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Correlation between olfactory receptor basal activity and odor response

An observational study

Lun Xu, MD[a,b], Qi Dai, MD[a,b], Yiqun Yu, MD[a,b], Hongmeng Yu, MD[a,c,*]

Abstract

Olfactory receptors (ORs) are the largest group of G-protein-coupled human receptors responsible for detecting and distinguishing odors. However, the fundamental mechanisms underlying OR responses remain poorly understood. This study aims to evaluate the basal activity of mouse and human ORs in the Hana3A cell line and examine the correlation between their basal activity and response characteristics to odor stimuli. Using a luciferase assay on the Hana3A cell line, the results showed that the 10 mouse ORs with the highest basal activity levels were positively correlated with their total response to odor stimuli. However, there was no significant correlation between the basal activity of human-derived ORs and their total response to odor stimuli. These findings indicate that basal activity levels significantly influence OR responses to odors, as evidenced by the positive correlation in the 10 mouse ORs with the highest basal activity levels and their odor response. This supports the notion that the receptor binding cavity is crucial in determining OR responses to odors.

Abbreviations: GPCRs = G protein-coupled receptors, hORs = human-derived olfactory receptors, mOR = mouse olfactory receptor, MRR = molecular receptive range, OR = olfactory receptors, RTP = receptor transport protein, TAARs = trace amine-associated receptors.

Keywords: basal activity, hana3A cell line, luciferase assay, odor response, olfactory receptor

1. Introduction

As 1 of the 5 senses, olfaction is important in daily life. The sense of smell enables us to perceive and differentiate a vast array of odor molecules, relying on a combinatorial coding system where multiple olfactory receptors (ORs) interact with each odorant. The specificity and breadth of OR–ligand interactions influence odor recognition, and factors such as concentration variability and odor mixtures add complexity to this process.[1] Mammals detect and discriminate between multiple odors through a large family of G protein-coupled receptors (GPCRs), yet little is known about the molecular and structural basis of the odor response properties of individual ORs. Therefore, the research into the mechanisms of action of ORs and the search for treatments for related disorders are of great importance for clinical solutions to olfactory disorders.

The sense of smell cannot be generated without ORs. ORs belong to the class A rhodopsin family and play a critical role in detecting and distinguishing many odor molecules.[2] In addition to ORs, trace amine-associated receptors (TAARs) have been identified as important contributors to odor perception, particularly in recognizing volatile amines associated with social and environmental cues.[3] TAARs work alongside ORs to enhance the brain's ability to decode complex odor mixtures by broadening the range of detectable compounds and influencing behavioral responses.[4] This synergistic interaction between ORs and TAARs provides an additional layer of specificity in

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[a] Department of Otolaryngology, Ear, Nose & Throat Institute, Eye, Ear, Nose & Throat Hospital, Fudan University, Shanghai, People's Republic of China, [b] Clinical and Research Center for Olfactory Disorders, Eye, Ear, Nose & Throat Hospital, Fudan University, Shanghai, People's Republic of China, [c] Research Units of New Technologies of Endoscopic Surgery in Skull Base Tumor (2018RU003), Chinese Academy of Medical Sciences, Shanghai, People's Republic of China.

** Correspondence: Hongmeng Yu, Department of Otolaryngology, Ear, Nose & Throat Institute, Eye, Ear, Nose & Throat Hospital, Fudan University, 83 Fen Yang Road, Shanghai 200031, People's Republic of China (e-mail: hongmengyush@163.com).*

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odor coding, allowing for the nuanced perception of odors in diverse environments. Despite extensive research, the precise mechanisms governing OR–ligand interactions remain unclear. The ability of ORs to recognize odorants depends not only on ligand binding but also on activation dynamics. Moreover, odor perception is influenced by the interplay between ORs and other receptors, such as TAARs, further complicating the decoding of odor mixtures. The functional interplay between ORs and TAARs enhances the olfactory system's ability to distinguish odorants with high sensitivity and specificity. Structural analysis of TAARs has revealed key ligand-binding motifs, such as the D3.32W6.48Y7.43 motif, which is critical for amine odorant recognition.[5] While ORs and TAARs recognize different classes of odorants, their complementary roles suggest a potential synergistic mechanism in odor perception, particularly in distinguishing amine-containing compounds. Understanding these interactions is critical for advancing olfactory research and developing clinical interventions for olfactory disorders.

There are 2 possible challenges to OR–ligand interactions in odor recognition, and 1 challenge is identifying an odor at widely varying concentrations because the pattern of ORs responsive to an odor is expected to change quantitatively and qualitatively with odor concentration. This prediction stems from data on olfactory bulb glomeruli response patterns, which broaden with increasing concentration and have glomeruli that drop out of the response as concentration increases.[6,7] The second challenge is identifying an odorant when it is mixed with other odorants, which happens naturally when a second odor is present. Studies of odor perception demonstrate that this becomes more difficult as odor mixtures become more complex.[8]

It has been found that mouse OR 256-8 (mOR256-8), a member of the mOR256 subfamily, can be converted into a broad-spectrum responsive OR by exchanging 1 or several residues.[9] The present study found that the total response of some ORs was positively correlated with their basal activity, indicating ligand nondependent receptor activation. These studies suggest that the broad responsiveness of OR is dependent not only on ligand binding but also on its activation mechanism. Another broadly responsive OR (e.g., mOR256-3) requires a low activation threshold and a permissive binding pocket. Thus, it is likely that at least some ORs have a permissive binding pocket in which interactions would be more stochastic compared to other GPCRs.[10] Thus, the responsiveness of ORs to odorants is determined by ligand affinity and their intrinsic activation potential. One major challenge in olfactory research is understanding how ORs maintain specificity while responding to a broad range of odorants, particularly in complex environments with multiple odor molecules. This study provides insights into the fundamental mechanisms governing odor recognition by examining the basal activity and tuning width of ORs. Therefore, through the heterologous expression system of ORs, we studied the factors affecting the basal activity and tuning width of ORs and further explored the mechanism of action between ORs and ligands, providing a theoretical basis for basic research and clinical research in the field of olfaction.

