# Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features

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**Psychophysical studies indicate that structural features of odorants differentially influence their perceived odor. In the olfactory bulb (OB), odorants are represented by ensembles of activated glomeruli. Here we used optical imaging of intrinsic signals to examine how these structural features are represented spatially in the sensory map of the rat OB. We found that the dorsal OB contained two topographically fixed domains; constituent glomeruli in each domain could be activated by odorants with particular functional groups. Within each domain, other structural features such as carbon chain length and branching were represented by local differences in patterns. These results suggest that structural features are categorized into two classes, primary features (functional groups) that characterize each domain, and secondary features that are represented by local positions within each domain. Such hierarchical representations of different structural features correlate well with psychophysical structure–odor relationships.**



Sensory input to the olfactory system takes the form of molecular information carried in a huge variety of odorants. This information is received by sensory neurons in the olfactory epithelium and transmitted to the central olfactory system, the olfactory bulb and olfactory cortex.

Early psychophysical studies indicated a close relationship between structural features of odorants and their subjectively perceived odors, although knowledge of precise rules for structure–odor relationships is far from complete. The structural features that influence odor quality include type and position of functional groups, length and branching patterns of carbon chains, presence of unsaturated bonds and charges, and the overall shape and size of odorants[1–4]. Functional groups, known as 'osmophores', are typically the most important determinants of odor quality[3–7]. For example, a homologous series of fatty acids (R–COOH) has similar pungent, sour and sweat-like odors, whereas a homologous series of alcohols (R–OH) has fresh and sweet odors. Within the series of fatty acids, the perceived odors exhibit minor and gradual changes with progressive increase in carbon chain length[4]. To understand neuronal mechanisms responsible for odor perception, we examined how structural features such as functional groups and carbon chain length are represented in the central olfactory system of the mammalian brain. We are especially interested in how nonspatial information, such as the structural features of odorants, is spatially represented in the brain.

To perceive a huge variety of odorants, mammals have developed up to 1000 types of odorant receptors[8,9]. Individual olfactory sensory neurons probably express a single type of odorant receptor[10]. Sensory neurons expressing a given odorant receptor send their axons to a few topographically fixed glomeruli in the OB[11–13]. Individual glomeruli represent a single or at most a few types of odorant receptor(s). Thus, the glomerular sheet of the OB provides a sensory map with which the brain can identify which of the numerous odorant receptors have been activated by inhaled odorants.

In a few types of odorant receptors whose response specificity has been examined in detail, individual receptors respond to a range of odorants that share specific molecular features[10,14–16]. A single odorant can in turn be recognized by different types of odorant receptors, presumably because they exhibit multiple molecular features[10]. Thus there is a complex relationship between odorants and receptors. Nevertheless, odorant receptors can distinguish even small variances in odorant structures. A given odorant with certain molecular features is therefore coded by specific ensembles of activated odorant receptors in the olfactory epithelium, and by corresponding combinations of activated glomeruli in the OB[17].

How are different molecular features spatially represented in the glomerular sensory map of the OB? In the zebrafish, many odorants evoke distributed glomerular activity within specific regions of the OB[18,19]. A series of amino acids activates glomeruli in the lateral region, whereas bile acids activate those in the medial region. Within each region, small differences in odorant structure are represented by different combinations of activated glomeruli. Similarly, in mammalian OB, stimulation of the olfactory epithelium with fatty acids elicits glomerular and neuronal activity in focal regions[20–22]. In 2-deoxyglucose (2-DG) studies of metabolically active regions of the OB[22], increases in carbon chain length cause systematic shifts of the position of activated

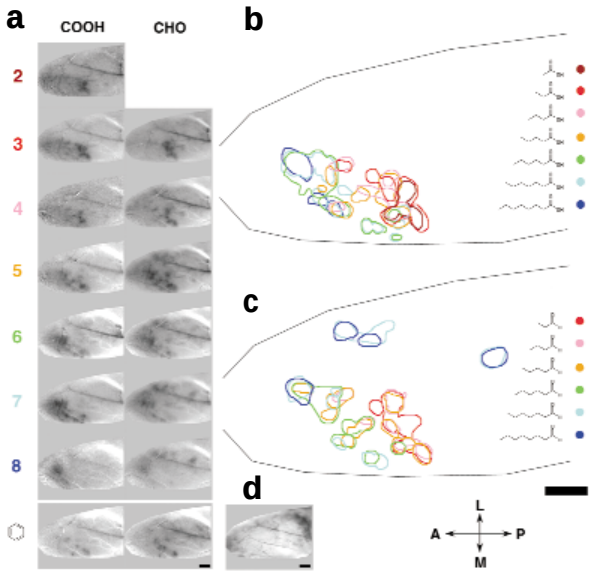



**Fig. 1.** A homologous series of carboxylic acids and aliphatic aldehydes activate glomeruli clustered in an anteromedial domain of the dorsal OB. (**a**) Optical images of intrinsic signals in response to aliphatic acids (1:10 dilution, COOH, left column) and aldehydes (1:50 dilution, CHO, right column). The number of carbon atoms in the stimulant molecule is indicated at the left of each picture. Bottom row, responses to benzoic acid (left) and benzaldehyde (right). All the responses were recorded from a single animal. (**b**) Superimposed optical maps of regions activated by aliphatic acids. (**c**) Superimposed maps for aliphatic aldehydes. The chemical structure of each odorant is shown to the right. Each color indicates a specific carbon chain length. Within the anteromedial domain, the position of activated glomeruli shifted anteriorly and laterally with increasing carbon chain length of acids (**b**) and aldehydes (**c**). (**d**) Blood vessel patterns in the imaged region. A, anterior; P, posterior; L, lateral; M, medial. Scale bars, 500 μm.

