OLFACTION

Mapping odorant receptors to their glomeruli

Wang et al. used transcriptomic profiles of olfactory sensory neurons to determine the identity of their odorant receptors and map the location of their corresponding glomeruli on the olfactory bulb surface. The method enables high-throughput molecular mapping of the glomerular layout and opens up new venues to understand olfactory processing.

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To detect odorants, the brain relies on specialized sensors: the olfactory sensory neurons (OSNs) of the olfactory epithelium, each of which expresses one out of many odorant receptor (OR) genes[1] (for example, about 350 in humans and about 1,100 in the mouse). OSN axons project to the olfactory bulb and converge into discrete spherical structures (50–100 µm in diameter) onto its surface; these structures are known as glomeruli, and are organized anatomically by OR type (Fig. 1). A given odorant triggers a specific spatiotemporal pattern of glomerular activation that reflects the stimulation of the corresponding ORs[2,3]. Throughout life, and shaped by experience, neural circuits across the brain use these patterns as inputs to construct olfactory percepts: from the subtlest notes of a flavorful Chianti to the smell of your neighbor's barbeque, they can all be traced back to patterns of glomerular activation.

Although the existence of the glomerular map has long been established, its function has remained mysterious. Across individual mice, glomeruli targeted by OSNs carrying the same OR type are located at stereotypical positions on the surface of the olfactory bulb[3–5] (s.d. of about 1–3 glomerular spacings). Such exquisite precision has tempted researchers to posit that the glomerular layout bears key developmental and/or computational roles. However, to date, it remains unclear how the specific location of a given glomerulus relates to its OR identity in a systematic, lawful fashion; in fact, only a few dozen ORs have been mapped to glomeruli. In this issue of *Nature Neuroscience*, Wang et al.[6] reconstructed a map of the glomerular locations of approximately half of the mouse ORs. Their approach is a compelling example of combining high-resolution transcriptomics and machine learning to analyze neural tissue organization. Together with another study[7], this work opens new exciting venues for determining how the glomerular map comes about during development, and for understanding what information it may hold about the algorithms that the brain uses to support olfactory perception.

In agreement with recent work[8], Wang and colleagues[6] first used single-cell RNA sequencing in the mouse olfactory epithelium to show that the gene transcription program of mature OSNs varies distinctively as a function of the type of OR that they express. OSNs that clustered together on the basis of similarity in their transcriptional profiles were identified post hoc to express the same OR, even when the OR genes were withheld from the analysis. The authors further used the transcriptional program of about 1,000 genes, differentially regulated across OSNs, to derive the receptor identity of each type of OSN using a machine-learning approach. The resulting predictions found the OR identity with varying degree of accuracy, depending on the frequency of OSNs sampled in the data (from 95% for the top 10 OSN types to about 50% across a population of around 650 OSN types). Overall, the predictions scored well above the expected chance level (which is 0.15% in the latter case). Interestingly, when making incorrect predictions, the classifier tended to pick receptors of similar protein sequence to the correct OR, placed within the same genomic OR cluster.

In a clever twist, Wang et al.[6] next exploited the tight correlation between the OSN transcriptional profile and receptor type to obtain an OR map of the glomerular layout. They also capitalized on the involvement of the OR in OSN axon path-finding: the OR mRNA is present in the OSN axon terminals. To begin, they used a spatial transcriptomics technique known as Slide-seq to determine the glomerular placement of 65 ORs spread along the anterior–posterior axis of the bulb. The authors further used this information, in conjunction with the OSN transcriptional profiles obtained from the epithelium, to train a classifier to predict the glomerular location for a given type of OSN (and, consequently, a given OR). The classification was performed in an iterative manner using the transcriptional profile (excluding the OR gene) and selecting all but one of the 65 reference glomeruli for training. Wang et al.[6] predicted the glomerular locations of the ORs sampled with high precision — within two to three glomerular spacings from their actual anatomical positions. Although spatial transcriptomics can map directly[7] the OR identity of all glomeruli, constraints related to the large number of OR types make this process laborious. Instead, using the same strategy, the authors further inferred the glomerular locations for all 654 ORs whose OSN transcriptional profiles they acquired in the olfactory epithelium, and built a comprehensive OR-defined glomerular map. Importantly, for a subset of receptors, they successfully verified their predictions using transgenic mice with specific ORs fluorescently labeled as well as multiplexed error-robust in situ hybridization (MERFISH).