In this study, the basal activity of mouse and human ORs in the constructed Hana3A cell line was assayed[11] and to correlate their basal activity and response characteristics with odor to provide a theoretical basis for elucidating the binding mechanism of ORs to ligands.

2. Materials and methods

2.1. Chemicals and OR constructs

Odorants were purchased from Sigma-Aldrich, St Louis. They were dissolved in dimethyl sulfoxide to make stock solutions at 1 mM and then freshly diluted in optimal minimum essential medium (MEM) (ThermoFisher, Waltham) to prepare the odorant stimuli. The OR constructs were kindly provided by Dr YY (Shanghai Fudan University, China).

2.2. Cell culture and transfection

In the experiments, Hana3A cells, a HEK293T-derived cell line that stably expresses receptor-transporting proteins (RTP1 and RTP2), receptor expression-enhancing protein 1 (REEP1), and olfactory G protein ($G\alpha_{olf}$), were used.[12] The cells were grown in MEM (Corning) supplemented with 10% (v/v) fetal bovine serum (ThermoFisher) and 100 μg/mL penicillin-streptomycin (ThermoFisher), 1.25 μg/mL amphotericin (Sigma-Aldrich), and 1 μg/mL puromycin (Sigma-Aldrich).

All OR plasmids were transfected into the cells using Lipofectamine 2000 (ThermoFisher). Before the transfection, the cells were plated on 96-well plates (NEST, Wuxi, Jiangsu, China) and incubated overnight in MEM with 10% fetal bovine serum at 37 °C and 5% CO_2. For each 96-well plate, 2.4 μg of pRL-SV40, 2.4 μg of CRE-Luc, 2.4 μg of mouse RTP1S, and 12 μg of receptor plasmid DNA were transfected. The cells were subjected to a luciferase assay 24 h after transfection.

2.3. Luciferase assay

Luciferase assays were performed using the Dual-Glo luciferase assay kit (Promega, Madison, WI) per the reference protocol.[11] OR activation triggers the $G\alpha_{olf}$-driven AC-cAMP-PKA signal cascade and phosphorylates cAMP response element-binding protein. Activated cAMP response element-binding protein induces luciferase gene expression, which can be quantified by luminescence [measured here using a biological luminescent plate reader (MD SPECTRAMAX L)]. The cells were co-transfected with firefly and Renilla luciferase, in which firefly luciferase was the reporter of cAMP. Renilla luciferase comprises an active simian virus 40 (SV40) promoter (PRL-SV40; Promega, Madison, WI), which controls cell viability and transfection efficiency. The ratio of firefly luciferase to Renilla luciferase was measured. Determination was performed as follows: 24 h after transfection, the medium was replaced with 100 μL odor solution (different doses), diluted in optimum MEM (ThermoFisher), and the cells were further incubated at 37 °C and 5%CO for 4 h. The odor response of each OR was repeated 4 times on the same plate, and the basal activity of each mOR was averaged from 4 parallel samples on the same plate. All ORs were detected at 300 μM odorant concentration. After incubating in lysis buffer for 15 min, add 20 μL twin-crystal luciferase reagent to each well of 96-well plate and measure firefly luciferase luminescence. Next, add 20 μL STOP-Glo luciferase reagent to each well, and measure Renilla luciferase luminescence. The parametric dose-response curves were fitted with GraphPad Prism 8.

2.4. Statistical analysis

All statistical plots in this study were expressed using the mean ± standard deviation (mean ± SD). All ORs cell experiments were done in 4 replicates for each group, and the mean of the 4 groups was taken for statistics. Basal activity statistics of ORs. The basal activity of ORs was measured by luciferase assay. The control group was empty vector pCI. 4 parallel samples were made for each group of odorant receptors, and the data were analyzed by Microsoft software. The mean value was used for sorting when screening for odorant receptor basal activity. ORs correlation analysis. The ORs detected in the previous step were ranked, and the top 10 and bottom 10 ORs were selected and stimulated with odorants, and their

total response values were measured. The results were analyzed using Microsoft software and GraphPad Prism 8 software, and the results were obtained by using the "Linear regression" and "Correlation" modules. A significant difference was indicated by $P < .05$ ($P < .05$ and $r > 0.8$: high correlation between variables; $P < .05$ and $.5 < r \leq 0.8$: moderate correlation between variables; $P < .05$ and $r \leq 0.5$: low correlation between variables). Statistical results were expressed using the mean ± standard deviation (mean ± SD).

2.5. Ethical statement

The Institutional Review Board of the Affiliated Fudan University approved the study protocol (Number: 2023-678). The Ethics Committee of the Affiliated Fudan University approved the collection and analysis procedures of all clinical samples.

