Spatial pattern of sensory cell terminals in the olfactory sac of the tiger salamander. I. A scanning electron microscope study

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INTRODUCTION

Different odorants or groups of odorants elicit different spatial patterns of excitation in the olfactory epithelium and bulb of different species (Moulton, 1976). Some of these patterns, or components of them, could result from the differential sorption of molecules across the olfactory surface (imposed patterning). Indeed, Mozell & Jagodowicz (1973) have shown that the relative retention times differed when different odours flowed across the frog olfactory mucosa. Another form of patterning could depend on the spatial organization of receptors according to their odour specificities (inherent patterning). But unless precautions are taken to eliminate imposed patterning, it is difficult to know to what extent inherent patterning occurs. Studies which have achieved this separation employ a punctate odour delivery device (Kauer & Moulton, 1974). When this device is used and odour maps are derived by recording directly from the olfactory surface of the tiger salamander, the intrinsic patterns are found to be composed of two elements: a broad anterior-posterior differentiation of response (Kubie & Moulton, 1979; Kubie, Mackay-Sim & Moulton, 1980) on which is superimposed a more detailed pattern. For a given odorant, the detailed pattern typically consists of an irregularly shaped area of increased sensitivity to the odorant focused around a small region of greatly enhanced sensitivity. The position and shape of each area is specific for each odorant so far tested (including isomers) and suggests that – on a spatial basis – the resolving power of the olfactory epithelium for different odorants may be much greater than previously suspected (Mackay-Sim & Moulton, 1980).

Given this new emphasis on functional mapping of the olfactory epithelium of the tiger salamander, it becomes increasingly important, as an aid to odorant mapping, to know the detailed morphology of the olfactory surface. In addition, although some of the features of odour maps are unlikely to have morphological correlates, there could conceivably be other characteristics that could account for other functional properties at a broad regional level. Existing morphological studies of the olfactory epithelium of the tiger salamander stress the importance of variations in the local composition of the olfactory epithelium. However, they do not provide
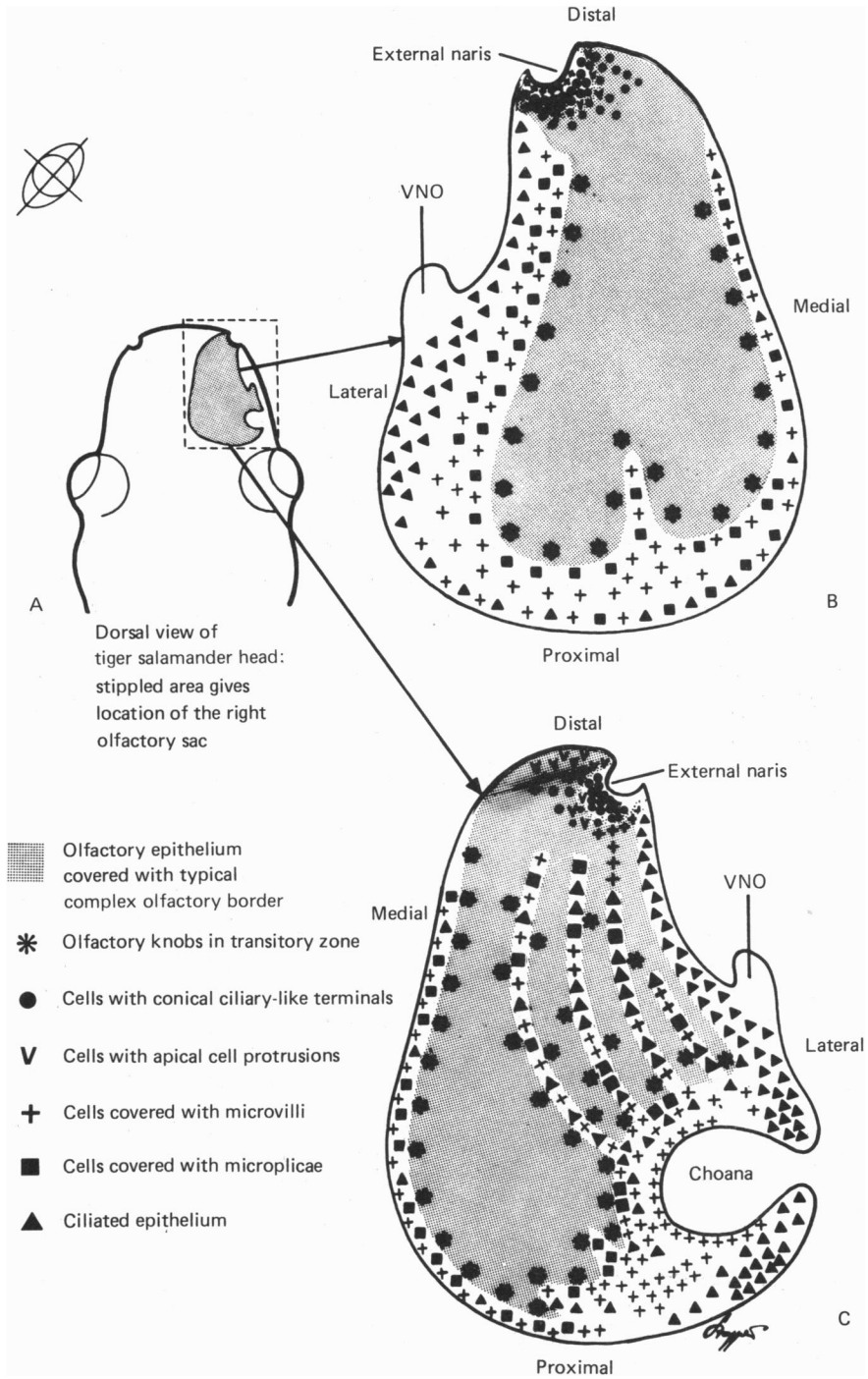
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Fig. 1. Schematic diagram of the location of the olfactory sac in the tiger salamander (A) and the spatial pattern of the different epithelial surfaces of its roof (B) and floor (C).

a detailed map of the olfactory surface (Graziadei & Monti-Graziadei, 1976). Therefore, the main objective of the present investigation is to provide such a map and to determine whether the surface morphology of the olfactory sac epithelium, as seen by scanning electron microscopy (SEM), reveals structural differences that correlate with any features of the spatial differentiation of response to odour as determined electrophysiologically.

