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Encoding the Odor of Cigarette Smoke

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Cellular/Molecular

Encoding the Odor of Cigarette Smoke

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The encoding of odors is believed to begin as a combinatorial code consisting of distinct patterns of responses from odorant receptors (ORs), trace-amine associated receptors (TAARs), or both. To determine how specific response patterns arise requires detecting patterns *in vivo* and understanding how the components of an odor, which are nearly always mixtures of odorants, give rise to parts of the pattern. Cigarette smoke, a common and clinically relevant odor consisting of >400 odorants, evokes responses from 144 ORs and 3 TAARs in freely behaving male and female mice, the first example of *in vivo* responses of both ORs and TAARs to an odor. As expected, a simplified artificial mimic of cigarette smoke odor tested at low concentration to identify highly sensitive receptors evokes responses from four ORs, all also responsive to cigarette smoke. Human subjects of either sex identify 1-pentanethiol as the odorant most critical for perception of the artificial mimic; and in mice the OR response patterns to these two odors are significantly similar. Fifty-eight ORs respond to the headspace above 25% 1-pentanethiol, including 9 ORs responsive to cigarette smoke. The response patterns to both cigarette smoke and 1-pentanethiol have strongly responsive ORs spread widely across OR sequence diversity, consistent with most other combinatorial codes previously measured *in vivo*. The encoding of cigarette smoke is accomplished by a broad receptor response pattern, and 1-pentanethiol is responsible for a small subset of the responsive ORs in this combinatorial code.

Key words: GPCR; olfaction; perception; sensory; physiology; smoking

Significance Statement

Complex odors are usually perceived as distinct odor objects. Cigarette smoke is the first complex odor whose *in vivo* receptor response pattern has been measured. It is also the first pattern shown to include responses from both odorant receptors and trace-amine associated receptors, confirming that the encoding of complex odors can be enriched by signals coming through both families of receptors. Measures of human perception and mouse receptor physiology agree that 1-pentanethiol is a critical component of a simplified odorant mixture designed to mimic cigarette smoke odor. Its receptor response pattern helps to link those of the artificial mimic and real cigarette smoke, consistent with expectations about perceptual similarity arising from shared elements in receptor response patterns.

Introduction

Natural odors are mixtures of many species of volatile chemicals, known as odorants. Odors are initially encoded as "combinatorial codes" consisting of the response patterns of receptor proteins located in the cilia of olfactory sensory neurons (OSNs) (Malnic et al., 1999; Nara et al., 2011). The majority of odorantresponsive receptors are the odorant receptors (ORs), which

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number in the hundreds in most mammals and \sim 1100 in mice (Buck and Axel, 1991; Niimura et al., 2014). Also important for odor detection are trace amine-associated receptors (TAARs). In mice, all but one of the 15 TAARs are expressed in OSNs where they are important for detecting volatile amines (Liberles and Buck, 2006; Ferrero et al., 2012; Johnson et al., 2012; Dewan et al., 2018). Each mouse OSN expresses just one allele of one OR or TAAR gene (Chess et al., 1994; Mombaerts, 2004; Liberles, 2015). This maximizes the distinctiveness of each OSN's response and allows each olfactory bulb glomerulus to be innervated only by axons of OSNs expressing the same OR or TAAR (Mombaerts et al., 1996), so that receptor response patterns can be represented faithfully in spatiotemporal patterns of glomerular activity.

We understand little about how OR and TAAR response patterns contribute to perception. For example, are response patterns similar for odors that have similar percepts? Are response

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T.S.M. has an equity interest in a company based on technologies used to measure responses to odors. The remaining authors declare no competing financial interests.

patterns to complex odors necessarily broad, or do antagonistic interactions between odorants at receptors (de March et al., 2020) cause complex odors to have narrow response patterns? Are response patterns determined by only a few of the odorants in a complex odor? Are there core sets of odorants and receptors that determine perception of odors? This idea of core sets of receptors for percepts is consistent with the ability of chemists to mimic the percept evoked by a complex odor with just a few of its odorants (Tamura et al., 2008) and with concentrationinvariant perception of odors (Friedrich and Korsching, 1997; Rubin and Katz, 1999; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried et al., 2002; Bozza et al., 2004; Storace and Cohen, 2017; Bolding and Franks, 2018). In this study, we investigated the receptor response pattern of a complex odor and the contribution of one of its odorants.

To focus our efforts on an odor significant to humans, we investigated cigarette smoke. Not only is it a common odor, it has clinical importance. In smokers and reformed smokers, the odor of cigarette smoke increases the desire to smoke (Cortese et al., 2015a,b). The link between the olfactory system and the brain's reward circuit has become hijacked by nicotine's activation of the reward circuit during exposure to cigarette smoke (Picciotto and Mineur, 2014; Balfour, 2015). This physiological response contributes to the difficulty smokers encounter when they attempt smoking cessation (Halpern et al., 2018; Hajek et al., 2019). Exposing freely behaving mice to cigarette smoke evokes a broad response pattern containing both ORs and TAARs. A small subset of these ORs comprise the response pattern to a low concentration of an artificial mimic of cigarette smoke odor. One of the odorants in this mimic, 1-pentanethiol, is especially important for human perception of artificial cigarette smoke odor and drives responses from several of the mouse ORs responsive to artificial cigarette smoke and to real cigarette smoke.

Materials and Methods

Materials. Odorants were obtained at the highest purity available. Indole and butyric acid were kind gifts from Firmenich SA. Thiophene and 1,3-cyclohexadiene were purchased from Alfa Aesar. All other odorants were purchased from Sigma Millipore. An artificial cigarette smoke odor designed to mimic the odor of cigarette smoke (Dravnieks et al., 1975) was formulated by mixing 26 odorants at the proportions shown in Table 1. This artificial cigarette smoke odor should not be confused with commercial products mimicking cigarettes or cigarette smoke. Such products are designed to mimic certain properties of cigarettes, such as visual appearance, but not their odor.

Human subject testing. The procedures used for human subjects were approved by the University of Kentucky Institutional Review Board. Male and female volunteers ages 18-50 from the Lexington, KY consented in writing to participate in this study. Smokers, pregnant women, persons with active sinus infections, persons with a history of smell or taste deficits, persons suffering from fragrance allergies or chemical sensitivity, persons with a history of migraines headaches, and persons with a diagnosed neurologic disorder were excluded.

Tests of similarity between artificial cigarette smoke odor and odorant depletion mixtures lacking one of the odorants in artificial cigarette smoke odor were done by 18 consenting subjects: 10 females and 8 males. A total of 27 odor mixtures were prepared: the full artificial cigarette smoke odor mixture and 26 mixtures, each lacking one of the 26 odorants in the full mixtures. The mixtures were prepared fresh on the day of testing and absorbed into cotton balls sealed in brown glass vials. Subjects were seated in front of Movex fume capture hoods used to prevent odors from filling the testing room and causing adaptation. Subjects were first asked to familiarize themselves with the artificial cigarette smoke odor. Using intervals of at least 1 min between odor

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|-----------------------|------------|--------------|---------------|
|-----------------------|------------|--------------|---------------|

| Odorant | CAS # | Volume (µl) |
|------------------------------|------------|-------------|
| Pyridine | 110-86-1 | 5 |
| Nicotinaldehyde | 500-22-1 | 15 |
| 5-Ethyl-2-methylpyridine | 104-90-5 | 30 |
| 2-Vinylpyridine | 100-69-6 | 5 |
| 4-Pyridinecarbonitrile | 100-48-1 | 5 mg |
| Methyl isonicotinate | 2459-09-8 | 30 |
| 2-Ethyl-3-methylpyrazine | 15707-23-0 | 20 |
| 2-Methoxy-3,5-methylpyrazine | 93905-03-4 | 4 |
| 2-Methoxyphenol | 90-05-1 | 15 |
| o-Cresol | 95-48-7 | 5 |
| 5-Methylfurfural | 620-02-0 | 15 |
| 2,5-Dimethylpyrrole | 625-84-3 | 30 |
| Phenylacetylene | 536-74-3 | 15 |
| lsoprene | 78–79-5 | 15 |
| 1,3-Cyclohexadiene | 592-57-4 | 5 |
| Indene | 95-13-6 | 15 |
| Allylbenzene | 300-57-2 | 15 |
| 2,3-Butanedione | 431-03-8 | 10 |
| Thiophene | 110-02-1 | 5 |
| 1-Pentanethiol | 110-66-7 | 2.5 |
| Allylamine | 107-11-9 | 2.5 |
| 1-Aminopentane | 110-58-7 | 2.5 |
| Butyric acid | 107-92-6 | 10 |
| 1-Hexanal | 66-25-1 | 5 |
| Trans, trans-2, 4-Hexadienal | 142-83-6 | 5 |
| Indole | 120-72-9 | 5 mg |

 $^{\prime 0}$ Indole and 4-pyridinecarbonitrile were dissolved at 0.5 mg/µl in DMSO and 10 µl of each added to the mixture.

sampling, each subject was given each of the depleted odorant mixtures in an order uniquely randomized for each subject. Subjects scored these mixtures using a method modeled after those of Keller and Vosshall (2016). The similarity of each odorant-deficient mixture to the full mixture was scored on a scale of 0 to 100, with 0 representing no similarity and 100 representing identical sensations.

Another 27 consenting subjects, 17 females and 10 males, were used to test whether individual odorants were discriminably different from the artificial cigarette smoke odor mixture. Subjects were seated in front of Movex fume capture hoods and asked to familiarize themselves with the odor of the full artificial cigarette smoke odor mixture. Using intervals of at least 1 min between odorants, each subject was given an odorant to sample and asked to rate its similarity to the full synthetic cigarette smoke odor mixture on a scale of 0 to 100. Subjects then also rated their perception of the pleasantness of each odorant, again using a scale of 0 to 100, with 0 being the most unpleasant odor imaginable and 100 being the most pleasant odor they could imagine. Each subject sampled the odors in a unique random order.

Detection of OR and TAAR responses in live mice. In this assay, odor stimulated expression of GFP from the activity-dependent S100A5 gene locus in the *S100a5-tauGFP* mouse (The Jackson Laboratory, stock #006709). The *S100a5-tauGFP*^(+/-) mice used for this project are from a stock back-crossed for 10 generations against C57BL/6J. Both sexes of mice, ages 7-12 weeks, were used. All procedures with mice were done according to protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky. GFP⁺ and GFP⁻ cell samples were collected by FACS of dissociated olfactory mucosae of heterozygous S100a5-tauGFP mice after stimulation with odor or with clean air. Because each OSN only expresses a single OR or TAAR gene, the OR and TAAR mRNAs specifically enriched in samples from mice stimulated with odorant but not in samples from mice stimulated with clean air must encode receptors responsive to the odorants tested. The reliability of this assay of receptor responses to odorants in freely behaving mice has been confirmed by in vitro studies of individual OR responses expressed in cultured cells (McClintock et al., 2014; de March et al., 2020).

