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Original Article

The olfactory mucosa of river catfish, *Eutropiichthys vacha* (Hamilton, 1822): a histochemical study

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Abstract: Miscellany in histochemical peculiarity of the olfactory mucosa was studied for localization of axons, mucopolysaccharides, glycogen, protein and lipid in schilbid catfish, *Eutropiichthys vacha* (Hamilton, 1822). Silver deposition was detected in the abundance of receptor cells in the olfactosensory epithelium and well as marked in the knob and dendrite process of primary receptor cells. The mucous cells with various stages of maturity containing different degrees of mucopolysaccharides were identified by employing the Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) test. This combined test imparted purple colour due to PAS for neutral mucin and blue colour for AB reaction due to the presence of acid mucin exclusively. The results of Best's carmine test indicated considerable amount of glycogen present in the receptor cells, basal cells and supporting cells in the olfactory mucosa. Basic protein and bound lipid were ascertained in the various cells of the epithelial lining and in blood cells of the central core were discussed with behavioural activities of the fish interested.

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Introduction

Olfaction is one of the most notable senses driving the basic types of behaviour in teleosts for communication with the aquatic environment. It is extremely important in fish participating in survival linked activities such as feeding, migrations, nest finding, reproductive strategies, parental behaviour and fright reaction to alarm substance (Camacho et al., 2010). Olfactory cues are ascertained by a pair of olfactory organs connected to the olfactory lobes of the brain and pertinent attitudes are released in any given species (Mana and Kawamura, 2002).

In recent years, a widespread attraction has been exhibited by researchers on morphology, structural organization and function of the olfactory organ to understand the mechanism of olfaction in teleosts. Cellular elements and their chemical constituents conceiving of the olfactory system are indispensable to annotation of olfactory function. Scanty studies have been made on histochemistry of the olfactory epithelium of fish (Singh and Singh, 1987; Pastor et al., 1991; Saito et al., 2004; Bettini et al., 2009; Ghosh and Chakrabarti, 2012, 2015; Kim et al., 2019) to localize the chemical peculiarity in different cell types.

In view of the paucity of knowledge regarding the chemical nature and functional significance of diverse cells lining the olfactory mucosa of schilbid catfishes in relation to mode of life and living, the present work has been undertaken on *Eutropiichthys vacha* (Hamilton, 1822) (Actinopterygii: Siluriformes: Schilbeidae), a carnivore surface feeder which feeds on insects, crustaceans, rotifers, mollusks and small fishes (Talwar and Jhingran, 1991; Nath, 1994).

Materials and Methods

Sample collection: A total of 24 adult specimens of both sexes *E. vacha* (measured 22±2.07 cm in total length) were captured from the river Ganga around Kalyani, West Bengal throughout the year 2018. The specimens were deeply anaesthetized with benzocaine (4 mg/L) and the olfactory organs were carefully

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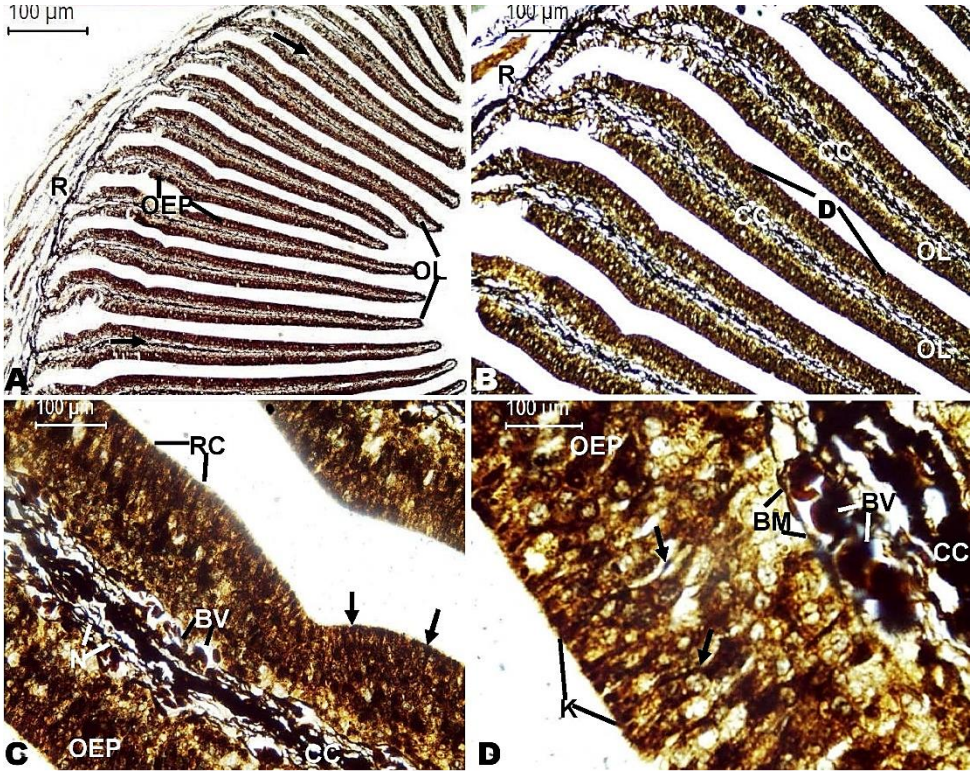

