Spatial Arrangement of Glomerular Molecular-Feature Clusters in the Odorant-Receptor Class Domains of the Mouse Olfactory Bulb

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Matsumoto H, Kobayakawa K, Kobayakawa R, Tashiro T, Mori K, Sakano H, Mori K. Spatial arrangement of glomerular molecular-feature clusters in the odorant-receptor class domains of the mouse olfactory bulb. J Neurophysiol 103: 3490–3500, 2010. First published April 14, 2010; doi:10.1152/jn.00035.2010. The glomerular layer of the mammalian olfactory bulb (OB) forms odorant receptor (OR) maps. Each OR map is structurally and functionally compartmentalized into zones (dorsal and ventral) and domains (D and DII in the dorsal zone). We previously reported that glomeruli with similar molecular receptive range properties formed molecular feature clusters at stereotypical positions in the rat OB. However, the spatial arrangement of the molecular feature clusters with regard to the OR zones and domains has not been systematically examined. In this study, we optically mapped the molecular feature clusters of glomeruli within the domain and zone framework of the OB using domain-visible class II GFP transgenic mice. In all mice examined, fatty acid-responsive cluster A was located in the lateral part of domain DI, whereas clusters B, C, and D were arranged in an anterior to posterior order within domain DII. We also found a new cluster of glomeruli that respond to fox odor trimethyl-thiazoline and its structural analogs (heterocyclic odorants that contain sulfur and nitrogen atoms within the ring). This cluster (named cluster J) was located posterior to cluster D within the DII domain. These results show that molecular feature clusters correspond to specific subsets of glomeruli in selective domains of the OR map, suggesting that the molecular feature clusters represent specific ORs that have similar molecular receptive range properties and functional roles.

INTRODUCTION

The surface of the mammalian olfactory bulb (OB) is covered by a few thousand glomeruli (Mori et al. 1999; Shepherd et al. 2004). Because individual glomeruli represent a single odorant receptor (OR), the glomerular sheet in the OB forms OR maps (Mori et al. 2006). An individual OB has two mirror-symmetric OR maps: the lateral map and the medial map (Nagao et al. 2000). However, the spatial organization within each OR map is not fully understood.

Gross groupings of glomeruli at stereotypical positions within each OR map have been observed using two distinct approaches. One approach is based on the classification of the OR that is represented by individual glomeruli. Mammalian ORs are classified into dorsal-zone (D-zone or zone 1) and ventral-zone (V-zone or zones 2–4) ORs (Zhang et al. 2004). D-and V-zone ORs are expressed by sensory neurons within the D-zone and V-zone of the olfactory epithelium, respectively. D-zone sensory neurons project their axons selectively to the D-zone of the glomerular sheet of the OB, whereas V-zone sensory neurons send their axons exclusively to the V-zone of the glomerular sheet (Yoshihara et al. 1997). Thus the glomerular OR map is organized into two largely segregated zones.

ORs are also classified in two classes: class I (fish-type) and class II (terrestrial-type) (Freitag et al. 1995; Glusman et al. 2001; Ngai et al. 1993; Zhang and Firestein 2002). Although almost all V-zone ORs are class II, D-zone ORs are further classified into D-zone class I (DI) ORs and D-zone class II (DII) ORs. Sensory neurons expressing DI ORs project their axons to glomeruli in the DI domain, whereas those expressing DII ORs project their axons to the DII domain of the OB D-zone. Thus the D-zone of the glomerular OR map comprises two segregated domains: DI and DII (Bozza et al. 2009; Kobayakawa et al. 2007; Tsuboi et al. 2006). The DII domain in the glomerular OR map is further subdivided into anterior and posterior subdomains (Imai et al. 2009). Together, these data show a clear spatial organization of domains and subdomains in the OR maps of the OB.

Another approach to study the spatial grouping of glomeruli is based on the characteristics of the molecular receptive range (MRR) or the odorant-selectivity of individual glomeruli. The map of the MRR, or spatial odor representation in the glomerular sheet, shows that individual glomeruli respond to a range of odorants that share common molecular features and that glomeruli with a similar MRR property gather to form molecular feature clusters at stereotypical positions in the glomerular sheet (Igarashi and Mori 2005; Johnson and Leon 2007; Mori et al. 2006; Takahashi et al. 2004a; Uchida et al. 2000). In addition, a recent imaging study showed coarse chemotopy but local diversity in odorant tuning (Soucy et al. 2009).

