Cellular/Molecular

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

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Mechanisms by which G-protein-coupled odorant receptors transduce information in insects still need elucidation. We show that mutations in the *Drosophila* gene for $G_q\alpha$ (*dgq*) significantly reduce both the amplitude of the field potentials recorded from the whole antenna in responses to odorants as well as the frequency of evoked responses of individual sensory neurons. This requirement for $G_q\alpha$ is for adult function and not during antennal development. Conversely, brief expression of a dominant-active form of $G_q\alpha$ in adults leads to enhanced odor responses. To understand signaling downstream of $G_q\alpha$ in olfactory sensory neurons, genetic interactions of *dgq* were tested with mutants in genes known to affect phospholipid signaling. *dgq* mutant phenotypes were further enhanced by mutants in a PLCβ (phospholipase Cβ) gene, *plc21C*. Interestingly although, the olfactory phenotype of mutant alleles of diacylglycerol kinase (*rdgA*) was rescued by *dgq* mutant alleles. Our results suggest that $G_q\alpha$-mediated olfactory transduction in *Drosophila* requires a phospholipid second messenger the levels of which are regulated by a cycle of phosphatidylinositol 1,4-bisphosphate breakdown and regeneration.

Key words: dgq; olfactory sensory neurons; electroantennogram; *plc21C*; *rdgA*; signal transduction

Introduction

In all animals studied, odorant detection is initiated by interaction of volatiles with seven transmembrane domain receptors. In vertebrates, these receptors are coupled to the olfactory G-protein (G_{olf}), which activates adenyl cyclase III and raises cAMP levels in sensory neurons resulting in the activation of a cyclic nucleotide gated ion channel followed by the opening of a chloride channel (Ache and Young, 2005). In contrast to this well established mechanism, the events that follow odor reception in invertebrates are still rather controversial. In insects, the role of G-protein-coupled sensory transduction is confounded by the fact that the highly conserved OR83b receptor heterodimerizes with conventional receptors (ORs) and the Manduca OR/OR83b dimer directly confers ligand stimulated nonselective channel activity in heterologous systems (Nakagawa et al., 2005). Cell culture and *in vivo* evidence that the *Drosophila* Or83b exhibits an unusual membrane topology with the proposed cytoplasmic G-protein binding region lying on the extracellular side has also been provided (Benton et al., 2006). These findings beg the analysis of the role of G-protein-coupled signaling during olfactory transduction in invertebrates.

Previous evidence supports a role for cAMP (Martin et al., 2001; Gomez-Diaz et al., 2004) and inositol 1,4,5-trisphosphate ($InsP_3$) as second messengers downstream of receptor activation in arthropods (Krieger and Breer, 1999). More recently, changes in the levels of membrane-bound phospholipids have been shown to alter the primary response to odorants in crustaceans (Zhainazarov et al., 2004). These studies together favor the possibility of differential activation of dual transduction systems involving both $G_s\alpha$ (via cAMP) and $G_q\alpha$ (via $InsP_3$ and other phospholipids) during olfactory sensing (Ache and Young, 2005). Moreover, expression of $G_q\alpha$ RNA was demonstrated in a subset of olfactory sensory neurons (OSNs) (Talluri et al., 1995) and its knockdown by targeted RNA interference (RNAi) resulted in behavioral deficits (Kalidas and Smith, 2002).

In the present study, we directly tested the role of $G_q\alpha$ in olfactory transduction through the electrophysiological measurements of antennal neuron responses in several classes of mutants in the *Drosophila* gene for $G_q\alpha$ (*dgq*). Null mutants in *dgq* are recessive lethal as expected from previous expression studies (Ratnaparkhi et al., 2002). Hence to measure olfactory responses from the adult olfactory organs of *dgq* null alleles, we used the mosaic analysis with repressible cell marker (MARCM) method

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(Lee and Luo, 1999) to produce green fluorescent protein (GFP) marked clones of *dgq* null antennal neurons. A developmental contribution of $G_q\alpha$ was ruled out by adult-specific rescue of the physiological deficits observed with a $G_q\alpha$ transgene. To understand signaling mechanisms downstream of $G_q\alpha$, the interaction of *dgq* mutants was tested with existing mutants and RNAi lines, predicted to affect phospholipid intermediates.

Materials and Methods

Fly stocks. The dgq^{221c} allele was generated by P-element excision of the *P{SUPor-P}Galpha49B[KG07290]* strain carrying an insertion in the 5′-untranslated region (UTR) of *dgq*; the dgq^{1370} allele was generated by ethyl methane sulfonate mutagenesis. Mutations were verified by their failure of complementation with *Df(2R)vg-C*, which uncovers the *dgq* locus and subsequent molecular analysis. *GqGAL4* and *UASdgqα3* stocks were provided by Sonal Srikanth [National Centre for Biological Sciences (NCBS), Bangalore, India] and Neha Agrawal (NCBS); details of the RNAi construct for *dgq* ($UASGq^{1F1}$) and *UASAcGq* transgenics are published (Ratnaparkhi et al., 2002; Banerjee et al., 2006). *plc21C* insertion allele ($plc21C^{P319}$) and the deficiency *Df(2L)p60A*, which uncovers *plc21C*, were obtained from S. Leevers (Cancer Research Institute, London, UK) (Weinkove et al., 1999). $rdgA^1$ and $rdgA^3$ stocks were provided by Dr. R. Padinjat (The Babraham Institute, Cambridge, UK) (Hardie et al., 2002). $Tub\text{-}PGAL80^{ts}$, $Tub\text{-}PGAL80^{ts}$ (McGuire et al., 2003) recombinant was provided by Albert Chiang (NCBS). *y,w*; $P\{ry{+}t7.2 = neoFRT\}^{42B}/CyO$, $Tub\text{-}GAL80\ FRT^{42B}$, *ey-Flp*; $hsGAL4^t/CyO$, and *Df(2R)vg-C/CyO* were obtained from the *Drosophila* Stock Center (Bloomington, IN). *Or22aGAL4* and *Or83bGAL4* strains were kindly provided by Leslie Vosshall (Rockefeller University, New York, NY) (Vosshall et al., 2000). RNAi lines for *rdgA* and *plc21C* were obtained from the VDRC stock center. Stocks were grown on standard cornmeal medium at 25°C. White prepupae [0 h after puparium formation (APF)] were collected and allowed to develop on moist filter paper. Wild-type pupae take ~100 h to eclose when grown at 25°C in our laboratory.

DNA sequencing. The *dgq* gene was amplified by the PCR from genomic DNA from dgq^{1370} larvae, cloned into a TOPO vector (Invitrogen, Carlsbad, CA) and sequenced by standard methods. The background strain used for generating dgq^{1370} was sequenced in the same region to verify that the mutation was not a result of a background polymorphism.

Reverse transcription and PCRs. Total RNA was isolated from 200 adult antennas following standard procedures. Primers complementary to exon 2 (forward primer, 5′-GGTCACCTCATCGAGCAAGC-3′) and exon 3 (reverse primer, 5′-GAGCAAGTCTGCATGCAACGG-3′) were used for reverse transcription and PCR analysis (RT-PCR) of *plcβ21C*, according to published procedures (Nair et al., 2007).

Clonal analysis. Clones of *dgq* mutants were generated using the MARCM method (Lee and Luo, 1999). dgq^{221c} and dgq^{1370} were recombined onto the FRT^{42B} chromosome. Females of genotype FRT^{42B}, $dgq^{221C}/CyOGFP$; *ey-Flp/TM6Tb* and FRT^{42B}, $dgq^{1370}/CyOGFP$; *ey-Flp/TM6Tb* were crossed to $Tub\text{-}GAL80\ FRT^{42B}/CyO$; *OR83bGAL4, UAS2XEGFP/TM6Tb.* Non-*CyOGFP*, non-*Tb* flies were used for electroantennogram (EAG) recordings and the presence of clones was monitored by expression of GFP in the third antennal segment. To visualize projections of OSNs into the antennal lobe, *Or22aGAL4;* FRT^{42B} dgq^{221c}/CyO; *ey-Flp/TM6Tb* and *Or22aGAL4;* FRT^{42B}, dgq^{1370}/CyO; *ey-Flp/TM6Tb* were mated with $Tub\text{-}GAL80\ FRT^{42B}/CyO$; *UASNsybGFP/TM6Tb* males. Non-*CyO*, non-*Tb* flies were dissected and stained using anti-GFP and mAbnc82.

Cuticle preparation. Adult antennas were removed and placed in a drop of Faure's solution (34% v/v chloral hydrate, 13% v/v glycerol, and 20 mg/ml gum-arabic) and allowed to clear overnight at 70°C. Sensilla were counted after projecting Nomarski images from the microscope onto the video monitor. The mean and SD of sensillar numbers were estimated from at least five antennas for each genotype.

Immunohistochemistry. Dissection and antibody staining of pupal antenna and brain as well as whole mounts of the adult brain were performed as described previously (Jhaveri et al., 2000). Fifteen micrometer frozen sections through the fly head were fixed in 4% paraformaldehyde in PBS for 30 min, washed extensively, and blocked in PBS containing 5% normal goat serum, 0.1% bovine serum albumin, and 0.03% Triton X-100 for 1 h at room temperature. Where mAbnc82 was to be used, the protocol of Laissue et al. (1999) was followed. The primary antibodies used were anti-Gq (1:200; SC-3912; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-GFP (1:10,000; Invitrogen), mAb22C10 (1:5; Developmental Studies Hybridoma Bank, Iowa City, IA), mAbnc82 (1:10; Developmental Studies Hybridoma Bank), and mouse anti-GFP (1:20; Sanmar Chemicals, Bangalore, India). Secondary antibodies were anti-mouse and anti-rabbit IgG conjugated to either Alexa 488 or Alexa 568 (1:200; from Invitrogen). Labeled samples were mounted in 70% glycerol and examined in the Bio-Rad (Hercules, CA) Radiance 2000 or an Olympus (Tokyo, Japan) FV1000, at 1 μm intervals; data were processed using Image J, Confocal Assistant 4.2, and Adobe Photoshop 5.5.

