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REVIEW

Mosaic activity patterns and their relation to perceptual similarity: open discussions on the molecular basis and circuitry of odor recognition

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Abstract

Enormous advances have been made in the recent years in regard to the mechanisms and neural circuits by which odors are sensed and perceived. Part of this understanding has been gained from parallel studies in insects and rodents that show striking similarity in the mechanisms they use to sense, encode, and perceive odors. In this review, we provide a short introduction to the functioning of olfactory systems from transduction of odorant stimuli into electrical signals in sensory neurons to the anatomical and functional organization of the networks involved in neural representation of odors in the central nervous system. We make emphasis on the functional and anatomical architecture of the first synaptic relay of the olfactory circuit, the olfactory bulb in vertebrates and the antennal lobe in insects. We discuss how the exquisite and conserved architecture of this structure is established and how different odors are encoded in mosaic activity patterns. Finally, we discuss the validity of methods used to compare activation patterns in relation to perceptual similarity.

Keywords: antennal lobe, olfactory bulb, olfactory percept, olfactory ensheating cells, sensory map, transduction.

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Some of the open questions in the field of olfaction were addressed in the symposium entitled 'Sensation, perception, and neural representation of the environment: olfaction as case study' held at the Argentinean Society for Research in Neuroscience Annual Meeting in October 2013. The problem of recognizing odors in a natural environment, in which the stimulus is not homogeneous in time and space, questioned the view of the olfactory system as a slowly responding one. Evidence was presented showing that insects can detect asynchrony in the presentation of components of a mixture in the millisecond range, implying that the ability of the olfactory system to segregate objects from background has a time resolution faster than previously thought (Szyszka *et al.* 2012; Stierle *et al.* 2013). It was also postulated that recognition of odors in a complex natural environment would need the olfactory circuit to function as a filter that adjusts the sensitivity of the system to different odors according to experience. This filter relies on circuit plasticity in the antennal lobes (i.e., strengthening of inhibitory interactions) that contribute to the salience of novel components in a mixture of odors (Locatelli *et al.* 2013). In addition, it was shown that inhibitory interactions in the second relay of the olfactory pathway act as gain control and contribute to the transformation from a dense combinatorial olfactory code into a sparse and highly specific odor code. Studies have helped point out that sparse coding facilitates further sensory computations and associative learning (Theunissen 2003; Olshausen and Field 2004; Froese *et al.* 2014).

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Abbreviation used: CNG, cyclic nucleotide-gated.

Three other topics discussed in this symposium will be more deeply reviewed in the following sections. First, we shortly introduce basic concepts regarding the olfactory transduction mechanism and discuss new evidence suggesting that this cascade is a locus for plasticity in the olfactory system. We then review available information on mechanisms of glomerular map formation with the focus on factors extrinsic to sensory neurons. We finally discuss the extent to which the perceptual quality of the odors and odor similarity can be predicted based on glomerular activation patterns.

The basics of olfactory systems

The olfactory system allows living organisms to sense chemical stimuli present in the surrounding environment. In contrast to the objective physical characterization of sensory stimuli relevant to the visual, auditory, and somatosensory systems, the characterization of stimuli relevant to the chemical senses is still under debate. On the one hand, research oriented to the fragrance industry tends to classify odor stimuli in odor classes using subjective measures based on similarity to arbitrarily defined standard odors (e.g., minty vs. nutty). On the other hand, basic research tends to classify odor stimuli using objective measures based on the physicochemical properties of odorant molecules (e.g., presence of specific chemical groups or hydrophobicity) and on the composition of mixtures, an approach that can be used only if the components of odor stimuli are known (Auffarth 2013). The description of odor stimuli in regard to the response of olfactory receptor proteins turns more complex in light of the existence of odorant-binding proteins that are secreted into the aqueous environment present in the olfactory cavities (e.g., the mucus in the nasal cavity of vertebrates or the lymph in the olfactory sensilla of insects). These proteins may associate with odorant molecules before they reach the olfactory receptor proteins in sensory neurons. Then it is conceivable that closely related odorant molecules in terms of physicochemical properties turn into dramatically different stimuli when they are associated with odorant-binding proteins, making the description of odor space much more complex.