3. Results

3.1. Screening the basal activity of 182 mORs

This study examined 182 mORs by luciferase assay to determine their basal activities. This study analyzed a subset of mORs, specifically the members of the mOR256 subfamily. The mOR256-X notation refers to distinct isoforms within this subfamily, each identified by a unique numerical designation (e.g., mOR256-3 and mOR256-8). The top 10 and bottom 10 ORs were selected according to the detection values. Table S1, Supplemental Digital Content, https://links.lww.com/MD/O716 provides information about the basal activity ranking of the 182 mORs. The basal activity of 182 mORs was examined, with the top 20 displayed in Figure 1A. In addition, the bottom 20 ORs are shown in the Figure 1B.

3.2. Screening the basal activity of 280 hORs

In the present study, luciferase assay detected the basal activity of 280 human-derived ORs (hORs). Based on their assay values, the top 10 and bottom 10 ORs were selected. Details regarding the basal activity ranking of the 280 hORs are presented in Table S2, Supplemental Digital Content, https://links.lww.com/MD/O717.

We detected the top 20 hORs, and the results showed that the basal activity values of hOR5J2, hOR52P1, hOR5G3, hOR2H2, hOR5M9, hOR3A2, hOR2A12, hOR52N1, and hOR2L8 were relatively high (Fig. 1C). Furthermore, the 20 hORs were detected after ranking, and the results showed that the basal activity values of hOR2T1, hOR52J3, hOR2J4, hOR52N2, and hOR52L2 were relatively low (Fig. 1D).

3.3. The basal activity of ORs is correlated with the response to odor

Ten ORs with the highest and lowest basal activity were selected from 182 mORs and 280 hORs for further study to explore the response relationship between their basal activity and odorant stimulation. The total reaction value to odorant stimulation is the sum of the reaction value to each odorant.

The top 10 mORs (mOR13-6, mOR18-1, mOR166-14, mOR256-17, mOR259-1, mOR139-1, mOR170-5, mOR182-11p, mOR204-33P, and mOR223-10) were stimulated by odors, the results showed that mOR13-6, mOR18-1, mOR166-14, mOR256-17, and mOR259-1 had relatively high total response to odor stimulus (Fig. 2A). In the same way, 10 mORs (mOR31-2, mOR32-4, mOR110-8, mOR183-11P, mOR245-7, mOR245-8, mOR125-5P, mOR136-5, mOR170-1, and mOR170-15) were stimulated by odors. The results showed that mOR245-7, mOR125-5P, and mOR136-5 had relatively low total response to odorant stimulation (Fig. 2B).

