The Stimulatory $\alpha_q$ Protein Is Involved in Olfactory Signal Transduction in Drosophila

Ying Deng1,2, Weiyi Zhang2,3, Katja Farhat2,4, Sonja Oberland5, Günter Gisselmann2, Eva M. Neuhaus5*

1 Sino-France Joint Center for Drug Research and Screening, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, P. R. China, 2 Cell Physiology, Ruhr-University Bochum, Bochum, Germany, 3 Bioduro (Beijing) Co. Ltd, Zhongguancun Life Science Park, Changping, Beijing, China, 4 Department of Cardiovascular Physiology, Georg-August University, Göttingen, Germany, 5 NeuroScience Research Center, Charité, Berlin, Germany

Abstract

Seven-transmembrane receptors typically mediate olfactory signal transduction by coupling to G-proteins. Although insect odorant receptors have seven transmembrane domains like G-protein coupled receptors, they have an inverted membrane topology, constituting a key difference between the olfactory systems of insects and other animals. While heteromeric insect ORs form ligand-activated non-selective cation channels in recombinant expression systems, the evidence for an involvement of cyclic nucleotides and G-proteins in odor reception is inconsistent. We addressed this question in vivo by analyzing the role of G-proteins in olfactory signal transduction using electrophysiological recordings. We found that $\alpha_q$ plays a crucial role for odorant induced signal transduction in OR83b expressing olfactory sensory neurons, but not in neurons expressing CO2 responsive proteins GR21a/GR63a. Moreover, signaling of Drosophila ORs involved $\alpha_q$ also in a heterologous expression system. In agreement with these observations was the finding that elevated levels of cAMP result in increased firing rates, demonstrating the existence of a cAMP dependent excitatory signaling pathway in the sensory neurons. Together, we provide evidence that $\alpha_q$ plays a role in the OR mediated signaling cascade in Drosophila.

Introduction

Mammalian odorant receptors (OR) comprise the largest family of seven-transmembrane spanning G-protein-coupled receptors which are characterized by extracellular N- and intracellular C-termini [1]. OR proteins are localized in the cilia of the olfactory sensory neurons where they stimulate a G\textsubscript{a}{s} mediated signal transduction cascade ultimately leading to neuronal depolarization [2,3]. 62 OR genes were shown to be expressed in the antennae, maxillary palps and larvae in Drosophila melanogaster [4]. Flies have therefore considerably fewer OR genes than vertebrates, most of which are expressed between 600–1300 ORs [5]. The odor response spectrum for most receptors was determined in vivo by electrophysiological recordings [6]. The organization of the peripheral olfactory system shows striking similarities to the mammalian olfactory system, the ORs recognize multiple odorants, the neurons express, with a few exceptions, one OR, and the axons of the olfactory neurons that express the same OR converge onto specific glomeruli in both, the insect antennal and the axons of the olfactory neurons that express the same.

Nevertheless, differences exist in the functional properties of vertebrate and invertebrate OR proteins. One key difference is the ubiquitously expressed insect receptor OR83b, which is conserved across insect species [7–9]. OR83b interacts with conventional ORs and is essential to transport them to the sensory cilia [10–12]. Moreover, although Drosophila ORs were identified by bioinformatic strategies to contain seven transmembrane domains, recent experimental investigations have revealed that the membrane topology of Drosophila ORs is distinct from conventional GPCRs, with the N-terminus of these receptors located in the cytoplasm [13]. Similarly, insect gustatory receptors also lack clear sequence similarity to G-protein-coupled receptors (GPCRs). The hypothesis that insect chemoreceptors could define a novel family of transmembrane proteins was further substantiated by the findings that heteromeric insect OR/OR83b complexes can form ligand-gated ion channels [14–16]. Contradictory results concern the nature of the underlying transduction mechanism. While rapid, solely ionotropic, and G-protein independent currents were described by Sato et al. [14], non-selective cation currents activated by means of an ionotropic and a metabotropic pathway, with OR83b being directly activated by intracellular cAMP or cGMP, have been described in a study by Wicher et al. [16].

The existence and nature of a signal transduction cascade downstream of insect ORs, especially in the sensory neurons, is therefore still partially unresolved question. Since they had been categorized as GPCRs during their initial identification, G-protein signaling has been investigated in odor elicited activation of insect neurons. $\alpha_q$ was found to be expressed in insect antennae, and although it is not specifically enriched in moth cilia [17–19], immunoreactivity was found in chemosensory hairs of Drosophila and Anopheles antennae [19,20]. Also other heterotrimeric G-protein subunits have been found in the Drosophila antennae [21]. Pharmacological studies in locust, cockroach and moth antennae revealed that G-proteins are involved in odor-evoked increases in

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* E-mail: eva.neuhaus@charite.de
in Drosophila olfactory neurons by RNAi leads to impaired performance in several odor induced behavioural assays [24] and flies expressing mutated Gαq showed reduced odorant evoked response in electrophysiological recordings [25]. Gαo was found to be required for maximal physiological responses to multiple odorants [26]. Also in gustatory neurons expressing receptors which also lack clear sequence similarity to GPCRs, experimental evidence exists for an at least modulatory role of heterotrimeric G-proteins in sugar perception [27–29]. On the other side, the results of a recent RNAi based study do not support a role for Gα proteins in odor sensitivity, although small influences of G-proteins on the odor evoked spike rates were observed for some odorants [30]. Genetic mosaic analysis used in this study showed that odor responses are normal in the absence of Gαq, which is required for normal CO2 responses [30].

We also aimed at determining if Drosophila ORs couple to G-proteins in the heterologous expression system and if G-proteins play a role in olfactory signaling in vivo. We found that the Drosophila Gαs protein, a close homologue to the vertebrate o-GDP), pertussis toxin (PTX), which inhibits specifically Go subunit, since the ab1C neuron does not express OR83b or GR21a and GR63a, but do not express the olfactory co-receptor OR83b [36,37]. Unexpectedly, CO2 responses were not altered in heat-shocked Or83b-Gal4;UAS-CTX flies compared to control flies (Figure 1 E).

