REVIEW

Molecular Tuning of Odorant Receptors and Its Implication for Odor Signal Processing

Johannes Reisert[1] and Diego Restrepo[2]

[1]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, USA and [2]Department of Cell and Developmental Biology and Neuroscience Program, University of Colorado Denver, Anschutz Medical Campus, 12801 East 17th Avenue, Aurora, CO 80045, USA

Correspondence to be sent to: Diego Restrepo, Department of Cell and Developmental Biology and Neuroscience Program, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO 80045, USA. e-mail: diego.restrepo@ucdenver.edu

Abstract

The discovery of the odorant receptor (OR) family by Buck and Axel in 1991 provided a quantum jump in our understanding of olfactory function. However, the study of the responsiveness of ORs to odor ligands was challenging due to the difficulties in deorphanizing the receptors. In this manuscript, we review recent findings of OR responsiveness that have come about through improved OR deorphanization methods, site-directed mutagenesis, structural modeling studies, and studies of OR responses in situ in olfactory sensory neurons. Although there has been a major leap in our understanding of receptor–ligand interactions and how these contribute to the input to the olfactory system, an improvement of our understanding of receptor structure and dynamics and interactions with intracellular and extracellular proteins is necessary.

Key words: electroolfactogram, odorant receptor, olfactory sensory neuron

Introduction

The field of olfaction was profoundly transformed by the finding that odorants are detected by hundreds of 7 transmembrane (TM) spanning G-protein-coupled odorant receptor proteins expressed in the cilia of olfactory sensory neurons (Buck and Axel 1991; Firestein 2005). The genome of each species includes a large number of odorant receptor (OR) genes that constitute the input to the olfactory system (Young et al. 2002; Godfrey et al. 2004; Malnic et al. 2004; Zhang et al. 2004) and current studies of olfactory perception are guided by the combinatorial coding hypothesis (Stewart et al. 1979; Kauer 1991; Malnic et al. 1999; Koulakov et al. 2007). An important insight into how the information about structural features enters the brain was gained by the finding that each olfactory sensory neuron (OSN) expresses one receptor protein and that all neurons expressing the same receptor send their axons to a small number of ovoid neuropil structures (glomeruli) in the olfactory bulb where the signal is transmitted to mitral and tufted cells (Mombaerts et al. 1996; Mombaerts 2004). Thus, the large number of odorants contained in a complex odor can be individually recognized at the level of the olfactory epithelium by ORs to become a temporally dynamic odor map at the glomerular layer of the olfactory bulb (Mori et al. 2006; Wachowiak and Shipley 2006; Johnson and Leon 2007) to be relayed to the olfactory cortex to form the odor percept.

If the role of olfactory sensory neurons is to deconstruct the incoming signal into information about structural features then a key step in understanding olfaction is to characterize the ligand specificity of olfactory sensory neurons expressing the same receptor and its subsequent transformation into action potential output. This process is evidently dependent on ligand specificity of ORs in situ in the cilia. In this review, we provide an appraisal of the understanding of tuning of OR proteins, responsiveness of olfactory sensory neurons to odors, and responses of olfactory sensory neurons expressing identified ORs. In our manuscript, we do not cover related topics that have been reviewed elsewhere. These include the chromatographic nature of the access of the odors to the olfactory epithelium (Mozell et al. 1987), perireceptor events (Getchell et al. 1984; Pelosi 2001), the nature of signal transduction and adaptation in olfactory sensory neurons (Schild and Restrepo 1998; Kleene 2008), the role of ORs in axon guidance (Imai and Sakano 2008), and the characteristics of odor maps at the glomerular surface of the bulb (Mori et al. 2006; Johnson and Leon 2007).

Brief description of changes in action potential firing elicited by stimuli in olfactory sensory neurons in situ

Ottoson's (1955) early fundamental "Analysis of the electrical activity of the olfactory epithelium" laid the foundation for much of the investigation of the odor-dependent activity in olfactory sensory neurons. He used electroolfactogram (EOG) recordings, a field potential response to odorants that represents an integrated response of olfactory sensory neurons in the neighborhood of the electrode tip (Scott and Scott-Johnson 2002). His findings already showed many of the hallmarks of the olfactory system, like a relatively narrow dose–response relation with the response saturating in around 1–2 decades of odorant concentration and a reduction in the response latency with increasing odorant concentration. With the later advent of metal or KCl-filled microelectrodes, it became possible to record single unit action potential activity instead of the ensemble response of many olfactory sensory neurons. Most early work was performed by recording the spike discharge from single (usually amphibian or tortoise) olfactory sensory neurons still situated in the nasal epithelium in the nasal cavity and application of the odorant in the vapor phase. Typically, spike activity increased with an increase in odor concentration and saturated at around 50 Hz. Often, a progressive decline in spike amplitude at high stimulus concentrations was observed during the spike train, which leads to a reduction of the number of action potentials being generated (Shibuya T and Shibuya S 1963; Gesteland et al. 1965; O'Connell and Mozell 1969; Mathews 1972; Gesteland and Sigwart 1977; Baylin and Moulton 1979). This work was quickly extended to extracellular in situ recordings from rat olfactory sensory neurons with comparable response properties in respect to spike pattern generation (Figure 1; Gesteland and Sigwart 1977), although these experiments were performed at room temperature, and not mammalian body temperature, as were later experiments (Sicard 1986). The basal spike rate in the absence of odorants in these in situ recordings ranged from 0.05 to 3 Hz (O'Connell and Mozell 1969; Mathews 1972; Getchell and Shepherd 1978; Baylin and Moulton 1979; Duchamp-Viret et al. 1999). Although this low spontaneous spike rate does not leave much dynamic scope for an odor-induced reduction in activity, it has nevertheless been observed (Gesteland et al. 1965; O'Connell and Mozell 1969; Holley et al. 1974; Getchell and Shepherd 1978; Kang and Caprio 1995; Duchamp-Viret et al. 1999). The basal spike rate is also not a static phenomenon but can be increased by the hormones leptin and insulin (Savigner et al. 2009). Interestingly, in the water-based catfish, olfactory sensory neurons show a higher basal spike rate and can show excitatory as well as inhibitory responses to amino acids (Kang and Caprio 1995).

