BioSystems 164 (2018) 94–101

Contents lists available at ScienceDirect

BioSystems

journal homepage: www.elsevier.com/locate/biosystems

Review article

Fundamental principles of the olfactory code

Veit Grabe, Silke Sachse*

Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Hans-Knoell-Str. 8, 07745 Jena, Germany

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form 13 October 2017
Accepted 16 October 2017
Available online 17 October 2017

Keywords:
Olfaction
Odorants
Olfactory receptors
Gene family
Combinatorial coding
Glomeruli
Olfactory bulb
Antennal lobe

ABSTRACT

Sensory coding represents a basic principle of all phyla in nature: species attempt to perceive their natural surroundings and to make sense of them. Ultimately, sensory coding is the only way to allow a species to make the kinds of crucial decisions that lead to a behavioral response. In this manner, animals are able to detect numerous parameters, ranging from temperature and humidity to light and sound to volatile or non-volatile chemicals. Most of these environmental cues represent a clearly defined stimulus array that can be described along a single physical parameter, such as wavelength or frequency; odorants, in contrast, cannot. The odor space encompasses an enormous and nearly infinite number of diverse stimuli that cannot be classified according to their positions along a single dimension. Hence, the olfactory system has to encode and translate the vast odor array into an accurate neural map in the brain. In this review, we will outline the relevant steps of the olfactory code and describe its progress along the olfactory pathway, i.e., from the peripheral olfactory organs to the first olfactory center in the brain and then to the higher processing areas where the odor perception takes place, enabling an organism to make odor-guided decisions. We will focus mainly on studies from the vinegar fly *Drosophila melanogaster*, but we will also indicate similarities to and differences from the olfactory system of other invertebrate species as well as of the vertebrate world.

Contents

1. Defining the odor space

Almost all organisms respond to odors in their external environment. The ability to perceive odors is present in simple, single-celled organisms, such as bacteria and protista, as well as in complex metazoans, such as mammals, arthropods and molluscs. Chemoreception provides a large amount of information that is often very specific about food, danger and conspecifics interested in mating or defending their territory. Hence, most organisms rely on their olfactory system to guarantee survival and reproduction. The olfactory system handles an enormous and nearly infinite number of diverse stimuli. Every plant, every animal and even most inanimate objects have a smell. Seemingly odorless things, such as water or stones, represent exceptions. Many of these scents are meaningless or incidental, and may be perceived as pungent, disgusting, pleasant or neutral. Other fragrances, such those of flowers, are hardly of evolutionary importance for humans, but are essential for insects and for the flowers themselves.

The odor space consists of small, volatile, mainly fat-soluble, organic compounds with molecular weights between 26 and 300 Da (Fig. 1) (Mori and Yoshihara, 1995). The number of different odorant molecules that can be discriminated by the human olfactory system has been estimated at 400,000 (Mori and Yoshihara, 1995). A more recent study claims that humans can discriminate even more than one trillion odors (Bushdid et al., 2014). However, this assumption is strongly debated (Gerkin and Castro, 2015; Meister, 2015), and the actual number of olfactory stim-

* Corresponding author.
E-mail address: ssachse@ice.mpg.de (S. Sachse).

https://doi.org/10.1016/j.biosystems.2017.10.010

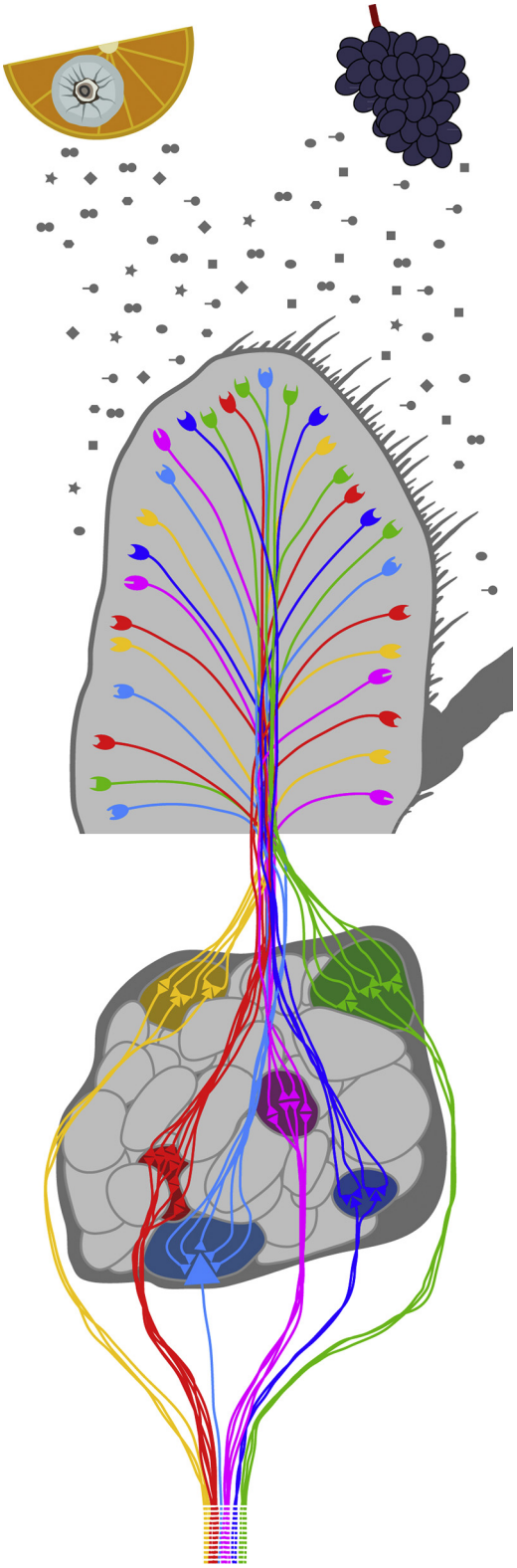

Fig. 1. First steps in olfactory coding.
Different odor sources, such as a moldy orange or grapes, emit diverse odor molecules into the air. These odor molecules interact in a combinatorial manner with the olfactory receptors (ORs) expressed in peripheral olfactory organs (here: an insect antenna). The binding of an odor molecule onto an olfactory receptor excites the corresponding olfactory sensory neuron. The neuronal excitation is then relayed to the first olfactory center, the insect antennal lobe or the vertebrate olfactory bulb, where the axons of the sensory neurons terminate. Notably, all sensory neurons that express the same OR converge onto the same olfactory glomerulus – the structural and functional units of the first olfactory brain center. This 1:1 convergence results in an odotopic map of the processing center.

uli that humans and also any other animal can discriminate is still unknown. Why is it so difficult to estimate the dimensionality of the odor space? All other sensory systems decode a clearly defined stimulus array consisting of, e.g., tones that vary in frequency and loudness, or color stimuli differing in wavelength and intensity. The odor space, however, comprises a virtually endless stimulus array (Ruddigkeit et al., 2012) that cannot be classified according to the positions of odorants along a single dimension that is a single physical parameter, preventing any easy characterization and definition of the features and boundaries of the space. For decades, scientists have attempted to categorize odors and to establish specific odor classes, but no one has come up with a comprehensive, widely accepted olfactory classification system (Kaeppler and Mueller, 2013). In order to understand how the odor space is encoded by the brain, one must know the range, resolution and limitations of the olfactory system. To obtain these parameters, researchers have carried out many psychophysical and neurophysiological experiments. These studies have helped to elucidate the fundamental principles underlying how the odor space is encoded and translated into neuronal activity and to create an internal neuronal representation in the form of an accurate neural map in the brain.

