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Relationship between Peripheral Receptor Code and Perceived Odor Quality

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Abstract

The discrimination of thousands of odorants is mediated by several hundred olfactory receptors (ORs). It is generally accepted that the main strategy in encoding odor quality is a combinatorial receptor code scheme, in which odorants are discriminated by different sets of ORs. In the present study, we classified 12 test odorants by their receptor codes and perceived odor qualities to examine whether odorants showing similar receptor codes are also similar in their odor qualities. Similarities of receptor codes between odorants were estimated by the overlapping responses of murine isolated olfactory sensory neurons. In contrast, we conducted a human sensory test to classify the test odorants according to their odor qualities. Despite the difference in species, the groupings of the test odorants were well conserved between receptor code and odor quality. These findings indicate that odorants that are discriminated by murine receptor codes are perceived as different odors by humans and further suggest that similarity of receptor codes correlates with that of odor quality, at least in our test odorants at the concentrations tested.

Key words: calcium imaging, cluster analysis, multidimensional scaling, olfactory sensory neuron

Introduction

Humans and other mammalian animals can detect and discriminate numerous odorants through distinct anatomical structures beginning at the olfactory epithelium (OE) in the nose (Touhara 2002; Buck 2004). The olfactory sensory neurons (OSNs) in OE detect odorants by expressing olfactory receptors (ORs). ORs belong to the large gene family of G protein–coupled receptors (Buck and Axel 1991). The total 913 murine ORs are classified into 241 subfamilies (Godfrey et al. 2004), whereas human 339 ORs are classified into 172 subfamilies (Malnic et al. 2004). Although mice have approximately 3 times as many ORs as humans, 150 subfamilies (65% and 87% of the subfamilies in mice and humans, respectively) are common to both species (Godfrey et al. 2004). In a phylogenetic tree that is constructed by one member of each human and murine subfamily, 21 of 24 major branches contain both human and murine ORs (Godfrey et al. 2004). These results and others (Young et al. 2002; Zhang and Firestein 2002) suggest that many human and murine ORs share functional similarities.

Activation of a single OSN directly reflects that of a single OR because each OSN expresses only one member of the OR gene family (Chess et al. 1994; Serizawa et al. 2000). This is known as the one neuron–one receptor rule (Serizawa et al. 2004). Because OR genes were discovered, ligands for ORs have been explored using various heterologous expression systems (Wetzel et al. 1999; Kajiya et al. 2001; Saito et al. 2004) and isolated OSNs (Malnic et al. 1999; Touhara et al. 1999). Due to the technical difficulty of establishing a high-throughput OR expression system, a detailed molecular receptive range has been revealed for several ORs. For example, OR-I7 has specific tuning for aliphatic aldehyde with a backbone chain of 7–10 carbons, while displaying a high tolerance for certain molecular features (e.g., unsaturated 8-carbon aldehyde) (Araneda et al. 2000, 2004). mOR-EG has been shown to recognize eugenol, vanillin, and other structurally related odorants (Kajiya et al. 2001; Katada et al. 2005). These previous findings demonstrate that ORs have a relatively broad tuning for odorants. On the other hand, some ORs can discriminate a pair of enantiomeric carvones, which possess the same molecular structures except for the chiral portion (Hamana et al. 2003). Thus, ORs discriminate subtle differences in

molecular structure, while being activated by structurally related odorants.

These extensive studies on individual ORs indicate that a single OR recognizes multiple odorants and a single odorant activates multiple ORs. Every odorant therefore has a unique combination of responses from several ORs. The receptor code, a combination of ORs for an odorant, has been proposed to encode odor quality (Malnic et al. 1999; Kajiya et al. 2001). Recent advances in the understanding of odor coding may open the possibility of a biological approach to evaluating odor quality. Indeed, a recent study (Bieri et al. 2004) has examined the activation patterns of isolated rat OSNs in response to fragrance compounds, sandalwood oil and synthetic sandalwood molecules, to compare their receptor codes. However, the relationship between similarity of receptor code and similarity of odor quality is poorly understood for most odorants.