Single unit electrophysiology of CTX expressing flies

EAG responses give only summated information of signal transduction events in olfactory neurons. In the next set of experiments we investigated how the activity of olfactory neurons was affected by CTX expression. We performed single-unit electrophysiology and measured the responses of olfactory neurons in the ab1 sensillum, which contains in total four neurons (ab1A-D) responding to different odorants (Figure S3). The ab1C neuron in the ab1 sensillum contains the chemosensory receptors GR21a and GR63a, but not the olfactory co-receptor OR83b [36,37]. The ab1 sensillum therefore contains an internal control cell, since one of the neurons does not express the transgene when using the OR83b-Gal4 driver line. Application of ethyl acetate, a known stimulus for the ab1A neuron, to the ab1 sensillum of control flies resulted in an increased spike rate, while the spike rates did not increase in ab1 sensilla of CTX expressing flies. The control spike trains shown in the figure come from non heat shocked Or83b-Gal4;UAS-CTX flies, which were indistinguishable from wt flies. Moreover, the ab1A, ab1B and ab1D neurons in CTX expressing flies showed no spontaneous spiking (Figure 2 B, E). As control, we determined the CO2 response of the same sensillum, since the ab1C neuron does not express OR83b or other ORs, but instead GR21a and GR63a together forming the CO2 receptor [36,37]. Ab1C neurons showed no alteration in the spontaneous firing rates, and CO2 responses were found to be unaffected (Figure 2G, D), similar to the CO2 response in EAG recordings (Figure 1 E). Odorants activating the remaining neurons, 2,3-butandione for ab1B and methylsalicylate for
**Figure 1. Screening G-proteins for participation in olfactory signal transduction.** (A) UAS constructs of different Gα-proteins, mutated Gα-proteins and G-protein affecting toxins were expressed in the sensory neurons of the third antennal segment using an Or83b-Gal4 driver line, last bars present heat shocked Or83b-Gal4;UAS-CTX flies (CTX hs) and Or83b-Gal4;UAS-CTX flies without heat shock (CTX). EAG amplitudes in response to application of undiluted ethyl acetate were recorded (n=10 flies were recorded). The difference between wt and heat shocked CTX flies was statistically checked by the unpaired Student’s t test, **P<0.01.** (B) EAG amplitudes of different G-protein mutant flies (n>10 flies were recorded), expression of UAS constructs were driven by Or83b-Gal4. Odorant used were ethyl acetate, cyclohexanol and benzaldehyde, each in concentrations of 10⁻³, 10⁻², 10⁻¹, and undiluted. The fly strains tested were wt, PTX, Gzs wt, Gzs-GTP (marked in red), Gsq-GTP (marked in yellow), Gxo-GTP, Gxo-GDP, Gxo-wt (from left to right for each odorant dilution). If several doses were tested on the same antenna, lower doses were presented first, inter-stimulus intervals were at least 30 s. Significant differences were only observed for the responses of Gα-lof expressing flies towards cyclohexanol and low concentrations of benzaldehyde (shown in more detail in Figure S2A). Flies were statistically checked (pairwise) by unpaired Student’s t tests; significance levels were set according to the Bonferroni post hoc test for k = 4 means,*P<0.0125,**P<0.0025. (C) EAG amplitudes (in mV) upon exposure to different concentrations of 4 odorants in wt flies, Or83b-Gal4;UAS-CTX flies (CTX), heat shocked Or83b-Gal4;UAS-CTX flies (CTX hs), and heat shocked wt flies (wt hs). The differences between CTX hs flies and wt, wt hs, CTX flies were statistically checked (pairwise) by unpaired Student’s t tests; significance levels were set according to the Bonferroni post hoc test for k = 4 means, **P<0.0025. (D) EAG amplitudes of heat shocked UAS-CTX flies without Or83b-Gal4 driver (black bars) compared to non heat shocked flies (wt hs). (E) EAG amplitudes of heat shocked Or83b-Gal4;UAS-CTX flies (CTX hs, p = 0.04), Or83b-Gal4;UAS-CTX (CTX, p = 0.02), UAS-CTX (p = 0.26), Or83b-Gal4 (p = 0.29), and wt flies to application of CO₂ (according to significance levels for k=5 means, * would have been assigned for P<0.01). Error bars represent s.e.m.
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ab1D showed the same effect as ethyl acetate in their inability to elicit increases in the spike rates in CTX expressing cells (Figure 2E).

To rule out that the effects are caused by general cellular toxicity of CTX, we expressed CTX specifically in the G21a expressing neuron in the ab1 sensillum, CTX expression did not change the response of the cells to application of CO₂, ruling out that CTX expression causes cell poisoning or even cell death (Figure 2F–H). The absence of general cell poisoning of CTX was in agreement with another study investigating frizzled signaling [35]. Moreover, CTX expression did not disturb general cellular morphology in the antennae of Gal4-Or83b; UAS-mCD8-GFP flies (Figure 2I) or the presence of olfactory receptors in the dendrites of the sensory neurons (Figure 2K).

We also studied expression of CTX under the control of an OR specific Gal4 driver to target only a specific type of olfactory neuron. We used the OR22a-Gal4 driver line to drive expression of CTX in the ab3A neuron of the ab3 sensillum, which contains in addition a second type of neuron (ab3B) expressing OR85b. The OR22a expressing ab3A neuron in control flies (OR22a-Gal4;UAS-CTX) responded to 2-heptanone and hexanol with an increased spike rate (Figure 2L, N), which was not observed in CTX expressing cells (in heat shocked OR22a-Gal4;UAS-CTX flies) (Figure 2M, N). The ab3B neuron in the same sensillum serves as positive control since it does not express OR22a (and is therefore not expressing CTX in heat shocked OR22a-Gal4;UAS-CTX flies) and responds to the same substances. Spiking rates in these cells were not changed in the heat-shocked mutants compared to mutant flies that were not heat shocked (Fig. 2N).

GTPase defective Gαs causes delayed desensitization of the odorant response

The experiments with CTX clearly point to a crucial role of Gαs in olfactory signal transduction. In the following experiments we expressed mutated Gαs subunits in olfactory neurons to further investigate this point. Since complete absence of Gαs causes embryonic lethality in knock-out flies due to arrested development [34], investigation of olfactory perception in knock-out flies was...
Gα in Drosophila Olfactory Signal Transduction

A) Ctrl.

B) Or83b-CTX

C) Ctrl.

D) Or83b-CTX

E) 

F) Ctrl.

G) Gr21a

H) 

I) Or83b-mCD8-GFP-CTX

J) Or83b-CTX

K) Or83b-CTX

L) Ctrl.

M) Or22a-CTX

N)
not possible. We therefore tested Or83b-Gal4; UAS-Gαs-CTX expressing flies in single sensillum recordings. These flies express a GTPase defective Gαs in the sensory neurons, in addition to the endogenous Gαs. The mutation leads to a prolonged activation of the G-protein and can be regarded as constitutively active. Recording the spike rates upon application of ethyl acetate to the ab1 sensillum revealed that expression of GTPase defective Gαs caused a prolonged duration of the spike activity compared to the control flies (Figure 3A). A similar effect was observed upon application of 2,3-butadienone (Figure 3B). The prolonged duration of the period of increased firing in the mutant flies can likely be attributed to the deficiency in GTPase activity of the activated G-protein subunit.

Since cAMP was shown to increase the stimulus-dependent sensillar potential amplitude and the spontaneous action potential frequency in trichoid sensilla of the hawkmoth Manduca sexta [38], we specifically analyzed whether the responses might differ at the beginning of the application. We therefore analyzed the first 1000 ms of the response in a post-stimulus histogramm (Figure S4), grouping the spikes in 50 ms intervals. Also on this expanded time scale we could not see a significant rise in the mutant compared to wt control flies.

Recombinantly expressed Drosophila ORs activate heteromeric G-proteins

For the understanding of the role of Gαs in odorant signal transduction it is important to investigate whether Drosophila odorant receptors can activate heterotrimetric G-proteins in addition to their ion cannal function. To investigate the functional interaction between ORs and G-proteins, we tested whether co-expression of the Drosophila Gαs-proteins could improve signaling in the recombinant HEK293 expression system, and whether this effect shows subunit specificity. To ensure efficient coupling of the Drosophila G-proteins to the endogenous Ca2+ pathway in HEK293 expression system, we constructed G-protein chimera composed of the N-terminus of human Gαs, lacking the last 44 amino acids, and the C-terminus of different Drosophila G-proteins (Figure 4A). The N-terminal GαsN16 part of the protein contains the binding site for the effector proteins, all chimeric proteins should therefore couple to the same signaling cascade, whereas the C-terminal part should resemble the coupling specificity of different Drosophila Gαs-proteins towards ORs. Similar constructs have already been described to enhance the signaling efficiency of vertebrate G-protein coupled receptors [39–41], and coexpression of similar chimeric G-proteins showed that the Drosophila sex peptide receptor couples to Gαs and Gγs, in the recombinant expression system [42].

