How We Smell: The Molecular Basis of Olfaction

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Abstract

Although smell is the oldest of animal senses, the molecular basis for how the olfactory system recognizes thousands of odors and translates them into odor perceptions has been a major enigma in neuroscience. Together, Richard Axel and I discovered a large (~1000) multigene family in mice that codes for an equal number of olfactory receptors (ORs). Since then, my lab has clarified the molecular basis of signal transduction via secondary messenger responses. We found that the olfactory epithelium is organized into four distinct non-overlapping zones and that each neuron expresses only one OR gene, revealing a combinatorial code. Interestingly, each OR can detect multiple odorants, and each odorant can recognize many different ORs. While signals are segregated in the olfactory epithelium and bulb, the olfactory cortex receives integrated inputs from multiple ORs. Later, my colleagues and I discovered two additional families of pheromone receptors, V1R and V2R, that were selectively expressed in the VNO. Our field has provided further insight into olfactory function by examining the stability of receptor choice, as well as how adaptation occurs. Knowledge of the olfactory system is of crucial importance, not only for curing human disorders associated with olfaction, but also for the understanding of our most primal sense.

Introduction

Smell is an ancient sense that all organisms possess, making its importance in olfaction undeniable. Even as newborns, we recognize our mother by her scent. It provides organisms with sensual pleasure, warns them against hazardous substances, and even allows them to identify foods, predators, and mates[1]. Furthermore, the sense of smell plays a vital role in memory, motivation, and even reproduction[1]. Olfactory dysfunction can result in a variety of smell-associated diseases. For example, people who have anosmia are unable to detect certain odors, demonstrating the importance of uncovering how smell perception occurs. Olfaction is a form of chemoreception that detects an array of volatile chemicals. In fact, humans possess incredible discriminatory abilities that allow them to identify thousands of diverse odorants[2]. Remarkably, only slight changes in the odorant's structure lead people to perceive a different scent[2]. In mammals, the main olfactory system detects an enormous variety of odors in the olfactory epithelium of the nasal cavity. These specialized sensory neurons detect odorants and then transmit signals to the olfactory bulb. The information is then relayed to the higher cortical processing areas of the brain, specifically the olfactory cortex. Interestingly, the olfactory system also contains an accessory olfactory system whose sensory receptors are located in the vomeronasal organ (VNO). The VNO is connected to the nasal cavity, and in contrast to the main olfactory epithelium, it has been shown to primarily respond to pheromones[3]. The detection of odorants was assumed to result from the attachment of odorants to ORs; however, no data existed to support this model. The scientific community was undecided about whether we possess a few receptors that can detect a multitude of odorants or, alternatively, a large number of receptors that respond to only one or a few odorants. Overall, before I began studying olfaction, very little was known about the olfactory system.

I became fascinated with olfaction because of the enigma of how mammals can detect thousands of odorous chemicals and how nearly identical chemicals can produce different odorant perceptions. In the 1980's, I began research on olfaction as a postdoctoral student in Richard Axel's lab. My first, and arguably most important, contribution toward the understanding of olfaction came when Richard Axel and I discovered a novel multigene family of ORs in the olfactory epithelium. These findings paved the way toward understanding how signal transduction occurs via G protein- coupled receptors. Although I left Axel's lab shortly after identifying the ORs, Axel and I independently discovered the organization of the olfactory system by examining the movement of sensory information from the olfactory epithelium to the cortex. In addition, my lab contributed to our understanding of how pheromone signaling occurs in the VNO. Altogether, my work has contributed to astonishing advances in the field of olfaction.

Olfactory Receptors

The scientific community has long assumed that specific receptors located in the olfactory cilia become activated by the binding of odorant molecules. Therefore, finding the receptor proteins would answer two important questions for olfaction: how the olfactory system responds to thousands of odorants and whether olfaction processing involves the use of a restricted or a large number of receptors. As a postdoctoral fellow in Richard Axel's lab, Axel and I set out to uncover the odorant receptors based on three assumptions that significantly narrowed our search. First, the odorant receptors most likely belong to a superfamily of G protein-coupled receptors (GPCRs). Second, since there are an enormous array of odorant molecules, the odorant receptors will also be diverse and therefore likely to be encoded by a large gene family. Lastly, odorant receptors should be limited to the olfactory epithelium. We were able to find ten ORs that were GPCRs that varied in amino acid sequence. The fact that they varied made sense because it meant that the receptors could interact with odorants possessing different structures. Our results supported all three of our assumptions, providing evidence that we had found receptors that encoded a multigene family. Later studies showed that we had found a huge multigene family in mice that consisted of ~1000 different ORs[4].