For immunochemical staining of adult antenna, the A3 segments were chopped in chilled PBS and fixed in 4% paraformadehyde with 3% Triton X-100 on ice for 5 h followed by three washes of 1 h each with PBS containing 2% Triton X-100, initially at room temperature and then at 4°C for 2 d. Samples were incubated for 3 d at 4°C in 1:5 dilution of mAb22C10 and anti-GFP (1:10,000). Antennas were washed three times for 1 h each in PBS containing 0.1% Triton X-100 after primary antibody incubation and treated with 1:200 dilution of anti-mouse and anti-rabbit IgG secondary antibodies conjugated to either Alexa 488 or Alexa 568, for 1 d at 4°C. After washes with PBS containing 0.1% Triton X-100, samples were mounted in anti-fading agent Vectashield (Vector Laboratories, Burlingame, CA), and examined in an Olympus FV1000 confocal microscope.

Electroantennogram recordings. Electrophysiological recordings were performed as described previously (Ayer and Carlson, 1991). Two- to 6-d-old female flies were immobilized on ice and wedged into an appropriately trimmed tapering ends of a yellow pipette tip allowing the head to protrude. Low melting point (58°C) myristic acid wax was used to hold the head onto the thorax. Glass capillaries (<1 μm in diameter) filled with 0.8% NaCl and connected to a $Ag/AgCl_2$ wire were used as electrodes. The reference electrode was inserted into the head capsule, whereas the recording electrode was placed in the basiconica rich region of the third antennal segment and connected to a high-impedance preamplifier (Electro 705; WPI, Sarasota, FL). Signals were viewed on a two-channel oscilloscope (TDS320; Tektronix, Beaverton, OR). Odorants, ethyl acetate (10^{-4}), butanol (10^{-2}), propionic acid (10^{-2}), benzaldehyde (10^{-3}), and iso amyl acetate (10^{-2}), of the highest purity available (Sigma-Aldrich, St. Louis, MO), were freshly diluted in liquid paraffin and used for odor delivery. Odor pulses were delivered into a constant stream of air for 2 s. An interval of 5 min was allowed after each odorant pulse to minimize effects attributable to adaptation. The peak-to-trough amplitude of the response was measured after each pulse of odor stimulation. When different genotypes were being tested, the unpaired *t* test was used to calculate significance. A value of $p < 0.01$ was taken to be significant.

Single-unit recordings. The reference electrode was inserted in the funicular tip of the antenna, and the recording electrode (<1 μm in diameter) was inserted into the base of an ab2 basiconic sensillum and connected to a high-impedance dual-channel preamplifier (model 750; WPI); identity of ab2 was ascertained by the characteristic spontaneous spike rate and by its location on the antenna. Signals were viewed on a two-channel oscilloscope (Tektronix TDS320). Odor pulses of 500 ms duration separated with an interstimulus interval of 10 s were given using an eight port custom-built olfactometer. Each sensillum was stimulated three times with 10^{-6}, 10^{-5}, and 10^{-4} dilution of ethyl acetate (Sigma-Aldrich), respectively, prepared fresh in liquid paraffin. The spike frequency was calculated using Labview 7.1 (National Instruments, Austin, TX) from an average of three trials. Recordings were obtained from numbers as described in the individual figure legends; minimally these were from six or more sensilla, taken from five or more flies.

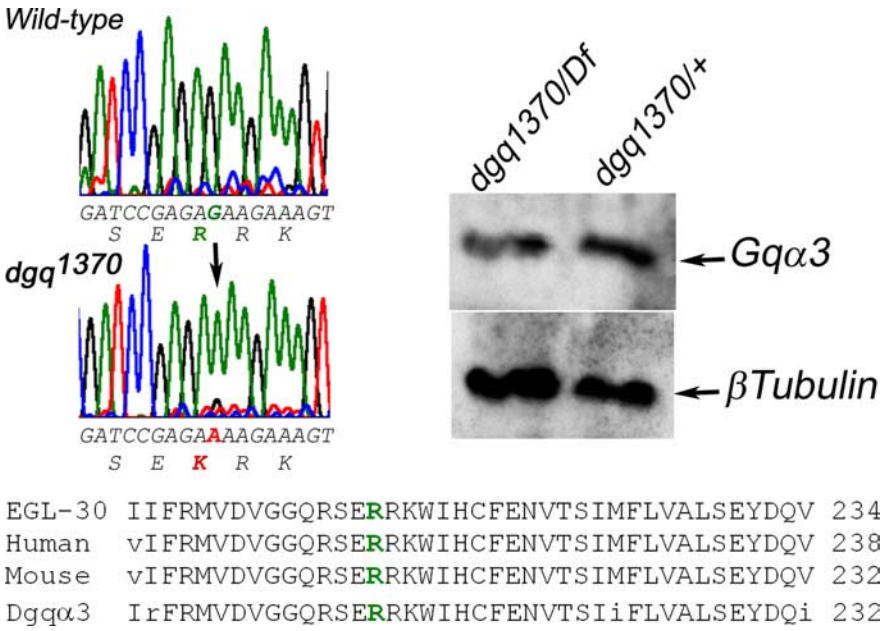

Figure 1. Identification of a single base pair change in *dgq*^{1370}. G→A change is found in genomic DNA isolated from *dgq*^{1370}. As a consequence, a highly conserved arginine residue in the switch region of $G_q\alpha$ is mutated to lysine. The $G_q\alpha$ sequences from *Caenorhabditis elegans* (EGL-30), human, mouse, and *Drosophila* are compared. Protein levels of $G_q\alpha3$ are not changed significantly in larvae of the genotype *dgq*^{1370}/*Df(vg-C)* compared with *dgq*^{1370}/± larvae.

Results

Mutants in the *Drosophila dgq* gene

The *dgq* gene is located at 49B on the second chromosome and encodes the only known functional Gq-like α subunit of heterotrimeric G-proteins in *Drosophila*. The gene encodes at least two functional isoforms, which arise by alternative splicing. Among these, *dgqα1* is expressed primarily in the adult eye (Lee et al., 1990), whereas *dgqα3* mRNA is expressed widely through development including the adult head and appendages (Talluri et al., 1995; Ratnaparkhi et al., 2002). Based on RNA expression analysis and behavioral studies with an RNAi construct (Kalidas and Smith, 2002), it has been suggested that a $G_q\alpha$ isoform might function downstream of olfactory G-protein-coupled receptors in antennal sensory neurons. To test this idea directly, we generated mutations in the *dgq* gene by excision of a P-element in the 5′-UTR of *dgq*, and by ethyl methane sulfonate mutagenesis. Molecular analysis demonstrated that the P-excision allele, *dgq*^{221c}, carries a lesion in the 5′ end of *dgq* spanning the translation start site in exon 3, thus rendering it null. Exons located 3′ to exon 3 are intact (Banerjee et al., 2006). Both *dgq*^{221c} and *dgq*^{1370} (induced by chemical mutagenesis) are lethal as homozygotes and as heterozygotes with *Df(2R)vg-C*, which uncovers the *dgq* locus. The lethality in *dgq*^{1370} fails to complement that of *dgq*^{221c}. Homozygous *dgq*^{1370} mutants produce a transcript with a G→A mutation at base pair 1933 (Fig. 1). This would result in a change from arginine (AGA) to lysine (AAA) at residue 207, which lies in the switch II helix region that is highly conserved among α subunits of all heterotrimeric G-proteins (Fig. 1). Structural studies show that this region undergoes a conformational switch on GTP binding, which is considered essential for effector function (Noel et al., 1993). Specifically, the arginines at positions 204 and 207 of Dgqα3 correspond to Arg205 and Arg208 of Gαi (Mixon et al., 1995). Both these residues have been shown to provide important stabilizing contacts after GTP binding with glutamates located further downstream. Presumably, the mutation in *dgq*^{1370} destabilizes the GTP-bound state of G_q and renders it effectively dead by preventing activation of the downstream effector. Normal levels of the mutant protein are present in *dgq*^{1370} homozygous larvae (Fig. 1).

Olfactory receptor neurons lacking *dgq* function exhibit reduced stimulus-evoked activity

Animals homozygous for the null alleles, *dgq*^{221c} and *dgq*^{1370}, die as either first- or early second-instar larvae. To study the odorant response from adult antennas, we generated animals with homozygous mutant clones on the antenna using the MARCM method (Lee and Luo, 1999). Mitotic recombination at the *FRT*^{42B} target site was induced using Flipase (*Flp*) driven by the *eyeless (ey)* promoter, which has been previously demonstrated to generate clones mostly in visual and olfactory sensory neurons (OSNs) and only minimally in the central brain (Zhang et al., 2003; Jefferis et al., 2004). Mutant clones identified by GFP driven by the OSN-specific "driver" *Or83bGAL4* (Larsson et al., 2004) were obtained in >80% of progeny of the appropriate genotypes (*FRT*^{42B}, *dgq*^{null}/*FRT*^{42B}, *tubPGAL80; ey-Flp/OR83bGAL4, UAS2XEGFP*) (Fig. 2*A*). Animals with strong GFP fluorescence in their antennas were chosen for the experiment, whereas animals with no visible GFP expression (i.e., no mutant clones) were control for effects attributable to background genotypes (Fig. 2*A*). Confocal sectioning of antenna bearing large clones, revealed significant mixing of marked and nonmarked cells suggesting that *ey-FLP* induces *FRT*-mediated recombination at multiple times during antennal development. The olfactory response was measured after pulsed odor delivery. EAGs (Fig. 2*A*) were obtained after each successive pulse of a concentration of each of five different odorants (Fig. 2*B–F*). Odorant concentrations used were chosen such that they lay within the range of the log linear response to that specific odorant, when tested on wild-type flies (data not shown). The response of wild-type (CS) and control flies bearing no detectable GFP-positive cells gave a comparable range of amplitudes for each of the odors tested ($p > 0.05$ in all cases except benzaldehyde and isoamyl acetate where $p > 0.01$; symbols in different colors in Fig. 2*B–F* represent different individuals). Antennas bearing clones of homozygous *dgq*^{221c} or *dgq*^{1370} tissue showed a response reduced to 3–10 mV compared with 8–16 mV in the controls (a comparison of the median responses of mutant and normal individuals showed significant difference; $p < 0.001$ in all cases). We ascertained that the mutation did not affect the morphology of the antenna by counting the three anatomically characterized sensilla (trichoidea, coeloconica, and basiconica); their numbers appeared normal in antenna with large clones homozygous for *dgq*^{221c} and *dgq*^{1370} (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). Antennal sensory neurons and their projections also appear normal as judged by immunohistochemical staining with mAb22C10 and anti-GFP shown in Figure 5, *J* and *K*.

