SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly Strains

Flies were maintained on conventional cornmeal-agar-molasses medium at 23-25°C and 60-70% relative humidity, under a 12 hr light: 12 hr dark cycle (lights on at 9 A.M.). The Canton-S strain originally from Barry Dickson was used as wild-type (Figures 1 and 2 and Figure S1). When tested as controls, UAS stocks were tested as hemizygotes after crossing to *w*^{1118}. Gal4 lines used in the feeding screen (Figure 3A) were manually preselected for restricted expression in the central nervous system, with a particular focus on the subesophageal zone, and were sourced from the Janelia Farm Fly Light collection (Jenett et al., 2012) at the Bloomington *Drosophila* Stock Center; the Kyoto *Drosophila* Genetic Resource Center; and an enhancer trap screen carried out at the Freie Universität (FU) Berlin (Siegmund and Korge, 2001) (provided by Annemarie Hofmann, FU Berlin). Other strains and sources: *57G09-Gal4* (Bloomington #46398); *57F03-Gal4,* (Bloomington #46386); *83F01-Gal4* (Bloomington #40364), *Gr43a*^{Gal4} (Miyamoto et al., 2012) (provided by Hubert Amrein, Texas A&M University); *Gr5a-Gal4* and *Gr66a-Gal4* (Wang et al., 2004b) (Kristin Scott, University of California, Berkeley); *Gr64f-Gal4* (Bloomington #57669); *Gr64a-Gal4* (Bloomington #57661); *Gr61a-Gal4* (Bloomington #57657); *Elav-Gal80* (Yang et al., 2009) (provided by Yuh Nung Jan, UCSF); *Tsh-Gal80* (Clyne and Miesenbock, 2008) (provided by Julie Simpson, HHMI-Janelia Farm Research Campus); *Vglut-Gal80* (Bussell et al., 2014) (Bloomington # 58448); *ChAT-Gal80* (Kitamoto, 2002) (provided by Toshihiro Kitamoto, University of Iowa); *UAS-inactiveTNT* (Bloomington #28839); *UAS-shi*^{ts} (Pfeiffer et al., 2010) (provided by Gerry Rubin, HHMI-Janelia Farm Research Campus); *UAS-CD8-GFP* (Bloomington #5130); *UAS-CD8-RFP* (Bloomington #32219); *UAS-CD8-GFP* (Bloomington #32186); *UAS-CD8-RFP*, *LexAop-CD8-GFP* (Bloomington #32229); *UAS-nrx-GFP1-10* and *LexAop-CD4-GFP11* (Chen et al., 2014) (provided by Larry Zipursky, UCLA); UAS-GCaMP6s (Bloomington #42746); *UAS-syt-GFP* (Zhang et al., 2002) (provided by Vanessa Ruta, The Rockefeller University); *UAS-Dscam-GFP* (Wang et al., 2004a) (provided by Wes Grueber, Columbia University), *UAS-myr-tdTomato* (Bloomington #32222) (provided by Vanessa Ruta, The Rockefeller University); *UAS-frt-[stop]-frt-CD8-GFP* and *UAS-frt-[stop]-frt-activeTNT* (von Philipsborn et al., 2011) (provided by Barry Dickson, HHMI-Janelia Farm Research Campus); *UAS-ReaChR:Citrine*

(Bloomington #53741)*; UAS-frt-[mCherry]-frt-ReaChR::Citrine* (Bloomington #53743); *Tub-frt-[Gal80]-frt* (Bloomington #38879); *LexAop-FLP* (Shang et al., 2008)*(*provided by Marco Gallio*,* Northwestern University*)*. Two different insertions of *UAS-active tetanus toxin* (TNT) were tested (*UAS-TNT-G*, Bloomington #28838; and *UAS-TNT-E,* Bloomington #28837). Both gave ingestion phenotypes when crossed to *57F03-Gal4*, but *UAS-TNT-G* was used only in the initial screen because it caused locomotion defects in the absence of *Gal4* (data not shown).

Transgenic Fly Production

Ir25a-Gal80 was generated by PCR amplification of the *Ir25a* enhancer fragment (chr2L: 4834318-4835333, antisense strand, 1016 bp,) from Canton-S genomic DNA with the following primers: Ir25a-F (5'-CGTCAATTGTTGTTGCTTGCTTGCC-3') and Ir25a-R (5'-TGCGTTTGTTTGTTTGCCCTAAAA-3'). The PCR product was cloned via the Gateway system (Life Technologies) into *pBPGal80Uw-6* (Pfeiffer et al., 2010). *57F03-LexA* was generated by PCR amplification of the *CG9918* enhancer fragment (chr3R: 14102809-14104846, antisense strand, 2038 bp) from Canton-S genomic DNA with the following primers: CG9918-F (5'-CAAGGAACGAGTTGCGAAAAGAGGC-3') and CG9918-R (5'-CGGCTGCGACTCGATGTCCCCTCAA-3'). The PCR product was cloned via the Gateway system (Life Technologies) system into *pBPnlsLexA-GADUw* (Pfeiffer et al., 2010). *57F03-Gal80* was generated by cloning the *CG9918* promoter fragment described above via the Gateway system into *pBPGal80Uw-6* (Pfeiffer et al., 2010). Transgenic lines were generated with the phiC31-based integration system (Groth et al., 2004) by Genetic Services Inc. The *Ir25a-Gal80* transgene was inserted into the attP40 genomic locus, and *57F03-LexA* and *57F03-Gal80* transgenes were inserted into the attP2 genomic locus.