Stimulation of olfactory sensory neurons with more than one odorant revealed that a given neuron is responsive to
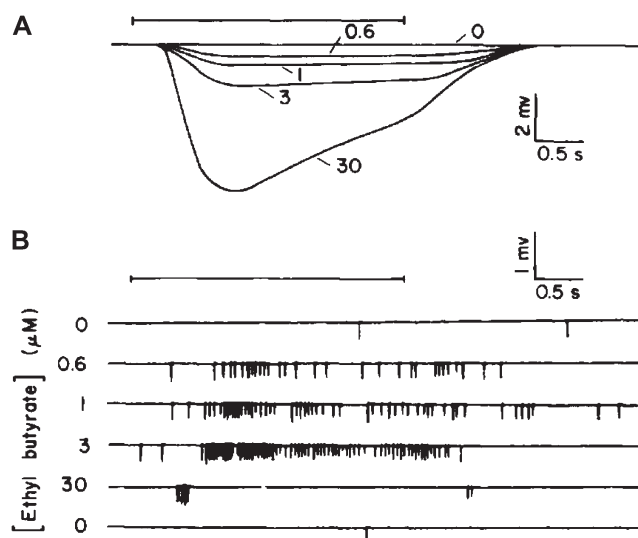
Figure 1 Odorant responses of rat olfactory sensory neurons in situ. Two electrodes were inserted though an opening in the dorsal surface of a rat head to reach the olfactory epithelium. A large diameter tip electrode recorded the electroolfactogram **(A)**, whereas a metal-filled sharp electrode recorded single olfactory sensory neuron action potential activity **(B)**. The odorant ethyl butyrate was delivered in the air phase for the duration of the bar at the top of each recording. Reprinted from Gesteland and Sigwart (1977) with permission from Elsevier.

multiple odorants, which can be structurally related or different (Sicard and Holley 1984; Duchamp-Viret et al. 1999, 2000). Many olfactory sensory neurons could respond to more than half of the presented odorants, underscoring their broad tuning. Importantly, a similar result was found in a recent thorough survey of responsiveness of single glomeruli in the dorsal surface of the olfactory bulb (Soucy et al. 2009). Responsiveness of single olfactory sensory neurons to multiple odors has been suggested to be due to multiple different "receptive sites" on an olfactory sensory neuron (Baylin and Moulton 1979), although Sicard and Holley argued that receptive properties could be present in a single receptor type (Sicard and Holley 1984). The sensitivity for a given stimulus could vary and, interestingly, olfactory sensory neurons showed different maximal receptor current or action potential frequency responses to different odorants (Firestein et al. 1993; Rospars et al. 2008).

By now, it is quite well understood how olfactory sensory neurons respond to and code for odorants. But the interaction of the odorant with the OR itself only began to be elucidated with the description of the family of ORs (Buck and Axel 1991) and is now beginning to unravel quickly.

Deorphanization of ORs

An obvious step after identification of a new receptor is its deorphanization through expression in a heterologous system. Initial attempts to perform deorphanization of the ORs were blunted by the lack of expression of receptors

in the plasma membrane in heterologous systems, presumably due to endoplasmic reticulum retention leading to degradation (McClintock and Sammeta 2003; Malnic 2007; Touhara 2007). To avoid this problem, Firestein and coworkers reasoned that adenovirus-mediated expression of an identified OR (rat I7) in olfactory sensory neurons in the intact epithelium would result in expression of the receptor in the cilia allowing measurement of odor responses by performing EOG recordings and calcium imaging in identified olfactory sensory neurons (Zhao et al. 1998; Araneda et al. 2000). Expression of rat I7 was successful and resulted in responses to a subset of odors that were significantly above responses in uninfected olfactory epithelia (Figure 2). The investigators found that I7 responds best to octanal but has a broad tuning curve responding to a wide variety of other compounds (Figure 2). The I7 receptor had high specificity for certain molecular features but high tolerance for others—a strategy that enables the olfactory apparatus to be both highly discriminating and able to recognize several thousand odorous compounds. The characterization of rat I7 by Firestein and coworkers remains one of the most thorough studies of responsiveness of an OR. Interestingly, Firestein and coworkers found that certain stimuli acted as antagonists for the I7 receptor (Figure 2B). The observation of antagonism has been confirmed for other ORs (Oka et al. 2004; Abaffy et al. 2007) raising the question whether an important factor in forming odor maps to natural odorants that are made up of hundreds of different molecules is antagonism by a subset of the odor components. Antagonistic odorants can potentially inhibit the perception of odorants as has been suggested as a potential mechanism for insect repellants (Ditzen et al. 2008) (but see Syed and Leal 2008). This poses a potential challenge for the understanding of the encoding of the quality of complex natural odors that are detected in the presence of a highly variable odor background (Doty 1986; Hudson 1999).

