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REVIEW

The role of the coreceptor Orco in insect olfactory transduction

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Abstract Insects sense odorants with specialized odorant receptors (ORs). Each antennal olfactory receptor neuron expresses one OR with an odorant binding site together with a conserved coreceptor called Orco which does not bind odorants. Orco is necessary for localization of ORs to dendritic membranes and, thus, is essential for odorant detection. It forms a spontaneously opening cation channel, activated via phosphorylation by protein kinase C. Thereafter, Orco is also activated via cyclic adenosine monophosphate (cAMP). Orco forms homo—as well as heteromers with ORs with unknown stoichiometry. Contradictory publications suggest different mechanisms of olfactory transduction. On the one hand, evidence accumulates for the employment of more than one G protein-coupled olfactory transduction cascade in different insects. On the other hand, results from other studies suggest that the OR–Orco complex functions as an odorant-gated cation channel mediating ionotropic signal transduction. This review analyzes conflicting hypotheses concerning the role of Orco in insect olfactory transduction. In conclusion, in situ studies in hawkmoths falsify the hypothesis that Orco underlies odorant-induced ionotropic signal transduction in all insect species. Instead, Orco forms a metabotropically gated, slow cation channel which controls odorant response threshold and kinetics of the sensory neuron.

Keywords Insect olfaction · Odorant receptor · Pheromones · Ionotropic receptor · Metabotropic signal transduction cascade

Abbreviations

cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
GR	Gustatory receptor
I_i	Ionotropic current
I_m	Metabotropic current
I_t	Transduction current
IP_3	Inositol 1,4,5-trisphosphate
IR	Ionotropic receptor
OR	Odorant receptor
Orco	Olfactory receptor coreceptor
ORN	Olfactory receptor neuron
PKC	Protein kinase C
PLCβ	Phospholipase Cβ
SNMP	Sensory neuron membrane protein
TM	transmembrane domain

Introduction

Insect olfactory receptor neurons (ORNs) express different types of chemosensory receptor families which were termed ionotropic receptors (IRs), odorant receptors (ORs), or gustatory receptors (GRs) (Benton et al. 2009; Nakagawa and Vosshall 2009; Croset et al. 2010; Isono and Morita 2010; Abuin et al. 2011; Sato et al. 2011; Zhang et al. 2011; Getahun et al. 2012). On the insect antenna olfactory sensilla are innervated by two or more ORNs each (Fig. 1) (Altner and Prillinger 1980; Keil and Steinbrecht 1984). An ORN expresses one to three ligand-binding receptors (ORs)

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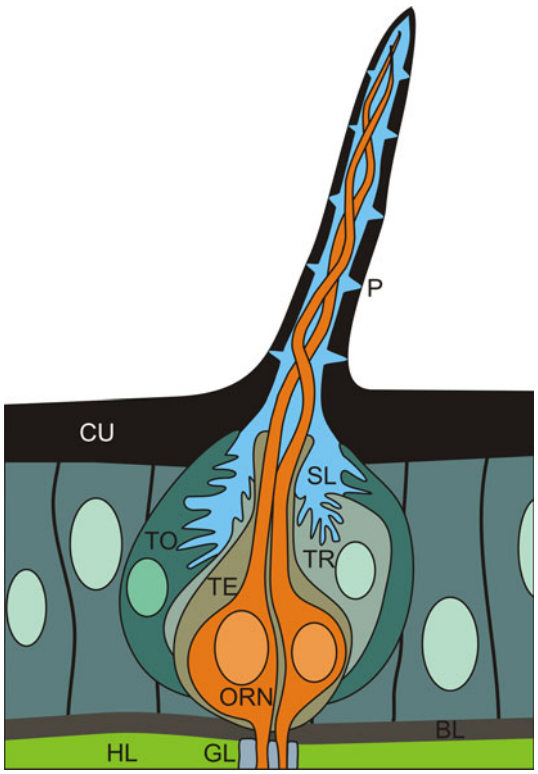

Fig. 1 Pheromone-sensitive trichoid sensillum. Two olfactory receptor neurons (ORNs) extend their outer dendrites into the sensillum lymph (SL) filled hairshaft. There, they contact pheromones which enter via the pores (P) in the cuticle (CU) of the hair-like sensillum. Supporting cells such as the tormogen (TO) and trichogen (TR) cells contribute to the structure of the sensillum and sensillum lymph contents. The thecogen cell (TE) isolates the inner dendrite and the soma of the ORN from the sensillum lymph, while a glia cell (GL) wraps the axon and isolates it from the hemolymph (HL), beyond the basal lamina (BL). Modified after Stengl 2010

together with one conserved ubiquitous coreceptor (Clyne et al. 1999; Vosshall et al. 1999; Krieger et al. 2002, 2003; Dobritsa et al. 2003; Elmore et al. 2003; Hallem et al. 2004a; Larsson et al. 2004; Couto et al. 2005; Goldman et al. 2005; Nakagawa et al. 2005; Benton et al. 2006; Hallem et al. 2006; Grosse-Wilde et al. 2010, 2011). While the classical ORs are extremely divergent seven-transmembrane domain (7TM) proteins, the coreceptor shares up to 94 % sequence identity with orthologues among insect species (Table 1) (Hill et al. 2002; Krieger et al. 2003; Pitts et al. 2004; Jones et al. 2005; Patch et al. 2009; Olafson 2013). Vosshall et al. (1999) first named the gene for this conserved receptor A45 and described it as a large (486 amino acids), more divergent member of the *Drosophila* OR-gene family with only 24 % sequence identity to the classical odorant receptors. In the fruit fly *Drosophila melanogaster* the protein encoded by A45 was named Or83b. In different insect species different names were assigned to the respective orthologues (Table 1). To simplify matters and to emphasize the importance of Or83b as coreceptor it was finally termed olfactory receptor–coreceptor: Orco (Vosshall and Hansson 2011). The authors suggested that the following requirements must be fulfilled to name a gene *orco*: The sequence identity to orthologues of other insect species is at least 50 %, mRNA and protein is expressed in the majority of chemosensory sensilla and the predicted protein size is larger than the size of conventional ORs, due to an insertion in the second intracellular loop.

In the majority of ORNs in different insect species Orco is expressed (Vosshall et al. 1999, 2000; Krieger et al. 2003; Larsson et al. 2004; Pitts et al. 2004; Jones et al. 2005; Nakagawa et al. 2005). In *D. melanogaster* it is present in apparently all OR-expressing ORNs that innervate trichoid and basiconic sensilla of the antennae and the maxillary palps, but it is neither expressed in coeloconic sensilla expressing IRs, nor in gustatory sensory neurons expressing GRs, nor in mechanosensory *Sensilla chaetica* (Larsson et al. 2004). In *Heliothis virescens* Orco is also expressed in trichoid and basiconic sensilla and in honey bees, additionally also in *Sensilla placodea* (Krieger et al. 2003). Furthermore, Orco is also expressed in gustatory tissues such as the proboscis and the legs of different mosquito species (Larsson et al. 2004; Melo et al. 2004; Pitts et al. 2004; Xia and Zwiebel 2006) and the proboscis of *H. virescens* (Krieger et al. 2002). However, it remains to be examined, if Orco is coexpressed with ORs in these gustatory tissues indicating an additional olfactory function of the tissues, or if Orco has a secondary function in gustation. So far, Orco appears to be specific for insect ORNs and no homologues for Orco or other members of the insect OR family have been found in crustaceans (Peñalva-Arana et al. 2009; Corey et al. 2013).

