Mesopot. J. Mar. Sci., 2016, 31(1): 15 - 28

Morphological and histochemical studies on the olfactory rosette of bagrid catfish, *Rita kuturnee* (Sykes, 1839)

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(Received: 25 June 2015 - Accepted: 15 March 2016)

Abstract - In the present study, the functional anatomy, histological features and histochemical localization of two enzymes *viz*., alkaline phosphatase (ALPase) and adenosine triphosphatase (ATPase) of the olfactory epithelium in *Rita kuturnee* were described. The structural organization of the olfactory epithelium was studied by using the histological and histochemical techniques. The morpho-anatomical study revealed that the paired olfactory chambers were situated at the dorsal-lateral sides of the snout, which was communicated outside by an anterior and posterior nasal openings. The elongated olfactory rosette lied at the bottom of chamber and supported with 54-56 lamellae on either side of the central narrow raphe. The histological analysis characterized the olfactory epithelium of lamellae which was made up of receptor, flagellated supporting, non-flagellated supporting, labyrinth, mucous and basal cells. Intense alkaline phosphatase and adenosine triphosphatase activity were evidenced in the receptor cells, supporting cells, basal cells and also in blood cells of the central core. Various cells lining the olfactory epithelium were correlated with the functional significance of the fish was concerned.

Keywords: *Rita kuturnee*, Olfactory organ, Anatomy, Histology, Histochemistry.

Introduction

Olfaction is one of the momentous chemosensory systems for fish and its functions are implicated in the procurement of food, detection of prey, recognition of sex, defense against predators, parental behavior and migration (Sinha and Sinha, 1990). The olfaction of fish was usually interrelated with the water ventilation by sniffing process (Nevitt, 1991). In teleost, water with chemicals enters into the olfactory cavity through nares; resulting the sensory receptors lining the olfactory mucosa forthwith contact to water contaminants. The receptor cells of the olfactory epithelium were stimulated when they come into contact with certain chemicals carried in water and transmit signals to the nervous system (Lara, 2008). The olfactory organs of the fish an exhibits extensive array of diversification relying upon systematic groups and ecological adaptations (Zeiske *et al*., 2009). A number of researchers have described the morpho-histological and cytoarchitectural pattern of various cells on the olfactory epithelium by using light microscope (Goel, 1978; Hara and Zielinski, 1989; Bandyopadhyay and Datta, 1996; Chakrabarti, 2005; Chakrabarti and Hazra Chowdhury, 2008; Ghosh and Chakrabarti, 2009; 2013; Patle and Baile, 2014a). However, very little information was available on neuroanatomy and enzyme histochemistry of the olfactory epithelium in teleost.

Therefore, it would be naturally worthwhile to elucidate the structural characterization and localization of alkaline phosphatase and adenosine triphosphatase in various cell types of olfactory epithelium in riverine carnivorous catfish, *Rita kuturnee* (Siluriformes, Bagridae) which feeds on molluscs, small fishes, insects, crustacea, worms..... etc.

Materials and Methods

Adult specimens of *R. kuturnee* (20 to 22 cm in total length) were procured from the river Ganga near Ambika Kalna, Burdwan district of West Bengal. Fishes were deeply anesthetized with an aqueous solution of tricaine methone-sulphonate (0.1% MS 222, Sigma Aldrich) and sacrificed following the guidelines given by the Institutional Ethical Committee. Intact olfactory organs were attentively dissected out from the olfactory chamber and further processed for respective studies.

Histological preparation:

The olfactory tissues were kept in aqueous Bouin's fixative for 16-18 h. After that the tissues were washed thoroughly with 70% ethanol, dehydrated with graded series of ethanol and cleared in xylene. Then the tissues were infiltrated in paraffin wax of 56-58°C under a thermostat vacuum paraffin-embedding bath for a period of 1 hr. Serial paraffin sections were cut at 4 μm thickness using a rotary microtome (Weswox). After routine histological process deparaffinized sections were stained with Delafield's Haematoxylin-Eosin (HE), Mallory's triple (MT) (Mallory, 1936) and Silver stain by Silver Impregnation Method (SIM) (Marsland *et al.*, 1954). After that the staining slides were mounted with DPX, observed and photographed under Olympus-Tokyo PM-6 compound microscope.