Figure 1. Photomicrographs of transverse sections of olfactory epithelium of *Eutropiichthys vacha* showing silver deposition in the neurons by Silver Impregnation Method (SIM). **(A)** Olfactory lamellae (OL) showing silver deposition in olfactory epithelium (OEP) and central core (CC) (arrows) radiated from raphe (R) (40X). **(B)** Showing silver reaction in the axonal processes of different receptor cells (D) lining the epithelium of OL. Note positive reaction in nerve fascicles of CC (100X). **(C)** Showing intense silver reaction in axonal processes of ciliated receptor cells (RC) and rod cells (arrows) of OEP. Note strong reaction in nerve fibres (N) and blood vessels (BV) in CC (400X). **(D)** Maximum localization of silver staining in primary RC with knob like structure (K) and synaptic contact in between primary and secondary RC (arrows) of OEP. Note intense silver staining in basement membrane (BM) and BV in CC (SIM) (1000X).

excised from the floor of the nasal cavity under a Zeiss Stemi 2000-C stereoscopic binocular microscope.

Histochemical analysis: Olfactory tissues were fixed in 10% neutral formalin for 16-18 h. After fixation, the tissues were washed well in 70% ethanol and dehydrated in graded ethanol series. The tissues were cleared in methyl benzoate and processed for paraffin embedding. Sections were cut as 8-10 µm thick using a rotary microtome (Weswox MT-1090A) and proceed for Silver Impregnation Method (SIM) for detection of axons (Marsland et al., 1954), Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for detection of neutral and acid mucins (Mowry, 1956), Best's Carmine (BC) method for detection of glycogen (Best, 1906), Mercury-Bromphenol Blue (MBB) method for detection of basic protein (Mazia et al., 1953) and Acetone Sudan Black (ASB) method for detection of bound lipid (Berenbaum, 1958). All the slides were observed and photographed using a Leica EC3 light microscope at different magnifications.

Results

Silver reaction: Silver reaction furnishes dark brown colour and ascertains various kinds of receptor cells in the apical surface of epithelium (Figs. 1A-B). Positive reaction of silver staining is observed in the axonal processes of ciliated and rod receptor cells and nerve fascicles in the central core (Fig. 1C). The axon of scanty primary receptor cells consequence with synaptic connection to the dendrite ends of secondary receptor cells. The basement membrane in between olfactory epithelium and central core and knob-like structure of receptor cells on the free epithelial surface shows positive silver reaction (Fig. 1D).

