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Architectural pattern of different cells lining the olfactory epithelium of long-whiskered catfish, *Sperata aor* (Hamilton)

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Abstract - The structural organization of the olfactory epithelium in *Sperata aor* was studied by light as well as scanning electron microscopy. The elongated olfactory organ comprised of 52-54 primary lamellae assorted on both sides of narrow median raphe. The middle dorsal part of the olfactory lamellae was characterised with linguiform processes. Sensory and non-sensory areas were distributed separately on each olfactory lamella. The sensory epithelium occupied in the middle linguiform process, whereas the basal part of lamellae was covered with non-sensory epithelium. The sensory epithelium was composed of morphologically distinct two types of receptor neurons either ciliated or microvillous. The non-sensory epithelium contained mucous cells and supporting cells with inconspicuous microridges. The orientation of different cells lining the olfactory epithelium was discussed in light of their functional significance.

Key words: Histoarchitecture, fine structure, olfactory organ, function, *Sperata aor*.

Introduction

Fish have distinctively localized and well developed olfactory organ, which is of great biological importance and plays an indispensable role in arousing the behavioral activities (Hara, 1971). Olfactory information is extremely important in the lives of fishes, including feeding, prey detection, predator avoidance, species and sex recognition, sexual behaviour and migration (Kleerekoper, 1967).

Olfaction results from stimulation of the sensory receptor cells in the olfactory apparatus, which is innervated by the olfactory nerve. The morpho-histology and structural organization of different cells lining the olfactory epithelium in fishes have been described by several workers (Ojha and Kapoor, 1973; Sinha and Sinha, 1990; Hansen and Zeiske, 1998; Ruzhinskaya, *et al*., 2001; Chakrabarti and Ghosh, 2009; 2010; Ma and wang, 2010; Ghosh and Chakrabarti, 2011; 2012).

These studies advocated the considerable morphological variability regarding the shape and location of the olfactory organ, number and arrangement of the olfactory lamellae, the orientation of sensory and non-sensory epithelium as well as an abundance of various receptor cells, which correlate with the enormous diversity of life-styles among fish.

The folding on the lamellae of the olfactory epithelium increases the surface area of the epithelium as well as the sensitivities and efficacy of the

olfactory organ (Zeiske *et al.*, 1976). There seems to be no record on correlation and cellular variation of the olfactory epithelium and their role in sensory reception in teleost under examine.

The objective of this study is to work out more closely the structural detailed and functional aspects of various cells lining the olfactory epithelium of *Sperata aor* (Siluriformes, Bagaridae), a predaceous freshwater catfish, which subsists on small fishes and worms.

Materials and Methods

Living mature fishes of *S. aor* (30 to 32 cm in length) were procured from local freshwater bodies of Burdwan, West Bengal, India. Fishes were anaesthetized with tricaine methone-sulphonate (MS 222; Sigma Chemical Co.) solution (100 mg/l) and sacrificed following the guidelines given by the Institutional Ethical Committee. Olfactory rosettes were dissected out from the dorsal side of the olfactory chamber under a stereo microscope and immediately processed for the histological and scanning electron microscopical (SEM) studies.

For the purpose of SEM study, the olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 20 min. The entire olfactory rosettes were carefully dissected out and the adhering mucus on the epithelial surface was removed by repeated rinsing with 1% Tween 40 solution. After being rinsed in 0.1 M phosphate buffer (pH 7.4), the tissues were infiltrated with 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4) for 24 h at 4°C.

After proper 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 phosphate buffer (pH 7.4) for 2 h. After secondary fixation the tissues were washed thoroughly in buffer, dehydrated through ascending series of acetone, followed by isoamyl acetate and then subjected to critical point drying method with liquid carbon-dioxide. After being dried the olfactory rosettes were mounted on metal stubs, coated with gold palladium to a thickness of approximately 20 nm and examined under a Hitachi S-530 scanning electron microscope.

For histological study, olfactory tissues were fixed in aqueous Bouin's fluid for 16 to 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 using a rotary microtome. After routine histological procedure the sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain.

Results

According to SEM examinations, the elongated olfactory rosette of *S. aor* consists of a series of 52 to 54 primary lamellae in each left and right side of the median narrow raphe. The lamellae are closely set and almost similarly oriented on either side of the raphe. The outer margins of the lamellae are attached to the wall of the olfactory chamber, while their inner margins are tied to the raphe (Fig. 1).

