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Neuroglia in the Optic Tectum of Teleosts.

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With 5 Figures

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Summary: The morphology of the neuroglial cells of the optic tectum of Cyprinid teleosts (*Cyprinus carpio* and *Barbus bocagei*) was analyzed using two variants of the Golgi method (Colonnier and Meyer) and electron microscopy. Although the description of the results is based on the three classical neuroglial types (ependymocytes, astrocytes and oligodendrocytes), their analysis shows that the differentiation between astrocytes and oligodendrocytes is complex. Owing to the occurrence of numerous elements with intermediate characteristics, it is suggested that in Teleosts both glial types (astrocytes and oligodendrocytes) correspond to functional variants of a single cell type.

Key words: Glia, Optic tectum, Carp, Barbel, Teleosts

Introduction

For many years, in research on the glial elements of the brain of fish the methods employing impregnation with metals have revealed the existence of long ependymal processes (Achúcarro, 1.915; Mirto, 1.895; Muller, 1.900; Narsen, 1.886; Ramón y Cajal, 1.972; Retzius, 1.893; Studnicka, 1900) that in some areas, among them the optic tectum, extend from the periventricular end to the pial surface. For Ramón y Cajal (1.972) these ependymal elements, or neuroepithelial cells, would be the only glial elements present in the optic tectum of certain lower vertebrates and would constitute the encephalic parenchyma. Shortly after, Achúcarro (1.915) reported the existence of neuroglia independent of the ependyma, "autonomous neuroglia", in some regions of the teleostean brain.

In early investigations the ependymal elements were suggested to have nutritional and supportive functions, which in mammals are ascribed to the neuroglia; other authors hypothesized that they might also be secretory elements (Achúcarro, 1.915; Agduhr, 1.932).

Regarding the ependymocytes, the perykarion has been studied in depth in vertebrates (Bellairs, 1.959; Brightman and Palay, 1.963; Duncan, 1.957; Klinkerfuss, 1.964; Schultz et al., 1.956), but the structure of the long ependymary process is less well known (Kruger and Maxwell, 1.966; Laufer and Vanegas, 1.974).

Regarding the remaining glial elements, light microscopy findings in the optic tectum of Teleosts have been of little relevance. By contrast, the glia was the first component examined in ultrastructural studies dealing with this mesencephalic center (Kruger and Maxwell, 1.966; 1.967).

Electron microscopy has shown that apart from ependymal processes there are also other processes, rich in fibrils, surrounding blood capillaries (Kruger and Maxwell, 1.967; Nakajima et al., 1.965); the processes belong to cells that appear to correspond to mammalian astrocytes (Maxwell and Kruger, 1.965a, b; Mugnaini and Walberg, 1.964). These cells constitute the most abundant glial elements in the brain of Teleosts although their fibrillar content is smaller than those of the astrocytes of higher vertebrates (Kruger and Maxwell, 1.967).

Reports have also been made of oligodendrocytes characterized by their dense cytoplasmic matrix and of the presence of extense systems of microtubules lacking in gliofilaments (Kruger and Maxwell, 1.967).

In brief, numerous problems concerning the glia of the Teleostean brain remain to be elucidated.

In the present work we analyse some aspects of the morphological characteristics of the neuroglial elements of the optic tectum of Cyprinids fishes by light and electron microscopy.

Material and Methods

The material consisted of the optic tecta of 15 specimens of *Cyprinus carpio* L. and another 15 specimens of *Barbus bocagei*, all of them adults, captured in the river Duero (Salamanca). After anaesthesia with MS-222 (Sandoz) at 0'03%, the optic tectum was removed in vivo and submerged in the corresponding fixative.