To confirm that lower EAG responses were indeed attributable to mutations in the *dgq* gene, a *UASdgqα3* transgene (Banerjee et al., 2006) was expressed in *dgq* mutants using the *GAL4/UAS* system (Brand and Perrimon, 1993). In these clones, *dgqα3*

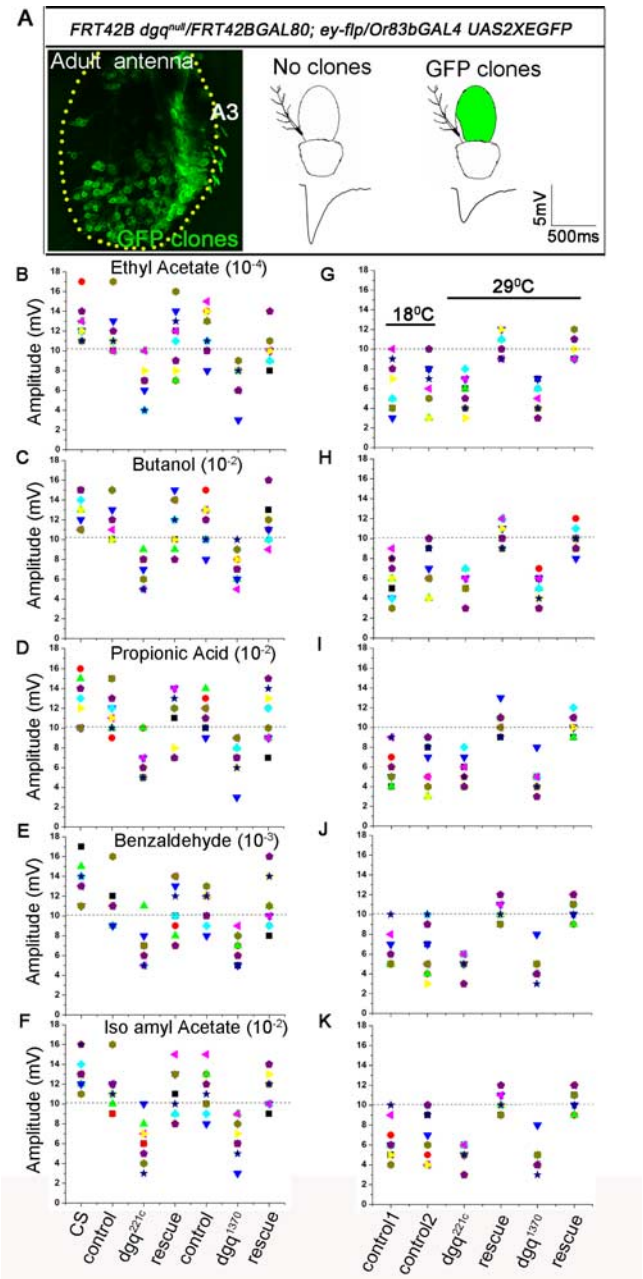

Figure 2. Electroantennograms from *dgq*-null clones have reduced amplitudes. ***A***, The MARCM technique was used to generate large GFP-positive clones in the third antennal segment from flies of the genotype *FRT*^{42B}*dgq*^{null}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP*. A 1 μm confocal section through the third antennal segment (A3) reveals the presence of clonal neurons (in green). Representative EAG traces from clonal antenna as well as internal controls (animals of the same genotype but with no visible GFP fluorescence in the third antennal segment) are shown. The diagrams indicate clones in each case (green shading). ***B–F***, Response profile of *dgq*^{null} clones and control siblings to a spectrum of odorants. Amplitudes of responses obtained from individual antennas of different genotypes are shown in colored symbols. In ***B–F***, CS is the wild-type strain; control, *FRT*^{42B}*dgq*^{null}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP* (with undetectable GFP positive clones); *dgq*^{221c}, *FRT*^{42B}*dgq*^{221c}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4,UAS2XEGFP*; and *dgq*^{1370}, *FRT*^{42B}*dgq*^{1370}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4,UAS2XEGFP* with large GFP mutant clones (see example in ***A***); rescue indicates animals with large *dgq*^{null} clones and a *UASdgq*α*3* transgene (*UASdgq*α*3/+; FRT*^{42B}*dgq*^{221c}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP* or *UASdgq*α*3/+; FRT*^{42B}*dgq*^{1370}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP*). Odor pulses are of 2 s duration followed by a gap of 5 min. Differences between the amplitudes for control and rescue strains are not significant for any of the odorant tested ($p > 0.1$). Both *dgq*^{null} strains are significantly different from the corresponding control strain ($p < 0.001$). ***G–K***, A ubiquitously expressed temperature-sensitive *GAL80* transgene was introduced in the rescue strain in ***B–F*** (*TubGAL80*^{tsx2}*/UASdgq*α*3; FRT*^{42B}*dgq*^{null}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP*). Animals reared throughout at 18°C are shown as control 1 and control 2 in ***G–K***. Recordings from 15 animals showed a range of responses from 2 to 10 mV as shown by the clones in ***B–F***. Experimental animals were shifted to 29°C immediately after eclosion for 5–6 d. *dgq*^{221c} or *dgq*^{1370} mutant animals without the rescue transgene (*TubGAL80*^{tsx2}*/+; FRT*^{42B}*dgq*^{null}/*FRT*^{42B}*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP*) reared at 29°C showed similar responses as control animals grown at 18°C. Rescue animals (*TubGAL80*^{tsx2}*/UASdgq*α*3; FRT*^{42B}*dgq*^{null}/*FRT*^{42B}*GAL80;ey-Flp/Or83bGAL4, UAS2XEGFP*) showed higher responses and rescued the phenotype.

was coexpressed with GFP by *Or83bGAL4*. Animals bearing *dgq*^{null} antennal clones in the background of *Or83bGAL4;UAS-dgq*α*3* responded with higher amplitudes in EAG recordings (Fig. 2*B–F*, data marked as rescue) (the median responses showed significant difference of $p < 0.005$, for all cases). To test whether the requirement for Dgq in odor sensing was sufficient during adulthood, we introduced a ubiquitously expressed temperature-sensitive *GAL80* transgene (*TubGAL80*^{ts}) (McGuire et al., 2003) into the rescue strains discussed above and grew them at permissive temperature (18°C) to silence GAL4 activity. Experimental animals were shifted to nonpermissive temperature (29°C) a few hours before eclosion to induce GAL4 expression and maintained at this temperature until tested, whereas controls were maintained at 18°C throughout. As shown in Figure 2, *G–K* (data marked as rescue), the *UASdgq*α*3* transgene rescues reduced EAG amplitudes even when expression begins in mature OSNs (by inactivation of the *TubGAL80*^{tsx2} transgene at 29°C very late in pupal development; $p < 0.001$). EAG amplitudes remain low in animals grown at 18°C (Fig. 2*G–K*, control 1 and control 2) or in animals transferred to 29°C as late pupae, but without the *UASdgq*α*3* transgene (Fig. 2*G–K*, *dgq*^{null} controls at 29°C). Thus, normal function of the *dgq* gene (and most probably the *dgq*α*3* splice variant) is necessary in adults for normal activity at the olfactory receptor neurons in response to odor stimuli.

It is known that a mutation in *dgq* affecting the visual splice variant (*dgq*α*1*) compromises the light response as measured by electroretinograms (ERGs) (Scott et al., 1995). As expected, *ey-Flp* also generated clones of *dgq* nulls in the retina (data not shown). As an independent confirmation of the clonal analysis, we measured ERGs from flies with visible antennal clones and found that responses were reduced to the same level as seen with the *dgq*^{1} allele. Moreover, *dgq*^{221c}/*dgq*^{1} and *dgq*^{1370}/*dgq*^{1} flies are viable and show compromised phototransduction, although not to the same extent as shown previously by *dgq*^{1}/*Df* flies (data not shown) (Scott et al., 1995). The residual visual response observed in *dgq*^{221c}/*dgq*^{1} and *dgq*^{1370}/*dgq*^{1} animals may arise from different genetic backgrounds of the three *dgq* alleles tested. Because the *dgq*^{1} allele affects only the visual splice variant of *dgq* (Scott et al., 1995; Ratnaparkhi et al., 2002), neither *dgq*^{221c}/*dgq*^{1} nor *dgq*^{1370}/*dgq*^{1} flies exhibited any defects in EAG measurements (data not shown). These data together with EAG recordings suggest that light and odor transduction in *Drosophila* exploit common mechanisms albeit using distinct transcripts of the *dgq* gene.

