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Thermotaxis in *Caenorhabditis elegans* Analyzed by Measuring Responses to Defined Thermal Stimuli

William S. Ryu and Aravinthan D. T. Samuel

Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138, and Rowland Institute for Science, Cambridge, Massachusetts 02142

In a spatial thermal gradient, *Caenorhabditis elegans* migrates toward and then isothermally tracks near its cultivation temperature. A current model for thermotactic behavior involves a thermophilic drive (involving the neurons AFD and AIY) and cryophilic drive (involving the neuron AIZ) that balance at the cultivation temperature. Here, we analyze the movements of individual worms responding to defined thermal gradients. We found evidence for a mechanism for migration down thermal gradients that is active at temperatures above the cultivation temperature, and a mechanism for isothermal tracking that is active near the cultivation temperature. However, we found no evidence for a mechanism for migration up thermal gradients at temperatures below the cultivation temperature that might have supported the model of opposing drives. The mechanisms for migration down gradients and isothermal tracking control the worm's movements in different manners. Migration down gradients works by shortening (lengthening) the duration of forward movement in response to positive (negative) temperature changes. Isothermal tracking works by orienting persistent forward movement to offset temperature changes. We believe preference for the cultivation temperature is not at the balance between two drives. Instead, the worm activates the mechanism for isothermal tracking near the cultivation temperature and inactivates the mechanism for migration down gradients near or below the cultivation temperature. Inactivation of the mechanism for migration down gradients near or below the cultivation temperature requires the neurons AFD and AIY.

Key words: thermotaxis; nematode; sensorimotor integration; thermosensation; behavioral models; navigation

In their classic study of thermotaxis in *Caenorhabditis elegans*, Hedgecock and Russell (1975) demonstrated that worms navigating spatial thermal gradients aggregate near their cultivation temperature (T_c). Within 3°C of this temperature, worms track isotherms, deviating from a given isotherm by as little as ~0.05°C. Hedgecock and Russell (1975) also harvested mutants that failed to aggregate at T_c and found that they could be classified as thermophilic (aggregating in warmer regions of a plate), cryophilic (aggregating in colder regions of a plate), or atactic (not aggregating at all). On the basis of this classification, Hedgecock and Russell (1975) suggested that competing thermophilic and cryophilic drives produce thermotactic behavior and that isothermal tracking occurs at their balance.

Mori and Ohshima (1995) identified neurons involved in thermotaxis by laser ablation. The resulting defects in behavior could be organized in the same manner as the mutant behaviors shown by Hedgecock and Russell (1975), namely thermophilic (obtained by lesion of the AIZ neuron), cryophilic (obtained by lesion of the AFD and/or AIY neuron), or atactic (obtained by lesion of the RIA neuron or multiple neurons). Thus, Mori and Ohshima (1995) proposed that the AFD and AIY neurons comprise the thermophilic drive, AIZ comprises the cryophilic drive, and RIA compares the two drives.

Although the labels thermophilic, cryophilic, and atactic describe aberrant aggregation patterns on spatial gradients, they do not explain how putative thermophilic and cryophilic drives direct the worm's movements toward the cultivation temperature. Also, it is unclear whether thermophilic and cryophilic drives are active during isothermal tracking or whether isothermal tracking represents a distinct mechanism.

The present study aimed to determine the mechanisms underlying thermotaxis, the manner in which they direct the worm's movements, and the roles of participating neurons. We studied the movements of worms as they performed thermotaxis by (1) tracking worms crawling on the surface of agar plates with defined spatial or temporal thermal gradients and (2) monitoring the swimming motions of a worm suspended in a droplet subjected to thermal stimuli. In addition to wild-type (N2) worms, we studied thermotaxis mutants that have been shown to have developmental defects in AFD or AIY neurons, which have been identified by Mori and Ohshima (1995) to be involved in thermotaxis.

We found strong evidence for a mechanism for migration down gradients toward the cultivation temperature that might correspond to the cryophilic drive of the prevailing model, but no compelling evidence for a mechanism for migration up gradients that might correspond to the thermophilic drive. The mechanism for migration down gradients modulates run duration but not run orientation, shortening runs in response to positive changes in temperature and lengthening runs in response to negative changes in temperature. The mechanism for isothermal tracking modulates run orientation to offset either positive or negative

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Correspondence should be addressed to Dr. Aravinthan Samuel, Cold Spring Harbor Laboratories, 1 Bungtown Road, Cold Spring Harbor, NY 11724. E-mail: samuel@cshl.org.

temperature changes. These data (the asymmetry between navigation above and below the cultivation temperatures and the different nature of the motor commands between migration down gradients and isothermal tracking) seem to undermine the prevailing simple model of competing cryophilic and thermophilic drives.

MATERIALS AND METHODS

Media and strains. *C. elegans* strains were grown and maintained as described by Sulston and Hodgkin (1988) (N2 was a gift from Craig Hunter, Harvard University, Cambridge, MA). The mutants *ttx-3 (ks5)* and *ttx-1(p767)* were obtained from Theresa Stiernagle at the *C. elegans* Genetic Center (University of Minnesota, Minneapolis, MN).

