Studies on the morphology of the olfactory organ in the freshwater teleost, *Labeo bata* (Hamilton)

S.K. Ghosh and P. Chakrabarti*

Fisheries Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713 104, West Bengal, India. *e-mail: dr.pchakrabarti@yahoo.in

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Abstract - The structural components of the olfactory apparatus of *Labeo bata* (Hamilton, 1822) were studied morphologically as well as histologically. The oval shaped olfactory rosette was comprised of 24-26 lamellae of different sizes on each side of the median raphe. Histologically each lamella was composed of two layers of olfactory epithelium separated by narrow central core which made up of loose connective tissues, nerve fibres and blood vessels. The sensory olfactory epithelium contained principally three types of receptor cells: primary, secondary and microvillous cells. The non-sensory epithelium was typified with a series of mucous cells, stratified epithelial cells and mast cells. Basal cells were situated at the base of the epithelium, adjacent to the central core. Various cells on the olfactory epithelium were correlated with their functional consequence of fish concerned.

Keywords: Functional anatomy, histoarchitecture, olfactory organ, *Labeo bata*.

Introduction

The olfactory organ in fish is of special interest because it is essentially a chemoreceptor and plays a meaningful role not only in locating food but also provides the necessary information about the surrounding environment. Olfaction is a momentous sensory modality of teleosts. Chemoreception performs a major role in the lives of fishes such as feeding, prey detection, predator avoidance, species and sex recognition, parental behavior, migration etc. (Hara, 1992). Olfactory cues are determined by the olfactory apparatus and proper behaviours are exempted in any given organism (Mana and Kawamura, 2002). Fish can ascertain and judge the water soluble chemicals principally by stimulation of the receptor cells during water ventilation over the olfactory epithelium (Cox, 2008). The structure and function of the olfactory organ in fish have been investigated by many researchers using light microscope (Bandyopadhyay and Datta, 1996; Sawad et al., 2006; Ghosh and Chakrabarti, 2009; 2012a; Chakrabarti and Ghosh, 2013) and electron microscope (Hansen and Zeiske, 1998; Diaz et al., 2002; Bhute and Baile, 2007; Ma and Wang, 2010; Kuciel et al., 2011; Ghosh and Chakrabarti, 2012b; 2013). However, lacuna still remains on the distribution, organization and characterization of different cells lining the olfactory epithelium of teleost. Therefore, an effort has been made in the present study to describe morphological characteristics and more closely the functional aspects of various cells on the olfactory epithelium of freshwater minor carp, Labeo bata (Cypriniformes, Cyprinidae). It is a bottom dwelling herbivore species, feeds on microscopic vegetable matter, algae, decaving organic substances etc.

Materials and Methods

Live mature specimens of L. bata (16-18 cm in total length) were collected from the local freshwater fish farm of Burdwan, West Bengal. Fishes were sacrificed within a few second of capture by decapitation following the guidelines given by the Institutional Ethical Committee. The olfactory rosettes were carefully dissected out from the floor of the nasal cavity under a stereo microscope and gradually processed for histological and scanning electron microscopical analysis. For histological studies, the olfactory tissues were immersed in aqueous Bouin's fluid for 16-18h. After fixation the tissues were washed repeatedly in 70% ethanol and dehydrated properly through an ascending series of ethanol. The tissues were cleared with xylene and embedded in paraffin wax of 56-58°C under a thermostat vacuum paraffin-embedding bath for a period of 1h and 20min. Sections were cut at 4µm thickness using a rotary microtome. After routine histological procedure deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. Staining slides were observed and photographed under Olympus-Tokyo PM-6 compound microscope. For Scanning electron microscopy (SEM), the olfactory apparatus were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1M phosphate buffer-pH 7.4 for 20min. Intact olfactory rosettes were dissected out and rinsed with 0.1M phosphate buffer-pH 7.4. The samples were again fixed with 2.5% glutaraldehyde buffered with 0.1M phosphate buffer-pH 7.4 for 24h at 4°C. After primary fixation, the tissues were rinsed in the same buffer for 10 min and post fixed in 1% osmium tetroxide (OsO₄) in 0.1M phosphate buffer-pH 7.4 for 2h. The tissues were washed thoroughly in buffer and dehydrated through an ascending series of acetone followed by isoamyl acetate and critical point dried with liquid carbon-dioxide. Dried olfactory rosettes were mounted on metal stubs, coated with gold palladium and viewed under scanning electron microscope. Hitachi S-530.

