Cellular/Molecular

A Large-Scale Analysis of Odor Coding in the Olfactory Epithelium

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Mammals can perceive and discriminate myriad volatile chemicals as having a distinct odor. Odorants are initially detected by odorant receptors (ORs) on olfactory sensory neurons (OSNs) in the nose. In the mouse, each OSN expresses one of ~1000 different OR genes. Although OSNs and their expressed ORs constitute the fundamental units of sensory input to the brain, a comprehensive understanding of how they encode odor identities is still lacking. To gain a broader and more detailed understanding of odorant recognition and odor coding at this level, we tested the responses of 3000 mouse OSNs to 125 odorants with diverse structures and perceived odors. These studies revealed extraordinary diversity, but also bias, in odorant recognition by the OSN, and thus OR, repertoire. They indicate that most OSNs are narrowly tuned to detect a subset of odorants with related structures and often related odors, but that the repertoire also includes broadly tuned components. Strikingly, the vast majority of odorants activated a unique set of OSNs, usually two or more in combination. The resulting combinatorial codes varied in size among odorants and sometimes contained both narrowly and broadly tuned components. While many OSNs recognized multiple odorants, some appeared specific for a given pheromone or other animal-associated compound, or for one or more odorants with a particular odor quality, raising the possibility that signals derived from some OSNs and ORs might elicit an innate behavior or convey a specific odor quality.

Introduction

The first step in odor perception is the detection of odorants by odorant receptors (ORs) on olfactory sensory neurons (OSNs) in the nasal olfactory epithelium (OE) (Buck and Axel, 1991; Buck, 2000). Each mouse OSN expresses 1 of ~1035 intact OR genes and thus transmits information to the brain derived from a single type of OR (Malnic et al., 1999; Niimura and Nei, 2005). While OSNs with the same OR are scattered in the OE, they all synapse in a few specific glomeruli in the olfactory bulb, thereby maintaining the segregation of inputs from different ORs (Ressler et al., 1993, 1994; Vassar et al., 1993, 1994; Mombaerts et al., 1996; Mombaerts, 2004), but how OR inputs are next organized in the olfactory cortex remains unknown (Haberly, 1998, 2001; Miyamichi et al., 2011).

OSNs expressing different ORs constitute the elementary units of olfactory sensory input to the brain in that each OSN synapses in the olfactory bulb. Knowledge of what OSNs recognize and how they are used to encode odor identities is therefore crucial to understanding how sensory inputs are processed in the brain to ultimately yield odor perceptions. Important insights into this question have come from studies of OSN and OR responses to varied odorants (Sato et al., 1994; Duchamp-Viret et al., 1999; Malnic et al., 1999; Araneda et al., 2004; Saito et al., 2009). However, due to the relatively small number of different OSN/OR–odorant combinations tested, a broad understanding of odor coding by the OSN/OR repertoire is still lacking.

Previous studies indicate that the OR family is used in a combinatorial manner to encode odor identities, with different odorants detected, and thereby encoded, by different combinations of ORs (Malnic et al., 1999; Kajiya et al., 2001). However, numerous important questions remain unanswered. These include the number of different ORs used to encode the identity of an odorant, whether that number differs among odorants, and whether the combinatorial receptor code of an odorant is composed of ORs that are "narrowly tuned" to a few odorants or "broadly tuned" to recognize many odorants. And, perhaps most perplexing, given that ORs are used in a combinatorial fashion, what determines the perceived odor of an odorant, whether it is perceived, for example, as minty instead of fishy?

To explore these questions, we conducted a large-scale analysis of OSN responses to a multitude of odorants with diverse structures and perceived odors. Since each OSN expresses only one OR gene, this approach allowed analysis of the OR as well as OSN repertoire. These studies indicate that the repertoire is extraordinarily diverse but also biased in its recognition properties. They further indicate that most OSNs/ORs are narrowly tuned but that the repertoire also contains broadly tuned components. Remarkably, the vast majority of odorants were recognized by a unique set of OSNs. Many OSNs responded only to odorants

Received March 13, 2011; revised April 22, 2011; accepted May 1, 2011.

Author contributions: K.N. and L.B.B. designed research; K.N. and L.R.S. performed research; K.N., L.R.S., X.Y., and L.B.B. analyzed data; K.N., L.R.S., and L.B.B. wrote the paper.

This work was supported by the Howard Hughes Medical Institute (L.B.B.); the Mitsubishi Kagaku Institute of Life Sciences, Japan (K.N.); and a grant from the National Institutes of Health (National Institute on Deafness and Other Communication Disorders) (L.B.B.). We thank Bettina Malnic for the Ric8b plasmid and Rich Boden and International Flavors and Fragrances for odorants. We are grateful to Peter Mombaerts for critical comments on this manuscript and to members of the Buck Laboratory for helpful comments and discussions.

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DOI:10.1523/JNEUROSCI.1282-11.2011

 0270-6474/11/319179-13$15.00/0

with a shared odor quality. Moreover, some recognized only a single odorant, pheromone, or other animalic odorant, raising the possibility that some OSNs/ORs might convey a specific odor quality or elicit an innate behavior.

Materials and Methods

OSN dissociation and calcium imaging

All animal procedures conformed to the Fred Hutchinson Cancer Research Center guidelines for the care and use of animals. OE tissue dissected from 6- to 12-week-old female C57BL/6J mice was rinsed in HBSS (Invitrogen), oxygenized for 20 min, and then incubated in 3 ml of prewarmed trypsin (0.01% in HBSS; Sigma-Aldrich) for 2 min at 37°C in a 3.5 mm plastic dish, after which 3 ml of 0.05% trypsin inhibitor (Sigma-Aldrich) containing 300 U of DNase I (Sigma-Aldrich) was added and the tissue incubated for 1 min at room temperature. After addition of 2 ml of DMEM (Invitrogen) containing 1% bovine serum albumin (Sigma-Aldrich; RIA grade), the tissue was minced with scissors, sieved with a 40 μm nylon mesh cell strainer (BD Biosciences), and then incubated with 8 μM fura-2 AM (Invitrogen) plus 100 μg/ml pluronic acid F-127 (Invitrogen) for 30 min at room temperature in the dark. After centrifugation for 5 min at 184 $\times$ *g*, cells were suspended in 120–160 μl of DMEM, plated on coverslips coated with 2.5 μl/coverslip of 1 mg/ml poly-D-lysine (Sigma-Aldrich), and allowed to settle for 40 min at room temperature in the dark.

Calcium imaging of the dissociated OSNs was done using a perfusion chamber mounted on an inverted microscope (Olympus; IX70) using a 10×/0.3 NA objective (Olympus; UplanFI) (Sato et al., 1994; Malnic et al., 1999). Fluorescence emission was determined every 4 s using a CCD camera (Hamamatsu; C4742-95-10NR) and a standard filter set [high Q filter set (R.P.I.): 470/40 nm excitation filter; 495 nm low-pass filter dichroic mirror; 525/50 nm emissions filter]. The cells were perfused with oxygenated HBSS solution (0.4 ml/min) in the perfusion chamber (Warner Instruments; RC-22) using a peristaltic pump. The data collection software used was MetaFluor (Molecular Devices).

Stimulation with odorants

Odorants were freshly diluted in HBSS using 100 mM stocks in DMSO. Thirteen mixtures (50 μM each chemical) were applied to cells sequentially (4 s/each mixture). Cells were then tested with single odorants from mixtures that had elicited a response. A positive response was initially determined by the timing of the response, the strength of the response (more than twofold higher than the noise amplitude of the baseline), and the shape of the response curve (sudden drop in fluorescence emission with gradual recovery). The typical shape of response curves was established by observing responses to repeated stimulation with 1 mM nonanoic acid. After testing with single odorants at 50 μM, stimulating odorants were often retested at lower concentrations (0.5, 5 μM). Finally, cells were exposed to KCl (87.4 mM) to assess their viability.

Odorants

Odorants of the highest purity available were purchased from Sigma-Aldrich, except where indicated by parentheses. Odorants from International Flavors and Fragrances (IFF) were obtained as a gift. Odorants contained in each mixture are shown below.

First odorant library: 125 odorants in 13 mixtures

The first odorant library included 125 odorants in 13 mixtures (used for all experiments except those that tested OSNs with 176 odorants and the responses of OSN446 and OSN454 in Fig. 5).

Mixture 1 (amines). Mixture 1 was as follows: 1-1, hexylamine; 1-2, heptylamine; 1-3, benzylamine; 1-4, bornylamine; 1-5, cyclohexylamine; 1-6, methylamine; 1-7, *n*-butylamine; 1-8, dimethylamine; 1-9, diisopropylamine; 1-10, dibutylamine; 1-11, trimethylamine; 1-12, cadaverine; 1-13, isobutylamine.

Mixture 2 (thiols). Mixture 2 was as follows: 2-1, 1-hexanethiol; 2-2, 1-heptanethiol; 2-3, benzyl mercaptan; 2-4, *p*-thiocresol; 2-5, *t*-butyl mercaptan; 2-6, *n*-butyl mercaptan; 2-7, 3-methyl-1-butanethiol.

Mixture 3 (alcohols). Mixture 3 was as follows: 3-1, 1-hexanol; 3-2, 1-heptanol; 3-3, *t*-butyl alcohol; 3-4, methanol; 3-5, *n*-butanol; 3-6, benzyl alcohol; 3-7, *p*-cresol; 3-8, alcohol C11 undecylenic; 3-9, tetrahydrolinalool; 3-10, Pamplefleur (IFF); 3-11, oxyphenylon (IFF).

