Serotonin Modulates Locomotory Behavior and Coordinates Egg-Laying and Movement in *Caenorhabditis elegans*

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ABSTRACT: Biogenic amines have been implicated in the modulation of neural circuits involved in diverse behaviors in a wide variety of organisms. In the nematode *C. elegans*, serotonin has been shown to modulate the temporal pattern of egg-laying behavior. Here we show that serotonergic neurotransmission is also required for modulation of the timing of behavioral events associated with locomotion and for coordinating locomotive behavior with egg-laying. Using an automated tracking system to record locomotory behavior over long time periods, we determined that both the direction and velocity of movement fluctuate in a stochastic pattern in wild-type worms. During periods of active egg-laying, the patterns of reversals and velocity were altered: velocity increased transiently before egg-laying events, while reversals increased in frequency following egg-laying events. The temporal coordination between egg-laying and locomotion was dependent on the serotonergic HSN egg-laying motorneurons as well as the decision-making AVF interneurons, which receive synaptic input from the HSNs. Serotonin-deficient mutants also failed to coordinate egg-laying and locomotion and exhibited an abnormally low overall reversal frequency. Thus, serotonin appears to function specifically to facilitate increased locomotion during periods of active egg-laying, and to function generally to modulate decision-making neurons that promote forward movement.
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INTRODUCTION

An important goal of reductionist neuroscience is to understand how specific proteins act within the context of the neuronal circuitry to control an animal's behavior. To understand a vertebrate nervous system at the molecular and cellular level is extremely difficult due to the extreme organizational complexity of vertebrate brains. However, for animals with less complex nervous systems, such as the nematode *Caenorhabditis elegans*, an understanding of the molecular and cellular basis of behavior is more easily obtained. The *C. elegans* nervous system is simple and well characterized at the anatomical level: an adult hermaphrodite contains only 302 neurons, each with a precisely determined and invariant position and cell lineage (Sulston and Horvitz, 1977; Sulston et al., 1983). This small nervous system is capable of per-

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ceiving and responding to a wide range of environmental conditions, including heavy and light touch (Driscoll and Kaplan, 1997), temperature, volatile odorants, osmotic and ionic strength, food, and other nematodes (Bargmann and Mori, 1997). Each of these sensory modalities in turn regulates many aspects of the animal's behavior, including the rate and direction of movement, the rates of feeding, egg-laying, and defecation, and the process of mating (Avery and Thomas, 1997). *C. elegans* is amenable to classical, molecular, and developmental genetic studies; thus, isolation, phenotypic characterization, and molecular analysis of behavioral mutants provides a promising avenue toward identifying the molecular events that underlie the animal's behavior (Bargmann, 1993). In addition, because each neuron can be identified by position, it is possible to infer the roles of individual neurons in nervous system function by determining the effect of cell-specific laser ablation on behavior (Bargmann and Avery, 1995).

Using these genetic and cell biological approaches, it has been possible to obtain many insights into the molecular and cellular basis of behavior. For example, studies of chemotaxis-defective and touch-insensitive mutants have elucidated molecular mechanisms underlying sensory transduction in olfactory and mechanosensory neurons (Bargmann and Mori, 1997; Driscoll and Kaplan, 1997). Genetics and cell ablation experiments have also provided detailed information about the molecular basis for several simple motor behaviors, including egg-laying, feeding, and defecation, which involve regulation of the contractile properties of a single specialized muscle group. However, because genes and neurons that affect higher-level aspects of nervous system function tend to have only subtle effects on behavior, much less has been learned about the neural basis for more complex motor patterns such as those involved in locomotion. Likewise, the mechanisms through which the decision to execute a given motor program is influenced by sensory information and the activity of other motor pathways are not well understood even in this simple organism.

