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ORIGINAL ARTICLE

Histological and histochemical studies on the olfactory rosette of *Mugil parsia* (Hamilton)

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The structure and functions of the olfactory organs in Mugil parsia *(Ham.) has been described. Histologically each lamella consists of supporting, olfactory receptor, basal, labyrinth and mast cells. The distribution and localization of acid and neutral mucins in the various cells of olfactory epithelium in* M. parsia *has been studied histochemically. Variations in the localization of glycogen in the different cells of the olfactory epithelium have been correlated with the functional significance of the region concerned in the fish studied.*

Key words: histoarchitecture, glycogen, mucopolysaccharide, localization, olfactory epithelium, *Murgil parsia*

INTRODUCTION

The study of the olfactory organ in fish is of paramount importance because it is essentially a chemoreceptor and plays a meaningful role not only in locating food but also in detecting the presence of odoriferous substances in the ecosystems. A number of researchers have studied the histological peculiarities of the olfactory epithelium in fish [3, 6, 9, 14–16]. However, lacunae still exist in some aspect of these studies relating to the olfactory epithelium of brackish water teleosts and there are few histochemical studies involving the identification and localisation of the various cellular contents occurring in the cell lining of the olfactory epithelium and their role in sensory reception in teleosts.

MATERIAL AND METHODS

Histology

Live mature fish of the *Mugil parsia* breed were collected from Digha fish farm, West Bengal. The heads of the *M. parsia* were fixed in aqueous Bouin's fluid for 18–20 hours and subsequently dissected from the dorsal side under a stereoscopic binocular microscope in order to dissect out the olfactory rosette. The tissues were then dehydrated properly through graded alcohols, cleared with xylene and embedded in paraffin. Sections were cut of 4–5 mm thick. The deparaffinised sections were brought to water and stained with Delafield's haematoxylin followed by counter-staining with eosin.

Histochemistry

For the histochemical studies the olfactory lobes were fixed in 10% neutral formalin. Subsequent to dehydration, transverse paraffin sections of 8–10 μm were cut and subjected to the following histochemical tests to evaluate the chemical nature of various cells lining the olfactory epithelium:
1. Periodic Acid Schiffs (PAS) in combination with Alcian Blue (AB) for the detection of neutral and acid mucins (PAS-AB) [13].
2. Best's carmine method for the detection of glycogen (BC) [5].

RESULTS

Histology

In *M. parsia* each olfactory organ has an olfactory rosette consisting of a primary lamella or lamellae. The outer margins of the lamellae are free, while their inner margins are attached to the raphe (Fig. 1). Each olfactory lamella consists of an olfactory epithelium and a central lamellar space, the central core

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Figure 1. Showing olfactory lamellae (OL) radiating from median raphe (R). Note the free outer margin of OL and inner margin attached to the R. (Scanning electron micrograph) × 50.
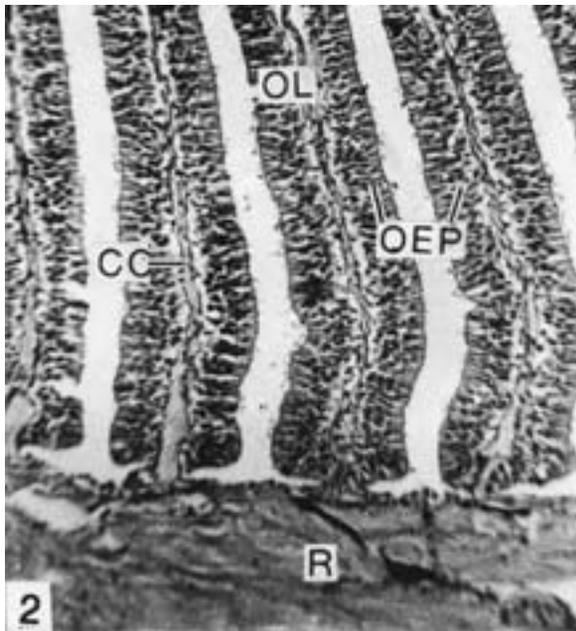

