Distributed representation of chemical features and tunotopic organization of glomeruli in the mouse olfactory bulb

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In the mammalian brain, similar features of the sensory stimuli are often represented in proximity in the sensory areas. However, how chemical features are represented in the olfactory bulb has been controversial. Questions have been raised as to whether specific chemical features of the odor molecules are represented by spatially clustered olfactory glomeruli. Using a sensitive probe, we have analyzed the glomerular response to large numbers of odorants at single glomerulus resolution. Contrary to the general view, we find that the representation of chemical features is spatially distributed in the olfactory bulb with no discernible chemotopy. Moreover, odor-evoked pattern of activity does not correlate directly with odor structure in general. Despite the lack of spatial clustering or preference with respect to chemical features, some structurally related odors can be similarly represented by ensembles of spatially distributed glomeruli, providing an explanation of their perceptual similarity. Whereas there is no chemotopic organization, and the glomeruli are tuned to odors from multiple classes, we find that the glomeruli are hierarchically arranged into clusters according to their odor-tuning similarity. This tunotopic arrangement provides a framework to understand the spatial organization of the glomeruli that conforms to the organizational principle found in other sensory systems.

GCaMP2 | calcium imaging | topographic map

For most external senses, sensory areas in the brain are spatially organized according to certain features of the stimuli. Visual and somatosensory information maps topographically in the thalamus and cortex (1, 2), and frequency tuning maps of sound are found in various stages from the cochlea to the auditory cortex (3). In chemical senses, the submodalities of taste are represented in different parts of the gustatory cortex (4). The topographic representation of sensory features allows the processing of information through parallel channels while preserving the next-neighbor relationship to enable computations such as contrast enhancement and fill-in (5).

How odor stimuli are mapped topographically in the olfactory system has been controversial. In the olfactory bulb, axons of olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) gene converge onto two stereotypically positioned glomeruli (6). The topographic map of the olfactory glomeruli shows remarkable stereotypy among animals of the same species and sometimes across species (6, 7). Numerous studies have examined how chemical features are represented in the olfactory bulb (8–18). The prevailing hypothesis posits that different chemical features are represented by compartmentalized regions in the olfactory bulb to form a “chemotopic” map (19, 20). The chemotopic hypothesis suggests that the olfactory glomeruli can be grouped according to the chemical features of the odorants that activate them and are spatially located in clustered regions (8–16, 21, 22).

This hypothesis, however, has been challenged. Quantitatively, its framework does not specify how many features are represented in a segregated manner, nor does it specify the size and permissible overlap among the clusters. Qualitatively, chemotopy requires nearby glomeruli be tuned to odors that share common molecular features, but recent experiments have provided little support of this notion (7, 23, 24). These studies have suggested that chemotopy at the fine scale does not exist and that the olfactory system violates the organization principle found in other sensory systems (7, 25, 26). Moreover, the topographic organization in the bulb is dispersed when the projection reaches the piriform cortex (PirC). Whereas the mitral cell in the bulb receives input from a single glomerulus, its projection into the PirC extends into a wide area, and a confined cortical region receives input from mitral cells across the entire bulb (27–29). Accordingly, odors evoke a sparsely distributed ensemble of neurons in the PirC (30, 31). This distributed odor representation removes an a priori reason for a spatial organization of the glomeruli. Serious question as to whether any organizational principle exists for the glomeruli has been raised.

In this study, we reevaluate the spatial representation of chemical features with systematic investigations of odor responses in the dorsal olfactory bulb in mice expressing the Ca2+ sensor GCaMP2 (32). These mice provide unprecedented sensitivity and single glomerular resolution that allow us to examine large numbers of odor stimuli and compare odor response patterns within individual animals. This approach overcomes several previous shortfalls by eliminating the requirement of collecting and assembling data from multiple animals, as in studies using [3H]2-deoxyglucose (2-DG) uptake or immediate early gene expression (8–11). It also allows us to use relatively low odor concentrations and to examine sparsely activated regions, which are likely neglected in previous studies. Our results find little support of chemotopy. Instead, we provide an alternative framework to explain the spatial organization of the glomeruli.

Results

Odor Response in G-CaMP2 Animals. We imaged compound heterozygotic OMP-tTA/tetO-G-CaMP2 mice (32), which expressed GCaMP2 in the OSNs without affecting their projection patterns (Fig. S1). GCaMP2 signals (∆F/F up to 25–40%) (Fig. 1) were much larger than intrinsic signals (AF/F ≤1%) and signals from synapticHuorin or Oregon Green (1–5%) (23, 24, 33). Using an automated olfactometer, we examined glomerular responses to ≈200 odors, among which ≈60 were selected for further study because they activated the dorsal glomeruli (Dataset S1). Different odors evoked distinct patterns of activity in 60–100 out of ≈200 glomeruli in the imaged area (Fig. 1 C–E). Responses were recorded across >1,000-fold change in odor concentrations (Fig. 1, Fig. S2, and Dataset S2), and the patterns to the same odor


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stimulation within individual animals were highly reproducible (Fig. S3).

