# Olfactory signalling in vertebrates and insects: differences and commonalities

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Abstract | Vertebrates and insects have evolved complex repertoires of chemosensory receptors to detect and distinguish odours. With a few exceptions, vertebrate chemosensory receptors belong to the family of G protein-coupled receptors that initiate a cascade of cellular signalling events and thereby electrically excite the neuron. Insect receptors, which are structurally and genetically unrelated to vertebrate receptors, are a complex of two distinct molecules that serves both as a receptor for the odorant and as an ion channel that is gated by binding of the odorant. Metabotropic signalling in vertebrates provides a rich panoply of positive and negative regulation, whereas ionotropic signalling in insects enhances processing speed.

Odorant
A chemical compound that stimulates the sense of smell. For terrestrial animals, odorants are small, volatile molecules; for aquatic animals, odorants are water soluble.

Pheromone
A chemical substance that is used for communication between members of the same species ('conspecifics'). It is released by an individual and detected by a conspecific.

G protein-coupled receptor
(GPCR). A member of a large family of membrane receptors that initiates a cellular response through G proteins. It threads through the cell membrane seven times, and the transmembrane segments adopt an α-helical secondary structure. Therefore, GPCRs are often referred to as heptahelical or 7-TM receptors.

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doi:10.1038/nrn2789
Published online
10 February 2010

The detection of chemical cues in the environment — which provide information on food, mates, danger, predators and pathogens — is essential for the survival of most animals. Depending on the biological function that they serve, these chemical substances are designated odorants or pheromones. The chemicals include small, volatile molecules, peptides and proteins, and gases such as carbon dioxide or oxygen, and can be detected at picomolar to millimolar concentrations. Considering the vast array of different chemicals, it is not surprising that different organisms use a large repertoire of distinct receptors, signalling pathways and anatomically segregated subsystems to sample their environment (FIG. 1). Indeed, several recent reports show that odorant receptors and olfactory transduction in vertebrates and insects are fundamentally different[1–3].

In both vertebrates and worms, odorants interact primarily with dedicated G protein-coupled receptors (GPCRs) on the membrane of a specialized sensory cell, thereby activating a signalling pathway that produces an intracellular messenger; this is termed metabotropic signalling. Ultimately, the biochemical signal is transduced into an electrical signal by the opening of ion channels. This olfactory signalling happens in cilia that extend from olfactory receptor neurons (ORNs), which are embedded in the olfactory epithelium. The repertoire of odorant and pheromone receptors in these species also includes guanylyl cyclases, enzymes that synthesize cyclic GMP (see Supplementary information S1 (box)), and members of the transient receptor potential (TRP) channel family[4–6]. In insects, most odorant receptors consist of a heteromeric complex that serves both as the receptor for the ligand and as the ion channel that is gated by binding of the ligand — a mechanism that is referred to as ionotropic. Whether the mechanism used by a particular organism is metabotropic or ionotropic has important consequences for the temporal encoding of odour, signal amplification (BOX 1) and feedback mechanisms that either enhance or terminate the response and adjust the cell's sensitivity.

In this Review, I compare the receptors and signalling mechanisms of vertebrate and insect olfactory systems, focusing on olfactory signalling in mammals and in *Drosophila melanogaster*. I begin with an overview of each system and then highlight the commonalities and differences between the systems.

## The vertebrate olfactory system

Olfaction happens in several olfactory subsystems of the nose (FIG. 1a). In the mammalian nose, five types of chemosensory GPCRs have been identified (TABLE 1): odorant receptors (ORs)[7], trace amine-associated receptors (TAARs)[8], two distinct vomeronasal receptors — V1R and V2R[9–11] — and formyl peptide receptors (FPRs)[12] (BOX 2). Most of what we know about these receptor families has been derived from structural and biochemical analysis of other GPCRs (see Supplementary information S2 (box)). The number of genes encoding chemosensory GPCRs varies considerably among species[13]. ORs are the largest family, with up to 2,130 genes having been discovered to date, whereas the other four families are generally much smaller (≤100 genes). The OR repertoire of different species ranges

between 125 OR genes in the fugu fish and 2,129 OR genes in the cow[13]; however, most vertebrate species have between 600 and 1,300 OR genes. An astonishingly large fraction of the OR genes in the genome are pseudogenes — that is, genes that have become non-functional during evolution. The fraction of total OR genes that represent pseudogenes varies between 12% (zebrafish) and 52% (humans and platypuses)[13].

