Supporting Information

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SI Materials and Methods

Strains. The wild-type strain (Bristol N2), *ttx-1(p767)*, and *ttx-3 (ks5)* mutant strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota). Strains used to map and clone *pg5* are the following: *SP24 dpy-5(e61) unc-54(e190) I, MT588 lin-31(n301) unc-4(e120) II, SP17 unc-32(e189) dpy-1(e1) III, DR105 unc-17(e245) dpy-20(e1282) IV, DR181 unc-60(m35) dpy-11(e224) V, SP413 unc-7(e139) lon-2(e678) X, GN70 pgIR5 (V, N2 > CB4856) V*. Transgenic strains used in this study include the following: ZC1974 *yxEx1006[Pttx-3::MYRTDKrd; Punc-122::gfp]*, ZC1990 *yxEx1022[Pglr-3::MYRTDKrd; Punc-122::gfp]*, ZC2002 *yxEx1034[Pgcy-8::MYRTDKrd; Punc-122::gfp]*, TV3494 *wyEx1348[Pgcy-8::caspase-3(p12)::nz; Pgcy-8::cz::caspase-3(p17); Punc-122::gfp]*, TV6718 *wyIs111;wyEx1046[Pttx-3::cfp;Pgcy-8::rab-3::mCh;Pttx-3::caspase-3(p12)::nz;Pttx-3::cz::caspase-3(p17); Punc-122::rfp]*, DCR404 *olaEx212[Pgcy-8::caspase-3(p12)::nz;Pgcy-8::cz::caspase-3(p17);Pttx-3::caspase-3(p12)::nz;Pttx-3::cz::caspase-3(p17); Punc-122::gfp]*, DCR2589 *ttx-3(ot22);wyEx1348[Pgcy-8::caspase-3(p12)::nz;Pgcy-8::cz::caspase-3(p17);Punc-122::gfp]*, DCR2500 *pkc-1(pg5);wyIs45;wyEx1348[Pttx-3::rab-3::gfp;Punc-122::rfp;Pgcy-8::caspase-3(p12)::nz;Pgcy-8::cz::caspase-3(p17);Punc-122::gfp]*, TV2231 *wyEx828[Pglr-3::caspase-3(p12)::nz;Pglr-3::cz::caspase-3(p17);Pglr-3::mCherry;Punc-122::gfp]*, DCR1258 *olaEx743[Podr-2b(3a)::gfp]*, PY7502 *Pceh-36::caspase-3;Pstrx-1::gfp*, *otls264 Pceh-36::RFP*.

Mutant Screen. Wild-type, L4 hermaphrodite larvae were exposed to ethyl methanesulfonate (50 mM) for 3 h at room temperature. Young adult nonclonal F2 progeny were grown at 20 °C in the presence of abundant food and tested on a linear (1 °C/cm) thermal gradient (1) at a starting temperature of 23 °C. Worms that migrated to the warmest region of the assay plate within 10 min were picked to individual growth plates, and their progeny were tested for thermotaxis defects.

Mapping *pkc-1(pg5)*. *pg5* mutants were backcrossed six times to N2 before mapping. *pg5*/+ heterozygotes showed no defect in thermotaxis, indicating that the mutation is completely recessive. *pg5* was mapped to chromosome V using traditional three-factor mapping with visible (Dpy, Unc) markers. Subsequent snip-SNP mapping placed the *pg5* locus on the right arm of chromosome V. snip-SNP mapping for *pg5* was performed using a chromosome V substitution strain, GN70 *pgIR5* (V, CB4856 > N2), in which chromosome V from N2 was substituted by chromosome V from the standard reference strain for SNP mapping, CB4856 (2).

Ablation Methods. A MicroPoint high-intensity pulsed dye laser system (Andor Technology) was focused onto targeted neurons, and a controlled series of 440-nm pulses was delivered to destroy the neuron. Ablated and mock-ablated worms were recovered and cultivated overnight for testing the following day.

KillerRed-mediated ablation was carried out by immobilizing three to five L4 stage animals at a time using nanoparticles (3). Basically, three to five animals were picked into a small drop of 0.1-μm diameter polystyrene microspheres on a 10% (wt/wt) agarose pad and covered with a coverslip. Each animal was exposed for 3–5 min, focusing a green laser source on the cell body of targeted neurons. After illumination, animals were rescued with M9 buffer and cultivated at 15 °C or 25 °C for 1 d before being tested in the behavior assay. Neurons were reimaged after 1 d to verify destruction of the targeted neuron.

Caspase-mediated cell ablations were performed using a reconstituted caspase system (recCaspase) as previously described (4). Briefly, the two recCaspase components were driven in single cells using neuron-specific promoters (*gcy-8* for AFD, *glr-3* for RIA, and *ttx-3* for AIY). Transgenic animals expressing these constructs were generated using standard methods. To assess and quantify the efficiency of cell ablation with recCaspase, the recCaspase components were crossed into integrated reporter lines that allow fluorophore visualization in the neuron of interest [*wyIs111* for AFD and AIY (5) and *wyIs93* for RIA (6)], and loss of reporter fluorescence was scored using a compound microscope (model DM5000 G; Leica). The percentage of animals with surviving neurons was calculated by dividing the number of reporter fluorophore-positive animals by the total number of animals scored. Lines were scored multiple times for each condition (three times for AFD and RIA, twice for AIY) with 20–30 control and 20–30 array-carrying animals examined per scoring. For animals with the recCaspase coinjection marker, 67.8 ± 1.1% of *wyIs93* animals displayed loss of RIA, 80 ± 8.4% of *wyIs111* animals displayed loss of AFD, and 65.9 ± 2.3% *wyIs111* animals displayed loss of AIY. For all three ablation conditions (AFD, RIA, AIY), in the control group without the recCaspase coinjection marker, no neuronal ablation was observed, as expected. The same recCaspase transgenic arrays used in assessing ablation efficiency were used for behavioral testing. AIY-ablated animals were selected based on the absence of CFP in AIY.

