Targeting highly expressed olfactory receptors to improve olfactory testing and training

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Dear Editor:

Olfactory testing and training constitute important foundations of diagnostic and therapeutic management of patients presenting with olfactory loss. The precise repertoire of human olfactory receptors (ORs) engaged by standard odorants in olfactory detection thresholds (n-butanol, phenylethyl alcohol or PEA), identification tests (using representative odor percepts), and training (e.g., PEA, citronellal, eucalyptol, eugenol) is surprisingly under-investigated, limiting our understanding of these methods. Notably, transcriptomic atlases of human ORs have been established by several research teams and have demonstrated a high degree of variability of the level of expressions among the 350 to 400 human ORs on an individual scale [(1-3)]. Indeed, only a subset of ORs are highly expressed, with the majority exhibiting low expression levels (Figure 1). However, most of these studies relied on a limited number of whole human olfactory mucosa samples (WHOMS), from donors with pathological conditions (such as ethmoidal adenocarcinoma) and with no information on their olfactory ability [(1,2)]. Therefore, these atlases might hinder the comparison of OR expression levels between individuals.
To date, only one study distinguished itself by including a larger number of WHOMS (from 26 adults, including 13 females and 13 males) with no history of olfactory loss or rhinological disease [(3)]. A key finding of this study is that these individuals shared a majority of highly expressed ORs, highlighting their potential functional significance in human olfaction [(3)]. Indeed, key food odorants are ecological relevant cues [(4)] and have been linked to highly expressed human ORs [(2)]. Moreover, animal studies have shown that the more widespread an OR is, the lower the detection threshold of its agonists might be [(5)].
Consequently, to address these gaps, our group started a research project named SMOTT (Single Molecule Olfactory Testing and Training) aiming at establishing a list of odorants that interact with highly expressed ORs (a group we referred to majORs in contrast to the low-expressed minORs), possibly improving olfactory assessment and rehabilitation. To give an analogy, it would be like testing a greater number of frequencies in an audiogram. Based on this specific atlas [(3)], we established a list of 80 majORs (those expressed above 80 copies / 20 ng RNA), accounting for approximately 80% of all ORs expressed in the human olfactory mucosa (Table S1) (Oral Communication, December Meeting on Smell and Taste, 6/7 December 2024, Basel). As one olfactory neuron expresses one type of OR, targeting those 80 majORs with specific odorant molecules might help to "screen" 80% of the olfactory mucosa surface. Then, based on a tested machine learning model [(6)] developed from the M2OR [(7,8)] database (which indexes all odorant-olfactory receptor interactions proved by in vivo, ex vivo and in vitro experiments), we performed an in silico study exploring the potential agonists of these majORs from a list of 5729 well-described odorants [(9)]. This resulted in a list of odorants ranked according to their ability to theoretically interact with many majORs, compared to those currently used for olfactory threshold testing and olfactory training (Table S2). It is noteworthy that PEA, for example, seems to activate only a few majORs, accounting for under 3% of the overall OR gene expression.

Conclusion

We hypothesize that 1) extending the spectrum of olfactory testing by targeting majORs might improve its accuracy and its correlation with different parameters (etiology, prognosis, olfactory-specific quality of life), 2) including these broad agonist odorants in an olfactory training protocol might improve patient outcomes by stimulating a greater number of remaining olfactory neurons, therefore increasing the olfactory signal that triggers both top-down and bottom-up mechanisms involved in olfactory training.