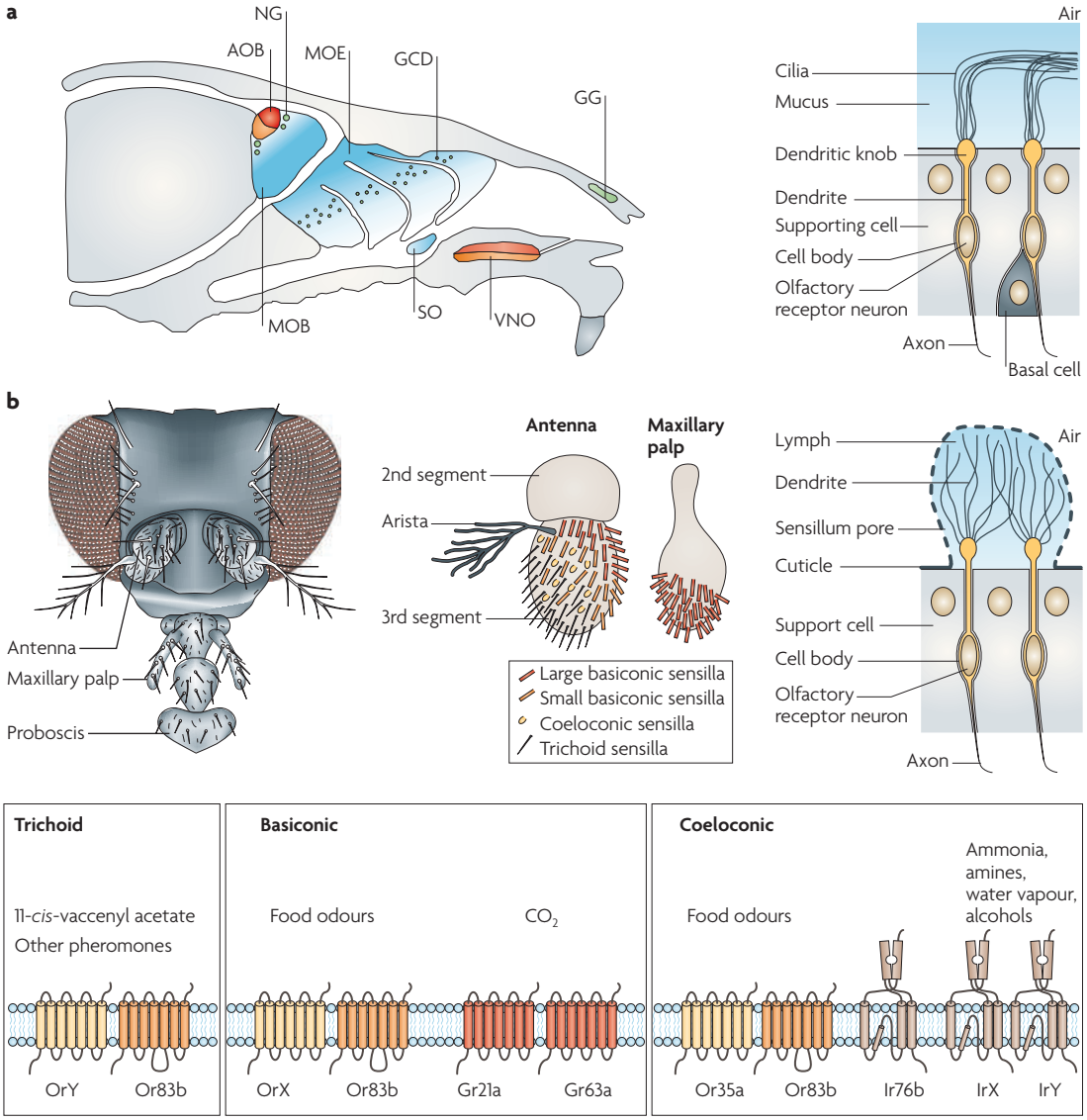



Figure 1 | **Olfactory subsystems in vertebrates and insects. a** | The vertebrate nasal cavity (left) contains several olfactory subsystems: the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the Grüneberg ganglion (GG), the septal organ (SO) and guanylate cyclase D-containing cells (GCDs) in the MOE. Sensory cells of the MOE and the SO project axons to glomeruli of the main olfactory bulb (MOB). Sensory cells of the GG and GCDs in the MOE send their axons to the necklace glomeruli (NG). Sensory cells of the VNO send their axons into the accessory olfactory bulb (AOB). Olfactory receptor neurons (ORNs) in the MOE (right) have one dendrite, which ends in a dendritic knob. From each dendritic knob, approximately 15 cilia extend into the nasal mucus. ORNs are surrounded by supporting cells and are constantly generated from basal cells. **b** | In insects, olfaction occurs in the third segments of the antenna and the maxillary palp (left). These organs are covered with sensory hairs — the sensilla (middle). Each sensillum hosts up to four ORNs. Insect ORNs are morphologically similar to vertebrate ORNs: the bipolar neuron gives rise to a single basal axon that projects to an olfactory glomerulus in the antennal lobe (right). At its apical side, the ORN gives rise to a single dendritic process, from which sensory cilia extend into the shaft of the sensillum. Three types of sensilla can be distinguished by their morphology and the chemicals to which their ORNs respond — basiconic, trichoid and coeloconic (bottom). In total, there are approximately 1,200 ORNs per antenna. The maxillary palp at the lower part of the head is a simpler structure than the antenna. It is covered by approximately 60 basiconic sensilla, each hosting two ORNs. For a comprehensive review of the architecture of fly chemosensory organs see REF. 91. The left panel in part **a** is modified, with permission, from REF. 163 © (2006) Macmillan Publishers Ltd. All rights reserved. The upper middle panel in part **b** is modified, with permission, from REF. 91 © (2007) Annual Reviews. The lower panel in part **b** is modified, with permission, from REF. 164 © (2009) Elsevier Science.

Box 1 | **Amplification and sensitivity of olfactory signalling**

**Vertebrates**

In general, G protein-coupled receptor (GPCR) signalling, such as that mediated by photoreceptors, amplifies a signal[138]. However, the principles governing olfactory signalling are quite different. Owing to the relatively low binding affinity of many odorants (micromolar range), the lifetime of the receptor–ligand complex is brief. Consequently, the probability that a receptor–ligand complex will meet a G protein and catalyse GDP–GTP exchange is low[72]. Why do most olfactory neurons not require high amplification at the receptor level? At micromolar odorant concentrations, more than 20 million odorant molecules arrive at a cilium every second[139]. Thus, although the probability that a single odorant molecule will activate the signalling pathway is minuscule, it is likely that a few odorant molecules will successfully evoke a response. By contrast, at low light levels, at which only a few photons reach the eye, amplification allows rod photoreceptors to detect and respond to single photons.

In the vomeronasal organ, concentrations of pheromone molecules above 0.1 pM can elicit a response[140,141]. At these low concentrations, only a few molecules per second are captured by a cilium. What are the biophysical requirements for such exquisite sensitivity? Receptors must bind the ligand with high affinity, increasing the lifetime of the ligand–receptor complex (seconds to minutes). During this time, the receptor may activate many hundreds of G proteins. However, active mechanisms are required to disable such stable ligand–receptor complexes. Receptor phosphorylation and β-arrestin capping may be an important route for response termination. In other cases, there may be no need for rapid inactivation, because temporal coding of successive stimuli does not matter.

**Insects**

Similar to vertebrate neurons, insect olfactory receptor neurons (ORNs) can be very sensitive, responding to the binding of a single molecule of a sex pheromone[142]. Insect ORNs, which have an ionotropic mechanism of action, also lack the amplification provided at the receptor and G protein level. How then can a single pheromone molecule activate an insect neuron? The open probability ($P_o$) of a ligand-gated channel is determined by its affinity for the ligand and, for nanomolar binding affinities, may reach unity on a timescale of seconds. Depending on the single-channel conductance, a single channel may readily carry currents in the order of a few picoamperes. The input resistance of vertebrate ORNs is high (2–8 GΩ) and a few picoamperes of inward current produce a voltage response that is sufficient to reach the threshold for triggering an action potential[143]. Similar mechanisms are seen in rod photoreceptors and sperm, which detect single photons and single molecules, respectively[108,144,145].

ORs are expressed in the main olfactory epithelium (MOE) of mammals and bind small, volatile 'odorous' molecules. The ORs are responsible for the classical sense of smell. Some ORs with unknown function are also expressed in other cell types and body regions, notably in the kidneys and sperm[14,15]. TAARs are also primarily found in the MOE, whereas the vomeronasal receptors and FPRs are expressed in other olfactory subsystems, such as the vomeronasal organ. The primary function of vomeronasal receptors and FPRs is to detect ligands associated with social cues. However, there may be exceptions to this rule, and the specific function of these receptors is not clearly defined (BOX 2; TABLE 1).

## Vertebrate ORs

Vertebrate ORs fall into at least nine groups (α, β, γ, δ, ε, ζ, η, θ and κ[16]). The α and γ groups (also referred to as class I and II ORs, respectively) underwent a large expansion in tetrapods. The other groups are present mainly in fish and amphibians, and they are absent in most land vertebrates. This suggests that α and γ ORs primarily detect airborne molecules, whereas the remaining groups detect water-soluble ligands[13], although this idea has not been tested experimentally. The expression of vertebrate ORs follows the one receptor–one neuron rule: each neuron expresses only one receptor gene[17].

***Chemoreceptive properties of ORs.*** Odorants vary in terms of size, shape, functional groups, charge, hydrophobicity and flexibility. These features are used by the olfactory system to recognize and discriminate between a wide array of chemical structures. Although the chemical 'universe' — the total number of odorants that can be detected — of any species is not precisely known, it probably scales with the size of the OR repertoire; for example, rodents (and even humans) may discriminate between several thousands or even tens of thousands of odorant molecules

The chemical receptive range of an OR has been addressed by several methods, including *in vitro* heterologous expression or *in vivo* overexpression of ORs[18–24], measurement of the correlation between receptor expression and ORN activity[19,25,26], and the expression of receptors from an endogenous locus in specific ORNs[27,28]. Several important concepts have emerged. Some ORs (termed 'generalists') have a broad receptive range, whereas others ('specialists') have a narrow receptive range. However, whether an OR is designated a generalist or specialist can depend on context, such as the concentration and number of tested odorants, and may not therefore be a useful characterization. Different odorants are recognized by unique but overlapping ensembles of ORs. Thus, the mammalian olfactory system uses a combinatorial strategy to encode chemical diversity[26].

Slight changes in the structure of an odorant (such as hexyl versus heptyl aldehydes) or its concentration can change the pattern of ORN activation. Some ORs can 'recognize' a specific functional group (such as an aldehyde group or alcohol group) in conjunction with other features of the ligand (such as a specific length of an aliphatic hydrocarbon chain or the presence of heterocycles)[22]. The dose–response relationship for a given odorant varies considerably among neurons and experiments[27–30]. For example, the dose–response relationship is steep in isolated ORNs[29,30] and shallower in intact tissue[27,31,32]. Perhaps ORs, even more so than other GPCRs, can exist in many conformations, with different signalling efficacies. Odorants can also act both as partial agonists[22] and as antagonists[33,34], adding another layer of complexity to olfactory coding.

