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Current Opinion in Neurobiology

Controversy and consensus: noncanonical signaling mechanisms in the insect olfactory system

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There is broad consensus that olfactory signaling in vertebrates and the nematode *C. elegans* uses canonical G-protein-coupled receptor transduction pathways. In contrast, mechanisms of insect olfactory signal transduction remain deeply controversial. Genetic disruption of G proteins and chemosensory ion channels in mice and worms leads to profound impairment in olfaction, while similar mutations in the fly show more subtle phenotypes. The literature contains contradictory claims that insect olfaction uses cAMP, cGMP, or IP3 as second messengers; that insect odorant receptors couple to $G_{\alpha}s$ or $G_{\alpha}q$ pathways; and that insect odorant receptors are G-protein-coupled receptors or odor-gated ion channels. Here we consider all the evidence and offer a consensus model for a noncanonical mechanism of olfactory signal transduction in insects.

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Current Opinion in Neurobiology 2009, **19**:284–292

This review comes from a themed issue on
Signalling mechanisms
Edited by Michael Ehlers and Gina Turrigiano

Available online 5th August 2009

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2009.07.015

Introduction

'What sense is it that informs this great butterfly of the whereabouts of his mate, and leads him wandering through the night? What organ does this sense affect? One suspects the antennae; in the male butterfly they actually seem to be sounding, interrogating empty space with their long feathery plumes. Are these splendid plumes merely items of finery, or do they really play a part in the perception of the effluvia which guide the lover?' — Social Life in the Insect World by JH Fabre [1]

Insects show robust and extremely sensitive behaviors that are elicited by chemical cues in a species-specific manner [2]. In the 1870s the French biologist Jean-Henri Fabre described the phenomenon that the female peacock moth releases invisible odor signals (termed 'pheromones' 50 years later [3]) to attract the male [1]. Surprisingly, in spite of a long and prolific history of research into insect olfaction (reviewed in [2,4]), the molecular mechanisms of insect olfactory signal transduction remain unclear.

In all animals, odor cues are detected by membrane receptors that signal the identity and quantity of chemicals in the environment by inducing electrical activity in primary olfactory sensory neurons (OSNs). Classic work in vertebrates indicated that odors stimulate adenylate cyclase activity [5,6]. This led to the subsequent identification of an olfactory-specific adenylate cyclase (ACIII) and $G_{\alpha}s$ protein ($G_{\alpha}olf$) [7] and later the discovery of a large family of genes encoding seven transmembrane domain G-protein-coupled odorant receptors (ORs) [8]. Genetic deletion of signaling components in the mouse severely disrupts olfactory function. Similarly clear results in the nematode *C. elegans* (reviewed in [4]) affirmed the universally accepted view that all animals smell through G-protein-coupled receptors (GPCRs) that activate canonical signaling pathways.

These evolutionary considerations have guided studies of insect olfactory signal transduction for several decades, leading workers in the field to assume that GPCRs and the signal transduction cascades activated by them will also operate in insects. However, the primary data to support these assumptions are surprisingly contradictory (Table 1). This article reviews the history of investigation into the problem and proposes a consensus model for a noncanonical mechanism of olfactory signaling in insects.

Pheromone-evoked physiological responses in insect olfactory neurons

Insects are equipped with two pairs of head appendages, the antennae, and maxillary palps, which are decorated with thousands of olfactory hairs called sensilla that in *Drosophila* each house between one and four OSNs (Figure 1) [2]. In other insects, a sensillum may house as many as 30 OSNs. Different classes of sensilla respond to different odor types (Figure 1b). Chemical cues pass through the pores in the sensillum wall, interact with ORs present on the membranes of sensory dendrites emanating from the OSN, and change the frequency of action potentials in these neurons. OSNs exhibit characteristic levels of spontaneous activity that depend on the specific odorant receptor expressed in the OSN and odors can either increase or decrease spiking frequency [9].

