The Primary Mechanism of Odor Perception

The latest findings and their application to the flavor and fragrance industry

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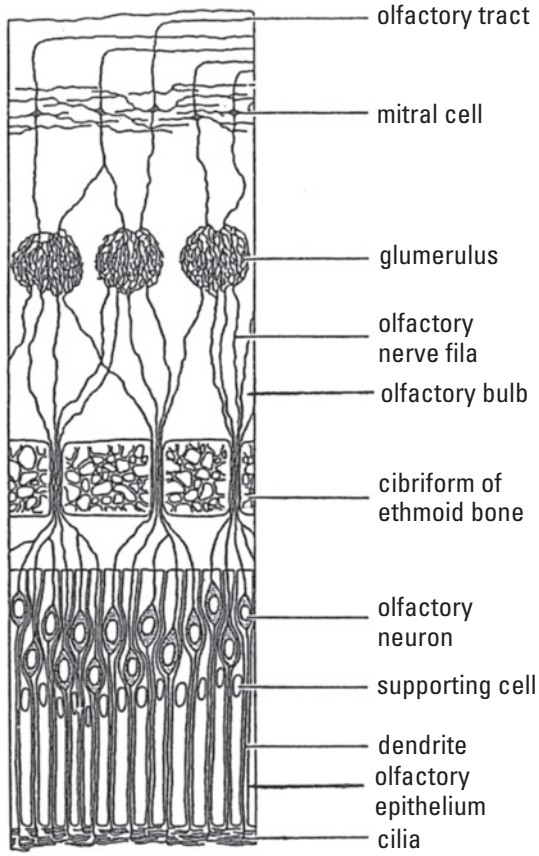
In Ohloff et al.'s "Scent and Fragrances", a chapter is devoted to the anatomy, physiology and biochemistry of scent receptors.[1] This chapter gives an excellent review of the knowledge about the subject up to 1995.

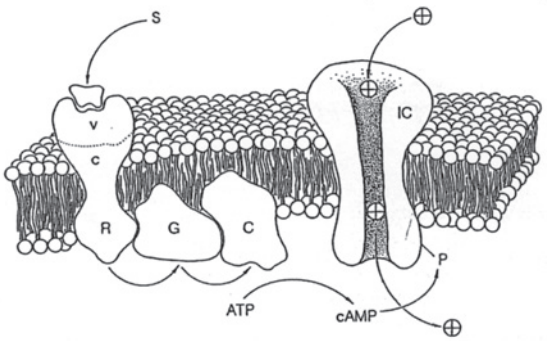
The *Regio olfactoria* contains up to 50 million primary sensory cells (*Nervus olfactorius*), accompanied by a much larger number of supporting and basal cells, which are permanently regenerated with a half-life of about 10 days. These sensory cells, which are bipolar neurons, are in contact with the outer atmosphere through an extension that ends in a bundle of cilia (*Cilia olfactoria*), which are embedded in the mucus that coats the surface of the olfactory mucosa (F-1). The olfactory cells form axons that are bundled (*Filia olfactoria*) to tracers the cribiform plate of the ethmoid bone, reaching the olfactory bulb (*Bulbus olfactorius*) of the brain, where they converge with post-synaptic cells to form synaptic structures called glomeruli. The first synapses are on the dendrites of the mitral cells. These synapses transmit olfactory information into the olfactory bulb of the brain (*Rhinencephalon*) and into parts of the brain where higher level processing of odors takes place (olfactory divergence). In addition to the olfactory nerves, the olfactory mucosa contains the trigeminal nerve (*Nervus trigeminus*), whose impulses from odorants are not transmitted to the olfactory bulb but are processed independently.

Sensory stimulation is initiated when an odorant comes in contact with parts of the receptor membrane in the cilia.[2] The static potential of a cell membrane is disrupted

Sketch of components of the olfactory nerve system[1] **F-1**



Schematic representation of the olfactory membrane[1] **F-2**



Molecular model for olfactory reception and signal transduction (Lancet [3-4], Anholt [5]), S Stimulus; R receptor protein with variable (v) and constant region (c); G GTP-binding protein guanidine triphosphate; C adenylate cylase; ATP adenosine-5′-triphosphate; cAMP cyclic adenosine-3′,5′-monophosphate; P protein kinase; IC ion channel

by a partial depolarization, and the stimulation transformed into action potentials that can be measured as an electroolfactogram (EDG).[3] This stimulation, which contains all information about the molecular properties of a stimulus in an encoded form, is transmitted through the *Tractus olfactorius* into the higher level centers of the central nervous system (CNS) and decoded into olfactory perceptions. The semantic description is the result of signal analysis and its comparison with a known odor pattern.

