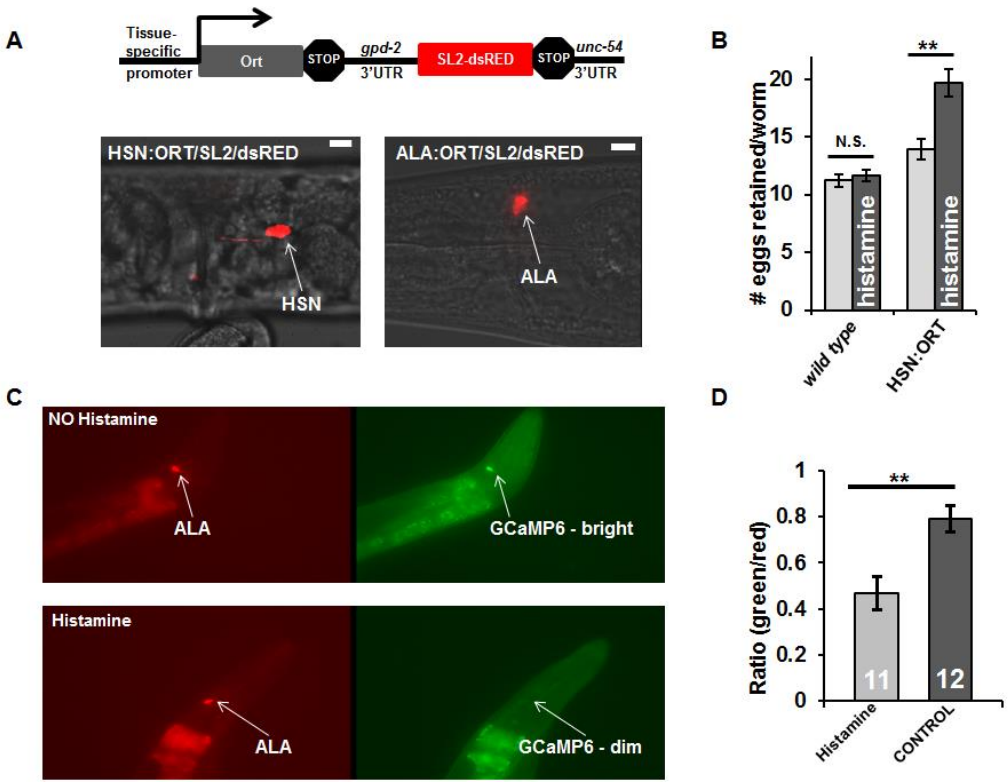


Figure S1: Validating ORT effectiveness is *C. elegans*. (**A**) The *Drosophila melanogaster* histamine-gated chloride channel, Ort, was fused to either the *egl-6* promoter (for HSN expression) or to the *ida-1* promoter (for ALA expression), upstream of the *gpd-2* 3'UTR, which contains an SL2 acceptor site, followed by dsRED and the *unc-54* 3'UTR. Expression of dsRED in the HSNs (one pictured) and the ALA neuron was observed in three transgenic lines for each genotype. Scale bar=5 μM. (**B**) Transgenic animals expressing Ort in the HSNs and exposed to histamine retained more eggs than transgenic sisters that were not exposed to histamine. (N≥43 animals, average of 4 lines, Student's t-test, **p<.005). (**C**) Animals that express Ort, the calcium indicator GCaMP6, and dsRED in the ALA neuron were grown in the presence or absence of histamine. The top panel (no histamine), shows bright GCaMP6 fluorescence in ALA and the bottom panel (histamine), shows reduced GCaMP6 fluorescence. (**D**) The ratio of ALA cell body GCaMP6 to dsRED fluorescence was lower when the animals were grown in the presence of histamine (histamine N=11, no histamine N=12, **P<.005, Student's t-test). Related to **Figure 1**.