Results

In L. bata, the paired olfactory organs are situated on the antero-dorsal aspects of the head, anterior to the eves in the depression of olfactory pits (Fig. 1). The olfactory chamber is communicated with the surrounding environment through two separate nasal apertures. Opening of nasal pit is separated by outwardly directed median nasal flap of skin forming anterior inlet and posterior outlet of the chamber. The openings lie close to each other (Fig. 1). The anterior opening is almost circular and remains wide open while the posterior outlet is of crescent shaped. Olfactory system comprises of olfactory rosette, olfactory bulb, olfactory nerve and olfactory lobe of forebrain (Fig. 2). The narrow extended olfactory tracts connect the olfactory bulbs and olfactory lobes of the forebrain. Under SEM observations, the olfactory rosette of L. bata is approximately oval in outline and composed of 24 to 26 primary lamellae radiating from both side of the slender median raphe (Fig. 3). The olfactory rosette holds the whole cavity of the olfactory chamber with convex ventral and concave dorsal surface. The lamellae are nearly quadrilateral in shape, adhered to the wall of the olfactory chamber by their inner ventral margins and to the raphe by their proximal terminate (Fig. 3). The lamellae in the middle region of the

olfactory rosette are abundant and their sizes gradually reduce towards both the anterior and posterior regions. The outer dorsal portions of lamellae bear well developed linguiform processes (Fig. 3). The sensory epithelium occupies a smaller area in the linguiform process whereas the broad Lateral surface of the olfactory lamella is disguised with non-sensory epithelium.

Histologically, the olfactory lamellae are arranged on raphe and made up of two layers of olfactory epithelium distinguished by narrow central lamellar space, the central core (Fig. 4). A well developed basement membrane is usually distinguishable which separates olfactory epithelium from the central core region (Fig. 8). The central core is filled with connective tissue stroma, bundle of nerve fibres (Fig. 7) and blood vessels (Fig. 5). The olfactory epithelium is divided into sensory region with primary, secondary and microvillous receptor cells and non-sensory region having large number of mucous cells, stratified epithelial cells and mast cells. Basal cells are scattered, lying in the deeper part of the olfactory epithelium above the basement membrane.

Primary neurons or receptor cells: Receptor cells are the sensory elements of the olfactory epithelium, supported with stratified epithelial cells (Fig. 5). Each cell is characterized with a cell body and long dendrite, stretched as a circumscribed process up to the epithelial surface (Figs. 6 and 7). The cell body contains deeply stained nucleus, situates deep in the epithelium. The axonal ends of primary neurons synapse with the dendrite tips of secondary neurons (Fig. 7).

Secondary neurons: The secondary neurons are mainly located below the primary neurons and marked by their elongated nuclei (Figs. 6 and 7). These cells are strongly basophilic. The axons of the secondary neurons run to the deeper region of the olfactory epithelium and pass into the central core of the lamella (Fig. 7).

Microvillous cells: Microvillous cells are placed more superficial layer in the epithelium, provided with faintly stained minute nuclei (Figs. 5, 6 and 7). They possess very short dendrites on the apical rim of the cell.

Mucous cells: Mucous cells are oval in shape, distributed in considerable abundance at the free margin of the olfactory epithelium mainly in the non-sensory area. The mature mucous cell exhibits a globular structure due to homogenous mucin and lies in the superficial layer (Figs. 4, 5 and 8).

Stratified epithelial cells: Stratified epithelial cells are distributed among the receptor cells of the surface epithelium (Figs. 5 and 6). The breadth of the cell decreases in proximal region and the conspicuous basophilic nucleus is situated distally. Cytoplasm is less granular and lightly stained (Fig. 6). These cells give the basic structure of the olfactory lamella.

Mast cells: Mast cell is small in size, more rounded with smaller aggregate of cytoplasm and has a polymorphous nucleus (Fig. 8).

Basal cells: Basal cells are few and lied above to the basement membrane, adjacent to the central core (Fig. 8). They are oval or pear shaped, possess distinct round nuclei and chromophobic cytoplasm (Figs. 6 and 8). Cytoplasmic prolongations from these cells do not extend up to the free epithelial surface. These cells actually form the reservoir for the formation of stratified epithelial and receptor cells as they migrate towards the upper part of the olfactory epithelium.



Figure 1. lateral view of head showing an anterior inlet (solid arrow) and posterior outlet (broken arrow) separated by nasal flap (arrow head).



