Step Response Analysis of Thermotaxis in *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* migrates toward a preferred temperature on a thermal gradient. A candidate neural network for thermotaxis in *C. elegans* has been identified, but the behavioral strategy implemented by this network is poorly understood. In this study, we tested whether thermal migration is achieved by modulating the probability of turning behavior, as in *C. elegans* chemotaxis. This was done by subjecting unrestrained wild-type, cryophilic, or thermophilic worms to rapid spatially uniform temperature steps (3°C), up or down from the cultivation temperature. Each of the three types of worms we analyzed showed a different pair of responses to the two types of steps. Comparison of wild-type and mutant response patterns suggested a model in which thermal migration involves a unique response to the gradient depending on the orientation of the worm relative to its preferred temperature. Overall, however, turning probability was modulated in a manner consistent with a role for turning behavior in thermal migration. Our results suggest that sensory systems for thermotaxis and chemotaxis may converge on a common behavioral mechanism.

Key words: C. elegans; **nematode; thermotaxis; spatial orientation; stochastic model; sensorimotor integration**

Introduction

Caenorhabditis elegans orients to both chemical (chemotaxis) and thermal (thermotaxis) gradients (Ward, 1973; Hedgecock and Russell, 1975), making it a promising experimental system for investigating the neuronal basis of spatial orientation. Previous studies have established a plausible behavioral mechanism for chemotaxis in *C. elegans* (Dusenbery, 1980; Pierce-Shimomura et al., 1999). Locomotion consists of periods of relatively straightforward movement punctuated approximately twice per minute by bouts of turning (Rutherford and Croll, 1979). Two main kinds of turns are recognized in *C. elegans*: "reversals," in which the animal moves backward for several seconds and then goes forward again in a new direction, and "omega turns," in which the animal's head bends around to touch the tail during forward locomotion, momentarily forming a shape like the Greek letter (Croll, 1975b). Statistical analysis reveals that reversals and omega turns occur in bursts that have been termed pirouettes (Pierce-Shimomura et al., 1999). Pirouette probability is modulated by the rate of change of chemical concentration (dC/dt): when dC/dt is <0, pirouette probability is increased, whereas when dC/dt is >0, pirouette probability is decreased. Thus, runs down the gradient are truncated, and runs up the gradient are extended, resulting in net movement toward the gradient peak.

The pirouette strategy in chemotaxis suggests a reasonable hypothesis for the behavioral mechanism of the migration phase of thermotaxis (Ryu and Samuel, 2002). Tracks of *C. elegans* in the migration phase closely resemble tracks of worms in chemotaxis assays, with episodes of straight crawling interspersed with sharp pirouette-like changes in direction in regions above and below the preferred temperature (T_p) (Mori and Ohshima, 1995). Thus, pirouettes might also serve to direct an errant worm back to T_p. If so, then when a worm moves away from T_p, turning probability should increase, whereas when a worm moves toward T_p, turning probability should decrease. To test the pirouette hypothesis for thermotaxis, we devised an apparatus that enabled us to subject freely moving worms to rapid temperature steps with minimal mechanical disturbance. Overall, turning probability was modulated in a manner consistent with a role for turns in thermal migration, although not always as predicted by the pirouette hypothesis in the strict sense. These results imply a new behavioral model for *C. elegans* thermotaxis. In addition, they suggest that in *C. elegans*, the sensory systems for chemotaxis and thermotaxis converge on a common behavioral mechanism.

Materials and Methods

Animals. C. elegans [Bristol N2; *ttx-1(p767)*, *ttx-3(ks5)*, *unc-86(e1416)*] were grown in mixed-stage cultures at 20°C on 1.7% agar-filled plates containing nematode growth medium seeded with *Escherichia coli* strain OP50 (Brenner, 1974). Experiments were performed on young adults from each strain (selected by relative size and absence of eggs).

Apparatus. The device for delivering rapid temperature steps is shown in Figure 1. A thin agarose film (~70 μm thick, 5.5 cm diameter) was formed by pressing molten agarose [low EEO (electro endo osmosis) electrophoresis grade; 5% aqueous; Fisher Scientific, Houston, TX] between two glass plates, separated by 70 μm shims. The agarose film was glued with a cyanoacrylate adhesive (Vetbond; B. Braun Medical AG, Emmenbrucke, Switzerland) beneath a hole in a Plexiglas plate, forming a trampoline-like assembly. The temperature of the agarose was controlled by placing it in contact with the surface of one of two insulated static fluid-filled chambers containing buffer at the desired temperature. The chambers were mounted on a smooth linear translator (data not shown) that enabled us to exchange chambers with minimal vibration.

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Chamber temperatures were monitored throughout the experiment by means of temperature probes mounted in the center of each chamber directly below the surface of the fluid as shown in Figure 1. In pilot experiments, a thermistor in contact with the top surface of the agarose showed that on exchanging chambers, temperature changed approximately exponentially with a half-time of 2.7 ± 0.11 sec (mean ± SEM; $n = 28$).

Solutions. The buffer solution in the chambers contained the following (in mM): 50 NaCl, 25 phosphate buffer, pH 6.2, 1 $CaCl_2$, and 1 $MgSO_4$. Osmolarity was adjusted to 340 mOsm/kg (sorbitol). At lower osmolarities (<150 mOsm/kg), worms became sluggish and often stopped for long periods during the assay (M. L. Moravec and S. R. Lockery, unpublished observations).

Step response assays. A single worm was transferred to an unseeded agar plate in a clean micropipette containing 1.5 μl of buffer. To remove bacteria, excess buffer was withdrawn and the worm was allowed to move well away from the transfer site. The worm was then transferred in the same way to the center of the agarose film equilibrated to the buffer in the 20°C chamber. After a 30 sec period for adaptation to the apparatus, the worm's behavior was observed for 5 min at 20°C (i.e., the cultivation temperature, T_c) and then for an additional 5 min at ± 3°C relative to T_c by bringing the surface of the fluid in the second chamber, at the new temperature, in contact with the agarose film. Because worm behavior has a strong random component, we chose to use a comparatively large temperature step to obtain a reasonable signal-to-noise ratio. Nevertheless, the lower and higher temperatures experienced by the worms in our assays (17 and 23°C, respectively) were well within the range of normal thermotaxis behavior (16–25°C) (Hedgecock and Russell, 1975) and well below the temperature of the thermal avoidance reflex (33°C) (Wittenburg and Baumeister, 1999). To control for mechanical artifacts, we performed sham steps in which both chambers contained buffer at 20°C. Behavior was recorded for real-time off-line analysis by means of a video camera attached to a stereomicroscope (12–16× magnification). All strains and temperature conditions were assessed by a single experimenter; all off-line analyses (see below) were performed by a second experimenter. Each worm was tested only once.