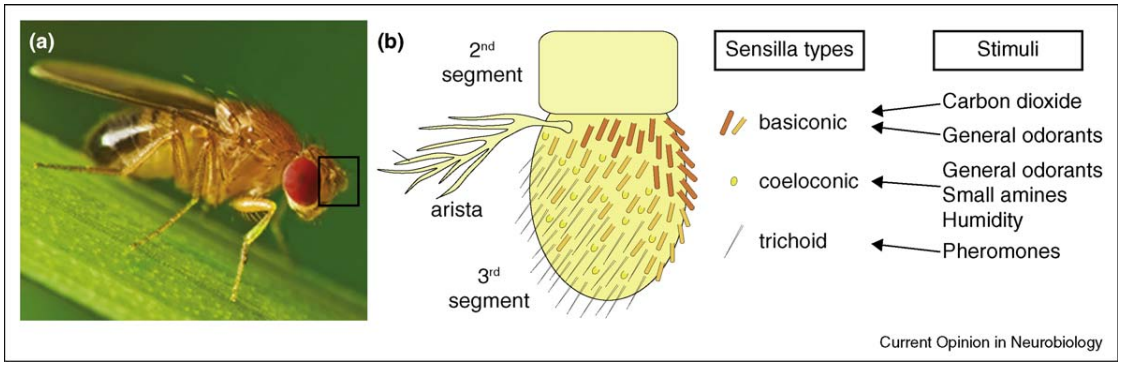
Table 1

Signaling systems implicated in insect olfactory transduction

Pathway/components	Effect/evidence	Reference
$G_\alpha q$: Ca^{2+}/IP_3/DAG/PLC		
$G_\alpha q$	Expressed in olfactory neurons	[15–18,19••,20]
$G_\alpha q$ (*dgq*) mutant/knockdown	Decreased response	[19••,28]
PKC activators	Activate AC_1 channel	[13]
DAG kinase (*rdgA*) mutant	Decreased response	[19••]
PI-TP	Expressed in olfactory neurons	[23]
PI-TP (*rdgB*) mutant	Decreased response	[23]
IP_3 kinase1	Expressed in olfactory neurons	[26]
IP_3 kinase1 overexpression	Altered response	[26]
IP_3	Odor-evoked increase	[10,11]
PLC	Expressed in olfactory neurons	[24]
PLC (*norpA*) mutant	Decreased response	[24]
PLCβ (*plc21C*) mutant	Decreased response	[19••]
$G_\alpha s$: cyclic nucleotides		
$G_\alpha s$	Expressed in olfactory neurons	[17]
$G_\alpha s$ mutant	Decreased response	[25,27]
AC and PDE	Expressed in olfactory neurons	[25]
AC mutant (*rut*)	Altered response	[25,27]
PDE mutant (*dnc*)	Altered response	[25,27]
cAMP	Odor-evoked increase	[10,52••]
cAMP	No odor-evoked increase	[10,12,51••]
cGMP	Odor-evoked increase	[12]
cGMP	Activates AC_1 channel	[13]
cAMP/cGMP	Activates OR83b	[52••]
cAMP/cGMP	Does not activate OR83b	[51••]
CNG channel	Expressed in olfactory neurons	[21]
CNG K + (*eag*) mutant	Decreased response	[22]
OR83b co-receptor		
Function requires OR83b	Imaging/electrophysiology	[34••,40,41,51••]
OR83b enhances function	Imaging/electrophysiology	[37•,42,43]
Function without OR83b	Imaging/electrophysiology	[47–50]
Function with G-protein	Imaging/electrophysiology	[48,49,52••]
Function without G-protein	Imaging/electrophysiology	[37•,42,43,50,51••]

Abbreviations: PKC, protein kinase C; DAG, diacylglycerol; PI-TP, phosphatidylinositol transfer protein; IP_3, inositol 1,4,5-trisphosphate; PLC, phospholipase C; CNG, cyclic nucleotide-gated; AC, adenylate cyclase; PDE, phosphodiesterase; cAMP, 3′–5′-cyclic adenosine monophosphate; cGMP, 3′–5′-cyclic guanosine monophosphate.