Figure 2. Dissected head showing olfactory rosette (OR), olfactory bulb (OB), olfactory nerve (ON) and olfactory lobe (L) attached with forebrain (B).

Morphology of the Olfactory organ in Labeo bata



Figure 3. Oval shaped olfactory rosette showing olfactory lamellae (OL) radiating from narrow raphe (R) by scanning electron microscopy. Note linguiform processes (arrows) of lamellae; $SEM \times 50$.



Figure 4. Olfactory lamellae (OL) based on raphe (R) showing olfactory epithelium (OEP) separated by the narrow central core (CC). Note the presence of receptor cells (RC) (solid arrows) and mucous cells (MC) (arrow heads) at the apical border of OEP; $MT \times 100$.



Figure 5. Sensory OEP provided with a number of elongated receptor cells (solid arrows) and scattered microvillous cell (MV) (arrow head). The non-sensory OEP comprised of a series of MC and stratified epithelial cells (SEC) (broken arrows). Note the presence of blood vessels (BV) in CC; HE \times 400.



Figure 6. OEP showing primary RC (solid arrows), secondary RC (broken arrows), microvillous cells (arrow heads) and SEC. Note the presence of basal cell (BC) adjacent to CC; $HE \times 400$.



Figure 7. Higher magnification of sensory OEP showing primary RC, secondary RC (broken arrows) and MV (arrow head). Solid arrows indicate the contact in between primary and secondary RC. Note prominent nerve fibres (N) in CC; HE × 1000.



Figure 8. OEP lined with RC (solid arrows), mast cells (arrow heads), MC and BC. Note the presence of basement membrane (BM) which separates CC from OEP; $MT \times 1000$.

Discussion

The olfactory organ of fish is lodged at the ethmoid region of the head and composed of many folds to form lamellae (Hara, 1975). The multilamellar olfactory organs of fish have an acute sense of smell in various aspects of life history and shows considerable diversity which reflects the degree of development and ecological habitats (Zeiske et al., 1992). Modification of the olfactory system may occur through adaptation to a specific environment. In L. bata two nasal openings are located close to one another, detached by outwardly directed nasal flap. Water perforates into the anterior nasal opening and passes out through the posterior outlet throughout onward movement of fish. The nasal flap is responsible for determine the water impinging on it, passing through the anterior inlet and for impeding it from gaining access into the posterior outlet (Oiha and Kappor, 1973). Kumari (2008) also observed similar olfactory mechanism in *Catla catla*. According to Burne (1909) crescent shaped posterior outlet is highly specific for carp seems to be too sweeping. The oval shaped olfactory rosette of L. bata consists of 24 to 26 lamellae arranged on both side of the median raphe which assumes Bateson's (1889) rosette type 3 and Burne's (1909) rosette column I. According to Teichmann (1954), the oval shaped olfactory organ descends under the category of "eye-nose" fish, which means that this type of fish bear similarly developed optic and olfactory sense. The predominantly developed linguiform processes of the olfactory lamellae in L. bata facilitate the water flow across the olfactory rosette. Water entering the anterior inlet is conducted directly over the central part of the rosette from where it passes to the interlamellar spaces. This corresponds to the findings of Ojha and Kapoor (1973) in the olfactory apparatus of Labeo rohita. The distribution of the sensory and non-sensory epithelia on the surface of the lamellae shows a great variety in different teleosts for adaptation to a specific environment (Yamamoto, 1982). In L. bata the sensory epithelium is restricted in the linguiform process whereas the broad lateral surface of the olfactory lamella is covered with nonsensory epithelium. This unique feature may be due to the fact that the sensory epithelium of linguiform process faces the flow of incoming water current and the receptor cells mobilizing different olfactory cues. Similar projections of the olfactory lamellae have been observed by Chakrabarti and Ghosh (2010) in the olfactory epithelium of Catla catla. The ecological niche inhabited by a given species has an immense impact on celluar organization of the olfactory mucosa (Hara, 1994). In L. bata the sensory olfactory epithelium contains three types of receptor cells: primary, secondary and microvillous cells whereas the non-sensory epithelium is characterized with mucous cells, stratified epithelial cells and mast cells. The dendrite of receptor cells runs to the surface of the lamella as a slender process are of unique attention as they form a part of the olfactory transduction mechanism and are stimulated by odour-bearing substances. Buck and Axel (1991) reported that the olfactory processing commences at the apical tip of receptor cells. Hino et al. (2009) postulated that fish could judge and detect the water soluble chemicals through the sensory receptor cells during water ventilation over the olfactory mucosa. The most interesting characteristic of the present investigation is the histological

