Supplemental Data

A Self-Regulating Feed-Forward Circuit Controlling *C. elegans* Egg-Laying Behavior

Supplemental Experimental Procedures

Egg-Laying-Behavior Assays

Egg-laying assays were performed by transferring individual worms to seeded NGM plates and allowing worms to lay eggs for 2 hours before counting the number of eggs. Liquid egg-laying assays were performed in varying osmolarity conditions in 96-well plates. 100 μl of melted 2% agarose in the indicated buffer were placed in each well and allowed to dry for 1~1.5 hours before starting the behavioral assay. Individual worms were placed on the solid agarose pad in each well and after about 1 min were covered with 50 μl of the same buffer. The number of laid eggs were counted after 15 mins for low osmolarity buffer OM10 (10mM HEPES pH=7.1) or 1 hour for high osmolarity buffer M9. Egg laying rates are presented as average ± SEM.

Serotonin Treatment

10 mg/ml serotonin in 2% agarose M9 buffer was prepared the day of the experiment. 100 μl of the serotonin agarose solution was placed into 96 well plates at room temperature and were allowed to dry for 1.5 hours. Single worms were transferred onto the serotonin agarose pads and covered with 50 μl of 10 mg/ml serotonin in M9 buffer for 30 mins before Ca^{2+} imaging.

Figure S1. Simultaneous Imaging of Egg-Laying Behavior and Neural Activity

Shown are images with combined transmitted visible and epifluorescent illumination revealing both vulval morphology and fluorescence emission from cameleon-expressing neurons. One egg-laying event occurred during the third frame. To better view the egg-laying events, the contrast was adjusted as shown in the bottom row.

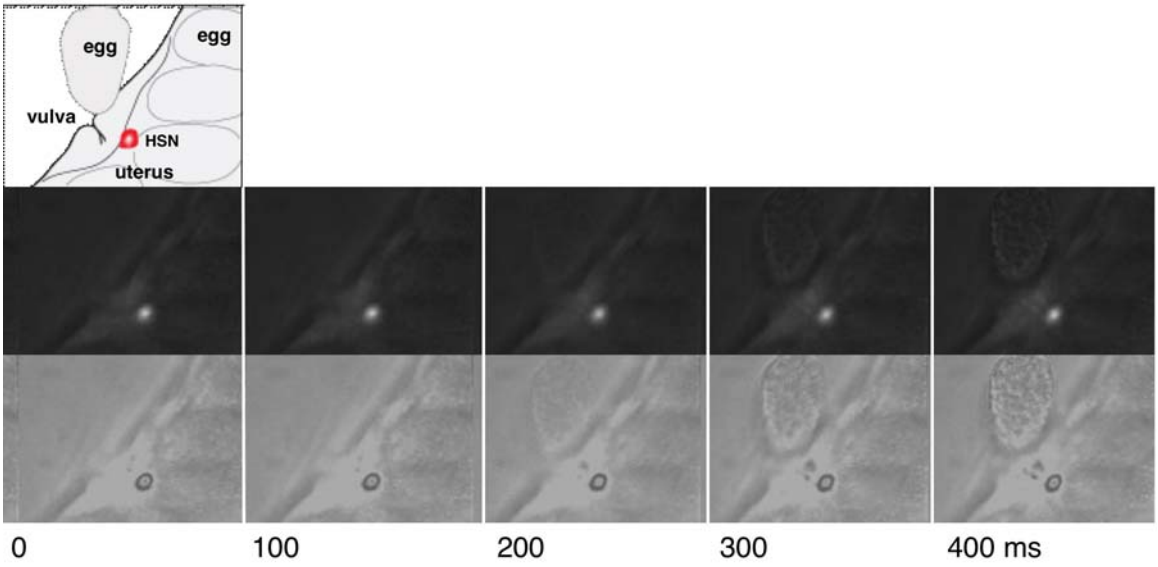

Figure S2. Vulval Muscles Are Not Essential for Motor-Neuron Activity

(A) Shown are sample traces of HSN and VC calcium transients in *egl-15* mutant animals, recorded under standard low osmolarity conditions.

(B) Histogram shows the average frequencies (± SEM) of calcium transients for the indicated cell in wild-type and *egl-15* mutant animals. The activity in the VC neurons of *egl-15* animals adapts more quickly than in wild-type, which accounts for the lower overall transient frequency.