Definitions of behaviors. Behavior was analyzed in terms of three main behavioral states (Croll, 1975a,b): forward locomotion, reversal, and omega turn. These three states accounted for 98% of the behavior. A fourth state, "other," was used as a catch-all state for the rare instances when behavior fell into none of the three main categories. The forward state was defined as anterior translation of the body associated with a head-to-tail body wave. The reversal state was defined as caudal translation of the body associated with a tail-to-head body wave. The omega-turn state was defined as head-to-body contact with the head moving forward. Note that our classification scheme omits deep bends, which resemble omega turns except that there is no contact between head and tail. This omission was unavoidable because deep bends are graded in amplitude between the bends of normal swimming movements and omega turns. As such, deep bends are difficult to discern objectively by eye.

In a three-state classification system, there are six possible state transitions. We used the following objective criteria to define the instant at which a given transition occurred: forward-reversal, first backward movement of the tail; reversal-forward, first forward movement of the head; forward-omega, the instant head-body contact was made; omega-forward, the instant head-body contact was broken; omega-reversal, first backward movement of the tail; and reversal-omega, the instant head-body contact was made.

State probabilities. The behavior of individual worms was scored manually by pressing computer keys to note the initial behavioral state and the times of state transitions. Blind scoring was unwarranted, because behavioral state transitions were clearly defined (see above) and generally unambiguous. We estimate that the scorer's reaction time was at most ~1 sec. Reaction time was unlikely to have affected our estimates of state probabilities because it adds an equal delay to the time of behavioral onset and offset. From the transition times for each worm, we constructed an ethogram showing the worm's behavioral state as a function of time in the assay (see Fig. 2). Ethograms for each strain and test condition were combined by computing in successive 10 sec bins, the average probabilities of finding a worm in the forward, reversal, and omega states. A 10 sec bin was chosen because it provides a good compromise between a high signal-to-noise ratio and the fine temporal structure of the behavior. Qualitatively similar results were obtained when the analysis was performed with bin sizes of 1, 2, and 5 sec. In each bin, state probability was computed as follows:

$$P_s = \frac{1}{W} \sum_{1}^{W} \frac{t_{s,w}}{\Delta t},$$

where P_s is the average probability of state s, W is the number of worms, $t_{s,w}$ is the amount of time worm w was in state s, and Δt is the bin width. Dwell times within each state were well fit by an exponential distribution. The main deviations from exponentiality were for the very brief dwell times (<2 sec). Deviations in this range were most likely attributable to time lags introduced by the observer.

Stochastic analysis. The fact that dwell times were distributed exponentially made it possible to analyze behavior stochastically. The assumption of three behavioral states (forward, reversal, and omega turn) implies six rate constants for the transitions between states. Like the state probabilities, the rate constants were computed in 10 sec bins. In each bin, the rate constant was computed as follows:

$$\alpha_{i,j} = \frac{F_{i,j}}{P_i},$$

where $\alpha_{i,j}$ is the rate constant for the transition from state i to j, $F_{i,j}$ is the frequency of the i-j transition (events per second), and P_i is the probability of state i. Computing rate constants for each time bin yielded the rate-constant time courses shown in Figure 5. Rate-constant calculations were checked in a "play-back" experiment in which the differential equations describing the time dependence of state probabilities were integrated numerically using the computed rate-constant time courses (see Fig. 5) to set the value of each rate constant at each time step in the integration procedure. In all cases, there was a satisfactory match between predicted and observed state probabilities (data not shown), indicating that there was sufficient resolution in the rate-constant time courses to reproduce state-probability data.

Statistics. Statistical significance of the effects of temperature steps on state probabilities was assessed (t test) by comparing the average change in forward probability after a temperature step with the average change in forward probability after a sham step. We did not test the significance of effects on the two turning states (reversal and omega-turn) because, as noted above, animals were either in the forward state or the turn state 98% of the time. The probability before the step was defined as the fractional dwell time in the forward state during the time window −180 to 0 sec, in which 0 is the time of the change. The probability after the step was defined as the fractional dwell time during the time window 20–200 (i.e., the approximated duration of the response). For biphasic step responses (see Fig. 3*B3*), we also computed fractional dwell time in the first bin (0–10 sec) after the step. Data are presented as means ± SEM. Statistical significance of the effects of temperature steps on transition rates was assessed using a Mann–Whitney test with the correction of noncontinuity for one degree of freedom. For each transition rate, experimental points falling in the time window 20–200 sec after the step were compared with the same set of points in the control experiment (no temperature step) for the same strain.

Results

To test whether temperature modulates turning probability as predicted by the pirouette hypothesis (Table 1), we placed individual worms on a thin agarose film in contact with the fluid surface of an open chamber filled with a buffer solution at the cultivation temperature (T_c, 20°C) (Fig. 1). After a 5 min baseline observation period, we quickly replaced the first chamber with a second one that contained the same buffer solution at either a second temperature (17°C in the "downstep" condition and 23°C

Table 1. Responses of wild-type and thermotaxis mutants to temperature steps as predicted by the pirouette hypothesis

Stimulus	Wild-type	Cryophilic (*ttx-3*)	Thermophilic (*unc-86*)
Downstep	Turn	Run	Turn
Upstep	Turn	Turn	Run

The term "turn" means an increase in the probability of the reversal and/or the omega states with a decrease in the probability of the forward state; conversely, "run" means an increase in the probability of the forward state with a decrease in the probability of the reversal and/or omega states.

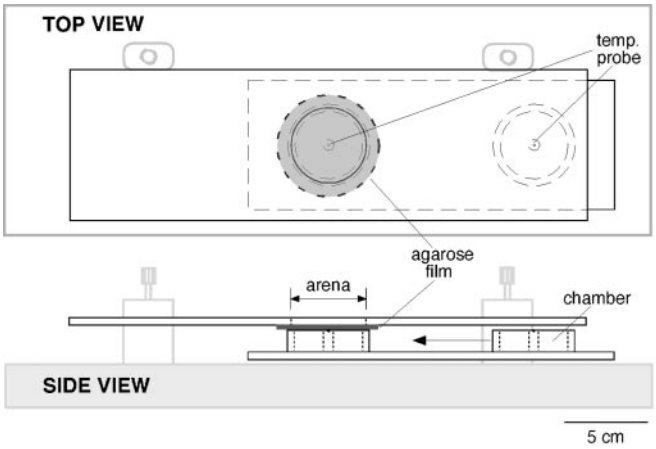

Figure 1. Apparatus for delivering temperature steps to unrestrained worms. A thin agarose film (gray circle) was glued beneath a hole in a Plexiglas plate, forming a trampoline-like arena. The worm was placed on the agarose film, the temperature of which was controlled by means of two insulated fluid-filled chambers containing buffer at different temperatures. Temperature steps were produced by sliding the chambers to the left, as shown by the arrow, with the agar film remaining stationary. Temperature was monitored throughout the experiment by probes placed in contact with the underside of the agarose film.

