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Gr64f is Required in Combination with other Gustatory Receptors for Sugar Detection in *Drosophila*

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Summary

The mechanisms by which the fruitfly, *Drosophila melanogaster*, detect sweet compounds are poorly understood; however, a subset of the family of 68 gustatory receptors (Grs) has emerged as the key receptors. These seven transmembrane receptors include Gr5a and at least one member of the six genes in the *Gr64* cluster (*Gr64a*), which are expressed in sugar-responsive neurons. Disruption of *Gr5a* prevents the detection of trehalose [1–3], while mutation of Gr64a impairs the responses to sucrose, maltose and glucose [4,5]. Recent studies suggest that these sugar receptors may require a co-receptor for function *in vivo* [4–6]; however, the identity of the putative co-receptor is not known. In the current work, we demonstrate that Gr64f is required in combination with Gr5a for the behavioral response to trehalose and for production of action potentials due to application of trehalose. Gr64f was also required in concert with Gr64a to rescue the defects in the sensitivities to sucrose, maltose and glucose, resulting from deletion of the entire *Gr64* cluster. These data suggest that *Drosophila* sugar receptors function as multimers and that Gr64f is required broadly as a co-receptor for the detection of sugars.

Results and Discussion

Eight Grs (Gr-S receptors) are co-expressed in gustatory receptor neurons (GRNs), which elicit an attractive behavioral response to sugars, making them prime candidates for functioning as sugar receptors [5,7,8]. One member of the Gr-S group, *Gr5a* is essential for trehalose sensation and is expressed in most sugar responsive GRNs [1–3,7,8]. A second Gr, *Gr64a*, is required for detecting sucrose, maltose and glucose [4,5]. However, Gr64a is not sufficient to induce sugar responses when misexpressed in cells that do not normally express this receptor [5]. Two *in vivo* studies suggest that trehalose sensation may require another gustatory receptor in addition to Gr5a, and this additional receptor may be encoded by the large *Gr64* gene cluster described below [5,6]. However, based on an *in vitro* analysis, it was concluded that Gr5a functions as a trehalose receptor in the absence of other Grs [3].

Gr64 cluster organization

The organization of the *Gr64* cluster is unusual in that it includes six tandemly arranged *Gr* genes (*Gr64a-f*), each separated by ~200 base pairs (Figure 1A). Given this organization, the presence of a single polyadenylation site following *Gr64f* and the report that RT-PCR products can be generated that span each pair of open reading frames, it has been suggested that *Gr64a-*

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f may be transcribed as a single polycistronic mRNA [6]. Alternatively, based on the products produced from 5' and 3' RACE, *Gr64a-f* may be transcribed as both single (*Gr64a*, *Gr64e* and *Gr64f*) and bicistronic mRNAs (*Gr64bc* and *Gr64de*) [4]. Thus, the transcriptional pattern of the *Gr64* cluster is unresolved. To explore the expression of *Gr64* gene set further, we used RT-PCR. We isolated multiple overlapping RT-PCR products encoding all combinations of three adjacent Grs (Figure 1B). Thus, all *Gr* RNAs encoded in the *Gr64* cluster are initially transcribed as RNAs that span a minimum of three *Gr64* open reading frames. We were unable to amplify larger cDNAs that included four to six genes. Nevertheless, the overlapping pattern RT-PCR products suggest that *Gr64a-f* may be expressed as a polycistronic mRNA, which is subsequently processed into smaller mRNAs, similar to that described for the *CheB42a*/*llz* locus [9].

Gr64f is required to sense trehalose together with *Gr5a*

Elimination of the entire *Gr64* cluster (*ΔGr64*) disrupts the responses to multiple sugars, including trehalose, but not to aversive compounds such as caffeine [6]. To determine which *Gr* gene in the *Gr64* cluster was required for sensing trehalose we employed a variation of a behavioral assay [10] in which flies were offered a choice between a sugar in agarose, or agarose only. The two types of agarose were mixed with either red or blue food dyes, and the colors of their abdomens were assessed. A complete preference for consuming the trehalose would result in a preference index (PI) of 1.0, while a failure to discriminate between the alternatives would yield a PI of 0.5. The wild-type control (w^{1118}) displayed a strong preference to consume trehalose (Figure 1C; PI=0.90±0.02). In contrast, *ΔGr64* flies did not show a preference for trehalose over the agarose alone, but retained the ability to detect fructose (Figure 1C;Table S1), similar to those reported previously [6]. *Gr64a* did not appear to be the essential *Gr* within the *Gr64* cluster since introduction of a transgene encoding *Gr64a* (*UAS-Gr64a*) [5] under control of the *Gr5a-GAL4*, which directs *Gal4* expression in most if not all sugar responsive GRNs [7,8], did not rescue the phenotype (Figure 1C; PI=0.46±0.07; Table S1). The lack of rescue did not appear to be due to ineffectiveness of *UAS-Gr64a*, since this transgene in combination with the *Gr5a-GAL4* rescued the ability to sense sucrose, glucose and maltose in mutant flies missing *Gr64a* and *Gr64b* [5].

