Knowing Your Nose: Discovering How We Smell

Krista Kusinski*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

[Role Playing: **Linda B. Buck**
Howard Hughes Medical Institute
Fred Hutchinson Cancer Research Center
Basic Sciences Division
1100 Fairview Avenue North
Seattle, Washington 98109]

Summary

Mammals can discern thousands of molecularly different odorants as well as changes in their concentrations. How the olfactory recognizes such a large number of smells was not very well understood. The initial perception of smell occurs in the olfactory epithelium, which transmits information to the major olfactory bulb, and ultimately to the olfactory cortex via olfactory sensory neurons. This is the basic structure of the olfactory system. We sought to find the underlying mechanisms and tools that allow for the translation of these chemical odorants into the perception of smell. Our studies focused on finding the separate families of olfactory receptors used in the olfactory epithelium to recognize the immense number of odorants. Our studies of the olfactory epithelium, the major olfactory bulb, and the OC have focused on how this immense amount of information from the olfactory sensory neurons is organized at various steps in the discrimination process. We have discovered a novel family of odorant receptors as well as many other subfamilies through genetic analysis. We have also discovered a highly organized stereotypical map in the olfactory epithelium and the major olfactory bulb, as well as distinct patterns of activation in the olfactory cortex. Through the ability to trace specific neuronal circuits, we have studied the distinct odorant receptors patterns when exposed to distinct odorant types or mixtures. Our research in the field of olfaction has led to many discoveries in both mechanisms of odor perception and how that perception is ultimately organized to perceive odor.

Introduction

Mammals have the ability to discriminate between tens of thousands of different odors that are structurally diverse. Concentrations of odorants can also be identified in mammals. The mechanism of transduction as well as the structural architecture in the olfactory pathway was virtually unknown until recently.
However, we did know the major anatomical structures involved in olfaction. The process of discrimination between different odors starts in the olfactory epithelium (OE), with the detection of odorants by the olfactory sensory neurons (OSN). The chemicals are received by odorant receptors (OR) which proceed through the major olfactory bulb (MOB) to individualized golermuli, and eventually to the olfactory cortex (OC) for reconstruction and discrimination of the odorant [4]. However, the cellular organization of the olfactory transduction pathway is still unknown.

Smell is transduced by converting environmental odors into chemical stimuli in the nervous system. The mechanism underlying the transduction most likely involves a ligand gated odorant receptor. However, because no ORs have been discovered so far, this mechanism is unclear. We hypothesize the odorant acts like a ligand to activate the receptor. Because these receptors are often uninvestigated, along with the olfactory system in general, the smell pathway is not well understood [8].

Along with these odorant receptors, the cilia of the olfactory neurons have proven to be important in smell transduction; we see that a loss of smell occurs after the removal of cilia in rats. Another significant finding showed that when rats were exposed to odorants there is an observed stimulation in the production of adenylyl cyclase as well as an increase in cAMP levels [8]. Adenylyl cyclase is dependent upon GTP, which is regulated by a G protein linked receptor. Based on this finding and the sheer number of possible odorants, we hypothesized that ORs may be G protein linked as a major superfamily in the G protein family having many subfamilies. Our lab sought to investigate both the cellular mechanism involving ORs in the OE as well as identifying the odorant mapping in the OE, MOB, and the OC.

Discovery of new multigene family

With the progress made in the field of technology as well as the discovery of new G protein-coupled receptors (GPCRs), our lab was able to extensively search for the ORs in the olfactory epithelium, which we believe were able to recognize and code for odorants.

We operated on three assumptions to design this experiment. One assumption was that the ORs were likely to belong to the same superfamily of GPCRs. A second, assumption was that ORs would show the same structure and level of diversity that the large number of odorants showed. Our final asssumption, was that the OR expression would be found only in the OE.

Through the use of PCR primers specific for these GPCRs in OE, we successfully cloned and amplified 18 members of a new novel multigene family of ORs which were specifically expressed only in the OE. GPCRs are known to display seven transmembrane domains, and our lab found the ORs to have these specific sequencing characteristics. We also observed several differences in the Ors that were similar to known GPCRs, such as a possible binding domain. These domains vary between ORs allowing for the recognition of a large number of distinct odorants. Through these results we were able to report the findings of a new novel multigene family of ORs [8].