Thermal gradients on surfaces. Linear thermal gradients were established on agar surfaces by placing Petri dishes in the middle of rectangular aluminum plates, the ends of which were fixed at different temperatures. Glycerol was used to ensure good thermal contact between the Petri dish and the metal surface. In one apparatus, thermoelectric controllers (TECs) were fixed to the ends of an aluminum plate ($5 \times 12.7 \times 0.32$ cm). The TECs (3 cm square, 50 W) were thermostatically controlled to 0.1°C with a linear–bipolar Proportional Command Integral Control controller (HTC-3000; Wavelength Electronics, Bozeman, MT). With this apparatus, arbitrary gradients (as steep as 5°C/cm) could be programmed. An apparatus of the type used by Hedgecock and Russell (1975) was also built. In this case, a linear gradient was established along a $60 \times 10 \times 1.3$ cm aluminum plate by bolting $10 \times 10 \times 6$ cm blocks to each end, immersing one block in a thermostatically controlled water bath, and immersing the other in an ice bath. The temperature at the agar surface was measured with a T-type thermocouple (0.1°C resolution). The agar plates reached thermal equilibrium within 10 min. Gradients were linear up to 1 cm from the edge of the Petri dish.

The choice of temperatures for cultivating and testing worms was not arbitrary. Worms cultivate well in the range 15–25°C (Sulston and Hodgkin, 1988). At temperatures <15°C, worms become lethargic. We did not test worms at temperatures >27°C to avoid eliciting heat-shock or pain responses (Wittenburg and Baumeister, 1999). Also, the temperature range for thermotaxis assays has traditionally been from 17 to 25°C in radial thermal gradients, and we sought comparable conditions.

Temporal thermal ramps were produced by controlling the temperature of a brass plate (11 cm in diameter, 0.32 cm thick) in contact with a TEC. Plates (9 cm) containing nematode growth medium (NGM) (Sulston and Hodgkin, 1988) were sealed to the brass plate with a thin layer of glycerol. Spatial temperature variation along the agar surface was negligible. Temperatures were stable to 0.1°C, and ramping rates up to 10°C/min could be achieved. In all temporal ramping experiments, we used the rate of 0.5°C/min. Ramp duration was 5 min and was linear for the middle 3 min. Worms were tracked only during the linear portion of the ramps.

All experiments were done in a temperature-controlled room set to a temperature between the limiting temperatures of the gradient. For example, for a gradient between 17 and 19°C, the room temperature was set to 18 ± 0.1°C. The numbers of worms analyzed in each experiment are listed in the figure legends.

Thermal gradients in droplets. Temporal thermal ramps in droplets were produced by controlling the temperature of an anodized aluminum plate ($43 \times 48 \times 5$ mm) in contact with a TEC. NGM buffer (60 μl; same inorganic ion concentration as NGM plates) was placed in a 4 mm circular hole in the plate, forming a transparent, hanging droplet. Temperatures were stable to 0.1°C; ramping rates up to 20°C/min could be achieved.

Crawling assays. Populations were synchronized by selecting eggs to OP50-seeded NGM plates (Sulston and Hodgkin, 1988). These plates were incubated at specific temperatures between 15 and 25°C. We monitored incubator temperatures for constancy (T_c of ±0.1°C). Immediately before each assay, ~20 young adult worms were washed in 10 ml of NGM buffer at room temperature (same inorganic ion concentration as NGM plates) and moved to fresh NGM plates without bacteria. These plates were then placed on the metal surface of the equipment described above. A glass cover on the NGM plate was used during assays to eliminate variations in humidity, temperature changes attributable to evaporative cooling, and air currents.

A CCD camera (wv-BP550; Panasonic, Secaucus, NJ) equipped with a 9–27 mm zoom lens (f/2.0) was used to image the worms. Worms were illuminated obliquely by a ring of superbright red light-emitting diodes, producing a dark-type illumination. Magnification was set such that the Petri dish filled the field of view. Video images were captured at 1 frame per second (fps) by a Scion Image LG-3 capture board (Scion Corp., Frederick, MD) on either a Macintosh computer (PowerMac G3; Apple Computers, Cupertino, CA) or a personal computer (Pentium III; Dell Computer Company, Round Rock, TX).

For the spatial assays, worms at a specific cultivation temperature were prepared as described above. A glass plate was used to cover the Petri dish to reduce convection. After waiting 15 min to ensure that the worms and plate had reached thermal equilibrium, video images of the plate were captured for 500 sec at 1 fps.

For the temporal assays, we waited for 1 min after the onset of the ramp before capturing images. During the subsequent linear part of the ramp, images were captured for 120 sec at 1 fps.

Worm trajectories were scored by hand using the imaging program Scion Image (Scion Corp.) Worm paths within 1 cm of the plate edge were not scored to avoid artifacts arising from thermal edge effects. Numerical analysis was performed with conventional and customized algorithms using Matlab (MathWorks Inc., Natick, MA).

Swimming assays. Worms cultivated at specific temperatures were prepared as described above; single worms were placed in the droplet. Worms were imaged using a stereomicroscope and a CCD camera. Worms swam vigorously, but sinking to the bottom of the curved droplet, they stayed in the center of the field of view. Bends that terminated the swimming run were scored by eye. We did not sort large bends based on the degree or direction of bending. For swimming worms, we did not score reversals of the direction of wave propagation; reversals were rare, and they did not contribute significantly to the statistics of run termination.