Unfortunately, although adenoviral transduction of ORs in the olfactory epithelium has been used successfully by others (Touhara et al. 1999), this method has not proven to be reliable for deorphanization of ORs. An obvious problem is that all olfactory sensory neurons in the olfactory epithelium express an endogenous receptor and respond to odors and therefore the response of the virally transduced receptor happens on top of a background response to each of the odors tested. In addition, measurement of responses using the EOG, or measurement of odor responses through calcium imaging in individual olfactory sensory neurons expressing the exogenous receptor, is not amenable for high throughput assay of odor responses. A solution to the problem of background responses to endogenous receptors is the creation of an animal model system with "empty sensory neurons" lacking expression of the endogenous ORs. This was implemented by Carlson and coworkers in *Drosophila* to probe ligand specificity of individual ORs (Hallem et al. 2004). Such a model is useful in the appraisal of the factors affecting responsiveness of identified ORs in situ. Hitherto, an equivalent approach has not been possible in vertebrate olfactory sensory neurons.

A complementary approach to identify ligands for ORs amenable to high throughput screening is expression in a heterologous system. Considerable progress has been made in solving the problem of retention of ORs in the endoplasmic reticulum thereby attaining high throughput assays of OR responsiveness in heterologous systems. Krautwurst et al. (1998) reported the generation of an expression library containing a large and diverse repertoire of mouse olfactory receptor sequences in the TM II–VII region. From this library, 80 chimeric receptors were tested against 26 odorants after transfection into HEK-293 cells. Three receptors were identified to respond to micromolecular concentrations of carvone, (–) citronellal, and limonene, respectively. This methodology has recently been employed quite successfully to deorphanize 52 mouse and 10 human ORs. Interestingly, the 10 human ORs were significantly more sensitive compared with the mouse ORs. But only 23% of the tested mouse ORs and 4% of the human ORs showed responses to at least one of the 93 test odorants (Saito et al. 2009). The reason for this different response rate of mouse and human ORs is unclear. A potential problem could still be limited receptor cell membrane expression. Approaches that enabled more reliable expression of ORs in heterologous systems included modification of the amino acid sequence that promoted plasma membrane expression, and identification and coexpression of accessory proteins that promote plasma membrane expression of the ORs. The specific approaches used to attain plasma membrane expression were reviewed thoroughly in recent publications (Malnic 2007; Touhara 2007). Rather, in the following sections, we discuss what the information obtained to date on OR responsiveness tells us about the input to the olfactory system, and identifies what future developments are necessary in order to understand how modification of the input to the system affects downstream neural signals and odor-guided behavior.

Understanding the basis for the interaction of ligands with ORs through site-directed mutagenesis and modeling

As part of their strategy to clone ORs Buck and Axel hypothesized that ORs will be significantly diverse, because ORs should belong to a large family of 7 TM-spanning receptors to bind a wide variety of structurally diverse molecules (Buck and Axel 1991). Indeed, this turned out to be the case with the original ORs they described displaying a high nonconservative diversity in TM domains 3–5, suggesting a potential region for ligand interaction. Henceforth, 3 main approaches were used to identify binding pockets or amino acids that confer ligand binding and specificity: firstly, comparative data mining of OR sequences between and across species

and secondly molecular modeling studies of ORs. At the beginning, both approaches were either hampered by the limited number of available OR sequences or by the lack of

