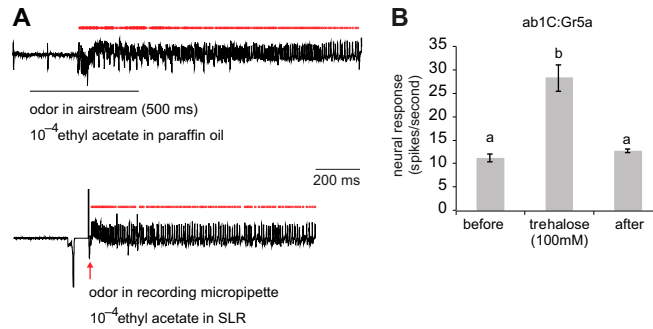
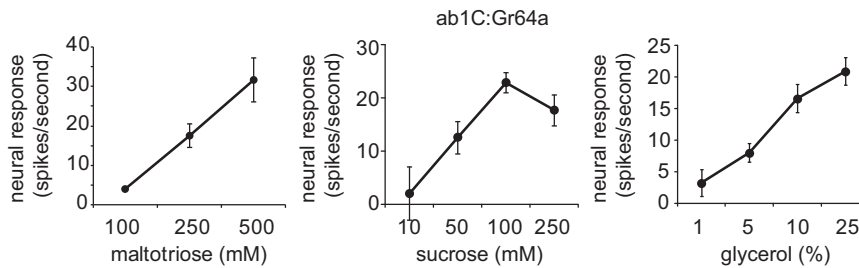


# Supporting Information

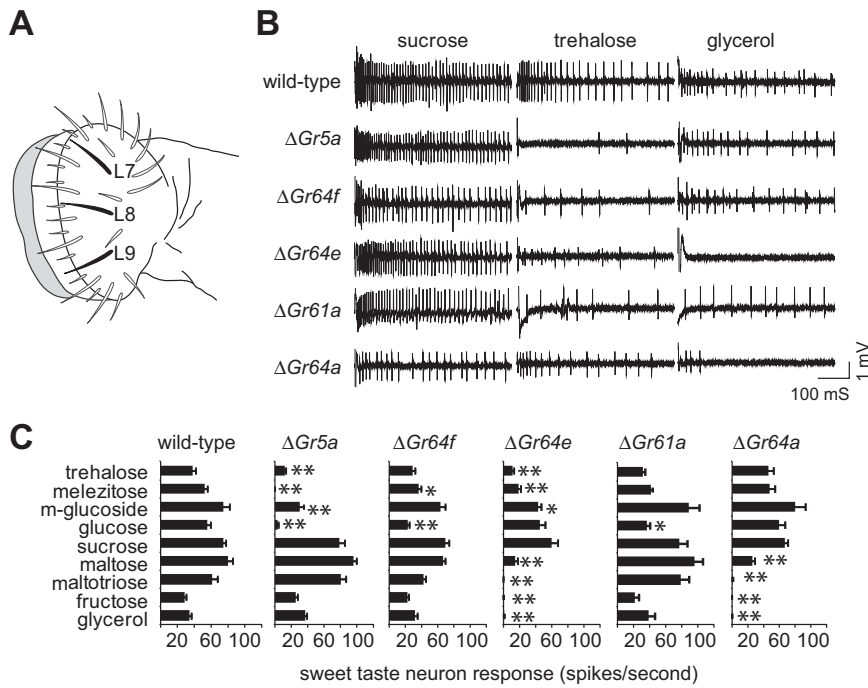
Freeman et al. 10.1073/pnas.1311724111



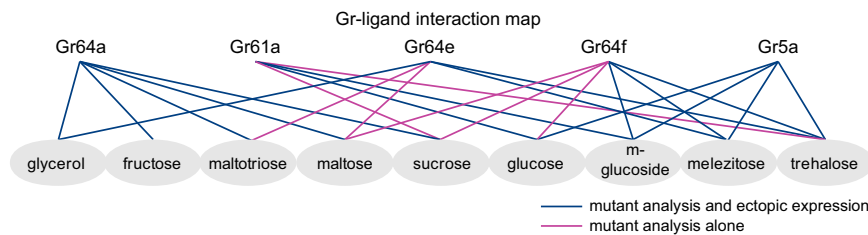
**Fig. S1.** (A) Representative traces from ab1 sensilla in control ( $w^{1118}$ ) flies using  $10^{-4}$  ethyl acetate in an airstream (*Upper*) or by placing it in the electrolyte of the recording electrode (*Lower*). Red dots indicate ab1A neuron spikes. (B) Mean responses of ab1C:Gr5a neurons to sensillum lymph ringer electrolyte before and immediately after recording with 100 mM trehalose. Each set of three recordings was taken from the same sensillum. Letters indicate statistical significance ( $P < 0.001$ ; one-way ANOVA with Tukey's post hoc test;  $n = 8$ ).



