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Evidence for orthogonal arrays of particles in the plasma membranes of olfactory and vomeronasal sensory neurons of vertebrates

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Summary

Plasma membranes of sensory neurons from the olfactory and vomeronasal neuroepithelia of the male rat and olfactory neuroepithelium of the tiger salamander (*Ambystoma tigrinum*) have been examined, using the freeze-fracture technique, for the presence and morphology of orthogonal arrays of particles (OAP).

Numerous OAP were scattered on the P-face of plasma membranes of the dendrites and cell bodies from rat vomeronasal sensory neurons. The OAP were 720 ± 200 nm^2 in area and they consisted of 4 to 20 particles whose centre-to-centre distance was about 7 nm. On the E-face, complementary orthogonal arrays of pits were observed. No OAP were detected in the olfactory sensory neurons of the rat.

In the dendritic and perikaryal plasma membranes of the tiger salamander olfactory sensory neurons, OAP 2230 ± 970 nm^2 in area were observed on the P-face. The OAP consisted of 12 to 36 particles. The centre-to-centre distance of the particles was about 7 nm. In the olfactory receptor cell plasma membranes of this species, OAP formed complexes of 2 to 28 individual OAP, the longitudinal axes of which were usually arranged in parallel. Complementary complexes of orthogonal arrays of pits were observed on the E-face.

Introduction

Olfactory and vomeronasal neuroepithelia are chemosensory stations, which are present in most mammals (Moulton & Beidler, 1967; Wysocki, 1979). The functions of the two epithelia appear to be, at least to some extent, independent of one another. The olfactory epithelium responds to a large variety of chemical stimuli, whereas physiological and behavioural studies on the vomeronasal neuroepithelium have demonstrated that it is mainly involved in perception of chemical stimuli related to sexual and reproductive regulation (Winans & Powers, 1977; Wysocki, 1979). In olfactory and vomeronasal neuroepithelia, bipolar sensory neurons are present, the axons of which establish

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synaptic contact in the glomeruli of the main and accessory olfactory bulb respectively (Cajal, 1911).

Orthogonal arrays of particles (OAP) (nomenclature according to Bordi & Perrelet, 1978) are plasma membrane specializations visualized by means of the freeze-fracture technique. They have been described by many authors in different cells from a variety of organs and species, including the nervous system of vertebrates (reviewed by Bordi & Perrelet, 1978; Hatton & Ellisman, 1981; Anders & Brightman, 1982). The first and only evidence for OAP in neuronal cells of vertebrates was described by Usukura & Yamada (1978), who observed OAP on the olfactory receptor cells in the newt. In contrast, Kerjaschki & Hörandner (1976) reported that OAP were only present in supporting cell membranes of mouse olfactory epithelium. The aim of the present study has been to investigate the presence and morphology of orthogonal arrays of particles in the olfactory and vomeronasal sensory neurons of the rat. In addition, the olfactory receptor cells of an amphibian, the tiger salamander, have been investigated in order to clarify the different findings reported by Kerjaschki & Hörandner (1976) and Usukura & Yamada (1978) regarding the presence of OAP in such cells.

Materials and methods

Vomeronasal and olfactory neuroepithelia of eight male Wistar rats and the olfactory neuroepithelia of four tiger salamanders (*Ambystoma tigrinum*) were investigated. The rats were anaesthetized with Nembutal and perfused intravascularly using 3.2% glutaraldehyde and 2.6% paraformaldehyde in cacodylate buffer (0.09 M, pH 7.35). The olfactory neuroepithelia of the tiger salamander were fixed after decapitation by immersion in the same fixative. The dissected olfactory and vomeronasal neuroepithelia were cut into pieces and kept in the fixative for several hours, washed in Hanks' solution and cryoprotected in a graded series of glycerol solutions (10, 20 and 30%). The samples were then frozen in liquid Freon 22 and stored in liquid nitrogen. Fracturing was done at -110° C in a Balzers 360 M unit at a pressure of 2×10^{-6} Torr. The fracture surface was shadowed with carbon–platinum at an angle of 45° and carbon was then evaporated at an angle of 90°. The replicas were cleaned in bleach and 40% chromic acid, then washed in distilled water.

