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Short-Term Adaptation and Temporal Processing in the Cryophilic Response of *Caenorhabditis elegans*

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Clark DA, Gabel CV, Lee TM, Samuel ADT. Short-term adaptation and temporal processing in the cryophilic response of *Caenorhabditis elegans*. *J Neurophysiol* 97: 1903–1910, 2007. First published December 6, 2006; doi:10.1152/jn.00892.2006. When navigating spatial thermal gradients, the nematode *C. elegans* migrates toward colder temperatures until it reaches its previous cultivation temperature, exhibiting cryophilic movement. The strategy for effecting cryophilic movement is the biased random walk: *C. elegans* extends (shortens) periods of forward movement that are directed down (up) spatial thermal gradients by modulating the probability of reorientation. Here, we analyze the temporal sensory processor that enables cryophilic movement by quantifying the movements of individual worms subjected to defined temperature waveforms. We show that step increases in temperature as small as 0.05°C lead to transient increases in the probability of reorientation followed by gradual adaptation to the baseline level; temperature downsteps leads to similar but inverted responses. Short-term adaptation is a general property of sensory systems, allowing organisms to maintain sensitivity to sensory variations over broad operating ranges. During cryophilic movement *C. elegans* also uses the temporal dynamics of its adaptive response to compute the time derivative of gradual temperature variations with exquisite sensitivity. On the basis of the time derivative, the worm determines how it is oriented in spatial thermal gradients during each period of forward movement. We show that the operating range of the cryophilic response extends to lower temperatures in *ttx-3* mutants, which affects the development of the AIY interneurons. We show that the temporal sensory processor for the cryophilic response is affected by mutation in the EAT-4 glutamate vesicular transporter. Regulating the operating range of the cryophilic response and executing the cryophilic response may have separate neural mechanisms.

INTRODUCTION

A major goal of systems neuroscience is to understand how neural circuits produce behaviorally relevant computation. The motile behavior of animals in defined sensory environments is naturally described as computation, i.e., transformations of sensory input into motor output carried out by layers of neural circuitry. An advantage of studying the nematode *Caenorhabditis elegans* is that all behaviorally relevant computation is carried out by a nervous system with only 302 neurons (White et al. 1986). *C. elegans* behaviors are also highly stereotyped, so that quantitative metrics of worm behavior in defined sensory environments may accurately describe the computations that are carried out by the neural circuits.

Thermotactic behaviors in *C. elegans* are particularly sophisticated (Hedgecock and Russell 1975). When *C. elegans* is cultivated at a specific temperature, it stores a long-term memory of its cultivation temperature, which we call the *thermotactic set point* (T_S). It has long been thought that the formation of T_S reflects associative learning between the cultivation temperature and detection of bacterial food (Ishihara et al. 2002; Kodama et al. 2006; Kuhara and Mori 2006; Mohri et al. 2005). However, we recently showed that adult-stage resetting of T_S is unaffected by the presence or absence of bacterial food and may depend only on long-term temperature experience (Biron et al. 2006). When the worm navigates spatial thermal gradients at temperatures higher than its previous cultivation temperature, the worm crawls down spatial thermal gradients until it reaches T_S, exhibiting what is called cryophilic movement. In a range of temperatures near T_S, the worm tracks isotherms in prolonged periods of forward movement. In a recent study, we examined the deterministic sensorimotor transformation that allows *C. elegans* to maintain isothermal alignment at temperatures near T_S (Luo et al. 2006). These observations indicate that the neural circuits for thermotaxis encode multiple sensorimotor transformations, for effecting cryophilic movement at $T > T_S$ and for effecting isothermal tracking at temperatures near T_S.

Here, we study the sensorimotor transformation that drives the cryophilic component of *C. elegans* thermotactic behavior. In isotropic environments, *C. elegans* motility consists of successive periods of forward movement (runs) interrupted by spontaneous reorientation maneuvers (sharp turns or reversals followed by turns, which are also called pirouettes) (Croll 1975; Pierce-Shimomura et al. 1999). The cryophilic response consists of extending (shortening) runs that are oriented toward colder (warmer) temperatures (Ryu and Samuel 2002). This strategy leads to net migration toward colder temperatures in the manner of a biased random walk. These features of the cryophilic response suggest the operation of two types of computation. First, the worm must determine whether the ambient temperature is above its thermotactic set point ($T > T_S$), the normal operating range of the cryophilic response. Second, during execution of the cryophilic response, the worm must analyze the orientation of each run with respect to the surrounding spatial thermal gradient to determine whether it is crawling toward warmer or colder temperatures and shorten or extend each run accordingly. Recent studies showed that subjecting worms to temperature changes in a spatially uniform manner evokes a cryophilic response that resembles their behavior on spatial gradients—negative (positive) temperature changes suppress (stimulate) reorientation maneuvers, thereby extending (shortening) runs (Ryu and Samuel 2002; Zariwala

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et al. 2003). These studies suggested that the cryophilic response involves a type of temporal sensory processing, but did not define the quantitative structure of this computation. In this study, we present a precise characterization of the temporal sensory processor that enables the worm to compute the orientation of its own movements in thermal gradients during the cryophilic response. By quantifying the movements of individual worms subjected to defined temperature waveforms, we show that the cryophilic response, across a broad range of thermosensory inputs, can be quantitatively and self-consistently explained by the temporal dynamics of short-term adaptation in temperature-evoked changes in the rate of spontaneous reorientation.