We coexpressed the respective chimeric G-proteins, OR33b and OR14a in HEK293 cells and performed Ca2+-imaging to record receptor elicited responses. The receptor under study was chosen, since it has been successfully used for Ca2+-imaging studies in recombinant expression systems previously [12,43,44]. We compared the number of cells responding to the application of cyclohexanone, a ligand for recombinantly expressed OR14a [44], to the amount of cells responding when the G-protein chimera were not coexpressed. For that we determined the ratio of cells responding with and without the chimeric G-protein. We coexpressed OR33b, OR43a and the respective G-protein chimera, or OR33b and OR43a without the G-protein determined the number of responding cells in each condition. The ratio of both data points revealed G-protein dependent differences. Although all chimeric G-proteins were able to moderately improve OR signaling, the Gαs chimera had the strongest effect (Figure 4B). All G-protein chimeras were expressed at comparable levels in the recombinant expression system (Figure 4C, D). Non-transfected cells were used as control to show that no endogenous Gαs-protein in HEK293 cells is detected under the same conditions. The presence of the G-protein chimera alone, with OR expression, did not result in any measurable Ca2+ increase upon odorant stimulation (data not shown). Next, we asked if the odor evoked Ca2+-signal and the enhancing effect by G-proteins was dependent on the presence of the OR33b subunit. For that we determined the ratio of cells responding with and without the chimeric Gαs-protein, when only OR43a was expressed and again determined the number of responding cells in each condition. We found that expression of OR43a alone in HEK293 cells was sufficient to generate cyclohexanone-dependent cellular responses, which were greatly enhanced by Gαs coexpression (Figure 4B). These findings provide additional indications that the receptor proteins indeed interact with G-proteins and that the odorant sensing subunit might be the site of interaction.

In another approach, we transfected HEK293 cells with odorant receptors (OR33b and OR22a), made membrane preparations and assayed [35S]GTPγS binding, which measures the level of G-protein activation following agonist occupation of a GPCR. We performed this assay on HEK293 cell membranes transfected with OR22a, OR33b and Drosophila Gαs. Membranes were stimulated by a mix of the OR22a ligands pentyl acetate and 1-octen-3-ol [6], which did not give any unspecific responses in this assay in HEK293 cells. Although these substances are not the best known ligands for OR22a [45], we identified them as best suited for the recombinant
experiments conducted here. Incubation with the cognate ligands lead to a significant increase in membrane bound radioactivity in comparison to control membranes, indicating that ligand binding to OR22a is indeed activating the G-protein (Figure 4E). Similar results were obtained when OR43a was coexpressed with OR83b (data not shown). Although ligand induced GTPγS binding was weaker in OR43a expressing cells, there was no indication for receptor specific effects in these experiments.

**Gαs is localized in the dendrites of the olfactory sensory neurons**

To understand the possible in vivo function of Gαs, it is necessary to find out whether it is expressed in olfactory neurons and where it is localized. This is a prerequisite for a direct activation by ORs and participation in the signal transduction cascade. We performed immunohistochemistry, RT-PCR, and western blotting to investigate the presence and localization of Gαs in the Drosophila antennae (Figure 5). Gαs mRNA could be detected in the third antennal segment by RT-PCR (Figure 5B). Drosophila Gαs shows strong homology to vertebrate Gαs at the C-terminus (Figure 5A). It could therefore be detected in the third antennal segment by western blotting using an anti-vertebrate Gαs C-terminus antibody (Figure 5C). In sections of Gal4-Or83b; UAS-mCD8-GFP flies we could localize Gαs in the dendrites of OR83b expressing neurons (Figure 5D).

**cAMP causes increased spike rates in olfactory sensory neurons**

Since Gαs most likely affects cAMP mediated signaling in the antennae, we tested whether an increase in intracellular cAMP in the sensory neurons would also cause an increase in the spike rates. We expressed the photoactivated adenylyl cyclase (PAC) from the flagellate Euglena gracilis in the olfactory neurons, a protein recently reported to yield light-induced changes in cAMP levels in Drosophila melanogaster [46]. Since the activity of PAC is strongly and reversibly enhanced by blue light, we recorded spike rates from ab2 sensilla in the dark and exposed the antennae to blue
The light exposure driven increase in cAMP resulted in an increase in the spiking rate of the neurons (Figure 6A). Light induced increases in spiking rates were detected in several types of sensilla (Figure 6B). The effect was reversible, and the return to normal spike rates occurred rapidly after turning the blue light off, this cycle could be repeated several times (Figure 6C). The activity of PAC in the olfactory neurons was very low without activation by blue light (Figure 6D). In ab2A, ab2B, ab3A and ab3B neurons, the light induced increase in spike rates was significantly higher than in wt flies, showing that the increase in spike rates upon blue light exposure depends on PAC expression (Figure 6E). PAC activation induced lower spike rates in ab2A neurons than the undiluted odorants ethyl acetate and 2,3-butanedione, which are strong activating odorants (Figure 6F). Since the excitation light has to pass the cuticle of the third antennal segment, and since the activation of PAC and generation of cAMP depends on the intensity of the light used [46], stronger neuronal excitation can be expected when light sources with higher intensities would be used. Cells activated by blue light could be further activated by odorant exposure (data not shown).

To test whether an increase in cAMP levels in the sensory neurons would lead to an increased spiking rate in OR83b knock-out flies (Or83b-/-), we generated a mutant fly expressing PAC on a OR83b knock-out background. Single sensillum recordings from the ab1 sensillum of Or83b-/- flies showed only one group of spikes representing the activity of the Gr21a neuron (Figure S5). The ab1 sensillum in wt flies showed four groups of spikes, corresponding to the four cell types projecting their dendrites in this sensillum (see Figure 2). When PAC was expressed by the Or83b-Gal4 driver in Or83b-/ flies, and the fly antennae were exposed to blue light, additional neuronal activity could be recorded (Figure 6G). Neither PAC expression alone (without stimulation by blue light), nor light exposure to Or83b-/ flies resulted in significant changes in spiking activity of the neurons (Figure S5). Also CO2 responses in PAC expressing Or83b-/ flies were not significantly changed, and odorant responses were still absent (Figure S5). We next sorted all spikes in a given time interval and analyzed the spike amplitude distribution (Figure 6H). We observed a similar spike distribution pattern in 453 spikes analyzed from 8 seconds recording of the ab1 sensillum of an Or83b-Gal4/UAS-PACa; Or83b2/Or83b2 (Or83b-/-) fly and 229 spikes from a wt fly (Figure 6H), indicating that PACa mediated cAMP rise in olfactory receptor neurons does efficiently recover spontaneous neuronal activity.

