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The fine structural organisation of the olfactory epithelium of *Cyprinus carpio* (Linnaeus): a scanning electron microscopic study

P. Chakrabarti, S. Hazra Choudhury

Department of Zoology, Burdwan University, Burdwan, West Bengal, India

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The fine anatomical structures of the olfactory epithelium of Cyprinus carpio *(Linnaeus) have been systematically studied with the help of the scanning electron microscope (SEM). The olfactory rosette is an oval structure composed of a number of lamellae arranged on a median raphe. A large part of the lateral surface of the rosette is covered with non-receptor epithelium, whereas the receptor epithelium occupies a much smaller area in the middle part. The non-receptor epithelium is covered with a tuft of ciliated supporting cells, among which the stratified epithelial cells and mucous cells are located. The receptor epithelium is represented by the flagellated and microvillus receptor and supporting cells. Different cells on the olfactory epithelium correlate with the functional significance of the fish concerned.*

Key words: SEM study, olfactory epithelium, *Cyprinus carpio*

INTRODUCTION

The olfactory organ of a fish shows considerable diversity, reflecting degree of development and ecological habitats [14]. *Cyprinus carpio* is a bottom-feeding omnivorous fish. The study of the olfactory organ of this fish is of paramount importance because it is essentially a chemoreceptor and plays a meaningful role not only in locating food but also in detecting the presence of odoriferous substances in the aquatic ecosystem. Although extensive information on the topological characteristics of the olfactory epithelium of different teleosts has been obtained through the electron microscope [2, 3, 6, 7, 9, 15], relatively little is known of the surface ultrastructures and modifications of the olfactory epithelium of bottom-dwelling carp. An attempt has therefore been made in the present study to characterise the surface architecture and the different functional aspects of the olfactory rosette of *Cyprinus carpio*, a bottom-dwelling cyprinodont.

MATERIAL AND METHODS

Healthy adults of the *Cyprinus carpio* species were collected from a local freshwater pond. The olfactory rosettes were perfused *in vivo* with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 5 min. The rosettes were then dissected out from the dorsal side under a stereoscopic binocular microscope. The adhering mucus was removed by rinsing in heparinised saline. After being rinsed in 0.1 M phosphate buffer (pH 7.4), the tissues were infiltrated with 2.5% glutaraldehyde for 24 h at 4°C. After fixation the tissues were removed, rinsed in the same buffer for 15 min and subjected to post-fixation in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4 for two hours. The tissues were washed in buffer and dehydrated through graded acetone followed by iso-amyl acetate and subjected to the critical-point drying method. After being dried, the olfactory rosettes were mounted on metal stubs, coated with gold and scanned in a Hitachi S-530 SEM. Some tissues were

Address for correspondence: P. Chakrabarti, Department of Zoology, Burdwan University, Burdwan 713104, West Bengal, India, tel: 0342-2656202, e-mail: pchakraborty.bu@gmail.com

also fixed in Bouins fluid for better understanding of the orientation of different cells. The tissues were then processed following a routine histological process and stained with haematoxylin and eosin.

RESULTS

The oval olfactory apparatus of *Cyprinus carpio* consists of a rosette of 25–26 primary lamellae. The outer margins of the lamellae are free, while their inner margins are attached to the raphe. The size and shape of the lamellae vary according to their position in the rosette. The mid-lamella is the largest in size and may be considered as typical. The dorsal margin of the lamella is provided with a linguiform process (Fig. 1). A large part of the lateral surface of the fold is covered with non-receptor epithelium, whereas the receptor epithelium occupies a much smaller area in the middle part. Histologically the surface zone of the non-receptor olfactory epithelium consists of stratified epithelial cells, ciliated supporting cells and goblet cells. The cilia of the supporting cells are distributed along the surface of the olfactory epithelium. Sensory cells are altogether wanting (Fig. 2). According to SEM studies the non-receptor epithelium is made up of patches of ciliated non-sensory cells, among which the polygonal or oval stratified epithelial cells (5–8 μm) are located (Figs. 3, 4). The apical surfaces of the stratified epithelial cells are provided with microridges. The microridges are unbranched and arranged in a concentric whorl, leaving long deep channels between them (Fig. 4). The supporting cells are joined to the adjacent cells, forming a double ridged structure. The supporting cells give the basic structure of the lamellae, having a microridge system on their surfaces. A few discrete areas measuring about 3–4 μm represent the opening of mucous cells. Secreted mucins are also deposited over the microridges of stratified epithelial cells (Fig. 4).

