The Olfactory Mucosa of Butter Catfish *Clupisoma garua* (*Siluriformes, Ailiidae*): An Ultrastructural Study

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Abstract

Objective: The olfactory system is one of the most important chemosensory systems for teleosts. The olfactory epithelium of freshwater catfish *Clupisoma garua* (*Siluriformes*, *Ailiidae*) was studied by the aid of light microscopy and transmission electron microscopy. **Materials and Methods:** Mature sex-independent fish species were collected from Hooghly River, a distributary channel of river Ganga. Following routine procedure of ultrastructural techniques, different sensory and nonsensory cells lining the olfactory epithelium were described. **Results:** The lamella contained two epithelia, with a central core sandwiched in between. Based on distribution pattern and structural characteristics of cell, each olfactory lamella was differentiated into sensory and nonsensory epithelium. The smaller sensory epithelium contained morphologically distinct ciliated, microvillous, and rod receptor cells, distinguished based on staining patterns, characteristic length, and architecture of their dendritic terminals. The nonsensory epithelium comprising the greater surface area was made up of labyrinth cells, mast cells, supporting cells, ciliated nonsensory cells, and basal cells. The cilia furnished a characteristic 9 + 2 microtubule arrangement. **Conclusion:** The functional significance of various cells on the olfactory epithelium was correlated with behavior of the fish concerned.

Keywords: Cellular structure, chemosensory system, *Clupisoma garua,* olfactory epithelium, transmission electron microscopy

Introduction

The olfactory organs of teleosts are of majestic biological concern as serve for crucial role in kin recognition, mate selection, food finding, predator avoidance, homing, and other behavioral activities.[1] Survive in aquatic habitat, without light but abundant with dissolved compounds, the chemosensory organs of fish exhibit remarkable adaptations according to ecological habitats and taxonomic levels.[2] The olfactory organ is apparently sheathed by epithelium which performs a momentous role in chemoreception.[3] Olfaction of fish is concerned to study as the olfactory organ directly interacts with the surrounding aquatic environment and marks the external chemical stimuli in various ways. The olfactory system accords when odorants affix to molecular receptors located on olfactory receptor neurons within the olfactosensory epithelium.[4] Many reports are available on the fine structure of the olfactory epithelium in a number of fish species.[5-12] The gross morphology, topology, and cellular organization of olfactory organ variegate considerably among teleosts. The number of olfactory lamellae and the distribution of receptor cells on the epithelial surface mediate the sensory distinctness of the fish.[13] The disposition and texture of olfactory cells among different teleosts have been extensively characterized and reported diverse types of sensory receptor cells having precise sort of attentiveness for distinct chemical cues. The term olfaction implies the system devised by the bipolar sensory neurons of the olfactory mucosa.[14]

Structural organization of the olfactory system is necessary to annotation of the olfactory execution. *Clupisoma garua* (Hamilton, 1822), a bottom dweller river catfish, feeds mostly on insects, mollusks, small fishes, decaying, and algal matter.[15,16] Only a few studies are reported on surface morphology and histology of olfactory organ in schilbid catfish.[17,18] Considering the dearth of information, the present

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study is an attempt to describe the structural organization of the olfactory epithelium in Garua bacha with its way of life and living by transmission electron microscopy.

Materials and Methods

Sample collection

During January to August 2019, 10–12 adult specimens of *C. garua* (ranging 14.12–18.87 cm total length) were procured from Hooghly River at Kalyani surrounding areas (West Bengal, India) by hand nets. Fishes were anesthetized with 0.2% 2-phenoxyethanol following the guidelines of the institutional animal ethics committee.