Figure 1



Insect olfactory sensilla. **(a)** Adult male vinegar fly, *Drosophila melanogaster*, on a blade of grass. Black box indicates the position of the chemosensory antennae. Adapted from a royalty-free photo (© Studiotouch #8408777, Fotolia.com). **(b)** Cartoon of one antenna, with the segments labeled and the position of three different types of chemosensory hairs on the third segment indicated, along with the classes of stimuli that activate neurons in these sensilla. Adapted from [34••], published by the Public Library of Science, which uses the Creative Commons Attribution License.

Modern studies into how odor cues activate insect OSNs began with Dietrich Schneider and colleagues, who used electrophysiology to record the electrical activity of the pheromone-tuned OSNs in the antenna of the male silkmoth [2]. Later biochemical work by Breer and colleagues indicated that pheromones induce rapid production of inositol 1,4,5-trisphosphate (IP3) [10,11] but found no evidence for the production of 3′–5′-cyclic adenosine monophosphate (cAMP) [10]. IP3 production required the activity of a pertussis-toxin sensitive G-protein-signaling pathway [11]. Ziegelberger *et al.* confirmed that cAMP was not produced, but detected pheromone-induced production of 3′–5′-cyclic guanosine monophosphate (cGMP) on a slower time scale more consistent with a role in modulating OSN sensitivity [12]. By patch clamping of the moth OSN dendritic membrane, Zufall and Hatt found a pheromone-gated nonselective cation channel (AC_1) that could also be activated by protein kinase C (PKC) activators and cGMP but not cAMP or IP3 [13]. They proposed a model of dual activation in which pheromones activate AC_1 to produce a rapid response via PKC and a more sustained response via cGMP [13]. Stengl found multiple pheromone-evoked currents in moth neurons operating at different time scales, the first a very rapid calcium current that declines in 100 ms that could not be blocked by PKC inhibitors, a second IP3-stimulated cation current that declines in less than 3 s, and a third inward current that was sustained over several seconds [14]. The molecular identity of the moth AC_1 and IP3-activated channels is still unknown.

Evidence for G-protein signaling in insect olfactory transduction?

These biochemical and electrophysiological studies implicating second messengers in insect olfactory signal transduction prompted a search for olfactory-enriched signaling proteins. G-protein subunits of $G_\alpha s$, $G_\alpha q$, and $G_\alpha o$ subtypes were found in OSNs in diverse insects (Table 1) [15–18,19••]. $G_\alpha s$ and $G_\alpha q$ were found to be enriched in sensory dendrites, implicating them in transduction mechanisms, but $G_\alpha o$ was localized only to the olfactory axon bundle, making it less likely that $G_\alpha o$ signaling is directly involved in transduction [17,20]. In addition to G proteins, olfactory cyclic nucleotide-gated and IP3-gated ion channels were described [14,21,22].

Starting in the 1990s, genetic analysis in *Drosophila* made it possible to test the functional relevance of these various signaling pathways in insect olfaction. Carlson and colleagues investigated the $G_\alpha q$ pathway and found reduced responses in flies mutant for PLC and associated phosphoinositide signaling components, but the effects were subtle and limited to subtypes of OSNs [23,24]. Alcorta and colleagues found evidence for both IP3 and cAMP in insect olfactory behavior, but again the phenotypes were quite subtle [25–27]. Flies in which $G_\alpha q$ was knocked down [19••,28] or deleted [19••] showed reduced electrophysiological and behavioral sensitivity to odors, and these $G_\alpha q$ defects synergized with mutations in DAG kinase and PLC [19••]. Recent *in vivo* work from Kain *et al.* [19••] represents the strongest evidence available that G-protein signaling coupled to phosphoinositides is required for maximal sensitivity of the insect odor response but not for the odor response itself. Kain and colleagues examined Drosophila $G_\alpha q$ (*dgq*) null OSNs generated either by genetic mosaic techniques or RNA interference and found a shift to lower sensitivity in the absence of *dgq*. This phenotype was enhanced when OSNs also lacked PLCβ21C or a diacylglycerol kinase encoded by the *rdgA* gene. The authors conclude that a phospholipid intermediate triggered by $G_\alpha q$ is crucial for optimal sensitivity of insect OSNs. We will revisit the question of G-protein signaling in the concluding remarks of this review.