appropriate high resolution crystal structures with sufficient resemblance of ORs and the knowledge of ligands. Nevertheless, these ''receptor driven'' methods yielded promising sets
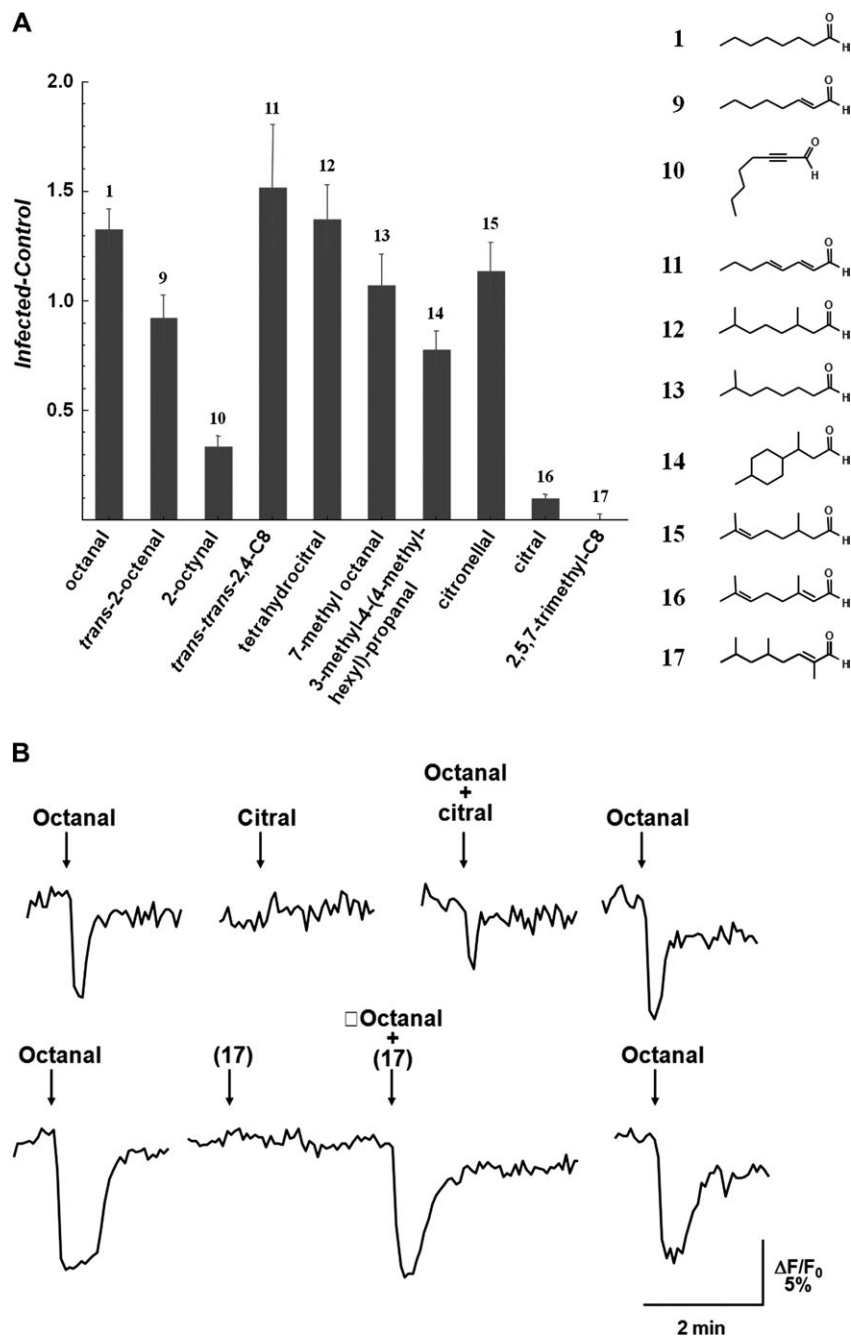
Figure 2   Responsiveness of OR I7 to C8 aldehydes. This figure is taken with permission from the study of Araneda et al. (2000) where they recorded odor responses for I7 through EOG recordings in rats whose olfactory epithelium had been transduced with an adenovirus that expressed rat I7 (Ad-I7). **(A)** Comparison of the EOG responses to analogs of octanal. All compounds are shown at 10^{-3} M except for citral (10^{-2} M). Citral, only at this high concentration, produced a small but significantly increased response ($P < 0.001$). At all concentrations tested, 2,5,7-trimethyl-2-octenal did not produce a significant increase in responses in infected animals. All other C8 analogs had increased responses ($P < 0.001$) at 10^{-3} M. Control responses were EOG responses from epithelium transduced with adenovirus that did not carry the coding region for I7. **(B)** Citral reduced octanal responses, as shown in Ca^{2+} imaging, in isolated olfactory neurons expressing I7 receptors. I7-expressing cells were recognized by the presence of GFP. Octanal (10 and 30 μM, top and bottom panels, respectively) produced an increase in Ca^{2+} as shown by the change in the emitted light. In the presence of citral (100 μM), top, but not in the presence of 2,5,5-trimethyl-2-octenal (100 μM), bottom, the response to octanal is reduced.

of potential ligand interaction sites (Singer et al. 1995; Pilpel and Lancet 1999). But because ligand–receptor pairs had not been identified, their validation remained indirect via comparison to other 7 TM receptors, mainly rhodopsin. The third more "ligand driven" approach relied on known ligands for specific ORs and their structural relatives to define relevant functional features of the ligand to map the odor-binding site and define requirements for the binding pocket in the OR. Finally, an increasing number of modeling studies have been complemented by site-directed mutagenesis and heterologous expression of ORs. This is important because the results of modeling provide testable predictions that can be probed through site-directed mutagenesis or other experimental approaches. Below we present a review of recent studies of the interaction of ligands with ORs. The interested reader should also look at the recent review by Katada et al. (2008).

The availability of the entire mouse and human OR repertoire enabled Man et al. (2004) to perform a comparison between orthologs (which should preserve their ligand specificity) and paralogs (which they assumed will have evolved a different odorant sensitivity and hence different amino acid sequences). An additional constraint was that locations of amino acids important in ligand binding should largely be conserved across ORs. Nineteen amino acid positions in TM helices 2–7 and 3 in the second and third extracellular loop were identified with most of them facing the lumen of a possible binding pocket in a structural OR model. When comparing 2 orthologous ORs across 5 species of the Japanese medaka fish, 14 residues in TM domains 3, 4, and 6 were identified to be involved in ligand binding (Kondo et al. 2002), 5 of which have also been identified by Man et al. In either study, the functional relevance and role of the identified residues could be validated indirectly by analogy to other well-studied 7 TM receptors with known ligands like rhodopsin and the D_2 dopamine receptor. In a model of the human OR1A1 and OR1A2 receptors (Schmiedeberg et al. 2007), 17 amino acids in TM2-7 were found to be involved in ligand binding, 12 of those were also described by Man et al. as were 4 of 10 amino acids that bind eugenol in the mOR-EG receptor (Katada et al. 2005).

The best studied OR–ligand pair both pharmacologically and structurally is the I7 receptor and its ligands and the first one where it was shown that a single point mutation in a heterologously expressed receptor protein (a Val to Ile substitution between the rat and mouse I7 receptors) can change the best ligand specificity from heptanal to octanal (Krautwurst et al. 1998). Singer (2000) based his receptor model on the then available 7.5 Å resolution structure of rhodopsin and found a binding pocket for octanal 10 Å from the extracellular surface, which was formed by TM domains 3–7. Two amino acids were identified, a lysine (Lys164) in TM4 and an aspartate (Asp204) in TM5, which were predicted to interact with the aldehyde group of octanal. The positively charged Lys146 accounted for most of the affinity for the aldehyde, possibly forming a Schiff base as retinal does in rhodopsin. In contrast, the negatively charged Asp204 hinders rather than promotes binding of octanal by competing for electrostatic interaction with Lys164. Five further residues in TM 5–7 were described to interact with the carbon chain of octanal by Van der Waals interactions. Predicted affinity for C6–C11 aldehydes matches reasonably well with electrophysiological data obtained from I7-positive olfactory sensory neurons.