Measurements and counts were carried out with the aid of a Leitz calibrated magnifying glass on micrographs at a final magnification of 75 000. The following parameters were determined: centre-to-centre distance of adjacent particles; number of particles per OAP; and size of OAP. The size of an OAP was defined as the area occupied by this OAP and it was calculated by multiplying

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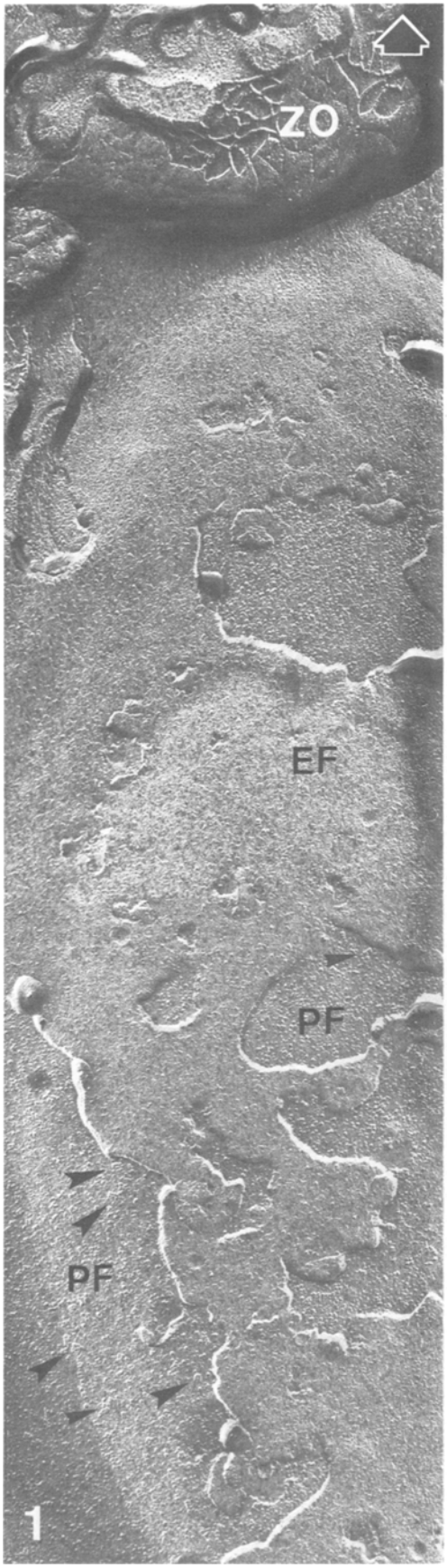
Fig. 1. Dendrite of a vomeronasal sensory neuron of the rat, partially covered by the E-face of the plasma membrane of the neighbouring supporting cell (EF). Note the meshwork of the *Zonula occludens* (ZO) and the presence of numerous orthogonal arrays of particles (arrowheads) on the P-face of the plasma membrane of the vomeronasal sensory neuron (PF). Thick arrow in all figures indicates the direction of shadowing. × 40 000.

Fig. 2. High magnification of the dendritic plasma membrane of a rat vomeronasal sensory neuron showing OAP (arrowheads) dispersed among background particles. × 128 000.