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Each mouse was housed individually in specially designed, heated (27°C) Plexiglas chambers (700 cm³) under a flow of 3.1 l/min of filtered air for 40 h to allow degradation of GFP evoked by prior odor exposure. Over the last 16 h of this 40 h period, the mice were stimulated by activating computer-controlled solenoid valves that divert the flow of filtered air to flush the headspace from a 50 ml Delrin vial containing 5 ml of either the DMSO vehicle or 25% 1-pentanethiol in DMSO. GFP fluorescence in an activated OSN peaks at 6-8 h after the onset of stimulation and because GFP has a 26 h half-life (Corish and Tyler-Smith, 1999) once an OSN responds to an odor it is marked for longer than the duration of the experiment. To stimulate with cigarette smoke, a 590 cm³ Plexiglas cylinder closed at the bottom and containing a lit 1R6F Research Cigarette (produced by the Center for Tobacco Reference Products at the University of Kentucky) was secured against the wire mesh bottom of each mouse chamber for 1 min and the airflow to each chamber was discontinued, allowing smoke to enter the mouse chamber. For control mice, the same cylinder was used but it contained no cigarette. This procedure began 16 h before death of the mice and was done 6 times with 30 min between stimulations. This same procedure was used for stimulation with artificial cigarette smoke odor, using 0.6 ml of the artificial cigarette smoke odor source mixture or a nonvolatile vehicle (DMSO) soaked into a cotton ball as the stimuli. At the completion of stimulation, olfactory mucosae were dissected and cells dissociated in a procedure involving papain, trypsin, deoxyribonuclease, and low calcium saline as described previously (Yu et al., 2005; Sammeta et al., 2007). Cells from three identically treated mice were pooled, and FACS was performed in the University of Kentucky Flow Cytometry and Cell Sorting Facility using an iCyt Synergy cell sorting system to collect GFP⁺ and GFP⁻ cell samples. Total RNA was isolated using the QIAGEN RNeasy Micro kit (catalog #74004). RNA quantity was measured using Affymetrix Mouse Clariom S arrays in the University of Kentucky Microarray Facility. The microarray data are available in Gene Expression Omnibus under accession number GSE146418. Data were initially processed using Affymetrix GeneChip Command Console software to generate globally normalized quantities for each gene transcript cluster. Additional processing to generate GFP⁺/GFP⁻ ratios from the microarray signal intensities was done in Microsoft Excel. These GFP⁺/ GFP⁻ enrichment ratios are equivalent to fold differences and help to normalize effect across the different abundances of mRNAs and across differences in constitutive activity of ORs and TAARs. The median signal intensity of 135 mature OSN-specific mRNAs (Nickell et al., 2012) was used to adjust for differences in the amount of mature OSNs across samples.

Experimental design and statistical analysis. Both human subject experiments were shared control designs where each condition was compared with a single common control, which in both cases was the full artificial cigarette smoke odor mixture. The test administrator was blind to the identities of the odors, each being identified only by a unique code on the odor vial. A different person performed statistical analyses of these data and was also blind to the identity of the odorants represented by the unique codes. Rating scores for each individual were converted to rankings and analyzed by estimation statistics for effect size using a shared control model for mean differences (http://www.estimationstats.com/#/analyze/shared-control). Effect sizes and CIs were calculated and are displayed. The p values reported for the effect sizes derive from Welch's unequal variance t test (Welch, 1947).

The *in vivo* assay design was a paired comparison of a group of 3 mice of either or both sexes exposed to odor to a sex- and age-matched group of 3 mice simultaneously exposed to filtered air, replicated 4 times. For analysis of these data, we used a Bayesian hierarchical model to obtain normalized measures of odorant effect, accounting for four sources of variation: basal receptor effect, odorant effect, nonspecific effect (change in both odorant and vehicle control), and random measurement error. For each odorant effect, the posterior mean divided by the posterior SD provides a measure (*Z* statistic) that is approximately normally distributed. Local false discovery rates (FDRs) (Efron, 2008; Stephens, 2017) were used to estimate the probability that each receptor was responsive to the odorant using a uniform mixture model. Based on the identification of responses from ORs whose agonists were identified

using independent methods, a 15% FDR was found to be a suitable level of risk for the identification of activated receptors (McClintock et al., 2014). Data for each receptor mRNA are reported as the GFP⁺/GFP⁻ ratio fold differences for odor-stimulated mice and for control mice. The overall response measure (delta) is the GFP⁺/GFP⁻ ratio fold difference for odorstimulated mice divided by the GFP⁺/GFP⁻ ratio fold difference for control mice. Responsive ORs show a large delta value because their mRNAs increase in the GFP⁺ sample while simultaneously decreasing in the GFP⁻ samples from odor-stimulated mice, but not in samples from control mice. Phylogenetic tree plots of OR sequence distance were generated in R. Dendrograms of the relationships between OR family size and the fraction of responsive ORs per family were generated using hierarchical clustering (Euclidean distance) functions from the R cluster library.

To compare receptor response patterns, we performed cluster analysis based on the following distance metric. To account for changes in overall magnitude of response, fold change differences on the log scale were quantile-normalized across experiments. A soft threshold was then applied so that only changes larger than twofold enrichment contributed to the distance. Euclidean distances between odor responses were then calculated. To assess whether odor responses were significantly similar, permutation tests were applied, forming a null distribution of odor distances by drawing 10,000 random permutations of the responses. This was done following the quantile normalization step, so that the distribution of responses was the same across all odorant response experiments. The permutation testing was implemented in R 4.0.2 (www.R-project.org).

Results

ORs and TAARs responsive to cigarette smoke

Cigarette smoke is highly complex, consisting of particulate matter, gases, and a wide variety of organic chemicals produced during the incomplete combustion of cigarettes (Rodgman and Perfetti, 2013). Among these are >400 structurally diverse volatile organic chemicals, known as odorants, making it one of the most complex odors known. Its complexity, which includes volatile amines, predicts that a broad array of ORs and TAARs would respond to it. To test this prediction, we used an in vivo assay that allows us to identify receptors responsive to any odor presented to freely behaving mice (McClintock et al., 2014). Expression of GFP from the S100a5 gene locus, which responds to odor-stimulated electrical activity in OSNs by rapidly increasing transcription (Fischl et al., 2014), marks responding OSNs. Because each OSN expresses only one OR or TAAR, capturing fluorescent and nonfluorescent OSNs by FACS followed by expression profiling to quantify all mRNAs allows us to measure in a single experiment every OR and TAAR. The mRNAs encoding responsive ORs show significant shifts from GFP⁻ samples to GFP⁺ samples in odor-stimulated mice compared with control mice stimulated with clean air, a fact confirmed by in vitro experiments (McClintock et al., 2014; de March et al., 2020).

Consistent with our predictions about large numbers of receptors responsive to complex odors, such as cigarette smoke, we detect responses from 144 ORs and 3 TAARs in freely behaving mice exposed to cigarette smoke (Fig. 1*A*; Table 2). These responses are specific to cigarette smoke and are not observed in mice exposed only to clean air (Fig. 1*B*). Sequence relationships divide ORs into two groups: terrestrial vertebrate-specific Class II ORs and the more evolutionarily ancient Class I ORs (Glusman et al., 2000). The ORs responsive to cigarette smoke are nearly all Class II ORs, with just one responsive Class I OR, Olfr619. The 143 Class II ORs responsive to cigarette smoke are widely distributed across the Class II portion of the OR sequence distance tree (Fig. 1*C*). This response pattern is robust, rich in breadth, and not concentrated around a few related or strongly responsive ORs (Fig. 1*C*). Instead, the response pattern consists



Figure 1. Receptors responsive to cigarette smoke *in vivo. A*, In mice, 144 ORs and 3 TAARs exceed the FDR criterion for a significant response to cigarette smoke. Delta is the cigarette smoke response relative to the clean air control response. FDR values capped at 10^{-10} . *B*, The distribution of responses, measured as the fold difference (FD) enrichment of receptor mRNAs in samples of active OSNs compared with inactive OSNs, identifies responses specific to cigarette smoke versus the clean air control (red). *C*, Responsive ORs are widely distributed across the Class II portion of the OR sequence distance tree. Only 1 Class I OR responds. Gold represents responses >5-fold. *D*, Clustering MOR families via similarity in family size and proportion of ORs responsive ORs, and this fraction has little weight in the clustering of OR families. Only OR families containing responsive ORs are shown. Circle size reflects family size.

of numerous peaks of strongly responsive receptors that are widely divergent in sequence. For example, the receptors whose response magnitude is fivefold or more include all three TAARs and 28 ORs that belong to 21 different OR families. Of the 186 Class II OR families in mice (Zhang and Firestein, 2002), 66 of them contain at least one responsive OR. Thirty-seven OR families have multiple responsive ORs, and families MOR204, MOR245, MOR114, and MOR225 contain five or more responsive ORs. However, these four families are all large, and the responsive ORs do not account for even a simple majority of ORs within them (Fig. 1*D*). The only instance where nearly all members of a multiple OR family are responsive to cigarette smoke is the MOR265 family, but it contains only two ORs (Fig. 1*D*).

The responsive TAARs include two closely related sequences, Taar7d and Taar7f, and the more distantly related Taar2. The odorant agonists of these three TAARs are not well defined, but TAAR7f is known to respond to N-methylpiperidine and N,Ndimethylcyclohexylamine, whereas TAAR7d also responds to N, N-dimethylcyclohexylamine (Liberles and Buck, 2006; Dewan et al., 2018). Of these two volatile amines, only N-methylpiperidine is known to be present in cigarette smoke (Rodgman and Perfetti, 2013). TAAR2 is one of several TAARs believed to be more responsive to primary amines, whereas the TAAR7 family may be more responsive to tertiary amines (Ferrero et al., 2012).

ORs highly sensitive to a mimic of cigarette smoke odor

Perception of the odor of cigarette smoke does not require all of the >400 odorants found in cigarette smoke. Odors resembling cigarette smoke odor have been produced using many fewer odorants (Dravnieks et al., 1975; Cortese et al., 2015b). An effective mimic reported by Dravnieks et al. (1975) contains 32 odorants known to occur in cigarette smoke or closely related in structure to an odorant found in cigarette smoke, with 26 core odorants and 6 odorants suspected of arising from chemicals or flavorings added to cigarettes. We formulated a mimic containing the 26 core odorants. The receptors highly sensitive to this