In this study, we have characterized the long-term pattern of locomotor behavior in *C. elegans* and identified neurons that correlate these patterns with the activity of the egg-laying motor program. Specifically, we demonstrate that two aspects of locomotor function, velocity and directional reversals, are random processes whose occurrence is temporally coordinated with the onset of egg-laying events. We also provide evidence that this temporal link between egg-laying and locomotion results from the modulation of decision-making brain interneurons by serotonin.

METHODS

Strains and Genetic Methods

Routine culturing of *C. elegans* was performed as described (Brenner, 1974). All worms analyzed in these experiments were young adults; fourth-stage larvae were picked the evening before the experiment and tracked the following morning after cultivation at 22°. We observed that animals tended to show higher locomotor activity immediately after being transferred to a fresh plate; thus, experimental animals were allowed to acclimate for at least 1 h before their behavior was analyzed. After this 1-h acclimation, additional time-specific differences in locomotor or egg-laying behavior were not detected.

The chromosomal locations of the genes studied in these experiments are as follows: LGII, *tph-1(mg280)*; LGIII, *bas-1*; LGV, *cat-4(e1114), egl-1(n986)*. For ablation experiments, we used the following strains: NC197 (*dpy-20(e1282)IV; wdIs4[dpy-20(+)unc-4::GFP]*, generously provided by David Miller); *akIs11*, which uses the *nmr-1* promoter to express ICE protease, which induces cell death, in a subset of command interneurons, not including AVB or AVJ, and *kyIs36*, which uses the *glr-1* promoter to express ICE protease in a subset of command interneurons, including AVB and AVJ (both generously provided by Villu Maricq).

Analysis of Locomotion Behavior

Locomotion behavior of individual animals on solid media (NGM agar) was recorded at room temperature (22°) for 4–8 h, as described (Waggoner et al., 1998) using an automated tracking system. For each recording, a six-column matrix was saved by the computer: hour, minute, second, millisecond, x-position of the worm's centroid, and y-position of the centroid. The animal's running mean velocity in a 10-s time window was calculated at 1-s intervals over the entire recording. To identify directional changes, we resampled the positional data at 0.1-mm intervals to convert the data into a path of contiguous line segments of constant size. We then computed turning angles as the smallest angle between adjacent segments. Analysis of the distribution of turning angles in wild-type animals revealed two distinct peaks. One peak, centered at 0 degrees, corresponded to animals continuing in approximately the same direction; the other peak, centered at 180 degrees, corresponded under to a large change in direction. Defining a directional change as a turning angle greater than 120 degrees, we manually inspected videotapes to determine the nature of the directional changes identified using our automated system. From 75 min of randomly selected wild-type footage, our automated system detected a total of 125 directional changes. Of these 124 corresponded to reversals (i.e., a switch from forward to backward movement), and 1 corresponded to a large turn. Because position was sampled over discrete intervals of distance, a few reversal events (12/136) that were detectable by eye were not detected by

the system, presumably because the distance moved backward by the centroid was smaller than the sampling interval.

To analyze N2 locomotion data, we observed 10 animals, 109 h, 366,384 velocity measurements, 7651 directional changes. For *egl-1*, we observed eight animals, 97 h, 234,005 velocity measurements, 6352 directional changes. For *tph-1*, we observed six animals, 61 h, 168,942 velocity measurements, 1777 directional changes. For *cat-4*, we observed six animals, 88 h, 247,961 velocity measurements, 3952 directional changes. For AVF$^-$ animals, we observed seven animals, 105 h, 262,843 velocity measurements, 4424 directional changes. For ASH$^-$ animals, we observed five animals, 62 h, 154,898 velocity measurements, 4124 directional changes. For *glr-1::ICE(AVB$^-$)*, we observed nine animals, 83 h, 199,395 velocity measurements, 1713 directional changes. For *nmr-1::ICE(AVB$^+$)*, we observed nine animals, 79 hours, 198,434 velocity measurements, 1217 directional changes.