In order to be detected, odorant molecules need to generate a response in olfactory sensory neurons, which have a cell body in the periphery. From the cell body, a dendrite that carries the molecular machinery to sense odorant molecules protrudes into the olfactory cavity of the vertebrate or into the sensilla in the antennae of insects, and an axon projects to the central nervous system to relay the information to higher order structures. Many molecular components of the transduction machinery involved in odor sensing have been identified in vertebrates and invertebrates. Because the mouse (*Mus musculus*) and the fly (*Drosophila melanogaster*) are the best studied species, the knowledge reviewed here refers mainly to them. The transduction modality of olfactory receptor proteins differs between vertebrates and insects, being metabotropic versus ionotropic, respectively. The vertebrate olfactory transduction pathway has been nicely reviewed (Su *et al.* 2009; Malnic *et al.* 2010) and is summarized in Fig. 1. The canonical olfactory pathway described in vertebrates is activated when odorant molecules bind to G-protein-coupled receptors (odorant receptors) localized in the plasma membrane of sensory neuron cilia. Each sensory neuron expresses one odorant receptor gene out of 600–1300 available genes, depending on the species

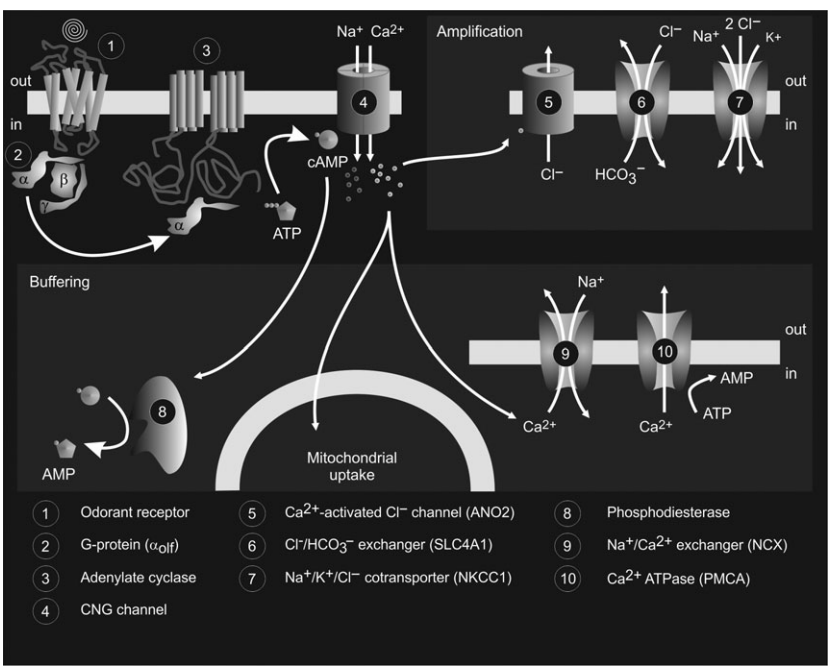

Fig. 1 Molecular components of the transduction signaling mechanism in the cilia of olfactory sensory neurons of vertebrates. Odorant molecules bind odorant receptors (1), which activate a G-protein (2) and this in turn activates adenylate cyclase (3). cAMP activates cyclic nucleotide-gated (CNG) channels (4) non-selective cation channels that produce membrane depolarization. Mechanisms that amplify the depolarization include a Ca^{2+}-activated Cl^- channel (5) and mechanisms that promote Cl^- accumulation in the intracellular space (6 and 7). Buffering mechanisms reduce the amplitude of the signal through cAMP degradation (8) and Ca^{2+} clearance. The latter mechanism involves Ca^{2+} extrusion to the extracellular space (9 and 10) and mitochondrial Ca^{2+} uptake through an unidentified path.