MATERIALS AND METHODS

The epithelium of the olfactory sac in 12 tiger salamanders (*Ambystoma tigrinum*) was studied. Prior to killing, most of the animals were anaesthetized with tricaine. Others were killed by cervical decapitation. The olfactory chambers were washed many times with balanced salt solution to remove the overlying mucous film. Following the saline wash, the olfactory sacs were perfused with either 3·2 % glutaraldehyde and 2·6 % paraformaldehyde in cacodylate buffer (0·09 M; pH 7·35) or 0·1 M cacodylate-buffered 3·5 % glutaraldehyde. No morphological differences could be obtained between these groups. The heads were then stored in the same fixatives for several hours to several days. Glutaraldehyde fixation was followed by saline washing. After washing, the olfactory sacs were dissected free from the head and divided into a dorsal and a ventral half (Fig. 1). Prior to post-fixation of the tissue in 1 % osmium tetroxide for several hours, the dorsal and ventral halves of the olfactory sacs were photographed. The light photomicrographs were used for orientation of the olfactory sac in the head and to determine overall configuration of the organ. Further preparation of the samples for SEM was similar to that described previously (Breipohl, Bijvank & Zippel, 1973; Breipohl, Bijvank & Pfefferkorn, 1974).

OBSERVATIONS

The olfactory organ of the tiger salamander is composed of a bilateral olfactory sac located distal to the eyes (Fig. 1A). Typical olfactory ciliated sensory cells covered the greater part of both ventral and dorsal surfaces of the sac (Fig. 1B, C); however, various other cell types were noted in different regions (Figs. 1B, C, 2).

The epithelial surface around the distally situated external naris was mainly characterized by cells covered with microvilli and cells with conical cilium-like terminals (Fig. 1B, C). These cells formed a border around the narial opening (Fig. 1B, C).

Proximal to the external narial epithelium, the surface of the roof and floor was composed of diverse types of cells. In this region, cells were observed with microvilli, with apical swellings and stalked, bleb-like protrusions. The latter cells were identified as secretory in nature (unpublished TEM observations). Also visible were cells with cilium-like conical profiles (Figs. 1, 3, 4, 7, 23). They were specific to this region and extended slightly into the sensory area. The diameter of the conical cilium-like profiles (Figs. 3, 6, 23) was larger than that of cilia. Randomly distributed typically ciliated cells also occurred in this area.