**Representation of Chemical Features.** We examined the representation of molecular features using odorants from eight chemical classes. Because the position of glomerulus expressing the same OR varied from animal to animal (34), all comparisons were conducted within individual mice. Although odors belonging to the same chemical class could activate a similar set of glomeruli, these glomeruli were also activated by odors from a distinct class (rows in Fig. 1E). Conversely, the response patterns to odors within the same chemical classes were different (columns in Fig. 1E), and odors sharing a common functional group were often found not activating the dorsal glomeruli at all (Dataset S1).

To visualize the spatial representation of chemical features in the bulb, we mapped the patterns of response to individual chemical classes. Within a single class, different odors activated glomeruli with no obvious spatial restriction. For example, cyclohexylamine and hexylamine preferentially activated the medial glomeruli, whereas isoamylamine and triethylamine activated a broad set of glomeruli (Fig. 2A). Using the strongest response of a glomerulus to any odorants of the amine group to represent its response to an amine, we found that amine was represented by glomeruli across the entire bulb (Fig. 2C, Amine). Similarly, in the ester group, isoamyl acetate, ethyl tiglate, and vinyl butyrate activated glomeruli distributed in distinct areas. Methyl propionate, on the other hand, activated broad regions (Fig. 2B). The overall representation of the ester group, therefore, was also broad (Fig. 2C, Ester).

Analysis of the eight chemical groups revealed no compartmentalized representation of individual functional groups (Fig. 2C). Each group activated glomeruli across the entire imaged area. At the single glomerulus level, no group of glomeruli was exclusively tuned to a single chemical class. As shown in Fig. 1E, almost all...
glomeruli were activated by odorants from six or more chemical groups (also see Fig. 2C). Thus, within the area we imaged, chemical features were represented by spatially distributed patterns.

Our observation contradicted the chemotopic hypothesis. Using our dataset, we performed statistical tests of the chemotopic hypothesis. We calculated the mean distance between the glomeruli activated by two odors. The mean distance values for all odor pairs were separated into two categories: the WITHIN group contained the values for two odors belonging to the same chemical class and the BETWEEN group for odors from two different chemical classes (Fig. 2D). According to the chemotopic hypothesis, the average BETWEEN group distance would be larger than the WITHIN group distance (i.e., \( d_{\text{BETWEEN}} - d_{\text{WITHIN}} \geq D \)), where \( D \) was a distance criterion for chemotopy. For \( D \geq 50 \mu m \), the minimal diameter of a single glomerulus, we found no statistical significance between the two distributions in 12 separate experiments (Fig. 2E and Fig. S4A–C). We also extended our test to the entire bulb. We found that, under a simplified model of chemotopy (Fig. S4C), the probability of seeing all eight chemical groups activating the imaged dorsal region was below \( 10^{-6} \) (Fig. S4D).

Our results, therefore, showed no chemotopy in the olfactory bulb. We wondered whether the discrepancy could be explained by technical differences, especially the less-sensitive methods and the required assembly of data from multiple animals through averaging in previous studies. We transformed our data by blurring and assembling them across different animals to obtain an averaged and threshold-filtered image for individual odors (Fig. S5A). The representational maps for individual odor classes obtained from this transformation indeed produced an impression of chemotopy (Fig. S5B–E).

**Representation of Odorants by Glomerular Population.** Because individual chemical features were represented by glomeruli distributed across large areas of the bulb, we wondered whether there were specific, nonspatial patterns of activity associated with an odor class. Individual odor could be represented by the population response visualized using dimension reduction methods such as principal component analysis (PCA) and multidimensional scaling (35). We performed PCA on the datasets and represented individual odor stimuli as vectors in the PCA space denoted by the first three principal components. The plots showed that different classes of odors were largely distributed in the PCA space (Fig. 3A and B). An odor belonging to one class was usually found mingled with odors from other classes. We observed that some odorants with structural similarity were found in clusters that contained mostly odors of a single class, especially for odors of the ketone or amine classes. The grouping of the odors was observed also in cluster analysis, another measure of pattern similarity (Fig. 3C). These observations suggested that a subset of odors sharing a common feature could be similarly represented by a group of glomeruli, although the glomeruli were scattered in the dorsal bulb.

**Correlation Between Odor Responses and Odorant Structures.** Did the clustering of odors in the PCA space indicate a correlation between population response and structural similarity? Organic compounds were multidimensional entities that could not be simply defined by a single chemical feature, such as a functional moiety. Examination of the patterns of activation according to a simple feature could not capture the complexity of the molecular structures. Because each organic chemical could be described by a set of 1,664 chemical descriptors, they have been used to provide a quantitative measure of structural difference between odors (36, 37). For example, methyl propionate and methyl butyrate shared a common ester moiety but differed in carbon length. Their descriptor profiles mostly overlapped but differed numerically at three positions (Fig. 4A). On the other hand, odorants belonging to different classes could be distinguished by more descriptors (Fig. 4B).

