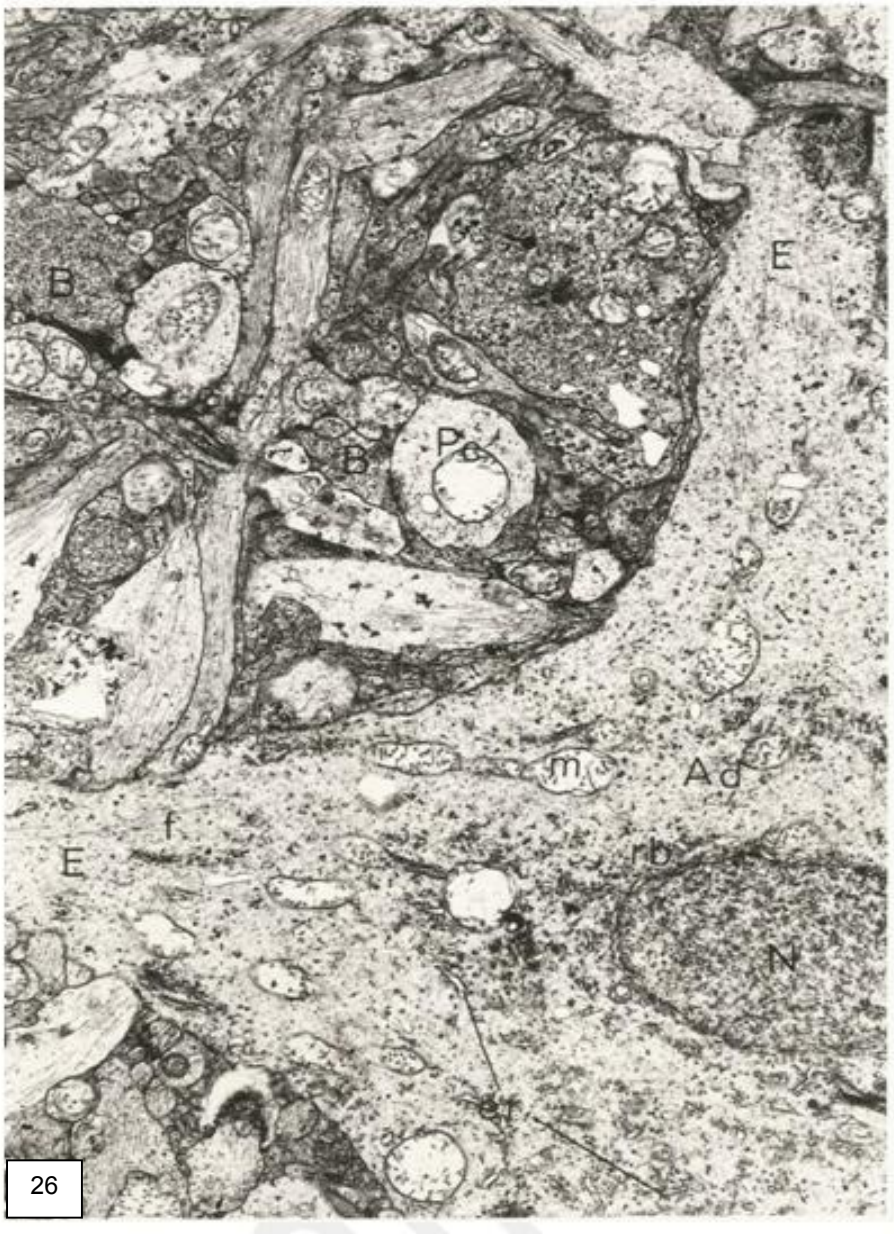


Fig. 26. Dislocated amacrine cell [Ad] with two expansions [E], ribosomes [rb], rough endoplasmic reticulum [er], mitochondria [m], short filaments [f], synaptic vesicles [v], bipolar cell [B], clear piriform amacrine cell [Pc]. Tangential section X 4650

3.1.7 Oligodendroglia

At the level of the inner plexiform and the optic fiber layers small cells with scarce cytoplasm were observed with many terminals from other cells around the plasma membrane. The cytoplasm exhibited many ribosomes, few rough endoplasmic reticulum vesicles and few mitochondria (Figs. 27, 28). The most evident structure were dense bodies surrounded by membranes similar to myelin figures. The nucleus is elongated with the chromatin close to the nuclear membrane with accumulations at the nuclear poles.