**Discussion**

Drosophila ORs are seven transmembrane proteins which were originally classified as G-protein coupled receptors. The receptors have an unusual membrane topology with an intracellular N- and extracellular C-terminus [13,47], and can function as ligand-gated ion channel in recombinant expression systems [14,16], raising the question of whether these receptors also use G-proteins for signal transduction. In the present study, we investigated whether Drosophila ORs couple to G-proteins. Screening an array of different over-expressed G-proteins, mutated G-proteins, and G-protein affecting toxins resulted in the identification of Gαs, a close homologue to the vertebrate olfactory G-protein Gαod, as being
important for odorant induced signal transduction in the olfactory sensory neurons. Sustained expression of cholera toxin (CTX), which ribosylates the Gαs subunit of the heterotrimeric G-protein and results in constitutive cAMP production, caused a reduction in electroantennogram (EAG) recordings and abolished firing rates of olfactory neurons in vivo. Moreover, expression of GTPase defective Gαs caused a prolonged duration of spike activity when compared to wt flies. Recombinantly expressed ORs could directly activate G-proteins (measured by odorant induced [35S]GTPγS binding to membrane preparations). Moreover, co-expression of chimeric Gαs with OR83b and OR43a in HEK293 cells lead to an increased cellular activation upon ligand exposure. Finally, the role of cAMP as a second messenger in the sensory neurons was further demonstrated by expression of photoactivated adenylate cyclase.

Figure 6. Increased cAMP levels can recover the spontaneous activity of olfactory neurons. (A) Increase in spike rates in single unit recordings from the ab2 sensillum in Or83b-Gal4; UAS-Pacα flies upon transition from dark to strong blue light excitation showing that cAMP increase stimulates an excitatory pathway in olfactory neurons, the increase was rapidly reversible. (B) Summaries of single unit recordings from the different neurons in the ab1, ab2 and ab3 sensilla. (C) Reversibility of the response to light shown by repeated 30 s on/off cycles in ab2A neurons. (D) Summaries of single unit recordings from ab1, ab2 and ab3 sensilla from wt (white bars) and Or83b-Gal4; UAS-Pacα flies (black bars) recorded in the dark. (E) Ratios of spike rates for ab2A, ab2B, ab3A and ab3B neurons recorded under blue light illumination vs. dark, showing the relative increases in spike rates in wt (black bars) and Or83b-Gal4; UAS-Pacα flies (white bars). (F) Comparison of the light induced spike rate in the ab2A neuron in Or83b-Gal4; UAS-Pacα flies (white bar) to spontaneous and odor induced spike rates in wt (black bars) flies. (G) Single unit recording of wt ab1 sensillum and of the blue light illuminated Or83b-Gal4/UAS-Pacα; Or83b+/Or83b− ab1 sensillum. (H) Analysis of the of spike amplitude distribution recorded from ab1 sensilla in wt and Or83b-Gal4/UAS-Pacα; Or83b+/Or83b− flies. Data shown represent about 450 spikes form 8 s recording. “A”–“D” indicate subpopulations of spikes attributed to neurons A, B, C and D. Differences between the indicated data points were statistically checked by the unpaired Student’s t test, *p<0.05; **p<0.01. Error bars represent s.e.m. doi:10.1371/journal.pone.0018605.g006
G-protein signaling in Drosophila olfactory neurons

In the initial screening procedure, CTX expressing flies showed strong reduction of odorant induced signals in EAG recordings. Single sensillum recordings revealed an absence of spontaneous neuronal activities in CTX flies, which was not caused by general cell poisoning effect of the toxin. Interestingly, background spiking is also absent in Or83b-/ flies, again not caused by unspecific cellular damage but by deficient receptor transport, indicating that disturbance of single components of the signaling cascade can lead to profound effects on neuronal spiking. After single sensillum recordings of Gs-GTP flies, which express a constitutively active mutated Gs-protein with decreased GTPase activity, we observed differences in the time course of odor induced spike rates compared to wt flies. The prolonged firing of the neurons in the mutant flies was likely caused by prolonged G-protein activation due to the deficiency in its GTPase activity. Taken together, the electrophysiological recordings of the mutant flies showed that Gs is involved in odorant detection in Drosophila olfactory neurons.

Yao and Carlson agree to a large extend to our finding that odor evoked initial spike rates of ab1A, ab2A, and ab3A were not altered by expression of GTPase defective Gs [30]. In addition, we found that expression of GTPase defective Gs caused a prolonged increase of the spike rates compared to the control flies, which is likely caused by inefficient shut off of the response due to the deficiency in GTP hydrolysis. Similar to the study published by Yao and Carlson [30], we did not find altered odor evoked EAG amplitudes or spike rates in Gs RNAi expressing olfactory neurons (data not shown). In our view this can be explained by inefficient reduction of G-protein levels. This is consistent with our observation that flies expressing Gs RNAi under a pan-neuronal driver are viable (data not shown), while Gs knock-out flies are not [34]. Mosaic analysis with a repressible cell marker showed that Gs-/- olfactory neurons had a decreased response to near-saturating concentrations of methyl butyrate and butyric acid, which can be rescued by Gs expression [30]. Although the contribution of Gs to odor responses was very modest in the study by Yao and Carlson [30], observable effects were reported.

Conflicting models for OR signaling have been suggested. Two studies found that OR and the ubiquitously co-expressed receptor OR83b form a ligand gated ion channel in heterologous expression systems [14,16], however one of these groups proposed that ORs may still couple to G-proteins [49]. Data from another recent report do not support a direct role of G-protein signaling for the initial response of olfactory neurons [30], although Gs was found to be required for maximal responses to several odorants [26] and in particular Gs is earlier found to be required by ab2A neurons for full sensitivity to low concentrations of odors [25]. The latter finding is consistent with the odor concentration-dependent differences we found in the Gs mutant flies. There is moreover compelling evidence from many studies for an involvement of the Gs effector enzyme PLC in signal transduction cascades in pheromone and odor transduction in moths [reviewed in [49]]. Precisely how the alternate mechanisms of odor transduction interact is unclear at the moment, but different odors and strengths of stimuli could in principle stimulate different transduction pathways to varying extent, and colocalized signal transduction cascades could allow adjustments in response to endogenous physiological rhythms, behavioral states, and stimulus properties [49]. Drosophila OR59b for example was shown to mediate excitatory and inhibitory responses in response to different odorants [6]. Different second messengers may therefore modulate the odor response depending on stimulus length and strength.

G-protein in signaling of recombinantly expressed ORs

In addition to the physiological experiments showing the involvement of Gs in odorant detection, we demonstrated that signaling of ORs in the HEK293 system is enhanced upon co-expression of a Drosophila Gs chimera. This finding provides additional indications that the receptors may interact with G-proteins. Drosophila receptors might even use a similar coupling mode as other GPCRs, since the G-protein chimera where only the C-termini of human Gs was exchanged with the respective Drosophila G-proteins were sufficient to enhance the signaling properties of the ORs. The fact that we found increased binding of [35S]GTPyS when ORs and G-protein were co-expressed in HEK293 cells indicates that direct coupling may occur, since no other components Drosophila olfactory neurons were co-expressed.

Recent work on recombinantly expressed ORs showed that inhibitors of heterotrimeric G-proteins (GDP-β-S), adenylyl cyclase, guanylyl cyclase, cyclic nucleotide phosphodiesterases and phospholipase C have negligible impact on OR43b responses, which lead the authors to the conclusion that G-protein signaling does not play a role in cell-based assays with insect ORs [15,50]. Activation of mammalian ORs in HEK293 cells leads to a transient inositol phosphate production followed by an increase in intracellular Ca2+ which can be detected at the single-cell level using the Ca2+-sensitive dyes and ratiometric imaging techniques [51,52]. Only when Galpha is co-transfected, vertebrate ORs can efficiently couple to a cAMP based signal cascade in recombinant expression systems [33]. Negligible effects of adenylyl cyclase, guanylyl cyclase and cyclic nucleotide phosphodiesterases do therefore not exclude the possibility of G-protein coupling, neither does GDP-β-S incubation, since the drug is barely membrane permeable. Although the membrane topology of the Drosophila ORs is nicely demonstrated [13,15], this experimental approach to signal transduction in the recombinant expression system does not unambiguously rule out a role for G-proteins but rather demonstrates that the insect receptors are ligand gated ion channels.

