SUPPLEMENTARY METHODS

Biochip Microfabrication: The microfluidic biochip was fabricated using soft lithography. The two-layer SU-8 mold was created using a two-step photolithography process. The thinner microchannels were patterned on the first SU-8 layer that was spun to a thickness equal to the worm's body diameter. The thinner microchannels included the worm trap, the 4-flow channels (stimulus channel, buffer channel and 2 control channels) and the comb-structure that connects the flush-channel to the worm trap. The thickness of 8-10 μ m larger than the worm body diameter. The thicker microchannels were patterned on the second SU-8 layer that was spun to a thickness of 8-10 μ m larger than the worm body diameter. The thicker microchannels included the worm inlet microchannel, the flush-microchannel and the outlet microchannels. PDMS was poured onto the SU-8 mold and allowed to cure at 65 °C for approximately 3 hours. The PDMS layer was peeled off from the SU-8 mold and punched using a sharpened, to form the fluidic inlets and outlets and finally bonded to a glass coverslip (#1.5) using air plasma (50 W, 250 mTorr, 35 s).

We fabricated four different SU-8 molds for conducting experiments with Day 1, 3, 4 and 5 worms respectively. The table below lists the thickness of the two SU-8 layers and the type of SU-8 used to pattern them for each SU-8 mold. In all cases, we process the SU-8 photoresist according to the recipe provided by MicroChem on its website.

	SU-8 Layer 1		SU-8 Layer 2	
Worm Age	Thickness (µm)	Туре	Thickness (µm)	Туре
Day 1	28	SU-8 2015	36	SU-8 2015
Day 3	32	SU-8 2015	42	SU-8 2015
Day 4	41	SU-8 2015	50	SU-8 2050
Day 5	50	SU-8 2050	60	SU-8 2050

Setup Automation Software: The LabVIEW code consists of the following three modules:

<u>Worm loading module</u>. This module is used to load single worms into the microtrap. White-light images of the microtrap are continuously analyzed in order to detect the presence of a worm, while the worm suspension solution is pressurized at 10 psi. The worm's presence is determined by comparing the average pixel intensity to a pre-defined threshold value. When a worm is identified the pressure at the inlet microchannel and the worm suspension is released.

Calcium imaging module. This module is used to locate the ASH neural body and record its response to the stimulus (1 M glycerol in this study). A coarse stack (5 μ m step size) of fluorescence images is initially acquired along the z-axis. Each image in the stack is first segmented using edge detection method. Regions with closed boundaries are then detected in order to locate fluorescent objects in the image. The ASH neuron is identified by comparing the size of the detected fluorescent objects with a pre-defined value. Next, a high-resolution z-stack (1 μ m step size) of fluorescence images is acquired below and above the focal plane of the neuron and the images are analyzed to locate the best focal plane. The best focal plane is located by identifying the z-plane that corresponds to the highest average fluorescence intensity of the neural body. For that best-focal plane, a time sequence of fluorescence images, that captures the response of ASH to the application and removal of the stimulus, is obtained. These images are stored in the memory for further analysis.

<u>Worm unloading module</u>. That module is used to unload worms from the microtrap by pressurizing the flush-channel. In a similar fashion to the worm loading module, white light images are analyzed in real-time to determine the removal of the worm from the microtrap. The removal of the worm is confirmed by comparing the average pixel intensity to a pre-defined threshold value. This is then followed by the release of pressure in the flush-channel.

Image and Data Analysis: We wrote a LabVIEW program to extract the mean FRET ratio from each fluorescent image. Each fluorescent image was split into two images, each one representing the YFP and CFP channels. Our program required four pieces of

information that were specified by the user: 1) the region of interest defining the boundary of the neuron in YFP channel, 2) the region of interest in the YFP channel within which the neuron moves, 3) the region of interest defining the background fluorescence of the worm in the YFP channel and 4) the initial coordinates of the neuron in each channel (YFP & CFP). Our program first calculates the difference between the initial coordinates of the neuron's centre in each channel. It uses this difference to correct for any misalignment between the YFP and CFP images. It then tracks the neuron in each channel and extracts the corresponding background subtracted mean fluorescence intensity. The FRET ratio is calculated as the ratio of mean fluorescence intensity of the neuron in the two channels.