Mixture 4 (esters). Mixture 4 was as follows: 4-1, phenyl ethyl isobutyrate; 4-2, terpinyl acetate; 4-3, ethyl benzoate; 4-4, benzyl acetate; 4-5, Fraistone (IFF); 4-6, Fructone (IFF); 4-7, methyl anthranilate; 4-8, phenoxy ethyl propionate; 4-9, Verdox (IFF); 4-10, ethyl acetate; 4-11, butyl acetate; 4-12, ethyl butyrate; 4-13, butyl butyrate; 4-14, *n*-pentyl acetate; 4-15, dihydromyrcenyl acetate (IFF); 4-16, Vanoris (IFF).

Mixture 5 (ethers). Mixture 5 was as follows: 5-1, 4-methylanisole; 5-2, Phenafleur (IFF); 5-3, butyl ethyl ether.

Mixture 6 (aldehydes). Mixture 6 was as follows: 6-1, isobutylaldehyde; 6-2, myrtenal; 6-3, octanal; 6-4, decanal; 6-5, undecanal; 6-6, 2-methylundecanal; 6-7, amyl cinnamic aldehyde; 6-8, hexanal; 6-9, *trans*-cinnamaldehyde; 6-10, Bergamal (IFF); 6-11, bourgeonal (IFF); 6-12, Helional (IFF); 6-13, 2-methyldecanal (IFF).

Mixture 7 (cyclic alkanes). Mixture 7 was as follows: 7-1, cyclohexanol; 7-2, Apopatchone (IFF); 7-3, cyclohexyl ethanol; 7-4, Coniferan (IFF); 7-5, Isocyclemone E (IFF); 7-6, patchone; 7-7, Verdone (IFF); 7-8, Cedramber (IFF); 7-9, cedrol; 7-10, Cyclacet (IFF); 7-11, Piconia (IFF); 7-12, Ambroxan (IFF); 7-13, Patchomint (IFF); 7-14, Talia (IFF).

Mixture 8 (terpenes). Mixture 8 was as follows: 8-1, L-carvone; 8-2, L-menthol; 8-3, pulegone; 8-4, L-carveol; 8-5, L-menthone; 8-6, L-menthyl acetate; 8-7, D-carvone.

Mixture 9 (vanillin-like). Mixture 9 was as follows: 9-1, aubepine; 9-2, eugenol; 9-3, heliotropine; 9-4, isoeugenol; 9-5, methyl salicylate; 9-6, palatone; 9-7, vanillin; 9-8, veramoss; 9-9, Vaniwhite (IFF).

Mixture 10 (camphors). Mixture 10 was as follows: 10-1, eucalyptol; 10-2, camphor; 10-3, isoborneol; 10-4, borneol; 10-5, L-fenchone.

Mixture 11 (azines). Mixture 11 was as follows: 11-1, indole; 11-2, skatole; 11-3, pyridine; 11-4, pyrazine; 11-5, 2-isobutyl-3-methoxypyrazine; 11-6, 2,5-dimethylpyrazine.

Mixture 12 (musks). Mixture 12 was as follows: 12-1, ω-pentadecalactone; 12-2, hexadecanolide; 12-3, musk ketone; 12-4, musk tibeten; 12-5, musk ambrette; 12-6, Ambrettolide (IFF); 12-7, Civettone (IFF); 12-8, Muscone (IFF).

Mixture 13 (ketones/others). Mixture 13 was as follows: 13-1, androstenone; 13-2, butyrophenone; 13-3, α-ionone; 13-4, ethyl butyl ketone (3-heptanone); 13-5, 2-heptanone; 13-6, isovaleric acid; 13-7, durene; 13-8, D-limonene; 13-9, 2-isobutylthiazole; 13-10, 2-*s*-butyl-4,5-dihydrothiazole; 13-11, *E*,*E*-α-farnesene (Bedoukian); 13-12, 2,5-dihydro-2,4,5-trimethylthiazole (PheroTech); 13-13, *E*-β-farnesene (Bedoukian).

Second odorant library: 176 odorants in 18 mixtures

The second odorant library included 176 odorants in 18 mixtures (not used for most experiments).

Mixture 1 (amines). Mixture 1 was the same as in the first library.

Mixture 2 (thols). Mixture 2 was the same as in the first library.

Mixture 3 (alcohols). Mixture 3 was the same as in the first library.

Mixture 4 (esters). Mixture 4 was the same as in the first library except 4-15 and 4-16, which were moved to mixture 17.

Mixture 5 (ethers and lactones). Mixture 5 was as follows: 5-1 through 5-3; 5-4, (−)-Ambroxide; 5-5, Safrole; 5-6, β-naphthyl isobutyl ether; 5-7, β-naphthol ethylether; 5-8, δ-octalactone (Lancaster Synthesis); 5-9, γ-undecalactone; 5-10, Toncarine (6-methylcoumarin).

Mixture 6 (aldehydes). Mixture 6 was the same as in the first library.

Mixture 7 (cyclic alkanes). Mixture 7 was the same as in the first library.

Mixture 8 (terpenes). Mixture 8 was the same as in the first library.

Mixture 9 (vanillin-like). Mixture 9 was the same as in the first library.

Mixture 10 (camphors). Mixture 10 was the same as in the first library.

Mixture 11 (azines). Mixture 11 was as follows: 11-1 through 11-6 from first library plus the following: 11-7, methylpyrrole; 11-8, 2-acetyl pyridine; 11-9, isoquinoline.

Mixture 12 (musks). Mixture 12 was the same as in the first library.

No mixture 13. Odorants from mixture 13 in the first library were separated into mixtures 16, 18, and 19 in this second library.

Mixture 14 (alcohols). Mixture 14 was as follows: 14-1, isoamylalcohol; 14-2, 2-hexanol; 14-3, 3-hexanol; 14-4, 2-heptanol; 14-5, 3-heptanol; 14-6, 4-heptanol; 14-7, 2-ethyl-1-hexanol; 14-8, geraniol.

Mixture 15 (alcohols). Mixture 15 was as follows: 15-1, raspberry ketone; 15-2, 2-phenylethanol; 15-3, furfuryl alcohol; 15-4, zigerone; 15-5, benzhydrol; 15-6, thymol; 15-7, bacdanol.

Mixture 16 (carboxylic acids). Mixture 16 was as follows: 13-6 from the first library plus the following: 16-1, butanoic acid; 16-2, isobutyric acid; 16-3, hexanoic acid; 16-4, heptanoic acid; 16-5, octanoic acid; 16-6, nonanoic acid; 16-7, adipic acid; 16-8, pimeric acid; 16-9, benzoic acid; 16-10, *p*-toluic acid; 16-11, tiglic acid.

Mixture 17 (esters). Mixture 17 was as follows: 4-15 and 4-16 from the first library plus the following: 17-1, isoamylacetate; 17-2, isopropyl hexanoate (TCI America); 17-3, butyl hexanoate; 17-4, diethyl succinate; 17-5, hexyl-2-furoate; 17-6, methyl cinnamate; 17-7, benzyl propionate; 17-8, Labdanol (isobutyl cinnamate); 17-9, isobornyl acetate.

Mixture 18 (ketones). Mixture 18 was as follows: 13-1 through 13-5 from the first library plus the following: 18-1, irone; 18-2, benzyl acetone; 18-3, cisjasmone.

Mixture 19 (others). Mixture 19 was as follows: 13-7 through 13-13 from the first library plus the following: 19-1, benzyl cyanide; 19-2, mesitylene; 19-3, stilbene.

Analysis of calcium imaging data

A total of 308 cells responded to at least one odorant mixture and were subsequently tested with KCl. Of those, 60 cells did not respond to KCl and another 31 cells were excluded from further analysis for other reasons (e.g., detachment, cell death, unstable fluorescence intensity, small mixture response relative to KCl response). Calcium imaging data (fluorescence intensity vs time in seconds) for individual cells were graphed using Excel software (Microsoft). Responses were analyzed by the fractional change in fluorescence intensity: $\Delta F/F_o$ or $(F - F_o)/F_o$, where F is the fluorescent light intensity at each point and F_o is the value of emitted fluorescent light before the stimulus application (baseline). The criterion used for a positive response was $\Delta F/F_o \geq 1\%$. Among 3000 KCl+ cells, 217 cells showed a robust response to one or more mixtures and were further analyzed in these studies. In a control experiment, 12 KCl-responsive cells were subjected to single-cell RT-PCR and a Southern blot of the amplified cDNAs hybridized to an OMP (olfactory marker protein) probe (Malnic et al., 1999). The probe hybridized to cDNA from 11 of 12 of the cells, indicating that the vast majority of KCl+ cells analyzed in these studies, if not all, were OSNs.

Functional analysis of Olfr42

The OR expressed in OSN226 was determined using single-cell RT-PCR (Malnic et al., 1999). The full-length coding sequence of Olfr42 was obtained from www.ncbi.nlm.nih.gov and used to amplify the sequence from C57BL/6J mouse genomic DNA using Pfu Ultra enzyme (Stratagene). The sequence was then cloned into the pCI expression plasmid (Promega) carrying the first 60 nt of bovine rhodopsin (Liberles and Buck, 2006), and DNA sequencing was used to verify the accuracy of the cloned sequence.

