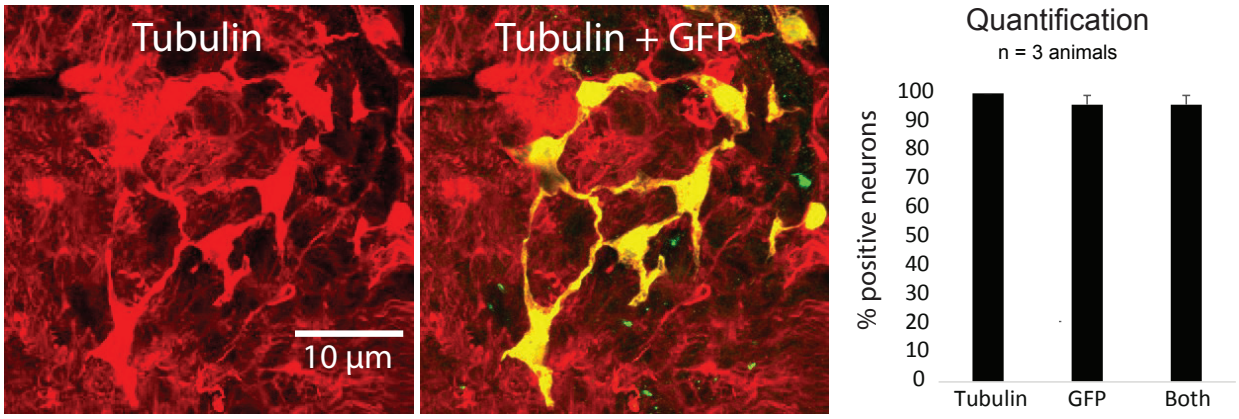

Figure S1. Percentage of transgenic neurons. Related to Figure 1
Left: Immunostaining with acetylated alpha-tubulin antibody only. Middle: Immunostaining with both GCaMP6s and acetylated alpha-tubulin antibodies. Right: Overlap between the two stainings. Error bars indicate SEM.

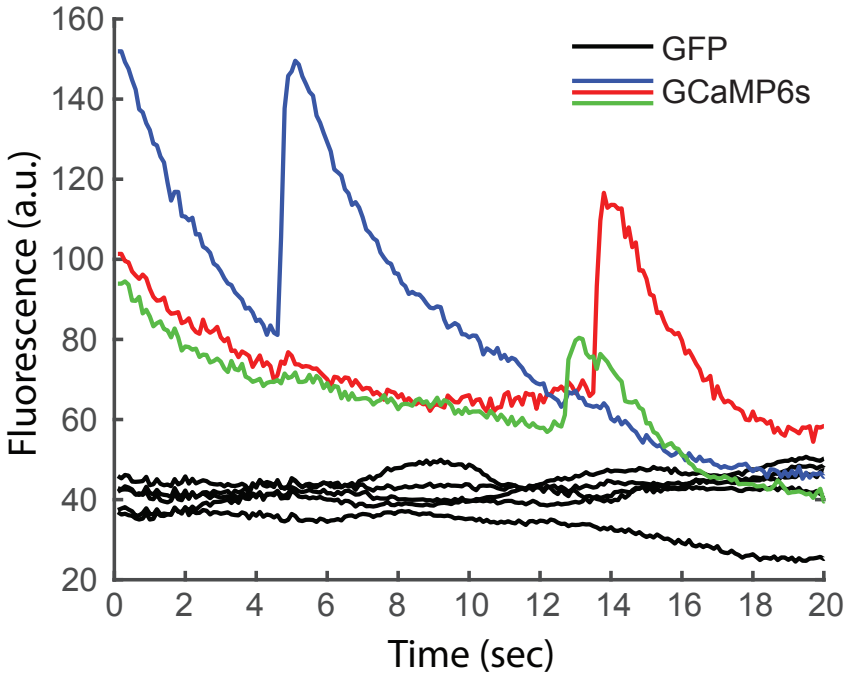

Figure S2. Fluorescence transients in cells expressing GFP compared to GCaMP6s. Related to Figure 1
The green, red and blue traces correspond to neurons expressing GCaMP6s which belong to the RP1, RP2 and CB groups, respectively. The black traces correspond to 5 neurons of another transgenic line expressing GFP in neurons. Both GFP and GCaMP6s signals were recorded during the same behavioral sequence: the last two pulses of a contraction burst, followed by about 15 seconds of elongation.

