VOL. 30 SEPTEMBER 2005

PERFUMER & FLAVORIST

The future of fragrance

Toward a Biotechnology of Scent Creation

What's in reach and what needs to be achieved for the olfactory revolution to become the fragrance revolution

Adam Elias,* Elias Fragrances Inc., and Nicolás Pírez and Justus V. Verhagen, Department of Biology, Boston University

It is a wondrous time to be involved in olfaction. Almost 15 years ago, the field was cracked open when recent Nobel Prize winners Linda Buck and Richard Axel discovered the genes that are responsible for encoding olfactory receptors — the proteins that bind to odorants, the proteins that in many ways are responsible for unraveling the mystery of the olfactory code (see The Breakthrough for abstract).[1] Since that time, much has been elucidated through genomic, cellular, molecular, physiological and anatomical studies of the mammalian olfactory system.[2-27] These studies have broadened understanding and revealed a complexity and elegance of function difficult to predict earlier. Furthermore, it is becoming increasingly clear that these developments will have an important role in the private sector, leading to various industry applications.[2] However, it is still unclear what exactly these new applications will be and how the transition from basic science to industry technology will occur.

The Question

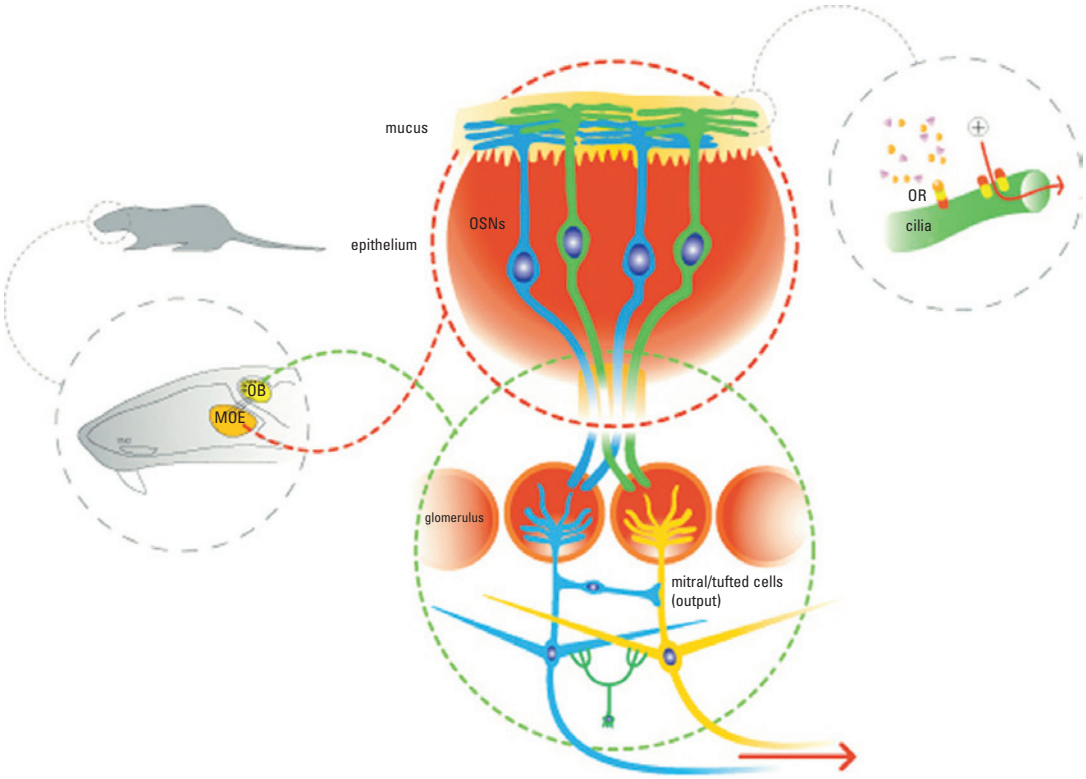
How can the molecular biology and neuroscience of olfaction help shine light on the relationship between the molecular properties of an odorant and its odor quality? This question invites many others, because in order to go from stimulus to perception all the intermediate steps need to be understood. Such steps include binding mechanisms and sensitivity of olfactory receptor (OR) and odorant, transduction processes that activate the olfactory sensory neurons, mechanisms that guide the sensory neurons' projections to the olfactory bulb (OB), local processing within the OB, and processing of odor information brain areas downstream from the OB. Understanding all of these steps is critical to unlocking the code — the good news is many of these steps already are partially understood.