Certain odorants can evoke a singular percept. Notably, slight alterations in the molecular structure can lead to substantial shifts in perceived odor quality (Laska and Teubner, 1999a,1999b). In addition, most odorants occur in the natural environment as only one of many odorants within large, complex blends; these blends increase the number of possible smells by orders of magnitude. For instance, a jasmine flower emits dozens of odorant molecules with various molecular structures (Mori and Yoshihara, 1995). The remarkable capacity of the olfactory system to recognize and discriminate this vast array of odors even at extremely low odor concentrations is comparable only to the immune system, which discriminates innumerable epitopes on antigen molecules or diverse peptides presented by MHC molecules. How is the olfactory system able to perceive this diverse array of stimuli?

2. Decoding odors with peripheral olfactory organs

To cope with the vast odor space, chemosensation developed into a complex system to perceive, identify, evaluate and transmit the gathered information from its environment. How this system was capable of resolving a nearly unlimited variety of odorants was, for a long time, unknown. Two major working hypotheses crystalized: Either a large number of olfactory receptors (ORs) each interacting with only a few odor ligands or a small number of ORs capable of interacting and responding to numerous odorants in diverse combinatorial manners could explain the phenomenon. The key to unraveling this enigma was the identification in 1991 of a novel multigene family that encodes ORs in rats (Buck and Axel, 1991) for which Linda Buck and Richard Axel were awarded the Nobel prize in physiology or medicine in 2004. This groundbreaking discovery led to an explosion in the number of analyses of the respective ORs along all branches of the tree of life. *Drosophila melanogaster,* today's major player in olfactory physiology research, was described, with 60 ORs (Clyne et al., 1999; Vosshall et al., 1999). Later on, ORs of numerous other organisms were identified. Among these were the honey bee, with 170 ORs (Robertson and Wanner, 2006), *Caenorhabditis elegans,* with 1300 ORs (Robertson and Thomas, 2006), humans, with 400 intact ORs (Niimura, 2012), mice, with over 1000 ORs (Niimura, 2012), and zebra fish, with 160 ORs (Niimura, 2012). However, how exactly this large gene family of ORs is capable of coding for the enormous number of odorants an organism might encounter in nature remained a question. Of course, only the combinatorial coding of the available receptors

could provide a proper explanation. At first, 30 receptors would have been sufficient to code for over 1 billion odorants even according to conservative models. However, a single type of OR has been found that is capable of eliciting a distinct response when activated without requiring any combinatorial coding. Research has propelled the vinegar fly, *Drosophila melanogaster*, and the house mouse, *mus musculus*, onto center stage, where they have become major actors in the effort to decipher the olfactory code and its olfactory pathways.

Flies and mice encounter an extremely complex environment, as reflected by the intricate structure of their olfactory system, which throughout the whole animal kingdom displays a strong bent toward conservation (Ache and Young, 2005; Eisthen and Nishikawa, 2002). Both vertebrates and invertebrates possess olfactory sensory neurons (OSNs) on their nose or antenna (Kaupp, 2010), where they express the ORs necessary for odorant detection (Fig. 1). In vertebrates, the olfactory epithelium is the area in which OSNs with their respective ORs come in contact with atmospheric volatiles; homologous tissues in insects and crustaceans are represented by the antenna or the antennule, respectively. These complex structures enable to translate the environmental odor information into neuronal activity, i.e. into a neural code that is further transferred to the brain.

Several principles of olfactory coding at various scales allow a strategically sorting of the odor information already before a single impulse of neuronal activity reaches the brain. In insects, an initial level of coding is represented by the different types of sensilla located in the various olfactory organs that contain OSNs responding to either pheromones (trichoid sensilla) (Kurtovic et al., 2007), food odors (basiconic sensilla) (Hallem and Carlson, 2006), acids (coeloconic sensilla) (Ai et al., 2010), oviposition cues (intermediate sensilla) (Dweck et al., 2013) or toxic odors (Stensmyr et al., 2012; Suh et al., 2004). In contrast, vertebrates do not have this separation; instead of various sensilla types, the epithelium of vertebrates is organized zonally (Ressler et al., 1993). A **second** level of coding found in insects lies in the pairing of OSN types within a single sensillum: neurons share the same space and have been described as influencing their partners' responsiveness at the dendritic level (Andersson et al., 2010; Su et al., 2012). This interaction of OSNs within a sensillum allows the insect a more precise orientation along a gradient as it increases the contrast between different odors. In insects, each OSN contains a specific protein receptor type in the membrane of the outer dendrite, together with a co-receptor that belongs either to the OR clade (co-receptor ORCo) (Larsson et al., 2004), or to the ionotropic glutamate receptor (IR) clade (co-receptors IR8a, IR25a and IR76b) (Benton et al., 2009).

The **third** level of coding allows the invertebrate system to discriminate between major groups of volatile odorants, with IRs mainly responding to ketones, acids and amines (Silbering et al., 2011) – similar to 'trace amine-associated receptors' (TAARs) in vertebrates (Liberles and Buck, 2006) – whereas ORs are activated mostly by acetates, aldehydes and aromatics (Hallem and Carlson, 2006). OSNs are accompanied by a set of supporting cells (thecogen, tricogen and tormogen in insects, and sustentacular and microvillar in vertebrates) which secrete various odorant binding proteins (OBPs) (Vogt et al., 1991) and odor-degrading enzymes (ODEs) (Younus et al., 2014) into the sensillum lymph or the mucus layer of the olfactory epithelium. OBP and ODE functions are not completely understood. Because OBPs are present in high concentrations in insect antennae and in vertebrate mucus (Pelosi, 1998), they contribute heavily to the sensitivity and temporal resolution (Leal, 2013). High neuronal speed and recovery rate, in turn, are critical, first for the sensitization processes of neurons that may occur through repetitive, subthreshold stimulation (Getahun et al., 2013), and then for affecting the habituation that can occur following repeated suprathreshold stimulations (Engel and Wu, 2009). Habituation keeps the OSN within its dynamic range and therefore prevents it from being saturated. Together, these parameters form a **fourth** level of coding, as each class of OSNs displays a diverse level of sensitivity to its key ligand (Pelz et al., 2006).