Unconventional topology and heteromeric assembly of insect odorant receptors

Understanding the molecular basis of odor responses in insects required the identification of insect ORs. After many years of failed GPCR homology-based searches for insect ORs, a combination of difference cloning [29] and genomic analysis [29–31] yielded a family of divergent seven transmembrane domain proteins. Subsequent functional analysis in flies confirmed that these membrane proteins indeed confer odor-specific responses in the antenna [9,32,33]. Carlson and colleagues made the important observation that an individual OR governed not only ligand-specificity, but also levels of spontaneous activity, activation kinetics, and whether a cell was inhibited or activated by a given odor [9]. On the basis of these results, Hallem *et al.* hypothesized that the insect OR is poised between an inactive state that is insensitive to G proteins and an active state that can lead to the activation of a G-protein-mediated signal transduction cascade [9]. Inhibitory odorants would lock the receptor into an inactive state and excitatory odorants would engage a G-protein-signaling pathway that increases the frequency of action potentials in the OSN.

Although insect ORs were widely assumed to be GPCRs, *in vitro* and *in vivo* structural analysis revealed that the membrane topology of insect ORs is inverted compared to conventional GPCRs [34••,35•,36•,37•]. Further, insect ORs have no amino acid homology to ORs in vertebrates or *C. elegans* or to any other class of GPCR. Accordingly, conventional binding sites for G proteins are not obviously present in the insect ORs. This implied that the insect olfactory system may utilize atypical molecular mechanisms, distinct from vertebrates and nematodes.

Aside from differences in OR protein sequence, insect differ from vertebrates in the expression of multiple ORs per cell — a ligand-binding OR and a second member of the gene family that is called OR83b in *Drosophila* and

called either OR2 or OR7 in other insects. OR83b and its orthologs in other insects are highly conserved across insect species [38–40] and is necessary for the trafficking of ORs to dendritic membrane *in vivo* [34••,41]. Throughout this review we will use the term OR83b to refer generically to the insect OR co-receptor. Biochemical studies showed that OR and OR83b proteins physically interact [34••,42], leading to the suggestion that OR83b functions as a co-receptor for the ORs, although OR83b does not itself respond to odors. Thus in contrast to the GPCR-type OR mediating odor responses in vertebrates, the insect OR appears to be a heteromultimeric receptor complex.

A diversity of receptor types and ligands in insects

Insects respond to a very wide array of chemical substances and recent advances in *Drosophila* have begun to explain how this is achieved [4]. The specific OR/OR83b subunit composition governs whether the neuron will respond to general odorants or pheromones (Figure 2a,b) [9,33,43]. Pheromone receptors are a subset of the OR gene superfamily that, along with OR83b, require a CD36 homolog called SNMP for function [44•,45•] (Figure 2b). How these proteins interact to modulate pheromone sensitivity is an active and exciting area of investigation. Finally, a completely new receptor family — the Ionotropic Receptors (IRs), divergent, insect-specific members of the ionotropic glutamate receptor family — was recently proposed to explain the odor sensitivity of OSNs housed in coeloconic sensilla [46••] (Figure 2c). These advances in receptor cloning were important in allowing the field to understand the molecular basis of odor recognition in a given OSN, but shed little light on how these receptors actually couple odor recognition with OSN activation.