Olfactory System Review

In the nasal epithelium, which lines the inside of the nose, there are olfactory sensory neurons (OSNs; F-1). The neurons have hair-like cilia, and on these cilia are densely packed proteins called olfactory receptors (ORs). These proteins are located in the membrane of the OSNs. When a certain odorant molecule enters the nose, it is exposed to the nasal epithelium and binds to an OR. This interaction leads to the opening of ion channels on the surface of the cell, allowing positive ions into the OSNs, thereby changing the electrical potential between the inside and outside of the cell. This change in membrane potential can induce an action potential: an electrical

*Previously with Boston University.

Schematic of the olfactory system **F-1**



Olfactory sensory neurons (OSNs) are located in the main olfactory epithelium (MOE) in the nose. Their cilia are located in the external layer of mucus and contain only one of ~1000 types of odorant receptors (ORs), which each bind to small subset of odorant molecules. OSNs of each type (here indicated by blue and green) massively converge onto only one or two specific glomeruli out of the many hundreds that are found in the olfactory bulb (OB). Mitral and tufted cells are the output neurons of the OB projecting to various structures in the central nervous system. An interneuron (green), located between these output neurons and thought to be important for interglomerular interactions, is also shown.

spike propagating throughout the rest of the neuron, ending with the release of chemicals called neurotransmitters, which allow communication with other neurons.

The receptor proteins in the olfactory system are special. People have paid much attention to the proteins' functions and properties because it is thought that the understanding of the olfactory code — how odors are encoded in the nervous system, and how they eventually lead to a perception — is intimately linked to the different ORs and how they respond to different odors. Each neuron contains many ORs, but expresses only one type.[3-4] Humans have around 1000 OR genes, each of which directs the production of a different OR protein.[5] The true marvel is that this family of genes is the largest gene super-family in mammals — three percent of the human genome, larger than any other gene family we have.[5]

The story gets even more interesting. Each OR protein binds a certain number of odorants, always of the same kind. Each odorant in turn binds a certain

The Breakthrough

Below is the abstract for "A novel multigene family may encode odorant receptors: a molecular basis for odor recognition" by Nobel winners Linda Buck and Richard Axel:

The mammalian olfactory system can recognize and discriminate a large number of different odorant molecules. The detection of chemically distinct odorants presumably results from the association of odorous ligands with specific receptors on olfactory sensory neurons. To address the problem of olfactory perception at a molecular level, we have cloned and characterized 18 different members of an extremely large multigene family that encodes seven transmembrane domain proteins whose expression is restricted to the olfactory epithelium. The members of this novel gene family are likely to encode a diverse family of odorant receptors.

fixed number of OR proteins. Since there are around 1000 receptors (one for each gene), each responding to multiple odorants, the information about any single odorant is contained within a combination of multiple types of active olfactory receptors and the resultant activity of the corresponding neurons.[3] This combinatorial code is a powerful paradigm for thinking about odor processing. To clarify, each receptor binds to certain compounds, not to certain odor qualities like lily, green, or musk.[6] The odor quality is more likely contained in the combination of the ORs binding and their corresponding neurons becoming activated, and later stages of brain processing.

Many researchers now think that the olfactory bulb contains some sort of spatial topography, which means that odors with similar molecular properties activate similar regions in the olfactory bulb.

New Techniques and New Developments

So, exactly how does this combinatorial code work, and what's the best way of figuring that out? In the epithelium, neurons containing specific OR types are relatively randomly distributed, although four basic zones have been found.[7-8] All of these sensory neurons located at the nasal epithelium send their projections (axons) to a structure in the brain called the olfactory bulb (F-1). This is the first relay station in the brain for olfactory information, the subject of intense investigation. And here's another astonishing discovery: all of the neurons that express the same type of OR, though located in different places in the epithelium, have been found to specifically converge onto only one or two out of many neural structures in the olfactory bulb called glomeruli.[9-10] This means that the combinatorial code is displayed quite dramatically and elegantly in the olfactory bulb, since each glomerulus activated by an odor involves the activation of only one kind of olfactory receptor protein. So if there were a way to see which glomeruli were active for a given odorant, and to know the corresponding OR expressed by the neurons that project to each activated glomerulus, one might understand the nature of the olfactory code in the olfactory bulb.