RESULTS

Migration toward cultivation temperature

Worms swim through fluids or crawl on surfaces using snake-like movements. We define a run as a period of uninterrupted forward motion (either swimming or crawling) in which bending waves propagate continuously from nose to tail. Crawling and swimming worms terminate runs in analogous manners: crawling worms terminate runs by abrupt reorientations that might be either turns or pirouettes (Fig. 1); swimming worms terminate runs by abrupt bends larger than those of the forward swimming rhythm (Fig. 1) or by reversing the direction of wave propagation. See Croll (1975) for an early behavioral study that connects abrupt bends of swimming worms with the abrupt reorientations of crawling worms.

To determine whether worms modulate the run duration in response to thermal stimuli, we measured run durations of worms exposed to rising and falling temperatures (ramps). In assessing the run duration of crawling worms, we scored both turns and pirouettes, because both terminate the previous run and choose a new orientation for the subsequent run. In assessing the run duration of swimming worms, we scored the frequency of large bends.

In assays of crawling worms, we ramped the temperature of entire plates, eliminating spatial gradients. We found that above T_c, the run durations in negative temporal gradients (i.e., falling temperatures) were lengthened, whereas the run durations in positive temporal gradients (i.e., rising temperatures) were shortened compared with the run durations in isotropic environments at temperatures within the range of the temporal ramps (Tables 1 and 2). Below T_c, run durations were the same regardless of whether the temperature rose or fell (Table 2). Run durations were exponentially distributed, suggesting Poisson statistics (Fig. 2). The rate of run termination in isotropic environments, attributable to background activity not evoked by temperature changes, rose monotonically with ambient temperature and by itself cannot drive aggregation at T_c (Table 1).

In assays of swimming, we ramped the temperature of droplets

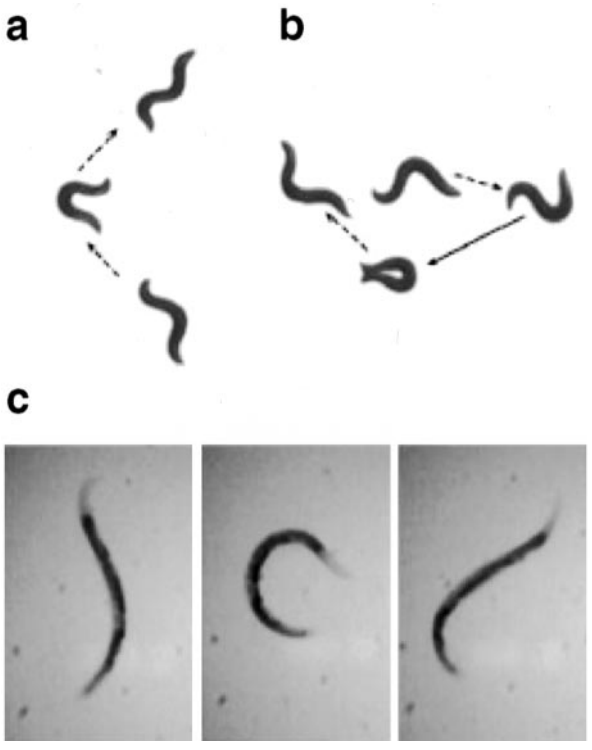

Figure 1. Run termination in crawling and swimming worms. Crawling worms terminate runs by turns (*a*) or by pirouettes (*b*), which involve reversals and coiling. *c*, Swimming worms terminate runs by abrupt bends larger than those of the rhythmic bends of forward swimming.

Table 1. Run durations of N2 worms cultivated at different temperatures crawling on agar surfaces at fixed temperatures (i.e., without temporal or spatial variation)

Cultivation temperature	Run durations at constant temperature (sec)		
	17°C	21°C	27°C
15°C	28 ± 2	22 ± 2	19 ± 1
22°C	29 ± 2	23 ± 1	20 ± 1
25°C	22 ± 1	18 ± 1	12 ± 1

Each measurement represents ~150 runs.

(60 μl) containing single worms. Confined to a droplet, a worm made visible swimming motions but did not leave the field of view. Run termination events were more frequent for swimming worms than for crawling worms. We measured the durations of all runs of populations of worms crawling on plates, and we scored the times of occurrence of run termination events of individual worms swimming in droplets (Fig. 3). We found that the thermotactic response for swimming worms was analogous to that of crawling worms. Above T_c, the number of run termination events per unit time was lower during negative ramps and higher during positive ramps compared with measurements using droplets of a fixed temperature within the range of the temporal ramps. Below T_c, the rate of run termination was the same regardless of whether the temperature rose or fell.

During runs, worms crawled at the same speed in both positive and negative temporal ramps (Table 3). Therefore, controlling run speed is not part of the thermotactic strategy. Run speed had a much weaker dependence on ambient temperature than the rate of run termination (compare Tables 1 and 3).

Table 2. Run durations of N2 worms cultivated at different temperatures crawling on agar surfaces subjected to temporal ramps

Cultivation temperature	Temporal ramps (0.5°C/min)	Run duration (sec)
15°C	17°C → 19°C	14 ± 1
	19°C → 17°C	33 ± 3
	24°C → 26°C	13 ± 1
	26°C → 24°C	23 ± 1
22°C	17°C → 19°C	24 ± 1
	19°C → 17°C	23 ± 1
	24°C → 26°C	14 ± 1
	26°C → 24°C	23 ± 2
25°C	17°C → 19°C	18 ± 1
	19°C → 17°C	16 ± 1
	21°C → 23°C	18 ± 1
	23°C → 21°C	16 ± 1

All ramps were at the same rate, 0.5°C/min. Each measurement represents ~200 runs. Gray shading indicates a significant bias toward lower temperatures (i.e., runs toward lower temperatures are significantly longer than runs toward higher temperatures). In statistical terms, gray labels only those experiments that have different mean run durations between positive and negative temporal ramps at the 99% confidence interval using Student's *t* test.