In the histological sections the middle region of the surface of the olfactory epithelium was mainly lined with receptor cells and a few mucous cells. The dendrite process of each primary receptor cell extended as a narrow cylindrical process up to the free epithelial surface. The dendrites of receptor cells are of varying length. The nucleus of the receptor cell is more or less oval (Fig. 5). Under SEM study the receptor epithelium was represented by dendrites of receptor cells, mucous cells with a mucus plug and supporting cells (Fig. 6). The supporting cells have a microridge system on their surfaces. The receptor cells are located in groups and are divided into two

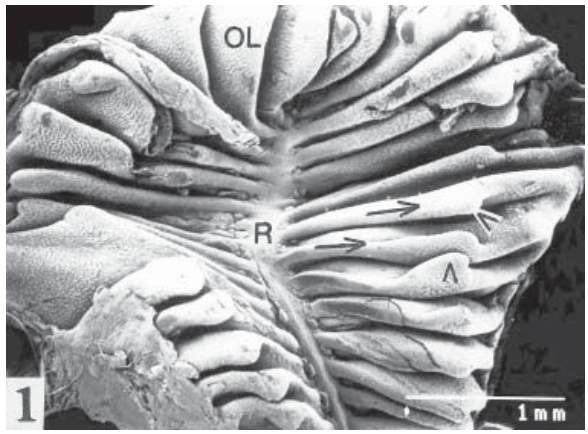

Figure 1. Different shapes of primary lamellae (OL) are shown radiating from the median raphe (R). Note the comparatively large lamellae in the middle region (arrows) and the linguiform process over the lamellae (arrow heads). Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. SEM × 50.

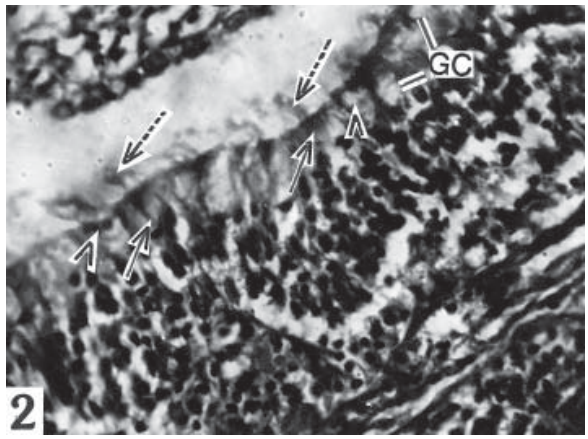

Figure 2. Histological section of non-receptor olfactory epithelium, showing ciliated supporting cells (solid arrows), stratified epithelial cells (SEC) (arrow heads) and goblet cells (GC). Note the presence of cilia on the surface of the epithelium (broken arrows). Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. Haematoxylin & Eosin × 400.

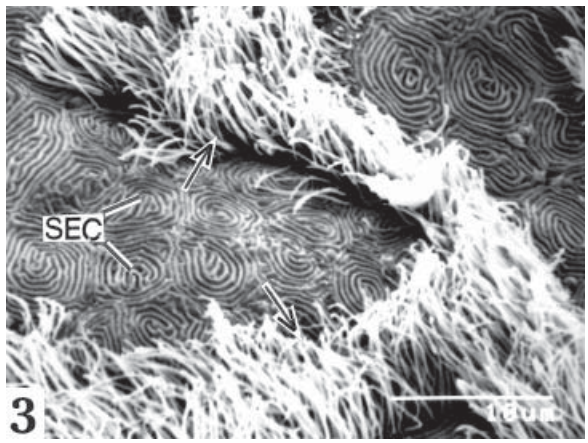

Figure 3. A tuft of ciliated non-sensory cells (arrows) is shown between oval or polygonal stratified epithelial cells (SEC). Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. SEM × 3200.