Functional analysis of Olfr42 was conducted in HEK293T cells grown in 96-well plates using methods previously described (Liberles and Buck, 2006) with the following modifications. Each well contained ~50,000 HEK293T cells (ATCC) cotransfected [using Lipofectamine and Plus Reagent (Invitrogen)] with 20 ng each of the OR plasmid, a Ric8b expression plasmid obtained from B. Malnic (University of São Paulo, São Paulo, Brazil) (Von Dannecker et al., 2006), a RTP1s expression plasmid containing the short version of RTP1 cloned from mouse OE cDNA (Saito et al., 2004; Zhuang and Matsunami, 2007), and the cAMP response element-secreted alkaline phosphatase (CRE-SEAP) reporter plasmid (BD Biosciences). Cells were incubated for 24 h at 37°C in serum-free media with or without test compounds, and then for 2 h at 65–70°C. An aliquot of supernatant from each well was then incubated (10–20 min, room temperature) with an equal volume of 1.2 mM 4-methylumbelliferyl phosphate (Sigma-Aldrich) in 2 M diethanolamine bicarbonate, pH 10.0, and fluorescence was measured with a CytoFluor 4000 plate reader (Applied Biosystems).

Results

A large-scale analysis of odor detection in the olfactory epithelium

To obtain a more comprehensive understanding of odor coding in the OE, we sought to analyze the responses of thousands of individual mouse OSNs to a large number and variety of odorants with diverse structures and perceived odors in humans. Since each OSN expresses only one OR gene and each OR gene is expressed, on average, in ~1/1000 OSNs, we reasoned that such an analysis could provide a broad view of odorant recognition not only by the OSN repertoire but also by the mouse OR family.

We first selected 125 odorants with diverse structures and perceived odors (in humans) and grouped them into 13 odorant mixtures according to structural features (Fig. 1). In some cases, these structural features correlate, at least to some extent, with perceived odors in humans: (1) amines (fishy, ammonia); (2) thiols (sulfurous); (3) alcohols (floral, fruity); (4) esters (fruity, floral); (5) ethers (floral); (6) aldehydes (aldehydic, citrusy); (7) cyclic alkanes (woody); (8) terpenes (green, minty); (9) vanillin-like (sweet); (10) camphors (camphor); (11) azines (pungent, animalic); (12) musks (musky); and (13) ketones/others (varied). Also included in the mixtures were a fox predator odor (13-12) (Day et al., 2004) and five mouse pheromones (Leinders-Zufall et al., 2000), one present in mixture 11 and the remainder in mixture 13.

To analyze the responses of OSNs to the odorants, we used calcium imaging (Malnic et al., 1999). Mouse OE cells were dissociated, loaded with the calcium indicator, fura-2, and then plated on glass coverslips. Individual OSNs were monitored for increases in intracellular calcium during sequential perfusion with the 13 odorant mixtures (containing 50 μM of each odorant) and then, in most cases, with single odorants (at 50 μM) from mixtures that had elicited a response. Many OSNs were subsequently tested with lower concentrations of stimulatory odorants (5 and/or 0.5 μM). Finally, cells were assessed for viability by exposure to 87.4 mM KCl, which induces calcium influx in living OSNs. Because of their limited survival time after isolation, OSNs that had responded to multiple mixtures were usually tested with single odorants from only some mixtures. Only OSNs that had responded to KCl ("KCl+ OSNs") were included in data analyses.

We tested 3000 KCl+ OSNs with the 13 odorant mixtures, a total of 39,000 potential OSN–mixture pairings and 375,000 potential OSN–odorant pairings. Of OSNs tested with elevated KCl, 308 responded to at least one mixture, but 60 were KCl− and another 31 OSNs were excluded from further analysis for other reasons (see Materials and Methods). Of the 3000 KCl+ OSNs, 217 (7.2%) responded to one or more mixtures and were suitable for analysis (Fig. 2). Of the 217 OSNs analyzed, 197 were subsequently tested with single odorants from activating mixtures and 169 of those responded to at least one odorant. In some cases, but not others, an odorant also stimulated an OSN at a lower concentration (5 and/or 0.5 μM), consistent with previous studies (Sato et al., 1994; Malnic et al., 1999; Bozza et al., 2002). Some OSNs failed to respond to single odorants from one or more stimulating mixtures. One possible explanation for this is that some mixture responses resulted from a summation of responses to multiple mixture components that alone did not stimulate a response at the concentration tested.

When 263 additional KCl+ OSNs were later tested with 18 mixtures containing 176 odorants, the percentage of mixture responsive OSNs increased to 13.2% (data not shown). This in-

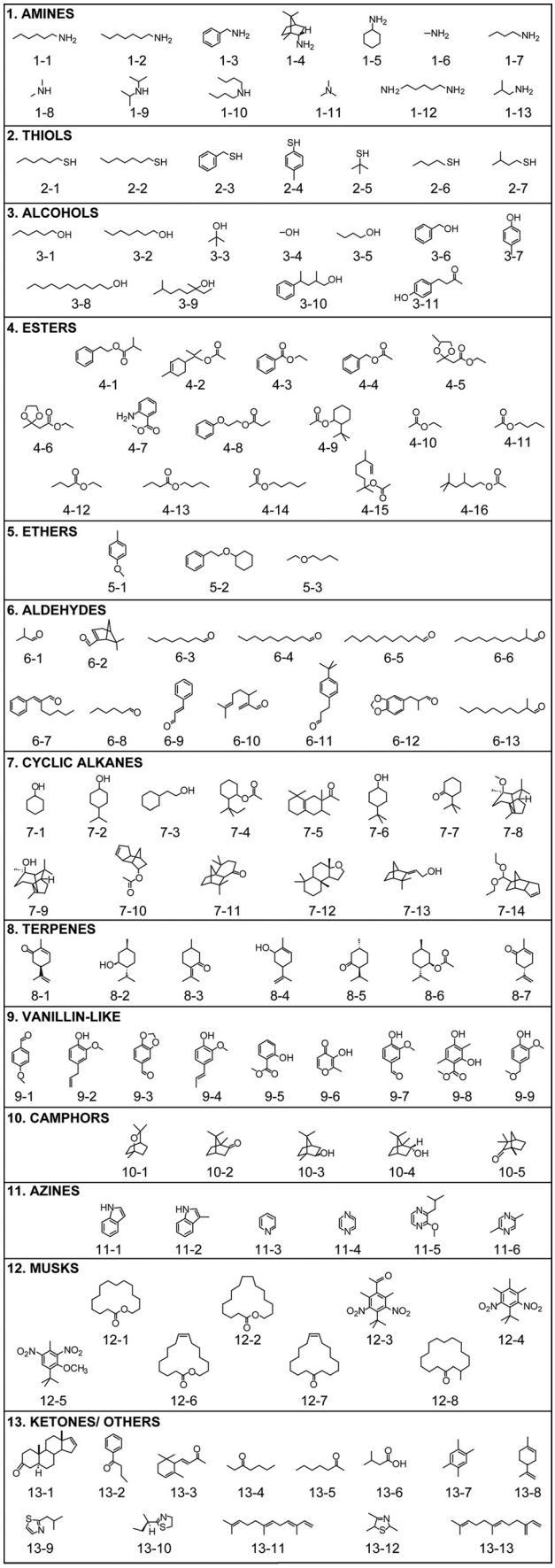

Figure 1. Odorants. A total of 125 odorants with varied structures and perceived odors in humans were grouped into 13 mixtures on the basis of structural features.

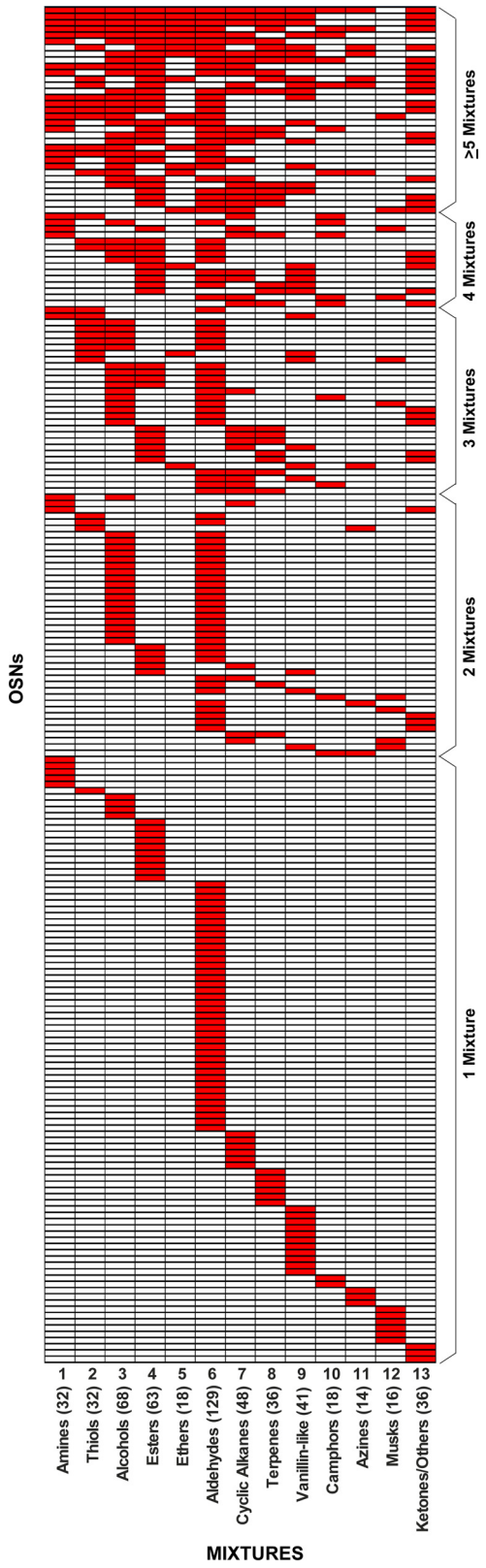

Figure 2. Responses of individual OSNs to odorant mixtures. This diagram shows the responses of 217 OSNs (rows) to the 13 odorant mixtures (columns). The red boxes indicate mixtures to which the neurons responded with an increase in intracellular calcium, as measured using calcium imaging. The mixtures contained 50 μM of each component odorant. The mixtures are indicated below with the number of OSNs activated by each mixture shown in parentheses. Each mixture stimulated a subset of OSNs, but OSNs varied in the number and combination of mixtures to which they responded.