The surface contour of the olfactory rosette shows the appearance of a pinnately compound leaf like texture. The middle dorsal portions of the

lamellae are furnished with linguiform processes.

The sensory epithelium occupies in the middle linguiform process whereas the basal part of the olfactory lamellae are covered with non-sensory epithelium (Fig. 1).

Histologically, the olfactory lamellae are composed of two layers of olfactory epithelium separated by a central lamellar space called central core, which constituted with loose connective tissue, nerve fibers and blood vessels (Figs. 2 & 3). A well developed basement membrane separates olfactory epithelium from the central core. The sensory olfactory epithelium is made up of a large number of primary and secondary receptor cells and few microvillous cells (Fig. 3).

The receptor cells are distinguished by their elongated-oval and darkly stained nuclei. However, the dendrite process of primary receptor cell extends as a narrow cylindrical process up to the free epithelial surface. The secondary receptor cells are mainly present below the primary receptor cells and they form synaptic contact with the primary receptor cells (Figs. 3 & 4).

Microvillous cells are small in number and without cilia, dispersed in between receptor cells along the free surface of the epithelium (Fig. 3). According to SEM observation, the apex of the linguiform area exhibits hairy appearance due to the presence of dense aggregation of ciliated receptor cells (Fig. 5).

In some regions, the sensory epithelium is embossed with microvillous receptor cells in between ciliated receptor cells showing sculpture appearance on the surface (Fig. 6).

Microvillous receptor cells are characterized by small dendrites. The transitional zone of sensory and non-sensory epithelium comprises few dendrite patches of receptor cells with supporting cells and blood cells (Fig. 7).

Mucous cells with mucin droplets are scattered among the supporting cells. Histologically, the surface of non-sensory epithelium is mainly composed of supporting cells with conspicuous nuclei and mucous cells (Fig. 8).

Under SEM study, the non-sensory epithelium is characterized by closely arranged supporting cells with inconspicuous microridges. The openings of mucous cells with mucin masses are located in between supporting cells (Fig. 9).

Discussion

The olfactory mucosa is located on the floor of the olfactory chamber and is often folded to form lamellae (Hara, 1975). The number and shape of the olfactory lamellae are related to the space available in the olfactory cavity of fish; thus they represent adaptations that maximize the sensory area under given restrictions (Zeiske, 1973: 1974).

The present study displays that the elongated olfactory rosette of *S. aor* consists of 52-54 primary lamellae in each left and right side of the median narrow raphe. This entitles it belongs to Teichmann's (1954) group of nose-fishes comprising solitary and nocturnal predators (Bannister, 1965). The distribution of the sensory and non-sensory epithelia on the surface of the lamellae exhibits a great variety in fish species for adaptation to a specific

environment (Yamamoto, 1982).

In the present study, the sensory epithelium is confined in the middle linguiform process while the basal parts of the olfactory lamellae are characterized 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.

The ecological niche inhabited by a given species probably has a great impact on its structure and cellular organization (Hara, 1994). Similar tongue like projections of the olfactory lamellae has been observed by Ojha and Kapoor (1973), Ghosh and Chakrabarti (2011) in freshwater carp, *Labeo rohita* and *Labeo bata*, respectively.

In the present study, the sensory epithelium of *S. aor* principally consists of two morphologically distinct types of receptor cells: ciliated and microvillous cells. They arise 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 enlist because they form a part of the olfactory transduction mechanism, are stimulated by odour bearing substances and also enable the fish to detect food.