considered. Among the best characterized species, the nematode *Caenorhabditis elegans* represents an exception to this rule (Gaillard *et al.* 2004). Each odorant receptor may bind a subset of related odorant molecules and each odorant molecule may bind to a subset of odorant receptors with different affinities. Binding of an odorant molecule to the odorant receptor activates a G-protein (Gαolf), which in turn activates adenylate cyclase, leading to an increase in the intracellular concentration of cyclic AMP (cAMP) and activation of cyclic nucleotide-gated (CNG) ion channels. The activation of CNG channels produces an influx of Na^+ and Ca^{2+} ions in sensory neurons and thus cellular depolarization (Pifferi *et al.* 2010). A variety of mechanisms that further amplify odor-induced depolarization have been described, including a Ca^{2+}-activated Cl^- conductance that mediates the efflux of Cl^- ions and Cl^- transporters that promote the accumulation of Cl^- in the lumen of cilia (Hengl *et al.* 2010). In addition, Ca^{2+} buffering mechanisms involving membrane transporters and mitochondrial uptake regulate the gain of the transduction pathway providing a broader dynamic range for improved coding of stimulus intensity (Fluegge *et al.* 2012). Non-canonical pathways for olfactory transduction have been described in subsets of sensory neurons, involving signaling through guanylyl cyclase-D (Leinders-Zufall *et al.* 2007) or transient receptor potential channels (Lin *et al.* 2007). In contrast to vertebrates, odorant receptors in insects are dimeric, show inverted membrane topography when compared with vertebrate receptors (i.e., the N-terminus of the protein is located in the cytoplasmic side) and function as ionotropic receptors which are gated directly by chemical stimuli, leading to neuronal depolarization (Silbering and Benton 2010). Activation of a metabotropic cAMP/cGMP-dependent pathway is believed to produce more sustained and larger current responses to stimuli (Wicher *et al.* 2008).

One interesting aspect that is beginning to be addressed is the potential of the olfactory transduction cascade to display plasticity. Olfactory deprivation leads to upregulation of elements of the transduction cascade, suggesting that sensory neuron responses to odorants can display plasticity at the cellular level (Coppola and Waggener 2012). Recent findings show that repeated exposure to an odorant can cause responsive sensory neurons to develop an increased sensitivity and faster kinetics, associated with parallel increments in the expression of CNG channels and phosphodiesterase (Fig. 1) (Cadiou *et al.* 2014). These observations are especially interesting in light of another recent discovery showing that the same sensory neuron can respond to structurally similar but different odorants with Ca^{2+} transients mediated by divergent signaling cascades, involving either adenylate cyclase or phospholipase C, or both (Yu *et al.* 2014), enormously expanding the available elements potentially involved in plasticity of sensory neuron responses. Further research using comparable techniques will clarify the differences between plasticity elicited by deprivation and by odor enrichment.

The central projections of sensory neurons expressing the same odorant receptor converge in one or a few discrete and specific areas – or glomeruli – in the first synaptic relay of the olfactory pathway (i.e., the olfactory bulb in the mouse and the antennal lobe in the fly), allowing for a topographic representation of odor stimuli (Fig. 2). Interestingly, specific