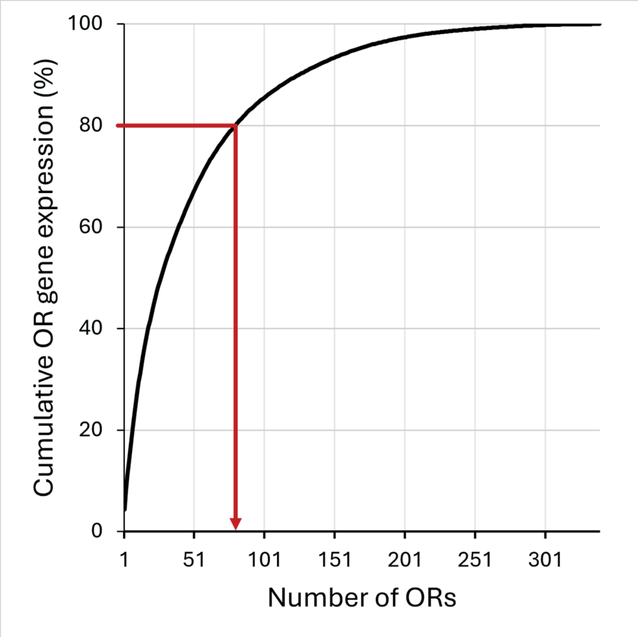

Figure 1. Cumulative expression rate of OR genes. The maximum corresponds to the total number of OR gene copies identified by transcriptomics in the olfactory mucosa according to the study by Verbeurgt et al. [3].

Abbreviations

OR: olfactory receptor; PEA: phenylethyl alcohol; WHOMS: whole human olfactory mucosa sample; SMOTT: single molecule olfactory testing and training; CID: Pubchem Compound Identifier; SMILES: Simplified Molecular Input Line Entry System.

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Authorship contribution

HB: study design, original draft writing, data analysis, final approval; JT: study design, editing, final approval; IPS: data analysis, editing and final approval; SB: study design, data acquisition, data analysis, editing and final approval.

Conflict of interest

None.

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SUPPLEMENTARY MATERIAL

Rank	majORs [1]	Average count of gene copies [2]	Relative gene expression (%) [3]	Cumulative gene expression (%)
1	OR7C1	1108	4,33	4,33
2	OR9G4	946	3,69	8,02
3	OR5A1	767	2,99	11,01
4	OR5P3	752	2,93	13,95
5	OR52N1	611	2,38	16,33
6	OR9A2	609	2,38	18,71
7	OR5K1	598	2,33	21,04
8	OR8D1	558	2,18	23,22
9	OR52H1	534	2,08	25,30
10	OR52B6	528	2,06	27,36
11	OR52I1	497	1,94	29,30
12	OR10G3	487	1,90	31,21
13	OR5P2	452	1,76	32,97
14	OR5A2	436	1,70	34,67
15	OR5AN1	377	1,47	36,14
16	OR2B11	365	1,42	37,57
17	OR4F16	361	1,41	38,98
18	OR11A1	324	1,26	40,24
19	OR2D2	322	1,26	41,50
20	OR52D1	317	1,24	42,73
21	OR13G1	312	1,22	43,95
22	OR56B1	294	1,15	45,10
23	OR10A6	286	1,12	46,22
24	OR51I2	286	1,12	47,34
25	OR52N4	261	1,02	48,35
26	OR2W3	255	1,00	49,35
27	OR2AE1	234	0,91	50,26
28	OR2A5	232	0,91	51,17
29	OR51I1	221	0,86	52,03
30	OR10AD1	221	0,86	52,90
31	OR2AT4	209	0,82	53,71
32	OR1K1	206	0,80	54,52
33	OR2S2	200	0,78	55,30
34	OR10H5	198	0,77	56,07
35	OR1Q1	192	0,75	56,82
36	OR4D9	191	0,74	57,56
37	OR5C1	188	0,73	58,29
38	OR9I1	187	0,73	59,02
39	OR4D1	182	0,71	59,74
40	OR5V1	182	0,71	60,45
41	OR51B4	179	0,70	61,14
42	OR51E1	170	0,66	61,81
43	OR10A4	168	0,65	62,46
44	OR52W1	167	0,65	63,11
45	OR2V2	166	0,65	63,76
46	OR1L6	165	0,64	64,40
47	OR51M1	162	0,63	65,03
48	OR6B1	160	0,63	65,66
49	OR4K13	158	0,62	66,28
50	OR2AG1	157	0,61	66,89
51	OR52N5	150	0,59	67,48
52	OR1L4	149	0,58	68,06
53	OR1D2	146	0,57	68,63
54	OR2AG2	143	0,56	69,19
55	OR6C4	141	0,55	69,74
56	OR1M1	138	0,54	70,28
57	OR1B1	135	0,53	70,80
58	OR6F1	134	0,52	71,33
59	OR13A1	127	0,50	71,82
60	OR7E24	125	0,49	72,31
61	OR10A5	122	0,48	72,79
62	OR6A2	119	0,46	73,25
63	OR2T12	117	0,46	73,71
64	OR2G6	114	0,44	74,15
65	OR2F1	111	0,44	74,59
66	OR8G1	108	0,42	75,01
67	OR10H1	107	0,42	75,43
68	OR7A5	105	0,41	75,84
69	OR51Q1	104	0,40	76,24
70	OR2A25	103	0,40	76,64
71	OR2T6	101	0,39	77,04
72	OR10K2	95	0,37	77,41
73	OR2T33	94	0,37	77,77
74	OR4K2	93	0,36	78,13
75	OR2M3	90	0,35	78,49
76	OR7A17	87	0,34	78,82
77	OR6N1	86	0,34	79,16
78	OR51B2	84	0,33	79,49
79	OR13J1	84	0,33	79,81
80	OR2J3	82	0,32	80,13