Scanning electron microscopical (SEM) preparation:

After dissecting the olfactory chamber the olfactory rosettes were immersed *in vivo* with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 20 min. Then the olfactory rosettes were carefully dissected, rinsed in 0.1 M phosphate buffer (pH 7.4), fixed with 2.5% glutaraldehyde for 24 hrs at 4°C. and post fixed with 1% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer (pH 7.4) for 2 hrs. Fixed tissues were washed repeatedly in buffer and dehydrated in ascending series of acetone followed by isoamyl acetate. The tissues were dried with critical point drier (Hitachi 8CP2), mounted on metal stubs, coated with gold palladium (20 nm thick) and observed under scanning electron microscope (SEM), (Hitachi S-530).

Enzyme histochemical preparation:

The olfactory organs were immediately fixed in cold absolute acetone for 16 hrs at 4 °C. The tissues were dehydrated in absolute acetone, cleared in benzene and embedded in paraffin wax of 52-54°C in vacuum medium for 45 min. Serial paraffin sections were cut at 8 μm thickness by following the Calcium-Cobalt method for detection of Alkaline Phosphatase (ALPase) activity (Gomori, 1951), Calcium method was applicated for the detection of Adenosine Triphosphatase (ATPase) activity (Padykula and Herman, 1955). Staining slides were viewed and photographed under the Olympus-Tokyo PM-6 compound microscope.

Results

Morpho-anatomy:

R. kuturnee possessed a pair of nasal pits or olfactory chambers located on dorsal-lateral sides of the snout, in front of the eyes (Fig. 1). Each olfactory chamber was communicated with the surroundings environment through the separate nasal apertures and opens to the exterior by two nostrils: an in-current (anterior) and an out-current (posterior) nostril. The two openings were situated at a distance from one another. The inlet and outlet were segregated widely by a nasal bridge of epidermis (Fig. 1). In *R. kuturnee*, the olfactory organ was lodged in the ethmoid region of the head at the bottom of olfactory chamber. Olfactory rosettes along with protuberant olfactory bulbs were conjoined to the forebrain by long olfactory nerves (Fig. 2).

Scanning electron microscopy (SEM) analysis:

The olfactory rosette was an elongated texture with a convex ventral and a concave dorsal surface, occupies most of the olfactory chamber (Fig. 3). A lengthy incapacious median raphe extended along the long axis of the rosette from its preceding to the succeeding end. The rosette was framed with 54-56 thin lamellae on each side radiated outwards from central raphe and exhibited a feather like style. The lamellae were parallel oriented on either side of the raphe. The magnitude of lamellae was differed in relation to their place in rosette. The lamellae were large in middle portion whereas their sizes gradually reduced towards both ends of the olfactory rosette. These were adhered to the wall of the olfactory chamber by their inner ventral edges and to the raphe by their proximal terminates. Olfactory lamellae were distinguished into sensory and non-sensory epithelium. The dorsal edges of the lamellae were characterized with critically grow flat erection covered with sensory epithelium at their distal ends (Fig. 3).

Histology:

The olfactory rosette of *R. kuturnee* was consisted of a series of olfactory lamellae emitted from raphe (Fig. 4). Each lamella was comprised of two layers of olfactory epithelium separated by broad central lamellar space, central core contained blood vessels, connective tissue fibres and nerve fibres (Fig. 5). The lamellae were covered with mixed sensory and non-sensory epithelium. The olfactory epithelium contained of the following types of cells.

Receptor cells were present in between the supporting cells and characterized by darkly stained oval or elongated nuclei situated deep in the epithelium (Figs. 5 & 7). Intense reaction of silver stain was observed in the sensory epithelium due to dense aggregation of receptor cells. However, the reaction was more pronounced in the knob like structure and dendrite process of the receptor cells (Fig. 6). The dendrite of each receptor cells extended as narrow and cylindrical process up to the epithelial surface and sometime few receptor cells form a swelling which protrudes above the epithelial surface (Fig. 8). Two types of supporting cells could be distinguished in the most superficial layer of the olfactory epithelium (Fig. 5). These were columnar flagellated and rounded non-flagellated supporting cells. The first type had a large oval nucleus with a clear chromatin material.

The distal limb of the cell was broad and its tip supported by stubby flagella (Figs. 7 & 8).

The second type of supporting cells were lightly stained, possesses a comparatively broader distal limb and a spherical nucleus (Fig. 7). The oval shaped labyrinth cells were dispersed in the epithelial surface with conspicuous nuclei towards the basal portions (Figs. 5, 7 & 8). Mucous cells of both secretory and non-secretory were few in number and scattered in between the supporting cells in the superior layer of the olfactory epithelium. They were round or ovoid in outline with faintly stained nuclei towards the basal ends (Figs. 5 & 7). Basal cells were grouped and located in the deeper part of the epithelium and smaller in size in comparison to other cell types. They were almost round in shape having centrally placed prominent nuclei (Fig. 7).