Orco is essential for dendritic localization of odorant receptors and, thus, is essential for odorant receptor-dependent odorant responses

While different genetic and physiological studies agreed that odorant response specificity of ORNs depends on the ligand-binding ORs (Dobritsa et al. 2003; Elmore et al. 2003; Hallem et al. 2004a, b) the role of the coexpressed Orco remained elusive. Orco was proposed to be important for the localization and stabilization of ORs in the dendritic membranes as membrane localization protein and possibly also as chaperon molecule allowing for correct protein folding of ORs (Larsson et al. 2004; Benton et al. 2006). Alternatively, it could also play a decisive role for the transient binding and transduction of odorants via a heteromeric OR–Orco receptor complex (Larsson et al. 2004). Evidence for a role of Orco as localization/stabilizing partner for ORs was provided first via mutant analysis. In *D. melanogaster* Orco null

Table 1 Orco orthologues of different insect species

Species	Original denotation	Accession number	Reference
Acyrthosiphon pisum	ApOr1	XM_001951611	Smadja et al. (2009)
Aedes aegypti	AaOr7	AY582943	Melo et al. (2004)
Aldrichina grahami	AgraOrco	HQ190955	Olafson (2013)
Anopheles gambiae	AgamGPRor7	AY363725	Hill et al. (2002)
Antheraea pernyi	AperR2	AJ555486	Krieger et al. (2003)
Apis mellifera	AmelR2	NM_001134943	Krieger et al. (2003)
Bactrocera dorsalis	BdOrco	EU621792	Zheng et al. (2012)
Bactrocera cucurbitae	Or83b	HM745934	Zheng et al. (2012)
Bombyx mori	BmorR2	AJ555487	Krieger et al. (2003)
Calliphora erythrocephala	CeryR2	AJ555538	Krieger et al. (2003)
Ceratitis capitata	CcOr83b	AY843206	Jones et al. (2005)
Chrysomya megacephala	CmegOrco	HQ315861	Olafson (2013)
Culex quinquefasciatus	CqOR7	DQ231246	Xia and Zwiebel (2006)
Diaphania indica	DiOR83	AB263114	Mitsuno et al. (2008)
Drosophila ananassae	DanaOrco	XM_001953308	Olafson (2013)
Drosophila melanogaster	Or83b	AY567998	Vosshall et al. (2000)
Drosophila yakuba	DyakOrco	XM_002096017	Olafson (2013)
Epiphyas postvittana	EpOR2	EU791887	Jordan et al. (2009)
Haematobia irritans irritans	HirrOrco	ACF21678	Olafson (2013)
Harpegnathos saltator	Hsal\Orco	EFN84180	Jones et al. (2011)
Helicoverpa armigera	OR83b	HQ186284	Zheng et al. (2012)
Helicoverpa assulta	HassOrco	EU057178	Yang et al. (2012)
Helicoverpa zea	HzOr83b	AY843204	Jones et al. (2005)
Heliothis virescens	HR2	AJ487477	Krieger et al. (2002)
Holotrichia oblita	HoblOrco	JF718662	Yang et al. (2012)
Holotrichia plumbea	Or83b	HQ110087	Zheng et al. (2012)
Locusta migratoria	LmigOrco	JN989549	Yang et al. (2012)
Lucilia sericata	LserOR1	HQ315862	Wang et al. (2012)
Manduca sexta	MsextaOR2	FJ546087	Patch et al. (2009)
Musca domestica	MdomOrco	JQ365179	Olafson (2013)
Mythimna separata	MsOR83	AB263111	Mitsuno et al. (2008)
Nasonia vitripennis	NvOr1	NM_001170994	Robertson et al. (2010)
Ostrinia nubilalis	OnOr2	GQ844877	Wanner et al. (2010)
Pediculus humanus corporis	PhumOrco	EEB12924	Yang et al. (2012)
Plutella xylostella	PxOR83	AB263117	Mitsuno et al. (2008)
Schistocerca gregaria	SgreOrco	JN989550	Yang et al. (2012)
Sitobion avenae	SaveOrco	GQ275379	Yang et al. (2012)
Spodoptera frugiperda	SfOR2		Smart et al. (2008)
Spodoptera litura	OR2	DQ845292	Zheng et al. (2012)
Stomoxys calcitrans	ScalOrco	EU622914	Olafson (2013)
Tenebrio molitor	TmolR2	AJ555539	Krieger et al. (2003)
Tribolium castaneum	*Tcas*Or16	AM689918	Abdel-Latief (2007)

mutants the localization of ORs to dendrites was severely disrupted in dorsal organs of larvae as well as antennae of *imagines* (Larsson et al. 2004). Rescue experiments restored the localization and maintenance of ORs at their dendritic localization. Thus, for both of these functions the presence of functional Orco was necessary (Larsson et al. 2004; Benton et al. 2006).

Since stable expression of ORs in ORN dendrites is essential for odorant detection, electrical responses of ORNs were impaired in Orco mutant flies as well as in flies

with RNAi-dependent knockdown of Orco (Larsson et al. 2004; Neuhaus et al. 2005). Not only in adult fruit flies odorant detection was impaired without Orco, but also Orco mutant larvae failed to respond to most odorants at the concentrations tested in chemotaxis experiments (Larsson et al. 2004). These olfactory impairments were restored in Orco-rescue experiments which allowed for localization and maintenance of ORs to dendritic membranes of ORNs. However, it remained to be determined whether Orco also is necessary for olfactory transduction.

Orco adopts an inverted membrane topology and heteromerizes with odorant receptors

Both, ORs and Orco are members of the large family of 7TM receptors. The canonical 7TM receptors couple to G proteins via a conserved binding motif also associated with the intracellular C-terminus. Computational and experimental evidence, however, suggested that ORs and Orco both adopt an inverse membrane topology with extracellular C-termini (Benton et al. 2006; Wistrand et al. 2006; Lundin et al. 2007; Smart et al. 2008; Guo and Kim 2010; Tsitoura et al. 2010). The OR–Orco receptor complexes appeared to associate via the conserved C-termini (TM4-TM7), possibly via interactions between the cytoplasmic loop IC3 of both proteins (Benton et al. 2006; Miller and Tu 2008; Harini and Sowdhamini 2012). Studies using heterologous expression systems showed that Orco as well as classical ORs from fruit flies occur as homo- and heteromers with unknown stoichiometry in the membrane (Neuhaus et al. 2005; German et al. 2013). These complexes are assumed to be associated further with other molecules such as sensory neuron membrane proteins (SNMPs) (Rogers et al. 1997, 2001a, b; Benton et al. 2007; Forstner et al. 2008; Jin et al. 2008; review: Vogt et al. 2009; German et al. 2013). The surprising inverted topology of ORs in addition to the heteromerization with Orco raised the question whether ORs really interact with G proteins during olfactory transduction. It remained to be determined, whether there are so far unknown intracellular G protein binding motifs present on ORs or Orco, or whether heteromeric OR–Orco complexes signal without the employment of G protein-dependent processes.

Orco is a non-specific cation channel that promotes spontaneous activity in olfactory receptor neurons

Independent of coexpression with ORs, Orco from different species formed a non-specific, spontaneously opening, Ca^{2+}-permeable cation channel in heterologous expression systems (Sato et al. 2008; Wicher et al. 2008; Jones et al. 2011; Sargsyan et al. 2011; Nolte et al. 2013). The ORNs from Orco-deficient mutant flies showed strongly diminished spontaneous activity and, therefore, Orco provides a dominant leak current. Such a leak current is a pacemaker current which drives hyperpolarized sensory neurons up to spike threshold and triggers spontaneous activity (Larsson et al. 2004; Benton et al. 2007; Deng et al. 2011). Since spontaneous membrane potential oscillations which underlie spontaneous activity are a prerequisite to temporal encoding, Orco might be necessary for temporal encoding in insect ORNs (Stengl 2010). "Temporal encoding", in contrast to "rate codes" carries information about odorant quality and quantity in the timing of the first spike within a population of neurons and not in the response rate of single neurons (Singer and Gray 1995; Laurent 2002; Junek et al. 2010; Nadasdy 2010; review: Stengl 2010). Thus, via affecting spontaneous activity of the ORNs, Orco could also affect response threshold and kinetics of odorant responses (Stengl 2010). The discovery of different Orco-dependent agonists and antagonists allowed to test this and other hypotheses of Orco function (Jones et al. 2011; Nichols et al. 2011; Pask et al. 2011, 2013 Bohbot and Dickens 2012; Chen and Luetje 2012; Jones et al. 2012; Taylor et al. 2012; Nolte et al. 2013; Röllecke et al. 2013). Employment of the Orco agonist VUAA1 (Jones et al. 2011) in trichoid sensilla of the hawkmoth *Manduca sexta* in situ revealed that Orco activation increases spontaneous activity as well as background activity between pheromone responses in pheromone-sensitive ORNs (Nolte et al. 2013). Furthermore, Orco-specific agonists and antagonists supported the notion that Orco determines spontaneous activity in ORNs of *Anopheles gambiae* (Jones et al. 2011, 2012) and *D. melanogaster* (Su et al. 2012).

In addition to Orco, ligand-binding ORs also affected spontaneous activity, since replacement or loss of ORs changed spontaneous activity patterns of ORNs in *D. melanogaster* (Dobritsa et al. 2003; Elmore et al. 2003; Hallem et al. 2004a). Furthermore, in heterologous expression systems *D. melanogaster* OR22a was spontaneously active (Wicher et al. 2008) and different *A. gambiae* ORs coexpressed with the same Orco-ion channel changed the sensitivity of the heteromeric complex to ion channel blockade (Nichols et al. 2011; Pask et al. 2013). While there is consensus that ORs and Orco directly interact it is not clear how the heteromeric complex functions (Nakagawa et al. 2012). It is still not resolved whether ORs affect the pore of Orco-ion channels indirectly, or whether they directly contribute to the ion channel pore. In addition, it is controversially discussed whether odorant binding to ORs gates the ion channel pore, allowing for an odorant-induced ionotropic signal transduction process (reviews: Nakagawa and Vosshall 2009; Stengl 2010).