Table S1. List of the 80 majORs according to the study by Verbeurgt et al. [(3)].

[1] major Olfactory Receptors; [2] according to Verbeurgt et al. [(3)]; [3] relative expression to the total OR genes. Total gene copies in WHOM is 25619.

	Molecule	CID	SMILES	% of activated majORs according to the machine learning model [6]	% of activated ORs (majORs + minORs) according to the machine learning model [6]	Predicted majORs
Odorants used in current olfactory testing and training	Phenyl ethyl alcohol	6054	C1=CC=C(C=C1)CCO	2.67	3,26	OR10A6, OR52D1, OR2J3
	Eucalyptol	2758	CC1(C2CCC(O1)(CC2)C)C	1.02	1,09	OR51B4, OR2J3
	Citronellal	7794	CC(CCC=C(C)C)CC=O	0.9	1,3	OR52N5, OR2J3
	Eugenol	3314	COC1=C(C=CC(=C1)CC=C)O	7.39	8,16	OR5P3, OR6F1, OR10G3, OR51B4, OR2J3, OR52N4
	n-Butanol	263	CCCCO	0.98	1,19	OR51E1, OR2J3
Examples of broad agonists for future Single Molecule Olfactory Testing and Training (SMOTT)*	Benzothiazole	7222	c1ccc2scnc2c1	79.27	98,80	OR7A5, OR7A17, OR5P2, OR5P3, OR10A5, OR6A2, OR7C1, OR2V2, OR9A2, OR1Q1, OR2D2, OR52N1, OR5A2, OR4D1, OR8D1, OR8G1, OR10AD1, OR1M1, OR10A6, OR10H5, OR13J1, OR1B1, OR5A1, OR5AN1, OR2A25, OR2B11, OR2M3, OR2T12, OR2T33, OR4D9, OR4K13, OR52W1, OR6N1, OR52I1, OR2AG2, OR1L4, OR2AE1, OR6F1, OR52H1, OR9I1, OR4F16, OR9G4, OR6B1, OR2AT4, OR10G3, OR2T6, OR5C1, OR13G1, OR6C4, OR4K2, OR2A5, OR1K1, OR2G6, OR2W3, OR2F1, OR52B6, OR13A1, OR10H1, OR5V1, OR51B2, OR51B4, OR2S2, OR51E1, OR1L6, OR51M1, OR52N5, OR51Q1, OR51I2, OR52D1, OR51I1, OR2AG1, OR10A4, OR1D2, OR56B1, OR2J3, OR52N4, OR11A1, OR5K1
	Galaxolide	91497	C[C@H]1C(C)(C)c2cc3c(cc2C1(C)C)[C@H](C)COC3.C[C@H]1C(C)(C)c2cc3c(cc2C1(C)C)[C@@H](C)COC3.C[C@@H]1C(C)(C)c2cc3c(cc2C1(C)C)[C@H](C)COC3.C[C@@H]1C(C)(C)c2cc3c(cc2C1(C)C)[C@@H](C)COC3	75.42	94,75	OR7A5, OR7A17, OR5P2, OR5P3, OR10A5, OR6A2, OR7C1, OR2V2, OR9A2, OR1Q1, OR2D2, OR52N1, OR5A2, OR4D1, OR8D1, OR8G1, OR10AD1, OR1M1, OR10A6, OR10H5, OR1B1, OR5A1, OR5AN1, OR2A25, OR2B11, OR2M3, OR2T12, OR4D9, OR4K13, OR6N1, OR52I1, OR2AG2, OR1L4, OR2AE1, OR6F1, OR52H1, OR9I1, OR4F16, OR9G4, OR6B1, OR2AT4, OR10G3, OR2T6, OR5C1, OR13G1, OR6C4, OR4K2, OR2A5, OR1K1, OR2G6, OR2W3, OR2F1, OR7E24, OR52B6, OR13A1, OR10H1, OR5V1, OR51B4, OR2S2, OR51E1, OR1L6, OR51M1, OR52N5, OR51Q1, OR51I1, OR2AG1, OR10A4, OR1D2, OR56B1, OR52N4, OR11A1, OR5K1
	2H-Pyran-2-one	68154	O=c1cccco1	44.12	53,77	OR7A17, OR5P2, OR5P3, OR10A5, OR6A2, OR2D2, OR52N1, OR5A2, OR4D1, OR8D1, OR10AD1, OR10A6, OR5AN1, OR2B11, OR2M3, OR4D9, OR2AG2, OR1L4, OR2AE1, OR9I1, OR4F16, OR9G4, OR6B1, OR10G3, OR6C4, OR4K2, OR2A5, OR2G6, OR13A1, OR5V1, OR2S2, OR1L6, OR51M1, OR52D1, OR2AG1, OR10A4, OR1D2, OR2J3, OR52N4, OR11A1, OR5K1
	Isopropyl crotonate	5354359	C/C=C/C(=O)OC(C)C	37.71	43,19	OR5P3, OR10A5, OR6A2, OR2V2, OR9A2, OR52N1, OR5A2, OR8G1, OR10AD1, OR10H5, OR5A1, OR2A25, OR2B11, OR2T12, OR2T33, OR52W1, OR52I1, OR2AG2, OR2AE1, OR52H1, OR4F16, OR6B1, OR13G1, OR2G6, OR2W3, OR2F1, OR10H1, OR51B4, OR51E1, OR52D1, OR51I1, OR2AG1, OR10A4, OR2J3, OR52N4, OR11A1
	2-Indanone	11983	O=C1Cc2ccccc2C1	33.11	40,05	OR5P3, OR10A5, OR2V2, OR9A2, OR2D2, OR5A2, OR8D1, OR8G1, OR10A6, OR1B1, OR2A25, OR2T12, OR2T33, OR4K13, OR6N1, OR52I1, OR2AG2, OR6F1, OR4F16, OR9G4, OR10G3, OR2T6, OR13G1, OR4K2, OR1L6, OR51M1, OR52N5, OR52D1, OR2AG1, OR2J3, OR11A1

