Supplemental Figure 1. Generation of the *Gr43a^{GAL4}* allele

(A) Organization of the wild-type the GAL4-targeted *Gr43a* locus. The*Gr43a* coding region was replaced with *GAL4* and *white^{mini}* by homologous recombination. The *white^{mini}* gene was removed via flanking loxP sites using CRE recombinase.

(B) Southern and PCR analysis of *Gr43a^{+}* and *Gr43a^{GAL4}* allele using probes and PCR primers indicated in (A). Genomic DNA was digested with SacI (S; 5' probe) and XhoI (X; 3' probe).

Supplemental Figure 2. Characterization of *Gr43a^{GAL4}* proventricular ganglion (related to Figure 1)

(A) Some projections of *Gr43a^{GAL4}* neurons pass through the brain along the esophagus and make a turn at the intersection of the proboscis and head capsule, where they join the labral nerve and enter the SOG (inlet, arrowhead).

(B) The presynaptic marker syt-HA is localized in projections to the midgut.

(C) The dendritic marker DenMark is enriched in innervations of the foregut lumen. Br, brain; Pb, proboscis; Es, esophagus; SOG, subesophageal ganglion; Fg, foregut; Cd, crop duct; Pv, proventriculus.

Supplemental Figure 3. Co-expression of *Gr43a^{GAL4}* and *Gr5a^{lexA}*
(related to Figure 1)

(A) The distal *Gr43a^{GAL4}* neurons (arrowhead), but not the central neurons used for Ca^{2+} imaging, co-express *Gr5a^{lexA}* in the foreleg (arrowhead).

(B) Some neurons co-express *Gr43a^{GAL4}* and *Gr5a^{lexA}* in the labial palp (arrowhead).

Supplemental Figure 4. Sorbitol is tasteless (related to Figure 5)

(A) Frequency of proboscis extension reflex (PER) upon stimulation of the foreleg with water, sorbitol (100mM) or fructose (100mM). Sorbitol failed to elicit PER. NS, not significant; *$p < 0.05$; ANOVA. Error bars represent standard error. n=8 experiments (4-9 flies per experiments).

(B) Ca^{2+} imaging of *Gr43a^{GAL4}* neurons in the forelegs when stimulated with water, sorbitol (100mM) or fructose (100mM). *Gr43a^{GAL4}* neurons do not respond to sorbitol. NS, not significant; **$p < 0.0001$; ANOVA. Error bars represent standard error. $9 \leq n \leq 11$.

Supplemental Figure 5. Distinct projections of the *Gr43a^{GAL4}* brain neurons

(A) Diagram of the projection pattern of the *Gr43a*^{GAL4} brain neurons. Solid and dotted lines represent projections to the anterior and posterior part of the brain, respectively. All cell bodies are located in the posterior superior protocerebrum, and project axons to the middle inferior lateral protocerebrum (anterior projections) and to the posterior superior protocerebrum (posterior projections).

(B) Images of *Gr43a*^{GAL4} neuronal projections. *Gr43a*^{GAL4} was used to express mCD8GFP (membrane marker, green) and syt-HA (presynaptic marker, red). The *Gr43a*^{GAL4} brain neurons show distinct projection pattern. Some axons project towards the anterior brain along the middle inferior lateral protocerebrum. Other neurons project to the posterior superior lateral and medial protocerebrum, and few axons cross the midline and extend into the opposite side of the brain. Both anterior and posterior projections have numerous axon terminals.

Supplemental movie 1. Calcium imaging of *Gr43a*^{GAL4} neuron in the foreleg.

The foreleg was stimulated with fructose (100mM) at 13.0 sec. Two frames per seconds.

Supplemental movie 2. Calcium imaging of *Gr43a*^{GAL4} neuron in the brain.

The brain was stimulated with fructose (100mM) at 72.0 sec. Movie is played 10 times faster. One frame per three seconds.