METHODS

Strains

C. elegans strains were cultivated following standard procedures (Brenner 1974). The *glr-1*(*ky176*) and *glr-2*(*ak10*) mutant strains were gifts of A.V. Maricq (University of Utah, Salt Lake City, UT). The wild-type N2 and all other mutant strains were obtained from the *C. elegans* Genetics Center (Minneapolis, MN).

Crawling assay

The movements of worms crawling on spatial temperature gradients were imaged using dark-field illumination of the surfaces of agar plates without food. Linear temperature gradients were established with an underlying aluminum platform under thermoelectric control (Ryu and Samuel 2002). To study the cryophilic response at $T > T_S$ and exclude isothermal tracking, we cultivated worms at 15°C and used gradients from 20 to 25°C. Likewise for studies at $T < T_S$, worms were cultivated at 25°C and tested on a gradients from 15 to 20°C. To analyze the movements of large numbers of worms at once, we used custom-written software for population-wide behavioral quantification written in MATLAB (The MathWorks, Natick, MA) that was based on particle-tracking algorithms by Crocker and Grier (1996). Agar plates carrying 20–40 worms were illuminated by a ring of LEDs, and video images were captured at 0.5 Hz for 30 min using a computer running LabVIEW (National Instruments, Austin, TX). Afterward, our population-wide behavioral quantification software was used to decompose individual worm trajectories into alternating periods of runs and reorientations. Run duration was calculated by flagging the reorientation at the start and end of each run as any change in the direction of worm movement by >45° in 4 s, a procedure that is insensitive to small-angle turns. Run orientation was calculated with respect to gradient direction.

Swimming assay

We monitored the movements of individual worms swimming inside droplets of NGM buffer while subjecting them to defined thermal stimuli (Ryu and Samuel 2002). To study cryophilic movement, we placed individual worms cultivated at 20°C inside 0.6 μL droplets sandwiched between two coverslips. The droplet was placed on a temperature-controlled stage held at a defined baseline temperature. The droplet temperature was modulated above the stage temperature by controlled exposure to infrared laser illumination (230 mW, λ = 1,480 nm, Furukawa Electric, Tokyo, Japan). The laser wavelength is near an absorption peak of water, which corresponds to an attenuation length of 0.4 mm, roughly the depth of the sandwiched droplet. We used a proportional integrative derivative feedback loop to define temperature waveforms with a computer running LabVIEW, continuously monitored droplet temperature with a microthermocouple (Physitemp Instruments, Clifton, NJ), and continuously regulated laser current (LDX-3500, ILX Lightwave, Bozeman, MT). Within about 3°C above the stage temperature, the system heats or cools the droplet as fast as nearly 0.3°C/s or fixes temperature with 0.02°C root mean square resolution. A computer captured images of the swimming worm at a rate of 10 Hz with a charge-coupled detector camera. Video was analyzed by a computer algorithm that detects reorientation maneuvers on the basis of body extension (Chung et al. 2006). During forward swimming, the end-to-end distance of the worm oscillates around large values. During turns, the end-to-end distance drops sharply and briefly. The algorithm measured body extension in each video frame and flagged each dip in body extension below a threshold set to roughly 50% of maximum extension, thus differentiating sudden deep bends and omega turns from periods of forward swimming. Each swimming assay of an individual worm lasted 5–15 min and involved the presentation of multiple stimulus waveforms. The algorithm does not distinguish forward swimming from reverse swimming. Counting reorientation events of swimming worms by eye, we found that turns typically outnumber reversals by >20:1, so the automated system efficiently extracts the majority of stimulus-evoked reorientation events.

RESULTS

The cryophilic response modulates the statistics of run duration

In previous experiments, we found that *C. elegans* exhibiting cryophilic movement at temperatures above its thermotactic set-point ($T > T_S$) extends runs when the surrounding temperature is decreasing and shortens runs when that temperature is increasing. This strategy is known as the biased random walk and leads *C. elegans* to cooler temperatures (Ryu and Samuel 2002). To more thoroughly quantify the cryophilic response of crawling worms, we analyzed the statistics of run duration as a function of run orientation of behaving worms navigating linear spatial thermal gradients with fixed 0.5°C/cm steepness at both $T > T_S$ and $T < T_S$ (Fig. 1*A*). When *C. elegans* crawls on spatial thermal gradients, it encounters the most rapid temperature changes when its runs are oriented directly up or down the gradient, but slower temperature changes when its runs are at an angle to the gradient. We found that the run durations of crawling animals are variable and exponentially distributed, suggesting that the statistics of run duration are Poisson (Fig. 1*B*). For wild-type animals at $T > T_S$, we found that the mean run duration (or, inversely, the stochastic rate of reorientation) is a smoothly varying function of the angle between run direction and gradient direction (Fig. 1*C*). In other words, the cryophilic response appears to be directly correlated with the temperature change associated with the direction of the worm's self-movement in the spatial thermal gradient. In contrast, when wild-type worms navigate at $T < T_S$, run duration does not vary with run direction. Wild-type worms are able to restrict their cryophilic response to the operating range of $T > T_S$.

For comparison, we quantified the cryophilic response of several mutants. Mutations in the gene *ttx-3*, which encodes a LIM homeobox gene and disrupts development of the AIY interneuron, lead to cryophilic aggregation—over time, *ttx-3* mutants will aggregate at the coldest point on a spatial thermal gradient irrespective of T_S (Hobert et al. 1997). We found that the cryophilic response of *ttx-3*(*ks5*) mutants on spatial thermal gradients has the same form as that of wild-type worms at $T > T_S$, with runs down the gradient tending to be longer than runs

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up the gradient (Fig. 1*C*). However, unlike wild-type worms, the *ttx-3* mutant worms also display the cryophilic response at $T < T_S$, extending runs toward colder temperatures and shortening runs toward warmer temperatures. Therefore the *ttx-3* mutation disrupts the worm's ability to regulate the operating range of its cryophilic response. We note that the magnitude of the cryophilic response exhibited by *ttx-3* mutants at $T < T_S$ also appears to be weaker than that at $T > T_S$, so it is also possible that part of the modulation of reorientation rate that underlies the cryophilic response is carried out directly by the AIY interneuron.