How much a specific OR contributes to the combinatorial 'code' for a given odorant is not known. However, in humans, the perception of androstenone and related steroids varies enormously between individuals — from unpleasant to pleasant to odourless. A genetic variation in an OR (OR7D4) alters this perception and accounts for a substantial portion of the individual variability in the perception of these steroids[35].

What are the characteristics of the OR binding site? The amino-acid residues that form a binding cavity have been determined by comparing the sequences of receptor orthologues and paralogues[36], by molecular dynamics simulation using the helix scaffold of rhodopsin as a template[37,38] and by mutagenesis and heterologous expression[20,21,39]. In general, these studies show that odorants interact

Chemical receptive range
The number and chemical characteristics of the ligands that bind to an odorant receptor. It may be narrow (for example, only aliphatic alcohols of a certain length) or broad (for example, several different functional groups).

Molecular dynamics simulation
A computational technique that uses numerical methods to predict the structure of a protein from its amino-acid sequence. It is also used to simulate the docking of a ligand to its receptor. As a starting point, previously solved protein structures (for example, of rhodopsin) are used as templates.

Table 1 | **Different classes of olfactory receptors in vertebrates and insects**

| Class | Receptors | Ligands | Oligomeric state | Localization |
|---|---|---|---|---|
| *Vertebrates* | | | | |
| GPCRs | OR | Odours | Monomer | Main olfactory epithelium, Grüneberg ganglion, vomeronasal organ and exogenic expression |
| | TAAR | Amines | Monomer | Main olfactory epithelium and Grüneberg ganglion |
| | FPR | Pathogen- and inflammation-related compounds | Unknown | Apical layer of vomeronasal organ |
| | V1R | Small, volatile molecules and sulphated steroids | Monomer | Apical layer of vomeronasal organ and main olfactory epithelium |
| | V2R | Peptides (ESP1 and MHC peptides), MUPs and sulphated steroids | Monomer and heteromer with H2-Mv proteins and B2M | Basal layer of vomeronasal organ and Grüneberg ganglion |
| Monotopic receptors (RTK type) | GCD | Extracellular: uroguanylin and guanylin<br>Intracellular: bicarbonate, $Ca^{2+}$ and neurocalcin-δ | Dimer | Main olfactory epithelium |
| | GCG | Unknown | Unknown | Grüneberg ganglion |
| *Insects* | | | | |
| Ionotropic '7-TM' receptors | OR | Food odours and pheromones | Heterodimer (OrX–Or83b) | Antenna (basiconic, trichoid and coeloconic sensilla) and maxillary palp |
| | GR | $CO_2$ | Heterodimer (Gr21a–Gr63a) | Antenna (basiconic sensilla) |
| Ionotropic 'glutamate' receptors | IR | Ammonia, amines, water vapour and alcohols | Multimeric | Antenna (coeloconic sensilla) |

B2M, β2 microglobulin; ESP1, exocrine gland-secreting peptide 1; FPR, formyl peptide receptors; GCD and GCG, guanylate cyclase type D and G; GPCR, G protein-coupled receptor; GR, gustatory receptor; Gr21a and Gr63a, *Drosophila melanogaster* gustatory receptors 21a and 63a; H2-Mv, non-classical class I major histocompatibility genes; IR, ionotropic receptor; MHC, major histocompatibility complex; MUP, major urinary protein; OR, odorant receptor; RTK, receptor tyrosine kinase; OrX–Or83b, heteromeric *D. melanogaster* odorant receptor composed of Or83b and another OR (OrX); TAAR, trace amine-associated receptor; 7-TM, seven-transmembrane; V1R and V2R, vomeronasal receptors type 1 and 2.

with residues in helices 2–7 of the OR. Approximately 22–85 candidate residues are predicted to form the binding pocket[36,40] and provide the structural diversity that underlies odorant recognition. Computational methods allow binding energies to be estimated[38], with values that are in good agreement with activation profiles determined experimentally[26]. In addition, these studies propose that specific 'fingerprint' sequences are characteristic of receptors binding a particular chemical class of ligands, such as aliphatic monocarboxylic acids or alcohols[38]. However, several factors limit the predictive value of these approaches (see Supplementary information S2 (box)). In the future, the availability of additional GPCR structures as templates will aid the reconstruction of odorant-binding sites and our understanding of the molecular mechanisms underlying specificity. Moreover, several proteins have been identified that enhance targeting of ORs to the cell surface[41–43]. These tools will greatly facilitate the study of ORs in heterologous cell systems. Clearly, further progress will require several high-resolution structures of ORs with and without a ligand bound to be obtained.

***The cyclic AMP signalling pathway of ORs.*** The activation of most ORNs involves a canonical cAMP signalling pathway[44] (FIGS 2,3). Binding of odorants activates the OR, which stimulates the rapid synthesis of cAMP by adenylyl cyclase III (ACIII) through a mechanism mediated by the olfaction-specific G protein, $G\alpha_{olf}$ (REF. 45). Subsequently, cAMP opens cyclic nucleotide-gated (CNG) channels[46]. These channels are highly permeant to $Ca^{2+}$ (REF. 47) and their opening increases the ciliary intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$)[48,49] and causes the opening of $Ca^{2+}$-activated $Cl^-$ channels (CaCCs)[50–52]. Because ORNs accumulate rather than export $Cl^-$ ions[53–55], the opening of $Cl^-$ channels leads to $Cl^-$ efflux that further depolarizes the cell. Recently, a family of classical CaCCs was identified[56–58]. A splice variant of one of these proteins, anoctamin 2 (also known as TMEM16B), is highly expressed in ORNs[59]. When heterologously expressed in oocytes, this channel gives rise to currents that are similar if not identical to that of the native channel, suggesting that anoctamin 2 contributes to OR-mediated signalling[54,59,60].

In the frog, CNG channels and CaCCs contribute equally to the OR-mediated current[44]. In rodents, the inward current carried by $Cl^-$ channels is up to 33-fold larger than that carried by CNG channels[60]. Thus, the principal role of CNG channels in rodents is to carry a small initial $Ca^{2+}$ current that provides the trigger for the much larger inward $Cl^-$ current. The opening of $Cl^-$ channels causes a nonlinear amplification of the depolarizing signal. The relationship between odour concentration and receptor current is steep[30,61] owing to the cooperative activation of the CNG channel and the CaCC[54,60,62,63].

***Recovery and adaptation.*** To encode the temporal properties of the stimulus and to be prepared for subsequent stimulation, rapid cessation of the ORN response is crucial. Moreover, after completion of the response, the sensitivity of sensory neurons is adjusted in a process called adaptation. Mechanisms that allow cells to recover from stimulation may also be involved in sensitivity regulation, making it difficult to experimentally dissect one from the other. ORNs display short- and long-term adaptation to brief or sustained odorant stimulation, respectively. Both modes of adaptation seem to be controlled by changes in $[Ca^{2+}]_i$ (REF. 64). Considering the central role of $[Ca^{2+}]_i$ in feedforward and feedback mechanisms (FIG. 3), it is surprising that the precise site of $Ca^{2+}$ action during adaptation remains to be identified.

