Bridging the Olfactory Code

The tangled history of odor understanding and the path toward an odor-predicting algorithm

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In 1969, John Amoore, an English biochemist, published a book titled *Molecular Basis of Odor*.[1] In it, the author proposed the mechanisms involved in odor detection, from the moment an odorant reaches the olfactory epithelium to the construction of the sensory perception by the brain. Amoore offered the following visualization: a long span of bridge along which he placed a number of the different branches of science related to olfactory perception. On one end of the bridge he placed the chemistry of the olfactive stimulus. He set the physiology of sensory perception on the opposite end. At different points along the bridge's length, Amoore placed a variety of other specialties, including volatile molecule analysis, molecular structure determination and biology. The author explained that the enormous spaces between these various points would be filled by scientific progress. Now, some 40 years later, one could say that the understanding of olfaction has nearly crossed this bridge. Yet the mechanism of primary olfactory reception, which is generally accepted by the international scientific community, remains a controversial point. The assumed model posits that an odorant is recognized by an olfactory receptor and represents one of the first steps across Amoore's bridge to olfactory perception.

The Shape Theory

Amoore claimed that odorants were recognized by olfactive receptors in the same way a lock recognizes a key. He established a classification of the different families based on the shape of the odorants. Amoore believed that the specificity between the odorant and the receptor was high: each molecule was to be recognized by only one receptor and, conversely, each receptor would mainly recognize just one kind of molecule. But it has since been shown that humans are able to perceive more than 10,000 unique smells, so the number of receptors that would be necessary in Amoore's theory would be impractically high. Recent experiments in this field have suggested a solution to this problem: human beings have approximately 350 functional receptors, but the specificity of the receptors is not as high as initially expected. Consequently, an odorant is capable of activating different kinds of receptors and each receptor is activated by different kinds of molecules. Rather than recognizing the specific shape of a molecule, some researchers believe they instead respond to molecular features known as osmophores, or the atoms responsible for aroma.

> A chess game is not understandable if just the moves at the corner of the board are regarded.
>
> —Wolfgang Köhler

To illustrate, one could imagine a garden in spring, with hundreds of thousands of odorant molecules floating in the air. These molecules come in different shapes and sizes and allow one to enjoy, for example, the marvelous fragrance of roses. In the olfactory epithelium, some of the molecules get trapped in the olfactory mucous, an extracellular fluid of aqueous nature. Due to this aqueous nature, not every molecule will be able to dissolve or diffuse in the fluid, which is a prerequisite for the activation of the olfactory receptors. The molecules that *are* able to move in this environment are those that exhibit a certain polarity and can dissolve in the liquid. The molecules that remain untrapped interact with the different types of receptors in the olfactory epithelium, in the hopes of activating one. A receptor is a strangely shaped bundle of seven cylinders. Each cylinder constitutes an amino acid sequence, forming a helicoid structure. To return to the key and lock metaphor, when the correct key is inserted into a pin tumbler lock, the pins line up level, and the cylinder can be turned to disengage the bolt. Similarly, a molecule is drawn by its dipole moment among the seven cylinders of the receptor protein, and if it fits appropriately, the protein conformation changes and a reaction is induced. It is quite probable that the relative turning movement of some of the α-helixes is what provokes the conformation change. (**See F-1**.)

There are approximately 350 different such "locks," olfactory receptors located in the cellular membrane that transmit from exterior to interior. Even if the exact mechanism of odorants' approach to receptors is not precisely known, one can suppose different possibilities. In the first scenario, as suggested earlier, those molecules presenting enough solubility in the mucus could pass through

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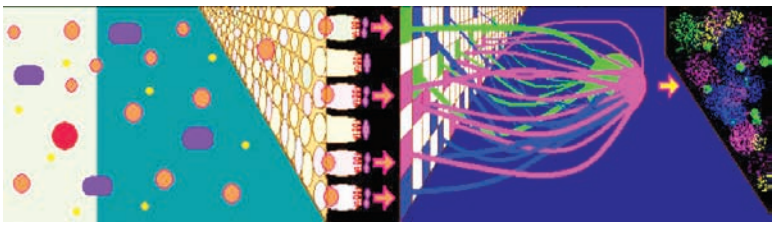
The left side of this illustration displays odorous molecules of different shapes as they travel toward receptor proteins; the center of the image shows several odorous molecules as they activate olfactory receptors, triggering a flow of electrical signals as indicated with arrows; finally, the illustration shows axons that converge in different sorts of glomeruli and, to the right, synaptic connections in the olfactory cortex

F-1



it and activate the receptors. In the case of a molecule that is not soluble enough, it could be assisted by the odorant-binding proteins, which would transfer it to the membrane. Once there, the molecule would dissolve and pass through the membrane surface until it entered the receptor laterally, thus activating it.