Once we were able to identify ORs specific to the OE we used those as a basis to study other aspects of transduction of odorants. Our studies in this field showed that two cyclic nucleotide-gated (CNG) ion channel subunits, rOCNC1 previously known and

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rOCNC2 which we found, were both necessary in order for odorant discrimination. This gave us another piece of information in the molecular basis for odorant discrimination [11].

Although we had a basis for studying olfaction this finding opened the door to many new questions in the field. The number of subfamilies, as well as the transduction mechanism, and the spatial mapping of ORs were all still unknown.

Olfactory Mapping in the OE and MOB

We found a unique zonal topographical mapping in the OE. Each OSN expressed only one OR per neuron (figure 1) [4]. We also observed different zones being expressed through what we had identified as separate subfamilies of ORs.

We used a series of probes on the OE to identify were specific OR genes were expressed. This zonal map had little or no overlap in the zonal boarders. Each zone specifically expressed only one subfamily of receptors, but within that zone the receptors were distributed randomly and the same receptors were not found in only one area. The regions displayed bilateral symmetry and were almost identical in different individuals. We believe that hybridization within zones may lead to the probe detecting different members of the same subfamily [20].

From previous studies we knew that many ORs may respond to the same odorant, but it was not clear how this information would be integrated from these large zones to give the perception of smell [4]. We were able to investigate this through analyzing patterns of OR gene probes *in situ* in the MOB. ORs, which were spatially segregated in their particular zones of the OE, synapse on the same glomeruli of the MOB (figure 1) [19]. Different spatial zones also synapsed with different glomeruli in the MOB suggesting that the initial zonal organization is maintained in the MOB [4]. Our belief was that this map was an epitope, where different ORs, which recognized the same determinant that may be in many different odorants, would all synapse on the specific glomeruli [19].

These discoveries gave us some insight as to how such a large group of diverse odorants undergoes initial organization in the OE and MOB. It also gave more insight into the idea that subfamilies of receptors exist. Several questions were raised, such as how the zonal organization develops and if the same receptors project to the same bulbar areas [19, 20].

Development of stereotyped map

We knew that each neuron expressed only one receptor and synapses with other neurons expressing the same receptor in the glomeruli. There are approximately 1000 genetically different ORs which can be expressed by the neurons. Each of these neurons travels to their designated glomeruli during development, creating a stereotyped map which is retained even in different individuals [19]. This