170

identification 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 synaptic connection between the primary and secondary receptor cells may extend from the epithelial surface to the central core. Chakrabarti and Hazra Chowdhury (2008) also observed secondary neurons in the olfactory epithelium of *Cuprinus carpio*. In the present investigation in contrast to the primary and secondary neurons, the microvillous receptor cells consist of minute dendrites. This also conforms to the observation 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. Zippel et al. (1997) suggested that microvillous cells in the olfactory epithelium of gold fish mediate responses to pheromones. Bhute and Baile (2007) also advocated that the microvillous cells perceive and process signals of pheromone, which is a significant proceeding of reproduction in Labeo rohita. On contrary, Bakhtin (1977) and Bannister (1965) reported that microvillous cells in the olfactory epithelial surface of Squalus acanthias and teleostean fishes are forerunners of ciliated receptor cells. Mucous cells of varying magnitude with secretory activity are profusely present in the olfactory epithelium of L. bata. The secreted mucin from the mucous cells may be help in the smooth flow of water circulation in the olfactory chamber by binding microscopic debris which is ejected out through the posterior nostril to keep free the sensory receptor cells. This is special adaptive feature of this bottom dwelling teleost. This is in compliance with the findings of Rahaman and Khan (1980) in the olfactory epithelium of Anabas testudineus and Bandvopadhvav and Data (1996) in the olfactory organ of Heteropneustes fossilis. The terminal mucus film may also act as an ion trap, which obstructs the invading of heavy metals into underneath organs (Banerjee, 1993). The stratified epithelial cells may give mechanical support to other sensory cells in the olfactory epithelium. Moulton and Beidler (1967) reported that the stratified epithelial cell has complex secretory and nutritional functions. Mast cells in the olfactory mucosa are believed to play an important role in reproduction of Rhesus monkey (Saini and Breipohl, 1977). Baltic trout (Bertmer, 1982) and Indian major carp. Labeo rohita (Bhute and Baile, 2007). Mast cells also can change metabolic activity of neurons and thereby the sensitivity of olfactory epithelium (Doving et al., 1980). The basal cells are located adjacent to the central core and having no cytoplasmic processes reaching to the free epithelial surface. These cells can function as stem cells for regeneration of lost or damaged receptor cells and stratified epithelial cells throughout the life (Yamamoto, 1982; Zeiske et al., 1992). Using tritiated thymidine followed by autoradiography, Thornhill (1970) and Graziadei and Metcalf (1971) have shown that the basal cells, apart from differentiating into stratified epithelial cells, also give rise to the olfactory receptor cells, which are continually replaced during life.

Conclusion

The olfactory rosette of *L. bata* consists of a series of lamellae due to their bottom dwelling habit and possesses well developed olfactory sense.

The process of olfactory sensations is elicited by the contact of odour particles with the receptor cells, plays an indispensible role in its feeding and other activities.

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172

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دراسة مظهرية للعضو السمعي لسمكة المياه العذبة Labeo bata (Hamilton)

أس. كي. غوش و بي. جاكرابارتي مختبر الثروة السمكية, قسم علم الحيوان, جامعة بوردوان, بوردوان 104 713, غرب البنغال, الهند

المستخلص – جرت دراسة التركيب المظهري والنسيجي للجهاز السمعي لسمكة Labeo bata وأتضح أن الشكل البيضوي للزهرة السمعية يتكون من 24 - 26 صفيحة دقيقة مختلفة الاحجام وتقع على جانبي الخط الوسطي. نسيجياً، تتكون كل صفيحة من طبقتين من النسيج الطلائي السمعي يفصلهما طبقة وسطية من نسيج رابط رخو وألياف عصبية وشعيرات دموية. النسيج الطلائي السمعي الحسي يحتوي على ثلاثة أنواع من الخلايا المستقبلة وهي الأولية والثانوية والمايكروفيلوس. أما النسيج الطلائي السمعي غير الحسي فيتكون من سلسلة من الخلايا المخاطية ونسيج طلائي طبقي مع خلايا سارية. الخلايا القاعدية توجد عند أسفل النسيج الطلائي بجوار الطبقة الوسطية. جرى ربط مختلف الخلايا السمعية بالوظيفة التي تقوم بها.