Detection of alkaline phosphatase (ALPase):

Higher amount of ALPase activity was found to be associated with the receptor cells, including dendrite processes and flagellated supporting cells (Fig. 9). Positive ALPase activity was also noticed in the nuclei of non-flagellated supporting cells and basal cells. Moderate localization of this enzyme was also observed in the blood vessels of the central core (Fig. 9).

Detection of Adenosine triphosphatase (ATPase):

High ATPase activity was discernible in the receptor cells and the upper part of flagellated supporting cells in *R. kuturnee* (Fig. 10). Maximum enzyme activity was associated with the border of olfactory epithelium. The basal cells in deeper region showed intense ATPase activity around the large nuclei. The nerve fibres and blood vessels in the central core also showed positive ATPase activity (Fig. 10).

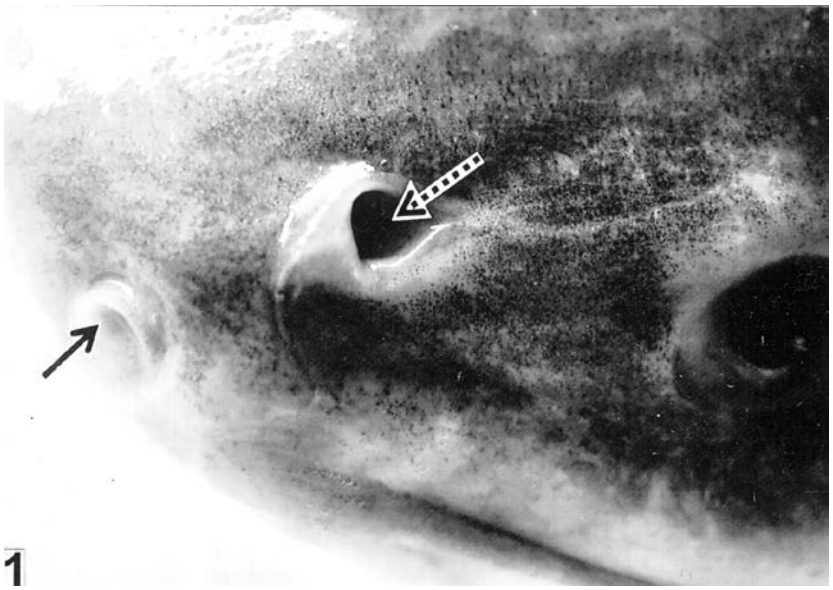

Figure 1. Dorsolateral view of head showing the anterior nostril (solid arrow) and posterior nostril (broken arrow) with wide gap.

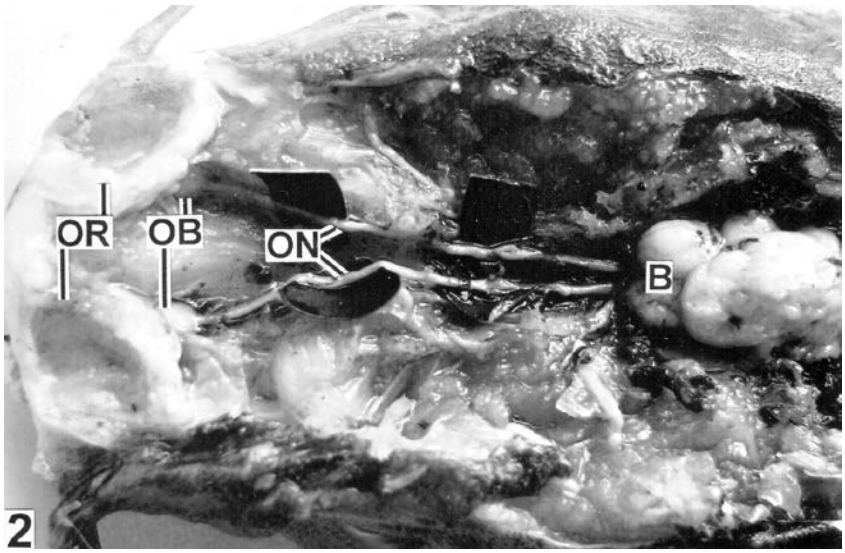

Figure 2. Dissected portion of head showing the connection of forebrain (B) with the olfactory rosettes (OR). OB represents olfactory bulb and ON indicates olfactory nerve.

