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Goα Is Involved in Sugar Perception in *Drosophila*

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Abstract

Detection of chemical compounds in food sources is based on the activation of 7 transmembrane gustatory receptors (GRs) in mammals and in insects such as *Drosophila*, although the receptors are not conserved between the classes. Different combinations of *Drosophila* GRs are involved in the detection of sugars, but the activated signaling cascades are largely unknown. Because 7 transmembrane receptors usually couple to G-proteins, we tried to unravel the intracellular signaling cascade in taste neurons by screening heterotrimeric G-protein mutant flies for gustatory deficits. We found the subunit Goα to be involved in feeding behavior and cell excitability by different transgenic and pharmacological approaches. Goα is involved in the detection of sucrose, glucose, and fructose, but not with trehalose and maltose. Our studies reveal that Goα plays an important role in the perception of some sweet tastants. Because the perception of other sweet stimuli was not affected by mutations in Goα, we also found strong indication for the existence of multiple signaling pathways in the insect gustatory system.

Key words: *Drosophila*, G-protein, Go, gustatory receptor, signaling

Introduction

The sense of taste enables the fruit fly, *Drosophila melanogaster*, to detect sugars in the environment, but our knowledge of the molecular mechanisms involved in detection of chemical signals is still fragmentary. The main gustatory organs of *Drosophila* are the 2 labial palps at the tip of the proboscis (Shanbhag et al. 2001). Taste chemoreception is mediated via sensory sensilla that are distributed in the proboscis, legs, and wings, as well as in the ovipositor (Stocker 1994; Singh 1997; Vosshall and Stocker 2007). These sensilla contain the dendrites of gustatory receptor neurons (GRNs), in which defined subsets of 60 gustatory receptor (GR) genes are expressed (Clyne et al. 2000; Dunipace et al. 2001; Scott et al. 2001; Robertson et al. 2003). The GR Gr5a is expressed in most sugar-responsive GRNs and was found to be necessary for the response to a small subset of sugars, particularly trehalose (Dahanukar et al. 2001, 2007; Ueno et al. 2001). Activation of *Gr5a*-positive neurons was found to be sufficient to elicit acceptance behavior (Wang et al. 2004; Marella et al. 2006). Gr64a is essential for the detection of multiple other sugars, including sucrose, glucose, and maltose (Dahanukar et al. 2007; Jiao et al. 2007; Slone et al. 2007). Strikingly, deletion mutants lacking both *Gr5a* and *Gr64a* do not show electrophysiological or behavioral responses to common sugars, indicating that these receptors are essential for the detection of sugars in general (Dahanukar et al. 2007). More recent studies demonstrate that Gr64f is a coreceptor, which is required for the detection of most sugars in concert with Gr5a and Gr64a, suggesting that GRs function as heteromultimers (Jiao et al. 2008).

GRs are 7 transmembrane proteins (Clyne et al. 2000; Robertson et al. 2003), which usually couple to guanine nucleotide–binding protein (G-protein)–linked signaling cascades. In mammals, the G-protein α-gustducin was shown to be involved in response to sweet tastants (McLaughlin et al. 1992; Wong et al. 1996). There was evidence presented that different G-protein subunits, Gγ1, Gsα, and Gqα, are involved in sugar reception in *Drosophila* (Ishimoto et al. 2005; Ueno et al. 2006; Kain et al. 2010). Further downstream in the signaling cascades, the phospholipase Cβ21C, the Inositol triphosphate (IP_3) receptor, as well as the adenylyl cyclase 78C were shown to play a role in the detection of sugars (Usui-Aoki et al. 2005; Ueno and Kidokoro 2008; Kain

et al. 2010). These findings imply the existence of more than one sugar-evoked signaling pathway in GRNs of *Drosophila*.

Our study was intended to unravel the intracellular signaling cascade in the gustatory neurons, with special emphasis to the role of the Gα protein subunit. Behavioral screening assays provided evidence that Goα is required for the behavioral response to sucrose. The electrophysiological responses to certain sugar stimuli were impaired in Goα-deficient flies, whereas responses to other sugars remained unaffected. Our results demonstrate that Goα plays an important role in the perception of certain sugars, and we propose that distinct G-protein–related pathways are involved in the recognition of sugar taste information in *Drosophila*.

Materials and methods

Fly stocks

Flies were raised at room temperature (23 ± 2 °C) on semi-defined medium (http://flybase.org) and were kept under a 12 h light:dark cycle. All *UAS-Gα RNAi* strains were purchased from VDRC (Dietzl et al. 2007). *Gr5a-GAL4* and *Gr66a-GAL4* were kindly provided by H. Amrein (Duke University Medical Center) and J. R. Carlson (Yale University); *UAS-Go*, *UAS-Go^{GDP}*, and *UAS-ptx* were a kind gift from A. Tomlinson (Columbia University). The NP3200/+ line was from the Drosophila Genomics Resource Center collection, Kyoto (number 104-410 (y[*] w[*]; P{*GawB*}NP3200/SM1)). We confirmed the integration site of P{*GawB*}NP3200 in the *Goα* gene locus via polymerase chain reaction (PCR) using primers as follows: *Go-fw* 5′-TGC TAA TGT GCT TTG GCT TG-3′, *Go-rv* 5′-GGA GGT AGG AGG GGG TAG AA-3′, and *GAL4-rv* 5′-TTA AGT CGG CAA ATA TCG CAT GC-3′ (Supplementary Figure S5). Wild-type (Canton S), *hsGAL4/CyO*, EP2154/EP2154, and *UAS-mCD8::GFP* flies, used as a source of GFP, were from the Bloomington Stock Center (Indiana University).

Chemicals

D-(+)-sucrose (minimum 99%) and D-(+)-glucose monohydrate (minimum 99%) were purchased from AppliChem. D-(+)-trehalose (minimum 99.5%) was received from Fluka. D-(+)-maltose (minimum 98%), D-(-)-fructose (minimum 99%), caffeine, quinine hydrochloride, potassium chloride (minimum 99%), choline dihydrogencitrate salt (minimum 99%), and choline base solution 50% (w/w) were obtained from Sigma-Aldrich. Pertussis toxin (PTX), *Bordetella pertussis*, was purchased from Calbiochem.

RT-polymerase chain reaction

For reverse transcription (RT)-PCR analysis, total RNA was isolated from 200 labella or 20 heads following the RNeasy procedure (Qiagen). For cDNA synthesis, 200–400 ng RNA was utilized using the iScript cDNA synthesis kit (BIO-RAD). Primers for RT-PCR were designed to span exon–exon boundaries to be aware of amplification of genomic DNA. Primer sequences were as follows: *Goα-fw* 5′-GGT GTC CAG GAGT GCT TCT C-3′, *Goα-rv* 5′-CTC AAC GAT GCC AGT GGT C-3′; *Giα-fw* 5′-GAG TAC AGC ACG CGA TAA GG-3′, *Giα-rv* 5′-CGA TAC TCC TCG CAT TCC TC-3′; *CG17760-fw* 5′-GAA TTC CCT ACG GTC AGC AG-3′, *CG17760-rv* 5′-GCC TGG TAA TCA CTC CGT TC-3′; *CG30054-fw* 5′-CAT ATG CGG CCA GGG AAT A-3′, *CG30054-rv* 5′-TGC ATT GCA AAA TTG TAT CC-3′; *Gα49B-fw* 5′-TGA AGG ATC TCG ATC GTG TG-3′, *Gα49B-rv* 5′-TCT TCT CTC GGA TCG CTG AC-3′; *Gα73B-fw* 5′-GAA CAT CCA CGA GTC CAT CC-3′, *Gα73B-rv* 5′-ATC ATA GCA GGC GCG AAT AC-3′; *Gsα-fw* 5′-TTC TTC AAA CCT ATG AGA GG-3′, *Gsα-rv* 5′-TCC TAC GCT CGT CCC GCT GG-3′; and *cta-fw* 5′-TTT GCT TGC TTT CGA TGT TG-3′, *cta-rv* 5′-CCA TGA ATG ATT CGC ATT TG-3′.

Quantitative real-time PCR

Quantitative real-time PCR (Q-PCR) experiments were performed using the iQ SYBR Green Supermix reagent and the iQ5 thermal cycler apparatus from BIO-RAD, with regard to the manufacturer's recommendations. cDNA samples were prepared following the RT-PCR method. Primer sequences were as follows: pair 1 (according to Figure 3A), *dGo1-fw* 5′-GCA GCC GCC AGG AGT CGC CT C-3′, *dGo1-rv* 5′-GAC AAC CGG TCG ATA TTG TTT A-3′; pair 2, *dGo2-fw* 5′-GCC ATC CAG CGA TCC AAA CA G-3′, *dGo2-rv* 5′-GAC AAC CGG TCG ATA TTG TTT A-3′; and pair 3, *Goα-fw* 5′-GGT GTC CAG GAG TGC TTC TC-3′, *Goα-rv* 5′-CTC AAC GAT GCC AGT GGT C-3′. The housekeeping gene product *rp49* was used to normalize the amount of *Goα* mRNA in the different cDNA populations tested. Primers were as follows: *rp49-fw* 5′-GCT AAG CTG TCG CAC AAA TG-3′, *rp49-rv* 5′-TGT GCA CCA GGA ACT TCT TG-3′. We amplified the PCR products for 40 cycles. The Q-PCR procedure was repeated for at least 3 times in independent runs. We calculated the different expression levels by applying the delta-delta Ct method (Livak and Schmittgen 2001).