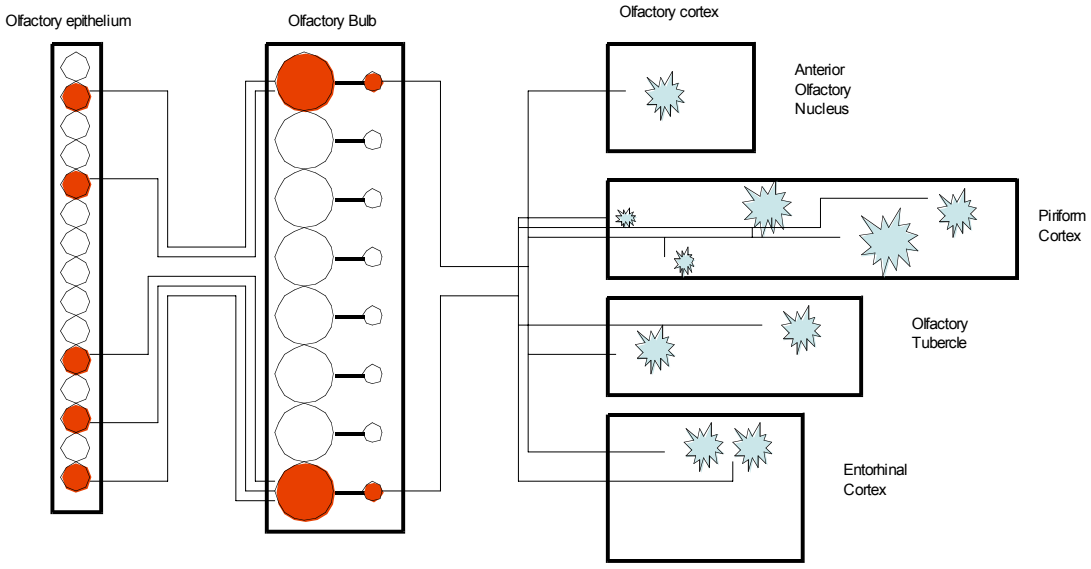

Figure 1. Organization of odorant receptors within the nervous system. Odorant receptors of the same kind are scattered in the olfactory epithelium, and each sensory neuron is responsible for one of these receptors. At the olfactory bulb, however, the axons from those sensory neurons from the same receptor type, all synapse on the same olfactory bulb mitral cell (small circles) within the olfactory bulb (large circles) to create a stereotyped map from the odorant receptor input. The information that was segregated in the olfactory bulb and epithelium, upon reaching the olfactory cortex inputs from different receptor types recognizing the same odorant overlap. This overlap can be seen in many different regions of the cortex and forms a stereotyped sensory map. The stereotyped sensory map allows for odorant information to be processed in different ways from the same odorant stimuli. Figure modified from Zou, Z. et al. 2001

stereotyped map means that each OR gene has a designated zone in the OE and is integrated in the MOB. However, the method by which this highly stereotyped map is achieved during development still remained unknown.

To investigate the development process through which this map is achieved, we studied the development of wildtype mice and mice which lacked an olfactory bulb. In wildtype mice development of the correctly organized ORs occurs with the onset of OR gene expressions before synapses are formed with the glomeruli. Also, mice lacking an olfactory bulb developed normally, meaning neurons still traveled to the correct region but did not synapse with anything because the bulb was lacking [23]. These findings suggested that the method of development did not depend on the retrograde transport from the correct glomeruli. This means there is a target-independent map which exists in the development of the OE and bulbar map. Other studies have provided findings that there is a coding region in the OR gene which, when deleted or altered in some way, results in the inability for the neuron to reach its targeted glomeruli [5]. This allows for some new insight into the ability of the axon to travel to the designated region on the MOB.

These findings allowed for insight into the ability for the OE map to develop before contact with the bulbar region. However, it leaves the question of how the correct neurons synapse in the expected glomeruli, and an even greater question of how the OSNs express the correct receptor out of 1000 possible genes [23].

Chromosomal organization of OR genes

We discovered previously the existence of a large multigene family of receptors in the olfactory system, and that each of these receptors is specific to certain neurons in the OE [8, 20]. Because we found our studies supported the existence of a target-independent mapping during development, [23] we sought to determine if there was a locus-dependent or locus-independent model on the mouse chromosome, which could allow for the selective expression of ORs in mice [22].

To investigate these two different models of chromosomal distribution, we studied the OR within the given zones of expression, as well as their sequence in the zones, and the chromosomal location of the expressed ORs [22]. In the four different zones of the OE, we worked with 21 different ORs and determined their chromosomal locations. Our studies showed that even though ORs were expressed in the same zone, the majority of the time they were located at a different chromosomal locus [22].

Later studies, which were added with improved knowledge of the mouse genome, would attribute the 931 different mouse OR genes to 51 different loci on 17 different chromosomes [9]. The completion of the human genome project allowed us to identify 339 OR genes, which reside on 51 different loci in 21 of the human chromosomes [14]. These studies showed that the majority of ORs in a subfamily were expressed at a single locus instead of separate loci. These findings suggest that the olfactory system is extremely complex and that, consistent with our previous speculation, subfamilies generally respond to similar odorant structures [9, 14, 8].

Odors are coded for by multiple ORs

We had always believed that one neuron expresses a single receptor based on three findings. Hybridization of OR gene probes in situ only occurred in approximately 0.1% of the neuron population. Only one allele for a specific OR gene was observed, and each olfactory neuron expresses only one type of OR gene [20]. The ability to discriminate a vast number of odorants had often been thought to be a combination of receptors which respond to the same odorants. However, without the appropriate means to study these pathways we were unable to provide evidence for this theory [13]. However, when the first odorant and OR specific pair were found, we were able to study the mechanism of odorant coding [13].

To investigate the molecular basis for the olfactory sensory pathways, we used a combination method to study related structures of identified ORs when exposed with a varied population of odorants [13]. Three major findings came out of this study. Firstly, individual ORs can recognize a variety of odorants. Second, individual odorants can be detected by a number of ORs. Finally, different sets of ORs recognize different odorants (figure 2) [13]. Small changes in concentration or in the molecular structure of odorants can lead to a different set of ORs being stimulated.

These findings show that a combinatorial of receptors coding occurs in mammals in odorant discrimination in the OE (figure 2). This highly complex network of neuronal connections underlies the mammalian ability to discriminate between thousands of distinct odors.