Semithin sections and transmission electron microscopic preparation

Olfactory organs were carefully excised from freshly scarified fish. Small fragments of olfactory rosette were fixed in modified Karnovsky's solution (2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4) for 4 h at 4°C and postfixed in 1% osmium tetroxide in the same buffer for further 2 h at room temperature. After thoroughly washed in phosphate buffer, the samples were dehydrated in a graded series of acetone and embedded in Epon-Araldite following standard protocols. After resin polymerization, transverse semithin sections were cut at 1 μm thickness with a glass knife using ultracut microtome (Leica EM UC7) and stained with toluidine blue.[19] Staining sections were examined under a light microscopic observation and photographed under ZEISS Primo Star trinocular microscope with Tucsen 5.0 MP digital microscopy camera at different magnification.

Ultrathin sections (silver to gold) were cut and mounted onto 300 mesh copper grids for transmission electron microscope examination. Sections were stained with 1% aqueous uranyl acetate and lead citrate and examined using a Tecnai G2 20S-Twin transmission electron microscope (FEI, The Netherlands) operating at 200 kV at Sophisticated Analytical Instrumentation Facility Centre, All India Institute of Medical Sciences, New Delhi - 110 029.

Results

Lamellae radiating from raphe comprise sensory and nonsensory regions [Figure 1a and b]. The sensory part of epithelia is in distinct portion rather than monotonously localized over the olfactory lamella. The lamella is made up of two layers of epithelium encircling a delicate stromal sheet, central core which contains connective tissues with blood vessels and nerve fibers [Figures 1a-d and 2e]. A distinctive basement membrane segregates olfactory epithelium from the central core [Figures 1c and d and 2e]. The mucosa covering the raphe is typified with nonsensory cells [Figure 3e and f]. The receptor cells are morphologically differentiated into ciliated, microvillous, and rod type, distinguished on the basis of staining intensities of the perikaryon, characteristic

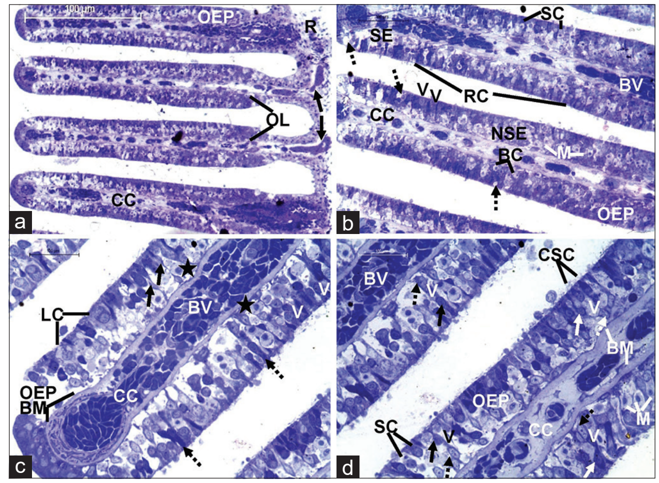

Figure 1: Photomicrographs of the semithin section of the olfactory lamella in *Clupisoma garua* stained with toluidine blue, (a) Section of olfactory lamellae (OL) radiated from raphe (R) showing olfactory epithelium and central core (CC) containing blood vessels (arrows) (×40), (b) Olfactory epithelium (OEP) furnishes sensory regions (SE) lined with receptor cells (RC) and nonsensory regions (NSE) typified with supporting cells (SC), labyrinth cells (arrowheads), mucous cells (broken arrows), mast cells (M), and basal cells (BC). Note the presence of blood vessels (BV) in central core (CC) (×100), (c) Magnifying olfactory epithelium (OEP) exhibits primary receptor cells (solid arrows), microvillous receptor cells (arrowheads), rod receptor cells (broken arrows), basal cells (asterisks), and labyrinth cell (LC). Note the presence basement membrane (BM), which distinguishes olfactory epithelium from central core (CC) containing large number of blood vessels (BV) (×1000), (d) Sensory olfactory epithelium shows synaptic contact (arrowheads) in between primary (solid arrows) and secondary receptor cells (broken arrows). Nonsensory olfactory epithelium contains mast cells (M), ciliated supporting (CSC), and nonciliated supporting cells (SC). Note presence of blood vessels (BV) in central core (CC), which is distinguished from olfactory epithelium by basement membrane (BM) (×1000)

length, apical morphology, and architecture of their dendrites. Olfactory cells are firmly equipped.