Organization of OR in the Olfactory Epithelium

The enormous size and diversity of the multigene family suggested that odor discrimination in mammals relies on the discriminatory abilities of a huge array of ORs. We wanted to know how ORs are organized in order to achieve such precise discrimination between odorants. In the olfactory epithelium, neurons that express the same odorant receptor gene, thus responding to the same odorant, might be randomly dispersed or clustered in one region[5]. We found that OR genes are expressed in topographically distinct

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patterns in the mouse olfactory epithelium[5]. Furthermore, the olfactory epithelium is divided into expression zones that are organized along the dorsal-ventral and medial lateral areas of the nasal cavity[5]. These zones are bilaterally symmetrical in the two cavities and are identical in each person[5]. Importantly, each OR gene is expressed in only one olfactory neuron, and those neurons are randomly distributed within one zone[6]. In particular, neurons expressing a given OR are restricted to one of four OR expression zones[7]. Also, within the zones, we did not observe any clustering of neurons expressing the same gene nor any other type of spatial organization[5]. These results imply that sensory information is broadly organized into spatial sets before being transmitted to the olfactory bulb. Our findings imply that olfactory neurons that express the same odorant receptors project to the same region of the olfactory bulb.

Once we found that neurons expressing the same OR gene are located in the same zone, while being randomly distributed in that particular zone, we wanted to investigate how a neuron chooses its OR gene. It was unclear whether OR genes were clustered or randomly arranged in the genome. We found that OR genes are clustered within multiple loci that are randomly distributed throughout the genome. Furthermore, our lab discovered that a single locus can contain genes expressed in different zones. Since gene expression within a zone is random, we postulate that a cell first makes a locus choice and then a receptor choice[8].

In later experiments, the sequencing of the human and mouse genome allowed us to determine the number of OR genes in each species. By searching the human genome database, we identified a total of 636 OR genes in humans, 339 of them intact and functional[9]. Additionally, we found that the human OR family is extremely diverse, as can be seen from our identification of 172 subfamilies[9]. Analysis of the location of OR subfamilies in chromosomes revealed that most subfamilies are encoded by a single chromosomal locus. Furthermore, OR genes are unevenly distributed among the different loci on human chromosomes[9]. Examining the mouse OR gene family, several striking similarities and differences between the two species became apparent. Mice possessed more than twice the number of OR genes as humans; nevertheless, both groups had many subfamilies in common[10]. Interestingly, mouse subfamilies tended to be larger in size than their human equivalents[10]. These findings signify that humans and mice recognize similar odorants, yet mice are superior at detecting these volatile molecules.

Olfactory Bulb

Once the organization of the olfactory epithelium was established, we wanted to examine the first major relay station of olfactory signaling in the brain: the olfactory bulb. Previous studies have shown that each olfactory neuron axon forms synapses with only a single glomerulus[11]. Thus, it is apparent that the olfactory system exhibits spatial segregation of sensory inputs from the epithelium to the olfactory cortex. Each glomerulus receives information from neurons expressing only one type of receptor, and surprisingly, glomeruli have nearly identical locations in different individuals[11]. A novel two-photon calcium imaging technique implemented by Richard Axel's lab was used to examine glomeruli activity. The response of 23 glomeruli to 16 different odorants showed that most glomeruli only respond to a small subset of odorants[12]. However, at higher odorant concentrations, more glomeruli were recruited. Unlike sensory neurons that are constantly replaced, the bulbar map remains constant over time, indicating its contribution to the stimulation of odor memories[2].

An important issue that we wanted to explore was how randomly scattered olfactory neurons within a zone are able to synapse at only a few sites in the olfactory bulb. We examined OR gene expression in both a developing mouse embryo and in mutant embryos in which the olfactory bulb was removed. Our studies showed that OR genes are expressed at about the same time during embryogenesis. Also, OR genes were normally expressed in mutant mice that lacked the olfactory bulb. These results rule out a retrograde hypothesis and instead reveal an independent development of the epithelial and bulbar maps[13].