To identify which of the five remaining genes in the *Gr64* cluster (*Gr64b-f*) was required for sensing trehalose, we generated or obtained flies predicted to reduce or eliminate expression of each gene. These included an insertion of a *Minos* transposable element [11] in the fourth exon of *Gr64e* (Figure 1A; $Gr64e^{M}$), and three *UAS-Gr64* RNAi lines corresponding to *Gr64b*, *Gr64d* and *Gr64f*, which were combined with the *Gr5a-Gal4*(Figure S2A). Since no fly lines were available to test the specific requirement for *Gr64c*, we used homologous recombination to delete a 429 base pair region encoding residues 81 to 509 in *Gr64c* (Figure S2A; *ΔGr64c*). The *white* marker gene, which was inserted at the site of the deletion, was flanked by two *loxP* sites, enabled the subsequent elimination of this marker gene by genetically introducing the Cre recombinase (Figures S2A and S2B; K2). Thus, only a 34 base pair *loxP* site remained at the site of the deletion in *ΔGr64c*. Since expression of some of the *Grs* would be predicted to be reduced rather than eliminated, we modified the behavior assay to improve sensitivity. When given a choice between 40 mM trehalose and 2 mM fructose, wild-type flies strongly preferred trehalose (Figure 2A; PI=0.91±0.03). However, *ΔGr64* flies favored fructose, resulting in a PI near 0 due to defect in sensing trehalose (Figure 2A; PI=0.07±0.04). Using this assay, we found that flies with a deletion, insertion or two siRNA transgenes targeting *Gr64b, c, d or e* displayed preferences for trehalose similar to wild-type (Figure 2A; Table S2A). In contrast, flies with the siRNA transgene that reduced expression of the *Gr64f* RNA (Figure S1A) displayed a partial but significant decrease in selecting 40 mM trehalose over 2 mM fructose (Figure 2A; PI=0.58±0.07).

The results with the *Gr64f* siRNA suggested that *Gr64f* might be the receptor that is required together with *Gr5a* to detect trehalose. This possibility was supported by a recent *in situ* hybridization study demonstrating extensive co-expression of *Gr64f* and *Gr5a* RNAs [4]. To test directly whether *Gr64f* is critical for the trehalose response, we generated a *UAS-Gr64f* transgene and expressed it under the control of *Gr5a-GAL4* in *ΔGr64* mutant flies. We found that expressing *Gr64f* in *Gr5a*-expressing cells (Figure S1B and Figure S1C) fully restored trehalose sensation in the behavioral assay (Figure 2B; PI=0.92±0.04). In contrast, when we used the *GAL4*/*UAS* system to express the two other genes targeted by RNAi, *Gr64b* or *Gr64d* (Figure S1B and Figure S1C), there was no rescue of trehalose sensation in *ΔGr64* flies (Figures 2B). Thus, the combination of experiments using RNAi, loss-of-function mutations, and rescue transgenes demonstrated that *Gr64f* was the gene that was co-required with *Gr5a* to sense trehalose.

A defect in the two-way choice test could reflect impairment in the production of action potentials in the GRNs or other defects such as perturbations in axon projections. To distinguish between these possibilities, we performed tip recordings, which assay action potentials in GRNs, which are produced in response to tastants. In *Drosophila*, sugar-responding GRNs are housed in bristles referred to as sensilla, which are distributed on the fly's tongue (labellum), as well on the wing margins, legs and female genitalia [12]. The sensilla include one mechanosensory neuron and two to four taste neurons, which elicit responses to either sugars, bitter compounds, salt or water [12]. The sensilla are generally characterized according to their length as L (long), I (intermediate) and S (short) type bristles, although the highest frequency responses to sugars are in L type sensilla [13]. Therefore, we applied trehalose to L type bristles and assayed the frequencies of action potentials produced in the GRNs (Figure 2C and 2D). In the absence of either *Gr5a* or *Gr64f* (e.g. *ΔGr64* flies), virtually no action potentials were produced upon presentation of either 100 mM trehalose, or even 300 or 900 mM (Figures 2C and 2D; Table S2B). However, when the two genes were co-expressed in *Gr5a* GRNs, such as in wild-type or in *ΔGr64* flies expressing *UAS-Gr64f* under the control of the *Gr5a-Gal4*, we observed high frequencies of action potentials in response to 100 mM trehalose, and slightly higher frequencies upon presentation of 300 or 900 mM trehalose (Figures 2C and 2D; Table S2B). At the very highest concentration of trehalose (900 mM), there were some action potentials produced in flies expressing *Gr64f*, but not *Gr5a* (*ΔGr5a*; Figure 2C; Table S2B). Nevertheless, the behavioral and electrophysiology results demonstrate that Gr5a and Gr64f are required together for normal detection of trehalose.

Gr64f is required in combination with *Gr64a* to detect sucrose, maltose and glucose

Gr64a is essential for sensing sucrose, maltose and glucose [4,5]; however, it is unclear whether it is the sole receptor in the *Gr64* cluster required for detection of these sugars. To address this question, we expressed the *UAS-Gr64a* transgene under control of the *Gr5a-GAL4*, in a *ΔGr64* background and performed two-way choice assays and tip recordings. Deletion of the full *Gr64* cluster eliminated the behavioral and electrophysiological responses to sucrose, maltose and glucose (Figure 1D, Figure 3 and Figure 4; Table S1, Table S3 and Table S4), in addition to the impairment in trehalose sensation described above. Introduction of *Gr64a* in *ΔGr64* flies did not restore the behavioral or electrophysiological responses to any of these sugars (Figure 1D, Figure 3 and Figure 4).