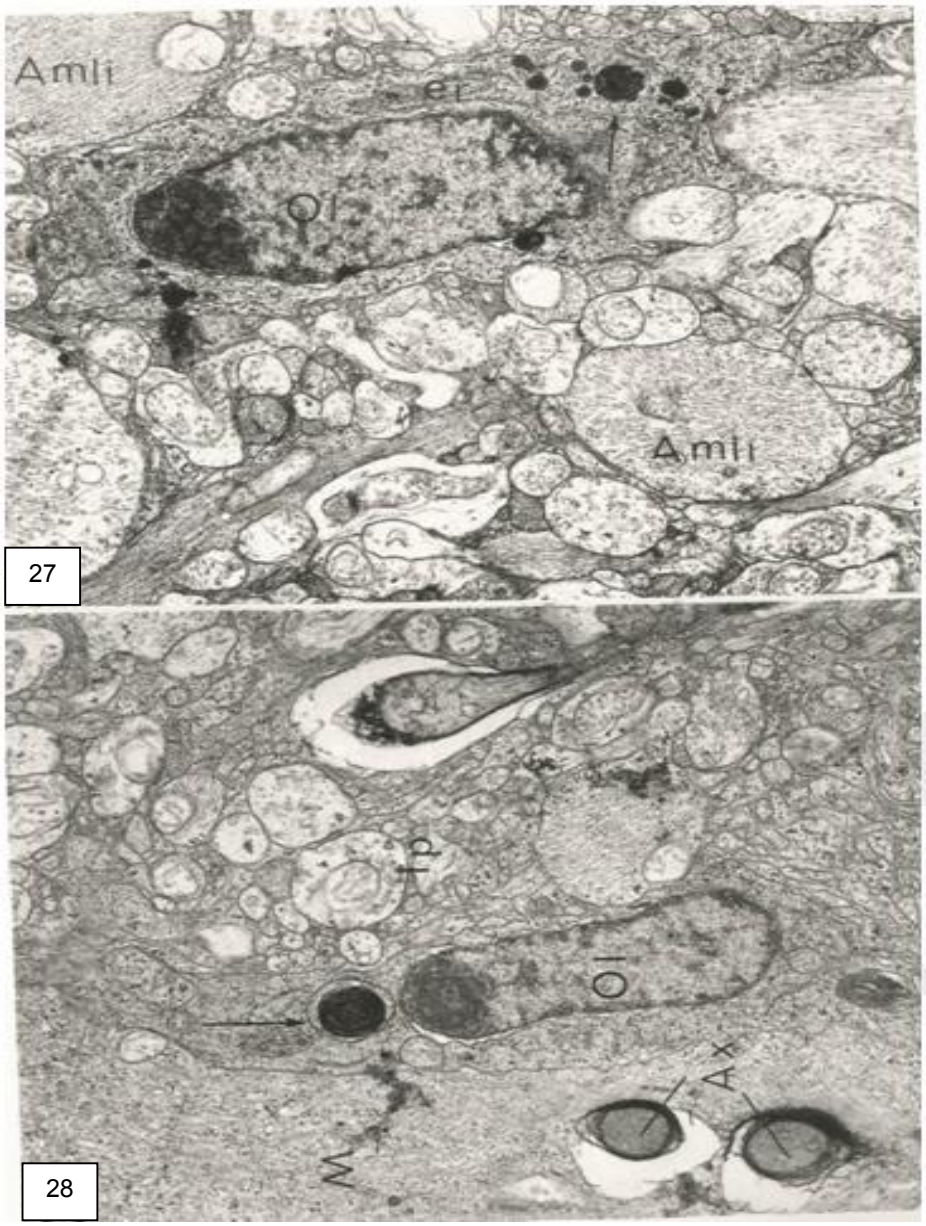


Fig 27. Oligodendrocytes [Ol], rough endoplasmic reticulum [er]. Dense bodies surrounded by membranes similar to myelin (arrows). Radial section X 10000
Fig 28. Oligodendrocytes [Ol], with dense bodies surrounded by membranes similar to myelin (arrows), myelinated axon [Ax] from optical nerve fiber, inner plexiform layer [Ip],: Müller cell fiber [M]. Radial section. X 8500

3.1.8 Glycogen localization by cytochemistry

3.1.8.1 Horizontal cells

The external horizontal cells (Hc) exhibits the highest density of glycogen granules in the cytoplasm (Figs. 29, 30, 31) as compared with other Hc and also in the ascendant expansions and their terminations in rod and cones (Fig. 32).

In external horizontal cells glycogen granules are seen as dense cumuli of different size in material fixed in glutaraldehyde only, without osmium post-fixation (Fig. 33). Control sections treated with salivary amylase showed holes of variable

size corresponding to glycogen granules digested by the enzyme (Fig. 34).

3.1.8.2 Stellate amacrine cells

Glycogen granules are uniformly distributed around nucleus and between cytoplasmic fibrils in stellate amacrine cells (Fig. 35) and also between microtubules in Amle external cells (Fig. 36). In the ascendant expansions of stellate amacrines glycogen is seen between packages of fibrils mainly in the periphery close to the plasma membrane, surrounded by undulated amacrines, close to BC (Fig. 37).