Periodic acid Schiff's reaction in combination with Alcian blue: Histochemical detection of neutral and acid mucopolysaccharides is denoted by PAS reaction

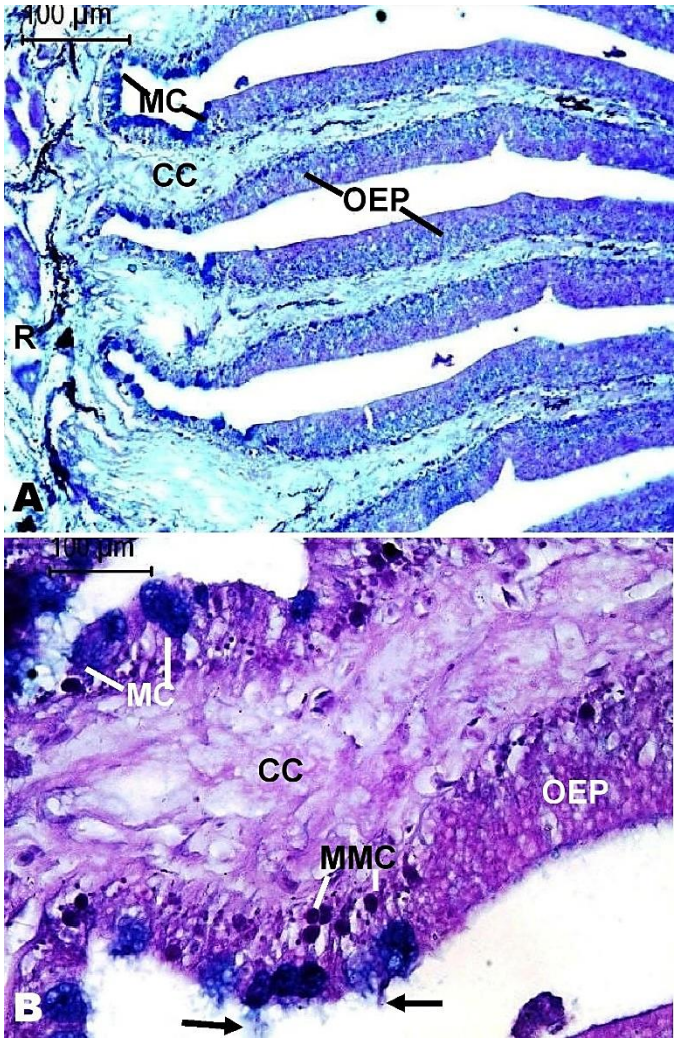

Figure 2. Photomicrographs of the olfactory lamella of *Eutropiichthys vacha* showing Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB). **(A)** Localization of acid mucin in the secretory mucous cells (MC) of olfactory epithelium (OEP). Central core (CC) displays moderate reaction. R marks raphe (100X). **(B)** Intense acid mucin in the secretory mucous cells (MC) and neutral mucin in non-secretory mucous cells (MMC) of OEP. CC shows moderate reaction. Arrows indicate luminal secretion (400X).