To probe a comprehensive, quantitative relationship between chemical structure and glomerular response, we first performed cross-correlation analysis of odor pairs using chemical descriptors and glomerular response patterns (Fig. 4C and D, respectively). Specifically, we used the optimized descriptor set (optimized set II) derived from mammalian OR responses (37) to build a similarity matrix among the odors. The matrix showed high similarity within chemical classes but low similarity between classes (Fig. 4C and Fig. S6A and B). The similarity matrix for glomerular responses, however, was more complex. We observed that odors from the same class displayed within-group similarity, which was consistent with PCA plot showing clustering of odorants of the same class. Such similarity, however, was not uniform. Some odors within the groups were as dissimilar to each other as they were to odors from other groups. In direct contrast to the similarity matrix for odor structures, we also observed high similarity between odors from different classes (Fig. 4D).

To directly assess the correlation between the two parameters, we computed pairwise similarity scores for odors from 12 animals and plotted the scores against their distances in odor space specified by optimized descriptor set II (Fig. 4E). A distribution concentrated around a diagonal line would indicate a tight
correlation between the response patterns and the structural similarity, but the scatter plot showed no such correlation (Fig. 4E). Analyses using optimized descriptor set I (36) or the full descriptor set produced the same result (Fig. S6).

Therefore, when analyzed against large number of odorants, there was no clear correlation between odor structures and the response patterns, suggesting that representation of odor features was not only distributed spatially but also in the population response of the glomeruli. Response similarity was not dictated by structural similarity of the odors, and vice versa.

Correlation Between Odor Tuning and Physical Distance for Glomerular Pairs. In the absence of chemotopy, was there an alternative organization principle for the glomeruli? We investigated the relationship between the spatial location of the glomeruli and their odor tuning properties. The similarity of odor tuning between a pair of glomeruli could be expressed as the Pearson correlation value between their response profiles. We computed the similarity score in odor tuning and plotted it against the physical distance between a pair of glomeruli and found a correlation in all experiments ($r = -0.4 \pm 0.15$; Fig. 5A–C). Analysis of $\approx$30,000 glomeruli pairs from 12 bulbi suggested higher similarity in odor tuning at shorter distance (Fig. 5D). Using a method developed by Meister and colleagues (7), we compared this distribution with the null hypothesis distribution assuming no relationship between tuning similarity and glomerular distance (Fig. 5E). We found a large deviation from the expected independence distribution (Fig. 5F). The most deviation was for pairs with distances $<500$ $\mu$m and similarity score $>0.7$ (an excess of 16.42% of the total glomerular pairs were above the expected null distribution). We noted that applying only high concentrations of odors in continuous successions, as in previous studies, significantly reduces the correlation (Fig. S7). This was likely due to the adaptation of OSNs to strong odor stimulation. Therefore, it was critical to use short odor delivery, low concentration, and to allow ample recovery time to accurately assess the responses.

Functional Organization of the Olfactory Glomeruli. We visualized the spatial relationship of the glomeruli by first performing cluster analysis of the glomeruli using the pairwise similarity scores for odor tuning. At different thresholds the glomeruli could be divided into different numbers of clusters. Color-coding glomeruli within the same cluster revealed that glomeruli with similar tuning properties formed loosely organized patches (Fig. 6A and B). Higher threshold resulted in larger clusters; each resulted from the merging of neighboring small patches. At the highest threshold, the glomeruli separated into two macrodomains along the anteromedial–posteriolateral axis (Fig. 6C–F).

Although the projection pattern of OSNs expressing the same receptor was stereotyped, there were large degrees of variation in the precise location of the glomeruli (7, 34). Therefore, it was unlikely that different animals had an identical map. Nevertheless, we found that the general patterns of the clustering were similar among individuals (Fig. S8A–F). Moreover, the patchy map within the same animal was relatively consistent when subsets of odors that included all major classes were selected to cluster the glomeruli (Fig. S8 G–I). We quantified the clusters at different threshold and found the number consistent across animals, as indicated by the relatively small error bars (Fig. 6G). Interestingly, at a given threshold, the number of glomeruli falling into each cluster was not distributed evenly (Fig. 6H). The size of the cluster (number of glomeruli in it) was inversely related to the number of such cluster. We found that the distribution was better fit by a power law than an exponential curve. This observation suggested that the patchy hierarchical arrangement of glomeruli according to tuning similarity could be scale free.

Discussion

Using a sensitive, transgenically expressed calcium sensor, we have systematically examined the representation of chemical features of the odorants within individual animals and at single glomerulus resolution in the dorsal bulb. We conclude that there is no direct relationship between the response pattern and odorant structure.
Odor features are generally represented by distributed sets of glomeruli. These observations are consistent with several studies that show individual glomeruli are not tuned specifically to a particular chemical feature (7, 23). Although these studies have suggested the lack of fine-scale chemotopy, our study provides a systematic analysis to argue that there is no chemotopic representation of chemical features in either fine or broad scales. Our data are limited to the dorsal area for technical reasons; extending the study to a wider area of the bulb, as well as a quantitative model of chemotopy, will provide more rigorous tests of the model.

We have reached a different conclusion from studies showing chemotopy because of the high sensitivity and the high resolution afforded by G-CaMP2. 2-DG mapping and intrinsic imaging experiments rely on broad patterns such as blood flow to measure glomerular activity and are likely to bias toward densely activated areas. Importantly, the activity maps derived from multiple animals, as in many previous experiments, are coarse owing to the local variability of the glomerulus position. As our simulations show, averaging a strongly activated glomerulus over a larger region diminishes its contribution in sparsely activated areas. A bias toward strongly and densely activated area may create the impression of a chemotopic representation.

