Published in final edited form as:

Nat Neurosci. 2019 August ; 22(8): 1306–1317. doi:10.1038/s41593-019-0442-z.

Mosaic representations of odors in the input and output layers of the mouse olfactory bulb

Honggoo Chae^{1, #}, Daniel R. Kepple^{1, 2, #}, Walter G. Bast¹, Venkatesh N. Murthy³, Alexei A. Koulakov^{1, 2, *}, Dinu F. Albeanu^{1, 2, *}

¹Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

²Watson School for Biological Sciences, Cold Spring Harbor, NY

³Department of Molecular & Cellular Biology and Center for Brain Science, Harvard University, Cambridge, MA

Abstract

The elementary stimulus features encoded by the olfactory system remain poorly understood. We examined the relationship between 1,666 physical-chemical descriptors of odors and the activity of olfactory bulb inputs and outputs in awake mice. Glomerular and mitral/tufted cell (MTC) responses were sparse and locally heterogeneous, with only weak dependence of their positions on physical-chemical properties. Odor features represented by ensembles of MTCs were overlapping but distinct from those represented in glomeruli, consistent with extensive interplay between feedforward and feedback inputs to the bulb. This reformatting was well-described as a rotation in odor space. The physical-chemical descriptors accounted for a small fraction in response variance, and the similarity of odors in physical-chemical space was a poor predictor of similarity in neuronal representations. Our results suggest that commonly used physical-chemical properties are not systematically represented in bulbar activity and encourage further search for better descriptors of odor space.

Keywords

glomeruli; mitral and tufted cells; intrinsic signals; two photon calcium imaging; distributed representation; topography; odor space; physical-chemical odor properties; LASSO

Introduction

Insight into the computations performed by sensory systems arises from understanding the stimulus features encoded by them, such as wavelength of light, frequency of sound¹, etc. Unlike other sensory modalities (i.e. vision, audition), it is not understood what properties of

*Correspondence: albeanu@cshl.edu or koulakov@cshl.edu.

[#]equal contribution

Authors contributions: A.A.K. and D.F.A. conceived the study. H.C., D.R.K., W.G.B., A.A.K. and D.F.A. contributed to the design of experiments and analysis. H.C. performed two photon imaging experiments. W.G.B. performed intrinsic optical imaging experiments. D.F.A., V.N.M. and A.A.K. supervised the project and wrote the manuscript with input from all authors.

Competing interests: The authors declare no competing interests.

odors are important in olfaction, and how they are processed by the olfactory system. The relationship between odor structure (chemical space), the spatial and temporal patterns of activity in the brain (neuronal space) and the perceived odor quality (perceptual space) has been elusive^{2–9}. As a result, currently, one cannot robustly predict neuronal activity patterns and perceptual attributes starting from the physical features of odor molecules.

Even the earliest step in olfaction, the interaction between a particular OR and ligands (odorants) has defied simple descriptions. Since the olfactory system derives percepts on the basis of responses of a large number of odorant receptor (ORs) types, the number of dimensions of the odor space could be large. Nonetheless, recent studies, using dimensionality reduction methods suggest that a relatively small number of odor physical-chemical descriptors (~30 out of ~1,600 to ~5,000 in the Dragon database¹⁰) capture odor similarity in all three spaces – chemical, neuronal and perceptual^{5,11–15}. It has been proposed that most variance in human perceptual space can be explained by considering low dimensional manifolds (~2–20), with dimensions related to behaviorally relevant features such as stimulus pleasantness, toxicity and/or hydrophobicity^{2,5,7,12,16}.

The spatial layout and wiring patterns of neural circuits can offer important clues about the underlying computations – for example, a retinotopic organization along with nearest neighbor interactions allowed the inference of local contrast enhancement in the retina¹⁷. In the vertebrate olfactory system, there is a reproducible and precise layout of OR identity in the glomerular layer of the olfactory bulb (OB)¹⁸. However, how this receptor-based layout translates to a functional map remains unclear^{13,19–25}.

Odor information arriving at glomeruli from the olfactory sensory epithelium is modified within the OB by local and top-down interactions. Two classes of output neurons, mitral and tufted cells (MTCs), convey information to several olfactory cortical areas. These two output channels differ in their inputs, morphology, intrinsic excitability, local connectivity, activity patterns, downstream targets and top-down feedback^{26–30}.

Inspired by progress in the characterization of odors through the use of a large number of physical-chemical properties, here we investigate whether these features are represented in the neuronal activity patterns in the input and output layers of the bulb. We studied how the activity patterns of glomeruli and MTCs in awake, head-fixed mice relate to a set of commonly used 1,666 physical-chemical odor properties. We asked four questions. First, how well are these physical-chemical properties of odors captured by the glomerular and MTC responses? Second, does similarity of odor molecules in the physical-chemical properties space correlate with the similarity of neuronal population responses? Third, does the physical location of individual glomeruli or MTCs depend on their physical-chemical receptive fields? Finally, what is the relationship between neuronal odor representations in the input and output layers of the bulb?

Results

We used imaging of intrinsic signals from the dorsal surface of the OB to probe the responses of glomeruli (n=5 mice, 10 bulb hemispheres/fields of view, 871 glomeruli) to an

array of chemically diverse odors (Supplementary Figure 1, Supplementary Table 1, Methods). Previous reports indicate that glomerular intrinsic signals approximate well the activity of presynaptic olfactory sensory neuron (OSN) terminals in the OB¹⁹. In separate experiments, we employed multiphoton microscopy to monitor the responses (GCaMP3/6, Methods) of mitral and tufted cells tilling the dorsal aspect of the bulb (n=8 mice, 13 hemispheres, 19 fields of view, 1,711 M/T somata, Supplementary Figures 1-3) to the same stimuli in awake mice across a range of concentration (Methods).

Odor responses of glomeruli and MTCs are poorly described by Dragon physical-chemical properties

Previous studies suggested that the olfactory system is tuned to extract specific physical-chemical features of odors^{11,12,31,32}. Here, we investigated whether a wide array of physical-chemical molecular parameters are good predictors of glomerular and MTC responses. We used the Dragon database¹⁰ to evaluate 1,666 physical-chemical properties to each odor in the panel. The responsiveness of each glomerular or MTC region of interest (ROI) to a particular property (called here the property response) was characterized as a Pearson's correlation between the odor responses of the ROI and the values taken by the property (property strength vector, PSV) across the 49 monomolecular odors (Figure 1a-c, Methods). The array of such correlations across the Dragon molecular properties is defined as the property response spectrum of that ROI (Figure 1d,g). This definition is analogous to calculating a neuronal receptive field in the visual or auditory systems, where the response strength of a given cell is correlated with certain stimulus features. The property response spectrum represents the strength of individual ROI response to an array of physical-chemical properties, thus playing the role of a molecular receptive field.

For both glomeruli and MTCs, the number of properties that single units responded to significantly (false discovery rate, FDR, $q < 0.1$ for the property response spectrum of each ROI considered, Methods) varied from ROI to ROI, with an average of 12.7 per glomerulus and 16.4 properties per MTC (Figure 1f, i). In general, individual responsive glomeruli and MTCs were only poorly tuned to the physical-chemical properties (Supplementary Figure 4a,c; ~26% of glomeruli and ~33% of MTCs showed no significant correlations), and the vast majority of glomerular and cell odor response-property correlation pairs was not significant (Figure 1d-i, Methods). Within the subset of significant glomerular and cell odor response-property pairs (~6%), correlations spanned both negative and positive values (absolute average: 0.35 ± 0.07 STD for glomeruli and 0.36 ± 0.08 STD for MTCs, Supplementary Figure 4e). Across different experiments, the tuning widths (number of significant properties per ROI) were similar (Supplementary Figure 4b,d).

We further used principal component analysis (PCA) to quantify the dimensionality of three data types that describe the physical and neuronal odor representations (Figure 1j,k,l). Approximately 17 dimensions were sufficient to account for 90% of variance in the values taken by Dragon molecular properties (PSV) across the 49 odors used (Figure 1l). Thus, many of these properties are redundant^{15,16}, and a 17D flat surface contains substantial amount of information (90%) on the molecular properties. The dimensionality of the physical-chemical descriptors depends on the odors included in the panel, and, in principle,

can be independent of the responses of olfactory neurons. In comparison, the responses of glomeruli to odors in our panel could be described within a 21D principal components (PC) space, and those of the MTCs in a 24D space at the same level of variance explained (90%, Figure 1j,k). We iteratively sampled increasing number of odors (up to 49, Methods) and observed that the dimensionality of the molecular properties, glomerular and MTC responses increased steadily. Thus, the quantities reported here represent lower bounds on these metrics of odor space. MTC responses generally had higher dimensionality than glomerular responses, consistent with previous reports of decorrelation of glomerular inputs within the bulb^{20,33–36} (Supplementary Figure 4f,g).

Do neuronal responses represent Dragon molecular properties efficiently? We used a computational technique we call principal component exchange (PCX), and projected the molecular properties (PSV) into the PCA space of neuronal responses (glomerular or mitral, Methods). We then computed the variance of molecular properties data captured by the neuronal responses PC space of increasing dimensionality (Figure 1j,k). If the molecular and neuronal responses PC spaces were identical, then glomerular (or mitral cell) PCs would capture the same fraction of variance in both neuronal responses and the molecular properties datasets. Instead, we find that glomerular and respectively mitral cell responses PCs explain almost the same fraction of variance in the molecular properties dataset as they do in randomly generated data (Methods, grey and black lines, Figure 1j,k). When projected to the PC space of molecular properties, glomerular and mitral (same for tufted, data not shown) cell responses, were also similar to random data controls in terms of amount of variance explained (Figure 1l). To better characterize the performance of PCX, we used surrogate data in which a known relationship is embedded. We systematically perturbed the glomerular odor response vectors by adding variable known amounts of noise. As expected, gradual injection of noise resulted in less-and-less variance explained by the glomerular PCs. Corrupting the glomerular responses signal to noise ratio (SNR) by more than 5 fold led to similar amounts of variance explained as by the molecular properties (Supplementary Figure 4h). Projecting mitral cell responses in the glomerular PC space, or glomerular responses in the mitral cells PC space captured less variance compared to the reference PC spaces (glomerular and mitral), but substantially more than random data as discussed later (Supplementary Figure 4h). Recording glomerular responses in anaesthetized mice, or sampling MTC responses within shorter intervals (0.5s to 1.5s) from stimulus onset, led to similar results (Supplementary Figure 5).

Overall, our data shows that neural responses of both glomeruli and OB outputs are poorly tuned to the physical-chemical properties analyzed, and instead reflect odor features that are not well-captured by these molecular properties commonly used in computational chemistry, and by previous studies in olfaction.

