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Distribution and Organization of Different Cells Lining the Olfactory Epithelium of the Indian Minor Carp, *Labeo bata* (Hamilton 1822): A Light and Scanning Electron Microscopic Analysis

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Abstract: The olfactory epithelium of adult *Labeo bata* (Hamilton) has been studied by light and scanning electron microscopy. The oval shaped olfactory rosette consists of 26 to 28 primary lamellae arranged on both side of the median leaf like raphe. The middle dorsal portion of the lamellae is provided with linguiform processes. This linguiform process is occupied by sensory epithelium and characterized by the presence of two types of receptor cells, ciliated and with microvillous cells. The apical and basal part of the olfactory lamellae are covered with non-sensory epithelium. The non-sensory epithelium is made up of stratified epithelial cells and mucous cells. The surface of the non-sensory epithelium is represented by stratified epithelial cells which are provided with unbranched microridges arranged in a concentric whorl. Variations in the cellular organization in the sensory and non-sensory olfactory epithelium have been highlighted in reference to the olfactory sense of the fish concerned.

Key words: Histology, surface architecture, function, olfactory epithelium, *Labeo bata*

INTRODUCTION

Olfaction is a major mediator of chemical signals and is involved in diverse teleost behaviors (Hara, 1992). In teleosts the olfactory components play an important role in food-finding, reproductive behaviour, nest-finding, detecting and avoiding predators or other perilous situations (Frabman, 1994). Olfaction is achieved by olfactory cells on the surface of lamellae (Hansen and Zeiske, 1998; Ma and Wang, 2010). The morpho-histology and the cytoarchitectural pattern of the different cells lining the olfactory epithelium vary considerably by light microscopic (Ojha and Kapoor, 1973; Bandyopadhyay and Datta, 1996; Mandal *et al*., 2005; Ghosh and Chakrabarti, 2009, 2010) and at the electron microscopic level (Zeiske *et al*., 1994; Mana and Kawamura, 2002; Baile *et al*., 2008; Chakrabarti and Ghosh, 2010a,b, 2011). These studies advocated the considerable morphological variability regarding the shape and location of the olfactory organ, number and arrangement of the olfactory lamellae, the distribution of sensory and non-sensory epithelium in relation to the diverse environments that the fish inhabit. However, there is also dearth of knowledge in some aspects of these studies relating to the olfactory sensation of cyprinidae. An effort has therefore, been made in the present investigation to describe the structural characterization and the functional significance of various cells lining the olfactory epithelium of *Labeo bata* (Hamilton 1822) (Cyprinidae, Cypriniformes) is a freshwater minor carp which plays a meaningful role in detecting the odoriferous substances in the freshwater ecosystem.

MATERIALS AND METHODS

Twenty healthy mature fishes of *L. bata*, ranging in size from 16 to 18 cm, were obtained from local freshwater bodies. The specimens were killed by an overdose of tricaine methone-sulphonate (MS 222) following the guidelines given by the Institutional Ethical Committee. The heads of the specimens were dissected out from the dorsal side of the olfactory chamber under a stereoscopic binocular microscope to take away the olfactory rosette.

For the purpose of SEM study, the olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 30 min. The rosettes were then dissected out and the adhering mucus on the epithelial surface was removed by repeated rinsing with heparinized saline (heparin sodium salt 10,000 IU mixeded in 0.67% NaCl solution). After rinsing in 0.1 M cacodylate buffer (pH 7.4), the tissues were again immersed in 2.5% glutaraldehyde buffered with 0.1 M

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cacodylate buffer (pH 7.4) for 24 h at 4°C. After fixation, the tissues were rinsed in the same buffer for 10 min and post fixed in 1% osmium tetroxide (OsO_4) in 0.1 M cacodylate buffer (pH 7.4) for two h. After secondary fixation the tissues were washed thoroughly in buffer, dehydrated through ascending series of acetone, followed by isoamyl acetate and subjected to critical point drying method with liquid carbon dioxide. The dried tissues were mounted on metal stubs, coated with gold palladium with a thickness of approximately 20 nm and scanned in a Hitachi, S-530 SEM.