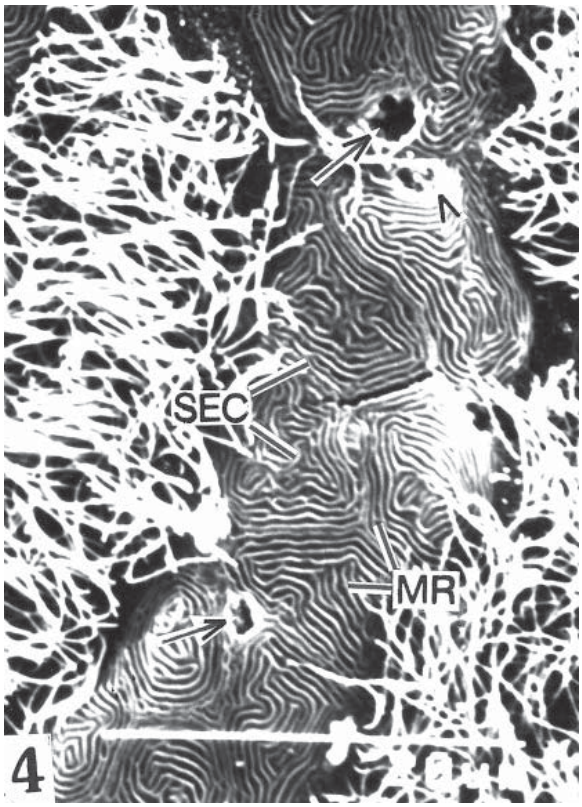

Figure 4. Surface of non-receptor epithelium showing polygonal or oval stratified epithelial cells (SEC) provided with unbranched microridges (MR) and leaving channels in between. Note the opening of the mucous cells (arrows) and retention of mucin (arrow heads) over SEC. Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. SEM × 4000.

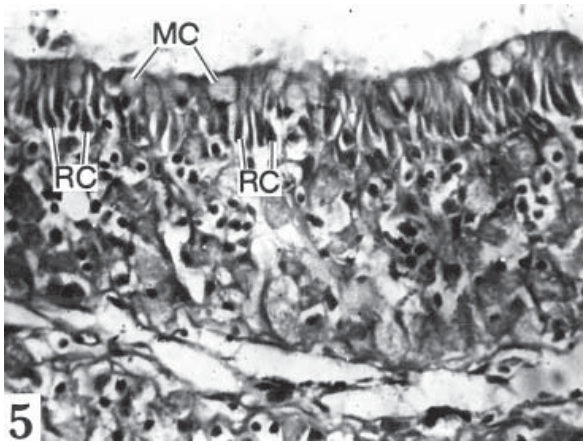

Figure 5. Surface zone of receptor epithelium, showing orientation of receptor cells (RC). Note cylindrical process of a dendrite of the RC towards the surface of the epithelium and the presence of mucous cells. Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. Haematoxylin & Eosin × 400.

types on the basis of the structure of their apical parts, whether flagellar or microvillar. The receptor of the microvillar cells are always somewhat submerged into the thickness of the flagellar receptor

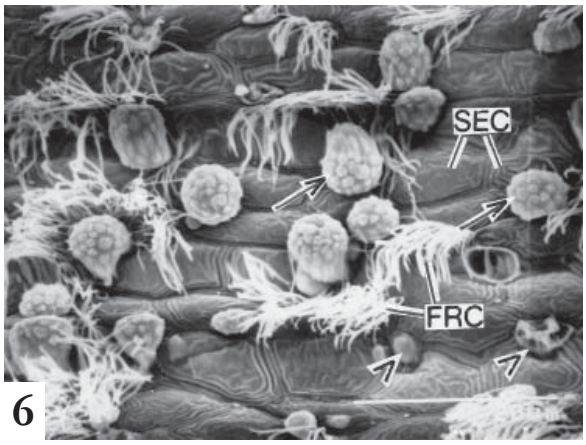

Figure 6. Receptor epithelium showing flagellar receptor cells (FRC) and microvillar receptor cells (MRC) (arrows). Note the presence of stratified epithelial cells (SEC) with indistinct microridges (MR) and mucous cells with mucus plug (arrow heads). Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. SEM × 4000.

layers (Fig. 6). In some areas the cylindrical primary receptor cells are provided with sensory cilia at the surface. Thus the receptor cell is directly exposed to the external environment. The branched dendrites of the secondary neurons synapse with the axonal end of the primary receptor cells. The terminal nerves of the secondary neurons extend to the basement membrane and pass out of it into the central core of the lamella when viewed under the SEM (Fig. 7).

DISCUSSION

Olfactory mucosa containing the olfactory sensory neurons is typically located on the floor of the olfactory chamber, which is often folded, forming olfactory lamellae [4]. The multilamellar peripheral olfactory organ in cyprinodonts provides an acute sense of smell and various aspects of their existence, such as feeding and reproduction, are mediated through olfactory cues [5]. The number and shape of the olfactory lamellae are related to the space available in the olfactory cavity of the fish and therefore represent adaptations which maximise the sensory area under a given restriction [12, 13]. The olfactory epithelia of the *Cyprinus* consists of 25–26 lamellae arranged into a rosette-like structure adapted to the maximum to the space available and similar to other cyprinid olfactory epithelia of type VI as identified by Yamamoto and Ueda [11].

