Online Methods

Calcium imaging. Young adult animals were glued to a 1.5% agarose pad (30 mM TAPS, pH 9) using cyanoacrylate glue (Nexaband S/C, Abbott Laboratories, Abbott Park, IL); animals were briefly cooled during the gluing process. A two-channel, gravity fed perfusion pencil (360µm tip), whose flow was controlled by programmable upstream valves (AutoMate Scientific, Berkeley, CA), was placed in close proximity (~0.5 mm) to the nose of each animal. The flow rate was 0.3 ml/min and the total bath volume was ~ 0.2 ml. Local perfusion, and thus rapid solution exchange ($t_{1/2}$=0.5 s), was achieved by locating the worm in a fluid plume between the inflow perfusion pencil and the outflow pipette. Solutions contained the indicated amount of NaCl plus (in mM): HEPES-NaOH pH 7.1 (10), $CaCl_2$ (1), $MgSO_4$(1); osmolarity was adjusted to 350mOsm with glycerol. Optical recordings were performed on a Zeiss Axiovert 135, using a Zeiss Plan-Apochromat 63x oil, 1.4 NA objective. The microscope was fitted with a Hamamatsu ORCA AG CCD camera (Hamamatsu Photonics, Bridgewater, NJ), an Optical Insights Dual-View beam splitter (Photometrics, Tuscon, AZ), and an electronic shutter (ASI, Eugene, OR). Images were acquired at 10-20Hz using MetaVue software (version 6.2r2, Molecular Devices, Sunnyvale, CA). Image stacks were analyzed using the Jamlyze program, as previously described[31]. The YFP/CFP emission ratio was computed as (YFP intensity)/(CFP intensity) – 0.65, where the latter term corrects for CFP bleed-through into the YFP channel. Photobleaching was corrected by fitting a single exponential to inactive portions of the ratio trace and dividing the latter by the fitted curve. Imaging data in Supplemental Fig. 2 & 5 were collected on a Zeiss Axioskop 2 upright microscope. In this setup both ASE neurons were imaged simultaneously using a customized manipulator that rotated the worm such that both neurons were in the same focal plane. A constant flow of the pre-stimulus solution was delivered to the recording chamber at a rate of 1 ml/min. Stimuli were delivered by a perfusion pipette (100µm tip) whose position was controlled by a motorized stage (Polytec/PI M-111.1 DG microtranslation stage). During stimulation, the tip of the pipette was moved from downstream of the animal to within 50µm of the worm's nose.

Selective activation of ASE neurons. The capsaicin imaging setup was identical to that used for NaCl imaging. The pre-stimulus saline was identical to the 40mM NaCl imaging solution except that it contained 0.25% EtOH. For the stimulus saline, a stock solution of capsaicin in EtOH was diluted in the pre-stimulus saline to 25µM (ASEL experiments) or 5µM (ASER experiments). We found that 25µM capsaicin was required to evoke consistent ASEL calcium transients in the *gcy-7*::TRPV1 strain, whereas 5µM was required to evoke consistent ASER calcium transients in the *gcy-5*::TRPV1 strain. The final EtOH concentration in the stimulus saline was always 0.25%. Behavioural responses to capsaicin were obtained using the previously described semi-restrained worm assay[29]. Briefly, animals were affixed to a 1.5% agarose pad (30mM TAPS pH 9) using a small drop of cyanoacrylate glue placed near the terminal bulb of the pharynx, submerged in the pre-stimulus saline, and allowed to recover for 3-4 minutes. Under these conditions, semi-restrained animals exhibit behavioural correlates of forward and reverse locomotion as well as omega bends[29]. The behaviour of individual worms was scored manually by pressing computer keys to record changes in behavioural state. The strains, solutions, and perfusion system were the same as those used in capsaicin imaging experiments. Experimental and control animals were tested in an alternating sequence, with the experimenter blind to animal condition.

Neuronal ablations. Cells were killed using a laser as described previously[30]. L1 animals (N2) were mounted on 2.5% agarose pads containing 5–7 mM of the immobilizing agent NaN_3. ASE neurons were identified by position. Animals were remounted 1–3 h after surgery to confirm the ablation. Animals with collateral damage were discarded. Sham-operated animals were treated in the same manner except that the laser was not fired. Step response behavior was tested 3 days after ablation.

Step-response assay. The step-response apparatus has been described[8]. Worms were placed individually on a thin porous membrane supported over a yoked pair of miniature inverted showerheads. Each showerhead emitted a saline solution with a different salt concentration. Stepwise concentration changes were delivered by sliding the showerhead assembly relative to the worm. The behaviour of individual worms was scored manually by pressing computer keys to record changes in behavioural state. Ablated and sham animals were tested in an alternating sequence, with the experimenter blind to animal condition. Solutions used in the assay were identical to solutions used during NaCl imaging experiments. These solutions induce larger downstep responses than reported previously[8]; we determined that the response increase was due to the presence of 1mM Ca^{+2} in the solutions used here (data not shown).

Behavioral statistics. The forward probabilities (*P*) for upstep experiments were transformed as 2arcsin(*P*) to compensate for the compression of variance at extremes of the probability scale[32]; this transformation is conservative because it increases the variance at the extremes. Statistical significance was assessed by a

two-factor repeated-measures ANOVA applied to forward probabilities during a post-stimulus analysis window. In addition, we made planned pairwise comparisons (*t*-tests) on mean behavioral-state probabilities at each time point in the analysis window. The significance level of each *t*-test was adjusted using the Bonferroni correction for multiple comparisons. To determine *a priori* the analysis window length to use in each ablation experiment condition (Fig. 4b-g and Supplemental Fig. 6), we identified the last post-stimulus time point at which there was a significant difference in post hoc comparisons (*t*-tests) between shams responding to a step and unstimulated control animals (a no-response group). The window extended from the first post-stimulus time point to this last significantly different time point. List of analysis window lengths: 50sec for 9 mM upstep (Fig. 4b, d & f), 40sec for 9 mM downstep (Fig. 4c, e & g), 60sec for 40 mM upstep (Supplemental Fig. 6a, c & e) and 50sec for 40 mM downstep (Supplemental Fig. 6b, d & f)

[31] Kerr, R. *et al.* Optical imaging of calcium transients in neurons and pharyngeal muscle of *C. elegans*. *Neuron.* **26**, 583-594 (2000).

[32] Winer, B.J., Brown, D.R. & Michels, K.M., *Statistica Principles in Experimental Design*, 3rd ed. (McGraw-Hill, Boston, 1991).