The intra-ocular portion of the optic nerve in the turtle *Mauremys caspica*

J. C. DÁVILA, S. GUÍRADO, A. DE LA CALLE AND F. MARÍN-GIRÓN

Department of Morphology, Faculty of Sciences, University of Málaga, 29071 Málaga, Spain

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INTRODUCTION

In spite of the large number of reports on the structure and ultrastructure of the mammalian optic nerve, including man (Yuri, 1960; Nara, 1961; Yamamoto, 1965; Hayreh & Vrabec, 1966; Anderson, Hoyt & Hogan, 1967; Cohen, 1967; Anderson, 1969, 1970; Anderson & Hoyt, 1969; Rhoades, Hsu & Parfett, 1979; Büssow, 1980), ultrastructural studies of the optic nerve in non-mammalian species, particularly in reptiles, are very scant. In reptiles, only incomplete studies of the optic nerve have been carried out. Inoue, Inoue, Nishimura & Shimai (1974) described the glial cells in reptilian optic nerves, using Golgi methods, and Kruger & Maxwell (1967) studied the optic nerve glial cells with the electron microscope. Davydova & Smirnov (1973) carried out degeneration techniques, and Davydova & Boyko (1976a, b) and Davydova, Goncharova & Boyko (1976) studied morphofunctional characteristics of the optic nerve of turtles, according to the number of axons and their degree of myelination. However, no investigator has yet undertaken a study of the intra-ocular portion of the optic nerve in a non-mammal.

The aim of the present work is to give a complete description of the intra-ocular portion of the optic nerve in the turtle *Mauremys caspica*, taking into special account the differences between this and the mammalian optic nerve. In addition, this material provides a good anatomical system for the study of glial cells and their relationships with the surrounding connective tissue.

MATERIAL AND METHODS

Optic nerves of 20 adult turtles, *Mauremys caspica*, were used. The animals were kept in a refrigerator (1–3 °C) until loss of reflexes (the timing of exposure was variable, ranging between 0.5–2 hours) and were then decapitated. Brain and optic nerves were removed after the skull was split. Seven pairs of optic nerves were processed for light microscopy. These were fixed with Bouin’s fixative and kept in propylene oxide and embedded in paraffin. Thick sections (10 μm) were stained with haematoxylin and eosin or by Holzer’s method for glial fibres. The remaining optic nerves were processed for electron microscopy. The nerves were immersed in Karnovsky’s fixative (2% paraformaldehyde and 2.5% glutaraldehyde) for 2 hours at room temperature. Pieces were postfixed in 1% osmium tetroxide for 1 to 2 hours at 4 °C. After dehydration in a graded ethanol series, the tissue was cleared in propylene oxide and embedded in Araldite. Semithin sections (1 μm) were stained with toluidine blue. Thin sections were mounted on grids and stained with uranyl acetate and lead citrate. Micrographs
Fig. 1. Photomontage of a sagittal section through the intra-ocular portion of the optic nerve. The internal and lateral limits of this region are bordered by a thin fairly basophilic layer (arrows). V, vitreous body; R, retina; CH, choroid; S, septum; gc, glial columns. Toluidine blue. × 295.

Fig. 2(a–c). Transverse sections through the intra-ocular optic nerve at retinal (a), choroidal (b) and scleral (c) levels. An increase of the cell number can be observed, as well as the regular arrangement of cells at scleral levels. gc, glial columns; S, septum. Haematoxylin and eosin. × 145.
Intra-ocular portion of turtle optic nerve

were obtained with a Zeiss photomicroscope, and a Philips EM 201 electron microscope.

RESULTS

Light microscopy

The intra-ocular portion of the optic nerve is limited internally by the vitreous body, laterally by the retina, choroid and sclera; externally its limit is the region where the nerve leaves the eye (Fig. 1).

Cellular elements are scarce at the retinal levels of the intra-ocular portion (Fig. 2a), but the number of cells increases at choroidal (Fig. 2b) and scleral levels (Fig. 2c), where they are aligned in columns parallel to the course of the axons (Fig. 1).

The internal border of the intra-ocular portion is formed by a thin layer, with somewhat more basophilia than the subjacent parenchyma, interposed between the axons and the vitreous body. Laterally, another layer can be observed which separates the optic nerve from the retina. This layer continues backwards forming a limiting sheet between the axons and the choroid-scleral connective tissue.

There is no connective network similar to the mammalian lamina cribrosa at the exit of the optic nerve.