Here is where neuroscience steps in. There are many ways to measure the activity of a neuron. One way is to stick an electrode inside or near it and measure the voltage changes, since voltage changes correspond with activity. But that's only one cell — what if you want to look at whole populations of neurons? For this purpose, different neural imaging techniques have been developed. These techniques primarily aid the study of the olfactory system of rats and mice, because their olfactory bulbs are quite developed and accessible and thus relatively easy to image. One method is called intrinsic signal imaging. Neurons reflect light differently when they are active; thus, by measuring a certain type of light reflectance change, one could attribute the shift to neural activity.[11-12] Another method is called 2-deoxyglucose (2-DG) imaging. This takes advantage of the fact that since active neurons take up 2-deoxyglucose at a higher rate, a measurement of the presence of this chemical can also be attributed to olfactory neural activity.[13] Recently, three new methods to image neural activity in general have shown great promise: calcium-imaging, voltage-imaging and genetically-encoded methods, all of which employ fluorescent molecules. For olfaction in particular, if there were a way to attach fluorescent molecules to the all of the olfactory sensory neurons, and a way to make them fluoresce more when the neuron is activated, then one could actually see when the neurons are active and which odors activate which glomeruli in a live animal. This would surely be a significant step closer towards understanding the code.

Well, this is exactly what has been done. With calcium imaging, special dyes have been developed over the past quarter century that change their fluorescence when binding to calcium. This property is very useful because there is an important influx of calcium into neurons that are active (calcium ions are involved in the mechanisms responsible for neurotransmitter release). Other investigators applied this technology to olfactory bulb imaging in the following manner: they first loaded calcium dye into the olfactory epithelium of a mouse. The dye entered the cells and was transported to the glomeruli in the olfactory bulb. When researchers presented an odor to a mouse, they could see which glomeruli became fluorescent, and to what extent.[14] Another method of visualizing olfactory activity employs voltage-sensitive dyes, which change their fluorescence when the voltage of a cell changes.[15] An even newer technique employs genetic engineering. A pH-sensitive fluorescent protein called synapto-pHlourin (spH) was genetically inserted into OSNs by substituting the gene responsible for the protein called

olfactory marker protein (OMP) with the new spH gene. All of the sensory neurons that usually express the OMP now express this fluorescent spH protein. Because of its attributes, spH appears near vesicles and neurotransmitters at the end of a neuron. When this neuron is activated (through the smelling of an odor), both neurotransmitter and spH are released outside of the cell. Thus, the difference in pH between the inside of the vesicle and the outside of the cell causes spH to fluoresce (F-2).[16]

What researchers have been finding from these studies is very intriguing. For one, many researchers now think that the olfactory bulb contains some sort of spatial topography. This means that odors with similar molecular properties activate similar regions in the olfactory bulb. This makes sense because ORs that are similar respond to odorants that are similar and also go to similar places in the olfactory bulb (F-1).[5,17] If this topography hypothesis is correct, then the understanding of what constitutes the parameters and distances in olfactory perceptual space can progress by seeing how molecular parameters are represented spatially in the brain. Since there is some relationship between molecular structure and odor quality, perhaps the olfactory bulb can be the next place to look to clarify that relationship.