Glomerular mapping of ORs and odorant responses, however, has been performed independently, and there has been no systematic comparison between the spatial arrangement of the OR-zones and domains and the molecular feature clusters. Indeed, only two studies have reported limited data on their spatial relationship (Bozza et al. 2009; Kobayakawa et al. 2007). In this study, we systematically analyzed the relationship between the spatial arrangement of OR-zones and domains and the molecular feature clusters of glomeruli in the mouse OB by combining optical imaging of intrinsic signals and olfactory cell adhesion molecule (OCAM) immunohistochemistry. To visualize the spatial arrangement of the domains,
we used class II-GFP transgenic mice in which glomeruli in the DII but not the DI domain were labeled with GFP. By optically imaging intrinsic signals, we measured the responses of glomeruli in the dorsal surface of the OB of the class II-GFP transgenic mice to a systematic panel of odorants. To visualize zonal organization, we labeled OB sections with an anti-OCAM antibody, which stains glomeruli in the V-zone but not in the D-zone. These data showed a clear spatial arrangement of molecular feature clusters within the framework of the DI and DII domains of the mouse OB.

A recent study showed that specific subsets of glomeruli in the DII domain mediate innate fear responses to predator odors, whereas selective subsets of glomeruli in the DI domain are involved in aversive responses to spoiled food odors (Kobayakawa et al. 2007), suggesting functional compartmentalization of OR maps in terms of odor-induced behavioral responses (Mori et al. 2009). For example, the innate fear response of mice to the fox odor trimethyl-thiazoline (TMT) is mediated by a specific subset of glomeruli located in the caudal part of the DII domain. However, the MRR properties of TMT-responsive glomeruli and surrounding glomeruli in the DII domain have not been explored using optical imaging. In the latter part of this study, we thus analyzed the MRR properties of glomeruli near the TMT-responsive glomeruli within the DII domain.

METHODS

Animal preparation

Imaging experiments were performed using 16 adult class II-GFP transgenic mice (7–13 weeks old; 20–33 g) that were generated by crossing mice containing the Cre-inducible GFP gene [ROSA-STOP-GFP (stock number 4077) reporter mice; Jackson Laboratory] with MOR23-cre mice (Kobayakawa et al. 2007). In the class II-GFP mice, class-II OR-expressing sensory neurons are labeled with GFP, and GFP-positive glomeruli and GFP-negative glomeruli in the D-zone of the OB are largely segregated into two distinct areas, the DII and DI domains, respectively (Kobayakawa et al. 2007). Animals were anesthetized with medetomidine (0.5 mg/kg, ip), ketamine (22.5 mg/kg, ip), and pentothal sodium (25 mg/kg, ip). Additional doses of pentothal sodium were given to maintain the anesthesia, if necessary. Animals were placed in a stereotaxic apparatus (SR-6N, Narishige, Tokyo, Japan). The skull overlying the dorsal surface of the OB was removed. Body temperature was maintained at 37.5°C using a homeothermic heatpad system (MK-900, Muromachi Kikai, Tokyo, Japan). Heart beat, respiratory rate, and the lack of pain reflexes were monitored continuously. All experiments were performed in accordance with the guidelines of the Physiological Society of Japan and the animal experiment committee of the University of Tokyo.

Odorants

Almost all odorants were purchased from Sigma-Aldrich (St. Louis, MO), Tokyo Chemical Industry (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan). A panel of 21 odorants was used for the first series of experiments (panel A): 3 aliphatic acids (propionic acid [3-COOH], valeric acid [5-COOH], heptanoic acid [7-COOH]) and 4 aldehydes (propionaldehyde [3CHO], valeraldehyde [5CHO], heptaldehyde [7CHO] and benzaldehyde [Bz-CHO]), 3 aliphatic alcohols (pentyl alcohol [5OH], heptyl alcohol [7OH] and nonyl alcohol [9OH]), 3 aliphatic ketones (di-n-propyl ketone [K3-3], n-pentyl methyl ketone [K5-1] and n-heptyl methyl ketone [K7-1]), 3 phenols (phenol [Phe], m-cresol [m-Cre] and o-ethyl phenol [o-Ephe]), 3 phenyl ethers (guaiacol [Gua], creosol [Creo] and anisole [Ani]), and 2 aliphatic-aromatic ketones (acetophenone [Aceph] and d-pulegone [d-Plg]). These odorants are known to activate glomeruli in the dorsal surface of the rat OB (Takahashi et al. 2004a).