For light microscopy studies, the optic tectum of ten carps and another ten barbels were processed according the Golgi-Colonnier (Colonnier, 1964) or Golgi-Meyer (Meyer, 1982)

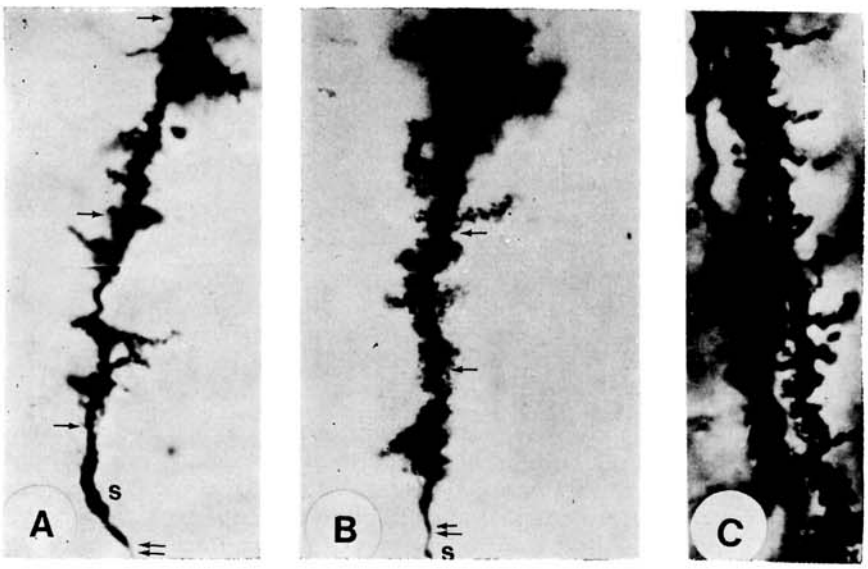

Fig. 1.

A. — Ependymocyte with inner perikaryon.
s: perikaryon; arrow: long ascending prolongation; double arrow: short descending prolongation. *Cyprinus carpio* Golgi-Colonnier. × 1 000.

B. — Ependymocyte with cell body limiting with the ventricle.
s: perikaryon; arrow: long ascending prolongation; double arrow: beginning of the ascending prolongation without branching. *Cyprinus carpio* Golgi-Meyer. × 1 000.

C. — Lateral branches of the ascendent prolongation of an ependymocyte. Golgi-Colonnier. × 2 000. *Barbus bocagei.*

procedures. Full details about the embedding and sectioning are given in Alonso et al. (1987).

For electron microscopy small portions (1 mm) were fixed in 2'5% glutaraldehyde in 0'18 M cacodylate buffer at pH 7'4 for 1 hour at 4 °C; then postfixed in osmium tetroxide (1%) in the same buffer and embedded according the Spurr technique (Spurr, 1.969). Using an Ultracut E (Reichert-Jung) ultramicrotome, semithin sections were obtained which after staining with toluidine blue were used for light microscopy and were kept as controls for electron microscopy. Ultrathin sections (40—60 nm) were contrasted with uranyl acetate and lead citrate for study under a Zeiss EM 109 electron microscope.

Results

Glial cells are an universal component of the central nervous system of vertebrates, although there are noteworthy differences regarding their characteristics and abundance in the different Classes. Despite these differences, the typical classification of the neuroglia used for mammals is commonly extended to all vertebrate species. This classification was used as a base for the results of our own study in which we analyse light and electron microscope observations separately.

Light microscopy

Ependymal glia

The ependimocytes are the most characteristic glial elements of the Stratum periventriculare (SPV) and comprise the only glial type that is consistently shown up by silver impregnation techniques. Their round bodies are located in the deep zones of the SPV and, depending on the siting of this perikaryon, two types of ependymocytes can be distinguished: those that have their cell body at a certain distance from the ventricular cavity and join this via a short,

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Figs. 2 and 3

Fig. 2.

