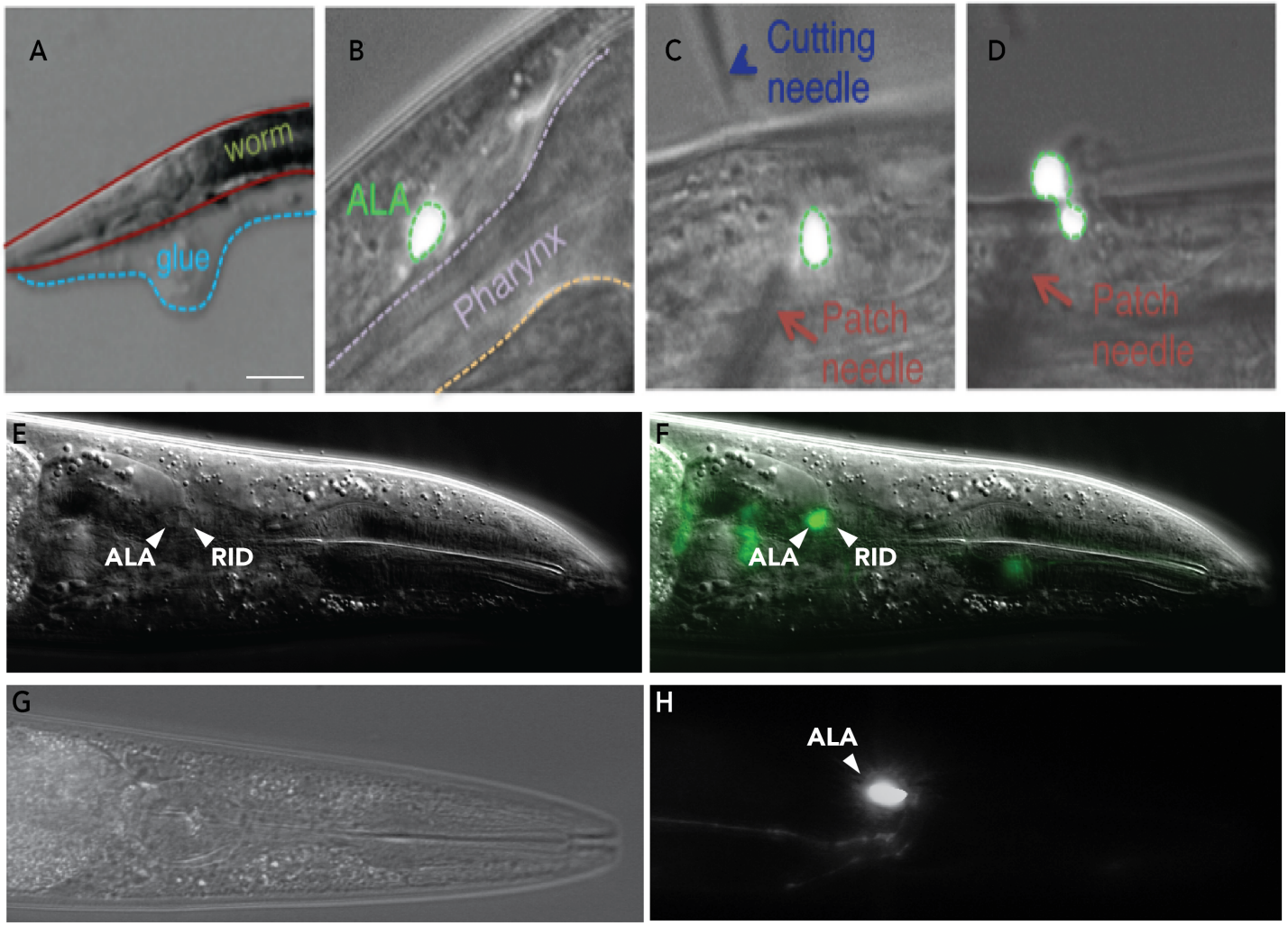

Figure S1, related to Figure 1. *flp-24* and *nlp-8* are expressed in ALA.
(**A-D**) Microdissection of the ALA neuron from mid-L4 larva. (**A**) A mid-L4 larvae (labeled "worm") was attached to a freshly made agar pad with dental glue along the ventral bodyline [S1]. (**B**) The ALA neuron was identified as dorsal to the pharyngeal isthmus and labeled with the *ceh-14* promoter driving GFP expression in the ALA neuron (green circle), the only dorsal head neuron expressing $P_{ceh\text{-}14}$*::gfp*. (**C**) A fine glass cutting needle (blue arrowhead) was used to cut open the dorsal worm body close to the vulva to release body pressure (not shown), and a small puncture was made in the dorsal head just big enough to release the ALA neuron. (**D**) A glass patch needle (red arrow) was used to collect the released ALA neuron. (**E**) DIC image of the ALA and RID neurons (white arrows). As previously reported, ALA is posterior to RID [S2]. (**F**) Overlay of *nlp-8 gfp* reporter expression on DIC image. *nlp-8* is expressed in ALA, as well as other neurons in the head [S3]. (**G**) Bright-field image of the ALA neuron. (**H**) *flp-24 gfp* reporter expression in ALA (white arrow) was indicated by GFP in young adult *C. elegans*. The ALA neuron is located dorsal to the pharynx between the anterior and posterior pharyngeal bulbs. Anterior is right. Dorsal is up. Scale bar represents 20 μm.