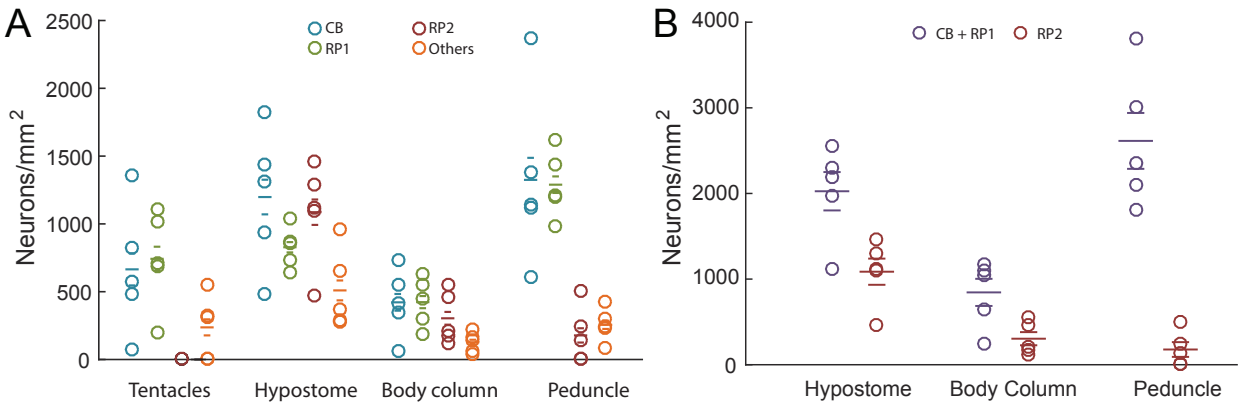

Figure S3. Distribution of neuronal density. Related to Figure 4
Neuronal density in different animal parts, according to each cell type (A) and when RP1 and CB are combined (B). A. The density of RP1 and CB neurons is higher in the hypostome and the peduncle than in the body column. The density of RP2 neurons is not significantly different between the peduncle and the body column, but higher in the hypostome. B. RP1 + CB versus RP2 give density patterns similar to the patterns reported in Epp and Tardent (1978) for ectoderm versus endoderm. n = 1023 neurons compared over 5 animals. Wide bars indicate mean, narrow bars indicate SEM.