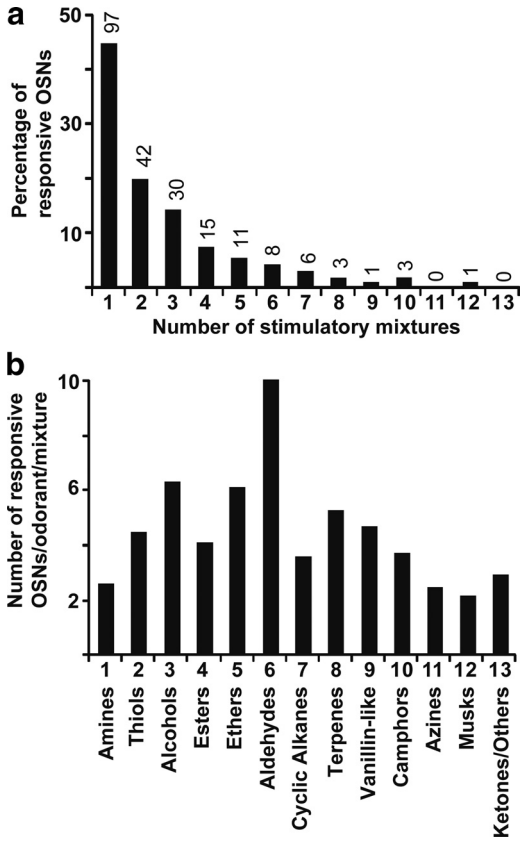

Figure 3. Quantitation of OSN responses to odorant mixtures. ***a***, Individual OSNs responded to 1–12 mixtures, but most responded to only 1 or a few mixtures. The number of neurons that responded to the indicated number of mixtures is shown above each bar. ***b***, Taking into account the number of odorants/mixture, the number of OSNs that responded to different mixtures varied, with the aldehyde mixture stimulating the most OSNs and the musk mixture stimulating the least.

crease in the percentage of responsive OSNs suggested that a large proportion of the KCl+ OSNs analyzed by these methods were capable of responding if tested with an appropriate odorant, although the exact proportion cannot be ascertained. Since each OR gene is expressed in roughly 1/1000 OSNs, it is likely that these studies queried a large proportion of the OR repertoire.

The repertoire is biased

These studies revealed several striking features of the mouse OSN repertoire. First, the repertoire is biased. Although each odorant mixture stimulated a subset of OSNs, some mixtures activated far more OSNs than others (Figs. 2, 3). Remarkably, 59% of the 217 OSNs activated by mixtures responded to the aldehyde mixture, whereas, in contrast, only 15% of OSNs responded to the amine mixture. Taking into account the number of odorants per mixture, the aldehyde mixture activated an average of 9.9 OSNs/odorant, whereas, at the other extreme, the musk mixture activated only one-fifth as many (2.0 OSNs/odorant) and the azine and amine mixtures only stimulated 2.3 and 2.5 OSNs/odorant, respectively (Fig. 3). Alcohols and terpenes activated intermediate numbers of OSNs (6.2 and 5.1 OSNs/odorant, respectively).

Bias at the level of single odorants was also evident but was most striking among the 81 OSNs tested with single aldehydes (Fig. 4). Echoing a report that octanal stimulates a high percentage of rat OSNs (Araneda et al., 2004), 33 of the 81 OSNs (40.7%) responded to octanal (6-3) and 30 (37%) responded to decanal (6-4), whereas other aldehydes stimulated 2–21 OSNs each. Bias was also seen among odorants from some other mixtures, although it was less extreme than that observed for aldehydes. For example, individual alcohols stimulated 0–11 of 34 OSNs tested and different esters activated 0–7 of 26 tested OSNs (Fig. 4).

The observed biases in OSN responses to different odorants could reflect bias in either the number of ORs that recognize different odorants or bias in the proportion of OSNs that express different ORs. To explore the source of the observed bias to octanal and decanal, we compared the response profiles of OSNs activated by these odorants in terms of their responses to, first, single aldehydes and, second, different mixtures. The 33 OSNs activated by octanal showed 28 different mixture/aldehyde response profiles and the 30 OSNs activated by decanal showed 26 different profiles (Fig. 4). Possibly because of variations in the level of expression of a given OR among OSNs, some OSNs expressing a particular OR may respond to two odorants at different thresholds, whereas others respond only to the lower threshold odorant (Bozza et al., 2002). Thus, OSNs with the same OR can show related, but different response profiles. For this reason, the actual number of ORs involved in the responses to octanal or decanal is unknown.

Nevertheless, the larger number of different response profiles seen for these two odorants than for other aldehydes indicates that there are likely to be more ORs that recognize octanal or decanal than other odorants, a conclusion similar to that reached for octanal in rat (Araneda et al., 2004). These results support the idea that there is bias not only in the OSN repertoire, but also the OR repertoire, and that there are likely to be many more ORs that recognize some odorants than others.

The repertoire exhibits extreme diversity

One of the most striking features of the OSN responses seen in these experiments was their extreme diversity. This diversity was apparent, first, in the responses of OSNs to the 13 odorant mixtures. The 217 mixture-responsive OSNs showed 93 different response profiles composed of responses to single mixtures or combinations of mixtures (Fig. 2).

OSN responses to individual odorants were also extremely diverse. For example, 81 OSNs tested with single aldehydes showed 36 different response profiles composed of responses to single aldehydes or combinations of different aldehydes (Fig. 4). Similarly, 34 OSNs tested with single alcohols showed 15 response profiles and 6 OSNs tested with different musk odorants showed 5 different profiles (Fig. 4).

The diversity of OSN responses seen in these experiments is consistent with the large size of the mouse OR repertoire and the extensive diversity seen in OR protein sequences (Zhang and Firestein, 2002; Godfrey et al., 2004). However, as already noted, OSNs with the same OR can show slightly different response profiles, possibly because of variations in the level of OR gene expression among neurons. Thus, while much of the response diversity seen in these experiments is likely to reflect diversity in the recognition properties of different ORs, some is likely to be due to variations in the response properties of OSNs expressing the same OR.

Most mouse OSNs are narrowly tuned

The large number of OSNs and odorants tested in these studies permitted analysis of the extent to which individual OSNs are narrowly tuned to recognize a relatively small number of related odorants versus broadly tuned to recognize a comparatively large number and variety of odorants.

These experiments indicate that the majority of mouse OSNs are narrowly tuned. Narrow tuning was apparent, first, from OSN responses to the odorant mixtures. Of 217 OSNs activated by mixtures, 44.7% (97 of 217) responded to only one mixture containing structurally related odorants (Fig. 3).

Narrow tuning was further evident in the responses of OSNs to individual odorants. Of the 97 OSNs that responded to only one odorant mixture, 76 subsequently responded to at least one odorant from that mixture. More than one-half of those OSNs [43 of 76 (56.6%)] responded to only one odorant and another 35.5% (27 of 76) responded to two to three odorants, whereas only 7.9% (6 of 76) responded to four to five odorants, and none responded to all odorants in the mixture.

In most cases, the odorants recognized by the narrowly tuned OSNs had related structures. Two examples shown in Figure 5 are OSN223, which selectively responded to four structurally related odorants from the vanillin-like mixture, and OSN366, which responded only to indole and skatole, two structurally related odorants from the azine mixture that share an animalic-fecal odor (Yokoyama and Carlson, 1979; Garner et al., 2007). Narrow tuning to structurally related odorants was also seen among OSNs that recognized more than one mixture. For example, one OSN (OSN166) responded to structurally related odorants in mixtures 7 and 10 (odorants 7-13, 10-2, 10-3, and 10-5) (data not shown). Two other examples are OSN175 and OSN319, each of which recognized aliphatic odorants with long carbon chains present in different mixtures (data not shown). The first responded to a undecylenic alcohol (3-8) and decanal (6-4), while the latter responded to heptane thiol (2-2), heptanol (3-2), and octanal (6-3) (data not shown).

These results suggest that the majority of mouse OSNs are narrowly tuned to recognize a relatively small assortment of odorants that share a particular structural motif. Narrow tuning clearly extends beyond the recognition of a single obvious structural motif, however, since individual OSNs responded to varied subsets of odorants with the same motif and, in some cases, the odorants recognized by an OSN did not share any obvious structural feature.