Odor similarity in physical odor space is a poor predictor of neuronal representations

Does the similarity between pairs of odors, calculated using the set of 1,666 physical-chemical properties reflect the similarity in neuronal representations of the same odors in either the input or output layers of the OB? To describe similarity in odor physical space, for each odor pair in the panel, we calculated the Euclidean distance between the normalized

molecular properties strengths associated with each odor in the panel as proposed by previous studies^{11–13,37}. To represent odor similarity in the neuronal representations, we used two metrics: the Euclidean distance between the ROIs responses to the same pair of odors, and Pearson's correlation in neuronal responses (pooling data across fields of view, Methods). For both glomeruli and MTCs, the pairwise odor similarity in the space defined by the physical-chemical properties had only poor and variable correlation with the similarity in neuronal representations (Figure 2a, b).

Perhaps, only a small subset of molecular properties has a robust relation to the neural responses. To address this question, for each odor pair, we built a sparse regression between the squared pairwise odor response distances and the squared differences between individual physical-chemical property values. We used a non-negative Least Absolute Shrinkage and Selection Operator (LASSO) algorithm that selects a sparse subset of non-zero properties from the full set to better explain the differences in neuronal responses³⁸ (Methods). The properties were selected to yield the best fit of pairwise distances in neuronal responses and physical-chemical space, by weighting the odor similarity calculated in the physical-chemical space. Using this approach, we identified small subsets of properties which are well-reflected in the neuronal responses. For example, using 10 molecular properties selected via LASSO, the correlation between the pairwise odor distances in physical-chemical and neuronal responses increased substantially (~0.60); we ran LASSO independently for the glomerular and MTC data sets, Methods). Including more properties into the analysis mildly improved the correlation (40 properties brought the correlation score to ~0.65, Figure 2c,d).

We further determined how these regression analyses and correlations generalize across different FOVs (FOV cross-validation), and odor pairs (odor cross-validation, Methods). Cross-validation across FOVs decreased only slightly the observed glomerular responses-to-physical space correlations, consistent with reproducible glomerular odor maps across individuals¹⁹. The same procedure however resulted in substantially lower correlations (~0.2) for the MTC responses, which may reflect differences in sampling MTCs along the dorsal aspect of the OB across fields of view. In contrast, the odor cross-validation procedure, or the combination of these two procedures, drastically diminished the correlations to the physical-chemical representations (at most ~0.10 for glomeruli and ~0.05 for MTCs, Figure 2c,d, Methods). Employing a greedy algorithm (Methods) with odor cross-validation for the MTCs data led to qualitatively similar results (Supplementary Figure 4j).

For both awake and anaesthetized glomerular data and awake MTC responses, we also performed the LASSO analysis on shuffled controls, where the Dragon properties were shuffled by odor identity, while keeping the neuronal responses unchanged (Figure 2e,f, Supplementary Figure 5e). Interestingly, searching for sparse subsets of shuffled properties with regularization increased the correlation between pairwise odor similarity in physical and neuronal spaces (for both glomerular and MTC responses). A drastic decrease in correlation occurred upon performing cross-validation across odors and fields of view (Figure 2e,f, Supplementary Figure 5e).

Thus, while correlations between similarity in physical-chemical and neuronal response spaces could be identified using subsets of molecular properties, they held little predictive power when new odor pairs or shuffled properties were tested, suggesting that such correlations emerge due to overfitting.

Relating the tuning of glomeruli and MTCs to molecular properties to their placement on the bulb

Previous experiments in the glomerular layer of the rodent OB have provided contrasting views of spatial organization. Some authors suggested that different classes of chemicals are represented in a spatially segregated manner, perhaps even in an ordered topographic manner^{25,39–41}. Others noted a great deal of local disorder, with no evidence of a smoothly-varying representation^{13,19,42}. To date, these spatial relations have not been assessed using physical-chemical properties of odors, such as those provided by Dragon. Although our analysis above has shown that glomeruli and MTCs sample a small subspace of the properties (Figure 1), some of these parameters are represented in the neuronal responses. Therefore, we investigated whether tuning to the molecular properties is spatially laid out in a systematic fashion at the level of glomeruli and MTCs.

For each property, we characterized the relationship between the tuning of glomerular and MTC responses, and the spatial location of glomeruli/cells along the anterior-posterior (AP) and medial-lateral (ML) axes of the bulb. The tuning of individual glomeruli or MTCs was described by their property responses. For the ensemble of ROIs monitored in each FOV, we computed the correlation between each ROI's location and its response sensitivity to each property, using false discovery rate (FDR) correction to account for false positives given the large number of properties. For each FOV (2D plane), every molecular property is described by the strength of its correlation along the AP and ML axes (Figures 3a-f, 4a,b, Methods).

To determine whether any specific directions on the bulb surface are well-aligned with independent combinations of the molecular properties, we performed PCA on the glomerular data to identify new relevant orthogonal reference axes (PC1 and PC2, Figure 3a, Methods). Across different animals, the first principal axis (PC1, blue, Figure 3) was consistently rotated approximately 40 degrees ($\text{Avg}=38.7\pm16.3$ STD) with respect to the AP direction. Several molecular properties (20) appeared correlated (positively and negatively, $\text{FDR } q<0.1$) with PC1 (Figure 3c,e, Supplementary Table 2), and could be identified robustly across animals (11 out of 12 hemibulbs). The properties correlated with the PC2 axis were much less consistent across samples (2 out of 12 hemibulbs, Figure 3d,f).

The responses of glomeruli are predictive of their location on the bulb surface at a coarse spatial scale

Could the positions of individual glomeruli on the bulb be inferred using the properties they are responsive to? We tested this hypothesis by building a sparse linear regression for individual glomerular positions based on their tuning (property response spectra) to the molecular properties. Regression was obtained as above, using LASSO and selecting a small subset of active properties (20) from the full set (Methods). To quantify the quality of prediction, we evaluated the prediction error for each glomerulus for PC1 and PC2

normalized by the average glomerulus spacing (AGS)¹⁹. We find that glomerular positions are defined more precisely along the PC1 versus PC2 axis (STD = 3.8 versus 4.7 AGS, Figure 3g,h). Importantly, shuffled controls (obtained by randomizing the identity of molecular properties, while not altering the glomerular responses) produced statistically indistinguishable outcomes in the prediction of glomerular positions by using shuffled property responses along newly computed PC1 and PC2 axes (Figure 3g,h). We also performed the same regression analysis using only the odor response spectra of individual glomeruli, and reached to quantitatively similar results (Figure 3g,h).

Overall, these results indicate that responses of glomeruli to odors are predictive of their coarse placement on the bulb surface consistent with previous reports relating the odor spectra and location of glomeruli^{19,25,41}. They also suggest that correlations observed between the topography of glomerular odor representations and the physical-chemical properties cannot be distinguished from effects of overfitting (chance level) due to the large number of Dragon properties.

Tuning of MTCs to molecular properties is not correlated with their spatial location

We further investigated the relationship between the location of OB output neurons and their tuning to molecular properties. In general, MTC responses in a field of view were locally heterogeneous, responding to chemically diverse odors (Supplementary Figure 6). Most sampled fields of view showed very few or no significant correlations at all (Figure 4a,b). Strikingly, in one FOV (#21, tufted cells), several properties were correlated with both AP and ML axes of the bulb. Given previous results⁴³, and the relatively small size of individual FOVs sampled (~300–500 μ m), we hypothesized that such correlations could arise due to similarity in the responses of groups of sister cells receiving inputs from the same glomerulus. If, for example, two of such sister cell groups are on opposite sides of a field of view, we may observe a correlation between the cells' locations and tuning arising from differences in the responses of these sister cell groups.

To identify putative sister cells, we clustered cells based on similarity in their responses to odors (Methods, Supplementary Figure 6). Clustering was performed within and across FOVs, since sister MTCs receiving primary input from same glomeruli are expected to be found on the dorsal aspect of the bulb in multiple animals. The results of clustering are displayed using a T-distributed Stochastic Neighbor Embedding (t-SNE) projection⁴⁴ in Figure 4c,d. After clustering, we collapsed the major clusters of cells into single 'average' cells, with both responses to odors and positions represented by the average value within each cluster (Figure 4e,f). When this approach was used, all significant correlations between tuning to molecular properties and cells' locations vanished (Figure 4h).

MTCs and glomeruli sample different molecular subspaces

We find that glomerular and MTC odor responses differ in their overall dimensionality, capture different amounts of variance with respect to tuning to the Dragon molecular properties, and display varying degrees of spatial correlation to these descriptors. We further investigated the differences in glomerular and MTCs sampling of the odor space.

First, using the PCX method, we compared the amount of variance in MTC and glomerular responses captured by the glomerular responses' PCs to the 49 stimuli. If MTC and glomerular response spaces were similar, the glomerular PCs would explain nearly the same fraction of variance in both the mitral cells and glomerular datasets. In contrast, glomerular PCs explained substantially less variance in the mitral versus glomerular responses (Figure 5a). Similarly, the PCs calculated for mitral cells odor responses were insufficient to describe the glomerular responses. However, the mitral cells PCs performed well in capturing tufted cell responses (Figure 5a).

One explanation for the differences in glomerular and mitral cell responses PC spaces is a sampling bias in probing the two layers, since our data includes a small population of mitral cells and glomeruli, albeit, from the same region of the bulb. In an attempt to reconcile the glomerular and mitral cell data, we tested a random selection model in which the responses of MTCs reflect the responses of individual glomeruli (Methods). Within this model, the discrepancy between glomerular and model mitral cell sampled spaces is small (green vs. dashed blue lines, Figure 5b) and not compatible with the experimental data (green vs. blue, Figure 5a).

We sought to identify an alternative model that could better explain the relation between glomerular and mitral cell odor spaces. We computed a rotation matrix in the odor space, which could convert the glomerular PCs to the mitral PC space. More precisely, if $\hat{R}_G$ is the matrix of glomerular responses (glomeruli x odors), we generated surrogate mitral cell responses $\tilde{R}_{MC}$ (mitral cells x odors) using the following equation:

$$\tilde{R}_{MC} = \hat{R}_G \cdot \hat{Q} \quad (1)$$

Here $\hat{Q}$ is the (odors x odors) rotation matrix ($\hat{Q}^T \hat{Q} = \hat{I}$) that relates the glomerular and mitral cells odor spaces. To derive $\hat{Q}$, we used singular value decomposition. We call this transformation the rotation model, since, to produce model mitral cell responses, it mixes glomerular responses to different odors with coefficients provided by rotation matrices (Methods). We find that the responses of model mitral cells (generated from glomerular responses, dashed blue lines, Figure 5c) are close to the variance produced by actual mitral cells responses (blue solid, Figure 5c), while those obtained from shuffled glomerular controls differ widely (Supplementary Figure 7). Thus, a simple rotation (equation 1) can generate mitral cell responses from glomerular responses and vice versa. The discrepancies between the rotation model and experimental data can be explained by a lower dimensionality PC space occupied by glomeruli (Figure 1g, h). Thus, surrogate mitral cell responses (Figure 5c, right) are left-shifted compared to the real cell responses because the dimensionality of the glomerular PC space is lower (21D vs. 24D, Supplementary Figure 4f,g). The rotation model reflects the possibility that MTCs sample a subset of the glomerular PCs, as well as other (top-down) stimulus related information (i.e. expectation, behavioral value, etc.) which are underrepresented in the glomerular input^{27,30,45}.

