240 CD
DOI: 10.1017/S143192760606154X
Microsc Microanal 12(Supp 2), 2006

FINE-STRUCTURAL ASPECTS OF VERTEBRATE OLFACTORY SIGNALING

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Cilia sprouting from the dendritic endings (**de** in Figs. 1-3) of bipolar sensory cells are the olfactory system's equivalent of rod and cone outer segments in the visual system. Vertebrate olfactory cilia have a short proximal part (**pc**) and a thin part (**dc**) that can be as long as 50 μm in mammals and even longer in lower vertebrates. The distal parts of the olfactory cilia form a carpet that lines the nasal lumen thus vastly enhancing the area susceptible to ambient odors [1]. Olfactory signaling begins when odors interact with special members of the GTP-binding protein, or G-protein, linked odor receptor superfamily. The stimulus-receptor interaction leads to activation of particular G proteins, the alpha-subunits of which in turn activate an adenylyl cyclase, eventually leading to an electrical activation of the receptor cell. Important components, notably odor receptors (Fig. 1), the G-protein subunits $G_{olf\text{-}alpha}$ (Fig. 2), Type III adenylyl cyclase, and the alpha-subunit of a special cyclic nucleotide-gated channel, of the olfactory signal-transduction apparatus have been localized fine-structurally to vertebrate olfactory cilia [1]. Others have not yet, notably chloride channels important to carry most of the current [2]. Localization of the components was demonstrated using antibodies to the proteins in question. Antibody binding was visualized with a second antibody (GAR=goat-anti-rabbit in Figs. 1-3) bound to a tag, usually colloidal gold, that found the first antibody. For all ultrastructural localization cryofixation was used of, often but not always paraformaldheyde-fixed tissue followed by freeze-substitution through acetone (not chemically fixed tissue) or methanol (chemically fixed tissue), cryo-embedding in Lowicryl K11M, and post-embedding immunocytochemistry [1, 3, 4]. To localize odor receptors, two rabbit-raised mono-specific polyclonal antibodies to two such receptors of two different rodent species, rat and mouse, were used. In both instances, and as predicted, immunopositive cells were rare; up to about 0.4% of the receptor cells were labeled (Fig. 1; [4]). In contrast, antibodies to all proteins activated upon the odor receptor interaction, successively $G_{olf\text{-}alpha}$, type III adenylyl cyclase, and cyclic nucleotide gated channels, bound to ciliary structures of most or all dendritic of both rat and mouse (Fig. 2). All of these proteins are mainly found in the distal parts of the olfactory cilia, and much less so in the dendritic endings and the proximal parts of the cilia. Finally, molecules that are likely involved in desensitization of the signal, G-protein receptor kinase 3 (GRK3), beta-arrestin-2, Ca^{2+}/calmodulin-dependent protein kinase II, CaMKII, and phosphodiesterase PDE1C2 was also found in receptor cell cilia but occurred in microvilli (**mv**) of neighboring supporting cells as well (Fig. 3; [4]).

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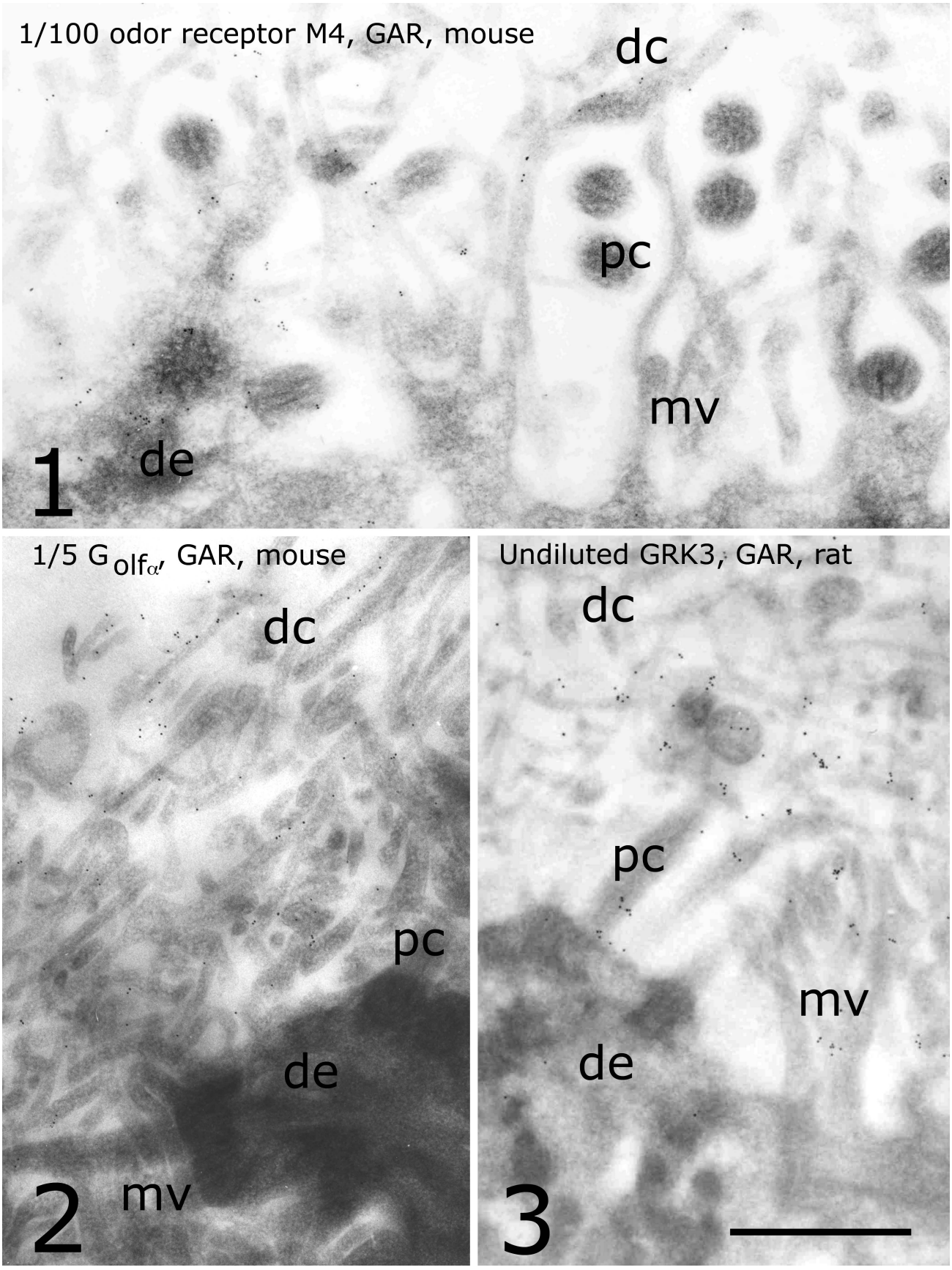
This material is based upon work supported by the National Science Foundation under Grant No. 0094709

Figures 1-3: Sections through the surface of mouse (Figs. 1 and 2) and rat (Fig. 3) olfactory epithelial tissue fixed with freshly depolymerized paraformaldehyde and cryoprotected before freeze-substitution. The sections were incubated with antibodies to an odor receptor (Fig. 1), $G_{olf\text{-}alpha}$ (Fig. 2) and GRK3 (Fig. 3) at indicated dilutions. The secondary probe was GAR-gold (10 nm in Figs. 1 and 2 and 15nm in Fig. 3). For other details and abbreviations see above. Scale bar=1 μm.



https://doi.org/10.1017/S143192760606154X Published online by Cambridge University Press