Orco-ion channels are metabotropically regulated

While Orco was not gated directly via odorants, its open time probability was increased after application of membrane permeable cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) analogues (Wicher et al. 2008). The Orco-ion channel from *D. melanogaster* possesses five protein kinase C (PKC) phosphorylation sites, which control its cyclic nucleotide-sensitivity (Sargsyan et al. 2011). Mutation of all five PKC phosphorylation sites almost completely abolished its gating via cyclic nucleotides. Thus, only after PKC-dependent phosphorylation Orco is directly activated via cAMP (Sargsyan et al. 2011; Getahun et al. 2013). In addition, Orco can already be activated in the absence of cyclic nucleotides after activation of phospholipase Cβ (PLCβ) and after activation of PKC (Sargsyan et al. 2011; Getahun et al. 2013) and hence, it is sensitive to intracellular Ca^{2+} concentrations. It remains to be examined whether Orco from other species such as moths shows the same metabotropic regulation as demonstrated for *D. melanogaster*. In cockroaches and moths pheromones rapidly and transiently elevated intracellular inositol 1,4,5-trisphosphate (IP_3) levels, indicating metabotropic activation of PLCβ. The IP_3-dependent rises in intracellular Ca^{2+} then stimulate PKC (Breer et al. 1990; Stengl et al. 1992; Boekhoff et al. 1993; Stengl 1993, 1994). Activation of PKC could possibly result in the activation of Orco and, therefore, in elevated background activity of ORNs due to Orco activation (Stengl 2010; Nolte et al. 2013). Whether metabotropic activation of Orco affects the phasic and/or slower tonic component of the phasic-tonic odorant response of ORNs remains to be examined in different insect species in situ. In addition, it remains to be determined whether odorants also activate PLCβ in *D. melanogaster*.

Odorant receptors mediate odorant responses also in the absence of Orco

Several publications consistently reported that only classical ORs bind odorants and are essential for specificity of the heteromeric OR–Orco complex (Elmore et al. 2003; Nakagawa et al. 2005; Neuhaus et al. 2005; Sato et al. 2008; Wicher et al. 2008; Jones et al. 2011; Nichols et al. 2011; Pask et al. 2011; Chen and Luetje 2012). When ORs from different species were expressed without Orco in heterologous expression assays they evoked ligand-specific responses (Wetzel et al. 2001; Sakurai et al. 2004; Nakagawa et al. 2005; Neuhaus et al. 2005; Grosse-Wilde et al. 2006; Smart et al. 2008; Deng et al. 2011). However, rather high odorant concentrations in the μM or mM range with long odorant exposures over seconds were required. The ORs apparently coupled to coexpressed or cell-endogenous G proteins. While *Drosophila* Schneider 2 (S2) and *Spodoptera frugiperda* 9 (SF9) cells express an endogenous Orco this is not the case for other cell lines used such as human embryonic kidney 293 (HEK293) cells (Kiely et al. 2007; Kiely 2008; Smart et al. 2008). Thus, Orco is not essential for odorant detection if ORs are successfully inserted in the plasma membrane. Nevertheless, odorant responses were enhanced, if Orco was coexpressed with general ORs (Nakagawa et al. 2005; Neuhaus et al. 2005; Smart et al. 2008). It remains to be determined, whether this Orco-dependent increase in sensitivity was due solely to the more frequent and more stable membrane insertion of ORs. Alternatively, it was hypothesized that OR–Orco heteromers are directly involved in olfactory transduction as odorant-gated ion channels (review: Nakagawa and Vosshall 2009).

Ionotropic versus metabotropic mechanisms of olfactory transduction

Odorant-dependent ionotropic signal transduction implies that the receptor which binds odorants is an ion channel that changes its open time probability upon odorant binding. A current is generated resulting in the odorant-dependent receptor potential. Ionotropic receptors such as auditory receptors are selected for speed and mediate electrical responses in the microsecond range. In contrast, metabotropic receptors which couple to G proteins operate on a different time scale and mediate responses in the millisecond range. They modify enzyme activities to change second messenger levels and are selected for sensitive signal detection, signal amplification, as well as expansion of the response range.

The employment of odorant-dependent ionotropic transduction pathways in different insects is still controversially discussed because, even in the same species, findings contradict each other. Studies mostly with focus on *D. melanogaster* were the first to provide evidence for OR–Orco heteromers as directly ligand-gated ion channels which underlie an ionotropic mechanism of olfactory transduction. The ORs from *A. gambiae* (AgamORs), *Bombyx mori* (BmorORs), and *D. melanogaster* (DmelORs) together with respective Orcos were heterologously expressed and patch clamp recordings as well as Ca^{2+} imaging studies were performed (Sato et al. 2008; Smart et al. 2008; Wicher et al. 2008). Even in the absence of odorant stimulation the receptor complexes as well as Orco alone mediated spontaneous Ca^{2+} influx reminiscent of receptor-dependent spontaneous activity of insect ORNs (de Bruyne et al. 1999, 2001; Dobritsa et al. 2003; Hallem et al. 2004a, 2006). The authors concluded that Orco

homomers as well as OR–Orco heteromers form spontaneously active ion channels. Application of odorants (10 μM bombykol, 100 μM pentyl acetate, 100 μM 2-methyl phenol, second-long stimulation) elicited a non-selective cation conductance only in the presence of Orco and ORs together (Sato et al. 2008). Thus, either, odorant binding to ORs increased the open probability of this OR–Orco receptor–ion channel complex, or, alternatively, odorant binding activated a metabotropic cascade which changed second messenger levels and thereby activated second messenger-dependent ion channels. To determine whether the receptor complex signals via G protein-coupled cascades, different pharmacological experiments were performed. In HeLa cells expressing DmelOR47a + DmelOrco no current was elicited in whole-cell patch clamp experiments with cAMP, cGMP, or IP_3 included in the patch pipette. In addition, no Ca^{2+} rises were elicited with membrane permeable cyclic nucleotide analogues in Ca^{2+} imaging experiments on HEK293T cells expressing DmelOR47a + DmelOrco or BmorOR-1 + BmorOrco. Moreover, after odorant stimulation no increases in intracellular cAMP were observed (Sato et al. 2008). Antagonists of metabotropic cascades such as U73122 (PLC antagonist) and GDP-βS (non-hydolysable form of GDP which inhibits G protein signaling) did not affect odorant-evoked currents through DmelOR47a + DmelOrco or BmorOR-1 + BmorOrco, respectively (Sato et al. 2008), and only moderately changed responses to 1 mM ethyl butyrate in DmelOR43b + DmelOrco transfected heterologous cells (Smart et al. 2008). Also in single sensillum recordings performed on *D. melanogaster* interference with metabotropic cascades only caused minor changes in odorant responses of different ORNs (ab1a, ab2a, or ab3a) (Yao and Carlson 2010). From these findings, it was concluded that the OR–Orco complex does not couple to G proteins. It remained to be determined whether conditions chosen for the pharmacological studies such as intracellular Ca^{2+} concentrations prevented respective activation of second messenger-dependent ion channels in the expression systems. In addition, it remained unknown whether parallel metabotropic cascades could substitute for the interruption of only one metabotropic signal transduction cascade.

To further challenge the hypothesis that OR–Orco heteromers form non-selective ion channels gated by odorants directly, the response kinetics of OR heteromultimers were examined in HeLa cells expressing DmelOR47a + DmelOrco or AgamOR2 + AgamOrco in a combination of Ca^{2+} imaging and patch clamp studies (Sato et al. 2008). The Ca^{2+} response latency to odorant stimulation was 240 ± 46 ms and the latency of the current response was 17.9 ± 3.1 ms for DmelOR47a + DmelOrco while the current response for AgamOR2 + AgamOrco was 28.5 ± 1.9 ms. These kinetics match the fastest metabotropic signal transduction cascades obtained when receptors, enzymes, and ion channels are tightly linked in signalosomes as shown for visual signal transduction (Hardie and Raghu 2001). To further examine whether the heteromeric receptor complex has ion channel properties and determines ion selectivity it was studied whether various ion channel blockers affect different OR–Orco complexes in a different way (Sato et al. 2008; Nichols et al. 2011; Pask et al. 2011, 2013; Röllecke et al. 2013). Since both bombykol-dependent inward currents and baseline Ca^{2+} levels were blocked by ruthenium red in *Xenopus* oocytes expressing BmorOR-1 + BmorOrco, but not in HEK293T cells expressing AgamOR2 + AgamOrco it was concluded that different OR–Orco complexes have different ion channel properties (Sato et al. 2008). This hypothesis was further confirmed in outside-out patch clamp studies employing oocyte and HEK293T membranes expressing DmelOR47a + DmelOrco, or AgamOR2 + AgamOrco (Sato et al. 2008). Currents were elicited with a slope conductance of 27 pS at −60 mV for DmelOR47a + DmelOrco and 20 pS for AgamOR2 + AgamOrco in an odorant-dependent fashion. No difference in the odorant-dependent currents was observed with or without ATP (1 mM)/GTP (100 μM) in the patch clamp pipette. From these studies, Sato et al. (2008) concluded that insect heteromeric OR–Orco complexes form ligand-gated ion channels underlying an ionotropic signal transduction cascade without any involvement of metabotropic cascades (Fig. 2a).