In the second series of experiments, we used 10 heterocyclic compounds [2,4,5-trimethyl-thiazoline (TMT), 2-methyl-thiazoline, 4-methyl-thiazole, 5-methyl-thiazole, thiazole, thiazolidine, thiophene, tetrahydrothiophene, pyrrole and pyrrolidine], and aliphatic and cyclic compounds (panel B). The aliphatic compounds were three aliphatic ketones (K3-3, K5-1, and K7-1), whereas the cyclic compounds were two phenols (Phe and m-Cre) and five aliphatic-aromatic ketones [Aceph, (+)-carvone, (-)-carvone, (+)-camphor, and d-Plg]. In addition, three heterocyclic compounds [2-sec-butyldihydrothiazole (SBT), 2-isopropyl-dihydrothiazole (IPT), and dehydro-exo-brevicomin (DHBl) present in male mouse urine (Tashiro and Mori 1999; Tashiro et al. 2008) were used in the second series of experiments. Aliphatic acids, aliphatic aldehydes, and aliphatic ketones were diluted 1/5, 1/50, and 1/20 (vol/vol) with odorless mineral oil, respectively. Anisole, TMT, thiophene, and tetrahydrothiophene were diluted 1/10.

Optical imaging of intrinsic signals

Detailed procedures for optical imaging have been described previously (Takahashi et al. 2004a; Uchida et al. 2000). Briefly, intrinsic signals (absorption changes at 705 nm) were optically recorded from the dorsal surface of the OB through a glass coverslip window of an agarose gel (2%) chamber. Images were collected using a CCD camera (CS8310, Tokyo Electric Industry, Tokyo, Japan) and digitized with an IBM/PC-compatible computer equipped with a video frame grabber board (Pulsar, Matrox, Quebec, Canada). A 4.2 × 3.1-mm region was imaged with a spatial resolution of 320 × 240 pixels. Images of surface blood vessels were photographed using 540-nm wavelength light and a CCD camera. The focusing depth was adjusted to 50–150 μm below the dorsal surface of the OB. For each recording trial, data were collected for 8 s with a frame length of 0.5 s (16 frames per trial). Odorant stimulation was applied from the beginning of the 5th to the end of the 16th frame. The interstimulus interval was 30 s. Odorants were prepared in glass test tubes either as a liquid diluted in mineral oil or in solid form. Odorant stimulation was performed by placing an odorant-containing test tube within 10 mm of the animal’s nostril. The order of odorant application was arbitrarily changed in each experiment. Each odorant was tested more than four times per animal.

Data analysis

Images were analyzed using IDL software (Research Systems, Boulder, CO). A differential image was used to eliminate nonspecific global darkening and high-frequency noise of the differential image (cut-off frequencies were σ = 60.0/μm for the high cut-off and σ = 1.0/μm for the low). To achieve a better signal-to-noise ratio, filtered images were averaged over 4–12 odorant presentations. The final images were imported into Adobe Photoshop for cropping and display. All right OBs were inverted and displayed on the left side. For superimposed displays of multiple odorant-evoked responses, the threshold was set at 0.035% reflectance change of the glomerular response, and the images were processed using a 3 × 3-pixel median filter.

Because the diameter range of glomeruli in the mouse OB is ~50–120 μm (Royet et al. 1988), each dim spot that showed more than a 0.035% reflectance change and a diameter >90 μm was defined as the response from one glomerulus. A number was assigned to each glomerulus. The response magnitude of an individual glomerulus was calculated by averaging the responses within a 90-μm-diam circle using MetaMorph software (Universal Imaging, West Chester, PA). Because of the local pile-up of glomeruli, we could not rule out the
possibility that a spot originated from a nearby glomeruli or from multiple overlapping glomeruli.

To get an objective measure of the degree of similarity of odorant-response properties between two different glomeruli, we calculated uncentered correlation coefficients for pairs of glomeruli, in a manner similar to that described in a previous report (Soucy et al. 2009)

\[ s_{AB} = \frac{\sum_{j=1}^{n} r_{ij}^{(A)} r_{ij}^{(B)}}{\sqrt{\sum_{j=1}^{n} r_{ij}^{(A)}^2} \sqrt{\sum_{j=1}^{n} r_{ij}^{(B)}^2}} \]

where \( r_{ij}^{(A)} \) = response of glomerulus A to odorant j, and \( n \) = number of odorants.