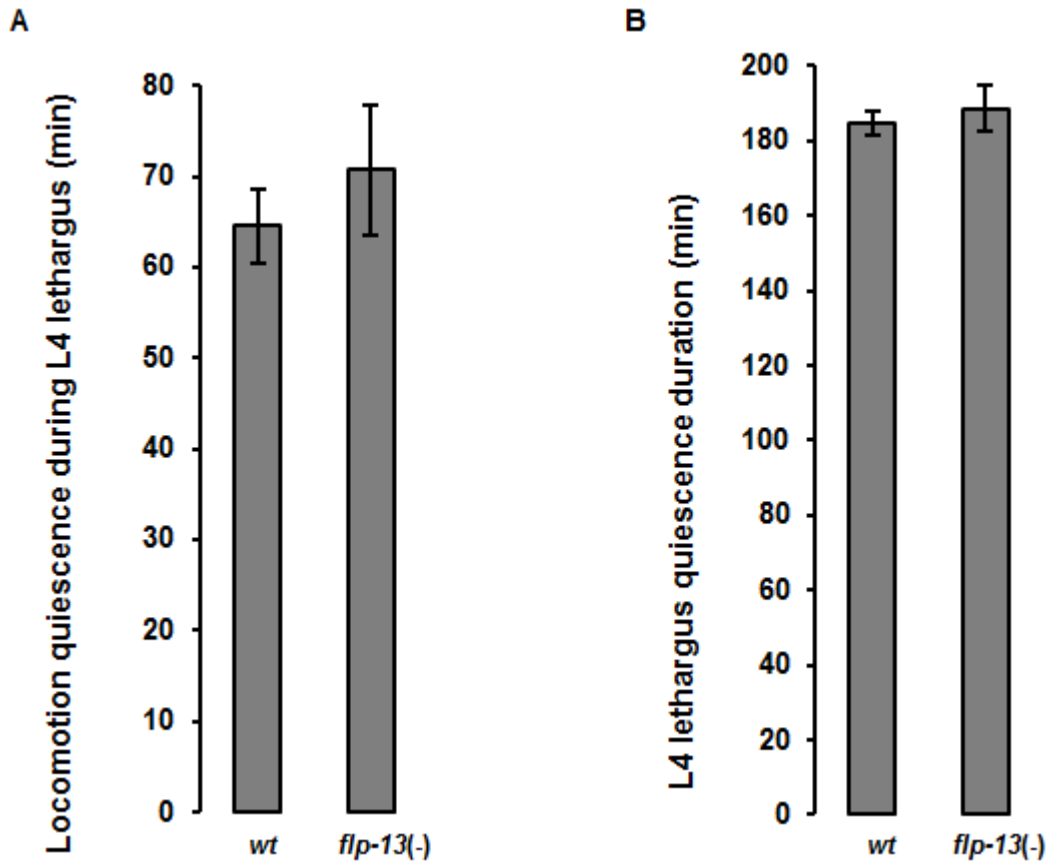
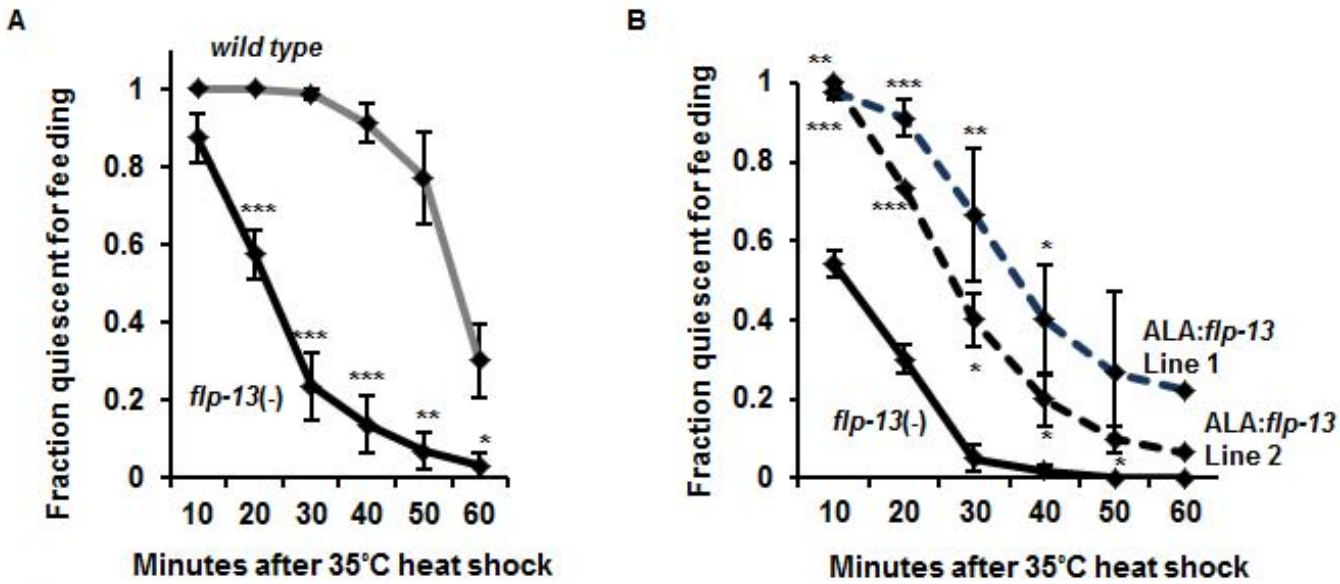