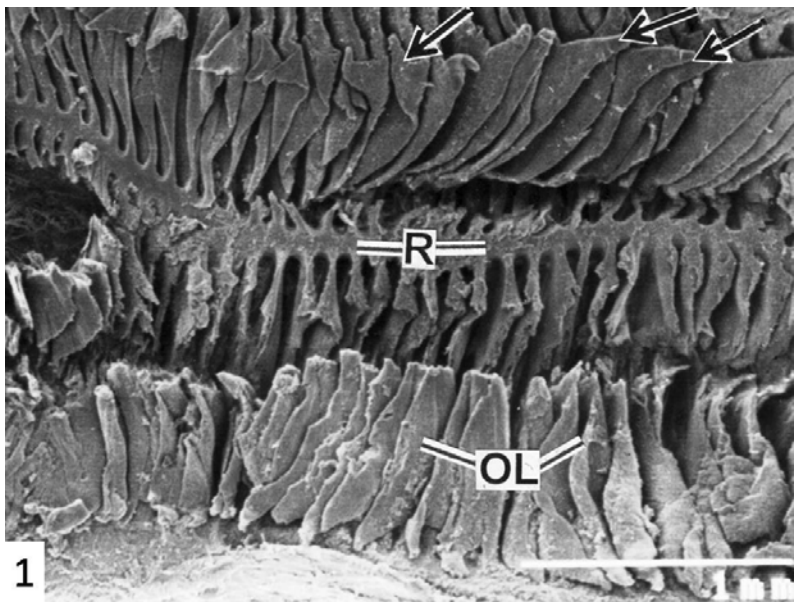

Figure 1. Elongated olfactory rosette exhibiting olfactory lamellae (OL) radiating from the median raphe (R). Note linguiform processes (arrows) of the lamellae; SEM × 50.

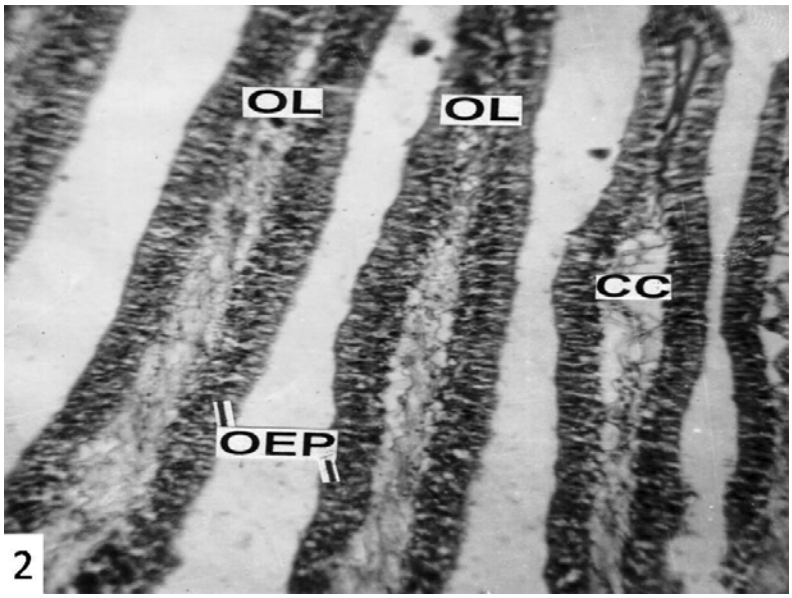

Figure 2. Section of olfactory lamellae (OL) showing olfactory epithelium (OEP) on either side of the central core (CC); MT × 100.

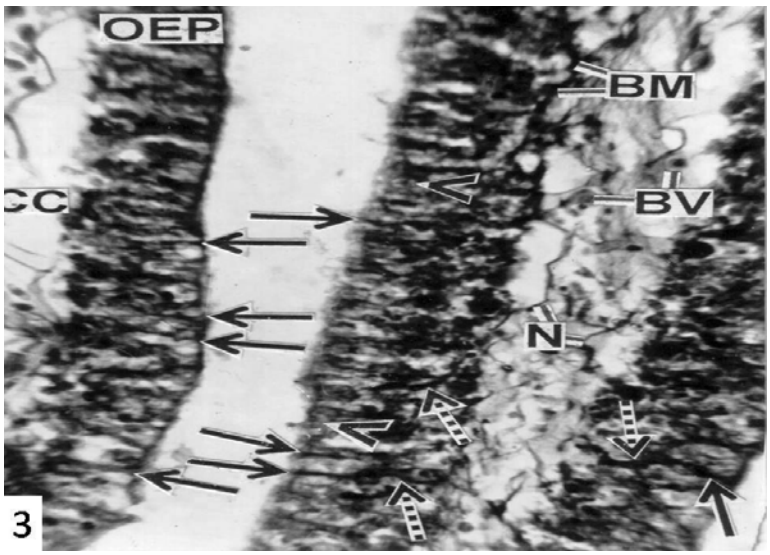

Figure 3. Sensory olfactory epithelium (OEP) lined with primary receptor cells (RC) (solid arrows), secondary RC (broken arrows) and microvillous cells (arrow heads). OEP is separated from central core (CC) by a basement membrane (BM). Note the presence of blood vessels (BV) and nerve fibres (N) in CC; HE × 400.

