Pflügers Arch - Eur J Physiol (2001) 441:579–586
DOI 10.1007/s004240000492

INVITED REVIEW

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Molecular physiology of odor detection: current views

Published online: 11 January 2001

Abstract This review outlines current views of the principles and mechanisms underlying olfactory signaling, and presents some thoughts on open questions and future perspectives in this field. We briefly introduce the structure and function of the olfactory system and its sensory neurons, which respond to appropriate odorants with distinct patterns of electrical activity and contribute to the phenomenon of odor coding based on their characteristic receptor repertoire and their specific interconnections with target neurons in the brain. The molecular mechanisms mediating the process of chemo-electrical signal transduction in sensory cells and their functional implications are discussed. As a prerequisite for the high sensitivity and fast kinetics of the transduction process, we propose that the functional elements of the transduction cascades are spatially arranged in multimeric complexes, organized by scaffolding proteins, which anchor the signaling networks to the membrane of olfactory sensory cilia.

Keywords Adaptation · Olfaction · Olfactory receptors · Scaffolding proteins · Second messenger cascades · Signal transduction

Introduction

The perception of smell is a construct of nose and brain together, which cooperatively encode and decipher the chemical properties of our physical environment [45]. Olfactory sensation begins with the binding of odorant molecules to specialized receptors decorating the membrane of olfactory sensory neurons. This interaction triggers complex intracellular reaction cascades leading to the transduction of chemical signals into a pattern of electrical activity, which is conveyed to the brain for further processing. Research has provided a wealth of information about the initial steps of olfaction. In the early 1990s, the discovery of a large gene family encoding odorant receptors [13], and the characterization of downstream signaling pathways fundamentally contributed to the current understanding of odor recognition and signal transduction in the nose, and thus was a major breakthrough in resolving this issue. Large parts of the model that has emerged from these findings appear conclusive, with little obvious need for additions or modifications, while others still bear a number of unresolved and often controversially discussed issues.

The cellular elements of olfaction

The main olfactory epithelium (MOE) in the nose is specialized to detect fluctuations in the concentration of a large diversity of air-borne molecules and to transduce this information into a stream of neuronal activity which is conveyed to the brain. The MOE consists of three principal cell types: olfactory sensory neurons, non-sensory supporting cells, and undifferentiated basal cells [64]. Olfactory sensory neurons are small bipolar neurons, projecting unbranched and unmyelinated axons, gathered in bundles of the olfactory nerve, through the cribriform plate to the olfactory bulb. Each axon connects to the dendritic trees of mitral and tufted cells in single glomeruli, which represent the primary input relay in the hierarchy of the olfactory brain. Towards the nasal cavity the olfactory sensory neurons extend a large dendrite, terminating near the surface of the epithelium in an apical knob, which carries a number of cilia (Fig. 1). The cilia are embedded in a thin layer of mucus which covers the epithelium, thus providing a specialized liquid compartment separated from the interstitial fluid by a barrier of tight junctions [53, 54, 58]. The cilia are regarded as highly specialized sensory organelles, responsible for the recognition and transduction of olfactory signals. This notion is based on various findings, including the obser-

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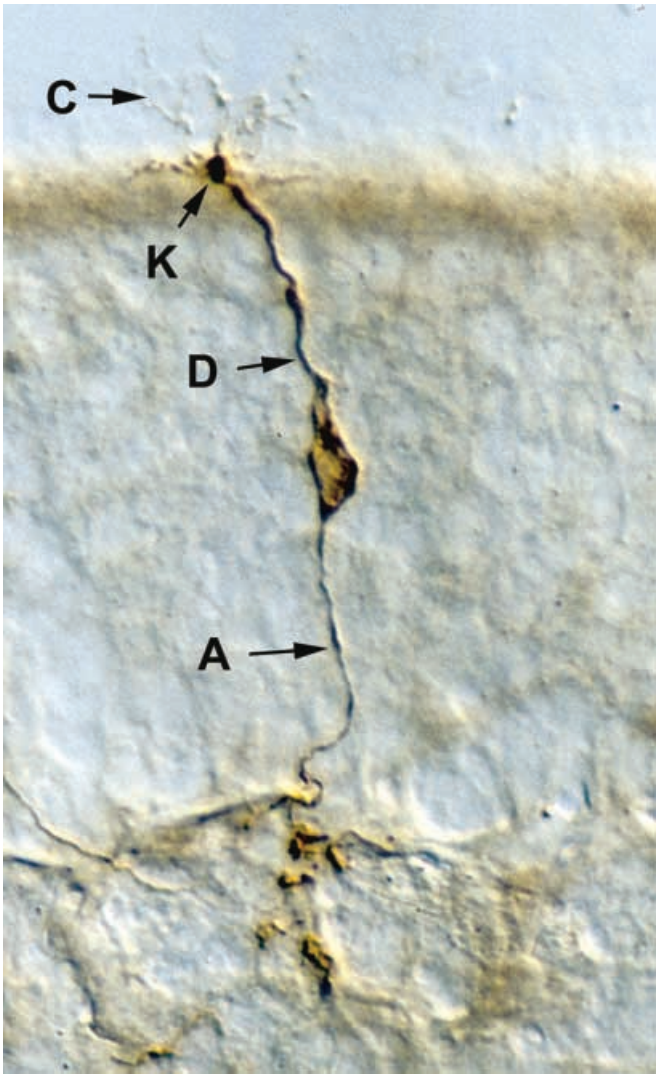