Figure S2: *flp-13* mutants are not defective in lethargus quiescence. Total L4 lethargus locomotion quiescence (**A**) and L4 lethargus quiescence duration (**B**) are not different between *flp-13*(*tm2427*) mutants and wild-type (wt) animals. Shown are the averages and SEM of ten pair wise analyses of wild-type and *flp-13* mutant animals using machine vision. (N=10, p>0.05, Student's t-test). Related to **Figure 2.**



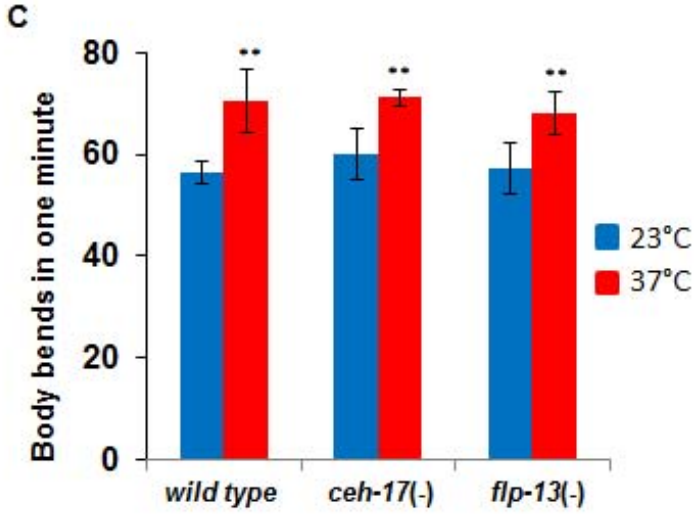


Figure S3: Restoration of *flp-13* in ALA rescues the feeding quiescence defects of *flp-13* mutants following heat stress. *ceh-17* and *flp-13* mutants are not defective in acute response to heat. (**A**) Animals were heat shocked for 30 minutes at 35°C (protocol 1). *flp-13*(*tm2427*) mutants display a defective feeding quiescence response following heat shock. (**B**) Two transgenic lines in which *flp-13* is expressed in ALA partially rescue these defects. (N>30 worms per trial, 2 trials for line 1 and 3 trials for line 2, *p<.05, **p<.005, ***p<.0005, Student's t-test). (**C**) First-day old adult worms were transferred individually onto unseeded NGM agar at room temperature (23ºC) and body bends were counted for one minute. The worm was transferred to an unseeded NGM agar plate that had been prewarmed in a 37ºC water bath and body bends were then counted again. (N=5 for each genotype, **p<0.01, two-tailed Student's t test). Related to **Figure 3**.