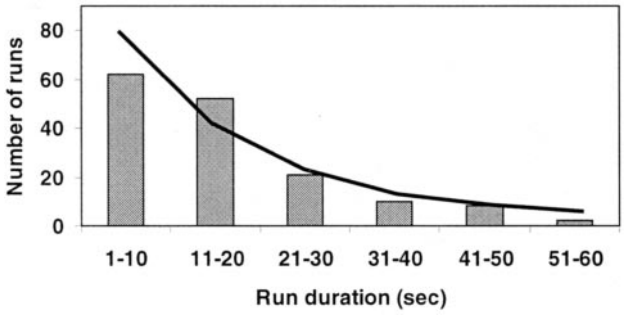

Figure 2. A sample histogram of run durations of N2 worms crawling in an isotropic environment (21°C). The data shown correspond to ~150 runs of worms raised at 22°C; the mean of this distribution corresponds to an entry in Table 1. The distribution is fit by a single exponential line: run termination might be a first-order kinetic process obeying Poisson statistics. The histogram was binned with 10 sec width.

In spatial gradients, worms might arrive more quickly at T_c by biasing reorientation or run curvature toward T_c rather than by picking run direction and curvature randomly. To address this issue, we studied the trajectories of worms migrating toward T_c on the surfaces of agar plates with spatial thermal gradients. On spatial gradients in which worms rapidly migrated down gradients toward T_c, abrupt reorientations did not direct worms toward the favorable direction (Fig. 4*a*), nor did worms steer by gradually turning toward T_c (Fig. 4*b*).

In our hands, when navigating even the steepest spatial gradients (4°C/cm) at temperatures below T_c, worms did not actively migrate toward T_c. Neither the run duration nor the run orientation was modulated to bias the worms up (or down) the gradients. Worms navigating at temperatures below T_c are atactic. Worms were able to aggregate near T_c by tracking isotherms on random arrival near T_c, but if they have a strategy for active migration up gradients toward T_c, it is undetectably weak using our assays.

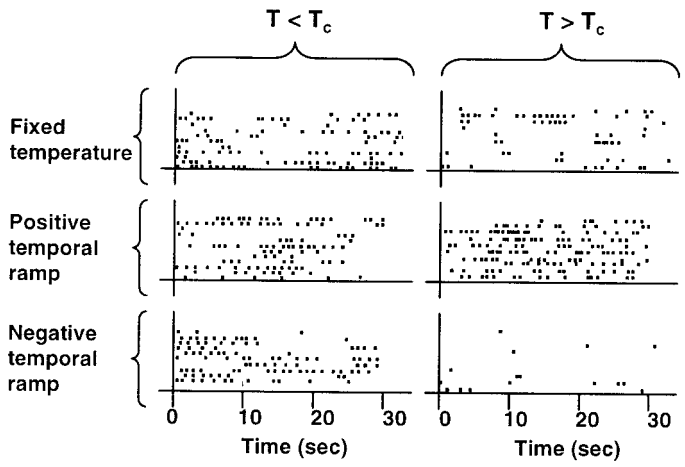

Figure 3. Times of occurrence of run termination events of N2 worms cultivated at 20°C swimming in droplets. Experiments at temperatures below T_c are at the *left*, and experiments at temperatures above T_c are at the *right*. Experiments at fixed temperatures are at the *top* (19°C for $T < T_c$; 24°C for $T > T_c$). Experiments subjecting the drops to positive temporal ramps of +4°C/min are in the *middle* (17–19°C for $T < T_c$; 22–24°C for $T > T_c$). Experiments subjecting the drops to negative temporal ramps of −4°C/min are at the *bottom* (19–17°C for $T < T_c$; 24–22°C for $T > T_c$). Within a *panel*, individual experimental trials are aligned along the vertical axis. Run termination events during a trial are indicated by *closed squares*; their horizontal positions indicate their times of occurrence from the beginning of each trial. (In the case of the ramp experiments, trials begin at the onset of the ramp.) All trials lasted 30 sec. For $T < T_c$, the rate of run termination was 0.26 ± 0.04 and 0.33 ± 0.04 Hz for positive and negative ramps, respectively; for $T > T_c$, the rate of run termination was 0.56 ± 0.03 and 0.08 ± 0.02 Hz for positive and negative ramps, respectively. The rate of run termination at fixed temperatures was 0.33 ± 0.08 and 0.22 ± 0.03 Hz for 19°C and 24°C, respectively. Values are means ± SEM.

Table 3. Run speeds of N2 worms cultivated at 22°C in isotropic environments of different temperatures and subjected to temporal ramps toward or away from 22°C

	Run speed (mm/min)
Constant temperature	
17°C	14 ± 2
21°C	15 ± 2
27°C	16 ± 2
Temporal ramps (0.5°C/min)	
17°C → 19°C	15 ± 2
19°C → 17°C	14 ± 2
24°C → 26°C	19 ± 4
26°C → 24°C	16 ± 3

Each statistic represents ~20 measurements. Run speed increases slightly at higher ambient temperatures but is not modulated as part of thermotactic strategy. These experiments conducted with worms cultivated at 15°C or 25°C yielded no detectable differences from these worms cultivated at 22°C (data not shown).