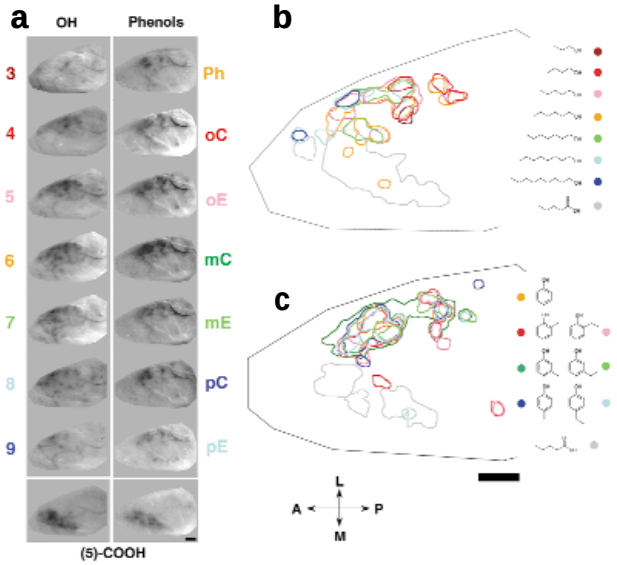



**Fig. 2.** A homologous series of aliphatic alcohols and phenols activates glomeruli clustered in a lateral domain of the dorsal OB. (**a**) Responses to a series of primary aliphatic alcohols (no dilution, OH, left column) recorded in one animal and phenol and its derivatives (no dilution, phenols, right column) recorded in another animal. The position of the anteromedial domain can be estimated by responses to pentanoic acid (n-valeric acid; (5)-COOH, bottom). The number of carbon atoms in the alcohol is indicated at the left. Phenol derivatives are indicated at the right. Ph, phenol; oC, *ortho*-cresol; oE, *ortho*-ethyl phenol; mC, *meta*-cresol; mE, *meta*-ethyl cresol; pC, *para*-cresol; pE, *para*-ethyl phenol. (**b**) Superimposed optical maps of regions activated by primary aliphatic alcohols. (**c**) Superimposed maps for phenols. As a reference, the areas activated by pentanoic acid (n-valeric acid) are shown by gray lines. Scale bars, 500 μm.



glomeruli within each focal region. Due to technical limitations, however, it has been difficult to systematically analyze the relationship between a variety of molecular features of odorants and their spatial representation in the OB.

Recently, the technique of intrinsic signal imaging in rat OB was introduced and used to map the activity of multiple odorants. Optical imaging techniques have high spatial resolution and can distinguish intrinsic activity signals in a single glomerulus[23]. Here we mapped intrinsic signals in the dorsal surface of rat OB evoked by a large panel of odorants (about 200) that varied systematically in structural features. The results demonstrated a topographic arrangement of molecular feature-specific domains in the glomerular sheet of the OB.

## Results

Using optical imaging, we recorded intrinsic signals evoked by a variety of odorants from a large area (about 4 mm × 2 mm) on the dorsal surface of the rat OB. Consistent with a previous study[23], the overall spatial patterns of glomeruli that were activated with each odorant were well conserved among animals. The glomerular activity ranged from 0.05 to 0.3% of the reflected light intensity. The intensity of glomerular activity was classified into four levels (weak, 0.050–0.070%; modest, 0.070–0.085%; strong, 0.085–0.100%; very strong, more than 0.100% changes in light intensity). For constructing the superimposed maps (Figs. 1–3 and 5), regions that showed more than 0.070% changes were outlined and shown in color.

### Domain organization and the role of functional groups

To examine molecular structure–response relationships, we first recorded responses to homologous series of fatty acids ($n$ = 13 rats), aliphatic aldehydes ($n$ = 13), alcohols ($n$ = 7) and straight chain alkanes ($n$ = 7). A range of fatty acids (R–COOH) that varied from two to eight carbons activated glomeruli that were clustered in the anteromedial part of the imaged region (Fig. 1a, left). As seen by superimposing the activated glomeruli, the fatty acid-responsive glomeruli were clustered in a circumscribed domain (anteromedial, Fig. 1b). Aliphatic aldehydes (R–CHO) that varied systematically in carbon chain length between three to eight also activated glomeruli in the anteromedial domain, although aldehydes with five or more carbons evoked additional glomerular responses in the lateral part (Fig. 1a, right, and c). Thus, each fatty acid or aldehyde activated at least a few glomeruli in the anteromedial domain. This was also the case for benzoic acid (Fig. 1a, bottom left, $n$ = 5) and for benzaldehydes (bottom right, $n$ = 4). In contrast, a homologous series of alkanes with five to ten carbons caused little activity in the entire imaged region (for example, Fig. 4a, hexane).

Primary alcohols (R–OH) with three to nine carbons elicited modest responses mainly in glomeruli in the lateral part of the dorsal OB (Fig. 2a, left). Superimposition of the activated glomeruli indicated that most were clustered in the lateral

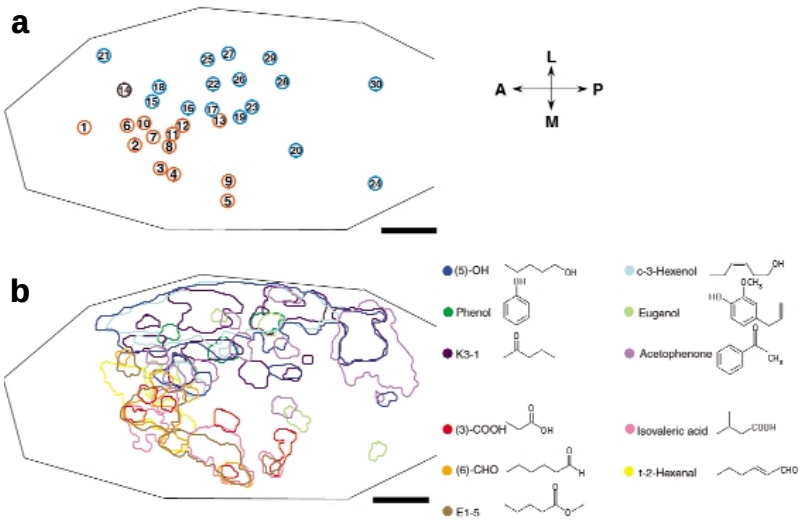



**Fig. 3.** Anteromedial and lateral domains are distinct in the molecular receptive range of their constituent glomeruli. (**a**) Location of the glomeruli shown in Table 1. (**b**) Superimposed optical maps for 11 representative odorants: pentanol ((5)-OH), cis-3-hexenol, phenol, eugenol, 2-pentanone (K3-1) and acetophenone activated glomeruli in the lateral domain. Propionic acid ((3)-COOH), 3-methyl butanoic acid (isovaleric acid), hexanoic acid (n-caproic acid) ((6)-COOH), trans-2-hexenal and methyl pentanoate (E1-5) activated the anteromedial domain. Scale bars, 500 μm.

domain, although a few were located within the fatty-acid-responsive anteromedial domain (Fig. 2b). Phenol has a hydroxyl group (–OH) attached directly to the benzene ring. Phenol and its derivatives evoked a strong glomerular response in the lateral portion of the imaged region, which largely overlapped with the alcohol-responsive lateral domain (Fig. 2a, right, and c).