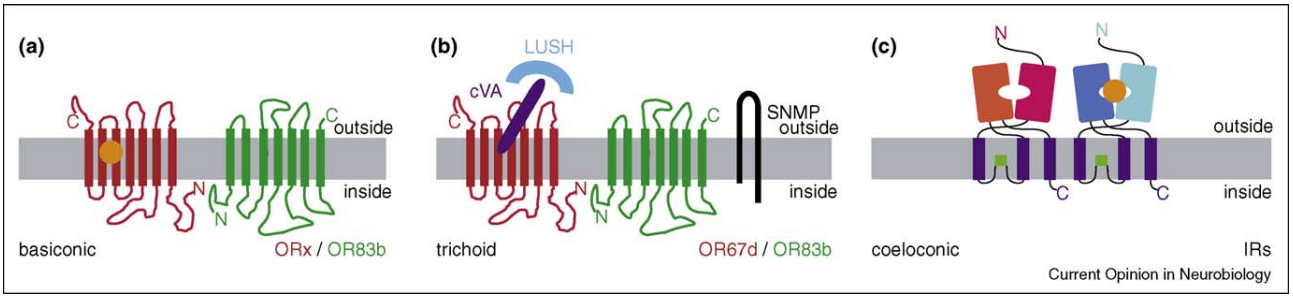
Insect odor transduction mechanisms probed through heterologous expression

To clarify how insect ORs are activated, multiple groups have turned to heterologous expression of these receptors in various cell types. Heterologous expression confers the benefits that ORs can be studied in isolation and subjected to pharmacological analysis, but conclusions must always be tempered because the receptors are not in their native environment in the insect OSN. Different groups chose different cell types — mammalian tissue culture cells or frog (*Xenopus laevis*) oocytes — and expressed ORs alone or with OR83b and with or without exogenous G proteins [37•,42,43,47–50,51••,52••].

Initially, several groups reported that insect ORs could function *in vitro* in the absence of the OR83b co-receptor but with high odor concentrations and the addition of exogenous G proteins (promiscuous $G_{\alpha}15$ or insect $G_{\alpha}q$) [47–50]. Upon addition of the appropriate odor, inward cation currents and an influx of extracellular calcium ions were detected [47–50]. Given the absolute requirement for OR83b for OR function *in vivo* [34••,40,41], the basis for how ORs function *in vitro* without OR83b is not currently understood. Some of these same groups later showed that cotransfection of ORs with OR83b significantly enhances the proportion of responding cells and also increases both odor sensitivity and the magnitude of the evoked response, even in the absence of exogenous $G_{\alpha}q$ [37•,42,43]. Touhara and colleagues [43] were the first to argue not only that OR/OR83b responses can occur in the absence of exogenous $G_{\alpha}q$ but also that the responses are biophysically different in the absence of G proteins.

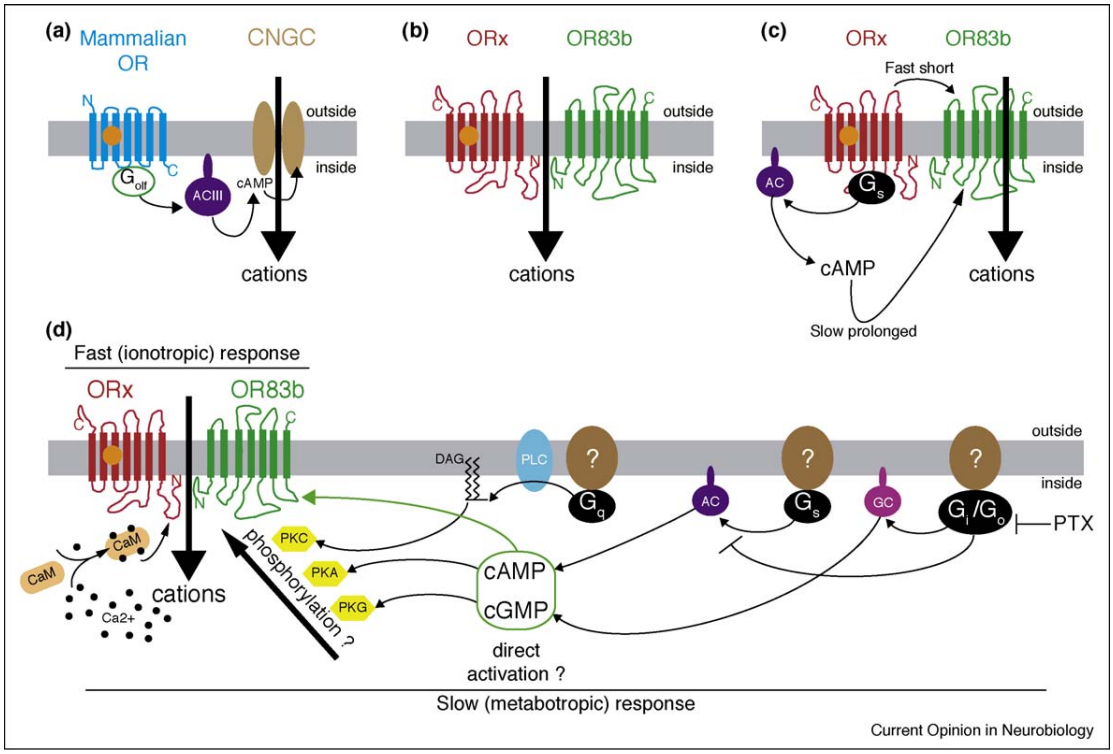
The most recent work in this field has directly questioned whether insect ORs function as GPCRs (Figure 3), and has yielded complex answers. Three groups, led by