Proximal to the region with the conical surface profiles, there was an epithelium with a typical olfactory border on both roof and floor (Figs. 8, 25). Here the sensory cilia covered the epithelial surface almost completely. In addition to the olfactory cilia, microvilli participated in the formation of the olfactory border. These microvilli originated not only from non-sensory cells, but also occasionally from the receptor
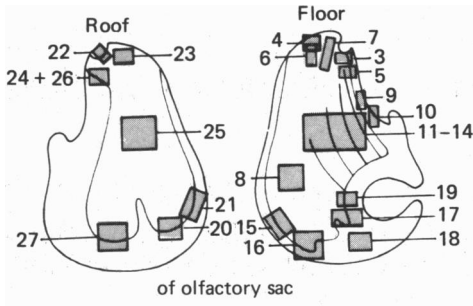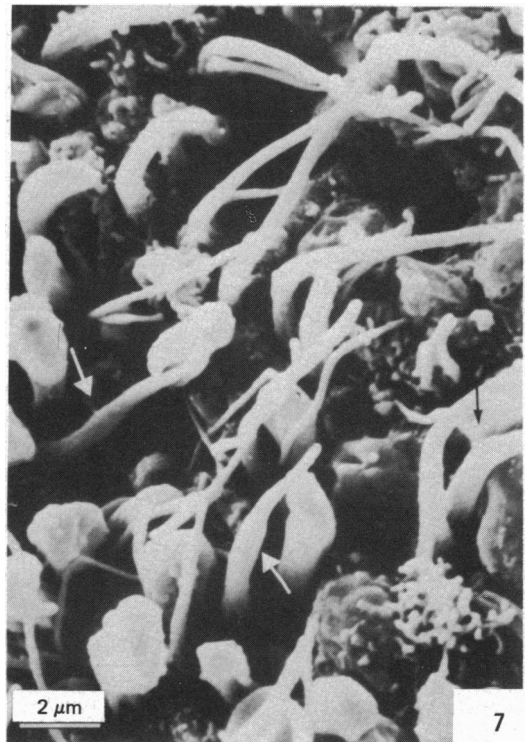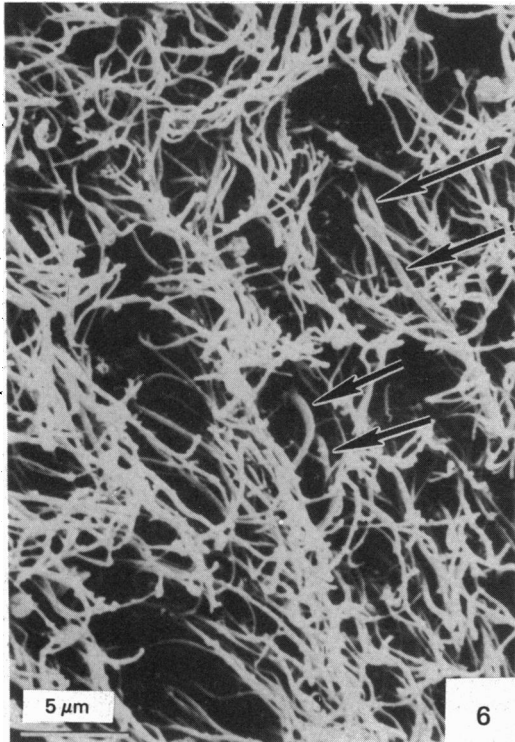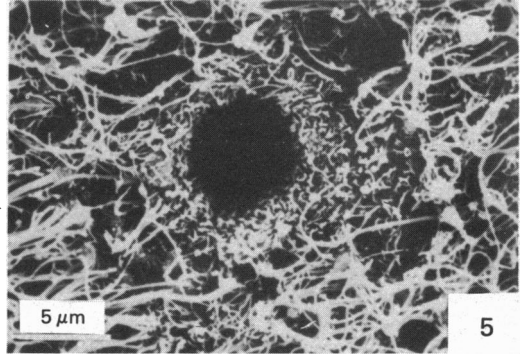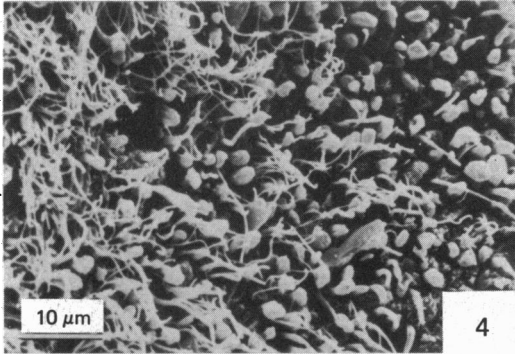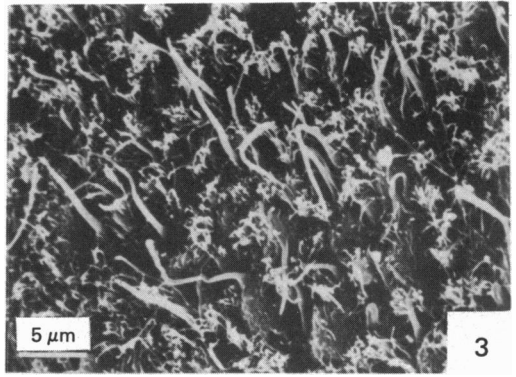
cells. The origin of the microvilli could best be seen in the transition zones from typical olfactory areas to the adjacent non-olfactory regions (Fig. 16). We have also observed glandular duct openings in these transition zones. However, they were hidden in the centre of the olfactory region by the long and numerous sensory cilia (unpublished TEM observations). The glandular duct cells typically showed numerous short microvilli evenly distributed over their apical poles (Fig. 5). The sensory cilia did not possess a distinctive thickened proximal part but gradually became thinner distally, a feature that could best be seen also in the transition zone from sensory to non-olfactory epithelia (Figs. 15, 16, 17–27). The surface of the olfactory cilia was not smooth, but showed irregularly distributed knob-like profiles of about 0·1 μm in diameter (Figs. 16, 19, 20).

The surface composition of the main olfactory area on the roof and floor of the olfactory sac was similar. However, on the floor of the sac, prominent stripes (or bands) free of sensory cells were present (Figs. 1C, 11–14). Such stripes, 3–7 in number, occurred on the lateral aspect of the sac floor. In contrast, only one short band, located in the proximal region of the sac, was noted on the roof (Fig. 2). The sensory cell-free bands on the floor of the sac extended to the epithelium surrounding the choana (Figs. 1C, 17). The surface of these epithelial stripes was composed of ciliated cells (Figs. 11–14), cells with microvilli (Figs. 11–13), and cells with microplicae (Fig. 14). Bands, which were mainly composed of ciliated cells, protruded above the level of the epithelial surface (Figs. 11, 13). Stripes formed of cells with microvilli and microplicae, containing only randomly interspersed ciliated cells, were depressed below the apical surface level of the adjacent epithelium (Figs. 12, 14).

The epithelium surrounding the choana was similar to that of the external naris in that it was populated by cells covered with microvilli. However, more proximo-lateral regions of the choana contained more ciliated cells than regions around the naris. Further, more glandular openings were observed around the choana (Figs. 17, 18).

In the transition zone between the epithelium that surrounded the choana and the sensory epithelium, isolated olfactory knobs could be seen in a similar pattern to that noted in the periphery of the stripes of cells covered with microvilli or microplicae (Figs. 15, 21). The medial region between roof and floor was lined with cells covered with microvilli, microplicae, and occasionally with cilia (Figs. 1B, C). The lateral area between roof and floor was covered with a ciliated epithelium (Fig. 9). Within this lateral area, large protrusions, isolated or grouped, were noted emerging between the ciliated cells (Fig. 10). The lateral region extended ventrally to the

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Fig. 2. Diagrammatic representation of the regions illustrated in Figs. 3–27. The numbers 3–19 refer to the different parts of the floor and 20–27 to those of the roof.

Fig. 3. Epithelial surface area with many conical, cilium-like profiles emerging from roundish apical bulgings of cells. Groupings of microvilli occur between these cells. × 1830.

Fig. 4. Surface area with many secretory active cells interspersed with conical, cilium like structures and the transition (left) to the olfactory epithelium proprium. × 1150.

Fig. 5. Glandular duct opening in the transitional region between the areas shown in Fig. 3 and the main olfactory area. × 2410.

Fig. 6. Transitional region between area shown in Fig. 4 and area with well developed olfactory border (Fig. 8). In addition to cilia of normal configuration, conical cilium-like structures are occasionally present (arrows). × 2670.