To address this issue, we classified 12 odorants by murine receptor code and odor quality, respectively, for humans and compared them with each other. In this study, sensory analyses were conducted with human subjects only, and no sensory tests were conducted with mice to determine whether mice are likely to perceive our test odorants as being olfactorily similar. However, taking into account that many human ORs have close relatives in mice (Young et al. 2002; Zhang and Firestein 2002; Godfrey et al. 2004), it is possible that olfactory functions are similar between humans and mice and that at least mice can smell our test odorants. Our analyses demonstrated that the classification of the test odorants, except for methyl salicylate, was conserved between receptor code and odor quality. This result suggests that odorants that activate a similar combination of ORs are perceived as similar odors by humans.

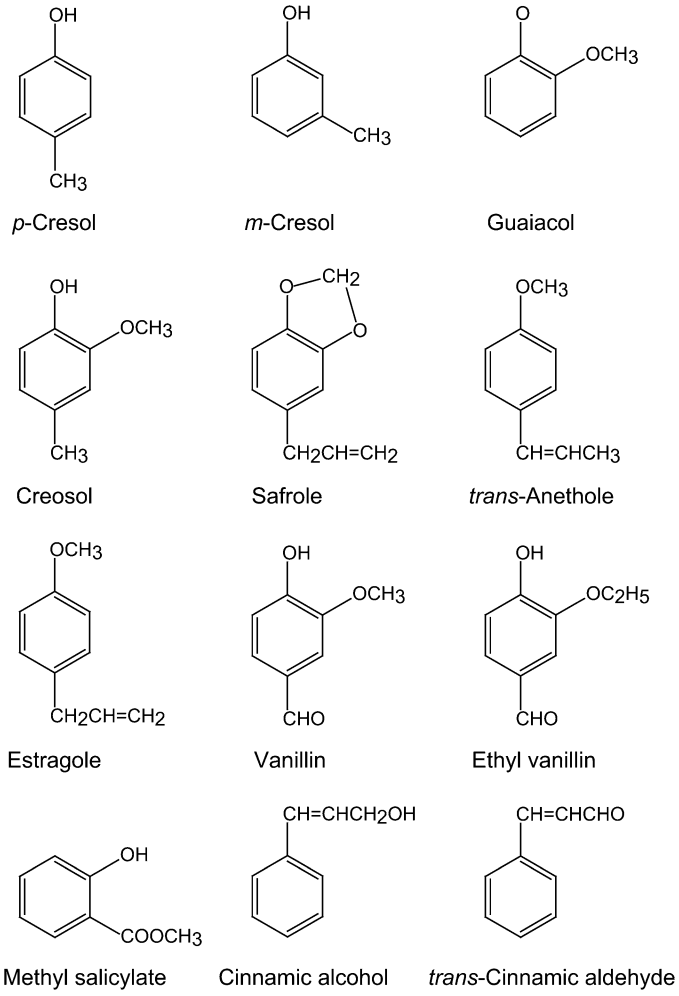

Figure 1 Molecular structure of odorants used in this study.

Materials and methods

Chemicals

Figure 1 shows the 12 odorants used in this study. We chose the test odorants from the point of view of odor quality, referring to Arctander's handbook (Arctander 1969), the Sigma-Aldrich Fine Chemical flavor and fragrances catalog, and the Good Scents Company's database (http://www.thegoodscentscompany.com/). According to these references, 4 odorants (*p*-cresol, *m*-cresol, guaiacol, and creosol) have medicinal and phenolic odors, 3 odorants (safrole, *trans*-anethole, and estragole) have anise and sweet odors, and 2 odorants (vanillin and ethyl vanillin) have vanilla and sweet odors. Methyl salicylate has wintergreen and mint odors. Two odorants (cinnamic alcohol and *trans*-cinnamic aldehyde) have cinnamon and spicy odors.

All odorants were purchased from Sigma-Aldrich (St Louis, MO). Odorant solutions of 100 μM were prepared in Ringer solution (in mM: 140 NaCl, 5.6 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 9.4 glucose, 2 Na-pyruvate, pH 7.4) for Ca imaging of murine OSNs. In the human sensory test, odorants were diluted to 1 M or 100 mM in propylene glycol, an odorless solvent. Three microliters solutions were prepared in 15-ml dark glass bottles capped with plastic screw caps.