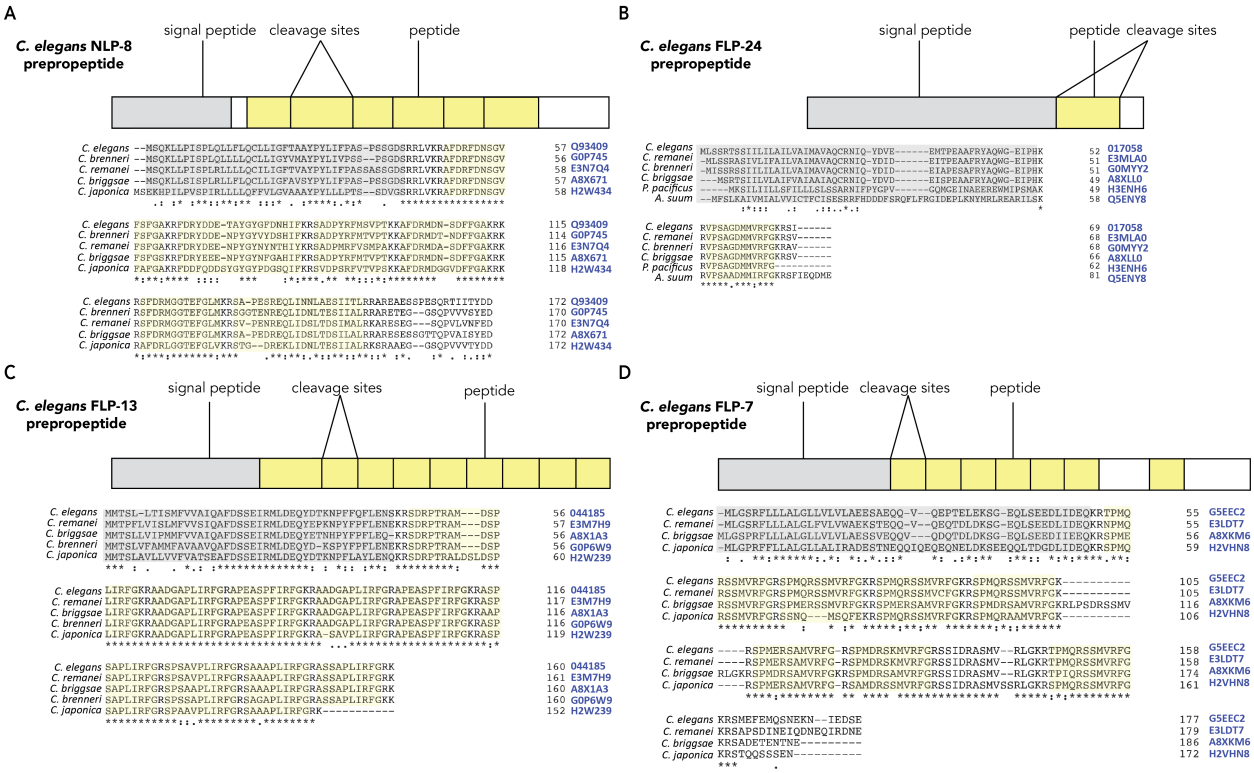

Figure S2, related to Figure 1. Propeptides of NLP-8, FLP-24, FLP-13, and FLP-7.

(**A**) *nlp-8* encodes a propeptide that generates six candidate mature neuropeptides (P1-P6). Previous publications indicated P1, P4, and P5 as candidate peptides [S3]. We propose that it is also possible for P2, P3, and P6 to serve as neuropeptides. Shown are amino acid sequences of NLP-8 in nematodes: *Caenorhabditis elegans* (Q93409), *Caenorhabditis brenneri* (G0P745), *Caenorhabditis remanei* (E3N7Q4), *Caenorhabditis briggsae* (A8X671), and *Caenorhabditis japonica* (H2W434). (**B**) *flp-24* encodes a propeptide that generates one mature neuropeptide. Shown are amino acid sequences of FLP-24 in nematodes: *Caenorhabditis elegans* (017058), *Caenorhabditis remanei* (E3MLA0), *Caenorhabditis brenneri* (G0MYY2), *Caenorhabditis briggsae* (A8XLL0), *Pristionchus pacificus* (H3ENH6), and *Ascaris suum* (Q5ENY8). (**C**) *flp-13* encodes a propeptide that generates nine mature neuropeptides (P1-P9): P2 and P4, P3 and P5 are repeated copies. Shown are amino acid sequences of FLP-13 in nematodes: *Caenorhabditis elegans* (O44185), *Caenorhabditis remanei* (E3M7H9), *Caenorhabditis briggsae* (A8X1A3), *Caenorhabditis brenneri* (G0P6W9), and *Caenorhabditis japonica* (H2W239). (**D**) *flp-7* encodes a propeptide that generates seven mature neuropeptides (P1-P7): P1 and P7 are repeated copies, as well as, P2, P3, and P4 are repeated copies, in confirmation of previous work [S4]. Shown are amino acid sequences of FLP-7 in nematodes: *Caenorhabditis elegans* (G5EEC2), *Caenorhabditis remanei* (E3LDT7), *Caenorhabditis briggsae* (A8XKM6), and *Caenorhabditis japonica* (H2VHN8). Neuropeptide annotation and sequence alignment were conducted via the *www.uniprot.org* alignment web server. Signal peptide: grey box; cleavage site: horizontal black line; neuropeptide: yellow boxes. "*" fully conserved residue, ":"strongly similar properties, and "." weakly similar properties.