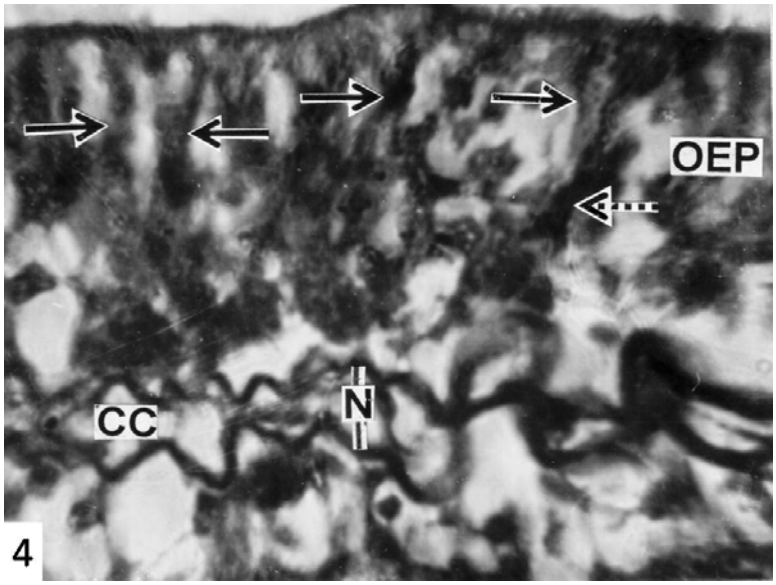

Figure 4. Higher magnification of the olfactory epithelium (OEP) displaying the dendrite process of primary RC (solid arrows) and secondary RC (broken arrow). Note prominent nerve fibres (N) in central core (CC); HE × 1000.

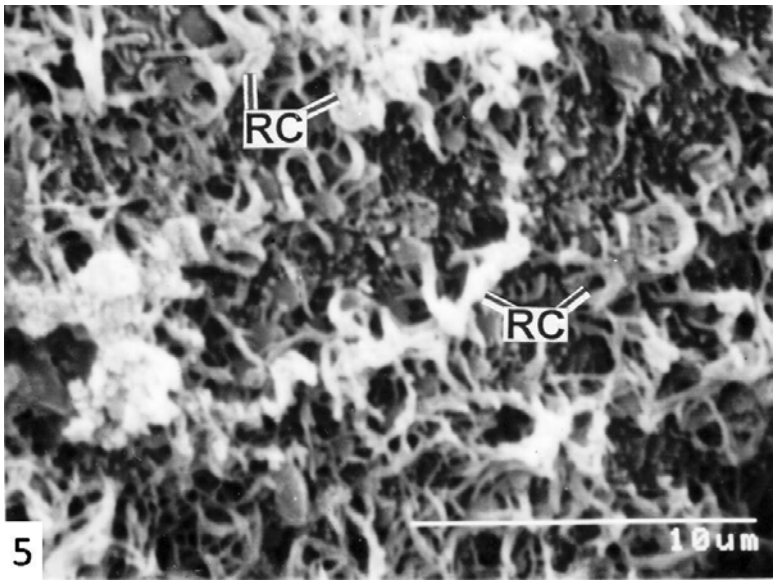

Figure 5. Sensory epithelium shows dense aggregation of ciliated receptor cells (RC); SEM × 4500.

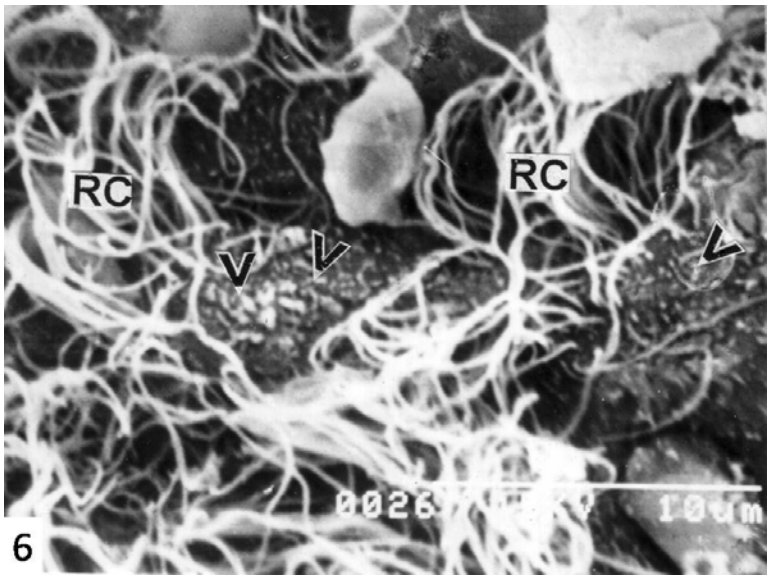

Figure 6. Higher magnification of sensory epithelium showing microvillous receptor cells (arrow heads) and dendrite patches of ciliated RC; SEM × 5000.