Light microscopy

The olfactory mucosa is characterized by morphologically distinct primary receptor cells, secondary receptor cells, microvillous receptor cells, rod receptor cells, and numerous nonsensory cells. Primary receptor cells are intensely stained characterized by their basophilic oval or elongated nuclei. They bear cylindrical dendrites which terminate at the free epithelial surface [Figure 1b-d]. Secondary receptor cells are located underneath the primary receptor cells, distinguished by their deeply stained extended nuclei and highly granular cytoplasm [Figure 1d]. The axons of scanty primary receptor cells show synaptic reference to the dendrite ends of the secondary receptor cells. Microvillous receptor cells are more exterior to epithelial lining, embossed with fine dendrons. They possess faintly stained round nuclei [Figure 1c]. Rod receptor cells are differentiated by their columnar appearance; contain thick extended dendrites towards the epithelial surface. These cells occurred in limited areas of sensory epithelium.

Nonciliated supporting cells contain distinct central nucleus and weakly stained cytoplasm [Figure 1b and d]. Ciliated supporting cells are more or less columnar in shape with a flat surface from which faintly visible cilia extend. Labyrinth cells are scattered randomly in the superficial layer of the olfactory epithelium [Figure 1d]. They are fairly larger, globular in appearance having conspicuous rounded nuclei [Figure 1b and c]. Mononuclear mast cells are oval-shaped having granular cytoplasm, distributed in the middle portion of epithelium [Figure 1b and d]. Small rounded basal cells with distinct central nuclei are scattered throughout the deepest layer of epithelium [Figure 1b and c]. Mucous cells are ovoid or circular in form and usually protrude at the free surface of the epithelium [Figure 1b].

Transmission electron microscopy

There are three identical types of receptor cells appearing in overlapping ordering in the sensory epithelium [Figure 2a]. The slender dendrite (3.9 ± 1.42 μm) of ciliated receptor cells is stretched arise from basal body and their width intensify progressively toward the nuclei [Figure 2b]. The cell body contains round to elongated nuclei and is located deep in the mucosa. The cytoplasm is more electrons condensed. Scanty receptor cells are identified without cilia, embossed with numerous microvillous projections (0.68 ± 0.31 μm) to the epithelial surface [Figure 2e and f]. The cell body is more outward in the mucosa in comparison to ciliated receptor cells and contains oval-shaped nucleus. The apical portion bears many adherent electron-lucent vesicles, and the heterochromatin matter remains dispersed throughout the nucleus [Figure 2f]. A characteristic rod cell is also present infrequently in the epithelium, which has a steady perch-like structure (1.02 ± 0.19 μm) on the epithelial lining [Figure 2d]. It comprises longitudinally arranged microtubules along long axis. Aggregations of mitochondria, scattered free ribosomes, centrioles, and electron-dense vesicles are encountered. A firmed junctional complex is formed with the neighboring supporting cells. The arrangement of microtubules in the ciliated and rod receptor cells exhibits the typical 9 + 2 arrangement in transverse section [Figure 2c].

Two types of supporting cells are identified: ciliated and nonciliated type [Figures 2a and 3a]. The nonciliated supporting cells are columnar in shape with round nucleus located at the lower portion of the cell [Figure 3b]. The ciliated supporting cells are dispensed in the surface of the mucosa, having broad flat apical surface characterized with number of cilia (0.5 ± 0.29 μm). These are emblematic kinocilia having 9 + 2 microtubular pattern. Vesicles with moderate electron density are observed in the apical cytoplasm, and mitochondria are scattered in the cytoplasm. Striated rootlets are found in the cell apex which gives rise to plenty of kinocilia. Labyrinth cells are globular in shape, packed with numerous mitochondria, and homogenous fine-grained particles [Figure 3a and b]. Basal cells are placed at the base of the mucosa just over the basement membrane [Figure 2e]. The cells are usually small with scanty cytoplasm, which contain various shaped large