Since Gr64f was needed along with Gr5a for the trehalose response, we considered the possibility that Gr64f was broadly co-required with other Grs for sugar sensation, and was necessary in concert with Gr64a to sense sucrose, glucose and maltose. Therefore, we introduced the *UAS-Gr64f* and *Gr5a-Gal4* transgenes in *ΔGr64* flies, in the presence and absence of *UAS-Gr64a*. When given a choice between 5 mM sucrose and 2 mM fructose, wild-type flies mainly consumed sucrose while *ΔGr64* flies preferred fructose due to a loss of sucrose

sensation (Figure 3A). When *Gr64f* was introduced into *ΔGr64* flies, sucrose sensation was not rescued (Figure 3A; Table S3). However, when *UAS-Gr64a* and *UAS-Gr64f* transgenes were both expressed in sugar-responding GRNs, we obtained a wild-type preference for sucrose over fructose (Figure 3A; Table S3). We also performed tip recordings and found that expression of *Gr64f* only in *ΔGr64* flies resulted in only a minimal increase in action potentials in response to 50 mM sucrose, which was not statistically significant (Figure 4A; Table S4). However, a wild-type frequency of action potentials was restored upon co-expression of *Gr64a* and *Gr64f*. Similarly, *Gr64a* and *Gr64f* were both necessary to produce a wild-type selection of 10 mM maltose over 2 mM fructose (Figure 3B), and a full electrophysiological response to 100 mM maltose (Figure 4B), although a low level of action potentials were produced upon introduction of *Gr64f* alone in the *ΔGr64* background (Figure 4B).

The receptor requirements for glucose detection were slightly different than for sensing sucrose or maltose. We found that expression of *Gr64f* alone in a *ΔGr64* background partially restored the preference for 25 mM glucose over 2 mM fructose (Figure 3C) and action potentials in response to 100 mM glucose (Figures 4C). Nevertheless, consistent with the results with sucrose and maltose, expression of *Gr64a* alone in *ΔGr64* did not improve the behavioral and electrophysiological responses to glucose (Figure 3C and Figure 4C), while introduction of both *Gr64a* and *Gr64f* restored a normal selection of glucose and wild-type frequency of glucose-dependent action potentials (Figure 3C and Figure 4C). The partial but significant rescue of the glucose response in *ΔGr64* by the *Gr64f* transgene alone is consistent with previous data that Gr5a contributes to glucose detection [4].

Since expression of *Gr64f* only in *ΔGr64* flies resulted in small increases in action potentials in response to 50 mM sucrose and 100 mM maltose (Figures 4A and 4B), we tested whether higher concentration of these sugars would increase further the electrophysiological responses. Neither 450 mM sucrose nor 900 mM maltose induced action potentials either in *ΔGr64* flies or in *ΔGr64* animals expressing *Gr64a* only (Figures 4A and 4B). In wild-type or in *ΔGr64* flies expressing both *Gr64a* and *Gr64f*, the higher concentrations of sucrose and maltose resulted in modest increases in action potentials over the lower levels of these sugars. Interestingly, in *ΔGr64* flies expressing *Gr64f* alone, there were significant increases in action potentials in response to higher concentration of sucrose and maltose, although the frequencies were still significantly lower than in wild-type (Figure 4, Table S4).

The combination of data presented here is consistent with the conclusion that Gr64f is a co-receptor that functions together with Gr5a for normal trehalose detection, and with Gr64a for the wild-type responses to sucrose, maltose or glucose. In addition, we suggest that Gr64f functions as a glucose co-receptor along with either Gr5a or Gr64a. Consistent with this latter proposal, the glucose response is defective upon mutation of either Gr5a or Gr64a, and fully eliminated in the Gr5a and Gr64a double mutant [4,5]. Furthermore, the observation that very high concentrations of sucrose and maltose result in significant increases in action potentials in *ΔGr64* flies expressing Gr64f suggests that Gr5a/Gr64f forms part of a low affinity receptor for these sugars. Despite the strict requirement for Gr64f for the responses to trehalose, sucrose, maltose and glucose, there is at least one sugar, fructose, which is detected independent of Gr64f since there is a fructose behavioral response in *ΔGr64* flies. The fructose receptor, which remains to be identified, does not appear to be expressed in L type sensilla, as few action potentials were detected in wild-type, even upon introduction of a 100 mM [4] or very high concentrations of fructose (300 mM; Figure S3). Nevertheless, the concept that the fructose receptor is distinct from other gustatory receptors is supported by a previous study demonstrating differential protease sensitivities of the fructose receptor, from the glucose and sucrose receptor [14].

Gr64f paired with Gr5a or Gr64a does not appear to be sufficient for eliciting sugar responses since misexpression of either of these pairs of *Gr* genes in bitter responsive GRNs or in a heterologous expression system is insufficient to produce sugar-sensitivity (Jiao and Montell, unpublished observations). These latter results differ from the finding that misexpression of the two CO_2 receptors, Gr21a and Gr63a, is sufficient for recapitulating CO_2 sensitivity [15, 16]. Thus, it is possible that the sugar-responsive Grs consist of multimers, which are more complex than dimers, or that sugar-responsive GRNs specifically express components required for sugar detection that are not expressed in other neurons.