Fig. 7. Transitional region between area shown in Fig. 3 and area with secretory cells (Fig. 4). Note appearance of presumed compound cilia (arrows). × 5000.
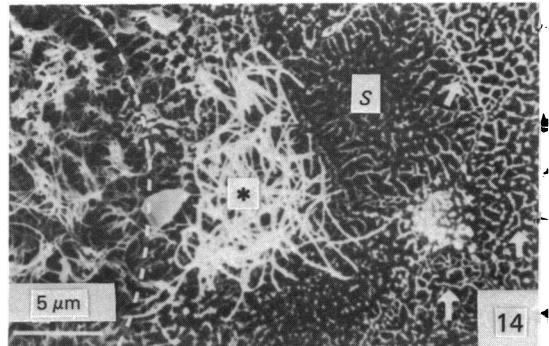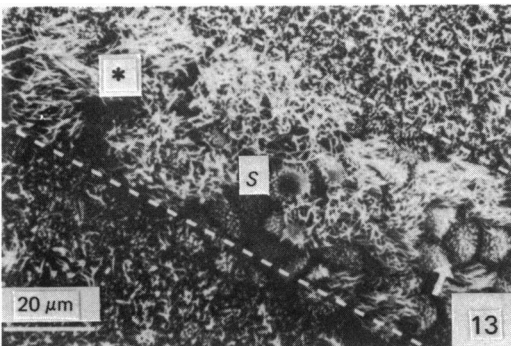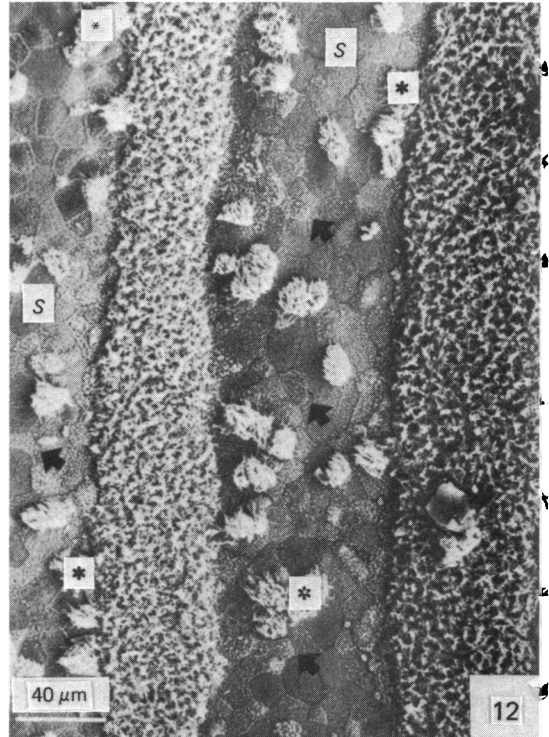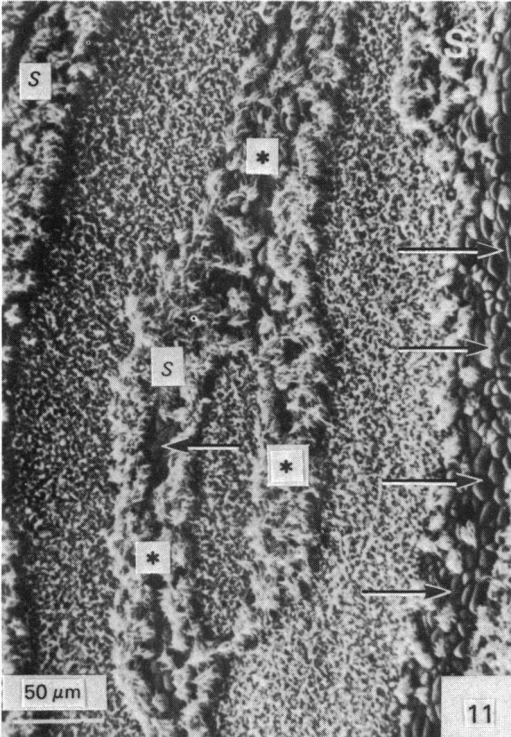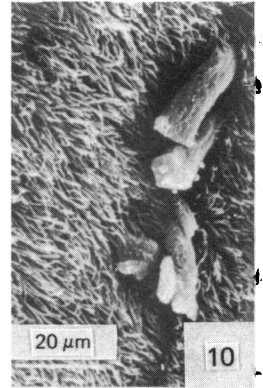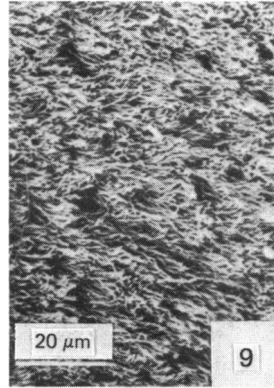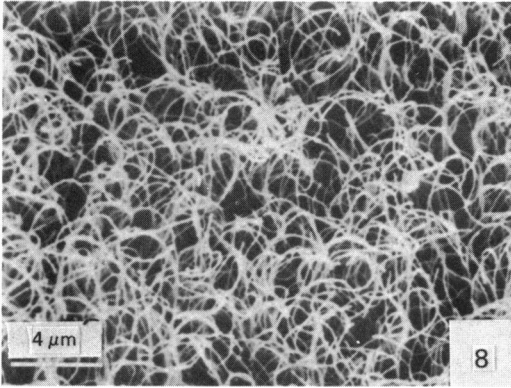
olfactory area while dorsally the ciliated epithelium was separated from the sensory area by a small zone of cells with microvilli and microplicae (Figs. 1 B, C, 2, 24, 26).

In addition to the above mentioned differences in the epithelial composition of the floor and roof, one other feature was observed. In one animal, near the external naris, an area was seen on the floor which was characterized by many microvillus-like profiles radiating in a rather orderly fashion from a central origin (Fig. 22).