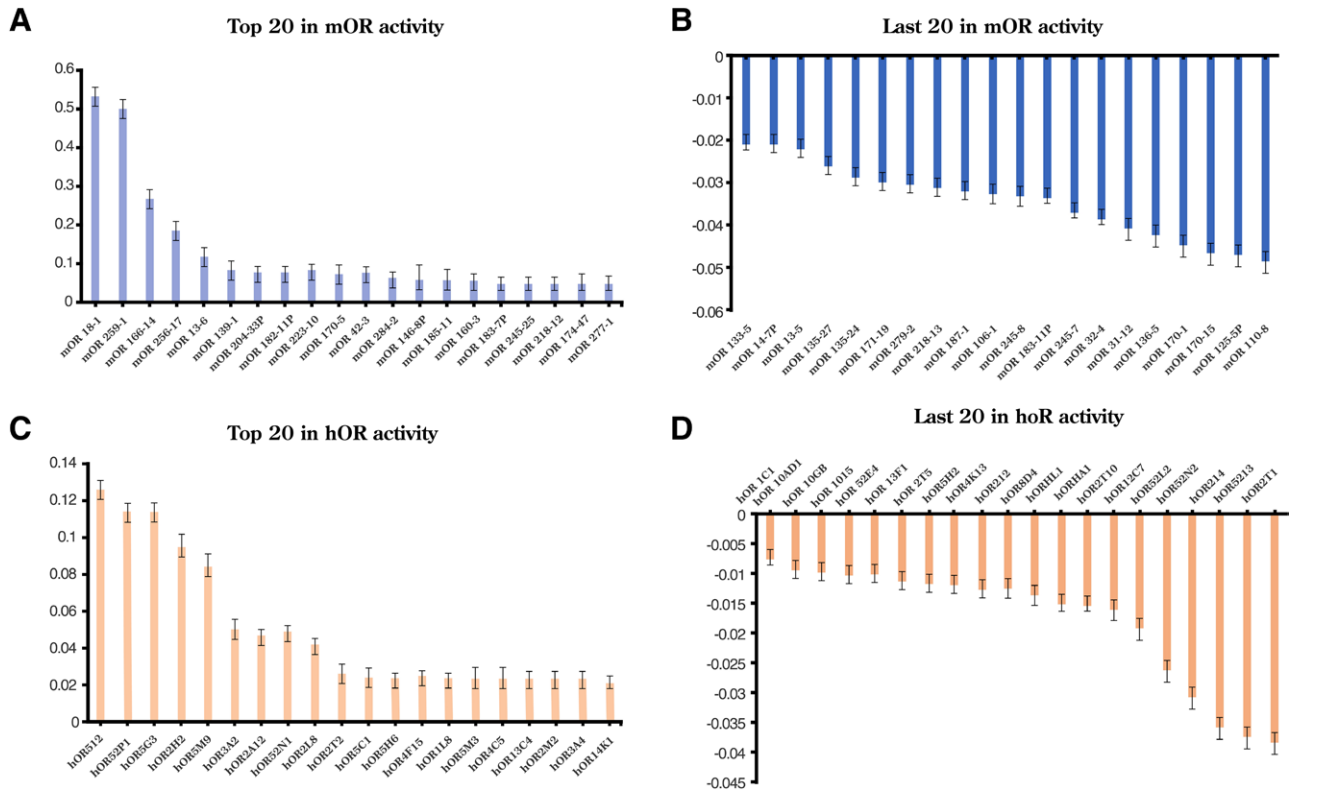

Figure 1. Basal activity of mouse and human ORs. (A) Basal activity level of the top 20 mice ORs. (B) Basal activity level of the last 20 mice ORs. (C) Basal activity level of the top 20 human ORs. (D) Basal activity level of the last 20 human ORs (mean ± SD, n = 4). ORs = olfactory receptors.

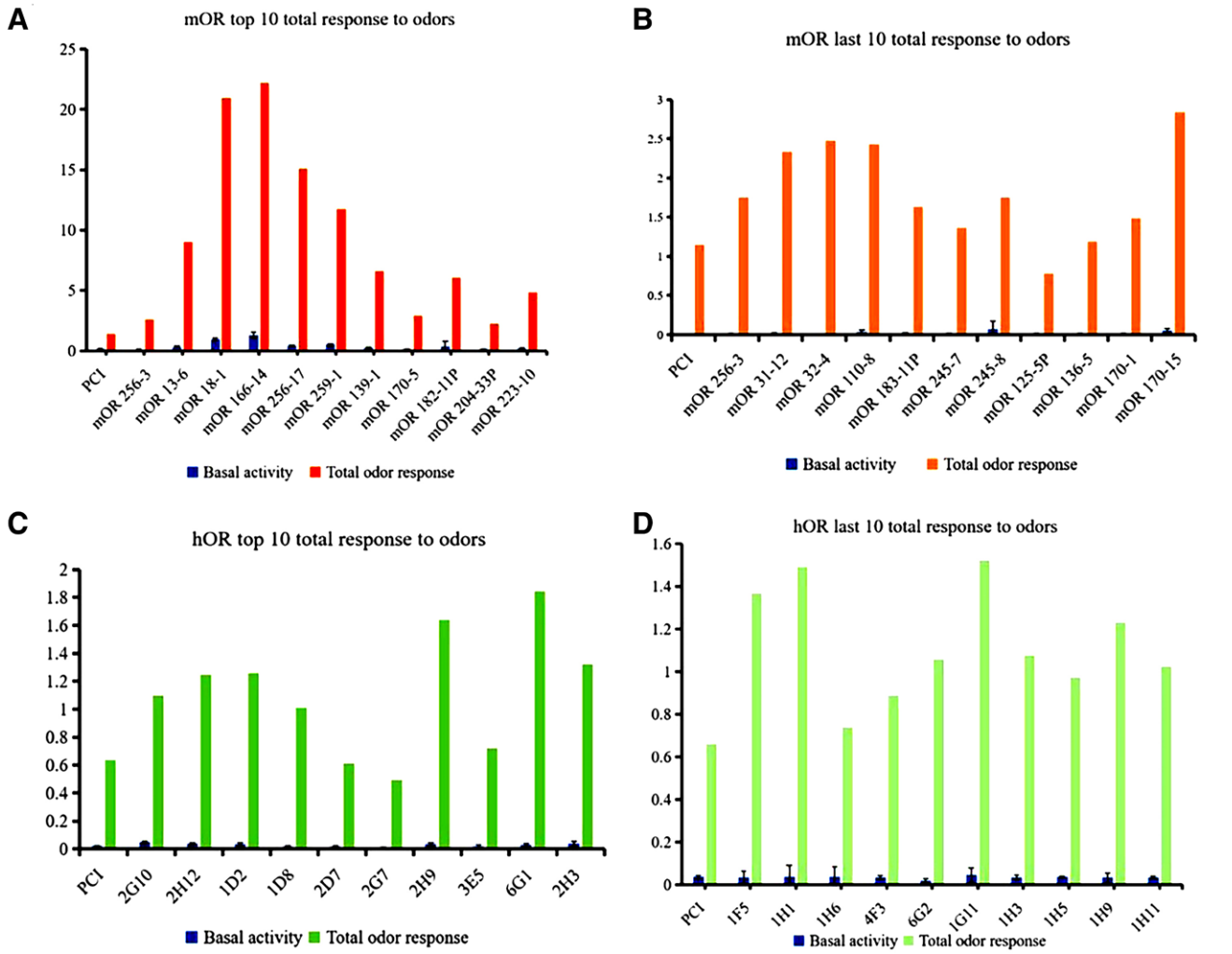

Figure 2. Total response to odorant stimulation of ORs with highest and lowest basal activity levels. (A) Total response of 10 mouse ORs with highest basal activity levels. (B) Total response of 10 mouse ORs with lowest basal activity levels. (C) Total response of the top 10 human ORs to odorant stimulation. (D) Total response of 10 human ORs with lowest basal activity levels (mean ± SD, n = 4). ORs = olfactory receptors.