For histological purposes, the olfactory rosettes were fixed in aqueous Bouin's fluid for 16-18 h and were dehydrated properly through ascending series of ethanol, cleared with xylene and embedded in paraffin wax of 56-58°C. Sections were cut at 4 μm thick. After routine histological procedure the sections were stained with Delafield's Haematoxylin-Eosin and Mallory's triple stain.

RESULTS

According to SEM studies, the oval shaped olfactory rosette of *L. bata* consists of 26 to 28 primary lamellae in each left and right rosette. The outer margins of the lamellae are free while the inner margins are attached to the leaf-shaped raphe (Fig. 1). The middle dorsal portions of the lamellae are provided with linguiform processes. The receptor epithelium occupies a smaller area in the middle linguiform process whereas on the large part of the lateral surface of the olfactory lamella is covered with non-receptor epithelium (Fig. 1, 2). Histologically, the olfactory lamellae are based on raphe and composed of two layers of olfactory epithelium separated by narrow central core which made up of loose connective tissue, nerve fibers and blood vessels (Fig. 3, 4). The sensory

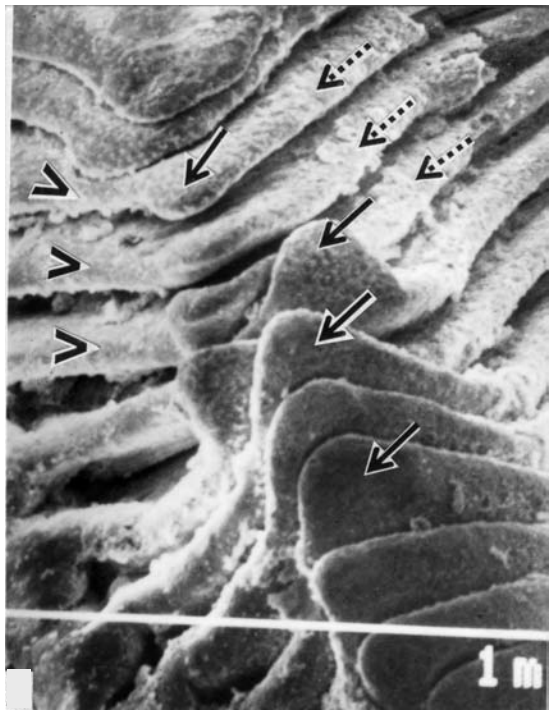

Fig. 2: Higher magnification of SEM view of olfactory lamellae showing receptor epithelium in the middle linguiform processes (Solid arrows) while the apical (arrow heads) and basal part (broken arrows) of lamellae are equipped with non-sensory epithelium (SEM)×200 X

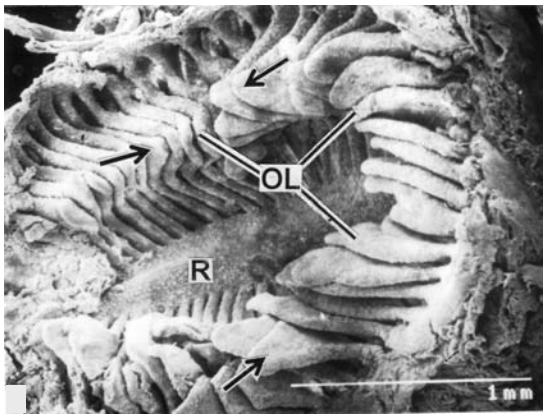

Fig. 1: Oval shaped olfactory rosette exhibiting olfactory lamellae (OL) radiating from median leaf like Raphe (R). Note linguiform processes (arrows) of lamellae (SEM)×50 X

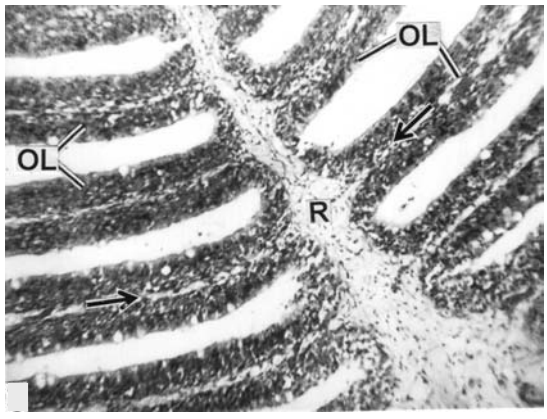

Fig. 3: Section of Olfactory Lamellae (OL) based on Raphe (R) showing olfactory epithelium which are separated by the narrow central core (arrows) (HE)×100 X