A. — Neuroglial-like cells (n) associated with a blood vessel (v). *Barbus bocagei* Golgi-Colonnier. × 1 500.
B. — Endothelial (e) and Neuroglial-like (n) cells. The latter are independent to blood vessel. *Barbus bocagei* Golgi-Meyer. × 2 000.
C. — Astrocyte-like (a) showing a vascular prolongation (arrow). *Barbus bocagei* Golgi-Meyer. × 1 000.
D. — Oligodendrocyte-like cell (o) associated with a neuronal body (s). *Barbus bocagei* Golgi-Meyer. × 1 000.
E. — Neuroglial cells (arrows) associated with a blood vessel (v). *Cyprinus carpio* Toluidine blue. × 1 500.
F. — Clusters of oligodendrocytes associated with neuronal cell bodies. arrow: oligodendrocyte-like; double arrow: neuronal body. *Cyprinus carpio* Toluidine blue. × 1 500.

Fig. 3.

A. — Periventricular portion of an ependymocyte cell body. N: nucleus; n: nucleolus; c: cilium. *Cyprinus carpio* × 9 000.
B. — Junction complex between the lateral areas of ependymocyte perikarya. a: zonula adherens; o: zonula occludens; d: desmosome. *Cyprinus carpio* × 20 000.

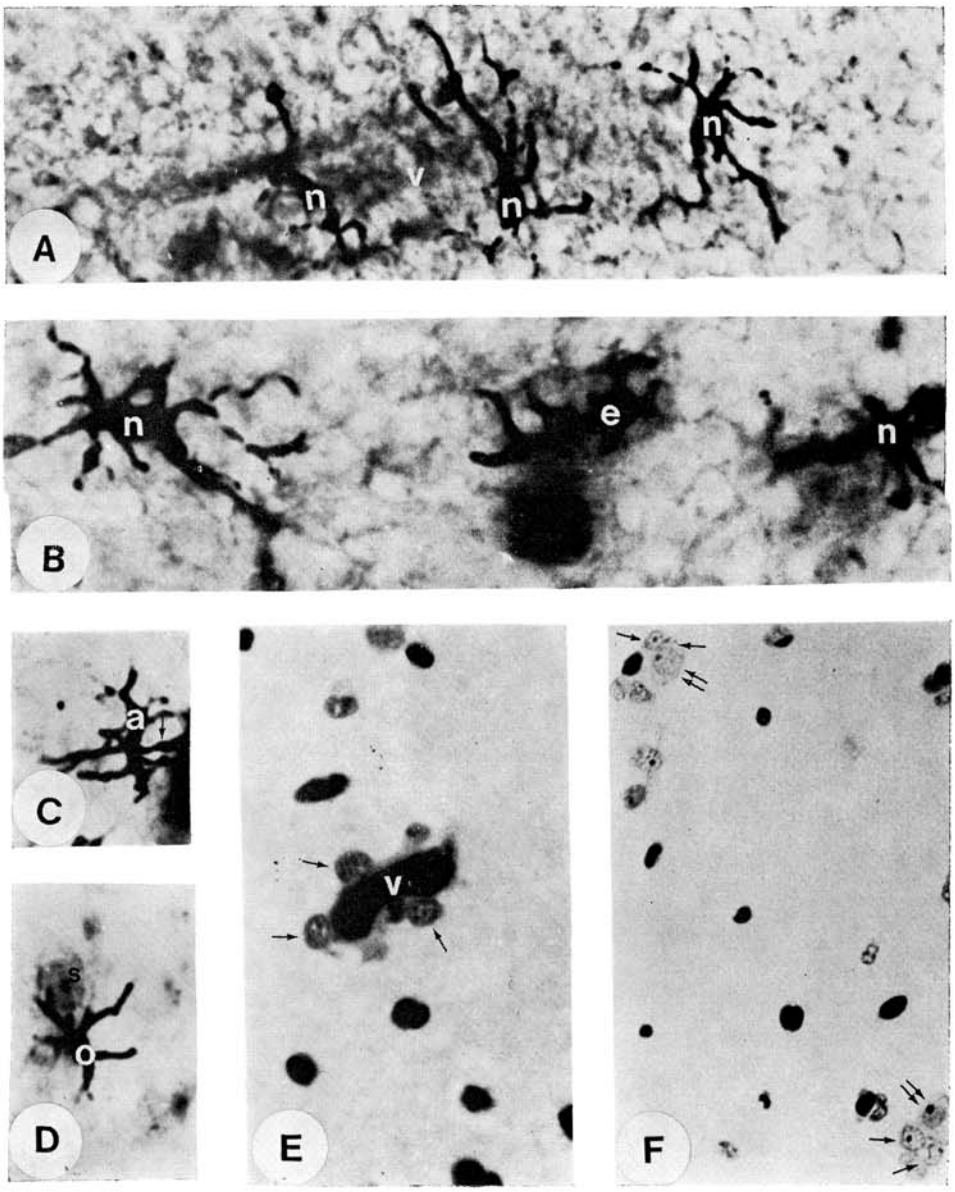

Fig. 2

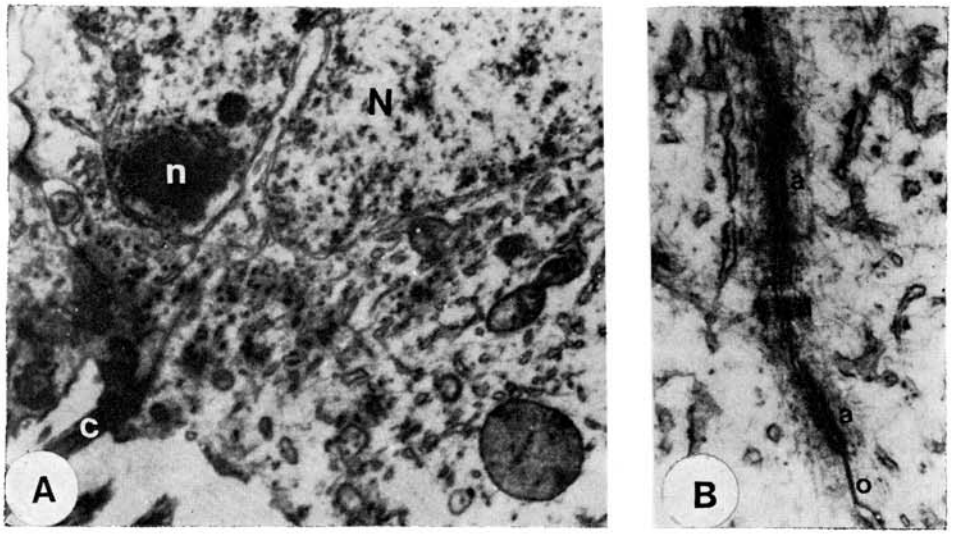

Fig. 3

relatively thick prolongation (Fig. 1a), and those whose cell body forms part of the ventricular limit (Fig. 1b).

In all cases, the ependymocytes have a prolongation that courses straight up to the pial surface (Figs. 1a, b) and emits large numbers of short fine branchs along its course (Fig. 1c). These ramifications are usually absent along the first part of the ascending prolongation, a feature that is more evident in the ependymocytes of perikaryon limiting with the ventricle (Fig. 1b).

Astroglia and Oligodendroglia

With light microscopy the differences between neuroglial elements of the astroglia and oligodendroglia types is particularly difficult to establish in the optic tectum of Cyprinid fishes. The specific silver impregnation for these cell types in higher vertebrates is inefficient and with to certain variants of the Golgi technique (Colonnier, Meyer) produces few, highly irregular images, generally of periventricular zones.