Figure 2. Showing olfactory lamella (OL) and raphe (R). Note the arrangement of various cells in the olfactory epithelium (OEP) and the orientation of connective tissue in the central core (CC) (H & E) × 100.
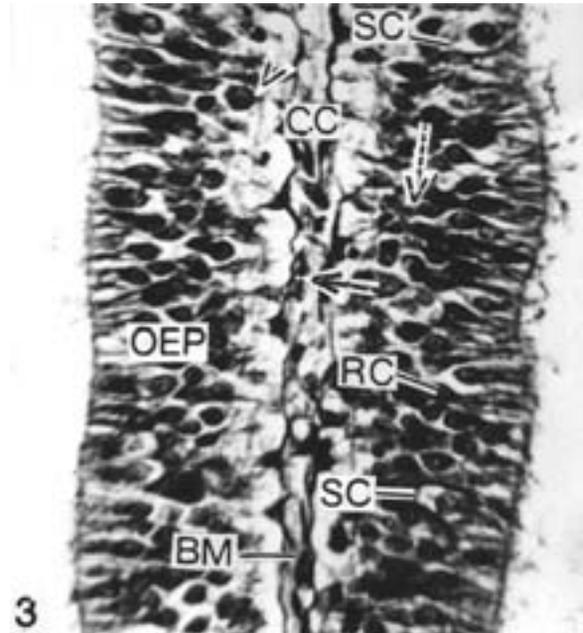

Figure 3. OEP and CC separated by basement membrane (BM). Note the presence of supporting cells (SC), primary receptor cells (RC) and secondary receptor cell (broken arrow) in the OEP. Note also basal cells (BC) (arrow head) and blood vessels (BV) (solid arrow) in CC (H & E) × 400.

The supporting or sustentacular cells

These are columnar and ciliated. Two morphological types of these cells can be distinguished in the olfactory epithelium of *M. parsia.* The first type (Fig. 3, 4) has a large oval nucleus with a clear chromatin material. A nucleolus is clearly discernible. The distal limb of the cells is broad and its tip supports stubby cilia (Fig. 3, 4). The second type of supporting cells (Fig. 4) are lightly stained narrow cells that occupy the extent of the epithelium from the basal lamina to the surface. The cytoplasm contains dust, such as chromatin particles and one or more nucleoli (Fig. 3). In *M. parsia* non-ciliated supporting cells are also found in the epithelium (Fig. 4). The non-ciliated supporting cells produce a serous secretion.

Primary neurons or receptor cell

These cells are the sensory elements of the olfactory epithelium and are accompanied by supporting cells. In *M. parsia* they are differentiated into primary and secondary neurons. Primary neurons are mainly present beneath the supporting cells and are differentiated by their rounded and deeply stained nuclei. The dendrite of each receptor cell is bipolar and extends as a narrow and cylindrical process as far as the free ciliated surface (Fig. 3, 4).
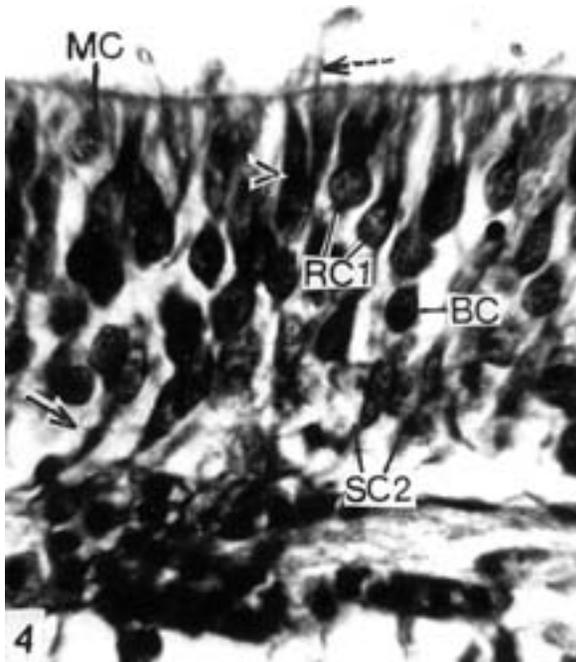
(Fig. 2). The lamellae are completely covered by sensory epithelium. A well-developed basement membrane is usually distinguishable (Fig. 3). The olfactory epithelium is very thick and mainly consists of 4 types of cells:
— supporting or sustentacular cells;
— primary neurons or receptor cells;
— secondary neurons or spindle-shaped cells;
— basal cells (Fig. 3).