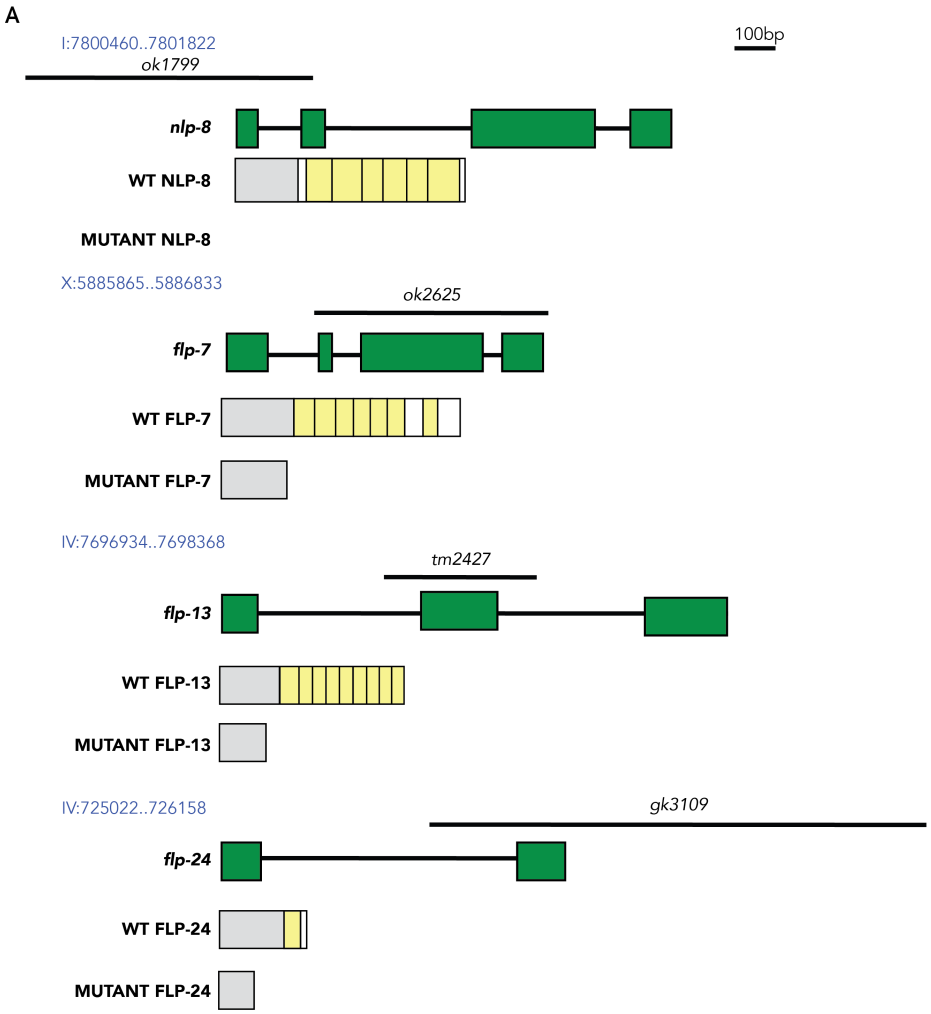

Figure S3, related to Figure 2. **Gene model with deletions in mutant alleles.**
Shown are gene models of *nlp-8, flp-7, flp-13*, and *flp-24* indicating the positions of the relevant deletion mutations, along with the structures of the wild-type proteins and the predicted mutant proteins, with domains annotated as in **Figure S2**. Horizontal black bars labeled with allele numbers indicate genomic deletions and green blocks represent exons of coding genes. Grey boxes indicate signal peptide and yellow boxes indicate mature neuropeptides. Orientation of genes and protein structures are 5' to 3', and N-terminal to C-terminal, respectively. Genomic positions are provided for each gene in blue.

