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A Transmission Electron Microscopic Study of the Olfactory Epithelium in Hill Stream Cyprinidiae, *Garra mullya* (Sykes)

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Abstract— *Olfaction is primarily produced by the stimulation of receptor cells on the olfactory organ's neuroepithelial surface, surrounded by olfactory nerve fibres. Numerous fish life processes, including migration, communication, feeding, schooling, defence, and reproduction, depend heavily on olfactory signals and cues. The olfactory and reproductory systems are interconnected structurally and functionally, and puberty-related alterations in the olfactory epithelium are documented. The olfactory epithelium, which covers a large portion of the surface of the olfactory rosette, a structure found within the olfactory chambers on the fish rostrum, is where the olfactory receptor cells are situated. Although ultra structural transmission electron microscopic studies of the olfactory organ and bulb are carried out by some investigators but very sparse information is available on hillstream fishes and that is why this work has been undertaken to detail the structure of olfactory system in G. mullya by electron microscopy. Microvillous olfactory receptor cells are placed compactly adjacent to the supporting cell showing a junction complex : the zonula-ocludens. Polygonal white cells are present in between the basal cells and supporting cells. Small polyhedral basal cells lie just above the basal lamina of olfactory epithelium. Basal cells may be working as stem cells for regeneration of lost or damaged non sensory and goblet cells*

Keywords— ***olfactory epithelium, TEM, hill stream fish, Garra mullya.***

I. INTRODUCTION

Hill streams are distinct aquatic habitats characterised by shallow, narrow channels, cold water, high altitude, various substrata, and strong water current. In order for hill stream fish to successfully adapt to this particular habitat, nature has given them a special anchorage mechanism to deal with rapid, shooting, and turbulent water flow (Ojha and Singh, 1992). The fauna is greatly influenced by current strength. Despite seasonal variations in water flow, it is always substantially higher than in plains' rivers and streams. Fish have therefore evolved sticky organs to survive the swift water flow. It is simple to categorise the fish that live in hill streams into two types. One group's members live temporarily in the highland streams and only particular times in their lives do they migrate up specifically to reproduce. These species climb simply using their muscles and do not display any unique changes. The other group has members who dwell permanently in hills' rivers and streams, and many of them have some adaptive traits. These alterations are primarily seen as sticky structures, which are typically found at the anterior end and on the fins (Arunkumar et al., 1990).

These fish are all bottom-dwelling organisms by necessity.

Bornean sucker, Balitora, Glyptosternum, Glyptothorax, and Pseudoecheneis are highly specialised species that have body structures that resemble leaves. Garra is a single genus, and its members exhibit every variation in body shape. Those who live in ponds and tanks have bodies that are roughly cylindrical, whereas those that live in swift-moving streams have bodies that are clearly flattened. Due to the lack of scales, the dorsal surface in front of the dorsal fin is smooth in species like Garra abhoyai and G.

rossicus. The body of G. mullya is covered in extremely well-developed cycloid scales. Thorax has a smooth, scale-free ventral surface (Saxena, 1959). The fish's exposed body surface is home to the organs that sense toxins in the water. Parker first used the phrase "common chemical sense" in 1912. Taste (gustation) and smell are the two senses that influence chemoreception the most (olfaction). The primary methods for locating and identifying chemical stimuli in the environment are as follows (Hara, 1994).

Olfaction is primarily produced by the stimulation of receptor cells on the olfactory organ's neuroepithelial surface. It is surrounded by olfactory nerve fibres (Hansen and Zeiske, 1998). Numerous fish life processes, including migration, communication, feeding, schooling, defence, and reproduction, depend heavily on olfactory signals and cues (Wilson, 2004; Camacho et al., 2010). All creatures depend on their ability to sense their chemical surroundings. Numerous creatures, from insects to mammals, use their highly developed olfactory systems to recognise, analyse, and encode chemo-stimuli that could include vital information for their survival, well-being, social relationships, and reproduction (Marc Spehr and Munger, 2009). The olfactory and reproductory systems are interconnected structurally and functionally, and puberty-related alterations in the olfactory epithelium are documented (Schreibman et al., 1984).