In addition, morphological segregation takes place because sensilla, which are not evenly distributed across the peripheral organs in all types of insects, show a type- and class-dependent clustering (de Bruyne et al., 1999; Grabe et al., 2016), forming a **fifth** level. In some species, this clustering leads to a potential prioritization of sensilla which are located near the tip. These mostly pheromone-sensitive sensilla encounter the odors earlier than the ones at the antennal base. In vertebrates, it is unclear if such a clustering exists.

Most of the previously mentioned parameters of peripheral olfactory coding are stable, but their behavioral implications can vary based on the internal state of the animal. It is known that circadian rhythms in moths reflect their antennal octopamine levels, and that these levels alter the moth's olfactory sensitivity in accordance with its crepuscular activity (Schendzielorz et al., 2015). Other studies hint at strongly aversive ligands, such as CO_2, in flies; these ligands can evoke an opposite behavioral response, depending on whether the fly is walking or flying (Wasserman et al., 2013).

Beyond the primary olfactory organs, which house the majority of OSNs in insects and vertebrates, a **sixth** level of coding is shaped through particular organs along the body of the insect. Those organs could be sensory hairs that code chemosensory input for precise ethological functions such as the evaluation of food through the labellum or other mouthparts in *Drosophila melanogaster* or in *Triboleum castaneaum* (Dippel et al., 2016; Fujii et al., 2015), or oviposition site search by the ovipositor in parasitic wasps (Yadav and Borges, 2017). An additional invagination of the antenna, called sacculus, segregates humidity sensing (Enjin et al., 2016). Temperature sensing is located on the arista, a branched hair-like flagellum on the antenna (Gallio et al., 2011).

In vertebrates, the vomeronasal organ is an accessory olfactory system which projects onto the accessory olfactory bulb and further onto the amygdala and bed nucleus of the stria terminalis and from there onto the hypothalamus (Brennan and Zufall, 2006). In rodents, the 'Grueneberg ganglion' detects low temperatures, alarm pheromones and kairomones leading to fear behavior (Bumbalo et al., 2017; Fuss et al., 2005).

3. The primary olfactory code

Tracing the path of OSNs on their way from the nose or the antenna to the central nervous system, the first neuropil encountered is the antennal lobe (AL) in insects, the olfactory lobe in crustaceans or the olfactory bulb (OB) in vertebrates (Figs. 1 2, **A**) (Ache and Young, 2005; Homberg et al., 1989). In most animals, these represent the primary olfactory centers where OSNs converge, targeting discrete spherical subunits of this neuropil – the olfactory glomeruli. Glomeruli vary strongly in number throughout various animal species, roughly corresponding to the number of ORs present in the species, namely, 52 glomeruli in vinegar flies, 140 in zebra fish, 165 in honeybees, 400 in ants, 1300 in giant robber crab, 3000 in migratory locusts, 1800 in mice and 5500 in humans (Braubach et al., 2012; Galizia et al., 1999a; Grabe et al., 2016; Krieger et al., 2010; Maresh et al., 2008; Schachtner et al., 2005; Zube et al., 2008). Underlying the fundamental principle of glomerular organization is the so-called odotopic map (Fig. 2**B**), on which each glomerulus receives input solely from the precise group of OSNs that express the very same OR, an OSN-type-to-glomerulus arrangement that is true for every glomerulus throughout vertebrate as well as invertebrate brains (Gao et al., 2000; Mombaerts et al., 1996; Vosshall et al., 2000). Each glomerulus codes the identity of a certain array of odorants provided by the receptive range