DISCUSSION

The present investigation has revealed that: (1) most of the olfactory sac of the tiger salamander is lined by olfactory epithelium, (2) the olfactory epithelium is uniform in appearance at the SEM level, (3) the cilia of the olfactory cells, in contrast to mammalian cilia, lack a thick proximal and thin distal segment, and (4) a variety of other cell types (non-sensory or sensory) are present which have particular locations on the floor and roof of the sac.

Although the olfactory border of the tiger salamander appears uniform at the SEM level, the sensory cilia are different from those of mammals. SEM examination revealed that the cilia of the tiger salamander taper continuously proximodistally. A similar type of cilium has been described on fish olfactory cells (Breipohl *et al.* 1973). In contrast, mammalian and frog olfactory cilia are characterized by a short, thick proximal and a long, thin distal segment (Reese, 1965; Andres, 1966; Moulton & Beidler, 1967). Reese proposed that the proximal segments are more motile than the thin distal ends. The olfactory cilia of tiger salamanders and fish could therefore have different motility when compared with mammalian and anurian cilia. However, this possibility remains to be documented. If movements of sensory cilia are reduced, the immediate environment (glandular secretions and non-sensory kinocilia) becomes of prime importance for competent olfaction or modulation of this sense or both. To date, there are several reports which provide evidence for regional patterning of response to odours in the olfactory mucosa and olfactory bulb and for the mapping of the epithelium onto the bulb of the tiger salamander and other species (Adrian, 1951; Le Gros Clark, 1951; Moulton, 1967; Mustaparta, 1971; Land, 1973; Breipohl, Zippel, Rückert & Oggolter, 1976; Kauer & Moulton, 1974; Kubie *et al.* 1980; Mackay-Sim & Moulton, 1980). Morphological studies (Graziadei & Monti-Graziadei, 1976) of the olfactory epithelium of the tiger salamander, although stressing the importance of variations in the local composition of this epithelium, do

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Fig. 8. Typical appearance of the olfactory border (floor) with whorl-like arrangement of the olfactory cilia. × 2500.

Fig. 9. Typical appearance of respiratory-like, ciliated surface, × 530.

Fig. 10. Surface of respiratory-like, ciliated epithelium with protrusions. × 820.

Fig. 11. Area of bands (*S*) within the olfactory epithelium (floor). The bands are composed of cells covered with cilia (*) and microvilli (arrows). × 220.

Fig. 12. Area of bands (*S*) within the olfactory epithelium (floor). The bands are composed of cells covered with microvilli (arrows), microplicae and cilia (*). The apical cell poles are flat. × 290.

Fig. 13. Detail of an area in Fig. 11 showing cells with cilia (*) and microvilli (arrow). Dotted line indicates border of non-olfactory epithelial band (S). × 580.

Fig. 14. Detail of an area in Fig. 12. A non-olfactory epithelial band with cells covered with microplicae (arrows) or cilia (*). Dotted line indicates border to olfactory epithelium. × 2000.
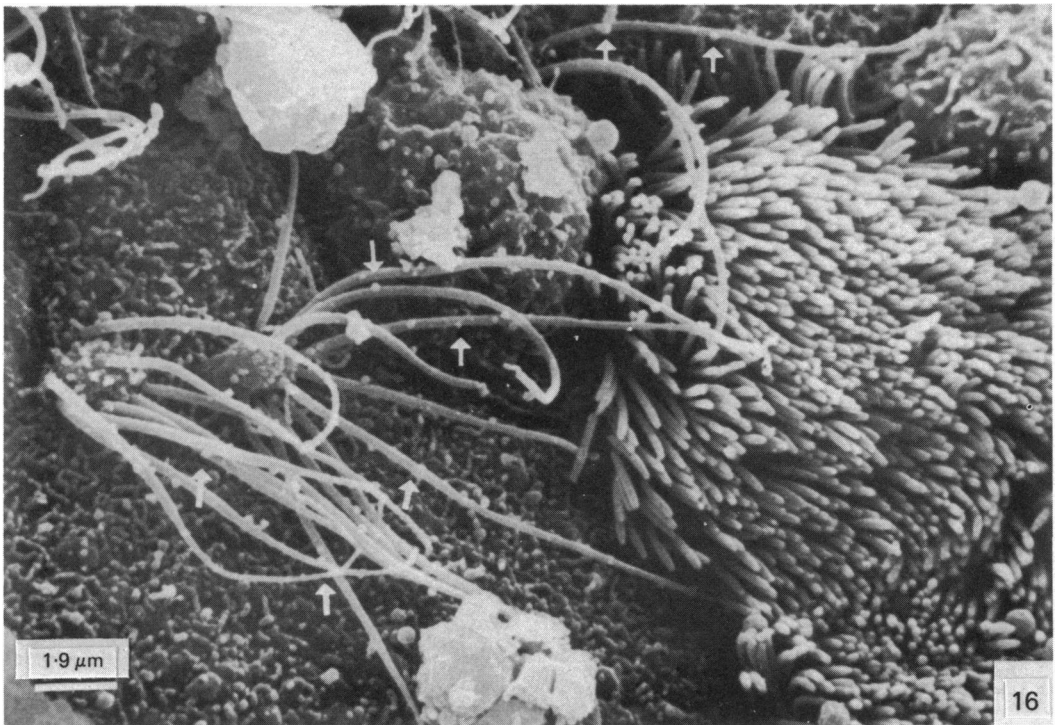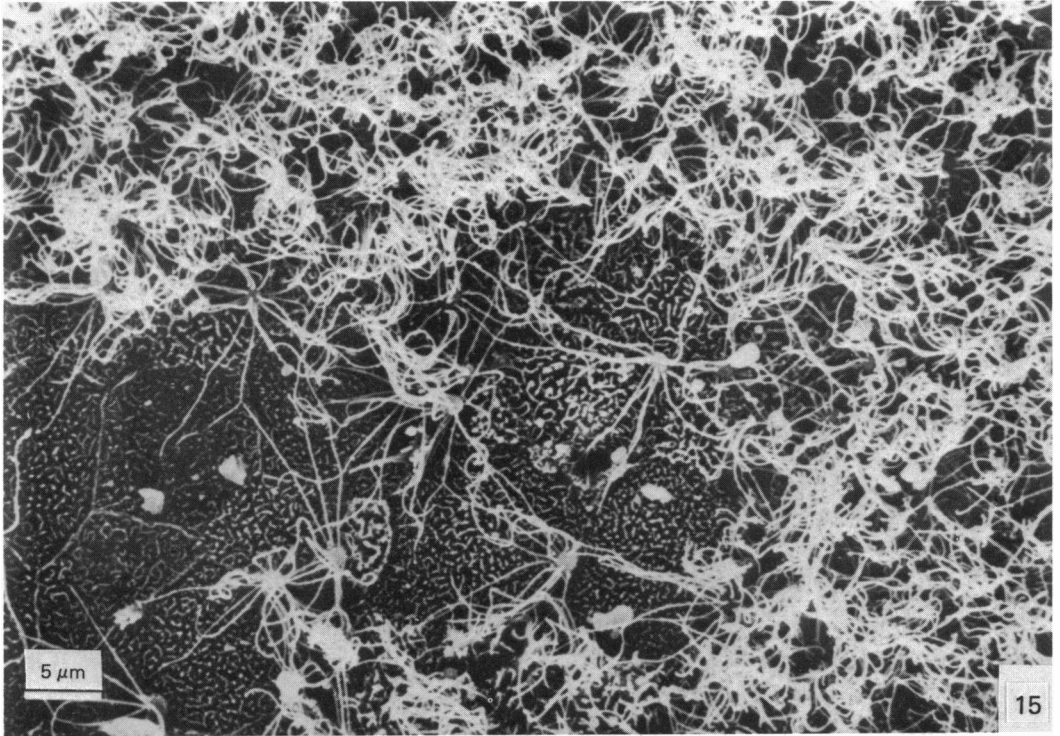
Fig. 15. Transitional area of olfactory epithelium to more proximally located epithelium composed of cells covered with microvilli and microplicae. × 1800.