Our model aims to relate the odor representations in the input and output layers of the OB. The model described by equation (1) uses a small number of parameters (odors x odors) to

mix glomerular responses to different odorants and yield MTC responses. An equivalent circuit level model (Methods) pools together inputs from multiple glomeruli to produce MTC responses⁴⁶ using a substantially larger number of parameters.

To further characterize the differences between glomerular and MTC response odor subspaces, we employed a statistical method called canonical correlation analysis (CCA)⁴⁷. CCA identifies a set of angles (Jordan principal angles) that describes the relationship between two subspaces (i.e. glomerular and mitral) embedded into the same multidimensional space. The cosine of the Jordan principal angle is the canonical correlation coefficient. For two 2D planes in 3D, for example, one of the Jordan angles is the angle between the planes. In spaces of higher dimension, the number of non-zero Jordan angles is larger than one (see Methods for a description of number, distribution and relationship between Jordan angles and degree of overlap between subspaces).

Calculating the distribution of Jordan principal angles between PC spaces of increasing dimensionality indicated that glomerular and mitral cell odor response spaces are distinct, but related (Figure 5d,e, Supplementary Figure 8, Methods). The distribution of Jordan angles between mitral cells and glomerular PC spaces (blue line in Figure 5d) was matched by a glomeruli vs. glomeruli distribution, when noise was added to one set of glomerular responses with SNR ~1.0. This analysis suggests that mitral cells mix inputs from glomeruli and additional information from other sources in roughly equal proportion.

Overall, we propose that the odor spaces of glomerular and mitral cell responses are related via a rotation transformation. This transformation mixes responses obtained for different odors with predictable real-value coefficients, and may reflect the interplay between local processing and top-down centrifugal inputs to the bulb.

Discussion

We sampled odor responses of OB inputs and outputs in awake naive head-fixed mice, and sought to relate this activity to the physical-chemical properties of odors. Our experiments show that odors activate glomeruli and MTCs in a mosaic, spatially dispersed manner, with poor relation to an extensive set of commonly used physical-chemical molecular properties. Specifically, odors with similar physical-chemical descriptors did not elicit similar activity in the neuronal representations. Molecular properties were insufficient to explain the overall variance in neural odor responses, and lacked predictive power for the placement of both glomeruli and MTCs. Comparing activity patterns across the input and output layers, we found that glomeruli and MTCs sample different stimulus subspaces, and identified a rotation transformation in odor space that can relate these two sensory representations.

Dimensionality of the OB odor responses

The estimates of the dimensionality of odor space vary widely, ranging from several hundred, based on the number of odorant receptor types, to just 2–10 based on human perceptual responses^{5,16}. This is different, for example, from our understanding of color vision. There, three types of cones receptors form the 3 dimensions on which any neuronal and perceptual visual representations can be built⁶. In our experiments, the responses of a set

of glomeruli and MTCs from the dorsal aspect of the bulb to a 49 stimuli panel could be well-described within a ~20D flat PC space (Figure 1). This is a lower bound on the dimensionality of bulb neuronal representations, since systematically increasing the number of odors included in the analysis led to steady increase in the dimensionality of responses (Supplementary Figure 4f). This ~20D PCA space is flat and significantly fewer dimensions may be needed if a curved manifold is used to fit the data^{5,48}.

We note that the responses of MTCs are somewhat higher dimensional than those of glomeruli (24D vs. 21D, Supplementary Figure 4f). This could be a signature of MTCs' integration of lateral signals across glomeruli which are not available to optical imaging on the dorsal surface, as well as of differences in sensitivity of the imaging methods used. In addition, local inhibitory inter-glomerular cross-talk, and top-down feedback and neuromodulatory input may amplify the dimensionality of MTC responses, consistent with previous work^{20,33–36}.

The relationship between molecular properties and neuronal responses in the bulb

Using Principal Component Exchange (PCX) analysis, we found that the PC spaces of properties and responses share very little overlap. Thus, both glomeruli and MTC responses appear to include information that is not related to odor molecular properties *per se*. Such information may reflect valence, previous experience and expectations, or behavioral information, including changes in stimulus sampling. Additional relevant properties (not included in the set of 1,666) could also drive the responses of the olfactory neurons. The dimensionality of molecular properties increased with the number of odorants used, an indication of under-sampling the physical-chemical odor space (Supplementary Figure 4f). Including ROIs from other aspects of the bulb, in addition to the dorsal surface may strengthen this relationship. Finally, neuronal responses may simply contain randomness that is unrelated to any useful signals, although the measured variability of responses to individual odors was less than 10%. Future experiments with mice engaged in different behavioral tasks, together with extended sampling of neuronal responses and further chemical space characterization will help disambiguate these possibilities.

Can odor properties predict OB odor responses?

Several studies have suggested that the odorant molecular properties can be used to predict the responses of neurons in the olfactory system. We addressed this question for both OB inputs and outputs by asking whether similarity in molecular properties of pairs of odors can predict the similarity of neuronal populations. We used a sparsening procedure (LASSO) that helps select the molecular properties most predictive of the neuronal responses. Approximately 10 properties were sufficient to establish a substantial correlation (~0.60) between pairwise odor distances in molecular properties and similarity in neuronal population responses. To test the robustness of this relationship, we employed cross-validation methods (Figure 2, Supplementary Figures 4,5) that rigorously separated testing from training data. We found that the properties predictive of similarity of either glomerular or MTC responses using training odor sets fail to generalize to new pairs of odors.

Our results differ from other published studies that found predictable relations between odorant molecular properties and activity in the early stages of the olfactory system in insects, fish, tadpoles and rodents^{11,12,49}, but see^{13,19,42}. These differences could arise due to several reasons. First, due to the number of odors used (~50), the relevant properties may not have been robustly established. This seems an unlikely explanation since other studies have used similar number of odors, and did not systematically examine different concentrations, as we did for the MTCs (Supplementary Figure 3, Methods). A second possibility is that previous analyses have focused primarily on relating physical-chemical odor space to patterns of activity in the anaesthetized preparations. Any relation between molecular properties and activity could be modulated by brain state. However, our experiments indicate that molecular properties explain a similarly low fraction in the variance of glomerular responses in both awake and anaesthetized animals (Supplementary Figure 5a-d). A third possibility, is that previous work may suffer from this same problem of overfitting and poor generalization, but the regression models were not tested using pairs of odors outside the training set. We also note that the relationship of olfactory neurons responses to these physical properties could be complex and highly nonlinear, and the algorithms used here may not capture it well.

Beyond a look-up table of physical-chemical properties

There has been considerable debate on whether there is a continuous and recognizable map of chemical space in the OB. Since microscopy offers spatial information, we asked whether the location of glomeruli or MTCs is related to their selectivity to molecular properties. Significant correlation between odor spectra (and Dragon properties) and the location of glomeruli was observed over a broad scale (~4–5 glomerular spacings, Figure 3). This is consistent with previous reports^{19,25,41} which identified large chemotopic domains on the bulb surface (~1 mm). The precision of these predictions is substantially lower (~6–8 fold) compared to the precision of the glomerular spatial layout across individuals (~0.5–1.0 glomerular spacings)¹⁹, but see⁵⁰. The orientation of the identified principal axis (PC1) may reflect specific interactions between the axon terminals of OSNs and gradients of axon guidance molecular cues during the formation of the glomerular map.

With respect to the predictive power of molecular properties on glomeruli placement, the results of the regression analysis should be subject to caution given the large number of Dragon properties tested. Indeed, shuffled controls (obtained by randomizing the identity of molecular properties) produced statistically indistinguishable outcomes in the prediction of glomerular positions by using shuffled property responses along newly computed PC1 and PC2 axes.

While tiling the dorsal aspect of the bulb, we used smaller fields of view for monitoring MTCs activity. This constrains our conclusions on spatial tuning of OB output neurons to properties to a finer scale (~0.5 mm). Weak, but statistically significant correlations between molecular properties and the location of somata were present only in a small number of fields of view. These correlations appear to be induced mainly by the presence of cells with highly correlated odor tuning, putative co-glomerular “sister” cells⁴³, that display similar

average odor tuning. When redundancies in responses were removed, any correlations between molecular properties and MTCs placement were lost (Figure 4).

Relating the glomerular and MTCs odor response spaces

Although ideally this problem should be addressed by observing MTC and glomerular responses simultaneously in the same preparation, and with same activity sensors, obtaining such data was beyond the scope of the present study. Instead, we took advantage of data acquired in different animals to determine whether the spaces sampled by the two olfactory processing layers differ in a systematic fashion. We found that populations of glomeruli and MTCs on the dorsal aspect of the bulb sample different subspaces with respect to the same panel of odors. In agreement, with previous work^{30,33,45}, our analyses (PCX, CCA, Figure 5) indicate that MTCs do not simply relay the glomerular inputs to higher olfactory centers, but substantially modify and diversify the OR input channels, as indicated by their higher dimensionality. Our data is consistent with a scenario in which glomeruli and mitral cell responses occupy intersecting, but distinct sensory odor spaces. Their different representations may reflect feedforward input from the olfactory epithelium, local bulbar processing and top-down input, which could sample information along different axes of the odor scenes. For example, mitral and tufted cells may filter out certain features of glomerular activity, but also integrate information that appears underrepresented at the glomerular level. A rotation transform across odor responses relates well these two spaces (Figure 5). This observation provides a potential framework for understanding the bulb input-output function in future studies aimed at probing simultaneously glomerular and MTC activity in naïve mice or during behavior.

Our data suggest that the physical-chemical properties used by us and others are not sufficient to fully represent the responses of olfactory bulb neurons. It seems that the molecular properties initially generated for computational chemistry studies do not capture stimulus features important for animals' sensory perception, and novel descriptors are needed to link chemical space to neuronal representations. Relevant descriptors may carry information pertaining to behaviorally relevant properties of odors encountered by animals in their ecological niche as previously proposed (i.e. hedonic value, edibility, survival)^{5,8,16}. Systematic exploration of the natural odor statistics may offer further insight on the structure of odor space.