Mutations in the gene *tax-4*, which encodes a cyclic nucleotide–gated channel expressed in the AFD thermosensory neuron as well as in other neuronal types, disrupt thermotaxis (Coburn et al. 1998). We found that *tax-4*(*p678*) mutant worms do not significantly modulate run duration as a function of run orientation at $T > T_S$ or at $T < T_S$ (Fig. 1*C*). These data are consistent with the atactic phenotype of *tax-4* mutants—their tendency to distribute randomly on spatial thermal gradients irrespective of previous cultivation temperature. Mutations in the gene *ttx-4* were previously reported to cause a thermophilic phenotype—a tendency to accumulate at the warmest regions of spatial thermal gradients—as well as several defects in chemotactic behavior (Okochi et al. 2005). The *ttx-4* gene encodes a member of the protein kinase C (PKC) family, an nPKC-epsilon/eta ortholog, that is expressed in many sensory neurons including the AFD thermosensory neurons, the AWC and AWA olfactory neurons, the ASE gustatory neurons, and the ASH osmosensory neurons (Okochi et al. 2005). However, we found no evidence for thermophilia caused by the *ttx-4* mutation. The *ttx-4*(*nj1*) mutants are atactic at $T > T_S$ and so do not modulate run duration as a function of run orientation at higher temperatures. Unlike wild-type worms, *ttx-4*(*nj1*) tend to slightly extend runs toward warmer temperatures at $T < T_S$, but this would not carry them all the way to the warmest point on a plate (Fig. 1*C*).

We obtained an interesting and subtle phenotype using worms carrying a mutation in the gene *eat-4*, which encodes a vesicular glutamate transporter involved in the synaptic output of several neuronal types. Mutations in *eat-4* disrupt several sensory modalities including osmolarity avoidance, touch avoidance, and chemotaxis (Lee et al. 1999). We found that worms carrying the *eat-4*(*ad819*) mutation exhibit a very slight cryophilic response at $T > T_S$; run durations are uncorrelated with run orientation, except those runs that are oriented directly down the gradient are slightly extended (Fig. 1*C*). Therefore the *eat-4*(*ad819*) mutation severely weakens, but does not quite abolish, the mechanism for generating the cryophilic response at $T > T_S$.

The effect of the *eat-4* mutation suggests that the neural circuit for the cryophilic response includes glutamatergic signaling pathways. Glutamatergic pathways were previously implicated in the worm's ability to modulate its reorientation rate when it executes area-restricted search behavior (Hills et al. 2004). Area-restricted search is defined as the brief episode of