Table 2. Receptors responsive to cigarette smoke

| OPERAGE MOR232-11 3.5.8 OHENDS MOR17-12 2.29 0H1278 MOR245-11 24.66 OHT1755 MOR172-20 3.53 OHENDS 2.23 0H1750* MOR107-18 20.63 OHT1750 MOR107-18 20.43 OHT22 MOR185-6 3.33 OHT1414 MOR22-1-3 2.23 0H736 MOR106-18 15.62 OHT1744 MOR23-17 3.22 OHT1700 MOR22-24 2.20 0H736 MOR164-1 10.23 OHT1725 MOR24-11 3.18 OHT1710 MOR13-1 2.20 0H747 MOR164-1 10.23 OHT1726 MOR167-1 3.21 OHT1740 MOR12-2 2.16 0H775 MOR167-5 9.22 OHT1726 MOR164-4 3.09 OHT740 MOR16-1 2.14 0H1744 MOR167-5 8.90 OHT1713 MOR167-1 3.06 OHT670 MOR16-1 2.14 0H1744 MOR167-1 3.06 OHT670 MOR14-1 2.11 0.116 <th>OR</th> <th>MOR name</th> <th>Delta</th> <th>OR</th> <th>MOR name</th> <th>Delta</th> <th>OR</th> <th>MOR name</th> <th>Delta</th> | OR | MOR name | Delta | OR | MOR name | Delta | OR | MOR name | Delta |
|--|-----------------------|-------------------------|-------|-----------------------|-----------------------|-------|-----------------------|-----------|-------|
| olf123c MOR245-11 24.46 Olf1135 MOR172-2 3.53 Olf14134 MOR23-5 22.23 0lf750 MOR105-18 20.43 Olf52 MOR185-6 3.33 Olf14144 MOR23-13 22.33 0lf756 MOR106-15 15.62 Olf1113 MOR24-16 3.52 Olf1714 MOR23-72 3.22 Olf1710 MOR23-51 2.20 0lf1634 MOR24-16 13.52 Olf1717 MOR13-51 3.20 Olf1710 MOR13-51 2.20 0lf1634 MOR246-11 0.28 Olf1744 MOR22-5 3.13 Olf1634 MOR17-5 3.22 Olf1744 MOR155 2.01 0.01 MOR17-5 3.22 Olf1744 MOR156-1 2.16 0.01 MOR17-5 MOR17-5 9.32 Olf1752 MOR156-1 3.06 Olf1730 MOR166-12 2.16 0.01 0.01 0.01 MOR166-12 2.16 0.01 0.01 0.01 0.01 0.01 MOR166-12 2.01 0.01 0.01 0. | Olfr488 | MOR204-15 | 25.75 | 0lfr1257 | MOR232-1 | 3.58 | Olfr905 | MOR167-1 | 2.29 |
| Ohr Ohr <td>0lfr1278</td> <td>MOR245-11</td> <td>24.46</td> <td>0lfr1135</td> <td>MOR177-2</td> <td>3.53</td> <td>0lfr1014</td> <td>MOR213-5</td> <td>2.28</td> | 0lfr1278 | MOR245-11 | 24.46 | 0lfr1135 | MOR177-2 | 3.53 | 0lfr1014 | MOR213-5 | 2.28 |
| Olfr30 MOR106-18 20.43 Olfr32 MOR186-6 3.33 Olfr4141 MOR204-13 2.23 Olfr304 MOR204-16 13.62 Olfr1048 MOR187-2 3.22 Olfr300 MOR212-4P 2.20 Tanz 13.7 Olfr1048 MOR187-13 3.20 Olfr304 MOR181-1 2.20 Tanz 13.7 Olfr144 MOR202-13 3.13 Olfr409 ¹¹ MOR10-12 2.20 Olfr376 MOR10-31 10.28 Olfr144 MOR202-13 3.13 Olfr409 ¹¹ MOR10-4 2.16 Olfr377 MOR20-73 8.32 Olfr352 MOR13-10 3.09 Olfr366 MOR10-12 2.16 Olfr130 MOR17-5 8.32 Olfr479 MOR20-713 8.22 Olfr479 MOR20-713 8.01 MOR20-713 8.01 MOR20-713 3.00 Olfr370 MOR141-1 2.11 Olfr149 MOR20-713 8.22 Olfr479 MOR20-713 8.01 MOR12-2 2.05 Olfr149 <td>0lfr1137^b</td> <td>MOR177-20</td> <td>20.69</td> <td>Olfr1495</td> <td>MOR266-9</td> <td>3.53</td> <td>Olfr433</td> <td>MOR123-1</td> <td>2.23</td> | 0lfr1137 ^b | MOR177-20 | 20.69 | Olfr1495 | MOR266-9 | 3.53 | Olfr433 | MOR123-1 | 2.23 |
| 0lf736 MOR204-16 15.62 0lf1123 MOR204-17 3.24 0lf1204" MOR232-6 2.21 Taar2 13.57 0lf1 MOR135-13 3.20 0lf20 MOR135-11 2.20 Olf1648 MOR184-1 10.42 0lf1477 MOR261-11 3.18 0lf1111 MOR181-1 2.17 0lf736 MOR164-1 10.23 0lf144 MOR261-1 3.13 0lf1491 MOR181-1 2.17 0lf173 MOR17-5 9.22 0lf1644 MOR17-5 3.09 0lf1740 MOR18-4 2.16 0lf184 MOR17-5 8.90 0lf1131 MOR17-14 3.09 0lf1780 MOR18-4 2.13 0lf164 MOR18-5 8.90 0lf170 MOR27-15 3.06 0lf170 MOR18-4 2.13 0lf164 MOR18-4 3.31 0lf1679 MOR18-4 2.13 0lf170 MOR18-4 2.13 0lf164 MOR22-6 7.37 0lf1538 MOR18-4 3.30 0lf1690 | Olfr750 | MOR103-18 | 20.43 | Olfr52 | MOR185-6 | 3.33 | Olfr441 | MOR261-3 | 2.23 |
| Olfreide MOR20-16 13.62 Olfrifolde MOR187-2 3.22 Olfrifolde MOR212-4P 2.20 Tara/ 13.70 Olfrif MOR185-13 3.20 Olfrifolde MOR185-2 2.20 Olfrifolde MOR185-11 10.28 Olfrif44 MOR202-5 3.13 Olfrif49 ¹⁹ MOR120-4 2.12 Olfrifolde MOR170-5 9.32 Olfrif04 MOR185-4 3.09 Olfrif64 MOR10-12 2.16 Olfrifold MOR170-5 8.32 Olfrif52 MOR130-10 3.09 Olfrif67 MOR161-1 2.14 Olfrif64 MOR262-713 8.22 Olfrif67 MOR262-715 3.06 Olfrif67 MOR141-1 2.11 Olfrif64 MOR262-713 8.22 Olfrif67 MOR262-713 3.06 Olfrif67 MOR141-1 2.11 Olfrif64 MOR22-71 7.03 Olfrif67 MOR22-71 3.03 Olfrif67 MOR141-1 2.11 Olfrif65 MOR12-7 2.90 Olfrif67 < | Olfr736 | MOR106-5 | 15.62 | 0lfr1123 | MOR264-17 | 3.24 | 0lfr1204 ^a | MOR232-6 | 2.21 |
| Tari2 IS37 Olfri MOR135-11 3.20 Olfr.201 MOR183-11 2.20 Olfr678 MOR163-1 10.28 Olfr1448 MOR202-5 3.13 Olfr491* MOR204-11 2.17 Olfr371 MOR244-11 9.97 Olfr1026 MOR165-4 3.12 Olfr490 MOR171-46 MOR16-12 2.16 Olfr313 MOR170-5 9.20 Olfr1131 MOR17-4 3.09 Olfr609 MOR104-1 2.16 Olfr1047 MOR185-13 8.22 Olfr313 MOR17-4 3.09 Olfr609 MOR164-1 2.14 Olfr1047 MOR24-19 3.06 Olfr51 MOR164-1 2.11 Olfr31 MOR24-27 7.03 Olfr497 MOR24-9 3.03 Olfr510 MOR24-3 2.08 Olfr36 MOR164-2 8.16 Olfr497 MOR24-9 3.03 Olfr510 MOR24-3 2.08 Olfr376 MOR14-1 6.33 Olfr197 MOR24-14 2.06 Olfr510 MOR15-1 | Olfr484 | MOR204-16 | 13.62 | 0lfr1048 | MOR187-2 | 3.22 | 0lfr1500 | MOR212-4P | 2.20 |
| OHT1054 MOR18H=2 10.42 OHT497 MOR261-11 3.18 OHT1111 MOR18H=2 2.20 OHT678 MOR164-1 10.28 OHT148 MOR106-4 3.12 OHT690 MOR171-46 2.16 OHT137 MOR177-5 9.32 OHT1046 MOR176-4 3.09 OHT690 MOR10-12 2.16 OHT44 MOR170-5 8.90 OHT131 MOR177-4 3.09 OHT697 MOR10-12 2.16 OHT44 MOR170-5 8.90 OHT32 MOR170-6 OHT676 MOR24-34 2.11 OHT614 MOR24-33 8.22 OHT497 MOR24-13 3.06 OHT670 MOR141-1 2.11 OHT636 MOR22-1 7.03 OHT497 MOR24-9 3.06 OHT670 MOR24-34 2.01 OHT464 8.81 OHT497 MOR24-14 2.06 OHT670 MOR24-14 2.06 OHT464 MOR14-1 6.32 OHT497 MOR24-14 2.06 0HT670 MOR24-14 | Taar2 | | 13.57 | Olfr1 | MOR135-13 | 3.20 | Olfr20 | MOR135-11 | 2.20 |
| OHR/B2 MOR16-1 10.28 OHr1448 MOR20-5 3.13 OHr647 ^h MOR204-11 2.17 OHr1277 MOR248-11 9.97 OHr1026 MOR195-4 3.12 OHr647 ^h MOR170-5 2.16 OHr844 MOR170-5 9.22 OHr1044 MOR185-4 3.09 OHr649 MOR106-12 2.16 OHr647 MOR185-3 8.57 OHr352 MOR185-10 3.09 OHr676 MOR164-1 2.11 OHr131 MOR24-33 8.36 OHr976 MOR24-10 3.06 OHr570 MOR16-11 2.11 OHr133 MOR24-13 8.22 OHr479 MOR24-14 3.01 OHr510 MOR24-34 2.11 OHr143 MOR151-1 7.37 OHr437 MOR24-13 3.03 OHr510 MOR24-34 2.08 OHr144 MOR151 MOR151 MOR151 MOR151 MOR151 2.09 OHr173 MOR161-1 2.01 OHr1450 MOR161-1 MOR151 MOR151 MOR152 <td>0lfr1054</td> <td>MOR188-2</td> <td>10.42</td> <td>Olfr437</td> <td>MOR261-11</td> <td>3.18</td> <td>0lfr1111</td> <td>MOR181-2</td> <td>2.20</td> | 0lfr1054 | MOR188-2 | 10.42 | Olfr437 | MOR261-11 | 3.18 | 0lfr1111 | MOR181-2 | 2.20 |
| Olf1277 MOR 24-11 9.97 Olfr 1026 MOR 196-4 3.12 Olfr 1930 MOR 176-6 2.16 Olfri 133 MOR 177-5 9.32 Olfr 104 MOR 185-4 3.09 Olfr 260 MOR 106-12 2.16 Olfri 147 MOR 188-3 8.57 Olfri 522 MOR 13-16 3.09 Olfri 676 MOR 24-10 3.09 Olfri 670 MOR 13-1 2.11 Olfri 15 MOR 267-13 8.22 Olfri 67 MOR 22-19 3.06 Olfri 670 MOR 24-13 3.01 Olfri 670 MOR 24-14 3.03 Olfri 670 MOR 24-14 2.04 Olfri 167 MOR 25-67 7.37 Olfri 578 MOR 24-9 3.03 Olfri 670 MOR 24-3 2.08 Olfri 167 MOR 15-1 C.53 Olfri 1033 MOR 16-1 2.11 2.06 Olfri 73 MOR 170-2 2.05 Olfri 167 MOR 16-1 C.14 6.33 Olfri 72 MOR 170-2 2.05 Olfri 167 MOR 16-1 C.14 MOR 16-3 2.97 <td>Olfr878</td> <td>MOR163-1</td> <td>10.28</td> <td>0lfr1448</td> <td>MOR202-5</td> <td>3.13</td> <td>Olfr491^b</td> <td>M0R204-11</td> <td>2.17</td> | Olfr878 | MOR163-1 | 10.28 | 0lfr1448 | MOR202-5 | 3.13 | Olfr491 ^b | M0R204-11 | 2.17 |
| Ohrinsa MORIT7-5 9.32 Ohrinu4 MORIT5-4 3.09 Ohrinu64 MORIT6-12 2.16 Ohrinu44 MORIT5-5 8.90 Ohrinu52 MORIT5-1 3.09 Ohrinu69 MORIT6-1 2.14 Ohrinu47 MORIT8-3 8.70 Ohrinu52 MORIT5-1 3.09 Ohrinu67 MORIT6-1 2.14 Ohrinu51 MORZ45-23 8.36 Ohrinu52 MORIT5-1 2.01 MORIZ67-15 3.06 Ohrinu MORI14-1 2.11 Ohrinu53 MORIG-2 8.16 Ohrinu58 MORIZ0-1 3.03 Ohrinu50 MORIT0-2 2.09 Ohrinu57 MORIG-1-4 6.83 Ohrinu57 MORIG-1-4 6.83 Ohrinu52 3.03 Ohrinu53 MORIT0-2 2.05 Ohrinu57 MORIG-1-4 6.83 Ohrinu33 MORI22-1 2.97 Ohrinu23 MORI20-1 2.05 Ohrinu50 MORI20-2 2.05 Ohrinu33 MORI92-2 2.97 Ohrinu24 MOR20-2 2.05 <t< td=""><td>0lfr1277</td><td>MOR248-11</td><td>9.97</td><td>0lfr1026</td><td>MOR196-4</td><td>3.12</td><td>Olfr930</td><td>MOR171-46</td><td>2.16</td></t<> | 0lfr1277 | MOR248-11 | 9.97 | 0lfr1026 | MOR196-4 | 3.12 | Olfr930 | MOR171-46 | 2.16 |