When a molecule reaches its objective, a conformational change in the protein provokes the flexion of the whole structure. This "shiver" activates a switch that triggers the fast movement of a convoy of minute electrical charges.

What has just been described is the fundamental mechanism that allows an odorant to bond to an olfactory receptor. These olfactory neurons are ciliar cells, which significantly boosts the total receptor surface. Knowledge of the molecular recognition mechanism may help one deduce some of the properties that molecules must have in order to exhibit odor properties. First, they must be volatile to reach the olfactory mucous. They must also be partially soluble in water to be able to diffuse in the epithelial mucous, and partially soluble in the lipidic chain in which the transmembrane protein is located. The importance of the lipophilicity of the molecules lies in the fact that they must fill the hydrophobic binding pocket of the receptor. In addition, they must be able to lower the surface tension of the external layer of the olfactory mucous to sink through it. Meanwhile, they must not be too small or too large. Molecules that are larger than approximately 300 Daltons are excluded. In general, the aliphatic compounds such as alcohols and esters decline in olfactive intensity in relation to their molecular size. Molecular length is another factor that determines the range of molecules that are capable of activating a certain receptor.[2] Molecules that accomplish their objectives bind to the active center of the helicoidal seven transmembrane protein by means of electrostatic interactions that cause a conformational change in the protein. As a result, some changes are induced to a G protein. The G protein is in the intracellular domain of the neuron; inside the neuron there is a protein that recognizes when the receptor has undergone a change in its shape. This behavior triggers a series of chain reactions that transmit electrical impulses at high speed to the olfactory bulb glomeruli.

The action mechanism of certain molecules and their respective enantiomers makes the functioning of the olfactory receptors more clear. It is experimentally verified that certain pairs of R and S enantiomers activate different receptors; as a consequence, they also activate different glomeruli in the olfactory bulb. This accounts for the various smells of enantiomers. For example, (R)-carvone smells like spearmint, while (S)-carvone smells like caraway.[3] Furthermore, it has been shown that each segment of peptides in the helicoidal structure of a transmembrane receptor can have different interactions with each enantiomer. Carvone is detected by many different receptors. Some respond to both enantiomers, while others engage only to the (R)- or (S)-. From a theoretical point of view, the calculus of the energy of the proteid complex for each enantiomer confirms this mechanism. In the case of carvone, there are selective receptors for each enantiomer. However, other pairs of enantiomers activate exactly the same types of receptors and, as a consequence, possess the same odor. This is the case with the (R)- and (S)-enantiomers of camphor. A deeper knowledge on the selectivity of certain biological receptors in relation to certain kinds of enantiomers could contribute to a better understanding of mechanisms of activation of these receptors.[4]

The Combinatorial Model

The combinatorial model explains quite accurately the mechanism of the primary olfactive reception. To verify a new theory it is advisable to use the falsation method proposed by the Austrian philosopher Karl Popper, refuting it with good arguments. If refuting the theory is not possible, it must be temporarily admitted as true.

The combinatorial model, with its stereochemical nature, is similar to the former model proposed by Amoore, but with the addition of some remarkable novelties. The interaction is in fact stereoelectronic rather than simply stereochemical. To be fair, when Amoore formulated his theory, the olfactory receptors had not been yet been discovered, and the mechanisms involved in the recognition of the odorants were still unknown.

In 1991, molecular biologists Richard Axel and Linda Buck of Columbia University in New York published a paper in *Cell* regarding the nature of the olfactory receptors, for which they were eventually awarded the 2004 Nobel Prize in Physiology or Medicine. In this paper they cloned and characterized 18 different members of a gene family that codified a group of proteins able to act as olfactory receptors.[5]

In March 1999 a group of investigators, including Buck and Bettina Malnic from the Harvard Medical School, and

Junzo Hirono and Takaaki Sato from the Life Electronics Research Center in Amagasaki, Japan, deciphered the mystery of how the brain picks up smells. They used a method in which they exposed mouse neurons to several different kinds of odorants. Via a technique of calcium visualization, the investigators detected which nerve cells were triggered by a certain smell. When an odorous molecule was bound to an olfactory receptor, the calcium channels in the cell membranes opened, and the calcium ions entered the olfactory neuron. The images of the calcium ions provided information on the flow of these elements. This is how the authors corroborated, as stated before, that an olfactory receptor can recognize several different odorous molecules and that an odorous molecule can be recognized by several different receptors. These results are of exceptional value to understand how the olfactory system works. These conclusions show that the different olfactory receptors operate using a combinatory code.