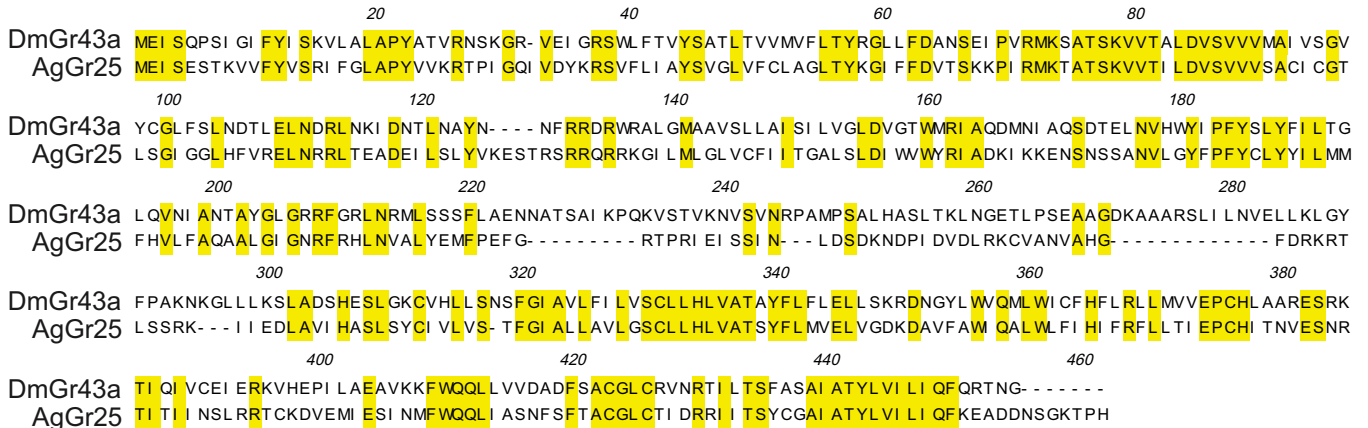
**Fig. S2.** Dose-dependent responses of ab1C:Gr64a neurons to indicated tastants ( $n = 6-12$ ).



**Fig. S3.** (A) Schematic of the fly labellum highlighting L-type sensilla that were used for electrophysiological recordings in black. (B) Sample traces of recordings with 100 mM sweet stimuli obtained from L-type sensilla. Genotypes are as follows:  $w^{1118}$  (wild type),  $\Delta EP(X)-5$  ( $\Delta Gr5a$ ),  $Gr64f^{MB12243}$  ( $\Delta Gr64f$ ),  $Gr64e^{MB03533}$  ( $\Delta Gr64e$ ),  $Gr61a^1$  ( $\Delta Gr61a$ ), and  $Gr64a^1$  ( $\Delta Gr64a$ ). (C) Mean responses of sweet taste neurons in L-type sensilla to indicated sugars tested at a concentration of 1 M and glycerol at 50%. \* $P < 0.05$ ; \*\* $P < 0.001$  (vs. wild-type; one-way ANOVA with Tukey's post hoc test;  $n = 6-12$ ).



**Fig. S4.** A ligand–gustatory receptor (Gr) interaction map indicating relationships identified by mutant analysis and ectopic expression in the ab1C neuron.



**Fig. S5.** Protein alignment of DmGr43a and AgGr25 with identical amino acids highlighted in yellow. Image was generated with MacVector and Gonnet alignment.