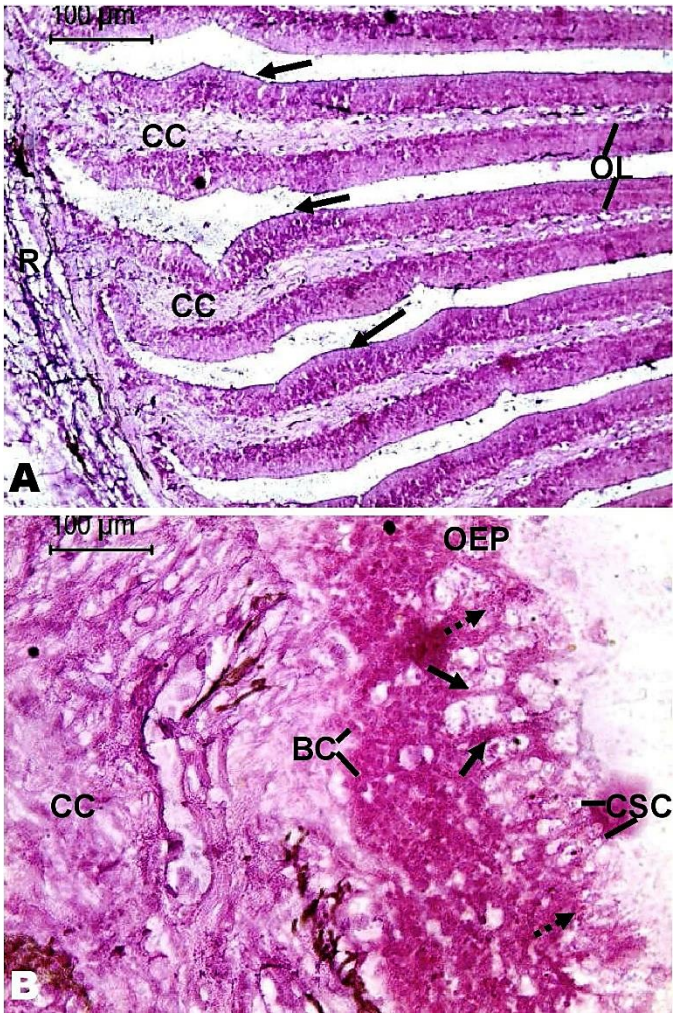

Figure 3. Part of horizontal section of olfactory epithelium of *Eutropiichthys vacha* showing Best's Carmine (BC) reaction for glycogen. **(A)** Intense localization of glycogen in the epithelial border (arrows) of olfactory lamella (OL). Central core (CC) and raphe (R) displays moderate to weak reaction (100X). **(B)** Olfactory epithelium (OEP) displays glycogen content in receptor cells (solid arrows), ciliated supporting cells (CSC), non-ciliated supporting cells (broken arrows) and basal cells (BC). CC exhibits moderate reaction (400X).

in combination with AB (PAS-AB) test. The positive reaction is due to the presence of 1-2 glycol groups in the mucous cells. This combined test furnishes a red colour due to PAS reaction for neutral mucin and blue colour for AB reaction confirms the presence of acid mucin (Fig. 2A). The secretory mucous cells at the surface of the epithelium and peripheral non-secretory mucous cells show intense blue and red colour assuring acid and neutral mucins, respectively. The secretory mucous cells are vacuolated and roughly reticulated. Loosely disposed connective tissues and elastic fibres in the central core exhibit moderate reaction (Fig. 2B). The secreted mucin also positively rebounds with AB test. Negligible activity is observed in the basal cells, supporting cells and receptor cells.

Best's Carmine reaction: The accrue of Best's carmine assay mark presence of glycogen in the receptor cells, basal cells, ciliated supporting cells and non-ciliated supporting cells of the epithelium as well as in the epithelial border (Figs. 3A-B). Diffuse granules are observed in the cytoplasm of basal cells and supporting cells. Moderate glycogen content is present