Fluorescence microscopy

NP3200/*UAS-mCD8::GFP* flies were mounted in 2% agarose, such that the labellum and the legs were uncovered. The GFP fluorescence was observed with a Leica DM6000 confocal fixed stage. We developed *Z* sections of approximately 0.4 μm, which were processed to construct maximum projections.

Immunohistochemistry

For the immunohistochemical localization of Goα in the labellum, we fixed heads from *Gr5a-GAL4/UAS-mCD8::GFP* flies in phosphate-buffered saline (PBS) containing 4%

paraformaldehyde and 0.2% Triton X-100 for 30 min on wet ice. After rinsing the heads 3 times for 10 min in PBS and 0.2% Triton X-100 (PBST), they were incubated with PBST containing primary antibodies rabbit anti-Goα (Thambi et al. 1989) in a dilution of 1:100 at 4 °C overnight. After washing for 3 times with PBST, we exposed the heads for 2 h at room temperature to secondary antibodies goat anti-rabbit immunoglobulin G coupled to Alexa-633 (Invitrogen). The heads were mounted with Mowiol/DABCO and analyzed by confocal microscopy with a Zeiss LSM 510 META.

Electrophysiology

Extracellular recordings of action potentials on single taste sensilla were performed with modifications of previously described methods (Hodgson et al. 1955; Wieczorek and Wolff 1989). Briefly, newly enclosed flies were transferred to vials with semidefined medium. Female flies, which were aged 4–12 days, were introduced to 2- to 100-µl pipette tips, such that the anterior portion of the head protrudes from the end of the tip. An indifferent electrode was inserted into the thorax of the flies. The labellar sensilla (L1, L2, and L3) were stimulated with a recording electrode that was filled with the respective test solution. Signals were remitted to an EPC7 patch clamp amplifier (List Medical) in the current clamp mode. We counted the number of impulses in a period of 500 ms, starting 200 ms after onset of stimulation. As electrolyte, we used 50 mM tricholine citrate in all test solutions, except for KCl, which was dissolved in distilled water. Tricholine citrate (50 mM) was chosen as electrolyte in the different sugar solutions, as this component was shown to inhibit exclusively the responses from water receptors (Wieczorek and Wolff 1989).

The pharmacological application of PTX was carried out as follows: Responses of L-type sensilla from wild-type flies were monitored at 20 and 100 mM sucrose, respectively. The same sensilla were incubated for 20 min with 10 µg/ml PTX diluted in 50 mM tricholine citrate followed by stimulation with 20 and 100 mM sucrose again. As a control, the described procedure was performed apart from delivery of PTX to the solution.

Behavioral assays

Two-choice preference tests were performed using modifications of previously described work (Tanimura et al. 1988). Newly enclosed flies were collected in vials with fresh fly food. Three- to six-day-old flies were starved for 20–24 h in vials containing 1% agarose allowing them the access to water. Forty to sixty of those flies were introduced to 6-well cell culture dishes that contained 2 alternating concentrations of sugar dissolved in 1% agarose. These sugar solutions additionally included either 2 mg/ml sulforhodamine B monosodium salt (Fluka) or 0.5 mg/ml erioglaucine (Sigma-Aldrich). After flies were fed for 2 h in the dark at room temperature, they were anesthetized and were then classified with respect to their abdominal color: blue (#b), red (#r), purple (#p), and colorless (#c). The preference index (PI) was calculated as follows: (#b + ½ #p)/(#b + #r + #p) or (#r + ½ #p)/(#b + #r + #p). The closer the PI is to one the higher is the preference for the blue and accordingly the red substrate. A PI of 0.5 indicates no preference for any substrate. The percentage of #c flies was lower than 10% in each test. At least 120 flies per strain were tested in 3–6 independent tests. The food coloring did not affect the behavioral response of the flies to the diverse sugar concentrations, as we switched the tastant–dye combination and saw no preference differences in comparison to those that were observed before. For the avoidance tests, flies were treated as described above. The 6-well cell culture dishes were alternatively filled with 2 mM maltose and 8 mM maltose alone or 8 mM maltose blended with 8 mM caffeine or 1 mM quinine. The PI was calculated as described above.

For the proboscis extension reflex (PER), 3- to 6-day-old flies were starved for 20–24 h in vials with 1% agarose. After anesthetization, the dorsal abdominal side of flies was fixed on a microscope slide by employing a solvent-free contact adhesive (Peho). Half an hour later, we used glass pipettes to saturate the flies with pure water until they did not extend their proboscis to the waterdrop. Then, the test solution, 100 mM sucrose, was applied to the first legs of the fly for 3 s, meanwhile monitoring the extensions of the proboscis. After a period of 1–2 min, the procedure was repeated for overall 5 times. In between, we checked the water saturation of the flies. Responses were scored as follows: a fly that extended its proboscis for at least 3 times after stimulation was counted as 1, a fly that responded less than 3 times to the stimulus was counted as 0.

Results

Expression of RNAi against *Goα* in *Gr5a* neurons affects the behavioral response to sucrose

To define an expression profile of Gα protein subunits in the labellum of the fly, we carried out RT-PCR analysis and detected mRNAs of all *Gα* subunits in the main gustatory organ except for *CG30054* (Supplementary Figure S1). In order to identify Gα protein subunits that participate in sugar perception, we performed 2-choice preference tests (Tanimura et al. 1988) with fly lines expressing RNAi directed against different G-protein subunits (*UAS-Gα RNAi*) (Dietzl et al. 2007) in sugar sensory neurons. The behavioral screening tests were performed with, if available, 2 independent *UAS-Gα RNAi* strains, in which the P{*UAS-Gα RNAi*} inserted in different integration sites of the chromosomes, whereas the expressed RNAi molecules were directed against the same G-protein mRNA. Given that *Gr5a* serves as a marker for the sugar neuron in each sensillum of the labellum (Marella et al. 2006), we expressed the interfering RNA against the different *Gα* subunits under control of the *Gr5a*

promoter (*Gr5a-GAL4*). As no fly line was available that expresses RNAi against mRNA of the G-protein subunit concertina, this subunit was not examined in the 2-choice screening assay. We discovered that flies expressing *Goα* RNAi in *Gr5a* cells (*Gr5a-GAL4;UAS-Goα RNAi*) exhibit a strongly decreased ability to distinguish between 5 mM sucrose and 2 mM sucrose (Figure 1). The control strains, wild type (CantonS), homozygous *Gr5a-GAL4*, and homozygous *UAS-Goα RNAi*, displayed a strong bias to consume the higher concentrated sucrose solution (Figure 1). Because mutants expressing RNAi against other Gα protein subunits in *Gr5a* cells retained a preference for 5 mM sucrose in the 2-choice assay, we focused our studies on *Goα* transgenic flies.

Goα RNAi–expressing gustatory neurons show reduced responses to sucrose

In order to validate the effect of RNAi against *Goα*, we performed *Goα* expression analysis in *Gr5a-GAL4* and *Gr5a-GAL4;UAS-Goα RNAi* flies and compared the expression levels with those from wild-type flies. Whereas the amount of *Goα* transcripts in wild-type and *Gr5a-GAL4* flies was equal, the *Gr5a-GAL4;UAS-Goα RNAi* mutants displayed a reduced amount of *Goα* transcripts (Figure 2A). For a further characterization of cells expressing RNAi against *Goα* (*Gr5a-GAL4;UAS-Goα RNAi*), we used an electrophysiological approach to test the neuronal activity of these cells. We generated sugar-induced action potentials in GRNs performing tip recordings from the L-type sensilla, L1, L2, and L3, as described (Hodgson et al. 1955) (Figure 2B). Sucrose was chosen as stimulus because this disaccharide is known to elicit strong physiological responses (Dahanukar et al. 2007). The control strains, *Gr5a-GAL4* and *UAS-Goα RNAi*, showed similar firing rates at sucrose concentrations ranging from 20 to 500 mM. In contrast, *Gr5a-GAL4;UAS-Goα RNAi* flies exhibited a significantly reduced spike rate (Figure 2C,D). A 100 mM sucrose solution elicited neural responses of 43 spikes per second in these flies, whereas the neurons showed 82 and 80 spikes per second in *Gr5a-GAL4* and *UAS-Goα RNAi* flies. To exclude the possibility that the impaired electrophysiological responses to sucrose in *Gr5a-GAL4;UAS-Goα RNAi* flies were due to developmental defects of *Gr5a* neurons, we utilized the heat shock–inducible GAL4 expression (*hsGAL4*) during the adult stage in the *UAS-Goα RNAi* background. *hsGAL4;UAS-Goα RNAi* flies were reared at 19 °C to minimize expression of RNAi against *Goα* during pupal development. To induce RNAi expression, 6-day-old adults were heat shocked at 37 °C for 30 min and were allowed to recover for 16 h at 25 °C. Adults of the genotype *hsGAL4;UAS-Goα RNAi* that were continuously kept at 25 °C served as control flies. Tip recordings from the L-type sensilla revealed that the heat shock–induced expression of RNAi against *Goα* results in decreased neuronal responses at various sucrose concentrations (Figure 2E,F). A heat shock at 37 °C for 30 min and subsequent recovery for 16 h at 25 °C had no effect on the sucrose-induced responses in wild-type flies (data not shown). We therefore conclude that Goα contributes to the process of gustatory transduction and the decreased responses to sucrose were most likely not caused by defects in neural development. Because the observed neuronal responses of *Gr5a-GAL4;UAS-Goα RNAi* and *hsGAL4;UAS-Goα RNAi* flies were consistent with the observed phenotype of *Gr5a-GAL4;UAS-Goα RNAi* flies in the 2-choice preference tests, these experiments strongly suggest an involvement of Goα in sucrose perception in *Drosophila*.