Oligo Name	Description	Sequence
		P*hsp-16.2*::*flp-13*
oNQ372	For-Promoter of hsp-16.2 in pPD49.83	GCCAAGCTTGCATGCCTG
oNQ373	For - Nested to oNQ372	CAGGTCGACTCTAGAGG
oNQ374	Rev - Promoter in pPD4.83	GGATCCGATGAGGATTTTC
oNQ354	For – Start of *flp-13*- Adds *hsp-16.2* tail	CTTCGAAAATCCTCATCGGATCCATGATGACGTCACTGCT
oNQ355	Rev –3' to the *flp-13* gene	GATAGAACAATTCATTTTTGTG
oNQ356	Rev - Nested to oNQ355	CAAGACGTCGAAGAAGAAG
		P*ida-1*:*flp-13*
oNQ44	For - >3kb upstream of *ida-1*	GCCTGCCTGTGCCAACTTACCTG
oNQ663	Nested to oNQ44	CAACTTACCTGCCTACCGTCTA
oNQ1133	For - Stat of *flp-13* - Adds *ida-1* tail	CCGCATCTCTGTGTCATCCGCATGATGACGTCACTGCTCACTATCTC
oNQ1134	Rev - Start of *ida-1* - Adds *flp-13* tail	GAGATAGTGAGCAGTGACGTCATCATGCGGATGACACAGAGATGCGG
		P*flp-13*:mCherry
oNQ1043	See Above	
oNQ1044	Nested to oNQ1044	TTGAACAACTTCCCCTTTATCC
oNQ1094	Rev - +12 to *flp-13* - Adds mCherry tail	TGCTTTTTTGTACAAACTTGTCATTGACGTCATCATGTTCGAATC
oNQ1095	For - Start of mCherry - Adds *flp-13* tail	GATTCGAACATGATGACGTCAATGACAAGTTTGTACAAAAAAGCA
oNQ472	See Above	
oNQ771	See Above	
		P*flp-13*:NLS:*gfp*
oNQ1043	See Above	
oNQ1044	See Above	
oNQ1074	Rev - +9 to *flp-13* - Adds NLS:*gfp* tail	GATTCGAACATGATGACGTCAATGACTGCTCCAAAGAAGAAG
oNQ1075	For - NLS:*gfp* in pPD122.13 - Adds *flp-13* tail	CTTCTTCTTTGGAGCAGTCATTGACGTCATCATGTTCGAATC
oNQ924	See Above	
oNQ925	See Above	
		P*egl-6*:ORT//SL2//dsRED
oNQ1319	For -*egl-6* promoter	TTTCCAGAGAGAACAGAGTCC
oNQ1320	Nested to oNQ1319	CCAGAGAGAACAGAGTCCTTA
oNQ1256	Rev - Stop of ORT - Adds *gpd-2/3* 3'UTR tail	GGGTCCTTTGGCCAATCCCGGCCGCTTAGATACTCGTAGAACACACACTTAG
oNQ1257	For - *gpd-2/3* 3'UTR in pRL304 - Adds ORT tail	TGGCATCTACTGGTGTGTGTTCTACGAGTATCTAAGCGGCCGGGATTGGCCAAAG