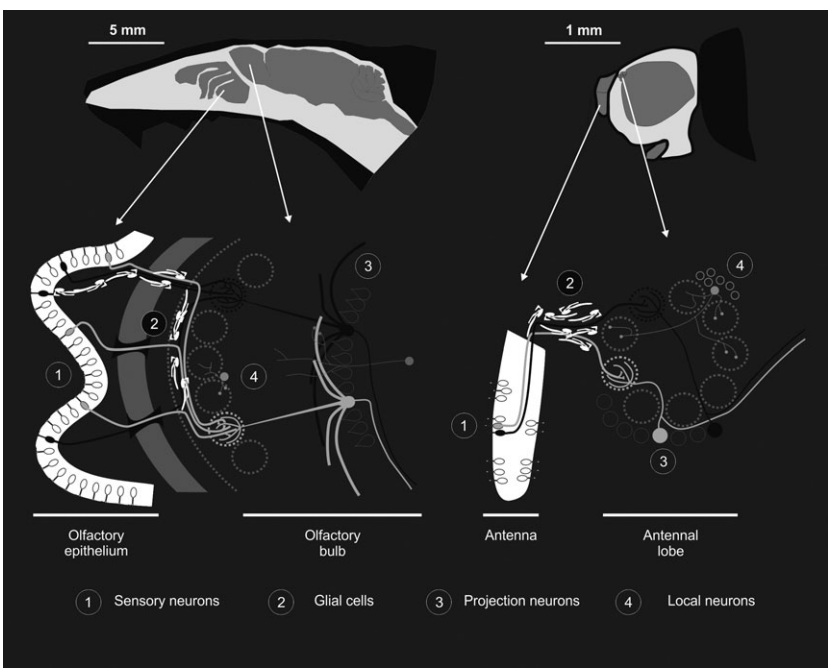

Fig. 2 Schematic representation of the olfactory circuitry at the level of the first olfactory relay for the mouse (left) and the fly (right). Olfactory neurons (1) expressing the same odorant receptor are shown in the same color (black or light gray), as well as corresponding projection neurons onto which they converge and synapse (3). Olfactory sensory neuron axons interact with glial cells along the olfactory nerve (2). Local neurons of the first relay are shown in gray and with dashed lines (4).

glomerular positions are reproducible from animal to animal, however, local permutations of glomeruli are observed if the map is analyzed at a sufficiently small spatial resolution (Strotmann *et al.* 2000). Given that even pure odorants can activate more than one odorant receptor and that each odorant receptor can be activated by more than one odorant, the stimulus identity is encoded by the combination of activated glomeruli. Interestingly, chemically related odorants show overlapping patterns of glomerular activation. Glomerular organization of olfactory circuits at the level of the first relay seems likely to have arisen as a case of convergent evolution, suggesting that this organization constitutes a successful solution for odor coding (Strausfeld and Hildebrand 1999). In addition to the spatial pattern, the temporal sequence of activation of different glomeruli also contributes to stimulus discrimination (Lei *et al.* 2004). The relevance of a temporal component in the olfactory code becomes evident when the olfactory task is challenged by the use of similar odors that elicit partially overlapping patterns. In that circumstance olfactory accuracy depends on odor sampling time (Rinberg *et al.* 2006). Interestingly, the sampling time for full expression of an activation pattern coincides with the time it takes the animals to express an olfaction based behavioral decision (Fernandez *et al.* 2009). Furthermore, blocking the temporal pattern by interfering with the local inhibitory network affects discrimination of similar but not different odors (Stopfer *et al.* 1997).

