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Dense Osmiophilic Material in the Surface of the Olfactory Bulb in the Teleost *Cyprinus carpio* L.

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With 4 Figures

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Summary: A dense osmiophilic structure was found associated to the basal lamina of the outer limiting glial membrane of the carp olfactory bulb. In certain sections this material appears as "beads" composed of small strand-like structures, and in others it seems to form a palisade-like structure. The possible origin and function of this structure is discussed.

Key words: Olfactory bulb, osmiophilic material, carp, teleosts.

Introduction

The presence of a connective covering of the central nervous system associated to the outer limiting glial membrane is a consistent feature in vertebrates (STERZI, 1901; KAPPERS et al., 1936), though its structure is modified in the various taxonomic groups (STERZI, 1901; FLEXNER, 1929). In this sense, important variations have been described in meningeal organization in several Teleost species (KAPPERS, 1925) and even between the spinal and cranial meninges of a single species (KUHLENBECK, 1973).

Ultrastructurally, the organization of the leptomeninges has mainly been studied in higher vertebrates (BONDAREFF et al., 1972; PETERS et al., 1976; McLONE, 1980) and there are few data relating to the same in lower vertebrates.

The present work analyzes a strongly osmiophilic structure, previously unreported in higher vertebrates associated with the basal lamina of the outer limiting glial membrane of the olfactory bulb of the carp.

Material and Methods

Adult specimens of *Cyprinus carpio* (Order Cypriniformes) were employed in this study. The animals had a length range of 24–40 cm and a weight range of 280–1300 gr. The fish were anaesthetized with MS-222 (Sandoz) at a concentration of 0.03%. Following "in vivo" removal, the olfactory bulbs were placed in 2.5% Glutaraldehyde in 0.18 M Cacodylate-buffer at pH 7.4 for 1–4 hours at 4°C. After this they were sectioned in small portions (≈ 1 mm) and washed in the same buffer with 10% sucrose, postfixed for 1 hour with 1% Osmium Tetroxide in the same buffer and washed again with Cacodylate buffer. After dehydration in a graded Acetone series, adding 1% Uranyl Acetate during the 70% Acetone phase, following by two washes in Propylene Oxide for 30 minutes, each of the pieces was embedded in Durcupan.

Using an Ultracut E (Reichert-Jung) ultramicrotome, semithin (0.5 μ m) sections were obtained which after stain-

ing with Toluidine Blue or Cresyl Violet were used for light microscopy and were kept as controls for electron microscopy. Ultrathin sections (400–500 Å) were contrasted with Uranyl Acetate and Lead Citrate for study under a Zeiss EM 109 electron microscope.

Results and Discussion

The external surface of the olfactory bulb of the carp, the so-called outer limiting glial membrane, is composed of glial elements identified as marginal astrocytes and their prolongations, which separate the layer of the fibers of the olfactory nerve from the surface of the bulb and which, in turn, rest on an evident basal lamina.

According to light microscopy, the study of the semithin sections stained with Toluidine Blue or with Cresyl Violet reveals the presence on the outer border of the olfactory bulb of a dense line which demarcates the limit with the surrounding meningeal connective (fig. 1).

Electron microscopy at low magnification permitted the identification of this limiting line with a strongly osmiophilic structure, with a bead-like appearance, located externally to the basal lamina of the outer limiting glial membrane of the olfactory bulb (fig. 2).

At greater magnifications variations may be seen in its appearance: in some cases the "beads" are rounded forms with a striated aspect composed of small strand-like structures densely packed and arranged parallelly (fig. 3); in other sections, this separation into small groups cannot be observed but rather the "beads" are closely packed and arranged perpendicularly to the outer limiting glial membrane forming a palisade-like structure of approximately 800–1000 Å in height (fig. 4). Each of the constituent

"strands" has a thickness of about 60–70 Å and seems to be composed of two highly electron-dense bands separated by a clearer zone (fig. 4); frequently, the dense bands corresponding to adjacent elements contact at some part of their course. This suggests that one might be dealing with longitudinal sections of tubular structures or may be solid cylindrical

structures whose external part, which would correspond to the dense bands, might have a composition other than that of the central portion, which is more electron-lucent.