| Olfrispin MOR170-S 8.90 Olfri 131 MOR173-4 3.09 Olfri809 MOR161-1 2.14 Olfri 1447 MOR 382-3 8.56 Olfri976 MOR24-10 3.09 Olfri876 MOR13-14 2.13 Olfri 16 MOR267-13 8.22 Olfri479 MOR267-15 3.06 Olfri870 MOR24-14 2.11 Olfri 16 MOR24-3 8.6 Olfri978 MOR24-9 3.03 Olfri619 MOR24-3 2.09 Olfri 16 MOR24-14 6.33 Olfri922 MOR161-3 3.00 Olfri90 MOR18-4 2.88 Olfri 36 MOR152-1 7.03 Olfri130 MOR24-31 2.97 Olfri430 MOR16-1 2.08 Olfri 36 MOR15-1 6.42 Olfri130 MOR16-2 2.93 Olfri430 MOR26-1 2.95 Olfri 366 MOR16-1 5.66 Olfri960 MOR16-2 2.93 Olfri430 MOR26-2 1.99 Olfri 365 MOR14-1 5.66 Olfri960 | Olfr153 | MOR177-5 | 9.32 | 0lfr1044 | MOR185-4 | 3.09 | 0lfr746 | MOR106-12 | 2.16 |
| Olf1047 MOR188-3 8.57 Olfr352 MOR136-10 3.09 Olfr376 MOR126-11 2.14 Olf1131 MOR245-23 8.36 Olfr376 MOR267-15 3.06 Olfr370 MOR161-1 2.11 Olf1203 MOR164-2 8.16 Olfr578 MOR159-4 3.03 Olfr510 MOR204-34 2.11 Olf1466 MOR225-17 7.03 Olfr497 MOR226-13 3.03 Olfr510 MOR14-2 2.09 Olfr350 MOR151-4 6.83 Olfr622 MOR159-4 3.03 Olfr497 MOR206-2 2.05 Olfr360 MOR15-2 6.35 Olfr1033 MOR122-13 2.97 Olfr482 MOR206-2 2.05 Olfr360 MOR16-2 6.20 Olfr660 MOR14-2 2.93 Olfr697 MOR26-5 2.04 Olfr379 MOR14-1 6.68 Olfr696 MOR20-23 2.87 Olfr306 MOR26-2 1.95 Olfr379 MOR18-1 5.64 Olfr506 MOR20-13 | Olfr894 | MOR170-5 | 8.90 | 0lfr1131 | MOR177-4 | 3.09 | Olfr809 | MOR108-4 | 2.16 |
| Olf1313 M0R245-23 8.36 Olf676 M0R24-10 3.06 Olf670 M0R16-14 2.13 Olf16 M0R267-13 8.22 Olf479 M0R27-15 3.06 Olf670 M0R14-1 2.11 Olf1710 M0R225-6P 7.37 Olf558 M0R204-9 3.03 Olf619 M0R20-7 2.09 Olf1496 M0R12-1 7.03 Olf6797 M0R204-9 3.03 Olf6190 M0R13-5 2.09 Olf1630 M0R159-1 6.42 Olf1718 M0R122-13 2.97 Olf642 M0R12-1 2.05 Olf1610 M0R162-2 6.20 Olf660 M0R14-2 2.93 Olf6190 M0R26-14 2.01 Olf7650 M0R16-1 5.64 Olf6790 M0R16-2 2.93 Olf6190 M0R26-1 2.91 Olf7630 M0R18-1 5.66 Olf725 M0R16-2 2.93 Olf6190 M0R26-2 1.93 Olf7630 M0R18-1 5.61 Olf7255 M0R20-1 2.76 | 0lfr1047 | MOR188-3 | 8.57 | Olfr352 | MOR136-10 | 3.09 | Olfr876 | MOR161-1 | 2.14 |
| Olfrifo MOR267-13 8.22 Olfr479 MOR267-15 3.06 Olfr870 MOR11-1 2.11 Olfr323 MOR164-2 8.16 Olfr538 MOR224-9 3.06 Olfr510 MOR204-34 2.11 Olfr1486 MOR127-1 7.03 Olfr497 MOR204-9 3.03 Olfr690 MOR17-2 2.09 Olfr675 MOR161-4 6.83 Olfr922 MOR161-3 3.00 Olfr173 MOR18-3 2.08 Olfr675 MOR159-1 6.42 Olfr1198 MOR225-13 2.97 Olfr189 MOR18-2 2.00 Olfr660 MOR17-2 2.95 Olfr1097 MOR26-2 2.05 Olfr676 MOR14-1 6.68 Olfr689 MOR170-8 2.91 Olfr1306 MOR26-7-14 2.01 Olfr676 MOR14-1 5.64 Olfr618 MOR16-3 2.87 Olfr486 MOR20-19 1.99 Olfr1206 MOR25-7 5.64 Olfr1306 MOR23-13 1.98 Olfr490 MOR12-1 1.98 | 0lfr1313 | MOR245-23 | 8.36 | Olfr976 | MOR224-10 | 3.06 | Olfr3 | MOR136-14 | 2.13 |
| Olf923 MOR164-2 8.16 Olf958 MOR224-9 3.06 Olfr510 MOR204-34 2.11 Olf11496 MOR225-6P 7.37 Olfr558 MOR159-4 3.03 Olfr619 MOR17-2 2.09 Olfr3675 MOR161-4 6.83 Olfr622 MOR161-3 3.00 Olfr173 MOR184-3 2.08 Olfr360 MOR19-1 6.42 Olfr103 MOR192-2 2.97 Olfr420 MOR204-14 2.06 Olfr365 MOR14-1 6.08 Olfr699 MOR170-8 2.91 Olfr510 MOR26-1 2.05 Olfr365 MOR14-1 6.08 Olfr699 MOR170-8 2.91 Olfr506 MOR26-14 2.01 Olfr365 MOR14-1 5.66 Olfr506 MOR24-23 2.87 Olfr306 MOR24-2 1.99 Jar7d 5.61 Olfr255 MOR124-1 2.76 Olfr486 MOR24-2 1.98 Olfr1103 MOR245-1 2.76 Olfr486 MOR24-19 1.98 <tr< td=""><td>Olfr16</td><td>MOR267-13</td><td>8.22</td><td>Olfr479</td><td>MOR267-15</td><td>3.06</td><td>Olfr870</td><td>MOR141-1</td><td>2.11</td></tr<> | Olfr16 | MOR267-13 | 8.22 | Olfr479 | MOR267-15 | 3.06 | Olfr870 | MOR141-1 | 2.11 |
| OlfT178 MOR225-6P 7.37 Olff358 MOR159-4 3.03 Olff619 MOR3-5 2.09 OlfT496 MOR127-1 7.03 Olff497 MOR20+9 3.03 Olff900 MOR170-2 2.09 Olff355 MORR161-1 6.42 Olff1708 MOR25-13 2.97 Olff422 MOR20-2 2.05 Olff1016 MOR174-2 6.35 Olff17033 MOR19-2 2.95 Olff1807 MOR20-2 2.05 Olff266 MOR166-2 6.02 Olff690 MOR170-8 2.91 Olff3106 MOR26-1 2.06 Olff247 MOR265-1 5.88 Olff891 MOR16-3 2.87 Olff486 MOR26-2 1.99 Olff132 MOR255-1 5.64 Olff1918 MOR16-3 2.87 Olff486 MOR20-2 1.98 Olff132 MOR257P 5.41 Olff106 MOR24-1 2.75 Olff146 MOR20-1 1.98 Olff1300 MOR18-4 5.26 Olff1706* MOR23-1 <td< td=""><td>Olfr923</td><td>MOR164-2</td><td>8.16</td><td>Olfr958</td><td>MOR224-9</td><td>3.06</td><td>Olfr510</td><td>MOR204-34</td><td>2.11</td></td<> | Olfr923 | MOR164-2 | 8.16 | Olfr958 | MOR224-9 | 3.06 | Olfr510 | MOR204-34 | 2.11 |
| Olfri496 MOR127-1 7.03 Olfr497 MOR204-9 3.03 Olfr900 MOR170-2 2.09 Olfr357 MOR161-4 6.83 Olfr492 MOR161-3 3.00 Olfr173 MOR18-3 2.08 Olfr360 MOR179-1 6.42 Olfr1198 MOR22-13 2.97 Olfr482 MOR204-14 2.06 Olfr1161 MOR174-2 6.35 Olfr1033 MOR199-2 2.95 Olfr1097 MOR206-2 2.05 Olfr565 MOR114-14 6.08 Olfr680 MOR146-2 2.93 Olfr510 MOR25-1 2.04 Olfr555 MOR148-1 5.66 Olfr618 MOR20-23 2.87 Olfr1306 MOR264-1 2.01 Olfr352 MOR148-1 5.64 Olfr355 MOR148-1 8.06 MOR245-1 2.75 Olfr486 MOR204-2 1.98 Olfr1032 MOR257-7 5.41 Olfr1306 MOR245-1 2.75 Olfr1306 MOR15-2 1.98 Olfr1032 MOR225-7 5.41 | 0lfr1178 | MOR225-6P | 7.37 | Olfr358 | MOR159-4 | 3.03 | Olfr619 | MOR31-5 | 2.09 |
| Olf875 MOR161-4 6.83 Olf922 MOR161-3 3.00 Olf1773 MOR18-3 2.08 Olf360 MOR159-1 6.42 Olf1108 MOR225-13 2.97 Olf482 MOR204-14 2.06 Olf1105 MOR16-2 6.35 Olf1103 MOR19-2 2.95 Olf1697 MOR205-1 2.05 Olf7676 MOR14-14 6.08 Olf899 MOR170-8 2.91 Olf1506 MOR245-15 2.04 Olf7679 MOR14-14 6.06 Olf7506 MOR25-15 2.04 Olf7506 MOR267-14 2.01 Olf6750 MOR14-1 5.66 Olf7506 MOR20-12 2.87 Olf17066 MOR20-12 1.99 Taa7d 5.61 Olf7295 MOR20-12 2.76 Olf1481 MOR20-2 1.98 Olf1102 MOR188-4 5.26 Olf11306 MOR230-3 2.74 Olf6908 MOR162-1 1.98 Olf1206 MOR24-1 7.00 Olf7133 MOR230-12 1.98 <tr< td=""><td>0lfr1496</td><td>MOR127-1</td><td>7.03</td><td>Olfr497</td><td>M0R204-9</td><td>3.03</td><td>Olfr900</td><td>MOR170-2</td><td>2.09</td></tr<> | 0lfr1496 | MOR127-1 | 7.03 | Olfr497 | M0R204-9 | 3.03 | Olfr900 | MOR170-2 | 2.09 |
| Olfräße MOR159-1 6.42 Olfr1198 MOR225-13 2.97 Olfr482 MOR204-14 2.06 Olfr1161 MOR174-2 6.35 Olfr1033 MOR19-2 2.95 Olfr1097 MOR206-2 2.05 Olfr366 MOR162-2 6.20 Olfr860 MOR14-2 2.93 Olfr851 MOR25-15 2.04 Olfr267 MOR265-1 5.88 Olfr899 MOR170-8 2.91 Olfr306 MOR245-15 2.04 Olfr355 MOR148-1 5.66 Olfr509 MOR276-2 1.99 Tar7d 5.64 Olfr918 MOR164-3 2.87 Olfr486 MOR204-19 1.99 Olfr1032 MOR199-1 5.61 Olfr205 MOR20-1 2.76 Olfr481 MOR204-2 1.98 Olfr1030 MOR188-4 5.26 Olfr1206 th MOR23-3 2.74 Olfr908 MOR165-2 1.98 Olfr206 th MOR24-1 5.00 Olfr1706 th MOR24-7 2.68 Olfr1303 MOR23-7 1.97< | 0lfr875 | MOR161-4 | 6.83 | Olfr922 | MOR161-3 | 3.00 | Olfr173 | MOR184-3 | 2.08 |
| Off T161 MORT24-2 6.35 Olf T053 MORT99-2 2.95 Olf T1097 MOR26-2 2.05 Olf T1056 MORT44-14 6.08 Olf T699 MORT4-14 2.01 Olf T6509 MOR265-1 2.05 Olf T6509 MOR265-1 2.01 Olf T6509 MOR265-1 2.01 Olf T6509 MOR265-1 2.01 Olf T630 MOR204-2 1.99 Tar7d 5.64 Olf T1918 MOR164-3 2.87 Olf T486 MOR204-19 1.99 Olf T132 MOR199-1 5.61 Olf T1305 MOR202-1 2.75 Olf T486 MOR231-13 1.98 Olf T1302 MOR245-7 2.68 Olf T1325 MOR105-2 1.98 Olf T1200 ⁶ MOR245-7 2.68 Olf T138 MOR252-9 1.97 Olf T420 ⁶ MOR245-7 2.68 Olf T138 MOR232- | Olfr360 | MOR159-1 | 6 42 | 0lfr1198 | MOR225-13 | 2 97 | 0lfr482 | MOR204-14 | 2.06 |
| Olfri056 MOR186-2 6.20 Olfrå60 MOR146-2 2.93 Olfrå51 MOR155-1 2.05 Olfrå67 MOR141-14 6.08 Olfrå89 MOR170-8 2.91 Olfrå509 MOR245-15 2.04 Olfrå67 MOR265-1 5.88 Olfrå81 MOR162-7 2.90 Olfrå509 MOR267-14 2.01 Olfrå55 MOR148-1 5.66 Olfrå70 MOR204-19 1.99 Taar7d 5.64 Olfrå186 MOR204-13 2.87 Olfrå86 MOR204-19 1.99 Olfr1020 MOR199-1 5.61 Olfr225 MOR204-1 2.75 Olfr1286 MOR204-13 1.98 Olfr1020 MOR188-4 5.26 Olfr1206 ^b MOR20-3 2.74 Olfr308 MOR259-9 1.97 Taar7f 5.19 Olfr1706 ^b MOR14-3 2.67 Olfr1308 MOR259-9 1.97 Olfr490 MOR24+1 5.00 Olfr1331 MOR259-3P 2.63 Olfr1308 MOR237-2 1.98 <t< td=""><td>0lfr1161</td><td>MOR174-2</td><td>6.35</td><td>0lfr1033</td><td>MOR199-2</td><td>2.95</td><td>0lfr1097</td><td>MOR206-2</td><td>2.05</td></t<> | 0lfr1161 | MOR174-2 | 6.35 | 0lfr1033 | MOR199-2 | 2.95 | 0lfr1097 | MOR206-2 | 2.05 |
| Olfr369 MOR14-14 6.08 MOR170-8 2.91 Olfr305 MOR245-15 2.04 Olfr474 MOR265-1 5.88 Olfr806 MOR162-7 2.90 Olfr509 MOR267-14 2.01 Olfr355 MOR148-1 5.66 Olfr506 MOR204-23 2.87 Olfr1396 ^b MOR204-19 1.99 Jar7d 5.64 Olfr305 MOR204-23 2.87 Olfr481 MOR204-12 1.98 Olfr1032 MOR199-1 5.61 Olfr295 MOR20-1 2.76 Olfr481 MOR204-2 1.98 Olfr1032 MOR188-4 5.26 Olfr1206 ^b MOR230-3 2.74 Olfr338 MOR259-9 1.97 Olfr1206 ^b MOR245-1 2.75 Olfr1338 MOR259-9 1.97 Olfr4707 MOR14-9 4.92 Olfr040 MOR167-3 2.67 Olfr11308 MOR259-3 1.97 Olfr490 MOR204-17 4.71 Olfr1303 MOR227-8P 2.60 Olfr145 MOR161-6 1.95 | 0lfr1056 | MOR186-2 | 6.20 | Olfr860 | MOR146-2 | 2.93 | Olfr851 | MOR155-1 | 2.05 |