The anatomy and molecular constitution of the cilia, as well as their biochemical activities, indicate the stimulation takes place on intrinsic glycoproteins of the type gp 95 in the olfactory membrane.[4] The variable part *v* of the receptor molecule *R* (F-2), which can have many active sites, binds most probably in a non-covalent manner with the stimulus. This reversible complexation, formed by van der Waals forces, Coulomb attractions or hydrogen bonds, initiates an allosteric change of the quaternary structure of *R*, which activates the GTP binding protein *G* (first messenger), which in turn initiates a cascade of enzymatic reactions.[5,6] Cycli AMP, activated by adenylatecyclase, is the second messenger, which opens the channel protein *P* through phosphorylation.[7] A direct opening of the ionic channel is not very probable.[8]

The depolarization of the olfactory membrane is connected to a rapid ion-exchange. During one enzyme cascade, thousands of ion channels are opened,

leading to signal amplification. This mechanism explains the sometimes extremely low detection threshold of odorants. In the related mechanism of vision, it was shown that one stimulated receptor molecule can activate up to one million second messenger molecules.[9] The discrimination of a nearly infinite number of odorants leads to mechanistic comparison to the recognition of antigens in the immune system. Antibodies are specific receptors that can signal the antigens in a manner similar to the way "odogens" i.e. (odorants) recognize "odobodies."[10,11]

Analysis of the olfactory membrane has revealed important compounds.[12] Adenylatecyclase shows 15 times higher activity than all other mammalian tissues, including the brain.[4] The activation of the enzyme is largely dependent on the presence of the GTP-binding protein (G). Actually, all odorants increase the cyclase activity .[5,6] Up to now, at least five different G-proteins have been shown to exist in the olfactory epithelium. Recently, a G-protein called golf has been identified from olfactory nerve cells in rats; it has been cloned. The gene for the α-subunit of this GTP-binding protein was sequenced, and an antibody of high specificity for the detection of golf was developed.[25] Golf is supposed to play an important role in the transduction of odors.

Binding studies, using labeled ligands, led to the discovery of proteins that bind camphor, anisole, pyrazine, benzaldehyde and 5-α-androst-16-en-3-one.[11-18] In most of these cases, the specificity of the bindings is low, raising some doubt as to their activity as receptors. However, monoclonal antibodies against two olfactory mucosae proteins that can bind derivatives of anisole and benzaldehyde have been developed.[19]

The discovery of an odorant binding protein (OBP) in the lateral nasal glands located next to the olfactory mucosa has provided a new insight into the biochemical mechanism of olfactory perception.[20] OBP occurs in high concentration in the olfactory mucosa and has a molecular weight of 19 kDa. Its gene has been cloned.[21] OBP belongs to a group of carrier proteins for the transport of small lipophilic molecules. It is postulated that OBP binds odorants stoichiometrically and carries them to the receptors in the olfactory membrane.[22] As an alternative to this transport mechanism, one could consider that OBP functions as a filter that protects the receptors from an over-saturation of odorants. Competitive binding studies indicate that OBP changes its conformation with increasing concentration of odorants, leading to a decrease in affinity for these compounds.[23] The similarity of OBP and the binding protein for retinol is another indication for the mechanistic analogy of the senses of odor and vision.[23] Studies on the mechanism of gene expression of sensory-active proteins in the mucosa in connection with their biological function are in progress.[24]

Enormous progress has been made in the isolation of individual genes that are active in the nose and nowhere else in the body. These unique genes sequenced belong to a huge multi-gene family that encodes seven-membrane domain proteins. Eighteen different clones were able to be grouped into these sub-families, which probably contain receptors for the major classes of odorants.[26] (See "The Findings of Buck and Axel.")

The Findings of Buck and Axel (1991-1999)

In 1991, Buck and Axel confirmed the presence of about 1,000 different odor receptors.[26] These receptors are seven trans-membrane domain proteins whose expression is restricted to the olfactory epithelium. In 2004, Buck and Axel received the Nobel Prize in medicine for this excellent foray into the frontiers of knowledge.