Furthermore, an odorant stimulus-response was conducted for the top 10 hORs (hOR52P1, hOR5J2, hOR2L8, hOR2A12, hOR52N1, hOR5M9, hOR2H2, hOR3A2, hOR2T2, and hOR5G3). The results showed that hOR2H2, hOR5G3, hOR2T2, hOR5J2, and hOR2L8 had relatively high total response to odors (Fig. 2C). Similarly, the results of the odor stimulation response on 10 receptors (hOR11A1, hOR11L1, hOR8D4, hOR2T1, hOR52N2, hOR52L2, hOR2T10, hOR2J4, hOR13C7, and hOR52J3) are shown in Figure 2D. The total response of hOR8D4, hOR2T1, and hOR2J4 to odors was relatively low.

A linear regression analysis was conducted on 20 mORs (mORs ranking top 10 and bottom 10) to reflect the correlation between screened OR basal activity and the total response to odors. The results showed that the total reaction of the top 10 mORs to odors was positively correlated with their basal activities (Fig. 3A). The total response was summarized for each mOR to 16 odors (300 μM). Linear regression analysis showed that r was 0.9441 ($P < .0001$), with a strong correlation. The total response of the 10 mORs with the lowest basal activity to odor was unrelated to their basal activity (Fig. 3B). Linear regression analysis showed that r was 0.4454 ($P = .1468$), without correlation. A linear regression analysis was also performed on these 20 hORs (top 10 and bottom 10 hOR rankings), which showed that the total response of the top 10 basal activity hORs to odor was not correlated with their basal activity (Fig. 3C) and a linear regression analysis yielded that the r was 0.5767, $P = .0810$, which was not correlated. The total response of the bottom 10 hORs regarding basal activity to odorants was not associated with their basal activity (Fig. 3D), and a linear regression analysis yielded an r of 0.1311, $P = .7008$, with no correlation.

4. Discussion

In this study, the basal activity of mORs (mOR18-1, mOR259-1, mOR166-14, mOR256-17, mOR13-6, mOR139-1, mOR204-33P, mOR182-11p, mOR223-10, and mOR170-5) was positively correlated with their effects on odor stimulation, and the higher the basal activity, the stronger the response to odor stimulation. However, there was no significant correlation between mORs with weak basal activity and hORs in response to odorant stimulation. This result may be related to the cell surface expression capacity of the OR and the binding mechanism of the OR to the ligand. Studies have shown that sensitive OR responding to a higher concentration of odors may not be the strongest responder, and vice versa.[13] Therefore, it is possible that the concentration of odors stimulating human ORs in the experiment did not reach the maximum response concentration. One of the reasons the structure and properties of ORs are still unknown is that it is challenging to express large numbers of ORs in heterologous expression systems. In these systems, ORs