	Molecule	CID	SMILES	% of activated majORs according to the machine learning model [6]	% of activated ORs (majORs + minORs) according to the machine learning model [6]	Predicted majORs
Combination of odorants	2H-Pyran-2-one Isopropyl crotonate 2-Indanone	68154 5354359 11983	O=c1cccco1.C/C=C/C(=O)OC(C)C.O=C1Cc2ccccc2C1	65.17	78,27	OR9G4, OR5A1, OR5P3, OR52N1, OR9A2, OR5K1, OR8D1, OR52H1, OR52I1, OR10G3, OR5P2, OR5A2, OR5AN1, OR2B11, OR4F16, OR11A1, OR2D2, OR52D1, OR13G1, OR10A6, OR52N4, OR2W3, OR2AE1, OR2A5, OR51I1, OR10AD1, OR2S2, OR10H5, OR4D9, OR9I1, OR4D1, OR5V1, OR51B4, OR51E1, OR10A4, OR52W1, OR2V2, OR1L6, OR51M1, OR6B1, OR4K13, OR2AG1, OR52N5, OR1L4, OR1D2, OR2AG2, OR6C4, OR1B1, OR6F1, OR13A1, OR10A5, OR6A2, OR2T12, OR2G6, OR2F1, OR8G1, OR10H1, OR2A25, OR2T6, OR2T33, OR4K2, OR2M3, OR6N1, OR2J3

Table S2. Examples of odorants used in current olfactory threshold testing and olfactory training and examples of broad agonists of majORs including a combination of three odorants that covers a large receptor space. Agonists predominantly activating trigeminal receptors have been excluded on the basis of scientific literature and/or usual sensory description.