Fig. 1 The morphology of olfactory sensory neurons. Microscopic image of a sensory neuron in the main olfactory epithelium of a transgenic mouse, which expresses green fluorescent protein (*GFP*) under control of the promoter of odorant receptor OR37A. The section was stained with an anti-GFP antibody, followed by immunoperoxidase detection. The GFP protein diffuses into all cellular compartments, thus making them visible. The cell extends an apical dendrite (*D*) towards the nasal cavity, terminating in a knob (*K*) from which a number of sensory cilia (*C*) protrude into the mucus. From its basal pole, the neurons project a single unbranched axon (**A**) to the olfactory bulb

vations that deciliated olfactory sensory neurons do not respond to odors [44]. It is further supported by immunochemical analyses, showing that putative odorant receptors [42a, 55] and key enzymes of the olfactory signaling cascades are primarily located in the cilia [1, 2, 14]. Furthermore, electrophysiological recordings have demonstrated that the chemosensory responsiveness of olfactory neurons is mainly based on the cilia [25, 50]. Finally, fluorescence imaging experiments demonstrate that odorant-induced Ca^{2+} signals initially occur in the cilia before they can be detected in the knob [46, 47]. The cilia probably contribute to the responsiveness of an olfactory cell by significantly increasing the accessible surface area of the chemosensory membrane, while keeping the volume rather small. The role of the dendritic knobs in signal transduction remains unclear.

Altogether, olfactory sensory cells are highly polarized neurons with distinct and spatially segregated functional domains, which are characterized by their morphological specialization and a unique molecular composition. The cellular mechanisms creating and maintaining this structural and functional compartmentalization remain largely elusive.

Odor-induced electrical responses

Responses of the olfactory epithelium to an odor stimulus can be recorded as slow voltage changes at the epithelial surface [28, 67, 77]. This electro-olfactogram (EOG) reflects changes of the transepithelial potential between the interstitial and the mucus fluid compartments, representing the summed activity of many olfactory sensory neurons. The EOG thus not only reflects the strength of the response of individual neurons, but also depends on the number of responding neurons in the recording area. EOGs therefore do not provide information about the responsiveness of individual sensory neurons. However, the simultaneous recording of odor-induced single-cell responses and the EOG from rat olfactory epithelia has recently been accomplished [21, 22]. This experimental approach allowed the detection of action potentials generated by single olfactory neurons in the context of the activity of the surrounding sensory epithelium.

The nature and kinetics of the ion conductances underlying the electrical responses of olfactory cells have been investigated by numerous studies over the last 10 years, involving intracellular recordings and patch-clamp analyses of isolated sensory neurons, as well as optical recordings using ion-sensitive fluorescent dyes (for a detailed review see [75]). These studies have revealed that many odorants elicit the activation of a Ca^{2+}-permeable non-selective cation conductance [27], followed by a non-linearly Ca^{2+}-dependent chloride conductance [41]. It has been suggested that, due to the specific ion concentrations in the mucus, this results in an influx of extracellular calcium followed by an outflux of intracellular chloride, which cooperatively depolarize the cilia membrane [71]. It has been assumed that the chloride conductance significantly amplifies the odor-induced depolarization of the cilia, and the non-linear Ca^{2+} sensitivity of this current is thought to introduce an excitation threshold to improve the signal-to-noise ratio of the transduction process [23, 39]. If the stimulus strength is sufficient, the depolarization of the cilia propagates by passive electrotonic spread, and finally triggers the generation of action potentials at the initial segment of the axon [89]. Repolarization of the cells seems to involve Ca^{2+}-dependent and fast inactivating potassium conductances. The extrusion of Ca^{2+} ions is mediated by Na^{+}/Ca^{2+} exchange mechanisms in cilia,

and probably involves Ca^{2+}-ATPases in knobs, dendrites, and cilia [19, 37, 56, 66, 70].