This kind of impregnation reveals numerous cells associated with blood vessels (Figs. 2a, b). In some cases, owing to their morphology and location, the cells are classified as endothelial elements (Fig. 2b). In others, they may be identified as perivascular astrocytes with an irregular perikaryon out of which arise some but not many prolongations with few branches (Fig. 2a). In semithin sections these perivascular elements exhibit a dense nucleus surrounded by very scanty cytoplasm (Fig. 2e). A common finding is very impregnated cells with characteristics similar to

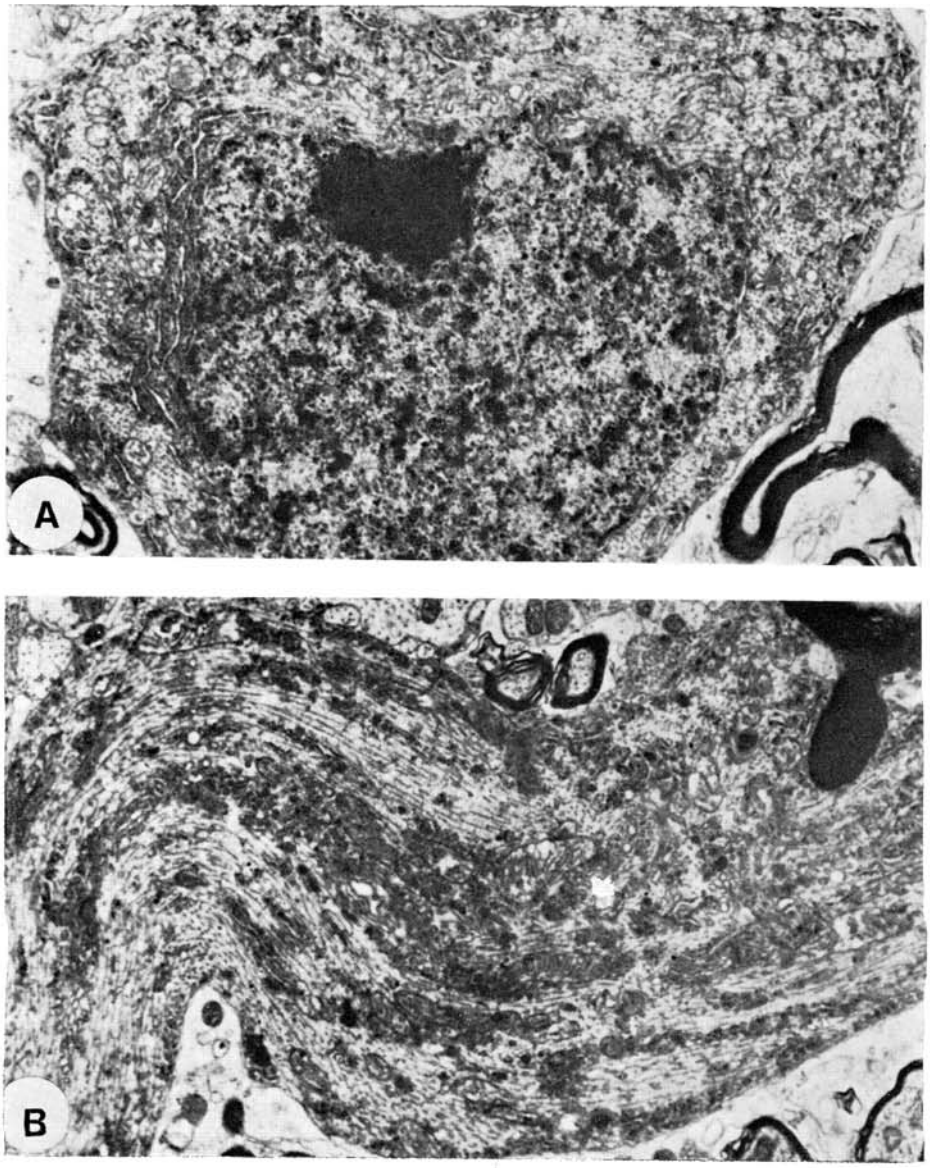

Fig. 4.

A. — Cell body of a dense oligodendrocyte. *Barbus bocagei* × 12000

B. — A very thick oligodendrocyte prolongation. *Barbus bocagei* × 12000.

those just described but unrelated to blood vessels (Fig. 2b) or connected to these via a vascular prolongation (Fig. 2c).