Complete genotypes of all fly strains used in the paper:

<u>Figure 1C</u>
Wild-type Canton-S males
<u>Figure 1D-1L</u>
Wild-type Canton-S males and females
<u>Figure 2A-2J</u>
Wild-type Canton-S males
<u>Figure 3A</u>
Females: w^{1118}/ w^{1118}*; UAS-TNT-G /+; +/+*
Males: From a collection of 228 individual neuronal-Gal4 lines
<u>Figure 3B-3D</u>
Males*:* w^{1118}*/Y; +/+; 57F03-Gal4/UAS-CD8-GFP*

Figure 3E-3J
Males:

w^{1118}/Y; +/+; UAS-shi^{ts}/+
w^{1118}/Y;+/+; 57F03-Gal4/ UAS-shi^{ts}
w^{1118}/Y; +/+; UAS-shi^{ts}/+
w^{1118}/Y;+/+; 57F03-Gal4/ UAS-shi^{ts}

Figure 4A-4C
Males:

w^{1118}/Y; UAS-frt-stop-frt-CD8-GFP/+;57F03-LexA-GAD, LexAop-FLP/83F01-Gal4

Figure 4D
Males:

Tub-frt-Gal80-frt/Y; +/+; 57F03-LexA-GAD, LexAop-FLP/83F01-Gal4, UAS-CD8-GFP

Figure 4E-4J
Males:

w^{1118}/Y; +/+; 83F01-Gal4/+
w^{1118}/Y; UAS-frt-stop-frt-TNT/+;57F03-LexA-GAD, LexAop-FLP/+
w^{1118}/Y; UAS-frt-stop-frt-TNT/+;57F03-LexA-GAD, LexAop-FLP/83F01-Gal4

Figure 5C
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; Gr66a-Gal4/+; 57F03-LexA-GAD/+

Figure 5D
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; Gr5a-Gal4/+; 57F03-LexA-GAD/+

Figure 5E
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; Gr64f-Gal4/+; 57F03-LexA-GAD/+

Figure 5F
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; Gr61a-Gal4/+; 57F03-LexA-GAD/+

Figure 5G
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; $Gr43a^{Gal4}$-Gal4/+; 57F03-LexA-GAD/+

Figure 5H
Males:

UAS-CD8-RFP, LexAop-CD8-GFP /Y; Gr64a-Gal4/+; 57F03-LexA-GAD/+

Figure 5I
Males:

w^{1118}/Y; Gr66a-Gal4/UAS-CD8-RFP; +/+

Figure 5J
Males:

w^{1118}/Y; Gr5a-Gal4/UAS-CD8-RFP; +/+

Figure 5K
Males:

w^{1118}/Y; Gr64f-Gal4/UAS-CD8-RFP; +/+

Figure 5L
Males:

w^{1118}/Y; Gr61a-Gal4/UAS-CD8-RFP; +/+

Figure 5M

Males:

w^{1118}/Y; Gr43a^{Gal4}/UAS-CD8-RFP; +/+

<u>Figure 5N</u>

Males:

w^{1118}/Y; Gr64a-Gal4/UAS-CD8-RFP; +/+

<u>Figure 5P</u>

Males:

w^{1118}/Y; LexAop-CD4-GFP11/+; 57F03-LexA-GAD/UAS-nrx-GFP1-10
w^{1118}/Y; Gr5a-Gal4/ LexAop-CD4-GFP11; 57F03-LexA-GAD/UAS-nrx-GFP1-10
w^{1118}/Y; Gr43a^{Gal4}/ LexAop-CD4-GFP11; 57F03-LexA-GAD/UAS-nrx-GFP1-10
w^{1118}/Y; Gr64f-Gal4/ LexAop-CD4-GFP11; 57F03-LexA-GAD/UAS-nrx-GFP1-10

<u>Figure 6A-6I</u>

Females:

w^{1118}/ w^{1118}; UAS-GCaMP6s /+; 83F01-Gal4/+

<u>Figure 7A</u>

Males:

w^{1118}/Y; UAS-ReaChR:Citrine /+; +/+
w^{1118}/Y; UAS-ReaChR:Citrine /Gr5a-Gal4; +/+
w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR:Citrine/+;57F03-LexA-GAD, LexAop-FLP /83F01-Gal4

<u>Figure 7B</u>

Males:

w^{1118}/Y; UAS-ReaChR:Citrine /Gr5a-Gal4; +/+
w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR:Citrine/+;57F03-LexA-GAD, LexAop-FLP /83F01-Gal4

<u>Figure 7C</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+

<u>Figure 7D</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /83F01

<u>Figure 7E</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+

<u>Figure 7F</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /83F01

<u>Figure 7G</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+

<u>Figure 7H</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /83F01

<u>Figure 7I</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+
w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /83F01

<u>Figure 7J</u>

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+
w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /83F01

Figure S1A-SE

Wild-type Canton-S males

Figure S1F-S1N

Wild-type Canton-S males and females

Figure S2A-S2F

Males:

w^{1118}/Y; UAS-inactiveTNT/+; +/+
w^{1118}/Y; UAS-TNT-E/+; +/+
w^{1118}/Y; UAS-inactiveTNT/+; 57F03-GAL4/+
w^{1118}/Y; UAS-TNT-E/+; 57F03-GAL4/+

Figure S2G

Males:

w^{1118}/Y; UAS-inactiveTNT/+; 57F03-GAL4/+
w^{1118}/Y; UAS-TNT-E /+; 57F03-GAL4/+
Elav-Gal80/Y; UAS-inactiveTNT/+; 57F03-GAL4/+
Elav-Gal80/Y; UAS-TNT-E /+; 57F03-GAL4/+
w^{1118}/Y; Ir25a-Gal80/UAS-inactiveTNT; 57F03-GAL4/+
w^{1118}/Y; Ir25a-Gal80/UAS-TNT-E; 57F03-GAL4/+
w^{1118}/Y; Tsh-Gal80/UAS-inactiveTNT; 57F03-GAL4/+
w^{1118}/Y; Tsh -Gal80/UAS-TNT-E; 57F03-GAL4/+
w^{1118}/Y; Vglut -Gal80/UAS-inactiveTNT; 57F03-GAL4/+
w^{1118}/Y; Vglut -Gal80/UAS-TNT-E; 57F03-GAL4/+

Figure S2H

Males:

Elav-Gal80/Y; +/+; 57F03-GAL4, UAS-CD8-GFP/+
w^{1118}/Y; Ir25a-Gal80/+; 57F03-GAL4, UAS-CD8-GFP/+
w^{1118}/Y; Vglut-Gal80/+; 57F03-GAL4, UAS-CD8-GFP/+
w^{1118}/Y; Tsh-Gal80/+; 57F03-GAL4, UAS-CD8-GFP/+