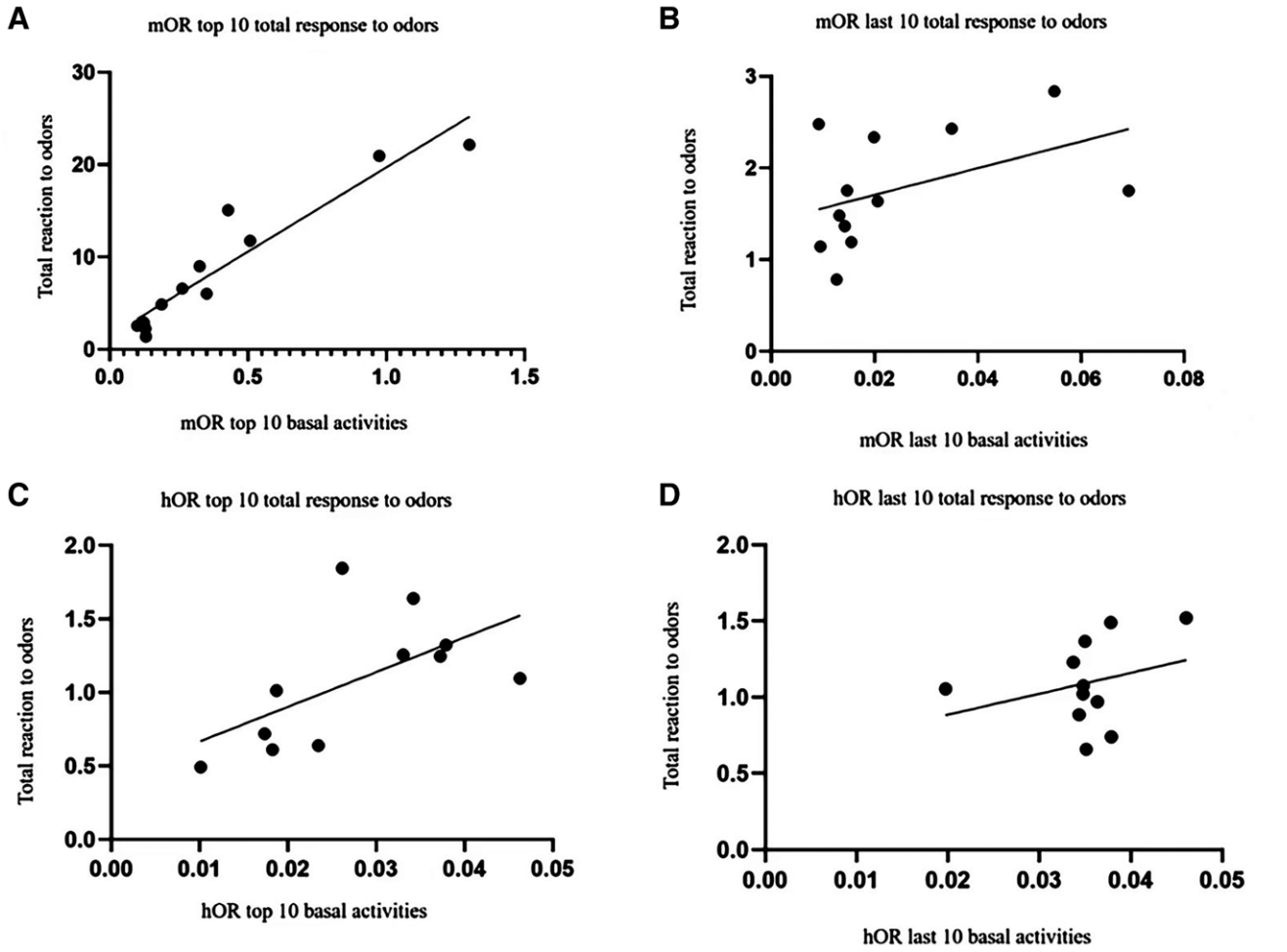

Figure 3. OR basal activity level is correlated with response to odors. (A) Linear regression analysis was performed between basal activity and total response to odors in the top 10 moue ORs. (B) Linear regression analysis on mouse ORs with lowest basal activity level. (C) Linear regression analysis was performed in the top 10 human ORs. (D) Linear regression analysis on 10 human ORs with lowest basal activity level. ORs = olfactory receptors.

are primarily localized in the cell's endoplasmic reticulum and only migrate to the cell membrane upon expression.[14,15] It is believed that the basal activity of ORs is related to their ability to be expressed on the cell membrane. Therefore, the ability of ORs to be on the membrane is one of the crucial factors determining their basal activity. When expressed in cells other than olfactory sensory neurons, ORs do not translocate efficiently to the cell membrane surface. The features that control this surface expression are thought to be determined by specific amino acids in the ORs sequence. This may depend on the interaction of ORs with RTP1S (which facilitates the translocation of ORs to the cell surface) or membrane composition.

Receptor transport protein (RTP)1 and RTP2 are thought to be chaperone proteins in OSNs that enhance the cell surface expression of many ORs when cotransfected into heterozygous cells.[16,17] When RTP1 and RTP2 genes were knocked out, most ORs were significantly deficient in expression, suggesting that these ORs require RTP1 and RTP2 to function.[18] It has been suggested that the retention of ORs in the cell is due to the structural instability of ORs, and this structural instability is because most ORs do not fold properly in heterologous cells and thus cannot be transported to the plasma membrane. This could also explain why many consensus ORs show robust cell surface expression, whereas most natural ORs show no detectable cell surface expression in heterologous cells.[19]

A study by Hiroaki Matsunami et al team found that in terms of amino acid residues conserved in ORs independent of RTP, NBW3.39 and 3.43 in the third transmembrane domain were associated with the expression of ORs. Substitution of 3.39E and 3.43L effectively improved the membrane localization of multiple ORs in mice and humans. NBW3.39E and NBW3.43L were less conserved in human ORs than in mouse ORs.[20] In the present study, the total response of hORs to odorants was unrelated, which might be related to the fact that hORs are more challenging to express in heterologous expression systems than mORs. In the broad-tuned receptor mOR256-3, specific OR residues appear to be involved in broadening the capacities of the binding pocket through a very labile toggle switch.[21]

The OR binds to the ligand by van der Waals forces, and its affinity is weak, while the nonOR GPCR binds by ionic bonding, and its affinity is strong. It was revealed that a new synthetic OR, the consensus OR, was designed by analyzing the OR amino acid sequence. The consensus OR efficiently translocated to the cell surface, while its agonist response was significantly enhanced.[16] Molecular dynamics simulations of the consensus OR revealed that designing salt bridges in the three-dimensional homology model of the consensus OR could further improve the expression of the consensus OR on the cell surface.

In addition to the ability of epithelial expression to affect basal activity, the OR response to odorants is also related to the nature of the binding cavity and the low activation barrier (binding affinity). The largest sequence variability among ORs lies in this binding site as well as the nature of the binding cavity,

which likely confers our ability to discriminate between different chemical odors.[22]

The size of the binding cavity and the amino acid residues within it can affect receptor-ligand binding. Previous studies have shown that the receptor response is dependent on the molecular volume of the odorant, suggesting that affinity and/or efficacy becomes optimal when the molecular volume of the odorant matches the size of its binding pocket within the receptor.[23,24] It has been shown that in the receptor hOR51E1, when residue H108 is mutated to the more significant, less hydrophilic residue H108F, the receptor response to both agonists is completely lost, and molecular modeling revealed that these residues are located in the upper part of the binding cavity and regulate the size of the cavity.[22] They control the accessibility of the agonist within the binding cavity by sensing the size of the hydrophobic part of the agonist, which regulates the effectiveness of the receptor response. In our study, the narrow-tuned receptor hOR2W3 had relatively high basal activity but responded poorly to odorants. One study modeled it and found low freedom to accommodate multiple ligands in its binding pocket, which may be a causal factor in determining the narrow-tuned OR.[25]

The specific odorant structures recognized by ORs are termed the molecular receptive range (MRR) and MRR breadth range of ORs,[26] where some ORs are narrowly tuned, and others are broadly tuned.[27]

It has been found that broad-tuned receptors mOR256-17 and narrow-tuned receptors mOR256-8 and mOR256-22 exhibit more significant differences at the amino acid level (54%–57% identity) and MRR.[28] The differences between the MRRs were in the specific odors recognized and the breadth of MRRs. This suggests that the degree of overlap of MRRs may be positively correlated with the degree of amino acid homology of ORs.[29] For the broad-tuned receptor hOR2W1, it was found that the model binding affinity values of agonists to hOR2W1 were lower than those of inhibitors of hOR2W1, which may be related to its low activation barrier (binding affinity).