Regarding the typical classification of the neuroglia the term of oligodendroglia may be assigned to all the glial elements that have very few prolongations, sometimes with fine branches on their ends (Fig. 2d). These cells may found isolated or, more commonly, closely related to neuronal somata. In the latter case, in semithin sections the perineuronal satellites exhibit a nucleus that is occasionally clearer than that of the astrocytes and has slightly more perinuclear cytoplasm (Fig. 2f).

Although the glial elements that we have been able to evidence by silver impregnation are mostly located in deep zones of the optic tectum, the normal morphological techniques employed show bodies of glial elements (associated with vessels, perineuronal satellites and free) in all tectal zones; however, they are particularly abundant in the SPV and in the Stratum Marginale (SM).

Electron microscopy

Ependymal glia

The cytoplasm of these cells is very rich in tonofilaments, particularly the peripheral zones of the cell body; polyribosomes and cisternae of smooth endoplasmic reticulum are also abundant (Fig. 3a). In the basal zones it is common to find portions of Golgi complex showing typical morphological signs of great

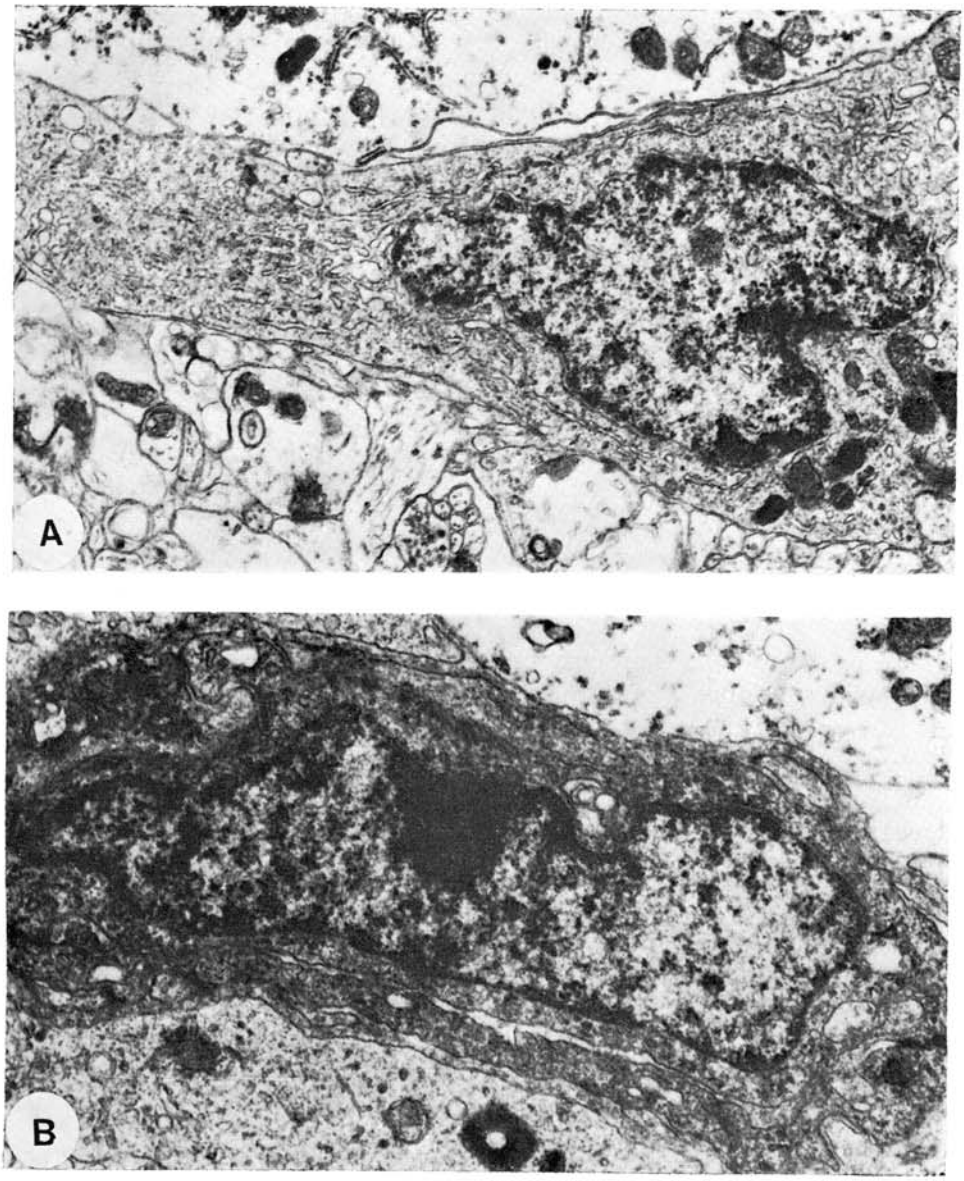

Fig. 5.

A. — Perineuronal satellite showing a low cytoplasmic density. *Cyprinus carpio*. × 12000.

B. — Perineuronal satellite showing a high cytoplasmic density. *Cyprinus carpio*. × 12000.

activity. The nucleus, containing chromatin arranged in thin accumulations and a prominent nucleolus, is located in the basal portion and is usually voluminous, sometimes with very deep indentations (Fig. 3a).

The ependymocytes project numerous cilliae to the ventricular cavity; the cilliae are anchored to the cytoplasm by deep cilliary roots (Fig. 3a).

Electron microscopy shows that the ependymocytes constitute an ependymal surface forming a closed stretch in which broad junction complexes between adjacent ependymocytes play an important role, the zonulae adherentes being specially numerous and large. (Fig. 3b).

Astroglia and Oligodendroglia

As in the case of light microscopy, electron microscopy findings do not permit an unequivocal distinction between elements similar to astroglia and oligodendroglia. However, certain ultrastructural details exists that, together with the localization of each element, facilitate such a classification.