Is there a spatial organization of the olfactory glomeruli? We show that the glomeruli are organized according to tuning similarity, regardless of how many chemical groups a glomerulus is tuned to. Our conclusion differs from an earlier study that concludes there is no obvious organization of the olfactory glomeruli (7). The discrepancy can be explained by the differences in probe sensitivity, odor concentration used, and how odors are delivered (Fig. S7). On the other hand, the tunotopic organization found in our study is consistent with a theoretical study that suggests that patchy clusters of similarly tuned glomeruli are likely to form to map the multidimensional odor space onto the 2D glomerular layer (38).

The predication has not been experimentally demonstrated or visually identified until now.

The tunotopic organization of the olfactory glomeruli may reflect the evolutionary history of the olfactory system. OR genes clustered in the same loci tend to have high sequence homology as a result of local expansion of receptor genes (39). Neurons expressing

Fig. 5. Correlation between glomerular tuning similarity and physical distance. (A and B) Scatter plots for the similarity in odor tuning between a pair of glomeruli against the physical distance between the pair (two different experiments). Blue solid and red dashed line shows the mean and median for distance (x axis), respectively. Cyan solid line and orange dashed line shows the mean and median values for similarity scores (y axis), respectively. The correlation coefficient values of linear regression fit (r) of the distribution are indicated. (C) Box plot showing the distribution of r values across 12 different experiments. Black circles indicate individual experiments. The P value is for testing against the null hypothesis that the mean of r is zero (t test). (D) A 2D histogram of distribution for glomerular pairs obtained from 12 experiments. Color indicates the amount of glomerular pairs falling into the each bin, expressed as the fraction of total glomerular pairs. (E) A 2D histogram of distribution of the glomerular pairs under the null hypothesis the glomerular odor tuning similarity is not correlated with physical distance. (F) Excess calculated from the subtraction of E from D.

Fig. 6. Hierarchical organizations of the olfactory glomeruli. (A) Cluster analysis of glomerular similarity in odor tuning. With cutoff at 0.3, the glomeruli are segregated into 18 clusters. Each cluster is color coded. (B) Map of glomeruli marked for their tuning similarity. The colors of the glomeruli match those of the clusters in A. Circles mark adjacent glomeruli that are in the same tuning cluster. (C and D) Cluster analysis with a cutoff value of 0.55 segregates the glomeruli into six clusters. Some of the small clusters shown in B fuse into the large clusters in D. (E and F) Cluster analysis with a cutoff value of 0.8 segregates the glomeruli into two major domains. (G) Histogram showing the number of clusters as a function of threshold. Data are from 12 independent experiments. Error bars show SE. (H) Distribution of cluster sizes. Each column represents the number of clusters with sizes falling into that bin. The number of clusters is normalized to the total number of clusters for each experiment at the specified threshold and is expressed as the percentage of total clusters. Cluster size (number of glomeruli per cluster) is normalized to the total number of glomeruli in each experiment and expressed as the percentage of all glomeruli. Threshold at 0.3 is shown, and the data points are fit with a power law curve (power = −1.07).
homologous receptor genes are likely to share common tuning properties, project to the vicinity of each other (40, 41), and form tunotopic clusters. The molecular evolution of the receptor genes, however, is unlikely dictated by specific chemical features to generate segregated representation of chemical structures. We note that the glomerular patches show a surprisingly nonuniform distribution; the size of the patches is inversely related to its number. This pattern of distribution has not been predicted and may reflect an uneven expansion of different receptor genes.

The tunotopic organization of the glomeruli explains the clumped patterns of glomerular activity by some odors. Because neighboring glomeruli tend to share similar odor tuning properties, some odorants can activate a spatially clustered set of glomeruli. However, such clustering should not be mistaken as chemotopy because each glomerulus is tuned broadly in terms of chemical features. As such, some odorants sharing common chemical features may evoke similar population responses whether or not the patterns are spatially clustered. The similarity in activity patterns is likely to be translated into similarity in perceptual experience and explains why some odorants belonging to a given chemical group share a similar perceptual quality (42).

We suggest that the arrangement of glomeruli in the olfactory bulb is fundamentally the same as other sensory maps. Visual, auditory, and somatosensory systems arguably are organized according to tuning similarities. The tuning similarities in these systems correlate with the physical properties (e.g., sound frequency) or the spatial relationship (e.g., retinotopy and somatotopy), whereas in olfactory tuning similarity is not correlated with odor structures.

The topographic arrangement in the olfactory bulb, therefore, has little to do with the mapping of chemical features of olfactory stimuli but likely facilitates neural computations. Placing glomeruli with similar odor-tuning properties in close proximity makes it possible to allow local neural circuits to disambiguate similar stimuli through mechanisms such as lateral inhibition (43, 44). This arrangement can provide a mechanism by which odor responses are sharpened and odorants evoking highly similar patterns can be distinguished. Thus, the olfactory bulb does not violate the organization principle found in other sensory systems.