The arrangement of ORs in the nose and bulb suggest that each OR recognizes multiple odorants. Although many attempts had been made to determine the odorant specificity of individual ORs, in 1999 our lab was finally able to solve this mystery. First, we identified ORs that possessed similar structures, yet responded to different odorants. We confirmed that one odorant is recognized by a multitude of ORs, and those different combinations of ORs respond to different odorants[6]. Together, these findings serve as evidence for a combinatorial receptor code for odors. Our results also explain why, with only a slight alteration in structure or concentration, humans perceive a change in odorants.

Olfactory Cortex

After shedding light on the functionality and organization of the olfactory epithelium and bulb, our lab wanted to know what happens in higher cortical areas of the brain that ultimately result in the perception of different odors. Therefore, our next goal was to investigate how sensory information is organized in the olfactory cortex. Using a genetic tracing system, we were able to visualize cortical neurons that receive signals from a particular OR[14].

Signal Transduction in the VNO

Our studies revealed several major findings. First, a stereotyped sensory map exists in the olfactory cortex, where signals from a specific receptor are relayed to specific clusters of neurons[15]. We also revealed combinatorial OR inputs in the olfactory cortex since overlap was observed in the posterior piriform and entorhinal cortex[15]. These overlapping patters are similar among individuals[16]. In contrast to the olfactory bulb, individual cortical neurons usually received input from a varied multitude of ORs[16]. My lab also showed that increasing odorant concentration causes an increase in the activation of cortical neurons[17]. Furthermore, structurally similar odorants have related representations in the olfactory cortex[17].

MOE and Signal Transduction

Besides uncovering the organization of the main olfactory system, we were also interested in examining the molecular basis of ORs. Past studies have shown that sensory transduction in the olfactory neurons is initiated by the binding of odorants to G protein coupled ORs. This binding stimulates adenylate cyclase III and the generation of cAMP, which, in turn, directly activates the cyclic nucleotide-gated (CNG) channels, resulting in an influx of calcium. Although most of the components of this signal transduction pathway have been cloned, the composition of the CNG channel remained uncertain. Previously, a CNG channel subunit, OCNC1, was cloned; however, differences between the functional properties of the subunit and CNG channel, led us to believe that the CNG channel might be composed of an additional subunit. In the rat olfactory epithelium, we found a novel subunit, OCNC2, which was actually 52% homologous to the OCNC1 subunit. Interestingly, the formation of hetero-oligomeric channels by the coexpression of OCNC1 and OCNC2 results in a signal transduction pathway very closely

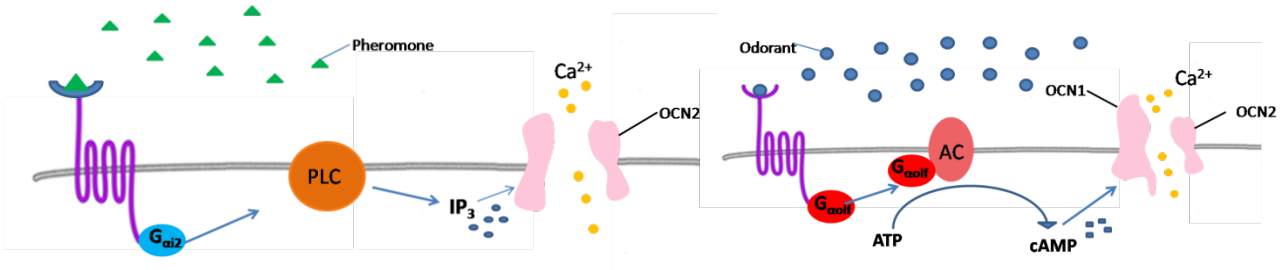

Figure 1: Signal Transduction in the MOE vs. VNO.
The binding of an odorant molecule to an OR in the MOE, results in a G protein coupled cascade where Gαolf activates adenylate cyclase to stimulate the production of the secondary messenger molecule, cAMP. In turn, cAMP activates the odorant CNG channel, which studies have revealed consists of OCN1 and OCN2, to open and let calcium rush in. On the other hand the binding of pheromone molecules to a pheromone

resembling the native CNG channel. For example, the sensitivity to low levels of cAMP in the native CNG channels is much closer to the coexpression of both subunits, rather than just OCNC1. These results help us create a more concrete picture of how sensory transduction occurs in the olfactory epithelium[18].