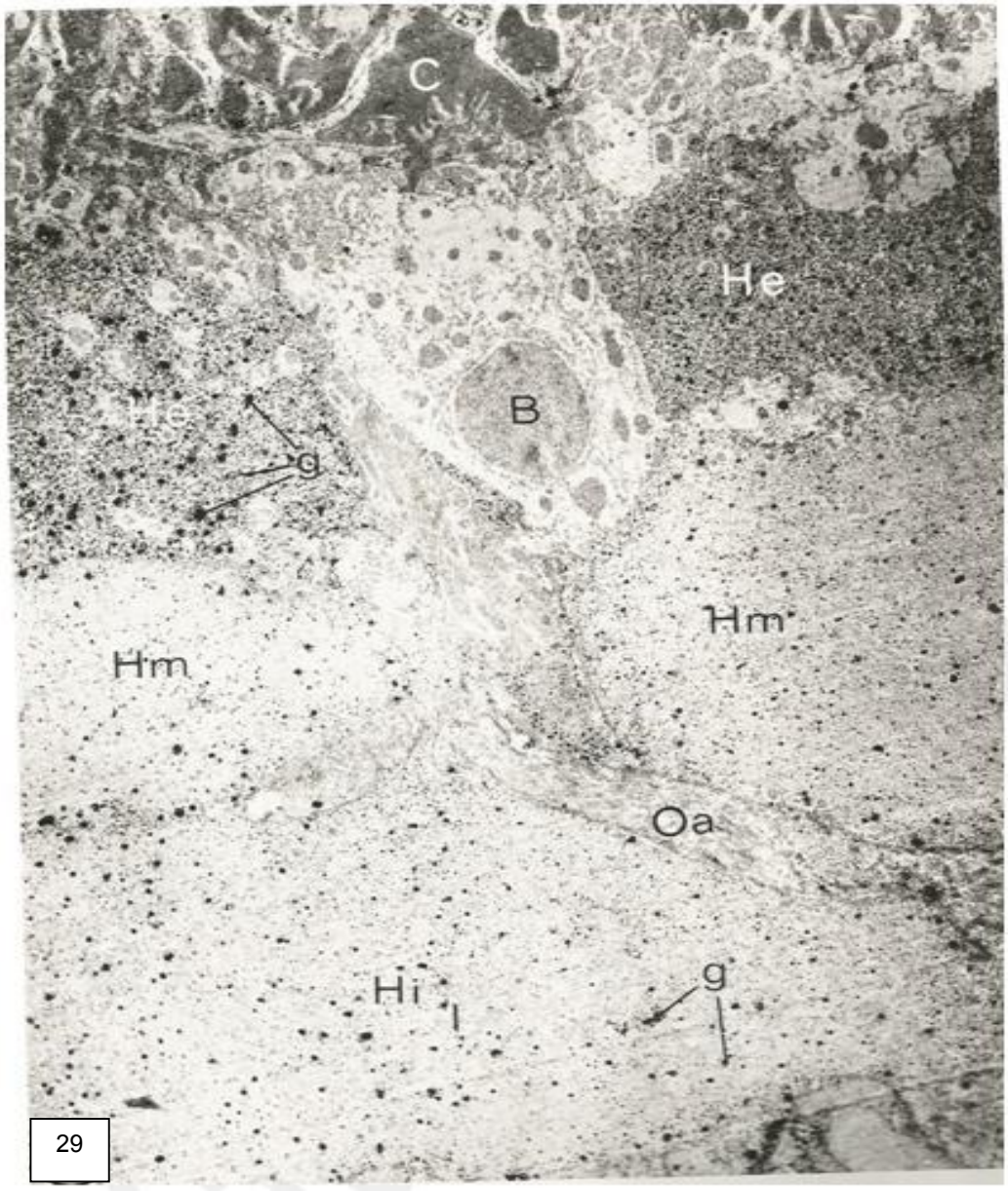


Fig. 29. All ultrastructural sections processed with periodic acid thiosemicarbizide for oxidized macromolecules. External [He], medium [Hm], internal [Hi], Horizontal cells with glycogen granules [g], bipolar cell [B], undulated amacrine cell [Oa], cone [C]. Radial section. X 7500