While there is general consensus that a heteromeric OR–Orco complex of unknown stoichiometry is formed there is no agreement on how this complex functions and whether it is employed for olfactory transduction in vivo, since non-physiologically high odorant concentrations were necessary to elicit odorant-dependent currents. Alternatively to Sato et al. (2008), Wicher et al. (2008) proposed that odorant stimulation of HEK293 cells heterologously expressing DmelOR22a + DmelOrco elicited a less sensitive ionotropic current (I_i) followed by a more sensitive metabotropic current (I_m) (Fig. 2b). The I_i did not rely on application of ATP and GTP, activated rapidly, reached its maximum current at 1 s and terminated at 10 s. The slower I_m activated after about 10 s, peaked at 60 s and terminated at 80 s. The I_i required higher odorant concentrations (0.1 μM) compared to I_m (1 nM). It was concluded that I_i is an ionotropic current due to odorant-dependent gating of the OR–Orco receptor–ion channel complex. It was suggested that next to I_i activation odorant binding to ORs in the heteromeric complexes activates a G protein cascade which increases intracellular cAMP levels (Wicher et al. 2008). The authors showed that cAMP levels rise after odorant stimulation in HEK293 cells expressing DmelOR22a + DmelOrco. Furthermore, cAMP could activate Orco homomers as well as heteromeric OR–Orco complexes. Thus, it

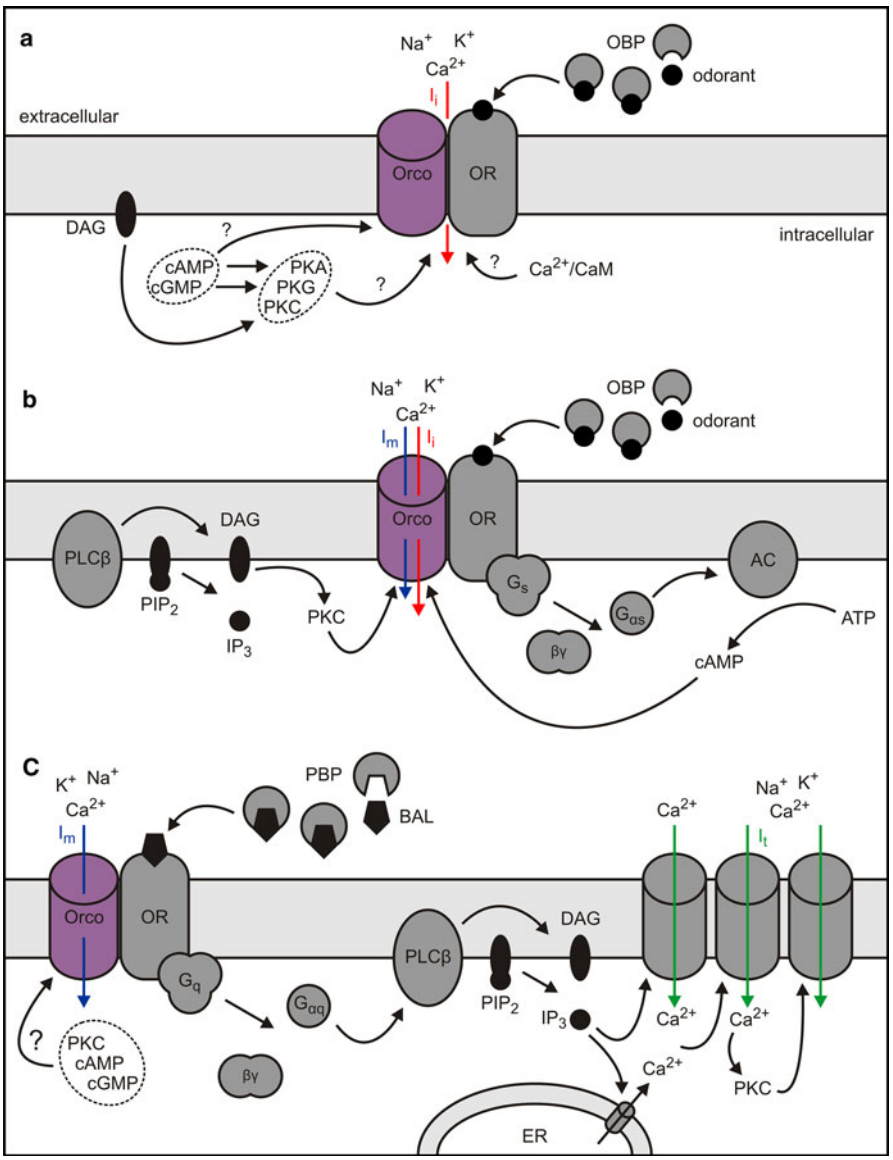

Fig. 2 Three different hypotheses of insect olfactory transduction are suggested mostly based upon work in the fruit fly (**a, b**) or the hawkmoth (**c**). **a** Solely ionotropic cascade possibly modulated metabotropically. The non-specific cation channel Orco heteromerizes with odorant receptors (ORs) and forms an odorant-gated receptor–ion channel complex underlying an ionotropic signal transduction process. Odorant binding to ORs triggers a current (I_i) which passes a pore formed by OR and Orco together (Sato et al. 2008). The ionotropic current is assumed to be modulated via metabotropic cascades of unknown metabotropic receptors which determine sensitivity and kinetics of the ionotropic odorant response (Nakagawa and Vosshall 2009). **b** Parallel ionotropic and metabotropic cascade. Odorant binding to heteromeric OR–Orco receptor–ion channels triggers first a less sensitive, faster ionotropic transduction current (I_i) and in parallel elicits a more sensitive, slower metabotropic transduction current (I_m) (Wicher et al. 2008). Odor-dependently the receptor complex couples to the trimeric G protein G_s activating adenylyl cyclases (AC). Increasing cAMP concentrations then activate Orco and elicit I_m only after previous phosphorylation of Orco via PKC. In addition, I_m is triggered via unknown phospholipase Cβ (PLCβ) activation PKC-dependently (Sargsyan et al. 2011). **c** Solely metabotropic cascade. Pheromone binding protein (PBP) controls transient pheromone binding to ORs which activates a G_q protein which in turn activates phospholipase Cβ (PLCβ) (review: Stengl 2010). The PLCβ-dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) generates inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The IP_3 rise increases intracellular Ca^{2+} concentrations first via rapid and transient activation of an IP_3-dependent Ca^{2+}-channel. The rapid Ca^{2+}-influx then gates Ca^{2+}-dependent cation channels which underlie the combined transient transduction current (I_t). Strong or long Ca^{2+} rises together with DAG activate a PKC. The PKC activates cation channels (I_t) and is assumed to phosphorylate Orco thereby increasing its conductance (I_m) and cyclic nucleotide-dependency. The I_m then changes spontaneous and background activity and thereby affects odorant response kinetics and sensitivity since it controls the membrane potential and the intracellular Ca^{2+} concentration (Stengl 2010; Nolte et al. 2013). *Question marks* indicate hypotheses without direct experimental evidence. *BAL* bombykal, *Ca^{2+}/CaM* Ca^{2+}/calmodulin, *ER* endoplasmatic reticulum, *OBP* odorant binding protein, *PKA* protein kinase A, *PKG* protein kinase G

was suggested that odorants activated G proteins which stimulate adenylyl cyclases, and that cAMP directly activated Orco resulting in I_m (Wicher et al. 2008). Interestingly, cAMP only increased the open time probability of Orco, if Orco was previously phosphorylated by PKC and activation of PLCβ or PKC was sufficient to activate Orco (Sargsyan et al. 2011; Getahun et al. 2013). The involvement of a PLCβ in fruit fly olfactory transduction is supported by the observation that $G_{\alpha q}$ deletion impaired odorant responses in the fruit fly (Kain et al. 2008). In addition, the involvement of adenylyl cyclase in olfactory transduction was confirmed by Deng et al. (2011), who also found evidence for other G protein-dependent cascades involved in fruit fly olfactory transduction.