Ca imaging of murine OSNs

Ca imaging assay was performed as previously described (Hamana et al. 2003). The animals were treated in accordance with Japanese law (No. 105) and the organization guideline for care and use of laboratory animals of the AIST Animal Experiment Committee. OSNs that were isolated from OE of male BALB/c mice (Japan SLC, Hamamatsu, Japan) were attached on a cover glass coated with Cell-TAK (BD Biosciences, Franklin Lakes, NJ). The isolated OSNs were loaded with fura-2, and Ca imaging recording was conducted with the AQUACOSMOS calcium imaging system (Hamamatsu Photonics, Hamamatsu, Japan). To test the viability of the OSNs, the response to high KCl (0.14 M) was measured. Odor stimuli and high KCl were applied for 4 s at intervals of at least 60 s.

Human sensory test

Similarities of perceptual odor qualities among 12 odorants were evaluated by a human sensory test. To avoid problems of individual differences in semantic interpretation, the direct sorting procedure was employed as described in previous reports (Lawless 1989; Lim and Lawless 2005). Eleven human subjects (10 male, 1 female, mean age = 30.3 years, standard deviation = 7.2 years) participated in this study. We instructed them not to eat, drink, chew gum, or smoke for at least 1 h prior to testing. The sensory test was divided into 2 sessions. The first session was conducted at a concentration of 100 mM, and the second session was performed at 1 M. There was a 10-min interval between the 2 sessions. The subjects took a sniff of stimuli in any order as many times as they liked and sorted them into groups based on similarity of odor quality. There was no constraint on the number of groups they could make. Written materials were provided for personal notes in order to help their memory of odor qualities. The subjects were allowed to take a rest when they felt fatigued during the sessions and resumed their task after a sufficient rest.

Data analysis of OSN responses

In Ca imaging assay, 110 OSNs responded to at least 1 odorant, and they were classified into 40 different response profiles as shown in Figure 3. For each OSN, a response was assigned a value of 1 and no response, a value of 0. Thus, all the responses, regardless of their response amplitude, showed a value of either 1 or 0. Assuming that OSNs showing the same response profile express the same OR, we estimated receptor code similarities for all possible pairs of test odorants by using 40 response profiles. A pair of odorants that has a similar receptor code is expected to share more OR responses. Therefore, we counted the number of profiles that responded or did not respond to both odorants in a pair. The number counted for each pair was used as the similarity index of a receptor code. Then, the similarity index was transformed to a dissimilarity index by subtracting the similarity index from 40. Thus, we made a pairwise distance matrix between the 12 odorants. The half matrix produced in this manner was analyzed by multidimensional scaling (MDS) and hierarchical cluster analysis with the statistical software SPSS (ver. 10.1.4J, SPSS Inc., Chicago, IL). The 2-dimensional solution was computed in MDS.

Data analysis of the human sensory test

In the sorting task, odorants that have a similar odor quality are placed more frequently in the same group. We therefore counted the number of subjects who sorted into the same group for all possible pairs of test odorants. The number counted for each pair indicates odor similarity. Then, odor similarity was converted to odor dissimilarity by subtracting the number counted for each pair from 11. Thus, we created a pairwise distance matrix between the 12 odorants. The half matrix produced in this manner was analyzed by MDS and hierarchical cluster analysis with the statistical software SPSS. The 2-dimensional solution was computed in MDS.

Results

Response profiles to 12 odorants

As a first step in evaluating similarities of receptor codes, we examined the responsiveness of isolated OSNs to the 12 odorants. Of 1143 OSNs examined, 110 responded to 1–8 of the test odorants (9.6%), whereas 1033 OSNs (91.4%) showed no odorant-induced response but were activated by high-KCl stimulus. Figure 2 shows examples of the responses of 3 isolated OSNs to the application of the 12 odorants. The OSN in Figure 2A was activated by 8 odorants among the test odorants. The OSN in Figure 2B recognized 4 odorants. The OSN in Figure 2C recognized *p*-cresol only.

Based on the differences in effective odorants for the OSNs, 40 different response profiles were obtained as shown in Figure 3. The OSNs of profile number 2 responded to both *p*-cresol and *m*-cresol, showing that these neurons could not discriminate the positions of the methyl group. The OSNs, illustrated by profile numbers 1 and 4, responded to either *p*-cresol or *m*-cresol. These neurons discriminated the positions of the methyl group. The differences in functional group, but not in position, were also discriminated by other OSNs assayed. The OSNs of profile number 31 were activated by vanillin, but ethyl vanillin failed to activate them. This result suggested that these neurons discriminated between methoxy and ethoxy groups. The differences in functional group between *trans*-cinnamic aldehyde and cinnamic alcohol were also discriminated by the OSNs of profile numbers 38 and 40.