| Diff 30 MORTA 1. Diff 30 MORTA 2. Diff 30 MOR267-14 2.01 Diff 355 MOR148-1 5.66 Olfr 506 MOR204-23 2.87 Olfr 309 ⁶ MOR207-14 2.01 Diff 355 MOR148-1 5.66 Olfr 505 MOR20-12 2.87 Olfr 486 MOR204-19 1.99 Jaar 7d 5.61 Olfr 505 MOR20-1 2.76 Olfr 486 MOR204-2 1.98 Olfr 102 MOR25-7P 5.41 Olfr 1306 MOR245-1 2.75 Olfr 1024 MOR231-13 1.98 Olfr 1200 MOR245-1 5.09 Olfr 1033 MOR245-7 2.68 Olfr 1032 MOR237-2 1.96 Olfr 200 MOR245-1 5.00 Olfr 1333 MOR245-7 2.68 Olfr 1160 MOR173-1 1.97 Olfr 400 MOR24-17 4.71 Olfr 1034 MOR227-8P 2.60 Olfr 1160 MOR173-1 1.97 Olfr 405 MOR18-2 4.27 Olfr 1034 MOR227-8P 2.60 Olf | Olfr769 | MOR114-14 | 6.08 | Olfr899 | MOR170-8 | 2.93 | 0lfr1306 | MOR245-15 | 2.03 |
| Internation Indication Internation Internation <thinternation< th=""> <thinternation< th=""></thinternation<></thinternation<> | 0lfr247 | MOR265-1 | 5.88 | Olfr881 | MOR162-7 | 2.91 | Olfr509 | MOR267-14 | 2.01 |
| Tar7d 5.64 Off 918 MOR14-12 2.83 Off 486 MOR204-19 1.99 Olf 1032 M0R199-1 5.61 Olf 7918 MOR20-1 2.76 Olf 486 MOR204-2 1.98 Olf 11032 M0R198-4 5.26 Olf 1106 ⁶ MOR245-1 2.75 Olf 11254 MOR231-13 1.98 Olf 11000 M0R188-4 5.26 Olf 1106 ⁶ MOR245-1 2.75 Olf 11325 MOR102-1 1.98 Olf 1280 ⁶ M0R248-1 5.00 Olf 11303 MOR245-7 2.68 Olf 11325 MOR102-1 1.98 Olf 1280 ⁶ M0R248-1 5.00 Olf 11303 MOR245-7 2.68 Olf 1138 MOR237-2 1.96 Olf 1490 MOR204-17 4.71 Olf 13131 MOR227-8P 2.60 Olf 1145 MOR137-1 1.97 Olf 1045 MOR185-2 4.27 Olf 1308 MOR245-22 2.59 Olf 1120 ⁴ MOR232-7 1.95 Olf 1045 MOR185-2 4.27 Olf 1424 | 0lfr855 | MOR148-1 | 5.66 | Olfr506 | MOR204-23 | 2.50 | Olfr1396 ^b | MOR276-2 | 1 99 |
| Narra Joh Onr Joh Non 13 Los Onr Joh Non 21 Joh Onr Joh Non 21 Joh Onr Joh Non 21 Joh Onr Joh Non 22 Joh Insta Non 22 Joh Non 22 Joh Joh< | Taar7d | | 5 64 | Olfr918 | MOR164-3 | 2.87 | 0lfr486 | MOR204-19 | 1.99 |
| Diff 182 MRR25 5.3 Diff 23 MR25 1.75 Diff 33 MR25 1.75 Diff 35 MR15 1.75 0lfr 1090 MOR225-7P 5.41 Olfr 136 MOR245-1 2.75 Olfr 1325 MOR21-1 1.98 Olfr 1090 MOR248-1 5.00 Olfr 1303 MOR245-7 2.68 Olfr 1338 MOR259-9 1.97 Olfr 200 MOR204-17 4.71 Olfr 1331 MOR259-3P 2.67 Olfr 1160 MOR173-1 1.96 Olfr 1040 MOR204-17 4.71 Olfr 1301 MOR259-3P 2.63 Olfr 145 MOR161-6 1.95 Olfr 1045 MOR185-2 4.27 Olfr 1308 MOR26-3 2.58 Olfr 1020 ^{ar} MOR232-7 1.95 Olfr 1045 MOR185-5 4.20 Olfr 1276 MOR26-1 2.54 Olfr 1202 ^{ar} MOR223-2 1.94 Olfr 1087 MOR283-10P 4.07 Olfr 1276 MOR26-1 2.53 Olfr 1477 MOR20-10 1.92 Olfr 1489 </td <td>Olfr1032</td> <td>MOR199-1</td> <td>5.61</td> <td>Olfr295</td> <td>MOR220-1</td> <td>2.07</td> <td>0lfr481</td> <td>MOR204-2</td> <td>1.99</td> | Olfr1032 | MOR199-1 | 5.61 | Olfr295 | MOR220-1 | 2.07 | 0lfr481 | MOR204-2 | 1.99 |
| Diff 102 More 2011 Diff 103 More 2011 Diff 103< | 0lfr1182 | MOR225-7P | 5 41 | 0lfr1316 | M0R245-1 | 2.76 | 0lfr1254 | MOR231-13 | 1.98 |
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| Olfr707 MOR114-9 4.92 Olfr904 MOR167-3 2.67 Olfr105 MOR173-1 1.97 Olfr490 MOR24-17 4.71 Olfr131 MOR259-3P 2.63 Olfr1160 MOR173-1 1.97 Olfr106 MOR171-3 4.61 Olfr1034 MOR227-8P 2.60 Olfr145 MOR161-6 1.95 Olfr1045 MOR185-2 4.27 Olfr1038 MOR26-3 2.58 Olfr980 MOR223-2 1.94 Olfr1087 MOR188-5 4.20 Olfr1276 MOR26-3 2.58 Olfr1208 MOR225-4 1.93 Olfr1087 MOR188-5 4.20 Olfr1276 MOR26-1 2.54 Olfr1208 MOR225-4 1.93 Olfr149 MOR205-10 2.53 Olfr1477 MOR202-10 1.92 Olfr1494 MOR266-1 2.48 Olfr94 MOR114-11 1.89 Olfr149 MOR205-1 2.46 Olfr312 MOR222-4P 1.86 Olfr149 MOR204-19 4.07 Olfr1243 MOR21 | Olfr1280 ^b | MOR248-1 | 5.00 | 0lfr1303 | M0R245-7 | 2.68 | 0lfr1338 | MOR259-9 | 1.90 |
| Offrago MoRT17 MoR114 MoR114 MoR114 | 0lfr777 | MOR114-9 | 4 97 | Olfr904 | MOR167-3 | 2.00 | 0lfr1160 | MOR173-1 | 1.97 |
| Off 160 MOREOT 17 Infinition MOREOT 17 MOREOT 19 MOREOT 17 MOREOT 17 MOREOT 19 | Olfr490 | MOR204-17 | 4 71 | 0lfr1331 | MOR259-3P | 2.67 | Olfr1189 ^b | MOR237-2 | 1.97 |
| Offrido Month's Hont Offrido Month's M | 0lfr160 | MOR171-3 | 4 61 | 0lfr1034 | MOR227-8P | 2.60 | Olfr145 | MOR161-6 | 1.95 |
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| Offride Inforce Inforce <t< td=""><td>Olfr982</td><td>MOR703-2</td><td>4.26</td><td>0lfr1099</td><td>MOR215 22 MOR206-3</td><td>2.59</td><td>01fr980</td><td>MOR222-7</td><td>1.95</td></t<> | Olfr982 | MOR703-2 | 4.26 | 0lfr1099 | MOR215 22 MOR206-3 | 2.59 | 01fr980 | MOR222-7 | 1.95 |
| Offrido Montoo J | 0lfr1087 | MOR188-5 | 4 20 | Olfr474 | MOR105-2 | 2.50 | 0lfr1208 | MOR225-2 | 1.91 |
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| Offrid MOR202 19 F.O. Offrid MOR102 19 F.O. Offrid MOR102 19 MOR202 19 MOR202 11 MOR202 11 MOR202 11 MOR202 11 MOR202 11 MOR201 12 MOR202 11 MOR201 12 | 0lfr1489 | MOR202-19 | 4.07 | 0lfr1030 | MOR196-2 | 2.10 | Olfr312 | MOR727_4P | 1.05 |
| Olfr112 Molt2191 Molt2191 Molt2191 2.35 Olfr00 Molt1112 Molt2191 Molt2144 Molt2191 Molt2191 Molt2144 Molt2191 Molt2144 Molt2191 Molt2144 Molt2191 Molt2144 Molt2191 Molt2144 Molt2191 Mo | 0lfr1122 | MOR264-1 | 4.07 | 0lfr294 | MOR719-5 | 2.40 | 01fr780 | MOR114_17 | 1.00 |
| Offride MOR204-33P 4.02 Offridat MOR214-4 2.37 Offridat MOR171-17 1.30 Olfr467 MOR204-33P 4.02 Olfr1434 MOR214-4 2.37 Olfr935 MOR171-11 1.79 Olfr819 MOR265-2_p 3.99 Olfr738 MOR106-3 2.35 Olfr414 MOR179-5 1.78 Olfr706 MOR283-11 3.91 Olfr1201 MOR20-2 2.33 Olfr859 MOR146-3 1.77 Olfr1307 MOR245-19P 3.82 Olfr803 MOR111-3 2.33 Olfr693 MOR283-2 1.77 Olfr1366 MOR130-2 3.78 Olfr1281 MOR248-18 2.30 Olfr693 MOR283-2 1.76 Olfr1272 MOR227-3 3.68 Olfr771 MOR14-8 2.29 Olfr444 MOR261-2 1.76 Olfr521 MOR101-2 3.67 Olfr815 MOR219-5 2.29 Olfr444 MOR261-2 1.76 | 0lfr11/0 | MOR177_6 | 4.07 | 011224 01fr12/13 | MOR212 5 | 2.35 | Olfr074 | MOR171_47 | 1.04 |
| Olfr819 MOR265-2_p 3.99 Olfr738 MOR106-3 2.35 Olfr41 MOR179-5 1.78 Olfr819 MOR265-2_p 3.99 Olfr738 MOR106-3 2.35 Olfr41 MOR179-5 1.78 Olfr706 MOR265-11 3.91 Olfr1201 MOR20-2 2.33 Olfr859 MOR146-3 1.77 Olfr1307 MOR245-19P 3.82 Olfr803 MOR11-3 2.33 Olfr705 MOR283-2 1.77 Olfr1366 MOR130-2 3.78 Olfr1281 MOR248-18 2.30 Olfr693 MOR283-8 1.76 Olfr1272 MOR227-3 3.68 Olfr771 MOR14-8 2.29 Olfr444 MOR261-2 1.76 Olfr511 MOR10-2 3.67 Olfr855 MOR219-5 2.29 Olfr470 MOR261-2 1.76 | Olfr467 | MOR204_33P | 4.05 | 01fr1434 | MOR214-4 | 2.37 | Olfr935 | MOR171-47 | 1.00 |
| Olfr706 MOR283-11 3.91 Olfr1201 MOR230-2 2.33 Olfr859 MOR175-5 1.76 Olfr706 MOR285-11P 3.91 Olfr1201 MOR230-2 2.33 Olfr859 MOR16-3 1.77 Olfr1307 MOR245-19P 3.82 Olfr803 MOR11-3 2.33 Olfr705 MOR283-2 1.77 Olfr1366 MOR130-2 3.78 Olfr1281 MOR248-18 2.30 Olfr693 MOR283-8 1.76 Olfr1272 MOR227-3 3.68 Olfr771 MOR14-8 2.29 Olfr444 MOR261-2 1.76 Olfr521 MOR10-2 3.67 Olfr855 MOR219-5 2.29 Olfr440 MOR204-22 1.76 | 011407 01fr810 | MOR265_2 n | 3 00 | 01fr738 | MOR106_3 | 2.37 | 0lfr1/1 | MOR170_5 | 1.79 |
| Olfr100 MOR205-11 3.51 Olfr1201 MOR205-2 2.33 Olfr055 MOR140-5 1.77 Olfr1307 MOR245-19P 3.82 Olfr803 MOR111-3 2.33 Olfr655 MOR283-2 1.77 Olfr1366 MOR130-2 3.78 Olfr1281 MOR248-18 2.30 Olfr693 MOR283-8 1.76 Olfr1272 MOR227-3 3.68 Olfr771 MOR14-8 2.29 Olfr444 MOR261-2 1.76 Olfr511 MOR101-2 3.67 Olfr815 MOR219-5 2.29 Olfr440 MOR24-22 1.76 | Olfr706 | MOR203-2_P MOR203-11 | 3.99 | 01fr1201 | MOR220-2 | 2.33 | 0117850 | MOR1/6-2 | 1.70 |
| Olfr1366 MOR130-2 3.78 Olfr1281 MOR248-18 2.30 Olfr693 MOR283-8 1.76 Olfr1272 MOR227-3 3.68 Olfr771 MOR114-8 2.29 Olfr444 MOR261-2 1.76 Olfr521 MOR101-2 3.67 Olfr835 MOR219-5 2.29 Olfr470 MOR204-22 1.76 | 0lfr1307 | MOR245-19P | 3.21 | Olfr803 | MOR111_3 | 2.33 | Olfr705 | MOR283_2 | 1.// |
| Olfr1201 Mon246-16 2.30 Ollf035 Mon266 1.70 Olfr1272 MOR227-3 3.68 Olfr771 MOR114-8 2.29 Olfr444 MOR261-2 1.76 Olfr521 MOR101-2 3.67 Olfr835 MOR219-5 2.29 Olfr444 MOR261-2 1.76 | 0lfr1366 | MOR120_7 | 3.02 | 01fr1791 | MOR7/8_18 | 2.33 | Olfr603 | MOR203-2 | 1.77 |
| Olfr521 MOR101-2 3.67 Olfr835 MOR2019-5 2.27 Ollfr470 MOR201-2 1.70 | 0111300 | MOR777_2 | 3.70 | 0117201 01fr771 | MOR11/LQ | 2.30 | Olfr/1/1 | MOR261_2 | 1.70 |
| | 0lfr521 | MOR101-2 | 3 67 | Olfr835 | MOR219-5 | 2.29 | Olfr470 | MOR201-2 | 1.70 |