The olfactory epithelium, which covers a large portion of the surface of the olfactory rosette (as revealed in scanning electron microscopic studies by Bagade et al 2021), a structure found within the olfactory chambers on the fish rostrum, is where the olfactory receptor cells are situated. Despite the fact that the rosette's size and structure vary widely between species, it often has a longitudinal ridge (raphe) with two rows of olfactory lamellae projecting from it, greatly expanding the surface area (Zeiske et al., 1992). Olfactory epithelium is a complex tissue comprising sensory and non-sensory epithelia. Sensory epithelium is located on the raphe and central region of the olfactory lamella in majority of the species. The sensory epithelium is present in patches in some species also (Yamamoto, 1992).

Ultra structural transmission electron microscopic studies of the olfactory organ and bulb are carried out by some investigators (Hara, 1975; Ichikawa, 1976; Ichikawa and Ueda, 1977; Kosaka and Hama, 1979 a, b; Zeiskie et al., 1979; Kleerekoper, 1982; Schreibman et al., 1986; Hansen and Zeiske, 1998). In this work we are reporting the structure of olfactory system in *G. mullya* by electron microscopy.

II. MATERIAL AND METHODS

Transmission Electron Microscopy(TEM) was carried out at EM facility centre of Department of Anatomy, All India Institute of Medical Sciences, New Delhi. Adult females of *G. mullya* were used for the TEM study. Olfactory rosette were fixed in ice cold glutaraldehyde (PH 7.2) for 24 hours. After fixation, it was washed 3-4 times in cold PBS (PH 7.45) for 30 min. interval and post fixed for 1 hour in 1% osmium-tetraoxide (PH 7.2, 0.1 m phosphate buffer). The tissues were stained with 0.5% uranyl acetate, dehydrated in graded series of alcohol, embeded in Araldite or styrene methancrylate. At various stages, semi-thin sections were stained with methylene blue solution to follow location and histological characteristics of the cell types. After selection of the location, blocks were trimmed and ultra thin sections were collected on grids. Then grids were stained with uranyl acetate and if necessory with lead acetate and observed under the TEM.

III. OBSERVATIONS

In *G. mullya*, transmission electron microscopic studies of sensory olfactory epithelium revealed that, the olfactory epithelium shows microvillous olfactory receptor cells (mORC), suppoting cells (SC), ciliated non sensory cells (cNSC) , white cells (WC) and basal cells (BC) (Fig.04).

Ciliated olfactory receptor cells (cORC) are bipolar neurons bearing a cell body towards apical surface and thin axon towards basal lamina (Fig.05). It consists of elongated nucleus with denser nucleoplasm. Rough endoplasmic reticulum (RER), lysosome (LY) and mitochondria (M) are aggregated at the apical region. Nucleus of receptor cell is compactly placed in between the basal cell (BC) and supporting cell (SC) (Fig.05).

Olfactory microvillous receptor cell (mORC) is placed compactly adjacent to the supporting cell by the appearance of a junction complex, the zonula-ocludens. Free olfactory surface of the dendrite bulges out to form smaller olfactory knobs (OK) which are more prominent in the micro-villous receptor cell (Fig.06). Olfactory knob (OK) of mORC bear numerous micro-villi (MV). Oval to elongated mitochondria (M) are aggregated at the apical part of the dendrite. Rough endoplasmic reticulum (RER) and golgi bodies (GB) are present in the cytoplasm. Apically it is broad while basally it is narrow. Clear vacuoles (V) are present below the olfactory knob (OK) in the cytoplasm of dendrite and smaller centrioles (ce) are observed. Nucleus is small, ovoid in shape and heterochromatin material remains scattered and distributed throughout the nucleoplasm (Fig.07). Axons of this cell forms axonal bundles which extend towards the basal lamina (Fig.08).