Fig. 16. Two olfactory knobs with long olfactory cilia in similar area to that in Fig. 15, but somewhat more proximally located. Note distal thinning of the olfactory cilia and small bleb-like structures on them (arrows). × 5300.
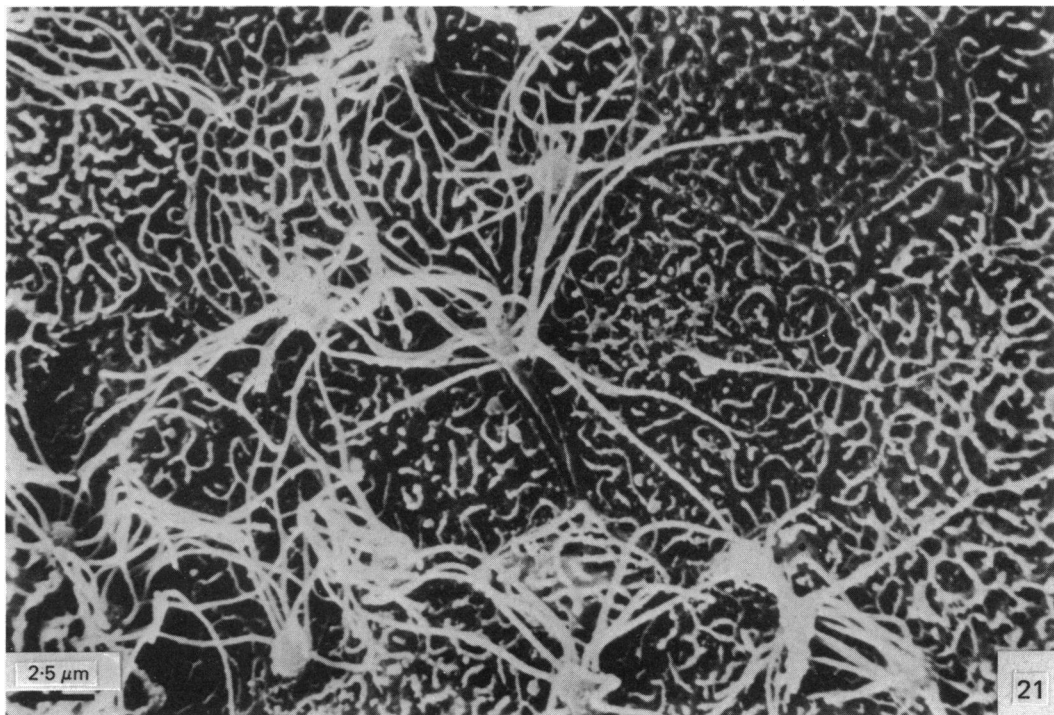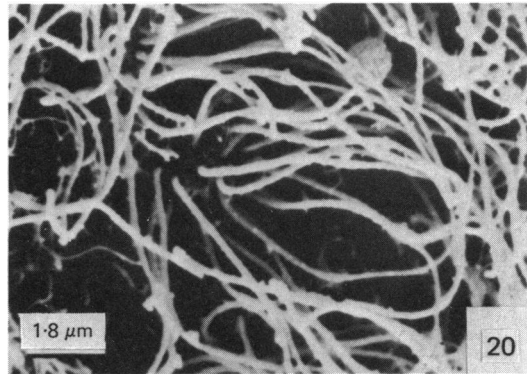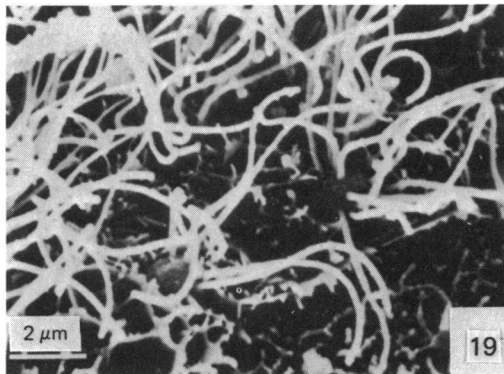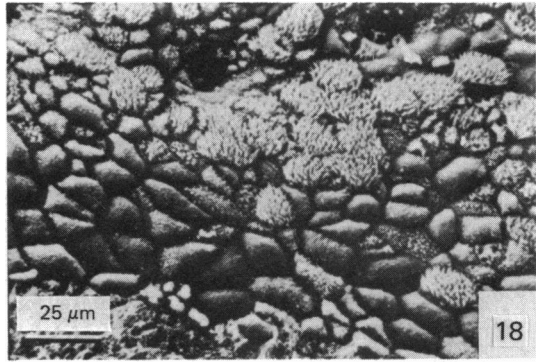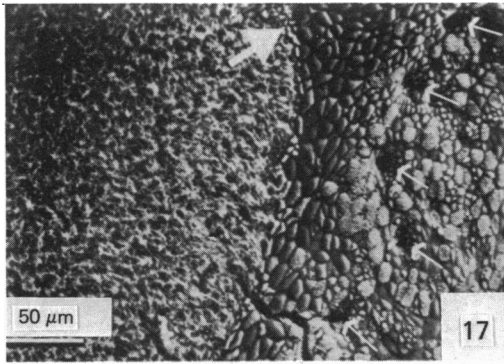
Figs. 17 to 21. For legends see p. 767.