Methods

Chronic windows for awake head-fixed intrinsic and multiphoton imaging.

Adult B6/129 and TBET-Cre X Ai38 GCaMP3.0 or Ai 95 GCaMP6f mice (four males and four females >80 days old, 25–40 g) were anesthetized with ketamine/xylazine (initial dose 70/7 mg/kg), supplemented every 45 minutes, and their heads fixed to a thin metal plate with acrylic glue. Heart beat, respiratory rate, and lack of pain reflexes were monitored throughout the procedure. Animals were administered dexamethasone (1 mg/Kg) to prevent swelling, enrofloxacin against bacterial infection (5 mg/Kg), and carprofen (5 mg/Kg) to reduce inflammation. To expose the dorsal surface of the OB for chronic imaging, a small craniotomy was made over both OB hemibulbs, using either a biopsy punch⁵¹ or thinning

the skull with a 27 high-speed dental drill (Foredom, Bethel, CT), and removing it completely. A 3 mm glass cover slip (CS-3R, Warner Instruments) was placed atop and sealed in place using Vetbond (3M), further reinforced with cyanoacrylate (Krazy Glue) and dental acrylic (Lang Dental). A custom-built titanium head-bar was cemented on the skull near the lambda suture as described previously^{26–30}. Carprofen (5 mg/Kg) was administered for two days following surgery. Animals were left to recover for at least 48 hours after surgery before imaging and further habituated before the imaging sessions. All animal procedures conformed to NIH guidelines and are approved by Cold Spring Harbor Laboratory Animal Care and Use Committee.

Odor stimulation.

A custom odor delivery machine was built to deliver up to 165 stimuli automatically and in any desired sequence under computer control of solenoid valves (AL4124 24 VDC, Industrial Automation Components). Pure chemicals and mixtures were obtained from Sigma and International Flavors and Fragrances. Odorants were diluted 3,000 and respectively 100 fold into mineral oil and placed in blood collection tubes (Vacutainer, #366431) loaded on a custom made rack and sealed with a perforated rubber septum circumscribing two blunt end needles (Mcmaster, #75165A754). Fresh air was pumped into each tube via one needle by opening the corresponding solenoid valve. The mixed odor stream exited the tube through the other needle and was delivered at ~1l/min via Teflon-coated tubing to the animal's snout. The concentration of the odors delivered to the mouse was measured using a photo-ionization device (PID; Aurora Scientific) and found to range between ~0.05–1 % saturated vapor pressure. The same PID was used to determine the time course of the odor waveform and the reliability of odor stimulation. A list of odors used in our experiments is provided in Supplementary Table 1. 49 out of the 57 stimuli used are monomolecular compounds, and were further included in the physical-chemical properties analysis. For the comparison of glomerular and MTC responses, the 1:100 dilution was used for same 49 odors. In a set of 6 M/T FOVs (3 mice), only the first 33 odors in the panel were used.

In general, for intrinsic optical imaging (IOI) experiments, in each stimulus trial, we presented 12s of air, followed by 24 seconds of odor delivery. The interval between trials was at least 45s and each stimulus was repeated 4–5 times. Data was obtained from 9 mice (5 awake mice, 5 females, 10 bulb hemispheres = hemibulbs, and 4 mice, 1 male, 3 females - 8 hemibulbs, for which responses were sampled in both awake and anaesthetized states). For two photon experiments, we scanned at 5–10 Hz/frame and covered a field of view up to ~350×500µm in the mitral and tufted cell layers. Before delivering odors, the OB was examined to gauge the quality of the surgery and select the regions of interest. The resting fluorescence of GCaMP3/6^{52,53} is low, but could be discerned by frame averaging (~10 frames). Resting images at different depths were obtained before choosing specific optical sections for further experiments. Once a specific optical section was chosen, a time sequence of 120–240 frames was acquired. During the first 10 seconds, fresh air was delivered, followed by odor stimuli (of matched flow rate to the fresh air to avoid mechanical olfactory sensory neuron activation) for 4 seconds. Finally, fresh air was delivered for 10 seconds. The inter-trial interval was 45 seconds. Each odor was typically delivered 3–4 times.

Intrinsic and multiphoton imaging:

We used computer controlled LEDs to shine far red light (780nm) for imaging intrinsic optical signals, a good proxy for presynaptic OSN activity^{19,54,55}, on the dorsal surface of the bulb, acquiring images at 25 Hz (Vosskuhler, 1300-QF CCD camera). For two photon imaging, we used a Chameleon Ultra II Ti:Sapphire femtosecond pulsed laser (Coherent) and a custom-built multiphoton microscope. The shortest possible optical path was used to bring the laser onto a galvanometric mirrors scanning system (6215HB, Cambridge Technologies). The scanning system projected the incident laser beam tuned at 930nm through a scan lens and tube lens to backfill the aperture of an Olympus 20X, 1.0 NA objective. Scanning and acquisition were performed using custom Labview based software (National Instruments).

Imaging odor responses in tufted and mitral cells of the OB

We used resting fluorescence and the presence of dark nuclei to identify tufted or mitral cells in the imaged field (Supplementary Figure 1d,f). We observed robust odor responses in the cell bodies of both tufted cells, identified based on the location of their somata in the external plexiform layer (EPL, Supplementary Figure 1d,e), as well as of mitral cells (Supplementary Figure 1f,g). Different odors led to different spatial (Supplementary Figure 1d,f) and temporal (Supplementary Figure 1e,g) pattern of activation of tufted and mitral cells. Responses to a given odor had diverse amplitudes and time courses in different cells (Supplementary Figure 1d-g). Conversely, a given cell responded to different odors with distinct amplitude and temporal dynamics and showed low trial to trial variability (Supplementary Figure 1e,g).

For each cell type, we calculated the mean fluorescence change during odor presentation and obtained an odor response spectrum (ORS) or odor tuning curve for 55 odors (of which 53 were monomolecular, Supplementary Table 1) per imaging session (Supplementary Figure 1d,g). As observed in the example ORS shown, different tufted (Supplementary Figure 1e) and mitral cells (Supplementary Figure 1g) responded in a distinctive manner to the odors used. Measurements of the odor waveform using a photo-ionization detector (PID) indicated that the temporal diversity of responses across odors was not due to differences in stimulus kinetics (data not shown).

To test our results across a range of concentration, we used two different oil dilutions (1:3,000 and 1:100) of each odor and sampled the responses of mitral and tufted cells in 19 different fields of view tiling the dorsal surface aspect of the bulb (out of these, for 6 fields of view, both dilutions were sampled). PID measurements of five randomly sampled odors indicate that these dilutions roughly span a range of 18 ± 5 fold in concentration (Supplementary Figure 2a). Clustering responses based on their temporal dynamics (hierarchical clustering) showed that sustained excitatory responses, as well as inhibitory responses were more frequent in mitral cells than in tufted cells (Supplementary Figure 2c,d).

Data analysis.

For intrinsic imaging experiments, responsive glomeruli were identified as previously described¹⁹. For mitral and tufted cells, ROIs were manually selected based on anatomy. Care was taken to avoid selecting ROIs on cell bodies overlapping with neuropil (MTC lateral dendrites). To facilitate detection of responding cells or regions, we calculated a ratio image for each odor (average of images in odor period minus average of images in pre-stimulus period, normalized by the pre-stimulus average). We further obtained a maximum pixel projection of all odor responses, assigning to each pixel in the field of view the maximum response amplitude across the odor panel used – this allowed us to visually identify odor responsive regions. These responsive regions of interest mapped to individual mitral/tufted cell bodies respectively in the fluorescence image and were selected for further analysis¹⁹.

Statistics.

All analyses were performed using Matlab (Mathworks) and Igor (Wavemetrics). The analyses performed included: paired t test, FDR correction, ANOVA, and Sign Rank Test. All tests were two-sided otherwise noted. For parametric tests data distribution was assumed to be normal, but this was not formally tested. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications^{19,24,43}. Randomization of conditions was not relevant to the study; data collection and analysis were not performed blind to the conditions of the experiments.

Odor responses.

To obtain odor responses, we computed the average fluorescence during the period of odor presentation for each trial i , F_{2i} , and the average fluorescence during the preceding air period F_{1i} . Responses were defined as statistically significant for $p < 0.1$ (2-way ANOVA). Because, in the subsequent analysis, additional more stringent statistical tests were performed, we reasoned that preliminary filtering of the data on the level of $p < 0.1$ is reasonable. For the subsequent analysis, we used relative response (dF/F) defined as:

$$\Delta_{cs} = (F_{2ct} - F_{1ct})/F_{1ct}, \quad (2)$$

where indexes c , s and t enumerate regions of interest (ROI, i.e. cells), odors, and trials respectively, while average is computed over the trials i .

For intrinsic optical imaging of glomeruli, we applied the same procedure as described previously¹⁹. Control ROIs drawn in non-responsive areas of the bulb were used to obtain a response signal threshold by comparison to odor responses in equal number of active glomeruli. Varying a signal threshold, the number of control ROIs that passed the threshold was compared to the number of responses in regions identified as active glomeruli to obtain a false positive ratio of < 0.1 . For both glomeruli and MTCs, ROI-odor pairs with non-significant response were set to 0.

Hierarchical clustering.

To identify mitral cell bodies with similar odor tuning, putative co-glomerular sister cells^{43,56,57}, we performed a cluster analysis based on the similarity between odor response spectra (average linkage, cutoff = 0.7). Each cluster with three or more members was taken to represent potential sister MTCs receiving common primary input from the same parent glomerulus and considered for further analysis.

Odor physical-chemical property response and property response spectra.

To determine the sensitivity of individual ROIs to various properties we evaluated the property response spectra, Φ_{cp} , which are the ROIs' chemical receptive fields. Each element of the matrix Φ_{cp} with indexes c and p indicates the strength of response of a given ROI, c to an odor physical-chemical property, p . Thus matrix Φ_{cp} describes the physical-chemical tuning of the set of ROIs. To compute Φ_{cp} , we first calculated the values of 1,666 physical-chemical properties for the odors used in our panel. This resulted in a property matrix P_{sp} . S enumerates the monomolecular odors (1–53 for MTCs and 1–49 for glomeruli), as above, while the second index p ranging between 1 and 1,666 denotes the physical-chemical properties. The property matrix, was obtained by downloading molecular structures for the monomolecular odors from PubChem and using an online set of algorithms Dragon to evaluate the physical-chemical properties. The full list of molecular descriptors (physical-chemical properties) used can be found here: http://www.taletе.mi.it/products/dragon_molecular_descriptor_list.pdf.