The results indicating that Gr64f is a broadly required receptor for both Gr5a and Gr64a are reminiscent of those with the *Drosophila* olfactory receptor (Or), Or83b, which is required as a co-receptor for other Ors [17]. In the case of Or83b, it appears to promote trafficking of Ors [17] and serves as a cation channel subunit in combination with other Ors [18,19]. Whether Gr64f functions in Gr receptor trafficking cannot yet be addressed, due to the absence of antibodies to Gr5a and Gr64a. Nevertheless, the results from the current study support the model that *Drosophila* sugar receptors function *in vivo* as heteromultimers, rather than as monomers as originally indicated for Gr5a [3]. Since elimination of Gr64f has no effect on the responses to bitter substances, the current data raise the possibility that there might exist a distinct co-receptor for the caffeine receptor, Gr66a [20], and other Grs that are essential for the detection of aversive compounds.

Experimental Procedures

Generation of the *Gr64c* knockout

Gene targeting of *Gr64c* was carried out using ends-out homologous recombination [21]. A 3.2 kb genomic region flanking the 5' end of *Gr64c* was amplified by PCR using the following primer pair: 5'-(Acc65I) AAAGGTACCTCCATCCAGTGGGATTGGTGTTCT-3' and 5'-(Acc65I-loxP) AAAGGTACCATAACTTCGTATAGCATACATTATACGAAGTTAT-ACCCAATCCTTGACTCACTCACCT-3'. A 2.6 kb genomic region flanking the 3' end of *Gr64c* was amplified using the primers: 5'-(NotI-loxP) AAAGCGGCCGCATAACTTCGT-ATAATGTATGCTATACGAAGTTATATTGCAGCTATGGACGTGGCTACT-3' and 5'-(NotI) AAAGCGGCCGCACAGCACCAGTTCGTAGC CCATTA-3'. The two arms were subcloned into pw35 [21] and sequenced to verify the correction orientations. Germline transformation (GenetiVision, Houston, TX) and the genetic crosses to generate the homologous recombinants (K1; Figure S2A) were carried out as described [22]. The K1 flies were mated with *y*,*w*;*noc*^{Sco}/CyO,P[w^{+mC}=Crew]DH1 (Flybase: BL1092) so that the *w* marker was excised by the Cre recombinase, creating the K2 flies (Figure S2A). The genotype of the K1 and K2 flies were verified by PCR (Figure S2B).

Genetics, Fly Stocks, and Constructs

To create the P[*UAS-Gr64f*] transgene, we amplified the coding region of *Gr64f* by RT-PCR from total labellar RNA. The cDNA was sequenced and subcloned into the pUAST vector [23], and germline transformation was conducted according to standard procedures. We have described the P[*UAS-Gr64a*] line previously [5]. The P[*Gr5a-Gal4*] [7] and *R1*;; *ΔGr64* [6] flies were obtained from Dr. H. Amrein and the *ΔGr5a* flies (*Δ19* line) [1] from Dr. J.R. Carlson. The *UAS* transgenes were crossed into the *R1*;; *ΔGr64* background harboring the P[*Gr5a-GAL4*] transgene. The *UAS-RNAi* lines 42517, 29422, and 44020 were obtained from the VDRC Stock Center (Vienna, Austria) and combined with the P[*Gr5a-Gal4*] transgene to target *Gr64b*, *Gr64d*, and *Gr64f*, respectively. The *Minos* insertion in *Gr64e*, *Gr64e*^{MB03533} (*Gr64e*^{M}), was from the Bloomington Stock Center.

RT-PCR Amplification of *Gr* RNAs

To perform the RT-PCR and real time RT-PCR, we dissected 50 or 200 labella respectively, and extracted total RNA using the TRIzol reagent (Invitrogen). The RNA samples were treated with DNaseI (Invitrogen) before performing the RT-PCR reactions. For real time RT-PCR, the RNA samples were further cleaned using the Rneasy mini kit (catalog no. 74104; Qiagen, Valencia, CA). A control without reverse-transcriptase was included to confirm that the RT-PCR product was derived from mRNA instead of genomic DNA. A OneStep RT-PCR Kit (catalog no. 210212; Qiagen, Valencia, CA) was used for the RT-PCR. Stratagene Mx3000P PCR motion and Brilliant II SYBR green QRT-PCR Master Mix Kit (catalog no. 600825; Stratagene, La Jolla, CA) was used for real time PCR. Fold-changes compared to the controls were calculated using the - ΔΔCt method [9].

Chemicals

Sucrose, glucose, maltose, fructose, tricholine citrate and sulforhodamine B were obtained from Sigma-Aldrich (St. Louis, MO); trehalose was from Fluka (St. Gallen, Switzerland); and brilliant blue FCF was from Wako Chemical (Richmond, VA).