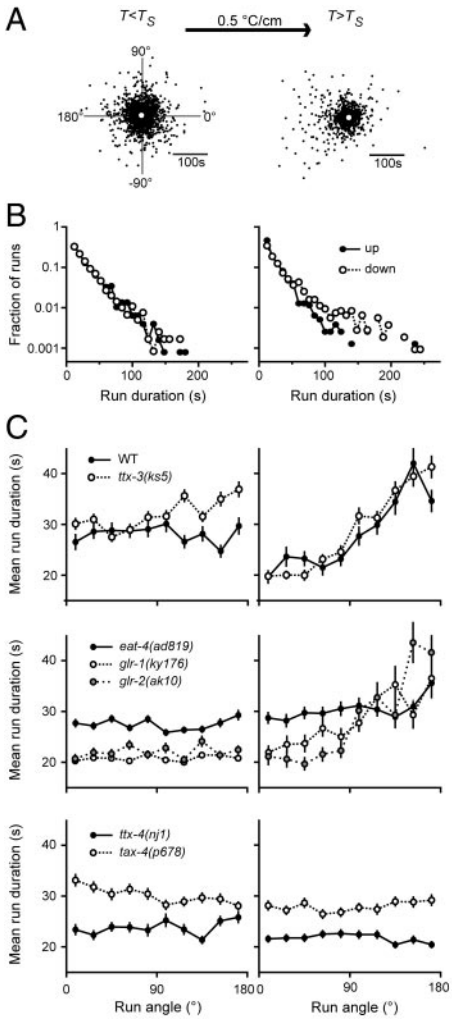

FIG. 1. Crawling assay of cryophilic movement. *A*: scatterplots show the correlation between run orientation and run duration for wild-type N2 worms navigating a linear spatial thermal gradient with 0.5°C/cm steepness. Starting point of all runs is set to the origin (white dot). Each black dot denotes the relative endpoint of each run: duration is indicated by distance from the origin (see scale bar) and run orientation is indicated by the angle with respect to the thermal gradient (defined to be 0° for worms crawling up the spatial gradient, shown by the arrow). For wild-type N2 worms tested above their cultivation temperature (T_S) (cultivated at 15°C and allowed to navigate on a gradient between 20 and 25°C, *right*) runs oriented down the gradient are extended and runs oriented up the gradient are shortened. In contrast, N2 worms tested below T_S (raised at 25°C and allowed to navigate on a gradient between 15 and 20°C, *left*), there is no measurable correlation between run orientation and duration. Each scatterplot represents run statistics collected from roughly 3,000 runs exhibited by nearly 200 worms (see METHODS). *B*: histograms of run duration as classified by run orientation with respect to the spatial thermal gradient. Data from runs up the gradient, i.e., run angles within 90° of the gradient direction from the scatterplots of *A*, are denoted by filled circles. Data from runs oriented down the gradient, all runs with angles >90° from the gradient direction, are denoted by open circles. At $T > T_S$ (*right*), the distributions of run duration are nearly exponential but there are more long runs directed down the gradient than up the gradient. At $T < T_S$ (*left*) the distributions of run duration are exponential and indistinguishable from one another. *C*: plots of mean run duration as a function of run orientation. *Top right*: cases of strong cryophilic response at $T > T_S$, wild-type and *ttx-3*(*ks5*) mutants. *Top left*: wild-type and *ttx-3*(*ks5*) at $T < T_S$; wild-type animals show no bias toward warmer or colder temperatures (data are fit to a constant with $P > 0.05$), whereas *ttx-3*(*ks5*) retain a cryophilic bias (data fit a constant with $P < 10^{-4}$). *Middle*: *eat-4*(*ad819*) mutant is nearly atactic at both $T > T_S$ and at $T < T_S$; *glr-1*(*ky175*) and *glr-2*(*sk10*) mutants retain strong cryophilic bias at $T > T_S$ but are atactic at $T < T_S$. *Bottom*: cases of weak or undetectable thermotactic responses at $T < T_S$ (*left*) and at $T > T_S$ (*right*) for *tax-4*(*p678*), *eat-4*(*ad819*), and *ttx-4*(*nj1*) mutants. In the *bottom panels*, mean run duration is fit to a constant ($P > 0.05$) for these mutants both at $T > T_S$ and at $T < T_S$. Between 30 and 70 worm-hours of data were collected for each genotype to obtain the statistics presented.

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short runs and high reorientation rate that occurs shortly after *C. elegans* is removed from bacterial food. After prolonged removal from food, *C. elegans* exhibits longer runs and lower reorientation rate, allowing the worm to end area-restricted search and disperse throughout its environment. Hills et al. (2004) discovered that mutations in either the *eat-4* vesicular glutamate transporter or the ionotropic glutamate receptor genes *glr-1* and *glr-2* disrupt the ability to execute area-restricted search. We investigated whether the same ionotropic glutamate receptors might also mediate the cryophilic response by analyzing the movements of *glr-1*(*ky175*) and *glr-2*(*sk10*) mutants on spatial thermal gradients. However, we found that neither mutation affects the worm's ability to execute its cryophilic response at $T > T_S$ or to suppress its cryophilic response at $T < T_S$. At $T > T_S$, both mutants exhibit long (short) runs when moving down (up) spatial thermal gradients. At $T < T_S$, neither mutant modulates run duration as a function of run orientation (Fig. 1*C*). These observations suggest that the glutamatergic signaling pathways that drive the cryophilic response may require glutamate receptors different from those encoded by *glr-1* and *glr-2*.

The cryophilic response exhibits high-temperature sensitivity over broad operating ranges

We sought to precisely characterize the dynamic range and the operating range of the cryophilic response using a recently devised swimming assay (Chung et al. 2006; Ryu and Samuel 2002). In our swimming assay, we subject individual worms to temperature changes in a spatially uniform manner. Swimming worms exhibit a cryophilic response by turning more (less) frequently when exposed to positive (negative) temporal changes in temperature (Fig. 2*A*). Sine-wave temperature stimuli often lead to sinusoidal modulations of turning rate as in Fig. 2*A*; because the highest (lowest) turning rate is during the warming (cooling) phase of the cycle, sine-wave stimuli can lead to a phase difference of about $\pi/2$, making the response resemble the time derivative of the stimulus.

We measured the operating range of the wild-type cryophilic response by subjecting worms cultivated at 20°C to sine-wave temperature stimuli at different baseline temperatures (Fig. 2*B*). We found that the amplitude of the cryophilic response (measured as the amplitude of a sine wave fitted to turning rate over the stimulus cycle) peaks near 23°C, drops gradually at higher temperatures, and drops sharply at <20°C (Fig. 2*C*). This is consistent with our earlier observations that *C. elegans* is atactic at $T < T_S$ (Fig. 1, or Ryu and Samuel 2002).

Next, we measured the dynamic range of the cryophilic response by subjecting wild-type worms to sine-wave temperature variations with different stimulus amplitudes (Fig. 2*C*). Variation in stimulus amplitude in the range of these experiments, from 0.05 to 1.5°C peak-to-peak amplitude, does not alter the phase difference between the stimulus and response sine waves. Sine-wave stimuli with fixed 30-s period and 23°C baseline saturate the cryophilic response with peak-to-peak amplitudes ≥0.5°C (corresponding to maximum rate of temporal change of about 0.05°C/s) and is half-maximal with peak-to-peak amplitudes near 0.3°C (corresponding to temporal changes of about 0.03°C/s), showing a significant response to 0.1°C peak-to-peak stimuli. Therefore the cryophilic response maintains a temperature sensitivity to temperatures changes as small as about 0.01°C/s over temperature ranges as broad as about 5°C.