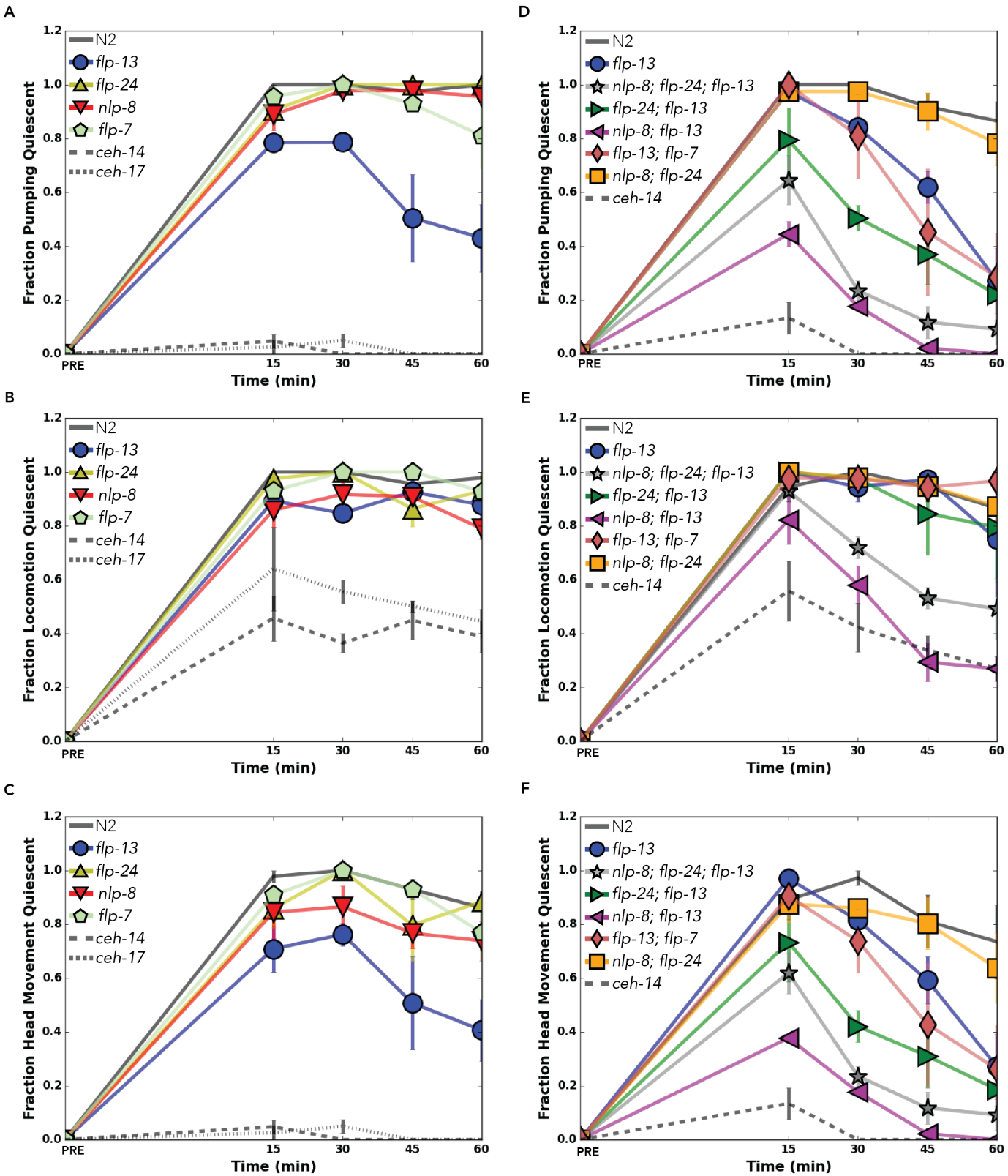

Figure S4, related to Figure 2. Variability in waking times post-heat shock of single, double, and triple neuropeptide mutants. (**A-F**) The fraction of single, double, and triple mutants pumping, locomotion, and head movement quiescent before (PRE) and up to one hour after heat shock. Behavior was scored at 15 minute intervals after heat shock. (**A**) & (**D**) *C. elegans* were scored as quiescent for pumping if there was no pumping during 10 seconds of observation. (**B**) & (**E**) *C. elegans* were scored as quiescent for locomotion if there was no centroid movement during 10 seconds of observation. (**C**) & (**F**) *C. elegans* were scored as quiescent for head movement if there was no head movement in the dorsal-ventral directions during 10 seconds of observation. Data represents the fraction of animals quiescent from three independent assays, where n≥33 *C. elegans* for each strain. Data shown as mean ± SEM.

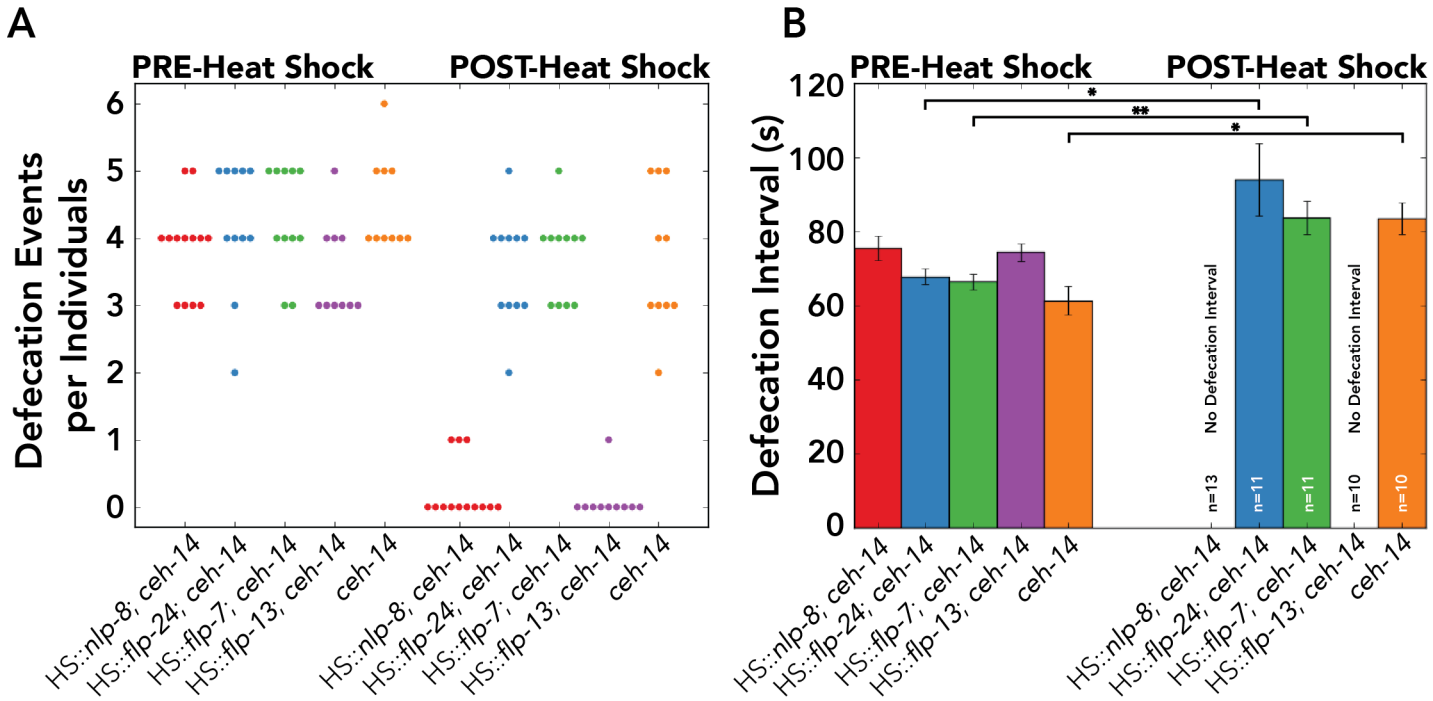

Figure S5, related to Figure 5. FLP-13 and NLP-8 reduce the total number of defecation events.
(**A**) Total number of defecation events for individuals five minutes before (PRE) and one hour after (POST) heat shock. Each dot represents the total number of defecation events for an individual during five minutes of observation. Overexpression of either *flp-13* or *nlp-8* inhibited defecation. (**B**) The time between defecation events represents the defecation interval. *ceh-14* animals exhibited a longer defecation interval post-heat shock compared to pre-heat shock (*ceh-14*: 61.3±3.9 seconds pre-heat shock compared to 83.5±4.3 seconds post-heat shock). No significant difference was observed post-heat shock between *ceh-14* and animals overexpressing *flp-24* or *flp-7* (HS::*flp-24, ceh-14*: 94.0±9.7 seconds post-heat shock; HS::*flp-7, ceh-14*: 83.7±4.5 seconds post-heat shock; compared to *ceh-14*: 83.5±4.3 seconds post-heat shock; $p \geq 0.4$). (**B**) Data shown as mean±SEM; n=total number of *C. elegans*; *p<0.05; **p<0.01; paired t-test.