Behavioral Assays

The two-way choice assays were performed according to procedures similar to those we have described previously [5]. In brief, 20–30 flies (3–6 days old) were starved for 24 hours on 1% agarose and then allowed to feed in 72-well microtiter dishes filled with two types of test mixtures in alternating wells. Each test mixture contained 1% agarose and either blue dye (0.125 mg/ml brilliant blue FCF) or red dye (0.2 mg/ml sulforhodamine B) and a test tastant. The combinations of test tastants were: 1) one sugar and no test tastant or 2) 2 mM fructose and a different sugar at a higher concentration (5–40 mM). After feeding for 90 min at room temperature in the dark, the flies were frozen at −20°C, and the numbers of flies with blue (N^B), red (N^R), or purple (N^P) abdomens were assessed by visual inspection. In those cases in which the colors were difficult to discern, the colors were determined using dissected guts. If the amount of red dye was between 50% and 150% of the blue dye, the color was scored as purple. If the red dye was >150% or <50% of the blue dye, the fly was counted as red and blue, respectively. All behavioral assays were performed 3 times. The PI values were calculated according to the following equation: $PI = (N^B + 0.5\ N^P)/N^{Total}$ or $(N^R + 0.5\ N^P)/N^{Total}$. As previously described, the dyes did not cause preference changes, as we showed recently [5]. The wild-type control used was w^{1118}, since this was the background that we used to generate the transgenic flies and *ΔGr64c* flies described above.

Electrophysiology

Tip recordings on L type labellar bristles (3 –to 6- day-old flies) were performed according to procedures similar to those described previously [20]. To provide a reference electrode and to stabilize the fly for the recordings, we inserted a glass capillary with Ringer's solution from the abdomen through the head into the labella. We used 30 mM tricholine citrate as the electrolyte in the recording electrode (10–20 μm diameter). The signals were collected and amplified from the recording electrode through a preamplifier (TastePROBE; Syntech, Kirchzarten, Germany) and a 100– to 3,000-Hz band-pass filter. Autospike 3.1 software (Syntech) was used to acquire the action potentials (12 kHz sampling rate) and to analyze the frequencies. All recordings using a given genotype and tastant were performed four to six times.

Data Analyses

All error bars represent SEMs. Unpaired Student's *t* tests were used to check for significant differences between the indicated pairs of data (*: $p < 0.05$; **: $p < 0.01$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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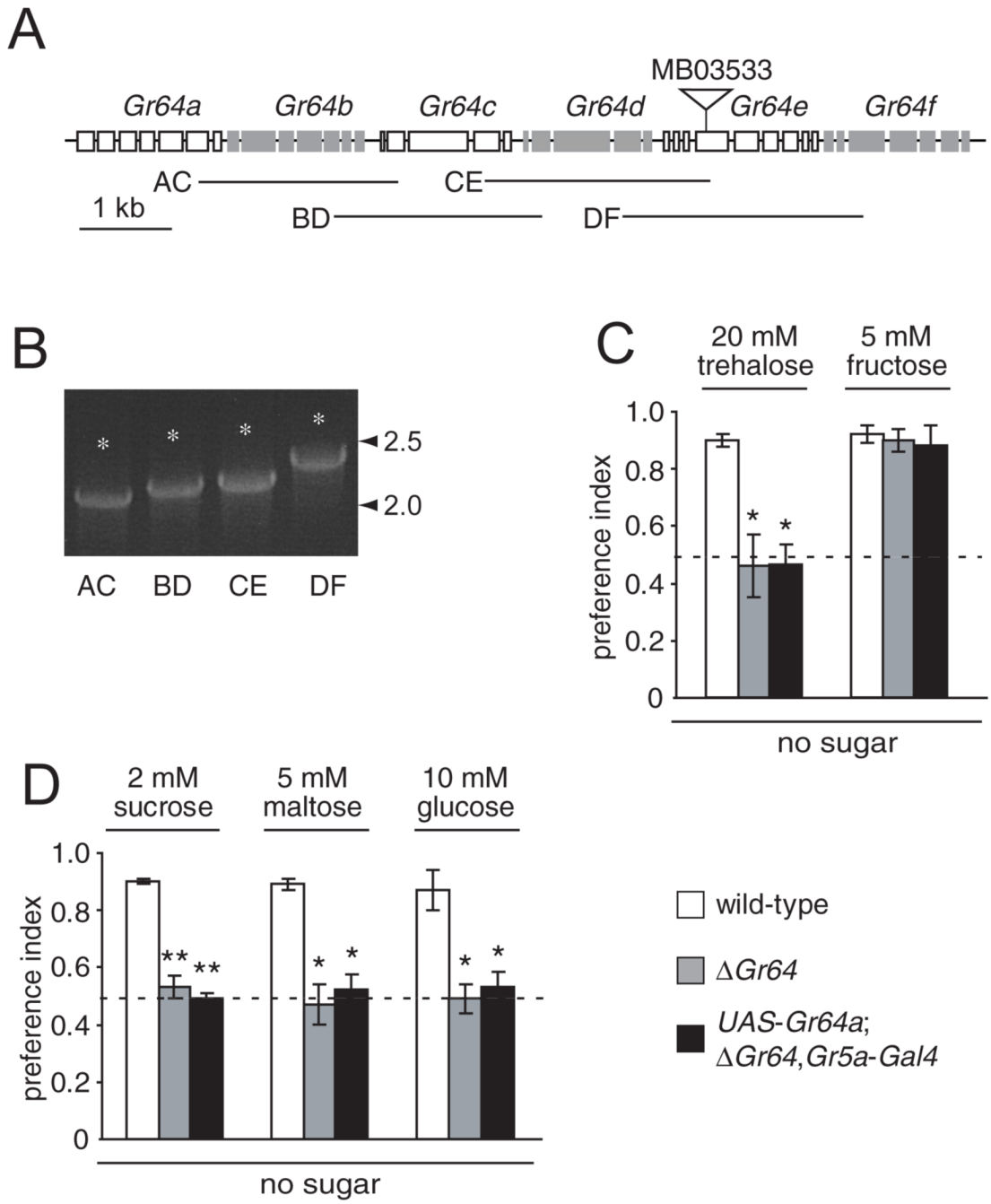