In 1995, Axel presented convincing visual evidence that neurons that activate one type of receptor — and therefore respond to a limited number of odors — project their axons to a small number of glomeruli in the brain.[27] Each neuron represents one type of receptor.

According to the described model of smell, mammals should be able to detect an extraordinarily large numbers of odors. Because "odors" interact with multiple receptors rather than individual ones, the possible combinations exceed by several orders of magnitude the number of odors animals can actually detect. Consequently, just as with other senses, the olfactory system offers a meager representation of the environment. Presumably, animals discriminate only those odors that are biologically important to their survival and reproduction. This view of olfactory perception shares several basic features with perception in other sensory systems. For example, in vision the brain analyzes an image by interpreting the individual components: form, location, movement, color. The unity of an image is accomplished by reconstructing the signals in the visual centers of the higher cortex.

In comparison, the brain analyzes an odor by dissecting the structural features of the scent. The odor is then reconstructed by the olfactory cortex.

But how does the olfactory cortex, which receives signals from the olfactory bulb, decode the map provided by the olfactory bulb? This question is one of the central and most elusive problems in neurobiology. It seems likely that some form of spatial segregation similar to that seen in the olfactory bulb, but undoubtedly far more complex, will be maintained as the signals project

into the cortex. This arrangement, however, merely places the problem of interpreting spatial information one level beyond the olfactory bulb, in the cortex. How does the cortex prompt the range of emotional or behavioral responses that smells often provoke?

To what extent is the recognition of odors in humans conscious or unconscious, and how much of behaviour or mood is governed by the perception of odors in our environment?

We have only begun to explore the logic of smell and how it can evoke the "vast structure of recollection." In 1999, Malnic et al. provided experimental evidence for previous proposals that the code for an odorant in the nose is a dispersed ensemble of neurons expressing different odorant receptors (ORs), whereas in the bulb, where many glomeruli are activated by one odorant, it is a specific combination of glomeruli whose spatial arrangement is identical in different animals.[28]

According to Buck and Axel, the detection of odorants is mediated by about 1,000 different G protein-coupled odorant receptors (ORs) encoded by a multi-gene family.[26] In the nose, neurons expressing a given OR are confined to one of four OR expression zones where they are randomly interspersed with neurons expressing other ORs. In the olfactory bulb, the axons of neurons expressing the same OR converge at fixed sites in only a few of the bulb's roughly 2,000 glomeruli.

This suggests that olfactory information is first organized into four large sets in the nose and then reorganized in the olfactory bulb into a sensory map, which is identical in different individuals. In both nose and bulb, information derived from ORs is strictly segregated; each olfactory neuron in the nose and each glomerulus in the olfactory bulb appear to be dedicated to input from one OR type.

Malnic et al. mentioned that some odorants are perceived as having different odors at different concentrations.[27] From their work, it seems probable that a change in the concentration of an odorant can change its receptor code. This, in turn, may lead to a change in odor quality.

One intriguing feature of olfactory perception is the dramatic effect that can be brought by a small change in odorant structure (example: normal aliphatic alcohols and acids). Manic et al.'s studies suggest that changes in the perceived quality of an odorant that result from an alteration in its structure may be a direct result of changes in its receptor codes.

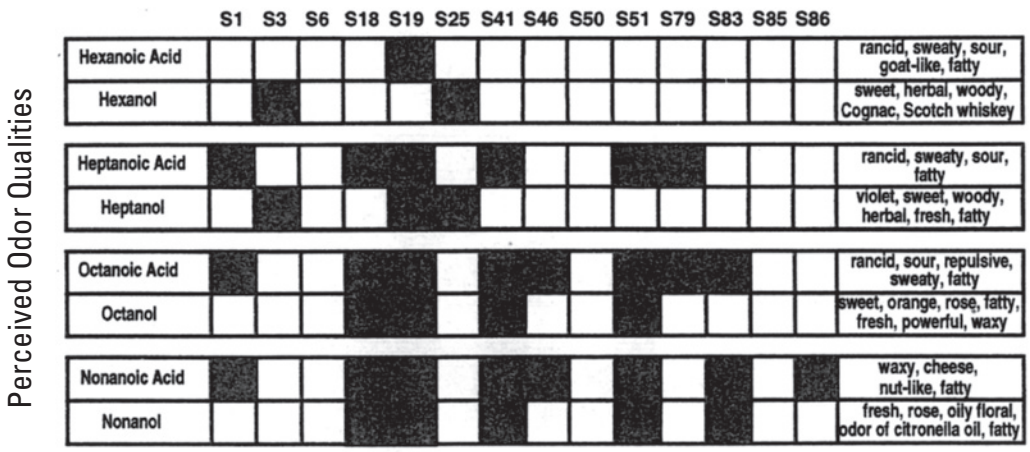
They also wrote:

