

From odor and pheromone transduction to the organization of the sense of smell

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Chemosensory neurons in the mammalian nose detect an array of odors and pheromones that carry essential information about the animal's environment. How the nose organizes this immense amount of information is a major question in sensory biology. New evidence suggests that there are several subpopulations of sensory neurons in the nose that project to different areas in the forebrain. Strikingly, evidence is now emerging that several of these neuronal subpopulations employ distinct second messenger cascades to transduce chemical stimuli. This new understanding of the heterogeneity of chemosensory transduction mechanisms offers the opportunity to use genetically altered animals to specifically target these subpopulations. Such approaches should enable researchers to examine the role that each of these subsystems could play in chemosensory-dependent behaviors.

The mammalian olfactory system recognizes a vast range of molecules that represent vital information about an animal's environment, including the location of prey and predators, the sexual, hormonal and reproductive state of mating partners, and the level of aggression in rivals. How does the nose organize this complex mixture of olfactory information? Recent evidence has shown that there are at least four spatially segregated subpopulations of sensory neurons within the nose, each projecting to different areas of the brain (Fig. 1), and each probably serving as somewhat distinct channels for different types of chemosensory information. There is increasing evidence that these discrete subpopulations of sensory cells also use different second messenger pathways for the transduction of chemosensory molecules into electrical signals. If so, characterization of the signal transduction machinery in these cells will permit the generation of genetically altered animals in which

each of these four information channels is rendered nonfunctional in a selective manner. This approach will facilitate the analysis of the precise roles that each of these subsystems play in odor-dependent behavior. Thus, the investigation of signal transduction mechanisms in the nose should remain at the forefront of research aimed at understanding the basic organization of the sense of smell.

cAMP versus $\text{Ins}(1,4,5)P_3$ in the main olfactory epithelium

An important step in the generation of anosmic knockout strains has been taken by Wong *et al.*¹, focusing on the largest group of ciliated olfactory receptor neurons (ORNs) in the main olfactory epithelium (MOE) (Fig. 1a,b). Previously, it was known that these neurons characteristically express one, or at most a few, members of the odor receptor family, in addition to several molecules of a cAMP-mediated signal transduction cascade including the G-protein G_{olf} , type III adenylyl cyclase (ACIII), and the cyclic nucleotide-gated (CNG) channel subunit OCNC1 (reviewed in Ref. 2). Gene knockout studies of OCNC1 and G_{olf} firmly established the essential role of both molecules in odor transduction^{3,4}. However, the adenylyl cyclase responsible for primary olfactory signaling had not been determined, because at least three different adenylyl cyclases (ACII, ACIII, and ACIV) are expressed in olfactory cilia¹.

To evaluate the role of ACIII, Wong *et al.*¹ disrupted the ACIII gene in mice. Interestingly, odor-induced electrical responses in the olfactory epithelium, measured by extracellular field potential recordings (known as an electro-olfactogram, or EOG), were completely eliminated in ACIII-null mice. Similarly, odor-dependent learning was impaired in these mice, providing definitive evidence for the crucial role of ACIII in odor detection. Wong *et al.*¹ then proceeded to test a model put forward by

the group of Heinz Breer in Stuttgart in the late 1980s (recently reviewed in Refs 5,6), in which fruity odors such as citralva are transduced primarily by cAMP, whereas putrid odors, such as isovaleric acid are transduced by a different second messenger, inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)P_3$]. In ACIII-null mice, responses to both odor groups were absent, indicating that all odors tested are transduced via ACIII in the MOE. The same conclusions were reached in a parallel study in which pharmacological antagonists of adenylyl cyclase inhibited EOG responses to both odor classes in mice in a fully reversible manner⁷. These results, together with those of previous studies^{3,4}, are conclusive evidence that, in the vast majority of ciliated ORNs of the MOE, G_{olf} -mediated activation of ACIII and CNG channel opening by cAMP are essential for the transduction of a wide variety of odors, including those that produced $\text{Ins}(1,4,5)P_3$ in biochemical assays. Thus, a model in which fruity odors are transduced by neurons using cAMP as second messenger and putrid odors are transduced by neurons using $\text{Ins}(1,4,5)P_3$ is no longer valid. However, a modulatory role for $\text{Ins}(1,4,5)P_3$ or other products of a phospholipase C (PLC) cascade in ciliated ORNs remains a viable hypothesis. Similarly, an involvement of $\text{Ins}(1,4,5)P_3$ in inhibitory odor responses, which are sometimes seen in these cells, should be tested.

A subset of ORNs in the MOE utilizing cGMP?