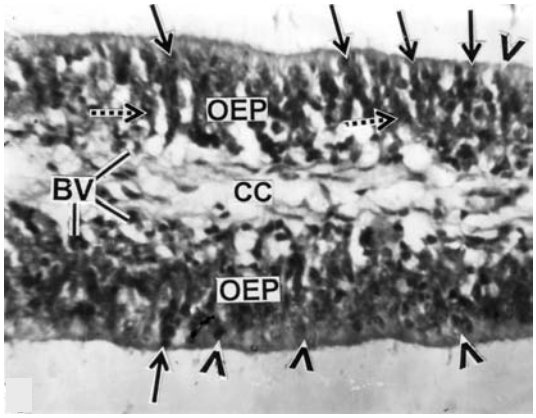

Fig. 4: Sensory Olfactory Epithelium (OEP) showing primary (solid arrows) and secondary receptor cells (broken arrows). Note the presence of supporting cells (arrow heads) in OEP and Blood Vessels (BV) in Central Core (CC) (HE)×400 X

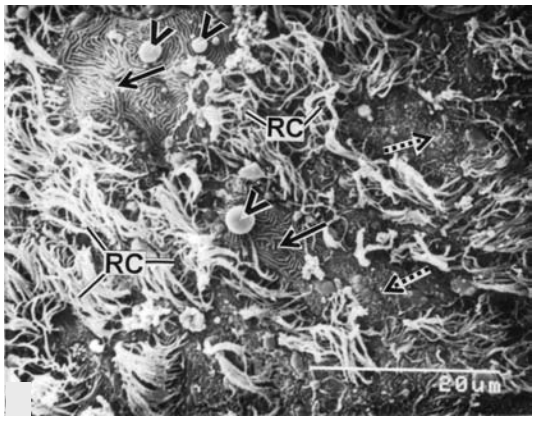

Fig. 6: Showing dendrite process of ciliated Receptor Cells (RC) and microvillous cells (broken arrows). Note the presence of mucin droplets (arrow heads) over the Stratified Epithelial Cells (SEC) (SEM)×4000 X

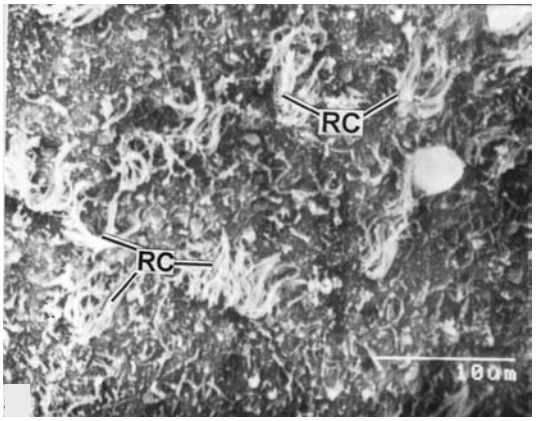

Fig. 5: Sensory epithelium showing dendrite process of ciliated Receptor Cells (RC), arranged in groups (SEM)×3500 X

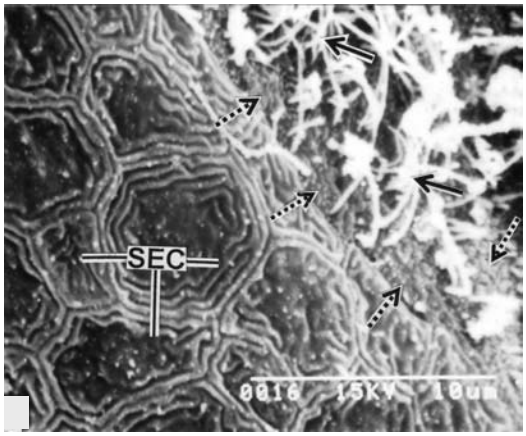

Fig. 7: Transitional zone of sensory and non-sensory olfactory epithelium showing ciliated receptor cells (solid arrows) interspersed with microvillous cells (broken arrows). Note the presence of Stratified Epithelial Cells (SEC) adjacent to receptor cells (SEM)×4500 X