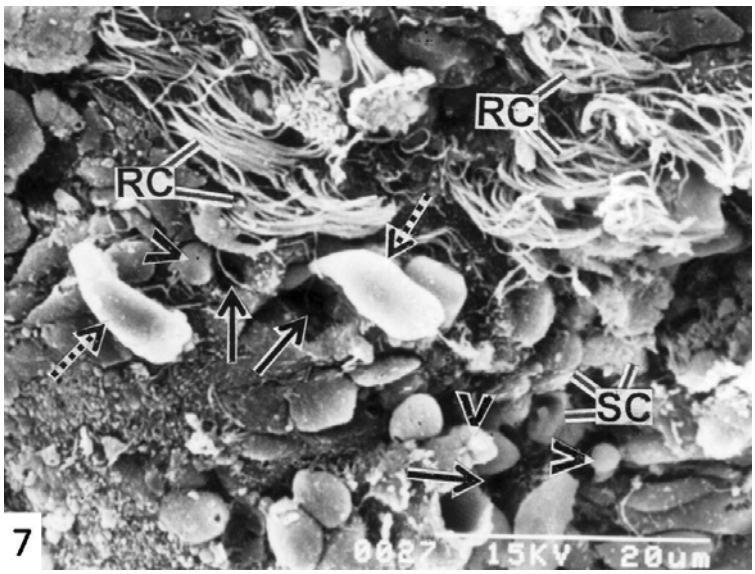

Figure 7. Transitional zone between sensory and non-sensory epithelium showing dendrite patches of receptor cells (RC) in between supporting cells (SC). Note the presence of mucous cells (solid arrows) and secreted mucin droplets (arrow heads) over the SC. Broken arrows indicate blood cells; SEM × 4000.

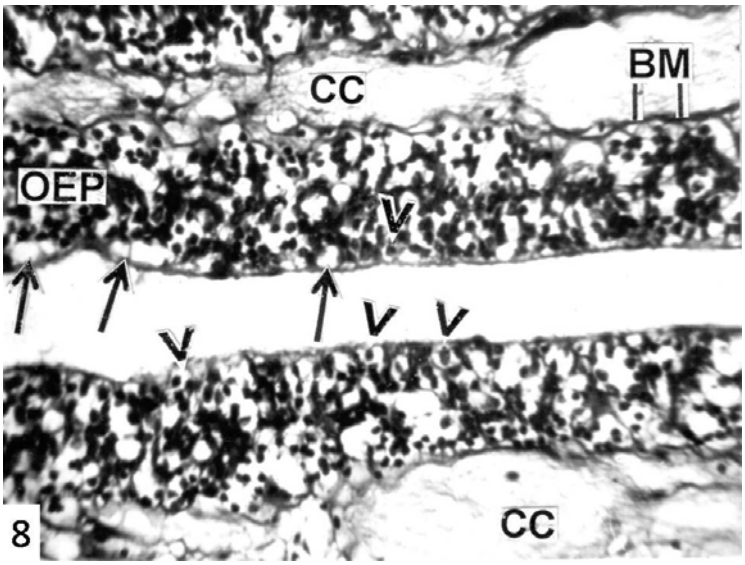

Figure 8. Non-sensory olfactory epithelium (OEP) provided with supporting cells (arrow heads) and mucous cells (solid arrows). Note OEP is disunited from central core (CC) by a prominent basement membrane (BM); MT × 400.