Figure 2



Diverse chemosensory receptors mediating olfaction in insects. **(a)** Basiconic sensilla are tuned to carbon dioxide detection using Gr21a/Gr63a (not shown) and general odorant detection using OR/OR83b complexes. The general odorant is indicated by the orange dot and interacts with the OR subunit in the complex. **(b)** Pheromones are detected with distinct OR/OR83b complexes that act in concert with a CD36 homolog called SNMP [44•,45•]. For the detection of *cis*-vaccenyl acetate (cVA), a soluble odorant-binding protein called LUSH is required [55••]. cVA is indicated by the purple ellipse and interacts with the OR67d subunit in the complex. **(c)** A newly described family of chemosensory receptors is encoded by variant ionotropic receptors (IRs) [46••]. IRs are expressed in coeloconic sensilla that detect general odorants, small amines, and humidity. Ligands (indicated by the orange dot) are presumed to be bound by extracellular domains of these receptors, but the nature of the IR receptor complex and what subunit(s) bind ligands remain to be determined.

Figure 3



Models of insect olfactory receptor signal transduction. **(a)** Canonical G-protein signaling in the mammalian olfactory system (ACIII, adenylate cyclase III; CNGC, cyclic nucleotide-gated channel). **(b)** Sato *et al.* [51••] propose that insect ORs form ligand-gated nonselective cation channels activated rapidly by odors in the absence of G-protein signaling. **(c)** Wicher *et al.* [52••] propose that the variable ORx subunit is a G-protein-coupled receptor and that OR83b is a cyclic nucleotide-gated ion channel. Odor activation of ORx triggers two pathways, a fast short ionotropic pathway and a slow prolonged metabotropic pathway. The metabotropic pathway involves G_s coupling of ORx, leading to the production of intracellular cAMP, which activates OR83b. **(d)** Integrative model of insect olfactory signal transduction proposed in this review article. See text for details. *Abbreviations*: CaM, calmodulin; PKC, protein kinase C; PKA, protein kinase A; PKG, protein kinase G; PLC, phospholipase C; AC, adenylate cyclase; GC, guanylate cyclase.

Touhara and Vosshall [51••], Newcomb [37•], and Hansson [52••], expressed ligand-specific ORs from various insects along with the corresponding OR83b co-receptor in mammalian or insect tissue culture cells or frog eggs.

Sato *et al.* expressed multiple different ORs along with *Drosophila* OR83b or its ortholog from moth, and mosquito in various heterologous cell types [51••] and characterized a very fast ionotropic response that persisted in the presence of PLC inhibitors and GDPβS, a general inhibitor of G-protein signaling. This led these authors to conclude that odor-evoked currents mediated by insect ORs are independent of known G-protein-signaling pathways. While Sato *et al.* found no evidence for odor-stimulated cAMP production, they did note that particular OR complexes showed odor-independent cyclic nucleotide sensitivity. Further they showed that the specific subunit composition governs the biophysical properties of the OR/OR83b receptor, strongly suggesting that these proteins function as a complex to form an odor-gated ion channel whose initial activation does not depend on G-protein signaling (Figure 3b).