Previous work has indicated that the expression of numerous axon guidance and cell adhesion genes in OSNs is regulated by the OR type that they express, or in conjunction with the OR choice[9]. Consistently, axon guidance and cell adhesion genes accounted for most of the ability of the classifier to distinguish between OSN types, and to predict the bulb locations of the OR–glomeruli pairs. The study revealed diverse gradients in the expression of axon guidance genes by comparing the positions of OR–glomeruli pairs along the anterior–posterior and dorsal–ventral axes of the bulb. These include ephrins, plexins and semaphorins gradients, all known players that shape the glomerular map during development[10], as well as genes that have not previously been implicated in OSN axon guidance. Altogether, these findings highlight the power of the approach used by Wang et al.[6] as a discovery tool for understanding the

mechanisms that underlie the construction of the glomerular map. The authors focused their investigation on the relationship between the OR identity and glomerular placement primarily in adult animals. Building on their findings, future work is well poised to identify the essential gene kit and the specific combination of gene expression gradients that are instructive during development for constructing the glomerular map to its high precision. This could be achieved, for example, by combining similar gene expression analysis throughout development in both OSNs and their postsynaptic glomerular moieties. In parallel, measuring and perturbing intrinsic OSN activity as a function of OR type will inform on how activity-dependent plasticity mechanisms and genetic programs interact to specify the glomerular map.

In agreement with early pioneering work[11,12], Wang et al.[6] found that the axons of OSNs expressing ORs of similar protein sequence converge to glomeruli that are close together on the bulb surface, within local neighborhoods (about 200 µm). A different study has also suggested that specific OR sequence motifs, which bear importance for shaping ligand selectivity, correlate with the spatial positioning of OR–glomeruli pairs along the dorsal aspect of the bulb[7]. Further investigation of whether spatial pockets of glomeruli of similar ORs, defined by specific sequence motifs, exist across different regions of the bulb may provide additional clues on the development and function of the glomerular map.

As the amino acid sequence of the OR constrains its binding affinity to ligands, could sequence similarity-based placement of glomeruli form the basis of a chemotopic map? Within the chemotopic framework of olfactory processing, odorants belonging to similar chemical classes activate nearby glomeruli and, conversely, nearby glomeruli have similar ligand specificities when compared to glomeruli that are far apart. A chemotopic-like arrangement is an attractive idea toward understanding how local glomerular-activation patterns represent odorants. Indeed, previous studies have reported that glomerular responses to certain chemically defined classes of odor (for example, aldehydes, alcohols and so on) are preferentially localized to spatial domains on the bulb surface, about 1 mm or larger in size[13]. However, as the authors discuss, the validity of a chemotopic glomerular layout continues to be highly debated. Independent studies have questioned its theoretical plausibility. This is due to hard constraints in mapping continuously a high-dimensional space (that is, odor space, which is parametrized by numerous chemical functional groups and other physical–chemical properties) onto the two-dimensional olfactory bulb surface on which the glomeruli are laid out. Such a gap in dimensionality leads, at best, to fractured chemotopic maps[3,14,15]. Previous work has also provided empirical evidence against a fine chemotopical organization of the glomerular layout. In particular, glomeruli that are sensitive to a given chemically defined class of odor (for example, fatty acids or ketones) tend to be interspersed by glomeruli that respond to entirely different structures[3], as also shown by Wang et al.[6]. Further, the response similarity of two glomeruli is almost entirely independent of their proximity (although, for a contrasting result, see ref. [16]). Interestingly, the interglomerular interactions within the bulb appear to follow both local and long-range synaptic connectivity rules[17]. As such, glomerular crosstalk is not locally constrained as in other sensory circuits. Thus, the processing of olfactory inputs may not necessarily depend on a chemotopic arrangement of the glomerular map. As this study has revealed the most comprehensive glomerular map to date, it paves the way