Figure 4. Demonstrating ciliated SC (arrow head) non-ciliated SC (SC$_2$), receptor cells 1 (RC$_1$), receptor cell 2 (RC$_2$) (solid arrow), basal cells (BC) and mast cell (MC) in OEP. Note the cilia of ciliated SC (broken arrow) (H & E) $\times$ 1000.

Secondary neurons or spindle-shaped cells

The secondary neurons are mainly present below the primary neurons and are distinguished by their elongated and oval nuclei. The axons of secondary neurons extend up to the basement membrane and pass out of it into the central core of the lamella (Fig. 4).

Basal cells

These cells are few and scattered, lying in the deeper part of the olfactory epithelium above the basement membrane. They are cuboidal, oval and rounded and contain distinct nuclei (Fig. 4). These cells form the reservoir for the formation of supporting and olfactory receptor cells.

Mast cell. Small in size and more rounded with a relatively smaller amount of cytoplasm and with a polymorphous nucleus (Fig. 4).

Labyrinth cell. These cells are scattered in the superficial layer of the olfactory epithelium. They are ovoid and rounded in appearance and their nuclei are present towards the basal ends (Fig. 4).

Central core of the Lamella. The central core of the lamella is lined on either side by olfactory epithelium (Fig. 2, 3). It is filled with loose connective tissue comprising collagenous, reticular and elastic fibres. A few mesenchymal cells are also found along

the basement membrane (Fig. 3). Apart from the connective tissue, blood vessels also occur in the central core (Fig. 3).

Histochemistry

PAS-AB. The combined PAS-AB reaction furnishes a bluish-purple colour of varying intensity according to the neutral and acid mucin content of the various cells in the olfactory epithelium. This combined test imparts a red colour as a result of the presence of neutral mucin and blue colour as a result of the presence of acid mucin exclusively. In *M. parsia* the intensity of the bluish-purple colour is discernible at its maximum intensity at the free border of the olfactory epithelium and the central core, confirming the presence of both neutral and acid mucins (Fig. 5, 6). The receptor cells, however, display a moderate reaction to this test (Fig. 6). The supporting cells show an intense PAS-AB reaction, confirming the presence of acid and neutral mucin (Fig. 6). The intense reaction of PAS-AB is also discernible in the mast cells located between the supporting cells. This indicates that they may have some secretory functions.
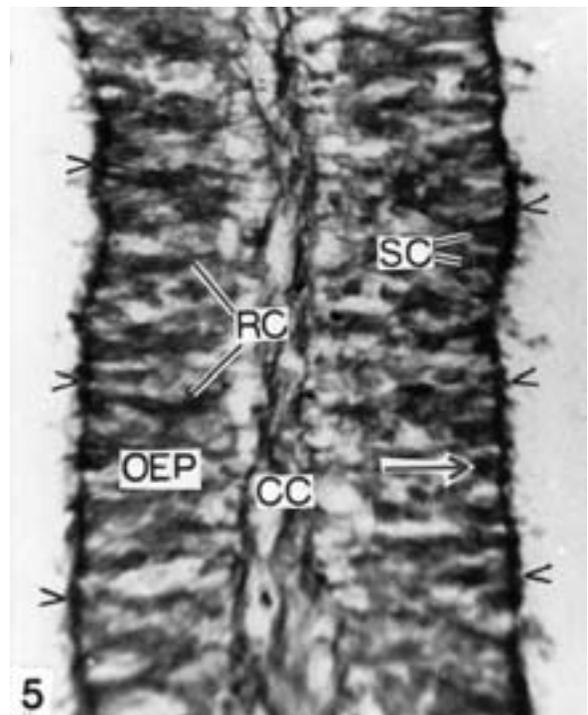