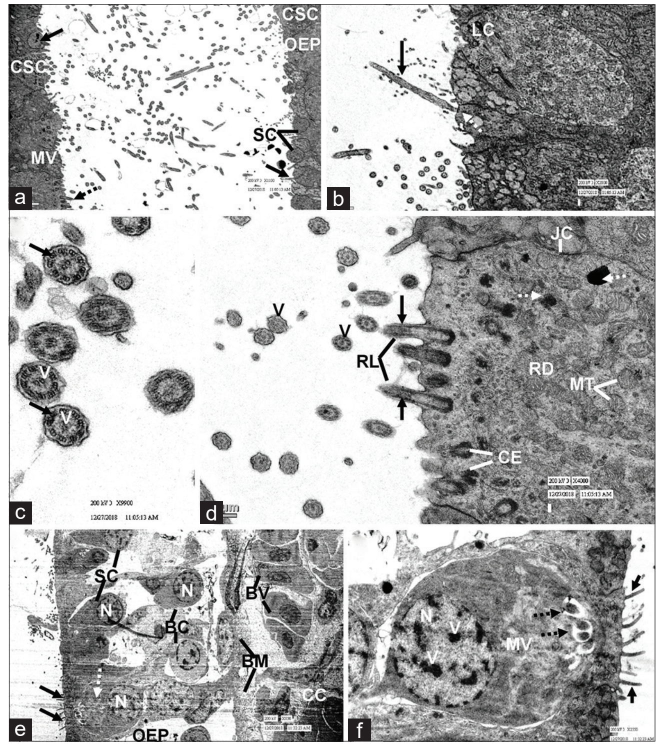

Figure 2: Photomicrographs of the olfactory epithelium of *Clupisoma garua* by transmission electron microscopy, (a) Olfactory epithelium (OEP) consists of ciliated receptor cells (solid arrows), microvillous receptor cells (MV), rod receptor cell (broken arrows), ciliated supporting cells (CSC), and nonciliated supporting cells (SC), (b) Dendrite of ciliated receptor cell (solid arrow) ending in basal body (broken arrow) and labyrinth cell (LC) packed with mitochondria, (c) Cross-section of cilia exhibits 9 pairs of outer microtubules (solid arrows) and 2 central ones (arrowheads), (d) Magnified view of rod cell (RD) shows parallel oriented microtubules (solid arrows) in rod-like cilia (RL), centrioles (CE), mitochondria (MT), and electron-dense vesicles (broken arrows). Arrowheads mark cross-section of rod and JC indicates firmed junctional complex, (e) Olfactory lamella distinguishes into olfactory epithelium (OEP) and central core (CC) by basement membrane (BM). Note the presence of basal cells, supporting cells (SC), and microvillous cell (broken arrow) having nuclei (N) in olfactory epithelium and blood vessels (BV) in central core (CC). Solid arrows mark the minute dendrons of microvillous, (f) Magnified MV embossed with numerous microvillous projections (solid arrows) illustrates electron-lucent vesicles (broken arrows) and nucleus (N) with dispersed heterochromatin matters (arrowheads)

nuclei [Figures 2e, 3a and c]. Mast cells have distinct nucleus, electron-lucent vesicles, rough endoplasmic reticulum, and roundish mitochondria all over the cell [Figure 3c]. Mucous cells are ovoid in appearance, confined in the nonsensory regions [Figure 3a], and packed with numerous large secretory vesicles. Due to tedious storing of secretory granules, nucleus together with other organelles lies at the floor of the cell [Figure 3d].

Raphe contains supporting cells bearing oval to elongated nuclei having fragmented heterochromatin in nucleoplasm and cytoplasm with rough endoplasmic reticulum, free ribosomes, mitochondria, and multitude of electron-dense granules [Figure 3e and f].

