SEM-STRUCTURE OF THE OLFACTORY EPITHELIUM IN NEWBORN MOLE-RAT (*Cryptomys* sp., Bathyergidae, RODENTIA)

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Received September 24, 1996
Accepted November 13, 1996

Abstract

Míšek I., F. Tichý, H. Burda: *SEM-structure of the Olfactory Epithelium in Newborn Mole-rat (Cryptomys sp.,* Bathyergidae, Rodentia). Acta vet. Brno 1996, 65:321-328.

The structure of the olfactory epithelium of the ethmoidal labyrinth was studied in three newborn Zambian common mole-rats (*Cryptomys* sp.) by means of scanning electron microscopy (SEM). The olfactory epithelium was about 20 μm high and its surface was covered by a thick tangle of olfactory stereocilia of 3-5 μm length. Five to eight stereocilia protruded radially from club-shaped endings (of about 1μm in size) of dendrites of sensory cells. These olfactory terminals were regularly distributed over the epithelium surface. Differentiating olfactory endings were not recorded. Distal segments of olfactory stereocilia as well as their mutual contacts and branching were observed only sporadically. The apical surface of the supporting cells carried microvili of a length up to 1 μm. Fibrous surface structures of the olfactory epithelium were covered by secrets of olfactory glands.

Sense organs, olfactory cilia, microvili, development

The olfactory epithelium has been studied at microscopical and electron-microscopical levels particularly in humans (e.g., Busuttil et al. 1977; Moran et al. 1982), laboratory mammals (e.g., Breipohl et al.1973ab; Waterman and Meller 1973; Yamamoto 1976) and domestic mammals (Kratzing 1970; Menco et al. 1976, 1978; Menco 1977). Much less attention has been paid in this respect to wild mammals (but see e.g., Mendoza et al. 1992). Reviews of morphological aspects of the olfactory epithelium in vertebrates have been provided by e.g. Breipohl et al. (1974ab), Graziadei (1971, 1973ab, 1974, 1975), Seifert (1970), Kerjaschki (1978), Usukura and Jamada (1978), Getchell (1986). Several studies have been dealing with development, regeneration, and chemism of olfactory neurons: Cuschieri and Bannister (1974, 1975), Kerjaschki and Horandner (1976), Menco (1988), Moulton (1974, 1975), Franceschini and Ciani (1991), and others.

All the above studies demonstrated that the structure of the olfactory epithelium in different vertebrate species is rather comparable. The olfactory epithelium is composed of sensory (receptor), supporting, and basal cells that differ in their structure, localization in the epithelium, and function.

Most studies dealt with receptor cells, particularly with their dendritic endings (olfactory knobs) and the cilia protruding from those endings. The numbers and length of the olfactory stereocilia are species-specific (Graziadei 1974; Menco 1977) and they are the main correlates of olfactory capacity. Ciliary membrane contains the receptor proteins (Getschel 1986). Stereocilia form mutual contacts and attach also microvilli of the supporting cells (De Lorenzo 1970; Menco 1977; Menco et al. 1978).

The function of the supporting cells is not quite clear. They were compared with the glia cells (Breipohl et al. 1974b). They may have also a secretory function (Yamamoto 1976). The ultrastructure of the supporting cells has been reported to be sex-specific (Mendoza et al. 1992).

Basal cells of the olfactory epithelium are generally considered to be elements which may differentiate into other types of cells. Besides that they form a layer isolating axons of receptor cells.

Within a framework of a study dealing with sensory biology of subterranean mammals we had an opportunity to examine olfactory epithelium of newborn African common mole-rats (genus *Cryptomys*). Regarding the lack of any information on olfactory epithelium in mole-rate of the family Bathyergidae in special and subterranean rodents in general, publication of the data provides an important databasis for further comparative and functional studies. Besides that a relatively very long prenatal development (about 98 days) combined with the birth in a rather altricial stage and a slow postnatal development of *Cryptomys* mole-rats (Burda 1989) make these rodents of particular interest for any developmental studies.