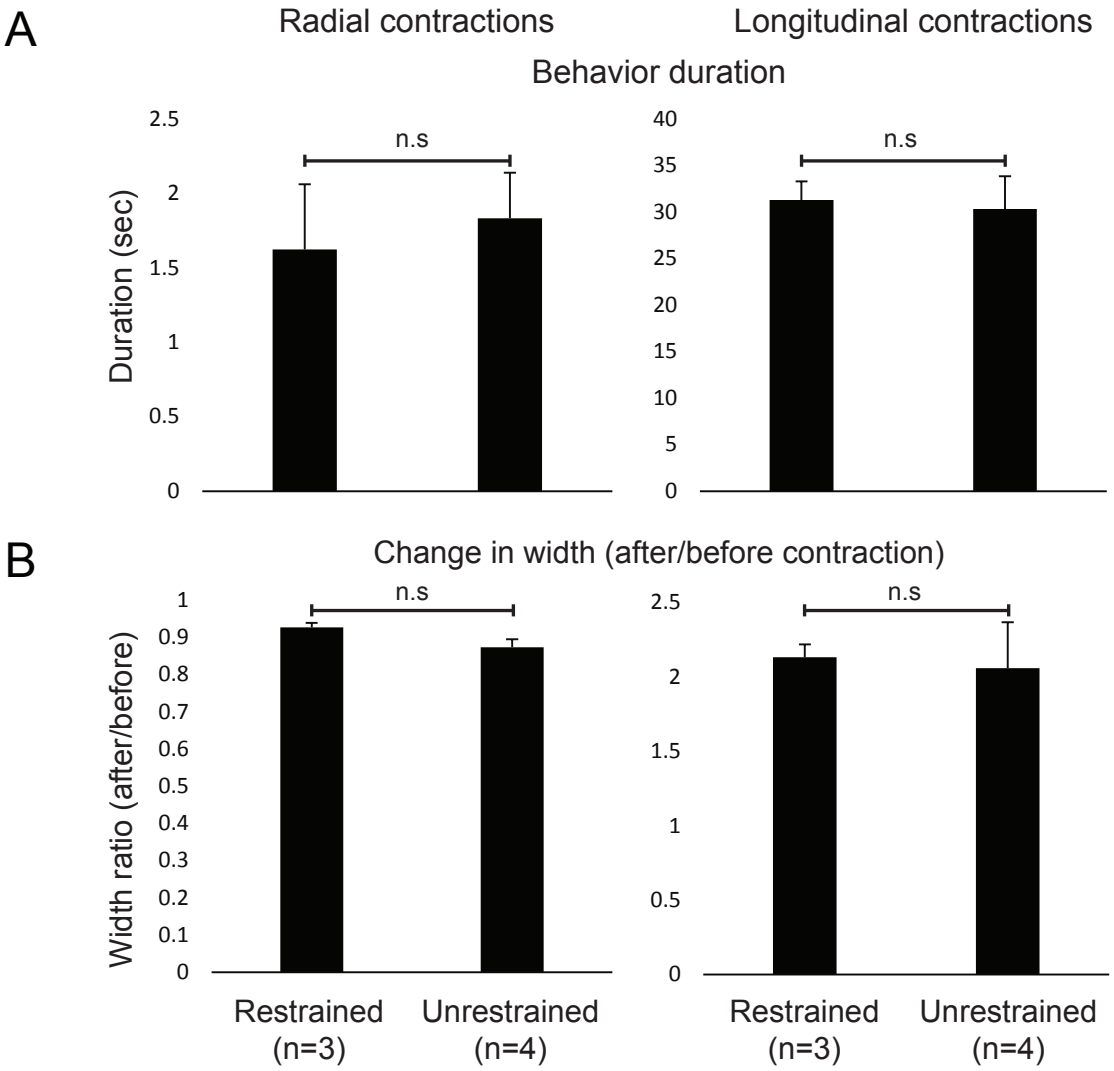

Figure S4. Differences in behavior between restrained and unrestrained preparation. Related to Figure 1
A) Duration of radial and longitudinal contractions in restrained vs unrestrained preparation. B) Change in width of the animal following radial and longitudinal contractions in restrained vs unrestrained preparation. Unpaired T-Test was used, with n.s = not significant (P>0.05).

Supplemental Experimental Procedures

Hydra maintenance

Hydra from AEP strain were maintained in the dark at 18C and were fed freshly hatched artemia nauplii once a week or more frequently when the colony needed to grow.

Transgenics

Transgenic lines were created according to [S1]. Accordingly, *Hydra* oocytes were injected with DNA (2.5 mg/mL) through a micropipet made with a pipet puller (Sutter Instruments model P-97) from borosilicate glass pipets (Sutter instruments, cat# BF100-50-10) and held with a microinjector (Narishige cat#IM-9B) controlled with a joystick micromanipulator (Narishige MN-151).

The DNA that was injected was a modified version of the pHyVec1 plasmid (Addgene cat#34789) [S2] where we replaced the GFP sequence (found between the pstl and EcoRI restriction sites) with a GCaMP6s sequence that was codon-optimized for *Hydra* (DNA2.0, Menlo Park, CA).

Just before the injection, 10 µl of DNA (2.5 mg/mL) were added to 6µl of phenol red and centrifuged for 10 min at max speed to collect debris at the bottom of the tube.