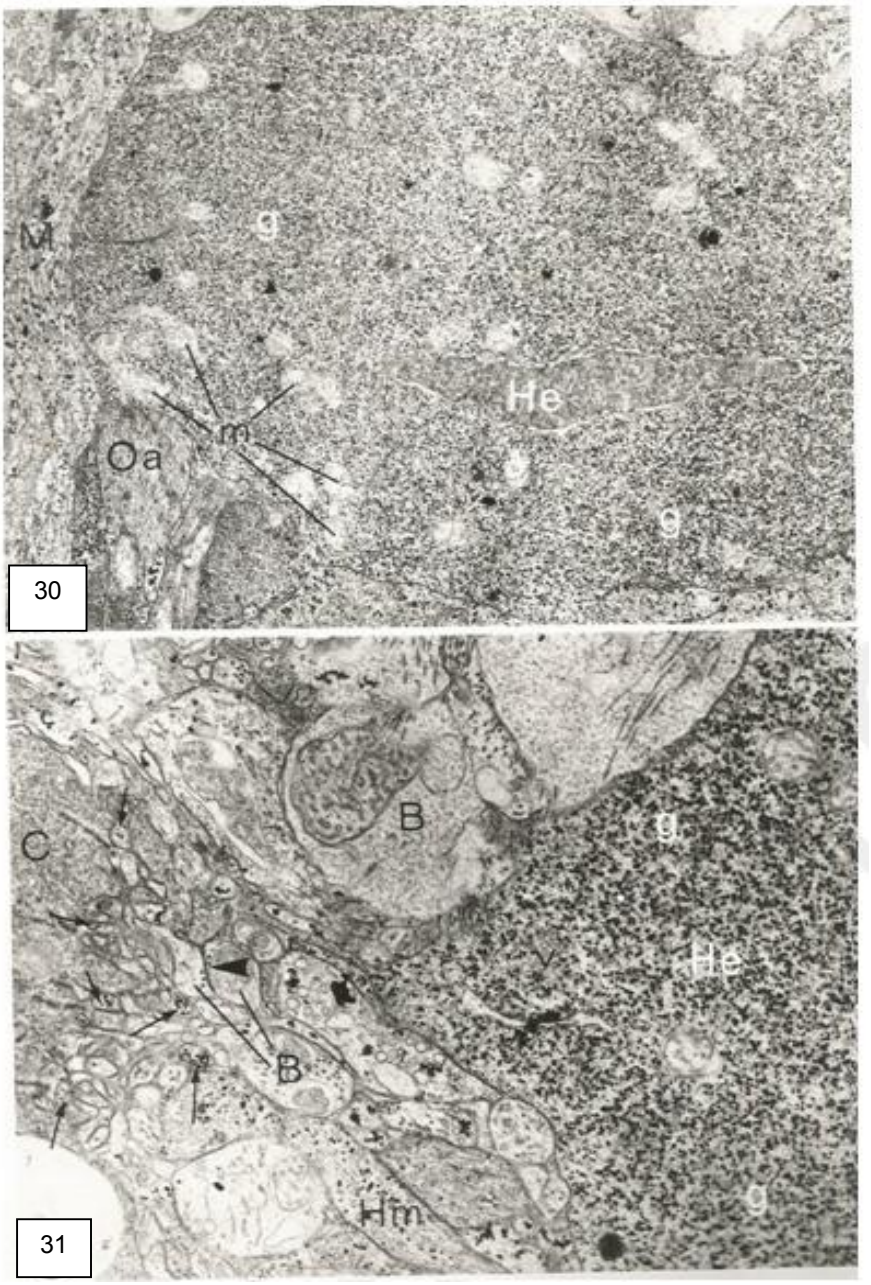


Fig. 30. External horizontal cell with abundant glycogen [g] granules of various sizes and numerous mitochondria [m], Müller cell fiber [M], undulated amacrine cell [Oa]. Radial section. X 10000

Fig. 31. External horizontal cell [He] and medium horizontal [Hm] with abundant glycogen [g] granules at the outer plexiform layer [triangle] and in cone [C] triad terminals [arrows], close to synaptic lamina, bipolar cell [B]. Radial section X 12500

3.1.8.3 Amacrine interstitial external cells

Numerous glycogen granules among parallel and cross sectioned microtubules with lower density than in external horizontal cells and amacrine stellate cells are observed in AmIe with membrane contacts among adjacent cells (Fig. 36).

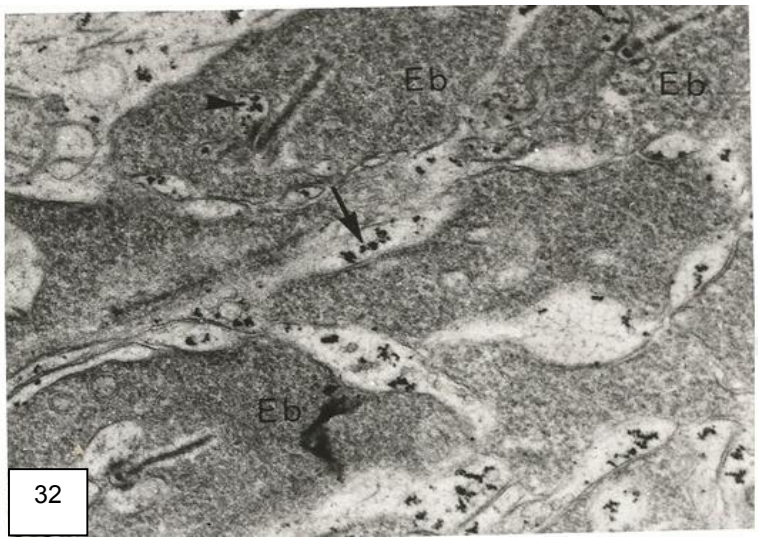


Fig. 32. Glycogen granules in the horizontal cell terminals [triangle] triads in rods beside a synaptic lamina and in the Müller cell fiber surrounding rod [arrow]. Sections processed with periodic acid thiosemicarbizide for oxidized macromolecules. Radial section X 25200