Materials and Methods

Three Zambian common mole-rats (*Cryptomys* sp., karyotype 2n=68, population Lusaka, Zambia, family Bathyergidae), born in captivity, were killed (through decapitation under narcosis) within one week after birth, i.e. in a neonate stage (cf. Burda 1990). The nasal septum was removed and the ethmoidal labyrinth thus exposed. The tissue was immediately washed three times for a total of 15 minutes in physiological saline solution. Then it was fixed in formol (1.33 mmol/l). The material was dehydrated in ethanol (0.6, 0.7, 0.96, and 1.0 mmol/l) and acetone. Finally, the tissues were dried at critical point, and gold-stained (BALZERS SCD 040). The specimens were examined and photographed under scanning electron microscope TESLA BS 300.

Results

There was not a distinct separation between the respiratory and olfactory regions in the nasal cavity of neonatal mole-rats.

On average 5-8 olfactory cilia were radially protruding from the dendritic endings of receptory cells (Fig. 1), covering the surface of the olfactory region as a dense network of fibrous cone-like structures. The single endings were spherical or club-shaped, up to 1 μm of length and were spaced on the epithelium surface about 3-5 μm apart from each other. Some groups of cilia were irregularly covered by a mucous layer (Fig. 3). Differentiating olfactory endings or regions of a regenerating epithelium were not observed. The olfactory cilia were 3-5 μm (up to 7 μm) long. They were about 0.1-0.2 μm thick at the base and tapered off conically. The terminal part of the cilium formed a thin undulated fibre-like structure (Fig. 3). Also mutual contacts and/or branching of single cilia could be recorded (Fig. 1).

The olfactory epithelium (the structure of which may be well studied in the specimen fracturs (cf. Fig. 2) was on average 20 μm high, composed of receptor, supporting, and basal cells.

The neurocytes of receptor cells were situated in the middle third of the epithelium height. They were irregularly oval, 3-6 μm long, and were seperated from each other by slender cylindrical supporting cells. From the cell-body, a dendritic projection protruded towards the epithelium surface. It was about 0.5-1 μm thick and ended by a club-shaped swelling - the so-called olfactory knob (Fig. 3). The supporting cell attached to the dendrite. In an opposite direction, towards the basal membrane, a thin (about 0.2 μm) terminally brush-like branching axon was projecting (Fig. 4).

The supporting cells of the olfactory epithelium were slender and pervading the whole epithelium height. They were about 4 μm wide at the epithelium surface and were tapering off to about 0.5 μm towards the basal membrane. The apical surface of the supporting cells was differentiated into microvili (about 0.5 μm) which were interwoven into the network of olfactory cilia. In some places, outlets (about 6 μm diameter) of olfactory glands were observed (Fig. 3).

Basal cells of the olfactory epithelium were irregularly oval or cone-shaped. They were