The distribution of the non-sensory and sensory epithelia on the surface of the lamellae shows a great variety in different fish species [10]. In the present study in *Cyprinus carpio* the non-sensory epithelia is

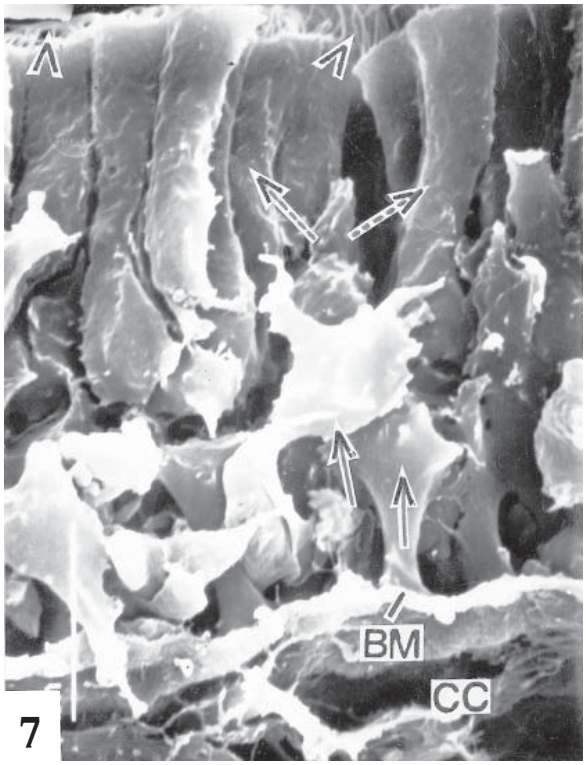

Figure 7. Cut portion of receptor epithelium showing primary receptor cells (RC) (broken arrows) provided with sensory cilia on the apical surface (arrow heads). Note the presence of secondary RC (solid arrows) with branched dendrites beneath the primary RC. BM indicates basement membrane and CC denotes central core. Scanning electron micrographs (SEM) of the olfactory rosette of *Cyprinus carpio*. SEM × 1600.

restricted to the lateral surface of the lamellae and the sensory epithelia occupies the smaller area in the middle part. In the non-receptor epithelium the tufted ciliated supporting cells are responsible for creating a water current in the olfactory chamber as well as the lamellar surface for better monitoring of the water quality by the receptor cells. The goblet cells are distributed between stratified epithelial and ciliated supporting cells. The secreted mucin from the goblet cells probably helps the smooth flow of water in the olfactory chamber by binding microscopic debris, which is ejected through the posterior nostril. This is in conformity with the findings of Rahmani and Khan [8] in the olefactory mechanism of *Anabas testudineus* and Bandyopadhyay and Datta [1] in *Heteropneustes fossilis*. Furthermore, the non-receptor epithelium consists of stratified epithelial cells provided with microridges arranged in a concentric whorl, leaving narrow depressions. Such microridges located on the stratified epithelial cells play a major role in the anchorage of thin mucus film over the epithelial membrane to protect the olfactory epithelium from different hazardous substances.

In the receptor epithelium of *Cyprinus carpio* two types of sensory dendrites have been observed, the flagellated and the microvillous. The present study reveals that the flagellated receptor cells dominate over the microvillous receptor dendrites. In *Cyprinus carpio* the flagellar receptor cells are grouped together and these cells are of special interest because they form part of the olfactory transduction mechanism, are stimulated by odour-bearing substances and also enable the fish to detect its food.

One of the most interesting features of the present study is the behaviour of the secondary neurons. The axons of the secondary neurons extend into the central core of the lamella. The synaptic connection between the primary and secondary neurons indicates that the sensitivity of receptor cells may extend from the epithelial surface to the central core. The number of supporting cells is found to be smaller than that of the receptor cells in the receptor epithelium. The supporting cells give the basic structure of the sensory lamellae, having a microridge system on their surface. The cilia of the olfactory epithelium facilitate the flow of the water current over the olfactory lamellae. This flow removes the remains of the stimulating substances and keeps the receptors ready for new stimuli.

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