Figure S3A-S3B

Males:

w^{1118}/Y; UAS-frt-stop-frt-CD8-GFP/+;57F03-LexA-GAD, LexAop-FLP/57F03-Gal4

Figure S3C

Males:

w^{1118}/Y; UAS-frt-stop-frt-CD8-GFP/+;57F03-LexA-GAD, LexAop-FLP/83F01-Gal4

Figure S4A

Males:

w^{1118}/Y; UAS-syt-GFP /+; 83F01-Gal4/+

Figure S4B

Males:

w^{1118}/Y; UAS-myr-tdTomato /+; 83F01-Gal4/UAS-Dscam-GFP

Figure S4C

Males:

w^{1118}/Y; UAS-CD8-GFP /Vglut-Gal80; 83F01-Gal4/+

Figure S4D

Males:

w^{1118}/Y; UAS-CD8-GFP /+; 83F01-Gal4/ChAT-Gal80

Figure S5B

Females:

w^{1118}/ w^{1118}; UAS-GCaMP6s /+; 83F01-Gal4/+

Figure S6A

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /+

Figure S6B

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR::Citrine /+;57F03-LexA-GAD, LexAop-FLP /57F03-Gal4

Figure S6C

Males:

w^{1118}/Y; UAS-frt-mCherry-frt-ReaChR:Citrine/+;57F03-LexA-GAD, LexAop-FLP /83F01-Gal4

CAFE Assay

This assay was carried out as described (Ja et al., 2007), with the following modifications. The CAFE chamber was prepared by placing a moistened Kim wipe at the bottom of a polystyrene vial (Fisher #AS-519). The top of the vial was plugged with a one-hole rubber stopper (VWR #59581-265). Liquid food was delivered with a calibrated 5 µl glass capillary (VWR #53432-706) inserted in a trimmed pipette tip, placed into the rubber stopper. Liquid food consisted of 10% (w:v) sucrose (Fisher #S5-3) and 5% (w:v) yeast extract (Fisher #BP1422-500). To measure post-fasting food ingestion, 10 flies were "wet-fasted" for 24 hr as follows: flies were placed into an empty plastic vial, with no fly food but with a wet Kim wipe placed at the bottom of the vial as a source of water and humidity. They were then removed from this vial and given access to liquid food in the CAFE for 3 hr, which was placed in a 23-25°C and 50-60% relative humidity incubator. The drop in liquid level in the capillary was measured manually by holding the capillary next to a ruler and measuring the liquid meniscus in length with respect to the black calibration band on the capillary. The black band was located 55 mm above the bottom tip of the capillary and indicated the volume in the capillary, which holds 5 µl liquid. The measured length was then converted (1mm = 0.09 µl), with the total liquid food consumed by the group of flies in a given CAFE converted to µl liquid food consumed per single fly. For each genotype, 12-30 groups of 10 flies were tested.

CAFE Food Ingestion Screen

5-6 females homozygous for *UAS-TNT-G* were crossed to 3-5 males from individual Gal4 lines. Parents were removed from the cross after 3-4 days, and progeny were allowed to eclose. From crosses that produced viable and healthy progeny, 10 adult males were collected and kept in a fresh food vial for 24 hr before being wet-fasted as described above. If ≥50% of the flies died during fasting, the Gal4 line was classified as semi-lethal, and not tested in the CAFE assay. Flies from the Gal4 lines that survived wet-fasting were given access to liquid food in the CAFE for 3 hr. A Gal4 line was classified as feeding-defective if the liquid food consumed per fly was one standard deviation below the mean liquid food consumed by all the Gal4 lines tested. Positive Gal4 lines were retested twice, and those that showed a feeding defect in all 3 rounds of testing were classified as abnormal feeders.

Locomotion

Fly locomotion was tracked in a custom-made 70 mm circular arena (Bussell et al., 2014). The chamber was fabricated from white Delrin plastic (McMaster-Carr), custom-machined to uniform thickness to allow backlighting from a light board (Smith-Victor Corporation #A-5A), and topped with a piece of Plexiglas with a small hole for introducing flies. The arena has sloped sides with a maximum height of 3 mm, which encourages flies to walk in two dimensions. The low ceiling and sloped walls prevent flight and limit the ability of flies to walk on the ceiling (Simon and Dickinson, 2010). Video was recorded with a consumer camcorder (Sony, DCR-SR68) mounted above the arena in the laboratory (22-24°C; ambient humidity). For each trial, a single wet-fasted fly was aspirated into the arena, allowed to acclimate for 60 s and videos were recorded for 60 s. The movement of the fly was tracked using Noldus EthoVision XT video tracking software and distance travelled was computed with EthoVision XT analysis software. 10-15 individual flies per genotype were tested.

Negative Geotaxis

Groups of 10 wet-fasted flies were placed in an empty vial in ambient laboratory conditions (22-24°C; ambient humidity). A vial was briefly tapped to force all the flies to the bottom. Flies have an innate preference to walk upward, so this assay measures negative geotaxis as well as climbing ability. We scored the number of flies that crossed a black line at the height of 7 cm from the bottom of the vial in the 10 s after the vial was tapped. Negative geotaxis was

calculated as the percentage of flies that successfully crossed the line divided by the total number of flies tested. For each genotype, 15 groups of 10 flies were tested.

Proboscis Extension Assay

A wet-fasted fly was aspirated into, and immobilized in, a 200 μl micropipette tip. The tip of the pipet was cut with a razor blade to expose the fly proboscis. The micropipette tip was stabilized on a glass slide and placed under a dissecting microscope. Taste sensitivity was tested by manually touching the labellum for 2-3 s with a liquid drop using a 10 μl micropipette and visually assessing whether the fly extended its proboscis. Each fly was tested 3 times and counted positive for proboscis extension if it responded in at least 2 of 3 trials. 15 flies were tested for each genotype.

Solid Food Ingestion

Solid food consisted of 10% (w:v) sucrose (Fisher #S5-3), 5% (w:v) yeast extract (Fisher, CAT# BP1422-500), 1% (w:v) agarose (Promega #V3125), and 2% (v:v) green food dye (McCormick). 10 flies were wet-fasted and then given access to solid food in a polystyrene vial (Fisher #AS-519) for 15 minutes. Flies were scored as fully fed only if their abdomen was completely filled with green food. Flies with very faint green abdomens were counted as unfed. For each genotype 10 groups of 10 flies were tested.