The mouse and rat I7 OR share a high sequence similarity and their predicted structure also turned out to be similar (Hall et al. 2004), based on a higher 2.8 Å resolution crystal structure of rhodopsin. Again, Hall et al. found a binding pocket 10 Å from the extracellular surface formed by TM domains 3, 4, and 6 (but not 5) and Lys164 to be important in the binding of the aldehyde. They do not indicate a role of Asp204. Other residues involved in binding of the carbon chain only partly overlap with the above-mentioned residues. The tested aldehydes extended parallel to the membrane in the binding pocket, whereas lilial, a ligand they predicted, extended perpendicularly to the membrane. But because lilial neither acts as a ligand nor as an inhibitor physiologically, it was concluded that lilial represents a false positive. Post hoc refinement to the model allowed exclusion of lilial as an agonist.

It should be pointed out at this point that binding of the ligand in the binding pocket is required but not sufficient for receptor activation. Thus, any binding partner identified via modeling of the binding energy could potentially be an inhibitor or partial agonist. This can be helpful to validate and test particular models. Combining molecular modeling and comparison of ortholog–paralog pairs of receptors, Abaffy et al. (2007) investigated 2 receptors (mOR42-3 and the closely related mOR42-1), which respond to dicaboxylic acids of different chain lengths when expressed in Xenopus oocytes and monitored electrophysiologically. mOR42-1 is activated by C12 dicaboxylic acid, whereas mOR42-3 is not. Their docking studies implicated 8 residues in TM 3, 5, and 6 to determine ligand binding. Val113 in mOR42-3 (which is a serine in mOR-42-1) is thought to spatially constrain the binding pocket to limit the receptor's ability to respond to long chain dicaboxylic acids. When Val113 in mOR42-3 was mutated to a serine, this caused a shift of the response profile to mOR42-3 to longer carbon chains, including C12. But their modeling results revealed that C12 dicaboxylic acid does bind to mOR42-3, which suggests that it might be an inhibitor. Indeed, coapplication of C9 (a good agonist for mOR42-3) and C12 dicaboxylic acid leads to a smaller response in their functional essay compared with C9 dicaboxylic acid alone.

In an extensive study, Katada et al. (2005) mapped the mouse eugenol receptor (mOR-EG) by replacing side chains on eugenol and also mutating single amino acids of the receptor. They were, quite successfully, able to change either the receptor sequence or the structure of the ligand and

predict the activity of those new ligand–receptor pairs. They monitored the receptors' activities by expression in HEK293 cells and Ca^{2+} imaging. Ten, mostly hydrophobic (and non-conserved across ORs) residues in TM 3, 5, and 6 shaped the binding pocket. Interestingly, a serine (Ser113) in TM3 serves as a hydrogen donor to the ligand as has been described previously for other ORs (Floriano et al. 2004).

With more ORs being deorphanized, a combined approach became feasible. Armed with the knowledge of 29 odorant–OR pairs residues in TM3-6 were identified, which are likely to play a role in ligand binding in 23 of the 29 ORs (Khafizov et al. 2007) 11 of which have also been identified previously in data mining or modeling studies (Hall et al. 2004; Man et al. 2004).

The rat I7 OR was systematically mapped using a pharmacological approach with a large variety of odorants structurally related to its ligand octanal (Araneda et al. 2000; Peterlin et al. 2008) to identify the structures of the ligand relevant to receptor activation (or inhibition). Any changes to the aldehydic group abolished receptor activation. The aldehyde itself was not sufficient for receptor activation because short chain aldehydes (C4 to C6) did not activate rI7, neither did ligands with carbon chains longer than ~C11, implying a length filter of the binding pocket. rI7 also imposed a second requirement for activation: The ligand had to access a second binding pocket around 7 Å apart from the aldehyde binding site, which not only recognizes methyl groups but can also tolerate small hydrophobic cyclopropyl and cyclobutyl rings. Lack of binding of an odorant to this second binding pocket can render an agonist an antagonist.

A challenging aim is to predict a receptor's agonist based on its amino acid sequence. The predictive power of modeling is increasingly being used to suggest potential ligands, which can now increasingly be tested due to the improved ability to express ORs heterologously (see among others Singer 2000; Floriano et al. 2004; Katada et al. 2005; Abaffy et al. 2007; Schmiedeberg et al. 2007; Saito et al. 2009). This will prove to be a promising area to emerge more strongly in the coming years, but will require a concerted effort of modeling approaches and experimental validation, especially because potential ligands discovered by modeling might not necessarily activate the receptor (see above). Hence, this can address how a ligand interacts with an OR, but it might not necessarily predict the final percept of the odor (Triller et al. 2008).

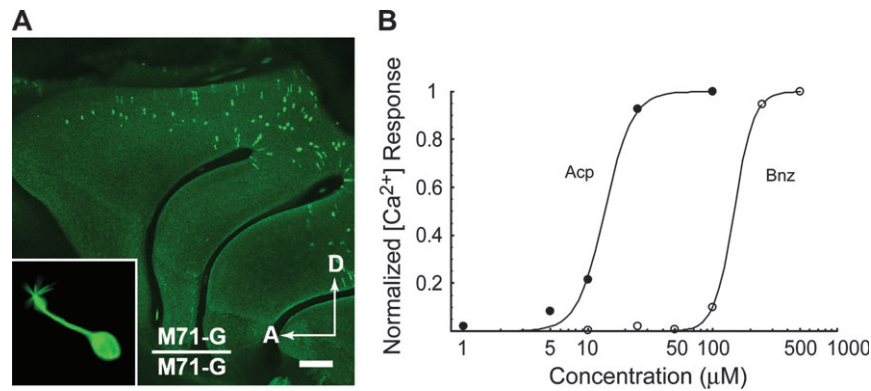## Responses of olfactory sensory neurons expressing identified ORs