Concerning the involvement of G protein cascades in fruit fly olfaction, more studies are necessary to resolve the contradictions between different publications. Possibly, different odorants and thus, different ORs might couple to different G proteins in various insect species (Breer et al. 1990; Ziegelberger et al. 1990; Boekhoff et al. 1993; Wicher et al. 2008). Alternatively, odorant- and G protein-dependent enzymes might require specific Ca^{2+} concentrations, which were not provided in respective experimental settings, as e.g. in the study by Sato et al. (2008) who used much lower Ca^{2+} concentrations than Wicher et al. (2008). It is possible that under these experimental conditions adenylyl cyclases could not be activated via odorant applications. In addition, studies failing to detect cAMP-dependent activation of Orco (Sato et al. 2008; Jones et al. 2011) possibly employed conditions which prevented phosphorylation of Orco required for its activation via cyclic nucleotides (Sargsyan et al. 2011). More experiments are necessary to further examine whether in *D. melanogaster* ORs couple to both PLCβ as well as to adenylyl cyclase signaling, or whether stress-dependently, or depending on odorant concentrations and behavioral state, different transduction cascades are employed as shown in moths (Stengl 2010).

In conclusion, based upon many studies from different laboratories in various insect species evidence is increasing that heteromeric OR–Orco receptor complexes couple to G proteins (Fig. 2b, c) and, thus, employ metabotropic cascades in insect olfaction (review: Stengl 2010). While there is agreement that Orco and ORs form a heteromeric receptor–ion channel complex it is still under debate whether this complex serves an ionotropic pathway of olfactory transduction in vivo.

Do insects employ odorant-induced ionotropic signal transduction in vivo?

The odorant-elicited Orco-dependent ionotropic currents (Fig. 2a, b, I_i) peaked and terminated in the range of seconds (Wicher et al. 2008). Thus, both Orco-dependent current components (I_i and I_m in Wicher et al. 2008; Fig. 2b) did not match time courses of phasic ORN responses in vivo which encode odorant quality and quantity within less than 100 ms (review: Stengl 2010). The slow time course of the ionotropic currents and the requirement of non-physiologically high odorant and pheromone doses in vitro (Sato et al. 2008; Smart et al. 2008; Wicher et al. 2008) could indicate that OR–Orco receptor–ion channel complexes do not change their conductance in the first 100 ms of the electrical odorant response in vivo. Possibly, Orco complexes serve another function such as gain control or modulation of the odorant response kinetics in a later time window of olfactory transduction (Stengl 2010).

To determine whether Orco plays a role for pheromone transduction in vivo in the intact hawkmoth *M. sexta*, tip-recordings from pheromone-sensitive trichoid sensilla were performed (Nolte et al. 2013). It was reasoned that perfusion of the Orco agonist VUAA1 (Jones et al. 2011) into the trichoid sensillum would potentiate responses to pheromone stimuli (bombykal) if Orco is indeed a functional part of a heteromeric pheromone receptor complex in vivo. Odorant binding to ORs would add up to VUAA1-binding to Orco, if both activate Orco-ion channels in the OR–Orco complex. Unexpectedly, no evidence for an Orco-dependent ionotropic mechanism was found in the hawkmoth. Despite the fact that VUAA1 activated hawkmoth-specific Orco in heterologous expression systems, it did not affect pheromone responses within the first 1,000 ms of each pheromone response (Nolte et al. 2013). However, VUAA1 elevated the background activity between pheromone applications within several seconds to minutes after pheromone stimulation, matching the slow time courses of odorant-dependent Orco activation in heterologous expression systems. In addition, the spontaneous activity of non-stimulated pheromone-sensitive ORNs was rapidly and strongly increased via VUAA1-dependent activation of Orco in the intact hawkmoth hinting at a function of Orco in the modulation of odorant response threshold and kinetics. Previously, it was shown that pheromone application activated a specific sequence of pheromone-dependent currents in the hawkmoth, which could be mimicked via IP_3 inclusion in the patch pipette (Stengl et al. 1992; Stengl 1993, 1994, 2010). First, a very transient pheromone-dependent current was activated which matched properties of an IP_3-dependent calcium current which declined within less than 50 ms (Stengl 1994). Nolte et al. (2013) now demonstrated that this first rapid pheromone-dependent current is not due to an Orco-dependent ionotropic current. Also, the second pheromone-dependent inward current does not mimic the properties of Orco, since it is activated and inactivated Ca^{2+}-dependently within several seconds. Rather, this

pheromone-dependent current appears to be a Ca^{2+}-dependent cation current which is gated by the influx of Ca^{2+} via the IP_3-dependent calcium current (Stengl 1993, 1994). Finally, the third pheromone-dependent inward current is gated via PKC-activation within seconds to minutes. It remains to be examined whether it is based upon Orco (Stengl 1993, 1994). Therefore, *M. sexta* appears to employ only metabotropic but no ionotropic mechanisms in pheromone transduction via pheromone-dependent activation of PLCβ (Fig. 2c). The PLCβ activation initiates the sequence of second messenger-dependent ion channels (as described above) which underlie the transduction current (I_t, Fig. 2c). Furthermore, the rise in intracellular Ca^{2+} then might upregulate Orco activity (I_m, Fig. 2c), possibly PKC-dependently, as shown for the fruit fly (Stengl et al. 1992; Stengl 1993, 1994, 2010; Sargsyan et al. 2011; Getahun et al. 2013; Nolte et al. 2013). Whether this metabotropic activation of Orco in the hawkmoth is mediated via the stress hormone octopamine supporting cAMP-dependent sensitization or via cGMP mediating adaptation still remains to be examined (Stengl 2010; Getahun et al. 2013). In addition, circadian clock-dependent mechanisms also appeared to regulate Orco activity since VUAA1-dependent activation of Orco differed daytime-dependently in hawkmoth ORNs (Schuckel et al. 2007; Nolte et al. 2013). It remains to be examined whether this daytime-dependent modulation of Orco activity is due to daytime-dependent activity of PLCβ or PKC, and/or due to circadian control of *orco* expression. In conclusion, it appears unlikely that Orco mediates odorant-induced ionotropic signal transduction in the hawkmoth in situ (Fig. 2c). Instead, in situ Orco might underlie slower processes of olfactory sensitization or adaptation of the odorant response, due to its metabotropic modulation (Stengl 2010; Getahun et al. 2013). It remains to be examined with in vivo experiments whether the same odorant-induced metabotropic signal transduction and the same role for Orco as slow pacemaker channel and not as rapid ionotropically gated channel also holds for other insects.

Selection pressure for insect olfactory transduction cascades favors metabotropic receptors

In the evaluation of different hypotheses of insect olfactory transduction one of the most important questions to ask is which evolutionary pressures shaped it. Was the predominant selection pressure for insect olfactory systems to maximize reaction speed up to the microsecond range, or to maximize sensitivity and response range expansion or was it essential to obtain both? Selection pressure should shape sex-pheromone detection, which is best studied in moths (Martin et al. 2011; Montagne et al. 2012). It could be reasoned that possibly odorant-induced ionotropic signal transduction is an adaptation to fast flight in insects. Although insects do not fly as fast as some birds, they reach higher speeds than slowly sliding slugs or the average speed of a walking four legged animal. For example, hawkmoths are fast and elegant flyers. The upwind flying hawkmoth that searches for the pheromone emitting female reaches velocities of about 3.5 m/s and its wing beat frequency is about 30 Hz (Tripathy et al. 2010). In flying insects both, flight velocity and wing beat frequency determine the sampling rate of odorants. With each downstroke of the wing the airflow between the brush of hair-like olfactory sensilla on the antenna is accelerated and odorant-carrying air pockets are exchanged. Thus, insects "sniff" periodically and can sample odorants about every 30 ms depending on their wing beat frequency (Justus et al. 2005; Ito et al. 2008; Tripathy et al. 2010). Sitting or walking insects might move and flick their antennae comparably to crustaceans, which sample at about 4 Hz (Atema 1995). The resulting intermittency of the odorant signal is a critical prerequisite for eliciting behavioral responses. Only an intermittent pheromone signal but not a continuous stimulation elicits arousal in the male moth and triggers and maintains the characteristic zig-zagging anemotaxis (Kennedy et al. 1981; Murlis and Jones 1981; Baker et al. 1988; Vickers and Baker 1992; Vickers 2000; Koehl 2006; Lei et al. 2009). Air turbulences twirl air currents into filaments of widely varying durations and wind velocities move the pheromone packages fast away from the pheromone pulse-emitting females. Therefore, the distance to the female moth is encoded in the mean frequency of pheromone filaments rather than in a gradual gradient of pheromone concentration. Apparently, male moths can distinguish the species-specific pheromone blend from wrong blend ratios within less than 100 ms comparing two consecutive "sniffs" with their antennae (review: de Bruyne and Baker 2008). In addition, during their zig-zagging upwind flight within 300 to 500 ms they change their flight pattern to cross wind-casting upon loss of pheromone detection (Baker et al. 1988). While little is known about required kinetics in other insects, the fastest response time required for the most important tasks of the moths' olfactory system is in the range of about 30 to 100 ms but not in the range of microseconds. Thus, these requirements for the olfactory system of insects make selection pressure for ionotropic processes unlikely.