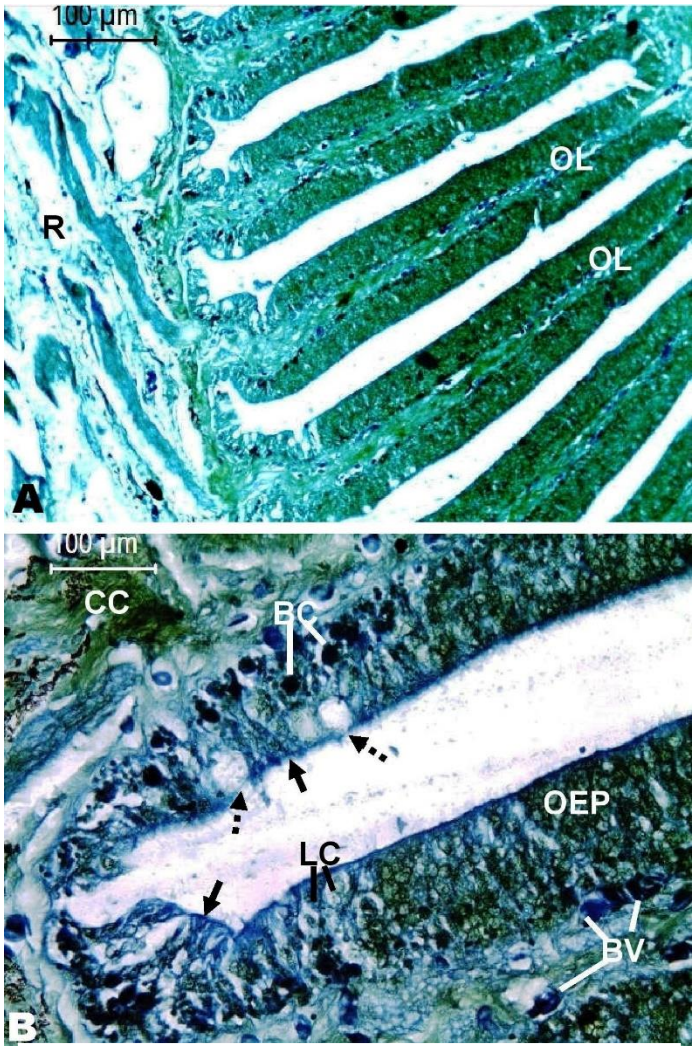

Figure 4. Photomicrographs of transverse sections of olfactory lamella of *Eutropiichthys vacha* showing Mercury-Bromphenol Blue (MBB) reaction for basic protein. **(A)** Distribution of protein in various layers of olfactory lamellae (OL) based on raphe (R) (100X). **(B)** Showing intense localization of protein in receptor cells (solid arrows) and basal cells (BC) of olfactory epithelium (OEP) and blood vessels (BV) of central core (CC). Note moderate reaction in labyrinth cells (LC) and weak reaction in mucous cells (broken arrows) (400X).

in the central core of the lamella.

Mercury-Bromphenol Blue reaction: The basal cells, labyrinth cells and receptor cells having cylindrical process up to the free epithelial surface are positive to mercury-bromphenol blue reaction furnishing a dignified concentration of proteins (Figs. 4A-B). The nuclei of basal cells are darkly stained. The mucopolysaccharide filing of mucous cells in the olfactory epithelium show proteinaceous nature (Fig. 4B). The puissant reaction of protein is also consorted with the blood cells of the central core.

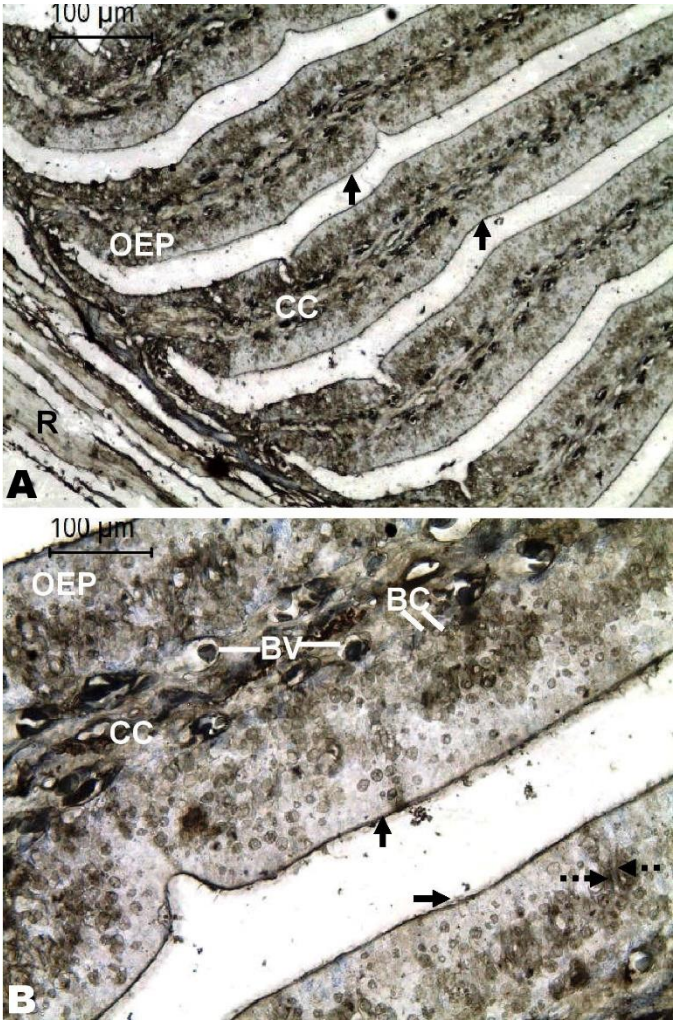

Figure 5. Photomicrographs of different regions of olfactory epithelium of *Eutropiichthys vacha* showing Acetone Sudan Black (ASB) reaction for bound lipid. **(A)** Distribution of lipids in the epithelial (OEP) lining (arrows) and central core (CC) of olfactory lamella radiated from raphe (R) (100X). **(B)** Showing sudanophilic materials in blood vessels (BV) of CC, basal cells and receptor cells (broken arrows) with sensory hairs (solid arrows) at surface of OEP (400X).