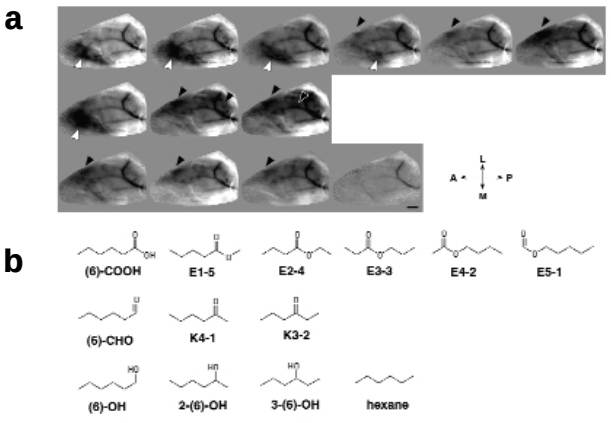
These results suggested that odorants that activate glomeruli in each domain share common molecular features. Most odorants effective in activating the anteromedial domain had either a carboxylic group (–COOH) or an aldehyde group (–CHO), whereas most odorants that activated the lateral domain shared a hydroxyl group (–OH).

To further characterize the role of functional group in the spatial pattern of response, we expanded the panel of stimulus odorants to include ketones (R–CO–R′, $n$ = 13) and esters (R–COO–R′, $n$ = 8). In addition, we expanded the panel to include odorants with relatively complex hydrocarbon structures, such as branching, double bonds or benzene rings. In a representative experiment (Table 1; Fig. 3), responses to the expanded panel (about 60 different odorants) were recorded from the same animal. By counting the number of glomeruli that were activated by at least one of the odorants, we estimated that the imaged region of the dorsal OB contained at least 120 glomeruli. Molecular receptive ranges (MRR) determined for 30 randomly selected glomeruli (Table 1) indicated two groups with completely different MRR in terms of functional groups. One group of glomeruli (1–13) was activated by aldehydes, acids and a subset of esters, whereas the other group of glomeruli (15–30) was activated by alcohols, phenols, ketones and a different subset of esters. A superimposed map (Fig. 3b) shows an almost segregated distribution of the two groups of glomeruli into the anteromedial and lateral domains. Although glomeruli located in the boundary portion between the anteromedial and lateral domains (such as glomerulus 14) were activated by acids, aldehydes and alcohols, such glomeruli with intermixed MRR were few in number.

Aldehydes, which contain a carbonyl group (–CO–) at the end of the molecule, activated glomeruli mainly in the anteromedial domain (Fig. 1a and c). Ketones contain the same carbonyl group, but positioned in the middle of the molecule, yet they activated mainly the lateral domain. This suggested that both type and position of the functional group is an important determinant for activating glomeruli in specific domains. To examine this possibility, we recorded responses to a panel of odorants that had six carbon atoms but showed systematic differences in the type and position of the functional group (Fig. 4b). Hexanal ((6)–CHO), 2-hexanone (K4-1) and 3-hexanone (K3-2) share the same carbon chain and also have a carbonyl group (–CO–), but the position of that group differs in each odorant (Fig. 4b, second row). Hexanal activated the anteromedial domain, whereas the two ketones activated the lateral domain (Fig. 4a, second row).

Esters (R–COO–R′) are produced by the chemical reaction of an alcohol (R′–OH) with an acid (R–COOH) and thus have two carbon chains (acid part, R and alcohol part, R′) attached to opposing sites on the functional group (–COO–). A series containing hexanoic acid and six carbon esters was used to examine the role of the position of the functional group (–COO–) as well

**Fig. 4.** A switch in the activated domain can be produced by a slight change in type and position of functional group. Stimuli were six carbon molecules with different functional groups at varying positions. (**a**) Optical maps of glomeruli activated by each odorant. Molecular formulae of stimulus molecules are shown in a corresponding position in (**b**). Areas that showed strong activity are indicated by arrowheads. Black arrowheads point to activated glomeruli in the lateral domain; white ones point to those in the anteromedial domain. Dilution of each odorant was 1:10 for acids, esters and ketones; 1:50 for aldehydes; no dilution for alcohols and alkanes. Scale bar, 500 μm.



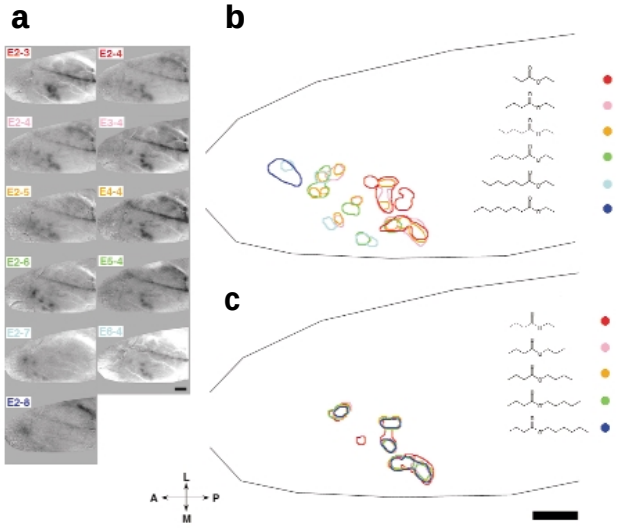



**Fig. 5.** Glomerular activity in the anteromedial domain elicited by a series of aliphatic esters. (**a**) Responses to a range of esters having different carbon chain lengths in the acid part (left column) or their alcohol part (right column). (**b**) Superimposed maps for esters having different chain length in their acid part. Within the anteromedial domain, the position of activated glomeruli shifted toward anteriorly and laterally with increasing chain length in the acid part of the esters. (**c**) Superimposed maps for esters having different chain lengths in their alcohol part. These esters showed similar activity maps. All the odorants were used with 1:10 dilution. Images in this figure and Fig. 1 were obtained from the same animal. Scale bars, 500 μm.

as the arrangement of two different carbon chains. Esters with more than four carbons in the acid part activated the anteromedial domain (Fig. 4a, E 1-5, E 2-4; Fig. 5), as was also the case for hexanoic acid ((6)-COOH). In contrast, esters with one or two carbons in the acid part and four or five carbons in the alcohol part activated the lateral domain (Fig. 4a, E 4-2, E 5-1; $n$ = 8). Propyl propanoate (E3-3), with three carbons in each part, activated glomeruli in both anteromedial and lateral domains (Fig. 4a, E 3-3). In this series, changing the position of the functional group (–COO–) from one end of the molecule (hexanoic acid ((6)–COOH)) to the other end (n-pentyl formate (E 5-1); Fig. 4b, first row) switched the activated glomeruli from the anteromedial to lateral domain, with an intermediate pattern for propyl propanoate (E3-3; Fig. 4a, first row). In contrast, in an analogous series of alcohols (Fig. 4a, third row), each molecule activated the lateral domain.

We further expanded the panel of stimulus odorants to include aliphatic compounds having –Cl, –Br, $–NH_2$, $–NO_2$, –NCS, –SH, –CN or $–SO_3H$ groups ($n$ = 5). Most of these molecules evoked little activity in the imaged region, except that 1-hexanethiol ($C_6H_{13}$–SH) evoked relatively weak activity in the lateral domain and hexylamine ($C_6H_{13}$–$NH_2$) in the anteromedial domain (data not shown).