Shortly after these experiments, we decided to examine how sensory transduction occurs in the VNO. To investigate the hypothesis that VNO and olfactory epithelium transduction occur via the same mechanism, we used in situ hybridization and Northern blot analysis to see whether mRNA encoding Gαolf, adenylate cyclase III, OCNC1, and OCNC2 are expressed in VNO neurons. Surprisingly, we found that out of all the major olfactory signaling molecules that we examined, only OCNC2 was expressed in VNO neurons. These results show that although the sensory transduction in these two systems is different, VNO sensory transduction, like the olfactory epithelium, likely involves CNG channels[19] (Figure 1).

After establishing the OCNC1 channel subunit as a key component of the olfactory CNG channel, my colleagues investigated activation and competition between cells via gene manipulation. Random inactivation of the X chromosome in females represents an intriguing situation in which two different cell populations exist in the same individual. Since the X-linked OCNC1 subunit is subject to random inactivation, another lab generated reporter-tagged OCNC1-deficient mice that allow a direct visualization of the neurons and their projections. Their findings showed that male mice, in which all neurons were mutant, retained the ability to create structurally normal olfactory epithelium and bulb.

However, in heterozygous female mice, the population of OCNC1-/+ neurons slowly depleted from the olfactory epithelium and resulted in altered projections to the olfactory bulb. Amazingly, this depletion of inactive, mutant cells is dependent on odorant exposure and can be reversed by odorant deprivation. Most likely, in OCNC1-/+ mice, the odorant-activated wild-type neurons would be able to acquire more neurotrophic factors, which would result in the depletion of the inactive, mutant neurons. These results demonstrate the "use it or lose it" theory, showing that in order to survive in a competitive environment, olfactory neurons must carry out their normal function. These findings have important implications to the development and organization of the olfactory system[20].

VNO and Signal Transduction

In contrast to the expanding knowledge of the main olfactory system, little was known about how pheromones are detected in the VNO. My colleagues, Catherine Dulac and Richard Axel, pioneered the researchon the molecular basis of the accessory olfactory system. They demonstrated that the VNO neurons contain significant levels of two G protein subunits distinct from the ones coupled to ORs: Gαo and Gαi2. In 1995, they identified a novel family of about 100 candidate pheromone receptors that were exclusively expressed in the Gαi2 subset of the VNO neurons (VNRs)[21]. Axel's lab found that a given neuron expresses only one pheromone receptor, similar to the ORs of the main olfactory system. However, the gene family they discovered was only expressed in 15% of the cells in the VNO, which implied the possible existence of another gene family in that area[21]. Previous studies had also shown that signal transduction via pheromone receptors involves increased levels of the secondary messenger system molecule IP321. Two years later, our lab found a different multigene family of about 140 members that code for candidate pheromone receptors expressed by the Gαo subset of VNO neurons (VRs)[22]. The VNRs are sometimes called V1R receptors, while the VRs are referred to as V2Rs. Interestingly, the VRs have a structure that differs from that of ORs and VNRs, implying that they bind ligands in a different manner. Similarly to other G-protein coupled receptors, ORs, VRs, and VNRs are seven transmembrane receptors; however, VRs possess an extremely long N-terminal extracellular domain compared to the ORs and VNRs[22,23].

Organization of MOE versus the VNO

The emergence of two families of pheromone receptors, in addition to the ORs, raised questions regarding the organization of the accessory olfactory system and how it differes from the main olfactory system. Vomeronasal neuron fibers, separately from olfactory epithelium neuron fibers, project to the accessory olfactory bulb, which is the initial processing center for pheromonal information. In 1987, studies showed that the VNO pathways circumvent higher cortical centers, and instead project directly to the amygdala and hypothalamus, eliciting behavioral responses. Axel's lab used gene targeting to visualize axonal projections from VNO neurons expressing a specific receptor in the accessory olfactory bulb. Conversely, from the main olfactory bulbar map, neurons expressing a specific receptor project to multiple glomeruli in the accessory olfactory bulb (Figure 2.). Furthermore, they found that smaller glomeruli usually received information from only one type of afferent, while larger glomeruli receive axons from neurons expressing a variety of receptors. Additionally, the accessory olfactory bulb contains a much smaller number of separate domains in comparison to the glomeruli of the main olfactory system, indicating its limited ability to elicit behavioral