Figure 5. Localisation of both acid and neutral mucin (ANM) in the SC, RC and MC (arrow) of OEP. Note intense ANM localisation along the border of OEP (arrow heads). (PAS-AB) $\times$ 100.
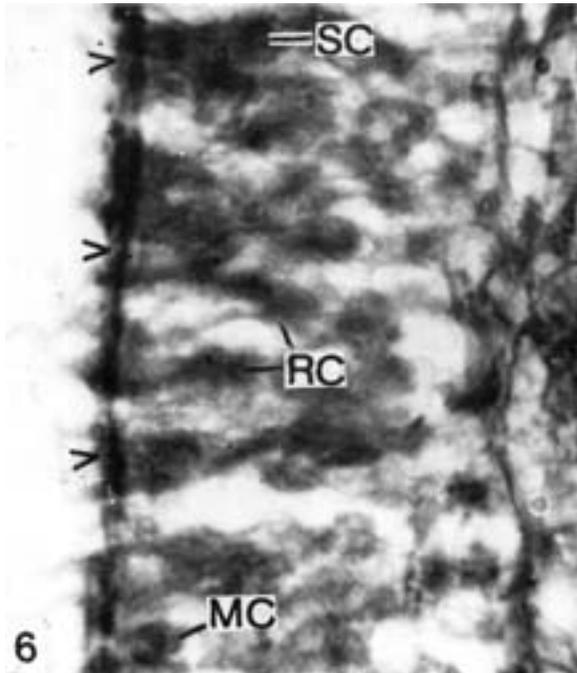
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Figure 6. Maximum localisation of ANM in the border of OEP (arrow heads). Note intense reaction in SC and MC. Note also the moderate reaction in RC. (PAS-AB) × 1000.
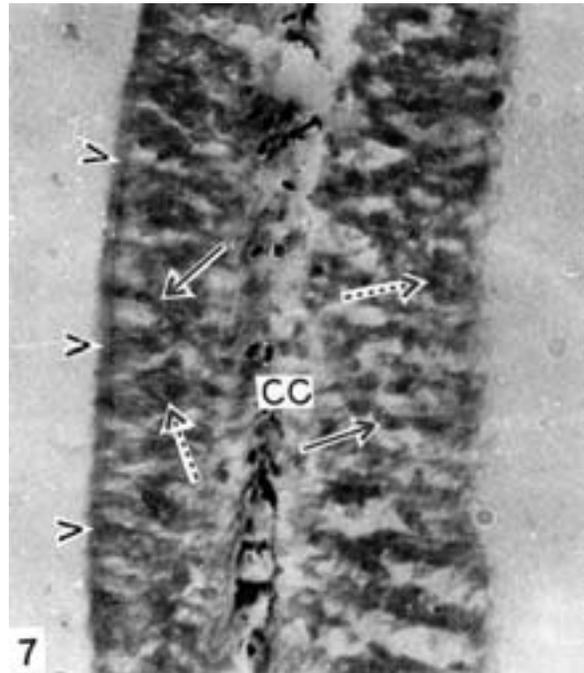

Figure 7. Localisation of glycogen in SC (broken arrows) and RC (solid arrows). Note the intense reaction in the epithelial border (arrow heads). (BC) × 400.

Glycogen. The results of Best's carmine test indicate an intense to moderate content of glycogen in the supporting cells, receptor cells and epithelial border of *M. parsia* (Fig. 7). However, maximum glycogen reaction is discernible in the supporting cells and epithelial border. The intense content of glycogen is present in the receptor cells of the olfactory epithelium (Fig. 7).

DISCUSSION

Histology

The nasal openings in *M. parsia* are situated close to one another. During forward swimming of the fish water enters the anterior nasal opening and passes out of the posterior. The present study reveals that the olfactory rosette of *M. parsia* is more or less oval in shape, a condition which stimulates Bateson's [4] rosette type 3, Burnes [7] rosette column 1 and Teichmann's [17] group 1.