In summary, the dorsal surface of rat OB contained two almost segregated domains; constituent glomeruli in each domain were characterized by a specific range of functional groups. The presence of the two domains at stereotyped positions was observed in all the OBs examined (14 right OBs and 10 left OBs in 24 rats).

### Molecular features represented within each domain

We next asked how molecular features other than functional groups influence the spatial patterns of activated glomeruli. Detailed observation of the spatial distribution of responses to fatty acids (Fig. 1a, left, and b) and aliphatic aldehydes (Fig. 1a, right, and c) showed that within the anteromedial domain, clusters of activated glomeruli shifted anteriorly and laterally with increasing carbon chain length[22,23]. In addition, a fatty acid and an aldehyde with the same carbon chain length elicited similar patterns of activated glomeruli within the domain (Fig. 1). This indicated that within the anteromedial domain, carbon chain length of fatty acids and aldehydes was coded by ensembles of glomeruli arranged locally and topographically, but in a largely overlapping manner. In all OBs examined ($n$ = 10), we consistently observed this gradual shift of activated glomeruli with increasing carbon chain length.

We examined the change in the spatial pattern of response within the anteromedial domain to an independent and a progressive change in the carbon chain length of the acid part (R) and the alcohol part (R′) of esters (R–COO–R′). When the carbon chain length of the acid part (R) was increased, the position of activated glomeruli shifted anteriorly and laterally in a manner very similar to fatty acids and aliphatic aldehydes (Fig. 5a, left, and b). In contrast, an increase in the carbon chain length of the alcohol part (R′) caused little shift of the position of activated

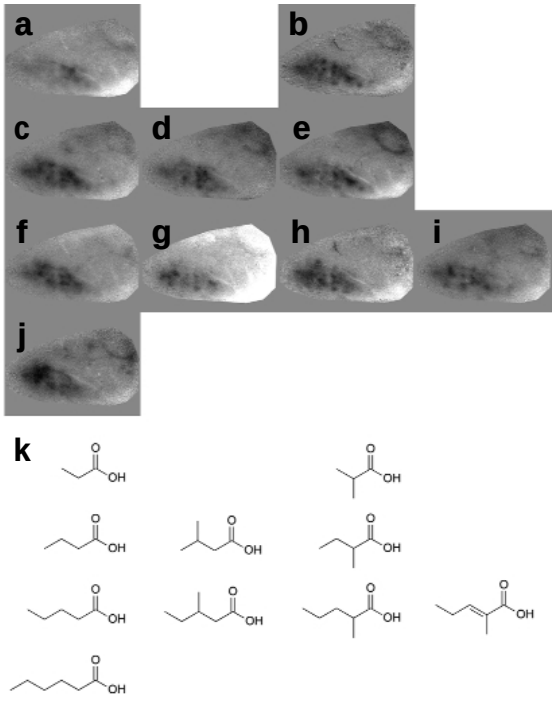



**Fig. 6.** Aliphatic acids with branched carbon chains and a double bond activated glomeruli in the anteromedial domain. (**a–j**) Optical maps of glomeruli in responses to aliphatic acids: propionic acid (**a**), 2-methylpropanoic acid (isobutyric acid) (**b**), butanoic acid (n-butyric acid) (**c**), 3-methylbutanoic acid (isovaleric acid) (**d**), 2-methylbutanoic acid (**e**), pentanoic acid (n-valeric acid) (**f**), 3-methylpentanoic acid (**g**), 2-methylpentanoic acid (**h**), trans-2-methyl-2-pentenoic acid (**i**), n-hexanoic acid (n-caproic acid) (**j**). Molecular formulae of odorants are shown in a corresponding position in (**k**). All the odorants were used with 1:10 dilution. Each acid caused a distinct spatial map of activated glomeruli. This figure and Fig. 2a, right column, were obtained from the same animal. Scale bar, 500 μm.

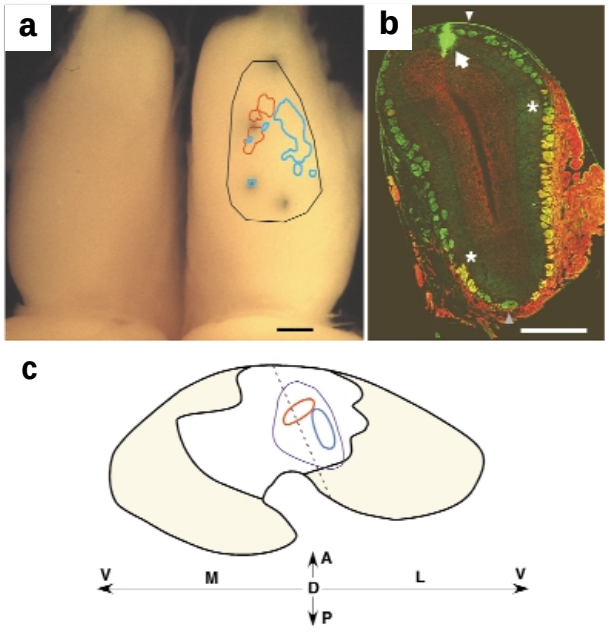



**Fig. 7.** Position of the anteromedial and lateral domains in the sensory map of the main OB. (**a**) Dorsal view of the OB. Imaged region (black contour) was overlaid on the OB with dye-injected points as the references. Regions activated by pentanoic acid (n-valeric acid) in the anteromedial domain are indicated by the red contour and those activated by phenols by the blue contour. (**b**) A coronal section labeled with OCAM antibody (red) and αN-catenin antibody (green). Boundary between OCAM-positive and -negative regions were indicated with asterisks. OCAM-negative glomeruli are located in the dorsal and medial parts of the OB. A dye-injected point is shown by the white arrow. Most dorsal and ventral points are shown by white and gray arrowheads. (**c**) Unrolled map of the glomerular layer of an OB. The most dorsal point is indicated by the dotted line. OCAM-positive zones are indicated by yellow shading. The imaged region is indicated by the purple contour. Almost all the imaged region localized to the OCAM-negative zone 1. Regions activated by acids or phenols are schematically indicated by red and blue, respectively. A, anterior; P, posterior; L, lateral; M, medial; D, dorsal; V, ventral. Scale bars, 1 mm.

glomeruli (Fig. 5a, right, and c). The spatial patterns of response evoked by an ester with a given carbon chain length in the acid part resembled that of the aliphatic acids and aldehydes with the same carbon chain length. These results suggested that odorant receptors represented in the anteromedial domain interact with the acid part, but not the alcohol part, of the carbon chain, in addition to the functional group (–COO–). Thus for polarized molecules like esters, the attachment position of the hydrocarbon chain in reference to a functional group was important.