Supporting cell (SC) is polygonal columnar cell which separates the mucous secreting goblet cell (GC) along the zonula adherens (ZA) Vacuoles, ER, and mitochondria are seen in SC (Fig.09). Many electron opaque vesicles (EOV) are accumulated in the apical cytoplasm of SC. These vesicles possess scanty secretary granules. The apical end of supporting cell protrudes above the epithelial surface having irregular short rod like cilia (C) and bears short irregular microvilli (MV). Various mitochondria (M) are present at the apical region of SC which consists of dense matrix with compact cristae. Few cristae of endoplasmic reticulum (ER) are also present in SC (Fig.10). Nucleus of SC is oval shaped and euchromatin material is disposed more near the nuclear membrane (Fig.10).

Ciliated non sensory cells (cNSC) are distributed in sensory as well as in non sensory regions of the olfactory lamella. These cells are interdigitate with adjacent SC and microvillous type protrusions are present between the cilia (Fig.11). These cells are columnar and broad towards the apical surface and provided with large number of longer cilia. There are typical kinocilia (KC) posessing 9+2 axial pattern of microtubules (Fig.12). It is noteworthy that each cilium carries two striated rootlets. Both longer and shorter rootlets run at an angle of 90^0 towards the mitochondrial zone. Both rootlets are striated by alternating light and dark bands. Mitochondria are distributed at the apical region of cell and their shape varies from oval to elongated type. Smooth endoplasmic reticulum (SER) are also observed below the mitochondria (Fig.12). Nucleus is round to elliptical in shape of the check-board pattern of chromatin. It is euchromatic with peripheral finer chromatin lumps (CL) and has a single outstanding nucleolus (NU) (Fig.13).

Mucous secreting goblet cells (GC) are distributed in the surface area of sensory and non-sensory region of olfactory epithelium. Goblet cell has a prominent golgi bodies (G) and well developed rough endoplasmic reticulum (RER). Cell membrane of goblet cell with the adjacent supporting cell is quite prominent (Fig.14).

White cell (WC) is oval to polygonal in shape. It is present in the middle zone of olfactory epithelium. It occur in between the basal cell and supporting cell. The nucleus of white cell is comparatively large with distinct nuclear membrane. Cytoplasmic granules are less. Mitochondria has degenerating cristae. Rough endoplasmic reticulum is dilated and shows a sign of deterioration (Fig.15).

Small polyhedral basal cell (BC) lie at the base of olfactory epithelium just above the basal lamina (BL) (Fig.16). Numerous basal cells are scattered in the sensory olfactory epithelium than in the non-sensory region (Fig.04). Nucleus (N) is oval with double nuclear membrane (NM) and is larger in relation to the cell size. Basal cells have numerous projections which enclose a bundle of receptor cell axons extending towards the basal lamina (Fig.16). Cytoplasm of basal cell is dense and contains mitochondria (M), rough endoplasmic reticulum (RER), golgi body (GB), electron lucent granules (G1), and electron dense granules (G2) (Fig.17 &18).

IV. DISCUSSION

In *G. mullya*, in transmission electron microscopic studies, in sensory region of olfactory epithelium, both ciliated olfactory receptor cells and microvillous olfactory receptor cells are found to be adjacent to the supporting cells and also to the ciliated non sensory cells. Sensory epithelium with few receptor cells in addition to supporting cells, ciliated non sensory cells and basal cells are reported in a number of teleosts (Yamamoto, 1982; Bandyopadhyay and Datta, 1998; Bhute *et al.*, 2007; Masram and Baile, 2014 and Waryani *et al.*, 2013). In between the basal and supporting cells, white cells are found in the middle of olfactory epithelium and also goblet cells are seen in the *G. mullya.* Similar findings are observed in *H. fossilis* (Dutta and Bandopadhyay,1997 and Masram and Baile, 2014).