Because the properties obtained were highly inhomogeneous in ranges and scales, we normalized the data as outlined below. If a property took both negative and positive values for the odors in the panel, we subtracted the mean value for this property and divided by the standard deviation across the odors in the panel. If a property was strictly positive (molecular weight, etc.), we examined the standard deviation of its logarithm (SDL). If SDL was larger than 1, we assumed the property is lognormally distributed. The corresponding property was replaced in matrix P_{sp} by its logarithm with the mean subtracted. If SDL was smaller than 1, we subtracted the mean from the property and divided the values by its standard deviation for the odors in the panel. Normalizing each property by the standard deviation, we minimized the influence of measurement units on the dynamic range of some properties which could introduce a subjective bias. By implementing this procedure, all properties were brought to zero mean and similar standard deviations. The resulting matrix was denoted $\tilde{P}_{sp}$. ~300 physical-chemical properties tool only zero-values for the odors in our panel and were not included in the analysis.

To compute the property response spectrum, the ROI's chemical receptive field across the properties, we used the following formula:

$$\Phi_{cp} = \sum_s \tilde{\Delta}_{cs} \tilde{P}_{sp} \quad (3)$$

For each region of interest c , a property response, defined as an entry in the property response spectrum, was equal to the correlation between the dF/F odor responses (normalized to unit standard deviation) of this ROI and the normalized property strength vector (PSV), given by the values taken by property p computed over the entire set of odors presented (enumerated by s).

To evaluate the significance of the correlation between a property and an ROI's response pattern, we use a p-value threshold of .05. p-values are calculated using MATLAB's built-in function `corr()`. This implementation evaluates the t statistic as: $t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$, where r is the correlation coefficient and n is the number of data points. The p-value is then twice the probability a t distributed variable exceeds t . We also applied False Discovery Rate (FDR) correction⁵⁸ for each ROI's property response spectrum at $q < 0.1$.

Odorant Response Similarity and Property Space Similarity.

We compute the correlation matrix of the relative responses (dF/F) Δ_{cs} and Δ_{ck} between each pair of odors, s and k , for all glomeruli or cells, c as:

$$C_{sk} = \frac{\sum_c (\Delta_{cs} - \bar{\Delta}_s)(\Delta_{ck} - \bar{\Delta}_k)}{\sqrt{\sum_c (\Delta_{cs} - \bar{\Delta}_s)^2} \sqrt{\sum_c (\Delta_{ck} - \bar{\Delta}_k)^2}} \quad (4)$$

Where $\bar{\Delta}_k = \frac{1}{N} \sum_{c=1}^N \Delta_{ck}$. Indexing the unique pairs of odors of C_{sk} with i , we have a vector of correlations, $\tilde{C}_i$. Using the property matrix $\tilde{P}_{sp}$ described above, we calculate the Euclidean distance matrix as:

$$D_{sk} = \sqrt{\sum_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2} \quad (5)$$

We calculate Euclidean distances in neuronal response space between each smell s and k as $N_{sk} = \sqrt{\sum_c (\Delta_{sc} - \Delta_{kc})^2}$. $\tilde{N}_i$ is the vectorized unique smell pairs of N_{sk} . Figure 2a,b (*Top*) plots $\tilde{D}_i$ against $\tilde{N}_i$. Following the same vectorization procedure as with the response correlations $\tilde{C}_i$, we vectorized the unique pairs of odors in matrix D_{sk} with $\tilde{D}_i$. Figure 2a,b (*Bottom*) shows a plot of $\tilde{D}_i$ versus $\tilde{C}_i$.

We searched for a subset of properties which generate property distances $\tilde{D}_i^2$ that correlate best with neuronal response distances $\tilde{N}_i^2$. For this, we used the LASSO algorithm³⁸. This algorithm minimizes the following equation :

$$\lambda \sum_p |\beta_p| + \left(\sum_i \left(\sum_c (\Delta_{sc} - \Delta_{kc})^2 \right)_i - \left(\sum_p \beta_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2 \right)_i \right)^2 \quad (6)$$

Where $()_i$ denotes the vectorization of the unique pairs of odors s and k described above. Manipulating each weight β_p (non-negative) to minimize the term

$\left(\sum_i \left(\sum_c (\Delta_{sc} - \Delta_{kc})^2\right)_i - \left(\sum_p \beta_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2\right)_i\right)^2$ creates a property space with square property distances $D_{sk}^2 = \sum_p \beta_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2$ that reconstruct the square neuronal response distances $N_{sk}^2 = \sum_c (\Delta_{sc} - \Delta_{kc})^2$. The first term of the LASSO objective function, $\sum_p |\beta_p|$, penalizes the use of nonzero weights (Supplementary Figure 4i). This forces the algorithm to choose the most parsimonious property space to reconstruct neuronal response space. Increasing the parameter, λ puts more pressure on each β_p to be zero. Figure 3c,d shows, for different number of non-zero properties, the correlations between distances in the weighted property space $\left(\sum_p \beta_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2\right)_i$ and distances in the neuronal response space $\left(\sum_c (\Delta_{sc} - \Delta_{kc})^2\right)_i$.

If all molecular property weights had values of 1, using LASSO, one would arrive at the correlation values shown in Figure 2a and b. To find a sparse and robust solution, the LASSO algorithm assigns zero value to the weights of most molecular properties. By varying a penalty parameter (λ) of the algorithm (Supplementary Figure 4i), we changed the number and relative contribution of the molecular properties included in computing the pairwise odor distances.

To cross-validate on new responses, we randomly selected and withheld half of the FOVs and performed LASSO regression on the remaining data (training set). Then, we recomputed the correlations between property distance and response distance in the withheld data (testing set). To determine how our results generalized for new stimuli, we removed one pair of odors from the panel (jackknife, leave one out) and performed the above analysis on the rest of the data. Then we calculated the distance between the two removed odors in the reduced property space found by LASSO regression. We repeated this procedure independently for each pair of odors i , with each repetition generating one property distance $\left(\sum_p \beta_p (\tilde{P}_{sp} - \tilde{P}_{kp})^2\right)_i$. Then, we found the correlation between the vector of these property distances and the neuronal response distances $\left(\sum_c (\Delta_{sc} - \Delta_{kc})^2\right)_i$. Thus, every prediction of an odor pair similarity was obtained based only on odor similarities calculated for all other odor pairs.

Finally, we cross-validated with new FOVs and new odors by combining the two previous cross-validation procedures. That is, we withheld one pair of odors and half of the FOVs, and then performed LASSO. Using the reduced property space found by LASSO, we then predicted the response distance of the two removed odors in the withheld response data.

The physical-chemical properties selected by LASSO regression were different for cross-validated and non-cross-validated curves in Figure 2. This is because data available in each case is different. For FOV cross-validation, a different set of properties emerged for each subset of selected FOVs in the training set. Similarly, for odor cross-validation, the distance between each pair of odors was calculated using a different data subset and led to distinct sets of best properties.

Greedy algorithm.

First, we found the property for which the Euclidean distance of each pair of odorants is best correlated with the odor response similarity, defined as the correlation between the M/T neuronal response profiles (cell response spectra) in all FOVs of these two odorants. Second, we searched through all remaining properties, and with each iteration added to the metric the property which most greatly increased the correlation between the physical-chemical property distance and neuronal response odor similarity. The process terminated once adding any new property decreased the correlation (Supplementary Figure 4j).

Correlations between ROI property response spectra and ROI locations.

To evaluate physical-chemical property-to-position correlations, we computed the Pearson's correlation coefficients between the locations of ROIs $\vec{r}_c = (x_c, y_c)$ and their property response spectra (receptive fields, Φ_{cp}). Here x_c and y_c are AP and ML positions of an ROI k on the surface of the bulb. If the average locations are $\bar{x} = \frac{1}{N} \sum_{c=1}^N x_c$ and $\bar{y} = \frac{1}{N} \sum_{c=1}^N y_c$, the Pearson's correlation for a property p with the position of the ROI along the AP axis is defined as:

$$R_{xp} = \frac{\sum_c (x_c - \bar{x})(\Phi_{cp} - \bar{\Phi}_p)}{\sqrt{\sum_c (x_c - \bar{x})^2} \sqrt{\sum_c (\Phi_{cp} - \bar{\Phi}_p)^2}} \quad (7)$$

where $\bar{\Phi}_p = \frac{1}{N} \sum_{c=1}^N \Phi_{cp}$. Similarly, the correlation of the property-to-ML position, denoted as 'y', correlation is defined as:

$$R_{yp} = \frac{\sum_c (y_c - \bar{y})(\Phi_{cp} - \bar{\Phi}_p)}{\sqrt{\sum_c (y_c - \bar{y})^2} \sqrt{\sum_c (\Phi_{cp} - \bar{\Phi}_p)^2}} \quad (8)$$

In addition to calculating the correlation values, we evaluated their statistical significance using MATLAB function 'corr'. The corresponding p-values were computed for each property: P_{xp} and P_{yp} . We applied False Discovery Rate correction⁵⁸ to each set of p-values and found the set of q-values Q_{xp} and Q_{yp} using the MATLAB function 'mafdr'. A property was assumed to be significantly correlated with A-P or M-L axes if the corresponding q-value was less than 0.1. The properties with significant correlations are shown in Figure 4a,b by color.

Predictions of glomerular position based on odor physical-chemical properties.

We tested whether glomeruli' tuning properties are predictive of their locations in the bulb. Different sets of properties were selected such as to yield the best match of glomerular positions for each field of view and reference axes (PC1 vs. PC2). To this end, for each field of view and for each bulb axis, we built a linear regression that should approximate the positions of glomeruli, c:

$$x_c = \sum_p \Phi_{cp} W_{xp} \quad (9)$$

$$y_c = \sum_p \Phi_{cp} W_{yp} \quad (10)$$

where W_{xp} and W_{yp} are sparse vectors of unknown coefficients that were found using the LASSO algorithm³⁸. We added a column of ones to the matrix Φ_{cp} to include a possible offset to the approximation of coordinates. We ensured vectors W_{xp} and W_{yp} have only 20 non-zero components. To validate the prediction built on the basis of glomerular receptive fields, we excluded a single ROI (glomerulus) from the dataset, further obtained regressions with the LASSO algorithm based on the remaining ROIs, and then used the removed glomerulus to test the quality of prediction (jackknife cross-validation). We repeated this procedure for all glomeruli in the dataset. We verified that changing the number of active properties does not affect our results substantially. The resulting predictions for glomerular positions were compared to the actually observed bulbar positions and the quality of predictions was evaluated by computing the distance between actual and predicted positions measured in terms of average glomerular size (AGS, 75 μm Figure 3g,h). The same analysis was performed on shuffled properties control, where the property strength vectors were shuffled by odor identity.