Many myelinated axons are intermingled among the more numerous unmyelinated axons throughout the intra-ocular portion (Fig. 1). It is at the level of the choroid that the axons become parallel to the major axis of the optic nerve.

The ventral pial and scleral connective tissues of the optic nerve are invaginated deeply into the nervous tissue, forming a prominent central septum that carries blood vessels (Figs. 1, 2). This septum, which is also found throughout the extra-ocular portion, is conspicuous at scleral levels but decreases at choroidal and retinal levels, remaining as a channel carrying vessels near the vitreal surface.

Electron microscopy

The intra-ocular portion of the optic nerve is bordered internally by a continuous mantle of glial tissue, which extends laterally to become continuous with the internal limiting sheet of the retina, and separates the axons from the vitreous body. This glial sheet consists of 10–15 layers of flattened cytoplasmic processes, densely packed with gliofilaments and sometimes with numerous short cisternae of smooth endoplasmic reticulum (Figs. 3, 4). A few cisternae of rough endoplasmic reticulum as well as some mitochondria are also present. These cytoplasmic processes originate from astrocytic perikarya located particularly in the central region (Fig. 3). The astrocytes have elongated nuclei, with the major axis parallel to the vitreal surface and the chromatin showing some peripheral condensations. The perinuclear cytoplasm is scanty and contains the usual organelles. From the cell poles long and thin processes arise, which run parallel to the vitreal surface to join the internal limiting sheet of the retina. Away from the central region of the glial mantle very few astrocytic nuclei can be seen, the mantle consisting mainly of the flattened cytoplasmic processes (Fig. 4).

Gap junctions are present between adjacent processes, most frequently near the vitreal surface (Fig. 3, inset). We have also observed endocytic vesicles at the surface of the astrocytic processes that abut onto the vitreous body (Fig. 4, inset).

Another population of glial cells is peripherally located, interposed between the axons of the intra-ocular portion and the retinal layers (Fig. 5). These cells, characterised as fibrous astrocytes, form a continuous wrapping that surrounds the
Fig. 3. Electron micrograph of the limiting glial sheet that separates the vitreous body ($V$) from the axons of the intra-ocular portion. An elongated astrocyte nucleus ($A$) occupies the deeper layer of this sheet. $\times 11840$. Inset: Gap junctions (arrows) between astrocytic processes at the vitreal surface. $gf$, gliofilaments. $\times 33360$.

Fig. 4. Detail of the limiting glial sheet between the optic nerve and the vitreous body. Several strata of endoplasmic processes, some with numerous profiles of smooth endoplasmic reticulum (triangles), constitute this layer. $V$, vitreous body. $\times 14895$. Inset: endocytic vesicle ($\star$) facing the vitreous. $\times 36140$.

Fig. 5. Electron micrograph of the limit between retina ($R$) and optic nerve. Perikarya and processes of astrocytes ($A$) form a continuous mantle that separates both regions. $\times 10300$. 

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intra-ocular part of the nerve at the retinal level. This glial mantle consists of both astrocytic perikarya and cytoplasmic processes running parallel to the axons. Some glial processes arising from these cells run toward the retina, whereas others penetrate between the optic nerve fibres.

The limiting glial sheet continues backwards at choroidal and scleral levels of the intra-ocular optic nerve. Here, however, the glial sheet consists mainly of cytoplasmic processes (Fig. 6), and astroglial perikarya are rarely observed. The astrocytic processes are perpendicular to the lateral surface of the nerve and they are completely filled with gliofilaments. Sometimes, stacked cisternae of rough endoplasmic reticulum are present (Fig. 7). Numerous indentations of the choroid–scleral connective tissue into the limiting glial sheet can be observed. Abundant patches of densities with the appearance of hemidesmosomes are found in the astrocytic plasma membrane that abuts on the basal lamina.

Besides forming part of these limiting sheets, glial cells are located between axons in the intra-ocular portion. We have observed two types of glial cells in the intra-ocular optic nerve, fibrous astrocytes and oligodendrocytes.

Astrocytes are few and scattered among the axons at the retinal level (Fig. 8) whereas their frequency increases at choroidal and scleral levels. Here, astrocytes are arranged to form columns parallel to the axons (Fig. 9). Astrocytes have a more electron-dense appearance than the surrounding axons. Ultrastructural features of these cells are similar to that of fibrous astrocytes in other parts of the central nervous system.