The number of OSNs that showed the same response profile varied from 1 to 17 as shown in the right-hand column of Figure 3. Several response profiles such as profile numbers 36 and 40 included a relatively large number of OSNs. In profile number 40, 17 OSNs responded to only *trans*-cinnamic aldehyde. Nine OSNs included in profile number 36 were activated by only methyl salicylate. In these cases, OSNs might express different kinds of ORs. One possible solution to this problem might be to identify what type of OR gene is expressed in each OSN, but technical limitations precluded the amplification of the OR gene from OSNs to which Ca imaging was applied. In this study, we therefore analyzed similarities of receptor codes on the assumption that OSNs presenting the same response profile express the same OR.

Similarity of receptor codes

We estimated receptor code similarities in all possible pairs of odorants from 40 different response profiles. The data were analyzed by MDS to investigate the degree of similarity

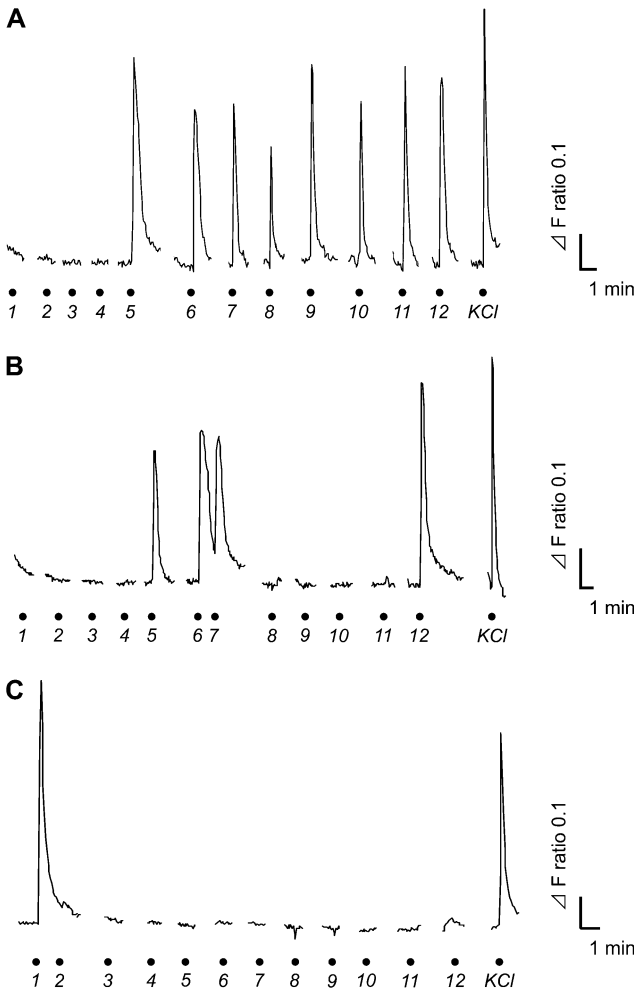

Figure 2 Responses of 3 isolated OSNs stimulated with 12 odorants. Odorants were applied for 4 s at the time indicated by the filled circle. High KCl (0.14 M) was applied to verify the viability of cells as a control. The test odorants used were 1, *p*-cresol; 2, *m*-cresol; 3, guaiacol; 4, creosol; 5, safrole; 6, *trans*-anethole; 7, estragole; 8, vanillin; 9, ethyl vanillin; 10, methyl salicylate; 11, *trans*-cinnamic aldehyde; and 12, cinnamic alcohol. **(A)** The OSN responded to 8 odorants. **(B)** The OSN responded to 4 odorants. **(C)** The OSN responded to only *p*-cresol. The OSNs exhibited various profiles of activity, from broadly tuned to narrowly tuned. All odorants were tested at 100 μM. The panels show the recorded profiles for profile numbers 26, 6, and 1 from Figure 3.

among receptor codes. Moreover, hierarchical cluster analysis of the same data was performed to classify the test odorants according to their receptor codes.