Working with *Drosophila* OR43b/OR83b, Smart *et al.* found that inhibitors of the $G_{\alpha}q$ pathway and the general G-protein inhibitor GDPβS did not block odor-evoked calcium increases, but changed the inactivation kinetics [37•]. This led the authors to conclude that while G-protein signaling is not required to activate the receptors, it may be involved in postactivation modulation.

In part agreeing with these results, Wicher *et al.* [52••] found that *Drosophila* OR22a/OR83b could be activated by odors to produce a very rapid ionotropic response that did not require G proteins. However, they also noted and studied in depth a considerably slower metabotropic response that depended on $G_{\alpha}s$ but did not involve $G_{\alpha}q$ pathways. Activation of the metabotropic pathway was shown to produce intracellular cAMP. OR83b was shown to be gated directly by cAMP or cGMP. Odor-evoked

currents persisted in the presence of a general inhibitor of G-protein signaling, GDPβS, but cells were less sensitive to odors. Thus this group concluded that ligand-binding ORs couple to $G_{\alpha}s$ to produce cyclic nucleotides, which in turn activate OR83b, which, they hypothesize, has the properties of a cyclic-nucleotide-gated nonselective cation channel. This model posits that the OR functions like a GPCR and that OR83b functions as an ion channel (Figure 3c), although direct interaction of insect ORs with G proteins remains to be shown.

Such dual activation is conceptually similar to the dual activation properties of moth OSNs found in early work by Zufall and Hatt [13] and Stengl [14], although the details of the relevant second messenger differ across all of these studies. Dual activation may work to extend the range of sensitivity to odors, with the ionotropic pathway operating at higher odor concentrations in a G-protein-independent manner and the metabotropic pathway being important to amplify signal strength at low odor concentrations. This would be consistent with the *in vivo* results from Kain *et al.*, which showed that eliminating $G_{\alpha}q$ signaling pathways reduced the sensitivity of OSNs but did not eliminate responses to odors [19••]. Although the time course of metabotropic activation found by Wicher *et al.* in heterologous cells was quite slow, it is conceivable that signaling is more rapid in native OSNs. For instance, it is possible that ORs and effector/modulator proteins can be physically linked in a signaling complex that affords more rapid activation than that achievable in heterologous cells. Strong evidence for such a signaling complex held together by scaffolding proteins has previously been shown for the *Drosophila* photoreceptor signaling system (see [53] for a review).

Prospects for a consensus model of insect olfactory signal transduction

How are we to reconcile all of these disparate findings? Is there a single unifying mechanism of insect olfactory signal transduction, or are there multiple pathways that depend on the specific OR and cell type being examined? In these concluding remarks, we summarize what we believe to be the points of consensus in the field and attempt to address the outstanding issues. The various possibilities for a consensus signaling model are schematized in Figure 3d.

While the proposal that insect ORs adopt a membrane topology opposite to GPCRs [34••] was initially greeted with skepticism, mounting structural [35•,36•,37•], and functional [37•,51••,52••] data now strongly suggest that insect ORs are a novel class of membrane receptor unrelated to GPCRs. There seems to be broad agreement that while the initial response to odors can occur in the absence of G-protein signaling [19••,37•,51••,52••], second messenger-mediated responses on a slower time scale may make these receptors more sensitive to odors [19••,37•,52••].