Oligo Name	Description	Sequence
oNQ1240	Rev - *unc-54* 3'UTR in pRL304	GGGAAGCGAGAAGAATCATAATG
oNQ1241	Nested to oNQ1240	GAGAAGAATCATAATGGGGAAG
		P*ida-1*:ORT//SL2//dsRED
oNQ44	See Above	
oNQ1254	For - Start of *ida-1* - Adds ORT tail	GCAGATCACCAGGATTATTATTTGGAACACCATGCGGATGACACAGAGATGCGG
oNQ1255	For - Start of ORT - Adds *ida-1* tail	CCGCATCTCTGTGTCATCCGCATGGTGTTCCAAATAATAATCCTG
oNQ1256	See Above	
oNQ1257	See Above	
oNQ1240	See Above	
oNQ1241	See Above	
		P*ida-1*:gCAMP6//SL2//dsRED
oNQ44	See Above	
oNQ663	See Above	
oNQ1233	For - Start of gCAMP6 in pRL304 - Adds *ida-1* tail	TCATCCGCATGCGCTTCTTCATGGTGTTCCAAATAATAATCCTGG
oNQ1234	Rev - Start of *ida-1* - Adds gCAMP6 tail	CCAGGATTATTATTTGGAACACCATGAAGAAGCGCATGCGGATGAC
oNQ1240	See Above	
oNQ1241	See Above	
		P*ida-1*:ChR2:*yfp*
oNQ44	See Above	
oNQ663	See Above	
oNQ1363	Rev - Start of *ida-1* - Adds ChR2 tail	CAGGGCGCCTCCATAATCCATGCGGATGACACAGAGATGCGG
oNQ1364	For - Start of Chr2 - Adds *ida-1* tail	CCGCATCTCTGTGTCATCCGCATGGATTATGGAGGCGCCCTG
oNQ472	See Above	
oNQ771	See Above	

Supplementary Table 1: Oligonucleotides used in this study. Most DNA construct was made using overlap-extension PCR, as previously described [S1]. Related to **Figure 1-4**.

Supplemental Experimental Procedures

Animal husbandry and strains

Animals were cultivated on NGM agar and fed the OP50 *E.coli* derivative strain DA837 [S2]. The following strains were used in this study: **N2** (Bristol), **EG4322** *ttTi5605 II; unc-119(ed3)III*, **IB16** *ceh-17*(*np1*)I, **TM2427** *flp-13*(*tm2427*)IV, **PS5009** *pha-1*(*e2132ts*)III; *him-5*(*e1490*)V; *syEx723*[P*hsp-16.2*:*lin-3*, P*myo-2*:*gfp*,*pha-1*(+)], **NQ566** *qnEx300*[P*flp-13*:NLS:*gfp*; P*ida-1*:mCherry; P*unc-122*:*gfp*], **NQ570** *qnIs303*[P*hsp-16.2*:*flp-13*, P*hsp-16.2*:*gfp*, P*rab-3*:*mCherry*], **NQ587** *flp*-13(*tm2427*)IV; *syEx723*[P*hsp-16.2*:*lin-3*,P*myo-2*:*gfp*, *pha-1*(+)], **NQ601** *flp-13*(*tm2427*)IV; *qnEx310*[P*ida-1*:*flp-13*; P*rab-3*:mCherry], **NQ602** *flp-13*(*tm2427*), **NQ705** *qnEx373*[P*ida-1*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ706** *qnEx374*[P*ida-1*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ707** *qnEx375*[P*ida-1*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ717** *flp-13*(*tm2427*)IV; *qnEx385*[P*ida-1*:*flp-13*; P*myo-2*:mCherry], **NQ746** *qnEx408*[P*egl-6*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ747** *qnEx409*[P*egl-6*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ748** *qnEx410*[P*egl-6*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ749** *qnEx411*[P*egl-6*:Ort//SL2//dsRED; P*myo-2*:*gfp*], **NQ750** *qnEx412*[P*ida-1*:Ort//SL2//dsRED; P*ida-1*:*GCaMP6*, *rol-6*(+)], **NQ751** *qnEx412*[P*ida-1*:Ort//SL2//dsRED; P*ida-1*:*GCaMP6*, *rol-6*(+)], **NQ752** *qnEx412*[P*ida-1*:Ort//SL2//dsRED; P*ida-1*:*GCaMP6*; *rol-6*(+)], **NQ761** *lite-1*(*ce314*)X; *qnEx420*[P*ida-1*:ChR2; P*myo-2*:mCherry], **NQ782** *flp-13*(*tm2427*)IV; *lite-1*(*ce314*)X; *qnEx420*[P*ida-1*:ChR2; P*myo-2*:mCherry]

The *flp-13(tm2427)* mutant, which was obtained from the national BioResource Center in Japan (PI: Shohei Mitani), was crossed three times to the wild-type N2 strain to create the strain NQ602.