Although Wong *et al.*¹ might have shut the door on the idea of dual transduction cascades in the main population of ciliated ORNs, other recent findings clearly show that not all of the chemosensory transduction in the nose is mediated by cAMP. It is known that ciliated neurons of the MOE comprise a heterogeneous population, with groups of cells displaying different immunochemical and histochemical

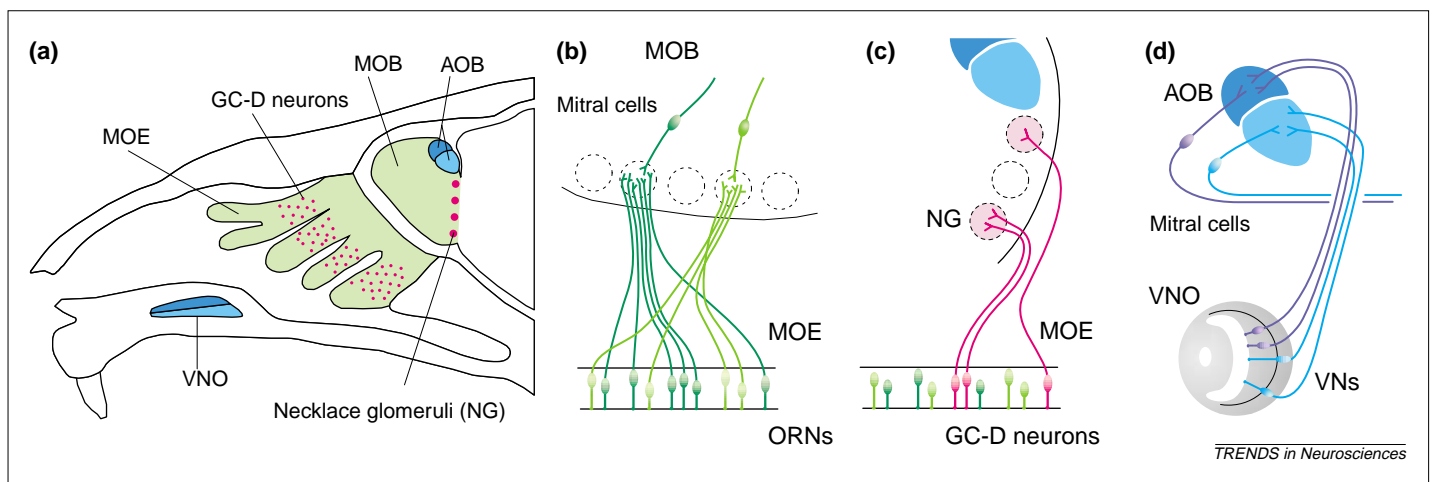


Fig. 1. Molecular and anatomical organization of chemosensory systems in the nose. (a) Midsagittal view of the rodent nasal cavity and forebrain. Olfactory receptor neurons (ORNs) of the main olfactory epithelium (MOE) project to glomeruli in the main olfactory bulb (MOB, green). GC-D neurons are also located in the MOE (pink), but project to a limited number of necklace glomeruli (NG) in the caudal MOB. Sensory neurons in the vomeronasal organ (VNO) project to the anterior (dark blue) and posterior (light blue) accessory olfactory bulb (AOB). (b) ORNs express one of approximately 1000 olfactory receptors (ORs), in addition to molecules of a cAMP signaling cascade, including G_{olf} , ACIII, and OCNC1. ORNs expressing the same OR converge on a small number of glomeruli; each glomerulus receives inputs from ORNs expressing the same OR. In the glomeruli, ORNs synapse with second order neurons, the mitral cells. (c) GC-D neurons, which also express PDE2 and a cone photoreceptor-type cGMP-gated cation channel, are interspersed amongst ORNs. It is unclear whether subpopulations of GC-D neurons project to individual NGs or if single GC-D neurons synapse with multiple NGs. (d) Two populations of vomeronasal neurons (VNs) have been identified in the VNO. The apical zone contains VNs (dark blue) that express one of approximately 170 putative pheromone receptors (V1Rs or V3Rs), in addition to the G protein $G_{\alpha_{12}}$ and the transient receptor potential (TRP) channel subunit TRP2. These VNs exclusively project to multiple glomeruli in the anterior AOB. The basal zone of the VNO contains VNs (light blue) that express members of another putative pheromone receptor family (V2Rs). These cells also contain TRP2, but express a different G protein, G_{α_o} , and project to the posterior AOB.

markers or unique patterns of gene expression. Perhaps the most intriguing of these subpopulations represents less than 1% of all ORNs and expresses a receptor guanylyl cyclase, GC-D (Ref. 8). It is clear that these neurons exhibit both unique anatomical and functional characteristics. Unlike most ORNs, which project their axons to one of hundreds of glomeruli on all aspects of the main olfactory bulb (MOB), GC-D neurons project to a small group of approximately nine glomeruli, the necklace glomeruli, which circumscribe the caudal MOB and are indirectly implicated in suckling behaviors (Ref. 9 and references therein) (Fig. 1a,c). By contrast to most ORNs, GC-D neurons continue to provide active input to their glomerular targets in OCNC1-null mice¹⁰. GC-D neurons express none of the transduction components that are characteristic of most ORNs, including ACIII, G_{olf} , OCNC1, the cAMP-specific phosphodiesterase PDE4A and the Ca^{2+} -calmodulin-sensitive PDE1C2 (Refs 9,11). However, GC-D neurons do express two proteins reminiscent of a cGMP-mediated transduction pathway: a CNG channel, similar or identical to the

cGMP-specific channel subunit expressed in cone photoreceptors, and a cGMP-stimulated PDE2 (Refs 9,11).