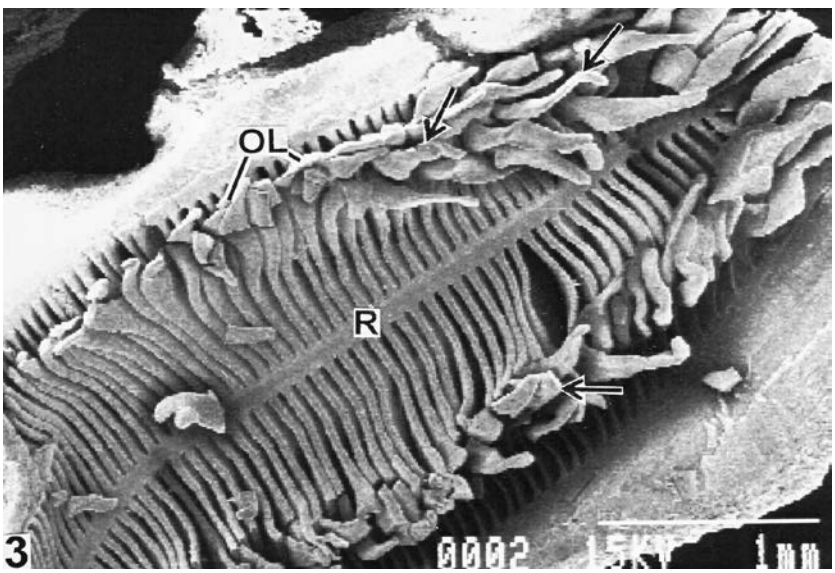

Figure 3. Elongated olfactory rosette with a series of olfactory lamellae (OL) radiating from central median raphe (R). Arrows mark the flat apical sensory region of OL by Scanning electron microscopy; SEM × 40.

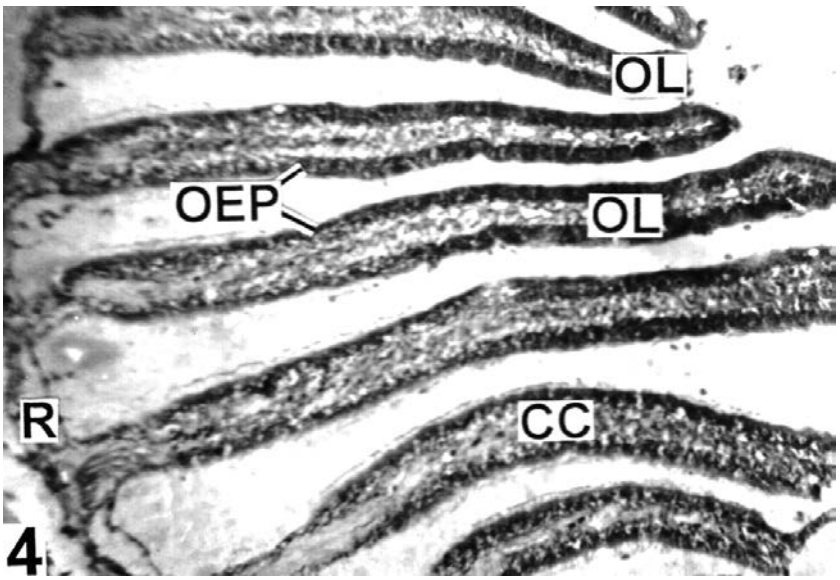

Figure 4. Slender olfactory lamellae (OL) attached with raphe (R) showing thin olfactory epithelium (OEP) separated by broad central core (CC); HE × 100.