Table S1, related to Figure 1. Protein-encoding genes detected in ALA at late-L4 stage larvae.
This table (attached as a separate Excel file) lists the expression of 8,133 genes in poly(A)$^+$ RNA isolated from two pools of ALA neurons, compared to previously determined gene expression levels in poly(A)+ RNA isolated from mixed-stage whole larvae [S1]. The data columns are as follows. "Gene" gives the full gene identifier (WormBase name, sequence name, and CGC name) of a gene; gene names were taken from WormBase release WS220. "ALA" and "larvae" denote the gene expression values (measured in RPKM) for a given gene observed either in pooled ALA neurons (this study) or in wild-type larvae [S1]. "ALA/larvae" gives the ratio of gene expression in pooled ALA neurons to gene expression in whole larvae, with an empirical pseudo-minimum for larval expression of 0.03 RPKM (the smallest non-zero RPKM value observed in the larval data set), used when no larval expression was actually observed (to avoid division by zero). "OMIM" denotes orthology to a human disease gene in the Online Mendelian Inheritance in Man database. Disease orthologies were computed by means of human-*C. elegans* orthologies in either WormBase release WS230 or in eggNOG 2.0's orthology groups, along with human gene-disease links downloaded from Ensembl (via EnsMart, using "*Homo sapiens* genes" data set GRCh37.p6) on 4/28/2012). "Protein size(s)" lists the sizes of protein products. "Protein feature" lists predicted features such as signal, transmembrane, coiled-coil, or low-complexity sequences, predicted respectively by the programs SignalP, TMHMM, Ncoils, and SEG. "TF" indicates whether a gene's product was predicted to be a transcription factor by J. Thomas, the Walhout laboratory, or the Gupta laboratory. "Bork KOG" lists orthology annotations by eggNOG 2.0. "PFAM domain" lists any such protein domains annotated in WormBase WS220. "WBPhenotype" lists any RNAi or mutant phenotypes annotated for a gene in WS220, with most phenotypes coming from mass RNAi screens. "NOT WBPheno" indicates that a gene was annotated as negative for such phenotypes in WS220. References for RNA-seq and annotation analyses are previously reported [S1]; references for phenotypic data are given in the WS220 release of WormBase.

Table S2, related to Figure 1. ALA expresses neuropeptide-encoding genes.
Five neuropeptide-encoding genes are ALA-enriched and have more than 10-fold higher expression in ALA neurons than their expression in whole larvae.

	Locomotion	Head Movement	Pumping
N2	-	-	-
flp-13	-	+	+
flp-24	-	-	-
nlp-8	-	-	-
flp-7	-	-	-
flp-24; flp-13	-	++	++
nlp-8; *flp-13*	+++	+++	+++
nlp-8; *flp-24*; *flp-13*	+++	+++	+++
flp-13; *flp-7*	-	+	+
nlp-8; *flp-24*	-	-	-
ceh-17	+++	+++	+++
ceh-14	+++	+++	+++

Table S3, related to Figure 2. Summary of neuropeptide loss-of-function results for locomotion, head movement, and pumping.

Degree of suppression of locomotion, head movement, and pumping behavior indicated: "+" weak suppression, "++" moderate suppression, and "+++" strong suppression.

Worm	N2	*flp-13*	*flp-24*	*nlp-8*	*flp-7*	*flp-24*; *flp-13*	*nlp-8*; *flp-13*	*nlp-8*; *flp-24*; *flp-13*	*flp-13; flp-7*	*nlp-8*; *flp-24*	*ceh-17*	*ceh-14*
1	14.7	**NR**	18.8	4.9	51.8	**NR**	7.3	16	1.5	38.5	1.2	3.4
2	12.0	12.3	45.0	2.4	10.1	14.3	14.2	1.0	17.1	15.8	2.0	1.0
3	16.0	50.5	40.9	18.6	14.4	16.2	11.4	1.4	29.0	26.0	1.0	2.2
4	16.0	1.0	15.2	28.8	**NR**	1.4	1.2	12.0	8.6	38.5	2.0	1.8
5	18.4	34.0	36.4	26.2	18.4	7.2	1.4	1.1	**NR**	1.0	8.5	1.9
6	26.3	14.9	11.6	8.6	**NR**	1.0	2.2	3.6	**NR**	13.7	2.7	16.6
7	33.1	**NR**	9.5	26.6	38.0	1.2	27.6	12.8	7.2	24.4	1.2	1.7
8	20.0	7.5	8.0	11.0	**NR**	11.1	9.1	3.8	24.9	7.3	11.5	2.5
9	31.3	2.9	6.4	21.0	9.0	1.7	2.4	1.7	**NR**	21.0	31.6	1.0
10	9.5	9.3	18.7	8.8	20.0	14.5	1.1	1.6	2.0	8.0	2.0	4.0

Table S4, related to Figure 3. Summary of neuropeptide loss-of-function results for avoidance.
Avoidance response times of individuals to 30% 1-octanol 30 minutes after heat shock. NR = No response.