Materials and Methods

G-CaMP2 mice were anesthetized by urethane, and odor-evoked responses were recorded under an upright microscope through thinned bone over the dorsal olfactory bulb. Odors were delivered by a custom-designed olfactometer. Analyses were performed using custom-written software in MATLAB. Detailed methods are described in the SI (Materials and Methods). Imaging data are available upon request.

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SI Materials and Methods

Mice and Imaging Experiment. The OMP-IRES-tTA (Jackson Laboratories stock no. 017754) and tetO-G-CaMP2 (Jackson Laboratory stock no. 017755) mice were described previously (1, 2). The tetO-G-CaMP2 mouse lines known as 12i and 5i, which exhibited strong fluorescence, were crossed to produce animals for this study. The compound heterozygotes were further crossed to P2-IREs-tauLuc2Z (Jackson Laboratory stock no. 006595) and MOR28-IREs-EGFP lines (2, 3). Animals were maintained in the Lab Animal Services Facility of Stowers Institute at 12-h:12-h light/dark cycle and provided with food and water ad libitum. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Stowers Institute and were in compliance with the National Institutes of Health Guide for Care and Use of Animals.

Odor Delivery. An olfactometer was constructed according to the design of Uchida and Mainen (4). Odor delivery was computer controlled with a custom-written software package developed using LabView (National Instruments). Odorants were freshly diluted in mineral oil on the day of the experiment and used within 24 h. Saturated odor vapor from each vial was diluted into a carrier stream of clean air through a manifold. Four mass flow controllers were used to maintain the flow rate of clean air and odor vapor via different channels. The total flow rate was maintained at 400 mL/min. Odor output was measured by an electron ionization detector (Aurora Scientific) before each experiment. Measured concentrations corresponded linearly with liquid dilution in the vials from 3 × 10⁻⁹ to 10⁻⁴ dilutions, consistent with previous observations (5). Odor concentrations were thus presented as percentage of saturated vapor calculated from liquid dilution and air dilution. Odor delivery time was 2 s, and odor sequence was randomized. Each odor was delivered from the lowest to the highest concentration in a sequence, with an 8- to 10-s interstimulus interval. An interval of more than 3 min is imposed before the next odor sequence. A subset of odors was delivered multiple times at random intervals to test the consistency of the responses. Other odors were delivered once for each concentration. In experiments shown in Fig. S8, multiple odors at 2.5% saturated vapor (S.V.) were delivered sequentially with an interstimulus interval of 10 s. Chemicals were purchased from Sigma-Aldrich or obtained as samples from International Flavors & Fragrances.

Optical Imaging. Two- to six-month-old G-CaMP2 mice were anesthetized by urethane, and the bone covering the olfactory bulb was thinned with dental tools. Odor responses were recorded by a Hamamatsu EM-CCD camera mounted on an Olympus BX50WI microscope using 2.5x (0.15 N.A.), 4x (0.10 N.A.), or 5x (0.25 N.A.) objectives. The frame rate was 8.5 Hz for image acquisition. A Xenon short arc XBO light source passed through a band-pass filter (450–490 nm; Olympus America) was used to excite the dorsal olfactory bulb. Images were binned 2 × 2, and the image size was 256 × 256 pixels.

Image Analysis. Imaging data analysis was performed using ImageJ (National Institutes of Health) with custom-written Java scripts to assist batch processing of the image files. To identify the activated glomeruli, all image stacks from the one experiment were aligned to an arbitrarily selected standard stack from the same experiment. The activated glomeruli were revealed by subtracting minimally projected background images from the aligned stack. Discrete areas corresponding to individual glomeruli were manually identified as regions of interest (ROIs). To identify all responding glomeruli in one experiment, the activated areas were compared across all odor stimuli. If an area responded to odor stimuli with consistent spatial and temporal patterns, it was considered as a single glomerulus. The maximal size of a ROI was set to be at 100 μm in diameter. ROIs obtained from all odor stimulations were pooled together to obtain a master ROI list. Measurement of response was performed on aligned image stacks without background subtraction to reflect the true ΔF/F. Mean pixel values of each ROI were used for analyses.

Data Analysis. A custom-written software package developed in MATLAB was used for signal processing and subsequent data analysis. The raw data trace was smoothed and baseline adjusted to obtain a ΔF/F trace. A peak-finding algorithm from a MATLAB package (http://terpconnect.umd.edu/~toh/spectrum/PeakFindingandMeasurement.htm) was implemented to automatically identify response peaks. The peak values were used for further analysis.

In chemotopic analyses, the peak responses of individual glomerulus to a specific odor were mapped to its locations. For analyses on odor groups, the maximal peak response to all odors within the odor group was used and mapped to the glomerular positions. For statistical test of the chemotopy hypothesis, the mean distance between two sets of glomeruli was calculated as:

\[ d_{ij} = \sqrt{\sum_{i,j} (x_{pi} - x_{pj})^2 + (y_{pi} - y_{pj})^2} \]

where \( d_{ij} \) denotes the average glomerulus distance between two odor-evoked activity patterns, with \( x_{pi} \) and \( y_{pi} \) the coordinates of the odor-activated glomerulus; \( p \) denotes the activated glomerulus for odor \( i \), and \( q \) the activated glomerulus for odor \( j \).

