Supplementary material

Behavioral Tests

A detailed protocol of the "drop test" assay is described in Hilliard et al., 2002. In brief, a drop of a solution is delivered near the tail of a moving animal. When the substance is a repellent the animal immediately ceased its forward movement and reverses, typically exiting the repellent after 2-3 seconds; if buffer alone is delivered, the animal will continue moving forward. The assay is conducted always on unseeded plates.

The response of a single worm to each drop delivered is recorded as either a positive or negative response. Responses are considered positive when the avoidance reflex is observed within 4 sec after the worm encounters the substance. An ISI (Inter Stimulus Interval) of at least two minutes is used between successive drops to the same worm, except in those adaptation experiments where the duration of the ISI is indicated. Each worm is tested with no more than 15 successive drops. The A.I. of individual animals is the fraction of positive responses over the total of trials (A.I. = + responses/total drops). The A.I. of a population is the mean of the A.I. of all the animals. (A.I. population = Σ A.I. of all the animals/total number of animals). A minimum of 10 animals was used in the population assays.

In the adaptation experiments that required prolonged exposure to the repellent, each animal on the unseeded plate was kept swimming in the repellent solution for the desired time by continuous delivery of the substance in the same modality used in the drop test.

Statistical Tests

We observed variability in calcium imaging results both within measurements made on the same animal, and between animals. Since sample preparation took the majority of the time, we elected to reduce total variability by making multiple measurements per animal. Linear mixed-effects modeling (Pinheiro and Bates, 2000) was used to estimate population means and compute significance between different treatments and genotypes. Limited bootstrap and jackknife analysis were performed to verify the robustness of the results. Since responses did not exactly follow a Normal distribution, we also verified findings of significance by computing the average response for each animal and then performing a non-parametric Kolmogorov-Smirnov test on these average responses. This highly conservative test confirmed all findings of significance ($p<0.05$), although values reported in the text are those from mixed-effects analysis, which we believe is most accurate.

Analysis of behavioral responses was performed with the Fisher Exact Test and/or chi-squared test, when multiple separate animals were stimulated (e.g. Figure 5E), or with the Kolmogorov-Smirnov test when an average response rate per animal was calculated (e.g. Figure 4E for glycerol avoidance index). Error bars for the standard error of the mean were computed using a Bayesian framework to estimate the systematic error and assuming a Bernoulli process to predict the stochastic error.

Statistical analysis was performed in the open-source statistical computing package R version 1.9.1 (The R Project, www.r-project.org) and in Octave (www.octave.org), an open-source package similar to Matlab.