Upon odor stimulation, olfactory sensory neurons respond with bursts of action potentials, the spike frequencies of which depend proportionally on stimulus strength over a wide range of concentrations. The latencies of this response can be as short as 100 ms for high stimulus concentrations [22]. It has been suggested that a stimulus-dependent differential recruitment of distinct neuron populations together with the induced spike frequency may be sufficient for coding the stimulus quality and intensity. However, as stated by Laurent [45], establishing an odor coding system requires proof of what aspects of the encoded signal the brain actually decodes.

Olfactory receptors and odor coding

The sense of smell is capable of discriminating between thousands of volatile chemical substances by perceiving them as distinct odors. The initial molecular mechanisms underlying this discriminative capacity of the vertebrate olfactory system have long been subject to numerous theories and controversial discussions. Is there a limited number of odorant receptors with low and overlapping specificity or a large number of receptors with distinct specificities for only one or a few odorant molecules? The discovery of a large family of G-protein-coupled odorant receptors [13] was an important step in resolving this issue. It was found that most olfactory sensory neurons express only one (or perhaps a few) odorant receptor subtype(s). An unusual mode of mutually exclusive monoallelic gene expression has been proposed for odorant receptors [15, 79]. Consequently, receptor expression profiles define subpopulations of olfactory sensory neurons with a defined receptor type and thus distinct response properties. Each of these subpopulations apparently projects to only a small, well defined set of target glomeruli in the olfactory bulb, thus translating the identity-dependent excitation pattern in the olfactory epithelium into a truly spatiotemporal activity pattern in the brain. Evidence for this view has been provided in vivo by the optical imaging of olfactory bulb activity in response to odors [26, 73].

Surprisingly, most olfactory sensory neurons are responsive to multiple pure odors, even if these compounds are considerably different in chemical structure. These findings suggest that the mammalian olfactory system uses a combinatorial coding system to discriminate between odors [20, 22, 51], in which the output of olfactory sensory neurons is defined by the odorant binding affinities of the receptor subtypes they express. Accordingly, each odorant or odorant mixture would be represented by a unique set of responsive neuron populations, which elicit distinct but overlapping patterns of activity in space and time in the corresponding glomeruli of the olfactory bulb [63, 83]. Importantly, such model should include a systematic concentration-dependent shift in the detection of odor qualities, because high stimulus concentrations would recruit additional populations of sensory neurons, and thus initiate a different pattern of neuronal activity. Compatible with this view, individual olfactory sensory neurons are often more discriminating between different odor compounds at near-threshold levels compared to higher concentrations [21, 22]. One might therefore consider the possibility that temporal aspects in the recruitment of olfactory sensory neurons by a given odorant stimulus may contribute to defining odor quality. Odorous compounds enter the nose with a stream of air, building up a diffusion gradient at the front of this stream and in the mucus. Different threshold levels of each population of sensory neurons for odorants could therefore contribute to the recognition of odor qualities by eliciting characteristic spatio-temporal response patterns, even at high stimulus concentrations. Such patterns could be recognized by the brain with its impressive capability of highly complex correlation analysis. However, this requires the olfactory detection machinery to have a sufficiently high temporal resolution, compatible with the extremely fast kinetics of the on- and off-set of the primary signaling processes [5, 8].