Figure 4A shows the MDS configuration for the 12 odorants in 2-dimensional space. The Kruskal stress value was 0.13. The distance represents dissimilarity; namely, odorants that are close together indicate that their receptor codes are similar. Four odorants (guaiacol, creosol, *m*-cresol, and *p*-cresol) were arranged close together. Vanillin and ethyl vanillin were distributed near each other. The positions of safrole and *trans*-anethole were close to that of estragole.

Profile number	*p*-Cresol	*m*-Cresol	Guaiacol	Creosol	Safrole	*t*-Anethole	Estragole	Vanillin	Ethyl vanillin	Methyl salicylate	Cinnamic alcohol	*t*-Cinnamic aldehyde	The number of same profile
1	●												4
2	●	●											3
3	●	●		●									1
4		●											4
5		●						●	●				1
6		●	●					●	●				4
7			●										1
8			●					●	●				2
9			●	●				●	●				1
10			●	●				●	●		●		1
11				●				●	●				5
12				●	●								1
13				●	●					●		●	1
14					●						●	●	2
15					●						●		1
16					●		●				●	●	1
17					●		●						5
18					●	●							2
19					●	●	●						3
20					●	●	●			●			3
21					●	●	●			●	●		1
22					●	●	●			●		●	1
23					●	●	●				●	●	1
24					●	●	●					●	6
25					●	●	●		●	●	●	●	2
26					●	●	●	●	●	●	●	●	1
27						●	●						1
28						●	●			●		●	1
29							●					●	1
30							●	●				●	1
31								●					3
32								●	●				7
33								●	●		●	●	2
34								●		●		●	1
35									●	●		●	1
36										●			9
37										●		●	1
38											●		3
39											●	●	6
40												●	17

Figure 3 Forty different response profiles tested with 12 odorants. The test odorants are shown at the top, and the number of identical response profiles is shown in the right-hand column. Responses are indicated by the filled circle, regardless of response amplitude.

The position of *trans*-cinnamic aldehyde was relatively close to that of cinnamic alcohol. Methyl salicylate was plotted between estragole and cinnamic alcohol.