Figure 1. Generation of the *Gr64* mutations and two-way choice tests using *ΔGr64* flies expressing a wild-type *Gr64a* transgene

(A) Organization of the *Gr64* locus. The six genes encoded in the *Gr64* cluster (*Gr64a-f*) are indicated. The exons are represented by the rectangles. MB03533 is a *Minos* transposable element inserted in the fourth exon of *Gr64e*. Three genes targeted by *UAS-RNAi* are shaded gray. (B) RT-PCR products generated using primers that span: 1) *Gr64a* and *Gr64c* (AC), 2) *Gr64b* and *Gr64d* (BD), 3) *Gr64c* and *Gr64e* (CE), and 4) *Gr64d* and *Gr64f* (DF). DNA markers (kb) are indicated to the right. All of the RT-PCR products span introns, and no products were observed that migrate at the positions of the predicted genomic products (indicated by asterisks). (C) Two-way choice tests using trehalose or fructose versus no sugar.

The wild-type control was w^{1118}. (D) Two-way choice tests using the indicated sugars. The fly lines were the same as in panel (C). n=3, *: $p < 0.05$, **: $p < 0.01$. Detailed statistics are provided in Table S1.

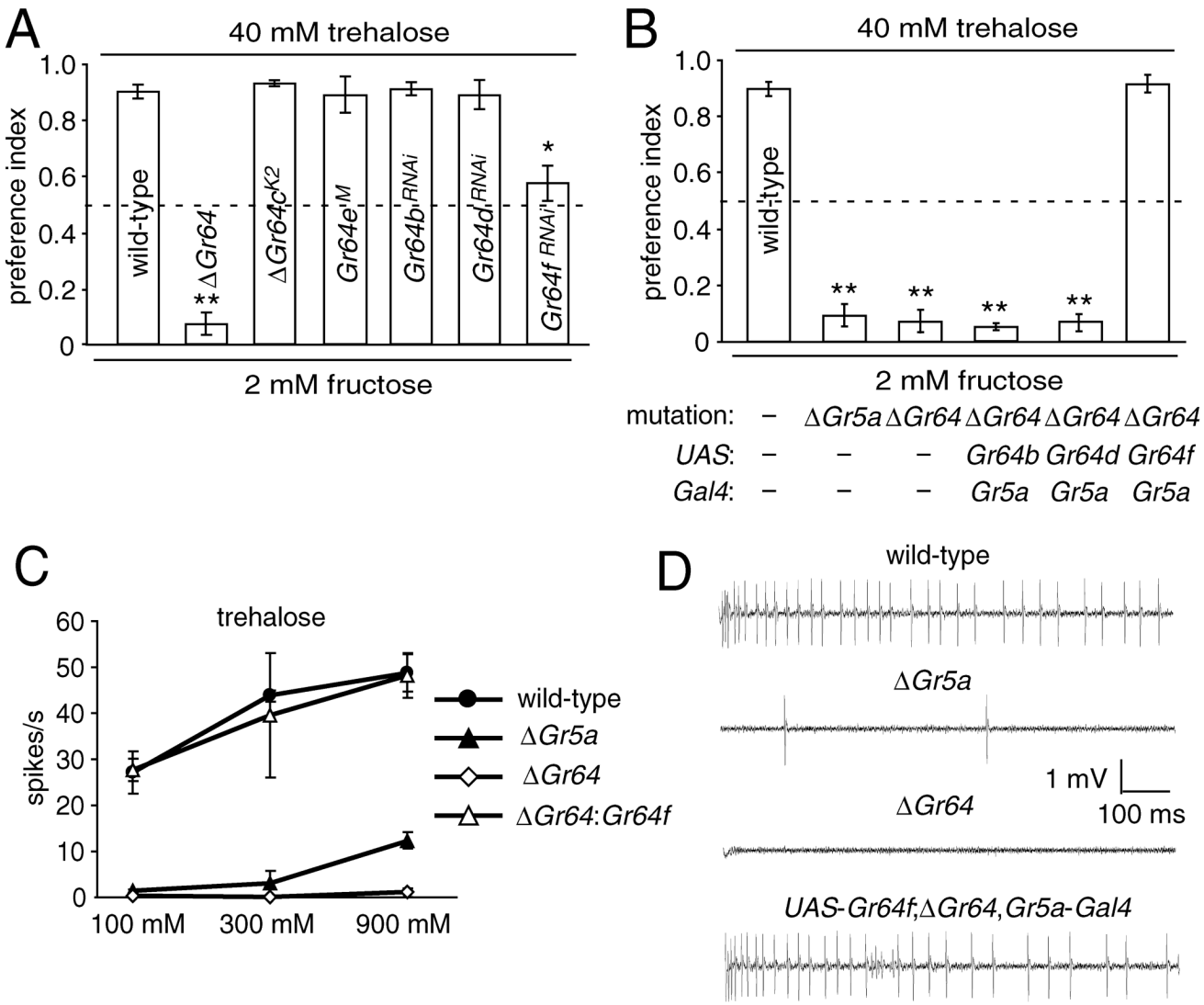

Figure 2. *Gr64f* is required to sense trehalose

(A) Two-way choice tests using 40 mM trehalose versus 2 mM fructose. The analyses were conducted on wild-type, the indicated mutants, or flies expressing the indicated *UAS-Gr64* RNAi transgenes under the control of the *Gr5a-Gal4*. (B) Expression of a *UAS-Gr64f* transgene using a *Gr5a-Gal4* rescued the preference for 40 mM trehalose over 2 mM fructose in a *ΔGr64* background. Flies expressing two other transgenes corresponding the genes targeted by RNAi, *UAS-Gr64b* or *UAS-Gr64d*, did not rescue the phenotype. (C) Average frequencies of action potentials (spikes/s) responding to 100 mM, 300 mM and 900 mM trehalose using the indicated fly lines. The averages were based on data collected between 50 msec and 1050 msec after application of the sugars. (D) Sample tip recordings using 100 mM trehalose. See Table S2 for statistics.