We also examined the effects of changing other parameters, such as adding a branch or introducing a double bond in aliphatic acids and aldehydes ($n$ = 7). These changes caused significant but relatively small changes in spatial patterns within the anteromedial domain (Fig. 6). These results suggested that the local position of glomeruli within the anteromedial domain might code for features of the carbon chains of fatty acids and aldehydes as well as the carbon chains that make up the acid part of esters.

In the lateral domain, a change in the carbon chain length of primary aliphatic alcohols (R–OH) caused a slight shift in the position of activated glomeruli (Fig. 2a, left, and b). The n-alcohols with shorter carbon chain length were effective in activating glomeruli distributed in relatively posterior portion, whereas those with longer carbon chain length activated more anterior glomeruli. A change in the position of a hydroxyl group in alcohols caused only a minor change within the lateral domain (Fig. 4a, third row (6)-OH, 2-(6)-OH and 3-(6)-OH).

Response patterns for phenols with various substitutions on benzene nucleus were compared by introducing a methyl, ethyl or propyl substitution in the ortho-, meta- or para-position (Fig. 2a, right, and c). Each compound caused a distinct pattern of activated glomeruli within the lateral domain.

### Arrangement of the two molecular feature domains

The position of the imaged region and the two domains were mapped on the OB (Fig. 7a). We then examined the spatial relationship between the two domains and the zonal organization of glomerular sheet in the OB. OCAM, an immunoglobulin superfamily cell adhesion molecule, is a marker for olfactory axons originating from zones II–IV of the olfactory epithelium[24]. These axons project to glomeruli in the caudolateral zones (zones II–IV) of the OB. OCAM-negative axons originate from zone I of the epithelium and project to glomeruli in the dorsomedial zone (zone I). From consecutive frontal sections, we constructed an unrolled map of glomerular layer (Methods; Fig. 7c). Almost all the imaged region (purple contour) belonged to OCAM-negative zone I. The anteromedial and lateral domains (red and blue contours, respectively, in Fig. 7c) were located within zone I. These results showed that within zone I, the glomeruli could be further parceled into local domains, each characterized by specific molecular features.

## Discussion

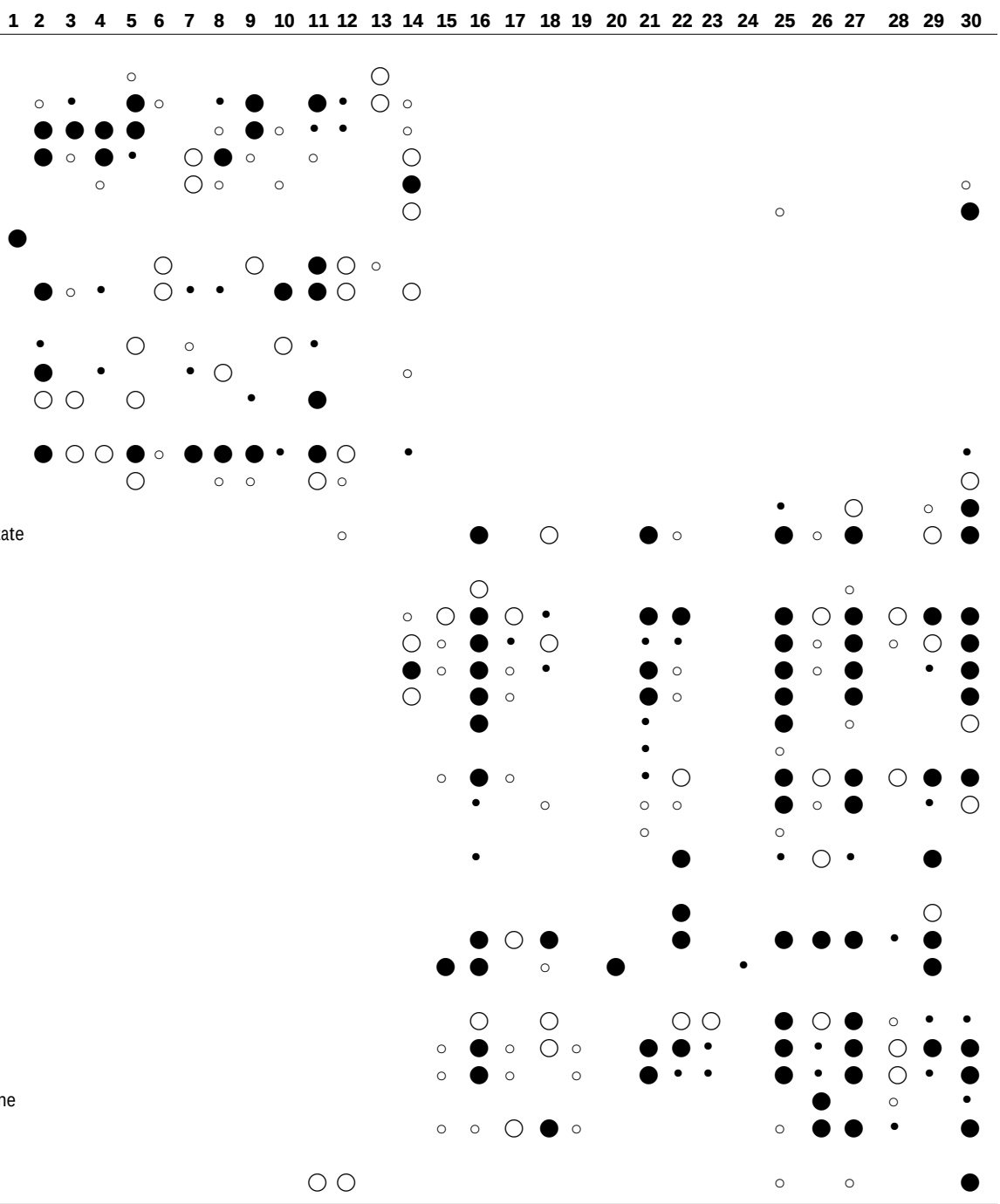
### Hierarchical representation of structural features

Our results demonstrated that various structural features of odorants differentially influenced the spatial map of activated glomeruli in the OB. Using a large panel of odorants, we found that structural features of odorants could be categorized into two classes that differentially affected the spatial map. Primary features are molecular profiles that characterize a domain. For the two domains in the dorsal OB, the primary features were functional groups and their position in the molecule. Secondary features are those that are represented by local arrangement of glomeruli within each domain. For both domains, the secondary features included the length and branching patterns of carbon chains.