Of note is the close relationship of this structure with the basal lamina of the outer limiting glial membrane, to which it seems to be anchored and which it accompanies even in its most complex folds such that occasionally it is possible to note fusions of this dense material corresponding to different parts of the basal lamina. These folds, or invaginations of the surface of the olfactory bulb, might correspond to sites of entrance of blood vessel from the meningeal connective to the bulb.

Furthermore, this strongly osmiophilic structure does not appear in all the specimens used by us and in those where it is found its presence is irregular; this is seen in the alternance between portions where it is perfectly observable, forming a kind of continuous envelope, and others where it disappears completely. Possibly, one is dealing with a material which can be readily eliminated with the usual manipulation of the pieces, which would at least in part account for both the scarcity of references in the literature and the discontinuity observed in the specimens in which its presence has been described. Regarding this, it should be additionally noted that in our own studies on the ultrastructure of the olfactory bulb and the optic tectum of *Barbus meridionalis* (unpublished results) this structure has not been observed.

References to structures which seem to correspond to the one described here are scanty but in the species in which their presence has been reported, their location in different parts of the encephalon and the uniformity of their appearance, together with their relationship to the basal lamina of the outer limiting glial membrane, seem to be significant. The first references to such structure found by the authors of this work is in a study by WESTERMAN and WILSON (1968) in *Carassius carassius* where they are described

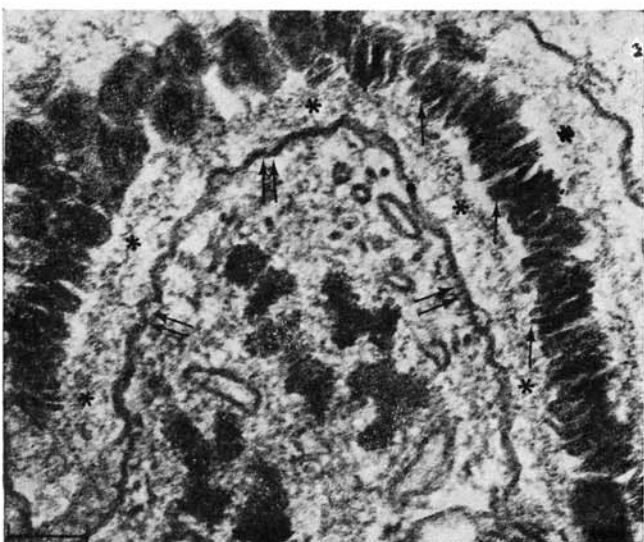
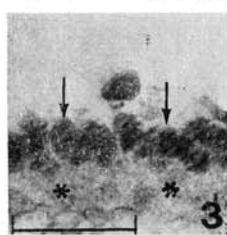
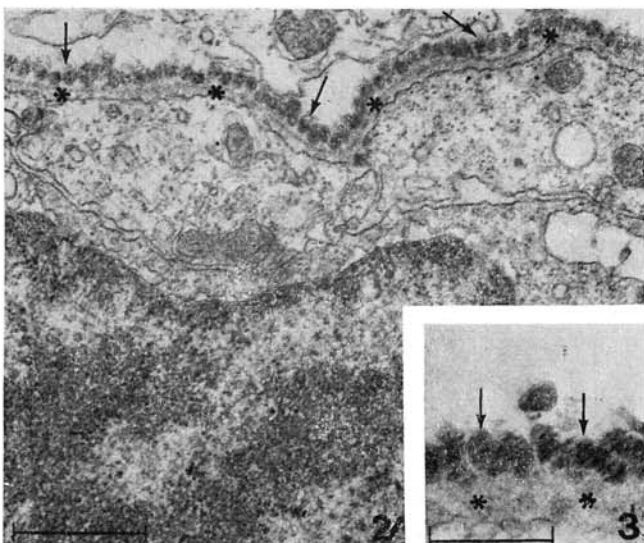
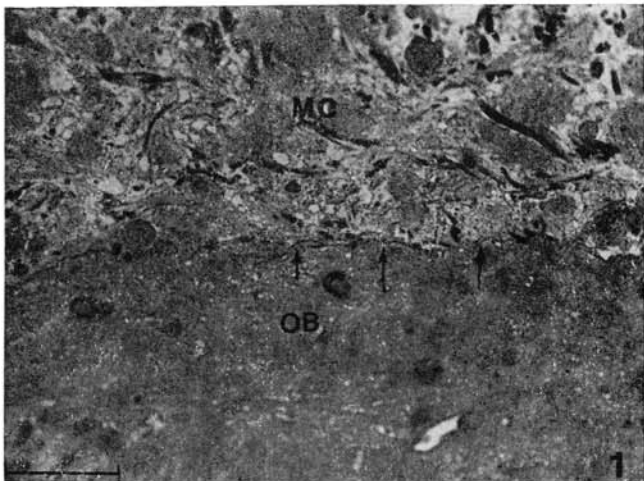