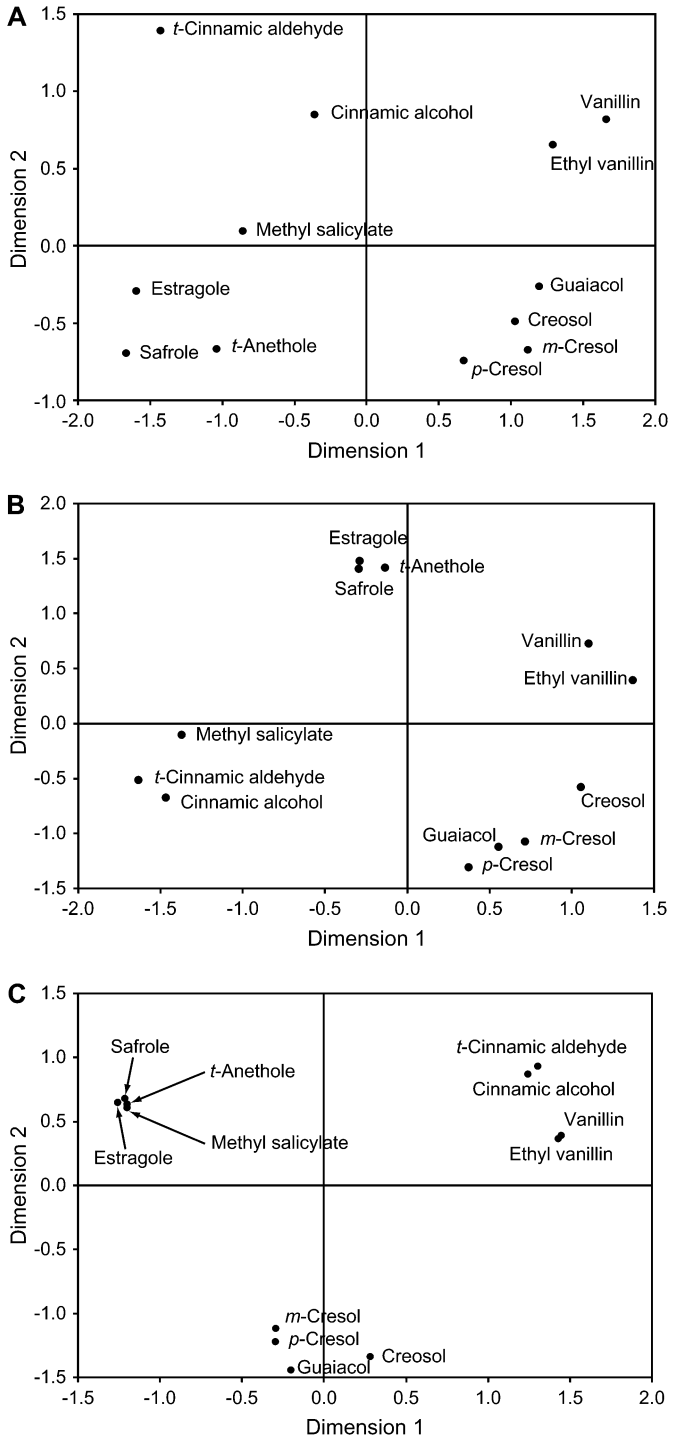

Figure 4 Distributions of 12 odorants in 2-dimensional space resulting from MDS. The distance represents dissimilarity. **(A)** The distribution of the test odorants based on the receptor codes. The stress value was 0.13. **(B)** Similarities of odor qualities at a concentration of 1 M. The stress value was 0.27. **(C)** Similarities of odor qualities at a concentration of 100 mM. The stress value was 0.29.

A hierarchical dendrogram is shown in Figure 5A. This cluster analysis based on similarities of receptor codes identified 2 apparent groups or 4 distinct groups. The clustering of the 12 odorants agreed well with the MDS configuration in the case of 4 groups but not in the case of 2 groups. Consistent with the MDS configuration, 4 odorants (guaiacol, creosol, *m*-cresol, and *p*-cresol) formed one cluster. Vanillin and ethyl vanillin were included in one cluster. The cluster of *trans*-anethole, estragole, and safrole emerged. One cluster