Fig. 3. E-face of the plasma membrane of a rat vomeronasal receptor cell. Note orthogonal arrays of pits (encircled) complementary to OAP. × 128 000.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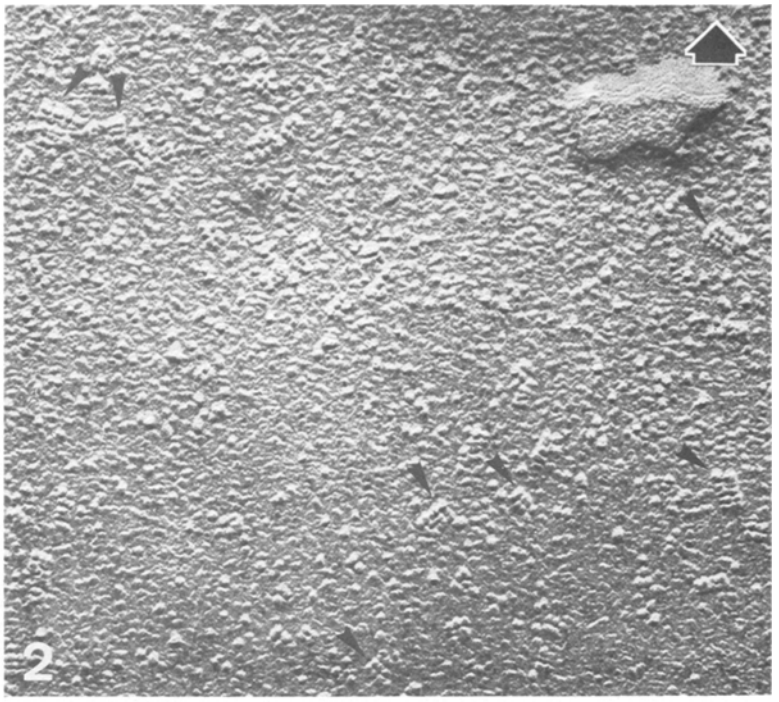
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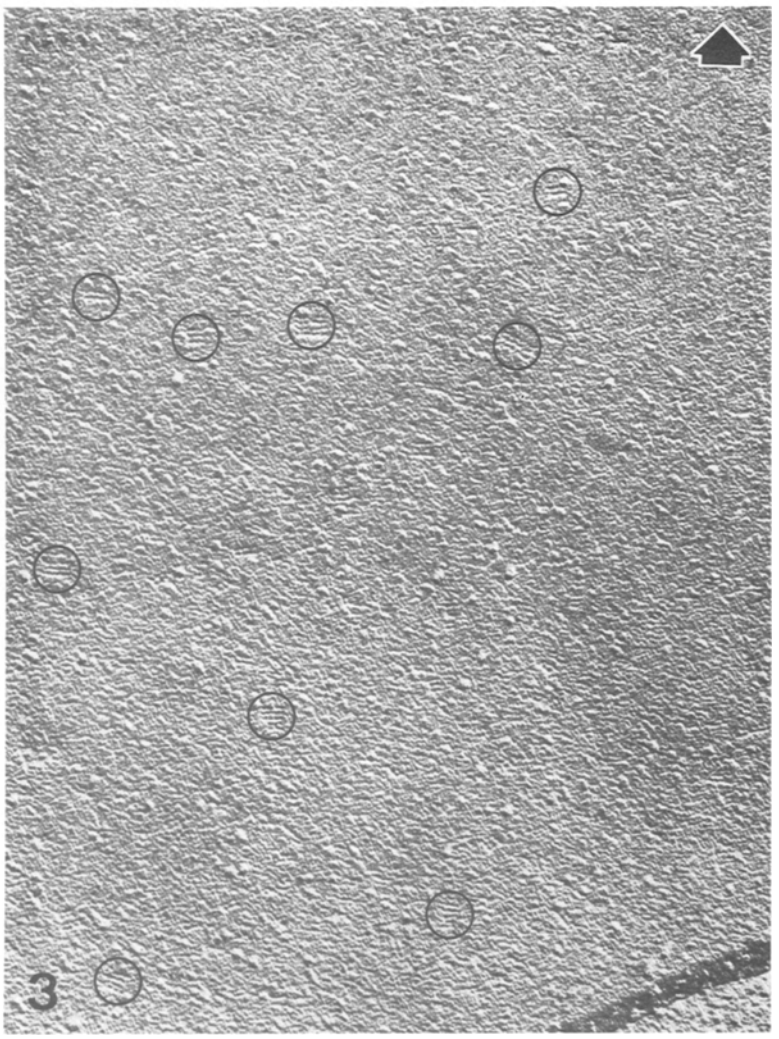
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Fig. 4. Region of the perikaryon of a rat vomeronasal sensory neuron. In the nucleus (N), pores (arrows) are visible while numerous OAP (arrowheads) are present on the P-face of the plasma membrane. × 80 000.

its length by its width. In each group, the size of the OAP was obtained from measurements based on 50 OAP belonging to 30 receptor cells in the rat vomeronasal neuroepithelium and 23 receptor cells in the olfactory neuroepithelium of the tiger salamander. In addition, since in the olfactory receptor cells of the tiger salamander, OAP formed characteristic complexes consisting of several OAP (Figs. 5,6), the number of these structures forming a complex were determined in the