Similar phenotypes were observed in another transgenic approach, where we overexpressed a constitutively inactive form of Goα (Go^{GDP}) in *Gr5a*-positive cells (*UAS-Go^{GDP};Gr5a-GAL4*). Go^{GDP} has a lower affinity for GTP and could be expected to have a dominant-negative effect, as the mutant form of the G-protein subunit competes with endogenous Goα (Katanaev et al. 2005). Dose–response curves of neuronal electrophysiological activities display that *UAS-Go^{GDP};Gr5a-GAL4* flies showed reduced electrical responses to various concentrations of sucrose (Supplementary Figure S2). Moreover, flies expressing *Go^{GDP}* in sugar neurons yielded significantly lowered PIs than the control lines in 2-choice preference tests (Supplementary Figure S2).

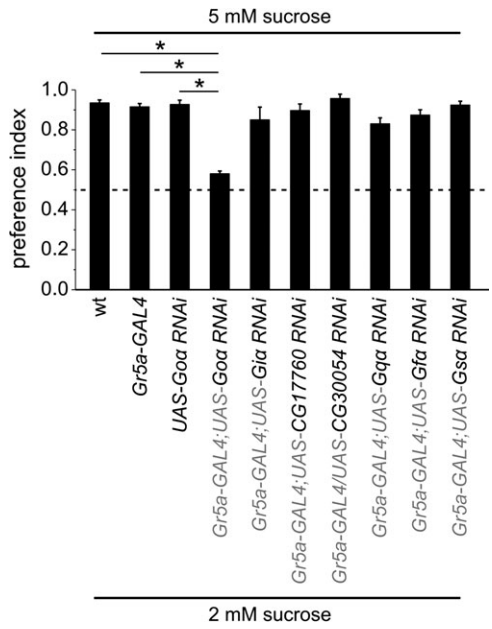

Figure 1 Behavioral screening tests with flies expressing RNAi against different G-protein mRNAs in *Gr5a*-positive cells. Flies had the choice between 2 and 5 mM sucrose. A PI of 1 indicates a total preference for the higher concentrated sucrose solution, whereas a PI of 0.5 indicates a lack of preference. Control strains were CantonS wild type (wt), homozygous *Gr5a-GAL4*, and homozygous *UAS-Goα RNAi*. Differences between the indicated data points were statistically checked by the unpaired Student's *t*-test, $*P \leq 0.05$. Error bars represent standard error of the mean.

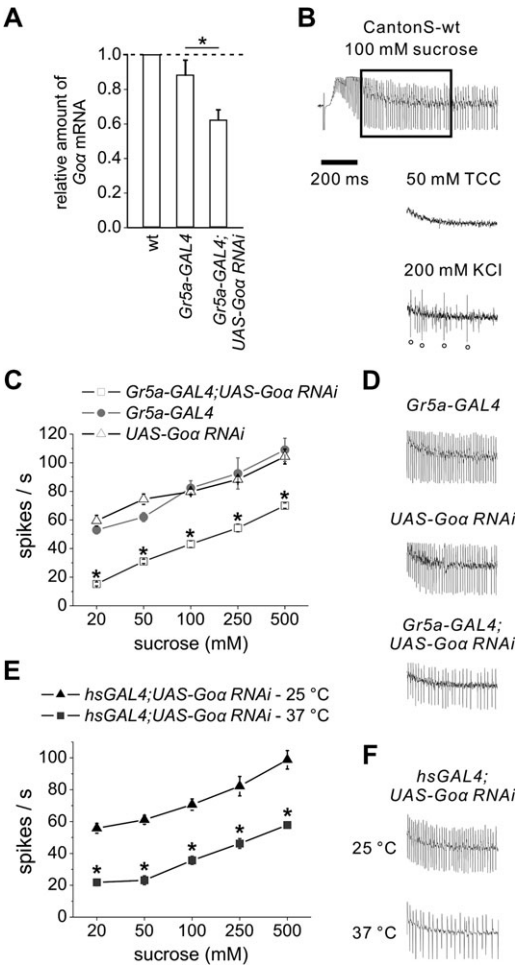

Figure 2 Electrophysiological properties of *Gr5a*-positive neurons expressing RNAi against *Goα*. **(A)** Expression analysis of *Goα* transcripts in labella from *Gr5a-GAL4;UAS-Goα RNAi* and *Gr5a-GAL4* via Q-PCR. The ribosomal gene product *rp49* was used to normalize the mRNA content among strains. A value of 1 refers to the expression level of *Goα* in wild-type (wt) flies. Error bars are standard error of the mean (SEM). Significance was calculated by the unpaired Student's *t*-test, $*P \leq 0.05$. **(B)** Representative traces of tastant-induced spike activities in wt flies recorded by tip recordings from L-type sensilla. Spikes were counted in a time period from 200 to 700 ms after onset of stimulation, as indicated by the black quadrangle in the CantonS wt trace. Unless otherwise indicated, the amplitudes and shapes of spikes depend on the specific stimulus. A 50 mM tricholine citrate (TCC) served as the electrolyte in all solutions apart from KCl, which was diluted in distilled water. A 200 mM KCl was applied as a test solution to each fly analyzed in order to check responses of the salt and water neuron. Dots mark the spikes generated by one of the stimulated neurons. **(C)** Sucrose-induced responses of sugar neurons that express RNAi against *Goα* mRNA were reduced. Shown were dose–response curves for *Gr5a-GAL4;UAS-Goα RNAi* (open square), *Gr5a-GAL4* (filled circle), and *UAS-Goα RNAi* (open triangle). For each sucrose concentration, $15 \leq n \leq 20$. Significant differences between data points were calculated using the unpaired Student's *t*-test, $*P \leq 0.05$. Error bars represent SEM. **(D)** Representative traces of neuronal responses in *Gr5a-GAL4*, *UAS-Goα RNAi*, and *Gr5a-GAL4;UAS-Goα RNAi* flies recorded upon stimulation of L-type sensilla with 100 mM sucrose. Stimuli were diluted in 50 mM TCC. **(E)** Neural responses to sucrose in noninduced and induced *hsGAL4;UAS-Goα RNAi* flies. Tip recordings from L-type sensilla of *hsGAL4;UAS-Goα RNAi*—25 °C (continuously kept at 25 °C, filled triangle) and *hsGAL4;UAS-Goα RNAi*—37 °C flies (heat shocked at 37 °C, filled square). For each sucrose concentration, $9 \leq n \leq 12$. Error bars represent SEM. The asterisks indicate statistically significant differences between the associated data points; $*P \leq 0.05$. **(F)** Original traces of noninduced and induced *hsGAL4;UAS-Goα RNAi* flies stimulated with 100 mM sucrose.

Neural and behavioral responses to sucrose are affected in mutant flies expressing reduced amounts of Goα

Due to the involvement of Goα in the development of heart epithelium and axon growth, a null mutation in *Goα* is lethal (Katanaev et al. 2005). In order to find mutants for a functional characterization, we queried the database for P-element insertions in the *Goα* gene locus because these insertions often result in reduced amounts of gene transcription. Two different Goα proteins, DGo1 and DGo2, have been identified that are transcribed from this gene locus (de Sousa et al. 1989; Schmidt et al. 1989; Thambi et al. 1989; Yoon et al. 1989). Two P-element insertions in a genomic intron of dGo2 and a potential promoter region of dGo1, P{*GawB*}NP3200 (Brand and Perrimon 1993) and P{*EP*}2154 (Rorth 1996), were selected for further analysis (Figure 3A). The P-element integration in EP2154/EP2154 flies results in absence of detectable dGo2 transcription, whereas both transcripts could be detected in heterozygous NP3200/+ transgenic flies by RT-PCR (Figure 3B). Q-PCR analysis revealed that the relative amount of total *Goα* mRNA was reduced to 57% in heterozygous NP3200/+ flies and 47% in homozygous EP2154/EP2154 flies (Figure 3C). dGo1 transcription was reduced to 70% and dGo2 transcription to 53% in NP3200/+ flies. The expression of the dGo2 transcript was almost completely suppressed in EP2154/EP2154 flies (0.0004%), whereas the amount of dGo1 equates to the wild-type level (Figure 3C). In addition, we analyzed the relative expression rates of these transcripts between labella and heads of wild-type flies. Relating to the amount in the heads, we found 24% of dGo1 and 82% of dGo2 expression in the labellum of the fly (Figure 3D).