In summary, when migrating toward T_c, worms modulate run duration but do not modulate run speed or orientation. When worms navigate at temperatures above T_c, they modulate the frequency of abrupt reorientations to migrate down the gradient: worms terminate runs more rapidly when moving away from T_c than when moving toward T_c. However, worms do not choose the angle of abrupt reorientations to direct runs in the more favorable direction. In contrast, when worms navigate at temperatures below T_c, we found no compelling evidence of a mechanism for migration up gradients toward higher temperatures.

Isothermal tracking

Worms crawling on a spatial thermal gradient track isotherms near T_c. On linear spatial gradients, they move along lines; on radial spatial gradients, they move in circles (Hedgecock and Russell, 1975; Mori and Ohshima, 1995). Hedgecock and Russell (1975) found that individual tracks are narrow (~0.05°C) but could occur as far as 3°C from T_c. A worm can track an isotherm in either direction, keeping the higher temperature to its left or its right.

We measured the trajectories of isothermal tracks on linear spatial gradients of varying steepness (Fig. 5). Worms zigzagged along isothermal tracks (i.e., when they crawled too far from the targeted isotherm, they turned back toward it). The frequency of the zigzag increased and the width of the zigzag decreased on steeper thermal gradients (Fig. 5*b,c*). Whatever the gradient steepness, worms responded by turning when they arrived at the temperature boundaries of the targeted isotherm. These boundaries were within ~0.05°C of that isotherm.

As found by Hedgecock and Russell (1975), worms tracked isotherms far from T_c (Fig. 6*a*). A single worm can track multiple isotherms. The movement of one such worm, which left one isotherm and resumed tracking on a parallel isotherm at a different temperature, is shown in Figure 6*b*. The distribution of temperatures at which a worm might track an isotherm is far broader than the width of a single isotherm. This is evidence that thermal preference is represented by the distribution of temperatures at which a worm might track isotherms, and not the temperature of any particular isotherm. Moreover, this distribution is not always centered on T_c (Fig. 6*a*). However, the observation pertinent to the present argument is that the width of the distribution is ~20–30 times broader than the width of a single track.

We suggest that isothermal tracking represents a behavioral mechanism that is active within a band of temperatures near T_c, and that it is not derived from the worm's behaviors when navigating at temperatures above or below T_c. Worms, when navigating at temperatures near T_c, track isotherms by avoiding temporal changes in temperature, turning to offset any changes exceeding ~0.05°C. In this case, as we have observed, a worm deviating from an isothermal track in steeper gradients would react more quickly (higher zigzag frequency) and within a shorter distance (smaller zigzag width). We suggest that the decision whether to track isotherms depends on the worm's comparison of the ambient temperature with T_c; the decision is yes if the worm is within 2–3°C of T_c.

Analysis of mutants

To determine the roles of individual neurons in the identified thermal circuit, we analyzed worms lacking AFD or AIY because of the developmental mutations *ttx-1* (Satterlee et al., 2001) and *ttx-3* (Hobert et al., 1997). The mutation *ttx-1* is thought to be specific to the AFD neuron. Although the mutation *ttx-3* is not specific to AIY, the other neurons it affects are outside the known thermotaxis circuit. Previous workers have characterized both *ttx-1* and *ttx-3* as defective in thermotaxis; we hoped that their defects would shed light on the wild-type mechanisms that comprise thermotaxis (see above).

Worms that lack AIY are cryophilic and do not track isotherms (Mori and Ohshima, 1995; Hobert et al., 1997). We found that the