In contrast, for insects as well as for other species it is extremely important to maximize odorant sensitivity and to expand the response range of ORNs. It was calculated that silkmoth ORNs can detect single pheromone molecules in a background of many different odorants (Kaissling and Priesner 1970; Kaissling 1987). Thus, insect olfaction maximized sensitivity especially of sex-

pheromone detection as the most important intra-specific odorant signal. Furthermore, the male's pheromone-sensitive ORNs have to span a wide range of pheromone concentrations. Male moths need to detect single pheromone molecules at great distances to the emitting female and they should not completely adapt and turn smell-blind when they touch the female's abdominal pheromone gland. Indeed, *M. sexta* trichoid sensilla innervating ORNs can distinguish pheromone concentrations over at least four log-units (Dolzer et al. 2003). They can sensitize as well as adapt, thereby enlarging their response range even further (Dolzer et al. 2003; Flecke et al. 2006, 2010; Flecke and Stengl 2009; Stengl 2010). Therefore, the olfactory system of moths is equipped to detect and distinguish odorant blends over a very wide concentration range and to allow for reaction velocities within the range of 30 ms. For the evolution of olfactory transduction mechanisms in insects and also in vertebrates it is apparent that selective pressures favored metabotropic over ionotropic receptors.

Conclusions

While there is general agreement that the highly conserved Orco locates and maintains ORs in the dendritic membrane of the sensory neurons, still there is no agreement how Orco functions during olfactory transduction in vivo. Accumulating evidence is provided for G protein-coupling of ligand-binding ORs but not of Orco, therefore, falsifying the hypothesis of Fig. 2a that all insects employ solely odorant-induced ionotropic signal transduction (Boekhoff et al. 1990, 1993; Breer et al. 1990; Laue et al. 1997; Wegener et al. 1997; Wetzel et al. 2001; Grosse-Wilde et al. 2006; Kain et al. 2008; Wicher et al. 2008; Chatterjee et al. 2009; Stengl 2010; Deng et al. 2011). In vivo experiments in the hawkmoth *M. sexta* showed that Orco activation during pheromone stimulation does not affect pheromone transduction, but shapes the spontaneous action potential rates and the tonic background activity between pheromone stimuli (Nolte et al. 2013). Thus, at least in the hawkmoth, the hypothesis of Fig. 2b is falsified. There is no evidence for a pheromone-induced ionotropic signal transduction pathway employing heteromeric OR–Orco complexes. Rather, Orco is a metabotropically regulated pacemaker channel (Fig. 2c) which affects odorant detection threshold and kinetics of the odorant response (Stengl 2010; Getahun et al. 2013; Nolte et al. 2013). Further in vivo experiments are needed to determine whether next to hawkmoths also the fruit fly and other insect species do not use odorant-induced ionotropic signal transduction under natural conditions with physiological odorant stimuli.

References

Abdel-Latief M (2007) A family of chemoreceptors in *Tribolium castaneum* (Tenebrionidae: Coleoptera). PLoS One 2(12):e1319

Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R (2011) Functional architecture of olfactory ionotropic glutamate receptors. Neuron 69(1):44–60

Altner H, Prillinger L (1980) Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. Int Rev Cytol 67:69–139

Atema J (1995) Chemical signals in the marine environment: dispersal, detection, and temporal signal analysis. Proc Natl Acad Sci USA 92(1):62–66

Baker TC, Hansson BS, Lofstedt C, Lofqvist J (1988) Adaptation of antennal neurons in moths is associated with cessation of pheromone-mediated upwind flight. Proc Natl Acad Sci USA 85(24):9826–9830

Benton R, Sachse S, Michnick SW, Vosshall LB (2006) Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. PLoS Biol 4(2):e20

Benton R, Vannice KS, Vosshall LB (2007) An essential role for a CD36-related receptor in pheromone detection in *Drosophila*. Nature 450(7167):289–293

Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB (2009) Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 136(1):149–162

Boekhoff I, Strotmann J, Raming K, Tareilus E, Breer H (1990) Odorant-sensitive phospholipase C in insect antennae. Cell Signal 2(1):49–56

Boekhoff I, Seifert E, Göggerle S, Lindemann M, Krüger BW, Breer H (1993) Pheromone-induced second-messenger signaling in insect antennae. Insect Biochem Mol Biol 23(7):757–762

Bohbot JD, Dickens JC (2012) Odorant receptor modulation: ternary paradigm for mode of action of insect repellents. Neuropharmacology 62(5–6):2086–2095

Breer H, Boekhoff I, Tareilus E (1990) Rapid kinetics of second messenger formation in olfactory transduction. Nature 345(6270):65–68

Chatterjee A, Roman G, Hardin PE (2009) G_o contributes to olfactory reception in *Drosophila melanogaster*. BMC Physiol 9:22

Chen S, Luetje CW (2012) Identification of new agonists and antagonists of the insect odorant receptor co-receptor subunit. PLoS One 7(5):e36784

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron 22(2):327–338

Corey EA, Bobkov Y, Ukhanov K, Ache BW (2013) Ionotropic crustacean olfactory receptors. PLoS One 8(4):e60551

Couto A, Alenius M, Dickson BJ (2005) Molecular, anatomical, and functional organization of the *Drosophila* olfactory system. Curr Biol 15(17):1535–1547

Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R (2010) Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genet 6(8):e1001064

de Bruyne M, Baker TC (2008) Odor detection in insects: volatile codes. J Chem Ecol 34(7):882–897

de Bruyne M, Clyne PJ, Carlson JR (1999) Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J Neurosci 19(11):4520–4532

de Bruyne M, Foster K, Carlson JR (2001) Odor coding in the *Drosophila* antenna. Neuron 30(2):537–552

Deng Y, Zhang W, Farhat K, Oberland S, Gisselmann G, Neuhaus EM (2011) The stimulatory $G\alpha_s$ protein is involved in olfactory signal transduction in *Drosophila*. PLoS One 6(4):e18605

Dobritsa AA, van der Goes van Naters W, Warr CG, Steinbrecht RA, Carlson JR (2003) Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 37(5):827–841

Dolzer J, Fischer K, Stengl M (2003) Adaptation in pheromone-sensitive trichoid sensilla of the hawkmoth *Manduca sexta*. J Exp Biol 206(Pt 9):1575–1588

Elmore T, Ignell R, Carlson JR, Smith DP (2003) Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. J Neurosci 23(30):9906–9912

Flecke C, Stengl M (2009) Octopamine and tyramine modulate pheromone-sensitive olfactory sensilla of the hawkmoth *Manduca sexta* in a time-dependent manner. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195(6):529–545

Flecke C, Dolzer J, Krannich S, Stengl M (2006) Perfusion with cGMP analogue adapts the action potential response of pheromone-sensitive *sensilla trichoidea* of the hawkmoth *Manduca sexta* in a daytime-dependent manner. J Exp Biol 209(Pt 19):3898–3912

Flecke C, Nolte A, Stengl M (2010) Perfusion with cAMP analogue affects pheromone-sensitive trichoid sensilla of the hawkmoth *Manduca sexta* in a time-dependent manner. J Exp Biol 213(Pt 5):842–852

Forstner M, Gohl T, Gondesen I, Raming K, Breer H, Krieger J (2008) Differential expression of SNMP-1 and SNMP-2 proteins in pheromone-sensitive hairs of moths. Chem Senses 33(3): 291–299

German PF, van der Poel S, Carraher C, Kralicek AV, Newcomb RD (2013) Insights into subunit interactions within the insect olfactory receptor complex using FRET. Insect Biochem Mol Biol 43(2):138–145

Getahun MN, Wicher D, Hansson BS, Olsson SB (2012) Temporal response dynamics of *Drosophila* olfactory sensory neurons depends on receptor type and response polarity. Front Cell Neurosci 6:54

Getahun MN, Olsson SB, Lavista-Llanos S, Hansson BS, Wicher D (2013) Insect odorant response sensitivity is tuned by metabotropically autoregulated olfactory receptors. PLoS One 8(3):e58889

Goldman AL, van der Goes van Naters W, Lessing D, Warr CG, Carlson JR (2005) Coexpression of two functional odor receptors in one neuron. Neuron 45(5):661–666