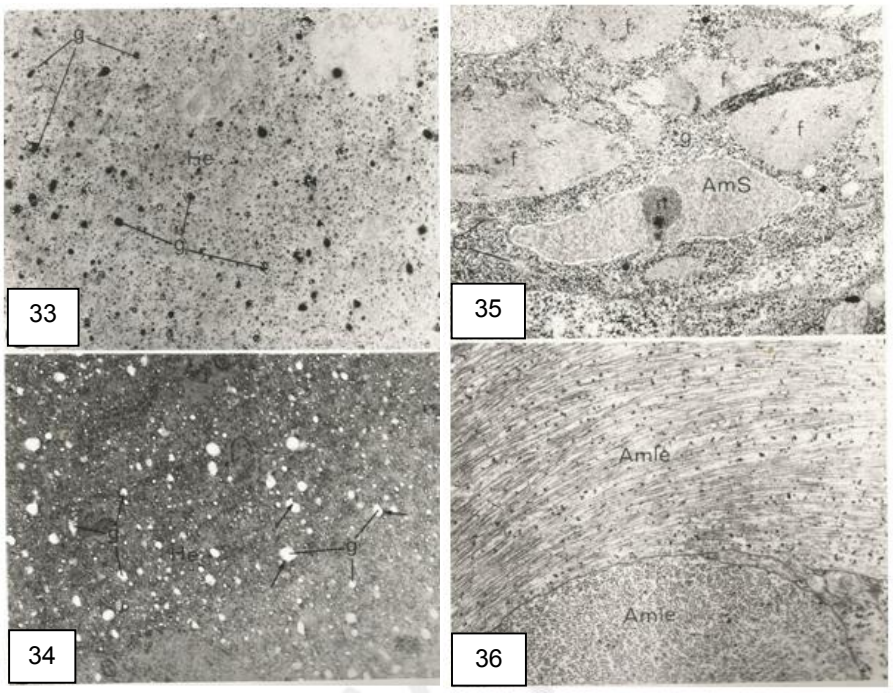
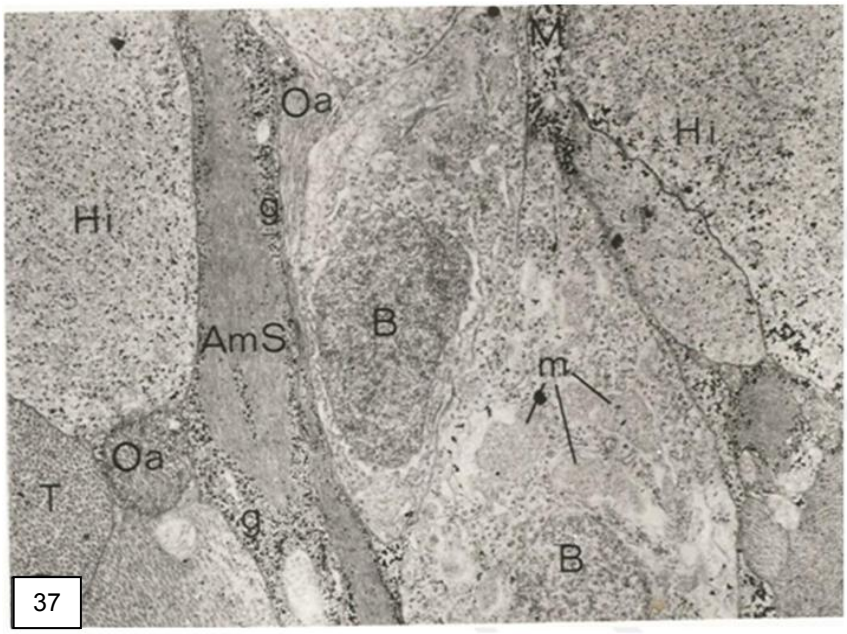


Fig 33. All ultrastructural sections processed with periodic acid thiosemicarbizide for oxidized macromolecules External horizontal cell [He] with abundant glycogen granules [g]. Radial section X 22500

Fig 34. External horizontal cell [He] with holes corresponding to digested glycogen [g] with salivary amylase [arrows]. Radial section. X 30500

Fig 35. Stellate amacrine cell [AmS], with nucleus [n] and prominent nucleolus, Golgi apparatus [G] abundant glycogen [g] granules between cytoplasmic fibrils [f]. Radial section X 20800

Fig 36. Amacrine interstitial external cells [Amle], with glycogen particles among longitudinal and transverse sections microtubules. Radial section. X 12500



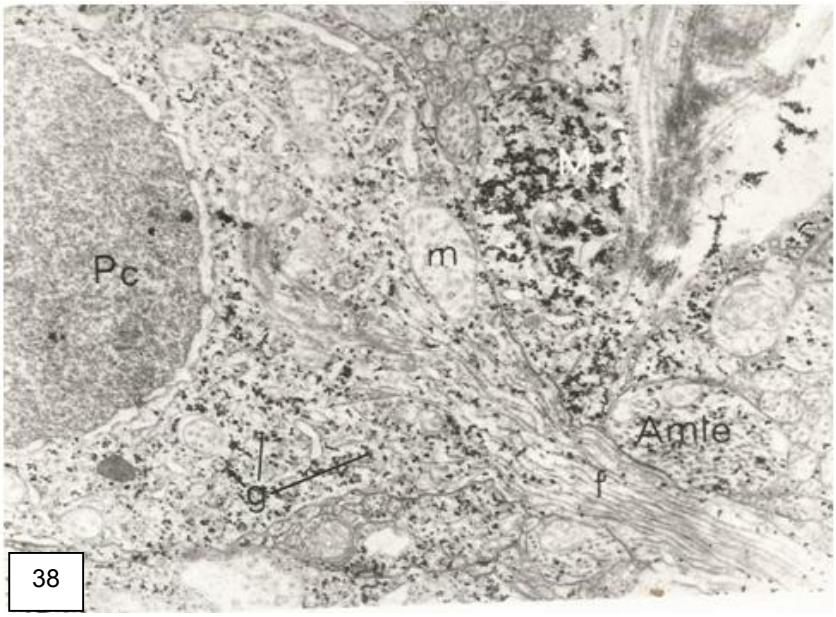


Fig 37. All ultrastructural sections processed with periodic acid thiosemicarbizide for oxidized macromolecules. Stellate amacrine cell ascendant expansion [AmS] with abundant glycogen [g] granules around central package of fibrils and close to the plasma membrane. Dark dense glycogen granules in internal horizontal cells [Hi],: undulated amacrine cell [Oa], tubular amacrine cell [T], bipolar cell [B] with mitochondria [m].: Müller cell fiber [M]. Radial section. X 7800

Fig 38. Clear piriform amacrine cell [Pc] with descendant expansion, filaments [f]; mitochondria [m] and glycogen granules [g]. Müller cell fiber [M] with abundant glycogen.: amacrine interstitial external cell [Amle]. Radial section. X 13500

3.1.8.4 Piriform amacrine cells

Clear amacrine piriform cells exhibit numerous glycogen granules among cytoplasmic microtubules and mitochondria with clear matrix (Fig. 38). Dark amacrine piriform cells showed the highest glycogen content in the retina, among very abundant elongated and big mitochondria with lamellar structure and clear matrix, as well as many clear vesicles of smooth endoplasmic reticulum. This amacrine cell is in membrane contact with the Müller cell fiber also with high glycogen content some in dense cumuli (Fig. 39).