Once the eggs were injected, they were placed in the dark at 18C for 2 weeks and then put back at room temperature on the bench. After a few days, eggs started hatching and the young hatchlings were fed for a couple of days before being screened for transgenic neurons. Transgenic *Hydra* generated this way are mosaic. Therefore, we kept these animals growing and asexually reproducing, and selected the offsprings according to the amount of neurons that were expressing the transgene (a procedure known as clonal propagation). We repeated this procedure until the amount of expression reached a maximum.

Based on this procedure, we expect each transgenic cell to have the same genome since only one injection of the plasmid occurred and that every animal was obtained from the same original individual by asexual reproduction. Also, the expression is driven by an actin promoter for which the use is likely to be similar in different neurons of the same population. It is important to note that the level of expression of the transgene changes dramatically over time. Indeed, it happens frequently that the amount of fluorescence of one animal goes from very high to very low within a few hours. This could be due to the fact that the expression of actin is regulated by factors affecting the entire animal (e.g. hormones) on a slow time scale. Therefore, time-lapse recordings are only performed when the level of fluorescence is high.

Imaging

Animals were placed between two coverslips (VWR cat# 89015-724 and VWR cat#16004-094) that are separated by one 100um spacer (Grace Bio-Labs cat#654006), which allows the animal to move while preventing any part of it from getting out of focus. Imaging was performed using a fluorescence dissecting microscope (Leica M165) equipped with a long-pass GFP filter set (Leica filter set ET GFP M205FA/M165FC) and a sCMOS camera (Hamamatsu ORCA-Flash 4.0) or an Olympus IX-70 inverted microscope equipped with U-MIWIB2 GFP filter cube and an EM-CCD (Hamamatsu EM-CCD C9100-12). In both setups, illumination came from a mercury arc lamp and the software micromanager [S3] controlled the microscope.

Classification of spikes (e.g. RP1, RP2, CB) was made with the help of a home-made MATLAB script that finds fluorescence spikes and displays a snippet of the video that corresponds to that event so that the user can classify each spike as being part of RP1, RP2 or CB.

The images were visualized with Fiji. In order to extract the fluorescence of the neurons, the cells were tracked using TrackMate [S4], and the data was then handled with MATLAB (The Mathworks, Natick, MA). It was not always possible to determine whether the tracked features were cells or swellings, and it is possible that in addition to cell bodies we also tracked such swellings.

Note that in our preparation, *Hydra* were placed between two coverslips and exposed to intense blue light from the arc lamp during imaging. Therefore, the animals were subject to strong tactile and photic stimulation which could affect our data. However, the observed frequency of rhythmic potentials and contraction bursts was similar to what was observed in previous studies where the animal was not restrained [S5,S6]. This indicates that the preparation did not affect the activity of the nervous system to a noticeable level.

Pseudocoloring

Because there is no method to stain neurons according to their functional type (e.g. RP1, RP2, CB), we used pseudocoloring in a few figures in order to label them according to their group. Accordingly, pseudocolored pictures were obtained by subtracting the frame before neurons were activated from the frame during which neurons were activated. The result of this subtraction was then added, in a specific color, to the frame before neurons were

activated. In the resulting picture, the activated neurons showed up in the specific color on a grey background made of all the signal that did not come from the neurons' activation.

Statistics

Cross-correlation

Every spikes of the RP1, RP2 and CB circuit were extracted from the movies and considered as spike trains. Then, cross-correlation was computed between these trains using the MATLAB function xcorr. The time lag used for cross-correlation measurement was 250 seconds.

To compare the number of spikes before vs after egestion, we ran a two-tailed paired T-test assuming equal variance using Microsoft Excel.

Comparison of morphological parameters

We used 2-tailed, paired student T-test. We indicated averages and standard errors on histograms and in the text.