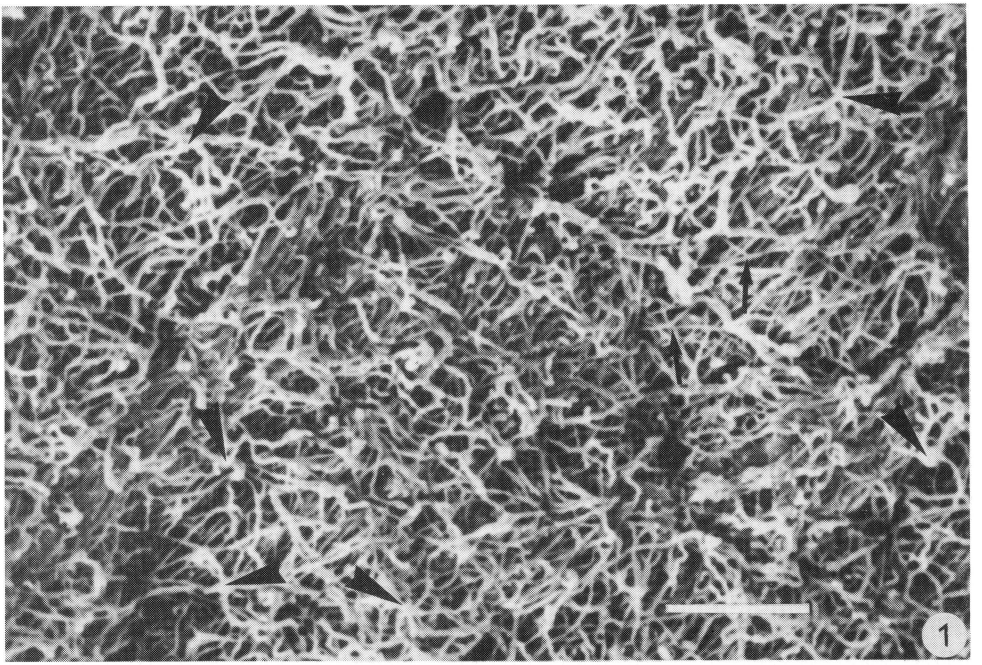

Fig. 1. The surface of mucosa in regio olfactoria. Olfactory endings (➤) with protruding cilia; Mutual contacts or ramification of distal segments of olfactory cilia (➡). Bar=5 μ

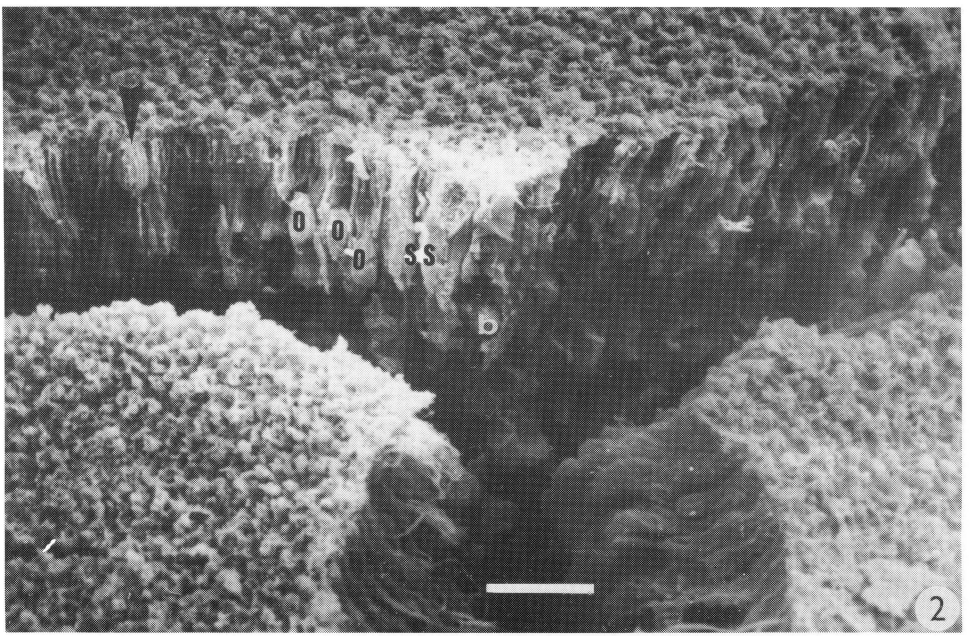

Fig. 2. The fracture of mucosa in regio olfactoria. Neurocytes of olfactory (receptor) cells (o); brush cell (➤); sustentacular cells (s); basal cell (b). Bar=10 μ