Odorant pairs were segregated into two groups: WITHIN group for odor pairs belonging to the same chemical class and the BETWEEN group for two odor belonging to different chemical classes. The chemotopy hypothesis was expressed as:

\[ d_{BETWEEN} - d_{WITHIN} \geq D \]

where \( D \) was the distance at which chemotopy was considered to exist.

Odor tuning similarity was calculated as:

\[ S_{ij} = \frac{\sum m r_{im} \cdot r_{jm}}{\sqrt{\sum m r_{im}^2 \sum m r_{jm}^2}} \]

Distance for the two glomeruli was expressed as \( D_{ij} = 1 - S_{ij} \), where \( i \) and \( j \) denote the identity of the glomerulus and \( r_{im} \) and \( r_{jm} \) correspond to the peak amplitude to \( m \)th odor for the two glomeruli. Similar calculation was used for calculating distance between two odor-evoked activity patterns, with \( r_{im} \) and \( r_{jm} \) corresponding to the response of the \( m \)th glomerulus to odors \( i \) and \( j \). Euclidean distance measurements gave rise to qualitatively the same conclusions. Cluster analysis and PCA were performed using the MATLAB Statistics Toolbox. PCA was performed with the z score of the response amplitude. 

Pairwise analysis of glomerular response similarity and physical distance. 

Pairwise glomeruli similarity value \( S_{ij} \) was plotted against the physical distance between the glomerular pair. Only pairs of glomeruli in the same olfactory bulb were used. Similarity scores were calculated using responses across all concentrations unless
Comparison between glomeruli responses and odor descriptors. Odors were expressed as vectors in odor space defined by the chemical descriptors (6, 7) or by the peak responses of a common set of glomeruli. Responses from the same animal to the same odorant at all concentrations were concatenated to form a single vector. In the descriptor representation, an odor was represented by either the full set of 1,664 descriptors or the two optimized subsets of 20 or 40 descriptors (6, 7).

Similarities were calculated using Pearson correlation value for the two representations. Pairwise response similarity vs. odor space distance was plotted for all odor pairs.

Fisher’s Combined Test of Chemotopy for Data in the Dorsal Bulb. For each olfactory bulb, an individual probability p was obtained from two-sample t test. To combine results from all bulbs, Fisher’s combined probability test was used to integrate extreme value probabilities into the χ² statistic as:

\[ X^2 = \sum_{i=0}^{k} \log_e \{ p_i \} \]  

[S1]

Then the overall P value was calculated from the χ² distribution with degree of freedom as 2k; k is the number of tests combined (12 total).

Test of Chemotopy for the Entire Olfactory Bulb. We build a simplified model of chemotopy to perform statistical analyses. We treat the entire bulb as a sphere with all of the glomeruli residing on the surface of the sphere. We first assume that every odor class is represented by distinct regions of the sphere with no overlap. For N classes of odorants, the surface area of the sphere is evenly divided into N equal sections. The surface area of each section is

\[ A_N = \frac{A_{\text{sphere}}}{N} \]  

[S2]

where surface area of a sphere is given by

\[ A_{\text{sphere}} = 4\pi r^2 \]  

[S3]

From our experimental results, we observed that all eight odor classes were represented in the dorsal area. We therefore consider the overlap case without changing the areas assigned to each odor class. We model each region as spherical to simplify the calculation. Consider a region that all sections overlap, with an area of \( A_{\text{overlap}} \). If the area of the overlap region is included in the area of each of the sections, the total area of each odor class section becomes

\[ A_N = \frac{A_{\text{sphere}}}{N} + \frac{N - 1}{N} \cdot A_{\text{overlap}} \]  

[S4]

where area of the overlapping region is given by

\[ A_{\text{overlap}} = \text{Overlap\%} \cdot A_{\text{sphere}} \]  

[S5]

In an extreme case, this situation could be visualized as a sphere cut radially into N equal sections, with a circular overlap region at the pole of the sphere shared by each section (Fig. S4C).

Our basic model, based on this scenario, permits us to assess the likelihood that randomly placed areas (corresponding to odor classes) will overlap in such a way. Specifically, we will take the circular overlap region to be at the pole of the sphere, then randomly place other circular regions (with the appropriate area \( A_N \) calculated above) onto the sphere and find the probability that all circular regions fully contain the overlap region (Fig. S4C).

In spherical coordinates, the surface area of each circular region, the area of a solid angle taken at the surface of the sphere, is given by

\[ A = 2\pi r^2(1 - \cos \alpha) \]  

[S6]

where \( \alpha \) is the angle from the center point to the edge of the area (Fig. S4C, Left). The angle \( \alpha_{ov} \) can now be described as a function of the overlap percent by setting Eq. S5 equal to Eq. S6:

\[ \text{Overlap\%} \cdot A_{\text{sphere}} = 2\pi r^2(1 - \cos \alpha_{ov}) \]

to obtain

\[ \alpha_{ov}(\text{Overlap\%}) = \cos^{-1}[1 - (2 \cdot \text{Overlap\%})] \]  

[S7]