Large-Format Behavioral Assay. A linear temperature gradient was generated on 22- × 22-cm agar plates by placing them on a black anodized aluminum platform (61 × 30 × 0.64 cm) bridged across the hot and cold aluminum reservoir blocks (10 × 30 × 3.8 cm). The cold reservoir temperature was maintained with a PID controller and H-bridge amplifier (Accuthermo), powered by a switching supply (Oven Industries) driving a thermoelectric cooler (TEC, Ferrotec) that pumped heat between the reservoir block and a liquid-cooled waterblock (Swiftech); the liquid was circulated with a pump and chiller unit (VWR). The hot reservoir was also controlled with PID feedback (Newport Electronics) that delivered current via a solid-state relay (Omega) to four resistive cartridge heaters (McMaster-Carr) embedded in the reservoir block. The temperature sensors (McMaster-Carr) used for both PID feedback loops were secured to the top plate near each reservoir block. Temperature at the agar gel surface was measured with a type T thermocouple probe (Physitemp) before and after each experiment to check for thermal drift; the same probe was used to systematically measure temperature in both horizontal dimensions on the surface. Temperature was constant in the direction perpendicular to the gradient.

The assay plate was illuminated by Super Bright LED bars. Movie S1 was captured using a 5-megapixel USB camera (Mightex) for 15 min at 2 fp using Mightex Camera Demo (V1.2.0). Trajectories were analyzed by adapting the MagatAnalyzer software package (7). To automatically flag reorientations and differentiate sharp turns and reversal turns, we considered both the posture of the animal and the movement of its center of mass. Rapid reorientations (sharp turns or reversal turns) were flagged when the heading change of the center of mass trajectory was >60° over 1 s. When the worm executes a sharp turn, the aspect ratio of the worm image decreases before the worm resumes forward movement in a new direction. When a worm executes a reversal, the aspect ratio remains high and the center of mass reverses its direction, backtracking along the original path. To quantify the aspect ratio, we took the ratio of the two eigenvalues of the covariance matrix of the pixel intensity recorded by the camera

(http://en.wikipedia.org/wiki/Image_moment). A larger ratio indicated that the worm was elongated and a smaller ratio indicated that the worm had assumed a more circular shape. Reversals were flagged as sudden reversals in trajectory within 20° of the reverse of the previous heading, maintaining an eigenvalue ratio of >1.8. Otherwise, reorientations were flagged as sharp turns.

High-Resolution Behavioral Analysis. A linear temperature gradient was generated on an agar surface using a smaller version (10 × 16 cm) of the apparatus that was used for high-throughput behavioral analysis. In this case, the entire temperature gradient assembly was placed on a Ludl Mac6000 Motorized Stage on an upright Nikon LV100 microscope. We used the Nikon Elements software package to manually control stage movements to keep the image of the worm in the center of the field of view and recorded the image of the worm at 4× magnification using an Andor Ixon Camera. The dorsal and ventral sides of each animal were manually labeled. Center-of-mass trajectories were calculated afterward using the recorded stage movements and images of the worm using custom scripts written in MATLAB (Mathworks).

Calcium Imaging. Worms were imaged on a custom-built temperature control stage using a spinning disk confocal microscope (Andor Technology), where a PID controller and H-bridge amplifier (Accuthermo) drove a thermoelectric cooler (TEC) (Newark) that pumped heat into and out of a thin copper plate (7.4 × 7.4 × 0.5 cm) with a liquid-cooled water block (Swiftech) acting as a thermal reservoir. A type-T thermocouple microprobe (Physitemp) was placed on the copper plate underneath a thin steel tab (7.4 × 2.5 × 0.013 cm). Transgenic worms expressing GCaMP6s (8) in AFD were placed on the steel tab directly above the temperature probe. Temperature was delivered by altering the PID controller's set point dynamically, using custom software written in LabVIEW (National Instruments). Because the temperature at the position of the worm could differ from the embedded thermocouple probe temperature, larval temperature was calibrated using a second probe taking the wworm's place while delivering the same temperature programs. Images from an EMCCD camera (Andor Technology) were recorded using either iQ (Andor Technology) or NIS Elements (Nikon Instruments) software.

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Fig. S1. Apparatus for behavioral and physiological analysis. (*A*) Schematic of spatial temperature gradient apparatus for tracking thermotaxis with stable cold and warm sides maintained to establish a linear gradient across the surface of a large agar gel. (*B*) Schematic of the microscope setup for calcium imaging. A custom temperature-controlled stage with objective focus feedback allows 3D confocal imaging during programmed temperature waveforms.

Movie S1. Overview of analysis. Playback of worms navigating a 22- × 22-cm plate under dark-field illumination, showing center-of-mass tracking of overall movement as well as high-resolution analysis of worm posture. Trajectories were segmented into alternating periods of forward movement, turns, and reversals on the basis of movement and posture as described in *Materials and Methods*. Playback is 15×.

Movie S1