Glomerular map formation

Odorant receptors and other components of the signal transduction machinery that are present in cilia of sensory neurons are also present in axon terminals and participate in the establishment of the topographic organization of glomeruli (Ressler *et al.* 1994; Vassar *et al.* 1994; Imai *et al.* 2006; Maritan *et al.* 2009). Known mechanisms underlying glomerular convergence have been nicely reviewed elsewhere (Takeuchi and Sakano 2014) and involve differential expression of axon guidance molecules by sensory neurons in an odorant receptor-dependent fashion. Two types of guidance molecules have been identified. Type I molecules are involved in the formation of a coarse map in the anteroposterior axis and their expression is likely regulated by cAMP generated by basal odorant receptor activity. Basal activity is independent of ligand binding and different odorant receptors are associated with different levels of basal cAMP production, which in turn regulates the level of type I molecule expression (Nakashima *et al.* 2013). A paradigmatic functional pair of type I molecules are Neuropilin 1 and Semaphorin 3A, a membrane receptor and a secreted chemorepulsive ligand for this receptor, respectively (He and Tessier-Lavigne 1997). Type II molecules are involved in local glomerular segregation and their expression is regulated by odor-induced cAMP signals (Serizawa *et al.* 2006). Examples of type II molecules are Kirrel2 and Kirrel3, membrane molecules that mediate homophilic adhesion (Schneider *et al.* 1995; Shen and Bargmann 2003). The differential regulation of type I and type II guidance molecules – that is, odor-independent versus odor-dependent – likely involves signaling through different G-proteins – Gs versus Golf- and downstream elements (Nakashima *et al.* 2013). In addition, glomerular position in the dorsoventral axis involves the participation of repulsive interactions mediated by Slit1/Robo2 and Semaphorin-3F/Neuropilin-2 – two other pairs of secreted chemotopic molecules and their membrane receptors (Cloutier *et al.* 2002, 2004; Walz *et al.* 2002; Cho *et al.* 2007). Two strategies have been used to identify mechanisms underlying map formation. The manipulation of molecular candidates exclusively in subpopulations of sensory neurons is useful to identify mechanisms that are intrinsic to these cells. Global genetic manipulations of specific candidate molecules also have been successful for the identification of mechanisms involved in map formation (Hasegawa *et al.* 2008). This second strategy in combination with the analysis of expression patterns of the candidate molecules being manipulated suggests that some mechanisms may be expressed by cells other than sensory neurons. Two evident loci emerge as candidates: (i) post-synaptic targets of sensory neurons and (ii) glial cells associated to sensory neurons. The literature available is highly biased to the report of mechanisms intrinsic to sensory neurons, whereas selective manipulations of candidate molecules in other cell types like the glia associated with sensory neurons or post-synaptic targets are much scarcer. Recent evidence showed that in the fly the extracellular signaling molecule Hedgehog, likely expressed by synaptic targets of sensory neurons, participates in sensory axon targeting, as sensory neurons show mistargeting when navigating an environment where Hedgehog was knocked down (Chou *et al.* 2010). A role for sensory neuron-associated glial cells in map formation has been postulated for many years. Evidence in support for that role comes from the description of a glia-rich sorting zone for sensory axons at the entrance of the antennal lobe in the moth Manduca sexta. Olfactory sensory neuron axons fail to fasciculate in the sorting zone of glia-deficient moths (Rössler *et al.* 1999). More recent evidence shows that Robo1 is expressed by the specialized ensheathing glial cells that surround olfactory sensory axons from the olfactory epithelium to the central nervous systems in vertebrates (Nguyen-Ba-Charvet *et al.* 2008; Aoki *et al.* 2013) and cooperates with Robo2 expressed by sensory neurons to achieve precise targeting of sensory axons. The lack of selective drivers for the glial cells associated with olfactory sensory neurons prevented the field from moving forward to test candidate molecules expressed by these olfactory ensheathing glia in vertebrates. Recent efforts to determine the marker expression profile of olfactory ensheathing glia

may help find such drivers (Vincent *et al.* 2005). Additional efforts to understand physiological properties of ensheathing glia *in situ* also will help to identify candidate molecules that may influence neuron–glia interactions involved in olfactory map formation (Rieger *et al.* 2007; Rela *et al.* 2010; Thyssen *et al.* 2013). The identification of tools for selective manipulation of candidate guidance molecules in ensheathing glia should be stimulated by very recent findings showing that selective loss of Semephorin-3A production in astrocytes results in abnormal targeting of sensory axons in the spinal cord (Molofsky *et al.* 2014). It is important to note that Semaphorin-3A deficiency exclusively in olfactory sensory neurons is associated with shifts in glomerular targeting (Imai *et al.* 2009), however, that result does not exclude the participation of glia-derived Semaphorin-3A in map formation.