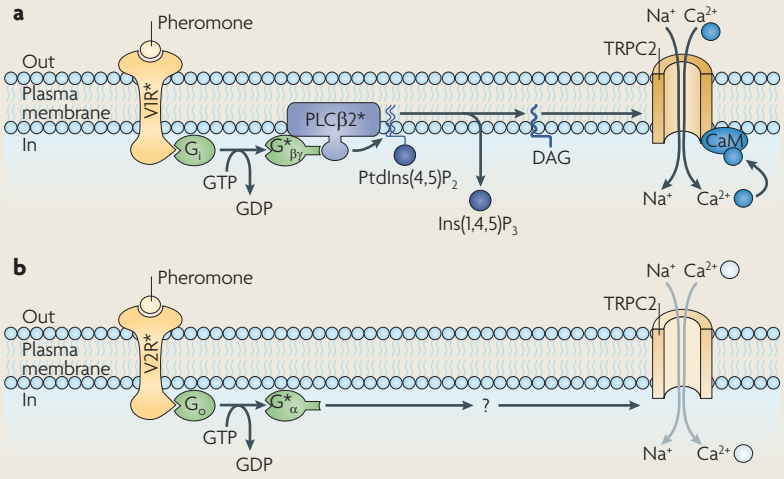
Response termination may occur at all stages of the OR signalling pathway (FIG. 3b). Proposed recovery mechanisms include receptor phosphorylation by protein kinase A (PKA) or G protein receptor kinase and subsequent 'capping' of the phosphorylated receptor by β-arrestin[65–67], inhibition of ACIII activity by $Ca^{2+}$–calmodulin (CaM)-dependent kinase II[68] and regulator of G protein signalling 2 (RGS2)[69], hydrolysis of cAMP by phosphodiesterase activity, desensitization of the CNG channel by $Ca^{2+}$–CaM-dependent processes[70], and removal of $Ca^{2+}$ by a $Na^+$–$Ca^{2+}$ exchanger[71]. The relative contribution of any one mechanism to recovery and adaptation is unknown.

The lifetime of the ligand–receptor complex may be too short (≤1 ms[72]) for the complex to be phosphorylated by a receptor kinase and capped by β-arrestin under standard conditions. However, such mechanisms may contribute to long-term desensitization during chronic stimulation[67].

ORNs express two phosphodiesterase isoforms: phosphodiesterase 1C (PDE1C), which is selectively localized to the ciliary lumen and is stimulated by $Ca^{2+}$–CaM[73], and PDE4A, which is distributed throughout the cell but absent from the cilia[74]. Unexpectedly, the response recovery of mouse ORNs in which the *Pde1c* gene has been disrupted is unaltered[75]. Termination of the response is significantly delayed only in mice deficient in both PDE1C and PDE4A. It is therefore likely that, in the absence of degradation in the cilia, cAMP rapidly diffuses into the dendritic knob, where it is degraded by PDE4A. Results obtained by bypassing PDE activity using caged cAMP analogues or pharmacological tools suggest that PDE activity does not contribute to short-term adaptation[76]. Similarly, adaptation in *Pde1c*$^{-/-}$ *Pde4a*$^{-/-}$ double-knockout mice is intact[75]. In *Pde1c*$^{-/-}$ mice, however, adaptation is impaired. Unexpectedly, odorant sensitivity was also reduced in these mice, a paradoxical phenotype given that PDE negatively regulates transduction by removing cAMP. Perhaps other components of the signalling pathways, and thereby the balance between activating and inactivating signalling steps, are disturbed in these mice.

The rate-limiting steps in response termination are the closing of the CNG channels and CaCCs. CNG channels are desensitized by $Ca^{2+}$–CaM-mediated feedback inhibition, which lowers the cAMP sensitivity[70]. Although all three olfactory CNG channel subunits[77] have CaM-binding sites[78–80], only a so-called 'IQ motif' in the B1b subunit renders the channel sensitive to CaM[80,81]. CaM

## Box 2 | **Pheromone signalling in vertebrates**




In vertebrates, different anatomical subsystems were thought to be dedicated to the detection and processing of odorants and pheromones. However, in recent years this functional separation of subsystems has become blurred[90]: common odorants can be detected by the vomeronasal organ (VNO)[146,147] and pheromone-like molecules can be detected by the main olfactory epithelium (MOE)[6,8,148,149]. Furthermore, pheromone receptors are expressed in the MOE[150], and signalling components of the MOE including olfactory receptors (ORs) are expressed in the VNO[151,152]. Finally, human pheromone receptors, when heterologously expressed in a cell line that hosts components of the classical cyclic AMP signalling pathway, mediate responses to several volatile odorants[153]. The VNO hosts three kinds of pheromone receptors that belong to the G protein-coupled receptor (GPCR) family: two distinct vomeronasal receptors (V1R and V2R)[9,10,11,154] and formyl peptide receptors (FPRs)[12].

V1R and V2R are expressed in non-overlapping apical and basal zones, respectively. The spatial organization of V1R and V2R matches the expression pattern of the G proteins $G\alpha_{i2}$ and $G\alpha_o$ (REFS 155,156), and axons from the apical V1R- and $G\alpha_{i2}$-expressing neurons project to the anterior part of the accessory olfactory bulb (AOB), whereas the basal V2R- and $G\alpha_o$-expressing neurons project to the basal part of the AOB. Many substances excite VNO neurons at picomolar to nanomolar concentrations[157]. Small, volatile molecules activate V1R-positive neurons[140]. By contrast, V2R-positive neurons are activated by small peptides[87,141,158–161]. Recently, sulphated steroids, another class of non-volatile chemicals, have been shown to potently activate the vast majority of VNO neurons[162].

The binding of the pheromone to a V1R receptor successively activates $G_i$, a G protein that is often involved in inhibitory signal transduction pathways; phospholipase Cβ2 (PLCβ2), which produces inositol-1,4,5-trisphoshate (Ins(1,4,5)$P_3$) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphoshate (PtdIns(4,5)$P_2$); and finally the transient receptor potential cation channel C2 (TRPC2; see the figure, part **a**). Activation of TRPC2 mediates $Na^+$ and $Ca^{2+}$ influx, leading to a depolarization. Recovery and adaptation may involve binding of $Ca^{2+}$–calmodulin (CaM) to TRPC2. The binding of pheromones to V2R receptors activates $G_o$, a trimeric G protein involved in diverse signal transduction pathways. In some V2R-expressing neurons, TRPC2 may be involved in generating depolarizing currents (see the figure, part **b**). However, because the signalling of major histocompatibility complex peptides in V2R-expressing neurons is intact in *Trpc2*$^{-/-}$ mice, other signalling mechanisms may exist.

The gene family of FPRs has seven members in mice[12]. Similar to OR or V1R genes, FPR genes display monogenic transcription and are not co-expressed with other vomeronasal chemoreceptors. FPRs respond to structurally unrelated peptides or proteins associated with inflammation or disease and are broadly tuned; thus, these chemosensory receptors may be involved in the identification of unhealthy conspecifics[12].

is pre-associated with the channel, allowing for rapid negative feedback[80]. However, adaptation was not impaired in ORNs expressing a CNG channel that lacks $Ca^{2+}$–CaM regulation but is otherwise intact[81].