Molecular biology, transgenics and integrations

DNA constructs were constructed using overlap-extension polymerase chain reaction (PCR), as previously described [S1]. Oligonucleotides used to make each construct are listed in **Table S1**.

The Ort and GCaMP6 constructs were made by first amplifying promoter sequences (of either *egl-6* or *ida-1*) from genomic DNA; the Ort cDNA from the plasmid pUAST-Ort [S3]; and the GCaMP6 sequence, *gpd-2*/*3* 3'UTR operon, dsRED, and *unc-54* 3'UTR all from pLR304 (A gift from Luis Rene Garcia). The relevant DNA fragments were then fused together by PCR. Other constructs were made by amplifying sequences from genomic DNA, GFP from the vector pPD95.75 (Addgene), NLS:gfp from the vector pPD122.13 (Addgene), mCherry from pCFJ90 (Addgene), and the heat-shock promoter from the vector pPD49.83 (Addgene).

Transgenic animals were created by microinjection [S4] using a Leica DMIRB inverted DIC microscope equipped with an Eppendorf Femtojet microinjection system. Either the wild-type strain N2 or the *unc-119* mutant strain EG4322 animals were injected with 2–50 ng/µl of each construct in combination with one of the following injection markers: 5ng/µl pCFJ90 (P*myo-2:mCherry*), 5ng/µl pPD118.33 (*Pmyo-2*:*gfp*), or 50–100ng/µl of P*unc-122*:gfp or pCFJ104 (P*rab-3*::*mCherry*). The DNA mix was adjusted to a final concentration of 150 ng/µl by adding 1 kb DNA ladder (New England Biolabs) or pCFJ151 (*unc-119*(+)). The integrated transgene *qnIs303* were constructed as previously described by UV irradiation of strains carrying the extrachromosomal transgene *qnEx303* [S5]. The integrated strain was out-crossed to the wild-type N2 strain four times before analysis.

Microscopy and fluorescence including calcium imaging

For GFP and differential interference contrast imaging, animals were mounted on 5% agar pads, immobilized with 15mM levamisole and observed through a 63X or 100X oil-immersion objective lens on a Leica DM5500B microscope. Leica LAS software was used to capture and analyze images.

Egg-retention assays

Both wild-type animals and four transgenic lines (NQ746, NQ747, NQ748, NQ749) were cultivated on agar plates that lacked peptone from the time of hatching and fed the *E. coli* bacterial strain DA837. To sustain animal growth, the plates were streaked with DA837 that had been grown as a lawn on other NGM plates, which did contain peptone. Worms were selected as L4s on the day prior to their analysis in the adult stage. First-day old adults were individually placed onto NGM plates lacking peptone, half of which were supplemented with 50 mM histamine and half of which were supplemented with 50 mM NaCl. After six hours of incubation at room temperature, the worms were placed on a glass slide in a droplet of buffer and cut open with a razor blade to release the eggs. Then the eggs were counted with the aid of a stereomicroscope.

Feeding quiescence after Heat exposure

On the day prior to the experiments, 15-25 L4 animals were transferred to Petri dishes containing 12 mL of NGM 1.7% agar seeded with DA837 bacteria. Two different experimental approaches were used to heat stress first day adult animals. Protocol 1: On the day of the experiment, the plates housing the worms were wrapped in parafilm and submerged in a 35˚C water bath for 30 minutes. During a single experiment, the various genotypes were staggered with regards to the time that they entered and exited the water bath. After removing the plates from the water bath, the worms were observed at room temperature (21-23°C) every 10 minutes for 60 minutes for the presence of pharyngeal pumping using a Leica MZ16 stereomicroscope at magnifications of at least 50X. Protocol 2: In experiments assessing the effect of exposure temperature on feeding quiescence, animals were subjected to precisely 30 minutes of heat at a specific temperature. To control the exposure temperature and duration, seeded NGM agar plates were pre-heated to the desired temperature for 20 minutes by submerging them in a water bath. Room temperature (21-23°C) first day adults were transferred to the pre-heated plates and immediately submerged in a water bath set at the same temperature for 30

minutes. Immediately after the heat exposure, the worms were transferred to room temperature plates seeded with bacteria and then assessed every 10 minutes for 60 minutes for the presence of pharyngeal pumping.