The anteromedial domain in the dorsal OB was delineated by the circumscribed region where constituent glomeruli were activated by at least one of the series of fatty acids (Fig. 1b). A majority of glomeruli in this domain were also activated by aliphatic aldehydes (Fig. 1c) and a subset of esters (Table 1; Fig. 3). The lateral domain can be defined as the region where constituent glomeruli can be activated by at least one of the series of aliphatic alcohols or phenols (Fig. 2). Glomeruli in this domain were also activated by ketones and a subset of esters. Although alkanes have the highest vapor pressure among aliphatic compounds[25], they evoked little activity in the imaged region, also supporting the idea that functional groups have an important role as primary features for activating the two domains.

Because individual glomeruli presumably represent a single type of odorant receptor[11–13], response specificity to odorants of a given glomerulus strongly reflects that of the represented receptor. Our results thus suggest that the odor-

**Table 1. Molecular receptive ranges of 30 randomly selected glomeruli (columns) in the same animal for 36 representative odorants (rows).**




The numbers are assigned to each glomerulus in order from anteromedial to posterolateral (**Fig. 3a**). The intensity of glomerular activity was classified into four levels (weak, 0.050–0.070%; modest, 0.070–0.085%; strong, 0.085–0.100%; very strong, more than 0.100% changes in light intensity) and indicated by symbols as follows: weak, ○; modest, ◯; strong, •; very strong, ●. The position of each glomerulus is indicated in Fig. 3a. Dilution of the odorants was 1:10 for acids, esters and ketones; 1:50 for aldehydes; no dilution for alcohols, phenols and terpene.

ant receptors represented within each of the two domains in the dorsal OB have a common site for interaction with the functional groups (primary features) of the ligand odorants. Extrapolating the present observation to other parts of the OB, it is possible that odorant receptors having a common primary feature receptive site are grouped together and represented by glomeruli located within a domain. Olfactory sensory neurons expressing odorant receptors with similar amino-acid sequence project their axons to neighboring glomeruli in the OB[26]. It will be of great interest to systematically compare the amino-acid sequences of receptors represented in the same domain.

Within each domain, secondary molecular features such as carbon chain length, branching, unsaturated bond, or position

or length of substitution on benzene nucleus were represented by local positions. Thus odorant receptors represented by glomeruli at different positions within each domain may differ in their receptive site for the secondary features. Interestingly, one of the secondary features, carbon chain length of aliphatic acids, aldehydes, alcohols and a subset of esters was systematically represented with a gradual shift of the position of activated glomeruli within each domain.

The MRR of individual glomeruli in the anteromedial domain typically covered fatty acids (R–COOH) and aliphatic aldehydes (R–CHO) with similar carbon chains (R). The MRR also covered a subset of esters (R–COO–R´) with similar carbon chains at their acid part (R) but not those with similar carbon chains at their alcohol part (R´; Fig. 4). This suggests that esters can be recognized by the anteromedial domain receptors because their functional group and acid part show primary and secondary features similar to those exhibited by fatty acids and aliphatic aldehydes.

Increasing odorant concentration recruits additional glomeruli[23]. This raises a possibility that concentration of odorants might have a significant effect on the domain organization and local spatial patterns of glomerular activities. To address this issue, we examined the spatial maps of activated glomeruli in responses to various concentrations (1, 1:10, 1:100 and 1:1000 dilutions) of some of the aliphatic aldehydes and fatty acids. We observed that overall spatial pattern of activated glomeruli did not change significantly in the imaged region, although the number and response intensity of activated glomeruli were reduced when the concentration of stimulus odorants was decreased (data not shown). This suggested that the basic domain organization and the local spatial pattern of glomerular responses remain relatively unchanged across a wide range of odorant concentrations.

### Spatial coding and structure–odor relationships

The present observation of the hierarchical representation of the primary and secondary molecular features correlated well with psychophysical studies on structure–odor relationships. First, odor quality is strongly influenced by functional groups, especially in odorants with relatively low molecular weight[3–7]. Second, changes in other parameters such as carbon chain length, branching or unsaturated bond cause only a slight change in odor quality[4,27]. Third, psychophysical discrimination ability between aliphatic aldehydes and alcohols with different carbon chain lengths correlates with the differences in carbon chain length, suggesting that odors of these molecules change gradually with the length of the carbon chain[27]. Indeed, the odor of fatty acids gradually shifts in quality from sour with a pungent note (short carbon chains) to rancid with fatty notes (relatively long carbon chains). Thus, the spatial map of glomeruli with domain organization might correlate with neuronal mechanisms responsible for odor quality perception. Each domain might relate to an olfactory submodality, and combinations of activated domains may relate to overall odor quality. More detailed differences in odor quality might be related to local spatial patterns within each domain.

In the present study, the mapping of odorant-evoked responses was limited to a subregion of the OB because optical imaging was possible only on the dorsal surface. To further examine the molecular feature–odor relationships, it will be necessary to map the spatial pattern of glomerular responses in all part of the OB using methods such as functional magnetic resonance imaging[28].

### Possible function of domains in the sensory map

Although our study was limited to the dorsal surface of the OB, many studies suggest that domain organization is present over the entire OB. Studies of 2-DG labeling or *c-fos* expression show that different odorants evoke activity in discrete patches in various regions of the OB[29–37]. Single-neuron recording studies show that mitral/tufted cells that respond to odorants having the same functional group are clustered in specific regions of the OB[20,21,38]. Furthermore, one 2-DG study shows that fatty acids with two to eight carbons evoke activity in four local regions in the rat OB[22]. The anteromedial domain in this study presumably corresponds to the 'dorsomedial' region in the rabbit OB, where a cluster of neurons can be activated by a series of fatty acids and aliphatic aldehydes[20,21].

What are the advantages of the domain organization for odor discrimination and perception? The information represented by the positions of activated glomeruli is further processed in the OB. For example, lateral inhibition and synchronous firing between mitral/tufted cells associated with different glomeruli may have important roles in odor information processing. An example of this would be to sharpen neuronal MRRs or to enhance behavioral odor discrimination ability[39–42]. These actions are mediated, at least in part, by the local circuits in the OB, including interneurons such as granule cells and periglomerular cells. Mitral/tufted cells located in a neighborhood within the same domain may interact with each other more effectively than more sparsely distributed mitral/tufted cells. Arranging the position of glomeruli in a gradual manner, as in the case of aliphatic acids, aldehydes and alcohols, according to carbon chain length, may help to enhance the ability to distinguish similar compounds by lateral inhibition. Thus, each domain might be a functional unit where molecular information about odorants that share a common primary feature is effectively processed. Local circuits within the domain may help to analyze in detail information about secondary molecular features.