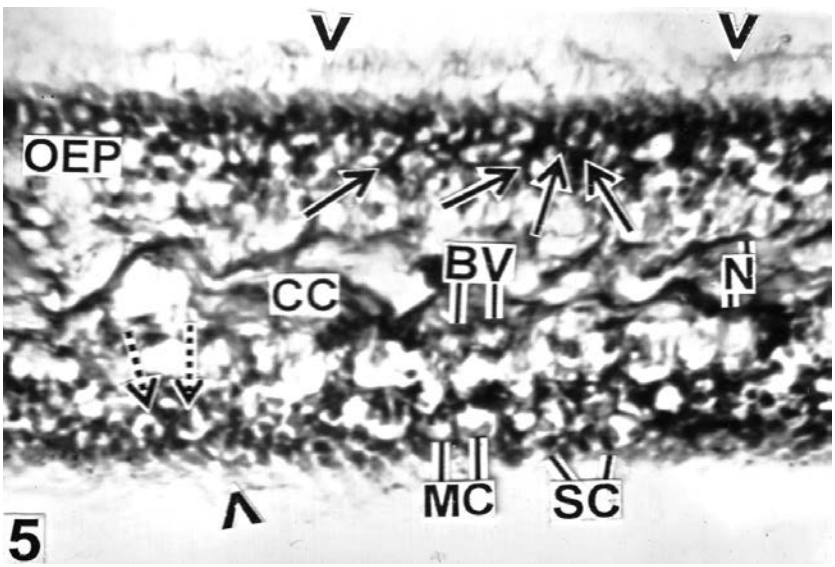

Figure 5. OEP showing flagellated supporting cells (arrow heads), stubby receptor cells (RC) (solid arrows), labyrinth cells (L) (broken arrows) and mucous cells (MC). SC indicates non-flagellated supporting cells. Note CC with nerve fibers (N) and blood vessels (BV); MT × 400.

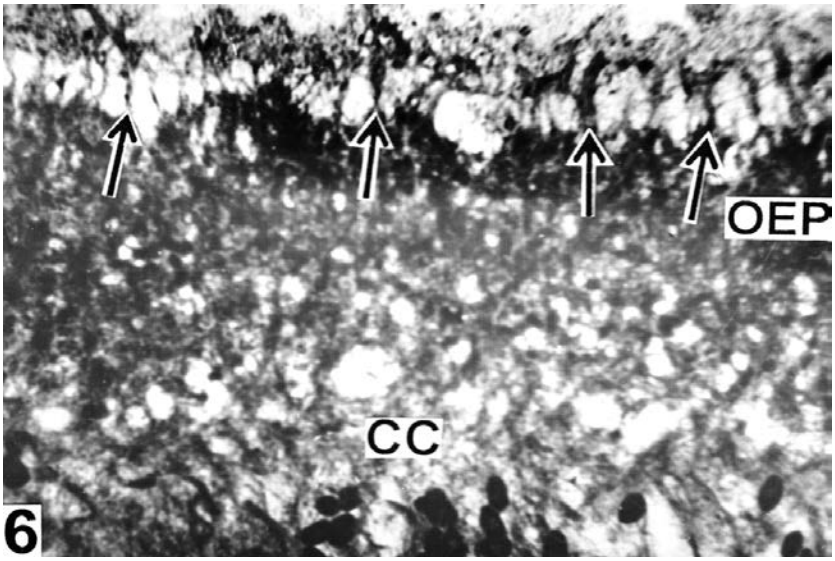

Figure 6. Sensory OEP shows intense silver reaction in the dendrite processes (arrows) of RC. CC points out central core; SIM × 400.

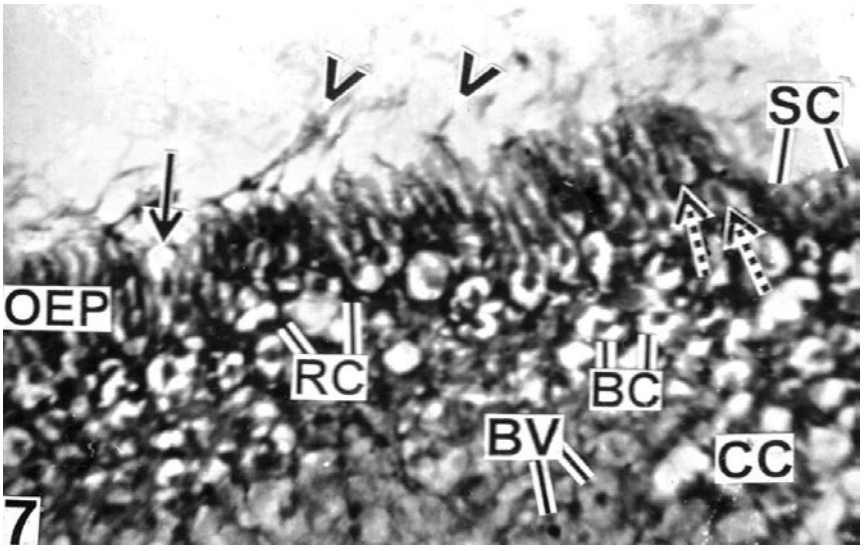

Figure 7. OEP typified with flagellated supporting cells (arrow heads), RC, MC (solid arrow), L (broken arrows) and group of basal cells (BC). Note the presence of non-flagellated supporting cells (SC) and CC with BV; HE × 600.