**Table S1. Gr combinations tested in ab3A neurons**

Gr combination in ab3A neuron	Genotype	Gq	Stimulus	Mean	SEM	<i>n</i>
Gr64a+Gr64f	UAS-Gr64a/UAS-Gr64f;	+	Trehalose	3	1.15	3
	UAS-Gq/Or22a-GAL4		Sucrose	0.00	0.00	3
Gr5a+Gr64f	UAS-Gr5a/UAS-Gr64f-2; UAS-Gq/Or22a-GAL4	+	Trehalose	1.33	0.33	3
			Melezitose	0.33	0.33	3
			<i>m</i> -glucoside	0.67	0.88	3
Gr64a+Gr43a	UAS-Gr64a/UAS-Gr43a; UAS-Gq/Or22a-GAL4	+	Sucrose	-1.00	0.00	3
			Glucose	0.50	0.29	4
			Fructose	0.75	0.48	4
Gr64a+Gr64e	UAS-Gr64a/UAS-Gr64e; UAS-Gq/Or22a-GAL4	+	Sucrose	0.25	0.25	4
			Glycerol	0.00	0.00	4
Gr64a+Gr64f+Gr64e	UAS-Gr64a/UAS-Gr64e; UAS-Gr64f/Or22a-GAL4	-	Trehalose	0.83	0.17	3
			Melezitose	1.17	0.60	3
			<i>m</i> -glucoside	0.67	1.76	3
			Glucose	0.67	0.33	3
Gr64a+Gr43a+Gr64e	UAS-Gr64a/UAS-Gr64e; UAS-Gr43a/Or22a-GAL4	-	Sucrose	0.33	0.33	3
			Fructose	2.00	0.00	3
			Glycerol	1.33	0.33	3
Gr64a+Gr21a+Gr63a	UAS-Gr64a/ $\Delta$ halo; UAS-Gr63a,UAS-Gr21a/Or22a-GAL4	-	Sucrose	-0.33	0.88	3
			Fructose	3.33	0.88	3
			Glycerol	0.00	1.00	3
Gr64a+Gr43a+Gr64e+Gr64f	UAS-Gr64a/UAS-Gr64e, UAS-Gr64f; UAS-Gr43a/Or22a-GAL4	-	Sucrose	2.00	0.58	3
			Maltose	5.25	0.85	4
			Maltotriose	2.25	0.48	4
Gr64a+Gr21a+Gr63a+Gr43a	UAS-Gr64a/UAS-Gr43a; UAS-Gr63a,UAS-Gr21a/Or22a-GAL4	-	Sucrose	2.88	0.52	4
			Maltose	3.38	0.90	4
			Maltotriose	1.75	0.48	4
Gr5a+Gr64e+Gr64f+Gr64e	UAS-Gr5a/UAS-Gr64e, UAS-Gr64f; UAS-Gr64b/Or22a-GAL4	-	Trehalose	0.88	0.59	4
			Melezitose	-1.00	0.91	4
			<i>m</i> -glucoside	0.00	0.00	4
				-0.50	0.74	4