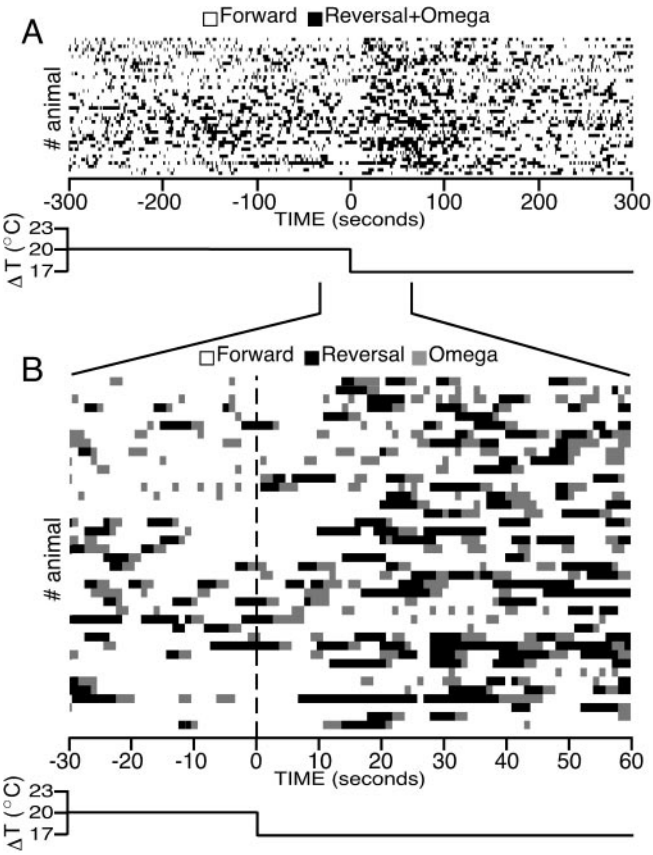

Figure 2. Ethograms for wild-type worms subjected to a downstep plotted on two different time scales. *A*, A complete experiment. Each row represents the data from one worm; thirty-nine worms are shown. White regions indicate the forward state; black regions indicate the reversal and omega-turn states. *B*, Expanded view of data near the time of the step in *A*. Here, black regions indicate the reversal state and gray regions indicate the omega-turn state. The temperature protocol is indicated below each graph.

in the "upstep" condition) or at the same temperature to control for possible mechanical artifacts. We then observed the behavior of the worm for an additional 5 min.

We classified the worm's behavior in terms of three mutually exclusive states: forward locomotion, reversal, and omega turn. Behavior was recorded by noting the times at which state transitions occurred, yielding a complete state history for each animal (Fig. 2). We tested wild-type worms and three different previously isolated thermotaxis mutants: cryophilic strain *ttx-3(ks5)* (Hobert et al., 1997), thermophilic strain *unc-86(e1416)* (Chalfie et al., 1985), and athermotactic strain *ttx-1(p767)* (Perkins et al., 1986). We chose this subset of thermotaxis mutants because they are known to move well and/or have relatively well circumscribed neuronal deficits. The behavioral records for each condition were analyzed by computing across individuals the probability that an animal was in the forward, reversal, or omega state as a function of time in successive 10 sec bins (Fig. 3). We observed no obvious nonspecific effects of the temperature steps, such as changes in instantaneous speed or the frequency of locomotory waves. Thus, the main behavioral effects appeared to be confined to changes in state probability.

Wild-type animals exhibit turns to downsteps and runs to upsteps

In wild-type animals, a downstep from T_c caused a transient (~2 min) decrease in forward state probability ($p < 0.001$) (Fig. 3*A2*). The decrease in forward probability was mirrored by an increase in the probability of both the reversal and the omega turn states. In control experiments, state probabilities were constant (Fig. 3*A1*). This indicates that there were no progressive effects of time and no mechanical artifacts attributable to moving the chambers to effect the temperature change. Thus, downsteps induced turning. The fact that the changes in state probability were transient suggests that probability is sensitive to changes in temperature but not to its absolute level. In contrast, an upstep elicited a sustained increase in forward probability ($p < 0.02$) (Fig. 3*A3*) and a concomitant decrease in reversal and omega-turn probability. In this case, the changes in state probability may reflect a dependence on absolute temperature; alternatively, adaptation to upsteps may be slower than adaptation to downsteps.

The pirouette hypothesis predicts that downsteps and upsteps induce turning in wild-type animals, because in both cases, the worm is shifted away from T_p (Table 1). Thus, the effect of downsteps on state probability is consistent with the pirouette hypothesis, whereas the effect of upsteps is not. However, as documented below, additional analysis of the response to upsteps suggested that runs are frequently preceded by a reversal-omega transition, a common behavioral motif in avoidance responses in *C. elegans* (Croll, 1975b; Culotti and Russell, 1978).

Cryophilic animals exhibit no response to downsteps and "turn-and-run" to upsteps

The gene *ttx-3* encodes a LIM homeodomain protein that is essential for normal thermotaxis in *C. elegans*. *ttx-3* is expressed in the neuron class AIY, a pair of interneurons that have been shown