**Histology**

In five mice, a blue dye (Chicago Sky Blue 6B, Tocris Bioscience) was iontophoretically injected after optical imaging into some of the active spots using a glass micropipette. Under deep anesthesia, animals were transcardially perfused with saline and then with 4% paraformaldehyde (Nacalai Tesque, Kyoto, Japan). The OB was dissected out, postfixed overnight at 4°C, and immersed in a 30% paraformaldehyde solution for ≥1 day at 4°C. Frozen sections of the OB (16 \( \mu \)m) were obtained using a cryostat. Consecutive coronal sections of the OB were stained using a rabbit polyclonal anti-OCAM antibody (Yoshihara et al. 1997), a rat monoclonal anti-GFP antibody (Nacalai Tesque, Kyoto, Japan), and a mouse monoclonal anti-synaptotagmin antibody (MAB5202, Chemicon). DI domain (GFP−/OCAM−), DII domain (GFP+/OCAM−), and V-zone (OCAM+) glomeruli were traced and reconstructed as a dorsal view. Reconstructed dorsal glomerular maps were superimposed by the optically determined glomerular response map using the position of marker dyes as a reference (Kobayakawa et al. 2007; Nagao et al. 2000).

**RESULTS**

Spatial arrangement of glomeruli molecular feature clusters in the framework of domain organization

The main purpose of this study was to examine the spatial arrangement of the molecular feature clusters of glomeruli within the domain and zone framework of the mouse OB. Using optical imaging of intrinsic signals and a systematic panel of stimulus odorants, we previously reported the spatial arrangement of four molecular feature clusters (clusters A–D) of glomeruli at the dorsal surface of the rat OB (Mori et al. 2006; Takahashi et al. 2004a). Although the shape and area of each cluster varied, the relative spatial arrangement of clusters A–D was conserved across animals. Thus, we first examined whether similar spatial arrangements of molecular feature clusters were present at the dorsal surface of the mouse OB. As will be described in detail (Figs. 2 and 3), molecular feature clusters A–D at the dorsal surface of the class II-GFP mouse OB resembled the spatial arrangement of those in the rat OB.

To examine the spatial arrangement of molecular feature clusters in the framework of domains and zones using the class II-GFP mouse OB, a positional marker dye was injected at several points that corresponded to odorant-activated glomeruli at the end of each optical imaging experiment. To visualize the domains and zones, coronal sections of seven OBs from five class II-GFP mice were immunostained with anti-OCAM and anti-GFP antibodies (Fig. 1A), and the dorsal view of the glomerular layer of the OB was reconstructed (Fig. 1B). The V-zone glomeruli are OCAM-positive (orange glomeruli) in Fig. 1A and filled circles in Fig. 1B), whereas D-zone glomeruli are OCAM-negative (Yoshihara et al. 1997). As shown previously (Bozza et al. 2009; Kobayakawa et al. 2007), the dorsal zone is further subdivided into the DI domain where most glomeruli are GFP-negative (Fig. 1B, ○) and the DII domain where most glomeruli are GFP-positive (green glomeruli in Fig. 1A, and light gray areas in Fig. 1B). The border between the DI and DII domains (indicated by a white rectangle in Fig. 1A) was determined (shown by dashed red lines in Fig. 1B). The optically determined glomerular response map was superimposed on the histologically defined map of the domains and zones using the position of marker dyes as a reference (Figs. 1 and 2).