Expresso Hardware and Data Acquisition

Each Expresso sensor bank comprises a printed circuit board with 5 Linear Optical Array Sensors (TAOS, TSL1406R) each consisting of 768 photodiodes and a microcontroller, which connects the system to a computer through a Universal Serial Bus port. In the Expresso, when a fly drinks liquid food from a glass capillary, the decrease in the liquid level is detected by a photodiode and is used to calculate instantaneous food ingestion (Figure S1A and S1B). Photodiodes are semiconductor devices that generate photocurrents upon light absorption (Rokos, 1973). The glass capillary containing the liquid to be fed to the fly was placed along the optical sensor. Backlighting was provided by a line of fifteen red LEDs (638 nm) located on the opposite site of the capillary. The backlighting was diffused with a 1.5875 mm thick sheet of white acrylic placed between LED backlights and the glass capillary. The sensor bank was covered with a light-tight box manufactured from black acrylic sheets using a laser cutter. A computer read the electrical signal generated by each photodiode and the light intensity at each pixel in the array was sampled at 8 Hz by a micro-controller (STMicroelectronics, STM32

F103RCBT6) on a development board (Leaf Labs, Maple Mini) attached to the Expresso sensor bank. The presence of liquid food in the capillary increases the light intensity measured by the optical sensor, such that the instantaneous position of the meniscus can be detected. When the light impinges on a pixel in the array it generates a photocurrent. For each pixel, the light intensity is measured as voltage (V), which is proportional to the intensity of the light impinging on the photodiode during the time window determined by the user (0.1 to 2 Hz). The data acquisition software receives the light intensity reading of each pixel from the micro-controller and computes the food-air boundary position (Figure S1A) by integrating all the light intensity values. The exact pixel position of the food-air boundary directly implies the current liquid level in the capillary. The pixel value can be converted to distance (e.g. mm) using the known pixel spacing (63.5 μm) between each photodiode. The current volume can be calculated by multiplying this distance with the fixed capillary cross sectional inner area (Figure S1B). When a fly consumes the liquid food in the capillary the food-air boundary moves from one pixel location to another (Figure S1A) and the consumed volume can be calculated accordingly (Figure S1B). Liquid level readings can be acquired at sample rates ranging from 0.1 to 2 Hz. with an accuracy of ±5.78 nl. The data acquisition software records the time vs. liquid level data for multiple sensor banks into a single file using the Hierarchical Data Format (HDF5) (http://www.hdfgroup.org/HDF5/).

Open source plans for Expresso sensor banks are available from IO Rodeo: http://public.iorodeo.com/docs/expresso/hardware_design_files.html.

The source code for Expresso data acquisition software can be downloaded from IO Rodeo: http://public.iorodeo.com/docs/expresso/device_software.html.

Expresso Data Analysis Software

The executable version of Expresso data analysis software can be downloaded at this link:

http://www.ticomo.ch/projects/expresso/download/analysis_software.html

The software inspects and analyzes liquid food level recordings that are collected by the Expresso data acquisition software. The analysis software detects bouts and aggregates data from selected regions in a recording, whole recordings, or groups of recordings with batch processing. The analysis algorithm automatically generates data sheets and graphical representations that visualize recorded liquid levels, confidence intervals for bout detection,

and detected bouts. Bouts and confidence intervals are detected in three steps (Figure S1C). Detected bouts are highlighted in green (Figure S1C, bottom trace).

1. Filtering. This first step removes invalid values and outliers from the raw data and de-noises the recorded signal. We use a wavelet decomposition to detect and remove background noise of the sensor (Donoho, 1995). The chosen method is robust to different experiment conditions and noise variations between different sensors. Briefly, we first eliminated the invalid values, where the sensor electronics return a liquid level value of -1. Next we determined the outlier values, where the measured liquid level transiently fluctuated greater than four standard deviations from the mean. These outliers were eliminated at the beginning and the end of the time series and replaced by interpolation elsewhere. Last, we denoised the raw data and eliminated periodic perturbations by soft-thresholding its wavelet coefficients using the universal threshold $\sqrt{2\ln(x)}$, (x=the length of the data).

2. Confidence. In the second step, events were identified by iteratively comparing z-scores (z) against a threshold ($Z\text{min}$).

$$e = z > Z\text{min}, z = \frac{x - \mu}{\sigma}, Z\text{min} = 1$$

For the first iteration, the filtered signal from step 1 is used for z-score computation, for further iterations previously detected events are eliminated before computing the z-score. The thresholding process is repeated until the variance of the remaining signal compares to the variance of a recording without meals (μ is the mean of the population; σ is the standard deviation of the population; x is the input signal).

3. Detection. In the third step, characteristic attributes are computed for each bout. This includes start and stop time for each bout, bout duration (d), total consumed liquid food volume (v), time between meals (i) and drinking rate. We filtered out events with a total consumed volume $v < V_{min}$ and merge neighboring events with $i < I_{min}$. By default V_{min} = 6 nl and I_{min} = 0 s. These values were determined based on technical specifications of the Linear Optical Array Sensor (TAOS, TLS1406R) and the Expresso data acquisition software. This method allowed us to distinguish the rapid decrease in liquid level in the capillary due to ingestion by the fly from the slow liquid level decrease due to evaporation

Expresso Assay Procedure

To test flies in the Expresso system, we used a custom-made feeding chamber fabricated from white Delrin plastic (Figure 1B). The feeding chamber has 5 compartments; each designed to hold a polystyrene cuvette (Lake Charles Manufacturing #189A) into which a single fly is placed. A sliding door is positioned between the cuvette holding the fly and the tip of the capillary, allowing synchronization of each trial for all 5 fly compartments. The Expresso sensor banks and the feeding chamber are placed in a transparent rack at a 45° angle. To measure fasting-induced food ingestion, flies were wet-fasted in groups of 10-20 and individually aspirated into the cuvettes attached to the feeding chamber. The sliding door was in the closed position at the beginning of each trial to allow flies to acclimate to the feeding chamber without access to the food capillary. When all flies were loaded into a cuvette, the Expresso data acquisition software was started and the sliding door was moved to the open position, allowing flies access to the capillary tips. Each trial lasted 33 minutes, and 10 flies were tested in parallel in two Expresso sensor banks run simultaneously. The Expresso system can be multiplexed to run 20 modules of 5 sensors in parallel, for 100 channels in total. Liquid food level recordings were batch-processed offline in the Expresso analysis software. When a fly did not consume a meal bout, the total meal bout volume was scored as 0 and latency to first meal bout was scored as the total time of the assay (=33 minutes). In Figure 2D, we calculated calorie intake for each fly based on the volume ingested and the concentration of the sucrose solution.