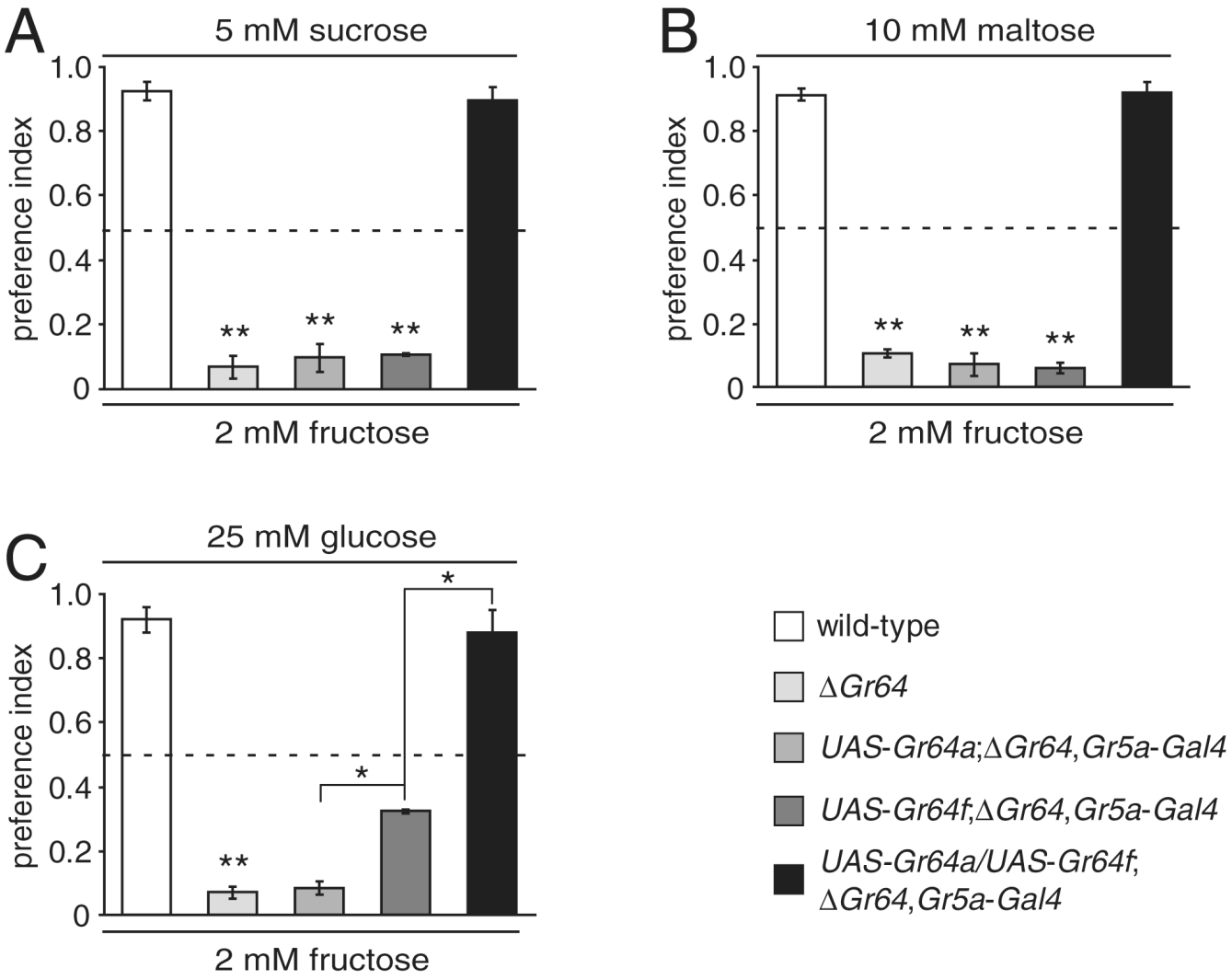

Figure 3. *Gr64f* was required for the behavioral responses to multiple sugars

The following fly stocks tested were allowed to choose between the indicated sugar and 2 mM fructose: 1) wild-type (w^{1118}), 2) *ΔGr64*, and 3–5) *ΔGr64* flies harboring the *Gr5a-GAL4* transgene together with *UAS-Gr64a* and/or *UAS-Gr64f* transgenes. (A) 5 mM sucrose. (B) 10 mM maltose. (C) 25 mM glucose. The statistics are presented in Table S3.

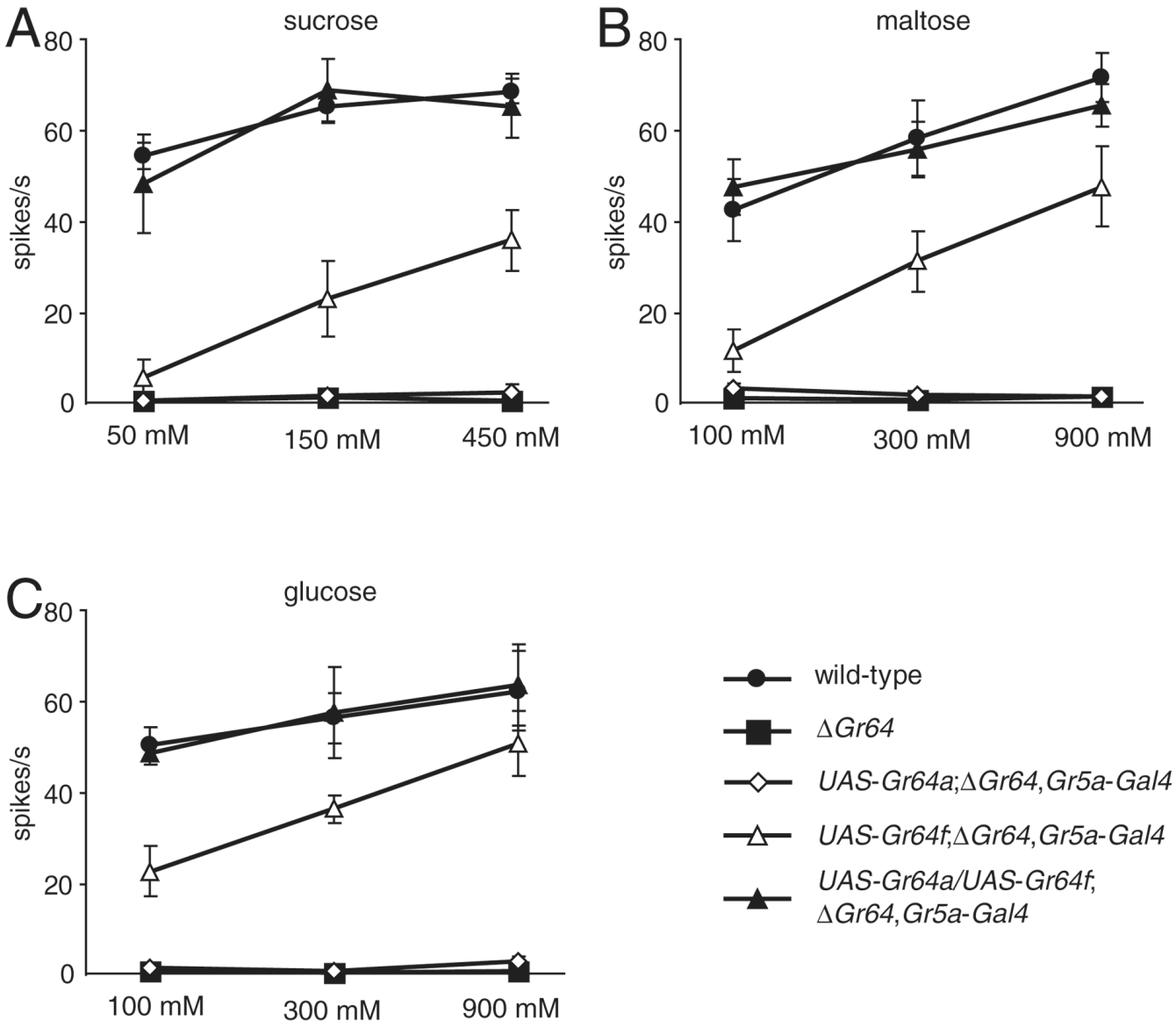

Figure 4. *Gr64f* was required for action potentials resulting from application of sucrose, maltose or glucose

Tip recordings were performed on L type sensilla of the indicated genotypes. The average frequencies of action potentials (spikes/s) were based on data collected between 50 msec and 1050 msec after application of the sugars. (n= 4–6, *: $p < 0.05$, **: $p < 0.01$). Three concentrations as indicated were used to assay the action potentials generated following application of: (A) sucrose, (B) maltose and (C) glucose. The statistics are listed in Table S4.