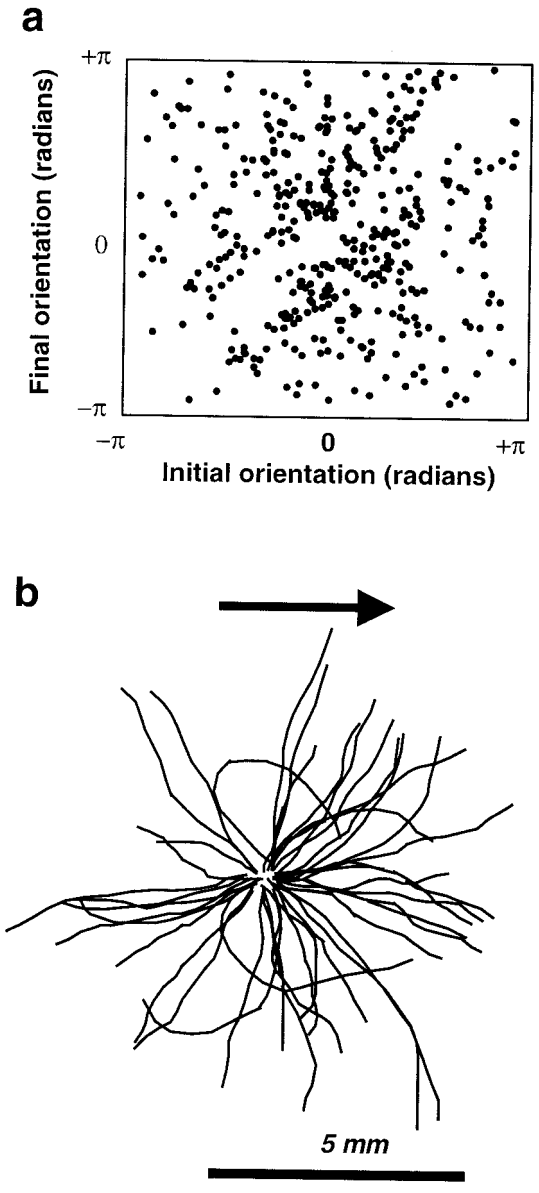

Figure 4. *a*, Initial and final run orientations interrupted by single abrupt reorientations ($n = 413$). Here, N2 worms cultivated at 22°C navigated a linear spatial gradient between 24 and 26°C of steepness 0.4°C/cm. The direction down the gradient was 0 radians. Final run orientations were not more clustered at 0 radians than initial run orientations, as would be the case if the angle of abrupt reorientation was not random and angle selection was weighted toward the favorable direction of movement. Along both axes there is a greater frequency of events between $-\pi/2$ and $+\pi/2$ radians, but this is an artifact: only abrupt reorientations in which both initial and final runs were longer than approximately three body lengths were used. This criterion enabled accurate measurement of run orientation, but on such steep gradients also reduced events corresponding to run orientations less than $-\pi/2$ or greater than $+\pi/2$. However, the relevant feature of this plot that survives this artifact is that the dispersion around 0 radians, the desired direction, is apparently the same for the final as for the initial run orientations. A numerical analysis of this data set shows that the rms deviations from 0 radians for the initial and final run orientations are 1.4 radians and 1.5 radians, respectively: worms are not better oriented toward 0 radians after abrupt reorientations. Similar results were obtained for worms cultivated at 22°C and navigating gradients between 17 and 19°C (data not shown). *b*, Traces of runs of duration of ~20 sec. Here, N2 worms cultivated at 22°C navigated a linear spatial gradient between 24 and 26°C of steepness 0.4°C/cm, a steepness at which worms robustly migrated down gradients but did not always terminate runs within a few seconds when moving up gradients. For presentation purposes, we have centered all runs as emerging from a single point. The *arrow* indicates the direction down the gradient. More tracks are to the *right* ($n = 27$) than to the *left* ($n = 15$), but the essential data are the shape and not the number of the tracks. The number of tracks to the *right* is larger because in any trial there were more runs of 20 sec duration down the gradient than up the gradient attributable to the worm's strategy of shortening or lengthening runs up or down the gradient. Based on the shape of the individual tracks, we conclude that worms did not steer individual runs toward the direction of the gradient. Some runs were curved, but the curvature was randomly CW or CCW and was not correlated with the gradient. Similar results were obtained for worms cultivated at 22°C and navigating gradients between 17 and 19°C (data not shown).

Table 4. Run durations of N2 and *ttx-3 (ks5)* worms navigating isotropic environments and subjected to temporal ramps

	Run duration (sec)	
	N2	*ttx-3 (ks5)*
Constant temperature		
17°C	32 ± 3	23 ± 1
21°C	26 ± 2	16 ± 1
27°C	17 ± 1	9 ± 1
Temporal ramps (0.5°C/min)		
17°C → 19°C	24 ± 1	17 ± 1
19°C → 17°C	23 ± 1	24 ± 2
24°C → 26°C	14 ± 1	9 ± 1
26°C → 24°C	23 ± 2	23 ± 1

Each measurement represents ~200 runs. Here, all worms were raised at 22°C. Gray shading indicates a bias toward lower temperatures (longer runs in negative temporal gradients than in positive temporal gradients). Whereas the statistics for the N2 worms subjected to temporal ramps exhibit cultivation temperature-dependent plasticity (see Table 1), *ttx-3 (ks5)* statistics are independent of cultivation temperature, and the worm moves toward arbitrarily cold temperatures.

mechanism for migration down gradients toward lower temperatures that modulates run duration is constitutively active in worms lacking AIY because of the mutation *ttx-3* (Table 4). The mechanism for migration down gradients controls the movements of wild-type worms only at temperatures above T_c but controls the movements of *ttx-3* worms at all temperatures. It has come to our attention that stronger alleles than *ttx-3* have been isolated, but the phenotype of the mutant we analyzed, *ttx-3 (ks5)*, is robust in our assays and fully penetrant (affecting 100% of worms).

Laser ablation of AFD produces cryophilic or atactic worms (Mori and Ohshima, 1995). As we found for worms lacking AIY, worms lacking AFD because of the mutation *ttx-1* (Satterlee et al., 2001) have a constitutively active mechanism for migration down gradients toward lower temperatures (data not shown). More strikingly, however, *ttx-1* worms crawling on the surfaces of agar plates usually moved in clockwise (CW) or counterclockwise (CCW) circles (Fig. 7). Circling was independent of ambient temperature, presence of spatial or temporal thermal gradients, or T_c. Circling was not observed on cultivation plates in which worms crawl through bacterial lawns. Therefore, circling represents a defect in taxis, not a defect in locomotion, and suggests a flaw in a mechanism that normally controls the direction of turning or run orientation.