Odor responses in antennas are sensitive to levels of G_q

If $G_q\alpha$ is indeed stimulated by odor-bound receptors, it is expected that expression of a constitutively active $G_q\alpha$ subunit (AcGq3) (Ratnaparkhi et al., 2002) could affect the EAG response. To measure the effect of AcGq3 expression on olfactory transduction without compromising possible antennal development, a *hsGAL4* strain (*hsGAL4*^{t}) that has minimal basal expression at 25°C (Banerjee et al., 2006) was used. Flies of the genotype *hsGAL4*^{t}*/UASAcGq3* (and appropriate control genotypes) were reared at 18°C to minimize AcGq3 expression during pupal de-

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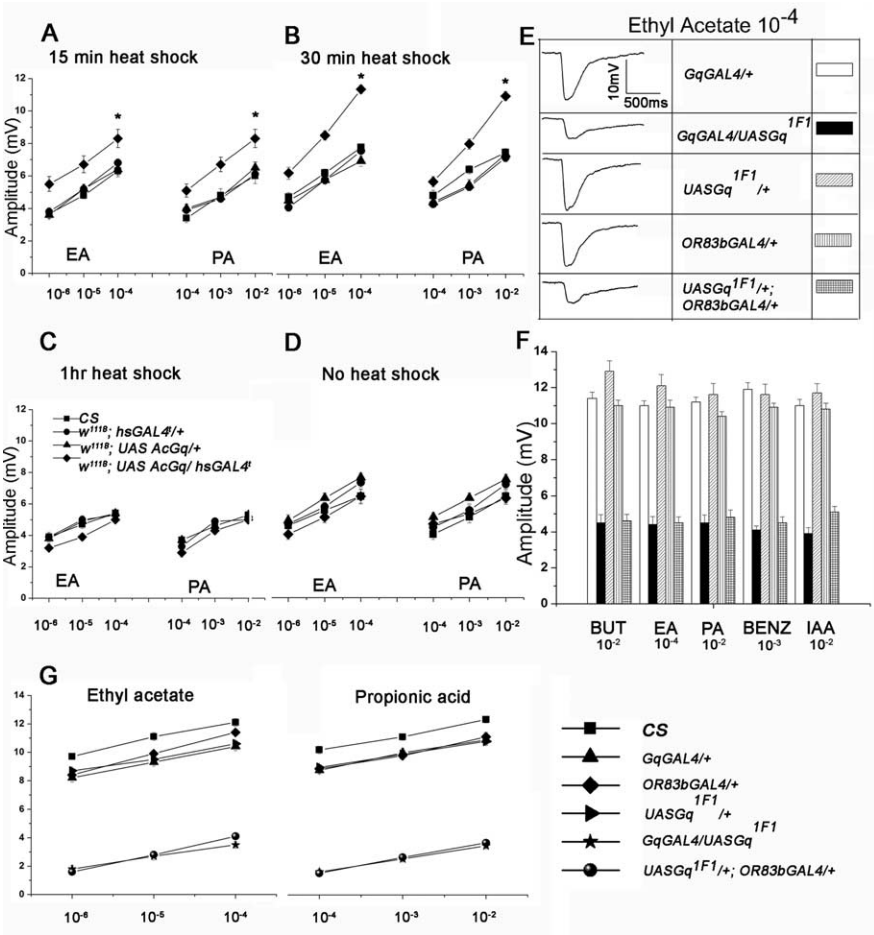

Figure 3. Odor responses in antennas are sensitive to levels of $G_q\alpha$ in sensory neurons. ***A–C***, Flies of the genotype *w*^{1118}; *hsGAL4*^{t}*/UASAcGq* and control animals *CS*, *w*^{1118};*hsGAL4*^{t}/+ and *w*^{1118};*UASAcGq*/+ were reared at 18°C throughout development. These animals exhibit a general reduction in EAG amplitudes compared with animals reared at 25°C (***D***). To induce AcGq expression, 4- to 5-d-old experimental and control animals were subjected to 37°C temperatures for 15 min (***A***), 30 min (***B***), or 1 h (***C***). EAG responses were measured at 10^{-6} to 10^{-4} dilution of ethyl acetate (EA) and 10^{-4} to 10^{-2} propionic acid (PA). Expression of AcGq significantly enhanced the amplitude of the EAG response at all concentrations of the two odorants tested (***A***, $p < 0.001$; ***B***, $p < 0.0001$) for heat shocks of 15 and 30 min. No enhancement of response was observed after a 1 h heat shock (***C***, $p > 0.1$). ***E***, Expression of an RNAi construct for *dgq* in the antenna reduces response to odorants. Representative EAG traces for ethyl acetate (10^{-4}) in controls (*GqGAL4/+*, *UASGq*^{1F1}/+ and *OR83bGAL4, UAS2XEGFP/+*) and experimental animals (*GqGAL4/UASGq*^{1F1} and *UASGq*^{1F1}*/+; OR83bGAL4, UAS2XEGFP/+*) are shown. ***F***, Average amplitude of EAG responses for butanol (10^{-2}) (BUT), ethyl acetate (10^{-4}) (EA), propionic acid (10^{-2}) (PA), benzaldehyde (10^{-3}) (BENZ), and iso-amyl acetate (10^{-2}) (IAA) in animals of genotypes shown in ***E***. Bars represent mean ± SEM ($n = 10$) for each genotype ($p < 0.0001$ for both genotypes). ***G***, Dose responses for ethyl acetate and propionic acid. Recordings from controls (*CS*, *GqGAL4/+*, *UASGq*^{1F1}/+ and *OR83bGAL4, UAS2XEGFP/+*) and experimental animals (*GqGAL4/UASGq*^{1F1} and *UASGq*^{1F1}*/+; OR83bGAL4, UAS2XEGFP/+*) are plotted. Response of the experimental animals is significantly different from controls ($p < 0.0001$).

velopment. It has been noted that all flies including wild-type controls produced smaller EAG responses when reared at 18°C compared with responses generated at 25°C (O. Siddiqi, unpublished observations) (Fig. 3*A–D*) [e.g., ethyl acetate (EA) at 10^{-4} induces an EAG of ~12 mV at 25°C but only ~6 mV at 18°C]. To induce AcGq3 expression, 4- to 5-d-old animals were heat shocked at 37°C for either 15 or 30 min. These flies, as well as similarly pulsed control genotypes, were allowed to recover for 1 h at 25°C and EAG responses were stimulated with three concentrations of ethyl acetate and propionic acid, representing basiconic and coeloconic hairs, respectively. Expression of AcGq3 significantly enhanced the amplitude of the EAG response at all concentrations of the two odorants tested (Fig. 3*A*,*B*) ($p < 0.001$). The enhancement was greater in flies that received a heat shock for 30 min ($p < 0.0001$). The AcGq3 transgene has a Q203L mutation (Ratnaparkhi et al., 2002), which in other Gα subunits, including Dgqα1, has been shown to slow down, but not abolish, their GTPase activity (Lee et al., 1994). Expression of this protein in OSNs enhances the normal EAG response, suggesting when $G_q\alpha$ molecules remain GTP-bound and therefore active for longer times after stimulation, there is greater activation of downstream signaling components. EAG responses were also tested from flies expressing AcGq3 after a 1 h heat shock, in which they appear reduced or similar to control values (Fig. 3*C*) ($p \gg 0.1$). An increased sensitivity of response at low levels of induction followed by a drop at higher levels is not unexpected because prolonged $G_q\alpha$ activation would be expected to deplete the membrane-bound lipid substrate phosphatidylinositol 4,5-bisphosphate (PIP_2) or lead to an adaptation of the transduction machinery.

Next, we measured EAG responses in flies with reduced $G_q\alpha$ levels by expressing a $G_q\alpha$ RNAi construct (*UASGq*^{1F1}) (Banerjee et al., 2006; Xia and Tully, 2007) in a majority of OSNs (*Or83bGAL4*) and in the expression domain of *dgq* using a *dgqGAL4* driver. This line was constructed by fusing 3.9 kb of *dgq* upstream sequence plus 3.4 kb of the 5′-untranslated region with the *GAL4* coding sequence. A significant reduction (4–5 mV) was observed in the amplitude of EAGs recorded from both *Or83b>GqRNAi* and *dgq>GqRNAi* strains compared with the individual parental strains ($p < 0.0001$) for all the odorants tested (Fig. 3*E*,*F*). The EAG response to two odorants, ethyl acetate and propionic acid (representative of basiconic and coeloconic hairs, respectively), was also reduced at higher dilutions (Fig. 3*G*) ($p < 0.0001$). Together with data from mutant clones, these results strongly support the idea that a *dgq* gene product transduces signals downstream of multiple odorant receptors in *Drosophila* antennal neurons.