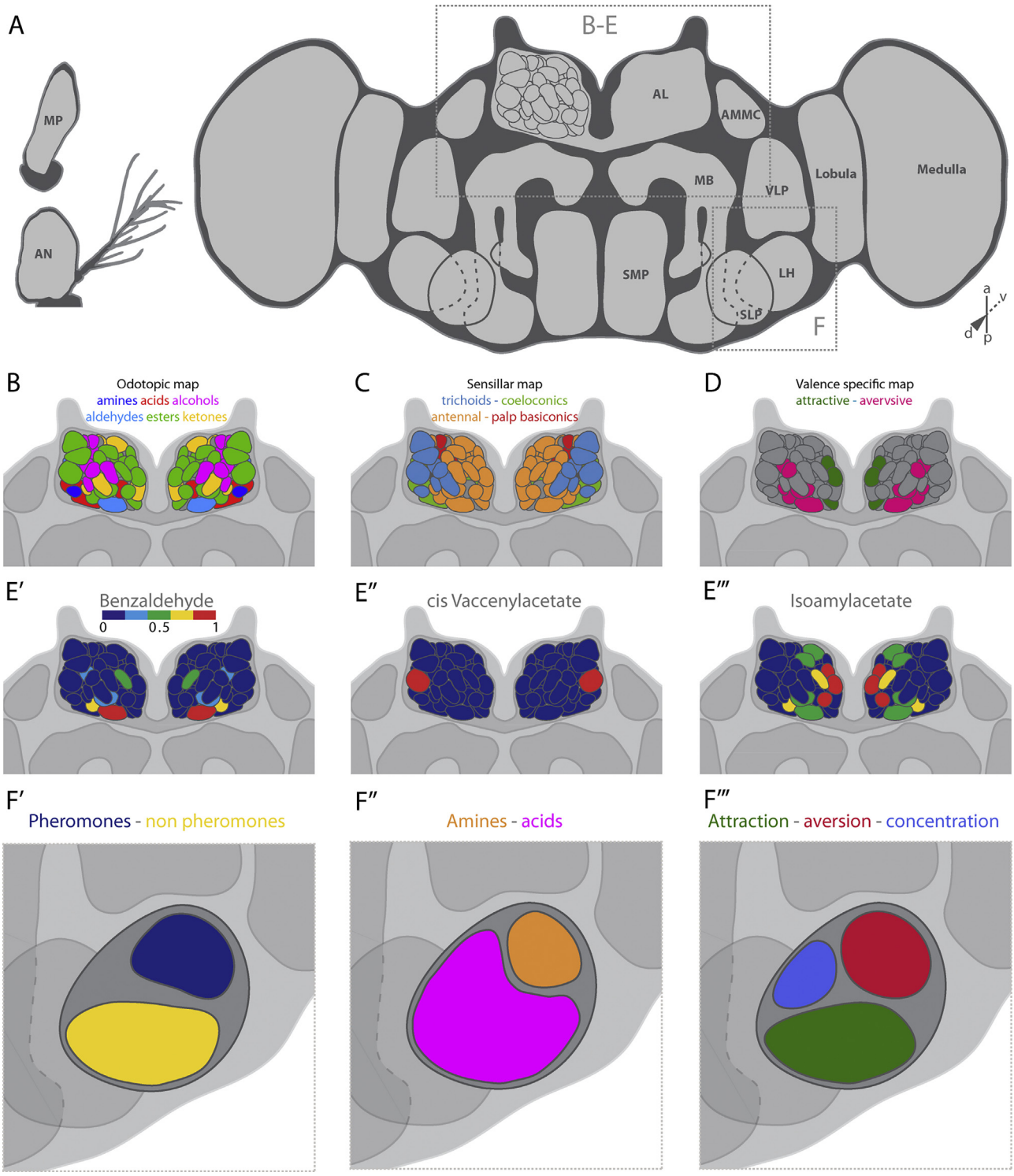

Fig. 2. Central olfactory coding.
A) Overview of the two peripheral olfactory organs of *Drosophila melanogaster*, the antenna (AN) and the maxillary palp (MP), and the neuropils in the central nervous system (AL – antennal lobe, AMMC – antennal mechanosensory and motor center, LH – lateral horn, MB – mushroom bodies, SLP – superior lateral protocerebrum, SMP – superior medial protocerebrum, VLP – ventrolateral protocerebrum); B) color-coded map of AL glomeruli whose OSNs' best-known ligand is an amine (dark blue), acid (red), alcohol (magenta), aldehyde (light blue), ester (green), or ketone (yellow); C) color-coded map of AL glomeruli receiving input from olfactory sensory neurons that originate from trichoid (blue), coeloconic (green), antennal basiconic (orange) or palp basiconic sensilla (red); D) color-coded map of glomeruli coding for attractive (green) and aversive odors (magenta) in the AL of *Drosophila*; E) false color-coded combinatorial activity pattern in the *Drosophila* AL in response to an odor puff of benzaldehyde (E'), the sex pheromone *cis*-vaccenylacetate (E") and isoamylacetate (E"'); F) overview of described functional segregations of the lateral horn based on whether the chemical properties of the activating odors are pheromones or food cues (F'), amines or acids (F"), attractive, aversive or concentrated (F"').

of the OR (or IR) expressed in its outer dendrites on the antenna, the olfactory epithelium, or elsewhere. How odor-evoked activity is then encoded in the central brain is well understood in *Drosophila melanogaster*. As most of these findings are assumed to be true in both vertebrates and invertebrates, for the reasons outlined earlier, we shall focus on the vinegar fly.

Odor identity in the combinatorial code is strongly conserved (Bhandawat et al., 2007) and propagated in the brain via a fixed number of direct chemical synapses per OSN and glomerulus to the projection neurons (PNs) in insects (Mosca and Luo, 2014) or mitral/tufted cells in vertebrates, which represent the output neurons of the first olfactory center. This fixed number of OSN synapses also shows various levels of convergence from OSNs to PNs (Grabe et al., 2016). These factors reflect the emphasis given to certain glomerular inputs over others throughout the code. Before these second-order PNs leave the AL on their way towards higher brain centers such as the protocerebrum, various computational processes take place.

The key players in this process are the local interneurons (LNs) in invertebrates or granule cells in vertebrates that connect diverse sets of glomeruli throughout the AL or OB (Chou et al., 2010; Ng et al., 2002; Seki et al., 2010). The neurons are mainly GABAergic or glutamatergic (i.e., inhibitory), while a few cholinergic (i.e., excitatory) LNs also exist; these seem to serve several functions (Shang et al., 2007). One prominent task of LNs is to gain-control the OSN input signals. OSNs receive input onto their presynapses leading to suppression via lateral GABAergic inhibition in case of strong activity in the system to retain the neurons in an activatable state (Root et al., 2008). In contrast to this global lateral inhibition, these interactions can also occur in quite specific ways. For example, food odors are able to reduce the innate aversion mediated by CO_2-responsive glomeruli by means of lateral inhibition (Turner and Ray, 2009). Chemical synapses have been offered as the primary explanation for these effects, but this view had to be further refined after gap junctions in this neuron population were shown to be present (Yaksi and Wilson, 2010).

A third variable in this gain control mechanism is the irregular sensitivity to the lateral inhibition of certain glomeruli via GABA (Hong and Wilson, 2015) again displaying a prioritization of single glomeruli in the combinatorial code through reduced noise and stronger contrast in those particular ones. This seemingly uniform global effect of LNs is further complicated by the fact that only about 28% of LNs innervate all glomeruli in flies (Chou et al., 2010). The non-uniform innervation density of interneurons correlates inversely with the number of uniglomerular PNs, especially in the case of odors with high ecological relevance. A small set of glomeruli that receive less lateral input via interneurons displays a significantly higher number of PNs projecting onto higher brain centers, and this creates a potentially more dominant output compared to the remaining glomeruli (Grabe et al., 2016). All such asymmetrically innervated glomeruli are known to code for highly relevant ecological cues, such as sex pheromones (Fig. 2E") and aggregation pheromones, as well as for highly noxious cues. Like the glomerulus coding for CO_2 aversion, these information pathways are often considered to be labeled lines, as they are highly specific for their respective task and they process the task functionally segregated from other glomeruli (Stensmyr et al., 2012). This segregation is in strong contrast to the way in which most other glomeruli code diverse information about food sources and elicit a combinatorial code, because their respective ORs respond to a variety of odors and single odors activate several glomeruli (Fig. 2E', E"'). The combinatorial code of activity in the various receptor channels leads to a coding capacity of 10^{15} different odors in the vinegar fly, if the most cautious estimation of plain binary activity combinations in the code is true (Münch and Galizia, 2016). Keeping in mind that the OR repertoire of the fly is among the smallest ever described, so one can guess about the coding capacity of the olfactory systems in other animal species (Bushdid et al., 2014). We have come a long way in deciphering this code of the AL in *Drosophila melanogaster*, but we still lack the complete picture, and the AL is just the second step from perception to behavior.

So how is this research actually conducted, considering that the recording and visualization of the ongoing processes is a wide field in itself? The temporal response properties of OSNs, LNs and PNs to odors have been well characterized through the years in vertebrates (Duchamp-Viret et al., 1999; Sato et al., 1994; Sicard and Holley, 1984) and in insects (Abel et al., 2001; Christensen et al., 1993; de Bruyne et al., 2001; Sun et al., 1993; Vareschi, 1971). Single-cell recordings have provided high temporal resolution, but only one neuron could be measured at a time. In order to investigate the spatial component of the olfactory code, different experimental approaches have been used as direct and indirect indicators for neuronal activity; these have allowed us to observe entire neuron populations simultaneously in the OB and the AL. Experiments using radioactively marked 2-deoxyglucose or expression of the immediate-early gene c-fos revealed that odors evoke spatially organized activity patterns, consisting of mosaics of activated glomeruli (Galizia and Menzel, 2001). However, because these measurements are composed only of single long-lasting odor stimulations in a single animal, they lack the temporal information, and comparisons within the animal for different odors cannot be made. Thus, optical recording methods have been developed to visualize both spatial and temporal aspects of olfactory coding in the living animal during stimulations with various odors. Several studies, using intrinsic signals (Meister and Bonhoeffer, 2001; Rubin and Katz, 1999; Uchida et al., 2000), voltage-sensitive dyes (Cinelli et al., 1995; Friedrich and Korsching, 1998; Spors and Grinvald, 2002) or calcium imaging (Friedrich and Korsching, 1997; Galizia et al., 1999b; Joerges et al., 1997; Sachse et al., 1999; Wachowiak and Cohen, 2001) have shown that odors are encoded as specific spatio-temporal 'across-glomeruli' patterns – the combinatorial code.