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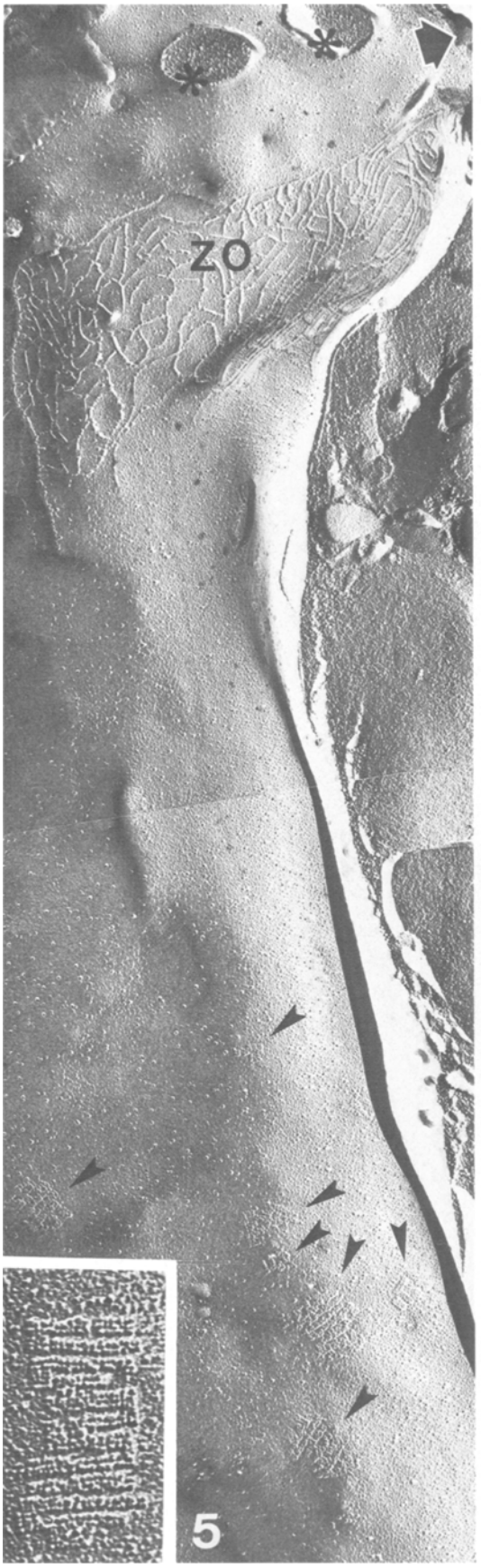
Fig. 5. Receptor cell of the olfactory epithelium of the tiger salamander. Note two fractured cilia (asterisks), a well-developed *Zonula occludens* (ZO) and numerous OAP (arrowheads) forming characteristic complexes. × 50 000. Inset shows a detail of a complex consisting of several OAP. × 106 000.

Fig. 6. E-face of the plasma membrane from the tiger salamander olfactory receptor cell at the level of the perikaryon, displaying numerous orthogonal arrays of pits, which form complexes of variable size. × 104 000.