## Methods

**Animal preparation.** Twenty four Sprague-Dawley rats (6 to 10 weeks old, 150–210 g) were used for the experiments. All procedures were performed in accordance with the animal care and use guidelines of the RIKEN Brain Science Institute Animal Committee. Anesthesia was induced by i.p. injection of medetomidine (0.5 mg/kg) and ketamine (67.5 mg/kg) and maintained by i.p. injection of pentothal sodium (25 mg/kg). A $4 \times 2$ mm area of skull overlying the dorsal surface of an OB was thinned with a dental drill. A vaseline well was built around the thinned skull. The well was filled with mineral oil (Sigma, St. Louis, Missouri) to maintain the transparency of the skull, and covered with a cover glass.

**Optical imaging of intrinsic signals.** Intrinsic signal imaging was done using standard methods[43]. Images of reflected light from the dorsal surface of the OB were collected using a CCD camera (CS8310, TELI, Tokyo, Japan) with tandem-lens macroscope arrangement, digitized and stored with Pentium PC using a frame grabber board (Pulser, Matrox, Canada). The images had a spatial resolution of $320 \times 240$ pixels (after binning pixels $2 \times 2$). In most experiments, we imaged a 4.2 mm $\times$ 3.14 mm region, giving a pixel size of 13.1 μm. Before imaging odor responses, blood vessel patterns were imaged using green illumination (540 nm wavelength). Intrinsic signals were imaged with 630 nm wavelength light. The focus was adjusted to 50–150 μm below the surface of the OB. For each recording trial, data were collected for 8 s with a frame length of 500 ms (16 frames per trial). Odor stimulation was applied from the beginning of fourth to the end of sixteenth frame. Each stimulus was separated by a 30 or 60 s interval. Odorants were prepared in a glass test

tube either in pure liquid form or with dilution in mineral oil. Odor stimulation was done by placing an odor-containing test tube within 5 mm of the animal's nostril. Each odorant was tested 3 to 10 times per animal. To check the consistency of the intrinsic signals during an experiment, responses to several standard odorants (for example, pentanoic acid, pentanal, phenol and eugenol) were tested repeatedly. An experiment was continued until the responses to control odor molecules changed in intensity. Experiments typically lasted for 3 to 10 hours, enabling us to examine many odorants in the same animal (up to 200 different odorants). Responses to each odorant were examined in at least three different animals.

Data analysis. Images were analyzed using IDL (Research Systems, Boulder, Colorado) and MetaMorph (Universal Imaging, West Chester, Pennsylvania) software. Odor-response maps were obtained by dividing the magnitude of signals acquired during stimulation (in most cases, frames 8 to 16) by that acquired before stimulation (frames 1 to 2). To achieve a better signal-to-noise ratio, responses were averaged over 3 to 10 odor presentations.

The intrinsic signals consisted of either several isolated spots with a diameter of around 100–200 μm or one or a few larger areas with relatively diffuse darkening. Within the larger area, we usually observed multiple peaks of darkening signal. In the present study, we assumed that each isolated spot or each isolated peak in the larger area corresponds to the activity of a single glomerulus (see ref. 23). However, we cannot rule out the possibility that a spot or peak of darkening signal originate from multiple closely located or overlapped glomeruli. Glomerular activity was quantified by a method reported previously[23] with slight modifications, as follows. First, we measured the mean pixel values around activity peaks (100–130 μm in diameter) for each glomerulus. Glomerular activity was calculated by subtracting the background value to correct the baseline. Background values were obtained by measuring mean pixel values within a few oval regions 200 to 500 μm surrounding the activity peaks. Background regions were selected not to contain blood vessels or intact bones. The glomerular activity usually ranged from 0.05 to 0.3% of the reflected light intensity. The areal extent of an activated glomerulus or region was defined as the region that showed activity larger than 50% of the maximum activity for each glomerulus. In some cases, areal extent was measured after removing the low spatial frequency component with Gaussian filter.

Odorants. Two hundred and five different compounds were used as odorants for stimulation. Odorants were purchased from Sigma, Tokyo Kasei Organic Chemicals (Tokyo, Japan), Nacalai tesque (Kyoto, Japan) or T.Hasegawa (Tokyo, Japan). Odorants used were 18 carboxylic acids including normal (n)-aliphatic acids with 2 to 10 carbon atoms (abbreviated to (2)- to (10)-COOH), 18 aldehydes including n-aliphatic aldehydes with 3 to 11 carbon atoms (abbreviated to (3)- to (11)-CHO), 21 aliphatic alcohols including primary alcohols with 3 to 10 carbon atoms (abbreviated to (3)- to (10)-OH), six n-alkanes with 5 to 10 carbon atoms, 32 ketones, 65 esters, 19 phenols including ortho(o)-, meta(m)-, para(p)-cresol, 2 terpene hydrocarbons, 2 lactons and 16 odorants with a hologen-, sulfide- or nitrogen-containing functional group. For secondary or tertiary alcohols, 2-hexanol and 3-hexanol were abbreviated to 2-(6)-OH and 3-(6)-OH. Normal aliphatic ketones, such as 3-hexanone (ethyl n-propyl ketone, $C_2H_5COC_3H_7$), were abbreviated to 'K 3-2', where each of two numbers indicate the carbon numbers in each side of a carbonyl group, and the large number was indicated first. Normal aliphatic esters, such as n-propyl acetate, were abbreviated to 'E 3-2', where the first number in the abbreviation indicates the carbon number of the alcohol, and the latter that of the acid part.

Histology. In some experiments, after the optical imaging, the bone overlying the imaged bulb was removed, and a blue dye, Chicago Sky Blue 6B (Tocris Cookson, Ballwin, Missouri), was iontophoretically injected with a glass micropipette at several points. Under deep anesthesia, animals were perfused with 4% paraformaldehyde. The OB was dissected out and examined for immunofluorescence staining. Cryostat sections (20 μm) were stained for OCAM and αN-catenin as described[44]. For detection of OCAM and αN-catenin, we used rabbit polyclonal antibodies to OCAM[24] and NCAT2, a rat monoclonal antibody to αN-catenin[44], respectively. An unfolded map of the glomerular layer of the OB was constructed as follows. A smooth line was traced along the center of the glomerular layer of every five sections (100 μm). The line was flattened by opening it at the most ventral point. OCAM-positive and -negative glomeruli and dye-injected points were then mapped on the flattened lines. The unfolded map was constructed by aligning the flattened traces of consecutive sections using the dorsal edge of the glomerular layer as a reference. The imaged region was determined with reference to the dye-injected points.