What has to be investigated in more detail is whether productive interaction with the G-protein only occurs when the receptors dimerize or whether monomers, and if so which part of the monomer, can activate the heterotrimeric G-protein. Our experiments in the HEK293 cell system suggest that the odorant sensing subunit of the receptor is sufficient for G-protein coupling, even though the effect of G-protein co-expression was stronger for heteromultimeric receptors. While ion channel characteristics depend on heteromeric complexes of a conventional insect ORs and the highly conserved OR83b family co-receptor [14], several reports support the activation of ORs in an OR83b-independent manner in different recombinant expression systems [12,44,50,54,55]. While further mechanistic models have to await investigations on the mode of receptor G-protein coupling in the olfactory system, it is tempting to speculate that Drosophila ORs couple to heterotrimeric G-proteins although having an inverse membrane topology compared to other GPCRs. Human adiponectin receptors (AdipoRs) and membrane progesterin receptors (mPRs), belonging to the PAQR (Progestin, AdipoQ-Receptor) family of proteins, are seven transmembrane receptors of a novel type that, similarly to Drosophila OR83b, share little sequence homology with other GPCRs [56,57]. Interestingly, AdipoRs have been shown to have intracellular N- and extracellular C-termini [56,58], but location of the termini of mPR is yet to be confirmed. Nevertheless, the fish mPR has been shown to be a plasma membrane protein whose activation leads to inhibition of adenyl cyclase in a pertussis toxin-sensitive manner, consistent with mPR being a novel type of GPCR [57].
Dual signaling mode in Drosophila chemosensory systems

Also Drosophila gustatory receptors (GRs) lack sequence homology with mammalian taste receptors or other GPCRs, but are distantly related to the large family of Drosophila ORs [59]. Although not unambiguously shown until now, ligand-gated channel properties of Drosophila GRs were suggested by in situ patch clamp recordings showing that an ion channel is directly gated by sucrose in fleshfly taste neurons [60]. Nevertheless, different G-protein mutant flies showed significantly decreased responses to sugars in comparison to control flies, but not a complete suppression of the responses [27–29]. The fact that CO₂ concentrations were also described in cultured olfactory receptor neurons of the moth Manduca sexta [62]. Endogenous circadian rhythms of cyclic nucleotides, generated by varying octopamine levels, contribute to the control of the intracellular Ca²⁺ concentrations in ORNs from Manduca [38,63], and generate more sensitive pheromone responses during the night. Whether cAMP levels are modulated with the endogenous circadian rhythm in Drosophila is not known at the moment, but could be investigated in future studies. It is unclear if the cAMP rise in Manduca increases the spontaneous activity of ORNs as this is not yet examined in the moth, but the PAC mediated cAMP modulation that we describe resulted in modulation of the spike rate in Drosophila ORNs, and suggests that this might also take place in other insects as already supposed by Monika Stengl [49].

The heteromeric OR/OR83b complexes could mediate spontaneous activity by forming constitutively open non-specific cation channels [14,48], and it was proposed that the insect receptor–ion channel complex functions in the control of membrane potential oscillations [48,49]. Since the open probability of the OR/OR83b complexes was shown to be regulated by cAMP [48], cAMP may regulate the membrane potential of the ORNs via the OR/OR83b complex.

We found here, that cAMP also induces increased spike rates in ORNs from OR83b k.o. flies, showing that there are more cAMP targets in the cells. Cyclic nucleotide–gated channels and Ih channels as potential cAMP targets are known to be expressed in the third antennal segment of a Drosophila [64–67] and mutants of the ether a go-go (egg) gene encoding a cyclic nucleotide–modulated K⁺ channel show olfactory deficits [68]. Interestingly, in sensory neurons in the basal layer of the mouse VNO K⁺ channels encoded by ether-a-go-go-related gene (ERG) function as key determinants of cellular excitability, and an increase in ERG channel expression extends the dynamic range of the stimulus–response function in basal vomeronasal sensory neurons [69]. Drosophila olfactory neurons usually display background firing in the absence of odors, cAMP dependent cag activation might provide an OR based mechanism of homeostatic plasticity in the periphery of the olfactory system, and could adjust the neurons to a desired output range.

Further evidences for G-protein signaling in Drosophila olfaction

An interesting observation is, that regulation of odor responses in Drosophila antennae by cell-autonomous circadian clocks is mediated by the levels of the G-protein receptor kinase GPRK2 [70], which influences the distribution of ORs in the sensory cells [71]. Rhythmic changes in spike amplitude were observed in-phase with the rhythms of odor-evoked EAG responses, but spontaneous or odor-evoked spike frequency of olfactory neurons did not change [70]. GPRK2 therefore has to influence an OR dependent signaling pathway downstream of the OR ion channel activity, possibly involving heterotrimeric G-proteins. Interestingly, we also have initial observations that the subcellular distribution of Gzs changes upon odorant exposure, with Gzs showing a marked redistribution from the dendrites of the sensory neurons to the base of the sensilla upon odorant exposure, providing further indication of an involvement of the protein in the signaling cascade (Figure S6).

There is other evidence for involvement of G-protein signaling in the Drosophila olfactory system. The exposure to nutrient-derived odors in Drosophila was shown to modulate life span in an Or83b dependent manner [72]. Longevity was extended when olfactory signaling was suppressed in Or83b-expressing neurons through the disruption of G-protein signaling [72], and it was speculated that G-protein function in an odor-induced signaling cascade in sensory neurons influences life span [72]. Moreover, accompanying microarray analysis revealed that aging and diet mostly affect expression of Drosophila odorant binding proteins and genes involved in regulating G-protein signaling.

Last but not least, there is more than single evidence that arrestsin modulate olfactory signal transduction in insects [73–76], indicating that the receptors retained some properties of classical G-protein coupled receptors.