Some OSNs are specific for animal-associated chemicals

The odorants tested in these studies included a small number that are associated

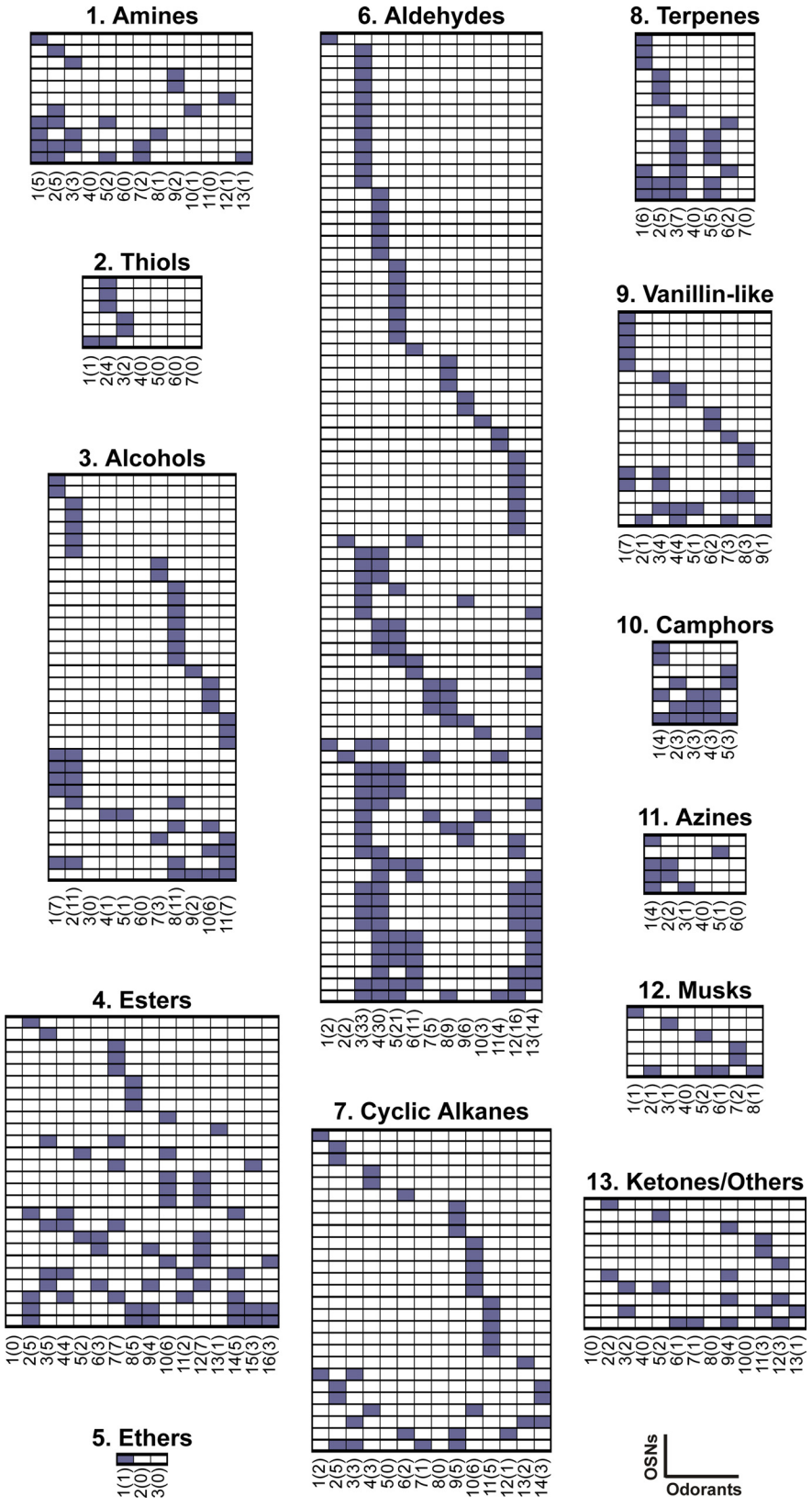

Figure 4. Responses of OSNs to single odorants. These diagrams show the responses of individual OSNs (rows) to single odorants (50 μM) (columns) from different odorant mixtures, as indicated. The blue boxes indicate odorants to which OSNs responded with an increase in intracellular calcium, as determined by calcium imaging. The number of OSNs that responded to each odorant in the mixture is shown in parentheses below. Individual OSNs varied in the number and combination of odorants to which they responded from the same mixture. Some odorants activated many OSNs, whereas others activated only one OSN or none.

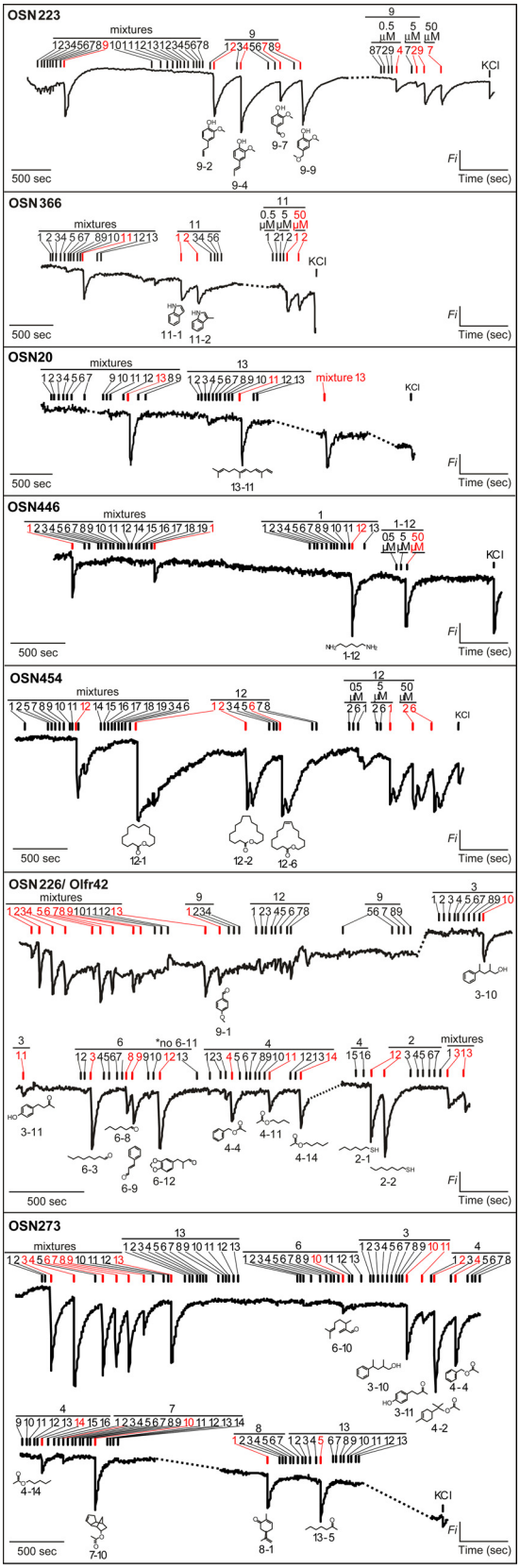

Figure 5. Calcium imaging was used to monitor changes in fluorescence intensity during sequential exposure of individual OSNs to 13 (or 18) odorant mixtures (with 50 μM each component odorant) and then to single odorants (50 μM) from one or more mixtures that had elicited a response. Lower concentrations of activating odorants (5, 0.5 μM) were also sometimes tested. A final response to 87.4 mM KCl confirmed OSN viability. OSN223 responded only to mixture 9 and then to four structurally related odorants from that mixture at 50 μM, but to

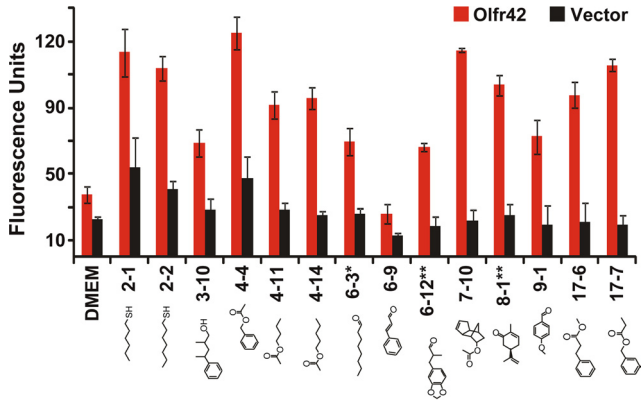

Figure 6. Olfr42 is a broadly tuned OR. After identifying Olfr42 as the OR expressed in OSN226, a broadly tuned OSN (Fig. 5), HEK293T cells were cotransfected with expression vectors encoding Olfr42 (or vector alone), RTP1s, and Ric8b together with a vector containing the cAMP reporter construct, CRE-SEAP. Responses to different odorants or to vehicle alone ("DMEM") were assayed using a fluorescent SEAP substrate ($n = 3$; each n in triplicate; results ± SEM). Odorants were tested at 10 μM, except where indicated (**30 μM; *100 μM). Cells expressing Olfr42 responded to 9 of 11 odorants that had activated OSN226 [not 6-8 (data not shown) and possibly 6-9], as well as to 4 other odorants that were not tested on the OSN (odorants 7-10, 8-1, 17-6, and 17-7).

with animals, at least some of which can elicit an innate response in mice. These included several mouse pheromones (11-6, 13-5, 13-10, 13-11, 13-13) (Leinders-Zufall et al., 2000), a predator odor present in fox feces, TMT (2,5-dihydro-2,4,5-trimethylthiazoline) (13-12) (Morrow et al., 2000; Kobayakawa et al., 2007), two odorants with a pronounced fecal odor [indole (11-1) and skatole (11-2)], one odorant that has the odor of decaying flesh [cadaverine (1-12)], and the odorants in the musk mixture, which are characterized by an animalic musky odor. One question was whether the OSNs that recognize these odorants also detect other odorants or whether they are instead highly specific.