Dimensionality of physical chemical properties and glomerular and MTC responses:

we sampled increasing number of monomolecular odors within our panel (up to 49) and performed principal component analysis to calculate the dimensionality (90% variance explained) of the properties (PSV) and of the glomerular and MTC odor responses. To estimate the robustness of this analysis, for each number of odors considered, we constructed a distribution of 1,000 odor sets and sampled random combinations of the possible odor subsets within the panel (Supplementary Figure 4f).

PCA space comparison using PC exchange (PCX) method:

To compare the odor spaces sampled by mitral cells versus glomeruli, we projected them onto each other's principal components. More explicitly, we consider the matrix of mitral cell responses, with element M_{ms} corresponding to mitral cell m responding to odor s , and similarly the response matrix with elements G_{gs} for glomerulus g and odor s . For each response matrix, singular value decompositions can be written as:

$$G = U_G D_G V_G^T, \quad M = U_M D_M V_M^T \quad (11)$$

where, $D_{G/M}$, and $V_{G/M}$ are the unitary matrix, diagonal singular value matrix, and eigenvector (PC) matrix respectively for the glomerular and MTC response matrices. If dimensions of the matrix G are $[N_G \times N_S]$, i.e. number of glomeruli by the number of odors, the dimensions of matrices U_G, D_G , and V_G are $N_G \times N_S$, $N_S \times N_S$, and $N_S \times N_S$ respectively. Here the number of odors is smaller than the number of glomeruli. We then computed projections of mitral cell responses onto the glomerular principal components,

M_G , and the projections of glomerular responses on the mitral cell principal components, G_M , as follows:

$$G_M = GV_M, \quad M_G = MV_G, \quad (12)$$

The variance of the glomeruli projections for each mitral cell principal component was calculated as:

$$\sigma_p^2 = \frac{\sum_g (G_{Mgp} - \mu_{Gp})^2}{N_G} \quad (13)$$

Where μ_{Gp} is mean of the glomerular responses on the mitral cell principal component p and N_G is the total number of glomeruli. Variance for mitral cells projected onto glomeruli can be found by replacing every instance of G with M and g with m .

The random data control used in Figure 1 is a random matrix, $N_G \times N_S$, constructed by sampling from a Gaussian distribution centered at 0 with STD = 1.

Resampling model:

In this model, we tested whether mitral cell responses are a re-sampling of glomeruli responses. To obtain the responses of one MTC to the odors in our panel, we randomly selected a glomerulus in the dataset and assumed that neuronal responses faithfully relay inputs from this glomerulus. We randomly selected glomeruli with repetition to generate a new response matrix with equal number of cells as the true mitral cell response matrix. We then used the PCA space comparison method described above to compare the resampled glomeruli to the true mitral cell responses.

Rotation model:

we tested whether mitral cell responses constitute a rotation of the glomeruli's sampling of odor space. Using the notation from the PCA space comparison method, we modeled surrogate mitral cell responses to be:

$$\tilde{M} = GQ \quad (14)$$

Where Q is a $N_S \times N_S$ rotation matrix, calculated as:

$$Q = V_G V_M^T \quad (15)$$

Because $G = U_G D_G V_G^T$ and $V_G^T V_G = I$, for the surrogate mitral cell responses (15) we obtain $\tilde{M} = U_G D_G V_M^T$. We further compared the rotated glomeruli response matrix with the mitral cell response matrix using the PCA space comparison method described above.

Rotation matrix Q cannot be viewed as a connectivity matrix. This rotation does not make predictions regarding the specific connectivity of individual glomeruli and mitral cells.

Instead, it enables us to compare the two olfactory bulb layers' sampling of odor space. This is because, in equation (15), matrix Q multiplies the glomerular responses G on the right thus mixing responses of glomeruli to different smells to obtain the surrogate mitral cell responses, $\tilde{M}$. Our model aims to relate glomerular and M/T response spaces using a minimum number of parameters.

A circuit level model would be required to pool together inputs from multiple glomeruli (versus pooling single glomerular responses across odors) to produce MTC responses⁴⁶. To obtain a weight matrix W that mixes glomerular responses for the same odor to obtain the same surrogate matrix, $\tilde{M}$ one would have to multiply the glomerular matrix on the left, i.e.

$$\tilde{M} = \hat{W} \cdot \hat{G} \quad (16)$$

Here, $\hat{W}$ is the (glomeruli x glomeruli) weight matrix which is much larger than matrix $\hat{Q}$. Because $\tilde{M} = U_G D_G V_M^T$ and $G = U_G D_G V_G^T$, the weight matrix can be identified:

$$W = U_G D_G V_M^T V_G D_G^{-1} U_G^T \quad (17)$$

These two equations can be viewed as feedforward network equations that produce mitral cell responses from glomerular activities. In comparison, equation (1) yields an equivalent, albeit more compact relationship between mitral and glomerular responses than equation (17).

Jordan principal angles:

A standard method for comparing the relation between multidimensional subspaces is to calculate the Jordan principal angles between them. In canonical correlation analysis (CCA), the *cosine* of the Jordan principal angles is the canonical correlation. Intuitively, CCA identifies the two maximally correlated vectors (called principal vectors) between the pair of subspaces of interest and calculates the angle between them (Jordan principal angle). The principal vectors are principal components for their corresponding subspaces. This process is iterated to identify all other remaining maximally correlated principal vectors (with the added constraint that they have to be orthogonal to the previously identified pairs of principal vectors) and to further calculate the corresponding Jordan angles between them. For example, the relationship between two lines intersecting in a multidimensional space is described by a single Jordan angle. For planes of higher than one dimension, their arrangement is described by more than one Jordan angle. For two randomly selected 2D planes, there are two Jordan angles: one angle is formed by the vectors perpendicular to these planes, and one angle that is always zero. Because 2D planes intersect along a line, the second Jordan angle is formed by two vectors belonging to two planes running along the intersection, and is, consequently, zero.

For two nD planes, the number of Jordan angles is n . If these planes are embedded into an N dimensional (ND) space ($N \geq n$), two planes placed arbitrarily with respect to each other (e.g. random), intersect along a space of dimension 0 if $2n \leq N$, like two lines on a 2D plane.

In this case, all Jordan angles are expected to be non-zero. For example, the relative arrangement of two 5D planes in general position in a 10D space is described by five non-zero Jordan angles. For $2n > N$, at least $2n - N$ Jordan angles are zero. For example, two 2D planes in 3D form two Jordan angles, one of which is zero. The same 2D planes in 4D, however, form two Jordan angles all of which, in general, are non-zero. Finally, for two ND planes embedded in N dimensions ($ND=N$), all N Jordan angles are zero, since these planes coincide (Supplementary Figure 8).

To calculate the Jordan principal angles between subspaces, we used the singular value decomposition method⁴⁷ (SVD). In this approach, SVD of the Gram matrix is computed for two PC bases in each subspace and the diagonal part of the diagonal matrix is used to identify the cosines of Jordan angles. We gradually increase the dimensionality of the two PC subspaces by using only PCs with highest variance (until explaining 90% of variance).

PCX compared to Jordan's angles:

We are not only interested in comparing dimensions but also the variance in those dimensions. For example, we would like to use a method which determines whether input responses are highly variant to an odorant dimension but then lowly variant to that dimension in the output responses. Standard use of Jordan principal angles does not distinguish between these cases. To identify such cases, instead of computing the Jordan principal angles between the two subspaces directly, we first consider further subspaces of both subspaces. We then progressively include principal components of both subspaces, iteratively re-computing the subspace principal angles for each addition.

More formally, for matrix $A \in \mathbb{R}^{N \times M}$ and $B \in \mathbb{R}^{P \times M}$ we can compute the SVD for both $A_{nm} = \sum_{ql} U_{nq}^a S_{ql}^a V_{ml}^a$ and $B_{pm} = \sum_{ql} U_{pq}^b S_{ql}^b V_{ml}^b$. The columns and rows of these SVD matrices are ordered such that the diagonal elements of S are descending in order. Thus, the principal components of V are ordered by the amount of variance they include in descending order. Then if $\theta(x, y)$ is a function which computes the subspace angles between matrices x and y , we find $\vec{k}_i = \theta\left(\sum_{l=1}^i \sum_p U_{nq}^a S_{ql}^a V_{ml}^a, \sum_{l=1}^i \sum_q U_{pq}^b S_{ql}^b V_{ml}^b\right)$ or $i = 1 \dots M$. Because the order of the principal components of each subspace depends on the variance, some of the elements of $\vec{k}_1, \vec{k}_2, \dots, \vec{k}_M$ may contain nonzero angles, indicating that the data is distinct in variance.

Data availability.

All data matrices representing glomerular and MTCs odor responses and physical-chemical descriptors for the odors in the panel, included in the analyses presented here are available on github: https://github.com/TeamAlbeanu/mosaic_representations.

Code availability.

Code used for analysis is available upon request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors would like to acknowledge members of the Koulakov and Albeanu labs and Fede Carnevale for critical discussions, and Martin Davis and Rob Eifert for technical support. This work was supported by the following funding sources: BBRF 2014 Young Investigator grant to H.C., WSBS PhD support to D.R.K, Swartz Fellowship to W.G.B, R01DC014366 to A.A.K., NSF IOS-1656830, NIH R01DC014487-03 to D.F.A. and R01DC017876-01 to A.A.K. and D.F.A.