Conclusions

Taken together, we provide here strong evidence for the involvement of Gzs in olfactory signal transduction in Drosophila and signaling of recombinant Drosophila ORs, although these proteins have been shown to have an unusual membrane topology and comprise a new class of ligand-activated non-selective cation channels [14,48]. Whether G-protein activation in the ORNs is mediated via ORs with intracellular C-termini or via a G-protein-binding domain at the N-terminus remains to be examined in future studies. There are experimental evidences for and against G-protein signaling in Drosophila olfaction. Our data are consonant with a recently proposed dual-activation model [77], due to which the primary response is generated by activation of ligand-gated ion channels, followed by a G-protein-mediated potentiation or modulation of the ionotropic response. Insects seem to have not only one principal odor transduction cascade, but different cascades which may allow for adjustment of the odor response depending on the endogenous rhythms, behavioural states, and the properties of the odorants [49]. Although further mechanistic models have to await investigations on the mode of receptor G-protein coupling in the olfactory system, it is tempting to speculate that Drosophila ORs couple to heterotrimeric G-proteins although having an inverse membrane topology compared to other GPCRs. This duality in signaling might ensure the precision of the underlying transduction mechanisms and allow the insect to cover a wide dynamic range, highly important due to the behavioral relevance of olfactory signaling in insects.
Methods

Drosophila stocks

All flies were maintained on conventional cornmeal-agar-molasses medium under a 12-hr-light:12-hr-dark cycle at 18°C or 25°C. The following fly stocks were kindly provided: OR83b-Ga4 [John Carlson, Yale University, USA], UAS-mCD8-GFP, Gr21a-Ga4, Or22a-Ga4 [Bloomington Stock Center], w[1118]; P[ry+17.2] = 70FLP]10 [Bloomington Stock Center #6930], UAS-Gαs-wt and UAS-Gαs-Q215L (Cahir O’Kane, University of Cambridge, England), UAS-Gαo-wt, UAS-GαoQ205L, UAS-Gαo-G203T, UAS/αs/Ctx and UAS/αs/Ctx/Ptx [Andrew Tomlinson, Columbia University, USA]; UAS-Gαq and UAS-GαqQ203L (Gaiti Hasan, Tata Institute of Fundamental Research, India); UAS-Gαi-wt, UAS-GαiQ205L and UAS-GαiRNAi (Juergen Knoblich, Institute of Molecular Biotechnology, Austria); UAS-Pacα (Martin Schwärzler, Freie Universität Berlin, Germany); Q83F/Or83F (Leslie Vosshall, Rockefeller University, USA). For the generation of UAS-Gαs-GFP flies, the transgenic construct was injected into yw embryo by the VANEDIS Drosophila injection service (Oslo, Norway) using standard procedures.

Construction of expression plasmids

The C-terminus of the human G-protein alpha subunit 16 (Gα16AC44) was amplified from an existing construct (pCISG16 [78]) by PCR using the following primers 5′-cggtgatcgcgccgctgtcagctc-3′; 5′-cggtgatcgcgccgctgtcagctcac-3′. The PCR fragment was cloned into the BamHI and EcoRV sites of the mammalian expression vector pCDNA3 (Invitrogen). Different Drosophila G-protein C-terminal fragments were generated from Drosophila head cDNA by polymerase chain reaction and fused into the EcoRV and Xbal sites of the pCDNA3-Gα16AC44 construct. The primers used for the PCR were: Gαs-wt, 5′-gctagccgagaaacctg-3′, Gαo-wt, 5′-cggtagatatacaatccttacgagacgagc-3′, Gαo-fw, 5′-cattgtagatatatataagg-3′, Gαq-fw, 5′-cgcttacagagacagcttttgagc-3′, Gαo-fw, 5′-gctctagtatagagcaatcttacgagacgagc-3′, Gαi-fw, 5′-cgaacagggacaaccaagggcagcgagc-3′, Gαi-fw, 5′-cggtagatatacaatccttacgagagc-3′, Gαi-fw, 5′-cggtagatatacaatccttacgagacgagc-3′, Gαi-fw, 5′-cggtagatatacaatccttacgagacgagc-3′.

Cell culture and transfection

Experiments using heterologous expression system were performed in HEK293 cells, which were maintained under standard conditions in MEM supplemented with 10% FBS, 100 units/ml penicillin and streptomycin, and 2 mM L-glutamine. Transfections were done using a standard calcium phosphate precipitation technique.

Single cell Ca2+ imaging

For calcium imaging experiments, HEK293 cells were transfected with OR43a, OR83b and the respective G-protein chimera or full length human Gα16 as control (ratio 1:2:4). Two days after transfection, culture medium was removed and replaced with Ringer solution. Cells were incubated (45 min) in Ringer solution containing 7.5 μM Fura-2-AM (Invitrogen) at room temperature. Ratiofluometric Ca2+ imaging was performed as described [51,79] using a Zeiss inverted microscope equipped for ratiometric imaging with a xenon arc lamp, a multiwavelength illumination system POLYCHROME II for excitation, a cooled charge-coupled device (CCD) camera, and WinNT based T.I.L.L.-Vision software to collect and quantify spatiotemporal Ca2+ dependent fluorescence signals (β40/β30 ratio). Cells were exposed to the odorant cyclohexane (300 μM) for 10 s using a specialized microcapillary application system that transiently superfuses all cells in the field of view from one of six user-selected capillary tubes, tube tips were in close proximity to the optical field. Ringer’s solution continuously superfused all the cells in the dish between application of test solutions. Images were acquired from up to 15 cells in a randomly selected field of view, and integrated fluorescence ratios (β40/β30 ratio) were measured.

[35S]GTPγS binding assay

Transfected cells were homogenized in 10 mM HEPES and 10 mM EDTA (pH 7.4) and membrane preparations were prepared. [35S]GTPγS binding was assayed in a final volume of 100 μl (pH 7.4), containing 10 mM HEPES, 100 mM NaCl, 10 mM MgCl2, 100 mM guanosine 5′-diphosphate (GDP) and 0.1 mM [35S]GTPγS. The incubation was started by the addition of membrane suspension (about 30 μg of membrane protein per reaction) and was carried out in triplicate for 30 min at 30°C. The reaction was terminated by addition of 3 ml of ice-cold filter wash buffer and rapidly vacuum filtered through glass fiber filters (Whatman GF/C). Filters were washed three times with 3 ml of wash buffer, transferred to scintillation vials, and counted in a Packard Tri-Carb liquid scintillation counter.

Western blotting and RT-PCR

The third antennal segments were collected from 100 flies (3–5 days old). RNA was isolated with TRIzol Reagent (Invitrogen) and cDNA was synthesized using MMLV reverse transcriptase (Invitrogen) and oligo (dT12-18) primer. Amplifications were performed with 1 ng cDNA and specific primer pairs for Gα4 (Gαs440-fw: 5′-TTCTTGAACCATGAGGAGG-3′ and Gαs661-rw: 5′-TCCTAGCGTCCGTTCC-3′). For western blotting, 200 antennae were homogenized in Laemmli buffer (30% glycerol, 3% SDS, 125 mM Tris/Cl, pH 6.8), resolved by 10% SDS-PAGE and transferred to nitrocellulose membrane (Protran; Schleicher & Schuell). Transfected HEK293 cells were harvested two days after transfection. Cells were homogenized and post-nuclear supernatants were mixed with Laemmli buffer, separated on 10% SDS-PAGE gels and blotted. The membranes were incubated with primary anti-Gα4 (Santa Cruz), with and without pre-incubation with a specific blocking peptide (Santa Cruz), or anti-Gα16 (sc-7416, Santa Cruz) antibodies and detection was performed with ECL plus (Amer sham) on Hyperfilm ECL (Amer sham).

Immunohistochemistry

Fly heads were cut, fixed for 3 hr in 4% paraformaldehyde at 4°C and subsequently incubated overnight in 25% sucrose in Drosophila Ringer’s solution (182 mM KCl, 46 mM NaCl, 3 mM CaCl2, 10 mM Tris-HCl, pH 7.2). Cryosections were performed to produce 12 μm sections. After blocking with 5% goat serum in Ringer’s, a polyclonal anti-Gα4 antibody (Santa Cruz), a polyclonal anti-Gα8 antibody (University of Texas-Southwestern Medical Center [80]), or anti-GFP antibody (Abcam) were applied to the sections overnight at 4°C. After washing, the secondary anti-goat antibody coupled to A48 (Invitrogen) was applied for 1 hr at room temperature. Pictures were taken with a Zeiss confocal microscope (LSM510 Meta; Zeiss).