Ab2a neurons from basiconic sensilla of *dgq*^{null} clonal antennas show a reduced sensitivity to ethyl acetate

Our results show that antennas mutant for *dgq* alleles exhibit reduced electrical activity to all odors tested. To confirm that the reduced EAG amplitudes in *dgq* clonal antennas were indeed attributable to a change in the response from individual sensory neurons, we measured the response to ethyl acetate from the well studied ab2 class of large basiconic hairs in animals of the genotype *FRT*^{42B}*dgq*^{null}*/FRT*^{42B}*GAL80; ey-Flp/Or83bGFP*. Ab2 sensilla are innervated by two OSNs of which the ab2a neuron expresses the Or59b receptor (Couto et al., 2005), which responds strongly to ethyl acetate (de Bruyne et al., 2001). We interrogated wild-type cells by stimulation with pulses of three dilutions of ethyl acetate and observed a dose-dependent increase in firing frequency (de Bruyne et al., 2001). We analyzed recordings ob-

tained from single ab2 sensillum, which lay within the regions of the antenna bearing large GFP clones. Morphologically similar clones were examined by confocal sectioning and found to have 256 ± 28 GFP-positive neurons (based on counts from five antennas). Because each antenna possesses ~1200 OSNs, we expected a 20–25% probability of finding a mutant neuron when sampled at random. We recorded from 18 ab2 sensilla present on antennas of the genotype $FRT^{42B}dgq^{1370}/FRT^{42B}GAL80$; *ey-Flp/Or83bGFP*. Among these, we obtained four sensilla in which the firing frequency of ab2a in response to pulses of ethyl acetate rose only minimally above the background firing rate when the odorant was delivered at dilutions of 10^{-6} and 10^{-5} (Fig. 4*A*). Their response at 10^{-4} dilution was reduced by ~50% compared with the controls (see below). As expected from the mosaic nature of the mutant antennas, in each of these animals a second ab2 hair responded normally to all concentrations of ethyl acetate (Fig. 4*B*). The remaining sensilla gave responses corresponding to controls as expected for neurons that are not included within the homozygous mutant patch (data not shown). Similar results were obtained with dgq^{221c} mutant sensilla (supplemental Fig. S2*A*, available at www.jneurosci.org as supplemental material). In contrast all (11 of 11) ab2 sensilla responded with a normal increase of the firing frequency on stimulation with increasing concentrations of ethyl acetate, when antennas with large GFP clones were taken from the control genotype $FRT^{42B}/FRT^{42B}GAL80$; *ey-Flp/Or83bGFP* (supplemental Fig. S2*B*, available at www.jneurosci.org as supplemental material). These data firmly implicate $G_q\alpha$ signaling as one of the mechanisms directly used for odor sensing in OSNs. The presence of a significant residual response even in the complete absence of $G_q\alpha$ (as in the null clones) does suggest that other mechanisms must exist within the same cell. The presence of two or more transduction mechanisms coupled differentially to a single odorant receptor might help explain some of the observed complexities of odorant receptor responses (Hallem et al., 2004; Hallem and Carlson, 2006).

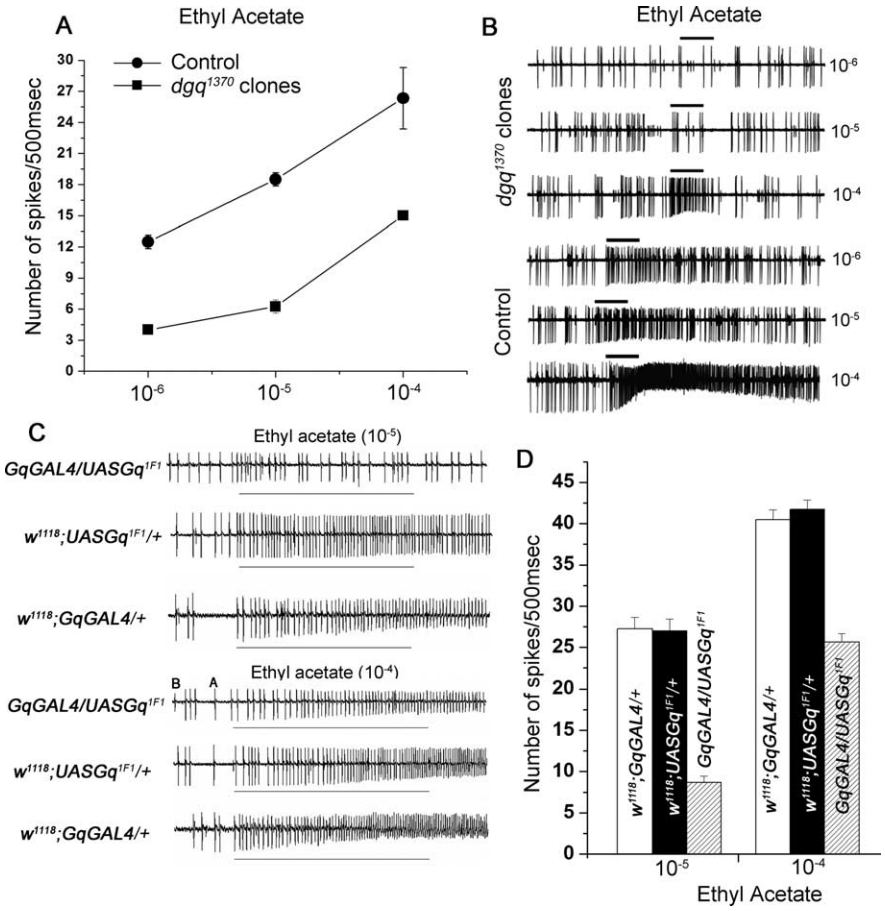

Figure 4. Odor response from single antennal neurons is reduced in $G_q\alpha$ mutants. ***A***, Single-unit recordings were measured from the ab2 class of large basiconic hairs in animals of the genotype $FRT^{42B}dgq^{1370}/FRT^{42B}GAL80$; *ey–Flp/Or83bGAL4, UAS2XEGFP* for different concentrations of ethyl acetate. Mean ± SEM from four mutant cells (squares; $n = 4$) and controls from the same antennas (see text; circles) at 10^{-6} to 10^{-4} dilution of ethyl acetate (***A***). The response of mutants cells is significantly different from controls ($p < 0.0001$). ***B***, ***C***, Representative traces for control and dgq^{1370} sensilla. The bar above each trace indicates the period of odor delivery (500 ms). In the control strains *GqGAL4/+* and $UASGq^{1F1}/+$, both dilutions of ethyl acetate elicit an increased frequency of firing from the "a" neuron. The frequency of response of ab2a is strongly reduced in $GqGAL4/UASGq^{1F1}$ compared with controls. ***D***, Mean ± SEM of the change in ab2a neuron spike frequency on odor stimulation from the indicated genotypes. A minimum of eight recordings were evaluated for each genotype. The firing frequency from $GqGAL4/UASGq^{1F1}$ animals is significantly different from controls at both concentrations of ethyl acetate ($p < 0.0001$).

Single-unit recordings were also obtained from $dgqGAL4>UASGq^{1F1}$ animals. In control flies of the genotypes *GqGAL4/+* and $UASGq^{1F1}/+$, the firing frequency of neuron A in ab2 sensilla increased visibly on the delivery of a 500 ms pulse of ethyl acetate (10^{-4} and 10^{-5} dilutions). This response was conspicuously reduced in ab2 hairs from flies of the genotype $GqGAL>UASGq^{1F1}$ (Fig. 4*D*,*C*, representative hairs).

The results we describe in *dgq* mutants could, in principle, arise from alterations in cellular metabolism because of changes in levels of lipid intermediates. However, this seems unlikely because the amplitudes and spontaneous neuronal firing rates of wild-type and mutant OSNs are indistinguishable from each other ($p \ll 0.001$) indicating that in *dgq* mutant OSNs action potentials can fire normally (supplemental Fig. S2*C*,*D*, available at www.jneurosci.org as supplemental material).

G_q is expressed in a majority of antennal sensilla

Functional analysis shows that a $G_q\alpha$ isoform, possibly Dgqα3, is required in multiple antennal sensory neurons for their response to a range of odorants. Although the presence of *dgqα3* RNA has been demonstrated in adult antennas, characterization of $G_q\alpha$ protein expression in developing and adult antennas has not been done (Talluri et al., 1995; Ratnaparkhi et al., 2002). Broad expression of $G_q\alpha$ in developing and adult antennal sensory neurons was confirmed by the following experiments. Initially, we looked at *dgqGAL4*-driven expression of *UAS2EXGFP*, which begins as early as 18 h APF in the differentiated neurons within the third antennal segment (Fig. 5*A*) and continues at 36 h APF when the projections into the antennal nerve become prominent (Fig. 5*B*, asterisk). Early expression of $G_q\alpha$ in the antennal system at 18 and 36 h APF was further confirmed by immunohistochemical staining with an antibody that recognizes the Dgqα3 isoform (Fig. 5*D*,*E*) (Ratnaparkhi et al., 2002). Expression continues in adults as seen by immunoreactivity to frozen sections of the antenna