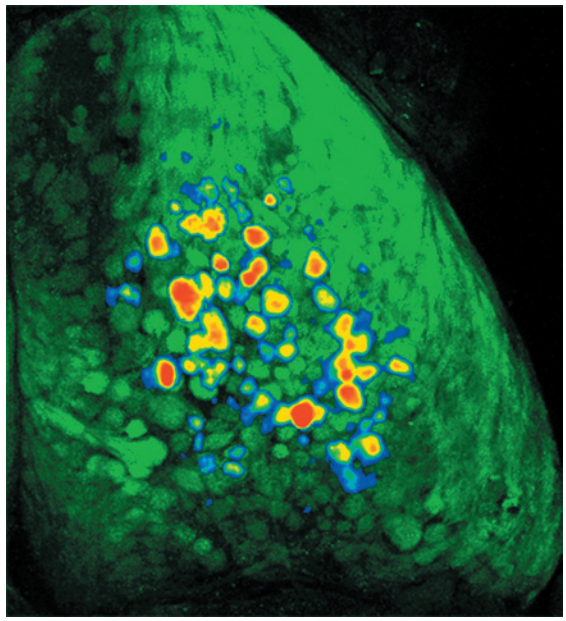
Applications to the Fragrance Industry

But how does all of this really affect the perfumer, the ultimate creator of a fragrance, or the chemist, the ultimate creator of a fragrant molecule? To begin with, in a perfect world, we would know which of the OR genes are non-functional, to which odorants the ORs bind, and how this binding affects the OSNs' neural activity. We would know to which glomeruli in the OB the OSNs project, and understand the spatio-temporal maps of the OB for each odorant and their mixtures as a function of concentration. We would also know how odor information is processed after the olfactory bulb, the exact functional connections with higher brain regions, and how those connections can explain secondary characteristics of olfactory perception, such as olfactory associated memory and emotion.

With this information, many enlightening developments could occur in the fragrance industry. For example, perhaps a company is looking for a way to mask malodors, say in human sweat. First, those odors would be identified through GC/MS olfactometry. Next, all of the human ORs would be screened to see which bound to those malodors. Then, various pleasant-smelling odors would be screened on those very ORs to see if there existed cases in which the simultaneous presentation of both the pleasant odor and the malodor as a mixture suppressed the activity of the original receptors that responded to the malodor. This suppression could be seen at the molecular level for individual OR responses, or could be seen at the OB level through imaging studies. The suppression could in fact be the physiological correlate of odor masking, something perfumers use universally. However, the difference here is that the science would precede the experimental outcome, something that does not currently happen in perfumery. Thus, for any given malodor, there could be a unique antagonist, or mixture of antagonists, that will molecularly (and thus perceptually) mask that malodor. Perfumers could directly use that knowledge to create novel fragrances that would be significantly superior to current masking technologies.

Odor masking is not the only possibility. Most fragrances are currently composed of complex mixtures, interacting with the olfactory system in quite complex ways. With the complete olfactory code unraveled, the mystery behind mixture effects would be demystified as well. In that case, it could be possible to create best-selling fragrances driven by prediction. What was it exactly about relative concentrations of compounds

A mouse olfactory bulb; image taken with a confocal microscope **F-2**



The background glomeruli are green from a genetically-encoded fluorescent protein called synaptopHlourin (spH). The image is overlaid with the activity map for the odorant methyl benzoate. The glomeruli in pseudo-color are the glomeruli activated by methyl benzoate (red most active, blue least active).

in Chanel No. 5 that affected the world in such a unique, profound way? Based upon knowledge of its unique activation of ORs on OSNs projecting to the OB, and those connections to higher level processing, it would be possible to construct novel fragrances that exhibit similar patterns of activation in the olfactory system. Classic, hit fragrances could be compared to the failures to see on a neurophysiological level exactly why two almost identical fragrance formulations produced different levels of popularity with the public.

Classic, hit fragrances could be compared to the failures to see on a neurophysiological level exactly why two almost identical fragrance formulations produced different levels of popularity with the public.

The above two examples are practical in nature, but the ramifications are much broader than that. With complete understanding of the olfactory system, people could think and go about fragrance creation in radically different ways. The relationship between molecular properties and odor qualities could be elucidated because all the steps involved in creating the perception would be understood. Odor quality description could be refined; the vocabulary that the F&F industry uses to communicate could be standardized so that one person could describe an odor percept and be sure the other person understood the meaning. This refinement of description could be achieved by employing neural activity measures. Furthermore, new conceptual paradigms could lead to new research endeavors to synthesize new fragrant chemicals. Chemists have long been interested in structure-activity relationships — that is, the molecular properties of a musk or ambergris note that make it distinctive — and how to use knowledge of those properties to predict and create novel musk or ambergris chemicals. Success has been only partial. Rules exist, but there are exceptions, and usually those rules only apply to a small subset of olfactory notes. With the development of a systematic neurophysiology and a psychophysics of smell, these smaller structure-activity rules could be expanded upon, so that synthesis of fragrance compounds could be fully hypothesis driven, less time-intensive and expensive, and more accurate.