Although expression of ORs in heterologous systems provides a plethora of information on ligand specificity for these proteins, the characterization of the responsiveness of the OR proteins in situ in the olfactory cilia is key to understanding olfaction. Great progress has been made in recording odorant-induced responses from *Drosophila* olfactory sensory neurons, which express a known OR. A given receptor could be narrowly tuned, responding only to a few of the 110 odorants presented or very broadly tuned, responding to nearly half of the test odorants. Action potential spike patterns elicited by different odorants could be quite diverse, including inhibitory responses, seen as the suppression of basal firing, being common (Hallem et al. 2004; Hallem and Carlson 2006).

Recording from vertebrate olfactory sensory neurons expressing a known receptor has seen less progress, possibly due to the more time-consuming experimental approach required. Hitherto, it necessitates the generation of a transgenic mouse, which coexpresses the fluorescent marker GFP with a particular receptor in an OSN for identification. Expression of a chosen OR in all olfactory sensory neurons using an olfactory marker protein-internal ribosomal entry site-OR construct is another possible alternative (Lane et al. 2005; Fleischmann et al. 2008), as is the expression of exogenous ORs in cultured olfactory sensory neurons (Chen et al. 2008). As mentioned above, the latter approaches can retain the endogenous OR of each cell, making the interpretation of odorant responses potentially more difficult.

Bozza et al. (2002) took the approach of coexpressing GFP in olfactory sensory neurons by altering the mouse M71 gene locus to code for bicistronic mRNAs expressing both tauGFP and either of three ORs: M71 itself, rat I7, or mouse I7 (see Figure 3A). Using Ca^{2+}-imaging, responses of isolated GFP-positive olfactory sensory neurons revealed acetophenone and benzaldehyde as agonists for M71. Dose–response relations were steep for both odorants, typically saturating within 1–2 log units of odorant concentration above threshold, with the average EC50 value for acetophenone being 5 times lower (20 µM) compared with benzaldehyde (100 µM) (Figure 3B). Interestingly, EC50 values for either odorant could vary 100-fold between individual M71 expressing olfactory sensory neurons. Swapping I7 in place of M71 confirmed octanal as an agonist for I7. However, in disagreement with reports by others using viral I7 overexpression and EOG recordings, they only observed small responses of I7 expressing olfactory sensory neurons to decanal and demonstrated that cinnamaldehyde and citral are I7 agonists (Araneda et al. 2000). The reasons for these discrepancies are unresolved but could originate from the different methodologies.

The wide variation in EC50 values for olfactory sensory neurons expressing the same OR are intriguing but could have been due simply to differences in the health of isolated neurons. This problem was tackled by Grosmaitre et al. (2006) who used an intact epithelium preparation to record electrophysiologically from identified olfactory sensory neurons, which in their case expressed the mOR23 OR. Application of the mOR23 ligand lyral at concentrations as low as 10 nM could elicit responses in some cells, whereas in others, responses were only generated at 1000-fold higher concentrations (10 µM). This great variation in sensitivity

Figure 3   Recording odorant-induced responses from olfactory sensory neurons expressing the M71 OR. **(A)** The introduction of IRES-tauGFP into the mouse genome following the coding sequence for the M71 OR allowed the identification and visualization of all M71-expressing olfactory sensory neurons, which are found in the most dorsal zone on the olfactory turbinates. Scalebar, 250 μm. **(B)** Screening of GFP-positive olfactory sensory neurons using Ca^{2+} imaging and a variety of odorants revealed the M71 agonists acetophenone (Acp) and benzaldehyde (Bnz). Note the ~10-fold shift difference in sensitivity. Modified from Bozza et al. (2002) with permission from the Society of Neuroscience.

of mOR23-positive olfactory sensory neurons was also reflected in the $K_{1/2}$ values obtained from Hill curve fits to peak response values of the lyral dose–response relation: They could vary from around 5 to 100 μM for individual cells. The dose–response relation was unexpectedly wide, typically spanning a 1000-fold concentration range from threshold to response saturation, which is at least atypical for dose responses obtained from olfactory sensory neurons with unknown ORs (Firestein et al. 1993; Ma et al. 1999; Reisert and Matthews 2001; Rospars et al. 2008) and also wider than previously reported for identified ORs (see above). Thus, olfactory sensory neurons in the intact epithelium display different sensitivities.

But how does an odor response in an olfactory sensory neuron relate to the response observed in the respective glomerulus in the bulb, which is innervated by olfactory sensory neurons expressing the same OR? Using a transgenic mouse line engineered to overexpress the mOR-EG OR and GFP under the mOR-EG promoter in olfactory sensory neurons that endogenously express mOR-EG Oka et al. (2006) constructed dose–response relations for eugenol (and other mOR-EG agonists) for both mOR-EG expressing olfactory sensory neurons and the glomeruli they innervate. Surprisingly, although eugenol and vanillin activated isolated olfactory sensory neurons (stimulated in the water phase) equally well with similar EC_{50}, the presynaptic Ca^{2+} of OSN nerve terminals in the glomeruli (stimulation in the gas phase, applied to the intact epithelium) to vanillin was greatly reduced and the dose response shifted to higher vanillin concentration when compared with eugenol. As suggested by the authors, the presence of mucus in the recording of the glomerular response might contribute to the observed discrepancies. It is also not known how the cell body Ca^{2+} responds in isolated olfactory sensory neurons, which is thought to originate from opening of voltage-gated Ca^{2+} channels in the cell body, translates to the action potential-mediated presynaptic Ca^{2+} response recorded in the glomeruli. Another

problem could be that the used odorants partition differently well or with different kinetics into the mucus.