One can hypothesize two possible models for chemosensory transduction in these cells. In the first model, a chemosensory ligand binds to the receptor domain of GC-D itself, stimulating cGMP production and opening of a CNG channel. In the second model, the ligand binds to an, as yet, unidentified G-protein-coupled receptor, leading to the modulation of cGMP levels by a PDE or by the regulation of GC-D. In this latter model, cGMP levels could be increased resulting in opening of the CNG channel, or decreased resulting in closing of the channel, in a manner reminiscent of the light response in photoreceptors. The targeting of genes, such as that encoding GC-D, will allow either the incorporation of histological reporters that are expressed in a cell-specific manner or the disruption of the gene and elimination of the functional protein. Such experiments will enable researchers to determine whether these cells comprise a homogeneous population in either the chemosensory receptor(s) they express or the transduction

machinery they utilize. They will also aid in the identification of ligands, either odors or pheromones, that activate these neurons, and will help reveal the chemosensory niche subserved by this ORN subpopulation.

Microvillous vomeronasal neurons are another story

Although the $\text{Ins}(1,4,5)P_3$ hypothesis has been disproved in ciliated ORNs of the MOE, there is mounting evidence that PLC is a key enzyme for signal transduction in microvillous sensory neurons in the nose. Most microvillous neurons are found in a separate chemosensory organ, the vomeronasal organ (VNO, Fig. 1a,d), in which they play an essential role in the detection of pheromones^{12,13}. A small subset of microvillous sensory neurons is also present in the MOE. Sensory cells in the VNO (known as vomeronasal neurons or VNs) can be divided into two main groups, both of which express a unique set of transduction-related molecules (Fig. 1d): (1) the most apical zone of the epithelium contains VNs that project to the anterior aspect of the accessory olfactory bulb (AOB) and express the G-protein $G_{\alpha_{12}}$ and a member of either the V1R or V3R families of putative pheromone receptors; (2) the basal zone contains VNs that project to the posterior aspect of the AOB and express the G-protein G_{α_o} and a member of the V2R family of receptors (see Refs 14,15, and references therein). Transduction components characteristic of most ORNs, such as ACIII, G_{olf} , and OCNC1, are absent from VNs (Ref. 16), although recent work has identified a member of the transient receptor potential (TRP) channel family, TRP2, in these cells^{17,18}. Interestingly, TRP2 is

highly expressed in the microvilli, implicating a role for this ion channel subunit in pheromone transduction¹⁷. Although it is unclear how TRP2 is activated, members of the TRP channel family are often coupled to PLC activation and can be gated by products of the phosphoinositide second messenger cascade¹⁹. Consistent with these findings, VNO responses to components of urine have been blocked by pharmacological inhibitors of PLC (Refs 13,20). Although these results are not yet conclusive, it seems increasingly probable that PLC is a key enzyme in the transduction of chemical information in microvillous VNs. The ability to record pheromone-stimulated functional activity in VNs (Refs 12,13), in addition to using genetic manipulation, should not only permit identification of the steps underlying pheromone detection but should also shed light on the functional need for different subpopulations of VNs.

Conclusions

The evidence summarized here shows that there are at least four subpopulations of chemosensory neurons in the mammalian nose, each with striking differences in their signal transduction machinery. Because of these molecular differences, knockout strains with selective signal transduction defects, such as those produced by Wong *et al.*¹, will provide invaluable tools for further investigations aimed at understanding the type of olfactory or pheromonal information processed by each of these subsystems. Of particular interest is how each of these four groups

of neurons encodes chemosensory information and what the consequences of the anatomical segregation of these subsystems for odor-dependent behavior might be. Finally, it should be noted that there are other neuronal subpopulations in the nose, such as those in the septal organ, whose function remains to be explored.

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Lipoprotein receptors: beacons to neurons?

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Lipoprotein receptors were originally considered simply as cellular transporters for cholesterol and other lipids. This view is rapidly changing. Signaling functions have recently been recognized in several members of the low-density lipoprotein receptor gene family. These Apolipoprotein E receptors are highly expressed in the developing and in the mature nervous system, in which they regulate crucial developmental processes and might also participate in synaptic neurotransmission.

In 1993 a team of scientists at Duke University reported the genetic association of the relatively common $\epsilon 4$ isoform of Apolipoprotein E (ApoE-4) with late-onset Alzheimer's disease (AD)¹. Initially, this finding raised considerable controversy because, at the time, available knowledge failed to provide an adequate explanation for the biochemical basis by which ApoE-4 might predispose its carriers to this debilitating neurodegenerative disease. However,

numerous subsequent studies have confirmed this initial finding, establishing *ApoE* as one of four currently identified and generally accepted genes that affect the onset of AD in man (reviewed in Refs 2,3).

ApoE was first described as a component of plasma lipoproteins, the carriers of lipids, such as triglycerides and cholesterol, in the circulation. In the liver, ApoE binds to two specific lipoprotein receptors, the low-density lipoprotein