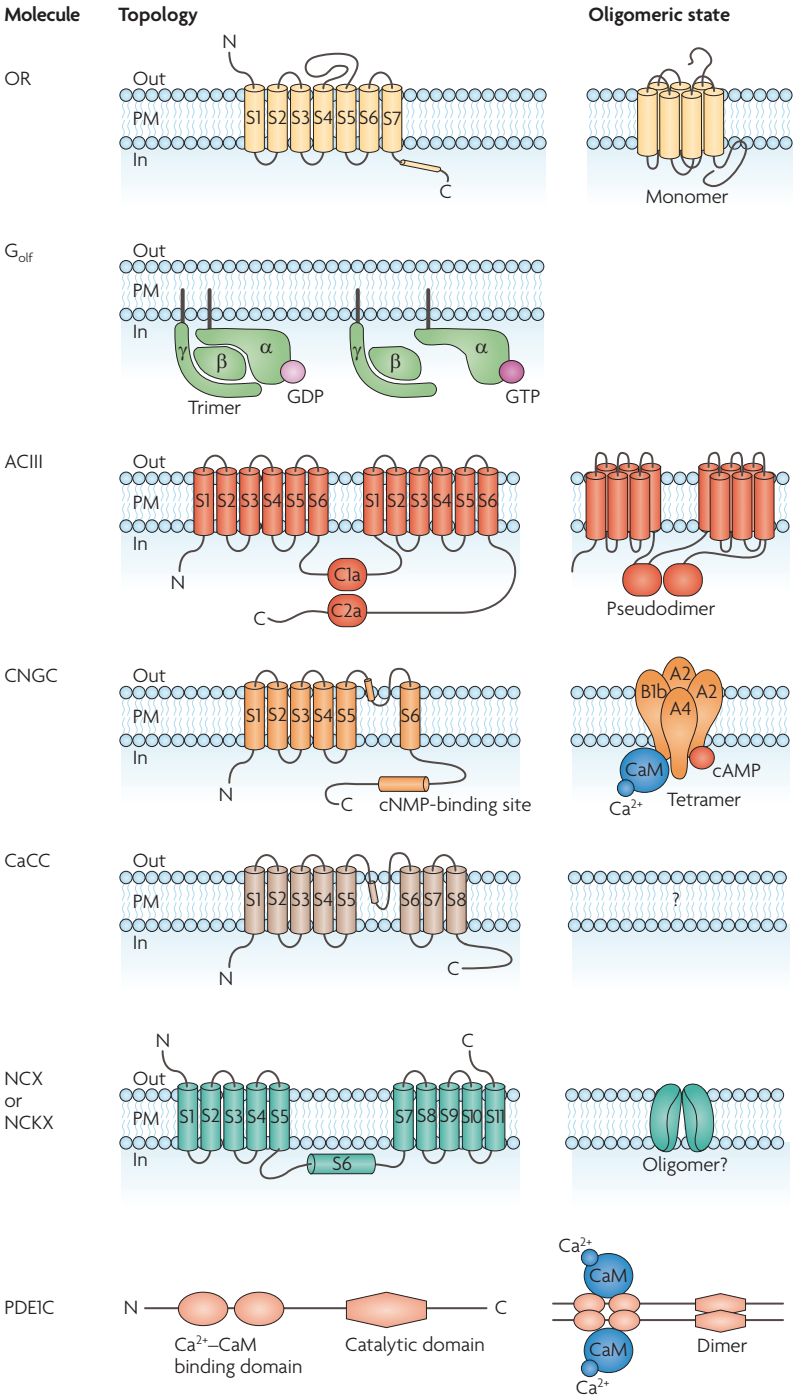



Figure 2 | **Molecules involved in mammalian olfactory signal transduction.** The topology and oligomeric state of molecules involved in mammalian olfactory signal transduction are shown. These include olfactory receptors (ORs), the trimeric G protein $G_{olf}$ (composed of subunits α, β and γ), adenylyl cyclase type III (ACIII), the olfactory cyclic nucleotide-gated channel (CNGC; composed of one B1b, one A4 and two A2 subunits), a $Ca^{2+}$- activated $Cl^-$ channel (CaCC), $Na^+$–$Ca^{2+}$ exchangers (NCX); $Na^+$–$Ca^{2+}$–$K^+$ exchangers (NCKX) and phosphodiesterase 1C (PDE1C). CaM, calmodulin; cNMP, cyclic nucleotide monophosphate; PM, plasma membrane.

Finally, $Ca^{2+}$ extrusion returns $[Ca^{2+}]_i$ to the resting state and closes CaCCs[71]. As 90% of the receptor current is carried by CaCCs, this is probably the most important recovery mechanism. Ion exchangers use the inwardly directed electrochemical gradient of other ions to export $Ca^{2+}$ from the cell. The NCX exchanger uses only the $Na^+$ gradient, whereas the NCKX molecule uses both a $Na^+$ and a $K^+$ gradient for $Ca^{2+}$ extrusion. At least three different NCX and three different NCKX molecules seem to be expressed in ORNs[59,82], but electrophysiological recording from dendritic knobs provides no evidence for NCKX-mediated $Ca^{2+}$ extrusion[60]. The olfactory marker protein (OMP) may also control $Ca^{2+}$ extrusion. *Omp*$^{-/-}$ mice display significantly delayed $Ca^{2+}$ clearance[83] that could be due to the absence of a protein complex that consists of OMP, CaM and a Bex protein[84]. However, another study concluded that $Ca^{2+}$ removal in cilia is not impaired by the absence of OMP[85]. $Ca^{2+}$ extrusion by the ($Ca^{2+}$)ATPases may be less important, because the pump efficiency of ($Ca^{2+}$) ATPases is generally lower than that of NCX or NCKX exchangers[84,86]. Thus, vertebrate OR signalling is both positively and negatively regulated by a rich network of intricate mechanisms.

## TAARs

TAARs were originally discovered in a search for the receptors of trace amines (such as tyramine, β-phenylethyl amine and octopamine) in the brain. Recently, TAARs were identified as chemosensory receptors that respond to amines[8]. Like ORs, TAARs are sparsely expressed in subregions of the MOE. Furthermore, TAAR-expressing neurons follow the one cell–one receptor rule and lack ORs. TAARs can increase cAMP levels in heterologous cells when stimulated with amine ligands, and TAAR-expressing neurons also express $G\alpha_{olf}$. Therefore, TAARs probably use a cAMP-signalling pathway.

Mouse TAARs specifically detect volatile amines found in urine — a rich source of social cues[87,88] that control reproductive behaviour and fertility, as well as other physiological responses. The TAARs that have been functionally tested each respond to a unique set of amine ligands. TAARs are evolutionarily conserved from lower vertebrates to humans[89], and they fall into three classes that are substantially expanded in fish[89]. Of these, class III TAARs do not have an aminergic ligand motif and probably respond to ligands other than amines.

## The olfactory system of insects

Olfaction in insects also happens in olfactory subsystems[90,91] (FIG. 1b). The repertoire of chemosensory receptors in flies is smaller than that in mammals. Three different kinds of chemosensory receptors have been identified in *D. melanogaster*: ORs (for which there are 60 OR genes) that are unrelated to vertebrate ORs, gustatory receptors (GRs, for which there are 73 GR genes) and ionotropic 'glutamate' receptors (IRs, for which there are 61 IR genes) (FIG. 1; TABLE 1). With one exception, all ORs are localized to the basiconic and trichoid sensilla. The GRs are expressed in taste organs throughout the body,