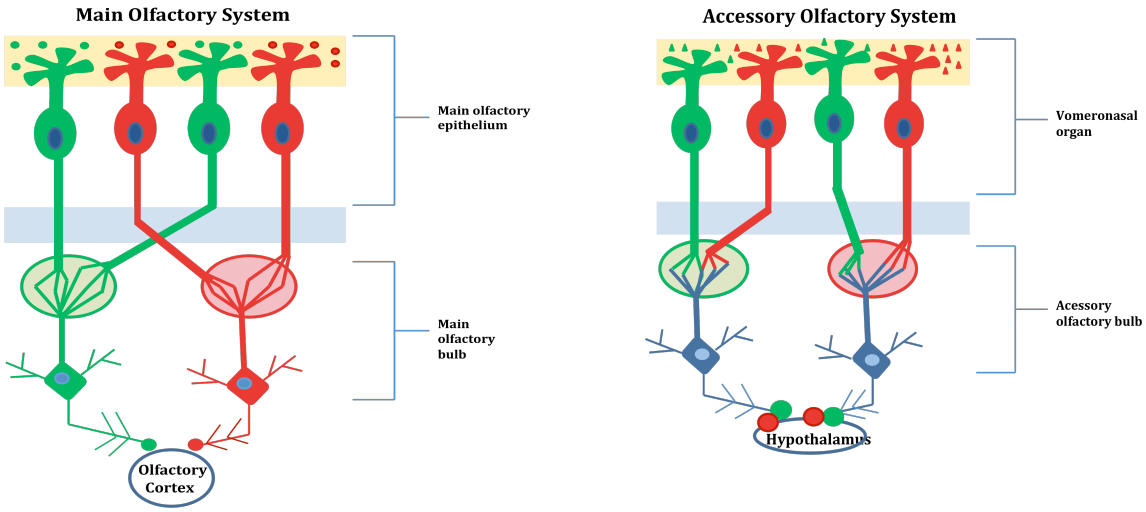

Figure 2: A Comparison Between the Main and Accessory Olfactory Systems.
Olfactory neurons, located in the olfactory epithelium respond to odorants, while the specialized neurons in the VNO usually detect pheromones. The axons of olfactory neurons that bind to the same odorants converge in the same glomeruli of the olfactory bulb. Conversely, individual glomeruli in the accessory olfactory bulb receive input from more than one type of sensory neuron. While the main olfactory system information is sent to higher cortical areas of the brain, such as the olfactory cortex, the signals from the accessory olfactory system bypass these areas and instead project to the hypothalamus.

changes. Meanwhile, the main olfactory system contains ~2000 glomeruli that regulate detection of odorants[24].

Nevertheless, the main and accessory systems are not as separate as it might appear. Neuropharmacological research has shown that VNO neurons can actually detect both pheromones and odorants. Not only did a number of single odorants, such as floral and musky, activate VNO neurons, but VNO neurons were even able to detect odorants at lower concentrations than the olfactory neurons[25]. A possible explanation for these remarkable findings is that some odorants, like pheromones, may stimulate behavioral responses[25]. To further investigate a possible commonality between odorants and pheromones, our lab examined the role of these chemical molecules on GnRH neurons, which are important regulators of reproduction[26]. Our experiments showed that GnRH neurons receive pheromone input from both pheromone and odor relay stations in the brain[26]. Also, a feedback loop exists in which GnRH neurons feedback to influence both odor and pheromone processing[26]. By demonstrating the olfactory system's influence on reproduction, these findings express its significance throughout the entire body.

Olfactory Switching

The observation that an olfactory neuron expresses only one receptor gene has instigated the desire to know what mechanism assures the expression of only one gene in a sensory neuron. Axel's lab shed light on this intriguing question by examining the stability of receptor choice. Their Studies show that immature olfactory sensory neurons which initially choose to express a mutant receptor can terminate its expression and switch at high frequencies to express a different receptor[27]. The expression of a functional receptor, on the other hand, signals the termination of switching. This logical mechanism assures that all mature neurons will express a functional receptor and that the choice of the receptor will remain stable for life[27]. These findings help explain why only one OR gene is chosen in addition to the receptor gene expressing only one allele[27,28].