Genetic tracing in OE

The ability to trace individual ORs through the olfactory system up to this point had been limited. Conventional methods of genetic tracing only allowed for local studies. Our studies lead us to believe that if the neurons themselves produced the tracer than we would be able to observe the transduction system in olfaction [10].

To test this, we chose to create a transgenic mouse which expressed wheat barley lectin (BL). The transport extended past the OE and into the OC, where BL was also detected in the piriform cortex. It can also be transported both in olfactory systems as well as pheromone neurons, allowing for some comparisons of the two systems and determine if the two may overlap at any point.

This method proved to be a successful tracer through out the neurons of the olfactory system. It allows for the detection of transneuronal tracers which can be genetically targeted in specific neurons [10].

Mapping in the OC

Our ability to trace OSN expressing specific OR genes, and the connections of these individual neurons to the higher region in the OC, has so far been limited given the research methods. However, with the new tracing

Odorants \ ORs	S 1	S 3	S 6	S 18	S 19	S 25	S 41	S 50	S 51	S 79	S 83	S 85	S 86
Hexanoic acid					×								
Heptanoic acid	×			×	×		×		×	×			
Octanoic acid	×			×	×		×		×	×	×		
Nonanoic acid	×			×	×		×		×		×		×
Pentanol		×											
Hexanol		×				×							
Heptanol		×			×	×							
Octanol				×	×		×		×				
Nonanol				×	×		×		×		×	×	
Bromobutanoic acid												×	
Bromopentanoic acid												×	
Bromohexanoic acid					×		×					×	
Bromooctanoic acid	×			×	×		×		×		×	×	
Hexanedioic acid												×	
Heptanedioic acid												×	
Octanedioic acid			×							×		×	
Nonanedioic acid			×					×		×		×	

Figure 2: Olfactory neuron recognition profile. The tested odorants are seen on the left side, and the odorant receptors which were responsive are seen on top. The filled boxes with an X indicated that the OR was responsive during stimulation. Figure is modified from Malnic et al. 1999.

ability we could now trace specific neuronal circuits from the desired receptor in the OE all the way to the synapses made in the OC [10].

To investigate the type of mapping in the OC, we used the genetic tracer method, described above, to trace desired neuronal circuits from the OR all the way to the OC [26]. Our results showed a stereotyped sensory map in the OC, where signals from specific receptors form clusters of activated cells which may receive input from a number of different ORs.The OR clusters overlap gives rise to the theory that single cortical neurons may receive input from a number of genetically different ORs. There is also bilateral symmetry in these OC areas and the placement of clusters is similar in different individuals meaning that these discoveries can be applied not just to one individual but to a number of different individuals [26].

Thousands of different neurons specific for a particular OR converge onto a specific cluster in the OC, which receives input from other ORs and also recognizes the specific odorant. After this initial organization in the OC sensory transmission is sent to other areas such as the neocortex and the limbic system. This is a possible explanation of why the mammalian olfactory system regulates and initiates so many systems and behaviors in the body [26].

Maps of odorants in the OC

The distribution of OR tagged neurons lead to the question, which of those neuronal clusters were activated during stimulation of a variety of different odorants. To study this idea, we studied how odors are spatially organized on the OC through the observations of the cortical activation through staining in the focused area of the anterior piriform cortex (APC) [25, 26]. The APC was previously seen as a major area for olfactory activity and elicited distinct patterns during OR genetic tracing [26].

We found that single odorants activate specific neurons in the APC, and each odorant activated a small amount of these neurons, which were unevenly distributed. These different odorants slightly overlapped but were very similar in different individuals. Odorants with related structures elicited similar patterns of neurons in the APC. These results imply that there might be some significant reason for which the mapping of the olfactory cortex exists in this manner [25, 26]

Activation of OC neurons

Previous findings had suggested that single cortical neurons integrate signals from a variety of different ORs, which detect the same odorant [26]. This mechanism, if proven correct, is most likely the first step in the reconstruction of the perception of odor, after its deconstruction which started at the OE. This theory requires that each neuron must receive input from a number of different ORs and that the activation of this cortical neuron is dependent upon the input from not just one, but the collection of the different ORs all coding for the same odorant [25]. In other words, α and β are two different odorants, the activation of the neuron in the OC on which they synapse is dependent upon simultaneous receptor input.