In five OBs from three class II-GFP mice, the border between the DI and DII domains was determined by observing the spatial arrangement of GFP-negative and -positive glomeruli at the dorsal surface using in vivo fluorescent imaging (Belluscio et al. 2002; Oka et al. 2006). The position of the boundary was superimposed on the optically determined glomerular response map in reference to the blood vessel patterns on the dorsal OB.
Using a panel of odorants consisting of aliphatic acids, aldehydes, aliphatic alcohols, aliphatic ketones, phenols, phenyl ethers, and aliphatic-aromatic ketones that are known to activate clusters A–D in the dorsal surface of the rat OB (Takahashi et al. 2004a), we optically mapped the glomerular responses at the dorsal surface of the OB of class II-GFP mice (Fig. 2). Aliphatic acids activated glomeruli in the antero-medial region of the imaged area (Fig. 2A). Glomeruli in the...
posterior part of the region tended to respond to aliphatic acids with short-carbon chains, such as 3COOH and 5COOH, whereas glomeruli in the anterior part of the region responded to all the aliphatic acids used in this study (n = 12 OBs). The clustering of aliphatic acid responsive glomeruli resembles the glomeruli in cluster A in the dorsal surface of the rat OB. Aldehydes activated glomeruli in the anteromedial and lateral regions of the imaged area (Fig. 2B; n = 10 OBs). Aliphatic alcohols activated glomeruli in the anterior part of the lateral region (Fig. 2C; n = 12 OBs), whereas aliphatic ketones activated glomeruli in the anterior and posterior parts of the lateral region (Fig. 2D; n = 12 OBs). Phenols and phenyl ethers activated glomeruli in the central part of the lateral region (Fig. 2, E and F; n = 12 OBs), and aliphatic-aromatic ketones primarily activated glomeruli in the posterior part of the lateral region (Fig. 2G; n = 12 OBs). The relative spatial arrangement of glomeruli that were activated in response to aliphatic alcohols, aliphatic ketones, phenols, phenyl ethers, and aliphatic-aromatic ketones resembles the activation patterns observed in the rat OB (Takahashi et al. 2004a).

The glomeruli responses of the rat OB D-zone have been mapped (Takahashi et al. 2004a) and provide a guide to defining similar boundaries in the mouse. For example, glomeruli in cluster A of the D-zone in the rat OB are located in the anteromedial region and respond to aliphatic acids and aldehydes. Cluster B is located in the anterior section of the lateral region, and these glomeruli respond to aliphatic alcohols and aliphatic ketones. Glomeruli in cluster C are located in the central part of the lateral region and respond to phenols and phenyl ethers, whereas cluster D is located in the posterior section of the lateral region, and these glomeruli respond to aliphatic ketones and cyclic ketones.

Based on the glomerular clustering topography used in the rat OB, we grouped the mouse D-zone glomeruli into four molecular feature clusters (A–D; Fig. 3): glomeruli (1–13) that responded to all the aliphatic acids used in this study (n = 12 OBs). The relative spatial arrangement of the molecular feature clusters in the DII domain form a molecular feature cluster. TMT is the TMT-responsive glomeruli and neighboring glomeruli in the DII domain (97% of all the fatty acid–responsive glomeruli in the D-zone, n = 7 OBs), whereas nearly all the aliphatic acids activated glomeruli in the DII domain (93, 99, 100, 98, and 99%, respectively). In contrast, aldehydes activated glomeruli in both DI (29%) and DII domains (71%).

Molecular feature cluster of TMT-responsive glomeruli

Specific subsets of glomeruli in the DII domain mediate the innate fear response of mice to the predator odor TMT (Kobayakawa et al. 2007). However, it is not well understood whether the TMT-responsive glomeruli and neighboring glomeruli in the DII domain form a molecular feature cluster. TMT is composed of a heterocyclic thiazoline ring that contains sulfur and nitrogen atoms. We thus used heterocyclic odors to stimulate the olfactory epithelium and examine the MRR properties of the TMT-responsive glomeruli and surrounding glomeruli. Figure 4 exemplifies the glomerular responses to these heterocyclic odors. All heterocyclic odors containing sulfur and nitrogen atoms in the ring (thiazoles and thiazolines) strongly activated several glomeruli in the most-caudal part of the DII domain, except for thiazolidine (Fig. 4B).
Heterocyclic odorants containing a single sulfur or nitrogen atom in the ring activated glomeruli that were primarily located in the central part of domain DII (Fig. 4C). These data indicated that the characteristic molecular feature of the odorants that activated TMT-responsive glomeruli in the caudal part of domain DII was a heterocyclic ring containing both sulfur and nitrogen atoms, which is characteristic of thiazoles and thiazolines.
ular responses to TMT, SBT, and 2-isopropyl-dihydrothiazole domain. To examine this possibility, we mapped the glomeruli containing a thiazoline-ring but DHB does not. Because SBT has a different molecular structure: SBT contains a thiazole- or thiazoline ring but is functionally related to SBT. DHB primarily activated glomeruli within the DII domain; light gray circles, glomeruli in the DII domain; dark gray circles, glomeruli in the V-zone. The boundary between the DI and DII domains is indicated by a red dotted line. Scale bar, 500 μm. B: responses to heterocyclic odorants containing both S and N atoms in the ring (thiazole-family). Ba-Bf: responses to TMT (Ba, red), 2-methyl-thiazoline (Bb, blue), 4-methyl-thiazole (Bc, light blue), 5-methyl-thiazole (Bd, green), thiazole (Be, yellow), and thiazolidine (Bf, pink). Bg: superimposed image of Ba-Bf. Glomeruli that were activated by each member of the thiazole-family odorants were color coded as indicated above and overlaid on the blood vessel pattern. C: responses to heterocyclic odorants containing an S or N atom in the ring (thiophene- or pyrrole-families). Ca–Cd: responses to thiophene (Ca, red), tetrahydrothiophene (Cb, blue), 1H-pyrrole (Cc, light blue), and pyrrolidine (Cd, green). Ce: superimposed image of Ca–Cd. All images were derived from the same OB (mouse B-1, left OB).