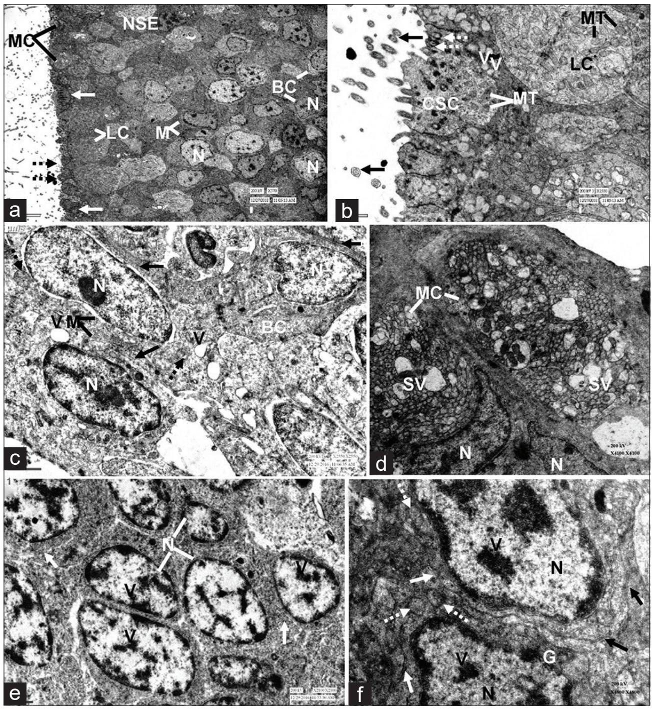

Figure 3: Photomicrographs of the olfactory epithelium of *Clupisoma garua* by transmission electron microscopy, (a) Nonsensory epithelium (NSE) typified with mucous cells (MC), labyrinth cells (LC), ciliated supporting cell (solid arrows), nonciliated supporting cells (broken arrows), mast cells (M), and basal cells (BC). N marks nuclei, (b) Nonsensory epithelium illustrates ciliated supporting cells (CSC), and labyrinth cells (LC) condensed with mitochondria (MT). Ciliated supporting cells show vesicles (arrowheads) and striated rootlets (broken arrows) and cross-section of cilia exhibits 9 + 2 doublets (solid arrows), (c) Mast cells (M) contain conspicuous nucleus (N), encircled by rough endoplasmic reticulum (solid arrows), mitochondria (broken arrows), and electron opaque vesicles (arrow heads). Basal cells (BC) contain lobular N, (d) Mucous cells consist (MC) of huge secretory vesicles (SV) and bottom most nuclei (N), (e) Raphe shows stratified epithelial cells provided with oval to elongated (N) having scattered heterochromatin materials (arrowheads) surrounded by endoplasmic reticulum (solid arrows), (f) Magnified stratified epithelial cells illustrate prominent nuclei (N) with dispersed heterochromatin (arrowheads). Note the presence of mitochondria (broken arrows), Golgi complex (G), and endoplasmic reticulum (solid arrows) adjacent to N

Discussion

The acute olfactory susceptibility of *C. garua* has been involved in different esteemed functions for exploring the surrounding environment in which they survive. Olfactory mucosa folded to conceive finger-like lamellae and optimally harmonized to furnish the large surface area regarding the potentiality of olfactory organ. The structural specialization and cellular composition of olfactory organ depends on the ecological niche inhabited by teleost species.[20] In *C. garua,* the organization of sensory and nonsensory regions is discontinuous in the olfactory epithelium classified as Type-II distribution pattern.[21]