We corrected the FRET ratio traces for photobleaching by removing the exponential trends before and after stimulation ¹. To do this, the trends before and after stimulation were curve fitted with exponential trendlines. We subtracted the curve fitted trendlines from the corresponding ratio traces and obtained new ratio traces that were compensated for photobleaching. We performed all the computations on the new ratio traces. For each trace, we calculated the percent change of the ratio relative to the average ratio during the first 5 s. We then calculated the slope, peak and duration of the rising phase at the onset of the stimulus.

Fourier Analysis: We analyzed the spectral content of the ASH calcium transients using a discrete fourier transform. To distinctly quantify the calcium oscillations from the spectral content of the ASH response, we first removed the contribution of the secondary peak corresponding to the off-response of ASH from the raw data. We then computed the fourier transform of the clipped ASH response curves (defined as y(t)) using the FFT subroutine in MATLAB, at 512 equally spaced frequencies from $f_0 = 0$ Hz to $f_{512} = 4$ Hz. The fourier transformed data was then used to calculate the 'normalized energy spectral density' using the following equation :



(1)

where, $Y(f_k) =$ discrete fourier transform of y(t)

N = 512 $f_1 = 0.008 \text{ Hz}$ $\frac{f_N}{2} = 2 \text{ Hz}$

We divided the energy spectral density curve (Fig. 4) into three different frequency bands – low, mid and high ²⁵. The lower frequencies (< 0.04 Hz) captured the primary ASH response, mid frequencies (0.04 - 1 Hz) captured the calcium oscillations and higher frequencies (> 1 Hz) captured the noise content in the data. To quantify the contribution of calcium oscillations to the ASH response curves, we calculated the energy spectral density contained within the frequency range of 0.04 - 0.2 Hz defined as the 'Band Power Ratio' as shown in the following equation :



where, $f_{mtn} \square = 0.04 \text{ Hz}$ $f_{max} \square = 0.2 \text{ Hz}$

The normalized energy spectral density and the band power ratio were then averaged across the two types of ASH calcium transients (with and without oscillations) from Day 1 worms (fig 4).

Manual recording from Day 5 worms: To validate the results acquired for Day 5 worms, we manually recorded the ASH response from these worms using the Metamorph software. Metamorph allowed us to access the 'enhanced gain' feature of the CCD camera and thus enabled the recording of ASH response from Day 5 worms that showed weak fluorescence. The enhanced gain feature allowed us to record from all Day 5 worms that were loaded in the device. We recorded the ASH response from 15 Day 5 worms (Supplementary Fig. 4a). The average values of the peak and slope (Supplementary Fig. 4b) of the rising phase in the ratio trace at the onset of the stimulus were similar to that obtained using the microfluidic platform.

SUPPLEMENTARY FIGURES

Supplementary Figure 1



Supplementary Figure 1. Effect of UV pre-exposure to the calcium transients of the ASH neuron from Day 1 worms. A 18 s pulse of glycerol (1 M) was used as a stimulus. Each trace represents percent change in the average FRET ratio from a population of 5 worms. Traces represent the responses obtained after 0 s, 40 s and 80 s UV pre-exposure.

Supplementary Figure 2



Supplementary Figure 2. Baseline intracellular calcium concentration in the ASH neuron in worms of different ages. These values were obtained by calculating YFP/CFP ratio prior to recording. The difference in the baseline calcium concentrations between any two worm ages was found to be within 2-7%. Error bars indicate standard error of mean (s.e.m). **P > 0.01 and *P > 0.1 (Student's *t*-test).

Supplementary Figure 3



Supplementary Figure 3. Algorithmic representation of the experimental protocol.





Supplementary Figure 4. ASH ratiometric transient from Day 5 worms in response to a hyperosmotic stimulus, recorded using Metamorph with an enhanced gain of 50x. (a) The curve represents an average of the ASH response from 15 Day 5 worms. The dashed line represents the presence of the stimulus. (b) The peak (left) and slope (right) of the on-response recorded using the microfluidic platform and Metamorph.