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1. Moncrieff, R. W. *The Chemical Senses* 3rd ed. (Leonard Hill, London, 1967).
2. Amoore, J. E., Johnston, J. W. Jr. & Rubin, M. The stereochemical theory of odor. *Sci. Am.* 210, 42–49 (1964).
3. Beets, M. The molecular parameters of olfactory response. *Pharmacol. Rev.* 22, 1–34 (1970).
4. Polak, E. H. Multiple profile-multiple receptor site model for vertebrate olfaction. *J. Theor. Biol.* 40, 469–484 (1973).
5. Rupe, H. & von Majewski, K. I. Ueber osmophore gruppen., II. Ueber die darstellung von diazoimiden. (triazoverbindungen). *Ber. Dtsch. Chem. Ges.* 33, 3401–3410 (1900).
6. Klopping, H. L. Olfactory theories and the odors of small molecules. *J. Agric. Food Chem.* 19, 999–1004 (1971).
7. Macleod, A. J. Chemistry of odours. *Symp. Zool. Soc. Lond.* 45, 15–34 (1980).
8. Buck, L. & Axel, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65, 175–187 (1991).
9. Mombaerts, P. Molecular biology of odorant receptors in vertebrates. *Annu. Rev. Neurosci.* 22, 487–509 (1999).
10. Malnic, B., Hirono, J., Sato, T. & Buck, L. B. Combinatorial receptor codes for odors. *Cell* 96, 713–723 (1999).
11. Ressler, K. J., Sullivan, S. L. & Buck, L. B. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79, 1245–1255 (1994).
12. Vassar, R. *et al.* Topographic organization of sensory projections to the olfactory bulb. *Cell* 79, 981–991 (1994).
13. Mombaerts, P. *et al.* Visualizing an olfactory sensory map. *Cell* 87, 675–686 (1996).
14. Zhao, H. *et al.* Functional expression of a mammalian odorant receptor. *Science* 279, 237–242 (1998).
15. Krautwurst, D., Yau, K.-W. & Reed, R. R. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* 95, 917–926 (1998).
16. Touhara, K. *et al.* Functional identification and reconstitution of an odorant receptor in single olfactory neurons. *Proc. Natl. Acad. Sci. USA* 96, 4040–4045 (1999).
17. Buck, L. B. The molecular architecture of odor and pheromone sensing in mammals. *Cell* 100, 611–618 (2000).
18. Friedrich, R. & Korsching, S. I. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron* 18, 737–752 (1997).
19. Friedrich, R. W. & Korsching, S. I. Chemotopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. *J. Neurosci.* 18, 9977–9988 (1998).
20. Mori, K., Mataga, N. & Imamura, K. Differential specificities of single mitral cells in rabbit olfactory bulb for a homologous series of fatty acid odor molecules. *J. Neurophysiol.* 67, 786–789 (1992).
21. Imamura, K., Mataga, N. & Mori, K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. *J. Neurophysiol.* 68, 1986–2002 (1992).
22. Johnson, B. A., Woo, C. C., Hingco, E. E., Pham, K. L. & Leon, M.

Multidimensional chemotopic responses to n-aliphatic acid odorants in the rat olfactory bulb. *J. Comp. Neurol.* 409, 529–548 (1999).
23. Rubin, B. D. & Katz, L. C. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 23, 499–511 (1999).
24. Yoshihara, Y. *et al.* OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons. *J. Neurosci.* 17, 5830–5842 (1997).
25. Lide, D. R. *CRC Handbook of Chemistry and Physics* (CRC, Boca Raton, Florida, 1996).
26. Tsuboi, A. *et al.* Olfactory neurons expressing closely linked and homologous odorant receptor genes tend to project their axons to neighboring glomeruli on the olfactory bulb. *J. Neurosci.* 19, 8409–8418 (1999).
27. Laska, M. & Teubner, P. Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes. *Chem. Senses* 24, 263–270 (1999).
28. Yang, X. *et al.* Dynamic mapping at the laminar level of odor-elicited responses in rat olfactory bulb by functional MRI. *Proc. Natl. Acad. Sci. USA* 92, 3371–3375 (1998).
29. Sharp, F. R., Kauer, J. S. & Shepherd, G. M. Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. *Brain Res.* 98, 596–600 (1975).
30. Sharp, F. R., Kauer, J. S. & Shepherd, G. M. Laminar analysis of 2-deoxyglucose uptake in olfactory bulb and olfactory cortex of rabbit and rat. *J. Neurophysiol.* 40, 800–813 (1977).
31. Skeen, L. C. & Hall, W. C. Efferent projections of the main and accessory olfactory bulb in the tree shrew (*Tupania glis*). *J. Comp. Neurol.* 172, 1–36 (1977).
32. Stewart, W. B., Kauer, J. S. & Shepherd, G. M. Functional organization of rat olfactory bulb analyzed by the 2-deoxyglucose method. *J. Comp. Neurol.* 185, 715–734 (1979).
33. Jourdan, F., Duveau, A., Astic, L. & Holley, A. Spatial distribution of 2-deoxyglucose uptake in the olfactory bulb of rats stimulated with two different odors. *Brain Res.* 188, 139–154 (1980).
34. Sallaz, M. & Jourdan, F. C-fos expression and 2-deoxyglucose uptake in the olfactory bulb of odour-stimulated awake rats. *Neuroreport* 4, 55–58 (1993).
35. Guthrie, K. M., Anderson, A. J., Leon, M. & Gall, C. Odor-induced increase in c-fos mRNA expression reveal an anatomical "unit" for odor processing in olfactory bulb. *Proc. Natl. Acad. Sci. USA* 90, 3329–3333 (1993).
36. Guthrie, K. M. & Gall, C. Functional mapping of odor-activated neurons in the olfactory bulb. *Chem. Senses* 20, 271–282 (1995).
37. Johnson, B. A., Woo, C. C. & Leon, M. Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. *J. Comp. Neurol.* 393, 457–471 (1998).
38. Katoh, K., Koshimoto, H., Tani, A. & Mori, K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. II. Aromatic compounds. *J. Neurophysiol.* 70, 2161–2175 (1993).
39. Yokoi, M., Mori, K. & Nakanishi, S. Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. *Proc. Natl. Acad. Sci. USA* 92, 3371–3375 (1995).
40. Laurent, G. Dynamical representation of odors by oscillating and evolving neural assemblies. *Trends Neurosci.* 19, 489–496 (1996).
41. Kashiwadani, H., Sasaki, Y. F., Uchida, N. & Mori, K. Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. *J. Neurophysiol.* 82, 1786–1792 (1999).
42. Mori, K., Nagao, H. & Yoshihara, Y. The olfactory bulb: coding and processing of odor molecule information. *Science* 286, 711–715 (1999).
43. Bonhoeffer, T. & Grinvald, A. in *Brain Mapping. The Method* (eds. Toga, A. W. & Mazziotta, J. C.) 55–97 (Academic, San Diego, California, 1995).
44. Uchida, N., Honjo, Y., Johnson, K. R., Wheelock, M. J. & Takeichi, M. The catenin/cadherin adhesion system is localized in synaptic junction bordering transmitter release zones. *J. Cell Biol.* 135, 767–779 (1996).