^aAlso responsive to artificial cigarette smoke odor and 1-pentanethiol.

^bAlso responsive to 1-pentanethiol.

^cAlso responsive to artificial cigarette smoke odor.

artificial cigarette smoke odor should represent receptors critical for forming a response pattern that is perceived by the brain as similar to the odor of cigarette smoke. Identifying these highly sensitive receptors would be complicated if some ORs have nonmonotonic dose-response relationships with their odorant agonists, a phenomenon predicted by observations of olfactory bulb glomeruli dropping out of glomerular response patterns as odorant concentration increases and confirmed by measures of OR responses *in vivo* (Friedrich and Korsching, 1997; Rubin and Katz, 1999; Meister and Bonhoeffer, 2001; Wachowiak and Cohen, 2001; Fried et al., 2002; Bozza et al., 2004; Hu et al., 2020; McClintock et al., 2020). To ensure that we identified sensitive ORs, we therefore tested artificial cigarette smoke odor under conditions where the odor concentration reaching the mice was substantially less than saturation. Under these conditions, four ORs gave significant responses (Fig. 2*A*). These responses were specific to artificial cigarette smoke odor and did not occur when the mice were exposed to clean air (Fig. 2*B*). Three of these ORs, Olfr1202, Olfr1204, and Olfr1257, belong to the MOR232 family, while Olfr705 belongs to the MOR283 family (Fig. 2*C*). These two OR families have 10 or more members, and both have multiple members responsive to real cigarette smoke, including all four ORs responsive to artificial cigarette smoke odor (Fig. 2*D*).