Limitations for Olfactory Research

Of course life isn't perfect, and the field of olfaction is far from a complete understanding of any one of the research areas that would allow the above scenarios to become fully realized. Only several odors that bind to specific ORs are currently known.[18] This is less than expected. The reality is that the usual way of getting that information — putting the OR in another cell, which immediately transports it to the cell surface for odorant testing — doesn't work. The OR protein doesn't transport to the surface of the cell.[18] In addition, we only know a handful of the locations from which ORs' respective neurons project in the bulb. With current OB imaging techniques, limitations abound. With most methods, one can only see the top of the OB, not the sides or the bottom. With techniques such as 2-DG, in which the whole OB is imaged, the spatial and temporal resolution is poor.

Furthermore, people know even less about olfaction in brain areas after the OB. Some have begun to trace the connections from the specific glomeruli in the OB to the olfactory cortex, but this work is in its early stages.[19, 20] Moreover, almost all of the information about the olfactory system now comes from other mammals, like mice and rats. Connecting insights to humans, especially in areas relating to perception, will be difficult at best. We may know that rotten, fishy smelling odors for humans activate certain areas of the mouse OB, but how do we know they smell rotten and fishy to a mouse?

Finally, in humans, odor perception can depend on the context. For example, DeAraujo et al. have shown that the same odor elicits a different percept when paired with the word "cheese" or "feet," and elicits different brain activity as well.[21] Multisensory context affects such as well (e.g. color-odor congruence or odor-taste learning). Human inter-individual variation exists too, in that their sensitivity to odorants not only varies globally but also can be induced (e.g. androstenone).[22] This all implies that although different individuals may have quite similar olfactory structures, there exists a certain degree of subjectivity between individuals in at least some stages of olfactory perception. And this subjectivity does not even take into account the olfactory associations and memory, unique to each individual, that occur above and beyond olfactory perception.

Potential Future

Nevertheless, the field has advanced more rapidly in the last 15 years than in any other time in history.

And incidentally, many industry applications need not wait for complete olfactory understanding. Already, agonist/antagonist relationships have been discovered for certain olfactory receptors, and more relationship discoveries are on the way.[23] Technologies to quickly identify the affinity of odors to a large number of olfactory receptors will most likely be developed. Moreover, a general understanding of the effects of mixtures on the olfactory system has dramatically improved in recent years, an essential ingredient towards the goal of quantitative and predictive fragrance creation.[24] The field of olfactory psychophysics now has the ability to build a truly objective and accurate database of information by focusing on metrics of olfactory discrimination to quantify similarity and dissimilarity rather than on traditional descriptive enumerations.[25] Olfactory bulb imaging studies have already begun to study quantitatively structure/odor relationships, and have provided insights that many chemists could benefit from in their own structure/odor studies.[26-27] In almost every field of olfaction, scientists are making new discoveries. Within the neuroscience community, olfaction is becoming one of the most promising areas of research. It is only a matter of time before the next fragrance classic will be achieved by combining biological insights and creativity from the fragrance industry.

Acknowledgements

The authors wish to thank Matt Wachowiak, assistant professor of biology at Boston University, for his helpful comments, edits and the image he provided for F-2. We also thank Pablo Maestre Galli for his schematic diagram of the olfactory system shown in F-1.

Address correspondence to Adam Elias, Elias Fragrances Inc., 999 East 46th St., Brooklyn, NY 11203; e-mail: aelias@gmail.com.

References

1. L. Buck and R. Axel, *A novel multigene family may encode odorant receptors: a molecular basis for odor recognition.* Cell, **65**(1), 175-187 (1991).

2. A.N. Gilbert and S. Firestein, *Dollars and scents: commercial opportunities in olfaction and taste.* Nat. Neurosci., **5**, Suppl:1043-1045, Review (2002).

3. B. Malnic, J. Hirono, T. Sato and L.B. Buck, *Combinatorial receptor codes for odors.* Cell., **96**(5), 713-723 (1999).

4. S. Serizawa, K. Miyamichi and H. Sakano, *One neuron-one receptor rule in the mouse olfactory system.* Trends Genet., **12**, 648-653, Review (2004).