To clarify whether thiazole- or thiazoline-responsive glomeruli form molecular feature clusters, we examined the MRRs of glomeruli responding to these odorants (Fig. 5). Glomeruli (23–30) that responded to thiazoles and thiazolines were located in the caudal part of domain DII and gathered to form a novel molecular feature cluster that we named cluster J. In the five OBs examined, cluster J was consistently located posterior to cluster D. Thus molecular feature clusters B, C, D, and J are located in order from the anterior to the posterior region of domain DII of the mouse OB.

Glomerular response to behaviorally relevant odorants contained in mouse urine

Mouse urine contains various odorants that induce specific behavioral responses (Lin et al. 2005; Novotny 2003). 2-sec-Butyl-dihydrothiazole (SBT) and dehydro-exo-brevicomin (DHB) are well-known urinary odorants that induce aggressive behavioral responses in male mice (Novotny et al. 1985). These odorants have different molecular structures: SBT contains a thiazoline-ring but DHB does not. Because SBT has a thiazoline-ring, we hypothesized that the mouse urinary odorant SBT may activate some glomeruli in cluster J of the DII domain. To examine this possibility, we mapped the glomerular responses to TMT, SBT, and 2-isopropyl-dihydrothiazole (IPT) (Liebich et al. 1977). SBT and IPT activated glomeruli in the most-caudal region of the DII domain (Fig. 6, Bb and Be). SBT-responsive glomeruli (4 and 6 in Fig. 6D) in cluster J also responded to TMT. Similar results were obtained in all 12 OBs tested.

We next examined glomerular response to DHB, an odorant that has molecular features distinct from the thiazole or thiazoline ring but is functionally related to SBT. DHB primarily activated glomeruli within cluster J, and some of the DHB-responsive glomeruli also responded to both TMT and SBT (for example, glomerulus 6 in Fig. 6D). In eight OBs examined, DHB consistently activated glomeruli near the TMT-responsive glomeruli within cluster J. These results suggest that odorants that do not have similar molecular features but provoke similar odor-induced behavioral responses can activate glomeruli in the same molecular feature cluster or even the same glomerulus. These results support the hypothesis that molecular feature clusters of glomeruli might be functionally related to specific odor-induced behavioral responses.

DISCUSSION

Molecular feature clusters in domains and zones

Each OR map of the glomerular sheet in the OB is organized into zones (Oka et al. 2003; Yoshihara et al. 1997) and domains
The glomerular map is also organized into molecular feature clusters based on the MRR properties of individual glomeruli (Johnson and Leon 2007; Mori et al. 2006). Although recent studies have suggested a relationship between zonal and domain organization and the spatial layouts of odorant-evoked glomerular responses (Bozza et al. 2009; Kobayakawa et al. 2007), studies to date have not systematically examined this relationship. In this study, we systematically examined the spatial relationship between molecular 

![Diagram of glomerular map]