1-Pentanethiol is important for the perception of artificial cigarette smoke odor

To determine which odorants matter most to the artificial mimic of cigarette smoke odor, we asked human volunteers to rate the similarity of artificial cigarette smoke odor to 26 mixtures, each lacking one of the 26 odorants in the full mixture. Only two of these depletion mixtures prove to be perceived by human subjects as significantly different from the full mixture. Mixtures lacking 1-pentanethiol and 2methoxyphenol are perceived as different from artificial cigarette smoke odor and from the depletion mixture most similar to artificial cigarette smoke odor (Fig. 3).

The importance of 1-pentanethiol and 2-methoxyphenol for the percept evoked by artificial cigarette smoke odor could arise via either of two very different mechanisms. The most straightforward mechanism is that these odorants evoke responses from ORs important to the cigarette smoke response pattern, and therefore by themselves evoke percepts resembling that of artificial cigarette smoke odor. Alternatively, many odorants are not only agonists at some ORs but are also antagonists at other ORs (Araneda et al., 2000; Spehr et al., 2003; Oka et al., 2004a,b; Sanz et al., 2005, 2008; Reisert, 2010), a phenomenon that is common in vivo (de March et al., 2020). Such antagonist effects could be critical for the perception of odor mixtures because of their ability to substantially alter the OR response pattern. To discriminate between these mechanisms, we asked human volunteers to rate the similarity and pleasantness of 14 individual odorants to artificial cigarette smoke odor. Odorants whose percepts resemble that of artificial cigarette smoke odor are unlikely to be important to the perception of cigarette smoke odor solely through receptor antagonism. The 14 odorants included 11 odorants present in the artificial cigarette smoke odor mixture, including the four odorants whose absence most affected perception of artificial cigarette smoke odor (1-pentanethiol, 2methoxyphenol, 2-ethyl-3-methylpyrazine, and 5-methylfurfural) and seven odorants whose absence had little effect on the perception of artificial cigarette smoke odor (2,4-hexedienal, methyl isonicotinate, pyridine, 2-methylphenol, allylamine, indene, and diacetyl). Other odorants were included to act as outliers (acetophenone, DL-limonene, and 2-menthene). These experiments identify 1-pentanethiol, 2ethyl-3-methylpyrazine, allylamine, and indene as having properties expected of an odorant acting as an important



Figure 2. ORs responsive to artificial cigarette smoke *in vivo.* **A**, In mice exposed to a low concentration of artificial cigarette smoke odor (artCSO), only four receptors respond, and they are all ORs. Delta is the artificial cigarette smoke odor response relative to the clean air control response. **B**, The distribution of responses, measured as the fold difference (FD) enrichment of receptor mRNAs in samples of active OSNs compared with inactive OSNs, identifies responses specific to artificial cigarette smoke odor versus the clean air control (red). **C**, Of the responsive ORs, three are closely related to each other and not related to Olfr705, the other responsive OR. **D**, All four of the ORs responsive to a low concentration of artificial cigarette smoke odor (artCSO) also respond to cigarette smoke (blue squares).



Figure 3. 1-Pentanethiol and 2-methoxyphenol matter most. Subjects (n = 18) rated 26 odor mixtures, each lacking one of the components of an artificial mimic of cigarette smoke odor, scoring the degree of similarity to the full mixture. The depleted mixture most similar to the full mixture lacks 2,4-hexedienal (#25), and only the mixtures lacking 1-pentanethiol (#20) and 2-methoxyphenol (#9) are significantly different from it. Odorants: 1, pyridine (t = 0.60, p = 0.553); 2, nicotinalde-hyde (t = 1.65, p = 0.101); 3, 5-ethyl-2-methylpyridine (t = 1.10, p = 0.278); 4, 2-vinylpyridine (t = 0.72, p = 0.474); 5, 4-pyridinecarbonitrile (t = 0.63, p = 532); 6, methyl isonicotinate (t = 0.29, p = 775); 7, 2-ethyl-3-methylpyrazine (t = 2.34, p = 0.024); 8, 2-methoxy-3,5-methylpyrazine (t = 0.32, p = 0.747); 9, 2-methoxyphenol (t = 2.78, p = 0.008); 10, 2-methylphenol (t = 0.39, p = 0.696); 11, 5-methylfurfural (t = 2.28, p = 0.028); 12, 2,5-dimethylpyrrole (t = 1.81, p = 0.078); 13, phenylacetylene (t = 1.41, p = 0.166); 14, isoprene (t = 1.30, p = 0.201); 15, 1,3-cyclohexadiene (t = 1.38, p = 174); 16, indene (t = 1.28, p = 0.207); 17, allylbenzene (t = 1.50, p = 142); 18, diacetyl (t = 1.21, p = 0.231); 19, thiophene (t = 0.82, p = 0.474); 20, 1-pentanethiol (t = 0.39, p = 0.004); 21, allylamine (t = 1.01, p = 0.316); 22, 1-aminopentane (t = 0.85, p = 0.402); 23, butyric acid (t = 0.72, p = 0.475); 24, 1-hexanal (t = 0.72, p = 0.477); 25, 2,4-hexedienal; 26, indole (t = 0.62, p = 0.541). **p < 0.01.



Figure 4. Odorants similar to cigarette smoke include 1-pentanethiol. *A*, Subjects (n = 27) rated the pleasantness of 14 monomolecular odorants, finding that only six of these odorants were not significantly different from artificial cigarette smoke odor (mix). *B*, Four of these six odorants also failed to be dissimilar from artificial cigarette smoke odor. Odorants: 1, 1-pentanethiol (pleasantness t = 0.61, p = 0.538; similarity t = 2.10, p = 0.040); 2, 2-methoxyphenol (t = -4.30, p = 0.000); t = 3.88, p = 0.000); 3, 2-ethyl-3-methylpyrazine (t = -1.07, p = 0.291); 4, 5-methylfurfural (t = -4.10, p = 0.000); 5, diacetyl (t = -2.68, p = 0.009; t = 1.25, p = 0.218); 6, allylamine (t = -0.61, p = 0.542; t = 0.33, p = 0.744); 7, pyridine (t = -0.01, p = 0.990; t = 3.33, p = 0.002); 8, 2-methylphenol (t = -2.54, p = 0.015; t = 3.95, p = 0.000); 9, methyl isonicotinate (t = -5.59, p = 0.000; t = 6.86, p = 0.000); 10, indene (t = -1.08, p = 0.287; t = 2.02, p = 0.048); 11, 2,4-hexedienal (t = -4.21, p = 0.000; t = 8.24, p = 0.000); 12, acetophenone (t = -6.36, p = 0.000; t = 5.64, p = 0.000); 13, DL-limonene (t = -8.71, p = 0.000; t = 8.31, p = 0.000); 14, 2-menthene (t = -8.34, p = 0.000; t = 7.78, p = 0.000). **p < 0.01.

agonist in artificial cigarette smoke odor. They are not significantly different from artificial cigarette smoke odor in terms of perceived similarity or in terms of perceived pleasantness (Fig. 4). Overall, 1-pentanethiol is the only odorant whose absence has a significant effect on the perception of artificial cigarette smoke and also is perceived by itself as similar to cigarette smoke odor by humans.

ORs responsive to 1-pentanethiol include ORs responsive to cigarette smoke

If 1-pentanethiol is truly important for the perception of cigarette odors, both real cigarette smoke and artificial mimics, we should find that several ORs responsive to it are among the ORs responsive to cigarette smoke. To test this hypothesis, we exposed freely behaving mice to headspace air above a solution of 25% 1-pentanethiol. This dilution exposes the mice to a relatively high, but not saturating, concentration of 1-pentanethiol and should result in responses from a substantial number of ORs. This prediction proved correct, with 58 ORs responding to 1-pentanethiol and not to the clean air control (Fig. 5A,B; Table 3). Of the 58 responsive ORs, 9 also respond to cigarette smoke: Olfr176, Olfr491, Olfr1137, Olfr1189, Olfr1202, Olfr1204, Olfr1206, Olfr1280, and Olfr1396. Of these, Olfr1137 is of particular interest because its response to cigarette smoke is the third largest we observed. In addition, both Olfr1202 and Olfr1204 also respond to artificial cigarette smoke odor.

The ORs responsive to 1-pentanethiol are distributed in clusters across the OR sequence distance tree, with 28 OR families containing at least 1 responsive OR and 13 OR families containing multiple responsive ORs (Fig. 5D). This response pattern is less widely distributed than that of cigarette smoke, and in certain OR families a large fraction of their ORs respond to 1-pentanethiol (Fig. 5*E*). In particular, a majority of ORs in families MOR230, MOR275, and MOR234 are responsive. This finding suggests that the ORs in these families might be particularly sensitive to thiols, or perhaps more broadly sensitive to short chain odorants.

Among the mouse ORs responsive to 1-pentanethiol are several that are common to receptor response patterns to odors that give rise to percepts related to cigarette smoke in humans. For example, the four ORs highly sensitive to artificial cigarette smoke odor are all part of the response pattern to real cigarette smoke and the response pattern to 1-pentanethiol contains 9 ORs responsive to real cigarette smoke. These overlaps in responsive ORs reflect a degree of similarity in the overall OR response patterns for artificial cigarette smoke odor, 1-pentanethiol, and real cigarette smoke. To assess the importance of these degrees of similarity, we compared response magnitudes measured for all ORs and TAARS in the in vivo assay for nine different odors: the three odors tested herein and six odors whose responses we published previously (Fig. 6). As expected, the patterns of response magnitude are significantly similar between 1-pentanethiol and artificial cigarette smoke odor, consist-

ent with the finding that human subjects judge 1-pentanethiol to be the odorant component most important for perception of artificial cigarette smoke odor. Significantly similar pairs of response patterns also include the response patterns to concentrations of the same odor, a three-odorant mixture called a citrus accord, and the response patterns of two aldehydes, bourgeonal and undecanal. However, neither the 1-pentanethiol response pattern nor the artificial cigarette smoke odor response pattern is significantly similar to the response pattern to real cigarette smoke. These results suggest that the perception of two odors can in some cases be similar despite having large differences in OR response patterns.