Worm	N2	*flp-13*	*flp-24*	*nlp-8*	*flp-7*	*flp-24*; *flp-13*	*nlp-8*; *flp-13*	*nlp-8*; *flp-24*; *flp-13*	*flp-13; flp-7*	*nlp-8*; *flp-24*	*ceh-17*	*ceh-14*
1	0	0	0	0	0	1	0	4	0	0	0	3
2	0	0	0	0	0	0	0	0	0	0	1	0
3	0	0	0	0	0	0	0	4	0	0	3	1
4	0	0	0	0	0	4	0	3	0	0	3	0
5	0	0	0	0	0	0	0	4	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	2
7	0	0	0	0	0	0	0	4	0	0	0	3
8	0	0	3	0	0	0	0	4	0	0	0	3
9	0	0	0	0	0	2	0	0	1	0	1	0
10	0	0	0	0	0	0	0	0	0	0	3	4
11	3					0	0	3			4	3
12	0					0	0	0			3	0
13	0					0	0	0			3	0
14	0					0	0	0			2	4
15	0					0	3	0			2	4
16	0					0	2	0			0	4
17	0					0	0	1			0	3
18	0					0	0	0			0	3
19	0					0	0	0			0	2
20	0						0	0				
21	3						0	4				
22	0						3	0				
23	0						0	5				
24	0						0	0				
25							0	0				
26							0	3				
27								3				
28								0				
29								4				
30								3				

Table S5, related to Figure 3. Summary of neuropeptide loss-of-function results for defecation.
Number of defecation events for individuals during five minutes of observation 30 minutes after heat shock-induced sleep.

Worm	**HS::*nlp-8; ceh-14***	**HS::*flp-24; ceh-14***	**HS::*flp-7*; *ceh-14***	**HS:: *flp-13; ceh-14***	***ceh-14***
1	46.8	8.8	1.2	**No Response**	3.0
2	**No Response**	8.2	1.5	**No Response**	1.5
3	**No Response**	17.3	1.0	**No Response**	1.0
4	1.4	42.8	1.5	14.4	2.2
5	22.0	44.0	1.3	**No Response**	1.0
6	23.7	40.0	2.0	**No Response**	4.0
7	22.6	23.1	1.5	26.3	1.2
8	**No Response**	**No Response**	2.0	**No Response**	1.2
9	12.3	10.8	1.7	**No Response**	0.9
10	**No Response**	**No Response**	1.4	**No Response**	1.0
11	**No Response**	18.2	1.5	**No Response**	1.5

Table S6, related to Figure 6. Time required for an avoidance response and number of non-responders after overexpression of FLP-24, FLP-7, FLP-13, and NLP-8.

Avoidance response times of individuals to 30% 1-octanol one hour after heat shock induced overexpression of neuropeptide genes.

Supplemental Experimental Procedures

Single ALA neuron dissection and transcriptome profiling

Individuals from strain TB513 (P_{ceh-14}*::gfp*) at the mid-L4 larval stage were hand-picked and glued on an agar pad for microdissection as previously described [S1] using the approach of Lockery and Goodman [S5] for neuronal dissection. GFP-tagged ALA neurons were individually collected with an unpolished patch-clamp tube that served as a pipette, transferred to a prelubricated microcentrifuge tube (**Figure S1A-D**), and snap-frozen with liquid nitrogen. Frozen tubes containing individual ALA neurons were kept at -70°C until their RNA was amplified as described by Schwarz et al. [S1], using the approach of Dulac and Axel [S6]. RT-PCR, RNA-seq, and computational analysis of individual neurons were done as in Schwarz et al. [S1]. To obtain RNA-seq data by Illumina sequencing, aliquots of RT-PCR from individual cells were collected into two pools (four cells and five cells). All RNA-seq reads were single-end, and originally 50 nt in length. Raw reads were quality-filtered as in Schwarz et al. [S1]. They were then truncated *in silico* from 50 nt to 38 nt, the read length for previously published control data from mixed-stage whole larvae [S1]. This truncation allowed the ALA reads to be mapped and quantitated using exactly the same pipeline that had been used for larval data, and thus allowed more exact comparisons between ALA and larvae. After quality filtering and truncation but before mapping, RNA-seq data from the two pools of wild-type ALA comprised 1,164,892,280 nt in 30,655,060 reads and 1,520,526,262 nt in 40,013,849 reads. Of these, 25.2% could be mapped to WS190 protein-coding gene models (i.e., 17,798,207 out of 70,668,909 reads). This relatively low rate is consistent with our previous observations in single-cell RNA-seq of linker cells, in which we found that human cDNA (probably acquired as human RNA during the manual dissection of individual *C. elegans* cells), linkers, and unmappable reads comprised a significant fraction of the final RT-PCR products [S1]. We used existing whole wild-type larval RNA-seq data [S1] as controls for housekeeping versus ALA-enriched genes. Expression values for genes were computed as in Schwarz et al. [S1]. They were defined by pooling reads from both mid-L4 ALA neuron sets into a single set of expression values, doing likewise for both whole-animal mixed-stage larval RNA-seq sets from Schwarz et al. [S1], and computing ALA/larval ratios of gene activity. We detected expression of 7,698 and 4,068 genes in the two ALA pools separately, and 8,133 genes collectively.

Data Availability

RNA-seq reads for the two pools of wild-type mid-L4 ALA neurons are available in the NCBI Sequence Read Archive (SRA), under accession number SRP038903 (*http://www.ncbi.nlm.nih.gov/sra/SRP038903*). RNA-seq reads for the two pools of whole *C. elegans* mixed-stage wild-type N2 larvae were previously published by Schwarz et al. [S1], and are available in the NCBI SRA under accession number SRA058596 (*http://www.ncbi.nlm.nih.gov/sra/SRA058596*).