5 µm

22

3 µm

23

10 µm

24

10 µm

25

2·9 µm

26

3·3 µm

27
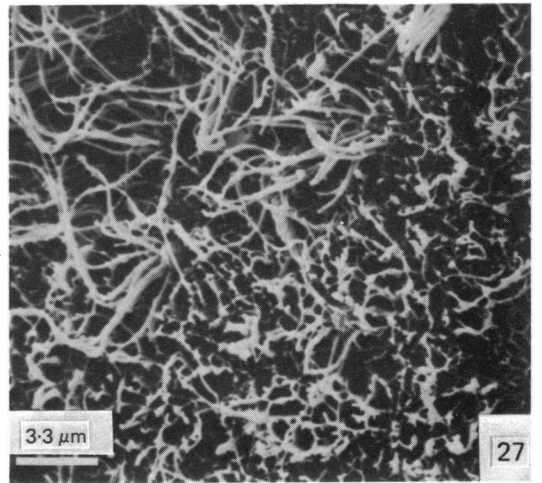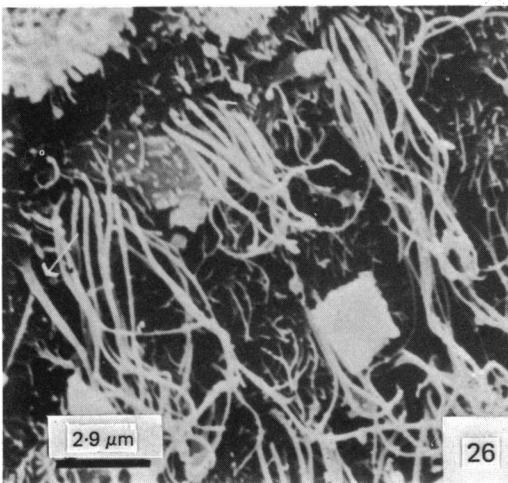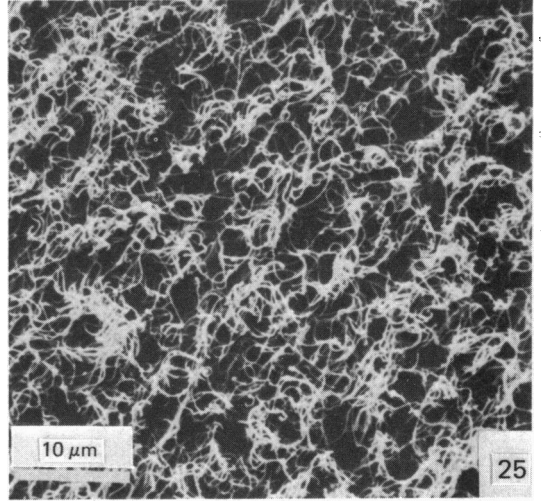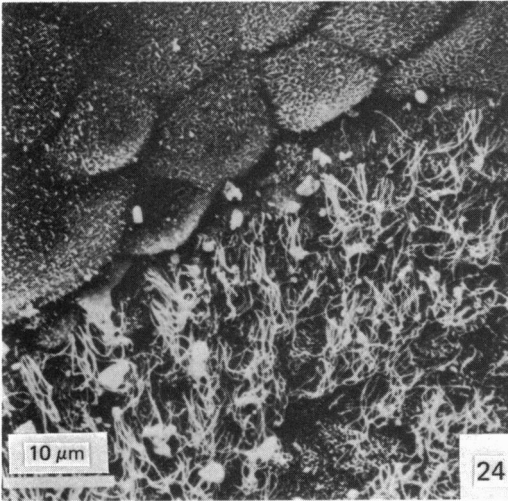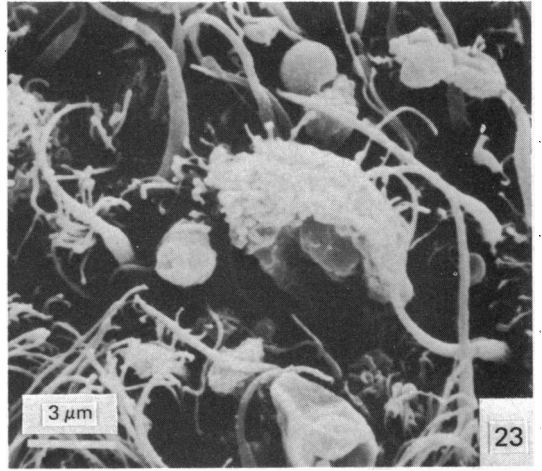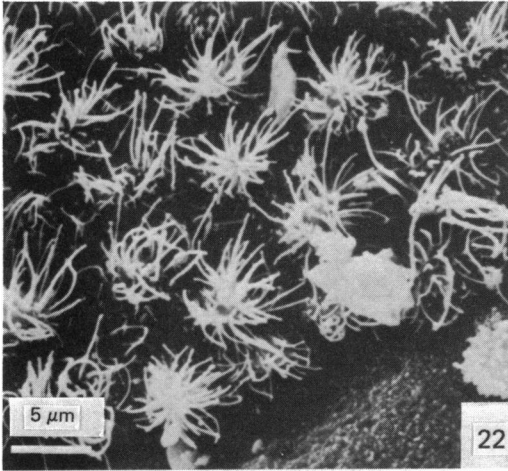
not correlate with data that indicate regional variation in olfactory sensitivity obtained by electrophysiological investigations. The present SEM study reveals a clear variation in the overall composition of the epithelial surface of the olfactory sac of the tiger salamander although it does not indicate any variation in areas covered with typical olfactory epithelium. The most anterior region of the olfactory sac was characterized by cells with protruding surfaces, which appear to be secretory in nature (unpublished observations). Intermingled between these presumably secretory cells, long conical ciliary-like profiles were noted in the olfactory sac of the tiger salamander. These profiles resembled the compound cilia, or rod-shaped terminals, found in the olfactory organ of fish (Bannister, 1965; Schulte & Holl, 1971; Breipohl *et al.* 1973; Breipohl, Zippel & Oggolter, 1977; Breucker, Zeiske & Melinkat, 1979) and chicks (Laugwitz, 1980). Whether the conical profiles are sensory in nature remains to be determined.