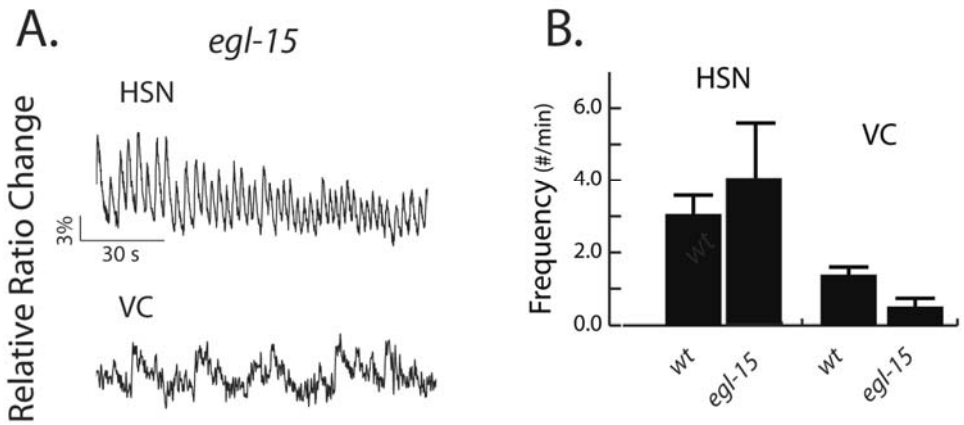

Figure S3. Effect of Osmolarity on vm1 and vm2 Calcium Transients

(A) Two vm1 or vm2 cells are imaged simultaneously in each worm, and the calcium traces are represented on the same lines. Scale bars are as indicated. r^2, the standard variation percentage indicating the degree to which the muscles of the same type are temporally correlated, is calculated and indicated. The statistical p values are indicated if significant.

(B) Histogram of spike frequencies (mean ±SEM).

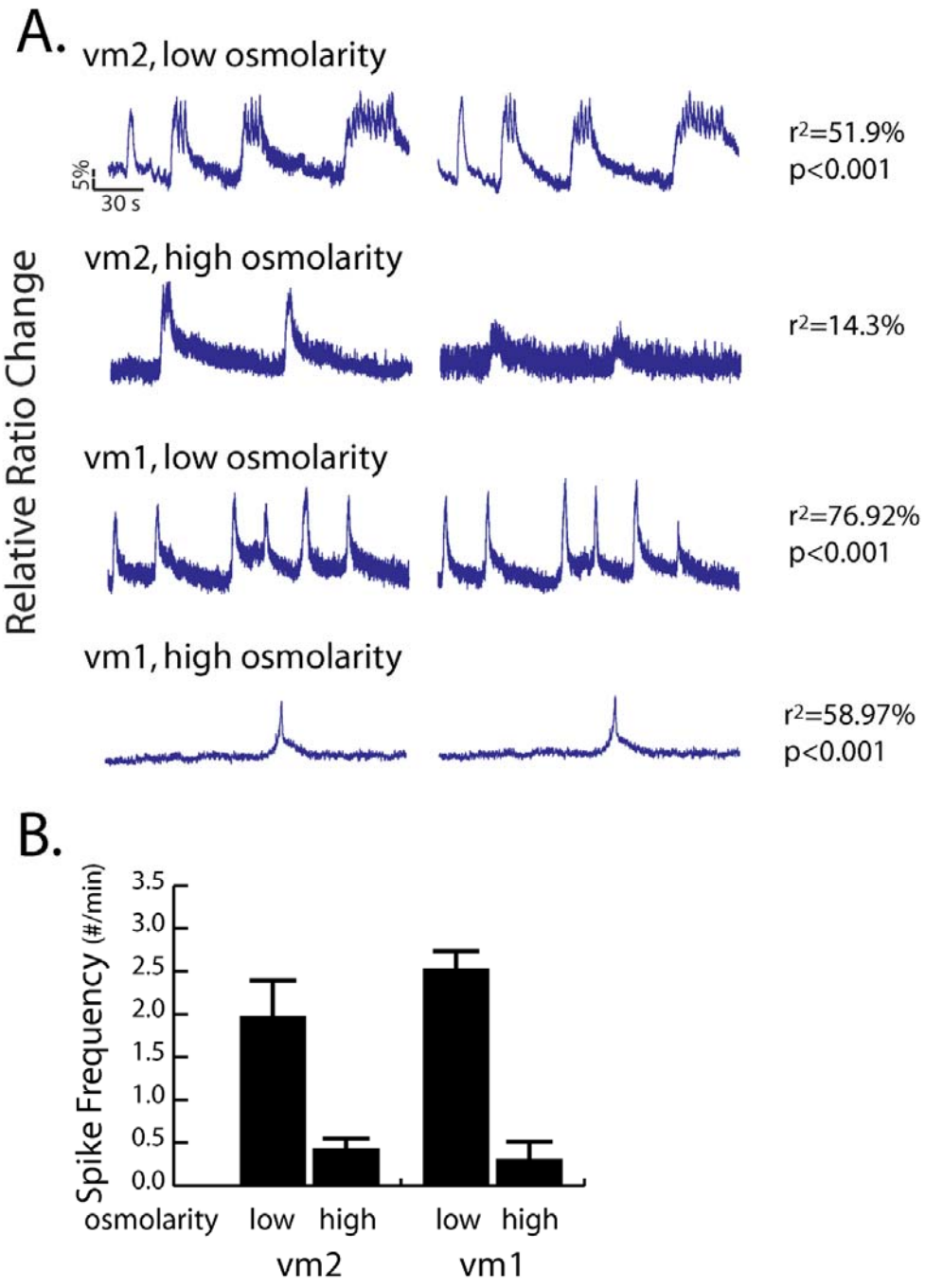

Figure S4. Vulval-Muscle Calcium Transients in Acetylcholine-Deficient Mutants

Shown are sample calcium traces of temperature-shifted *cha-1(y226)* mutant animals in standard low-osmolarity conditions.

(A) Simultaneous recording of two vm1 muscle cells in a single *cha-1* mutant animal.

(B) Simultaneous recording of two vm2 muscle cells in a single *cha-1* mutant animal.

(C) Histogram showing average ± SEM calcium-transient frequencies (compare to histogram in Figure S3).