Acetone Sudan Black resaction: This histochemical test has been employed for the detection of bound lipid associated with various cells of the olfactory lamella. The colours procured are generally black or grey. The basal cells, receptor cells along with sensory hairs at the epithelial surface showing lipoid nature. This method gives deep colouration of blood vessels in central core (Figs. 5A-B).

Discussions

The olfactory organ is the only organ of fish in which receptor neurons are directly exposed to aquatic

environment and vulnerable to water contaminants. In *E. vacha*, the acute localization of silver stain in the axons of the primary receptor cells, make synaptic contacts with the dendrites of the secondary neurons. The axons of the secondary neurons which enter into the central core are also intensely stained with silver reaction. This intimates that the impulses received by the terminal dendrites of primary receptor cells ultimately convey impulses to the central core for final transduction of impulses to the brain. This finding is accordance with that of olfactory epithelium of *Etroplus suratensis* (Chakrabarti and Ghosh, 2013) and *Catla catla* (Ghosh and Chakrabarti, 2015).

The histochemical investigations advocate that the olfactory mucosa of *E. vacha* comprehends mucopolysaccharides, glycogen, protein and lipid. PAS-AB positive materials are detected in the mucous cells. The presence of mucous cells in the present species makes their olfactory epithelium secretory in nature. In the present observation, the predominance of acid mucopolysaccharides in the epithelial lining prevents the friction against foreign particles, which enter into the olfactory chamber through water. The secreted mucin which covers the surface of the olfactory epithelium provides protection to delicate sensory hairs against osmotic effects of water (Hopkins, 1926) and forms a favourable environment for ionic and molecular diffusion (Chakrabarti, 2005). PAS-AB positive material is a complex mucopolysaccharides or glycoprotein in nature (Fullmer, 1965).

It has been noticed that diffuse glycogen occurs in the cytoplasm of supporting and basal cells of *E. vacha*. The movement of cilia of the supporting cells maintains a continuous directional flow along the surface epithelium, which is an active process, requires energy and presence of glycogen particles is the main source of such energy. The presence of glycogen in the receptor cells facilitating the conduction of the sense of smell and glycogen probably acts as a substrate for the source of energy required for the activities. The presence of glycogen also been reported by Ojha and Kapoor (1972) in *Channa punctatus* and Chakrabarti (2005) in *Mugil parsia*. Glycogen in the basal cells in the fishes under study may help in maintaining their metabolic status as well as provide energy so essential for sustaining their motility.

The mucous cells, receptor cells, labyrinth cells and basal cells are rich in protein. The contents of the mucous cells exhibit positive mercury-bromphenol blue reaction, confirming the proteinaceous nature which synthesis neutral glycoproteins. The basal and labyrinth cells in the olfactory epithelium exhibit intense to moderate reaction for protein probably for various metabolic as well as physiological activities (Ghosh and Chakrabarti, 2015). The presence of protein has reported in the elements of olfactory epithelium of hillstream teleosts (Singh and Singh, 1987).

It has been observed that the most lipids found in the epithelial border of the present species. The acetone sudan black method demonstrated that the presence of lipids mostly concentrated in the basal cells and moderately distributed along dendrites of receptor cells may be a sign of myalinated sheath in the axons of receptor cells and probably help in the impulse transduction process. Evans and Hara (1977) reported that phospholipids occur in the dendrite process of receptor cells of the olfactory epithelium in fishes. Mostly concentrated lipid material in blood cells of central core is required as a source of endogenous energy including its involvement for the physiological activities.

Acknowledgments

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