Electrophysiology

The electroantennogram (EAG) responses of wt and G-protein mutant flies were recorded as previously described [12,81]. Briefly, 2–3-day-old flies were mounted in truncated micropipette tips with the anterior portion of the head protruding from the end of the tip.
The indifferent electrode was inserted into the haemolymph of the head capsule. The recording electrode was placed on the frontal surface of the anterior aspect of the antenna. After obtaining a stable baseline, EAG recordings were initiated by a short pulse of odor (0.3 s), applied into an air stream that was directed toward the antenna. Duration of the pulse was electronically controlled. Total airflow was 2.8 l/min (tube diameter = 7 mm). The fraction of the stimulus or the respective blank controls was 0.4 ml/min, which was injected into a background stream of 2.4 l/min. When no stimulus was applied, a respective compensatory airstream was injected to keep the input air pressure on the fly constant. 50 µl of the odorant solutions were pipetted on filter paper strips that were inserted in 5 ml plastic serological syringes. The volume of the pipettes was adjusted to 4 ml, through which the stimulus airstream was directed during the application. Since the stimulus airstream was injected into the background stream before application to the antenna, the odorant containing gas phase was diluted to 14% prior application. The final odorant concentration in the gas phase that reaches the antenna can not be determined precisely, only different dilutions of the odorant can therefore be compared. CO2 was applied in a similar manner resulting in a final concentration of 14% due to the dilution with the background air stream. All odorants were purchased from Sigma in the highest available purity and were dissolved in paraffin oil at the given concentrations. Odorants used were cyclohexanol, benzaldehyde, heptanone and ethyl acetate. Inter-stimulus intervals were 30 s to avoid adaptation of the olfactory system to the stimuli.

The procedures of extracellular single-unit recording was essentially similar as previous report [82]. Canton-S flies aged < 1 week were used for wt recordings. hs-flp; Or83b-Gal4, UAS<αt>CTX flies were recorded after standard heat shock (one hour, 38°C) at age 4-5 days. Electrical activity of the neurons was recorded extracellularly by placing an electrode filled with sensillum lymph ringer in the base of the sensillum. A reference electrode filled with the same ringer was placed in the eye. Signals was amplified using a patch-clamp amplifier (L/M-PC, List-Medical Electronic, Darmstadt, Germany) in voltage-clamp mode and fed into a computer via a 12-bit analog/digital converter (Digidata 1200A; Molecular Devices, CA, USA). Impulses during the 1 s period before stimulation and the 1 s during stimulation were counted off-line using the Mini Analysis software (Synaptosoft, Decatur, GA, USA). The spikes were sorted into different groups according to their amplitude as previously described [83]. Each sensillum was tested with multiple odorants, and no more than two sensilla were analyzed per fly. Blue light exposure was accomplished with fluorescence illumination channel of the microscope using appropriate filters.

Supporting Information

**Figure S1** RT-PCR analysis of Gα subunit expression in *Drosophila antenna*. [A] RT-PCR results revealed expression of all Gα subunits transcrip- tants in the antenna, with a higher expression level of Gαq1, Gαq3, Gαs, CG17766 and CG17760 as compared to that of Gαf, CG3004 and CG30054. Gαq3 and the retina specific Gαq1 are transcribed from Gαf9B (CG17759) (Talluri et al., 1995). The different lanes correspond to: 1:Marker; 2:Gαf; 3:Gαq1eyC-1; 4:Gαq3-1; 5:Gαq1eyC-2; 6:Gαq3-2; 8:Gαs; 8:CG30054, 9:CG17766; 10: CG17760; 11:CG3004. (primer sequences are provided in Text S1). [B] Heterotrimeric G-protein α subunits are divided into 4 classes based on their sequence similarity and downstream effectors. Together with some well-identified Gα subunits from other species, a phylogenetic tree of Gα subunits was generated by the MegAlign program using the Clustal V method. As shown, 3 classes of vertebrate Gα proteins can also be identified from *Drosophila*. The ungrouped G-proteins are Gαf, CG3004 and CG17766, which show low similarities with the classified Gα subunits. Phylogenetic tree of Gα subunits from *Drosophila melanogaster, Mus musculus, Homo sapiens, Anopheles gambiae, Panulirus argus, and Rattus norvegicus*. (TIF)

**Figure S2** EAG recordings of flies with impaired G-protein signaling. [A] EAG amplitudes (in mV) of Gαq mutant flies expressing constitutively active (GTPase deficient) Gαq (n>10 flies were recorded), expression of the UAS construct was driven by Or83b-Gal4. Odorants used were ethyl acetate, cyclohexanol and benzaldehyde, each in concentrations of 10^-4, 10^-3, 10^-2, and undiluted. The differences between Gαq flies and wt flies were statistically checked (pairwise) by unpaired Student’s t tests; significance levels were set according to the Bonferroni post hoc test for k=4 means per odorant, *P=0.0125, **P=0.0025, p values are given on top the respective bars. [B] EAG amplitudes (in mV) of wild-type flies (control), wild-type flies where standard ringer solution was microinjected in the third antennal segment (ringers), and flies where CTX (diluted in ringer solution, 100 ng/ml) was injected in the third antennal segment (CTX). Error bars represent s.e.m. (TIF)

**Figure S3** Higher magnification of single sensillum recording from ab1 sensillum in wt flies. Spikes corresponding to activation of the different neurons (ab1A-ab1D) are labeled. (TIF)

**Figure S4** Expression of constitutively active Gαq. [A] Shown are traces from the ab1 sensillum of wt and Or83b-Gal4; UAS-Gαq-GTPflies during the 1000 ms ethyl acetate application period. [B] Summary of the responses in single unit recordings of ab1A neurons in Or83b-Gal4; UAS-Gαq-GTPflies, expressing a GTPase deficient Gαq mutant. Specifically the initial increase in spike rates upon application of ethyl acetate (1:100) was analyzed, number of spikes in 50 ms is counted and shown here. Differences between data points were statistically checked by the unpaired Student’s t test, p values are indicated on top of each bar. Significance levels were set according to the Bonferroni post hoc test for k=20 means, P<0.0025; the initial response in the first 50 ms was significantly different between both flies. Error bars represent s.e.m. (TIF)

**Figure S5** Control single sensillum recordings with PAC flies. Shown are traces from the ab1 sensillum from Or83b/-flies; light exposed Or83b/-flies showing that the sensillum does not respond to light exposure; PAC expressing OR83b/-flies showing no increase in spike rate due to PAC expression; PAC expressing OR83b/-flies exposed to CO2 showing that CO2 responses are normal; PAC expressing OR83b/-flies exposed to odorants, showing that PAC expression does not restore any odorant induced activity in the neurons; PAC expressing OR83b/-flies exposed to light, showing increase in spike rate due to PAC mediated cAMP increase. (TIF)

**Figure S6** G-protein redistribution upon odorant exposure. [A] Higher magnification pictures of Gα, staining (red), showing that Gαq is localized in the dendrites of the sensory neurons in odorant deprived animals, but is translocated to the cell body and the basis of the sensilla upon odorant exposure. [B] To confirm the observed redistribution of activated Gαq, a fly line was
generated expressing a Gαx-GFP fusion protein under control of the UAS promoter (OR103a-G4; UAS- Gαx-GFP), a schematic drawing of the fusion construct is provided. Fusion of GFP to either C- or N-terminus is critical for G-protein α subunits, since the NH2 terminus is important for association with the Gα subunits and the COOH terminal is required for interaction with receptor, but functional Gαx-GFP fusion proteins were obtained by inserting GFP into an internal loop. Expression of a similar fusion protein in olfactory neurons (Gal4-OR103a-UAS-Gαx-GFP), resulted in green fluorescence in the sensory neurons, and was identified in the third antennal segment by immunoblotting. In odorant deprived animals, the fusion protein was localized in the cell bodies and in the sensilla, but in odorant exposed flies Gαx-GFP could no longer be detected in the dendrites of the sensory neurons, indicating displacement of the fusion protein from the plasma membrane throughout the cytoplasm of the cells.