Moreover, the response of ORs to odorants may also be related to the nature of the odorant. It has been shown that depending on the ligand polarity, the binding pocket in the broad-tuned receptor hOR2W1 supports hydrophobic contacts with nonpolar amino acid residues, which is considered to be the primary mode of ligand interaction with broadly regulated ORs, supporting multiple binding modes through opportunistic interactions.[10] In our study, the basal activity of hOR2W1 was found to be relatively high, but its response to odorants was not very high, perhaps because hOR2W1 is not sensitive to these odorants.

Studies have shown that odor recognition follows a combinatorial coding scheme and that primary coding may help modulate the sensitivity of receptors to odor recognition.[30,31] According to the prediction of Hiroaki Matsunami team, they are expected to face each other in the extracellular portion of the transmembrane region, forming a vestibular location at the entrance to the orthotropic binding cavity.[32] The specificity of the binding cavity of the narrow-tuned receptors like mOR256-8 may be higher than that of the broad-tuned receptors. A molecular dynamics simulation and machine learning study revealed that changing amino acid residues can significantly alter the functional expression of OR in heterologous cells.[16] It also helps to search for the corresponding ligands of different ORs.[22,33,34] All of the above describes what happens when OR is expressed in a heterologous system, but it may differ in a natural cellular environment.[35] The interaction of mammalian ORs with mixtures depends on the cellular environment, which critically determines the response characteristics of the OR and, thus its affinity for the ligand, which is in agreement with many other GPCR studies.[36] Research on the activation mechanism of ORs has been progressing steadily, but no model has fully explained this phenomenon. In particular, it is not clear whether the chemical structure of the molecule is the main factor or due to the underlying activity of the receptor itself for the specific process of receptor activation by odor molecules. Perhaps because the phenomenon of receptor recognition and binding of ligands and receptor activation by bound ligands may be different processes, more rigorous and detailed follow-up studies are needed to clarify this issue.

Therefore, high-resolution analysis of chemical sensory receptor structures is essential for the in-depth study of ORs. Thanks to significant efforts in pharmacology, cell and molecular biology, neurobiology, and modeling, OR structure research has become more scientific, rational, and effective. This progress is built upon previous studies, extracting and integrating key information across fields and research into the specific mechanisms between ligands and receptors. The goal is to establish a cell-based volatile odor detection and identification platform, which forms the foundation for OR-based volatile odor sensors.[37] Ultimately, high-resolution chemosensory structures will aid in revealing the molecular mechanisms of receptors in this class of proteins.

Author contributions

Conceptualization: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Data curation: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Formal analysis: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Funding acquisition: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Investigation: Lun Xu, Qi Dai, Yiqun Yu.
Methodology: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Project administration: Lun Xu.
Resources: Lun Xu.
Software: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Supervision: Lun Xu, Yiqun Yu, Hongmeng Yu.
Validation: Qi Dai, Yiqun Yu, Hongmeng Yu.
Visualization: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Writing – original draft: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.
Writing – review & editing: Lun Xu, Qi Dai, Yiqun Yu, Hongmeng Yu.

References

[1] Wheeler PL, Murphy C. Olfactory measures as predictors of conversion to mild cognitive impairment and Alzheimer's Disease. Brain Sci. 2021;11:1391.
[2] Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell. 1991;65:175–87.
[3] Niimura Y, Matsui A, Touhara K. Extreme expansion of the olfactory receptor gene repertoire in African elephants and evolutionary dynamics of orthologous gene groups in 13 placental mammals. Genome Res. 2014;24:1485–96.
[4] Nara K, Saraiva LR, Ye X, Buck LB. A large-scale analysis of odor coding in the olfactory epithelium. J Neurosci. 2011;31:9179–91.
[5] Guo L, Cheng J, Lian S, et al. Structural basis of amine odorant perception by a mammal olfactory receptor. Nature. 2023;618:193–200.
[6] Fried HU, Fuss SH, Korsching SI. Selective imaging of presynaptic activity in the mouse olfactory bulb shows concentration and structure dependence of odor responses in identified glomeruli. Proc Natl Acad Sci USA. 2002;99:3222–7.
[7] Bozza T, McGann JP, Mombaerts P, Wachowiak M. In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. Neuron. 2004;42:9–21.
[8] Poupon D, Fernandez P, Archambault Boisvert S, Migneault-Bouchard C, Frasnelli J. Can the identification of odorants within a mixture be trained? Chem Senses. 2018;43:721–6.
[9] Yu Y, de March CA, Ni MJ, et al. Responsiveness of G protein-coupled odorant receptors is partially attributed to the activation mechanism. Proc Natl Acad Sci USA. 2015;112:14966–71.
[10] Charlier L, Topin J, Ronin C, et al. How broadly tuned olfactory receptors equally recognize their agonists. Human OR1G1 as a test case. Cell Mol Life Sci. 2012;69:4205–13.
[11] Saito H, Kubota M, Roberts RW, Chi Q, Matsunami H. RTP family members induce functional expression of mammalian odorant receptors. Cell. 2004;119:679–91.