Next, we aimed to investigate the neuronal responses of the P-element lines upon sucrose stimulation using tip recordings. The electrophysiological responses of the GRNs of both NP3200/+ and EP2154/EP2154 flies were significantly decreased at each sucrose concentration tested in comparison to the control strain (Figure 3E,F). In a rescue experiment, we complemented the NP3200/+ mutant, which is a GAL4 enhancer trap strain, by driving the expression of wild-type dGo2 under control of the enhancer-specific GAL4 activation (Brand and Perrimon 1993). Q-PCR experiments indicate that the amount of dGo2 transcripts in the rescue line (NP3200/*UAS-Go*) attained the amount of dGo2 in wild-type flies (Figure 3C). The reduced neuronal activity in NP3200/+ flies was likely caused by the low *Goα* expression levels because the impaired phenotype of these flies was partially rescued by dGo2 expression (Figure 3E,G). At each sucrose concentration tested, the rescue line displayed a significantly increased spike rate in comparison

to the deficient NP3200/+ flies. Notably, the neural responses of NP3200/*UAS-Go* were highly similar to those of wild-type flies at higher sucrose concentrations.

Two-choice preference tests revealed that solutions with high sucrose concentrations can restrictedly be distinguished from solutions with low sucrose concentrations by NP3200/+ and EP2154/EP2154 flies (Figure 3H). The impaired behavioral response of NP3200/+ was rescued by transgenic expression of dGo2 in *Goα*-positive cells (NP3200/*UAS-Go*) (Figure 3H). We additionally performed an established PER assay (Dethier and Goldrich-Rachman 1976), in which we applied a 100 mM sucrose solution to the first legs of the fly and monitored the induced extensions of the proboscis. Twenty-four percent of the NP3200/+ flies displayed a PER to 100 mM sucrose, whereas 93% of the wild-type flies showed a PER in response to stimulation with sucrose (Supplementary Figure S3). Taken together, these data support the participation of Goα in sucrose perception.

PTX impairs the behavioral and electrophysiological responses to sucrose

PTX is well known to inhibit specifically Goα and Giα function, as it catalyzes ADP-ribosylation of the G-protein

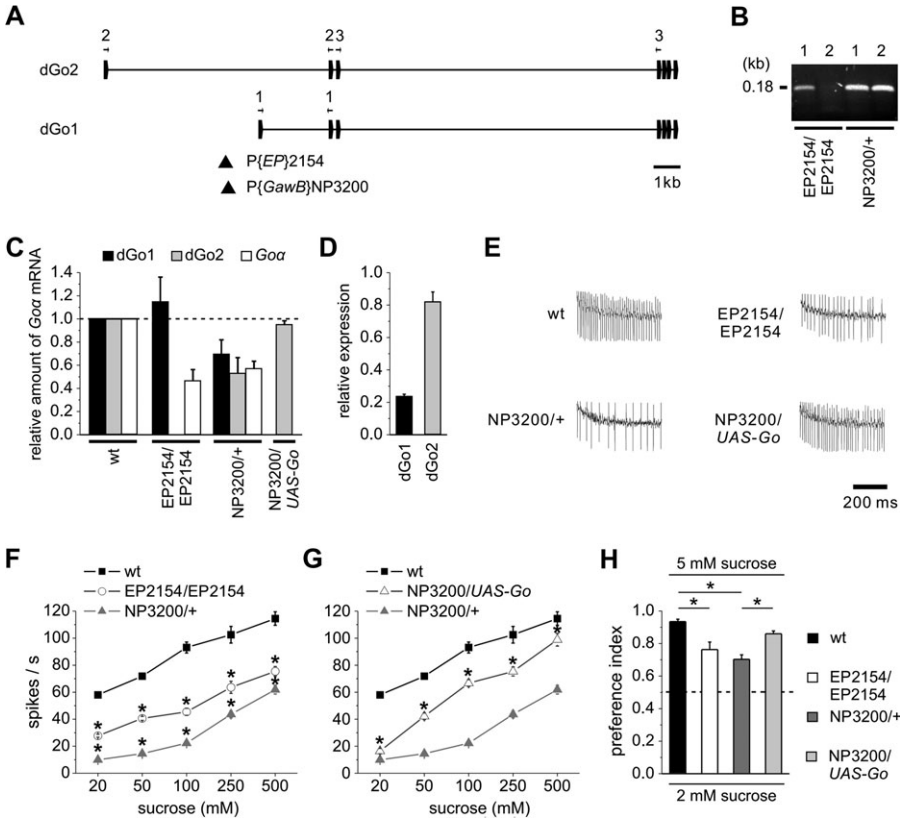

Figure 3 Electrophysiological and behavioral responses of flies containing P-element integrations in the *Goα* gene locus. **(A)** Integration site of P{*EP*}2154 and P{*GawB*}NP3200. Shown is the structure of the *Goα* coding region, indicating exons (filled arrows) and untranslated (lined) regions. The 2 P-elements, indicated by triangles, inserted into a genomic intron downstream of the dGo2 transcriptional start and a possible promoter region of dGo1, respectively. Distinct pairs of oligonucleotides were employed to detect the individual transcripts (pair 1: dGo1; pair 2: dGo2) and both of them simultaneously (pair 3: *Goα*) by PCR from *Drosophila* adult head cDNA. The illustration of the oligonucleotides is not drawn to scale. **(B)** RT-PCR from adult head cDNA of EP2154/EP2154 and NP3200/+ to analyze the expression of dGo1 (1) and dGo2 (2) in the mutant strains. **(C)** Q-PCR analysis showing relative expression level of dGo1, dGo2, and total *Goα* in EP2154/EP2154, NP3200/+, and NP3200/*UAS-Go*. The ribosomal gene product *rp49* was used to normalize the mRNA content among the analyzed strains. A value of 1 refers to the expression level of the associated *Goα* transcripts in wild-type (wt) flies. Error bars indicate standard error of the mean (SEM). **(D)** Comparison of the expression level of dGo1 and dGo2 between labella and heads of wt flies via Q-PCR. A value of 1 refers to the expression level of the associated transcript in the head. **(E)** Representative traces of wt, EP2154/EP2154, NP3200/+, and NP3200/*UAS-Go* flies recorded upon stimulation of L-type sensilla with 100 mM sucrose. Stimuli were diluted in 50 mM tricholine citrate. **(F)** Dose–response curves of wt (filled square), EP2154/EP2154 (open circle), and NP3200/+ (filled triangle). A range of concentration from 20 to 500 mM sucrose was used to test neuronal responses via tip recording. For each concentration, $10 \le n \le 16$. Error bars represent SEM. Data points of EP2154/EP2154 and NP3200/+ were statistically compared with those from wt flies using the unpaired Student's *t*-test; $^*P \le 0.05$. **(G)** Dose–response curves of wt (filled square), NP3200/+ (filled triangle), and NP3200/*UAS-Go* (open triangle). For each concentration, $10 \le n \le 12$. Error bars represent SEM. Data points of NP3200/+ and NP3200/*UAS-Go* were statistically compared using the unpaired Student's *t*-test; $^*P \le 0.05$. **(H)** Two-choice preference tests with wt, EP2154/EP2154, NP3200/+, and NP3200/*UAS-Go*. Flies were exposed to 2 and 5 mM sucrose solutions. A PI of 0.5 indicates preference for neither of both substances. Error bars are SEM. Significance was calculated by the unpaired Student's *t*-test, $^*P \le 0.05$.

subunit (Moss and Vaughan 1988). In *Drosophila*, Goα is the only cellular target for PTX because it is the only G-protein subunit with a cysteine residue on the fourth position starting from the C-terminus, where ADP-ribosylation occurs (West et al. 1985). Using a combination of a transgenic and a pharmacological approach, we tested whether PTX impairs the electrophysiological and behavioral responses to sucrose. First, we created flies that expressed the transgene *ptx* delimited to sugar neurons (*UAS-ptx/+;Gr5a-GAL4/+*) and investigated nerve responses of those flies employing tip recording. The responses to sucrose concentrations between 20 and 500 mM were significantly lower than those of the parental lines (Figure 4A). Remarkably, the strongest effect of PTX-induced inhibition of Goα was observed upon stimulations with 20 mM sucrose, with a spike frequency reduction to approximately 15% (Figure 4A). A 2-choice preference test revealed that *UAS-ptx/+;Gr5a-GAL4/+* flies completely lost the ability to discriminate between 2 and 5 mM sucrose (Figure 4B).