In 2006, S.D. Liberles and Buck published a paper in which they explicated the discovery of a second type of chemosensory receptor in the olfactory epithelium: TAAR receptors, associated with trace amines. These receptors recognize traces of amines that have been reported in urine as related to stress and pheromones. Their mission does not appear to be one of olfactory recognition but rather the detection of social signals. This new kind of receptor possesses a function similar to that of the vomeronasal organ in other animals.[6] Buck et al. also observed that slight changes in the chemical structure of the molecules result in the activation of different kinds of receptors; e.g. octanol presents a greasy, aldehydic, orange smell while the octanoic acid has a sweat smell.

The implications can be considered if one imagines a system consisting of 26 keys and 26 locks, each with a letter from A to Z. These locks are partially specific, which means that they can be opened by more than one key. In how many ways could we classify the keys using this system? The possibilities are as follows:

Combinations of 26 elements taken:
one at a time = 26
two at a time = 325
three at a time = 2,600

These calculi provide almost 3,000 ways of classification. Something similar could happen with odorants. Humans have approximately 350 sorts of receptors; in how many ways can the olfactory system classify the molecules, abiding with this analogy? The answer:

Combinations of 350 elements taken:
one at a time = 350
two at a time = 61,075
three at a time = 7,084,700

With a limited number of receptors one can recognize thousands of different odors. Just as words can be composed of very few letters or a larger number of letters, a molecule might activate just a few receptors or many of them. However, it should be noted that in a word letters can be repeated, and their order matters. In the case of smell, "letters" do not repeat, but order does not matter. In a mathematical language, one would say the olfactive system handles combinations without repetition. This olfactive alphabet has approximately 350 letters, and each "word" represents a unique olfactory sensation in the brain. This mechanism allows one to have an idea of the great discriminatory power of the described model.

The way a ligand (odorant) binds to an olfactory receptor is widely documented.[7,8] There is a minimum energy threshold under which the activation of the olfactory receptor is not executed.[9] The possibility of the intervention of metallic cations in the formation of the bond between the odorous molecule and the protein could reveal the reason that alcohols smell less strongly than their corresponding homologues, thiols. This is because the groups (-SH) have a greater affinity for metallic cations. Despite these observations, the possible participation of metallic ions in the primary olfactory reception is not clear yet.[10,11]

When a perfumer or a flavorist formulates a product it is sometimes very difficult to foresee the sensory result of making slight changes in the formula, distortions that may be the effect of inhibitory aspects.

That each olfactory receptor has a receptive range for different kinds of odorants has been verified. The selection occurs according to the characteristics of the odorant molecules, whether by structural similarity or by chemical global chemistry.[2] An inhibition effect has been observed when molecules compete for a union site. In pharmacology, the antagonism phenomenon is widely known.[12,13] When a perfumer or a flavorist formulates a product it is sometimes very difficult to foresee the sensory result of making slight changes in the formula, distortions that may be the effect of inhibitory aspects.

Despite recent revelations, there remain some properties of olfaction that are difficult to explain with the current knowledge of its mechanisms. For instance, why do chemical compounds with similar structures produce flavors with very different intensities? Hoping to explain some of these properties, in 1996, Luca Turin, an Italian biophysicist, resurrected the theory of molecular vibration that was originally suggested by Malcolm Dyson in the first half of the 20th century.

According to Turin, olfactory receptors detect molecular vibrations of infrared energy. This theory is based on the supposition that olfactory receptors function as tiny spectroscopes located in the olfactory epithelium. Turin maintains that olfactory receptors are able to transport electrons via an inelastic tunnel effect that has an incidence on the odorant molecule, transforming electrical energy into specific signals that are translated into emotions and sensations in the brain. He further

maintains that when an odorant binds to an olfactory receptor, a flow of electrons commences, derived from an energetically rich molecule—a small biological battery (NADPH). If the vibrational energy equals the energy difference between the maximum and minimum levels of the receptor, a G protein is activated, which allows for the transduction of the electrical impulse to the olfactory bulb through the neurons. According to Turin, in this mechanism a disulphide bond is reduced between both proteins. A metallic cation (Zn^{2+}) acts as a cofactor of the quoted mechanism.[14,15]

From the current knowledge of the mechanisms of olfaction it may be affirmed that the vibrational theory is not adequate to explain the primary olfactory reception.[16–18] Turin has been able to construct interesting hypotheses on different aspects of the olfactory system, like the possibility of an intervention of metallic cations in the olfactory mechanism. Nevertheless, the evidence shows that the vibrational theory is not capable of explaining how the olfactory system works.