**Table S2. Genotypes and sources of flies used in experiments**

Fly stocks	Genotype and/or source	Figures	References
Wild-type	<i>w[1118]</i>	3A; S3	
Gr21a-GAL4	Kristin Scott		1
Gr63a-GAL4 on III	BDSC (no. 9942)		2
Gr63a-GAL4 on II	BDSC (no. 9943)		2
$\Delta$ Gr63a	BDSC (no. 9941)		2
UAS-Gr5a	A.D. laboratory		3
UAS-Gr61a	A.D. laboratory		3
UAS-Gr64a	A.D. laboratory		3
UAS-Gr64b	BDSC (no. 27324)		4
UAS-Gr64c	A.D. laboratory		
UAS-Gr64d	A.D. laboratory		
UAS-Gr64e	A.D. laboratory		3
UAS-Gr64f	A.D. laboratory		3
UAS-Gr59c	Carlson laboratory		5
$\Delta$ Gr5a	$\Delta$ EP(X)-5	3 A, B, and C; S3	6
$\Delta$ Gr64f	<i>Gr64f[MB12243]</i> , BDSC (#27883)	3 A, B, and C; S3	
$\Delta$ Gr64e	<i>Gr64e[MB03533]</i> , BDSC (#23628)	3 A, B, and C; S3	7
$\Delta$ Gr61a	<i>Gr61a[1]</i>	3 A, B, and C; S3	3
$\Delta$ Gr64a	<i>Gr64a[1]</i>	3 A, B, and C; S3	3
ab1C:Gr5a-2x (same as ab1C:Gr5a)	<i>UAS-Gr5a-8/UAS-Gr5a-8; Gr63a-GAL4/Gr63a-GAL4</i>	1 B-D and F; 2 A and C; 3 B and C; S1	
ab1C:Gr5a-1x	<i>UAS-Gr5a-8/+; Gr63a-GAL4/+</i>	1 C and E; 2B	
Gr63a-GAL4	<i>UAS-Gr5a-8/+; Gr63a-GAL4/+</i>	1 C and E	
Gr21a-GAL4	<i>Gr21a-GAL4/+; UAS-Gr5a-3/+</i>	1E	
+Gr63a	<i>UAS-Gr5a-8/UAS-Gr5a-8; Gr63a-GAL4/Gr63a-GAL4</i>	1F	
-Gr63a	<i>UAS-Gr5a-8/UAS-Gr5a-8; <math>\Delta</math>Gr63a,Gr63a-GAL4/<math>\Delta</math>Gr63a, Gr63a-GAL4</i>	1F	
ab1C	<i>w[1118]</i>	2A; 4 A, C, and E; S1	
ab1C:Gr64f	<i>UAS-Gr64f-2/UAS-Gr64f-2; Gr63a-GAL4/Gr63a-GAL4</i>	2 A and C; 3 B and C	
ab1C:Gr64e	<i>UAS-Gr64e-3/UAS-Gr64e-3; Gr63a-GAL4/Gr63a-GAL4</i>	2 A and C; 3 B and C	
ab1C:Gr64b	<i>UAS-Gr64b/Gr21a-GAL4; UAS-Gr64b-2/Gr63a-GAL4</i>	2 A and C; 3 B and C	
ab1C:Gr61a	<i>UAS-Gr61a-2/Gr21a-GAL4; Gr63a-GAL4/UAS-Gr61a-4</i>	2 A and C; 3 B and C	
ab1C:Gr64a	<i>UAS-Gr64a-4/UAS-Gr64a-4; Gr63a-GAL4/Gr63a-GAL4</i>	2 A and C; 3 B and C; S2	
ab1C:Gr64c	<i>Gr21a-GAL4/UAS-Gr64c-B; Gr63a-GAL4/UAS-Gr64c-2</i>	2 A and C	
ab1C:Gr64d	<i>Gr21a-GAL4/UAS-Gr64d-4; Gr63a-GAL4/UAS-Gr64d-3</i>	2 A and C	
ab1C:Gr5a+Gr64f	<i>Gr21a-GAL4/UAS-Gr5a-8; Gr63a-GAL4/UAS-Gr64f-3</i>	2B	
ab1C:Gr5a+Gr64e	<i>Gr21a-GAL4/UAS-Gr5a-8; Gr63a-GAL4/UAS-Gr64e-2L</i>	2B	
ab1C:Gr5a+Gr64a	<i>UAS-Gr5a-8/UAS-Gr64a-4; Gr63a-GAL4/Gr63a-GAL4</i>	2B	
ab1C:Gr5a+Gr61a	<i>UAS-Gr5a-8/UAS-Gr61a-2; Gr63a-GAL4/Gr63a-GAL4</i>	2B	
ab1C:Gr5a+Gr64b	<i>Gr21a-GAL4/UAS-Gr5a-8; Gr63a-GAL4/UAS-Gr64b-2</i>	2B	
ab1C:Gr5a+Gr64c	<i>Gr21a-GAL4/UAS-Gr5a-8; Gr63a-GAL4/UAS-Gr64c-2</i>	2B	
ab1C:Gr5a+Gr64d	<i>UAS-Gr5a-8/UAS-Gr64d-4; Gr63a-GAL4/Gr63a-GAL4</i>	2B	
ab1C:Gr59c	<i>Gr21a-GAL4/Gr63a-GAL4;UAS-59c-9d,UAS-Gr9c-9d</i>	4 A-C	
+Gr63a	<i>Gr21a-GAL4/Gr63a-GAL4;UAS-59c-9d,UAS-Gr9c-9d</i>	4C	
-Gr63a	<i>UAS-Gr59c-14d/UAS-Gr5c-14d; <math>\Delta</math>Gr63a,Gr63a-GAL4/<math>\Delta</math>Gr63a, Gr63a-GAL4</i>	4C	
ab1C:DmGr43a	<i>UAS-Gr43a-8d/Gr21a-GAL4; Gr63a-GAL4/UAS-Gr43a-5d</i>	4 D and E	
ab1C:AgGr25	<i>Gr21a-GAL4/Gr63a-GAL4; UAS-AgGr25-3D/UAS-AgGr25-3L</i>	4E	

BDSC, Bloomington *Drosophila* Stock Center.

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