The responses to 20 mM sucrose were also monitored from L-type sensilla of wild-type flies that were preincubated with 10 μg/ml PTX for 20 min. Strikingly, neuronal responses were diminished to 5 spikes per second after the incubation with PTX (Figure 4C,D), whereas flies that were incubated with control solutions showed no effect on the firing rate elicited by 20 mM sucrose (data not shown). We also tested responses to 100 mM sucrose, a physiological concentration that is present in many fruits (U.S. Department of Agriculture, Agricultural Research Service 2006) and found these responses reduced to 73% after treatment with PTX (Figure 4C,D).

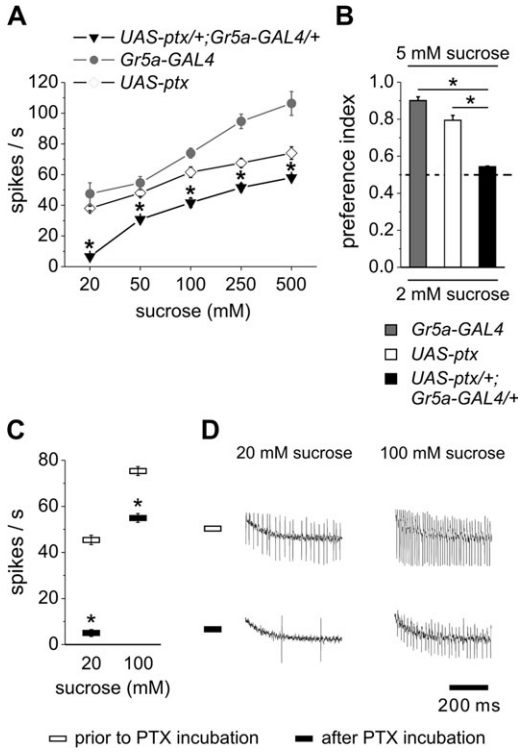

Figure 4 PTX impairs both the neuronal and behavioral response to sucrose. **(A)** Dose–response curves of *Gr5a-GAL4* (filled circle), *UAS-ptx* (open rhombus), and *UAS-ptx/+;Gr5a-GAL4/+* (filled triangle). For each sucrose concentration, $10 \le n \le 14$. Error bars represent standard error of the mean (SEM). The asterisks indicate statistically significant differences between *UAS-ptx* and *UAS-ptx/+;Gr5a-GAL4/+* flies calculated by the unpaired Student's *t*-test, $^*P \le 0.05$. **(B)** Two-choice preference tests with *Gr5a-GAL4*, *UAS-ptx*, and *UAS-ptx/+;Gr5a-GAL4/+*. Flies were tested at 2 mM sucrose versus 5 mM sucrose. A PI of 0.5 indicates a lack of preference for one substance. Error bars represent SEM. Significance was calculated by the unpaired Student's *t*-test, $^*P \le 0.05$. **(C)** Pharmacological application of PTX. Neural responses of L-type sensilla from wild-type (wt) flies were monitored at 20 and 100 mM sucrose (open square). Tested sensilla were incubated for 20 min with 10 μg/ml PTX diluted in 50 mM tricholine citrate. After the incubation period, the response to sucrose was checked again (filled square). Error bars represent SEM. Significance was calculated by the unpaired Student's *t*-test, $^*P \le 0.05$. **(D)** Original traces of wt flies before (open square) and after (filled square) treatment with PTX.

Goα is localized in taste organs

RT-PCR experiments revealed that *Goα* is expressed in the labellum of the fly (Supplementary Figure S4). We visualized *Goα*-positive neurons by expression of a membrane-located GFP reporter (*UAS-mCD8::GFP*) under control of the GAL4 enhancer trap strain NP3200. *Goα* expression was found in taste neurons of the labial palps and in the distal segments of the legs (Figure 5A,B). At least one neuron per sensillum was *Goα* positive, as each sensillum of the labellum exhibited GFP fluorescence. Using a higher magnification, we observed that L- and S-type sensilla were potentially innervated by 4 kinds of neurons because all 4 GFP-positive cells seem to extend their dendrite into the same sensillum. These are probably S1, L2, L1, and W cells, indicating that *Goα* promoter activity may be present in sugar-, bitter-, salt-, and water-responding neurons (Figure 5C). Immunohistochemical staining of labella from *Gr5a-GAL4/UAS-mCD8::GFP* flies with Goα antibodies revealed that *Gr5a* and *Goα* were expressed in overlapping subsets of GRNs (Figure 5D–G). In whole-mount preparations, we observed fluorescence exclusively in the dendrites of *Gr5a*-positive neurons (Figure 5E,F).

Responses to various sugars are dependent on different signaling pathways

In addition to sucrose, we examined the neuronal activation in *Goα* mutant flies initiated by other sweet stimuli: maltose, trehalose, glucose, and fructose. Previous studies on wild-type flies have shown that the response spectra of *Gr5a* neurons to these sugars show wide variation in their stimulus-induced spike frequencies. Solutions of sucrose and maltose (100 mM) elicited more than 60 spikes per second, whereas solutions of glucose, trehalose, and fructose displayed rather moderate firing rates at the same concentration (Dahanukar et al. 2007). If the detection of all sugars depends on a Goα-related pathway, one would expect that the neural responses to all sugar stimuli were depressed in

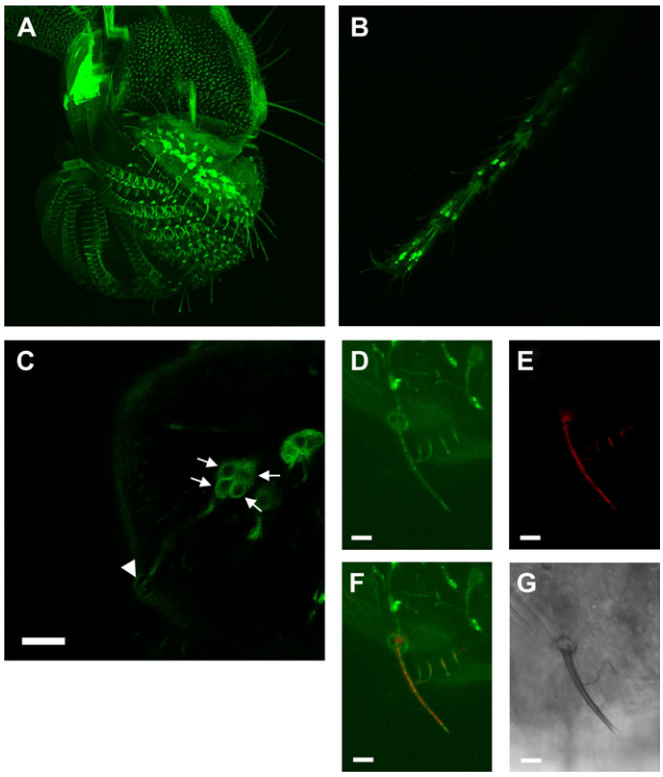

Figure 5 Expression pattern of *Goα* in the gustatory system. **(A, B)** NP3200 expression pattern visualized by GFP. (A) Labella and (B) tarsi of NP3200/UAS-*mCD8::GFP* flies were examined by confocal microscopy. Shown here are maximum projections of a 0.4-µm stack series. **(C)** GFP signals were observed at the base of all sensilla in the labellum. Arrows indicate single neurons in a cluster of GRNs that innervate the same sensillum. The arrowhead marks the base of the sensillum. Scale bar: 10 µm. **(D–G)** Immunofluorescence staining of whole-mount labella. Scale bars: 5 µm. (D) GFP fluorescence of a labellum from *Gr5a-GAL4/UAS-mCD8::GFP* (green). (E) Immunofluorescent image of a labellum stained with anti-Goα antiserum (red). (F) Merged image of (C and D). (G) Transmitted light image of the labellar sensillum.

EP2154/EP2154 and NP3200/+ flies. The electrical responses of sugar neurons from EP2154/EP2154 and NP3200/+ transgenic flies that were elicited by various concentrations of maltose and trehalose were identical to those of wild-type flies, whereas the spike rates elicited by different concentrations of glucose and fructose were significantly decreased (Figure 6A,B). The perception of maltose and trehalose does therefore seem not to be dependent on a Goα-related pathway. Similar observations were made with transgenic flies that expressed RNAi against *Goα* under control of the *Gr5a* promoter (*Gr5a-GAL4;UAS-Goα RNAi*) (Figure 6A,B). Remarkably, the extent of depression was greater at lower concentrations of glucose and fructose in EP2154/EP2154, NP3200/+, and *Gr5a-GAL4;UAS-Goα RNAi*, whereas the responses to 500 mM glucose and fructose were comparable with the control strains.