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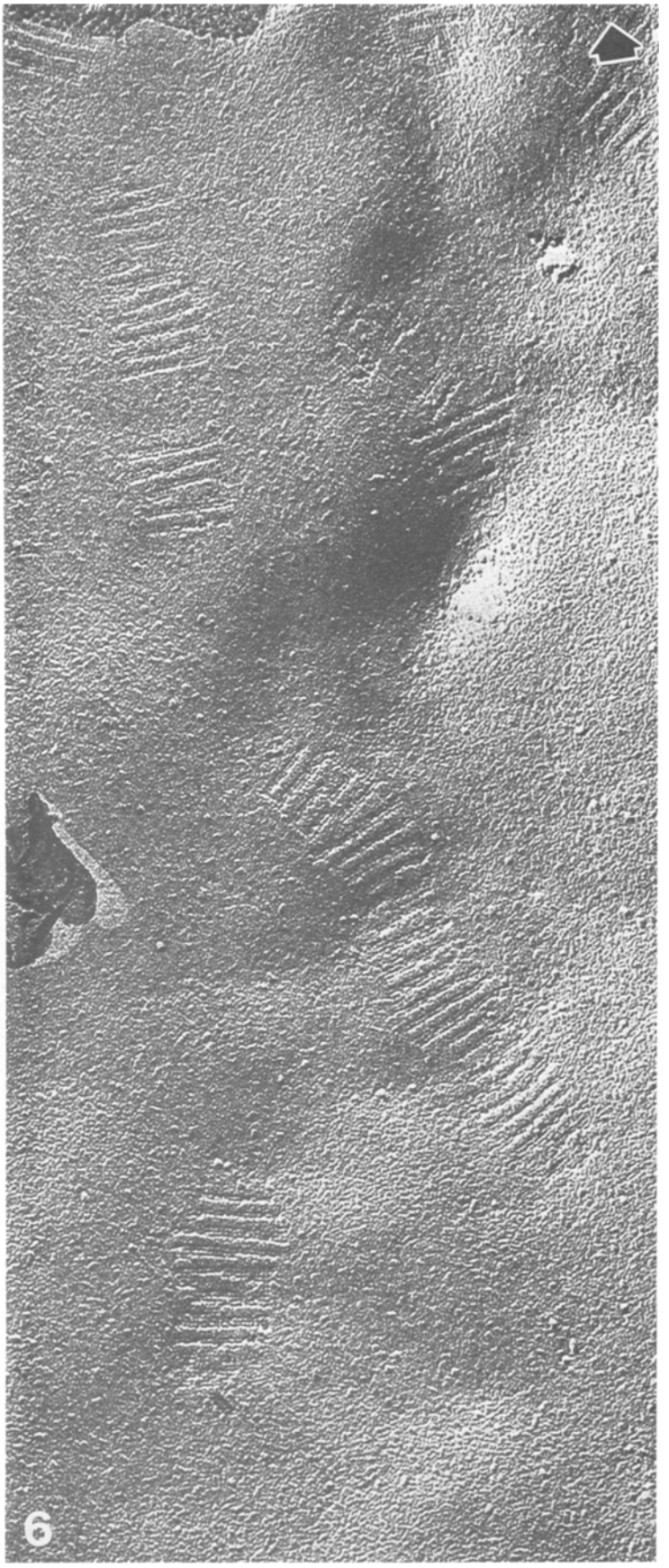

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olfactory receptor cells of this species. In all, 44 complexes of OAP belonging to 23 receptor cells were investigated.

For estimation of the density of individual intramembranous particles (IMP) the method previously described by Miragall & Mendoza (1982) was employed.

Results

Olfactory and vomeronasal neuroepithelia are composed chiefly of supporting, neuronal and basal cells.

Rat

In the vomeronasal receptor cells, OAP were generally observed on the P-face of the plasma membranes (Figs. 1,2,4). To confirm that these plasma membranes really belonged to receptor cells, the method previously described by Miragall & Mendoza (1982) to discriminate between receptor and supporting cells in the rat vomeronasal neuroepithelium was used. This method consisted of determining the number of individual intramembranous particles per μm^2 (IMP/μm^2) from portions of the lateral plasma membranes of receptor and supporting cells readily identifiable at the apical region of the epithelium. The P-faces of the receptor cell plasma membranes displayed 3200 ± 500 IMP/μm^2, whereas P-faces of supporting cell plasma membranes displayed only 2200 ± 300 IMP/μm^2. These values were compared to those obtained from portions of plasma membranes located more deeply in the epithelium and hence more difficult to identify. Therefore, in the rat vomeronasal neuroepithelium, P-faces of plasma membranes displaying more than 2700 IMP/μm^2 and OAP, like those in Figs. 1, 2 and 4, unequivocally belonged to receptor cells. The OAP were 720 ± 200 nm^2 in area and they consisted of 4 to 20 particles whose centre-to-centre distance was about 7 nm. OAP were scattered on dendrites (Fig. 1) and perikarya (Fig. 4). On the E-face of the neuronal plasma membranes, complementary orthogonal arrays of pits were observed (Fig. 3) which were difficult to identify due to their small size and scattered distribution. No OAP were detected in olfactory receptor cells.