Grosse-Wilde E, Svatoš A, Krieger J (2006) A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem Senses 31(6):547–555

Grosse-Wilde E, Stieber R, Forstner M, Krieger J, Wicher D, Hansson BS (2010) Sex-specific odorant receptors of the tobacco hornworm *Manduca sexta*. Front Cell Neurosci 4:22

Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wicher D, Hansson BS (2011) Antennal transcriptome of *Manduca sexta*. Proc Natl Acad Sci USA 108(18):7449–7454

Guo S, Kim J (2010) Dissecting the molecular mechanism of *Drosophila* odorant receptors through activity modeling and comparative analysis. Proteins 78(2):381–399

Hallem EA, Ho MG, Carlson JR (2004a) The molecular basis of odor coding in the *Drosophila* antenna. Cell 117(7):965–979

Hallem EA, Nicole Fox A, Zwiebel LJ, Carlson JR (2004b) Olfaction: mosquito receptor for human-sweat odorant. Nature 427(6971): 212–213

Hallem EA, Dahanukar A, Carlson JR (2006) Insect odor and taste receptors. Annu Rev Entomol 51:113–135

Hardie RC, Raghu P (2001) Visual transduction in *Drosophila*. Nature 413(6852):186–193

Harini K, Sowdhamini R (2012) Molecular modelling of oligomeric states of DmOR83b, an olfactory receptor in *D. melanogaster*. Bioinform Biol Insights 6:33–47

Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zwiebel LJ (2002) G protein-coupled receptors in *Anopheles gambiae*. Science 298(5591): 176–178

Isono K, Morita H (2010) Molecular and cellular designs of insect taste receptor system. Front Cell Neurosci 4:20

Ito I, Ong RC, Raman B, Stopfer M (2008) Sparse odor representation and olfactory learning. Nat Neurosci 11(10):1177–1184

Jin X, Ha TS, Smith DP (2008) SNMP is a signaling component required for pheromone sensitivity in *Drosophila*. Proc Natl Acad Sci USA 105(31):10996–11001

Jones WD, Nguyen TA, Kloss B, Lee KJ, Vosshall LB (2005) Functional conservation of an insect odorant receptor gene across 250 million years of evolution. Curr Biol 15(4):R119–R121

Jones PL, Pask GM, Rinker DC, Zwiebel LJ (2011) Functional agonism of insect odorant receptor ion channels. Proc Natl Acad Sci USA 108(21):8821–8825

Jones PL, Pask GM, Romaine IM, Taylor RW, Reid PR, Waterson AG, Sulikowski GA, Zwiebel LJ (2012) Allosteric antagonism of insect odorant receptor ion channels. PLoS One 7(1):e30304

Jordan MD, Anderson A, Begum D, Carraher C, Authier A, Marshall SD, Kiely A, Gatehouse LN, Greenwood DR, Christie DL, Kralicek AV, Trowell SC, Newcomb RD (2009) Odorant receptors from the light brown apple moth (*Epiphyas postvittana*) recognize important volatile compounds produced by plants. Chem Senses 34(5):383–394

Junek S, Kludt E, Wolf F, Schild D (2010) Olfactory coding with patterns of response latencies. Neuron 67(5):872–884

Justus KA, Carde RT, French AS (2005) Dynamic properties of antennal responses to pheromone in two moth species. J Neurophysiol 93(4):2233–2239

Kain P, Chakraborty TS, Sundaram S, Siddiqi O, Rodrigues V, Hasan G (2008) Reduced odor responses from antennal neurons of $G_q\alpha$, phospholipase Cβ, and rdgA mutants in *Drosophila* support a role for a phospholipid intermediate in insect olfactory transduction. J Neurosci 28(18):4745–4755

Kaissling KE (1987) Stimulus transduction. In: Colbow K (ed) R. H. Wright lectures on insect olfaction. Simon Fraser University Press, Burnaby, BC, pp 1–190

Kaissling KE, Priesner E (1970) Die Riechschwelle des Seidenspinners. Naturwissenschaften 57(1):23–28

Keil TA, Steinbrecht RA (1984) Mechanosensitive and olfactory sensilla of insects. In: KR C, Hiromu A (eds) Insect ultrastructure vol 2. Plenum Press, New York, pp 477–516

Kennedy JS, Ludlow AR, Sanders CJ (1981) Guidance of flying male moths by wind-borne sex-pheromone. Physiol Entomol 6(4):395–412

Kiely A (2008) Functional and structural analyses of an olfactory receptor from *Drosophila melanogaster*. Dissertation, University of Auckland

Kiely A, Authier A, Kralicek AV, Warr CG, Newcomb RD (2007) Functional analysis of a *Drosophila melanogaster* olfactory receptor expressed in Sf9 cells. J Neurosci Methods 159(2): 189–194

Koehl MA (2006) The fluid mechanics of arthropod sniffing in turbulent odor plumes. Chem Senses 31(2):93–105

Krieger J, Raming K, Dewer YM, Bette S, Conzelmann S, Breer H (2002) A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. Eur J Neurosci 16(4):619–628

Krieger J, Klink O, Mohl C, Raming K, Breer H (2003) A candidate olfactory receptor subtype highly conserved across different insect orders. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 189(7):519–526

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB (2004) Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43(5):703–714

Laue M, Maida R, Redkozubov A (1997) G-protein activation, identification and immunolocalization in pheromone-sensitive *sensilla trichodea* of moths. Cell Tissue Res 288(1):149–158

Laurent G (2002) Olfactory network dynamics and the coding of multidimensional signals. Nat Rev Neurosci 3(11):884–895

Lei H, Riffell JA, Gage SL, Hildebrand JG (2009) Contrast enhancement of stimulus intermittency in a primary olfactory network and its behavioral significance. J Biol 8(2):21

Lundin C, Kall L, Kreher SA, Kapp K, Sonnhammer EL, Carlson JR, Heijne G, Nilsson I (2007) Membrane topology of the *Drosophila* OR83b odorant receptor. FEBS Lett 581(29):5601–5604

Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei H, Hildebrand JG (2011) The neurobiology of insect olfaction: sensory processing in a comparative context. Prog Neurobiol 95(3):427–447

Melo AC, Rutzler M, Pitts RJ, Zwiebel LJ (2004) Identification of a chemosensory receptor from the yellow fever mosquito, *Aedes aegypti*, that is highly conserved and expressed in olfactory and gustatory organs. Chem Senses 29(5):403–410

Miller R, Tu Z (2008) Odorant receptor c-terminal motifs in divergent insect species. J Insect Sci 8:53

Mitsuno H, Sakurai T, Murai M, Yasuda T, Kugimiya S, Ozawa R, Toyohara H, Takabayashi J, Miyoshi H, Nishioka T (2008) Identification of receptors of main sex-pheromone components of three Lepidopteran species. Eur J Neurosci 28(5):893–902

Montagne N, Chertemps T, Brigaud I, Francois A, Francois MC, de Fouchier A, Lucas P, Larsson MC, Jacquin-Joly E (2012) Functional characterization of a sex pheromone receptor in the pest moth *Spodoptera littoralis* by heterologous expression in *Drosophila*. Eur J Neurosci 36(5):2588–2596

Murlis J, Jones CD (1981) Fine-scale structure of odor-plumes in relation to insect orientation to distant pheromone and other attractant sources. Physiol Entomol 6(1):71–86

Nadasdy Z (2010) Binding by asynchrony: the neuronal phase code. Front Neurosci 4:51

Nakagawa T, Vosshall LB (2009) Controversy and consensus: noncanonical signaling mechanisms in the insect olfactory system. Curr Opin Neurobiol 19(3):284–292

Nakagawa T, Sakurai T, Nishioka T, Touhara K (2005) Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307(5715):1638–1642

Nakagawa T, Pellegrino M, Sato K, Vosshall LB, Touhara K (2012) Amino acid residues contributing to function of the heteromeric insect olfactory receptor complex. PLoS One 7(3):e32372

Neuhaus EM, Gisselmann G, Zhang W, Dooley R, Stortkuhl K, Hatt H (2005) Odorant receptor heterodimerization in the olfactory system of *Drosophila melanogaster*. Nat Neurosci 8(1):15–17

Nichols AS, Chen S, Luetje CW (2011) Subunit contributions to insect olfactory receptor function: channel block and odorant recognition. Chem Senses 36(9):781–790

Nolte A, Funk NW, Mukunda L, Gawalek P, Werckenthin A, Hansson BS, Wicher D, Stengl M (2013) In situ tip-recordings found no evidence for an Orco-based ionotropic mechanism of pheromone-transduction in *Manduca sexta*. PLoS One 8(5): e62648