Ciliated cells ontogenetically proceeds the microvillous receptor cells (Zielinski and Hara, 1988), but according to some, ciliated and microvillous receptor cells develop from identical stem cells and the development of the centriole is arrested in microvillous receptors. Dense gathering of golgi complex in receptor cells in *G. mullya* indicates secretary nature of receptor cells. Presence of stimulatory neuropeptide GnRH in the olfactory receptor cells and their projections to the olfactory bulb is revealed in *Cirhinus mrigala* (Biju *et al.*, 2003) suggesting the role of olfactory receptor cells in transduction of environmental cues and further transmitting it through brain-pituitary-gonadal axis. In the present study olfactory receptor cells are also characterized in *G. mullya* which may have a role in signal transduction in further areas of brain. Similar findings are explained by (Chakrabarti *et al.*, 2011; Waryani *et al.*, 2013, Masram and Baile, 2014).

In *G. mullya*, microvillous olfactory receptor cells develop into knobs bearing numerous microvilli which show which is a characteristic feature of kinocilium is reported in *Schizothoraichthys progastus and Schizothorax richardsoni* (Singh *et al.*, 1995) and *H. fossilis* (Bandyopadhyay and Datta, 1998). In *G. mullya*, junctional complex the zonula ocluden is prominent between microvillous receptor cell and supporting cell which is also reported by Theisen (1972) and, Bandyopadhyay and Datta (1998); Geme and Doving (1969) in *Lota lota* and Dutta and Bandopadhyay (1997) in *H. fossilis* stated that the zonula ocluden protects the epithelial lining in the fresh water fish.

Thick ciliated supporting cells with oval nuclei are seen placed on the side of microvillous receptor cells. These cells can be distinguished from the ciliated receptor cells because of the round shape of nucleus and absence of olfactory knob in ciliated supporting cells. Presence of vesicles suggests the secretary nature of supporting cells. The abundance of mitochondria may have a role in the production of energy involved in a secretary function for these cells. Similar findings are reported by Masram and Baile, (2014) and Mokhtar *et al.*, (2014), Thornhill (1967), Bandyopadhyay and Datta (1998).

In *G. mullya,* zonula adherens separating the supporting cell and goblet cells is seen. It is reported by Thornhill (1967), Bandyopadhyay and Datta, (1998) and Mokhtar *et al.*, (2014) they and stated that zonula adherens acts as a sieve to prevent passage of substances through the interlamellar spaces.

In *G. mullya*, ciliated non sensory cells have typical kinocilia possessing 9+2 axial pattern of microtubules. It is noteworthy that each cilium carries two striated rootlets as reported by Banister (1965). In *G. mullya*, goblet cells is clearly identified. These cells are filled with golgi complex and rough endoplasmic reticulum. Secretion of goblet cells helps in facilitating the odorant removal (Horning and Mozell, 1981and Masram and Baile, 2014). *G. mullya* is a hill stream fish. Secretion of goblet cell may help in decreasing the friction of water in the chamber as well as protecting the epithelium from coming in contact with the hazardous material to some extent (Dutta and Bandopadhyay (1997). White cell is reported in the neuroepithelium of *Rainbow trout* (Evans *et al.*, 1982), and in olfactory epithelium of *H. fossilis* (Bandyopadhyay and Datta, 1998; and Masram and Baile, 2014). Similar cell is seen in the olfactory epithelium of *G. mullya.* Basal cells in the fish under study are present towards the basal region just above the basal lamina. These cells work as stem cells for regeneration of lost or damaged non-sensory and goblet cells, (Frabman, 1994; and Suzuki *et al.*, 1991). These cells regenerate the olfactory epithelium and are characterized by relatively short life span according to Zeiske et al., (1992) and Yamamoto *et al.*, (1982). The receptor cells may be replaced throughout the life by these proginator basal cells. This view is supported by presence of rough endoplasmic reticulum in the cytoplasm of these cells.