DISCUSSION

Our results do not easily reconcile with current opinion that thermotaxis is a balance between separate thermophilic and

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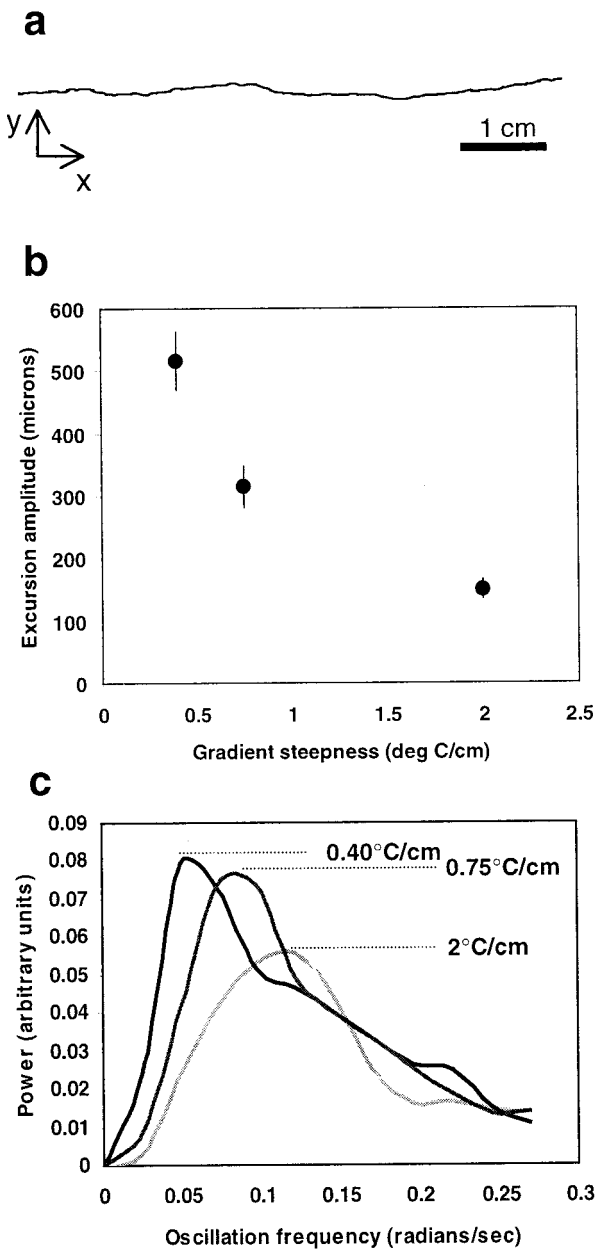

Figure 5. *a*, A single isothermal track of an N2 worm cultivated at 22°C on a linear thermal gradient of steepness 0.4°C/cm. *b*, Isothermal tracks were narrower as the steepness of thermal gradients increased. One metric of the width of isothermal tracks is $\langle y^2 \rangle^{1/2}$, the mean square deviation from the midline of the track. Here, excursion amplitude is defined as the mean $\langle y^2 \rangle^{1/2}$ of ~50 isothermal tracks of N2 worms cultivated at 22°C on three different gradients (0.4, 0.75, and 2°C/cm). Note that the metric of $\langle y^2 \rangle^{1/2}$ (i.e., 1 SD) is not the overall side-to-side width of a track. A better estimate is $4\langle y^2 \rangle^{1/2}$, which corresponds to the width in which the worm spends 95% of its time during a track. The corresponding temperature boundaries of isothermal tracks (the product of gradient steepness and $4\langle y^2 \rangle^{1/2}$) is 0.1 ± 0.01°C. *c*, In steeper gradients, the frequency of the side-to-side movement increased. Here, we show the normalized power spectrums of the isothermal tracks described in Figure 4*b*.

cryophilic drives (Thomas, 1995; Mori and Ohshima, 1997; Mori, 1999). Instead, we suggest that thermotaxis involves two mechanisms: (1) a mechanism for tracking isotherms and (2) a mechanism for migration down gradients toward lower temperatures. In wild-type thermotaxis, we found no evidence for a mechanism for migration up gradients toward higher temperatures.

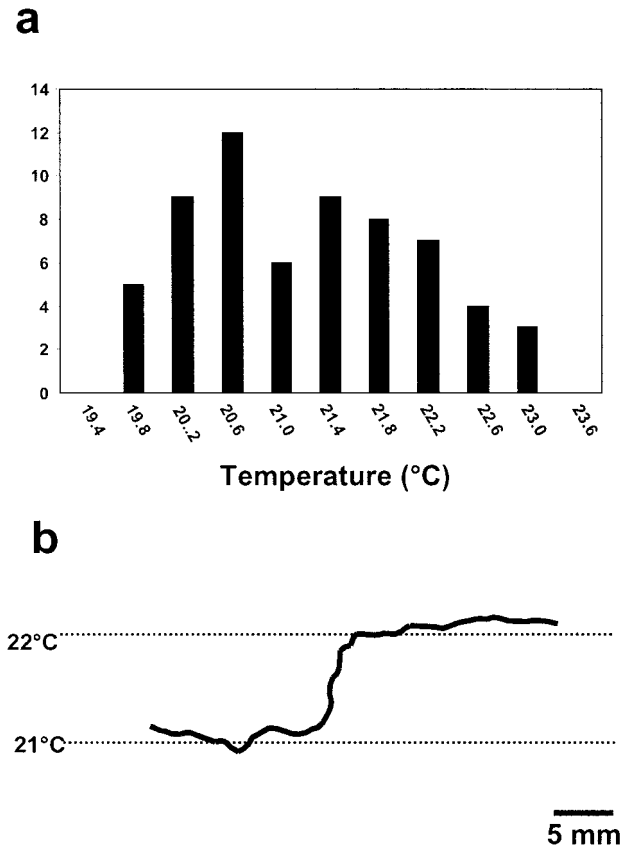

Figure 6. *a*, N2 worms cultivated at 22°C tracked isotherms in a range within 2°C of that cultivation temperature. The histogram shows the positions of the isotherms of ~40 worms drawn from a single plate tracked on a linear thermal gradient of steepness 0.4°C/cm and binned at a width of 0.4°C. *b*, Individual worms often stopped tracking one isotherm and resumed tracking a different one. This trace is an unusually clear example: the worm initially tracked at 21°C, fell off the isotherm, and continued to track at 22°C.