Tiger salamander

In the olfactory neuroepithelium, OAP 2230 ± 970 nm^2 in area were clearly observed on the P-face of the plasma membranes of most receptor cells at the level of the dendrites (Fig. 5) and perikarya (Fig. 6). The OAP consisted of 12 to 36 particles. The centre-to-centre distance of the particles was about 7 nm. The OAP formed complexes of 2 to 28 individual OAP, the longitudinal axes of which were usually arranged in parallel (Figs. 5,6). These complexes were up to 0.70×0.14 μm in size (Fig. 6). On the E-face of the plasma membranes, patterns of pits complementary to these complexes of OAP were readily identified (Fig. 6).

Discussion

In the C.N.S. of vertebrates, OAP have been reported to be present in glial and ependymal cells but have never been seen on neuronal plasma membranes (Dermietzel, 1973, 1974; Landis & Reese, 1974, 1981; Privat, 1977; Anders & Brightman, 1979, 1982; Hatton & Ellisman, 1981, 1982; Gotow & Hashimoto, 1982). Moreover, OAP have also been observed in different sensory organs of vertebrates or their associated cells. Thus, they were found in Mueller cells of the retina (Reale *et al.*, 1974, 1978; Raviola, 1976), in the perilymphatic cells of the spiral ligament (Reale & Luciano, personal communication), in the receptor-cell-free epithelium of the rat vomeronasal organ (Miragall *et al.*, 1979), and in the supporting cells of the mouse olfactory epithelium (Kerjaschki & Hörandner, 1976). Up to now the only exception has been represented by the receptor cells of the newt olfactory epithelium (Usukura & Yamada, 1978).

Despite some peculiarities in the olfactory and vomeronasal receptor cells of vertebrates, as for example their capacity to turnover (Barber & Raisman, 1978; Graziadei & Monti Graziadei, 1978) or the location of their perikarya in a neuroepithelium rather than in a sensory ganglion (Graziadei & Monti Graziadei, 1979), these cells are still recognized as neurons (Graziadei & Monti Graziadei, 1979). In the present investigation, OAP were observed in the vomeronasal receptor cell membranes of the rat and in the olfactory receptor cell membranes of the tiger salamander. These findings corroborate those of Usukura & Yamada (1978) for the olfactory receptor cells of the newt, and bring new unmistakable evidence concerning the presence of OAP in neuronal plasma membranes of vertebrates. In contrast to the vomeronasal receptor cell membranes of the rat, OAP are neither present in the rat olfactory receptor cell membranes as reported here, nor are they present in those of the mouse as reported by Kerjaschki & Hörandner (1976). This finding can be included among the structural differences between vomeronasal and olfactory receptor cells in rodents, in addition to, for example, the absence of sensory cilia and the presence of a well-developed perikaryon containing abundant smooth endoplasmic reticulum in the vomeronasal receptor cells (Moulton & Beidler, 1967; Graziadei, 1977), differences in the concentration of intramembranous particles and thickness of the cell coat of the sensory endings (Breipohl *et al.*, 1982; Mendoza & Breipohl, 1983), and differences in the configuration and number of strands of the tight junctions (Miragall *et al.*, 1983).

There is general agreement that OAP are not intercellular junctions, since they are also present in many non-junctional plasma membranes (Landis & Reese, 1974; Rash *et al.*, 1974; Ellisman *et al.*, 1976; Hanna *et al.*, 1976; Robenek & Greven, 1980), but their functional significance is unknown. It has even been suggested that OAP may have different functions in different systems (Anders & Brightman, 1979; Hatton & Ellisman, 1981). Equally, one cannot exclude the possibility that complexes of OAP in olfactory receptor cells of the tiger salamander may have a different function than that of individual OAP observed in vomeronasal receptor cells of the rat. Usukura & Yamada (1978), who found similar complexes of OAP in olfactory receptor cells of the newt,

assumed that these structures 'may function in the regulation of osmotic pressure and selective ion transport'. The presence of OAP in the olfactory receptor cells of the newt, as reported by Usukura & Yamada (1978), and those of the tiger salamander, as reported here, together with their absence in the olfactory receptor cells of the mouse (Kerjaschki & Hörandner, 1976) and rat, as demonstrated in the present study, indicates that the occurrence of OAP in plasma membranes of olfactory sensory neurons is species dependent. Further investigations on the appearance and distribution of these structures in sensory neurons of different olfactory subsystems and in different species may help elucidate the functional significance of these membrane specializations.

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