V. CONCLUSION

In transmission electron microscope, sensory region of the olfactory epithelium shows ciliated olfactory receptor cells with bipolar neurons. Ciliated receptor cells (cORC) are responsible for detection of bile salts and amino acid odorants as reported by different workers.

Microvillous olfactory receptor cells are placed compactly adjacent to the supporting cell showing a junction complex, the zonula-ocludens. Olfactory knobs bear numerous microvilli and oval to elongated mitochondria are aggregated at the apical part.

Polygonal columnar supporting cells separate the goblet cells along zonula-adherens. Many electron opaque vesicles accumulate in the apical cytoplasm. The apical end of supporting cell protrudes above the epithelial surface having irregular short cilia and microvilli. Oval shaped nucleus and euchromatin material is disposed more near the nuclear membrane.

Ciliated non sensory cells are distributed in sensory as well as in non sensory regions of the olfactory lamella. These cells interdigitate with adjacent supporting and microvillous type of protrusions which are present between the cilia. They have typical kinocilia possessing 9+2 axial pattern of microtubules. Each cilium carries two striated rootlets by alternating light and darkbands. cNSC ventilate the olfactory chamber by synchronous beating of cilia.

Mucous secreting goblet cells are distributed in the surface area of both sensory and non sensory regions of olfactory epithelium. Its secretion might help to facilitate the odorant removal and decrease the friction of water in the olfactory chamber and also to protect the epithelium from coming in contact with hazardous material to some extent.

Mucous secreting goblet cells are distributed in the surface area of both sensory and non sensory regions of olfactory epithelium. Its secretion might help to facilitate the odorant removal and decrease the friction of water in the olfactory chamber and also to protect the epithelium from coming in contact with hazardous material to some extent.

Polygonal white cells are present in between the basal cells and supporting cells in middle zone of the olfactory epithelium. Small polyhedral basal cells lie just above the basal lamina of olfactory epithelium. Basal cells may be working as stem cells for regeneration of lost or damaged non sensory and goblet cells.