In the Realm of the Olfactory Bulb

In the olfactory bulb, thousands of electrical messages come from the neurons of the olfactory epithelium and converge in meeting points, or spherical collectors, called glomeruli. Each glomerulus harvests the information of a certain odorant range that shares similar molecular characteristics, input from a single type of olfactory receptor.

Glomeruli with similar molecular ranges are located near others, constituting clusters. The three-dimensional distribution of the glomeruli in the olfactory bulb is not random, but rather obeys a molecular logic that allows the olfactory "software" to read odorant family patterns.[19] The three-dimensional disposition of glomeruli inside the olfactory bulb appears to be a key factor in allowing that software to process the information.

An illustration: With the aid of topographical maps of the olfactory bulb, it has been demonstrated that the essential oils of fennel and clove activate glomeruli clusters near those activated by alquilamines, which are responsible for the bad odor of food in poor conservation conditions.[20] As a result, the function of the alquilamine clusters is inhibited. In other words, the glomeruli activated by fennel and clove odors—mainly comprised of anethol and eugenol, respectively—stop the transmission of bad food odor via mitral neurons. This explains the important historical role clove and fennel have played as culinary ingredients, particularly in premodern times. The evolution of the olfactory system provides many examples of this meeting of function and necessity.

Electrical impulses that originate from the same type of receptor group travel together through neuronal highways called axons. If one could visualize the many "sparks" issuing from the approximately 350 different receptor types, one could actually track the intensity differences of each impulse over a given fraction of time. This virtual intensity corresponds fairly well to the olfactory intensity with which we perceive the different odors. A photograph of the situation would allow one to contemplate the different kinds of receptors activated in one precise instant. This information is important to the computation of chemical signals of the odorous molecules.

Synaptic "Glittering"

Information travels through synaptic connections between mitral neurons and the olfactory cortex. There, it is processed by an extraordinary computer, the brain, which uses the most incredible software, constituted by a large number of neuronal networks that transmit valuable information. To watch these synaptic scintillations, or glittering, would be a fantastic show. In the cortex, together with the hippocampus and other parts of the brain, millions of bits of information are interchanged in fractions of a second. The inputs from a given glomerulus diverge to multiple olfactory cortex areas. This divergence of inputs of the olfactory receptors allows a parallel processing of the signals that are combined or modulated in different ways before being sent to various functional regions of the brain.[21]

The olfactory cortex is structured in layers where the information is decoded from the bottom to the top. The lower layers have their evolutionary origin in ancestors of Homo sapiens. Thanks to this structure, based on the layer hierarchy, different aspects of the olfactive abstractions are processed and identified as a specific odor in the top layers.

Toward an Odor-predicting Algorithm

F-1 illustrates the various stages of olfactory understanding discussed herein. Despite the description of this journey requiring several pages, olfactory perception is actually produced in brief fractions of time. In this way, living beings are able to constantly exchange information with their environment. This interactivity is a key factor to their survival.

The olfactive research that has been done to date has affirmed that olfaction is the result of the specialization of neurons and that the functionality of the nose is as a detector of odorants. It is this author's opinion that the debate between the shapist and vibrational olfactory theories has definitely reached an end; there remain many questions regarding the olfactory software of the brain and other aspects of olfaction on the air—but this we will leave for another time.

Of course, olfactory perceptions do not exclusively obey the chemical stimulus of the odorous molecules: they are just one segment of total sensory perception, or gestalt, as the psychologist Wolfgang Köhler conceived it. In such a perception there is a confluence of stimuli coming from other senses, such as vision, touch and taste, or from the trigeminal nerves.[22] Olfactory perceptions are outcomes of a highly synthetic process that is further modulated by memory, expectation, context and emotional state.[23]

In the future, this combinatory model of olfactive understanding will reveal how each of the approximately 350 kinds of glomeruli in the olfactory bulb recognizes the varying molecular structures of odorants. With that

knowledge, humans will finally possess an olfactory map that will be contrastable with the sensory smell maps proposed by several authors.[24] A better understanding of the dimensions of the perceptual olfactory space from the sensory point of view, knowing how the brain processes the information, greater insights into the physiological facets of smell, and determination of just how receptors and glomeruli are activated will advance the long-standing dream of achieving an odor-predicting algorithm. In such a future, perfumers and R&D chemists may be able to design tailored odorous molecules, making them smell exactly as they wish.