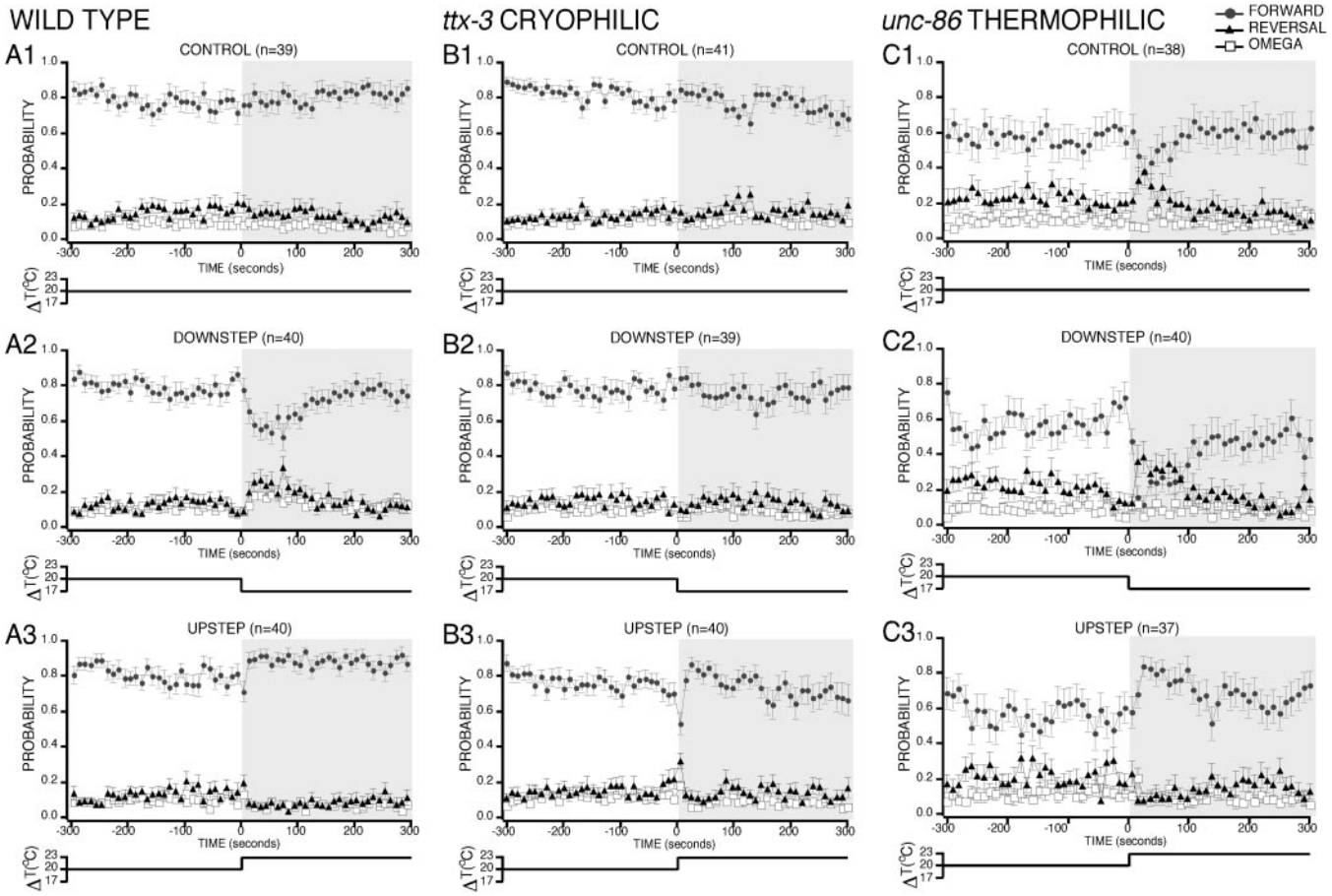

Figure 3. Behavior of wild-type, *ttx-3*, and *unc-86* mutant worms in response to step changes in temperature. The average probability of finding a worm in each of three behavioral states [forward (circles), reversal (triangles), or omega turn (squares)] is plotted in 10 sec bins versus time. Worms were subjected to one of three test conditions: a sham temperature step as described in Materials and Methods (top row), a downward step (downstep) from 20 to 17°C (middle row), or an upward step (upstep) from 20 to 23°C (bottom row). The temperature protocol is indicated below each graph. Within graphs, the shaded region represents the poststimulus period. Data are presented as means ± SEM; *n* indicates the number of worms in each group.

by laser ablation to be essential for normal thermotaxis (Mori and Ohshima, 1995). The gene is also expressed in three other pairs of neurons that are not part of the thermotaxis network and two neurons in the pharynx, the *C. elegans* feeding organ (Altun-Gultekin et al., 2001). *ttx-3* mutants migrate to temperatures below the cultivation temperature ($T_p < T_c$) (Hobert et al., 1997) as do animals in which AIY is ablated (Mori and Ohshima, 1995). In terms of thermotaxis, the main effect of the *ttx-3* mutation appears to be disruption of the normal projection pattern of the neurites of AIY neurons (Hobert et al., 1997).

A downstep from T_c had no effect on forward state probability in *ttx-3* ($p > 0.50$) (Fig. 3*B2*). In contrast, an upstep produced a biphasic response, a transient decrease in forward probability (time, 0–10 sec; $p < 0.001$) (Fig. 3*B3*) followed by sustained increase in forward probability (time, 20–200 sec; $p < 0.05$) (Fig. 3*B3*). The changes in forward probability were mirrored by converse changes in the probability of the reversal state. We conclude that upsteps in *ttx-3* elicit a two-part behavioral sequence: turn-and-run.

The pirouette hypothesis predicts that a downstep in a cryophilic mutant will produce runs, whereas an upstep will produce turns because a downstep shifts a cryophilic worm toward T_p, whereas an upstep shifts it away from T_p (Table 1). The observation that an upstep produces a turn-and-run sequence is consistent with the pirouette hypothesis, because the animal initially turns in response to the upstep. The tendency to follow turns with runs could be an adaptation for efficient avoidance of nonpreferred temperatures. The absence of a response to a downstep is, of course, inconsistent with the pirouette hypothesis in the strict sense. Together, however, the turn-and-run response to upsteps and the absence of a response to downsteps are consistent with the overall cryophilic phenotype of *ttx-3*. This is because an animal that turns when temperature goes up but does not turn when temperature goes down tends to drift toward lower temperatures.

Thermophilic animals exhibit turns to downsteps and runs to upsteps

The gene *unc-86* encodes a POU homeodomain protein that is essential for normal egg-laying, mechanosensation, and thermotaxis (Chalfie and Sulston, 1981; Finney et al., 1988). *unc-86* is expressed in 27 neuron classes, including AIZ, a pair of interneurons that are essential for normal thermotaxis (Mori and Ohshima, 1995). In *unc-86* mutants, AIZ neurons presumably do not form properly (Finney et al., 1988). Animals mutant for *unc-86* migrate to temperatures above the cultivation temperature ($T_p > T_c$), as do animals in which AIZ is ablated.

In *unc-86* mutants, a downstep from T_c produced a transient (~4 min) decrease in forward state probability (Fig. 3*C2*) and a concomitant increase in reversal probability. However, in control experiments, we also observed a decrease in forward probability (Fig. 3*C1*). We believe that the control response most likely reflects reversals induced by small mechanical vibrations that un-