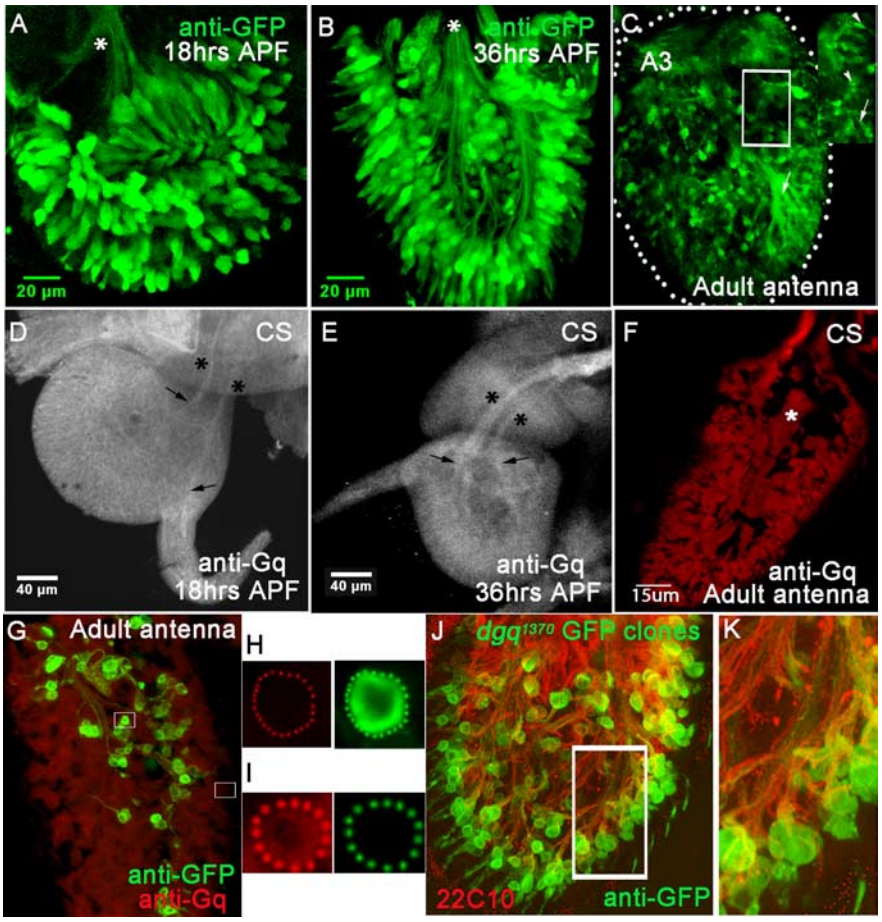

Figure 5. $G_q\alpha$ is expressed in OSNs of developing and adult antenna. ***A–F***, At 18 h (***A***, ***D***) and 36 h (***B***, ***E***) APF, expression of *dgq* is detected in the third antennal segment by reporter expression in the *dgqGAL4>UAS2XEGFP* (***A–C***) as well as by anti-Dgq immunostaining (***D–F***). Expression is detected in neuronal processes (* in ***A***, ***B***, and arrows in ***D***, ***E***) as well as in some non-neuronal cells. Dgq expression is detected in 15 μm frozen sections of adult antenna visualized with anti-Dgq antibody (***F***). GFP expression is also seen in the third (A3) antennal segments of the *dgqGAL4>UAS2EXGFP* adults (***C***). The inset in ***C*** shows a region of the antenna enriched for sensilla basiconica (***G***) dgq^{221c} OSNs are deficient for Dgq protein. Frozen sections (15 μm) of antennas of $FRT^{42B}dgq^{221c}/FRT^{42B}$*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP* double labeled with anti-Dgq and anti-GFP. ***H***, Merge of 6×1 μm confocal stacks. Pixel intensities in the red channel within individual cells were measured in multiple 1 μm sections. Cells labeled with GFP (dgq^{221c}/dgq^{221c}) showed a mean value of 3.63 ± 1 ($n = 5$), whereas the unmarked cells ($dgq^{221c}/+$) gave a value of 34.6 ± 2 ($n = 5$; $p < 0.0001$). Representative images (demarcated in the white boxes in ***G***) of a dgq^{221c} homozygous neuron (***H***) and a control $dgq^{221c}/+$ neuron (***I***) are shown for both red and green channels. ***J***, dgq^{null} adult antennas show normal OSN morphology. Antennas from $FRT^{42B}dgq^{1370}/FRT^{42B}$*GAL80; ey-Flp/Or83bGAL4, UAS2XEGFP* adults with large GFP-positive clones were stained for mAb22C10 (red) and anti-GFP (green). A magnified view of the region marked by a white box in ***J*** is shown in ***K***.

(Fig. 5*F*). Careful examination of confocal sections through *dgqGAL4>GFP* antenna stained with antibodies against GFP revealed a strong expression in a majority (but probably not all) OSNs (Fig. 5*C*). We further confirmed that the large basiconic sensilla that were analyzed in the single unit recordings described above were included in the *dgq* expression domain. From the broad pattern of expression observed in Figure 5, *C* and *F*, it seems likely that $G_q\alpha$ is also present in non-neural support cells surrounding the OSNs.

To confirm absence of $G_q\alpha$ in dgq^{221c} clonal OSNs, frozen sections (15 μm) of antennas were double labeled with anti-Dgqα3 and anti-GFP and analyzed by confocal microscopy (Fig. 5*G*). Figure 5*G* is a merge of several confocal stacks to demonstrate the presence of the clones. We selected sections covering single GFP-labeled neurons and quantified the pixel intensities resulting from anti-Dgqα3 immunoreactivity in each cell (Fig. 5*G*, boxed-in areas). A representative cell (Fig. 5*H*) demonstrates that the level of staining in homozygous mutant cells (marked with GFP) was no different from background levels. Non-clonal cells (not marked with GFP) show significant levels of Dgqα3 immunoreactivity (Fig. 5*I*). This result demonstrates that *dgq* clonal OSNs are indeed deficient for Dgq protein. Loss of Dgq protein, however, does not affect their morphology or axonal projections as observed by double labeling with a neuron-specific antibody mAb22C10 (Fig. 5*J*,*K*). Moreover, dgq^{null} mutant OSNs target correctly to the cognate antennal glomeruli (supplemental Fig. S3, available at www.jneurosci.org as supplemental material).

plc21C functions downstream of *dgq*

In the canonical receptor and G-protein-coupled signaling pathway, the enzyme phospholipase Cβ (PLCβ) is activated on GTP binding to $G_q\alpha$, which cleaves the membrane bound phospholipid, PIP_2, to generate soluble $InsP_3$ and membrane-bound diacylglycerol (DAG). The *Drosophila* genome has two genes encoding for PLCβ-*norpA* (Bloomquist et al., 1988) and *plc21C* (Shortridge et al., 1991). In a previous study, we found that dgq^{221c} and $plc21C^{P319}$ mutant alleles have synergistic effects on larval viability and adult flight (Banerjee et al., 2006), indicating that PLCβ21C can function together with *Drosophila* $G_q\alpha$. The $plc21C^{P319}$ mutant allele is a hypomorph, with a P-insert in the first intron, and is homozygous viable, whereas $plc21C^{p60a}$ is a small deficiency that removes the 5′ end of *plc21C* plus a neighboring essential gene *p60* (Weinkove et al., 1999). A reduction of *plc21C* RNA levels in $plc21C^{P319}$ homozygotes and $plc21C^{p60a}$ heterozygotes was confirmed by RT-PCR (Fig. 6*C*). $UASplc21C^{557}$ is a recently generated UASRNAi strain reported as specific for *plc21C* (http://stockcenter.vdrc.at/).

EAG responses of $plc21C^{P319}$ homozygotes and $plc21C^{P319/p60A}$ combinations were reduced to <6 mV for all chemicals tested (Fig. 6*A*). The EAG responses to ethyl acetate (from basiconic sensilla) and propionic acid (from coeloconic sensilla) were found to be reduced at several concentrations (Fig. 6*B*) ($p < 0.0001$). *plc21C/+ and dgq/+* heterozygotes resulted in a small but significant decrease in EAG responses compared with wild-type (CS) controls (decrease is ~2.5 mV, $p < 0.01$ in the case of *plc21C/+*, and ~3 mV, $p < 0.0005$ for *dgq/+*).

A combination of *plc21C/dgq* resulted in a 7–9 mV reduction in EAG responses compared with wild-type controls (Fig. 6*A*). This reduction is significantly greater than the value expected to arise from a mere additive effect of *plc21C/+* and *dgq/+* heterozygotes (for statistical validation, see legend to Fig. 6*A*). Although other explanations for this haploinsufficent interaction are formally possible, we favor the explanation that PLCβ21C interacts with $G_q\alpha$ in normal olfactory transduction. This observation was supported by the significantly reduced EAG response

to all five odorants obtained in an RNAi-driven OSN-specific downregulation of *plc21C*, and by downregulation of *plc21c* in the *dgq* expression domain (*GqGAL4/RNAiplc21c*^{557}) (Fig. 6*A*). EAG responses of the *plc21C* RNAi strain were reduced further on introduction of *UASGq*^{1F1} (RNAi*Gq*) (Fig. 6*A*). In the double mutant *dgq-plc21C* RNAi strain, EAG responses were reduced to ~2 mV (which is below the single RNAi lines) but are still not abolished completely. Moreover, expression of *plc21C* RNAi in the OSNs resulted in a significant reduction of the spike frequency of ab2a neurons on stimulation by ethyl acetate (Fig. 6*D*) ($p < 0.001$). Thus, odor transduction in ab2a neurons requires both $G_q\alpha$ and Plc21C.

Because small, but significant, odor responses remain in all *dgq* and *plc21C* mutant genotypes tested, these data further highlight the likelihood of other signaling mechanisms that collaborate with $G_q\alpha$ during odor detection. As reported previously, EAG responses recorded from a null allele for the second PLCβ, *norpA*^{P24}, appear close to normal for the five odorants tested (Riesgo-Escovar et al., 1995) (data not shown). The introduction of *norpA*^{P24} in *plc21C/dgq* animals had no additional effect in reducing EAG amplitudes (data not shown). *norpA* thus has no significant effect on antennal physiology in contrast to its proposed effect on signaling in OSNs located on the maxillary palp (Riesgo-Escovar et al., 1995) and the major role played by this gene in visual transduction (Bloomquist et al., 1988).