Eq. S7 gives us the angle \( \alpha \) of the overlap region as a function of the overlap percent. We can also determine \( \alpha \) for our other circular regions by setting Eq. S4 equal to Eq. S6:

\[ \frac{A_{\text{sphere}}}{N} + \frac{N - 1}{N} \cdot A_{\text{Overlap}} = 2\pi r^2(1 - \cos \alpha_{CR}) \]

where after substitutions and algebra, we obtain \( \alpha \) as a function of the number of odor class regions \( N \) and the overlap percent \( \text{Overlap\%} \):

\[ \alpha_{CR}(N, \text{Overlap\%}) = \cos^{-1}\left\{ 1 - \frac{2}{N} \cdot (N - 1) \cdot \text{Overlap\%} \right\} \]  

[S8]

In radial coordinates where the center of each area is expressed as \((r, 0, \phi)\), the radius \( r \) is held constant, and any change in the azimuth angle \( \phi \) gives identical results. Therefore, the probabilities with which an area \( A_N \) contains the overlap area \( A_{\text{Overlap}} \) can be expressed in terms of \( \theta \), which describes the angular distance between the pole and \( A_N \) for the area representing a given class of odor (Fig. S4C). The probability of a single randomly placed odor class region fully containing the overlap region whose center is located at the sphere’s pole \((r, 0, 0)\) can be calculated when the center of the odor class region given by \((r, 0, \phi)\) must satisfy

\[ 0 \leq \alpha_{CR} - \alpha_{ov} \]  

[S9]

which gives rise to

\[ P_1 = \frac{\alpha_{CR} - \alpha_{ov}}{\pi} \]  

[S10]

Therefore, the probability of \( N \) randomly placed odor class region fully containing the overlap region is

\[ P_N = \left( \frac{\alpha_{CR} - \alpha_{ov}}{\pi} \right)^N \]  

[S11]

The probability \( P_N \) as a function of \( N \) and \( \text{Overlap\%} \) can be calculated using Eqs. S7, S8, and S11.

Testing Effect of High-Concentration Odor Exposure on Correlation Between Glomerular Distance and Tuning Similarity. The overall similarity scores in our experiments (Fig. 5) are higher than those in the Soucy et al. study (8). This can be explained by the difference in probe sensitivity and the odor panels used. We have used only odors that activate the dorsal glomeruli, whereas the
calculation by Soucy et al. included many odors that did not activate the dorsal bulb. High probe sensitivity also allowed the detection of relatively weak responses. Because nonzero values in odor response vector tend to increase similarity score, the higher similarity scores observed in our study can be explained by these two factors. However, the change in score distribution could not explain the substantially stronger correlation between tuning similarity and the closeness of the glomeruli observed in our study. We reasoned that the discrepancy might be explained by the fact that our analyses used lower odor concentrations as well as a different temporal sequence of odor stimulation. Repeated, long exposures to relatively high-concentration odors, as in earlier studies (5, 8–17), might have resulted in the suppression of responses from some glomeruli. To test this conjecture, we performed experiments by sequentially exposing the animals to a panel of odors at high concentrations (2.5% s.v.). The results are shown in Fig. S8.