Both mechanisms for tracking isotherms and for migration down gradients toward lower temperature respond to positive and negative changes in temperature, but the two mechanisms are active in different temperature regimes and control the worm's movements in different manners. The mechanism for isothermal tracking is active at temperatures near T_c. This mechanism controls run orientation, responding to positive and negative changes in temperature by prompting turns to offset the changes. The mechanism for migration down gradients toward lower temperatures is active at temperatures above T_c. This mechanism controls run duration but not run orientation, responding to positive or negative changes in temperature by shortening or lengthening runs.

A calcium-binding protein, expressed in AFD, AIY, and other neurons, is essential for isothermal tracking (Gomez et al., 2001). Laser ablation of AFD or AIY abolishes isothermal tracking (Mori and Ohshima, 1995). We found that mutant worms with a developmental defect in AFD have a defect in turning that might indicate a flawed mechanism for isothermal tracking, because this mechanism normally controls turns and turn angles. Together, these results suggest that the mechanism for isothermal tracking includes AFD and AIY.

In mutants with developmental defects in AFD or AIY, the mechanism for migration down gradients toward lower temperatures is active at all temperatures irrespective of T_c. We suggest

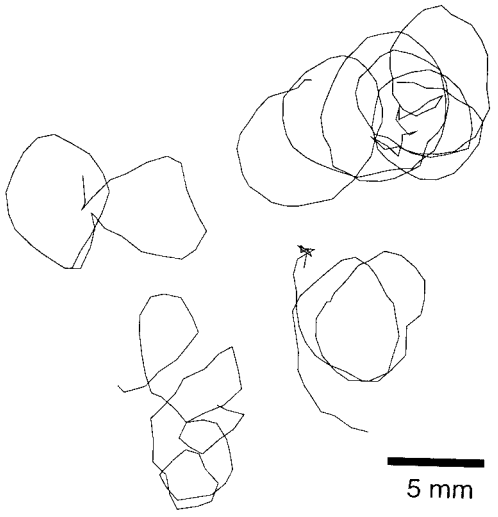

Figure 7. Trajectories of crawling *ttx-1* worms. Circling is either CW or CCW and is independent of ambient temperature or spatial gradients.

that the mechanism for isothermal tracking (involving AFD and AIY) is needed to suppress the mechanism for migration down gradients at temperatures near or below T_c. We do not know which neurons comprise the mechanism for migration down gradients toward lower temperatures.

Pierce-Shimomura et al. (1999) studied chemotaxis by tracking worms on defined salt gradients. Their results are similar to ours for migration down gradients toward lower temperatures. In both mechanisms for migration on chemical gradients and migration down thermal gradients, worms initiate abrupt reorientations to terminate runs that carry them away from their target. This shared feature suggests that the mechanisms for migration on chemical gradients and migration down thermal gradients might have other similarities (e.g., parallel connections to the same motor neurons).

Others have reported both thermophilic and cryophilic phenotypes attributable to mutation or laser ablation (Hedgecock and Russell, 1975; Mori and Ohshima, 1995). Thermophilia has been explained as damage to the cryophilic drive; cryophilia has been explained as damage to the thermophilic drive. However, after considering the mechanisms that underlie normal thermotaxis, these explanations no longer seem adequate. Cryophilia, like that of mutants lacking AFD or AIY, can be explained by constitutive activity of the mechanism for migration down gradients. However, because normal thermotaxis does not exhibit a mechanism for migration up gradients, there is no similar alteration of the normal mechanisms of thermotaxis that might explain thermophilia. It is possible to speculate about more subtle alterations of the two normal mechanisms that underlying thermotaxis that might cause worms to aggregate at temperatures higher than T_c. For instance, worms might add a constant offset to their memory of T_c, and thus may track isotherms (and thus aggregate) at temperatures warmer than the true T_c.

Also, the worm's preference for T_c has been explained as the balance between thermophilia and cryophilia. This explanation is also no longer adequate. Isothermal tracking reflects a capacity to compare ambient temperature with T_c: worms have a long-term memory of T_c; they track any isotherm near T_c. While tracking isotherms, worms react to small temperature changes by modulating run orientation, turning to offset these changes. In our view, the worm's behavior near T_c is not derivative of its behaviors above and below T_c. The behavior near T_c is qualitatively different from its behaviors above and below T_c: above T_c, the worm modulates run duration and not run orientation to migrate down gradients; below T_c, there is no active thermotactic mechanism.

Long-term memory of T_c determines the regimes of activity of the mechanisms for tracking isotherms and migration down gradients. Both mechanisms appear to make short-term comparisons of temperature, enabling responses to positive and negative temperature changes. However, these mechanisms control the worms' movements in different manners, the mechanism for migration down gradients controls only run duration; the mechanism for tracking isotherms controls run orientation. Elucidating the relationships between these two mechanisms, determining their shared and distinct neural circuit elements, and determining which neuron(s) are the primary transducers of temperature (specifically which neurons compare ambient temperature with a long-term memory of T_c and which neurons make short-term comparisons of temperature) are future goals uncovered by the present work.

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