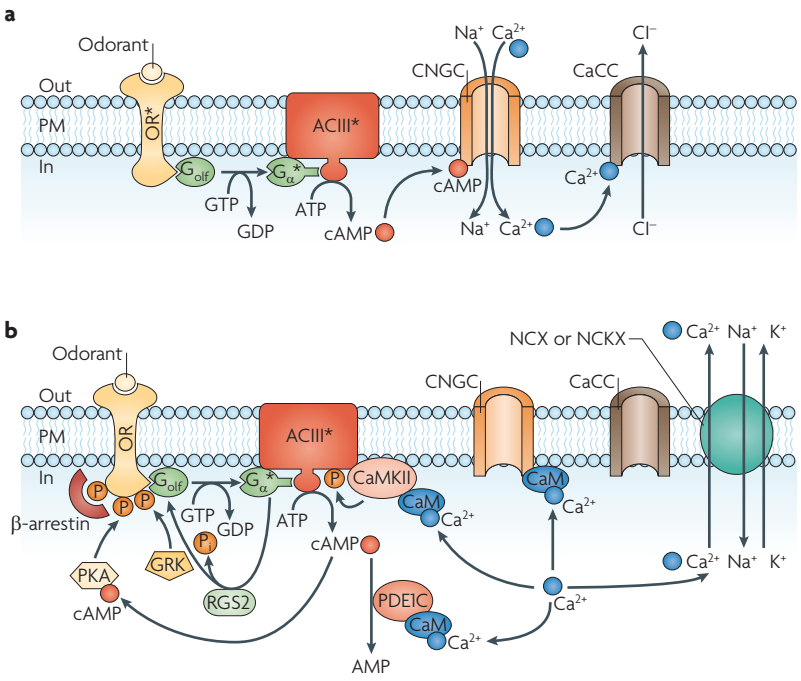



Figure 3 | **Signal transduction in mammalian olfactory receptor neurons. a** | The odour-induced signal transduction pathway. The binding of an odorant to the olfactory receptor (OR) successively activates the trimeric, olfaction-specific G protein ($G_{olf}$), adenylyl cyclase type III (ACIII), the olfactory cyclic nucleotide-gated channel (CNGC; composed of one B1b, one A4 and two A2 subunits) and a $Ca^{2+}$-activated $Cl^-$ channel (CaCC). Activation of both channel types finally leads to depolarization. **b** | Recovery and adaptation involves several $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathways. $Ca^{2+}$ controls the activity of the CNGC, ACIII and phosphodiesterase 1C (PDE1C). Moreover, export of $Ca^{2+}$ by $Na^+$–$Ca^{2+}$ exchange terminates signalling. OR activity seems to be terminated by several phosphorylation reactions and by the binding of β-arrestin to the phosphorylated OR. Asterisks indicate the activated form of the molecule. cNMP, cyclic nucleotide monophosphate; CaM, calmodulin; CaMKII, $Ca^{2+}$–calmodulin-dependent kinase type II; GRK, G protein-coupled receptor kinase; NCX, $Na^+$–$Ca^{2+}$ exchanger; NCKX, $Na^+$–$Ca^{2+}$–$K^+$ exchanger; PM, plasma membrane; PKA, protein kinase A; RGS2, regulator of G protein signalling 2.

and Gr21a and Gr63a are also expressed in $CO_2$-sensing basiconic sensilla. The IRs are primarily expressed in coeloconic sensilla[3].

## Insect ORs

There were early hints that insect and vertebrate ORs are distinct from one another. Although vertebrate chemosensory receptors share some sequence similarity with other GPCRs, insect receptors do not. Unsurprisingly, extensive cloning efforts based on sequence similarity failed to identify the elusive insect ORs. However, a bioinformatics approach that scanned the *D. melanogaster* genome for candidates with multiple transmembrane segments unveiled receptors with seven-transmembrane regions that were specifically expressed in olfactory organs[92–94]. The fly OR repertoire is considerably smaller than that of mammals, consisting of 62 ORs[95].

It quickly became clear that insect ORs are different from mammalian GPCRs. The insect receptors adopt a membrane topology that is the reverse of GPCRs[96–98]. Moreover, most fly olfactory neurons express two distinct receptors: a universal co-receptor, Or83b, and one of the common ORs[99]. Co-expression of common insect ORs with Or83b or its orthologues in mammalian cell lines or *Xenopus laevis* oocytes greatly enhanced the cellular response to ligands compared with the expression of common ORs alone, suggesting that the two form a functional unit[1,100]. Indeed, oligomerization of receptors to form a functional pair may be a common theme in insects. For example, GR21 and GR63 form a $CO_2$ sensor (without Or83b). Given that OR or GR pairs form a single receptor, the one receptor–one neuron hypothesis also applies to insects, although there are notable exceptions[101,102].

Two recent papers showed that insect ORs are ionotropic receptors that are directly gated by odorants[1,2]. Although both studies agreed that fly ORs form heteromeric ligand-gated ion channels, the experimental findings and conclusions of the two studies were very different (FIG. 4). One study[1] reported only a fast ionotropic response and found no evidence for the involvement of G proteins or intracellular messengers such as cAMP, cGMP or inositol-1,4,5-trisphoshate. By contrast, the other study[2] suggested that common insect ORs activate the synthesis of cAMP through a G protein, and that this in turn activates Or83b, which serves as a cAMP-gated ion channel. The second paper concluded that the G protein-mediated pathway provides the amplification needed for low odorant concentrations, whereas at high concentrations the direct ionotropic pathway is activated. Controversial issues in this field are discussed below.

***Homomeric versus heteromeric expression.*** The Or83b receptor is the most conserved insect OR and is expressed in all but one type of sensory neuron. Or83b is not directly involved in odour recognition[103]. Rather, it associates with the common 'tuning' ORs in the early endomembrane sorting pathway and escorts the OR–Or83b complex to the cilia. Consistent with this function, in mutants that lack Or83b, dendritic localization of common insect ORs is abolished, along with cellular responses to many odorants[99]. Thus, Or83b may serve both as a chaperone that assists in receptor trafficking and targeting and as a cognate co-receptor of the tuning OR. However, some *in vitro* studies in heterologous cells[104–106] reported odorant-stimulated responses when a common insect OR was expressed alone. Similarly, an odorant-induced rise of cAMP was detected in heterologous cells expressing Or22a, and cAMP-evoked currents were recorded only in cells expressing only Or83b[2]. It was therefore concluded that Or22a serves as a G protein-coupled odorant receptor and Or83b as a cAMP-gated ion channel. Notably, co-expression of Or22a and Or83b did not significantly enhance cAMP production or odorant-induced currents, suggesting that the respective function of each receptor is preserved in the homomer and — in principle — does not require a heteromeric complex. At present, it is unclear how these *in vitro* studies can be reconciled with the requirement for both a common OR and Or83b for OR signalling in insects[99] and other heterologous expression systems[1,100].

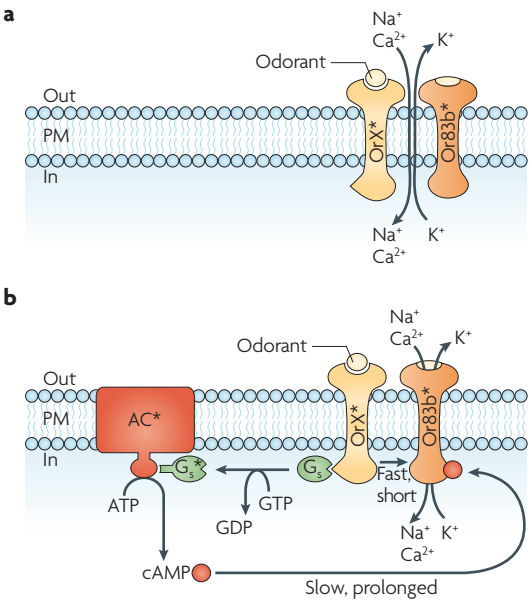



Figure 4 | **Models of odorant signalling pathways in insects. a** | One model[1] suggests that the odorant receptor forms an ion channel that is opened directly in response to the binding of odorants. The receptor consists of a common receptor (OrX) and a co-receptor (Or83b). This model does not specify the location of the channel pore. The simplicity of the model, however, does not imply that there are no other feedback or modulatory mechanisms: they are just not known. **b** | An alternative model[2] suggests that there are two pathways by which odour-induced depolarization can be generated. Upon odorant binding, activity is transferred to the Or83b subunit either by a direct (fast and short) or indirect (slow and prolonged) pathway. In the direct pathway, odorant binding directly opens a channel formed by the Or83b subunit, generating a fast and short depolarization; in the indirect pathway, activation of a G protein ($G_s$) and an adenylyl cyclase (AC) leads to cyclic AMP production. Upon binding of cAMP to Or83b, the channel opens and generates slow and prolonged depolarizing currents. The asterisks indicate the active form of the molecule. PM, plasma membrane.