To confirm these data on a behavioral level, we performed 2-choice preference tests, in which flies had the choice between 5 mM sucrose and 13 mM maltose or alternatively between 5 mM sucrose and 75 mM trehalose. Wild-type flies did not prefer these maltose or trehalose solutions compared with the sucrose solution, which is in correspondence with the fact that the sugar concentrations elicited similar spike rates in these flies. NP3200/+ flies, which displayed the strongest phenotype in the electrophysiological responses to sucrose, preferred maltose and trehalose over sucrose in this test, indicating that the observed defective behavioral response is confined to an impaired sucrose perception in the *Goα* mutant flies (Figure 6C).

Caffeine and quinine perception is unaffected in *Goα* mutants

Bitter neurons express the receptor Gr66a (Thorne et al. 2004; Wang et al. 2004), which is required for the behavioral and physiological responses to caffeine (Moon et al. 2006). As *Goα* is also expressed in *Gr66a*-positive neurons, we investigated the behavioral responses to caffeine and quinine in flies defective in Goα signaling. In the performed avoidance tests, maltose was chosen as control solution because the electrophysiological response to this disaccharide was not affected in those flies. The behavioral tests revealed that flies expressing either RNAi against *Goα* (*UAS-Goα RNAi*) or *ptx* (*UAS-ptx*) in *Gr66a*-labeled cells (*Gr66a-GAL4*) show normal responses to caffeine and quinine (Figure 7A). The majority of flies avoided the solution with the bitter substance, indicating that the detection of this substance was not impaired. EP2154/EP2154 and NP3200/+ flies also did not display any anomaly in response to caffeine or quinine, because these flies had PIs comparable to wild-type flies (Figure 7A). Next, we investigated responses of bitter neurons from EP2154/EP2154 and NP3200/+ flies using tip recordings from S6 sensilla. Caffeine and quinine solutions evoked similar responses in the *Goα* mutant strains and the control strain (Figure 7B). It is therefore unlikely that Goα is required for the caffeine or quinine response, but we cannot exclude that the protein subunit is involved in the detection of other bitter compounds.

Discussion

Goα is involved in the reception of sucrose

The current analysis shows that the G-protein subunit Goα plays an important role in the reception of the carbohydrates sucrose, glucose, and fructose in *Drosophila*. We have systematically analyzed behavioral and electrophysiological responses to sugars in different *Goα* transgenic flies and found these responses significantly altered in comparison to control strains. Notably, the strongest effect on the sucrose responses was observed at the lowest sucrose concentration tested (20 mM) in all transgenic or pharmacological approaches to affect Goα function. As the differences of neuronal activities in the mutant flies remained significant in

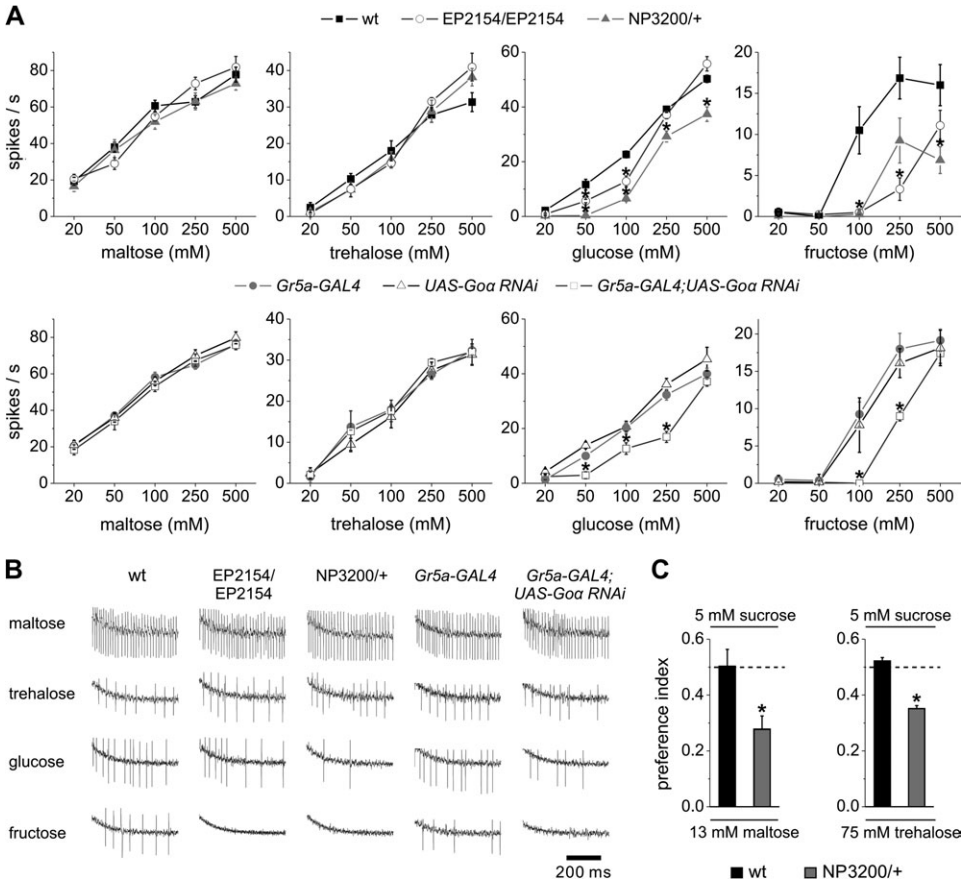

Figure 6 Neural and behavioral responses to multiple sugars in wild-type (wt) flies and *Goα* mutant strains. **(A)** Dose–response relationship of sugar responses of wt, EP2154/EP2154, NP3200/+, *Gr5a-GAL4*, *UAS-Goα RNAi*, and *Gr5a-GAL4;UAS-Goα RNAi*. The selected panel of sugars was tested at a range of concentration from 20 to 500 mM. For all stimuli, $12 \leq n \leq 18$. Significance was calculated by the unpaired Student's *t*-test, $^*P \leq 0.05$. Error bars represent standard error of the mean (SEM). **(B)** Sample traces of wt, EP2154/EP2154, NP3200/+, *Gr5a-GAL4*, and *Gr5a-GAL4;UAS-Goα RNAi* in response to 100 mM maltose, trehalose, glucose, and fructose. **(C)** Two-choice preference test with wt and NP3200/+ flies. Flies had the choice between 5 mM sucrose and either 13 mM maltose or 75 mM trehalose. A PI close to 0.5 indicates preference for neither of both substances. Error bars are SEM. Significance was calculated by the unpaired Student's *t*-test, $^*P \leq 0.05$.

comparison to control strains even at the highest concentrations used (500 mM), we conclude that a Goα-related pathway is involved in the reception of low and high concentrations of sucrose.

Both Goα proteins play a role in the reception of sucrose

Drosophila has 2 Goα proteins, DGo1 and DGo2, that are generated from the same gene by alternative splicing of transcripts (de Sousa et al. 1989; Schmidt et al. 1989; Thambi et al. 1989; Yoon et al. 1989). These transcripts share the last 6 of overall 7 exons, whereas the first exon is found at different genomic regions in both variants; relative expression rates in the proboscis are 24% for dGo1 and 82% for dGo2. Because expression of dGo1 is unaffected in EP2154/EP2154 flies, loss of DGo2 must be responsible for the observed phenotypes in this strain. On the other hand, dGo2 transcription was affected to a smaller extent in NP3200/+ flies although the observed phenotypes are slightly stronger, indicating that DGo1 also plays a role in the signaling cascade. The simplest interpretation of our results is that both isoforms can complement each other, which is also in correspondence with the rescue experiments using *UAS-Go* strains that contain dGo2 cDNA, which lead to a partial, but not complete rescue of the sugar responses.

Both Goα proteins differ in only 7 amino acids in the N-terminal region; the GTPase domains should therefore not show any difference in their function. Because the amino acid sequence of the N-terminal region is crucial for post-translational modifications that result in an anchoring of the α subunit to the plasma membrane (Jiang and Bajpayee 2009), we analyzed the sequence of both Goα proteins with regard to the consensus sequences for myristoylation and palmitoylation. Both proteins share the required sequences for these lipid modifications (data not shown); further studies are therefore required to explain the differences in function of the isoforms.