References

1. Kaas JH Topographic maps are fundamental to sensory processing. *Brain Res. Bull* 44, 107–112 (1997). [PubMed: 9292198]
2. Amoore JE, Palmieri G. & Wanke E. Molecular shape and odour: pattern analysis of PAPA. *Nature* 216, 1084–1087 (1967). [PubMed: 6075249]
3. Bushdid C, Magnasco MO, Vosshall LB & Keller A. Humans can discriminate more than 1 trillion olfactory stimuli. *Science* 343, 1370–1372 (2014). [PubMed: 24653035]
4. Haddad R, Lapid H, Harel D. & Sobel N. Measuring smells. *Curr. Opin. Neurobiol* 18, 438–444 (2008). [PubMed: 18824102]
5. Koulakov AA, Kolterman BE, Enikolopov AG & Rinberg D. In search of the structure of human olfactory space. *Front Syst Neurosci* 5, 65 (2011). [PubMed: 21954378]
6. Meister M. On the dimensionality of odor space. *Elife* 4, e07865 (2015). [PubMed: 26151672]
7. Schiffman SS Physicochemical correlates of olfactory quality. *Science* 185, 112–117 (1974). [PubMed: 4834219]
8. Su C-Y, Menuz K. & Carlson JR Olfactory perception: receptors, cells, and circuits. *Cell* 139, 45–59 (2009). [PubMed: 19804753]
9. Zarzo M. & Stanton DT Understanding the underlying dimensions in perfumers' odor perception space as a basis for developing meaningful odor maps. *Atten Percept Psychophys* 71, 225–247 (2009). [PubMed: 19304614]
10. Tetko IV et al. Virtual computational chemistry laboratory--design and description. *J. Comput. Aided Mol. Des* 19, 453–463 (2005). [PubMed: 16231203]
11. Haddad R. et al. A metric for odorant comparison. *Nat. Methods* 5, 425–429 (2008). [PubMed: 18376403]
12. Haddad R. et al. Global features of neural activity in the olfactory system form a parallel code that predicts olfactory behavior and perception. *J. Neurosci* 30, 9017–9026 (2010). [PubMed: 20610736]
13. Ma L. et al. Distributed representation of chemical features and tunotopic organization of glomeruli in the mouse olfactory bulb. *Proc. Natl. Acad. Sci. U.S.A* 109, 5481–5486 (2012). [PubMed: 22431605]
14. Saito H, Chi Q, Zhuang H, Matsunami H. & Mainland JD Odor coding by a Mammalian receptor repertoire. *Sci Signal* 2, ra9 (2009). [PubMed: 19261596]
15. Keller A. & Vosshall LB Olfactory perception of chemically diverse molecules. *BMC Neurosci* 17, (2016).
16. Secundo L, Snitz K. & Sobel N. The perceptual logic of smell. *Curr. Opin. Neurobiol* 25, 107–115 (2014). [PubMed: 24440370]
17. Barlow HB Summation and inhibition in the frog's retina. *J Physiol* 119, 69–88 (1953). [PubMed: 13035718]
18. Mombaerts P. Axonal wiring in the mouse olfactory system. *Annu Rev Cell Dev Biol* 22, 713–37 (2006). [PubMed: 17029582]
19. Soucy ER, Albeanu DF, Fantana AL, Murthy VN & Meister M. Precision and diversity in an odor map on the olfactory bulb. *Nat Neurosci* 12, 210–20 (2009). [PubMed: 19151709]

20. Cleland TA Early transformations in odor representation. *Trends Neurosci* 33, 130–9 (2010). [PubMed: 20060600]
21. Mori K. & Sakano H. How is the olfactory map formed and interpreted in the mammalian brain? *Annu Rev Neurosci* 34, 467–99 (2011). [PubMed: 21469960]
22. Murthy VN Olfactory maps in the brain. *Annu Rev Neurosci* 34, 233–58 (2011). [PubMed: 21692659]
23. Bozza T, McGann JP, Mombaerts P. & Wachowiak M. In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* 42, 9–21 (2004). [PubMed: 15066261]
24. Uchida N, Takahashi YK, Tanifuji M. & Mori K. Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. *Nat. Neurosci* 3, 1035–1043 (2000). [PubMed: 11017177]
25. Johnson BA & Leon M. Chemotopic odorant coding in a mammalian olfactory system. *J. Comp. Neurol* 503, 1–34 (2007). [PubMed: 17480025]
26. Burton SD & Urban NN Greater excitability and firing irregularity of tufted cells underlies distinct afferent-evoked activity of olfactory bulb mitral and tufted cells. *J. Physiol. (Lond.)* 592, 2097–2118 (2014). [PubMed: 24614745]
27. Fukunaga I, Berning M, Kollo M, Schmaltz A. & Schaefer AT Two distinct channels of olfactory bulb output. *Neuron* 75, 320–329 (2012). [PubMed: 22841316]
28. Igarashi KM et al. Parallel mitral and tufted cell pathways route distinct odor information to different targets in the olfactory cortex. *J. Neurosci* 32, 7970–7985 (2012). [PubMed: 22674272]
29. Nagayama S, Takahashi YK, Yoshihara Y. & Mori K. Mitral and tufted cells differ in the decoding manner of odor maps in the rat olfactory bulb. *J. Neurophysiol* 91, 2532–2540 (2004). [PubMed: 14960563]
30. Otazu GH, Chae H, Davis MB & Albeanu DF Cortical Feedback Decorrelates Olfactory Bulb Output in Awake Mice. *Neuron* 86, 1461–1477 (2015). [PubMed: 26051422]
31. Amoore JE Stereochemical and vibrational theories of odour. *Nature* 233, 270–271 (1971). [PubMed: 4938369]
32. Schiffman SS Contributions to the physicochemical dimensions of odor: a psychophysical approach. *Ann. N. Y. Acad. Sci* 237, 164–183 (1974). [PubMed: 4529466]
33. Gschwend O. et al. Neuronal pattern separation in the olfactory bulb improves odor discrimination learning. *Nat. Neurosci* 18, 1474–1482 (2015). [PubMed: 26301325]
34. Wiechert MT, Judkewitz B, Riecke H. & Friedrich RW Mechanisms of pattern decorrelation by recurrent neuronal circuits. *Nat. Neurosci* 13, 1003–1010 (2010). [PubMed: 20581841]
35. Cleland TA & Linstner C. On-Center/Inhibitory-Surround Decorrelation via Intraglomerular Inhibition in the Olfactory Bulb Glomerular Layer. *Front Integr Neurosci* 6, 5 (2012). [PubMed: 22363271]
36. Arevian AC, Kapoor V. & Urban NN Activity-dependent gating of lateral inhibition in the mouse olfactory bulb. *Nat Neurosci* 11, 80–87 (2008). [PubMed: 18084286]
37. Yablonka A, Sobel N. & Haddad R. Odorant similarity in the mouse olfactory bulb. *Proc. Natl. Acad. Sci. U.S.A* 109, E2916–2917; E2918–2919 (2012). [PubMed: 22915580]
38. Friedman J, Hastie T. & Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw* 33, 1–22 (2010). [PubMed: 20808728]
39. Belluscio L. & Katz LC Symmetry, stereotypy, and topography of odorant representations in mouse olfactory bulbs. *J. Neurosci* 21, 2113–2122 (2001). [PubMed: 11245695]
40. Friedrich RW & Korsching SI Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron* 18, 737–752 (1997). [PubMed: 9182799]
41. Mori K, Takahashi YK, Igarashi KM & Yamaguchi M. Maps of odorant molecular features in the Mammalian olfactory bulb. *Physiol. Rev* 86, 409–433 (2006). [PubMed: 16601265]
42. Cleland TA & Sethupathy P. Non-topographical contrast enhancement in the olfactory bulb. *BMC Neurosci* 7, 7 (2006). [PubMed: 16433921]

43. Dhawale AK, Hagiwara A, Bhalla US, Murthy VN & Albeanu DF Non-redundant odor coding by sister mitral cells revealed by light addressable glomeruli in the mouse. *Nat Neurosci* 13, 1404–1412 (2010). [PubMed: 20953197]
44. Maaten L. van der & Hinton, G. Visualizing Data using t-SNE. *Journal of Machine Learning Research* 9, 2579–2605 (2008).
45. Linster C. & Cleland TA Neuromodulation of olfactory transformations. *Curr. Opin. Neurobiol* 40, 170–177 (2016). [PubMed: 27564660]
46. Fantana AL, Soucy ER & Meister M. Rat olfactory bulb mitral cells receive sparse glomerular inputs. *Neuron* 59, 802–814 (2008). [PubMed: 18786363]
47. Björck Åke & Golub, G. H. Numerical methods for computing angles between linear subspaces. *Math. Comp* 27, 579–594 (1973).
48. Zhou Y, Smith BH & Sharpee TO Hyperbolic geometry of the olfactory space. *Sci Adv* 4, eaq1458 (2018). [PubMed: 30167457]
49. Wang G, Carey AF, Carlson JR & Zwiebel LJ Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U.S.A* 107, 4418–4423 (2010). [PubMed: 20160092]
50. Zapiec B. & Mombaerts P. Multiplex assessment of the positions of odorant receptor-specific glomeruli in the mouse olfactory bulb by serial two-photon tomography. *Proc. Natl. Acad. Sci. U.S.A* 112, E5873–5882 (2015). [PubMed: 26450880]

References for Methods:

51. Adam Y. & Mizrahi A. Long-Term Imaging Reveals Dynamic Changes in the Neuronal Composition of the Glomerular Layer. *J. Neurosci* 31, 7967–7973 (2011). [PubMed: 21632918]
52. Zariwala HA, Kepecs A, Uchida N, Hirokawa J. & Mainen ZF The limits of deliberation in a perceptual decision task. *Neuron* 78, 339–351 (2013). [PubMed: 23541901]
53. Chen T-W et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* 499, 295–300 (2013). [PubMed: 23868258]
54. Gurden H, Uchida N. & Mainen ZF Sensory-evoked intrinsic optical signals in the olfactory bulb are coupled to glutamate release and uptake. *Neuron* 52, 335–345 (2006). [PubMed: 17046695]
55. Vincis R, Lagier S, Van De Ville D, Rodriguez I. & Carleton A. Sensory-Evoked Intrinsic Imaging Signals in the Olfactory Bulb Are Independent of Neurovascular Coupling. *Cell Rep* 12, 313–325 (2015). [PubMed: 26146075]
56. Buonviso N. & Chaput MA Response similarity to odors in olfactory bulb output cells presumed to be connected to the same glomerulus: electrophysiological study using simultaneous single-unit recordings. *J. Neurophysiol* 63, 447–454 (1990). [PubMed: 2329354]
57. Kikuta S, Fletcher ML, Homma R, Yamasoba T. & Nagayama S. Odorant response properties of individual neurons in an olfactory glomerular module. *Neuron* 77, 1122–1135 (2013). [PubMed: 23522047]
58. Benjamini Y. & Hochberg Y. Controlling The False Discovery Rate - A Practical And Powerful Approach To Multiple Testing. *J. Royal Statist. Soc., Series B* 57, 289–300 (1995).