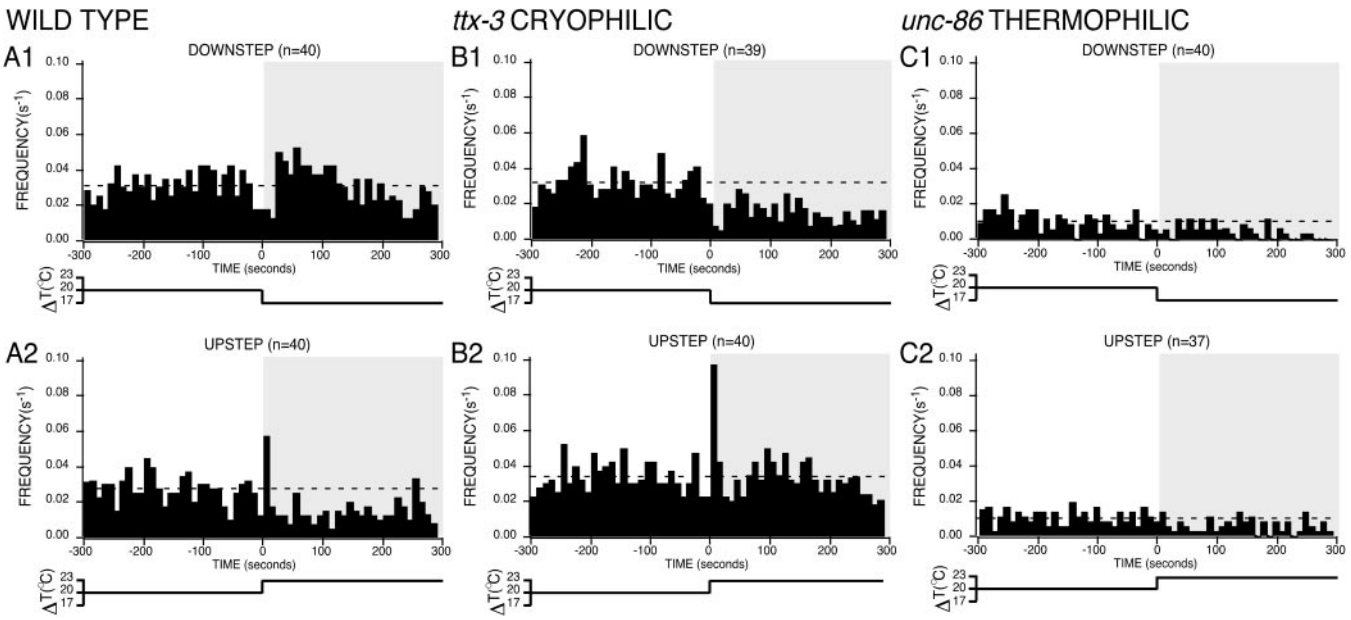

Figure 4. The effect of temperature steps on the frequency of avoidance responses in wild-type, *ttx-3*, and *unc-86* animals. Worms were subjected to one of three test conditions: a sham temperature step (data not shown), a downward step (downstep) from 20 to 17°C (top row), or an upward step (upstep) from 20 to 23°C (bottom row). Avoidance-response frequency, defined as the number of reversal-omega transitions per animal per second, is plotted versus time in 10 sec bins. The temperature protocol is indicated below each graph. Within graphs, the shaded region represents the poststimulus period. The dotted line shows the average frequency in the prestimulus period.

avoidably occurred when the chambers were moved, because vibrations are known to induce reversals in *C. elegans* (Rankin, 1990). Nevertheless, the decrease in forward probability in response to a downstep was larger and longer lasting than the mechanical artifact ($p < 0.001$) (Fig. 3, compare *C1* and *C2*). We conclude that in *unc-86*, the changes in state probability after a downstep are in part a specific response to temperature. The difference between wild-type and *unc-86* animals in control experiments (Fig. 3, compare *A1* and *C1*) suggests that *unc-86* may be more sensitive to mechanical stimulation than wild-type animals. If so, this is somewhat surprising, because *unc-86* lacks the mechanosensory neurons for the response to mild touch (Chalfie and Sulston, 1981). It is conceivable that other mechanosensory neurons, such as those responsible for nose touch (Kaplan and Horvitz, 1993), are sensitized in *unc-86*, but we have no independent evidence for this interpretation. An upstep in *unc-86* produced a transient (~4 min) increase in forward probability that was mirrored by converse changes in the probability of the reversal state ($p < 0.001$) (Fig. 3*C3*). The fact that this response is in the opposite direction from the mechanical artifact indicates that it is a specific response to temperature.

The pirouette hypothesis predicts that a downstep in a thermophilic mutant will elicit a turn and an upstep will elicit a run, because a downstep shifts a thermophilic worm away from T_p, whereas an upstep shifts it toward T_p (Table 1). This is precisely the pattern of responses observed for *unc-86*. We conclude that the responses of *unc-86* to downsteps and upsteps are consistent with the predictions of the pirouette hypothesis.

Responses of *ttx-1* animals were ambiguous

For completeness, we also tested the mutant *ttx-1(p767)*, a well studied thermophilic mutant that exhibits a mixed cryophilic–athermotatic phenotype (Mori and Ohshima, 1995). The AFD neurons are the likely thermosensory neurons in *C. elegans*. *ttx-1* worms lack the cilium-like structure on the sensory endings of the AFD neurons, suggesting a defect in sensory transduction (Perkins et al., 1986).

In *ttx-1*, a downstep produced a transient decrease in forward state probability mirrored by an increase in reversal probability. Interpretation of these data were complicated by the fact that in control experiments, we also observed a small transient decrease in forward state probability. Although this decrease in forward state probability was smaller than the one seen in downsteps ($p < 0.02$; data not shown), this suggests that at least part of the downstep response is a mechanical artifact. Upsteps in *ttx-1* produced no change in state probabilities (data not shown).

Because the decrease in forward state probability observed in control experiments is not significantly different from the upstep response ($p > 0.20$), it is unclear whether the absence of a response to upsteps represents an insensitivity to upsteps or the cancellation of equal but opposite mechanical and thermal responses. Resolution of these ambiguities will require separate experiments to determine how mechanosensory and thermosensory inputs add.

Upsteps elicit avoidance responses in wild-type and cryophilic animals

C. elegans avoids noxious stimuli such as high osmolarity, heavy metals, and high temperature (Culotti and Russell, 1978; Sambongi et al., 1999; Wittenburg and Baumeister, 1999). Avoidance responses often consist of a two-step motor program, a reversal followed immediately by an omega turn, which can reorient the animal by as much as 180°. As such, they could contribute to dispersal from a nonpreferred temperature.

To determine whether avoidance responses underlie any of the step responses in Figure 3, we computed the frequency of reversal-omega transitions as a function of time (Fig. 4). We found that the reversal-omega transition frequency exhibited a prominent spike immediately after the upstep in wild-type animals and the cryophilic mutant *ttx-3* but not in the thermophilic

mutant *unc-86* (Fig. 4, bottom row). The proportion of wild-type animals emitting at least one reversal-omega transition in the 10 sec bin immediately after the upstep step was 50% (20 of 40), significantly higher than expected based on the average number of wild-type control worms emitting a reversal-omega transition per 10 sec (data not shown; 24%; $\chi^2 = 20.17$; $p \ll 0.001$). Similarly, the proportion of cryophilic animals emitting at least one reversal-omega transition in the bin immediately after the upstep was 70% (28 of 40), significantly higher than expected based on the average number of cryophilic control worms emitting a reversal-omega transition per 10 sec (data not shown; 26%; $\chi^2 = 48.56$; $p \ll 0.001$).

The presence of a spike in the avoidance response data of wild-type animals indicates that the response to an upstep in the first 10 sec after the stimulus is often a turn. This observation, together with the fact that wild-type animals exhibit runs starting in the period from 10 sec after the upstep to the end of the experiment (Fig. 3*A3*), suggests that upsteps in wild-type animals elicit the turn-and-run strategy, as we saw in *ttx-3* (Fig. 3*B3*). Thus, both strains reorient in the same way when the temperature is shifted away from T_p in the positive direction. The turn-and-run strategy could, in principle, be used for reorientation when confronted with downsteps as well as upsteps. However, this was not the case, because neither wild-type nor *unc-86* exhibited a spike in avoidance responses after downsteps, although both strains exhibited a robust turning response (Fig. 3*A2*,*C2*). This result suggests that *C. elegans* exhibits distinct behavioral strategies when the shift away from the T_p is in the positive or negative direction.