olfactory epithelium is composed of a considerable number of primary and secondary receptor cells and supporting cells (Fig. 4). However, the dendrite process of each primary receptor cell extends up to the free epithelial surface. The dendrite of secondary receptor cells mainly present below the primary receptor cells (Fig. 4). Under SEM observation the apex of the linguiform area exhibits the ciliated dendrite process of receptor cells which are arranged in groups (Fig. 5). In some areas of the receptor epithelium few microvillous receptor cells are present in between ciliated receptor cells and are provided with inconspicuous microvilli. In addition some stratified epithelial cells are packed in between receptor cells (Fig. 6). In the transitional zone of sensory and non-sensory epithelium microvillous cells are discernible at the base of ciliated receptor cells which are also surrounded by stratified epithelial cells (Fig. 7).

Histologically, the surface zone of non-sensory epithelium is basically comprised of stratified epithelial cells with prominent nuclei and mucous cells (Fig. 8). According to the SEM study the non-sensory epithelium

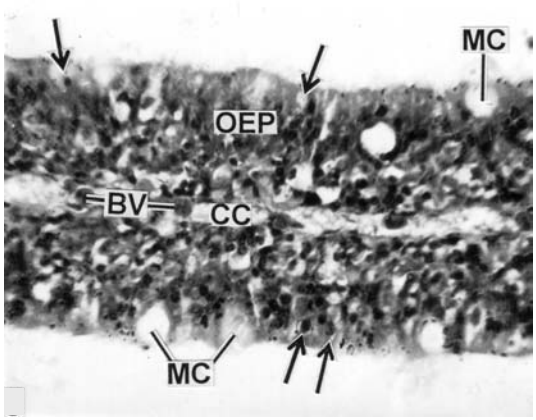

Fig. 8: Histological structure of non-sensory epithelium comprised of stratified epithelial cells (arrows) and Mucous Cells (MC). Note the presence of Blood Vessels (BV) in the Central Core (CC) (MT)×400 X

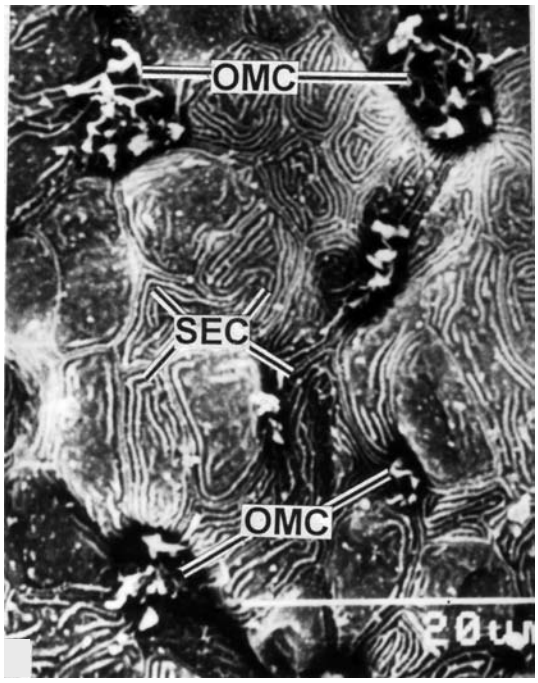

Fig. 9: Surface view of non-sensory epithelium showing Stratified Epithelial Cells (SEC) provided with unbranched microridges. Note the presence of Opening of Mucous Cells (OMC) with mucin mass in between SEC (SEM)×3500 X

is represented by stratified epithelial cells intercalated with the opening of mucous cells (Fig. 9). The apical surface of the stratified epithelial cells is provided with

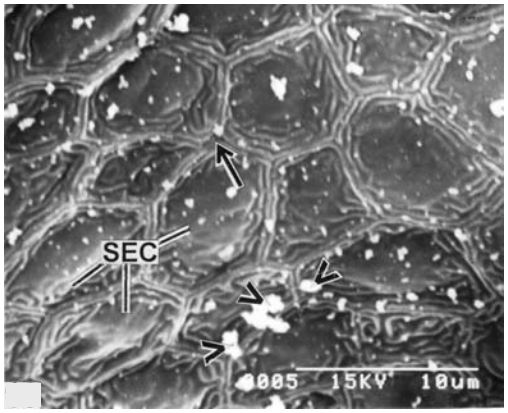

Fig. 10: Surface of raphe represented by compactly arranged Stratified Epithelial Cells (SEC) with inconspicuous microridges. Note the presence of opening of mucous cell (solid arrow) and secreted mucin droplets (arrow heads) over SEC (SEM)×4000 X

unbranched microridges arranged in a concentric whorl. Secreted mucin droplets are deposited in the opening of mucous cells (Fig. 9). The raphe is represented by compactly arranged stratified epithelial cells. The inconspicuous unbranched microridges on the apical surface of the epithelial cells are also arranged in a concentric whorl (Fig. 10).