Supplemental plasmid sequence. Related to Figure 1

The pHyVec1 - GCaMP6s plasmid was made by inserting a codon-optimized (DNA2.0, Menlo Park, CA) GCaMP6s sequence between PstI and EcoRI in the pHyVec1 vector. Accordingly, the codon optimized sequence was (fasta format):

>GCaMP6s

CTGCAGCCCCGGTAGAAAAAATGGGTAGTCATCACCATCATCACCACGGAATGGCATCAATGACAGG
AGGACAGCAAATGGGTCGTGATTTGTATGACGACGATGATAAAGATTTGGCTACTATGGTTGATTCAA
GCCGCCGTAAATGGAATAAGACTGGTCATGCTGTACGAGCTATTGGAAGGTTAAGTTCCCTTGAAAAT
GTTTATATAAAAGCAGATAAGCAGAAAAATGGTATCAAAGCTAATTTTCATATACGACATAACATTGA
AGATGGTGGTGTTCAACTTGCATATCACTATCAACAGAATACTCCTATTGGCGACGGACCTGTTTTACT
TCCTGATAATCATTATCTATCTGTCCAATCAAAACTGTCTAAAGACCCAAACGAAAAACGTGATCATA
TGGTTCTTTTGGAATTTGTGACGGCTGCCGGTATTACATTAGGGATGGATGAGCTTTACAAAGGGGGT
ACCGGTGGTAGTATGGTTTCGAAAGGAGAAGAGTTGTTTACTGGAGTAGTACCAATTCTGGTTGAATT
AGACGGAGATGTTAATGGTCATAAATTTTCAGTTTCTGGAGAAGGGGAAGGAGATGCTACATATGGAA
AACTTACGCTTAAGTTCATATGCACCACAGGAAAATTGCCTGTTCCATGGCCCACCTTGGTTACAACAT
TAACATATGGTGTCCAATGTTTTAGTAGATATCCAGATCATATGAAACAACATGACTTTTTCAAATCTG
CAATGCCAGAAGGCTATATTCAAGAGAGAACTATATTTTTTAAGGATGATGGAAACTATAAGACAAGA
GCTGAAGTGAAATTTGAAGGAGATACTTTAGTGAATCGCATAGAACTTAAAGGTATAGACTTTAAAGA
GGATGGCAATATTTTAGGTCACAAATTAGAGTACAATCTACCTGATCAACTAACAGAGGAACAGATTG
CAGAGTTCAAAGAGGCATTTTCACTTTTTGATAAAGATGGCGATGGAACAATCACTACTAAGGAATTA
GGTACTGTAATGAGATCACTTGGTCAAAATCCTACTGAAGCGGAATTACAAGATATGATTAACGAAGT
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ACCGTGATACTGAAGAGGAAATTAGAGAAGCATTTGGTGTATTTGACAAAGATGGAAACGGTTACATT
TCTGCTGCTGAACTAAGACATGTTATGACAAACTTAGGAGAAAAACTGACTGATGAAGAGGTCGACG
AAATGATAAGAGAAGCCGATATTGATGGAGATGGTCAAGTTAATTACGAAGAGTTTGTTCAAATGATG
ACAGCAAAGTAAGAATTC

Supplemental References

S1. Juliano, C.E. (2014). Generation of Transgenic Hydra by Embryo Microinjection. J. Vis. Exp. *91*, e51888.

S2. Dana, C.E., Glauber, K.M., Chan, T.A., Bridge, D.M., and Steele, R.E. (2012). Incorporation of a horizontally transferred gene into an operon during cnidarian evolution. PLoS One *7*, e31643.

S3. Edelstein, A., Amodaj, N., Hoover, K., Vale, R., and Stuurman, N. (2010). Computer control of microscopes using μManager. Curr. Protoc. Mol. Biol. *92*, 14.20.1-14.20.17.

S4. Schindelin, J., Arganda-carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., *et al.* (2012). Fiji : an open-source platform for biological-image analysis. Nat. Methods *9*, 676–682.

S5. Passano, L.M., and McCullough, C.B. (1964). Co-ordinating systems and behaviour in Hydra. I. Pacemaker system of the periodic contractions. J. Exp. Biol. *41*, 643–664.

S6. Passano, L.M., and McCullough, C.B. (1965). Co-ordinating systems and behaviour in Hydra. II. The rhythmic potential system.