Nowadays, functional imaging of calcium currents in model organisms is mainly carried out with genetically engineered calcium indicators (GECI), such as GCaMP6 (Chen et al., 2013), which exhibit a highly improved signal-to-noise ratio. Using these techniques, the combinatorial odor code could be confirmed for the AL of *Drosophila*. Furthermore, it could be demonstrated that the hedonic valence of odors is represented at the AL level. Certain glomeruli could be identified that respond more frequently to attractive odors, while others are mainly activated when an odor is aversive to the animal (Fig. 2**D**) (Knaden et al., 2012).

4. Odor categorization of higher processing centers

How is the odor information modulated and transferred to higher brain centers? And which features of an odor stimulus are encoded at this high processing level? The olfactory information from the first olfactory center – the insect AL or the vertebrate OB – is relayed via output neurons (PNs in insects and mitral/tufted cells in vertebrates) to higher processing centers. At the level of the first olfactory center, odors are coded as an across-glomeruli code (as mentioned above) according to which each odor activates an odor-specific combination of glomeruli. However, whereas at low olfactory-processing levels, odor specificity is encoded, at the level of high olfactory-processing centers, certain odor features, such as odor intensity, hedonic valence or specific behavioral value are represented, as revealed in recent studies (Sachse and Beshel, 2016). Hence, the specificity of the odor stimulus seems not be part of the odor code in higher-order brain centers.

In insects, the output neurons (PNs) target two different regions in the higher brain, namely, the mushroom body calyx and the lateral horn (Vosshall and Stocker, 2007). The mushroom body can be seen as a structure that is analogous to the piriform cortex in mammals (Su et al., 2009), whereas the lateral horn shares many similarities with the mammalian amygdala (Miyamichi et al., 2011; Sosulski et al., 2011). Neuroanatomical studies in *Drosophila* have revealed that the axonal branching of PNs is highly stereotypic from animal to animal with respect to the target areas in these two higher brain centers (Jefferis et al., 2007; Marin et al., 2002; Wong et al., 2002). The mushroom body has been a major subject of research over several decades, due to its well-substantiated role in learning and memory (Heisenberg, 2003), and many studies have tried to dissect its underlying neuronal circuitry (Aso et al., 2014a,2014b; Takemura et al., 2017). Interestingly, while there is a strong convergence and reduction in neuronal numbers from OSNs to the AL to PN output neurons, a strong divergence and vast increase in numbers is observable when moving from the PN to the mushroom body. Here, the ~150 PNs synapse onto ~2500 Kenyon cells, the intrinsic neurons of the mushroom body (Aso et al., 2009). The relatively high firing threshold and the crosswise interconnections among PNs and Kenyon cells speak in favor of an odor-coding principle called 'sparse code,' which means that very few Kenyon cells are responsive only to a distinct odor (Luo et al., 2010; Turner et al., 2008), a principle that appears to favor associative olfactory learning (Fiala, 2007). Taken together, both at the level of the AL and at the level of the mushroom body, odor information is processed and the information about a particular odor is distributed across relatively large numbers of neurons.

The lateral horn has been the focus of recent studies, and scientists are paying increasing attention to its neuronal wiring and odor coding properties. In contrast to the somewhat random PN connectivity in the mushroom body calyx (Caron et al., 2013), the target areas of PNs in the lateral horn reveal a specific pattern, namely, zonal clustering, which implies that each zone responds to a particular set of odorants (Fisek and Wilson, 2014; Marin et al., 2002; Tanaka et al., 2004; Wong et al., 2002). As mentioned above, these zones represent and most likely encode specific features of odors. Evidence for such patterns has come from the map of the axonal projections of PNs in the lateral horn that has revealed clear spatial segregation in PNs responding to pheromones or food odors (Fig. 2**F'**) (Jefferis et al., 2007) as well as in regions that are innervated by PNs activated by attractive amines or aversive acids (Fig. 2**F"**) (Min et al., 2013). Furthermore, the use of functional imaging and the monitoring of the odor response properties of PNs in the lateral horn have disclosed three spatially distinct regions that represent opposing hedonic valences, i.e., attractive or aversive odors as well as odor intensity (Fig. 2**F"'**) (Strutz et al., 2014). Hence, at the level of higher processing centers, odor specificity seems to have been lost, and odors are categorized according to their behavioral relevance. Whether odors are similarly categorized in higher brain centers in mammals – that is, according to behavioral value – remains unknown.

In conclusion, the olfactory system has evolved a highly sophisticated strategy to encode the vast array of odor ligands that occur in our natural environment. Our review summarizes the relevant steps from the combinatorial code initiated at the peripheral olfactory organs (antenna or nose) over the OR-specific convergence in the form of olfactory glomeruli in the AL or OB to higher processing centers. These coding steps subsequently allow odors to be perceived and thereby facilitate perceptual decision-making. Hence, the olfactory system has evolved a highly sophisticated strategy to encode the vast array of odor ligands that occur in our natural environment.

Acknowledgments

We are grateful for the editorial assistance of Emuly Wheeler. This work was supported by the Max Planck Society.