Fig. 1. Semithin section showing a dense line (arrows) at the intersection between the olfactory bulb (OB) and the meningeal connective tissue (MC). Cresyl Violet. Bar = 10 μm .

Fig. 2. Bead-like structure (arrows) associated to the external surface of the basal lamina (asterisks) of the limiting glial membrane. Bar = 1 μm .

Fig. 3. Striate aspect of the "beads" (arrows) seen in fig. 2 (asterisks = basal lamina). Bar = 0.5 μm .

Fig. 4. Palisade-like aspect of the osmiophilic material (single arrows) associated to the basal lamina (asterisks) in a folding zone of the bulbar surface. The double arrows point to the plasmatic membrane of an astrocyte in mitosis. Bar = 0.1 μm .

associated with the outer surface of the pia and are interpreted as a probable "torn of fine strands of tissue which run between the arachnoid and pial layers". Our team later located the structure in the olfactory bulb of *Cyprinus carpio* (VELASCO, 1980) and it also appears in figure 10 of a work on the optic tectum of *Carassius auratus* (STEVENSON and YOON, 1982). In all these cases the images are similar and the arrangement of the structure is identical with respect to the basal lamina, which together with the fact that it has only been located in different parts of the encephalic surface of specimens belonging to the Order Cypriniformes, Family Cyprinidae, suggests that one is dealing with a consistent structure, at least in the connective covering of the encephalic surface of the members of this Family of Teleosts.

Despite the slight similarity at low magnifications with other osmiophilic structures reported in neuropilar portions of the Teleost encephalon (RUBIO et al., 1982, 1985), it is of interest that the structure described by us is completely different both in morphology and in ordering as well as its location.

The fact that this dense material appears with the different fixing methods employed — Osmium Tetroxide at 2.5% with a mixture of Potassium Dichromate and Calcium Chloride (WESTERMAN and WILSON, 1968); 2.5% Glutaraldehyde and later postfixation with 1% Osmium Tetroxide (VELASCO, 1980; and the technique used in this work); or a mixture of 2.5% Glutaraldehyde and 3% Paraformaldehyde with postfixation in Osmium Tetroxide at 1% (STEVENSON and YOON, 1982) — limits the possibility that one is dealing with a fixation artefact.

The consistent close association of this osmiophilic material both with the basal lamina of the outer limiting glial membrane and with the basal lamina corresponding to some parts of the meningeal connective is suggestive of several possibilities: it constitutes an anchorage site between the outer limiting glial membrane and the meninx; it is a secretory product of the meningeal connective; or, the structure is a modification of the basal lamina of the outer limiting glial membrane.

Furthermore, the active synthesis and secretion of protein substances reported in the neuroepithelium of the outer limiting glial membrane in certain higher vertebrates (COHEN and HAY, 1971; McLONE, 1980) — substances whose leptomeningeal location is comparable to that observed by us in the carp — could indicate that the structure described in the present work is the result of the extracellular condensation of secretory substances of the cells of the outer limiting glial membrane.

Further studies employing ultracytochemical techniques both in developing and adult specimens are necessary to elucidate the origin and nature of this material.

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