Expresso Validation

The Expresso system was validated by comparing human and automated scoring of feeding behavior (Figure S1D and S1E). This was carried out by videotaping fly feeding behavior in the Expresso simultaneously with automated food ingestion recordings. The tip of the capillary was recorded at a rate of 1 frame per s (fps) with a CCD camera (Point Grey, GS2-GE-20S4M-C) equipped with an Infinimite Alpha lens and a 2X magnifier (Infinity Optics) mounted above the behavior chamber. The human observer scored the position of the fly's proboscis relative to the tip of the capillary, and noted the beginning and end of such contact. The fly was considered to be drinking liquid food only if its proboscis was extended into the tip of the capillary, and the fly was pumping food into its pharynx. From 50 trials each lasting 15-16 minutes, a human observer scored each frame from the 41 trials in which the fly consumed at least one meal. In total we analyzed 249 individual feeding events (Figure S1D).

Optogenetic Photoactivation

We used a custom-made light box carrying a 530 nm high power LED (LUXEON STAR LEDs, SR-05-M0100). LED intensity and pulse timing were regulated by an Arduino Uno Board (Smart Projects) controlled system designed based on the previously described circuit (Inagaki et al., 2013) powered by a 30 V power supply (X-Power DC Power Supply, 305D).

To test for light-induced proboscis extension, a single fly was aspirated into a 1 cm radius circular chamber. The fly was videotaped from the top to capture the extension of the proboscis with a CMOS camera (EM1400M, BigCatch Eyepiece Digital Camera For Microscope) attached to a dissecting microscope. We used an IR long-pass filter (Edmund Optics) to avoid detection of the LED light and captured high-speed videos (20-30 fps) using ToupView (BigCatchUSA) image acquisition software under infrared (IR) light illumination. In each trial, the fly was recorded for 30 s without LED light illumination followed by 30 s with LED light delivered at 1 Hz with pulse duration of 500 ms. Proboscis extension was assessed manually from the video. A fly was scored as positive when it extended its proboscis in response to photoactivation one or more times during the 30 s photoactivation period. The percent of flies showing proboscis extension was plotted per genotype and treatment (Figure S5D).

To test light-induced sugar ingestion, a single fly was aspirated into a 200 µl cut micropipette tip and the head immobilized using a UV-curing glue (KOA 300, KEMXERT CORP). The micropipette tip was stabilized with a metal rod connected to a rotatable probe clamp (MXC, Siskiyou Corporation). The fly was positioned in close proximity with its proboscis 0.2 mm to 0.3 mm away from a glass capillary filled with 1 M sucrose solution that was attached to a microinjector (Drummond, Nanoject II) (Figure 7C and 7D). At the start of each experiment, 200 nl to 350 nl 1M sucrose was ejected. The movement of the microinjector and the capillary was controlled by a micromanipulator (Burleigh, EXFO, PCS-6000). The fly was videotaped from the side to capture the extension of the proboscis by a CCD camera (Point Grey, GS2-GE-20S4M-C) equipped with an Infinity Lens (68 mm focal length) and a 2X magnifier (Infinity Optics). We used an IR long-pass filter (Edmund Optics) to avoid detection of the LED light and captured high-speed videos (30 fps) using the Flycap2 (Point Grey) image acquisition software under IR light illumination. In each trial, the fly was recorded for 30 s without LED illumination and then 30 s with LED illumination delivered at 1 Hz with pulse duration of 500 ms. To quantify ingestion, we measured the distance of the meniscus of 1M sucrose to the

base of the capillary before and after photoactivation (Figure S6E and S6F). To obtain an estimate of the liquid consumed from this measurement, we first generated a standard curve by using the Nanojet II to deliver liquid volumes from 0 nl to 750 nl in units of 50 nl. Using nonlinear regression, a second order polynomial curve was fitted to the data with the following formula (R^2=0.9884).

$$y = 42.21 + 1.134x - 0.0006629x^2$$

Using this formula, 1M sucrose volume consumed by each fly was estimated and plotted per genotype and treatment (Figure 7J and Figure S6F).

Immunostaining and Microscopy

For whole mount staining, brains, ventral nerve cords, and rostrums were dissected in phosphate-buffered saline (PBS, calcium- and magnesium-free; Lonza BioWhittaker #17-517Q) and incubated in 4% paraformaldehyde (PFA) in PBS for 20-30 minutes at room temperature on an orbital shaker. Tissues were washed 3-4 times over 1 hr in PBS containing 0.1% Triton X-100 (PBT) at room temperature. Samples were blocked in 5% normal goat serum in PBT (NGS-PBT) for 1 hr and then incubated with primary antibodies diluted in NGS-PBT for 24 hr at 4°C. The next day, samples were washed 5-6 times over 2 hr in PBT at room temperature and incubated with secondary antibodies diluted in NGS-PBT for 24 hr at 4°C. On the third day, samples were washed 4-6 times over 2 hr in PBT at room temperature and mounted with Vectashield Mounting Medium containing DAPI (Vector Labs, #H-1200) on SuperFrost plus glass slides (Fisher Scientific #12-550-15) between two glass bridge coverslips. The samples were covered by a glass coverslip on top and sealed using clear nail polish. For staining of frozen sections, fly heads were removed with a sharp razor blade under carbon dioxide anesthesia, and lined up at the bottom of a disposable base mold (VWR Scientific #M475-4) containing Tissue-Tek OCT (VWR Scientific #62550-01), then placed in dry ice for 30 minutes. 10-15 μm antennal sections were cut using a cryostat (Thermo Scientific, Microm-HM550) and collected on SuperFrost Plus glass slides. The slides were dried at room temperature for 30 minutes, and then fixed in 2% PFA in PBS for 10 minutes at room temperature. Samples were washed 3 times 5 minutes in PBS and blocked with NGS-PBT for 1 hr at room temperature before being incubated in primary antibodies diluted in NGS-PBT at 4°C overnight. The next day, samples were washed 3 times 5 minutes in PBS, incubated in diluted secondary antibodies for 2-3 hr at room temperature, washed again 3

times 10 minutes in PBT and mounted with Vectashield Mounting Medium (VectorLabs #H-1200). Images were acquired at 512 x 512 pixel resolution at 1 μm intervals using an inverted Zeiss LSM 780 laser scanning confocal microscope and Zeiss digital image processing software ZEN. Maximum projection images of Z-stacks were generated in the ImageJ open source image-processing package (http://rsbweb.nih.gov/ij/).