References

Abel, R., Rybak, J., Menzel, R., 2001. Structure and response patterns of olfactory interneurons in the honeybee, *Apis mellifera*. J. Comp. Neurol. 437, 363–383.
Ache, B.W., Young, J.M., 2005. Olfaction: diverse species, conserved principles. Neuron 48, 417–430.
Ai, M., Min, S., Grosjean, Y., Leblanc, C., Bell, R., Benton, R., Suh, G.S.B., 2010. Acid sensing by the Drosophila olfactory system. Nature 468, 691–695.
Andersson, M.N., Larsson, M.C., Blaženec, M., Jakuš, R., Zhang, Q.-H., Schlyter, F., 2010. Peripheral modulation of pheromone response by inhibitory host compound in a beetle. J. Exp. Biol. 213, 3332.
Aso, Y., Grübel, K., Busch, S., Friedrich, A.B., Siwanowicz, I., Tanimoto, H., 2009. The mushroom body of adult drosophila characterized by GAL4 drivers. J. Neurogenet. 23, 156–172.
Aso, Y., Hattori, D., Yu, Y., Johnston, R.M., Iyer, N.A., Ngo, T.-T.B., Dionne, H., Abbott, L.F., Axel, R., Tanimoto, H., Rubin, G.M., 2014a. The neuronal architecture of the mushroom body provides a logic for associative learning. eLife 3, e04577.
Aso, Y., Sitaraman, D., Ichinose, T., Kaun, K.R., Vogt, K., Belliart-Guérin, G., Plaçais, P.-Y., Robie, A.A., Yamagata, N., Schnaitmann, C., Rowell, W.J., Johnston, R.M., Ngo, T.-T.B., Chen, N., Korff, W., Nitabach, M.N., Heberlein, U., Preat, T., Branson, K.M., Tanimoto, H., Rubin, G.M., 2014b. Mushroom body output neurons encode valence and guide memory-based action selection in Drosophila. eLife 3, e04580.
Benton, R., Vannice, K.S., Gomez-Diaz, C., Vosshall, L.B., 2009. Variant ionotropic glutamate receptors as chemosensory receptors in drosophila. Cell 136, 149–162.
Bhandawat, V., Olsen, S.R., Gouwens, N.W., Schlief, M.L., Wilson, R.I., 2007. Sensory processing in the Drosophila antennal lobe increases reliability and separability of ensemble odor representations. Nat. Neurosci. 10, 1474–1482.
Braubach, O.R., Fine, A., Croll, R.P., 2012. Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (Danio rerio). J. Comp. Neurol. 520, 2317–2339.
Brennan, P., Zufall, F., 2006. Pheromonal communication in vertebrates. Nature 444, 308–315.
Buck, L., Axel, R., 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65, 175–187.
Bumbalo, R., Lieber, M., Schroeder, L., Polat, Y., Breer, H., Fleischer, J., 2017. Grueneberg glomeruli in the olfactory bulb are activated by odorants and cool temperature. Cell. Mol. Neurobiol. 37, 729–742.
Bushdid, C., Magnasco, M.O., Vosshall, L.B., Keller, A., 2014. Humans can discriminate more than 1 trillion olfactory stimuli. Science 343, 1370–1372.
Caron, S.J.C., Ruta, V., Abbott, L.F., Axel, R., 2013. Random convergence of olfactory inputs in the Drosophila mushroom body. Nature 497, 113–117.
Chen, T.W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., Looger, L.L., Svoboda, K., Kim, D.S., 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300.
Chou, Y.-H., Spletter, M.L., Yaksi, E., Leong, J.C.S., Wilson, R.I., Luo, L., 2010. Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. Nat. Neurosci. 13.
Christensen, T.A., Waldrop, B.R., Harrow, I.D., Hildebrand, J.G., 1993. Local interneurons and information processing in the olfactory glomeruli of the moth *Manduca sexta*. J. Comp. Physiol. A 173, 385–399.
Cinelli, A.R., Hamilton, K.A., Kauer, J.S., 1995. Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging.III. Spatial and temporal properties of responses evoked by odorant stimulation. J. Neurophysiol. 73, 2053–2071.
Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J., Carlson, J.R., 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron 22, 327–338.
Dippel, S., Kollmann, M., Oberhofer, G., Montino, A., Knoll, C., Krala, M., Rexer, K.-H., Frank, S., Kumpf, R., Schachtner, J., Wimmer, E.A., 2016. Morphological and transcriptomic analysis of a beetle chemosensory system reveals a gnathal olfactory center. BMC Biol. 14, 90.
Duchamp-Viret, P., Chaput, M.A., Duchamp, A., 1999. Odor response properties of rat olfactory receptor neurons. Science 284, 2171–2174.
Dweck Hany, K.M., Ebrahim Shimaa, A.M., Kromann, S., Bown, D., Hillbur, Y., Sachse, S., Hansson Bill, S., Stensmyr Marcus, C., 2013. Olfactory preference for egg laying on citrus substrates in drosophila. Curr. Biol.: CB 23, 2472–2480.
Eisthen, H.L., Nishikawa, K.C., 2002. Convergence: obstacle or opportunity? Brain Behav. Evol. 59, 235–239.
Engel, J.E., Wu, C.-F., 2009. Neurogenetic approaches to habituation and dishabituation in Drosophila. Neurobiol. Learn. Mem. 92, 166–175.
Enjin, A., Zaharieva, E.E., Frank, D.D., Mansourian, S., Suh, G.S.B., Gallio, M., 2016. Humidity sensing in drosophila. Curr. Biol. 26.
Fiala, A., 2007. Olfaction and olfactory learning in Drosophila: recent progress. Curr. Opin. Neurobiol. 17, 720–726.
Fisek, M., Wilson, R.I., 2014. Stereotyped connectivity and computations in higher-order olfactory neurons. Nat. Neurosci. 17, 280–288.