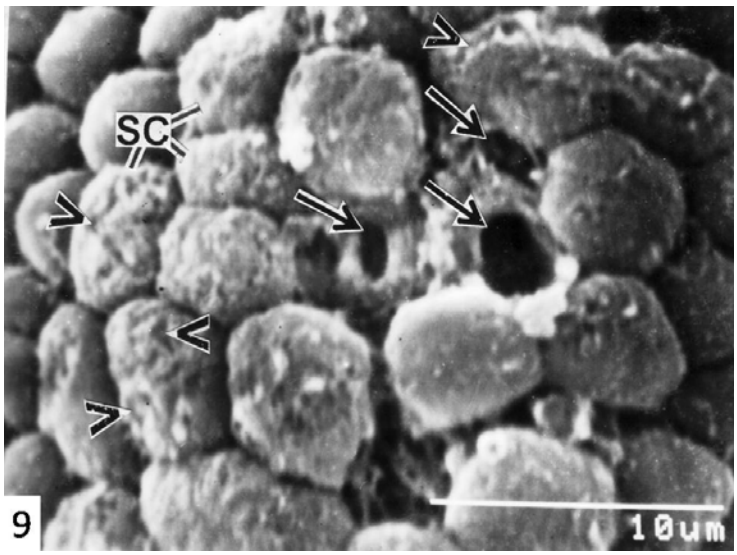

Figure 9. Surface view of non-sensory epithelium showing closely arranged supporting cells (SC) with inconspicuous microridges (arrow heads). Note the opening of mucous cells (solid arrows) with mucin mass in between SC; SEM × 5000.

It is well established that the receptor cells present in the olfactory epithelium are able to detect chemical changes in the surrounding environment (Datta and Das, 1980).

In the present observation, the ciliated receptor cells correspond to the type I cells of Yamamoto and Ueda (1978). In contrast to the ciliated receptor cells, the microvillous receptor cells have a slightly sunken apex and consist of minute 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. Zippel *et al.* (1997) reported ciliated and microvillous receptor cells in the olfactory epithelium of gold fish. They suggested that microvillous receptor cells mediate responses to pheromones, where as ciliated cells mediate responses to amino acids.

The most interesting feature of the present study is histological identification of secondary receptor cells in addition to primary receptor cells 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. Ojha and Kapoor (1973) also found secondary neurons in the olfactory epithelium of *Labeo rohita*. Graziadei and Metcalf (1971) postulated that new neurons replace the old and degenerating ones and establish fresh synaptic contact in the olfactory bulb.

In the transitional zone of sensory and non-sensory epithelium, few ciliated receptor cells in between supporting cells are responsible for better monitoring of the water quality even up to this zone. Furthermore, the non-sensory epithelium consists of supporting cells provided with inconspicuous microridges on their apical surface that help in holding mucus film over the epithelium and in protecting the sensory receptor cells from different hazardous substances.

The mucin secreted by mucous cells probably constitutes an important medium in which the 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 Bandyopaghyay and Datta (1996) in the olfactory function of *Heteropneustes fossilis*.

Conclusion

Sperata aor is a highly predaceous catfish which feeds on small fishes and worms, very much dependent on its olfactory sense. Therefore, the high density of ciliated receptor cells in the elongated olfactory epithelium is of special interest to enable the fish to detect food. On contrary, the microvillous receptor cells mediate responses to pheromones in regulation of reproduction.

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نمط بناء الخلايا المختلفة المبطنة للنسيج الطلائي الشمي للسمك القط الطويل الشارب *Sperata aor* (Hamilton)

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المستخلص - لقد درس التنظيم التركيبي للنسيج الطلائي الشمي في *Sperata aor* بالمجهر الضوئي والمجهر الألكتروني الماسح. يتكون عضو الشم المتطاول من (52-54) صفائح أولية مرتبة على جانبي حاجز وسطي ضيق. يتميزالجزء الوسطي الظهري للصفائح الشمية بوجود بروزات لسانية الشكل. تتوزع المناطق الحسية وغير الحسية بصورة مستقلة على كل صفيحة شمية. يحتل النسيج الطلائي الحسي وسط البروز اللساني، بينما يتغطى الجزء القاعدي من الصفائح بنسيج طلائي غير حسي. يتكون النسيج الطلائي الحسي من وحدات عصبية مستقبلة بهيئة طرازين مميزين مظهريا، إما مهدبا أو بشكل زويبغات دقيقة. يحتوي النسيج الطلائي غير الحسي على خلايا مخاطية وخلايا دعامية مع وجود حروف دقيقة غير واضحة. لقد نوقش إتجاه الخلايا المبطنة للطلائية الشمية على ضوء أهميتها الوظيفية.