Figure 1. Tuning of glomeruli and mitral/tufted cells responses to Dragon physical-chemical properties.

a. Odor responses of three example glomeruli and mitral cells. The responses (dR/R, dF/F) are shown for a panel of 49 monomolecular odors. Non-significant responses were set to zero (Methods).

b. Six example molecular property strength vectors (PSV) across same odors as in (a); HNar (Narumi harmonic topological index), nBZ (number of benzene rings), nS (number of sulfur atoms), T(O..O) (sum of topological distances between oxygen atoms), X1A (average

connectivity index of order 1), Espm15u (spectral moment of order 15 from edge adjacency matrix).

c. Property responses (PR) given by the Pearson correlation coefficients between the odor responses (**a**) of the three example glomeruli (*top*) and mitral cells (*bottom*), and the 6 example molecular property strength vectors (**b**) calculated over 49 odors in the panel.

d, g. Example correlations (property response spectra, PRS) between odor responses and molecular properties for two example glomeruli (**d**) and mitral cells (**g**).

e, h. Distribution of the glomerular response-molecular property (**e**) and M/T cell response-molecular property (**h**) pairwise Pearson's correlations. Significant correlations (**e**, $n=13,446$; **h**, $n=27,992$) are shown in black and non-significant ones in grey (false discovery rate, two-sided t-test, $p<0.05$, FDR, $q<0.1$, Methods).

f, i. Histogram of the number of molecular properties that individual glomeruli (**f**, $n=13,446$) and M/T cells (**i**, $n=27,992$) respond to, above the significance threshold (two-sided t-test, $p<0.05$, FDR, $q<0.1$, Methods), with an average of 12.7 per glomerulus and 16.4 per M/T cell.

j, k, l. Results of principal component analysis, PCA for glomerular (**j**), M cells responses (**k**) and molecular properties (**l**) ($n=871$ glomeruli, $n=639$ mitral cells, $n=1,320$ properties). Percent of variance explained is shown as a function of the number of included principal components, PCs. (**j**) Percent variance explained of glomerular (green), mitral cells (blue) odor responses, molecular property strength vectors (grey) and random data controls (black) shown as function of the number of included glomerular responses principal components. (**k,l**) Percent variance explained of glomerular (green), mitral cells (blue) odor responses, molecular property strength vectors (grey) and random data controls (black) shown as function of the number of included mitral cell responses principal components (**k**) and of molecular properties principal components (**l**).

Figure 2. Pairwise odor similarity comparison across physical-chemical and neuronal response odor representations.

a. Pairwise odor Euclidean distance across Dragon physical-chemical properties versus distance between glomerular responses, expressed as the Euclidean distance ($n=1,176$ odors pairs, $R=-0.18$, *Top*) and Pearson correlation ($n=1,176$ odors pairs, $R=-0.15$, *Bottom*).
b. Pairwise odor Euclidean distance across Dragon physical-chemical properties versus distance between M/T cell responses, expressed as the Euclidean distance ($n=1,176$ odors pairs, $R=-0.17$, *Top*) and Pearson correlation ($n=1,176$ odors pairs, $R=-0.20$, *Bottom*). Note

that average odor response Euclidean distances for glomeruli and M/T cells representations are expected to differ since they are determined by the absolute strength of intrinsic and fluorescence signals.

c. Least absolute shrinkage and selection operator (LASSO) regression based on a subset of physical-chemical properties selected from the 1,666 set, describing the relationship between odor pairwise similarity across properties versus glomerular responses; (*light green*) all imaged hemibulbs were used as a training set for optimizing the regression; (*dark green*) half of the fields of view were used as a training set and the remaining half for cross-validation (FOV cross-validation); (*pink*) all imaged hemibulbs were used for training, while one pair of odors was iteratively left out during training and added back subsequently for cross-validation (jackknife, odor cross-validation); (*red*) half of the fields of view were used for training and the remaining half for cross-validation; in addition, one pair of odors was iteratively left out during training and subsequently added back for cross-validation (jackknife, FOV and odor cross-validation).

d. Least absolute shrinkage and selection operator (LASSO) regression based on a subset of physical-chemical properties selected from the 1,666 set, describing the relationship between odor pairwise similarity across properties versus M/T cell responses; (*light green*) all FOVs of imaged M/T cells were used as a training set for optimizing the regression ($R=0.68$); (*dark green*) half of the fields of view were used as a training set and the remaining half for cross-validation (FOV cross-validation, $R=0.2$); (*pink*) all imaged FOVs of mitral and tufted cells were used for training, while one pair of odors was iteratively left out during training and added back subsequently for cross-validation (jackknife, odor cross-validation, $R=-0.06$); (*red*) half of the fields of view were used for training and the remaining half for cross-validation; in addition, one pair of odors was iteratively left out during training and subsequently added back for cross-validation (jackknife, FOV and odor cross-validation, $R=-0.08$); dotted line corresponds to an instantiation of the LASSO regression, when only 10 physical-chemical properties were allowed to take non-zero weights.

e., f. Using LASSO to describe the relationship between odor pairwise similarity across properties and glomerular (e) or mitral cell (f) responses using shuffled molecular properties; the properties strength vector associated with a given odorant is randomly swapped with the properties strength vector of another molecule in the panel without manipulating the neuronal responses.

Figure 3. Weak spatial correlations between glomerular positions and molecular property responses.

a, b. Pearson correlations between individual property responses ($n=1,320$ properties) and the placement of glomeruli along the anterior-posterior (AP) and medial-lateral (ML) axes of the bulb. Each dot represents the Pearson's correlation value of an individual molecular property. Performing PCA on the cloud of molecular property correlations computes the orientation of the first principal component (PC1, blue) with respect to the AP and ML anatomical axes. Properties shown by blue and black dots correspond to significant and non-

significant correlations with the PC1 axis (two-sided t-test, $p < 0.05$, FDR $q < 0.1$). Red dots show properties significantly correlated with PC2 axis (orthogonal on PC1, two-sided t-test, $p < 0.05$, FDR $q < 0.1$). Orientations of the PC1 and PC2 axes in 12 hemibulbs (bulb hemispheres) are shown by blue and red lines.

c, d. Pearson's correlations between individual molecular properties and glomerular positions along the PC1 and PC2 axes for each hemibulb. In each panel, properties are re-sorted with respect to the strengths of correlation. For the panel of odors used, several properties (~300) did not take non-zero values, and were not included in the analysis.

e, f. Number of hemibulbs in which a given molecular property is significantly correlated with the glomerular position along PC1 (**e**) and PC2 (**f**) (two-sided t-test, $p < 0.05$, FDR $q < 0.1$). A set of properties is consistently correlated with the PC1 axis (at most 11 out of 12 hemibulbs). Correlations along PC2 are less consistent across samples (at most 2 out of 12 hemibulbs).

g, h. Histogram of normalized displacement error vectors between the location of observed and predicted glomerular locations along PC1 (blue) and PC2 (red) axes. The predictor was obtained using a LASSO algorithm (jackknife cross-validation) to build a sparse linear regression based on 20 molecular properties (Methods). Prediction error is shown along PC1 (*Left*, STD=3.8 average glomerular size, AGS) and PC2 axes (*Right*, STD=4.7 AGS). Black traces correspond to prediction errors obtained for shuffled molecular properties control analyses; in this control, the properties strength vector associated with a given odorant is randomly swapped with the properties strength vector of another molecule in the panel without manipulating the neuronal responses. Green traces correspond to prediction errors obtained by running the regression analysis on the glomerular odor response spectra.

Figure 4. Lack of correlation between mitral/tufted cell placement and physical-chemical odor properties.

a, b. Pearson's correlation coefficients between M/T cell locations with respect to the anterior-posterior (AP, *Left*) and medial lateral (ML, *Right*) axes within fields of view, FOVs (rows) and their response sensitivity to individual properties across the physical-chemical properties analyzed. Only significant correlations are shown (two-sided t-test, $p < 0.05$, FDR, $q < 0.1$). FOVs were sorted for mitral and respectively tufted cells for both nominal dilutions (1:100 and 1:3,000).

- c.** T-distributed Stochastic Neighbor Embedding (t-SNE) projection of responses of all sampled mitral (blue) and tufted (red) cells. Each dot represents an individual M/T cell from one of the 19 FOVs imaged (1,205 mitral cells and 506 tufted cells).
- d.** Same t-SNE projection as in (c) with different putative co-glomerular clusters (n=15) shown by different colors. Tufted cell responses are represented by black circles.
- e.** Odor responses (dF/F) of tufted cells from the example FOV (#21) sorted by size of functional clusters (Methods). Horizontal bars (*Top*) mark clusters of putative co-glomerular sister cells.
- f.** Positions of tufted cells in the example FOV. Color dots mark clusters of putative sister cells. Larger colored dots correspond to the average location of putative sister cells within a given functionally defined cluster.
- g, h.** Example field of view illustrating the correlation of physical-chemical properties with placement of cells along the anterior-posterior (AP) and medial-lateral (ML) axes of the bulb before (**g**) and after (**h**) removal of putative co-glomerular sister cells via odor tuning based clustering. Each point represents an individual molecular property (n=1,320). Properties significantly correlated (two-sided t-test, $p < 0.05$, FDR $q < 0.1$) with either AP or ML axes are displayed as blue (n=160) and red (n=288) dots respectively; black dots represent non-significant correlations.

Figure 5. Comparison of glomerular and M/T cells odor response spaces.

a. (Left) Percent variance explained of glomerular (green), mitral (blue) and tufted (red) cells responses shown as function of the number of included glomerular principal components, PCs. (Right) Percent variance explained of glomerular (green), mitral (blue) and tufted (red) cells odor responses shown as function of the number of included mitral cells responses principal components.

b. Using a re-sampling model to explain the discrepancy between glomerular and mitral cell spaces. Model mitral cells responses were generated by taking samples from the glomerular

responses. (*Left*) Percent variance explained of glomerular (green), mitral (blue) and simulated model mitral (dashed blue line) cells responses shown as function of the number of included glomerular principal components. Simulated mitral cell response variance (dashed blue line) appear similar to the explained glomerular variance (green) and deviate from experimentally observed mitral cell responses (solid blue). (*Right*) Percent variance explained of glomerular (green), mitral (blue) and simulated glomerular (dashed green line) responses shown as function of the number of included mitral cells response principal components.

c. Model mitral cells responses were generated by rotating glomerular responses in odor space. Similarly, model glomerular responses were generated by rotating mitral cell responses. (*Left*) Percent variance explained of glomerular (green), mitral (blue), simulated model mitral (dashed blue line) cells and model glomerular (dashed green line) responses shown as function of the number of included glomerular principal components. (*Right*) Percent variance explained of glomerular (green), mitral (blue), simulated mitral cell (dashed blue line) and model glomerular (dashed green line) responses shown as function of the number of included model mitral cells response principal components.

d. Jordan principal angles between the glomerular and mitral cell odor response subspaces (*Left*) and random subspaces of same dimensionality (*Right*) as a function of number of principal components included in the analysis. Principal components are sorted in descending order of explained variance. To maintain contrast in the color scheme rendering the number of counts in the 2D histogram, the 0 bin angle entry in the 2D histogram was re-scaled.

e. Jordan principal angles (radians) between glomerular and mitral cell odor response subspaces (blue) sorted in descending order with respect to the amount variance explained by their corresponding principal vectors (principal components) in the two subspaces (these are the vectors between which the Jordan angles are calculated); the principal components which together account for 90% of the variance were included; Black trace corresponds to principal angles between random subspaces embedded in the same 49 dimensions; Different shades of green and gray correspond to principal angles between the glomerular subspace and a noise-added version of itself; varying amounts of known noise were added to the glomerular responses to change the signal-to-noise ratio (SNR); note that adding increasing amounts of noise resulted in higher value principal angles.