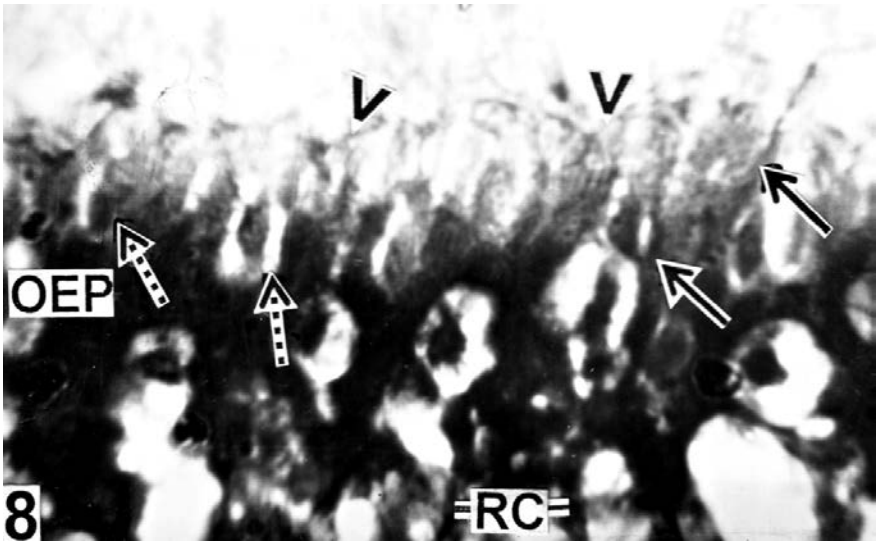

Figure 8. Higher magnification of OEP lined with flagellated SC (arrow heads), L (broken arrows) and RC with narrow cylindrical processes (solid arrows); MT × 1000.

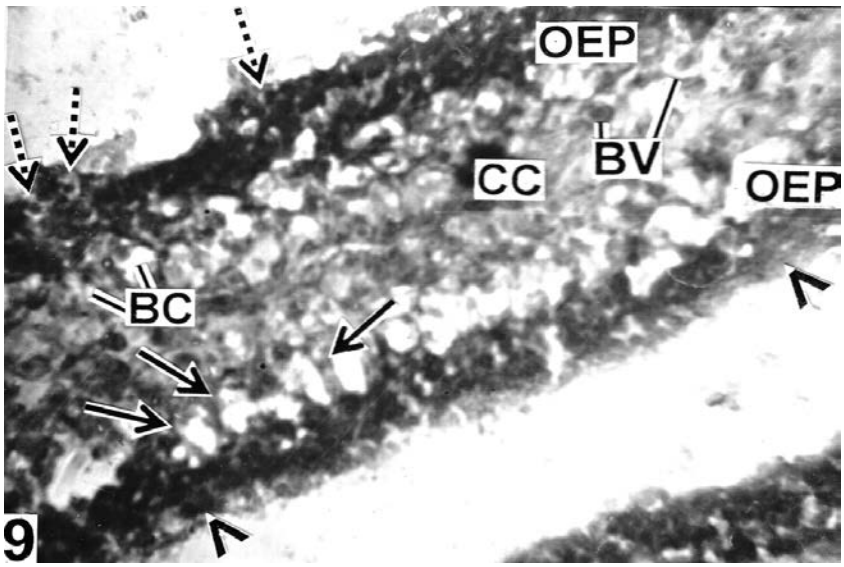

Figure 9. Displaying the localization of ALPase in receptor cells (RC) (solid arrows), flagellated supporting cells (arrow heads), non-flagellated supporting cells (broken arrows) and basal cells (BC) of olfactory epithelium (OEP). Note moderate enzyme activity in blood vessels (BV) of central core (CC); ALPase × 400.