Since insect ORs have no homology to GPCRs, how would these proteins be integrated into a signaling framework that includes modulation by G proteins? A straightforward model to reconcile all of these data would posit that OR/OR83b forms a nonselective cation channel that fluxes cations including calcium upon odor binding by the ligand-specific OR in the subunit. This initial, rapid ionotropic response would be a property of the OR/OR83b protein alone (Figure 3b,c). The influx of calcium would trigger a slower metabotropic response to produce postactivation modulation of the OR/OR83b complex and increase sensitivity by increasing the open probability of the receptor (Figure 3c,d). Since it seems unlikely to us that the OR/OR83b complex directly interacts with G proteins, we are left with the problem of how G proteins are activated secondary to OR/OR83b activation. One possibility is that as yet undescribed membrane receptors (brown circles in Figure 3d) are costimulated by OR/OR83b activation, thus triggering conventional G-protein signaling. Another possibility is that a solely intracellular signaling network acts to stimulate G proteins directly. There are at present no available data to support either model and more work is needed to clarify exactly how and where G proteins act in insect olfactory signaling. Further, the conflicting evidence for an involvement of $G_{\alpha}q$ versus $G_{\alpha}s$ signaling pathways must be resolved. One possibility is that different OR/OR83b complexes couple to different signaling pathways. Alternatively, some of the observations in heterologous cells may not reflect the coupling properties of insect ORs *in vivo*.

Although many details are lacking, a blended ionotropic/metabotropic model would be consistent with the early observations made by Zufall and Hatt [13] and Stengl [14] on a pheromone-gated ion channel that has a rapid primary response not modulated by second messengers, and secondary responses that are sensitive to pharmacological perturbation of second messenger pathways. On the basis of subsequent work, it is conceivable that the AC_1 current is a property of a pheromone-sensitive OR/OR83b complex in the moth.

While neither the ORs nor OR83b has any obvious homology to cyclic-nucleotide-binding domains, they could conceivably have a novel nucleotide activation domain. Perhaps more likely, cyclic nucleotides could act indirectly by activating kinases that phosphorylate the OR/OR83b complex (Figure 3d). The intracellular domain of OR83b is enriched in multiple consensus phosphorylation sites by calcium-activated and cyclic-nucleotide-activated kinases (Pellegrino and Vosshall, unpublished data), making post-translational modification plausible for this receptor.

Such a model of blended ionotropic/metabotropic modulation is typical for ion channels as a class of signaling proteins. Nearly all ion channels are subject to extensive

post-translational modification by phosphorylation or lipid, nucleotide, or calcium–calmodulin binding to alter open probability and inactivation kinetics. For example, the TRPV1 capsaicin-sensitive ion channel is directly gated by chemicals and heat, but can be potentiated by GPCR-mediated second messenger pathways that either phosphorylate the ion channel or produce phosphoinositides that directly modulate TRPV1 (for review see [54]). Thus our consensus model is only surprising in light of the old and, as we argue, incorrect assumption that insect ORs are GPCRs.

Why might insects have evolved a noncanonical mechanism to translate odor binding to OSN activation? One obvious cost of an ionotropic mechanism is the loss of signal amplification provided by GPCRs. In contrast, an obvious benefit is the speed of signaling permitted by direct activation independent of second messenger pathways. Our consensus model would accommodate both modes of signaling, perhaps acting at different ranges of odor concentration.

Much remains to be done to provide truly convincing data for this integrated model of olfactory signal transduction. The evidence that the OR/OR83b complex forms an odor-gated ion channel is still quite preliminary. If these are ion channels, the ion-conducting pore must be identified, be it formed at the OR/OR83b interface (Figure 3b) or solely within OR83b (Figure 3c). Structural information on the stoichiometry of the OR complex as well as a high-resolution X-ray crystal structure will go a long way toward solving the ongoing debate on the mechanism of action of this class of receptors. If OR and OR83b are subject to post-translational modification subsequent to the initial ionotropic activation, these sites of modification must be identified by a comprehensive structure–function analysis. Finally, all of these hypotheses must be validated in an *in vivo* preparation with careful electrophysiology, pharmacology, and genetics. Exciting days lie ahead in this field.

Acknowledgements

Work in the authors' laboratory is supported by the National Institutes of Health (DC006711 and DC008600), the Howard Hughes Medical Institute, and funded in part by a grant to R Axel and LBV from the Foundation for the National Institutes of Health through the Grand Challenges in Global Health Initiative. The authors thank Kazushige Touhara, Dieter Wicher, and Bill Hansson for constructive comments on the manuscript.

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- • of special interest
- •• of outstanding interest

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