Supplemental Experimental Procedures

Molecular Biology

GAL4 knock-in vector is based on CMC105 vector (Larsson et al, 2004). To remove mini-white gene from the targeted locus, loxP sequences (5'-GGATCTATAACTTCGTATAGCATACATTATACGAAGTTATAGATCC-3') were inserted in both 5' and 3' flanking regions of *white*^{mini} gene. *GAL4* coding sequence was amplified by 5'-GGGCCTAGGACAAGCGCAGCTGAACAAGC-3' and 5'-GCTAGCAGAACTAGTGGATCTAAACGAG-3' primers from SM1 vector, inserted outside of loxP elements. Thus, this vector contains the *GAL4* and *white*^{mini} gene, as well as a I–SceI site, flanked by FRT sites.

Homologous Recombination

Gene targeting using homologous recombination was performed as described by

Rong and Golic (Rong and Golic, 2000). Upstream (5' arm; 3284627-3280654) and downstream (3' arm; 3279046-3275109) regions of *Gr43a* were amplified by Elongase (Invitrogen) from Oregon-R DNA. These fragments were cloned into the *GAL4* knock-in vector.

Virgin female flies carrying targeting construct insertions on 3rd chromosome were crossed to w^{1118}*;70FLP; 70I–SceI*, and 3 day–old progeny were heat shocked at 38°C for 60 min. Approximately 2100 white–eyed adult virgin females were crossed to w^{1118}*;70FLP* males and progeny were heat shocked as described above. Of the 58 red–eyed flies that were obtained, five were gene replacements. Putative *Gal4* knock-in events of candidate strains were molecularly analyzed by PCR from genomic DNA and Southern analysis. To test for loss of the *Gr43a* coding sequence, we performed PCR using primers annealing to sequences within the *Gr43a* coding region (3279821-3280539) and the 5' upstream arm (11109764–11110454). *Gal4* gene replacement was confirmed by Southern analysis. Genomic DNA was digested with SacI or XhoI, fragments were separated on 1 % agarose gels by electrophoresis and transferred to nictorcellulose membranes (Amersham, Inc.), which were hybridized with probes derived from regions upstream of the 5' arm or downstream of the 3' arm (corresponding to nucleotides 3286234–3285765 and 3274950–3274200, respectively). To remove potential secondary DNA mutations, the $Gr43a^{Gal4\text{-}white\ mini}$ strain was backcrossed six times to w^{1118}. Finally, the *loxP* flanked $white^{mini}$ gene was excised by Cre recombinase to yield $Gr43a^{Gal4}$.

Internal sugar measurements

Adult males were frozen after the feeding procedure. For the assays, their heads were homogenized in water. Non-soluble debris was removed by centrifugation at 14000 rpm for 10 minutes. Fructose measurements were performed on the supernatant using the Seliwanoff's test (Nunes et al., 2008). The aliquot of head homogenates were mixed with twice the volume of 0.05% resorcinol in 6N HCl. Mixture was centrifuged at 14,000 rpm for 10 minutes, supernatant was heated 95 ˚C for 10 minutes and absorbance was determined at 484 nm using a Genesys 20 spectrophotometer (Thermo Scientific). Background value (absorption prior heat treatment) was subtracted. Although Seliwanoff's test is highly specific to fructose, abundant glucose and trehalose in *Drosophila* raises the background, which we adjusted by subtracting corresponding absorbance values for glucose and trehalose. Glucose and trehalose were measured by

the InfinityTM Glucose Hexokinase Reagent (Thermo Scientific). Head homogenates were incubated with the reagent for 10 minutes at 37 ˚C, and absorbance at 340 nm was measured. For trehalose measurement, 0.04 U/mg (head) of pig kidney trehalase (Sigma) was incubated with head homogenates for 10 minutes at 37 ˚C, before the aliquot was incubated with the reagent.