DISCUSSION

The olfactory epithelium shows considerable diversity, reflecting the degree of development and ecological habitats (Zeiske *et al*., 1992). The present study reveals that the elongated olfactory rosette of *L. bata* consists of 26 to 28 olfactory lamellae arranged on either side of the leaf-like median raphe and can be classified under Bateson (1890) rosette type-3 or Burne (1909) rosette column I. According to Teichmann (1954), the oval type of olfactory organ falls under the category of eye-nose fish which means that this category of fish possesses similarly developed optic and olfactory faculties. The surface of the olfactory lamellae of *L. bata* can be distinguished into sensory and non-sensory regions. The sensory receptor epithelium is restricted in the middle apical tongue like portions of the lamellae while the apical and basal part of the lamellae is provided with non-sensory epithelium. This arrangement may be due to the fact that the tongue shaped area of sensory epithelium faces the flow of incoming water current and the receptor cells mobilizing different olfactory cues. Similar tongue like projections of the olfactory lamella have been

observed by Chakrabarti and Ghosh (2010a, b) in the olfactory epithelium of *Catla catla*. Zielinski and Hara (1988) and Hara and Zielinski (1989) also identified definite aggregations of ciliated receptor cells and confirmed their olfacto-sensory functions. Ojha and Kapoor (1973), Kumari (2008) remarked almost similar type of olfactory lamella in Indian major carp *Labeo rohita* and *Catla catla*.

In the present study, in *L. bata* the sensory epithelium mainly consists of two morphologically distinct types of receptor cells: ciliated and microvillous cells. They occur together but in different proportions. Zeiske *et al.* (2003) also observed that the ciliated and microvillous receptor cells also occur together in the olfactory organ of genus *Acipenser* but in different proportions in different species. The present study reveals that the ciliated receptor cells dominate over the microvillous receptor cells. The ciliated receptor cells are of special interest because they form a part of the olfactory transduction mechanism, are stimulated by odour bearing substances and also enable the fish to detect food. In the present observation, the ciliated receptor cells correspond to the type I cells of Yamamoto and Ueda (1978). In the present investigation in contrast to the ciliated receptor cells, the microvillous receptor cells have a slightly sunken apex and consist of min dendrites. This also conforms to the findings of Camacho *et al.* (2010) in the olfactory epithelium of sturgeon. The microvillous receptor cells might form a different olfactory transduction mechanism for pheromones or amino acids. Bhute and Baile (2007) also advocated that the microvillous receptor neurons perceive and process signals of pheromone which is an important step of breeding in *Labeo rohita*. On the other hand Bakhtin (1977) and Bannister (1965) reported that microvillous cells in the olfactory surface of *Squalus acanthias* and teleostean fishes are predecessors of ciliated receptor cells.

In the transitional zone of sensory and non-sensory epithelium few scattered ciliated receptor cells are responsible for better monitoring of the water quality even up to this zone. Furthermore, the non-sensory epithelium consists of stratified epithelial cells provided with unbranched linearly arranged microridges on their apical surface that help in holding mucus film over the epithelium and in protecting the sensory receptor cells from mechanical injury or from different hazardous substances. The mucous cells are distributed between the stratified epithelial cells of the non-sensory epithelium. The mucus covering the olfactory lamellae constitutes an important medium in which odorants are diffused. On the other hand the mucin probably helps in binding microscopic debris and keeps the sensory cells ready for new stimuli. This is in conformity with the findings of Rahmani and Khan (1980) in the olfactory mechanism of *Anabas testudineus*.

Labeo bata being a freshwater minor carp subsists on microscopic vegetable matter, algae, decaying organic matter etc. Therefore, the dense population of various receptor cells in the ovoid olfactory organ adapted accordingly in relation to food and feeding habit of the fish.

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