Friedrich, R.W., Korsching, S.I., 1997. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. Neuron 18, 737–752.
Friedrich, R.W., Korsching, S.I., 1998. Chemotopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. J. Neurosci. 18, 9977–9988.
Fujii, S., Yavuz, A., Slone, J., Jagge, C., Song, X., Amrein, H., 2015. Drosophila sugar receptors in sweet taste perception, olfaction and internal nutrient sensing. Curr. Biol.: CB 25, 621–627.
Fuss, S.H., Omura, M., Mombaerts, P., 2005. The Grueneberg ganglion of the mouse projects axons to glomeruli in the olfactory bulb. Eur. J. Neurosci. 22, 2649–2654.
Galizia, C.G., Menzel, R., 2001. The role of glomeruli in the neural representation of odours: results from optical recording studies. J. Insect Physiol. 47, 115–130.
Galizia, C.G., McIlwrath, S.L., Menzel, R., 1999a. A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy. Cell Tissue Res. 295, 383–394.
Galizia, C.G., Sachse, S., Rappert, A., Menzel, R., 1999b. The glomerular code for odor representation is species specific in the honeybee Apis mellifera. Nat. Neurosci. 2.
Gallio, M., Ofstad, T.A., Macpherson, L.J., Wang, J.W., Zuker, C.S., 2011. The coding of temperature in the drosophila brain. Cell 144, 614–624.
Gao, Q., Yuan, B., Chess, A., 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. Nat. Neurosci. 3, 780–785.
Gerkin, R.C., Castro, J.B., 2015. The number of olfactory stimuli that humans can discriminate is still unknown. eLife 4, e08127.
Getahun, M.N., Olsson, S.B., Lavista-Llanos, S., Hansson, B.S., Wicher, D., 2013. Insect odorant response sensitivity is tuned by metabotropically autoregulated olfactory receptors. PLoS One 8, e58889.
Grabe, V., Baschwitz, A., Dweck Hany, K.M., Lavista-Llanos, S., Hansson Bill, S., Sachse, S., 2016. Elucidating the neuronal architecture of olfactory glomeruli in the drosophila antennal lobe. Cell Rep. 16, 3401–3413.
Hallem, E.A., Carlson, J.R., 2006. Coding of odors by a receptor repertoire. Cell 125, 143–160.
Heisenberg, M., 2003. Mushroom body memoir: from maps to models. Nat. Rev. Neurosci. 4, 266–275.
Homberg, U., Christensen, T.A., Hildebrand, J.G., 1989. Structure and function of the deutocerebrum in insects. Annu. Rev. Entomol. 34.
Hong Elizabeth, J., Wilson Rachel, I., 2015. Simultaneous encoding of odors by channels with diverse sensitivity to inhibition. Neuron 85, 573–589.
Jefferis, G.S.X.E., Potter, C.J., Chan, A.M., Marin, E.C., Rohlfing, T., Maurer, C.R., Luo, L., 2007. Comprehensive maps of drosophila higher olfactory centers: spatially segregated fruit and pheromone representation. Cell 128, 1187–1203.
Joerges, J., Küttner, A., Galizia, C.G., Menzel, R., 1997. Representation of odours and odour mixtures visualized in the honeybee brain. Nature 387, 285–288.
Kaeppler, K., Mueller, F., 2013. Odor classification: a review of factors influencing perception-Based odor arrangements. Chem. Senses 38, 189–209.
Kaupp, U.B., 2010. Olfactory signalling in vertebrates and insects: differences and commonalities. Nat. Rev. Neurosci. 11, 188–200.
Knaden, M., Strutz, A., Ahsan, J., Sachse, S., Hansson, B.S., 2012. Spatial representation of odorant valence in an insect brain. Cell Rep. 1, 392–399.
Krieger, J., Sandeman, R.E., Sandeman, D.C., Hansson, B.S., Harzsch, S., 2010. Brain architecture of the largest living land arthropod, the Giant Robber Crab Birgus latro (Crustacea, Anomura, Coenobitidae): evidence for a prominent central olfactory pathway? Front. Zool. 7, 25.
Kurtovic, A., Widmer, A., Dickson, B.J., 2007. A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature 446, 542–546.
Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., Vosshall, L.B., 2004. *Or83b* encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43, 703–714.
Laska, M., Teubner, P., 1999a. Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes. Chem. Senses 24, 263–270.
Laska, M., Teubner, P., 1999b. Olfactory discrimination ability of human subjects for ten pairs of enantiomers. Chem. Senses 24, 161–170.
Leal, W.S., 2013. Odorant reception in insects roles of receptors, binding proteins, and degrading enzymes. Annu. Rev. Entomol. 58, 373–391.
Liberles, S.D., Buck, L.B., 2006. A second class of chemosensory receptors in the olfactory epithelium. Nature 442, 645–650.
Luo, S.X., Axel, R., Abbott, L.F., 2010. Generating sparse and selective third-order responses in the olfactory system of the fly. Proc. Natl. Acad. Sci. 107, 10713–10718.
Münch, D., Galizia, G.C., 2016. DoOR 2.0: comprehensive mapping of Drosophila melanogaster odorant responses. Sci. Rep., 6.
Maresh, A., Rodriguez Gil, D., Whitman, M.C., Greer, C.A., 2008. Principles of glomerular organization in the human olfactory bulb –Implications for odor processing. PLoS One 3, e2640.
Marin, E.C., Jefferis, G.S.X.E., Komiyama, T., Zhu, H., Luo, L., 2002. Representation of the glomerular olfactory map in the Drosophila brain. Cell 109, 243–255.
Meister, M., Bonhoeffer, T., 2001. Tuning and topography in an odor map on the rat olfactory bulb. J. Neurosci. 21, 1351–1360.
Meister, M., 2015. On the dimensionality of odor space. eLife 4, e07865.
Min, S., Ai, M., Shin, S.A., Suh, G.S.B., 2013. Dedicated olfactory neurons mediating attraction behavior to ammonia and amines in Drosophila. Proc. Natl. Acad. Sci. 110, E1321–E1329.
Miyamichi, K., Amat, F., Moussavi, F., Wang, C., Wickersham, I., Wall, N.R., Taniguchi, H., Tasic, B., Huang, Z.J., He, Z., Callaway, E.M., Horowitz, M.A., Luo, L., 2011. Cortical representations of olfactory input by trans-synaptic tracing. Nature 472, 191–196.
Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J., Axel, R., 1996. Visualizing an olfactory sensory map. Cell 87, 675–686.
Mori, K., Yoshihara, Y., 1995. Molecular recognition and olfactory processing in the mammalian olfactory system. Prog. Neurobiol. 45, 585–619.
Mosca, T.J., Luo, L., 2014. Synaptic organization of the Drosophila antennal lobe and its regulation by the Teneurins. eLife 3, e03726.
Ng, M., Roorda, R.D., Lima, S.Q., Zemelman, B.V., Morcillo, P., Miesenbock, G., 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. Neuron 36, 463–474.
Niimura, Y., 2012. Olfactory receptor multigene family in vertebrates: from the viewpoint of evolutionary genomics. Curr. Genomics 13, 103–114.
Pelosi, P., 1998. Odorant-binding proteins: structural aspects. Ann. N. Y. Acad. Sci. 855, 281–293.
Pelz, D., Roeske, T., Syed, Z., Bruyne M. d. Galizia, C.G., 2006. The molecular receptive range of an olfactory receptor in vivo (Drosophila melanogaster Or22a). J. Neurobiol. 66, 1544–1563.
Ressler, K.J., Sullivan, S.L., Buck, L.B., 1993. A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell 73, 597–609.
Robertson, H.C., Thomas, J.H., 2006. The putative chemoreceptor families of C elegans. WormBook 6, 1–12.
Robertson, H.M., Wanner, K.W., 2006. The chemoreceptor superfamily in the honey bee, Apis mellifera: expansion of the odorant, but not gustatory, receptor family. Genome Res. 16, 1395–1403.
Root, C.M., Masuyama, K., Green, D.S., Enell, L.E., Nässel, D.R., Lee, C.-H., Wang, J.W., 2008. A presynaptic gain control mechanism fine-Tunes olfactory behavior. Neuron 59, 311–321.
Rubin, B.D., Katz, L.C., 1999. Optical imaging of odorant representations in the mammalian olfactory bulb. Neuron 23, 499–511.
Ruddigkeit, L., van Deursen, R., Blum, L.C., Reymond, J.-L., 2012. Enumeration of 166 billion organic small molecules in the chemical universe database GDB-17. J. Chem. Inf. Model. 52, 2864–2875.
Sachse, S., Beshel, J., 2016. The good, the bad, and the hungry: how the central brain codes odor valence to facilitate food approach in Drosophila. Curr. Opin. Neurobiol. 40, 53–58.
Sachse, S., Rappert, A., Galizia, C.G., 1999. The spatial representation of chemical structures in the antennal lobes of honeybees: steps towards the olfactory code. Eur. J. Neurosci. 11, 3970–3982.
Sato, T., Hirono, J., Tonoike, M., Takebayashi, M., 1994. Tuning specificities to aliphatic odorants in mouse olfactory receptor neurons and their local distribution. J. Neurophysiol. 72, 2980–2989.
Schachtner, J., Schmidt, M., Homberg, U., 2005. Organization and evolutionary trends of primary olfactory brain centers in Tetraconata (Crustacea+Hexapoda). Arthropod Struct. Dev. 34.
Schendzielorz, T., Schirmer, K., Stolte, P., Stengl, M., 2015. Octopamine regulates antennal sensory neurons via daytime-Dependent changes in cAMP and IP3 levels in the hawkmoth manduca sexta. PLoS One 10, e0121230.
Seki, Y., Rybak, J., Wicher, D., Sachse, S., Hansson, B.S., 2010. Physiological and morphological characterization of local interneurons in the Drosophila antennal lobe. J. Neurophysiol. 104.
Shang, Y., Claridge-Chang, A., Sjulson, L., Pypaert, M., Miesenböck, G., 2007. Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. Cell 128, 601–612.
Sicard, G., Holley, A., 1984. Receptor cell responses to odorants: similarities and differences among odorants. Brain Res. 292, 283–296.
Silbering, A.F., Rytz, R., Grosjean, Y., Abuin, L., Ramdya, P., Jefferis, G.S.X.E., Benton, R., 2011. Complementary function and integrated wiring of the evolutionarily distinct drosophila olfactory subsystems. J. Neurosci. 31, 13357–13375.
Sosulski, D.L., Lissitsyna Bloom, M., Cutforth, T., Axel, R., Datta, S.R., 2011. Distinct representations of olfactory information in different cortical centres. Nature 472, 213–216.
Spors, H., Grinvald, A., 2002. Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb. Neuron 34, 301–315.
Stensmyr, M.C., Dweck, H.K.M., Farhan, A., Ibba, I., Strutz, A., Mukunda, L., Linz, J., Grabe, V., Steck, K., Lavista-Llanos, S., Wicher, D., Sachse, S., Knaden, M., Becher, P.G., Seki, Y., Hansson, B.S., 2012. A conserved dedicated olfactory circuit for detecting harmful microbes in drosophila. Cell 151, 1345–1357.
Strutz, A., Soelter, J., Baschwitz, A., Farhan, A., Grabe, V., Rybak, J., Knaden, M., Schmuker, M., Hansson, B.S., Sachse, S., 2014. Decoding Odor Quality and Intensity in the Drosophila Brain., pp. 3.
Su, C.-Y., Menuz, K., Carlson, J.R., 2009. Olfactory perception: receptors, cells, and circuits. Cell 139, 45–59.
Su, C.-Y., Menuz, K., Reisert, J., Carlson, J.R., 2012. Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature 492, 66–71.
Suh, G.S., Wong, A.M., Hergarden, A.C., Wang, J.W., Simon, A.F., Benzer, S., Axel, R., Anderson, D.J., 2004. A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature 431, 854–859.
Sun, X.J., Fonta, C., Masson, C., 1993. Odour quality processing by bee antennal lobe interneurons. Chem. Senses 18, 355–377.
Takemura, S.-y., Aso, Y., Hige, T., Wong, A., Lu, Z., Xu, C.S., Rivlin, P.K., Hess, H.F., Zhao, T., Parag, T., Berg, S., Huang, G., Katz, W., Olbris, D.J., Plaza, S., Umayam, L., Aniceto, R., Chang, L.-A., Lauchie, S., Ogundeyi, O., Ordish, C., Shinomiya, A.,