The most noteworthy characteristics of the astrocyte-like elements are the scantiness of the cytoplasmic matrix and their low number of organelles (a few mitchondria, endoplasmic reticulum, microtubules and a variable number of dense bodies). The packets of gliofilaments are much less numerous than in higher vertebrates, decreasing drastically on the prolongations as these become more distant from the cell body. The prolongations of these glial elements may contact each other by gap junctions and in the surface zones of the SM it is common to find narrow junctions between astrocyte prolongations and the terminal processes of ependymocytes.

The formation of tightly fitting astroglial envelopes associated with synaptic structures can be observed, specially when the post-synaptic element is a dendritic spine.

The glial elements with characteristics comparable to those of the oligodendrocytes of higher vertebrates have the following as their ultrastructural features: a cytoplasmic matrix of varying density but in all cases denser that of the cytoplasm of astrocytes; numerous free ribosomes; well-developed smooth endoplasmic reticulum, and an irregular nucleus with a heterochromatic aspect. The presence of an extense system of microtubules is also common (Figs. 4b, 5a, 5b). Dispersed throughout the optic tectum are some thick prolongations, easily visible under the light microscope, with the same ultrastructural characteristics as the above-described somata (Fig. 4a).

The perineuronal satellites, which are extremely abundant in the optic tectum of Teleosts, exhibit the characteristics just described for the oligodendrocytes; they are closely related to the neuronal somata but no kind of anchoring between their membranes can be observed (Figs. 5a, b).

In general both in the perineuronal satellites and in the oligodendrocytes it is possible to establish a gradient in their cytoplasm density; this ranges from low densities, similar to those of astrocyte cytoplasms, to elements with a very high cytoplasmic density (Figs. 5a, b). The oligodendroglial characteristics are more pronounced in the dark elements, while the clear ones are sometimes difficult to distinguish from the astrocytes.