5. G. Glusman, I. Yanai, I. Rubin and D, Lancet, *The complete human olfactory subgenome.* Genome Res., **11**(5), 685-702 (2001).

6. C. Sell, *Cracking the Code: How does our sense of smell work.* Perfum. Flavor., **28**(2) (2003).

7. R. Vassar, S.K. Chao, R. Sitcheran, J.M. Nunez, LB Vosshall and R. Axel, *Topographic organization of sensory projections to the olfactory bulb.* Cell, **79**(6), 981-991 (1994).

8. R. Vassar, J. Ngai and R. Axel, *Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium.* Cell, **74**(2), 309-318 (1993).

9. T. Bozza, P. Feinstein, C. Zheng and P. Mombaerts, *Odorant receptor expression defines functional units in the mouse olfactory system.* J. Neurosci., **22**(8), 3033-3043 (2002).

10. H.B. Treloar, P. Feinstein, P. Mombaerts and C.A. Greer, *Specificity of glomerular targeting by olfactory sensory axons.* J. Neurosci., **22**(7), 2469-2477 (2002).

11. M. Meister and T. Bonhoeffer, *Tuning and topography in an odor map on the rat olfactory bulb.* J. Neurosci., **21**(4), 1351-1360 (2001).

12. N. Uchida, Y.K. Takahashi, M. Tanifuji and K. Mori, *Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features.* Nat. Neurosci., **3**(10), 1035-43 (2000).

13. B.A. Johnson, C.C. Woo and M. Leon, *Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb.* J. Comp. Neurol., **393**(4), 457-71 (1998).

14. M. Wachowiak and L.B. Cohen, *Representation of odorants by receptor neuron input to the mouse olfactory bulb.* Neuron, **32**(4), 723-35 (2001).

15. H. Spors and A. Grinvald, *Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb.* Neuron, **34**(2), 301-15 (2002).

16. T. Bozza, J.P. McGann, P. Mombaerts and M. Wachowiak, *In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse.* Neuron, **42**(1), 9-21 (2004).

17. P. Feinstein and P. Mombaerts, *A contextual model for axonal sorting into glomeruli in the mouse olfactory system.* Cell, **117**(6), 817-831 (2004).

18. P. Mombaerts, *Genes and ligands for odorant, vomeronasal and taste receptors*. Nat. Rev. Neurosci., **5**(4), 263-278, Review (2004).

19. Z. Zou, L.F. Horowitz, J.P. Montmayeur, S. Snapper, and L.B. Buck, *Genetic tracing reveals a stereotyped sensory map in the olfactory cortex.* Nature, **414**(6860), 173-179 (2001).

20. Z. Zou, F. Li and L.B. Buck, *Odor maps in the olfactory cortex.* Proc. Natl. Acad. Sci. USA, **102**(21), 7724-7729 (2005).

21. I.E. de Araujo, E.T. Rolls, M.-I. Velazco, C. Margot and I. Cayeuxet, *Cognitive modulaton of olfactory processing*. Neuron, **46**, in press.

22. C.J. Wysocki, K.M. Dorries and G.K. Beauchamp, *Ability to perceive androstenone can be acquired by ostensibly anosmic people.* Proc. Natl. Acad. Sci. USA, **86**(20), 7976-7978 (1989).

23. Y. Oka, M. Omura, H. Kataoka and K. Touhara, *Olfactory receptor antagonism between odorants.* EMBO. J., **23**(1), 120-6 (2004).

24. S. Firestein, *A code in the nose.* Sci. Signal Transduction Knowledge Environment, **227**, pe15, Review (2004).

25. P.M. Wise, M.J. Olsson and W.S. Cain, *Quantification of odor quality.* Chem. Senses, **25**(4), 429-43, Review (2000).

26. Y.K. Takahashi, M. Kurosaki, S. Hirono and K. Mori, *Topographic representation of odorant molecular features in the rat olfactory bulb.* J. Neurophysiol., **92**(4), 2413-27 (2004).

27. Y.K. Takahashi, S. Nagayama and K. Mori, *Detection and masking of spoiled food smells by odor maps in the olfactory bulb.* J. Neurosci, **24**(40), 8690-8694 (2004). ■