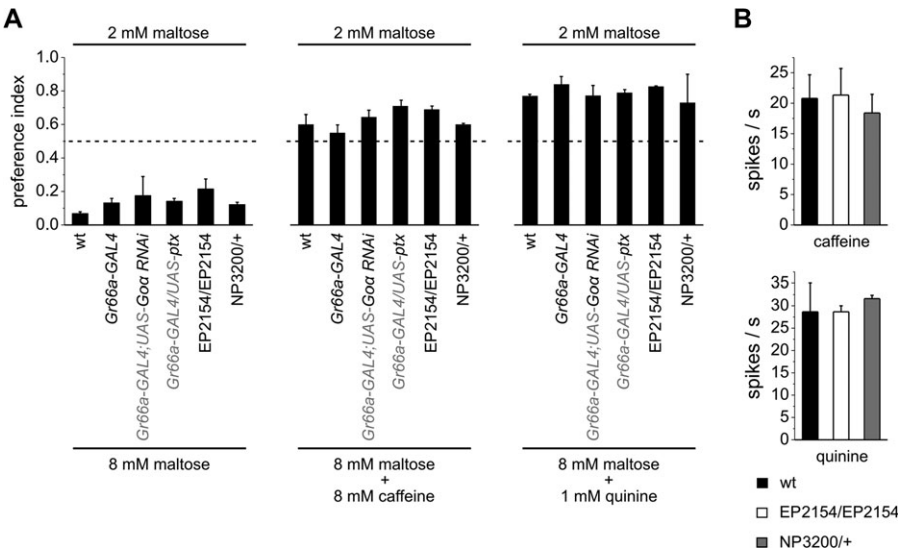

Figure 7 Avoidance tests with *Goα* transgenic flies. **(A)** Flies had the choice between 2 mM maltose and 8 mM maltose alone or 8 mM maltose blended with 8 mM caffeine or 1 mM quinine. A PI of 1 indicates total preference for 2 mM maltose, whereas a PI of 0.5 indicates a lack of preference. The behavioral response of EP2154/EP2154, NP3200/+, and flies expressing either *Goα* RNAi or *ptx* under control of the *Gr66a* promoter was analyzed. Control strains were wild type (wt) and *Gr66a-GAL4*. Error bars represent standard error of the mean (SEM). **(B)** Neuronal responses from wt, EP2154/EP2154, and NP3200/+ flies upon stimulation with either 10 mM caffeine or 10 mM quinine. For each substance, $6 \leq n \leq 8$. Error bars represent SEM.

Goα signaling cascades

The *Drosophila* Goα also appears to be important for various neuronal functions. Recent studies indicate that Goα contributes to olfactory reception in the fruit fly (Chatterjee et al. 2009). It is moreover important in the development of the organism because Wnt and PCP Fz pathways can be transduced by trimeric G-protein complexes that contain this subunit (Katanaev et al. 2005). Goα is also required for the formation of heart epithelium in *Drosophila* (Fremion et al. 1999). Heterotrimeric Go is in general the most abundant G-protein in the brain and has been implicated in various functions such as embryogenesis, learning and memory, sensory organ development, and neurodegenerative diseases such as Parkinson and Alzheimer's disease to schizophrenia (reviewed in Jiang and Bajpayee 2009). In line with the ubiquitous expression in the brain, Goα protein subunit seems to be expressed in all taste neurons of the gustatory system, although only the sugar responses were affected in the mutants. Even though Goα seems to not to interact with all taste receptors, the protein might fulfill other functions in the neurons, besides the transduction of GR activation. Because the current understanding of its signaling mechanisms and its effectors is still either incomplete or unknown, these functions are at the current state of knowledge difficult to predict on a molecular level.

In *Caenorhabditis elegans*, Goα seems to act upstream of a DAG kinase in a serotonergic signaling pathway, where it antagonizes the Gq-phospholipase C beta DAG signaling cascade (Miller et al. 1999). *Caenorhabditis elegans* Goα was shown to be involved in sensory signal transduction because it is important for olfactory adaptation (Matsuki et al. 2006). Whether this PLC antagonizing effect may also exist in other species such as *Drosophila* is to date unknown. Goα could be involved in an IP_3-activated signaling cascade because the application of inhibitors of the PLC-mediated IP_3 transduction cascade yielded reduced responses to various sugars of GRNs in the flesh fly, *Boettcherisca peregrina* (Koganezawa and Shimada 2002). Moreover, 2 independent transgenic approaches revealed that IP_3 signaling is indispensable for the reception of sucrose and trehalose in *Drosophila* (Usui-Aoki et al. 2005).

A second putative signaling mechanism may involve direct ion channel modulation by Goα proteins. PTX-sensitive Goα proteins can also modulate Ca^{2+} channels in different mammalian neurons (Hille 1994) and L-type Ca^{2+} channels via muscarinic receptors in myocytes (Valenzuela et al. 1997). Goα can also activate K^+ channels in several signaling systems and in different organisms (VanDongen et al. 1988; Peleg et al. 2002).

Multiple gustatory signaling mechanisms in *Drosophila*

Despite their 7 transmembrane structure, *Drosophila* GRs lack sequence homology with mammalian taste receptors or other G-protein coupled receptors but are distantly related to the large family of *Drosophila* olfactory receptors (ORs) (Dunipace et al. 2001). It has been shown for the olfactory system of *Drosophila* that ORs form functional heterodimers, consisting of 1 of the 61 ligand-specific ORs and the coreceptor OR83b (Larsson et al. 2004; Neuhaus et al. 2005). *Drosophila* ORs are 7 transmembrane proteins, which appear to have an extracellular C-terminus and an intracellular N-terminus (Benton et al. 2006; Lundin et al. 2007) and

function as ligand-gated ion channels (Sato et al. 2008; Wicher et al. 2008). Even so, *Drosophila* olfactory signal transduction involves the heterotrimeric G-proteins Gqα and Goα (Kain et al. 2008; Chatterjee et al. 2009).

Although not unambiguously shown until now, *Drosophila* GRs may also exhibit ligand-gated channel properties besides activation of G-proteins. The existence of a G-protein–independent signaling pathway in insect sugar neurons was already suggested by in situ patch clamp recordings showing that an ion channel is directly gated by sucrose in the flesh fly (Murakami and Kijima 2000). Such ionotropic properties of GRs would provide a reasonable explanation why we did not observe an elimination of sucrose-induced responses in our electrophysiological experiments with *Goα* mutant flies. Similar results were obtained in analogous experiments with *Gγ1* null mutants that showed a significantly decreased response to sucrose in comparison to control flies, but not a complete suppression of the sugar-evoked response (Ishimoto et al. 2005). According to a recently proposed dual-activation model (Nakagawa and Vosshall 2009), the primary response to sucrose in *Drosophila* could be generated by activation of sucrose-gated ion channels composed of different GRs, followed by a G-protein–mediated potentiation of the ionotropic response. The fact that CO_2 detection, mediated by a heterodimer consisting of GR21a and GR63a expressed in the fly antenna, requires Gqα and Gγ30a in vivo provides additional evidence for GR-mediated signal transduction via a metabotropic mechanism (Yao and Carlson 2010).

According to this model, Goα would play a modulatory part in the physiological detection of sugars. As demonstrated before, the severest defects of *Goα* mutants in tip recordings were observed at low sucrose concentrations, supporting a role of Goα in potentiating receptor function.

Role of other G-proteins in the perception of sugars

Goα was found to be involved in the perception of sucrose, glucose, and fructose, whereas trehalose and maltose seem to be independent from a Goα-related pathway, suggesting that there are heterogeneous pathways for the detection of sugars in *Drosophila*. These findings complement previous studies, in which a Gsα- and a Gqα-related pathways were suggested for trehalose perception (Ueno et al. 2006; Kain et al. 2010). Ueno et al. (2006) performed an electrophysiological approach and found significantly reduced spike rates in *Gr5a-GAL4/UAS-Gsα RNAi* and heterozygous *Gsα* null mutants upon stimulation of L-type sensilla with low trehalose concentrations. In a behavioral assay, the authors accessorily found a depressed intake of sucrose, fructose, and glucose in these flies. Using a different approach to study gustatory behavior, we found that *Gr5a-GAL4;UAS-Gsα RNAi* flies performed like wild-type flies in 2-choice preference tests. Despite the existence of minor differences that might be explained by the different experimental paradigms, one can conclude that Gsα-, Gqα-, and Goα-related pathways are involved in sugar perception of the fly.

The different G-protein pathways could be activated via different types of sugar receptors, although until now no type of G-protein is assigned to a certain GR in *Drosophila*. Whereas a Gr5a-initiated pathway in response to trehalose may involve Gsα and/or Gqα coupling in vivo, sucrose could activate a Goα-related pathway via other GRs, most likely involving a receptors of the *Gr64* cluster. Because GRs were shown to interact (Jiao et al. 2008), different types of receptor heteromultimers may couple preferentially to different types of heterotrimeric G-proteins, depending on their subunit composition.

Activation of different, stimulus- and concentration-dependent signaling pathways in sugar-sensitive cells was also observed in mammals (reviewed in McCaughey 2008). Whereas sucrose elicits, for example, an intracellular increase of the Cyclic adenosine monophosphate and subsequent Ca^{2+} influx, saccharin stimulation induces an intracellular increase in the secondary messenger IP_3 and results in Ca^{2+} release from internal stores (Bernhardt et al. 1996). However, there is evidence that the second pathway can also be activated by sucrose in mice (Zhang et al. 2003).

This report may help to understand the role of GR signaling in the insect gustatory system. Insight into the molecular determinants underlying receptor/G-protein interactions may help to fully understand the mechanisms and consequences of processing taste stimuli in future studies.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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References

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

Bernhardt SJ, Naim M, Zehavi U, Lindemann B. 1996. Changes in IP_3 and cytosolic Ca^{2+} in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. J Physiol. 490(Pt 2):325–336.