Expression of "ORs" in other tissues

A surprising fact about OR genes is that they comprise approximately 3% of the genes in a rodent (Young et al. 2002; Godfrey et al. 2004; Zhang et al. 2004). Given the use of a large fraction of the genome by this gene family, it is not surprising that a subset of these receptors have been found to be expressed in other tissues. The ectopic expression of ORs in spermatozoa (Parmentier et al. 1992) was used to study hOR17-4 (Spehr et al. 2003). Ligands (e.g., bourgeonal and lilial) were identified and their attractant role in sperm chemotaxis established. Undecanal was found to be an effective inhibitor of hOR17-4, suppressing both the bourgeonal-induced Ca^{2+} response in spematazoa and bourgeonal-guided chemotaxis. But hOR17-4 does not only function as a sperm chemodetector but also as a "conventional" OR in humans (Spehr et al. 2003). Fukuda and Touhara followed up on these findings by making the interesting observation that multiple ORs are expressed in individual sperm cells in mouse (Fukuda and Touhara 2006). Recently, expression of mRNAs encoding for six ORs (Olfr78, Olfr90, Olfr1373, Olfr1392, Olfr1393, and Olfr NP_TR6JSE50FPA) was detected in kidney by amplification of reversed transcribed cDNA by the polymerase chain reaction (Pluznick et al. 2009). These investigators also found evidence for expression of elements of the canonical olfactory transduction pathway (G_{olf} and adenylate cyclase type III) in kidney and postulated that the olfactory receptors and elements of the transduction cascade are involved in regulation of glomerular filtration rate. Given that 3% of all genes in rodents (and ~1% in humans! Malnic et al. 2004) are ORs, it is not unlikely that future work will identify other ORs involved in diverse functions in other tissues. An interesting question is whether the

differences in microdomain environment in the different tissues would result in differences in ligand binding to the ORs.

Specific hyposmias and polymorphisms in individual ORs

The seminal work of John Amoore on thresholds to single compounds in human subjects revealed marked variability in human sensitivity to odors (Amoore 1965, 1967). In a theoretical study, Lancet et al. (1993) speculated that variability in sensitivity to specific odorants could be due to polymorphisms in ORs affecting odor-binding affinity. One of the most striking differences in sensitivity to odors in humans is in the perception of the smell of androstenone and androstadienone, compounds that range in perception by humans from offensive, to pleasant, to odorless depending on the individual (Wysocki and Beauchamp 1984). In a recent study, Keller et al. (2007) screened the responsiveness of 335 putative human odor receptors to a panel of 66 odors in a heterologous expression system. They identified OR7D4 as a receptor that responds to androstenone and androstadienone with affinities in the micromolar range. Interestingly, 2 mutations were identified in humans (R88W and T133M) that result in lower affinity for these compounds. The results of Keller and coworkers contradict a widely held view that variability in individual receptors is unlikely to cause large behavioral effects on odor sensitivity. Yet, variability in individual receptors can cause large behavioral effects. Whether mutation of a single receptor reducing the affinity for a ligand results in marked hyposmia for an odorant depends on whether other receptors bind the odorant with an affinity similar to that of the mutated OR. If there are no other receptors with high affinity for the odorant then the effect will be severe hyposmia. If there are other receptors with similar affinity, then the effect of OR mutation on detection would not be noticeable (Lancet et al. 1993; Koulakov et al. 2007). A parallel psychophysical study in a human population including 391 subjects found that individuals heterozygous or homozygous for either of these 2 mutations tended to perceive androstenone and androstadienone as less intense compared with controls. The study of Keller and coworkers strongly suggests that this perceptual variability for androstenone and androstadienone that has been termed specific anosmia is indeed due to genetic variability in specific ORs. Because a single odorant often interacts with multiple ORs, mutation in one receptor is unlikely to cause specific anosmia for an odor, but more likely a specific hyposmia or a change in the odor image. Androstenone, as a steroid, might be unusual in the way it is perceived. Thus, it is likely that complex traits underlie most observed variations in odor perception. Indeed, a study by Lancet and coworkers (Menashe et al. 2007) shows that enhanced odorant sensitivity (hyperosmia) to isovaleric acid by a subset of human subjects is a complex genetic trait contributed by both receptor and other mechanisms of the olfactory pathway.

Do all ORs respond directly to odors?