Temperature steps affect rates of transition between behavioral states

The data from Figure 3 describe how populations of worms behave in response to temperature steps, but they do not specify the behavior of individual worms. The behavior of individual worms has a significant random component (see Materials and Methods, Fig. 2). Thus, to address the question of individual worm behavior, we adopted a stochastic approach in which behavior is analyzed in terms of the probability that an individual worm will make a transition between behavioral states. The probability that a worm in state i will make a transition to state j is given by the following:

$$P_{i,j} = \alpha_{i,j}\Delta t,$$

where $\alpha_{i,j}$ is the transition rate constant (i.e., the probability per unit dwell time in state i that a worm will make the transition to state j) and Δt is a small time interval. In a three-state system there are six rate constants. To understand how the behavior of individual worms was modulated by temperature, we computed the value of each rate constant as a function of time relative to the temperature step in 10 sec bins. The rate constant analysis was performed for four different conditions: downsteps and upsteps in wild type and *ttx-3*, the two strains in which there was no mechanical artifact.

The results of one such analysis are depicted in Figure 5, which shows the wild-type rate-constant time courses for downsteps in Figure 3*A2*. For comparison, we also computed the rate constants during the corresponding control experiment in Figure 3*A1*. Regions between the experimental and control rate-constant curves are shaded either black, to indicate where the rate constant in the downstep experiment was greater than the rate constant in the control experiment, or gray, to indicate where the rate constant in the downstep experiment was less than in the control experiment.

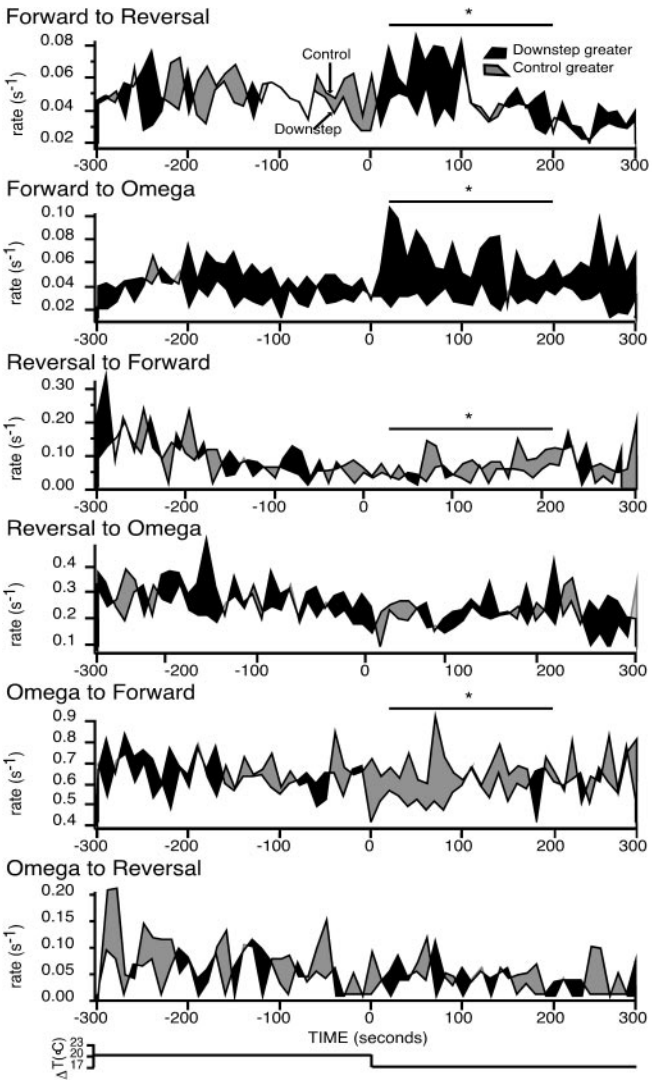

Figure 5. The effect of a temperature step on the six rate constants for transitions between behavioral states in wild-type animals. Each graph shows the effect on the rate constant for the indicated transition (e.g., forward to reversal) as a function of time relative to the temperature step. Rate constants were computed as described in Materials and Methods. Two traces are shown: the rate constant in response to a downstep and the rate constant in a control experiment using a sham step. Regions between the downstep and the control rate constant curves are shaded either black to indicate where the rate constant in the downstep experiment was greater than the rate constant in the control experiment, or gray to indicate where the rate constant in the control experiment was greater than in the upstep experiment. The temperature protocol is shown at the bottom of the figure. Asterisks indicate significant differences from control in the time window indicated by the horizontal line. Note that a different vertical scale is used in each graph. Data are from the experiment shown in Figure 3, *A1* and *A2*.

Four rate constants showed significant effects (Mann–Whitney; $p < 0.05$). After the downstep, the forward-reversal rate (α_{fr}) and the forward-omega rate (α_{fo}) increased, whereas the reversal-forward rate (α_{rf}) and omega-forward rate (α_{of}) decreased. All four effects were consistent with the observed modulation of state probabilities (Fig. 3*A2*) because they promote the probabilities of the reversal and omega states at the expense of the forward state.

The results of the four rate-constant analyses are summarized in Table 2 and Figure 6. We draw three main conclusions regarding the stochastic mechanisms of the temperature responses in *C. elegans*. First, a change in the probability of a state can be the result of changes in entry rate (e.g., the reversal state in the wild-type upstep condition), exit rate (e.g., the forward state in the

Table 2. Effect of temperature steps on average rate constants for behavioral state transitions in wild-type and *ttx-3* animals

Transition	Experiment	Wild type	*ttx-3*
Forward-reversal	Control	0.038 ± 0.002	0.059 ± 0.003
	Downstep	0.052 ± 0.003*	0.030 ± 0.002*
	Upstep	0.021 ± 0.001*	0.061 ± 0.002
Forward-omega	Control	0.026 ± 0.001	0.029 ± 0.001
	Downstep	0.060 ± 0.003*	0.024 ± 0.002
	Upstep	0.026 ± 0.001	0.022 ± 0.001*
Reversal-forward	Control	0.065 ± 0.006	0.120 ± 0.004
	Downstep	0.029 ± 0.003*	0.055 ± 0.004*
	Upstep	0.094 ± 0.011	0.109 ± 0.009
Reversal-omega	Control	0.195 ± 0.006	0.232 ± 0.010
	Downstep	0.218 ± 0.010	0.128 ± 0.007
	Upstep	0.232 ± 0.013	0.275 ± 0.012*
Omega-forward	Control	0.648 ± 0.016	0.610 ± 0.016
	Downstep	0.539 ± 0.010*	0.423 ± 0.013*
	Upstep	0.687 ± 0.026	0.646 ± 0.019
Omega-reversal	Control	0.033 ± 0.005	0.019 ± 0.004
	Downstep	0.034 ± 0.004	0.028 ± 0.004
	Upstep	0.014 ± 0.004	0.045 ± 0.006*

Rate constants were averaged in the time window 20–200 sec after the temperature step (mean ± 1 SE). Asterisks indicate significant differences relative to control rate constants averaged across the same time window (Mann–Whitney; $p < 0.05$).