3.1.8.5 Müller cell fiber

Müller cell fiber is radially oriented from the external to the internal end of the retina. The cytoplasm shows abundant glycogen granules many in dense cumuli, numerous dilated smooth and rough vesicles of endoplasmic reticulum and many mitochondria of lamellar structure and clear matrix. The nucleus is localized below the level of stellate amacrine cells, showing indentations frequently. This cellular element has very high glycogen content in the retina (Figs. 40, 41) similar to the dark piriform amacrines and external horizontal cells (Figs. 29, 30, 31).

3.1.8.6 Bipolar and ganglion cells

Bipolar cells classified as neurons have scarce glycogen in cytoplasm, in dendrites and terminal endings at inner plexiform layer (Figs. 29, 37). In ganglion cells, glycogen is seen as abundant granules of different size among cytoplasmic fibrils, dense bodies and mitochondria of clear matrix. This ganglion are cells also surrounded by the Müller cell fiber high in glycogen density and myelinated axons (Fig. 42).

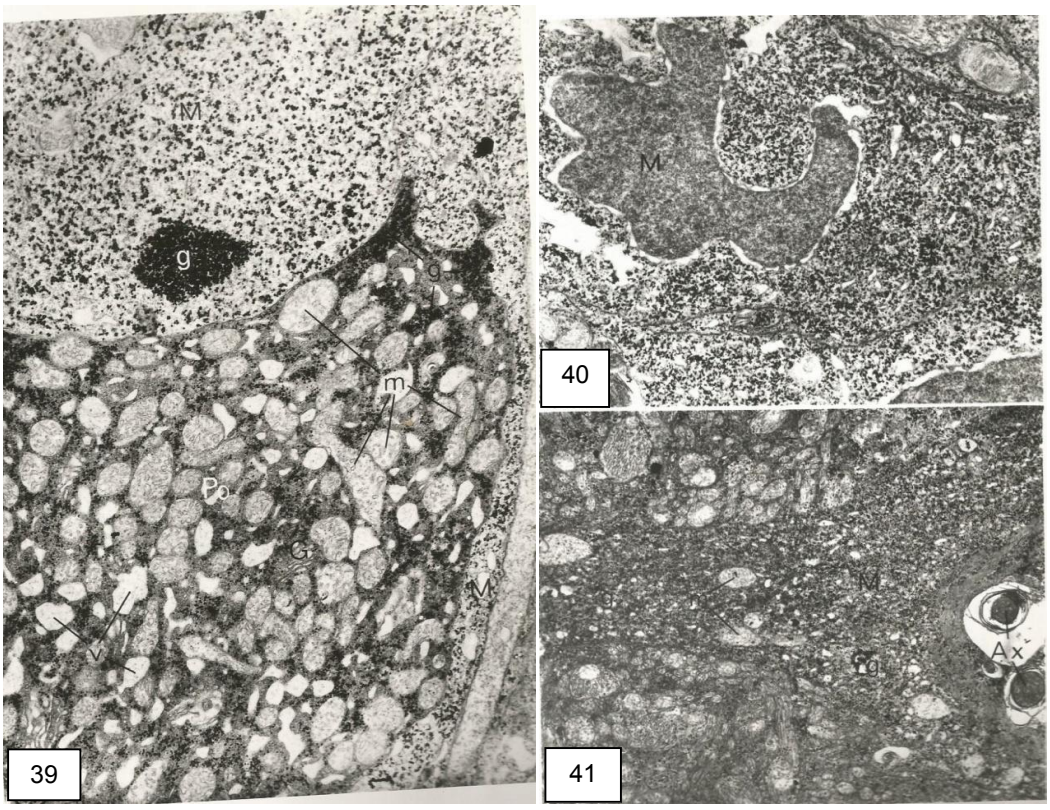


Fig. 39. All ultrastructural sections processed with periodic acid thiosemicarbizide for oxidized macromolecules Dark piriform amacrine cell [Po] with high density of glycogen [g] granules, with abundant elongated mitochondria [m], Golgi apparatus [G], vesicles of smooth endoplasmic reticulum [v], Müller cell fiber [M]. Radial section. X 12500

Fig. 40. Müller cell fiber [M] with nucleus and very abundant, dense glycogen granules. Radial section X 12500

Fig. 41. Müller cell fiber [M] above myelinated axons [Ax] from optical nerve layer, mitochondria [m], glycogen granules [g]. Radial section. X 7500