Downstream signaling mechanisms

The initial step in activating olfactory sensory neurons is the binding of the odorous ligand to an appropriate receptor in the cilia membrane. Olfactory receptors are probably subjected to conformational rearrangements upon ligand binding, similar to the light-induced structural changes of rhodopsin [32], thereby transmitting the signal from the extracellular to the cytoplasmic face of the membrane. There is a general consensus that most odorants activate receptors that are linked to the stimulation of adenylyl cyclase type III (AC3) [2] by a specific G_s-like alpha-subunit, G_{olf} [35]. The anosmic phenotype of AC3- and G_{olf}-deficient mice is compatible with a central role for adenylyl cyclase and $G_{\alpha s}$-like G proteins in olfactory signaling [3, 85]. Activation of AC3 elicits cyclic adenosine-3′,5′-monophosphate (cAMP) levels in the cilia to rise, which in turn triggers the gating of cyclic-nucleotide-gated non-selective cation channels (CNCs). CNCs are heterotetrameric channel complexes [80], which are highly permeable to Ca^{2+} [23], and are probably composed of two ion-conducting α-subunits, CNCα3 and CNCα4, and two regulatory CNC-β1b-subunits [6]. The gating of CNCs accounts for the initial component of the odor-induced electrical response, and mice deficient in CNCα3, which is essential for forming functional CNCs, suffer from general anosmia [12, 88]. The influx of cations through CNCs depolarizes the cilia membrane, elevates the intracellular Ca^{2+} concentration, and in turn triggers a Ca^{2+}-activated chloride conductance which significantly amplifies the electrical signal. The chloride channel mediating this current remains to be identified.

Biochemical studies have provided evidence that other olfactory signaling pathways may exist. It was

observed that some potent odorants, including lyral, lilial, trimethylamine, and isovaleric acid, failed to elicit a biochemically detectable increase of cAMP concentration in isolated olfactory cilia [10, 81]. It was argued that these odorants might activate only a very small fraction of olfactory sensory neurons, making the elicited second messenger response undetectable in a biochemical assay [30]. However, it was found that the same "non-cAMP odorants" elicit a substantial increase of inositol 1,4,5-trisphosphate (IP_3) concentration with similar rapid and transient kinetics [5, 8, 33, 72]. The notion that a set of odorants indeed activates the IP_3 pathway in isolated olfactory sensory neurons was confirmed by calcium imaging analysis, showing that a calcium transient induced by lilial was completely blocked by U73122, a specific inhibitor of phospholipase C [65]. The same inhibitor had no influence on calcium signals induced by citralva, which is mediated by the cAMP cascade. Single-cell polymerase chain reaction (PCR) experiments indicated that lilial-responsive neurons express a phospholipase C of the β2-subtype (PLC-β2), suggesting that this isoform is involved in generating IP_3 in response to an odor stimulus. IP_3-gated ion channels have been considered as possible downstream targets of odorant-induced increases of IP_3 concentration [17, 49]. The molecular identity of these signaling elements, however, remains uncertain. Recent evidence indicates that receptor activation is linked to phospholipase C by G proteins other than G_{olf}. Immunoprecipitation of activation-dependent photolabeled G_α subunits and the attenuation of odor-induced IP_3 responses by specific antibodies indicate that a $G_{\alpha o}$-like G protein may be involved [74].

Our understanding of the chemosensory transduction process is further complicated by the finding that, in addition to cAMP- and IP_3-mediated pathways, guanylyl cyclases might be involved (for review see [29]). It was found that a subset of olfactory sensory neurons expresses membrane-bound guanylyl cyclase D (GC-D), along with cGMP-stimulated phosphodiesterase (PDE2) [36]. These neurons are randomly dispersed throughout the olfactory epithelium, converging on a small subset of glomeruli in the so-called necklace region of the olfactory bulb. It was speculated that GC-D-expressing olfactory sensory neurons may be involved in intraspecies communication, such as the odorant detection associated with mother bonding or suckling behavior in rodents [29]. Furthermore, soluble guanylyl cyclases are expressed in olfactory sensory neurons, possibly involved in nitric-oxide- and/or carbon-monoxide-mediated modulation of olfactory signaling [9, 34]. Recently, an olfaction-specific S100-related protein, P26olf, was identified [59, 60]. P26olf and other putative Ca^{2+}-dependent activators of guanylyl cyclase (GCAPs) [62], together with the high sensitivity of CNCs for cGMP, provide additional possibilities for converging signaling pathways.