> Humans can detect some odorants at a much lower concentration than they can others. One possible explanation for this phenomenon is suggested by our finding that different odorants can be recognized by different number of ORs and by different percentage of olfactory neurons. Thus, the size or complexity of an odor code might be an important determinant of how easily an odorant can be detected, perhaps reflecting the cumulative intensity of signals transmitted to the olfactory bulb.

Differences in the sizes of odor codes might also be relevant to the existence of selective perceptual deficits (specific anosmias) to some odorants but not to others. If an odorant is recognized by only one OR, mutations in that OR would result in specific anosmia for the odorant. If an odorant is recognized by multiple ORs, specific anosmia would not occur unless all of the relevant ORs were mutated. In this case, mutation of one OR that recognizes the odorant would, however, change its code, perhaps giving rise to perceptual differences among individuals, which are known but not understood.

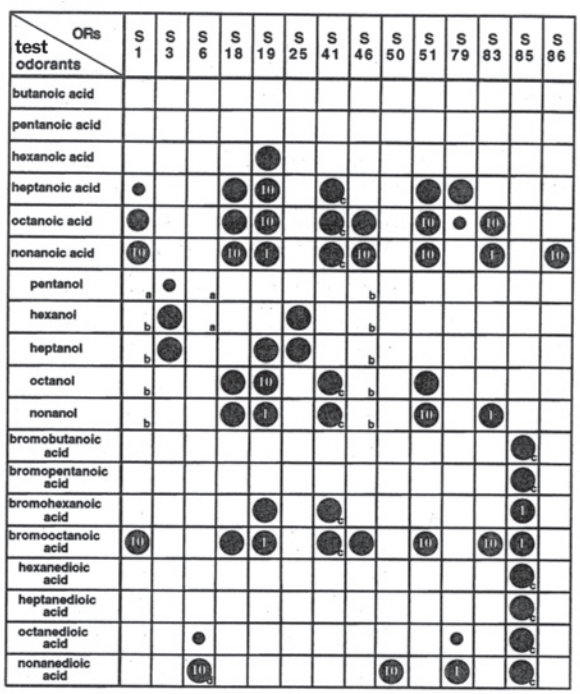
What is the molecular basis of odor quality? Interestingly, a single odorant is often perceived as having several different odor "qualities." When two odorants are mixed, both odorants can often be perceived in the mix. This implies that a perceived

Comparison of the receptor codes for odorants that have similar structures but different odors[27] **F-3**



aliphatic acids and alcohols with the same carbon chains were recognized by different combinations of Ors, thus providing a potential explanation for why they are perceived as having strikingly different odors; perceived odor qualities shown on the right were obtained from Arctander (1969), The Good Scents Company (www.execpc.com/~goodscnt//index.html), and The Chemfinder Web server (chemfinder.camsoft.com)

The recognition profiles of 14 olfactory neurons and the ORs they expressed[27] (test odorants and ORs identified) **F-4**



a, not tested; b, tested at 10 μM, but not 100 μM; c, not tested at 10 μM or 1 μM; d, not tested at 1 μM; filled circles indicate responses to 100 μM odorants, with smaller circles indicating a relatively weak response (less than half the change in fluorescence intensity elicited by KCl); responses that were also obtained at 1 or 10 μM odorant are indicated by a 1 or 10 outside the filled circle

odor quality can result from a subset of the ORs that recognize the mix. This in turn raises the possibility that, in the extreme case, a single OR might convey an odor quality. One way to explore this question would be to ask whether there are ORs that recognize only odorants with a particular odor quality. For example, the two ORs that recognized only aliphatic alcohols in our experiments (S3 and S25) might be candidates for ORs that convey a "woody" or "sweet" quality (F-3 and F-4).