Activation patterns and percepts

One of the major challenges in olfaction sciences is to understand the mechanisms by which the detection of an odorant by the olfactory sensory neurons is translated into an odor percept in the central nervous system (Gottfried 2010). In non-verbal experimental animals amenable to neural recordings, odor perception can be recognized through odor-elicited behaviors. This allows the researcher to measure the neural representation of the odor across different layers of the olfactory circuit in relation to the behavioral output, with the goal of understanding when and how the neural activity pattern elicited by the odor contributes to a specific percept. For this aim, odor-elicited activity patterns are studied in three successive domains of the olfactory pathway: (i) the olfactory sensory neurons, (ii) the second-order neurons (projection neurons in insects, mitral/tufted cell in vertebrates), and (iii) the third-order neurons (Kenyon Cells of insects and pyramidal cortical neurons of vertebrates) (Su *et al.* 2009). As mentioned before, the first two domains are notable examples of a population coding scheme in which odors are represented by a dense combinatorial code distributed across several neurons. In contrast, odor representation in the third domain uses a sparse coding scheme which means that a small and highly specific subset of neurons participates in the pattern elicited by each particular odor (Jortner 2012).

The complexity and the coding power of the multidimensional population code used in early stages of the olfactory circuit raises the question of whether perceptual similarity between two odors can be predicted from the representation of the odor in the first two layers of the olfactory circuitry. As a general rule, odors encoded by very similar and highly overlapping patterns show behavioral generalization, which is taken as indicative that both odors have similar perceptual qualities and thus the animal does not discriminate among them. On the other hand, when two odors are encoded by different and non-overlapping patterns, animals can easily discriminate among these two odors. The prediction in regard to discrimination or generalization becomes more difficult when odor patterns have a partial degree of overlap. Recent studies in Drosophila have nicely shown that the probability that two odors have the same perceptual quality can be quantitatively predicted based on the similarity of the respective activity patterns across olfactory sensory neurons (Kreher *et al.* 2008). Moreover, the ability to gradually alter the pattern of an odor by turning off specific sensory neurons led to demonstration of a direct relationship between the degree of change in the pattern and the degree of change in the perceptual quality of the odor (Parnas *et al.* 2013). Interestingly, studies in which perceptual quality of the odors could be studied in relation to patterns in the first- and the second-order olfactory neurons, have shown that perceptual quality can be more accurately predicted based on the readout of the activity patterns across the second-order neurons, consistent with the fact that these neurons are located one step forward in the olfactory processing and thus closer to behavior than sensory neurons. Odors that have distinct representations at the sensory neuron level, but appear similarly represented in the second-order neurons are generalized in a behavioral test, consistent with a categorization role of the olfactory processing that takes place from the first- to the second-order olfactory neurons (Niewalda *et al.* 2011). Studies in moths have shown that behavioral discrimination between biologically relevant odor blends and the odor components is reached in second-order neurons after non-linear processing of the input into the antennal lobe (Kuebler *et al.* 2012). In honey bees, two related mixtures of odors that show considerable overlap in the antennal lobe and are generalized during the first trials of differential conditioning can be discriminated after a strong conditioning protocol. Interestingly, the representations of these mixtures across projection neurons are more different in trained animals than in naïve animals (Fernandez *et al.* 2009). This result not only provides evidence that the ability to discriminate between two odors is related to the differences in their representation in the antennal lobe, but also that this difference is modulated by experience (Sandoz *et al.* 2003; Yu *et al.* 2004; Rath *et al.* 2011).

The choice of a metric to compare and determine similarity between activity patterns is not easy. The most used comparison technique to obtain a quantitative determination of similarity between activation patterns encoded in multidimensional spaces is based on Euclidean distances. However, it is worthwhile to point out that the use of Euclidean distance or any other related mathematical reduction applied to the analysis of patterns has to be cautiously revised because it may oversimplify the complexity of the combinatorial code and may distort the perceptual relation between two odors. A number of cases that expose this problem are enumerated.