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EXPLAINATION OF FIGURES

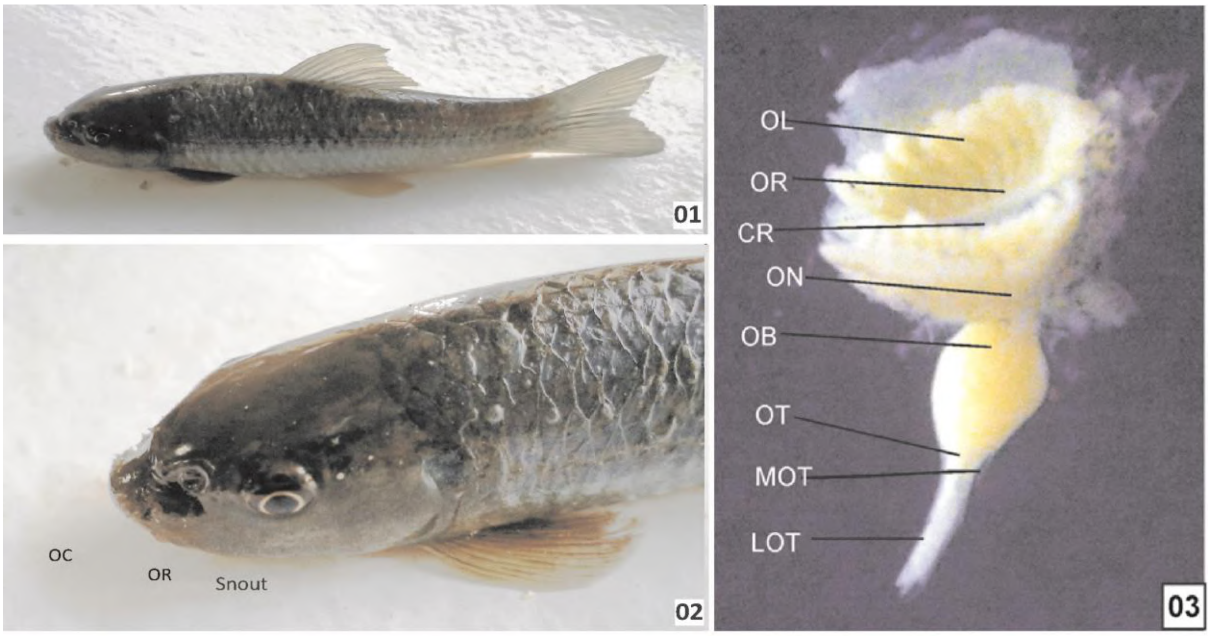

Fig. 1: Lateral view of fish Garra mullya (Sykes). Fig. 2: Lateral view showing olfactory rosette (OR) in the olfactory chamber (OC). Fig. 3: Whole view of olfactory organ showing olfactory rosette (OR) with olfactory lamellae (OL) connected by short olfactory nerve (ON) to the olfactory bulb (OB) andolfactory tract (OT) showing lateral olfactory tract (LOT), medial olfactory tract (MOT).