**Fig. 5.** The spatial arrangement of cluster J in which glomeruli strongly respond to thiazoles and thiazolines. A: positions of the 31 glomeruli in the DII domain that were activated by at least 1 odorant in stimulus panel B (Fig. 5B). The imaged region covers the dorsal surface of the OB (left OB of mouse B-1). Clusters B (glomeruli 1–9), C (glomeruli 10–13), D (glomeruli 15–21), and J (glomeruli 23–30, light blue) were arranged in order from the anterior to posterior region of the DII domain. The boundary between the domains is shown by a red dotted line. Scale bar, 500 μm. B: the MRR of 31 glomeruli (columns) in the left OB of mouse B-1 for 20 different odorants (rows). The intensity of glomerular activity was classified into 4 groups (weak response, 0.035–0.055%, small dots; modest response, 0.055–0.075%, medium dots; strong response, 0.075–0.095%, large dots; very strong response, >0.095%, the largest dots). Almost all glomeruli were assigned to clusters A–D and J. Glomeruli in cluster J strongly responded to heterocyclic odorants containing both S and N atoms in the ring.
feature clusters of glomeruli and domain organization using domain-visible class II-GFP mice and OCAM-immunohistochemistry.

Our data showed that the spatial arrangement of molecular feature clusters A–D in the mouse OB resemble those reported for the rat OB, suggesting that the spatial arrangement of molecular feature clusters is largely conserved between rats and mice. These findings are consistent with a previous 2-DG study that showed that overall glomerular response maps to odorants with specific molecular features were similar between rats and mice (Johnson et al. 2009).

In this study, the data clearly show that molecular feature cluster A is consistently located in the lateral region of the DI domain, whereas clusters B, C, D, and J are invariably ordered from the anterior to posterior region of the DII domain at the dorsal surface of the mouse OB (Fig. 7). Thus fatty acid-responsive glomeruli in cluster A represent a subset of class I ORs, whereas glomeruli in clusters B, C, D, and J represent subsets of class II ORs. The stereotypical spatial arrangement of the molecular feature clusters within the framework of OR domains and zones suggests that molecular feature clusters of glomeruli largely represent specific subsets of ORs that have similar MRR properties.

The fixed spatial relationships between clusters A–D and domains are consistent with a recent study that showed the molecular mechanisms that underlie glomerular positioning in the antero-posterior axis in the DI and DII domains (Imai et al. 2009). These data also indicate that the MRR properties of DI glomeruli (in cluster A) are distinct from those of DII glomeruli (in clusters B, C, D, and J). This distinction might relate to the fact that class I ORs are functionally and evolutionally distinct from class II ORs (Freitag et al. 1995; Glusman et al. 2001; Hirota et al. 2007; Ngai et al. 1993; Zhang and Firestein 2002; Zhang et al. 2004).

Previous mapping studies without the reference of domain and zone boundaries have described the chemotopy of OB maps as being quite coarse (Bozza et al. 2004; Soucy et al. 2009). This study underlined the importance of referring the domain and zone organization in mapping the glomerular responses to odorants and showed a clear spatial organization of molecular feature clusters. For example, when we focus on aliphatic acid– and aldehyde-responsive glomeruli within the DI domain (Figs. 2 and 3), the carbon chain length of aliphatic acids and aldehydes was systematically represented with a gradual shift of the position of activated glomeruli in the cluster A, which was consistent with the previous studies (Meister and Bonhoeffer 2001; Takahashi et al. 2004a; Uchida et al. 2000). However, this was not really the case to aliphatic aldehyde–responsive glomeruli within the DII domain (Figs. 2 and 3). In addition, our results provided strong evidence that pairs of glomeruli located within the same domain (pairs of glomeruli within DI-domain or within DII-domain) showed significantly higher similarities of odorant-response properties (correlation coefficient, 0.51 ± 0.01; 606 pairs in Fig. 3B) than those positioned in different domains (pairs of glomeruli one in DI-domain and the other in DII-domain; 0.01 ± 0.01; 429 pairs; Supplemental Fig. S2). Furthermore, two glomeruli that were located in close proximity but in distinct domains tended to show quite different MRR properties (e.g., glomeruli 13 and 14 in Fig. 3B). These results indicate the importance of zone and domain frameworks in understanding the functional organization of the odor maps in the OB.