Astrocytes located at scleral levels exhibit special features. They have very elongated somata (Figs. 10, 11), orientated mainly at right angles to the axons (Fig. 11). Nuclei are oval to fusiform with the chromatin forming small clumps. The perinuclear cytoplasm is scanty and extends towards the cellular poles forming one or two long, thick processes. Perikarya and processes are packed with gliofilaments but other organelles are scarce (Fig. 12).

Transverse sections at the scleral level of the intra-ocular optic nerve show that astrocytic processes occupy a large area and form a network, through which the nervous fibres pass (Figs. 10, 11). This population of astrocytes occupies a position corresponding to that of the lamina cribrosa of mammals.

Many indentations and dense regions resembling hemidesmosomes (Figs. 14, 15) are observed in the zones where glial processes reach the septal and choroid–scleral connective tissue.

Oligodendrocytes are much less frequent than astrocytes. They appear isolated among the axons and do not form part of the limiting structures. The most conspicuous features of these cells are their pronounced electron density and the eccentrically located nucleus (Fig. 12).

Oligodendrocytes in the intra-ocular portion of the nerve are accompanied by numerous myelinated axons, which are either scattered or arranged in small groups

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Fig. 6. Low magnification micrograph of the intra-ocular portion at choroidal level. The limiting glial sheet (LGS) is mainly formed by astrocytic processes (ap), which reach this sheet in a perpendicular plane. CH, choroid. × 5265.

Fig. 7. Detail of the glial sheet that separates the choroidal connective tissue from the optic fibres. The astrocytic processes are filled with filaments (gf) and, sometimes, with cisternae of rough endoplasmic reticulum (rer). Numerous indentations of connective tissue into the glial sheet are observed (arrows). ×13440.
among the unmyelinated axons. Myelinated axons exhibit irregular profiles in cross section, the axoplasm containing microtubules and microfilaments, as well as some round mitochondria. Various degrees of myelinisation can be observed in the axons within the intra-ocular optic nerve, but their size is greater than the unmyelinated axons.

The number of myelinated fibres increases gradually from the intra-ocular to the extra-ocular portion, but there is no marked increase in the number of myelinated fibres in the border between the two regions.
As mentioned above, a deep septum of connective tissue extends throughout the intra-ocular portion. Numerous astrocytic processes reach the septum perpendicularly (Fig. 13), forming a glial mantle between the connective tissue and the nervous parenchyma. Many indentations of the septal connective tissue into the limiting glial sheet considerably increase the surface of the interface between them (Fig. 14). In addition to cellular indentations, numerous dense areas similar to hemidesmosomes can be observed in the plasma membranes adjacent to the basal lamina (Figs. 14, 15).

DISCUSSION

The optic nerve of *M. caspica* is divided into two regions; an intra-ocular and an extra-ocular portion. The intra-ocular portion of the nerve has been widely studied in mammals (Blunt, Wendell-Smith & Baldwin, 1965; Hayreh & Vrabec, 1966; Wendell-Smith, Blunt & Baldwin, 1966; Anderson *et al.* 1967; Anderson, 1969, 1970; Minckler, McLean & Tso, 1976; Quigley, 1977; Büssow, 1980). However, this region has not been studied in reptiles and other lower vertebrates.

The division between intra-ocular and extra-ocular portions in the mammalian optic nerve is marked by the presence of a connective structure, the lamina cribrosa, described by Fuchs (1916). On the basis of the existence of a lamina cribrosa, Hayreh & Vrabec (1966), in *Macaca mulatta*, divided the intra-ocular portion into three regions: prelaminar, laminar and postlaminar.

In mammals, astrocytes in the prelaminar regions of the optic nerve form a three dimensional, basket-like network that surrounds the nerve bundles and divides them into fasciculi. This astrocytic network continues through the lamina cribrosa and the extra-ocular optic nerve. At the scleral level, the connective tissue of the lamina cribrosa forms a scaffolding for the passage of nerve axons, and reinforces the back of the eye at the site of the nerve exit. Astrocytes in the laminar region are reduced to a thin mantle that surrounds the nerve bundles, separating them from the connective tissue of the lamina cribrosa (Hogan, Alvarado & Weddell, 1971).