The olfactory epithelium consists of supporting, olfactory receptor, basal, mast and labyrinth cells. In *M. parsia* the supporting cells are provided with a stubby ciliated structure. In *Notopterus notopterus* the supporting cells have short and faintly visible cilia [8]. Trujilo-Cenoz [19] also observed the ciliated supporting cells in the olfactory epithelium of *Fizrovia lineata,* Iwai and Nakamura [11] in *Thunnus obesus*

and Ojha and Kapoor [15] in *Labeo rohita.* These cilia are believed to increase the free surface of the supporting cell. In *M. parsia* the number of ciliated supporting cells is found to be smaller than that in the receptor cells in the outer third region of the lamella. However, Moulton and Beidler [12] reported that the supporting cell has complex secretory and nutritional functions. In *M. parsia* the non--ciliated supporting cells may produce a serous secretion which maintains the continuous directional flow of the mixed secretion along the surface of the epithelium. This flow removes the remains of the stimulating substances and keeps the receptors ready for new stimuli. The cilia of the olfactory epithelium facilitate the flow of the water current over the olfactory lamellae of the olfactory chamber of *M. parsia.*

In *M. parsia* the basal cells occupy a position in the lower region of the olfactory epithelium immediately above the basement membrane and have no cytoplasmic processes which reach the free surface. Using tritiated thymidine followed by autoradiography, Thornhill [18], Graziadei and Metcalf [10] showed shown that the basal cells, apart from differentiating into supporting cells, also give rise to olfactory receptor cells, which are continually replaced during life. Andres [1, 2] also suggested that the basal cells are the precursors of regenerating re-

ceptor cells. In *M. parsia* the receptor cells are grouped together with supporting cells between them. These olfactory receptor cells are bipolar neurons whose dendrites are derived peripherally. The sensory hairs of receptor cells are of special interest because they form part of the olfactory transduction mechanism and are stimulated by odour-bearing substances. One of the most interesting features of the present study is the histological identification of spindle-shaped secondary neurons. The axons of secondary neurons extend into the central core of the lamella. In the present study the fact that the synaptic connection between the primary and secondary neurons is not prominent may be due to degenerating dendrite processes. The sensitivity of the receptor cell may change in the euryhaline *M. parsia* when they migrate into seawater from freshwater or vice versa. Shortly after entering freshwater the fish are covered with a thick layer of mucus, which may protect the sensory cells directly exposed to the environment from the sudden changes in osmotic pressure.

The labyrinth cells in the olfactory are similar to those in the chloride cells, which serve as excretory cells for osmoregulation and ion regulation in fish gills and pseudobranch. In this way, they may cause the olfactory organs to function optimally in water of different salinities.

In the present study the mast cells in the olfactory epithelium of *M. parsia* are thought to have caused fluctuations in the production of mucus in the olfactory mucosa.

Histochemistry

The histochemical nature of mucin in the olfactory epithelium of *M. parsia* has been studied by employing a PAS-AB histochemical test. In the present observation the predominance of acid mucopolysaccharide in the epithelial border of *M. parsia* prevents friction against foreign particles, which enter into the olfactory chamber through water. In addition, the coating of mucus forms a favourable environment for ionic and molecular diffusion. Intense acid mucopolysaccharide reaction in the mast cells in *M. parsia* is due to the presence of a profuse amount of heparin, which probably plays a positive role in keeping the olfactory epithelium moist and keeping clear and protecting the sensory epithelium from any foreign particles. Various cells lining the olfactory epithelium of *M. parsia* exhibit an intense reaction to glycogen, probably for various metabolic as well as physiological activities. However, the maximum gly-

cogen reaction is discernible in the supporting cells of *M. parsia*. The movement of the cilia of the supporting cells maintains a continuous directional flow along the surface of the epithelium, which is an active process, requiring energy for which the presence of glycogen is the main source. The glycogen reaction in the receptor cells may be related to the transduction of impulses.

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