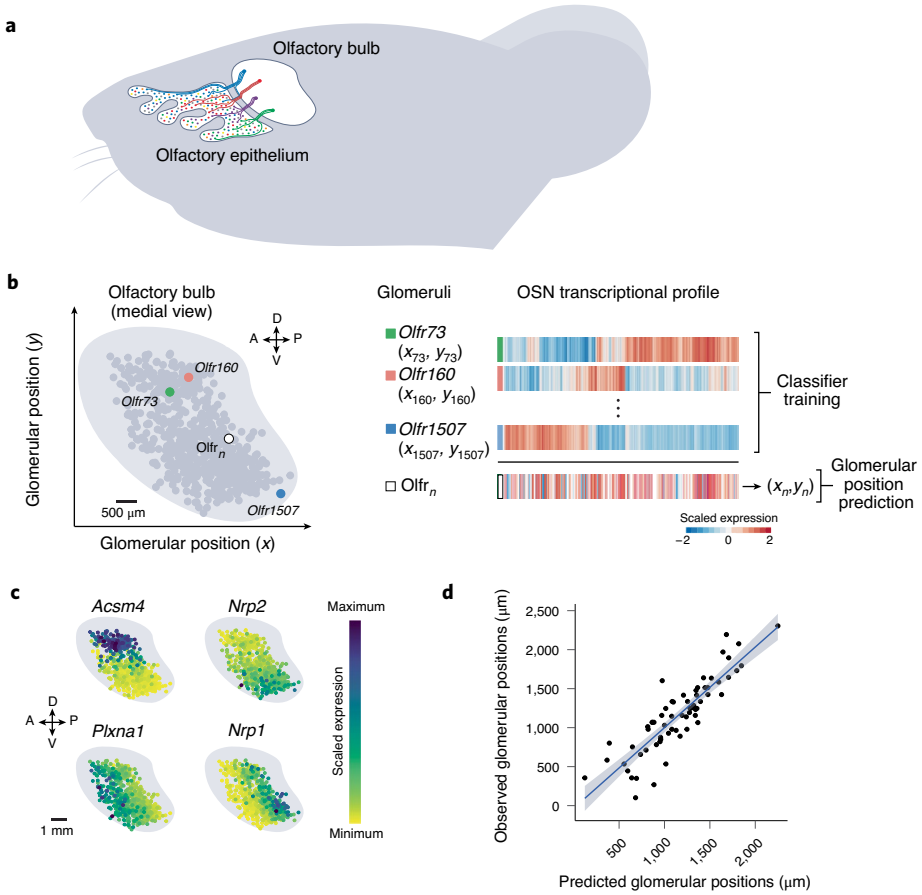

Fig. 1 | Mapping ORs to their glomeruli using OSN gene expression profiles. a, OSNs in the olfactory epithelium project their axons and form glomeruli in stereotyped locations on the olfactory bulb surface. OSNs expressing the same type of OR are shown in the same color. **b**, Each OSN type, expressing a given OR, can be described by its unique transcriptional profile. The experimentally identified glomerular positions (left) and OSN transcriptomes of an example set of ORs (for example, *Olfr73*, *Olfr160* and *Olfr1507*) (right) can be used to infer the (x_n, y_n) position of any given glomerulus on the bulb surface as function of the OSN-OR (Olfr$_n$)-type gene-transcription program. A, anterior; D, dorsal; P, posterior; V, ventral. **c**, Four example glomerular (OSN) expression gradients of genes on the bulb surface (*Acsm4*, *Nrp2*, *Plxna1* and *Nrp1*). **d**, Accuracy in predicting the glomerular positions along the anterior–posterior axis of the bulb using the gene transcription profiles of OSN types. Blue line, linear regression line; gray shadow, 95% confidence interval. Model performance is shown for a set of 65 glomeruli whose positions were experimentally determined using spatial transcriptomics while probing for a subset of the ORs. Images adapted from ref. [6], Springer Nature America, Inc.

for the systematic examination of which physical–chemical features of odorants, if any, are mapped systematically on the bulb surface.

Could a comprehensive glomerular map of ORs help to discover principles that relate the odor space to olfactory perception? Expanding Wang et al.'s approach to find the OR identity of all bulb glomeruli will enable setting up public databases[6,7] for common referencing and promote synergy in the mechanistic investigation of olfactory processing. For example, the OR-defined glomerular layout is ideal for anchoring ongoing efforts to connect the identity of ORs with mapping and predicting their ligand selectivity by monitoring OSN (glomerular) responses to large sets of odorants. Similarly, it may aid in the discovery of the brain-wide logic of downstream neural circuits that process olfactory information by tracing and cross-referencing inputs from multiple subsets of ORs. In conjunction with developments in patterned optogenetic activity manipulation[18] and high-throughput mapping of neural connectivity[19], the OR–glomerular scaffolding will help us to figure out the input–output connectivity rules and, ultimately, in our understanding of the computations that are performed by the olfactory system. Combining such strategies with behavioral analysis promises to bring us closer to finding a basis set for odor sensing, and to reveal the algorithms that the brain uses to construct olfactory percepts. ❐

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Competing interests

The authors declare no competing interests.