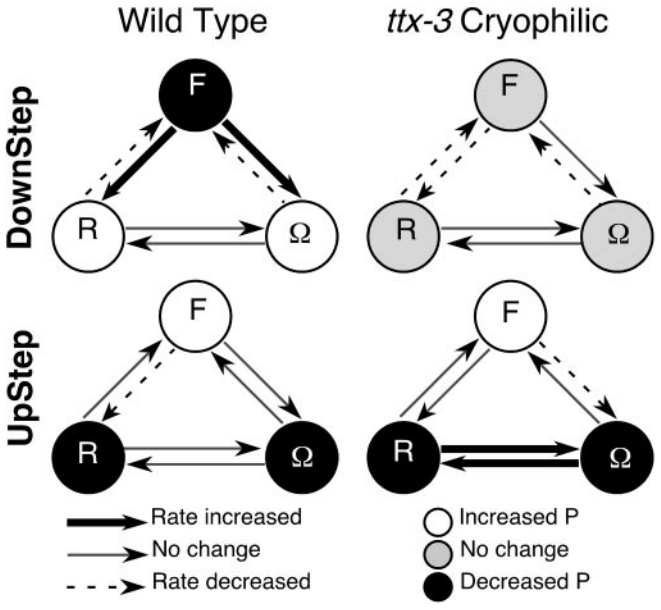

Figure 6. Summary of the effects of temperature steps on rate constants in wild-type and *ttx-3* mutants. Circles represent behavioral states; shading within circles indicates the effect of the temperature step on the probability of the indicated state (F, forward; R, reversal; Ω, omega turn) as shown in the key. The thickness and line style of arrows indicates the effect of the temperature step on rate constants. The rate constant data of Figure 5 are summarized at the top left; similar data (summarized in Table 2) were used to construct the other three panels. Note that the diagrams on the bottom refer to the run phase of the turn-and-run response, such as the response depicted in Figure 3*B3* (20–200 sec).

wild-type upstep condition), or both (e.g., the forward state in the wild-type downstep condition). Second, each of the six rate constants is sensitive to temperature, because each rate constant was modified in at least one condition. Third, modulation of behavior is distributed across multiple rate constants. This conclusion follows from the observation that in three of four cases in Figure 6, more than one rate constant exhibited a significant change in amplitude relative to control. Together, these results suggest that temperature may have widespread effects in the neural network for thermotaxis in *C. elegans*.

Discussion

The pirouette hypothesis

Using a novel apparatus to subject worms to rapid temperature steps, we tested whether pirouettes are modulated by temperature in a manner consistent with a contribution to spatial orientation during the migration phase of thermotaxis. Our results are consistent with the pirouette hypothesis in four of six circumstances we examined: downsteps in wild-type and thermophilic strains and upsteps in cryophilic and thermophilic strains. In each case, turning was either facilitated or suppressed as predicted by the hypothesis. Our results are, on first appearance, inconsistent with the pirouette hypothesis in the strict sense with regard to the other two cases we examined: upsteps in wild-type mutants and downsteps in cryophilic mutants. However, closer inspection of the data revealed that turning is modulated in a manner consistent with thermal migration even in these two cases.

The first inconsistency in the strict sense is with regard to upsteps in wild-type animals, in which the pirouette hypothesis predicts turns (Table 1) but we observed runs (Fig. 3*A3*). However, despite this discrepancy at the level of probabilities of behavioral states, we noted that a second-order feature of turning behavior, the frequency of reversal-omega transitions (i.e., avoidance responses), showed a marked spike immediately after the upstep, with 50% of animals emitting at least one such transition in the first 10 sec after the stimulus (Fig. 4*A2*). Thus, the response of wild-type animals to upsteps involves avoidance responses followed by runs, a behavioral pattern that is ultimately consistent with the pirouette hypothesis, because an avoidance response is a type of turn. This interpretation is supported by noting that cryophilic worms, whose turning behavior is consistent with the pirouette hypothesis at the level of state probabilities (Fig. 3*B3*), also exhibited a spike in avoidance responses (Fig. 4*B2*) followed by runs (Fig. 3*B3*).

The second inconsistency in the strict sense of the pirouette hypothesis is with regard to downsteps in cryophilic mutants, in which the pirouette hypothesis predicts runs (Table 1), but we observed no change in state probabilities (Fig. 3*B2*). We propose that cryophilic worms nevertheless migrate toward cooler temperatures for two reasons. First, their tendency to turn when the temperature rises is still intact, and possibly enhanced relative to wild-type worms (Fig. 3, compare *A3* and *B3*; Fig. 4, compare *A2* and *B2*). Second, cryophilic worms, unlike wild-type and thermophilic worms, show no tendency to turn when the temperature drops (Fig. 3*B2*). This means they are free to drift in the direction of cooler temperatures. Emitting runs on encountering a temperature drop would, of course, be a more efficient strategy, but in principle, either the absence of a response to downsteps or an enhanced avoidance of upsteps is sufficient for the cryophilic phenotype.

The overall similarity between pirouette functionality in chemotaxis and thermotaxis suggests that in *C. elegans*, sensory systems for spatial orientation in different modalities may converge on a common behavioral mechanism. However, additional experiments will be required to show that pirouettes are also modulated by smaller temperature steps that might match more

Table 3. Behavioral strategies for thermal migration in response to four combinations of direction of locomotion and rate of temperature change

Direction relative to preferred temperature	$\Delta T/dt$	Experiment	Behavioral strategy
Away	−	Downstep wild type Downstep thermophilic	Turn
Away	+	Upstep wild type Upstep cryophilic	Turn-and-run
Toward	−	Downstep cryophilic	No response
Toward	+	Upstep thermophilic	Run

Plus signs indicate $\Delta T/dt > 0$; minus signs indicate $\Delta T/dt < 0$. Behavioral strategies are defined in the legend of Table 1.

closely the amplitude of temperature changes encountered by worms in realistic spatial gradients. In addition, it will be important to show that the pirouette responses are quantitatively sufficient for thermal migration and to determine the relative contributions of other behaviors, such as deep bends.

Behavioral mechanisms of thermal migration in *C. elegans*

How do worms migrate up or down a thermal gradient? The computational tasks inherent in thermal migration can be organized according to two factors: the animal's direction of locomotion relative to T_p (toward or away) and the sign of the instantaneous rate of change of temperature (positive or negative); these two factors yield four unique stimulus conditions. A summary of our results in light of the four possible stimulus conditions in thermal migration is shown in Table 3. In assigning strains to particular rows in the table, we relied on the fact that a thermophilic animal, by definition, behaves as if it were always below T_p regardless of its T_c, whereas a cryophilic animal behaves as if it were always above T_p regardless of its T_c. Wild-type worms in our experiments were, of course, tested at T_p. Thus, a downstep in the case of a thermophilic animal corresponds to the stimulus condition away−, whereas an upstep corresponds to the stimulus condition toward+, and so on for the other strains and temperature steps.