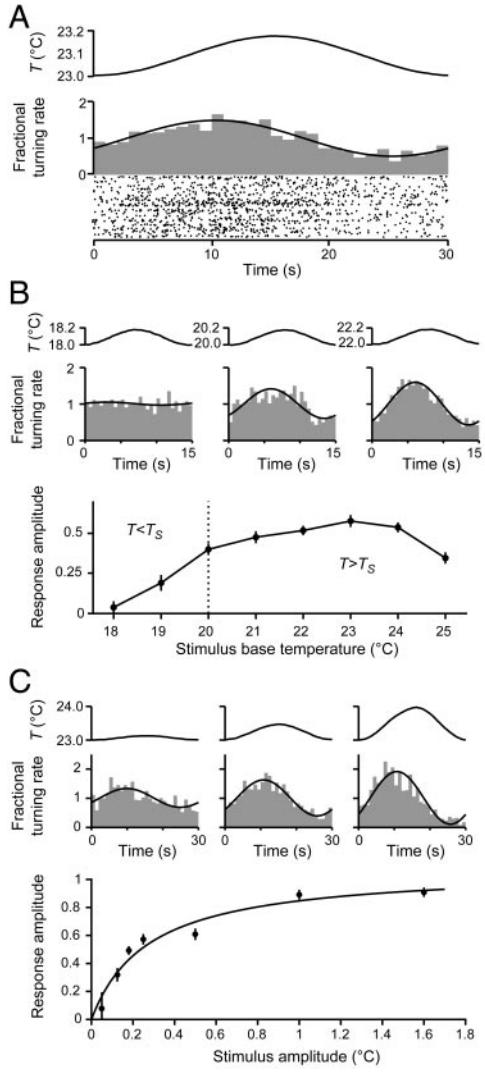

FIG. 2. Dynamic and operating range of cryophilic movement. *A*: wild-type N2 worms were cultivated at 20°C, placed in droplets, and subjected to cycles of a defined temperature stimulus (*top*). Video of the swimming worm during the temperature stimulus was analyzed for the occurrence of turns using a machine-vision algorithm (see METHODS). Raster plot shows the occurrence of turns throughout several stimulus cycles. From the raster plot, we computed the response as the histogram of turning rate as a function of stimulus phase normalized by average turning rate. *B*: operating range of the cryophilic response. Wild-type worms cultivated at 20°C were subjected to sine-wave temperature stimuli with different baseline temperatures, but with fixed 15-s period and 0.2°C peak-to-peak amplitude. Data from representative trials near 18, 20, and 22°C are shown at *top*. Response amplitude is presented as a function of baseline temperature, showing that wild-type worms display strong cryophilic response at temperatures $>T_S$, demarcated by the dotted line. In all, 156 worms were used in this experiment, with 17–20 worms used in each measurement. *C*: dynamic range of the cryophilic response. Stimulus and response are shown for worms subjected to 30-s sine-wave stimuli with 0.1, 0.5, and 1.0°C peak-to-peak amplitudes (*top*). Entire dynamic range of the cryophilic response to 30-s sine-wave stimuli is shown in the *bottom*. Response amplitude is presented as a function of stimulus amplitude, showing that the cryophilic response saturates with amplitudes >0.5°C. In all, 103 worms were used in this experiment, with 6–28 worms used in each measurement.

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The cryophilic response is bidirectional and adaptive

Based on the crawling assays, a cryophilic response was observed for wild-type worms only at $T > T_S$, a weak cryophilic response was observed for *eat-4* mutants at $T > T_S$, and a cryophilic response was observed for *ttx-3* mutants at all temperatures. In our next experiment, we quantified the temporal dynamics of the cryophilic response of swimming worms subjected to precisely defined temperature steps. We found that wild-type worms subjected to a 0.4°C temperature upstep at $T > T_S$ exhibit a transient elevation of turning rate, which rises to a maximum after about 5 s before returning to the prestimulus value after about 15 s. A 0.4°C downstep leads to a similar but inverted response (Fig. 3*A*). These biased responses to upsteps and downsteps are consistent with cryophilic movement on spatial thermal gradients, in which increasing (decreasing) temperature shortens (extends) runs. Therefore the cryophilic response is *1*) *bidirectional*, in that both increases and decreases in temperature raise and lower the probability of reorientation, respectively; and *2*) *adaptive*, in that after a temperature step the probability of reorientation gradually returns to its baseline level with well-defined temporal dynamics. We also measured the sensitivity of the wild-type response to smaller-amplitude steps and found that the cryophilic response to temperature steps rises with increasing step sizes in the range 0.05–0.2°C and plateaus in the range 0.2–0.4°C (Fig. 3*B*). Therefore the worm's sensitivity to step stimuli is similar to its sensitivity to gradual sine-wave stimuli.