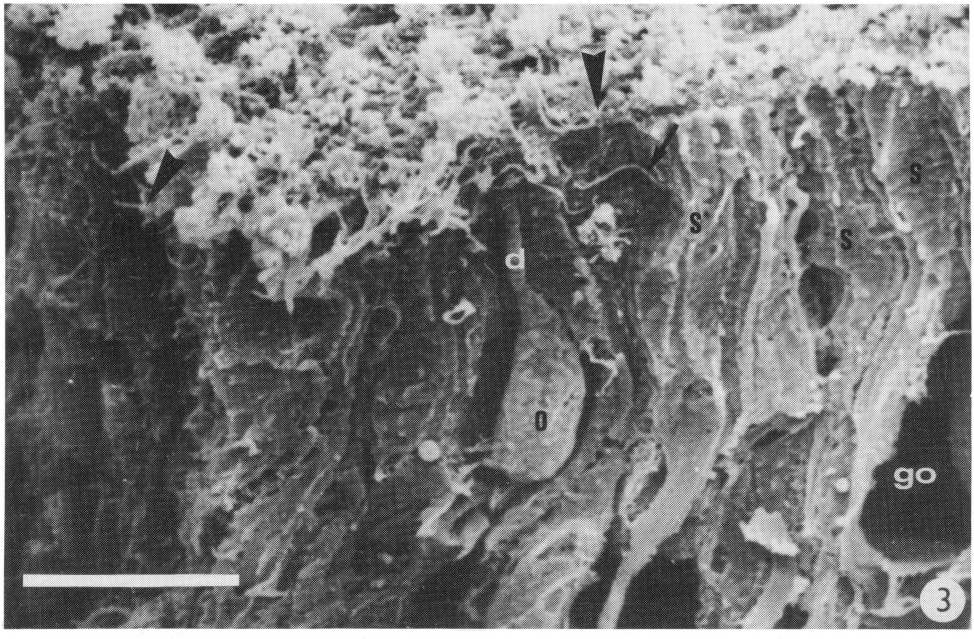

Fig. 3. Detailed view of fracture of the epithelium in regio olfactoria. Neurocyte of the olfactory (receptor) cell (o) with dendrite (d); olfactory endings (➤) with cilia; sustentacular cells (s); distal segment (➡); outlet of the olfactory gland (go). Bar =10 μ.

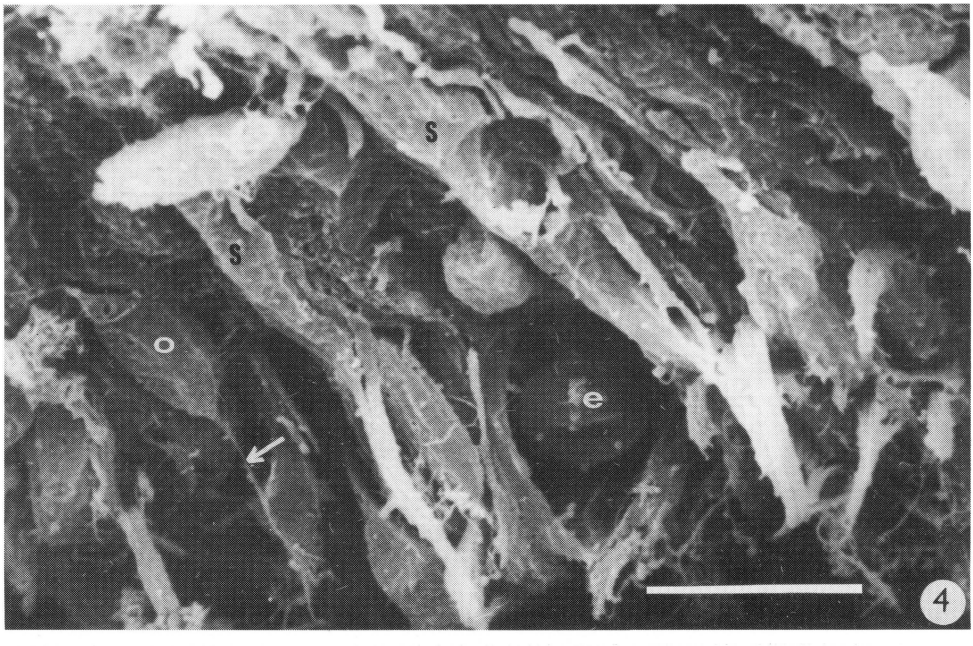

Fig. 4. The detail of fracture of the epithelium in regio olfactoria.Neurocyte of the olfactory (receptor) cell (o) with axon (➡); sustentacular cells (s); erythrocyte (e). Bar=10 μ.