A substantial number of examples shows that animals are able to generalize among different intensities of the same stimulus. In regard to odors, such ability is relevant when an animal is navigating in an odor plume that may guide the search of a mate or a food source (Vickers *et al.* 2001). In such a case, it is to be expected that animals are able both to recognize the odor and also be sensitive to odor intensity. In the context of olfactory conditioning, generalization across odor intensities has been mainly observed when animals are trained with low odor concentration and tested with high odor concentration (Bhagavan and Smith 1997; Pelz *et al.* 1997). However, on the basis of Euclidean distance this generalization cannot be predicted. High odor concentration provides more intense and complex activation patterns than low odor concentration (Sachse and Galizia 2003) and the Euclidean distance between patterns elicited by both concentrations would provide a distance consistent with qualitatively distinct odors. In this case, it might seem that either correlation coefficient or angular distance between patterns might be better predictors of similarity. However, generalization between odor intensities is not observed from high to low intensities (Bhagavan and Smith 1997). Thus, predictions about generalization or discrimination of different odor concentrations solely based on Euclidean distance or correlation coefficient between patterns might yield ambiguous conclusions.

Both Euclidean distance and correlation coefficient between two complex coding patterns reduce the comparison of the patterns to a single value. Any of these values establish a reciprocal relationship: odor A is similar to odor B, as much as odor B is similar to odor A. If Euclidean distance reflects a degree of perceptual similarity, then generalization between A and B should too be symmetrical. However, there are examples of pairs of odors that show asymmetrical generalization. That is, animals trained to odor A show a given degree of generalization to B, that is different to the degree of generalization to odor A when the animals have been trained to odor B (Guerrieri *et al.* 2005). The previous example about generalization or discrimination between low and high odor concentrations might be considered a special case of asymmetrical generalization. Again, this inconsistency shows that the generalization between the odors cannot be predicted by solely by analysis of Euclidean distance or correlation coefficient.

In a previous paragraph, we mentioned studies in which discrimination learning increases the separation between activation patterns of odors associated with different outcomes (Fernandez *et al.* 2009; Rath *et al.* 2011). There are, however, other reports in which discrimination learning was not associated with changes in the representation of odors at the level of sensory neurons or second-order neurons (Peele *et al.* 2006; Barth *et al.* 2014). These examples constitute cases in which the degree of discriminability between two odors cannot be predicted based on the separation of their representations.

When Euclidean distances or correlation coefficients between two odors are calculated, all glomeruli or neurons participating in the combinatorial patterns receive the same relative weight. In other words, each glomerulus or neuron is accounted as an orthogonal dimension that contributes to the identity and perceptual quality of the odor as much as any other glomerulus or neuron. This concept is notoriously wrong, however. It is known that some neurons are very narrowly tuned, some others are broadly tuned and yet others are in between (Hallem and Carlson 2006). Thus, it is reasonable to consider that neurons with different degrees of specificity must have a very different impact in the perceptual quality of a pattern. A similar consideration is valid for glomeruli or neurons which are dedicated to detection of specific odorants and thus are hardwired to a particular behavior – the so called 'labeled lines' (Sachse *et al.* 2007; Semmelhack and Wang 2009; Stensmyr *et al.* 2012). The activation of a labeled line in a given pattern is expected to notably modify the perceptual quality of the odors although the pattern has changed in only a few or just one component.

As conclusion, any metric based on algorithms that equalize all elements coding the input and that ignores the fine and certainly heterogeneous architecture of the structure that builds the read-out of the olfactory population coding, will provide only a limited approximation to the perceptual quality of an odor. Experiments using stimulation of single elements of the population code with high spatial and temporal resolution (Smear *et al.* 2013) in combination with a more profound knowledge of the connectivity with the post-synaptic elements are necessary to make better interpretations of activation patterns encoding olfactory stimuli.

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