In order for vertebrate and invertebrate olfactory sensory neurons to follow repeated odor pulses at frequencies of up to 10 Hz, the odor-induced responses must be rapidly inactivated. A variety of molecular mechanisms may contribute to the deactivation process [43, 52]. For example, the olfactory adenylyl cyclase AC3 is inhibited by Ca^{2+} through phosphorylation by Ca^{2+}/calmodulin kinase II [84], and Ca^{2+}/calmodulin-dependent phosphodiesterases may accelerate the hydrolysis of cAMP [4, 7, 87]. Intracellular Ca^{2+} also decreases the affinity of CNCs for their ligand cAMP [40, 42], suggesting that channel inactivation by calcium, possibly involving Ca^{2+}/calmodulin, may contribute to the signal termination process. Furthermore, there is evidence that the stimulus-induced second messenger response is terminated by the phosphorylation of odorant receptors via G-protein-coupled receptor kinases (GRKs) and β-arrestin-2, which cooperatively uncouple receptor/G-protein interactions [4a, 75a, 4b, 18, 68]. This process may involve the activation of GRKs by the second-messenger-dependent protein kinase A in cAMP-mediated signaling, while protein kinase C supposedly participates in IP_3-mediated signaling [11]. β-Arrestin-induced receptor internalization via clathrin-coated pits may further contribute to the inactivation process [69].

The sensitivity and speed of olfactory signaling

Chemosensory neurons in the rat vomeronasal organ detect distinct pheromone compounds with extremely high sensitivity [48]. For sensory neurons of the main olfactory epithelium, the detection of even single molecules has been postulated [57] – although controversy surrounds this issue [30, 31]. Despite this dispute, there is no doubt about the possibility of an extremely sensitive chemoelectric transduction machinery in olfactory sensory neurons. The detection of single odorant molecules, however, seems less relevant for vertebrates, while detecting concentration gradients with the greatest possible resolution might be of greater importance. From this point of view, it would be interesting to see whether one or a few additional odor molecules above a previously experienced concentration can make a difference to the output of an olfactory sensory neuron.

A prerequisite for obtaining high detection sensitivity is a short latency between stimulus application and the electrical response of the sensory neuron. For phototransduction, it has been shown that increased latencies of receptor cell responses to single photons results in reduced macroscopic response amplitudes, because the summation of quantal responses to individual photons is distributed over a broader range of time [78]. Similarly, in olfaction fewer odorant molecules may be needed to elicit cilia membrane depolarization above threshold when the latency of quantal responses transduced by each activated receptor is minimized. Indeed, rapid kinetics have been observed for odorant-induced activation and inactivation in olfactory sensory neurons. The time course of these processes is comparable to that of phototransduction, although the absolute values are difficult to compare because stimulus delivery and the interaction