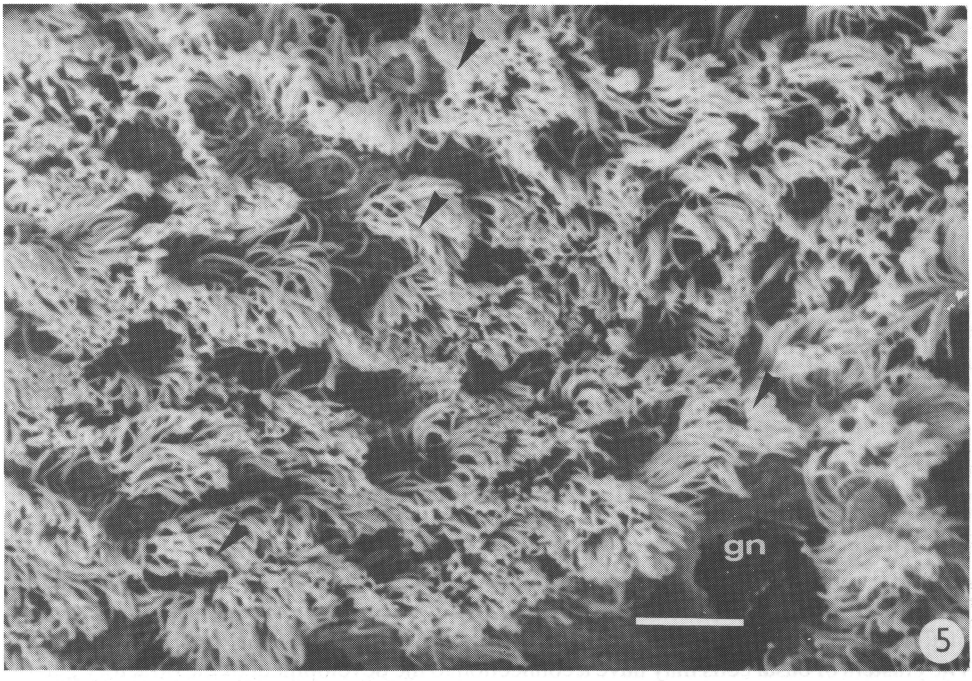

Fig. 5. The surface in regio respiratoria. Clusters of kinocilia (➤); outlet of the olfactory gland (gn). Bar=5 μ.

situated at the basal membrane with relatively large distances between neighbouring basal cells. Sporadically, however, in the vicinity of glandular outlets larger clusters of basal cells could be recorded. Basal cells were often related to axons of the receptor cells.

Barrel-shaped cells (Fig. 2) could be seen sporadically in the upper third of the olfactory epithelium between the apical segments of the supporting cells. A slender projection protruded from the bottom part of these cells towards the basal membrane.

The surface of the respiratory epithelium was covered by kinocilia (about 4 μm long) stretching as tufts outwards from the apical regions of the epithelium cells. Most cilia were bent in rostral direction. Between kinocilia, outlets of nasal glands were opening (Fig. 5).

Discussion

As stated above in the introduction to this paper, nothing is know about the structure of the olfactory epithelium in subterranean rodents. Even the functional capabilities of the olfactory sense in bathyergid mole-rats have not been exactly studied thus far, in spite of the fact that such information would be of great importance for many sociobiological models regarding e.g. the way how these rodents find food and recognize their kins (cf. Burda et al. 1990; Burda 1995).