In *M. caspica*, the intra-ocular portion does not present any structure of a connective tissue nature which could be regarded as homologous with the mammalian lamina cribrosa. Astrocytes of the intra-ocular optic nerve are disposed essentially in a manner similar to mammals. At the scleral level the number of astrocytes increases and there are some structural differences between these cells and the astrocytes of the retinal and choroidal levels. They represent a long flattened soma that lies perpendicular to the major axis of the nerve. Their long and thick processes extend at right angles to the axons, often reaching the peripheral and septal connective tissue, forming numerous hemidesmosomes and indentations. These structural characteristics and arrangement are not observed in other regions of the optic nerve of *M. caspica*, and we suggest that astrocytes at the scleral level may represent a framework similar to the mammalian lamina cribrosa.

In the intra-ocular portion of the optic nerve of *M. caspica* we have found both fibrous astrocytes and oligodendrocytes. Cone & MacMillan (1932) did not report oligodendrocytes in the prelaminar region of the cat optic nerve while Blunt *et al.* (1965), using silver methods, reported only astrocytes in the prelaminar and laminar regions of the cat. Wendell-Smith *et al.* (1966) in the cat, Anderson (1969, 1970) in the monkey and man, Hogan *et al.* (1971) in man, and Büssow (1980) in the cat and monkey, describe astrocytes as the unique glia in the intra-ocular portion of the optic nerve in the prelaminar and laminar regions. Blunt *et al.* (1965) suggested a direct
relationship between the absence of oligodendrocytes and the lack of myelinated axons in the intra-ocular portion just above the lamina cribrosa. In Mauremys, we found an abundance of myelinated axons in the intra-ocular portion of the optic nerve, as well as many typical oligodendrocytes.

Astrocytes constitute most of the glial cells in the intra-ocular portion of the optic nerve of Mauremys. These fibrous astrocytes are identical to those described in mammals and they have been characterised using the criteria of Mugnaini & Walberg (1964), Wendell-Smith et al. (1966), and Peters, Palay & Webster (1976). They are scattered or aligned in columns parallel to the axons. This organisation is similar to the arrangement in mammalian optic nerves (Wendell-Smith et al. 1966; Hogan et al. 1971; Büssow, 1980). In addition astroglia forms the limiting glia that separates the axons from the vitreous body, retina, choroid and sclera.

In Mauremys the limiting glial sheet adjacent to the vitreous body consists of astroglial processes with frequent gap junctions between them. This glial sheet has been described in mammals (Hayreh & Vrabec, 1966; Anderson, 1969; Hogan et al. 1971; Büssow, 1980) but in mammals the number of strata is smaller. Laterally, separating the intra-ocular optic nerve from the retinal layer, there exists a group of astrocytes forming a limiting glial sheet. This glial sheet is named the intermediary tissue of Kuhnt in mammals (Hogan et al. 1971), and is continuous externally with the limiting glial sheet adjacent to the choroid and sclera. In Mauremys, we found a similar disposition of the glial cells; they form limiting structures at retinal, choroidal and scleral levels.

The glial sheet, interposed between the choroid–scleral connective tissue and the axons of the optic nerve, is characterised by the large number of regularly arranged dense areas in the plasma membrane adjacent to the basal lamina. These structures are similar to the junctions between extracellular substance and subpia described as hemidesmosomes by Brightman & Reese (1975). In addition to hemidesmosomes, the glial sheet is penetrated by many indentations of the connective tissue that considerably increase the interface between them. At the scleral level, both hemidesmosomes and indentations are more abundant in the limiting glial sheet that separates the axons from the connective tissue, which supports the hypothesis that astrocytes at this level may play the role of a framework similar to the lamina cribrosa in mammals.

**SUMMARY**

The cytoarchitecture of the intra-ocular optic nerve was investigated by light and electron microscopy. Glial cells in the intra-ocular portion are disposed among the axons, either scattered or forming columns, and bordering the nervous parenchyma. Astroglia forms the limiting glia that separates the optic nerve from the vitreous body, retina, choroid and sclera. Indentations of choroid–scleral tissue are observed in the limiting glial sheet as well as an abundance of hemidesmosomes in the astrocytic plasma membranes that abut onto the basal lamina. At the scleral level there is no connective tissue structure similar to the mammalian lamina cribrosa. However, astrocytes in this region are regularly arranged in thick columns and their processes cross throughout the nerve perpendicular to the axons, forming a framework through which the nerve fibres pass. Numerous myelinated and unmyelinated axons are present in the intra-ocular portion. Oligodendrocytes are also present in close relation with myelinated axons.
A deep connective tissue septum arises from the ventral region of the optic nerve and extends along the intra-ocular portion.

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REFERENCES