In comparing the present findings with the spatial patterns of excitation elicited by different odours, the most relevant electrophysiological data are the odour maps and two-point comparisons derived by direct punctate stimulation of the olfactory surface in the tiger salamander (Kubie *et al.* 1980). These data show that most odorants are more effective stimulants either anteriorly or posteriorly. Unfortunately, the considerable animal-to-animal variation that exists in the sac and the different shapes of epithelial maps preclude the possibility of locating the exact margins of electrophysiologically responsive mucosa. In general, however, recordings were made from positions on the ventral mucosa identified in Figure 1 as typical olfactory epithelia. There are no features in this region that could account for the antero-posterior differentiation in responsiveness to odorants. However, a tendency for the anteromedial area to be less sensitive to most odorants than other regions might be related to the greater concentration of bands composed of heterogeneous types of cells in that region (Fig. 1). Whatever their functional properties might be, the

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Fig. 17. Transition from olfactory epithelium (left) to epithelium that typically surrounds most parts of the choana (right). Note glandular openings (thin arrows). Large arrow indicates origin of non-olfactory epithelial band. × 160.

Fig. 18. Higher magnification of epithelial area in Fig. 17 (right). × 400.

Fig. 19. Border of olfactory epithelium as visible in Fig. 17 but under higher magnification. Note olfactory knobs surrounded by cells covered with microplicae and microvilli. × 5000.

Fig. 20. Area as in Fig. 19 but on roof. More than 10 olfactory cilia can be seen originating from the olfactory knob. × 5330.

Fig. 21. Area as in Fig. 15 but on the roof. Note differences in number and length of the olfactory cilia per given olfactory knob and note splitting of the intercellular clefts by the olfactory terminals. × 4000.

Fig. 22. The most distal area of the roof around the external naris depicting microvillus-like profiles radiating in orderly fashion from central origins. × 2330.

Fig. 23. Distal olfactory sac epithelial surface. Comparable area to that shown in Fig. 4 but on roof. × 3330.

Fig. 24. Transitional region from distal non-sensory (upper half) and sensory olfactory area (lower half). × 800.

Fig. 25. Olfactory border of the roof. × 1200.

Fig. 26. Higher magnification of the transitional region in Fig. 24. Note grouping of suspected normal olfactory cilia and occasional conical cilium-like structures (arrow). × 3420.

Fig. 27. Proximal transitional region from olfactory (upper left) to non-sensory epithelium (lower right) on roof. Note the well developed olfactory knob surrounded by cells with long microvilli and (lower right corner) transition to cells covered with microplicae. × 3080.

existence of these bands implies that recordings from this area might be expected to show greater variance than recordings from areas where the epithelium has a more uniform appearance. Similarly, on the dorsal epithelium the indentation of the posterior margin of the region covered with typical olfactory cells warns the electrophysiologist that special care should be taken in mapping this region. On the other hand, although smaller in total extent, the dorsal mucosa appears to be free of bands of heterogeneous cells and may therefore be expected to yield odour maps having less variance between animals.

Based on present morphological observations and existing literature, it should be stressed that physiological studies should be mindful of the variable cell composition of the olfactory sac because recording from areas separated by minute distances could lead to considerably different results.

SUMMARY

The olfactory sac of adult tiger salamanders (*Ambystoma tigrinum*) was assessed by scanning electron microscopy and the hitherto unknown variety of the olfactory sac cells has been documented. Regional differences in respect to the composition of the apical epithelial surface border in the olfactory sac have been recorded. Transitions between the olfactory epithelium proper and epithelia with different surface structures have been described. It has been shown that both the roof and the floor of the olfactory sac are mainly covered by sensory epithelium with a surface of uniform appearance. However, the sensory area of the floor is interspaced with non-olfactory epithelial bands. Variations were also noted in regard to secretory cells, cells with conical profiles, microplicae arrangements and populations of microvilli and kinocilia. These differences have been discussed in relation to electrophysiological data which suggest a regional patterning in the response to odours.

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