The olfactory epithelium spreads over the whole ethmoidal labyrinth and the adjacent part of the nasal septum, i.e., over almost the whole caudal half of the nasal cavity. This large area of the olfactory region may prove to be of significance. However, it is known that the extension of the olfactory region depends upon the age of the animal and that it is not necessarily a measure of olfactory capabilities (Menco 1977, 1988). A large diversification of the olfactory surface represents also a remarkable finding. The density of

olfactory endings and the numbers of olfactory cilia are correlated (Menco 1977). Usually, the younger individuals posses about 20-30 per cent more olfactory cilia than adults (Mulvaney and Heist 1971; Menco et al. 1978). The olfactory capabilities may be dependent also on the height of the olfactory cilia. The height of 5 μm as found here for neonate mole-rats is rather low compared to literature data on some other species (Seifert 1970; Menco 1977).

The majority of olfactory cilia protruding from the dendritic endings of receptor cells corresponds to the proximal segments. This is concordance with previous findings in fetal sheep (Tichý et al. 1995). We assume that most distal segments develop only in later postnatal stages. The existence of contacts between olfactory cilia protruding from different endings was described also for some other species (Menco 1977; Menco et al. 1978; Usukura and Jamada 1978; Tichý et al. 1995).

Although a continuous development of apical segments of sensory cells has been regularly described in various animal species, even during the postnatal development (e.g., Waterman and Meller 1973; Cuschieri and Bannister 1975; Kerjaschki and Horandner 1976; Menco 1988), we have not recorded any sign of such differentiations.

While the surface area of the olfactory epithelium is relatively very large (see above), its height (20 μm) as stated in the present paper is surprisingly low as compared with data on other species. Qualitatively, as far as the structure form, and arrangements of particular cell types are concerned, there are, however, no apparent differences to other mammalian species. Nevertheless, the basal cells, which are considered stem elements for development of all other cell types (e.g., Moran et al. 1982), are comparatively very sparsely distributed. The clusters of basal cells may have a connection to the development of the olfactory glands (Cuschieri and Bannister 1975).

We may assume that the olfactory epithelium in neonate common mole-rats is structurally still immature. Although this conclusion would be in concordance with the altricial stage of naked mole-rats, it shoud be stated at this point that other sensory organs (like the inner ear and the eye) are apparently structurally and morphometrically mature already in neonate *Cryptomys* (our yet unpublished results, and Lindenlaub and Burda 1993). Hence further studies in adult mole-rats are needed to decide whether the described state of the olfactory epithelium represents an immature stage or already an adult state „frozen" in a juvenile-like stage.

Rastrovací elektronová mikroskopie čichového epitelu u novorozeného rypoše (*Cryptomys* sp., Bathyergidae, Rodentia)

Cílem práce bylo zjištění struktury čichové sliznice na úrovni SEM v období těsně po narození z hlediska její maturace a funkčnosti. Na souboru tří jedinců ve stáří 7 dnů po narození byla studována struktura čichového epitelu v rozsahu labyrintus ethmoidalis. Bylo zjištěno, že čichový epitel je v tomto stadiu vývoje cca 20 μm vysoký a jeho povrch je pokryt hustou spletí čichových řasinek délky 3-5 μm. Řasinky paprsčitě odstupují v počtu 5-8 z kyjovitě utvářených zakončení dendritů smyslových buněk. Tato čichová zakončení jsou na povrchu epitelu rozmístěna pravidelně a dosahují velikosti cca 1 μm. Diferencující se čichová zakončení nebyla nalezena. Distální segmenty čichových řasinek lze pozorovat jen sporadicky. Ojediněle dochází k vzájemnému kontaktu nebo větvení distálních segmentů. Apikální povrch podpůrných buněk pokrývá soubor mikroklků délky do 1 μm. Vláknité povrchové struktury čichového epitelu obaluje sekret glandulae olfactoriae. Podle zjištěných morfologických výsledků je zřejmé, že maturace čichového epitelu není u sledovaných mláďat ještě ukončena. Z hlediska funkčnosti čichové sliznice a tedy i uplatnění čichu ve sledovaném stáří lze předpokládat, že plně funkční je čichová sliznice až v pozdější periodě hnízdního vývoje.

Acknowledgement

This work was supported by grant No. 206/93/0492 of the Grant Agency of the Czech Republic.

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