References

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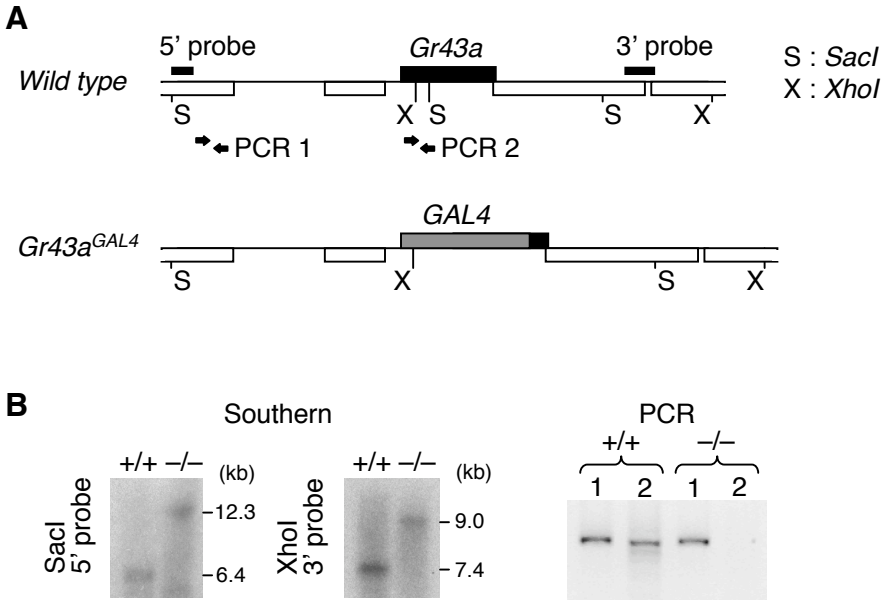
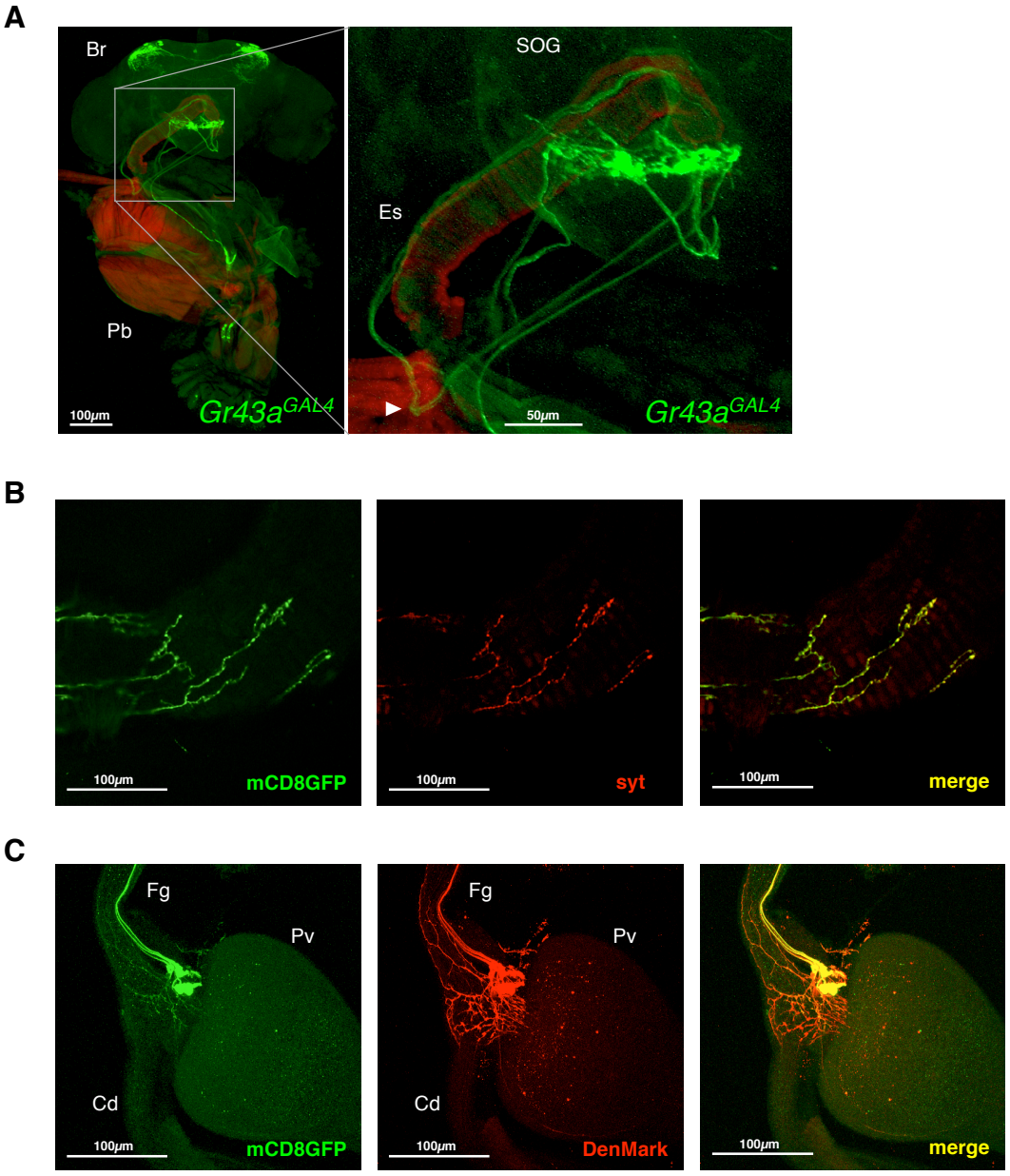
Rong, Y. S., and Golic, K. G. (2000). Gene targeting by homologous recombination in Drosophila. Science *288*, 2013-2018.