The sensory epithelium is characterized with three types of morphologically distinct forms: ciliated receptor cells, microvillous receptor cells, and rod receptor cells for receiving olfactory sensation from aquatic environment. Existence of ciliated or microvillous receptor cells are common in the olfactory lining of fishes whereas the rod receptor cells occur in some teleosts..[6,22,23] Several sensory cells can be considered as different functional and structural entities with diverse sensitivities to external stimuli.[21] The ciliated receptor cells correspond to Type I cell,[24] whereas microvillous to those of Type II cells[25] and rod cells of those Type IV cells.[26] Receptor cells bearing odorant receptors expose the stimuli and relay the information to the olfactory bulb.[5] *C. garua*, a bottom dweller, the long and well-developed dendrite process of the ciliated receptor cell, enables the fish to smell and encounter the environmental stress. The attentive aspect of the present study is the detection of secondary neurons in addition to primary neurons and the presence of synaptic connections between these two types of neurons in the olfactory epithelium. The axons of the secondary neurons may extend into the central core of the lamellae, which suggests that the impulses received by the dendrite of primary receptor cells ultimately send impulses to the central core and carried over into the brain finally. Ojha and Kapoor[27] also found secondary neurons in the olfactory epithelium of *Labeo rohita*. Graziadei and Metcalf[28] postulated that new neurons replace the old and degenerating ones and establish fresh synaptic contact in the olfactory bulb. The microvillous receptor cells perceive and process signals of pheromone, which is an important step of breeding in *L. rohita*.[29] On the other hand, Bakhtin[30] and Bannister[31] stated that microvillous cells in the olfactory surface of *Squalus acanthias* and teleostean fishes are predecessors of ciliated receptor cells. Datta and Bandopadhyay[32] reported that rod cell is not a regular type of cell, and the formation of rod may be due to fusion of cilia of ciliated region. Hernadi[33] proposed that the occurrence of the rod-shaped olfactory neuron has been observed in the presence of a new physiological condition. The presence of neurotubules in dendrite of all receptor cells might have the main role for detecting of odorants.[34] Datta and Bandopadhyay[32] mentioned that the microtubules are probably responsible for maintaining the shape of the dendrite process and channelizing different transport materials to a particular site.

The enlarge part of the nonsensory epithelium typified with ciliated supporting cells has no sensory function, but they possibly help in mechanical dissociation. The movement of cilia assists in driving out the mucin mass poured out from the mucous cells.[35] The cilia drive streams of incoming water with dissolved chemicals between the olfactory lamellae and over the olfactosensory epithelium. The supporting cells embossed with kinocilia have to propel water and/or mucus over the lamella. Other than the receptor cilia, kinocilia have dynein arms, which furnish their motility.[36] Aggregation of mitochondria in the cell suggests that they are providing the needed energy for the beating of cilia. The nonciliated supporting cells have been suggested to perform several functions: secretory, absorbing, and glial.[5,24,33,37] The labyrinth cells' cell type is similar to chloride cells which probably are involved in electrolyte transport in fish gills and pseudobranch.[38] Mast cells in the olfactory mucosa are believed to play an important role in reproduction of *L. rohita*[29] and Baltic trout.[39] They can change metabolic activity of receptors and thereby the sensitivity of olfactory epithelium. The rough endoplasmic reticulum and mitochondria also suggest its functional state. The basal cells are conceited to be the progenitor cells of the receptor and supporting cells.[36] The occurrence of basal cells in the deeper part of the epithelium abets to sustain the mucosa during normal cell turn over or necrobiosis. Mucous cells discharge mucin which protects the epithelial surface from mechanical corrosion and helps in binding the microscopic particles to keep the sensory receptor cells ready for receiving new stimuli. Hornung and Mozell[40] advocated that the secretion assists in facilitating the odorant discharge.

Conclusion

The olfactory organs of *C. garua* are presumed to achieve functional adducing in olfaction to recognize various kinds of olfactory stimuli. The occurrence of different types of receptor cells on sensory epithelium is able to judge the chemical changes in the surrounding aquatic ecosystem. For better understanding the chemoreception related to ecological habit of organism, more experimental studies are to be suggested.

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Conflicts of interest

There are no conflicts of interest.

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