Of the five mouse pheromones, only three elicited a response in OSNs and those three stimulated a total of five OSNs. Four of the OSNs responded not only to pheromones, but also other odorants. However, one OSN (OSN20) responded to only one mixture and to only one compound in that mixture, the pheromone α-farnesene (13-11) (Fig. 5). It is conceivable that OSNs that respond to pheromones as well as other odorants are involved in the perception of the pheromone as an odorant, whereas an OSN such as OSN20, which may be specific for a pheromone, is involved in the generation of instinctive responses to pheromones detected in the OE. In this regard, it may be relevant that α-farnesene, a pheromone in male urine that accelerates female puberty onset, can activate some neurons in the olfactory cortex that communicate with hypothalamic gonadotropin-releasing hormone (GnRH) neurons that regulate reproductive hormones (Boehm et al., 2005).

Another case in which we observed extreme odorant specificity that might be relevant to animal behavior was in responses to the odorants skatole and indole. These two closely related odorants are present in feces, both have fecal odors, and skatole is believed to give feces its characteristic odor (Yokoyama and Carlson, 1979; Garner et al., 2007). Among the neurons responsive to

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fewer odorants at lower concentrations. Narrow tuning was also seen for several OSNs that recognized animalic odorants (OSN366, indole/skatole; OSN20, α-farnesene; OSN446, cadaverine; OSN454, musk odorants). In contrast, broad tuning was evident for OSN226 and OSN273, which responded to multiple odorant mixtures and to single odorants with varied structures.

Table 1. Most odorants are recognized by a unique set of OSNs

Mixture	No. odorants/mixture	No. OSNs tested with single odorants	No. odorants recognized by ≥1 OSN	No. odorants recognized by ≥2 OSNs	No. odorants recognized by only 1 OSN	No. different OSN combinations
(1) Amines	13	11	10	6	4	10
(2) Thiols	7	6	3	2	1	3
(3) Alcohols	11	34	9	7	2	8
(4) Esters	16	26	15	14	1	15
(5) Ethers	3	1	1	0	1	1
(6) Aldehydes	13	81	13	13	0	13
(7) Cyclic alkanes	14	27	12	10	2	12
(8) Terpenes	7	14	5	5	0	5
(9) Vanillin-like	9	18	9	6	3	8
(10) Camphors	5	7	5	5	0	4
(11) Azines	6	5	4	2	2	4
(12) Musks	8	6	7	2	5	5
(13) Ketones/others	13	11	9	6	3	8
Total	125	247	102	78	24	96

This table shows data for each odorant mixture, including the number of odorants in the mixture recognized by more than one OSN and the number of different combinations (sets) of OSNs that recognized individual odorants in the mixture. Most odorants were recognized by a unique combination of OSNs. No. different OSN combinations, the number of different sets of OSNs activated by single odorants in the mixture.

these odorants, we found one (OSN366) that detected only one mixture and only two odorants in that mixture, indole and skatole (Fig. 5).

We also identified one OSN (OSN293) that was highly specific for cadaverine (1-12), the odorant with the odor of decaying flesh (data not shown) (Fig. 4). Interestingly, this was the only OSN that responded to cadaverine among all those examined. Another OSN highly specific for cadaverine (OSN446) was identified when additional OSNs were tested with 176 different odorants (Fig. 5). One question is whether indole, skatole, and cadaverine, all of which are repulsive to humans, elicit innate responses, such as avoidance, in mice.

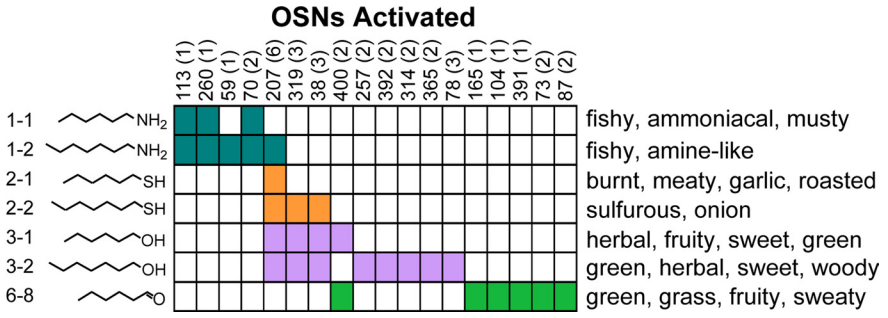

Figure 7. Structurally related odorants are recognized by different combinations of OSNs. *n*-Aliphatic odorants with six or seven carbon atoms and different functional groups (amino, thiol, hydroxyl, or aldehyde) (rows) each elicited responses in a different combination of OSNs (columns), providing an explanation for their ability to generate diverse odor perceptions in humans. The identification number of each OSN is shown above followed by the number of mixtures to which the OSN responded in parentheses. Odor descriptors for each odorant are shown at right. OSNs that responded to mixture 1, 2, 3, or 6, but were not tested with odorants from the mixture or did not respond to any of its component odorants are not shown.

As already noted, only a small proportion of OSNs responded to the musk mixture. Of the six OSNs that subsequently responded to individual musk odorants, three responded not only to musks but also to other types of odorants. However, the other three (OSNs 216, 339, and 355) all responded to only the musk mixture and each of those responded to a single musk odorant (12-1, 12-3, or 12-7) (data not shown) (Fig. 4). Another OSN from the set tested with 176 odorants (OSN454) responded to only the musk mixture and then to several different musk compounds (Fig. 5). As with indole, skatole, and cadaverine, it remains to be seen whether or not these animalic odorants stimulate specific responses in mice.

Although it cannot be excluded that the OSNs that responded to these animal-associated odorants also recognize other odorants that were not tested, these results raise the possibility that there might be some pheromones or other animalic odorants that are recognized by highly specific OSNs and ORs that provide signals to the brain that stimulate innate responses.

The repertoire contains broadly tuned components

Surprisingly, these studies revealed that a small proportion of mouse OSNs are broadly tuned. In contrast to the majority of OSNs examined, these OSNs responded to a relatively large number and variety of odorants.

The existence of broadly tuned OSNs was suggested, first, by the responses of some OSNs to odorant mixtures. Of 217 OSNs responsive to mixtures, 29 OSNs (13.4%) responded to 5–9 of the 13 mixtures and, remarkably, 4 OSNs (1.8%) responded to 10–12 mixtures (Figs. 2, 3).

Although it was not possible to test those OSNs with single odorants from all active mixtures, those tested with odorants from several mixtures were informative. For many of those OSNs, the stimulatory odorants shared a particular structural feature, such an extended carbon chain or an aldehyde or ester group (data not shown). However, other OSNs, such as OSN226 and OSN273, were activated by some odorants that shared a structural motif and others that did not (Fig. 5), suggesting the possible involvement of less obvious physicochemical characteristics.

Further analysis of one broadly tuned OSN, OSN226, demonstrated that the broad tuning of this OSN derived from broad tuning of the OR it expressed. OSN226 responded to 10 odorant mixtures and to 12 single odorants with which it was tested (Fig. 5). Using single-cell RT-PCR, we identified the OR gene expressed in this neuron as Olfr42. When we cloned Olfr42 and expressed it in HEK293T cells, we found that the OR responded to 9 of 11 of odorants that had stimulated OSN226 as well as to 4 additional odorants (Fig. 6). This is consistent with a previous study showing that a different mouse OR is broadly tuned (Grosmaitre et al., 2009).

Most odor codes are unique and combinatorial

Previous studies have indicated that different odorants are detected, and thereby encoded, by different combinations of ORs. The present studies allowed analysis of the extent to which this combinatorial scheme extends to a larger number and variety of odorants than were previously tested.

In these studies, we tested 125 odorants, 102 of which activated one or more OSNs. Comparison of OSNs activated by single odorants from the same mixture showed that the vast majority of odorants [96 of 102 (94.1%)] stimulated a unique set of OSNs (Table 1). Moreover, while some odorants were recognized by only one OSN, the majority [78 of 102 (76.5%)] were recognized by a combination of different OSNs (Table 1). For example, the 13 different aldehydes stimulated 13 different combinations of OSNs. Similarly, each of the 15 esters that activated OSNs stimulated a different set of OSNs, with 14 of 15 stimulating more than one OSN (Table 1). These findings indicate that the principle of combinatorial coding extends to a wide variety of odorants with different types of structures and perceived odors. It also shows how this principle, in combination with the extreme diversity of OSN odorant recognition, can generate a multitude of unique codes that permit a vast number of odorants to be discriminated.

Analysis of *n*-aliphatic odorants with six or seven carbon atoms and different functional groups (amino, thiol, hydroxyl, or aldehyde) showed that, despite their similarity, each odorant was recognized by a unique combination of OSNs (Fig. 7). As in a previous study of *n*-aliphatic odorants with other functional groups (Malnic et al., 1999), a change in either carbon chain length or functional group changed the combination of OSNs recognizing an odorant (its "combinatorial code"). Given the relatedness of human and mouse OR families (Zhang and Firestein, 2002; Godfrey et al., 2004; Malnic et al., 2004), human ORs are presumably used in a similar fashion, providing an explanation for the ability of these odorants to elicit different odor perceptions in humans (Malnic et al., 1999).

The size of the code varies among odorants

One question raised by previous studies, but unanswerable because of their smaller scale, was how large the "code" is for various odorants. What proportion of OSNs and ORs are used to encode the identities of individual odorants?

The data collected in the present studies indicate that the size of the code can vary extensively among odorants. The number of OSNs activated by different odorants from the same mixture (excluding odorants that stimulated no OSNs) was 1-11 for alcohols, 1-7 for esters, 2-33 for aldehydes, 1-6 for cyclic alkanes, and 1-7 for vanillin-like odorants (Fig. 4). These results suggest that, even among structurally related odorants, some odorants may be encoded by 10-30 times as many OSNs, and likely ORs, as others.