Olafson PU (2013) Molecular characterization and immunolocalization of the olfactory co-receptor Orco from two blood-feeding muscid flies, the stable fly (*Stomoxys calcitrans*, L.) and the horn fly (*Haematobia irritans irritans*, L.). Insect Mol Biol 22(2): 131–142

Pask GM, Jones PL, Rutzler M, Rinker DC, Zwiebel LJ (2011) Heteromeric anopheline odorant receptors exhibit distinct channel properties. PLoS One 6(12):e28774

Pask GM, Bobkov YV, Corey EA, Ache BW, Zwiebel LJ (2013) Blockade of insect odorant receptor currents by amiloride derivatives. Chem Senses 38(3):221–229

Patch HM, Velarde RA, Walden KK, Robertson HM (2009) A candidate pheromone receptor and two odorant receptors of the hawkmoth *Manduca sexta*. Chem Senses 34(4):305–316

Peñalva-Arana DC, Lynch M, Robertson HM (2009) The chemoreceptor genes of the waterflea *Daphnia pulex*: many Grs but no Ors. BMC Evol Biol 9:79

Pitts RJ, Fox AN, Zwiebel LJ (2004) A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. Proc Natl Acad Sci USA 101(14):5058–5063

Robertson HM, Gadau J, Wanner KW (2010) The insect chemoreceptor superfamily of the parasitoid jewel wasp *Nasonia vitripennis*. Insect Mol Biol 19(Suppl 1):121–136

Rogers ME, Sun M, Lerner MR, Vogt RG (1997) SNMP-1, a novel membrane protein of olfactory neurons of the silk moth *Antheraea polyphemus* with homology to the CD36 family of membrane proteins. J Biol Chem 272(23):14792–14799

Rogers ME, Steinbrecht RA, Vogt RG (2001a) Expression of SNMP-1 in olfactory neurons and sensilla of male and female antennae of the silkmoth *Antheraea polyphemus*. Cell Tissue Res 303(3): 433–446

Rogers ME, Krieger J, Vogt RG (2001b) Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. J Neurobiol 49(1): 47–61

Röllecke K, Werner M, Ziemba PM, Neuhaus EM, Hatt H, Gisselmann G (2013) Amiloride derivatives are effective blockers of insect odorant receptors. Chem Senses 38(3): 231–236

Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, Tanoue S, Yasukochi Y, Touhara K, Nishioka T (2004) Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc Natl Acad Sci USA 101(47): 16653–16658

Sargsyan V, Getahun MN, Llanos SL, Olsson SB, Hansson BS, Wicher D (2011) Phosphorylation via PKC regulates the function of the *Drosophila* odorant co-receptor. Front Cell Neurosci 5:5

Sato K, Pellegrino M, Nakagawa T, Vosshall LB, Touhara K (2008) Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452(7190):1002–1006

Sato K, Tanaka K, Touhara K (2011) Sugar-regulated cation channel formed by an insect gustatory receptor. Proc Natl Acad Sci USA 108(28):11680–11685

Schuckel J, Siwicki KK, Stengl M (2007) Putative circadian pacemaker cells in the antenna of the hawkmoth *Manduca sexta*. Cell Tissue Res 330(2):271–278

Singer W, Gray CM (1995) Visual feature integration and the temporal correlation hypothesis. Annu Rev Neurosci 18:555–586

Smadja C, Shi P, Butlin RK, Robertson HM (2009) Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the pea aphid, *Acyrthosiphon pisum*. Mol Biol Evol 26(9):2073–2086

Smart R, Kiely A, Beale M, Vargas E, Carraher C, Kralicek AV, Christie DL, Chen C, Newcomb RD, Warr CG (2008) *Drosophila* odorant receptors are novel seven transmembrane domain proteins that can signal independently of heterotrimeric G proteins. Insect Biochem Mol Biol 38(8):770–780

Stengl M (1993) Intracellular-messenger-mediated cation channels in cultured olfactory receptor neurons. J Exp Biol 178:125–147

Stengl M (1994) Inositol-trisphosphate-dependent calcium currents precede cation currents in insect olfactory receptor neurons in vitro. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 174(2):187–194

Stengl M (2010) Pheromone transduction in moths. Front Cell Neurosci 4:133

Stengl M, Zufall F, Hatt H, Hildebrand JG (1992) Olfactory receptor neurons from antennae of developing male *Manduca sexta* respond to components of the species-specific sex pheromone in vitro. J Neurosci 12(7):2523–2531

Su CY, Menuz K, Reisert J, Carlson JR (2012) Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature 492(7427):66–71

Taylor RW, Romaine IM, Liu C, Murthi P, Jones PL, Waterson AG, Sulikowski GA, Zwiebel LJ (2012) Structure-activity relationship of a broad-spectrum insect odorant receptor agonist. ACS Chem Biol 7(10):1647–1652

Tripathy SJ, Peters OJ, Staudacher EM, Kalwar FR, Hatfield MN, Daly KC (2010) Odors pulsed at wing beat frequencies are tracked by primary olfactory networks and enhance odor detection. Front Cell Neurosci 4:1

Tsitoura P, Andronopoulou E, Tsikou D, Agalou A, Papakonstantinou MP, Kotzia GA, Labropoulou V, Swevers L, Georgoussi Z, Iatrou K (2010) Expression and membrane topology of *Anopheles gambiae* odorant receptors in lepidopteran insect cells. PLoS One 5(11):e15428

Vickers NJ (2000) Mechanisms of animal navigation in odor plumes. Biol Bull 198(2):203–212

Vickers NJ, Baker TC (1992) Male *Heliothis virescens* maintain upwind flight in response to experimentally pulsed filaments of their sex-pheromone (Lepidoptera, Noctuidae). J Insect Behav 5(6):669–687

Vogt RG, Miller NE, Litvack R, Fandino RA, Sparks J, Staples J, Friedman R, Dickens JC (2009) The insect SNMP gene family. Insect Biochem Mol Biol 39(7):448–456

Vosshall LB, Hansson BS (2011) A unified nomenclature system for the insect olfactory coreceptor. Chem Senses 36(6):497–498

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96(5):725–736

Vosshall LB, Wong AM, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102(2):147–159

Wang X, Zhong M, Wen J, Cai J, Jiang H, Liu Y, Aly SM, Xiong F (2012) Molecular characterization and expression pattern of an odorant receptor from the myiasis-causing blowfly, *Lucilia sericata* (Diptera: Calliphoridae). Parasitol Res 110(2):843–851

Wanner KW, Nichols AS, Allen JE, Bunger PL, Garczynski SF, Linn CE, Robertson HM, Luetje CW (2010) Sex pheromone receptor specificity in the European corn borer moth, *Ostrinia nubilalis*. PLoS One 5(1):e8685

Wegener JW, Hanke W, Breer H (1997) Second messenger-controlled membrane conductance in locust (*Locusta migratoria*) olfactory neurons. J Insect Physiol 43(6):595–603

Wetzel CH, Behrendt HJ, Gisselmann G, Stortkuhl KF, Hovemann B, Hatt H (2001) Functional expression and characterization of a *Drosophila* odorant receptor in a heterologous cell system. Proc Natl Acad Sci USA 98(16):9377–9380

Wicher D, Schäfer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS (2008) *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452(7190):1007–1011

Wistrand M, Kall L, Sonnhammer EL (2006) A general model of G protein-coupled receptor sequences and its application to detect remote homologs. Protein Sci 15(3):509–521

Xia Y, Zwiebel LJ (2006) Identification and characterization of an odorant receptor from the West Nile virus mosquito, *Culex quinquefasciatus*. Insect Biochem Mol Biol 36(3):169–176

Yang Y, Krieger J, Zhang L, Breer H (2012) The olfactory co-receptor Orco from the migratory locust (*Locusta migratoria*) and the desert locust (*Schistocerca gregaria*): identification and expression pattern. Int J Biol Sci 8(2):159–170

Yao CA, Carlson JR (2010) Role of G-proteins in odor-sensing and CO_2-sensing neurons in *Drosophila*. J Neurosci 30(13):4562–4572

Zhang HJ, Anderson AR, Trowell SC, Luo AR, Xiang ZH, Xia QY (2011) Topological and functional characterization of an insect gustatory receptor. PLoS One 6(8):e24111

Zheng W, Zhu C, Peng T, Zhang H (2012) Odorant receptor co-receptor Orco is upregulated by methyl eugenol in male *Bactrocera dorsalis* (Diptera: Tephritidae). J Insect Physiol 58(8):1122–1127

Ziegelberger G, van den Berg MJ, Kaissling KE, Klumpp S, Schultz JE (1990) Cyclic GMP levels and guanylate cyclase activity in pheromone-sensitive antennae of the silkmoths *Antheraea polyphemus* and *Bombyx mori*. J Neurosci 10(4):1217–1225