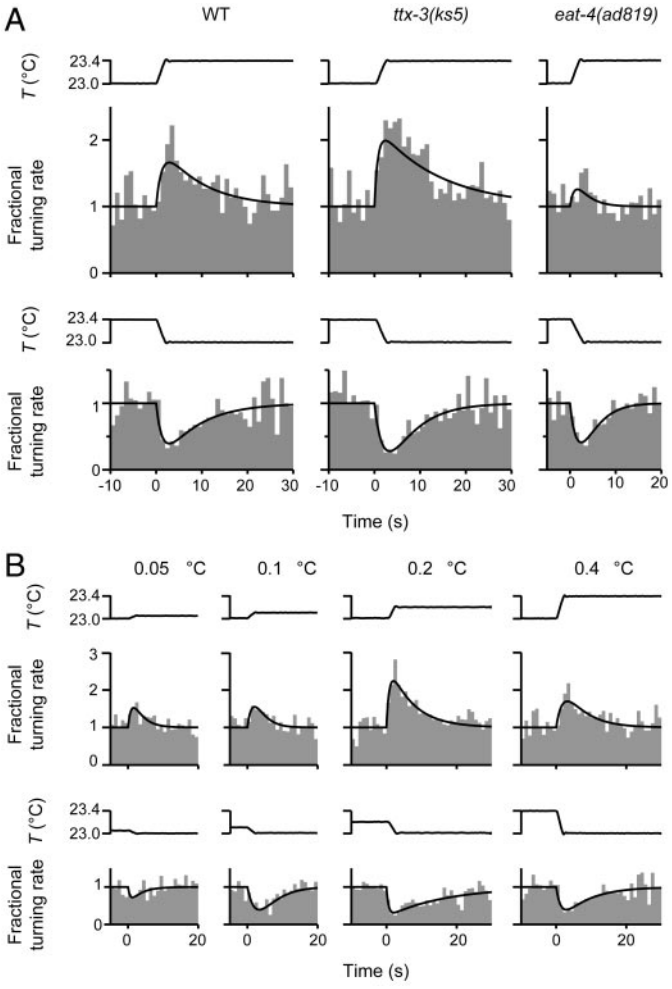

FIG. 3. Wild-type and mutant response to step stimuli. *A*: wild-type N2 and mutant worms cultivated at 20°C were subjected to upstep and downstep stimuli from 23°C. *Top panel* in each condition shows the temperature measured at the droplet with stimulus onset at $t = 0$ s; the *bottom panel* shows the fractional modulation in turning rate caused by the stimulus. Each step response is fit by least-squares to the sum of 2 exponentials from the functional form we used to predict the experimental measurements of Fig. 4 (see Supplementary Materials[1]). Between 36 and 50 worms were used to measure the response in each condition. *B*: wild-type worms cultivated at 20°C were subjected to upstep and downstep stimuli of different sizes from 23°C. Cryophilic response is detectable for steps as small as 0.05°C (fit to a constant: $P < 0.05$). Response grows with step sizes of 0.1 and 0.2°C, but plateaus with step sizes of about 0.4°C.

[1] The online version of this article contains supplemental data.

For comparison, we characterized the temporal dynamics of the step responses of *ttx-3*(*ks5*) mutants. At $T > T_S$, the *ttx-3* mutant exhibits the same temporal dynamics in the rate of reorientation after temperature steps as that of wild-type worms (Fig. 3*A*). The main difference between the *ttx-3* mutant and wild-type worms appears to be that wild-type worms inactivate the cryophilic response at $T < T_S$. The *ttx-3*(*ks5*) mutation does not appear to directly affect the temporal sensory processor that generates the cryophilic response itself. We were intrigued by the very slight cryophilic response at $T > T_S$ exhibited by the *eat-4*(*ad819*) mutant in our crawling assay, and so we subjected individual *eat-4*(*ad819*) mutant worms to temperature steps in our swimming assay. We found that the response of *eat-4*(*ad819*) mutant worms after temperature steps appears to be both weaker and shorter than that of wild-type worms (Fig. 3*A*). The residual cryophilic response of the *eat-4* mutant in the swimming assay is consistent with its very weak cryophilic response in the crawling assay.

The cryophilic response acts as a band-pass filter for sine-wave stimuli

The cryophilic response is adaptive, which explains how the worm maintains an exquisite sensitivity to small temperature changes over a broad operating range. However, the time course of the adaptive response also follows stereotyped dynamics, which may confer specific computational properties. In particular, the bidirectional and adaptive cryophilic response to step stimuli should act as a band-pass filter when viewed in the frequency domain: *1*) sine-wave stimuli with periods much shorter than the duration of the step response should evoke only small responses as successive responses of opposite sign cancel each other out, *2*) sine-wave stimuli with much longer periods (with much slower changes in temperature) should also evoke responses of small magnitude, and *3*) a sine wave with a period comparable to the duration of the step response should evoke the maximum response. We measured the amplitude of the response to sine-wave stimuli with 0.2°C peak-to-peak amplitude and different stimulus frequencies and indeed established these properties of a band-pass filter, with a maximum response corresponding to stimulus periods of nearly 15 s, the duration of the response to 0.2°C steps, and response roll-off at longer and shorter stimulus periods (Fig. 4*A*).

In a linear approximation of the cryophilic response, the worm's response to a sine-wave stimulus with any stimulus period should be mathematically reconcilable with the worm's

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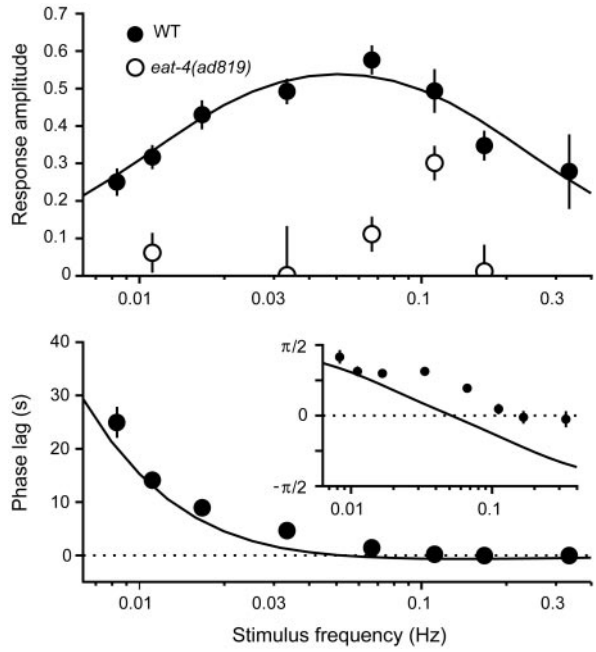