[12] Zhuang H, Matsunami H. Evaluating cell-surface expression and measuring activation of mammalian odorant receptors in heterologous cells. Nat Protocols. 2008;3:1402–13.
[13] Hu XS, Ikegami K, Vihani A, et al. Concentration-dependent recruitment of mammalian odorant receptors. ENeuro. 2020;7:ENEURO.0103–19.2019.
[14] Belloir C, Miller-Leseigneur M-L, Neiers F, Briand L, Le Bon A-M. Biophysical and functional characterization of the human olfactory receptor OR1A1 expressed in a mammalian inducible cell line. Protein Expr Purif. 2017;129:31–43.
[15] Noe F, Frey T, Fiedler J, Geithe C, Nowak B, Krautwurst D. IL-6-HaloTag(®) enables live-cell plasma membrane staining, flow cytometry, functional expression, and de-orphaning of recombinant odorant receptors. J Biol Methods. 2017;4:e81.
[16] Fukutani Y, Tamaki R, Inoue R, et al. The N-terminal region of RTP1S plays important roles in dimer formation and odorant receptor-trafficking. J Biol Chem. 2019;294:14661–73.
[17] Wu L, Pan Y, Chen G-Q, Matsunami H, Zhuang H. Receptor-transporting protein 1 short (RTP1S) mediates translocation and activation of odorant receptors by acting through multiple steps. J Biol Chem. 2012;287:22287–94.
[18] Sharma R, Ishimaru Y, Davison I, et al. Olfactory receptor accessory proteins play crucial roles in receptor function and gene choice. ELife. 2017;6.
[19] McCaffrey K, Braakman I. Protein quality control at the endoplasmic reticulum. Essays Biochem. 2016;60:227–35.
[20] Fukutani Y, Nakamura Y, Muto N, et al. Hot spot mutagenesis improves the functional expression of unique mammalian odorant receptors. Int J Mol Sci. 2021;23:277.
[21] de March CA, Yu Y, Ni MJ, et al. Conserved residues control activation of mammalian G protein-coupled odorant receptors. J Am Chem Soc. 2015;137:8611–6.
[22] Bushdid C, de March CA, Fiorucci S, Matsunami H, Golebiowski J. Agonists of G-Protein-coupled odorant receptors are predicted from chemical features. J Phys Chem Letters. 2018;9:2235–40.
[23] Saberi M, Seyed-Allaei H. Odorant receptors of Drosophila are sensitive to the molecular volume of odorants. Sci Rep. 2016;6:25103.
[24] Zhang L, Yuan Y, Ren T, Guo Y, Li C, Pu X. Shining light on molecular mechanism for odor-selectivity of CNT-immobilized olfactory receptor. Sci Rep. 2018;8:7824.
[25] Haag F, Ahmed L, Reiss K, Block E, Batista VS, Krautwurst D. Copper-mediated thiol potentiation and mutagenesis-guided modeling suggest a highly conserved copper-binding motif in human OR2M3. Cell Mol Life Sci. 2020;77:2157–79.
[26] Araneda RC, Kini AD, Firestein S. The molecular receptive range of an odorant receptor. Nat Neurosci. 2000;3:1248–55.
[27] Reed RR. After the holy grail: establishing a molecular basis for Mammalian olfaction. Cell. 2004;116:329–36.
[28] Abaffy T, Matsunami H, Luetje CW. Functional analysis of a mammalian odorant receptor subfamily. J Neurochem. 2006;97:1506–18.
[29] Li J, Haddad R, Chen S, Santos V, Luetje CW. A broadly tuned mouse odorant receptor that detects nitrotoluenes. J Neurochem. 2012;121:881–90.
[30] Zwicker D. Primacy coding facilitates effective odor discrimination when receptor sensitivities are tuned. PLoS Comput Biol. 2019;15:e1007188.
[31] Wilson CD, Serrano GO, Koulakov AA, Rinberg D. A primacy code for odor identity. Nat Commun. 2017;8:1477.
[32] Bushdid C, de March CA, Topin J, Do M, Matsunami H, Golebiowski J. Mammalian class I odorant receptors exhibit a conserved vestibular-binding pocket. Cell Mol Life Sci. 2019;76:995–1004.
[33] Jabeen A, de March CA, Matsunami H, Ranganathan S. Machine Learning Assisted approach for finding novel high activity agonists of human ectopic olfactory receptors. Int J Mol Sci . 2021;22:11546.
[34] Caballero-Vidal G, Bouysset C, Grunig H, et al. Machine learning decodes chemical features to identify novel agonists of a moth odorant receptor. Sci Rep. 2020;10:1655.
[35] Corey EA, Zolotukhin S, Ache BW, Ukhanov K. Mixture interactions at mammalian olfactory receptors are dependent on the cellular environment. Sci Rep. 2021;11:9278.
[36] Michel MC, Charlton SJ. Biased agonism in drug discovery-is it too soon to choose a path? Mol Pharmacol. 2018;93:259–65.
[37] Kida H, Fukutani Y, Mainland JD, et al. Vapor detection and discrimination with a panel of odorant receptors. Nat Commun. 2018;9:4556.