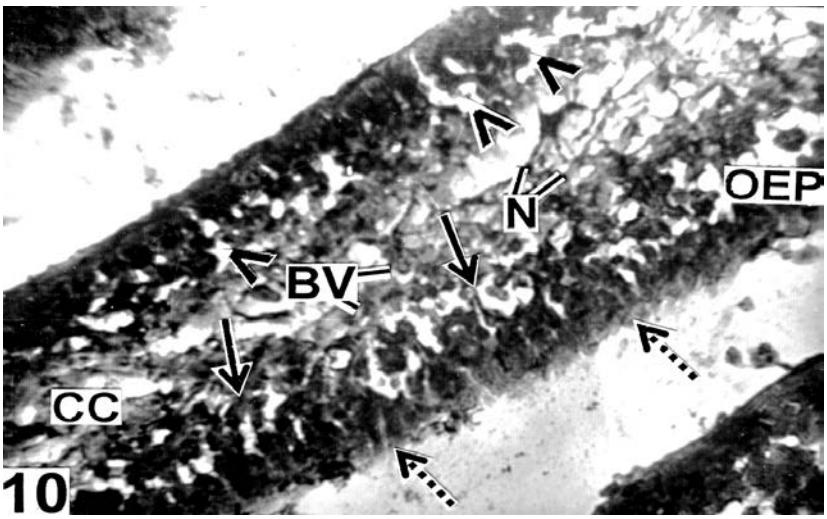

Figure 10. Showing ATPase activity in RC (solid arrows), flagellated supporting cells (broken arrows) and BC (arrow heads) of OEP. Note nerve fibres (N) and BV in CC exhibit positive enzyme activity; ATPase × 400.

Discussion

In most fish, the sense of smell was highly developed and was probably used more in the location of food than sight. Feeding behaviors were thought to be influenced by chemorecption among teleosts. Fishes were grouped into two categories *viz*., nocturnal and diurnal in relation to searching and procurement of food (Popova, 1967). In the latter group, the vision performed the chief role to capture the prey whereas the acute olfactory sensitivity played an indispensable role in feeding and prey detection in former group. In *R. kuturnee*, the olfactory chamber was communicated with the external aquatic environment through two separate nasal apertures: anterior inlet and posterior outlet. Water entered into the olfactory chamber through the inlet nostril, passed over the olfactory epithelium and went out via outlet nostril observed also by El-Attar and Al-Zahaby (2010) in silver carp. Suitable ventilation of the olfactory chamber was needed to bring the odorants to the olfactory mucosa for perceiving the chemical signal of the aquatic ecosystem (Balanger *et al*., 2003). Fish possessed a good sense of smell and were able to detect odour with the help of a pair of olfactory rosettes connected to the olfactory lobes of the brain by means of olfactory tracts (Singh, 1977; Ghosh *et al*., 2015). The elongated rosette of *R. kuturnee*, with 54 - 56 lamellae arranged on either side of the long and narrow raphe, could be classified with Bateson's (1889) rosette type 2 or under Burne's (1909) rosette column II. Total olfactory area was larger than the retinal area, signifying that the noses were better developed than the eyes. *R. kuturnee* was thus a macrosmatic species and belonged to Teichmann's (1954) group III, i.e., the "nose fishes" whose olfaction was superior in comparison to vision. Lamellae were arranged in a feather like pattern and the dorsal edges of the lamellae were characterized with flat

erections which slow down the water flow over the olfactory mucosa and facilitated better interaction of odorants particles with receptor cells.

Olfactory mucosa of *R. kuturnee* was folded to form a series of lamellae and optimally adapted to accommodate the large surface area in the limited space. The large surface area of the olfactory epithelium enhanced the sensitivity and efficiency of the olfactory organ (Zeiske *et al*., 1976; Patle and Baile, 2014b). The ecological niche occupied by a fish had a great impact on its structural characterization and specialization of olfactory epithelium (Kuciel *et al*., 2011). The olfactory epithelium of *R. kuturnee* consisted of receptor, flagellated supporting, non-flagellated supporting, labyrinth, mucous and basal cells. The olfactory processing initiated at the apical tip of olfactory receptor cells (Buck and Axel, 1991). In the present investigation, the receptor cells present in olfactory epithelium of *R. kuturnee* were able to detect chemical changes in the surrounding aquatic environment. Therefore, the well developed dendrite process of the receptor cell which terminated at the free surface of the epithelium enabled the fish to smell its food and mobilizes different olfactory cues even in muddy surrounding (Datta and Das, 1980; Sarkar and De, 2011).