FIG. 4. Frequency range of the cryophilic response. Wild-type and *eat-4*(*ad819*) worms were subjected to sine-wave stimuli with periods ranging from 3 to 120 s, with fixed 0.2°C peak-to-peak amplitude and 23°C base temperature. Each turning rate response was fitted to a sine wave and we recorded amplitude and phase. We plot response amplitude as a function of stimulus frequency (*top*), as well as phase difference between the stimulus and response sine waves, measured in seconds (*bottom*) and radians (*bottom inset*). Solid line predicts the response to a sinusoidal stimulus from the 0.2°C step response measurements of wild-type worms in Fig. 3*A* using standard methods (Dayan and Abbott 2001). A total of 234 N2 and *eat-4* worms were used in this experiment, with 11–28 worms used in each frequency measurement.

response to the step stimulus. The caveat to applying a linear approximation is that the cryophilic response exhibits certain nonlinearities; in particular, the cryophilic response has a specific operating range and is saturated by large stimuli. Nevertheless, with this caveat in mind, we sought to test whether responses to small stimuli of comparable size and baseline temperature—sine waves with 0.2°C peak-to-peak amplitude and steps of 0.2°C near 23°C—could be reconciled using a linear model (Dayan and Abbott 2001). We examined the two behaviorally relevant computational features of the cryophilic response: *1*) whether knowing the step response could yield the band-pass properties of the sine-wave response and *2*) whether it could predict the phase difference between sine-wave stimulus input and response output.

First, we applied a simple mathematical characterization of the worm's cryophilic response to temperature steps. The cryophilic response to a temperature step of magnitude T_0 delivered at $t = 0$ is a time-varying function, $\Delta R_{step}(t)$, that describes the modulation in reorientation rate. For simplicity, we approximate the cryophilic response to a temperature step as the difference of two exponentials (Fig. 3*A*)

$$\Delta R_{\text{step}}(t) = \alpha T_0[\exp(-t/\tau_1) - \exp(-t/\tau_2)] \quad (1)$$

At long times, $\Delta R(t) \rightarrow 0$, which reflects adaptation of the reorientation rate to prestimulus levels. The parameters α, τ_1, and τ_2 may be estimated by fitting *Eq. 1* to the experimental data in Fig. 3*A*.

Next, using the step response with experimentally measured parameters, we estimated the form of the sine-wave response. The sine-wave stimulus may be expressed as $T \sin(\omega t)$, but may also be expressed as the superposition of many small steps delivered in successive units of time dt. The size of each small step is equal to the time derivative of the sine-wave input multiplied by dt or $T\omega \cos(\omega t)$dt. In a linear model, the overall response to the sine-wave stimulus may be calculated by adding up the responses to all of the individual steps that constitute the history of the stimulus

$$\Delta R_{\text{sin}}(t) = \int_{-\infty}^{t} \Delta R_{\text{step}}(t - t')\omega T \cos(\omega t')\mathrm{d}t' \quad (2)$$

Integrating *Eq. 2* enables us to quantify the size of the cryophilic response as a function of sine-wave frequency

$$|\Delta R_{\text{sin}}| = \frac{|\omega\alpha T||\tau_1 - \tau_2|}{\sqrt{1+\omega^2\tau_1^2}\sqrt{1+\omega^2\tau_2^2}} \quad (3)$$

Equation 3 describes a band-pass filter that fits our experimental measurements of the cryophilic response over the range of our experimental measurements (Fig. 4*A*).

With sine-wave stimuli of long period (so that the timescales of cryophilic response and adaptation matter less) a sine-wave stimulus will induce the highest (lowest) turning rate near the highest (lowest) slope of the stimulus waveform—i.e., the phase difference between the stimulus and response sine waves will approach $\pi/2$, making the response resemble the time derivative of the stimulus. Our linear model—integrating *Eq. 2* and extracting the phase difference between the sine-wave input and response output—produces the time-derivative effect exhibited by the experimental measurements in the low-frequency limit, although it is a poor predictor of the phase difference at higher frequencies (Fig. 4*B*).

We conclude that in the domain of small stimulus amplitudes and gradual rates of temperature change, a linear model of the cryophilic response reconciles the experimentally measured step response with the experimentally measured sine-wave response. In particular, the linear model reproduces the behaviorally relevant computational features of the cryophilic response, which is to allow *C. elegans* to effectively compute the time derivative of gradual temperature variations over a time interval extending about 15–20 s into the past. Moreover, this linearity is consistent with the modulation of run duration of behaving worms on a linear gradient (Fig. 1).

The step response of the *eat-4*(*ad819*) mutants is asymmetric between upsteps and downsteps, which makes difficult the same linear modeling that we applied to wild-type animals. Moreover, because the *eat-4* response is much weaker than that of wild-type, it is difficult to know whether the step response of *eat-4* mutants actually adapts more quickly than wild-type or takes place at the normal rate but has a smaller peak. We did find that fitting the cryophilic response of *eat-4* mutant worms to *Eq. 1*, using either upsteps or downsteps, produced shorter time constants and smaller amplitudes than we obtained with wild-type worms. If so, the peak response of *eat-4* mutant worms to sine-wave stimuli should be smaller and occur at higher frequency than wild-type worms, which matches experimental measurement (Fig. 4*A*).

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DISCUSSION

Motile organisms with limited computational abilities must devise strategies to effectively use sensory information to navigate their environments. Here, we studied the case of cryophilic movement exhibited by *C. elegans*: *C. elegans* crawls down thermal gradients by extending (shortening) runs that are oriented down (up) gradients. To bias run duration toward colder temperatures, *C. elegans* analyzes the orientation of each run by integrating its own movement in spatial thermal gradients with ongoing temporal sensory processing. When it senses warming it reorients more frequently; when it senses cooling it reorients less frequently (Ryu and Samuel 2002).