In all cases, Statistical comparisons were made between genotypes tested simultaneously. Experiments were performed by investigators blinded to the genotypes of the animals.

When using Ort, wild-type animals and three transgenic lines (NQ705, NQ706 and NQ707) were grown in the absence of histamine on NGM plates lacking peptone and then transferred to plates with or without histamine (50mM) as L4 larvae the day before analysis. Plates that lacked histamine were supplemented with 50mM NaCl in order to balance the osmolarity with that of the histamine plates. For all other experiments, animals were grown on and transferred to standard NGM growth plates.

Locomotion quiescence after Heat exposure

To measure locomotion quiescence, we monitored first day adult worms cultivated on an agar surface in concave polydimethylsiloxane (PDMS) wells seeded with DA837 [S6, S7] following heat exposure. First, NGM plates were preheated in a water bath for 20 minutes to the desired heat exposure temperature. Animals grown on room temperature plates were transferred to the pre-warmed plates and heat shocked for 30 minutes. Following heat shock, single worms were transfered to the agar surface within a PDMS well. The loaded microchip was placed in a 50 ml Petri dish, along with a wet Kimwipe, to prevent desiccation, and then the Petri dish was closed and wrapped with Parafilm. Using a USB 2.0 Monochrome Industrial Camera (The Imaging Source®), dark-field images were taken every ten seconds for 90 minutes. Images were analyzed with the frame subtraction algorithm [S8-S10] using custom MATLAB software. For the experiments using ORT, the agar was supplemented with 50mM histamine.

FLP-13- and LIN-3-induced quiescence

To over-express *lin-3*, we used the strain PS5009 [S11], which we crossed into the *flp-13*(*tm2427*) background. In each genetic background – wild-type and *flp-13*(*tm2427*) – transgenic hs:*lin-3* animals were exposed to a 30-minute heat shock at 33°C by wrapping plates of first-day old adults in Parafilm and submerging in a water bath. After a 2-hour recovery in a 20˚C incubator, they were assessed for feeding quiescence. When comparing *lin-3* over-expression in the *flp-13*(*tm2427*) background with a background where *flp-13* was rescued in the ALA neuron, we crossed NQ587 animals with NQ601 and analyzed the F1 progeny. Those carrying the extrachromosomal array *qnEx310* displayed red fluorescence in the nervous system and were compared to animals that did not carry *qnEx310* and were therefore non-red. The over-expression analysis was carried out as described above.

To over-express *flp-13,* we heat shocked NQ570 animals, which carry an integrated array (*qnIs303)* expressing *flp-13* from the inducible heat-shock promoter from the gene *hsp-16.2*. We exposed animals to a 30-minute heat shock at 33°C and then assessed for feeding and locomotory quiescence every hour for 8 hours.

Quantitative PCR

First-day adult animals were collected prior to heat shock and then at various time intervals (0, 15, 30, 45, 60, 120, and 180 minutes) following a 37˚C heat shock for 30 minutes. Total RNA was collected from each group of worms using an RNAeasy mini kit (Qiagen), and cDNA was synthesized using the SuperScript one-step RT-PCR system (Invitrogen). We performed three or more biological replicates in each experiment, and for each biological replicate we used the average of two technical real-time PCR replicates. Real-time PCR was performed using Taqman Gene Expression Mastermix on an Applied Biosystems 7500 platform at the core services within the Penn Center for AIDS Research, an NIH-funded program (P30 AI 045008). Oligonucleotides used are listed in **Table S1.** Relative mRNA was determined by the delta-delta method [S12] by normalization to the expression of the gene *pmp-3*, which has been shown to show little expression variance [S13].