***Kinetics and waveform of the current response.*** When stimulated with brief puffs of odorants, insect ORs exhibited transient current responses with a simple waveform characterized by a short delay (≤30 ms), a rapid rise and a slower decay to baseline[1]. The short delay together with the current fluctuations in excised inside-out patches suggested that insect ORs form a ligand-gated channel complex. By contrast, the odorant-induced current responses reported in a different study[2] consisted of a small, rapid and transient response followed by a prolonged, larger component. The rapid and slow components were attributed to a direct ionotropic and a GPCR-based metabotropic mechanism, respectively. Odorants also evoked $Ca^{2+}$ responses, suggesting that the ionotropic receptors are $Ca^{2+}$ permeant. In both reports, the decline of currents evoked by brief odorant puffs is unexpectedly slow (up to 10 s): ligand-gated channels usually close instantaneously once the ligand has been removed (even the metabotropic response of vertebrate ORNs completely recovers in 1–2 s[76]). Perhaps the ionotropic mechanism of insect ORs is distinct from that of classical ionotropic receptors at neuronal synapses; that is, perhaps insect ORs stay active even after the ligand has been removed.

***Is Or83b a cAMP-gated ion channel?*** As described above, these two recent studies reached different conclusions, on the basis of different results, concerning the mode of action of insect ORs. Further work is required to resolve this issue. However, I would argue that, for several reasons, the proposal that Or83b is a CNG channel is less compelling. First, the odorant-induced rise of cAMP was detected by co-expression of either hyperpolarization-activated cation (HCN) or olfactory CNG channels that served as cAMP sensors. Under physiological conditions, CNG channel currents are highly outwardly rectifying, owing to $Ca^{2+}$ blockage of more permeant $Na^+$ ions[63]. By contrast, the odorant-induced CNGA2-mediated currents recorded in this study under presumably physiological conditions were not outwardly rectifying[2]. Second, the membrane-permeant analogues 8-Br-cAMP and 8-Br-cGMP stimulated currents in Or83b-expressing cells at extracellular concentrations of ≥10 nM. By contrast, classical mammalian CNG channels require at least 0.1–1 mM extracellular concentrations of these analogues for activation, and the most cAMP-sensitive mammalian CNG channel opens at micromolar concentrations of cAMP in excised-patch recordings[63]. Thus, the ligand sensitivity of the presumed Or83b channel must be much higher than that of classical CNG channels — probably in the picomolar range. In fact, novel CNG channels with 25–100 nM ligand sensitivity have recently been described[107,108]. Nevertheless, in my opinion, unequivocal demonstration of CNG channel activity requires cAMP-gated currents to be recorded in excised inside-out membrane patches, or the use of caged cAMP in the whole-cell configuration.

Third, Or83b lacks known motifs for a pore region or a cyclic nucleotide-binding domain, although mutations in a putative pore motif in S6 changed the ion selectivity[2]. Moreover, high signal amplification by a second messenger is not required for sufficient sensitivity[72,108] (BOX 1). Finally, dual activation of OrX–Or83b complexes by ligand and cAMP poses a host of conceptual difficulties. Activation would require both high-affinity ligand-binding sites that stimulate cAMP synthesis at low concentrations of odorant and low-affinity sites that activate the channel directly at high concentrations of odorant. Alternatively, odorants may initially act as partial agonists (see Supplementary information S2 (box)), and cAMP may fully open the channel.

The physiological importance of a slow and sustained cAMP odour response in a rapidly moving fly is also unclear. On stimulation, *D. melanogaster* receptor neurons increase their action potential frequency within a few hundred milliseconds, and most responses

cease within a few seconds[109]. Therefore, perhaps the native receptor and channel properties *in vivo* are different from the properties of the heterologously expressed complex.

***Open questions.*** The two studies raise interesting questions as to the mechanism of channel activation. Is the channel formed by one or by both subunits? Does Or83b co-determine the ligand affinity and selectivity of OrX? Initial experiments suggest that the ion selectivity, rectification and pharmacology of the channel depend on the subunit composition and that the respective members of the Or83b family from *D. melanogaster*, the silk moth *Bombyx mori* and the malaria mosquito *Anopheles gambiae* have different electrical properties[1]. What is the stoichiometry between OrX and Or83b? How is the electrical response terminated? The receptor heteromers form non-selective channels that also pass a $Ca^{2+}$ current. Does the $Ca^{2+}$ influx control sensitivity and response termination as in vertebrate ORNs? Does the receptor, similar to many neuronal ionotropic receptors, desensitize in the presence of the ligand?

## Chemosensory properties of insect ORs

The relatively small number of chemosensory receptors in insects compared with the number in vertebrates has allowed researchers to systematically study the chemical receptive range of individual sensilla and ORNs, providing some important insights. For example, ORNs are spontaneously active and can be either further activated or inhibited by ligands[110,111]. Similar to GPCRs, insect ORs may be partially active when no ligand is bound, and some odorants may act as inverse agonists (see Supplementary information S2 (box)) to suppress baseline activity; however, the antagonistic action of a specific ligand in the presence of other ligands has also been observed, suggesting that ligands can act as antagonists or as inverse agonists[110,112]. Whether a ligand activates or inhibits an ORN is dictated by the OR expressed[113]. This high baseline activity, which can be enhanced or suppressed, is recapitulated in heterologously expressed OrX and Or83b receptors[1,2] and provides an additional dimension with which to encode odorants.

There is no compelling evidence for a functional distinction of generalist versus specialist receptors in insects. At high ligand concentrations, the response profiles of most ORNs are smooth and broad. At low concentrations, the profiles sharpen considerably, and a given receptor responds to only one or two ligands[112]. Thus, the concentrations used may determine whether a receptor is classified as generalist or specialist in a continuum of specificity.

Odorants can also elicit different temporal response patterns[109,113], depending on both the odorant molecule and the receptor type. For example, the kinetics of response termination for a receptor varies between odorants. This may be due to the rate of dissociation of the odorant from the receptor[102]. Temporal coding of a stimulus also enhances the ability to recognize and discriminate odours[114].

Odorant-binding protein
A member of a diverse family of proteins that have been proposed to serve either as odorant scavengers or carriers that deliver the odorant or pheromone to the receptor.

## Ionotropic glutamate receptors

Recently, 61 members of a novel family of chemosensory receptors that are expressed in the dendrites of ORNs innervating coeloconic sensilla have been identified[3]. The receptors, designated IRs, are related to ionotropic glutamate receptors, although the two receptor families are divergent[115] and IRs lack the residues that are important for glutamate binding. Although the functional properties of IRs have not been studied in heterologous expression systems, their localization and structural features suggest that they are chemosensory receptors that may function as ligand-gated ion channels. The discovery of IRs strengthens the concept that insect and vertebrate olfaction are fundamentally different, in that insect odorant receptors function primarily as ionotropic receptors.

Although insect ORs and IRs may both be ionotropic, their oligomeric structures are probably different. Up to five IRs and only two ORs can be co-expressed in an ORN, each probably forming a functional receptor. Two IRs (IR8a and IR25a) are ubiquitously expressed in coeloconic ORNs — a situation that is reminiscent of the co-receptor function of Or83b. If IRs represent channel subunits, their assembly into tetrameric or pentameric complexes would create an enormous combinatorial diversity of receptors. Furthermore, if all subunits in a complex bind to a ligand and are able to gate the channel pore, cooperativity among subunits may tune channel activity to a narrow range of odorant concentrations.