Is there a functional logic to these differences, such as larger codes for food odors? Using "odor type" classifications from online resources, the most stimulatory odorants among tested alcohols, esters, aldehydes, cyclic alkanes, and vanillin-like compounds were classified as green/citrus, fruity, aldehydic (bitter, fatty, waxy), herbal, and anisic (sweet), respectively, while the least stimulatory were classified as camphor/alcoholic/fermented, fruity, spicy, amber/woody, and spicy/minty. These results do not suggest any functional logic to differences in the number of OSNs that recognized different odorants from the same mixture, at least not in reference to perceived odors in humans.

Table 2. Odorants recognized by the same OSN often share an odor quality

	No. OSNs	
Odor descriptor	≥2 odorants	1 odorant
Citrus	18	9
Fruity	8	2
Aldehydic	1	1
Sweet	2	6
Fishy, ammonia	3	2
Minty, mentholic	1	5
Camphor, woody	1	6
Animal, fecal	1	0
Musty	1	0
Phenolic	1	1
Floral	0	1
Sulfurous, onion	0	1
Green	2	6
Musk	0	3
No shared descriptor	10	n.a.
Total	49	43

This table shows data obtained from 92 OSNs that were tested with single odorants from every mixture to which they had responded and were activated by at least one odorant from each of those mixtures. Forty-nine of the 92 OSNs responded to two or more odorants. The odorants recognized by 39 of 49 of those OSNs all shared an odor quality or descriptor. Those that shared more than one descriptor (e.g., citrus and waxy or citrus, waxy, floral, and aldehydic) are listed under a single descriptor (e.g., citrus). The numbers of OSNs that recognized only one odorant and had different odor descriptors are shown at right. n.a., Not applicable.

As already discussed, the aldehyde, ester, and alcohol mixtures stimulated many more OSNs on a per odorant basis than did the amine, musk, and azine mixtures. In addition to being classified as belonging to particular odor types, individual odorants can be assigned one or more "odor descriptors" (odor qualities or subqualities). Although the tested aldehydes, esters, and alcohols have multiple odor descriptors, many of the tested aldehydes and esters are described as "citrus" or "fruity," descriptors also given to some of the alcohols. In contrast, most amines have "fishy/ammonia" odors, musks have musky odors considered to be animalic, and the tested azines are described as animalic, fishy, or green. This suggests that there may be a slight bias toward structural classes of odorants that include those with citrus or fruity odors. The relatively small proportion of OSNs that recognize odorants with animalic odorants could be of greater significance, however, since some odorants of that class could conceivably serve as social cues that elicit specific physiological responses or behaviors.

How do combinatorial codes convey odor qualities?

One question raised by these and previous studies is how an odorant's combinatorial code conveys its odor quality. Is it possible that some ORs can convey a particular odor quality, such as minty, or different subqualities of the same odorant? If so, one might expect to find some OSNs that recognize only a single odorant or odorants that share an odor quality. Although it is impossible to determine whether this is the case without testing every possible odorant with human ORs, the present studies did uncover some interestingly relationships between odorants and mouse OSNs, which express ORs related to those found in humans.

First, as already discussed, some OSNs recognized certain animal-associated odorants, such as cadaverine or individual musk odorants, but no other tested odorants. Second, the odorants recognized by some OSNs shared not only a structural motif but also an odor quality or odor descriptor in humans. Among 92 OSNs that were tested with single odorants from every mixture to which they had responded and were activated by at least one odorant from each of those mixtures, 49 responded to two or

more odorants. Of those, 39 of 49 (79.6%) recognized odorants that all shared an odor descriptor (Table 2, Fig. 8). These findings raise the intriguing possibility that, at least in some cases, a particular OR may convey a specific odor quality or subquality, such as minty or fishy.

However, many of the odorants shown in Figure 9 were recognized not only by such seemingly "odor-specific" OSNs but also by OSNs that responded to other odorants with unrelated odors. Moreover, as already discussed, some odorants with related structures but very different odors were recognized by partially overlapping sets of OSNs (Fig. 7).

Studies using human ORs and larger panels of odorants will ultimately be required to assess how ORs give rise to human odor perceptions. However, like other proteins, ORs found in human and mouse are related, suggesting that they are likely to have related ligand specificities. The above findings raise the possibility that, while there might be ORs that convey a particular odor quality, there may be many more ORs that do not do so.

Discussion

Here, we conducted a large-scale analysis of odorant recognition in the mouse olfactory epithelium, where individual OSNs and their expressed ORs constitute the fundamental units of sensory input to the brain. By imaging the responses of 3000 OSNs to 125 diverse odorants, we potentially tested as many as 375,000 OSN–odorant pairings. Given that each OSN expresses only 1 of ~1000 different OR genes, it is likely that these experiments queried the odorant recognition properties of a large proportion of mouse ORs. While humans have only ~350 ORs (Niimura and Nei, 2005), the OR families of the two species have related ORs and OR subfamily structures (Zhang and Firestein, 2002; Godfrey et al., 2004; Malnic et al., 2004), suggesting that the basic principles uncovered in the present studies are likely to be relevant to human odor perception.

Diversity and bias in the OSN repertoire

Previous studies have shown that different OSNs and ORs respond to different sets of odorants (Sato et al., 1994; Duchamp-Viret et al., 1999; Malnic et al., 1999; Araneda et al., 2004; Saito et al., 2009), as do different glomeruli in the olfactory bulb (Uchida et al., 2000; Belluscio and Katz, 2001; Johnson et al., 2002; Soucy et al., 2009). By testing a much larger combination of potential odorant–OSN interactions than previous studies, the present studies permitted a more comprehensive analysis of odorant recognition in the mouse OE.

These studies revealed an extraordinary level of diversity in the odorant recognition properties of individual OSNs. Remarkably, 217 OSNs that responded to odorant mixtures showed 93 different response profiles composed of responses to different odorants or combinations of odorants. Extreme diversity was also seen in the responses of OSNs to single odorants. For example, 81 OSNs responded to 36 different sets of aldehydes and 26 OSNs responded to 17 different sets of esters.

These studies also uncovered significant bias in the OSN repertoire with regard to the recognition of different odorant structural classes as well as to odorants of the same class. Some odorant mixtures activated up to 3–5 times as many OSNs as others, and, among odorants in the same mixture, some stimulated 5–10 times as many OSNs as others and, in one case, 16 times as many. Mixtures that stimulated the most OSNs contained more odorants with the food-related odor descriptors, citrus or fruity, than did mixtures that activated the fewest OSNs. However, no such func-

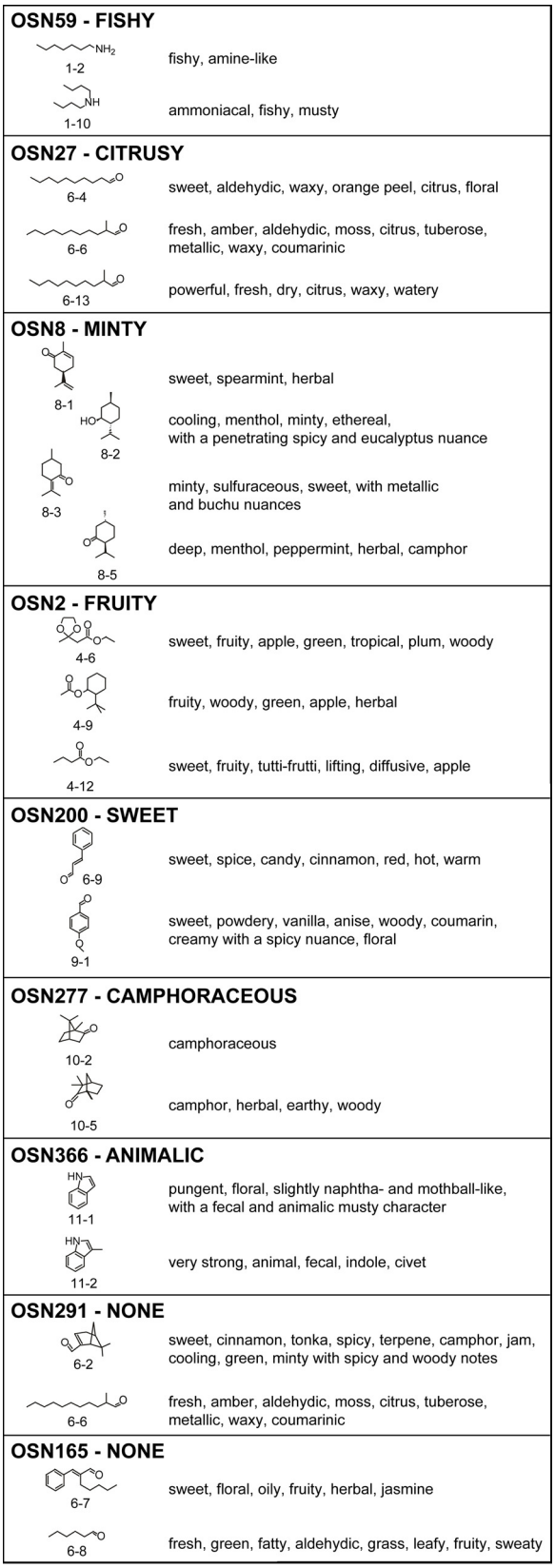

Figure 8. Individual OSNs can recognize related odorants with similar or dissimilar odors. Shown here are the odorants recognized by a series of different OSNs that recognized odorants with related structures. In many cases, the odorants detected by an OSN shared one or more odor descriptors (e.g., fishy), but this was not always the case, as indicated after the identification number of the OSN at left.