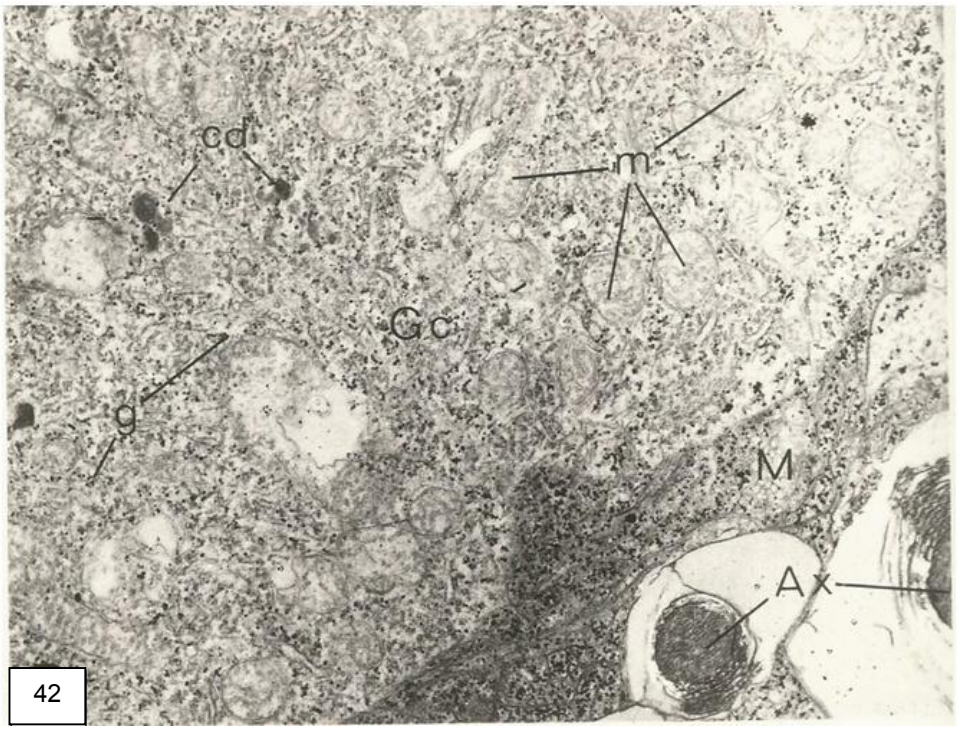


Fig. 42. Ganglion cell [Gc], with mitochondria [m], dense bodies [cd] and abundant glycogen granules [g].: Müller cell fiber [M], myelinated axons [Ax] from optical nerve layer. Sections processed with periodic acid thiosemicarbizide for oxidized macromolecules. Radial section. X 13000

4. DISCUSSION

Stellate amacrine cells with ascendant, lateral and descendant expansions were described in the retina inner granular layer [1]. A stellate amacrine cell similar to Cajal description was reported in teleost retina [20]. Piriform amacrines without axons and association amacrines or nerve cells with branched axons at the level of piriform amacrines in the retina were published [1]. Also neuronal type piriform amacrines and glial type amacrines considered astrocytes were reported in fish retina [2]. In this work clear piriform amacrines may be homologous to association amacrines cells found by Cajal [1, 21]. The difference between two types of piriform amacrines, clear and dark amacrines may be explained by the glutaraldehyde fixation methods, contrary to the one type of amacrines described previously in primate retina [4]. The present work revealed that pairs of dark and clear piriform amacrine cells with fussed areas in contact membranes were not originated at random since the Müller cell fiber surrounds both cell bodies completely avoiding the contact area, suggesting a functional contact between them similar to horizontal cell contacts [15]. Amle cells were found below stellate amacrine cell layer with many functional contacts between them similar to the unions between horizontal cells and stellate amacrine cells [15]. Amli cells also presented functional contacts with the terminal ending of bipolar cells in the inner plexiform layer with synaptic lamina close to the membranes. In the present paper, dense vesicles in cell terminals probably adrenergic vesicles were found at the inner plexiform layer, in contact with external interstitial amacrines, clear piriform amacrines, bipolar cell terminals and ganglion cell dendrites which might play role in the glycogen metabolism in the retina. Fluorescent microscopy and cytochemical methods showed catecholamines in the inner plexiform layer [22] that might correspond to the terminals with dense vesicles found in the present work, and have a role in retina light adaptation processing glycogen as a source of energy [23]. Oligodendroglia cells exhibits many myelinated figures and probably have a function around optical nerve fibers in transmission of the electrical potential similar to their function in the central and peripheral nervous system. The

highest glycogen density were in the external horizontal cells close to photoreceptors and in the Müller cell fiber probably to give energy to rods and cones in the visual process. Interestingly in horizontal cells lateral terminals Na^+ K^+ ATPase was described to explain S-potentials between horizontal cells in the retina [24]. This electrical synapses found in great numbers in all functional contacts between other retinal cells in this work, may also have the Na^+K^+ATPase, a faster connection than chemical synapses that would increase the velocity of the visual process in the vertebrate retina.