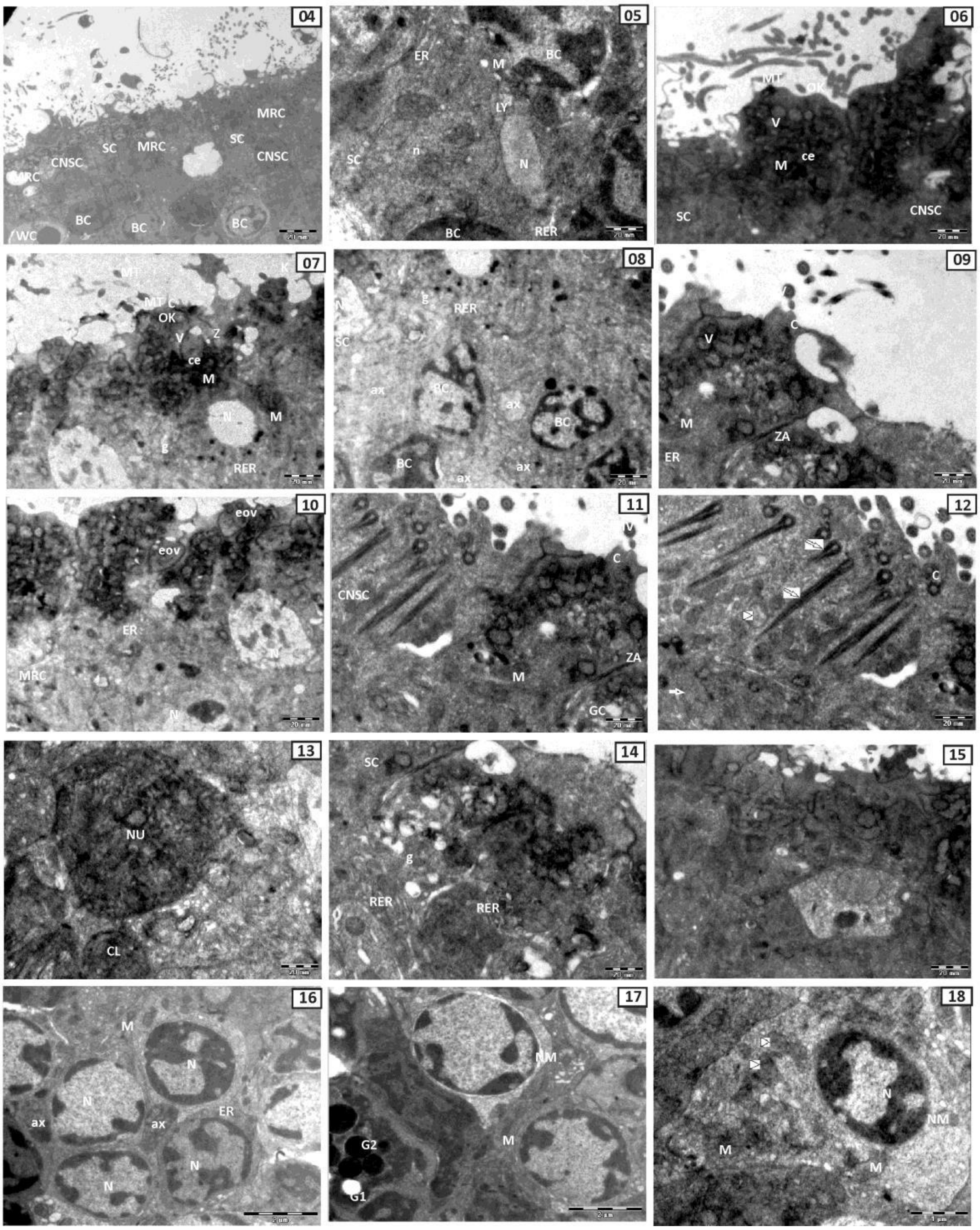

Fig. 04: TEM of sensory epithelium showing microvillous receptor cell (MRC), ciliatednon sensory cell (CNSC), supporting cell (SC), white cell (WC) and basal cell (BC) Fig. 05: TEM of olfactory receptor cell (CRC) showing elongated nucleus (N), rough endoplasmic reticulum (RER), lysosome (LY), mitochondria (M) and supporting cell (SC) showing oval nucleus (N) and euchromatin material disposed more near the nuclear membrane, endoplasmic reticulum (ER) are in the cytoplasm. Fig. 06 & 07: TEM of microvillous receptor cell showing microvilli (MV) with microtubules (MT), olfactory knob (OK), centriole (ce), clear vacuoles (V), nucleus (N),mitochondria (M), golgi complex (g) and rough endoplasmic reticulum (RER). Note- Spiral zonula occluden (Z) is compactly adjuscent with supporting cell. Fig. 08: TEM of sensory region showing nucleus of supporting cell (SC), microvillousreceptor cell (MRC) and exonal bundles between the adjuscent the basal cells (BC) extendover the basal lamina. Fig. 09 & 10: TEM of supporting cell (SC) showing cilia (C) with microvilli (MV), electron opeque vesicle (eov), granulae vesicle (V), mitochondria (M), endoplasmic reticulum (ER) and nucleus (N). Fig. 11: TEM of magnified supporting cell (SC)

showing short cilia (C) with microvilli(MV), electron opeque vesicle (eov), mitochondria (M) with cristae and saperate by gobletcell (GC) and ciliated non sensory cell by zonula adherant (ZA). Fig. 12: TEM of ciliated non sensory cell (CNSC) showing long cilia © with kinocilia(9+2 arrangement) (KC), short and long striated rootlets (arrow), mitochondria (arrowhead) and smooth endoplasmic reticulum (thick arrow). Fig. 13: TEM of ciliated nion sensory cell showing large nucleolus (NU) and chromatinlumbs (CL). Fig. 14: TEM of goblet cell (GC) showing golgi complex (g) and rough endoplasmic reticulum (RER). Fig. 15: TEM of white cell (WC) showing large oval nucleus with nuclear membrane (NM), mitochondria (M) and rough endoplasmic reticulum (RER). Fig. 16 & 17: TEM of basal cell showing large oval nucleus (N), axon bundle (ax), mitochondria (M), rough endoplasmic reticulum (RER), electron lucent granule (G1) andelectron dense granules (G2). Fig. 18: TEM of magnified basal cell (BC) showing large nucleus (N) with nuclear membrane (NM) and dense cytoplasm contains mitochondria (M), rough endoplasmic reticulum (RER) and golgi complex (g) (arrow head).