GRASP Visualization

To examine native GRASP fluorescence (Figure 5P), brains were dissected in cold (4°C) PBL (0.075 M lysine, 0.1 M sodium phosphate buffer [pH 7.4]), fixed for 30 minutes in 4% PFA in PBL at 25°C and washed 3 times 15 minutes in PBS + 0.3% Triton X-100. Samples were mounted as described above. Native GRASP signal was visualized with an Ultima two-photon laser scanning microscope (Bruker Nanosystems) equipped with galvanometers driving a Chameleon Ultra II Ti:Sapphire laser. Images were acquired using an Olympus 60x water immersion objective at 512 x 512 pixel resolution and 1 μm intervals. The fluorescence signal was detected with GaAsP photodiode detectors.

GRASP signals were quantified in ImageJ by measuring the mean grey value of a 40 x 40 pixel region of interest (F). For $F/F_{background}$ calculations, the GRASP fluorescence (F) was divided by the background fluorescent signal ($F_{background}$).

All GRASP experiments were carried out with *57F03-LexA*, and GRASP signal was measured where IN1 interneuron arbors overlap with taste neuron afferents in the anterior subesophageal zone. No GRASP signal was detected in other regions of the brain (data not shown).

Antibodies

Primary antibodies (supplier, dilution): rabbit anti-GFP (Torrey Pines # TP401, 1:2000); mouse anti-GFP (Molecular Probes #A-11120, 1:100); mouse anti-Brp (Developmental Studies Hybridoma Bank nc82; 1:20); rat anti-elav (Developmental Studies Hybridoma Bank, 1:100); rabbit anti-DsRed (Clontech #632496, 1:500); rabbit anti-Ir25a (Benton et al., 2009)(Vosshall Lab, 1:2000). The following secondary antibodies were used at a dilution of 1:500: AlexaFluor 488 goat anti-rabbit (Invitrogen #11008), AlexaFluor 488 goat anti-mouse (Invitrogen #11001); AlexaFluor 488 goat anti-chicken (Invitrogen #11039); Cy3 goat anti-mouse (Jackson

ImmunoResearch #115-165-166); Cy3 goat anti-rabbit (Jackson ImmunoResearch #111-165-144); AlexaFluor 568 goat anti-rat (Invitrogen #A11077).

Functional In Vivo Calcium Imaging with GCaMP6s

All functional imaging experiments in Figure 6 were performed on an Ultima two-photon laser scanning microscope (Bruker Nanosystems) equipped with galvanometers driving a Chameleon Ultra II Ti:Sapphire laser. Emitted fluorescence was detected with a GaAsP photodiode (Hamamatsu) detector. Images were acquired with an Olympus 60×, 0.9 numerical aperture objective at 512 pixel × 512 pixel resolution. For fast-scanning volumetric imaging, the laser was directed through an 8 kHz resonant scanning galvanometer and the objective was controlled by a piezo-electric Z-focus. Z-planes were defined to encompass the entire volume of the neuronal arbor of IN1 neurons. 10 planes were recorded, spaced ~2-3 μm apart and the entire volume was imaged at a rate of ~5 Hz. Fast-scanning volumetric imaging of the entire IN1 neuronal arbor was necessary to minimize the effects of the fly's movement on the recorded fluorescence signal. The fluorescence values reported are therefore the average fluorescence of the Z-projected volume and represent the average activity of the IN1 population. Because of the dense innervation by the IN1 processes and the unavoidable motion present in a living fly, it was not possible to distinguish the activity in individual IN1 processes.

Flies were prepared as previously described (Ruta et al., 2010). Briefly, flies were temporarily anesthetized using CO_2 (for <30 s), and then tethered to a piece of tape covering a hole in the bottom of a modified 35 mm petri dish using a human hair placed across the neck. The proboscis was glued in a partially extended position using UV-curable glue (Loctite), keeping the labellum free of glue. A small hole was cut into the tape, precisely above the head, to allow the top of the head capsule to extend above the plane of the tape. A dot of UV-curable glue was applied to the eyes to restrict head movement. The dish was then filled with External Saline solution (108 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 8.2 mM $MgCl_2$, 4 mM $NaHCO_3$, 1 mM NaH_2PO_4, 5 mM trehalose, 10 mM sucrose, 5 mM HEPES pH7.5, osmolarity adjusted to 275 mOsm) and the head capsule was opened by carefully cutting and folding back the flap of cuticle covering the dorsal-anterior portion of the head, including the antennae. Obstructing trachea, antennal nerves and Muscle16 were removed as described previously (Yoshihara, 2012).

Tethered Sugar Feeding

To simultaneously record fly behavior during imaging, a Point Grey Firefly Camera with Infinity Lens (94 mm focal length) was focused on the fly, which was illuminated by IR LED lights. Video was captured at 30 fps. Laser-scanning onsets and offsets, visible in the video recordings due to laser illumination through the head-capsule were used to align videos with imaging data. For the imaging experiments in Figure 6A-6D and 6H, 1-3 day old female flies were removed from fly food, and placed into an empty vial containing a wet Kim wipe for 16-20 hours. After this period of wet fasting, the flies were desiccated for 4-6 hr by transferring them to an empty vial containing a Kim wipe and desiccant (Drierite, stock# 23001). Therefore the total period of fasting prior to imaging was 20-26 hours. Flies were tethered for imaging as described above and positioned on the microscope. A drop of water or 1 M sucrose solution was presented using a pulled capillary pipette attached to a microinjector (Drummond, Nanoject II) to deliver sucrose solution or water in precise volumes (20 nl). To ensure that drops were uniformly spherical, and that the liquid did not wick down the sides of the capillary, we applied dental wax to the outside of the glass capillary.