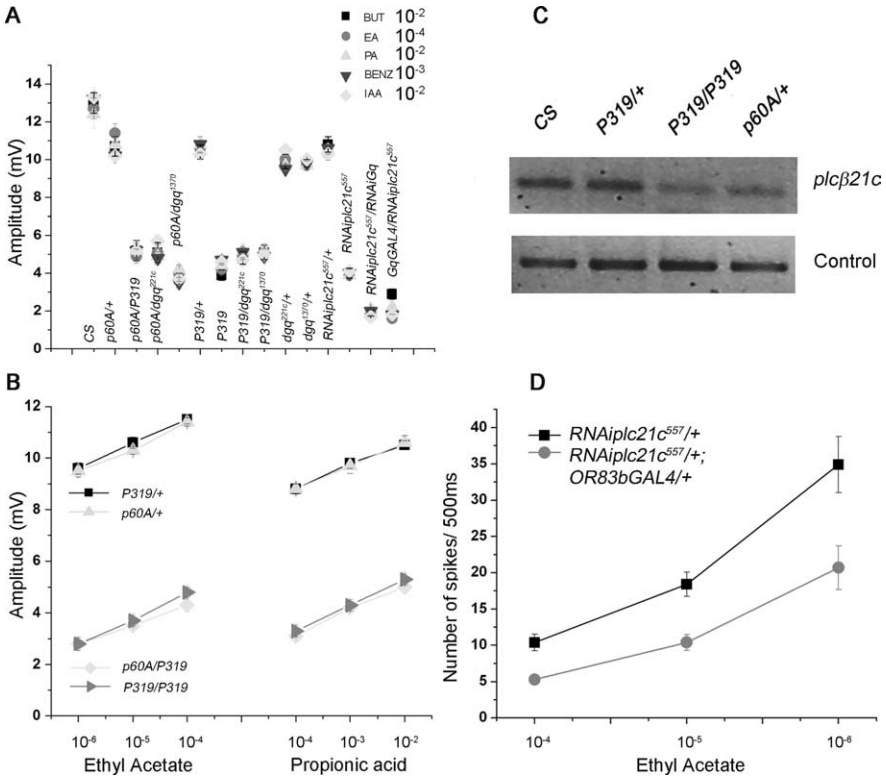

Figure 6. Trans-heterozygote interactions between alleles of *dgq* and *plc21C*. ***A***, Average amplitudes of EAGs for five odorants are shown for the indicated genotypes. *plc21C*^{p60A}/+ (*p60A*/+), *plc21C*^{P319}+ (*P319*/+), as well as *dgq*^{221c}/+ and *dgq*^{1370}/+ show a small reduction in response compared with the CS controls ($p < 0.001$). Responses of trans-heterozygote combinations *p60A/dgq*^{221c}, *p60A/dgq*^{1370}, *P319/dgq*^{221c}, *P319/dgq*^{1370}, and *p60A/P319* were significantly different from heterozygous controls ($p < 0.0001$ in all cases). The observed reduction in response of the trans-heterozygotes was significantly different ($p < 0.01$ in all cases) from the expected drop if this resulted from the lowered response of the two heterozygotes. Reduction was also observed in *RNAiplc21C*^{557} (*UASRNAiplc21C*^{557}/+; *OR83bGAL4*/+), *RNAiplc21C*^{557}/*RNAiGq* (*UASRNAiplc21C*^{557}/*UASGq*^{1F1}; *OR83bGAL4*/+), and *GqGAL4/RNAiplc21C*^{557} animals compared with control *UASRNAiplc21C*^{557}/+ animals ($p < 0.0001$). Symbols represent the mean ± SEM ($n = 10$ for all genotypes). ***B***, Dose responses for ethyl acetate and propionic acid for *p60A/P319* and *P319/P319* and controls *plc21C*^{p60A}/+ (*p60A*/+), *plc21C*^{P319}+ (*P319*/+). Values are means ± SEM ($n = 10$); $p < 0.0001$. ***C***, RT-PCRs from antennal RNA isolated from CS, *plc21C*^{P319} heterozygotes and homozygotes, and heterozygotes of the *plcβ21C* deficiency, *plc21C*^{p60a}/+. RNA levels were normalized by amplifying *rp49* from the same samples. ***D***, The change in spike frequency of ab2a neurons to varying concentrations of ethyl acetate is shown. Animals in which OSNs express RNAi for Plc21C (*RNAiplc21C*^{557}/+; *Or83bGAL4*/+) show a significant reduction ($p < 0.001$) in response at all odor concentrations compared with the controls (*RNAiplc21C*^{557}/+). Each trace is the average response (±SEM) of nine sensilla from five individuals.

Genetic interaction of *dgq* mutant alleles with *rdgA*, a DAG kinase mutant

The results so far show that signaling downstream of olfactory receptors in the *Drosophila* antennas requires Dgqα3 and PLCβ21C. To identify which of the two second messengers generated by $G_q\alpha$ activation of PLCβ21C, DAG or $InsP_3$, are required for the electrical response to odorants, we tested mutants that function in the two arms of the pathway. The *rdgA* gene codes for an ATP-dependant DAG kinase that converts DAG to phosphatidic acid, which in turn is a precursor for PIP_2 formation (Inoue et al., 1989). Homozygotes for two *rdgA* mutant alleles *rdgA*^{1} and *rdgA*^{3} show reduced (~4 mV) EAG responses to all five odorants tested (Fig. 7*A*), whereas heterozygotes appear close to normal (~10 mV). *rdgA*^{1} homozygotes also show reduced EAG responses to multiple concentrations of two odorants tested, ethyl acetate and propionic acid (Fig. 7*B*). Interestingly, unlike the effect of *rdgA* mutants on ERG termination kinetics (Garcia-Murillas et al., 2006), we do not observe slower termination of the EAG response in *rdgA* mutants (Fig. 7*D*). Moreover, unlike eyes of 1-d-old *rdgA*^{1}/*rdgA*^{1} adults, which exhibit strong retinal degeneration, both *rdgA* alleles have normal antennal morphology even 6 d after eclosion (data not shown). Thus, the reduced EAGs observed are not a consequence of antennal neuron degeneration.

Next, the EAG response was tested after introducing a single mutant copy of *dgq* in *rdgA* homozygotes (*rdgA/rdgA;dgq*/+). Interestingly, we found that reduction of Dgq levels to 50% by introduction of a single copy of the *dgq*^{null} allele rescued the EAG responses of *rdgA* homozygotes bringing them back to near normal (Fig. 7*C*). The extent of rescue varies with specific *dgq* and *rdgA* mutant allele combinations. Strongest effects were seen in *rdgA*^{1}/*rdgA*^{1}; *dgq*^{1370}/+ animals ($p < 0.0001$). Reducing *dgq* function by expression of the RNAi construct *UASGq*^{1F1} also rescued the EAG defects produced by both *rdgA* mutant homozygous alleles. The rescue of EAG responses in *rdgA* mutants by *dgq* mutants was further verified by testing RNAi lines specific for *rdgA* and *dgq* in appropriate combinations (Fig. 7*C*). EAG responses were significantly higher in the presence of RNAi constructs for both *rdgA* and *dgq* ($p < 0.0001$) (Fig. 7*C*) compared with the response from individual RNAi lines (Fig. 3*E*,*F* for $G_q\alpha$ RNAi). A possible explanation for these data is that failure of conversion of DAG back to phosphatidic acid and PIP_2 through *rdgA* encoded DAG kinase compromised the generation of an odor-evoked response but does not cause degeneration of OSNs.