Strains

Wild-type *C. elegans* strain was N2 (Bristol). Mutant strains obtained from the *Caenorhabditis* Genetics Center (CGC) including RB1990 *flp-7(ok2625) X*, and VC1309 *nlp-8* (*ok1799*) *I* were provided by the *C. elegans* Gene Knockout Project at OMRF (*http://www.mutantfactory.ouhsc.edu*). VC1971 *flp-24(gk3109) III* was provided by the *C. elegans* Reverse Genetics Core Facility at the University of British Columbia, part of the *C. elegans* Gene KO Consortium (*http://www.celeganskoconsortium.omrf.org*). Strain FX02427 *flp-13 (tm2427) IV* was obtained from the National Bioresource Project (*http://www.shigen.nig.ac.jp/c.elegans/mutants/).* Extrachromosomal arrays were: *rtEx227* (P_{nlp-8}::*gfp*) [S3], and transgenes generated in the course of this study, described below**.**

Mutant Strains and Alleles

PS6813: *flp-13(tm2427)* made from FX02427, outcrossed 3X
PS6814: *flp-24(gk3109)* made from VC1971, outcrossed 5X
PS6911: *nlp-8*(*ok1799*) made from VC1309, outcrossed 2X
RB1990: *flp-7(ok2625)*
TB528: *ceh-14(ch3)*. All references to *ceh-14* mutants refer to this allele
PS6991: *nlp-8*(*ok1799*); *flp-24*(*gk3109*) made from PS6911, PS6814
PS6994: *flp-24*(*gk3109*); *flp-13*(*tm2427*) made from PS6814, PS6813
PS6993: *nlp-8*(*ok1799*); *flp-13*(*tm2427*) made from PS6911, PS6813
PS6992: *nlp-8*(*ok1799*); *flp-24*(*gk3109*); *flp-13*(*tm2427*) made from PS6994, PS6993
PS7084: *flp-13*(*tm2427*); *flp-7(ok2625)* made from PS6813, RB1990

Transgenic Lines

Heat-shock transgenic strains:

Conditional expression of cDNAs was achieved by generating a fusion of the coding sequence of a gene under study to the *hsp-16.41* promoter [S7]. A synthetic DNA fragment consisting of the *hsp-16.41* promoter, DNA coding sequence, and each gene's endogenous 3'-UTR was generated using fusion PCR [S8]. For amplification of the coding sequence (*flp*-7, *flp-13, flp-24*, and *nlp-8* open reading frames), mixed-stage populations of wild-type animals were harvested for RNA extraction and subsequently reverse-transcribed into cDNA as previously described [S1]. Their corresponding 3'-UTR regions were amplified from wild-type mixed stage animal lysates. The *hsp-16.41* [S7] promoter region was amplified from plasmid pPD49.83 (AddGene). The fusion PCR product was verified by sequencing. Open reading frames and 3'-UTRs match the sequences of spliced transcripts as shown in WormBase (WS252). These constructs were injected with P_{myo-2}*::dsRed* as a co-injection marker and bluescript (KS+, Agilent) as carrier for construction of extrachromosomal arrays [S9], using the concentrations as indicated below.

Reporter Expression Transgenic strains:
PT4: *him-5(e1490); lin-15(n765); rtEx227[lin-15(+), P_{nlp-8}::gfp]*. [S3]
PS6896: *unc-119 (ed3); syEx1422[P_{flp-24}::GFP(25ng/ul), P_{ver-3}::mCherry (25ng/ul), unc-119(+) (50ng/ul)]*. [S10]
TB513: *dpy-20(e2017);chIs513[P_{ceh-14}::GFP, dpy-20(+)]*. [S11]

Heat-shock transgenic strains:
PS6835: *syEx1404SP$_{hsp16-41}$::flp-13(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6563: *syEx1286[P $_{hsp16-41}$::flp-24(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6571: *syEx1294[$P_{hsp16-41}$::flp-7(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6658: *syEx1323[$P_{hsp16-41}$::nlp-8(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.

Heat-shock transgenic strains in *ceh-14* background
PS6845: *ceh-14(ch3)*; *syEx1404[$P_{hsp16-41}$::flp-13(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6829: *ceh-14(ch3)*; *syEx1286[$P_{hsp16-41}$::flp-24(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6856: *ceh-14(ch3); syEx1294[$P_{hsp16-41}$::flp-7(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.
PS6830: *ceh-14(ch3); syEx1323[$P_{hsp16-41}$::nlp-8(10ng/ul), P_{myo-2}::dsRed(10ng/ul), KS+(90ng/ul)]*.

DNA Fragment	**Forward primer 5' to 3'**	**Reverse primer 5' to 3'**
flp-24 promoter	ACGCCTAACGCATGCCTCTTAC	AAAAGGCGCGCCCGATGTGCGCG ACGACAACAT
Phsp-16.41	ACGTTGAGCTGGACGGAAAT	GCTAGCCAAGGGTCCTCCT
flp-7 ORF	AGGAGGACCCTTGGCTAGC ATGCTTGGATCCCGCTTC	TCAAGGTGTTTGCATGTACTTGTT TATTCGCTGTCCTCGATGTTC
flp-7 3' UTR	GAACATCGAGGACAGCGAATAAACA AGTACATGCAAACACCTTGA	AACAGGCGTCGGTTCTTTATTT
flp-13 ORF	AGGAGGACCCTTGGCTAGCATGATGA CGTCACTGCTCACT	TTATTTTCTGCCAAAACGAATG
flp-13 3' UTR	CATTCGTTTTGGCAGAAAATAAATTC ACTTTTTGATCTTTCTTTGTGTG	CCGGATAGAACAATTCATTTTTG TGAA
flp-24 ORF	AGGAGGACCCTTGGCTAGCATGTTGT CGTCGCGCACATCGTCCATCAT	TCAGATGCTTCTTTTTCCAAATC
flp-24 3' UTR	CGATTTGGAAAAAGAAGCATCTGATA ATATACCATCTACCGGACTTCTTAT	TTTAACACACACAAAACGGTTTA TTTCTGTT
nlp-8 ORF	AGGAGGACCCTTGGCTAGCACAAAAG CGACATGAGTCAGAA	CAACGAACAATCATCACCTATGA CGATTGA
nlp-8 3' UTR	CACCTATGACGATTGAACTTCTTGAAC AACTGG	AAATGTCAGATTTTATTCACAAA CG