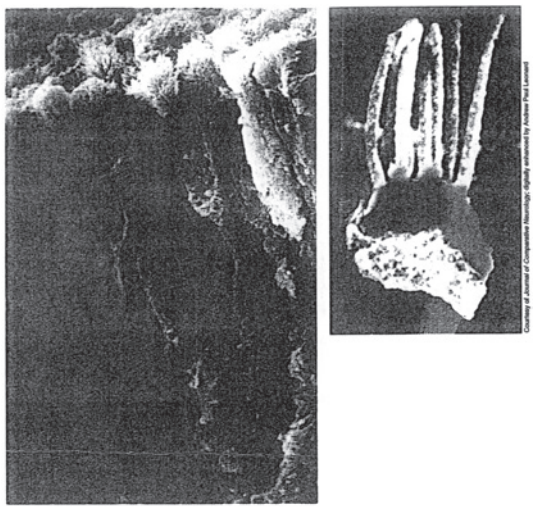
What appears strange in the findings of Malnic et al. is that (F-3 and F-4):

- Butanoic acid and pentanoic acid were not recognized by the ORs, which were investigated
- There does not seem to be a specific OR that recognizes an alcohol or an acid (that means a functional group does not interact with one specific OR)
- One specific OR (S85) recognizes all bromo- and dicarboxylic acids, but no monocarboxylic acids
- Dicarboxylic acids, which are virtually odorless to humans (because of their low volatility), are recognized by a only few ORs (especially S85)

Analysis of Existing Knowledge

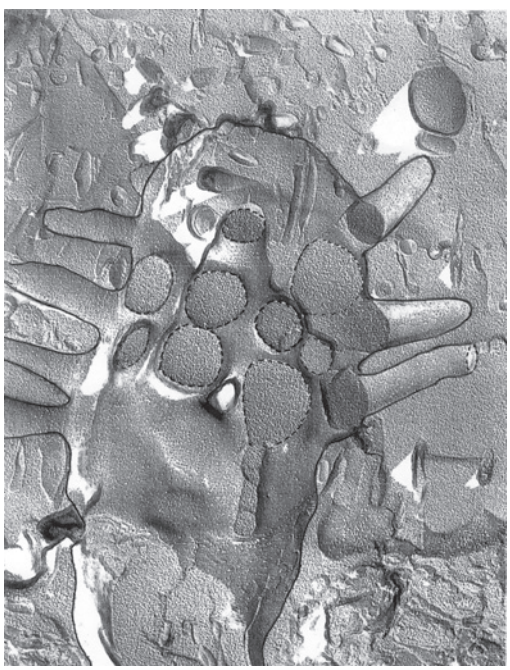
Although the primary interaction between an odorant and a biological system occurs at the cilia in the mucus, relatively little attention has been paid to the possible mechanism of this interaction. First, it seems worthwhile to recall the arrangement of the biological system of the epithelium. According to aforemen-

Sensory neuron in the human olfactory epithelium (left) with hair-like cilia protruding (right) **F-5**



the sensory neuron is surrounded by supporting cells and sits over a layer of neuronal stem cells, which generate new olfactory neurons during an organism's life; the hair-like cilia (right) are shown magnified 17,500 times; receptors located on cilia bind to odor molecules; images were taken by R.M. Costanzo and E.E. Morrison of Virginia Commonwealth University

An electromicroscopical photography of an imprint of one dendrite with cilia in the olfactory epithelium of a rat **F-6**



about 50,000 times; photo by B.Ph.M. Menco, psychological dept., University Utrecht

tioned and other published studies, the olfactory epithelium:[32,33]

- Has a surface of some two to three square centimeters (200-300 million μ^2)
- It contains olfactory neurons, supporting cells, dendrites with cilia
- There exist tens of millions olfactory neurons
- The dendrites (diameter up to 10 µ) have at the end 10-20 cilia (length 1-2 µ)
- The cilia are embedded in a mucus layer of about 50 µ thickness
- The mucus layer contains water and odor binding proteins

The cilia in the mucus often are represented as a plate with spaghetti, whereas pictures taken with an electron microscope show that the end of the dendrites with cilia more look like a hand with fingers (F-5 and F-6). Exact calculations reveal that the cilia have a length of about 1 µ, whereas the thickness of the mucosa is about 50 µ. As a consequence, there is a lot of space for the odorant to overcome to reach the olfactory receptors.