A different kind of switching can occur in odorant preference. Specialized sensory neurons are usually specialized for either an attractant or repulsive behavior. However, experiments show that the AWCON olfactory neuron in *C. elegans* is capable of directing both attraction and repulsion[29]. Normally, AWCON neurons exhibit attractant behavior. However, a mutation in a receptor-like guanylate cyclase that contributes to the production of cGMP and typically localizes to AWCON neurons, results in a reversed odor preference[29]. The repulsive behavior can be easily rescued through genetic manipulation of the protein kinase C pathway, and even through behavioral conditioning[29]. These results show the relative ease of changing odorant preference.

Olfactory Adaptation

Along with discovering the ability of organisms to switch an odorant preference, current research on the olfactory system has focused on explaining adaptation. Humans experience adaption, or increased desensitization, when they temporarily cannot distinguish a particular odor after prolonged exposure to it. Adaptation involves both a slow and a rapid response; however, the precise mechanism behind both of these responses had been largely unknown. Cornelia Bargmann's lab, using AWC attractant olfactory neurons in *a C. elegans* model, discovered that rapid adaptation occurs with the phosphorylation of signaling molecules, specifically the beta subunit of the cGMP-gated channel[30]. On the other hand, long-term adaptation requires the nuclear translocation, and therefore a functional nuclear localization signal, of EGL-4 30. EGL-4 mutants showed an inability to adapt, which suggests that the cGMP-dependent protein phosphorylation decreases the activity of the signaling mechanism after long exposure to odorants[30]. Another molecular study conducted by Kelliher et al. revealed the effect of an ablated CNGA4 subunit of the CNG channel on adaptation and odor discrimination[31]. CNGA4 was specifically chosen because its prior deletion in mice resulted in CNG channels with decreased affinity for cAMP[31]. In comparison to the WT controls, mutant mice could only discriminate singular odorants at increased concentrations[31]. Also, importantly, they were unable to detect the same odorants in the presence of adapting background odors[31].

These results signify that the function of the CNGA4 subunit, via its ability to regulate rapid Ca2+ -dependent modulation of the CNG channel, is necessary for odor adaptation in mice[31].

Conclusion

The sense of smell is a necessity for a number of organisms, starting from the simplest invertebrates. My contributions to olfaction have had a revolutionary impact on the understanding of our oldest sense. By discovering the multigene family of ORs in the olfactory epithelium, Richard Axel and I solved the mystery of how the brain can discriminate between 10,000 different odors. Since then, my lab has helped make breakthrough advances in the field by contributing to the molecular and organizational level of the olfactory system. My colleague Axel concurrently contributed to the developing model of olfaction. He also showed that each olfactory neuron only expresses one type of odorant receptor. In addition, my lab contributed to elucidating the organization and function of the accessory olfactory system. While Axel and Dulac identified a novel family of pheromone receptors in the VNO, my lab also discovered a second family of pheromone receptors that differ in structure. Recently my lab identified the existence of a second class of chemosensory receptors in the epithelium which we called trace amine-associated receptors (TAARS)[32]. These ORs exist for detecting volatile amines[32]. Furthermore, in the past year, our lab has discovered a third family of VNO receptors in mice that actually belong to a formyl peptide receptor (FPR) family; however, these FPRs are only expressed in VNO neurons[33].

In addition to uncovering the enigma behind the organization and function of the olfactory system, we are interested in finding treatments for various smell disorders. The ability to smell tends to decrease with age. People with hyperosmia have an abnormally acute sense of smell, which might be caused by over stimulated OR. Thus, a possible study could involve partially blocking or modifying specific ORs to limit the strength of the signal transmission. Likewise, people who suffer from hyposmia which is the decreased ability to smell, might benefit from OR stimulation. Since the olfaction is closely linked to gustation, it is important to find treatments for smell disorders in order for people to not lose their appetites.

The discovery of odorant receptors not only contributes to our overall understanding of how humans perceive various smells, but it also opens the way to new treatment options for people suffering from olfactory diseases. For example, people who suffer from anosmia can be treated via activation of ORs. Conversely, we can help people with hyperosmia by blocking the ability of some ORs to function. More research still needs to be done in order to aid humans who have olfactory dysregulations.

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