In Drosophila, the pheromone 11-cis-vaccenyl acetate (cVA) affects a variety of behaviors including aggression, male recognition, and sexual behavior (Xu et al. 2005; Ejima et al. 2007). Interestingly, detection of cVA by *Drosophila* requires not only the OR OR67d but also the odorant-binding protein (OBP) LUSH (Xu et al. 2005). OBPs are proteins found at high concentration in the sensillum lymph bathing the dendrites of olfactory sensory neurons in insects and in the mucus layer bathing the cilia of olfactory sensory neurons in vertebrates. OBPs are capable of binding a large number of diverse ligands, and their role in olfaction was unknown, although they had been proposed to participate in transport of the odor through the mucus, protection of the odor from degradation, or facilitating odor degradation. In Drosophila, Xu and coworkers had made the surprising observation that deletion of LUSH not only abolished cVA responses of olfactory sensory neurons in T1 trichoid sensilla, but also decreased by a factor of 400 the basal rate of firing of these olfactory sensory neurons in the absence of the ligand (Xu et al. 2005). This raised the question whether LUSH itself activates the olfactory sensory neurons. In a recent publication, Laughlin et al. (2008) show that the binding of cVA elicits rearrangement of the C terminus of LUSH (see Figure 4). They generated a mutant of LUSH (LUSH^{D118A}) whose C terminus in the absence of a ligand is structurally similar to the cVA-LUSH complex. Interestingly, the mutant LUSH^{D118A} stimulates olfactory sensory neurons in the absence of a ligand. This study raises the question whether it is the C terminus of LUSH that is recognized by the OR (or by accessory proteins such as sensory neuron membrane protein). Although the precise role of LUSH in the interaction with OR67d is not settled, and it is not known whether release of the odorant from LUSH and subsequent binding to the receptor is involved in the detection process, this study raises the question whether a subset of pheromones (and/or odorants) interacts with the ORs indirectly after binding to OBPs. Recognition through conformational changes in OBPs could result in significant increases in sensitivity and could affect the speed of the kinetics of interaction of odors with the receptor complex. The role of OBPs in vertebrate olfaction is less clear, because ORs typically have similar ligand-binding properties when investigated in the intact olfactory epithelium or in a heterologous expression system in the absence or presence of OBPs. Also note that all above modeling studies have been performed without the requirement of potential odor-binding proteins.

Conclusion

The understanding of the receptor–ligand interactions of ORs and their contribution to the input of the olfactory system has taken a major leap in the last 10 years. Although there are still problems with a subset of the receptors, and not all studies
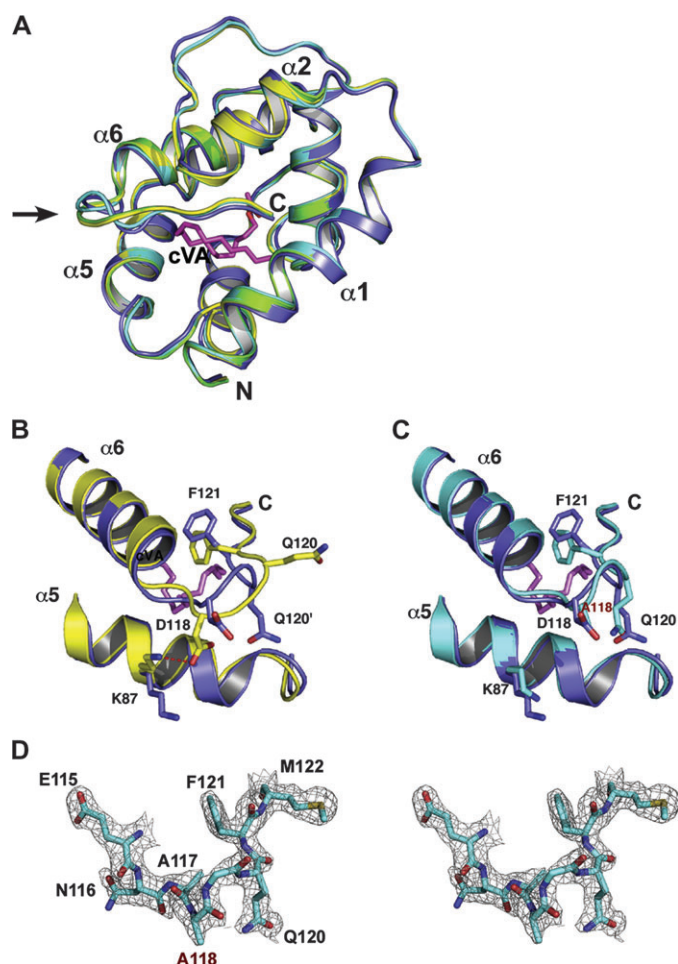
Figure 4 Structure of LUSH[D118A] Mutant Protein Mimics cVA-Bound LUSH Protein. **(A)** Ribbon diagram comparing one monomer from the asymmetric unit of the LUSH–cVA complex (blue) with the LUSH[D118A] (cyan), apo-LUSH (green), and LUSH–butanol complex (yellow). The cVA is shown as a stick model in magenta. Arrow indicates position of structural changes in the C-terminal loop shared by LUSH[D118A] (cyan) and LUSH–cVA (blue) but distinct from the LUSH–butanol complex (yellow) and apo-LUSH (data not shown). **(B)** Comparison of the structures of the 117–121 loop in the LUSH–butanol (yellow) and LUSH–cVA (blue) complexes. **(C)** Comparison of the structures of the same regions between LUSH–cVA (blue) and apo-LUSH[D118A] (cyan). LUSH–cVA and LUSH[D118A] adopt similar conformations. **(D)** Stereo representation of the electron density defining the loop between residues E115–M122 in the LUSH[D118A] structure solved without cVA. The position of A118 is indicated in red. The electron density is from a $2F_o-F_c$ map contoured at 1σ. Reproduced with permission from Laughlin et al. (2008).

sample olfactory space thoroughly, the expression of ORs in heterologous systems has become practical, and studies of receptors expressed in situ have become possible. These studies have shown that although some ORs are fairly specific in their ligand-binding specificity, many are broadly tuned, responding to particular features of the stimuli. Much future work is necessary to attain a thorough understanding of ORs. Three-dimensional structure and dynamics of ORs would greatly advance our understanding of the receptor–ligand interactions. A better understanding of the interactions with receptors and intracellular scaffolding proteins as well as external proteins involved in perireceptor events is necessary. Ultimately, the odorant–receptor interaction is only the initial step in olfaction and it is important to understand how these interactions cause activity of specific olfactory glomeruli in situ and beyond.

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