Discussion

The ependymocytes are the only glial component of the optic tectum of Teleosts that is perfectly visible with the light microscopy; according to light and electron microscopy observations their characteristics coincide with the descriptions of this element offered by other authors working with different species of Teleosts (Bellairs, 1.959; Brightman and Palay, 1.963; Ciani et al., 1.975; Duncan, 1.957; Klinkerfuss, 1.964; Kruger and Maxwell, 1.966; Lara, 1.982; Laufer and Vanegas, 1.974; Leghissa, 1.955; Schultz et al., 1.956; Stevenson and Yoon, 1.982; Vanegas et al., 1.974).

Generally, silver impregnations seem to be rather ineffective for the study of the glial elements of the optic tectum of Teleosts. Methods that provide good results in the olfactory bulb of the same species (Alonso, 1.987; Velasco, 1.980) are not conclusive in the optic tectum. It is possibly due to this that for some time the ependymocytes, perfectly visible using the Golgi method, were thought to be the only glial type existing in the optic tectum of lower vertebrates (Ramón y Cajal, 1.972) until Achúcarro (1.915) demonstrated the existence of "autonomous neuroglia" in lower vertebrates. However, classification into the same types as those seen in mammals is questionable. In this sense, some authors continue to postulate the existence of a single glial type (Zadunaisky et al., 1.963) while others believe that the distinction of different types of neuroglia in lower vertebrates is difficult (Maturana, 1.960; Mugnaini and Walberg, 1.964; Robertson et al., 1.963; Schultz et al., 1.956). Other authors make distinctions between ependymal and non-ependymal gliocytes (Sensharma and Amrendra, 1.981) and still others have found sufficient characteristics to establish a homology with mammalian astrocytes and oligodendrocytes (Ciani et al., 1.975; Garrido,

1.978; Ito, 1.971; Kruger and Maxwell, 1.967; Laufer and Vanegas, 1.974; Maxwell and Kruger, 1.965a, b).

Light microscope staining techniques such as the Mallory stain (PATH) or toluidine blue in semithin sections point to the existence in the optic tectum of elements with the characteristics of astrocytes, both associated with blood vessels and disperse throughout the neuropil; these techniques, however, only show up the distribution of cell bodies.

The variants of the Golgi method used in the present work reveal, although inconsistently, the morphology of some neuroglial elements that are quite different to those typical of higher vertebrates. Outstanding is the scarceness of prolongations of astrocyte-like elements, often making it impossible to differentiate between astrocytes and oligodendrocytes.

In some cases, the ultrastructural characteristics of astrocytes and oligodendrocytes are clearly different (Lara, 1.982); by contrast, there are large numbers of glial elements in which the characteristics of one or another glial type are confused to a greater or lesser extent. At ultrastructural level, the existence of more or less well-defined astrocytes and oligodendrocytes together with elements that are difficult to classify seems to be related to the gradient in cytoplasmic density. In the present work we have considered as astrocytes only the elements that have well-defined characteristics typical of this glial type, assigning the intermediate elements to the category of clear oligodendrocytes.

The fact that the amount of gliofibrills decreases gradually as one goes down the vertebrate scale (Kruger and Maxwell, 1.967), makes the classification of glial elements even more difficult.

By contrast, astrocyte prolongation are easy to distinguish owing to the low density of their matrix and their low number of organelles, although the gliofibrills bundles are very scanty (Lara, 1.982; Laufer and Vanegas, 1.974).

From these observations we conclude that the concepts of astroglia and oligodendroglia, well-established in higher vertebrates, are not directly applicable to the optic tectum of Teleosts and it is thus necessary to broaden the range of variability up to the intersection of the characteristics of both glial types. Accordingly, our conclusions agree with the authors who have recognized the difficulty in making this classification (Maturana, 1.969; Mugnaini and Walberg, 1.965; Robertson et al., 1.963; Schultz et al., 1.956). However, at the same time we consider that the existence of intermediate cell types could indicate that the astroglia and oligodendroglia in these vertebrates are functional differentiations of the same cellular type.

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