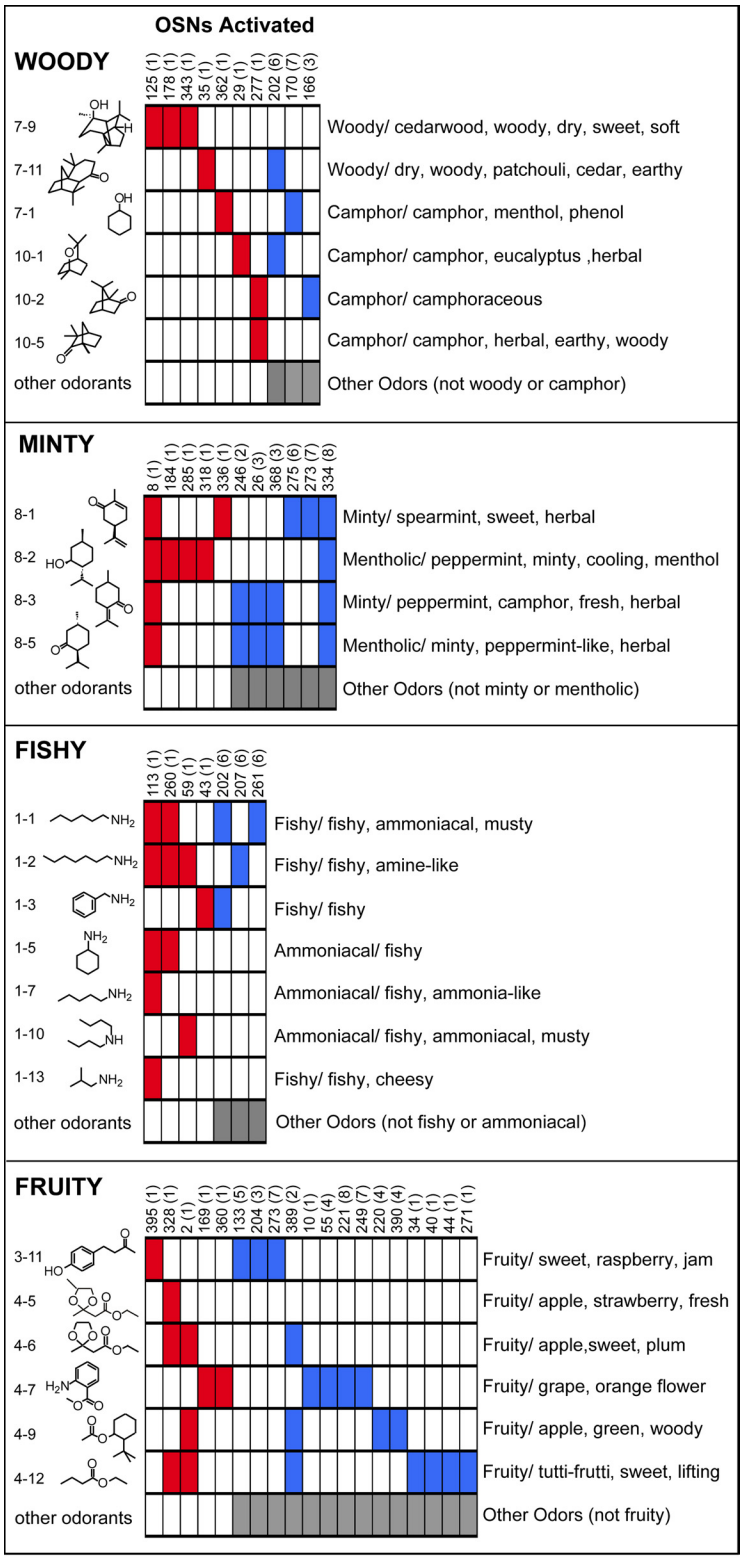

Figure 9. Individual odorants can be recognized by a combination of highly specific and broadly tuned OSNs. Some OSNs (columns) responded exclusively to one or few odorants (rows) of a particular odor type (woody/camphor, minty/mentholic, fishy, or fruity), as indicated by red boxes. As shown by the blue boxes, many of the same odorants were also recognized by OSNs that responded to odorants of unrelated odor types. Shown above is the identification number of each OSN followed by the number of mixtures to which it responded in parentheses.

tional link emerged from comparisons of the most and least stimulatory odorants in individual mixtures.

Together, these results suggest that the most important attribute of the OSN/OR repertoire is likely to be its recognition diversity. It is this diversity that allows the olfactory system to not only detect but also discriminate a wide variety of odorants, including those indicative of edible versus poisonous plants, locales appropriate versus inappropriate for nesting, and, among other animals, friend versus foe.

The repertoire contains both narrowly and broadly tuned components

These studies indicate that the majority of mouse OSNs are narrowly tuned to recognize a subset of odorants that share one or more structural features. In many but not all cases, odorants recognized by the same OSN also shared one of several odor subqualities ascribed to the odorants by humans, such as citrus or minty. It is also clear, however, that narrow tuning extends beyond the recognition of a single structural motif, since individual OSNs responded to some odorants with a given motif, but not others.

In addition to the major population of narrowly tuned OSNs, these studies uncovered a small proportion of OSNs that are broadly tuned to recognize a larger number and variety of odorants. Functional analysis of an OR expressed in one such neuron confirmed that its broad tuning derived from a single OR, consistent with previous analysis of another broadly tuned mouse OR (Grosmaitre et al., 2009). Although the selective advantages of narrowly versus broadly tuned ORs are not presently clear, it is conceivable that broadly tuned ORs make an important contribution to the animal's ability to discriminate closely related odorants by increasing the combinations of ORs that recognize different odorants.

Interestingly, some of the narrowly tuned OSNs identified in these studies appeared to be highly specific for an animal-associated compound. Among these were OSNs that selectively recognized the male mouse pheromone, α-farnesene, which accelerates female puberty onset, the fecal odorants indole and skatole, the rotting flesh odorant cadaverine, or individual musk odorants. The existence of such OSNs raises the possibility that certain OSNs/ORs may have the capacity to elicit innate physiological or behavioral responses. Consistent with this idea, exposure of female mice to α-farnesene was previously found to activate neurons in the olfactory cortex that are in contact with hypothalamic neurons that regulate reproduction (GnRH neurons) (Boehm et al., 2005). Also supporting this idea is the finding that removal of OSNs in one part of the OE ablates innate avoidance/fear responses to a fox predator odor while leaving the animal's ability to detect the predator

odor intact (Kobayakawa et al., 2007). Thus, some OSNs that recognize an animalic odorant may elicit an innate response, whereas others result in its perception as a common odorant.

Most odor codes are unique and combinatorial

Previous studies indicated that different odorants are detected, and thus encoded, by different combinations of ORs (Malnic et al., 1999; Kajiya et al., 2001). The present studies show that this combinatorial coding scheme extends to a wide variety of odorants with diverse structures. The vast majority of the odorants that elicited an OSN response activated a unique set of OSNs. Moreover, 77% of those odorants stimulated two or more OSNs.

The remarkable diversity in the combinations of OSNs that recognized different odorants underlines the enormous potential of combinatorial coding in permitting the discrimination of a multitude of odorants. As seen previously (Malnic et al., 1999), even highly related odorants with different odors were recognized by different combinations of OSNs, emphasizing the significant potential for combinatorial coding not only for discriminating odorants but also for generating diverse odor perceptions.

Odor codes and perception

How do combinations of activated OSNs give rise to the perceived odor of an odorant? Odorants are often described as having a strong central character or "quality" as well as additional "notes" or "subqualities." Is it the combination of ORs that generates each of these characteristics or might different characteristics be conveyed by different ORs? While it is impossible to definitively answer this question without testing every single human OR with every possible odorant, the present studies did reveal associations between odorants and mouse OSNs that might be relevant to odor perception in humans, particularly given the relatedness of human and mouse ORs.

Two observations suggest a potential link between individual OSNs/ORs and perceived odor characteristics. First, some OSNs, including some that recognized animalic odorants, responded to only a single odorant. Second, numerous OSNs recognized only odorants that shared an odor quality or descriptor. Although it cannot be excluded that these OSNs could have responded to unrelated, but untested odorants, these findings are consistent with the idea that individual OSNs/ORs might have the ability to convey particular odor qualities. Given that many or most mouse OSNs appeared to be narrowly tuned to recognize structurally related odorants, these findings are also in accord with the ability of some structurally related odorants to elicit similar odor perceptions in humans (Fig. 9). However, arguing against a link between OSNs/ORs and perceived odors, a number of mouse OSNs recognized odorants with very different odors and, in addition, certain *n*-aliphatic odorants with extremely different odors were recognized by partially overlapping combinations of OSNs.

While studies of human ORs will ultimately be required to understand the contributions made by individual ORs to odor perceptions, it is likely that human and mouse ORs, like other proteins, behave in a similar manner in the two species. If so, what hints can be gleaned from the present studies with regard to the functions of individual ORs in odor perceptions? Although some mouse OSNs appeared to recognize odorants with a shared odor quality in humans, the results noted above suggest a model in which perceived odor qualities or subqualities emerge not from individual ORs, but rather from the combination of ORs activated. However, another possibility that cannot be excluded is that at least some ORs may be capable of conveying a particular odor characteristic, but their ability to do so is context dependent, with the context being the combination of ORs that are coactivated in response to the odorant. In this highly speculative scenario, input from a particular OR might be essentially quenched when it is coactivated with some other ORs, but not others. However, unraveling how combinations of ORs generate diverse odor perceptions remains a challenge for the future that will require not only more knowledge of human OR specificities but also an understanding of how sensory inputs derived from combinations of ORs are organized and processed in the brain.

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