Grouping behavioral responses by stimulus condition reveals two significant features in the data. First, it shows that in our experiments, each stimulus condition was met with a unique behavioral strategy. This observation suggests that the four stimulus conditions are at some level perceptually distinct. Second, it shows that mutant worms responded in a manner qualitatively identical to that of wild-type worms under the same stimulus conditions (Table 3, rows 1 and 2). This result suggests that the mutant phenotype in each case derives from the uncoupling of T_c and T_p rather than from a disruption of the behavioral mechanisms for achieving T_p. However, both conclusions require additional testing. In particular, it needs to be determined whether wild-type animals in the toward− and toward+ conditions exhibit, respectively, the strategies of no response and run.

Analysis of *C. elegans* thermotaxis via controlled temporal stimulation is just beginning. Our results differ in several respects from those of (to our knowledge) the only previous study of this kind in *C. elegans* (Ryu and Samuel, 2002). In the previous study, wild-type animals were placed above or below the T_p and subjected to slow ramps (0.5°C/min) in the positive or negative direction. Thus, as in this study, all four possible stimulus conditions were presented. These authors found that above T_p, worms subjected to positive ramps (away+) exhibited turns, whereas worms subjected to negative ramps (our toward−) exhibited runs. This pattern differs from the responses we observed in that we found no response to the toward− condition. Both patterns are, of course, consistent with migration to T_p, although the pattern obtained using ramps would be more efficient. However, below T_p, the previous study found no strategy for thermal migration, because there was no difference in turning rate in response to positive ramps or negative ramps, the toward+ and away− conditions, respectively. Here, however, we observed a pattern of responses that is clearly consistent with migration to T_p, with toward+ inducing runs and away− inducing turns. At present, the reasons for the differences between the two studies are unclear, but they could be attributable to significant differences in the temperature stimulus and definitions of turning behavior used in the two studies.

The neural network for thermotaxis

The set of neurons known to be required for normal thermotaxis, together with previous anatomical reconstructions of the *C. elegans* nervous system (White et al., 1986), supports a role for the regulation of turning in thermal migration. Laser ablation studies have identified one (or possibly two) pair of sensory neurons and five pairs of interneurons that are essential for normal thermotaxis (Mori and Ohshima, 1995). Ablation studies of the mechanosensory reflexes in *C. elegans* (touch and tap withdrawal) have identified six other interneurons involved in regulation of the relative probability of forward locomotion and reversals (Chalfie et al., 1985; Wicks and Rankin, 1995; Zheng et al., 1999). (Interneurons that regulate omega turns have not yet been identified.) The reconstructions indicate that the interneurons of the thermotaxis network make widespread and redundant anatomical connections to the interneurons for forward locomotion and reversals. For example, the interneurons AIB, RIB, and RIM in the thermotaxis network (Mori and Ohshima, 1995) synapse on both AVA and AVB, the command interneurons for reversal and forward movement, respectively (Chalfie et al., 1985). If these anatomical connections are physiologically active, they could be the neuronal substrate for the regulation of forward and backward locomotion by the thermosensory network.

The stochastic analysis of the effects of temperature steps on behavioral state places constraints on the relationship between activity in the thermosensory network and activity in the network for forward locomotion and reversals. Stochastic analysis revealed that alterations in state probability were the result of changes in entry rate, exit rate, or both. In wild-type animals, for example, the decrease in forward probability after a downstep involves both an increase in the likelihood of making a transition out of the forward state and a decrease in the likelihood of making a transition out of either of the turn states. The thermotaxis network might therefore be expected to simultaneously suppress activation of forward locomotion neurons and increase activation of reversal neurons. The stochastic analysis also predicts that the time course of the changes in neuronal activity will reflect the time course of the changes in rate constants. In this sense, our approach of analyzing step response data stochastically provides a useful level of analysis that is intermediate between behavioral genetics and neurophysiology. Such an approach should accelerate our understanding of the linkage between genes, neurons, and behavior.

References

Altun-Gultekin Z, Andachi Y, Tsalik EL, Pilgrim D, Kohara Y, Hobert O (2001) A regulatory cascade of three homeobox genes, *ceh-10*, *ttx-3* and *ceh-23*, controls cell fate specification of a defined interneuron class in *C. elegans*. Development 128:1951–1969.

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94.
Chalfie M, Sulston J (1981) Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev Biol 82:358–370.
Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S (1985) The neural circuit for touch sensitivity in *Caenorhabditis elegans*. J Neurosci 5:956–964.
Croll NA (1975a) Behavioural analysis of nematode movement. Adv Parasitol 13:71–122.
Croll NA (1975b) Components and patterns in the behavior of the nematode *Caenorhabditis elegans*. J Zool 176:159–176.
Culotti JG, Russell RL (1978) Osmotic avoidance defective mutants of the nematode *C. elegans*. Genetics 90:243–256.
Dusenbery DB (1980) Responses of the nematode *Caenorhabditis elegans* to controlled chemical stimulation. J Comp Physiol 136:127–331.
Finney M, Ruvkun G, Horvitz HR (1988) The *C. elegans* cell lineage and differentiation gene *unc-86* encodes a protein with a homeodomain and extended similarity to transcription factors. Cell 55:757–769.
Hedgecock EM, Russell RL (1975) Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci USA 72:4061–4065.
Hobert O, Mori I, Yamashita Y, Honda H, Ohshima Y, Liu Y, Ruvkun G (1997) Regulation of interneuron function in the *C. elegans* thermoregulatory pathway by the *ttx-3* LIM homeobox gene. Neuron 19:345–357.
Kaplan JM, Horvitz HR (1993) A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 90:2227–2231.
Mori I, Ohshima Y (1995) Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature 376:344–348.
Perkins LA, Hedgecock EM, Thomson JN, Culotti JG (1986) Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev Biol 117:456–487.
Pierce-Shimomura JT, Morse TM, Lockery SR (1999) The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. J Neurosci 19:9557–9569.
Rankin CH (1990) *Caenorhabditis elegans*: a new model system for the study of learning and memory. Behav Brain Res 37:89–92.
Rutherford TA, Croll NA (1979) Wave forms of *C. elegans* in a chemical attractant and repellent and in thermal gradients. J Nematol 11:232–240.
Ryu WS, Samuel AD (2002) Thermotaxis in *Caenorhabditis elegans* analyzed by measuring responses to defined thermal stimuli. J Neurosci 22:5727–5733.
Sambongi Y, Nagae T, Liu Y, Yoshimizu T, Takeda K, Wada Y, Futai M (1999) Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in *Caenorhabditis elegans*. NeuroReport 10:753–757.
Ward S (1973) Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. Proc Natl Acad Sci USA 70:817–821.
White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos Trans R Soc Lond B Biol Sci 314:1–340.
Wicks SR, Rankin CH (1995) Integration of mechanosensory stimuli in *Caenorhabditis elegans*. J Neurosci 15:2434–2344.
Wittenburg N, Baumeister R (1999) Thermal avoidance in *Caenorhabditis elegans*: an approach to the study of nociception. Proc Natl Acad Sci USA 96:10477–10482.
Zheng Y, Brockie PJ, Mellem JE, Madsen DM, Maricq AV (1999) Neuronal control of locomotion in *C. elegans* is modified by a dominant mutation in the GLR-1 ionotropic glutamate receptor. Neuron 24:347–361.