Nunes, R.D. de Oliveira, R.L. and Braz, G.R. (2008). A novel method for measuring fructose ingestion by mosquitoes. J. Vector Ecol*. 33,* 225-231

Supplemental Figure 1

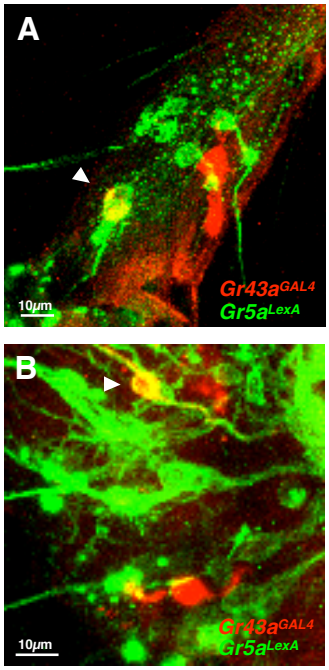


Supplemental figure 1



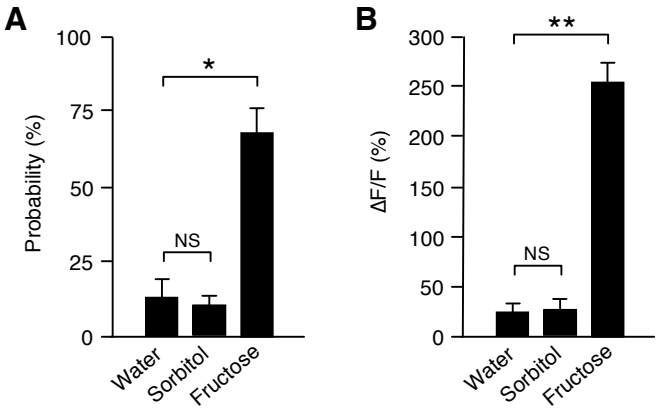
Supplemental figure 2

Supplemental Figure 3



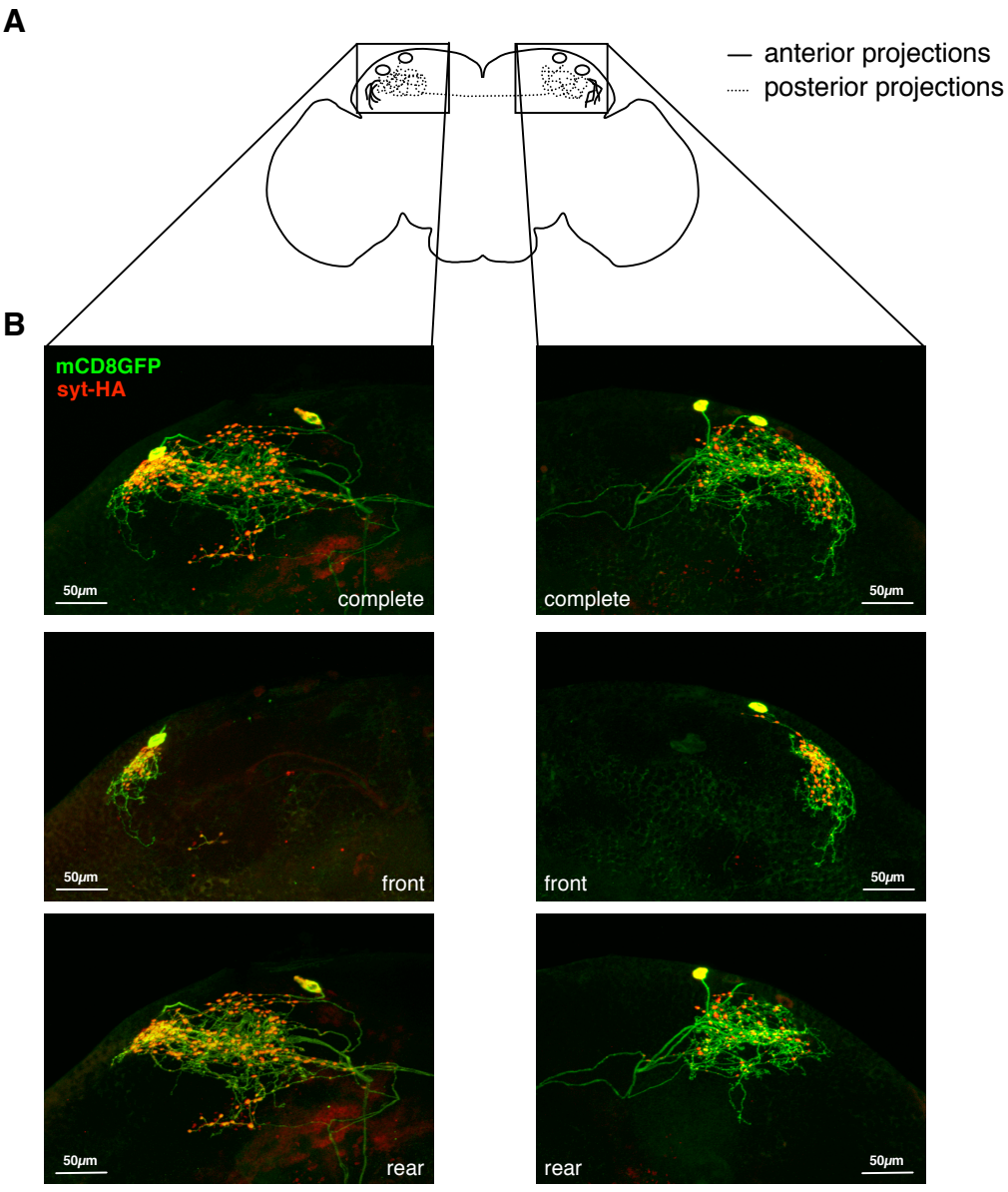
Supplemental figure 3

Supplemental Figure 4



Supplemental figure 4

Supplemental Figure 5



Supplemental figure 5