Run duration in *C. elegans* follows Poisson statistics and the worm continuously adjusts the probability of reorientation on the basis of sensed temporal changes in temperature as it navigates spatial thermal gradients (Fig. 1). Here, we showed that the cryophilic response to temperature changes is bidirectional and symmetric: the worm responds to positive (negative) temperature changes by increasing (lowering) its reorientation rate. We also showed that the cryophilic response to temperature change is adaptive: the reorientation rate returns to its prestimulus value after about 15 s. Short-term adaptation solves two problems at once. First, it allows *C. elegans* to maintain its sensitivity to small temperature changes over broad operating ranges. Second, the temporal dynamics of adaptation allows *C. elegans* to effectively compute the time derivative of gradual temperature variations. Moreover, the band-pass properties of the cryophilic response, a direct result of the temporal dynamics of adaptation, are tuned to analyze intervals of nearly 15 s, which is approximately the duration of the average run of crawling worms. Therefore it appears that the temporal dynamics of adaptation is designed to analyze the orientation of each run during the random walk of crawling worms. In other words, the mechanisms underlying the cryophilic response are matched to the behavioral task that the worm is required to perform.

The computational structure of the cryophilic response can be divided into two components: *1*) the worm must decide whether $T > T_S$, the normal operating range of the cryophilic response; and *2*) the worm executes the cryophilic response by continuously computing the time derivative of temperature changes and modulating the probability of reorientation accordingly. The first computational component of the cryophilic response is disrupted by mutation in the gene *ttx-3*, which disrupts the AIY interneuron and causes worms to extend the operating range of the cryophilic response to all temperatures without disrupting its time course (Hobert et al. 1997). Therefore the AIY interneuron appears to contribute to a pathway mediating the operating range of the cryophilic response. The second computational component of the cryophilic response is disrupted by the *eat-4*(*ad819*) mutation, which disrupts a vesicular glutamate transporter (Lee et al. 1999); these mutants exhibit a cryophilic response with different temporal dynamics, a cryophilic response that is both weaker and briefer than the normal response at $T > T_S$. These data suggest that a glutamatergic pathway mediates the temporal dynamics of the cryophilic response. Taken together, our observations suggest, to some extent, that the two computational components of the cryophilic response have separate neural substrates.

The functional organization of the neural circuit for thermotaxis is not yet understood (Chung et al. 2006; Mori and Ohshima 1995). The *eat-4* gene does not appear to be expressed in the AFD thermosensory neuron or in the AIY and AIZ interneurons that operate downstream of AFD (Lee et al. 1999). Moreover, the cryophilic response does not require signaling through the *glr-1* and *glr-2* glutamate receptor genes, which are expressed in the AVA, AVB, AVD, AVE, and PVC interneurons and are required for area-restricted search behavior (Hills et al. 2004). Nevertheless, our observation using the *eat-4* mutation might be exploited to map additional neural correlates of thermotactic behavior, by examining other neurons, in particular other sensory neurons, that use glutamate as a neurotransmitter, or by studying the effects of other glutamate receptors on the cryophilic response.

It was also previously suggested that *C. elegans* has an alternate mechanism for thermophilic movement, but this is a current controversy (Ito et al. 2006). We note that our assay quantifies the active performance of individual worms as they navigate spatial thermal gradients, whereas the standard assay—the one used by Okochi et al. (2005) to characterize the putatively thermophilic *ttx-4* mutant—quantifies the number of worms at the warm edge or cold center of a radial thermal gradient on an agar plate after an extended period of time. It is difficult to know how *ttx-4* mutant worms might achieve thermophilic accumulation as measured in the assay used by Okochi et al. (2005) without exhibiting active thermophilic performance as measured in our assay. One possibility is that atactic worms, if they are able to ignore the radial spatial thermal gradient, might be scored as thermophilic if they happen to crawl to the boundaries of the agar plate in the assay used by Okochi et al. (2005). Although it is difficult to pinpoint the reasons for the discrepancy, we suggest that quantifying the trajectories of individual worms during behavioral performance provides data that may be more rigorously interpreted than data obtained by quantifying worm location after the navigational behavior has been carried out.

Any system that carries out temporal signal processing may be said to have memory. The cryophilic response system has short-term analog memory in the sense that, before adaptation, the worm continues to respond to stimulus even after it has ended. The cryophilic response system is able to perform analog computation, calculating the time derivative of the input signal within a particular frequency range, by exhibiting adaptation to its prestimulus level with stereotyped temporal dynamics. We note that the cryophilic response of *C. elegans* is reminiscent of another well-studied computational system in biology, the chemotactic response of the bacterium *Escherichia coli* (Block et al. 1982). At the level of behavioral strategy, bacterial chemotaxis and nematode cryophilic movement are both biased random walks. Here, we demonstrated additional similarities at the level of computation—both organisms exploit the temporal dynamics of short-term adaptation to compute the time derivative of sensory inputs. It is remarkable that both systems have converged on the same navigational and computational solutions to move up and down gradients in their respective sensory environments. This may be related to recent suggestions that different biological systems, because of certain shared constraints such as modularity, may converge on similar answers to similar computational challenges (Kashtan and Alon 2005). It appears that two

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evolved networks—the neural circuits of *C. elegans* and the biochemical networks of *Escherichia coli*—have converged on short-term adaptation as the means of calculating time derivatives during biased random walks.

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