Because the tip of the pulled glass capillary was broken manually, resulting in 20 nl drops of different diameter, it was not possible to generate a universal standard curve for the volume of food ingested across different trials. Instead, in Figure 6H, we viewed the video frames of each meal-bout, and compared the drop size at the beginning and end of ingestion. By visual estimation, we binned the decrease in drop size into four categories to estimate the volume ingested. The categories were 20 nl (when the fly completely consumed the sucrose drop), 10 nl (when the fly drank approximately half of the drop), <10 nl (when the fly drank minute amounts as judged by visible cibarial pumping), and 0 nl (when the proboscis touched the drop, but the fly did not drink). Figure 6H includes only experiments from which we were able to estimate the volume ingested in all stimulus presentations for a single fly. These imaging experiments were divided into discrete trials each lasting 54.4 s, and the total imaging time varied from 21 minute to 33 minutes. In each trial, the capillary pipette tip was positioned near the fly's proboscis using a motorized micromanipulator (Scientifica). The 20 nl drop was touched to the proboscis to offer the fly the option of drinking. The capillary was withdrawn after complete consumption, or in cases where the fly consumed only part of the drop, or failed to consume any liquid, the capillary was withdrawn after an average of 6.7 s and a maximum of 20 s. Blue food coloring was added to the solution and fly abdomens were inspected after

each experiment to confirm ingestion. Only flies that successfully ingested sucrose during the imaging experiments were included in data analysis. We also monitored the spontaneous movements of the flies shown in Figure 6H and confirmed that they were actively moving their legs or proboscis in the last three trials of the experiment. Moreover we have found sensory responses in this imaging preparation to be stable for more than 50 minutes in a previous study (Cohn et al., 2015). Thus, the gradual decrease seen in IN1 activity is in response to the cumulative consumption of sucrose, and the development of satiety.

For the imaging experiments in Figure 6E-6G, 1-3 day old female flies were removed from fly food, and placed into an empty vial containing a wet Kim wipe for 22 hours. After this period of fasting, flies were transferred to two different types of new vials for 30-90 minutes prior to being prepared for imaging. To generate satiated flies, fasted flies were transferred to an empty vial containing a Kim wipe soaked in 1M sucrose solution containing 3% (v:v) red food coloring (McCormick). To generate flies that were fasted for 22-24 hours, fasted flies were transferred to an empty vial containing a Kim wipe soaked in water containing 3% (v:v) red food coloring (McCormick). Red food color was included to verify that flies had ingested the sucrose. Flies were tethered for imaging as described above and positioned on the microscope. To exclude the possibility that decreases in GCaMP6s signal were due to photobleaching or deterioration in the health of the sample, we measured the baseline fluorescence for several minutes before a stimulus was ingested. Across the animals analyzed, baseline GCaMP6s fluorescence in the first ~5 minutes before the sugar stimulus presentation was essentially unchanged (Mean baseline variance of F/F_0: 0.013, SEM: ± 0.0054) (see Figure 6E). After 5 minutes of baseline recording, 20 nl 1M or 100mM sucrose stimuli were offered to the fasted or fed flies for consumption using the same methods described above. GCaMP6s fluorescence was recorded for at least 7 more minutes continuously after the sugar stimulus was presented, making the entire imaging experiment 12 minutes long. Blue food coloring was added to the solution and fly abdomens were inspected after each experiment to confirm ingestion of sucrose during the imaging experiments. We also estimated the proportion of the 20 nl sucrose drop that was consumed in these experiments by looking at the video recordings of flies during the imaging experiment. In all cases more than 2/3 of the volume was ingested. We can therefore exclude the possibility that the lack of sustained activity seen with 1 M sucrose ingestion in fed flies and 100 mM sucrose ingestion in fasted flies is merely a

consequence of insufficient ingestion (Figure 6E-6G). Ingestion durations is shown at the bottom of each GCaMP6s trace as a histogram of all the flies tested (Figure 6E-6G).

Image Processing and Data Analysis

All image processing was done using FIJI/ImageJ (NIH). Further analysis was performed using custom scripts in ImageJ, Microsoft Excel, and Matlab. Fluorescence intensity was calculated by manually drawing a region of interest around the neuronal arbor of IN1 in the Z-projection of the volume collected at each time point (see Figure S5B). When necessary, to correct for motion during in vivo imaging, recordings were stabilized using the StackReg ImageJ plugin. The normalized time series of GCaMP6s fluorescence in Figure 6C was aligned to the time point 1 s before the stimulus was applied for each replicate. The time series of the traces beginning 1 s before the stimulus and ending 3 s after the stimulus were averaged and displayed. For $\Delta F/F_0$ calculations in IN1 arbors, the difference between the pre-stimulus value (F_0=average of 5 frames ending 5 frames before stimulus) and post-stimulus value (defined as the peak stimulus evoked across 30 frames after stimulus; or if the experiment ended before 30 frames, the maximum number of frames available after the stimulus was present) was divided by the pre-stimulus value ($\frac{\Delta F}{F_0} = \frac{F_{max} - F_0}{F_0}$).

For the imaging experiments in Figure 6E-6G, the normalized time series of GCaMP6s fluorescence was aligned to the sugar stimulus. The time series of the traces beginning ~300 s before the stimulus and ending ~420 s after the stimulus were averaged and displayed. For $\Delta F/F_0$ calculations in IN1 arbors, the difference between the pre-stimulus value (F_0=average of first 1080 frames before the stimulus) and post-stimulus value (defined as the peak stimulus evoked across 300 frames after the sugar stimulus) was divided by the pre-stimulus value ($\frac{\Delta F}{F_0} = \frac{F_{max} - F_0}{F_0}$). Persistent activity after the sugar stimulus was calculated by using the Area Under the Curve (A.U.C) function in Prism software.

For the imaging experiments in Figure 6H, GCaMP6s fluorescence signal was normalized to the initial F_0 and plotted together with the estimated volume ingested during the experiment per individual fly. The normalized changes in $\Delta F/F_0$ were binned into 4 groups based on the percent trial number, and averaged to show the changes in GCaMP6s signal in response to consecutive sucrose ingestion.