## Insect pheromone receptors

The most well-understood *D. melanogaster* pheromone is *cis*-vaccenyl acetate (cVA). Insect pheromone receptors — unlike those of the mammalian nose — belong to the superfamily of ORs. A single OR (Or67d) is responsible for sensing cVA[116,117]. However, Or67d requires both Or83b and another membrane protein, sensory neuron membrane protein (SNMP), for proper function[118,119] (see Supplementary information S3 (figure)). Although this receptor complex is activated at high cVA concentrations *in vitro*[116,118,119], an odorant-binding protein (OBP) facilitates activation *in vivo*. One such OBP, LUSH, is formed in the lymph of a subset of trichoid ORNs, including cVA-sensitive ORNs[120]. Mutants that lack this OBP do not respond to cVA[121]. cVA is deeply buried inside LUSH, and it is the cVA-occupied LUSH that activates neurons[122]. LUSH is an inactive ligand, perhaps a weak partial agonist[121] that is converted to a full agonist on cVA binding.

## Commonalities and differences: a summary

As described above, vertebrates and insects use similar strategies to recognize and discriminate odours (TABLE 2). Both have several large families of receptors to detect odorants, although the mammalian OR repertoire is considerably larger than that of insects. Moreover, the tuning of ORs (including some that are more broadly tuned and others that are more specific) and the action of odorants as agonists, antagonists or inverse agonists are also processes that are shared by mammals and insects. However, the high baseline

Table 2 | **Commonalities and differences of olfactory receptors in vertebrates and insects**

| Characteristic | Vertebrates | Insects |
|---|---|---|
| Class | GPCR | Non-GPCR |
| Repertoire | Large, variable | Smaller, constant |
| Topology | Heptahelical | Inverse heptahelical |
| Activation | Metabotropic | Ionotropic |
| Pseudogene fraction | High | None to low |
| Stoichiometry | Monomers | Heteromers |
| One receptor–one neuron rule | Yes | Yes* |
| Gene selection | Stochastic | Deterministic |
| Expression pattern | Zonal and random | Zonal and random |
| Instructive role | Yes | Unknown |
| Ectopic expression | Yes | Unknown |
| Inhibitory action of odorants | Rare | Common |
| Convergence of axons to glomeruli | Yes | Yes |
| Glomeruli per receptor type | Variable, ≤2 up to 20 | ~1 |

GPCR, G protein-coupled receptor. *There are notable exceptions to this rule, which have been excluded from this table for clarity.

receptor activity and inhibitory action of ligands seems to be a general feature of insect ORs, whereas it is an exception for mammalian ORs.

In vertebrates, each neuron expresses only a single receptor gene. Insect ORNs express between two (ORs) and four (IRs) different receptors. However, if we assume that many insect ORs assemble into a unique receptor complex[1–3], the one receptor–one neuron rule is also valid for insects in a functional sense. This rule forms the logical basis of the combinatorial strategy of odorant recognition.

The expression of vertebrate ORs is organized in several overlapping zones that are continuously arranged along the dorsoventral axis of the MOE[123–125]. The distribution of ORs in their respective zone has been described as random or stochastic. Similarly, in the *D. melanogaster* antenna, insect ORs segregate in different zones along the proximal–distal and dorsal–ventral axes[94]. Again, functionally identical sensilla are randomly distributed in each zone. The functional importance of the zonal organization is not precisely known.

There is also overwhelming evidence that vertebrate ORNs expressing a given OR send their axons to one or two glomeruli in the medial and lateral halves of the olfactory bulb (OB)[17,126]. Stimulation results in a glomerular pattern of activity that is unique for each odorant, referred to as an odour map[127]. The equivalent of the OB in insects is the antennal lobe. The OB and the antennal lobe are organized in a surprisingly similar way[128], underscoring the common principles that govern odour recognition in vertebrates and insects.

Despite these commonalities, there are several differences (TABLE 2). Mammalian and insect ORs differ greatly in their sequences, share no common ancestors and adopt a different membrane topology. Moreover, the signalling mechanisms are entirely different: mammalian ORs are GPCRs, whereas insect ORs are ligand-gated ion channels. The ionotropic signalling mechanism is well suited to the tracking of rapid changes in odour concentration and quality by a rapidly flying insect. In insects, ORNs hosting the same OR gene target a single glomerulus, and the number of ORs is similar if not identical to the number of glomeruli. In mammals, the number of glomeruli is considerably larger (in humans there are around 400 ORs and 6,000 glomeruli[129]). In this respect, the mammalian system is more flexible.

Both mammalian and insect ORNs must choose which OR gene to express from sizeable repertoires. The mammalian repertoires of functional ORs are large (300–1,300 ORs), whereas insect OR repertoires are much smaller (50–160 ORs). Both deterministic and stochastic models have been proposed to explain the choice of a receptor gene. In mammals, the choice of an OR is thought to follow a stochastic mechanism followed by a negative-feedback inhibition[130,131]. By contrast, in *D. melanogaster*, deterministic selection is accomplished by a molecular 'zip code' comprising three classes of regulatory elements that specify expression in the correct organ, activate OR genes in a subset of ORNs and restrict expression to a unique class of ORNs in that organ[132,133]. The reason why mammals and insects adopted different selection mechanisms is unclear. However, the larger OR repertoires in mammals, and consequently enhanced complexity, may have required a different selection procedure[13].

Although the mechanisms — adaptation to different environments and genomic drift due to gene duplication and deletion — underlying evolutionary changes in OR genes are similar in mammals and *D. melanogaster*[13], the result is different. The repertoire of OR genes in *D. melanogaster,* other insects and their ancestral species has been amazingly constant, whereas the repertoire of ORs varies extensively among different mammalian orders.

Finally, in mammals, ORs serve an instructive role that determines the projection of the ORN axon to a specific glomerulus[126,134]. Homophilic or heterophilic interactions between axons that involve ORs or OR-containing complexes cause axons to coalesce into a glomerulus[135,136]. Alternatively, the stimulation of cAMP synthesis by ORs may be involved in axonal sorting[137]. Such an instructive role of ORs has not been reported for insects.

## Future directions

We have observed considerable advances in our understanding of how organisms register and distinguish molecules in the olfactory system. The complete set of the principal molecules in the canonical cAMP signalling pathway of vertebrates has been identified, and the cellular signalling events are known with reasonable precision. What is lacking is a complete quantitative model that takes into account the restrictions imposed by the biophysical and kinetic properties of each signalling component, particularly with regard to short- and long-term adaptation. Moreover, we need a rigorous quantitative understanding of the molecular receptive range of receptors. Many technical issues limit our ability to generalize from and compare previous conclusions. Substantial advances will require atomic-resolution three-dimensional structures of receptors with different ligands bound. Advancing the concept of a 'conformational' space of a receptor will greatly help us to decipher the coding strategy on a more quantitative level by dissecting the contributions of each level of olfactory processing from the receptor to higher sensory areas in the brain.

Although insect ORNs dispense with an intricate biochemical signalling machinery, there is no doubt that feedback mechanisms must terminate and modulate the response to the odorant. Perhaps the discussion of metabotropic versus ionotropic olfactory signalling in insects will lead to unexpected insights into the modulation of a seemingly simple system such as that of insect ORs.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### Acknowledgements
I thank H. Fried and A. Aho for preparation of the figures and H. Krause for preparing the manuscript. I am particularly grateful to O. Ernst, Humboldt-University, Berlin, Germany, for the GPCR structures in the Supplementary information. Because of space limitations, I was unable to cite all relevant primary literature.


### Competing interests statement
The authors declare no competing financial interests.

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