To test this model, we studied the response of OC neurons in mice when they were exposed to different combinations of odors. When the mice were exposed to two different odorants at two different times they showed little response. But when mice were exposed to the odorants as a mix they showed significant activation suggesting that they will not respond to one odor by its self but rather a mixture of the odorants. In each of the odor pairs 30% of the cortical neurons did not respond to a single odorant but responded to the odorant pair [25].

These findings suggest that the activation of the cortical neuron requires that each odorant in an odorant is recognized by a combination of receptors and that this cortical activation depends on the input from more than one of these ORs (figure 2) 25].

Still more to discover

In 1991 we reported of a novel family of receptor genes in the OE [8]. This discovery revolutionized our ability to study the olfactory system and lead to many more discoveries. One of these discoveries was that small number of OSNs lacked the G protein found in all other ORs in the OE. This leaves the question of whether there is a second class of receptor genes in the olfactory [11].

To study this possibility we searched for a second possible GPCR in the ORs of mice. Through use of primers, complementary DNA, and PCR we observed two GPCR genes –*Taar7d* and *Taar9-,* which were expressed in some of the OSNs. These genes encode for members of the trace amine-associated receptor (TAAR) family. TAARs, until this point, have been seen as unrelated to the OR family [11]. This new discovery could prove an important factor in new findings in the field of olfaction because they provide another pathway for discrimination.

Expression of TAARs, after analysis using probes on various different tissue samples, indicated the expression may be limited to the OE only. To study the expression of TAARs on the OE we used specific primers for the *Taar* gene of mice. These studies showed that all *Taar* genes were recognized on the OE except for *Taar1*. The patterns of expression for the *Taar* probes were similar to the expression pattern of ORs on the OE. Each *Taar* gene was expressed on a small subset of neurons in the OE. Each OSN expressed only on TAAR receptor, and the individual TAARs were scattered within a specific zone of the OE [11].

In studying the chemosensory activity of TAAR genes we observed, contrary to our original hypothesis, instead of odorants the ligands for these receptors are amines. Three of these ligands, which we observed to create a TAAR response, were components of mouse urine. We also observed that TAARs increase cAMP when activated by ligands. This study showed that even though our previous findings indicated that only ORs were present in the OE, there is another type of chemosensory receptor present. This indicates a similar pathway of transduction as a possible model of olfaction [11].

These studies suggest that some urine TAARs can be used as a means to detect social behavior, and may result in a behavioral response [11]. Further studies are needed in order to clarify the exact role which the TAAR receptors play in the olfactory system as well as their spatial mapping, and mechanism for transduction.

Conclusion

The olfactory system remained for a long time a mystery. With our discovery of the OR gene family, a door, which allowed for more tools in the field, was opened [8]. With the new technology developing rapidly in the scientific world we were able to not only search for the genes of the ORs of mice, but also categorize them into subfamilies and position them on specific genomes and loci [9, 22]. Not only could we do this in mice but we could also identify the OR genes in humans with the completion of the human genome project [14].

We also discovered 4 highly specific zones in the OE which contained subfamily of receptors. Also, each OSN contained only one OR gene, and those OR expressing the same gene were not concentrated in a given region of the OE [20]. We also found that a highly organized epitope map existed in the MOB of mice, and this map was target-independent in pattern specification [19, 23]. Through a new method of genetic tracing we were able to see that there was a stereotyped sensory map in the OC [10, 26]. We also observed that a number of receptors are all activated by the same odorant and that input from both ORs may be necessary for cortical activation [13, 24].

Our discoveries in the field of olfaction could not have been possible without the advances in methods and technological equipment. These advances made our contributions to the field possible [6].

Possibilities to keep discovering

Our studies have presented numerous findings on the olfactory system. These have all contributed to building a model in which we might be able to fully understand the process of olfaction. There are still many pieces of the olfactory puzzle that are left unanswered and many more questions which arise from each new discovery.

The organization of the OC leads to some understanding of how we start to reconstruct the perception of smell which is initially destructed in the OE [25, 26]. However, the mechanism for this final

reconstruction of smell is still, at this time, unknown. Also, the odor mapping the OC raises a number of unanswered questions such as the difference of OR inputs and neuronal activity [25]. There are several models present, but none of them have been defined or proven thus far. The discovery of new chemosensory receptor family in the OE leads to another round of research to define the mechanism and significance in olfactory sensation of this new type of receptor [11].

Our studies and the work done by our fellow researchers in the field have provided us with valuable mechanisms to discover the initial mechanics of olfaction. However, the world of the nose still has some aspects which are unknown.

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