Measuring locomotion in response to heat

To determine that each genotype was not defective in their ability to sense heat we transferred worms from each genotype (*wild type*, *ceh-17*(-), and *flp-13*(-)) from room temperature plates to either room temperature plates or plates that were pre-heated in a water bath to 37°C. Body bends were counted for one minute immediately following transfer.

Optogenetic experiments

Two days prior to the experiment, NGM agar plates were fully seeded with a bacterial suspension containing all-trans-retinal (ATR) at a concentration of 2mM. Control plates were seeded with a bacterial suspension that lacked ATR. On the day prior to the experiment, fourth larval stage animals were transferred individually onto these seeded NGM plates. During the placement of the animals on the plates, the animals were briefly exposed to white light but then quickly placed in complete darkness overnight in a 20ºC incubator. First day adult animals were exposed to red light for one minute, then blue light for one minute, and then red light again for one minute. Either pharyngeal pumping or anterior body bends were counted during each of the three one-minute intervals. Data collected for each animal for each experimental condition were averaged. Note that because the animals were not stimulated mechanically and were in the presence of bacteria, their baseline locomotion was significantly slower than that of animals that had been stimulated mechanically in the absence of bacteria (e.g. Figure S3).

Supplemental References

S1. Nelson, M.D., and Fitch, D.H. (2011). Overlap extension PCR: an efficient method for transgene construction. Methods in molecular biology *772*, 459-470.

S2. Davis, M.W., Somerville, D., Lee, R.Y., Lockery, S., Avery, L., and Fambrough, D.M. (1995). Mutations in the Caenorhabditis elegans Na,K-ATPase alpha-subunit gene, eat-6, disrupt excitable cell function. The Journal of neuroscience : the official journal of the Society for Neuroscience *15*, 8408-8418.

S3. Liu, W.W., and Wilson, R.I. (2013). Transient and specific inactivation of Drosophila neurons in vivo using a native ligand-gated ion channel. Current biology : CB *23*, 1202-1208.

S4. Stinchcomb, D.T., Shaw, J.E., Carr, S.H., and Hirsh, D. (1985). Extrachromosomal DNA transformation of Caenorhabditis elegans. Molecular and cellular biology *5*, 3484-3496.

S5. Mello, C., and Fire, A. (1995). DNA transformation. Methods in cell biology *48*, 451-482.

S6. Yu, C.C., Raizen, D.M., and Fang-Yen, C. (2014). Multi-well imaging of development and behavior in Caenorhabditis elegans. Journal of neuroscience methods *223*, 35-39.

S7. Nelson, M.D., Trojanowski, N.F., George-Raizen, J.B., Smith, C.J., Yu, C.C., Fang-Yen, C., and Raizen, D.M. (2013). The neuropeptide NLP-22 regulates a sleep-like state in Caenorhabditis elegans. Nature communications *4*, 2846.

S8. Belfer, S.J., Chuang, H.S., Freedman, B.L., Yuan, J., Norton, M., Bau, H.H., and Raizen, D.M. (2013). Caenorhabditis-in-drop array for monitoring C. elegans quiescent behavior. Sleep *36*, 689-698G.

S9. Zimmerman, J.E., Raizen, D.M., Maycock, M.H., Maislin, G., and Pack, A.I. (2008). A video method to study Drosophila sleep. Sleep *31*, 1587-1598.

S10. Raizen, D.M., Zimmerman, J.E., Maycock, M.H., Ta, U.D., You, Y.J., Sundaram, M.V., and Pack, A.I. (2008). Lethargus is a Caenorhabditis elegans sleep-like state. Nature *451*, 569-572.

S11. Van Buskirk, C., and Sternberg, P.W. (2007). Epidermal growth factor signaling induces behavioral quiescence in Caenorhabditis elegans. Nature neuroscience *10*, 1300-1307.

S12. Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402-408.

S13. Hoogewijs, D., Houthoofd, K., Matthijssens, F., Vandesompele, J., and Vanfleteren, J.R. (2008). Selection and validation of a set of reliable reference genes for quantitative sod gene expression analysis in C. elegans. BMC Mol Biol *9*, 9.