Brand AH, Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 118:401–415.

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

Clyne PJ, Warr CG, Carlson JR. 2000. Candidate taste receptors in *Drosophila*. Science. 287:1830–1834.

Dahanukar A, Foster K, van der Goes van Naters WM, Carlson JR. 2001. A Gr receptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. Nat Neurosci. 4:1182–1186.

Dahanukar A, Lei YT, Kwon JY, Carlson JR. 2007. Two Gr genes underlie sugar reception in *Drosophila*. Neuron. 56:503–516.

de Sousa SM, Hoveland LL, Yarfitz S, Hurley JB. 1989. The *Drosophila* Go alpha-like G protein gene produces multiple transcripts and is expressed in the nervous system and in ovaries. J Biol Chem. 264: 18544–18551.

Dethier VG, Goldrich-Rachman N. 1976. Anesthetic stimulation of insect water receptors. Proc Natl Acad Sci U S A. 73:3315–3319.

Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, et al. 2007. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature. 448:151–156.

Dunipace L, Meister S, McNealy C, Amrein H. 2001. Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. Curr Biol. 11:822–835.

Fremion F, Astier M, Zaffran S, Guillen A, Homburger V, Semeriva M. 1999. The heterotrimeric protein Go is required for the formation of heart epithelium in *Drosophila*. J Cell Biol. 145:1063–1076.

Hille B. 1994. Modulation of ion-channel function by G-protein-coupled receptors. Trends Neurosci. 17:531–536.

Hodgson ES, Lettvin JY, Roeder KD. 1955. Physiology of a primary chemoreceptor unit. Science. 122:417–418.

Ishimoto H, Takahashi K, Ueda R, Tanimura T. 2005. G-protein gamma subunit 1 is required for sugar reception in *Drosophila*. EMBO J. 24: 3259–3265.

Jiang M, Bajpayee NS. 2009. Molecular mechanisms of Go signaling. Neurosignals. 17:23–41.

Jiao Y, Moon SJ, Montell C. 2007. A *Drosophila* gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. Proc Natl Acad Sci U S A. 104:14110–14115.

Jiao Y, Moon SJ, Wang X, Ren Q, Montell C. 2008. Gr64f is required in combination with other gustatory receptors for sugar detection in *Drosophila*. Curr Biol. 18:1797–1801.

Kain P, Badsha F, Hussain SM, Nair A, Hasan G, Rodrigues V. 2010. Mutants in phospholipid signaling attenuate the behavioral response of adult *Drosophila* to trehalose. Chem Senses. 35(8):663–673.

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

Katanaev VL, Ponzielli R, Semeriva M, Tomlinson A. 2005. Trimeric G-protein-dependent frizzled signaling in *Drosophila*. Cell. 120:111–122.

Koganezawa M, Shimada I. 2002. Inositol 1,4,5-trisphosphate transduction cascade in taste reception of the fleshfly, *Boettcherisca peregrina*. J Neurobiol. 51:66–83.

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

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25:402–408.

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

Marella S, Fischler W, Kong P, Asgarian S, Rueckert E, Scott K. 2006. Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. Neuron. 49:285–295.

Matsuki M, Kunitomo H, Iino Y. 2006. Goalpha regulates olfactory adaptation by antagonizing Gqalpha-DAG signaling in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 103:1112–1117.

McCaughey SA. 2008. The taste of sugars. Neurosci Biobehav Rev. 32: 1024–1043.

McLaughlin SK, McKinnon PJ, Margolskee RF. 1992. Gustducin is a taste-cell-specific G-protein closely related to the transducins. Nature. 357:563–569.

Miller KG, Emerson MD, Rand JB. 1999. Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in *C. elegans*. Neuron. 24: 323–333.

Moon SJ, Kottgen M, Jiao Y, Xu H, Montell C. 2006. A taste receptor required for the caffeine response *in vivo*. Curr Biol. 16:1812–1817.

Moss J, Vaughan M. 1988. ADP-ribosylation of guanyl nucleotide-binding regulatory proteins by bacterial toxins. Adv Enzymol Relat Areas Mol Biol. 61:303–379.

Murakami M, Kijima H. 2000. Transduction ion channels directly gated by sugars on the insect taste cell. J Gen Physiol. 115:455–466.

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

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

Peleg S, Varon D, Ivanina T, Dessauer CW, Dascal N. 2002. G(alpha)(i) controls the gating of the G-protein-activated K^+ channel, GIRK. Neuron. 33:87–99.

Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proc Natl Acad Sci U S A. 100(Suppl 2):14537–14542.

Rorth P. 1996. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. Proc Natl Acad Sci U S A. 93:12418–12422.

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

Schmidt CJ, Garen-Fazio S, Chow YK, Neer EJ. 1989. Neuronal expression of a newly identified *Drosophila melanogaster* G protein alpha o subunit. Cell Regul. 1:125–134.

Scott K, Brady R Jr, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell. 104:661–673.

Shanbhag SR, Park SK, Pikielny CW, Steinbrecht RA. 2001. Gustatory organs of *Drosophila melanogaster*: fine structure and expression of the putative odorant-binding protein PBPRP2. Cell Tissue Res. 304:423–437.

Singh RN. 1997. Neurobiology of the gustatory systems of *Drosophila* and some terrestrial insects. Microsc Res Tech. 39:547–563.

Slone J, Daniels J, Amrein H. 2007. Sugar receptors in *Drosophila*. Curr Biol. 17:1809–1816.

Stocker RF. 1994. The organization of the chemosensory system in *Drosophila melanogaster*: a review. Cell Tissue Res. 275:3–26.

Tanimura T, Isono K, Yamamoto MT. 1988. Taste sensitivity to trehalose and its alteration by gene dosage in *Drosophila melanogaster*. Genetics. 119:399–406.

Thambi NC, Quan F, Wolfgang WJ, Spiegel A, Forte M. 1989. Immunological and molecular characterization of Go alpha-like proteins in the *Drosophila* central nervous system. J Biol Chem. 264:18552–18560.

Thorne N, Chromey C, Bray S, Amrein H. 2004. Taste perception and coding in *Drosophila*. Curr Biol. 14:1065–1079.

Ueno K, Kidokoro Y. 2008. Adenylyl cyclase encoded by AC78C participates in sugar perception in *Drosophila melanogaster*. Eur J Neurosci. 28: 1956–1966.

Ueno K, Kohatsu S, Clay C, Forte M, Isono K, Kidokoro Y. 2006. Gsalpha is involved in sugar perception in *Drosophila melanogaster*. J Neurosci. 26:6143–6152.

U.S. Department of Agriculture, Agricultural Research Service. 2009. USDA National Nutrient Database for Standard Reference, Release 22 [Internet]. Nutrient Data Laboratory Home Page, Available from: http://www.ars.usda.gov/ba/bhnrc/ndl.

Ueno K, Ohta M, Morita H, Mikuni Y, Nakajima S, Yamamoto K, Isono K. 2001. Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene Gr5a. Curr Biol. 11:1451–1455.

Usui-Aoki K, Matsumoto K, Koganezawa M, Kohatsu S, Isono K, Matsubayashi H, Yamamoto MT, Ueda R, Takahashi K, Saigo K, et al. 2005. Targeted expression of IP_3 sponge and IP_3 dsRNA impairs sugar taste sensation in *Drosophila*. J Neurogenet. 19:123–141.

Valenzuela D, Han X, Mende U, Fankhauser C, Mashimo H, Huang P, Pfeffer J, Neer EJ, Fishman MC. 1997. G alpha(o) is necessary for muscarinic regulation of Ca^{2+} channels in mouse heart. Proc Natl Acad Sci U S A. 94:1727–1732.

VanDongen AM, Codina J, Olate J, Mattera R, Joho R, Birnbaumer L, Brown AM. 1988. Newly identified brain potassium channels gated by the guanine nucleotide binding protein Go. Science. 242:1433–1437.

Vosshall LB, Stocker RF. 2007. Molecular architecture of smell and taste in *Drosophila*. Annu Rev Neurosci. 30:505–533.

Wang Z, Singhvi A, Kong P, Scott K. 2004. Taste representations in the *Drosophila* brain. Cell. 117:981–991.

West RE Jr, Moss J, Vaughan M, Liu T, Liu TY. 1985. Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site. J Biol Chem. 260:14428–14430.

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

Wieczorek H, Wolff G. 1989. The labellar sugar receptor of *Drosophila*. J Comp Physiol A Sens Neural Behav Physiol. 164:825–834.

Wong GT, Gannon KS, Margolskee RF. 1996. Transduction of bitter and sweet taste by gustducin. Nature. 381:796–800.

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

Yoon J, Shortridge RD, Bloomquist BT, Schneuwly S, Perdew MH, Pak WL. 1989. Molecular characterization of *Drosophila* gene encoding Go alpha subunit homolog. J Biol Chem. 264:18536–18543.

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ. 2003. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell. 112: 293–301.