Behavioral assays

Behaviors were scored at 20°C. Sixteen hours before the start of the experiment, L4 larvae were picked so that only the behaviors of young adult animals were scored. Unless otherwise noted, between 10 and 25 animals were scored per assay. Pumping was conservatively scored as any movement of the pharyngeal grinder. Locomotion was scored as movement of the animal's centroid in the forward or reverse direction. Head movement was scored as any dorsal-ventral displacement of the animal's head from the posterior of the second pharyngeal bulb to the anterior tip. Pumping quiescence, locomotion quiescence, and head movement quiescence were scored by 10 seconds of direct observation of individual animals by an experimentalist that was blinded to genotype. Machine vision underestimates movement, and may conflate head movement and locomotion. Our experimental design, which used a motorized stage, also eliminated handling artifacts like dish-tap or transfer of animals that would otherwise confound experiments.

Defecation was scored as follows – individuals were placed onto a tracking microscope with 5x magnification for 5 minutes of video recording. Immediately after these recordings, worm pumping rate was scored by placement on another dissecting microscope with 55x magnification, and 10 seconds of video recording were taken. Defecation and pumping rate were manually scored by examining the 5 minute and 10 second video recordings respectively. This was done pre- and post-heat shock.

Avoidance behavior was scored as follows: individuals were placed onto a fresh and thinly seeded plate: 20 µl of saturated OP50 was spread evenly around the plate 16 hours before the experiment. Video recordings were taken on a (5x) tracking microscope. Individuals were presented with 30% 1-octanol before and one-hour after heat shock [S12,S13]. The response interval was manually scored by examining video recordings made pre- and post-heat shock. While scoring defecation and avoidance the experimentalist was not blinded to genotype.

Heat shock protocol

For all behaviors, unless otherwise specified: animals were placed onto a Petri plate containing 9 mL of NGM that was seeded only in the middle with 50 µl of saturated OP50 in LB, behaviors were scored before heat shock, and after heat shock at specified times. For all behaviors, only those animals on the OP50 lawn were scored. Petri plates were coded by a third party unless otherwise specified. Coded Petri plates were placed on a motorized stage to eliminate dish-tap and other behavior-modifying handling. The lid was taken off to prevent condensation, and obstruction of the view, but another glass was placed 2.3 cm above the plate so that gusts of wind did not affect behavior. Behavior was scored in the five minutes before heat shock. Parafilm was placed around the dish to create a waterproof seal.

Protocol for stress-induced sleep

Heat shock was used for our stress-induced sleep experiments. In particular, sealed plates were placed in a 35°C water bath for 30 minutes as in Hill et al. [S14,S15]. We found this temperature and length of heat shock most consistent for pumping quiescence, head movement quiescence, locomotion quiescence, and timing of the increased response latency. After heat shock (POST), plates were immediately placed on the motorized stage as before heat shock (PRE). In particular, the Petri dish lids were taken off, and replaced with a shielding glass. The plates were not touched for the next 60 minutes, as a motorized stage was used to prevent dish-tap artifacts. Extensive handling of animals could lead to inconsistent results. If a motorized stage is unavailable then we suggest placing Post-heat shock plates on a large glass-slide which rests on a dissecting scope. In this case, the large glass-slide could be gently moved and the behavior of individuals could be scored (this should minimize handling).

Pumping, locomotion, and head movement were scored at 15-minute intervals for 60 minutes after heat shock. We found 30 minutes after heat shock to be the most robust and consistent time point for stress-induced sleep. Avoidance behavior was scored only at 30 minutes. To score defecation events we used a 33°C water bath for 30 minutes. This protocol gave us the most consistent results and best dynamic range between N2 and *ceh-14* or *ceh-17* (**Figure 3**; **Table S5**). We scored defecation behavior 30 minutes after heat shock.

Our stress induced-sleep protocol differs from Nelson et al. [S15] in a number of ways. We handle the animals less and we concentrate on a single robust and consistent time point after heat shock (30 minutes). Further in regards to the difference in our locomotion result (**Figure 2**), we employ different methods of scoring locomotion than Nelson et al.: 1) we differentiate between head movement and locomotion, and 2) we concentrate on one time point after heat shock (30 minutes) rather than reporting the total time quiescent one hour after heat shock. We think that these two reasons, in addition to different methods of handling and strength of heat shock may account for differences in our results.

Heat shock-induced neuropeptide overexpression

Sealed plates were placed in a 33°C water bath for 30 minutes [S15,S16]. After heat shock (POST), condensation was removed from the top lid, and they were placed on the lab bench agar-side up; this was done at 20°C. After 20 minutes, the plates were placed agar-side down on a motorized stage as before heat shock (PRE). In particular, the Petri dish lids were taken off, and replaced with a shielding glass. The plates were not touched for the next 160 minutes. Our overexpression experiment uses extrachromosomal arrays which are expressed in many cells, and we assume that these cells have the machinery necessary to process the neuropeptides. We also assume that these peptides reach the right target in the right amount. It is unknown if results from these experiments are hypermorphic or neomorphic. Pumping, locomotion, and head movement was scored at 30 minute intervals for 3 hours after heat shock (POST). Avoidance and defecation were scored one hour after heat shock (POST).

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