Some Ideas About the Primary Processes of Olfaction

Having studied the aforementioned publications in more detail, one may philosophize and/or speculate about the primary processes of human olfaction. First, chemical compounds have to be volatile to reach the internal nasal area or the olfactory epithelium. That is to say, human beings can only smell airborne molecules, called odorants. Of course one can inject solutions of non-volatile chemicals on and in the epithelium of mammals and get olfactory responses but that does not mean that humans normally can smell these compounds.[28] Thus it is unlikely that they can smell higher aliphatic dicarboxylic acids.

Airborne odorants reach the surface of the nose's olfactory epithelium via inhalation. The epithelium's surface is a 50 µ-thick mucosa consisting of a watery layer with several types of glycol proteins.[21-25] Via diffusion an equilibrium exists between the concentration of the odorants in the air above the mucosa and the concentration of the odorants in the surface (of the epithelium).

Odorant-binding proteins are contained within the mucosa.[16-21] As mentioned before, this binding between odorant and proteins may be weak van der Waals forces, Coulomb attractions or hydrogen bonds. This reversible complexation could be in

equilibrium with airborne odorants and fresh air, as follows:

airborne odorant (AO) + binding protein (BP) === odorant bound protein (OBP) + air (A)

Thus, it will be clear that (fresh) air drives this equilibrium to the left, and the odorant molecules are washed away by air.

In a second step, the odorant bound proteins (OBP) could be transported through the mucus to the cilia on the end of a dendrite in the mucosa. The primary biological event of olfaction may occur at the cilia. The odorant bound protein could transfer the odorant to a binding G protein (OR), the so-called seven-membrane domain protein also in a reversible interaction:[26,28]

odorant bound protein (OBP) + G-protein (R) === binding protein (BP) + odorant receptor (OR)

The odorant can be "measured" for its electronicity and stereocity at or in the odorant receptor. It seems as if the odorant receptor palpates the complete odorant molecule. Strong electron-releasing or -attracting functional groups in odorants like carboxylic acids, amines and sulfur compounds could be recognized and discriminated. The sterical parts of the odorant-molecule may also be palpated by different ORs, the same way a blind person instantly translates Braille into words.

Because about 350 functionally different odor receptors exist, it seems quite possible that, overall, human beings can recognize and discriminate each functional and sterical part of an odorant molecule.[32-33] Moreover, by combinatorial receptor codes for odorants humans could be able to discriminate thousands of different odor qualities.[28] The signal amplification and transduction from the activated odor receptors to the brain is well-known.[2-11]

Applications of Buck and Axel's Findings

One may wonder how the findings of Buck and Axel can be applied in flavor and fragrance research and modern perfumery. If it is possible to isolate the genes that code for odorant receptors (ORs), several possibilities are opened. First, the genes (about 350) can be separated and multiplied. With each separated gene, the individual odorant receptor protein (OR) can be prepared. Each OR can be fixed on a suitable support in vitro, such as with a synthetic membrane. A light-emitting protein may be added to the ORs in such a way that the complex fluoresces after light-exposure if there is an interaction of the OR and an odorant molecule. This "synthetic" biological system affords a method for measuring olfactive properties of odorant molecules in vitro.

With this system the following investigations are possible:

- Making a library of all the different ORs, which interact with an odorant at a certain concentration (patterns for about 5,000 different odorant molecules)
- Testing the olfactive qualities of a new odorant molecule by comparing its activated ORs with those in the library (human subjective olfactive evaluation is no longer necessary)
- Confirming the odor intensity by testing at various concentrations
- Determining the active ORs for malodorant molecules (to find substituting molecules,
- Blocking of malodors such as sweat odor [valeric acid])
- Finding the patterns of activated ORs of odorant mixtures such as natural isolates and perfume compositions (to improve reconstitution and to search for missing odorant molecules)

The aforementioned investigations can of course be extended with more possibilities.

Some biotechnological companies are already carrying out experiments according to these routes. In the United States, the companies Linguagen (1995) and Senomyx (1998) are working with odor and taste receptors. Food and beverage companies like Campbell Soup and Coca Cola, and flavor & fragrance companies such as Givaudan and Floridienne have invested more than US$100 million in these companies. Chemcom (2000) in Belgium is active in the area of odor receptors and odorants, especially for preparing of blockers of malodorants (investment €6 million).

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