Fig. S1. Projection of OSNs in tetO-G-CaMP2/OMP-IRES-tTA mice. (A) Wide-field fluorescent images from the bisected heads of a compound heterozygote OMP-IRES-tTA/tetO-G-CaMP2/P2-IRES-tauLacZ mouse (A1) and a P2-IRES-tauLacZ control mouse (A2). In the control mouse, faint autofluorescent signal is observed in the main olfactory epithelium. (B) LacZ staining of the same animals. P2 OSN projections are examined from both the left and right bulbs. Images of the left bulbs are flipped to the same orientation as the right bulb. Arrowheads indicate the P2 glomeruli. (C) MOR28 projections are visualized in compound heterozygote OMP-IRES-tTA/tetO-G-CaMP2/MOR28-IRES-EGFP mouse (C1) and a control MOR28-IRES-EGFP (C2) mouse. Both the left and right bulbs are shown from ventral sides, with anterior part of the bulb pointing upward. The four bright green spots are the MOR28 glomeruli. Upper Right: Background green signals are from G-CaMP2.
Fig. S2. Odor-evoked responses in the G-CaMP2 animals. (A) Image frames show the dorsal bulb response patterns during 2-s applications of the odorant amyl acetate. Six frames from image stacks are shown representing the beginning, duration, and end of the responses. The images shown are subtracted from the background fluorescence. Time stamps from the start of odor delivery (at 0 s) are indicated in the images. Image size is 1.625 × 1.625 mm$^2$. (B) Response trace for two glomeruli (72 and 81). The positions of the glomeruli are indicated in A. Duration of odor application is shown in the black bar above the traces. (C–E) The corresponding heatmaps show the ΔF/F response to amyl acetate (C), methyl valerate (D), and pentanone (E) for all of the glomeruli. Vertical axes indicate time. Examples shown in C–E are from a different experiment shown in A and B. The color of the pixel indicates the intensity of the response for a glomerulus.
Fig. S3. Consistency in odor responses. (A) Heatmaps showing the peak glomerular response to three separate applications of amyl acetate (AA) at eight concentrations. Each column indicates the response from a single glomerulus, and each row indicates the response of all glomeruli to a single concentration of the odor. (B) Heatmap showing the peak glomerular responses to three separate applications of vinyl butyrate (VBE) at eight concentrations. (C) Peak glomerular response heatmaps to two separate applications of phenetole (PNE). Color bar in A shows the $\Delta F/F$ value for each glomerulus to each odor application. (D) Cross-correlation among odor response patterns for the subset of odors shown. Correlation values are calculated using response from all glomeruli across the odor concentrations.
Fig. S4. Statistical test of the chemotopy hypothesis. (A) Distribution histogram of distance between glomeruli activated by odor pairs belong to the same chemical class (Upper; WITHIN) or different classes (Lower; BETWEEN), with the data filtered at different thresholds (no threshold: T = 0; threshold at 30% maximum: T = 0.3*max; threshold at 50% maximum: right = 0.5*max). (B) The P values for the statistical tests using the Fisher combined probability test method. The P values for d_BETWEEN − d_WITHIN ≥ D, as a function of D, are plotted. Color lines indicate the P values for data filtered with no threshold (black), 30% maximum (red), or 50% maximum value (blue). Gray lines indicate the results for 12 individual experiments. (C) Illustration of a simplified model of chemotopy extended to the entire bulb and the overlap test (SI Materials and Methods). The olfactory bulb is treated as a sphere. Left: Gray-shaded area indicates where imaging experiments are conducted, and cyan-shaded area indicates the area representing a class of odorants. α_{ov} and α_{cr} denote the angle between the axis and the edge of the overlap cone and the class region, respectively. θ indicates the angle between the two axes, which will determine the degree of overlap. Right: Schematic showing a scenario that the glomerular sets representing different odor classes (three are shown) all overlap at the imaged area. (D) Probability for N groups chemicals (n = 4, 8, or 16) to overlap in the imaged area. Different curves show the size of the overlapped area, expressed as the percentage of the total sphere (black, 2%; red, 5%; blue, 10%).
Fig. S5. Transformed representation of odors and odor classes. (A) Process of transforming individual experiments into a combined representation of a single odor. Vinyl butyrate (VBE) is used for illustration. Twelve separate imaging results (three shown) are rotated, aligned, averaged, and thresholded at 25% of maximum to produce an averaged representation of VBE. (B–D) Averaged response patterns to ester (B), aldehyde (C), and ketone (D) molecules as the representations of individual odor classes. Odors shown are methyl propionate (MPE), methyl butyrate (MBE), methyl valerate (MVE), valeraldehyde (VAH), hexanal (HXH), heptanal (HPH), 2-pentanone (PTO), 2-hexanone (HXO), and 2-heptanone (HPO). (E) Averaged patterns for acid, amine, and ether.
Fig. S6. Glomerular response similarity and odorant structure similarities. (A and B) Heatmap represents the pairwise correlation matrix among 59 odors using the optimized descriptor set I (A) or full descriptor set (B). Odor IDs are marked along the axes. Their names and structures can be found in Dataset S1. Odors are arranged according to chemical classes. (C and D) Scatter plots in which the similarity in glomerulus response for an odor pair is plotted against the distance between the two in chemical space described by optimized descriptor set I (C) or full set (D). Data are pooled from 12 experiments. Distances are calculated as the cosine distance for the descriptors.
Fig. S7. Lack of correlation between glomerular tuning and physical distance for odorants delivered at high concentration. (A) 2D histogram showing the relationship between glomerular response similarity and physical distance for glomerular pairs. The similarities are calculated using response to odors at 2.5% S.V. Color indicates the fraction of glomerular pairs falling into the each bin. (B) Joint distribution of two marginal distributions for the two axes in A indicates the distribution under the null hypothesis. (C) Excess calculated from subtracting B from A. The excess of glomeruli with similarity score >0.7 and distance <300 µm only constituted 2.66% of the pairs with distance <500 µm and 0.88% of the total pairs. (D) As scattered plot. Blue solid line shows the mean and red dashed line shows the median for distance (x axis). Cyan solid line shows the mean and orange dashed line shows the median values for similarity scores (y axis).
Hierarchical organizations of the olfactory glomeruli. (A–F) Cluster analysis of glomerular similarity from a different experiment. (A and B) With cutoff at 0.12, the glomeruli are segregated into 18 clusters, and the glomeruli are marked for their tuning similarity. Glomeruli that are in the same tuning class and also physically close to each other are circles. (C and D) Cluster analysis with a cutoff of 0.3 segregates the glomeruli into six clusters. Some of the small clusters shown in B fuse into the large clusters in D. (E and F) Cluster analysis using a cutoff value of 0.6 segregates the glomeruli into two major clusters. (G–I) Cluster analysis of glomerular response similarity into 18 clusters using different set of odors. Maps of tuning similarity clusters onto their physical location (Upper) and clusters (Lower) for 59 odors (G), 36 odors (H), and 27 odors (I). Clusters that are consistent among all three odor sets are circled.
Dataset S1. Odor list

Dataset S1 (XLSX)

The list contains odor names, their functional group, and their ability to elicit response from the dorsal olfactory bulb. Odors used to map spatial representation of chemical features were listed with their odor ID and chemical structures.

Dataset S2. Numerical data for 12 separate experiments

Dataset S2 (XLS)

Numerical data for all imaging experiments used in this paper.