5. CONCLUSIONS

Dark and clear piriform amacrines cells were described according to their glycogen content. A novel cell named dislocated amacrine cell is described. Many terminals with dense vesicles are probably adrenergic vesicles and it is postulated they play a role in glycogen metabolism to give energy to the retina visual process. Glycogen distribution was described in all retinal cells by cytochemical methods, the highest glycogen density in external horizontal, stellate amacrine, dark piriform amacrines and Müller cells. Electrical synapses were found in great numbers in all functional contacts between retinal cells, together with lateral process of horizontal cells and may also have the Na^+K^+ATPase, a faster connection than chemical synapses that would increase the velocity of the visual process in the vertebrate retina.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This work was performed under the statement format for the use of Animals in Ophthalmic and Visual Research.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Ramón y Cajal S. La rétine des vertébrés. La Cellule. 1892;9:121-255. Facsimile edn. Trab. Lab. Invest. Biol. 1933;28:1-144. Facsimile edn. XIV Concilium Ophtalmologicum Hispania (Madrid). 1933; 144.
2. Villegas G, Villegas R. Neuron-glia relationship in the bipolar cell layer of the fish retinae. J. Ultrastruct. Res. 1963;8:89-106.
3. Kidd K. Electron microscopy of the inner plexiform layer of the retina in the cat and the pigeon J. Anat. Lond. 1962;96:179-188.
4. Dowling J, Boycott BB. Organization of the primate retina: Electron microscopy Proc. of the Roy. Soc. B. 1966;166:80-111.
5. Chen S, Li W. A color-coding amacrine cell may provide a blue-off signal in a mammalian retina. Nat Neurosci. 2012; 15:954-956.
6. Marc RE, Anderson JR, Jones BW, Sigulinsky CL, Lauritzen JS. The AII amacrine cell connectome: A dense network hub. Front Neural Circuits. 2014; 8:104.
   DOI: 10.3389/fncir.2014.00104
7. Kim JS, Greene MJ, Zlateski A, Lee K, Richardson M, Turaga SC, et al. EyeWirers. Space-time wiring specificity supports direction selectivity in the retina. Nature. 2014;15:331-336.
8. Triplett JW, Wei W, Gonzalez C, Sweeney NT, Huberman AD, Feller MB, et al. Dendritic and axonal targeting patterns of a genetically-specified class of retinal ganglion cells that participate in image-forming circuits. Neural Dev. 2014;9:2.
   DOI: 10.1186/1749-8104-9-2
9. Joo HR, Peterson BB, Dacey DM, Hattar S, Chen SK. Recurrent axon collaterals of intrinsically photosensitive retinal ganglion cells. Vis Neurosci. 2013;30:175-182.
10. Tsukamoto Y, Omi N. Functional allocation of synaptic contacts in microcircuits from rods via rod bipolar to AII amacrine cells in the mouse retina. Comp Neurol. 2013;52: 3541-3555.
11. Pérez León JA, Osorio-Paz I, Francois L, Salceda R. Immunohistochemical localization of glycogen synthase and GSK3β: Control of glycogen content in retina. Neurochem Res. 2013;38:1063-1069.
12. Okubo A, Sameshima M, Unoki K, Uehara F, Ohba N. Ultracytochemical demonstration of glycogen in cone, but not in rod, photoreceptor cells in the rat retina. Ann. Anat. 1998;180:307-314.
13. Mantyh PW, Rogers SD, Allen CJ, Catton MD, Ghilardi JR, Levin LA, et al. Beta 2-

adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. J. Neurosci. 1995;15:152-164.
14. Pfeiffer B, Grosche J, Reichenbach A, Hamprecht B. Immunocytochemical demonstration of glycogen phosphorylase in Müller (glial) cells of the mammalian retina. Glia. 1994;2:62-67.
15. O'Daly JA. Ultrastructure of *Mugil brasiliensis* Teleost retina I: Cones, rods, horizontal, bipolar, piriform amacrines, tubular cells, undulate amacrine cells, outer and inner plexiform layers. British Journal of Medicine & Medical Research. 2017;19:1-16
16. Fahimi HD, Drochmans P. Essai de Standarization de la fixation au glutaraldehyde. II. Influence des concentrations en Aldehyde et de L'osmolarite. J. Microscopie. 1965;4:737-748.
17. O'Daly JA, Imaeda T. Electron microscope study of Wallerian degeneration in cutaneous nerves caused by mechanical injury. Laboratory Investigation. 1967;17: 744-766.
18. Seligman AM, Hanker JS, Wasserkrug HL, Dmochowski H, Katzoff L. Histochemical demostration of some oxidized macromolecules with thiocarbohydrazide (TCH) or thiosemicarbazidde (TSC) and osmium tetroxide. J. Histochem. Cytochem. 1965;13:629-639.
19. Rosa F, Johnson FB. Enzymatic Digestion in the Cytochemical demostration of Glycogen. J. Histochem. Cytochem. 1967; 15:14-20.
20. Selvin de Testa A. Morphological studies on the horizontal and amacrine cells of the teleost retina. Vision Res. 1965;6:51-59.
21. Cajal SR. Textura del sistema nervioso del hombre y los vertebrados. 1904;II(Chapter XXX):609-645.
22. Haggendal J, Malforms T. Identification and cellular localization of the catecolamines in the retina and choroid of the rabbit. Acta Physiol. Scand. 1965;64: 58-66
23. Brujan BD, Diaz Borges JM, Alvarez N. Relationship between the contents of adrenaline, noradrenaline, and dopamine in the retina and its adaptational state. Life Sciences. 1965;4:473-477.
24. O'Daly JA. ATPase activity at the functional contacts between retinal cells which produce S-potential. Nature. 1967; 216:1329-1331.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17735