Data Presentation and Statistical Analysis

For all boxplots in the paper, the median is indicated by the black line, bounds of the box mark the 1st and 3rd quartile, whiskers extend to 1.5 times the interquartile range, and outliers are indicated with dots.

Details of statistical analysis for non-linear regressions in Figures 1-2:

Figure 1I: One-phase exponential decay function was fitted to the raw data of bout rate change over time (Goodness of fit scores: 0 hr R^2= 0.7142, 6 hr R^2= 0.8003, 12 hr R^2=0.8166, 18 hr R^2=0.8502, 24 hr R^2= 0.8027).

Figure 1K: One-phase exponential increase function fitted to the raw data of cumulative food ingestion over time. (Goodness of fit scores: 0 hr R^2= 0.97, 6 hr R^2= 0.98, 12 hr R^2=0.99, 18 hr R^2=0.99, 24 hr R^2= 0.99).

Figure 2G: One-phase exponential decay function fitted to the raw data of bout rate change over time (Goodness of fit scores: 1 mM R^2= 0.8110, 10 mM R^2= 0.8198, 100 mM R^2=0.8176, 1 M R^2=0.9866).

Figure 2I: One-phase exponential increase function fitted to the raw data of cumulative food ingestion over time. (Goodness of fit scores: 1 mM R^2= 0.9779, 10 mM R^2= 0.9558, 100 mM R^2= 0.9975, 1 M R^2= 0.9866).

REFERENCES

Benton, R., Vannice, K.S., Gomez-Diaz, C., and Vosshall, L.B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell *136*, 149–162.

Bussell, J.J., Yapici, N., Zhang, S.X., Dickson, B.J., and Vosshall, L.B. (2014). Abdominal-B neurons control *Drosophila* virgin female receptivity. Curr. Biol. *24*, 1584–1595.

Chen, Y., Akin, O., Nern, A., Tsui, C.Y., Pecot, M.Y., and Zipursky, S.L. (2014). Cell-type-specific labeling of synapses In vivo through synaptic tagging with recombination. Neuron *81*, 280–293.

Clyne, J.D., and Miesenbock, G. (2008). Sex-specific control and tuning of the pattern generator for courtship song in *Drosophila*. Cell *133*, 354–363.

Cohn, R., Morantte, I., and Ruta, V. (2015). Coordinated and Compartmentalized Neuromodulation Shapes Sensory Processing in *Drosophila*. Cell *163*, 1742–1755.

Donoho, D.L. (1995). De-noising by soft-thresholding. Inf. Theory IEEE Trans. On *41*, 613–627.

Groth, A.C., Fish, M., Nusse, R., and Calos, M.P. (2004). Construction of transgenic *Drosophila* by using the site-specific integrase from phage φC31. Genetics *166*, 1775–1782.

Inagaki, H.K., Jung, Y., Hoopfer, E.D., Wong, A.M., Mishra, N., Lin, J.Y., Tsien, R.Y., and Anderson, D.J. (2013). Optogenetic control of *Drosophila* using a red-shifted channelrhodopsin reveals experience-dependent influences on courtship. Nat. Methods *11*, 325–332.

Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T., and Benzer, S. (2007). Prandiology of *Drosophila* and the CAFE assay. Proc. Natl. Acad. Sci. U. S. A. *104*, 8253–8256.

Jenett, A., Rubin, G.M., Ngo, T.-T.B., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B.D., Cavallaro, A., Hall, D., Jeter, J., et al. (2012). A GAL4-driver line resource for *Drosophila* neurobiology. Cell Rep. *2*, 991–1001.

Kitamoto, T. (2002). Conditional disruption of synaptic transmission induces male–male courtship behavior in *Drosophila*. Proc. Natl. Acad. Sci. U. S. A. *99*, 13232–13237.

Miyamoto, T., Slone, J., Song, X., and Amrein, H. (2012). A fructose receptor functions as a nutrient sensor in the *Drosophila* brain. Cell *151*, 1113–1125.

Pfeiffer, B.D., Ngo, T.-T.B., Hibbard, K.L., Murphy, C., Jenett, A., Truman, J.W., and Rubin, G.M. (2010). Refinement of tools for targeted gene expression in *Drosophila*. Genetics *186*, 735–755.

von Philipsborn, A.C., Liu, T., Yu, J.Y., Masser, C., Bidaye, S.S., and Dickson, B.J. (2011). Neuronal control of *Drosophila* courtship song. Neuron *69*, 509–522.

Rokos, G.H.S. (1973). Optical detection using photodiodes. Opt. Quantum Electron. *5*, 351–366.

Ruta, V., Datta, S.R., Vasconcelos, M.L., Freeland, J., Looger, L.L., and Axel, R. (2010). A dimorphic pheromone circuit in *Drosophila* from sensory input to descending output. Nature *468*, 686–690.

Shang, Y., Griffith, L.C., and Rosbash, M. (2008). Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the *Drosophila* brain. Proc. Natl. Acad. Sci. U. S. A. *105*, 19587–19594.

Siegmund, T., and Korge, G. (2001). Innervation of the ring gland of *Drosophila melanogaster*. J. Comp. Neurol. *431*, 481–491.

Simon, J.C., and Dickinson, M.H. (2010). A new chamber for studying the behavior of *Drosophila*. PloS One *5*, e8793.

Wang, J., Ma, X., Yang, J.S., Zheng, X., Zugates, C.T., Lee, C.-H.J., and Lee, T. (2004a). Transmembrane/juxtamembrane domain-dependent Dscam distribution and function during mushroom body neuronal morphogenesis. Neuron *43*, 663–672.

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

Yang, C.-H., Rumpf, S., Xiang, Y., Gordon, M.D., Song, W., Jan, L.Y., and Jan, Y.-N. (2009). Control of the postmating behavioral switch in *Drosophila* females by internal sensory neurons. Neuron *61*, 519–526.

Yoshihara, M. (2012). Simultaneous recording of calcium signals from identified neurons and feeding behavior of *Drosophila melanogaster*. J. Vis. Exp. JoVE *62*, e3625.

Zhang, Y.Q., Rodesch, C.K., and Broadie, K. (2002). Living synaptic vesicle marker: Synaptotagmin-GFP. Genesis *34*, 142–145.