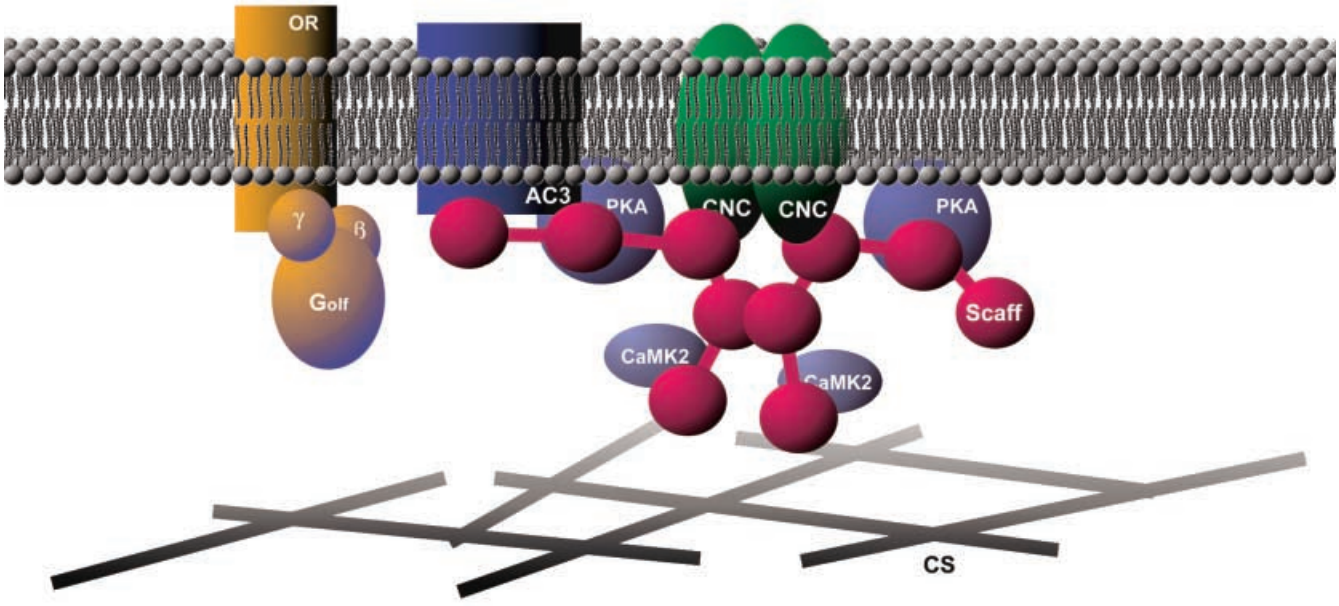

Fig. 2 Olfactory signaling complexes. Hypothetical model of signaling complexes in the cilia membrane of olfactory sensory neurons. Adenylyl cyclase type 3 (*AC3*), cyclic-nucleotide-gated channels (*CNC*), and modulating elements such as protein kinase A (*PKA*) and Ca^{2+}-dependent calmodulin kinase II (*CaMK2*) are thought to be spatially arranged in multimeric signaling complexes which are linked to the cytoskeleton (*CS*) by putative multivalent scaffolding proteins (*Scaff*). The resulting membrane-associated "transducisome" complex could significantly contribute to the rapid kinetics of primary reactions in olfactory signaling elicited by dissociating heterotrimeric G proteins (G_{olf}, β, γ) upon odorant receptor (*OR*) activation

between the odorant and the receptor introduce a significant temporal uncertainty compared to photoactivation.

Applying high odorant concentrations to olfactory sensory neurons elicits a characteristic response. A brief decrementing burst of action potentials with a duration of around 50 ms is followed by a silent phase which can last for up to several seconds [22]. The silent phase probably results from a strong odor-induced depolarization of the cell, trapping the action-potential-mediating voltage-gated sodium channels in their closed inactive configuration. Recovery from the silent phase thus requires repolarization of the cell. In contrast, low stimulus concentrations elicit a spike train that follows the stimulus for the entire phase of odor delivery. As an example, stimulation for 2 s with 3.5×10^{-7} M anisole was found to induce spiking frequencies almost tenfold lower compared to stimulation with 2×10^{-5} M anisole. The total number of spikes induced by the low stimulus concentration, however, was five times higher at equal stimulus duration [22]. This observation poses an interesting query for the next level of olfactory computation, because, obviously, the system requires a rather narrow window for signal integration in order to extract the stimulus strength from the action potential frequency. As mentioned above, the spiking behavior of olfactory sensory neurons is capable of following a pulsed stimulus application up to 4–10 Hz. Together with the remarkably short latencies of odorant-induced spiking at high stimulus concentrations (≅100 ms), these observations reflect the extremely rapid kinetics of activation and inactivation in olfactory primary processes, which is in good agreement with the kinetics of odor-induced second messenger responses observed by stopped-flow technology [5, 8].

Subcellular compartmentalization and signaling complexes

It has been suggested that the recruitment of signaling proteins to the cell membrane, and their arrangement in preformed supramolecular complexes are necessary for the effective activation of downstream signaling processes [38]. This topic was extensively studied using photoreceptor cells of the fruitfly, which respond to single photons with unitary membrane potential changes [61, 78, 82, 86]. It has been demonstrated that the multivalent scaffolding protein INAD is required to ensure the sensitivity and temporal resolution of this process, most likely by coordinating the signaling molecules into a multimeric complex – termed transducisome [82] or signalplex [86].

Considering the high sensitivity and temporal resolution in vertebrate olfaction, one might expect to find a similar molecular architecture in the chemosensory compartments of olfactory sensory neurons. This notion is sustained by the fact that many olfactory signaling proteins are distinctly located in the sensory cilia. We speculate that scaffolding proteins, such as PDZ-domain proteins [24], A kinase-anchoring proteins (AKAPs) [16], and lipid microdomains [76], might play an important role in olfactory cilia. Such molecular scaffolds, which often contribute to anchoring signaling proteins to specific sites in the cell membrane, could also be involved in organizing chemosensory microdomains in

olfactory cilia (Fig. 2). It will be an intriguing challenge for future research to unravel the principles and mechanisms of specific mutual interactions between the signaling molecules in olfaction. This issue seems of crucial importance for a better understanding of the chemoelectrical signal transduction machinery in olfactory sensory neurons.

Acknowledgements We thank Patricia Duchamp-Viret, Jörg Strotmann, Rebecca Elsässer, and Jörg Fleischer for critical comments on the manuscript, and Sidonie Conzelmann for kindly providing the microscopic image in Fig. 1.

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