Sigmund, C., Takemura, S., Tran, J., Turner, G.C., Rubin, G.M., Scheffer, L.K., 2017. A connectome of a learning and memory center in the adult Drosophila brain. eLife 6, e26975.
Tanaka, N.K., Awasaki, T., Shimada, T., Ito, K., 2004. Integration of chemosensory pathways in the *Drosophila* second-order olfactory centers. Curr. Biol. 14, 449–457.
Turner, S.L., Ray, A., 2009. Modification of CO2 avoidance behaviour in Drosophila by inhibitory odorants. Nature 461, 277–281.
Turner, G.C., Bazhenov, M., Laurent, G., 2008. Olfactory representations by drosophila mushroom body neurons. J. Neurophysiol. 99, 734–746.
Uchida, N., Takahashi, Y.K., Tanifuji, M., Mori, K., 2000. Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. Nat. Neurosci. 3, 1035–1043.
Vareschi, E., 1971. Duftunterscheidung bei der honigbiene – einzelzell-Ableitungen und verhaltensreaktionen. Z. Vergl. Physiol. 75, 143–173.
Vogt, R.G., Prestwich, G.D., Lerner, M.R., 1991. Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. J. Neurobiol. 22, 74–84.
Vosshall, L.B., Stocker, R.F., 2007. Molecular architecture of smell and taste in drosophila. Annu. Rev. Neurosci. 30, 505–533.
Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., Axel, R., 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96, 725–736.
Vosshall, L.B., Wong, A.M., Axel, R., 2000. An olfactory sensory map in the fly brain. Cell 102, 147–159.
Wachowiak, M., Cohen, L.B., 2001. Representation of odorants by receptor neuron input to the mouse olfactory bulb. Neuron 32, 723–735.
Wasserman, S., Salomon, A., Frye Mark, A., 2013. Drosophila tracks carbon dioxide in flight. Curr. Biol. 23, 301–306.
Wong, A.M., Wang, J.W., Axel, R., 2002. Spatial representation of the glomerular map in the *Drosophila* protocerebrum. Cell 109, 229–241.
Yadav, P., Borges, R.M., 2017. The insect ovipositor as a volatile sensor within a closed microcosm. J. Exp. Biol. 220, 1554–1557.
Yaksi, E., Wilson, R.I., 2010. Electrical coupling between olfactory glomeruli. Neuron 67, 1034–1047.
Younus, F., Chertemps, T., Pearce, S.L., Pandey, G., Bozzolan, F., Coppin, C.W., Russell, R.J., Maïbèche-Coisne, M., Oakeshott, J.G., 2014. Identification of candidate odorant degrading gene/enzyme systems in the antennal transcriptome of Drosophila melanogaster. Insect Biochem. Mol. Biol. 53, 30–43.
Zube, C., Kleineidam, C.J., Kirschner, S., Neef, J., Rössler, W., 2008. Organization of the olfactory pathway and odor processing in the antennal lobe of the ant Camponotus floridanus. J. Comp. Neurol. 506, 425–441.
de Bruyne, M., Clyne, P.J., Carlson, J.R., 1999. Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J. Neurosci. 19, 4520–4532.
de Bruyne, M., Foster, K., Carlson, J.R., 2001. Odor coding in the *Drosophila* antenna. Neuron 30, 537–552.