Discussion

The data obtained in this project, when interpreted in the context of our prior understanding of the initial encoding of odor signals, support five conclusions. (1) ORs responsive to 1-pentanethiol are important elements of the pattern of ORs responsive to cigarette smoke and related odors. In mice, support for this assertion is found in the ORs whose responses are shared across 1-pentanethiol, artificial cigarette smoke odor, and real cigarette smoke. In humans, support is found in the impact of removing 1-pentanethiol from artificial cigarette smoke odor and the similarity in perception between artificial cigarette smoke odor and 1-pentanethiol. (2) The cigarette smoke receptor response pattern is robust and broad. Even the 28 ORs most strongly responsive to it are widely divergent sequences distributed across 21 OR families. (3) The cigarette smoke OR response pattern is the first in vivo response pattern observed to include both ORs and TAARs, consistent with the presence of odorants containing amine groups among the >400 odorants found in cigarette smoke (Rodgman



Figure 5. ORs responsive to 1-pentanethiol *in vivo.* **A**, In mice, 58 ORs respond to headspace air above a solution of 25% 1-pentanethiol. Delta is the 1-pentanethiol response relative to the clean air control response. FDR values capped at 10⁻¹⁰. **B**, The distribution of responses, measured as the fold difference (FD) enrichment of receptor mRNAs in samples of active OSNs compared with inactive OSNs, identifies responses specific to 1-pentanethiol versus the dean air control. **C**, The responsive ORs are distributed in several clusters across the Class II portion of the OR sequence distance tree. **D**, Of the responsive ORs, nine are also responsive to cigarette smoke (blue squares). **F**, Clustering MOR families via similarity in family size and proportion of ORs responsive to 1-pentanethiol. Only a few OR families contain large fractions of responsive ORs. Only OR families containing responsive ORs are shown. Circle size reflects family size.

| OR | MOR name | Delta | OR | MOR name | Delta | OR | MOR name | Delta |
|-----------------------|-----------|-------|-----------------------------|-----------|-------|-----------------------------|-----------|-------|
| Olfr328 | MOR275-2 | 41.90 | 0lfr1261 | MOR234-3 | 3.34 | Olfr1280 ^b | MOR248-1 | 2.46 |
| Olfr195 | MOR184-5 | 21.08 | Olfr491 ^b | MOR204-11 | 3.31 | 0lfr1232 | MOR233-18 | 2.44 |
| Olfr224 | MOR275-3 | 20.31 | Olfr329-ps | MOR275-6P | 3.19 | 0lfr1299 | MOR248-8 | 2.44 |
| Olfr1183 | MOR230-6 | 20.25 | Olfr124 | MOR256-3 | 3.13 | 0lfr1256 | MOR231-1 | 2.43 |
| Olfr330 | MOR275-1 | 10.52 | <i>Olfr1204^a</i> | MOR232-6 | 2.97 | 0lfr1427 | MOR239-4 | 2.38 |
| Olfr1193 | MOR226-1 | 10.19 | Olfr31 | MOR274-1 | 2.89 | Olfr192 | MOR183-x | 2.37 |
| Olfr1395 | MOR277-1 | 8.76 | Olfr1195 | MOR230-4 | 2.86 | Olfr331 | MOR275-4 | 2.36 |
| Olfr325 | MOR275-5 | 8.38 | Olfr48 | MOR232-5 | 2.80 | 0lfr1262 | MOR234-1 | 2.35 |
| 0lfr1279 | MOR245-12 | 7.69 | Olfr193 | MOR183-7P | 2.78 | 0lfr1260 | MOR232-2 | 2.26 |
| Olfr286 | MOR286-2 | 7.39 | Olfr183 | MOR183-2 | 2.76 | 0lfr1209 | MOR230-7 | 2.25 |
| Olfr1284 | MOR245-13 | 7.29 | Olfr723 | MOR247-4 | 2.71 | 0lfr1288 | MOR245-9 | 2.17 |
| 0lfr1212 | MOR233-17 | 7.19 | Olfr165 | MOR279-1 | 2.71 | 0lfr1321 | MOR264-26 | 2.16 |
| 0lfr1394 | MOR280-1 | 5.46 | Olfr191 | MOR183-5P | 2.68 | Olfr724 | MOR247-2 | 2.12 |
| Olfr1189 ^b | MOR237-2 | 4.55 | Olfr1263 | MOR234-2 | 2.66 | Olfr176 ^b | MOR184-8 | 2.11 |
| Olfr1396 ^b | MOR276-2 | 4.21 | 0lfr1342 | MOR258-3 | 2.66 | Olfr15 | MOR256-17 | 2.11 |
| Olfr175-ps1 | MOR184-1 | 4.02 | Olfr1359 | MOR256-35 | 2.65 | <i>Olfr1202^a</i> | MOR232-7 | 2.09 |
| 0lfr1186 | MOR230-5 | 4.00 | Olfr1265 | MOR228-2 | 2.63 | Olfr464 | MOR240-2 | 2.08 |
| Olfr1137 ^b | MOR177-20 | 3.60 | 0lfr1200 | MOR225-12 | 2.63 | 0lfr1242 | MOR231-5 | 2.03 |
| Olfr1206 ^b | MOR230-3 | 3.42 | Olfr476 | MOR204-3 | 2.58 | | | |
| Olfr1199 | MOR230-8 | 3.41 | 0lfr1205 | MOR230-1 | 2.52 | | | |

| Table 3. OKs responsive to 1-pentar |
|-------------------------------------|
|-------------------------------------|

^aAlso responsive to cigarette smoke and artificial cigarette smoke odor.

^bAlso responsive to cigarette smoke.

and Perfetti, 2013). (4) Class I ORs are not important to the perception of cigarette smoke. Class II ORs constitute all but one of the ORs responsive to cigarette smoke and all of the ORs responsive to 1-pentanethiol and artificial cigarette smoke odor. (5) Our data provide the first example of odors evoking similar percepts while sharing ORs in their *in vivo* response patterns. This is an anticipated result, but we are not confident this correlation will always prove true and urge caution before presuming that it does.

1-Pentanethiol is important to the perception of odors related to cigarette smoke, and ORs responsive to it are part of the OR response pattern to cigarette smoke and artificial cigarette smoke



Figure 6. Response pattern similarity. Cluster analysis displays relative similarity between *in vivo* receptor response patterns to nine odors tested in this study or published previously (de March et al., 2020; McClintock et al., 2020). Statistical analysis identifies three significantly similar response patterns, including that of 1-pentanethiol (Pentanethiol) and artificial cigarette smoke odor (Artificial cigarette). The receptor response pattern to cigarette smoke (Real cigarette) is not similar to any of the other response patterns. The citrus accord odors (Citrus), which are mixtures of three odorants, differ by 20-fold in the concentrations used as stimuli (5% vs 100%).

odor. Our human testing data confirm that 1-pentanethiol evokes a percept similar to artificial cigarette smoke odor; and correspondingly, the mouse OR response patterns between these two odors are more similar than chance. However, ORs responsive to 1-pentanethiol explain only a small part of the receptor response to real cigarette smoke, a portion insufficient to produce significant similarity between the overall response patterns of 1-pentanethiol and real cigarette smoke. Not surprisingly, 1pentanethiol must be just one of several odorants important to the cigarette smoke response pattern. The identities of the others remain to be discovered.

The OR response pattern for 1-pentanethiol is also potentially indicative of ORs that respond to specific features of odorant molecules. This is especially true of families MOR230, MOR275, and MOR234 where a large fraction of the members of the family respond to 1-pentanethiol. Whether these groups of related ORs share sensitivity to certain simple thiols, or more broadly to many types of short chain odorants, pose interesting questions for future study. We do know that these are not the only families of ORs responsive to thiols. Olfr1509, a mouse OR responsive to a more complex thiol odorant, (methylthio)methanethiol (Duan et al., 2012), is not one of the ORs responsive to 1-pentanethiol, and other members of the same OR family (MOR244) also did not respond to 1-pentanethiol in our experiments.

Cigarette smoke is the first complex odor whose in vivo receptor response pattern has been measured. The breadth of the receptor response pattern evoked by cigarette smoke and the fact that the strongest responses are distributed across the diversity of Class II OR sequences and the TAARs are instructive. This is not simply because these complex odors contain so many species of odorants, although this is a contributing factor. The 1-pentanethiol OR response pattern by itself contains more than one-third of the number of receptors responsive to cigarette smoke and more than one-third of the number of OR families with members responsive to cigarette smoke. This breadth is consistent with previous findings that the response patterns of isoamyl acetate, bourgeonal, whiskey lactone, acetophenone, 2,5-dihydro-2,4,5-trimethylthiazoline, and carvones include ORs from several unrelated OR families (Hamana et al., 2003; Jiang et al., 2015; de March et al., 2020). We conclude that this breadth of responsivity is a characteristic feature of OR response patterns, not only for complex odors but also for individual odorants. This conclusion is consistent with evidence that specific anosmias are relatively rare, even while hyposmias are fairly common and generally consist of reduced detection thresholds for certain odorants (Trimmer et al., 2019). Loss or mutation of the OR most sensitive to an odorant would lead to reduced sensitivity to the odorant, but not absence of detection, because other ORs less sensitive to the odorant will respond as concentration rises (Sato-Akuhara et al., 2016; Dewan et al., 2018). This understanding that most odorant responses consist of several unrelated ORs is also instructive about OR evolution. OR evolution must have been driven primarily by selective forces favoring expansion and diversification over forces selecting for ORs highly sensitive to, and specific for, individual odorants. This would allow OR evolution, and by extension the general olfaction function provided by the main olfactory epithelium, to keep pace with changing and expanding odor environments.

With >400 odorants, all thought to be capable of evoking responses from multiple receptors, why isn't the response to cigarette smoke even broader than what we observe? Some of this discrepancy is presumably because of some of the odorants in cigarette smoke being present at low concentrations incapable of producing responses detectable in the assay used. However, responses to mixtures of odorants have long been known to be less than the sum of the responses to the individual odorants in the mixture (Laing and Francis, 1989; Livermore and Laing, 1996; Poupon et al., 2018). Antagonism of ORs by odorants, which is very common in mixtures of odorants (de March et al., 2020), almost certainly also contributes. These odorant interactions at receptors result in response patterns that make discerning individual odorants in mixtures difficult and favor perceiving complex odors as distinct objects rather than a combination of the elements of the mixture (Thomas-Danguin et al., 2014). Cigarette smoke is certainly perceived as a distinct object rather than its component elements, and is one of the most common odors we humans encounter.

Odors that have similar percepts sufficient to belong to the same odor object categorization may do so because they share responses from some ORs. The OR responses shared between cigarette smoke, artificial cigarette smoke odor, and 1-pentanethiol support this hypothesis. Indeed, perceptual similarity is known to correlate with the degree of similarity in neural activity at multiple levels of the mammalian olfactory system, and this is expected to arise from similarity in OR response patterns (Wilson, 2009; Gottfried, 2010; Pashkovski et al., 2020). However, our evidence is not yet sufficient to have confidence that this hypothesis will prove correct for all cases of similar percepts, in part because our evidence is a correlation between human perception and mouse receptor physiology. In addition, we cannot rule out the possibility that cortical pattern completion processing mechanisms might sometimes find similarity between odors that do not have overlapping OR response patterns but instead have overlap at higher levels of olfactory signal processing. Furthermore, the inverse hypothesis of OR response pattern similarity necessarily resulting in perceptual similarity is almost certainly false. Instances of highly similar odorants that differ substantially in their percepts, although they evoke strongly overlapping patterns of activity in OSNs or olfactory bulb glomeruli, strongly predict that similar OR response patterns can give rise to distinct percepts (Linster et al., 2001; Hamana et al., 2003). A specific example revealed in our comparison of receptor response patterns is the similarity of mouse OR response patterns for bourgeonal and undecanal, which humans

perceive as being quite distinct. Our findings support the idea that OR response pattern similarity is a basis for perceptual similarity, perhaps even when response pattern similarity is just a small fraction of responsive receptors, but with the caveat that the CNS is also capable of producing distinct percepts even from significantly overlapping OR response patterns.

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