The olfactory epithelium of *R. kuturnee* was consisted of flagellated and non-flagellated supporting cells intermingling with the receptor cells. Supporting cells gave the basic structure of the lamella. The flagellated supporting cells created a moderate and weak current over the olfactory lamellae, presumably assisting in water renewal and the transport of stimulant molecules to the receptor cells (Ghosh and Chakrabarti, 2009). On contrary, the non-flagellated supporting cells were believed to give mechanical support to other sensory cells. They might produce a serous secretion which maintains a continuous directional flow of the mixed secretion along the surface of the epithelium. The flow removed the remains of the stimulating substances and kept the receptors ready for new stimuli. The mixed fluid may protected the epithelial surface from excessive inflow of water and loss of electrolytes. The supporting cells had been suggested to perform several functions *viz*., secretory, absorbing and glial (Hansen and Zeiske, 1998; Bhute and Baile, 2007). In between the sensory receptor cells, supporting cells formed a mosaic shielding them from mechanical abuse (Patle and Baile, 2014b). The labyrinth cells on the surface of epithelium might be served as excretory cells for osmoregulation and ion regulation. In this way they took part in maintaining ion homeostasis in different salinities of water. Shirai and Utida (1970) reported that the labyrinth cells might be involved in electrolyte transport because they were structurally similar to chloride cells found in fish gills. Ruzhinskaya *et al*. (2001) also demonstrated the presence of typical chloride cells in the olfactory epithelium of *Acipenser maerii*, *A. ruthenus*, *Salmo gairdneri*, *Carassius auratus*, *C. carassius*, *Perca fluviatilis* and *Oreochromis mossambicus*. They also reported that these cells were present in the areas both of indifferent and sensory epithelium and provided active transport of ions between the inner and outer media to maintain ion and osmotic homeostasis.

Mucous cells secreted mucin which protects the olfactory mucosa from external hazards and also lubricated the surface of epithelium to smooth flow of water during ventilation. The mucus layer might also serve as an ion

trap, which delayed the access of heavy metals and salts to underlying organs (Waryani *et al*., 2013). The basal cells were situated in the deeper layer of the olfactory epithelium and assumed to be the progenitor cells of the receptor and supporting cells (Zeiske *et al*., 1992). According to Frabman (1994), the basal cells might act as stem cells for regeneration of lost or damaged non-sensory and goblet cells. Receptor cells with cylindrical dendrites displayed strong ALPase reaction in *R. kuturnee* was probably due to its positive role in transportation of various chemicals from olfactory epithelium to the central core. Further, the ALPase activity in the basal cells as well as supporting cells in the olfactory epithelium might be involved in the degradation of nucleotides and coupled with various kinds of metabolic processes. Banerjee and Mittal (1975) while dealed with the histochemistry of giant cells located in the skin epidermis of *Clarias batrachus* and reported that the perinuclear areas of the aforesaid cells were metabolically active. The intense ALPase activity in the blood cells of the central core probably assisted the transportation of various macromolecules. This corresponded to the findings of Ghosh and Chakrabarti (2014) in the olfactory epithelium of *Labeo bata*. The ATPase activity in the receptor cells including their dendrite processes was related to the transmission of various nerve impulses. Shantha and Nakajima (1970) observed the presence of ATPase in the receptor cells of the olfactory mucosa of rhesus monkey possibly involved in the process of olfactory sensations elicited by the contact of odour particles with these receptor cells while ATPase activity in the basal cells might be involved in the process of mitotic activity for the replacement of regenerating receptor and other cells of the olfactory epithelium. Intense localization of ATPase had also been reported in the dendrite processes of receptor cells along with epithelial bordering and the basal cells of the olfactory organ in *Catla catla* (Ghosh and chakrabarti, 2015). The basal cells were assumed to be the progenitors of the receptor and supporting cells (Zeiske *et al*., 1992; Mandal *et al*., 2005). Further, ATPase activity in supporting cells might be coupled with some kind of metabolic processes. The localization of ATPase in blood cells of central core implied its involvement for the physiological activities. Further, ATPase activity in the nerve fibres of central core probably had an important functional significance in transportation of various chemicals and nerve impulses to the brain.

Conclusion

R. kuturnee is a freshwater, carnivorous, bottom dwelling teleost subsists on molluscs, small fishes, insects, crustaceans, worms etc. with multilamellar olfactory organ which characterized with dense mat of receptor cells has an acute sense of smell in recognition of food and performing other essential activities. However, further studies on Transmission Electron Microscopy (TEM) and experimental works on the olfactory epithelium of *R. kuturnee* are recommended in corroborating the present findings.

Acknowledgements

The authors are thankful to Dr. S. Ray, Head, Department of Zoology, The University of Burdwan, for providing essential laboratory facilities.

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