Table S1 G-protein mutant strains used in this study.

| Gene family | Strain name | Characteristics |
|-------------|-------------|-----------------|
| Gα subunits | Gal4-OR103a-UAS-Gαx-GFP | Green fluorescence in the sensory neurons |

References

1. Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65: 175–187.
2. Firestein S (2001) How the olfactory system makes sense of scents. Nature 413: 213–218.
3. Rosenblatt GV, Moon C (2002) G proteins and olfactory signal transduction. Annu Rev Physiol 64: 189–222.
4. Vosshall LB, Wong AS, Axel R (2000) An olfactory sensory map in the fly brain. Cell 102: 147–159.
5. Neri M, Niimura Y, Nozawa M (2008) The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. Nat Rev Genet 9: 951–963.
6. Hallem EA, Moyzis RK, Carlson Jr. JR (2004) The molecular basis of odor coding in the Drosophila antenna. Cell 117: 963–979.
7. Jones WD, Young TA, Kluss B, Lee KJ, Vosshall LB (2005) Functional conservation of an insect odorant receptor gene across 250 million years of evolution. Curr Biol 15: R119–R121.
8. 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: 519–526.
9. 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 U S A 101: 5038–5043.
10. Larson MC, Domingos AI, Jones WD, Chiappe ME, Ameen H, et al. (2004) Or46b3 encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43: 703–714.
11. Nakagawa T, Sakurai T, Nishioka T, Tsuchiya K (2005) Insect sex pheromone signals mediated by specific combinations of olfactory receptors. Science 307: 1638–1642.
12. Neuhäusel EM, Gießmann G, Zhang W, Dooley R, Stortkuhl K, et al. (2006) Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. 2 Nat Neurosci 8: 15–17.
13. Benten R, Sachse S, Michnick SW, Vosshall LB (2006) Atpylic membrane topology and heterogenic function of Drosophila odorant receptors in vivo. PLoS Biol 4: e20.
14. Sato K, Pelligrino M, Nakagawa T, Nakagawa T, Vosshall LB, et al. (2008) Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452: 1002–1006.
15. Stewart R, Kivel A, Beale M, Vargas E, Carragher B, et al. (2000) Drosophila odorant receptors are novel seven transmembrane domain proteins that can signal independently of heterometric G proteins. Insect Biochem Mol Biol 30: 770–780.
16. Tichy D, Agisola HJ, Sehler S, Gniepel M, Heinemann SH, et al. (2006) Differential receptor activation by cockroach adenosine type 2A receptors produces differential effects on ion currents, neuronal activity, and locomotion. J Neurophysiol 95: 2314–2325.
17. Laue M, Maida R, Redkozubov A (1997) G-protein activation, identification and immunolocalization in pheromone-sensitive sensilla trichodea of moths. Cell Tissue Res 288: 149–158.
18. Miura N, Atsumi S, Tabunoki H, Sato R (2005) Expression and localization of three G protein alpha subunits, Go, Gi, and Gs, in adult antennae of the silkworm Bombyx mori. J Comp Neurol 483: 143–152.
19. Tallant S, Bhatt A, Smith DP (1995) Identification of a Drosophila G protein alpha subunit (dGαq-α3) expressed in chemosensory cells and central neurons. Proc Natl Acad Sci U S A 92: 11475–11479.
42. Yapici N, Kim YJ, Ribeiro C, Dickson BJ (2008) A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature 451: 33–37.

43. Wetzal CH, Oles M, Wellerdeick C, Kuczkiowski G, Gisselmann G, et al. (1999) Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and Xenopus laevis oocytes. J Neurosci 19: 7426–7433.

44. Wetzal CH, Behrendt HJ, Gisselmann G, Stortknull KF, Hovemann B, et al. (2001) Functional expression and characterization of a Drosophila odorant receptor in Drosophila melanogaster. J Neurobiol 46: 1544–1563.

45. Schultz Lang S, Schwan M, Seifert R, Strunkel T, Kateriya S, et al. (2007) Fast manipulation of cAMP level by light in vivo. Nat Methods 4: 39–42.

46. Lundin C, Kall L, Kreher SA, Kapp K, Sonnhammer EL, et al. (2007) Membrane topology of the Drosophila OR3B odorant receptor. FEBS Lett 581: 446–450.

47. Fischer D, Schaffner R, Baerenfreund R, Stensmyr MC, Heller R, et al. (2008) Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452: 1007–1011.

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

49. Wellerdieck C, Krieger J, Feistner T, Schaffner R, Stensmyr MC, Heller R, et al. (2008) Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452: 1007–1011.

50. Forstner M, Breier H, Krieger J (2009) A receptor and binding protein interplay in the detection of a distinct pheromone component in the silkworm Antherea polyphemus. Int J Biol Sci 5: 745–757.

51. Jordan MD, Anderson A, Begum D, Carraher C, Authier A, et al. (2009) Olfactory receptor neurons of the hawkmoth Manduca sexta. J Neurophysiol 96: 2866–2877.

52. Forstner M, Breier H, Krieger J, Feistner T, Schaffner R, Stensmyr MC, Heller R, et al. (2008) Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452: 1007–1011.

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

54. Wellerdieck C, Krieger J, Feistner T, Schaffner R, Stensmyr MC, Heller R, et al. (2008) Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 452: 1007–1011.

55. Spern M, Gisselmann G, Poplawski A, Viemann J, Wetzal CH, et al. (2003) Identification of a testicular odor receptor mediating human sperm chemotaxis. Science 299: 2054–2058.

56. Offermanns S, Simon MI (1995) G alpha 15 and G alpha 16 couple a variety of receptors to phospholipase C. J Biol Chem 270: 15175–15180.

57. Offermanns S, Simon MI (1995) G alpha 15 and G alpha 16 couple a variety of receptors to phospholipase C. J Biol Chem 270: 15175–15180.

58. White JF, Grodnitzky J, Louis JM, Trinh LB, Shihoacj J, et al. (2007) Identification of the class A G protein-coupled neurotransmitter receptor NTSl alters G protein interaction. Proc Natl Acad Sci U S A 104: 12199–12204.

59. Baumann A, Frings S, Godde M, Seifert R, Kapp UB (1994) Primary structure and functional expression of a Drosophila cyclic nucleotide-gated channel present in eyes and antennae. EMBO J 13: 5040–5050.

60. Baumann A, Frings S, Godde M, Seifert R, Kapp UB (1994) Primary structure and functional expression of a Drosophila cyclic nucleotide-gated channel present in eyes and antennae. EMBO J 13: 5040–5050.