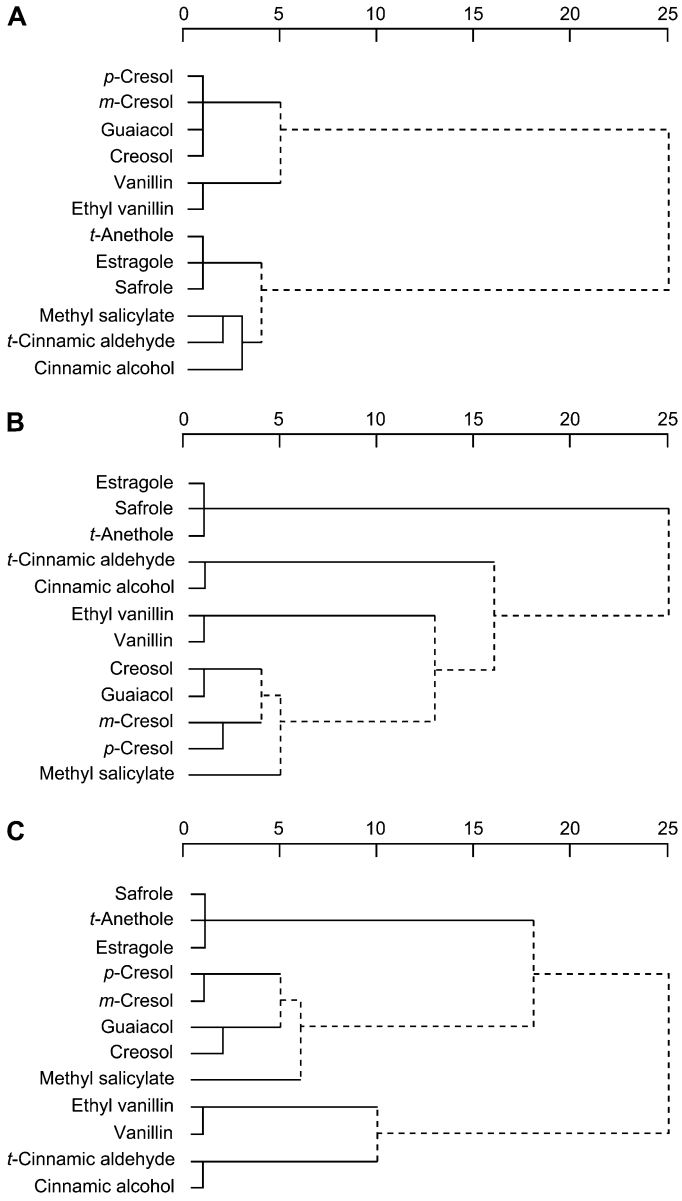

Figure 5 The results of hierarchical cluster analysis of 12 odorants for the receptor codes **(A)**, odor qualities at a concentration of 1 M **(B)**, and odor qualities at a concentration of 100 mM **(C)**. The dotted lines indicate the clusters that emerged in this study. The receptor codes of the test odorants were classified into 4 groups. Odor qualities were categorized into 6 groups at both odorant concentrations.

consisted of methyl salicylate, *trans*-cinnamic aldehyde, and cinnamic alcohol. Overall, the classification by cluster analysis was in accordance with the arrangement of test odorants in 2-dimensional space (comparing Figures 4A and 5A). We determined that the receptor codes of the test odorants were classified into 4 groups that emerged in the cluster analysis.

Similarity of odor qualities

The cluster analysis divided the receptor codes of the 12 odorants into 4 groups (Figure 5A). To examine whether the grouping of the test odorants by receptor code reflects odor quality, similarities of perceived odor qualities among 12 odorants were evaluated by the direct sorting method. MDS and hierarchical cluster analysis were applied to the data of perceptual similarity. Considering that perceived odor quality varies when stimulus intensity is changed (Gross-lsseroff and Lancet 1988), we presented odor stimuli to subjects at concentrations of 1 M and 100 mM.

Figure 4B,C shows the MDS configurations for high and low odorant concentrations, respectively. The distance represents dissimilarity; namely, odorants that are close together indicate that their perceived odor qualities are similar. The Kruskal stress values were 0.27 at a concentration of 1 M and 0.29 at a concentration of 100 mM. Stress values of more than 0.2 indicate a poor fit; therefore, our data might not be clearly structured in 2-dimensional space. Our MDS analyses revealed that 11 of the 12 odorants were similarly arranged at both odorant concentrations. Estragole, safrole, and *trans*-anethole were located close together. Vanillin and ethyl vanillin were close to each other. Cinnamic alcohol and *trans*-cinnamic aldehyde were distributed near each other. Four odorants (guaiacol, creosol, *m*-cresol and *p*-cresol) were arranged close together. The only exception was methyl salicylate, which was plotted close to *trans*-cinnamic aldehyde and cinnamic alcohol at a concentration of 1 M (Figure 4B). In contrast, it was located close to estragole, safrole, and *trans*-anethole at 100 mM (Figure 4C).

We performed cluster analysis to clarify the grouping of odor qualities of the 12 odorants. Figure 5B,C shows hierarchical dendrograms at concentrations of 1 M and 100 mM, respectively. The analyses separated the 12 odorants into 6 clusters, members of which were identical at both odorant concentrations. Of the 6 clusters, 4 consisted of 2 odorants: *trans*-cinnamic aldehyde and cinnamic alcohol, vanillin and ethyl vanillin, creosol and guaiacol, and *m*-cresol and *p*-cresol, respectively. Two clusters for creosol, guaiacol, *m*-cresol, and *p*-cresol were again linked at the next step of the clustering process. One cluster consisted of 3 odorants (estragole, safrole, and *trans*-anethole). Methyl salicylate formed a cluster by itself. These results indicate that the perceived odor qualities of 12 odorants were divided into 6 groups and did not alter by a 10-fold concentration change.

Comparison of murine receptor code with odor quality for humans

Between-cluster analyses of receptor codes and odor qualities (Figure 5), 7 of the 12 odorants (vanillin, ethyl vanillin, *trans*-anethole, estragole, safrole, *trans*-cinnamic aldehyde, and cinnamic alcohol) were classified identically. Odor qualities for 4 odorants (guaiacol, creosol, *m*-cresol, and *p*-cresol) were classified into 2 clusters, whereas these odorants were included in one cluster regarding their receptor codes. However, 2 clusters that emerged by odor quality were linked at the next step of the clustering process, suggesting that the odor qualities of the 2 clusters are relatively similar. The greatest difference between receptor code and odor quality occurred with methyl salicylate. By receptor code, methyl salicylate formed one cluster with cinnamic alcohol and *trans*-cinnamic aldehyde. In contrast, the odor quality of methyl salicylate was segregated from that of other test odorants. We concluded that the classification of the test odorants, except for methyl salicylate, was conserved between receptor code and odor quality.