References

1. JE Amoore, *Molecular basis of odor*, Thomas, Springfield, IL (1970)
2. R Araneda, A Kini and S Firestein, The molecular receptive range of odorant receptor, *Nat Neurosci*, 3(12) (2000)
3. D Krauwurst, Human olfactory receptor families and their odorants, *Current topics in flavour and fragrance research*. P Kraft and KAD Swift, eds. Wiley, Hoboken, NJ (2008)
4. N Nandi, Study of chiral recognition of model peptides and odorants: carvona and camphor, *Current Science*, 88(12), 1929–1937 (2005)
5. L Buck and R Axel, A novel multigene family may encode odorant receptor: a molecular basis for odor recognition, *Cell*, 65 175–187 (1991)
6. L Buck, A second class of chemosensory receptors in the olfactory epithelium, *S.d: Liberles*, 442(10), doi: 10.1038./nature05066 (2006)
7. S Katada, T Hirokawa, Y Oka, M Suwa and K Touhara, Structural basis for a broad but selective ligand spectrum of a mouse olfactory receptor: mapping the odorant binding site, *J Neurosci*, 25(7) 1806–1815 (2005)
8. R Haddad, L Carmel, N Sobel, D Harel, Predicting the receptive range of olfactory receptors, *Computational Bio*, 4(2;e18), 18 (2008)
9. P Hummel, N Vaidehi, W Floriano, S Hall and W Goddard, Test of binding threshold hypothesis for olfactory receptors: explanation of differential binding of ketones to the mouse and human orhologs of olfactory receptor 912-93, *Protein Sci*, 14 703–710 (2005)
10. M Zarzo, The sense of smell: molecular basis of odorant recognition, *Biological Reviews*, 82 455–479 (2007)
11. J Wang, ZA Luthey-Schulten and KS Suslick**,** Is the olfactory receptor a metalloprotein? *Proceedings of the National Academy of the Sciences United States of America*, 100(6) (2003)
12. Y Oka, A Nakamura, H Watanabe and K Touara, An odorant derivate as an antagonist for an olfactory receptor, *Chemical Senses*, 29(9), 815–822 (2004)
13. Y Oka, M Osmura, H Katoaka and K Touhara, Olfactory receptor antagonism between odorants, *The EMBO Journal*, 23(1) (2004)
14. L Turin, A spectroscopic mechanism for primary olfactory reception, *Chemical Senses*, 21(6) 773–791 (1996)
15. L Turin, Letter to editor, *Chemical Senses*, August, 33(8), 683–684 (2008)
16. TP Hettinger, Scent and alchemy: The paperback, *Chemical Senses*, 33(6) 575–579 (2008)
17. AJ Taylor and DD Roberts, *Flavour perception.* Wiley-Blackwell, Hoboken, NJ 132–134 (2004)
18. J Brookes, F Harstoutsiou, AP Horsfield and AM Stoneham, Could humans recognize odor by phonon assisted tunnelling? *ArXiv*, physics/0611205, 22 (2006)
19. K Mori, K Takahashi, K Igarashi and M Yamaguchi. Maps of odorant molecular features in the mammalian olfactory bulb, *Physiology Rev*, 86 409–433 (2006)
20. Y Takahashki, S Nagayama and K Mori, Detection and masking of spoiled food smells by maps in olfactory bulb, *J Neuroscience*, 24(40) 8690–8694 (2004)
21. L Buck, Unraveling the sense of smell, *Nobel Lecture*, Howard Hughes Medical Institute, Fred Hutchinson Cancer Research (2004)
22. CS Sell, Scent through the Looking Glass, *Perspectives in Flavour and Fragrance Research*, P Kraft and KAD Swift, eds., Wiley, Hoboken, NJ (2005) 67–88
23. DA Wilson and RJ Stevenson, *Learning to smell*. The Johns Hopkins University Press, Baltimore, MD (2006)
24. M. Zarzo, Understanding the underlying dimensions in perfumer's odor perception space as a basis to develop meaningful odor maps, *Attention, Perception and Psychophysics*, 71(2) 225–247 (2009)

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