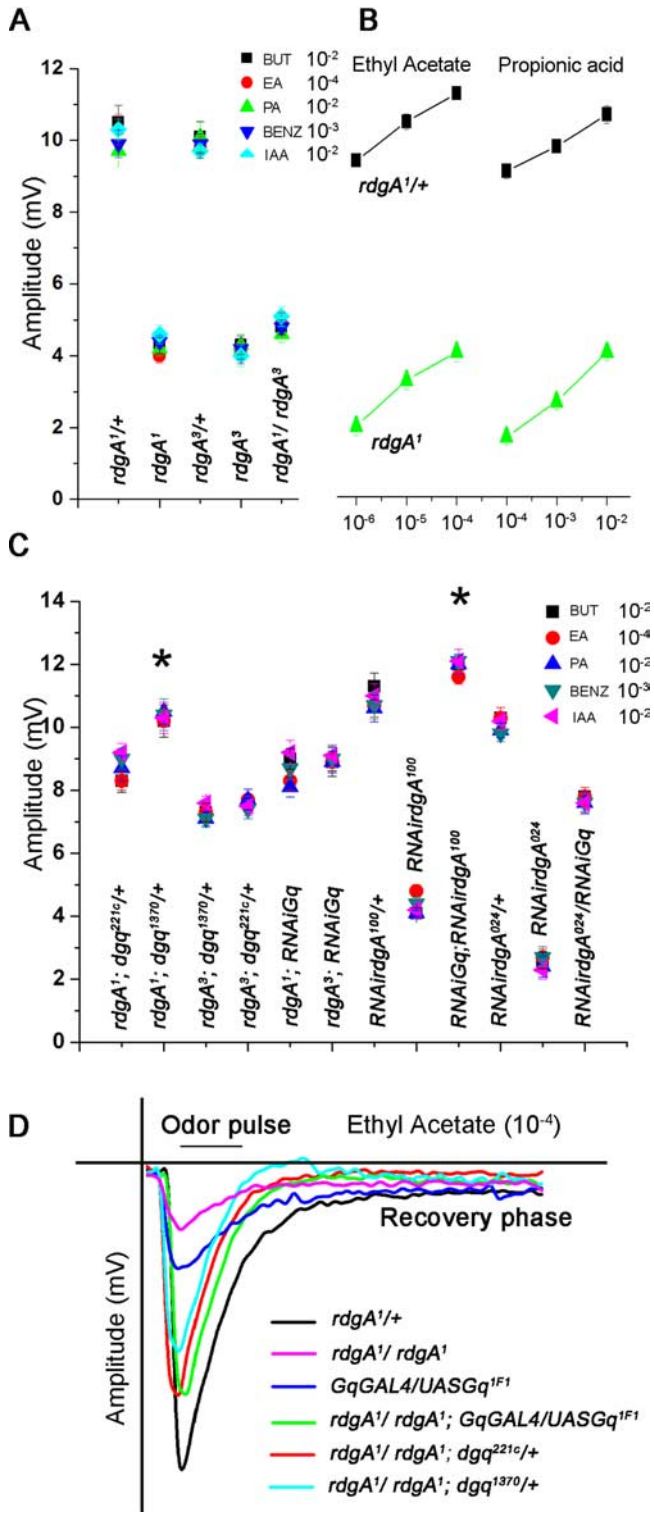

Figure 7. Trans-heterozygote combinations of *dgq* and *rdgA* mutant alleles exhibit rescue of reduced EAGs observed in *rdgA* mutants. ***A***, *rdgA*^{1} (severe allele) and *rdgA*^{3} (weaker allele) homozygotes show reduced (~4 mV) EAG responses to all five odorants tested compared with heterozygotes (*rdgA*^{1}/+ and *rdgA*^{3}/+); $p < 0.0001$. ***B***, Dose responses of *rdgA*^{1}/+ and *rdgA*^{1} homozygotes to ethyl acetate and propionic acid. The response of *rdgA*^{1}/*rdgA*^{1} animals are reduced significantly ($p < 0.0001$) at all odor concentrations. ***C***, Reduction of $G_q\alpha$ levels can rescue EAG amplitudes of *rdgA* mutant homozygotes. Genotypes with strongest rescue are marked with asterisks. *rdgA*^{1}; *RNAiGq* (*rdgA*^{1}/*rdgA*^{1}; *GqGAL4/UASGq*^{1F1}) and *rdgA*^{3}; *RNAiGq* (*rdgA*^{3}/*rdgA*^{3}; *GqGAL4/UASGq*^{1F1}) rescues *rdgA* mutants comparable with *dgq* mutants. Expression of two different RNAi constructs in OSNs *RNAirdgA*^{100} (*UASRNAirdgA*^{100}/*OR83bGAL4*) and *RNAirdgA*^{024} (*UASRNAirdgA*^{024}/+; *OR83bGAL4*/+) showed lower responses compared with control animals, *UASRNAirdgA*^{100}/+ and *UASRNAirdgA*^{024}/+ ($p < 0.0001$). Expression of RNAi constructs for both *Gq*α and *rdgA* in OSNs *RNAiGq; RNAirdgA*^{100} (*UASGq*^{1F1}/+; *UASRNAirdgA*^{100}/*OR83bGAL4*) and *RNAiGq; RNAirdgA*^{024} (*UASRNAirdgA*^{024}/*UASGq*^{1F1}; *OR83bGAL4*/+) showed higher responses ($p < 0.0001$) compared with *UASRNAirdgA*^{100}/*OR83bGAL4* and *UASRNAirdgA*^{024}/+; *OR83bGAL4*/+. ***D***, Representative EAG traces for genotypes tested in ***A*** and ***C***.

Reduction in the signaling flux through $G_q\alpha$ (by lower Dgq levels in *dgq*^{null}/+ genotypes or by $G_q\alpha$ RNAi expression) compensates for this defect and rescues the olfactory defect of *rdgA* (see Discussion).

Because there is a significant body of data from studies with other organisms suggesting that $InsP_3$ is a second messenger in invertebrate olfactory transduction (Ache and Young, 2005), we measured EAG responses from single copies of the two *dgq* alleles with two different mutations of the $InsP_3$ receptor as heterozygotes (*dgq*^{null}/+; *itpr*^{EMS}/+). *itpr*^{sv35} is a null (Joshi et al., 2004), whereas *itpr*^{ka901} can act as a gain-of-function allele (Srikanth et al., 2006). The effect of these alleles on antennal olfactory responses has not been investigated before. No significant change in the response to any chemical was observed in the eight strains tested (data not shown). These data are in agreement with a previous study from *Drosophila*, in which EAGs measured from the antennas of viable alleles for the $InsP_3$ receptor were normal (Deshpande et al., 2000). Thus, primary olfactory responses from the *Drosophila* antennas do not require the $InsP_3$ receptor but are dependent on a DAG kinase (*rdgA*).

Discussion

We have shown that mutations in the G-protein $G_q\alpha$ result in reduced sensitivity of *Drosophila* antennal sensory neurons to several different chemical stimuli. Genetic analysis suggested that its downstream effector is phospholipase Cβ encoded by the *plc21C* gene. PLCβ enzymes catalyze breakdown of the membrane-bound lipid PIP_2 to generate DAG and $InsP_3$. Reduced EAG amplitudes in DAG kinase mutants (*rdgA*) and their rescue by lowering $G_q\alpha$ levels, argue that the extent of PIP_2 depletion after $G_q\alpha$ and PLCβ activation is responsible for this arm of olfactory transduction. Altered PIP_2 levels have been shown to gate or modulate membrane conductances in other contexts (Zhang et al., 2003). The presence of a residual olfactory response in antennal neurons that are null for *dgq* supports existence of an additional transduction mechanism.

Olfactory transduction in *Drosophila*: single or multiple pathways?

Odorant detection in animals occurs through a diverse class of G-protein-coupled receptors (Buck and Axel, 1991). In mammalian olfactory sensory neurons, it appears that all odorant receptors activate a single G-protein G_{olf} and generate one second messenger, cAMP (Jones and Reed, 1989; Belluscio et al., 1998). In *Drosophila dgq* mutants, we find a reduced response to all five odorants tested, indicating that multiple odorant receptors activate $G_q\alpha$. Ethyl acetate, isoamyl acetate, and benzaldehyde stimulate receptors located in the basiconic sensilla, whereas butanol and propionic acid activate neurons innervating the coeloconic sensilla (Hallem et al., 2004; Couto et al., 2005; Yao et al., 2005). It has been proposed that the basiconic sensilla primarily detect food odors and the coeloconics express receptors that respond to certain specialized odors (Laissue and Vosshall, 2008). Our results suggest that these functional distinctions are not based on a change in signal transduction machinery.

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The residual odor-induced response observed in $G_q\alpha$ mutant antennas and in single ab2a neurons argues for the presence of an alternate transduction mechanism(s) in OSNs. Odorants could stimulate either other G-proteins, which alter cyclic nucleotide levels (Gomez-Diaz et al., 2004), or a direct odor-gated ion channel, as in heteromers of Manduca OR/OR83b (Nakagawa et al., 2005) or both. Precisely how the alternate mechanism of odor transduction interacts with $G_q\alpha$-mediated transduction described here requires additional study. The strong genetic evidence presented here, supporting a role for a $G_q\alpha$-mediated signaling cascade during olfactory transduction, needs to be reconciled with recent data suggesting that *Drosophila* odorant receptors do not conform to the structure or topology typical of other G-protein-coupled receptors (Benton et al., 2006).

Based on the quality and strength of the odor stimulus it receives, each odorant receptor could stimulate different transduction pathways to varying extents. We propose that firing frequency of the OSN is an integration of this information, perhaps by cross-sensitization or desensitization of membrane conductances gated by the individual pathways. Interestingly, it has been demonstrated that a single odorant receptor (e.g., Or59b), present within the same neuron can mediate both excitatory and inhibitory responses as a consequence of stimulation by two different odorants (Hallem et al., 2004). Precedents for multiple signaling pathways in odor detection within a single organism are present in invertebrates (Ache and Young, 2005), although their existence within the same olfactory neuron has not been demonstrated so far.

Similarities of signaling intermediates in *Drosophila* olfaction and vision

The requirement of phospholipase C for odor-induced activity of OSNs in the maxillary palp highlights an overlap in signaling mechanisms during olfaction and vision (Riesgo-Escovar et al., 1995). Our data further highlight parallels in these two sensory systems. In *Drosophila* photoreceptors, signal amplification begins with activation of a number of G-protein molecules by a single photoisomerised rhodopsin molecule, followed by activation of one PLC molecule for each G-protein (Hardie et al., 2002). Although the current methods of measuring olfactory responses in *Drosophila* do not allow direct measurement of signal amplification, the enhanced EAG amplitudes observed in AcGq3-expressing animals and synergism between *dgq* and *plc21C* mutant alleles observed here supports the idea of signal amplification by $G_q\alpha$.

The *rdgA* gene has a role in both amplification and termination of the visual response (Hardie et al., 2002). Our observation that $rdgA^1$ and $rdgA^3$ homozygotes exhibit reduced EAG responses, in the absence of antennal neuron degeneration, suggest that the rate of DAG turnover to generate other intermediates leading to PIP_2 is important for membrane depolarization of OSNs. The absence of antennal degeneration predicts a low level of basal PLCβ activity in OSNs, and perhaps strong adaptive mechanisms that prevent activation of membrane channels in normal odor-rich environments. This needs to be investigated further. The fact that reduced EAGs can be rescued by a single copy of *dgq* mutant alleles, supports the existence of a cycle of phosphoinositide turnover in OSNs, in which reduced levels of $G_q\alpha$ help to slow down this cycle and thus balance the rate of PIP_2 regeneration in *rdgA* mutants. The observed suppression appears qualitatively similar to the interaction of *rdgA* and *dgq* during the amplification phase of *Drosophila* visual transduction in which *rdgA* mutants enhance the sensitivity of $dgq\alpha^1$ mutants as measured by the amplitude of quantum bumps (Hardie et al., 2002). Finally, either PIP_2 depletion, or a lipid intermediate presumably gates a depolarizing membrane channel. The identity of this channel can only be conjectured on at this stage. However, based on studies from other G-protein-coupled lipid signaling pathways, a transient receptor potential (TRP) family channel might be a possible candidate. Among this family, the two most well studied members in *Drosophila* are TRP and TRPL (TRP-like), both of which are enriched in photoreceptors. A previous study on the olfactory responses of *trp* mutants ruled out a role for this gene in primary sensory transduction of odor stimuli (Stortkuhl et al., 1999). Several members of this family exist in *Drosophila* and need to be investigated for their role in olfactory transduction.

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