Discussion

In this study, we examined the responsiveness of isolated murine OSNs to 12 odorants and classified the test odorants by their receptor codes. We also performed a human sensory test to classify the test odorants by their odor qualities. Except for methyl salicylate, the classification of the test odorants by receptor code agreed well with the classification by odor quality (Figure 5). These results support the fact that odor quality is encoded by a combination of activated ORs and further suggest that odorants that activate similar receptor codes present similar odor qualities.

First, it should be mentioned that different species were employed between experiments. We conducted the sensory test with human subjects due to difficulty in assessing odor similarities among 12 odorants in mice. In contrast, receptor code similarities were determined by the responses of murine OSNs to each odorant. The research design of this study raises the question of whether human sensory data are adequate for comparison with murine OSN responses because it remains unclear whether mice perceive our test odorants as well as humans. Genetic studies have revealed that the human and murine OR repertoire is similar and that many human ORs have close relatives in mice, suggesting that the majority of odorant features detectable by one species are also recognized by the other (Godfrey et al. 2004; Malnic et al. 2004). These findings raise the possibility that olfactory functions are similar, if not identical, between the 2 species. Indeed, all compounds examined in this study activated murine OSNs as well as being perceived by humans, indicating that at least mice could smell our test odorants. The present study also showed that discriminative odorants in murine receptor codes were perceived by humans as different odors.

Therefore, we believe that comparison between human sensory data and murine OSN responses is meaningful, though not ideal.

The data presented here suggest that the peripheral code generated in OE is relatively preserved though the central olfactory system modifies and integrates it. Olfactory information received by OSNs is transmitted to the olfactory bulb (OB), the first site for the processing of information in the brain, and further delivered to the olfactory cortex in which olfactory perception is assumed to be constructed (Mori et al. 1999, 2006; Buck 2004). The axons of OSNs expressing the same OR converge onto a few defined glomeruli in OB (Vassar et al. 1994; Mombaerts et al. 1996), indicating that the pattern of activated glomeruli reflects the combination of activated ORs. In OB, local neural circuits enhance the contrast of olfactory information by lateral inhibition (Yokoi et al. 1995) or impose differential temporal patterns on signals transmitted to the cortex (Laurent 1997). Thus, odor signals generated by OSNs are processed and integrated along the olfactory ascending route. In our study, the classification by odor quality obtained through the human sensory test is thought to be based on olfactory information that is processed and integrated by the central nervous system, whereas the receptor codes for the test odorants display the first sensory inputs. Nevertheless, the classification by receptor code was consistent with that by odor quality. Odor codes produced in OE are processed in the central olfactory system but may be comparatively maintained.

The classification of methyl salicylate disagreed considerably between receptor code and odor quality. Four odorants (guaiacol, creosol, *m*-cresol, and *p*-cresol) appeared to be more greatly separated regarding odor quality. Although species difference is one of the reasons, our heterogeneous sampling of OSNs also may contribute to these inconsistencies. It has been reported that OE can be divided into 4 distinct zones and that OSNs expressing a particular OR gene are randomly distributed within 1 zone (Ressler et al. 1993; Vassar et al. 1993; Sullivan et al. 1996). Moreover, a recent report has shown that the expression area in OE is unique to each OR gene (Miyamichi et al. 2005). Although we attempted to isolate OSNs from various areas of OE, it was difficult to ensure homogeneous sampling. In addition, the number of OSNs assayed might be insufficient for examining in detail similarities of receptor codes. These limitations of our method may cause the disagreement between receptor code and odor quality.

We examined the relationship between murine receptor code and perceived odor quality for humans using physiological and psychophysical approaches. Despite species difference, similarity of receptor codes correlated with that of odor quality. The results suggest that combinations of activated ORs encode odor qualities and that odorants, at least our test odorants, sharing more ORs in their receptor codes are more similar in perceived odor quality.

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