FIG. 6. Heterocyclic odorants that are present in mouse urine activated glomeruli in cluster J of the DII domain. A: the imaged region (enclosed by a black circle) was superimposed on the reconstructed map of the dorsal OB. Open circles, glomeruli in the DI domain; light gray circles, glomeruli in the DII domain; dark gray circles, glomeruli in the V-zone. The boundary between the DI and DII domains is indicated by a red dotted line. Scale bar, 500 µm. B: responses to 2,4,5-trimethyl-thiazoline (TMT) (Ba, red), 2-sec-butyl-dihydrothiazole (SBT) (Bb, blue), 2-isopropyl-dihydrothiazole (IPT) (Bc, light blue), and dehydro-exo-brevicomin (DHB) (Bd, green). Be: superimposed image of Ba–Bd. Glomeruli that were activated by each heterocyclic odorant are color coded as indicated above and overlaid on the blood vessel pattern. The boundary between the DI and DII domains is indicated by a red dotted line. C: a magnified view of the caudal region of the imaged area in domain DII (enclosed by a green line in A). Glomeruli are defined as described in METHODS. Glomeruli that responded to thiazoles and thiazolines were defined as part of cluster J (light blue). Scale bar, 500 µm. D: the MRR of 9 glomeruli in the magnified region in Fig. 6C.

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odorants, we found that a subset of TMT-responsive glomeruli in cluster J also responds to SBT and IPT. Interestingly, although these molecules have similar molecular features to TMT, they may relate to different behavioral responses. For example, SBT is a volatile component in male mouse urine and is important for inducing inter-male aggressive behavioral responses in mice (Novotny et al. 1985). This raises the possibility that cluster J glomeruli are involved not only in predator odor-induced fear responses but also in urine odor-induced inter-male aggressive responses. In agreement with this hypothesis, some glomeruli in cluster J responded to DHB, another volatile component in male mouse urine that induces inter-male aggressive behavioral responses (Novotny et al. 1985). DHB has molecular features that are apparently distinct from thiazoles and thiazolines (Fig. 6). Despite this, some glomeruli in cluster J were activated not only by DHB but also responded to TMT and SBT (Fig. 6). Thus, although cluster J is primarily characterized by responses to thiazoles and thiazolines, it contains glomeruli that respond to odorants that have molecular features distinct from thiazoles and thiazolines.

The glomeruli in cluster A of the DI domain are thought to be involved in spoiled food odor-induced aversive responses (Kobayakawa et al. 2007). The spoiled food odor contains aliphatic acids and alkylamines. We previously noted that a large subset of cluster A glomeruli respond not only to fatty acids but also to alkylamines, odorants whose molecular features are distinct from those of fatty acids (Takahashi et al. 2004b). These results thus raise the possibility that the molecular feature clusters in the DI and DII domains have evolved not only by the clustering of glomeruli that represent ORs with similar MRR properties but also by adopting those glomeruli that represent functionally related ORs, even though they may not necessarily detect similar molecular features.

In the DII domain, clusters B, C, D, and J were positioned from anterior to posterior in this order. Does the ordered positioning of clusters have functional meanings? Cluster J is located in close proximity to cluster D. This suggests the possibility that mitral and tufted cells associated with glomeruli in clusters J and D interact intensively via local neuronal circuits in the OB. Mouse urine contains various ketones and heterocyclic odorants that are functionally related to the olfactory recognition of male and female mice and even individual mice (Novotny et al. 2007; Restrepo et al. 2006). Present and our preliminary studies showed that some of the urine odorants activated glomeruli in clusters J and D [K5-1 (2-heptanone) in Fig. 2D, IPT in Fig. 6B, and 6-hydroxy-6-methyl-3-heptanone]. Thus urine odorant-responsive mitral and tufted cells in cluster J might strongly interact with those in cluster D. The integration and modification of urine odor signals via the local neuronal circuits might have important roles for further processing these signals in the olfactory cortex.

Mitral and tufted cells associated with TMT-responsive glomeruli in cluster J project their lateral dendrites not only to cluster D but also to wide regions of the DII domain. Some of the lateral dendrites project into the DII domain and even into the V-zone (K. Igarashi and H.M., unpublished observation). This suggests that these mitral and tufted cells may potentially interact via local interneurons with mitral and tufted cells associated with other glomeruli in domains DI and DII and V-zone (Takahashi et al. 2004b; Willhite et al. 2006; Yokoi et al. 1995). Further analysis of the spatial arrangement of the lateral dendrites of functionally identified mitral and tufted
cells in the frameworks of molecular feature clusters, OR-domains and OR-zones should clarify the function of interglomerular interactions via local neuronal circuits in the OB.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