Analysis of Egg-Laying Behavior

The time of egg-laying events and the intervals between them were determined from analysis of videotapes obtained using the automated tracking system. Quantitative analysis of the egg-laying pattern using this interval data was performed as described (Zhou et al., 1998). Briefly, egg-laying events in *C. elegans* are clustered, with periods of active egg-laying, or active phases, separated by long inactive phases during which eggs are retained. Both the duration of the inactive phases ("intercluster intervals") and the duration of intervals between egg-laying events in a cluster ("intracluster intervals") model as exponential random variables with different time constants (Waggoner et al., 1998). Thus, the probability density function for the intervals between events is

$$f_x(x) = k_1\lambda_1 e^{-\lambda_1 x} + k_2(p\lambda_2)e^{-(p\lambda_2)x}, \quad x \geq 0,$$

$$k_1 = \frac{p(\lambda_1 - \lambda_2)}{\lambda_1 - p\lambda_2}, \quad k_2 = \frac{\lambda_1(1-p)}{\lambda_1 - p\lambda_2}$$

where the intracluster time constant is $1/\lambda_1$ and the intercluster time constant is $1/p\lambda_2$. The parameters were determined using the maximum likelihood estimation technique described previously (Zhou et al., 1998). The expected variance of estimated parameters and time constants was determined by generating 100 independent sets of simulated egg-laying data using the model probability density function, and computing the standard deviation of the parameters estimated from these simulations.

Ablation of Neurons

To ablate the AVF neurons, we used the strain NC197 (genotype *dpy-20(e1282)IV; wdIs4(dpy-20(+)unc-4::GFP)*, generously provided by David Miller), which carries an integrated *unc-4::GFP* fusion. Both the SAB and AVF neurons express *unc-4::GFP*, and SABVL and SABVR are difficult to distinguish from AVFL and AVFR in the larvae. We therefore used a two-step laser ablation procedure to determine the effect of AVF ablation. In early first-stage larvae, SABVL and SABVR were ablated. We allowed these worms to recover overnight before ablating the AVFs the following day. On the third day, cell killing of all four neurons was verified by scoring for the absence of GFP-expressing neurons in the head region. SAB-only ablated worms were used as a control.

To ablate the ASH neurons, we first identified these neurons by filling with the lipophilic dye diI. Early first-stage larvae were placed in a solution of M9 and diI for 1 h, which labeled the amphid neurons ASK, ADL, ASI, ADF, ASH, and ASJ. The ASHs were identified and ablated based on their position; ablation was confirmed the following day using the same dye-filling procedure.

RESULTS

Analysis of Long-Term Patterns of Movement

Previous studies of the temporal pattern of egg-laying indicated that egg-laying shows a high degree of temporal clustering (Waggoner et al., 1998). However, casual examination of the egg distribution on a culture plate suggested that egg-laying events were likely to be spatially dispersed. To gain insight into how this spatial dispersal could occur, we first attempted to obtain a quantitative description of the pattern of locomotion in wild-type *C. elegans*. Using an automated tracking system, we were able to follow the movements of individual animals for long (>6 h) time periods. Using this system, it was possible to obtain a record of the animal's body position at regular intervals and derive from this time-coded positional data a number of characteristics of the animal's movement over a long time course [Fig. 1(a)].

We focused our attention on two aspects of locomotory behavior: velocity and directional changes. To compute an animal's translational velocity, positional data were sampled over intervals of constant time, and the distance traveled over each interval was divided by the interval duration. In this way, we obtained velocity measurements for wild-type animals over the course of several long recordings [Fig. 1(c)]. Fourier analysis of these recordings indicated that the velocity traces were aperiodic, suggesting that the fluctuations in velocity over time were stochastic [Fig. 1(d)]. However, a strong positive correlation was observed between velocity measurements hundreds of seconds apart, indicating that animals alternate between periods of high and low locomotor activity [Fig. 1(b)]. Taken together, these data suggested that locomotor

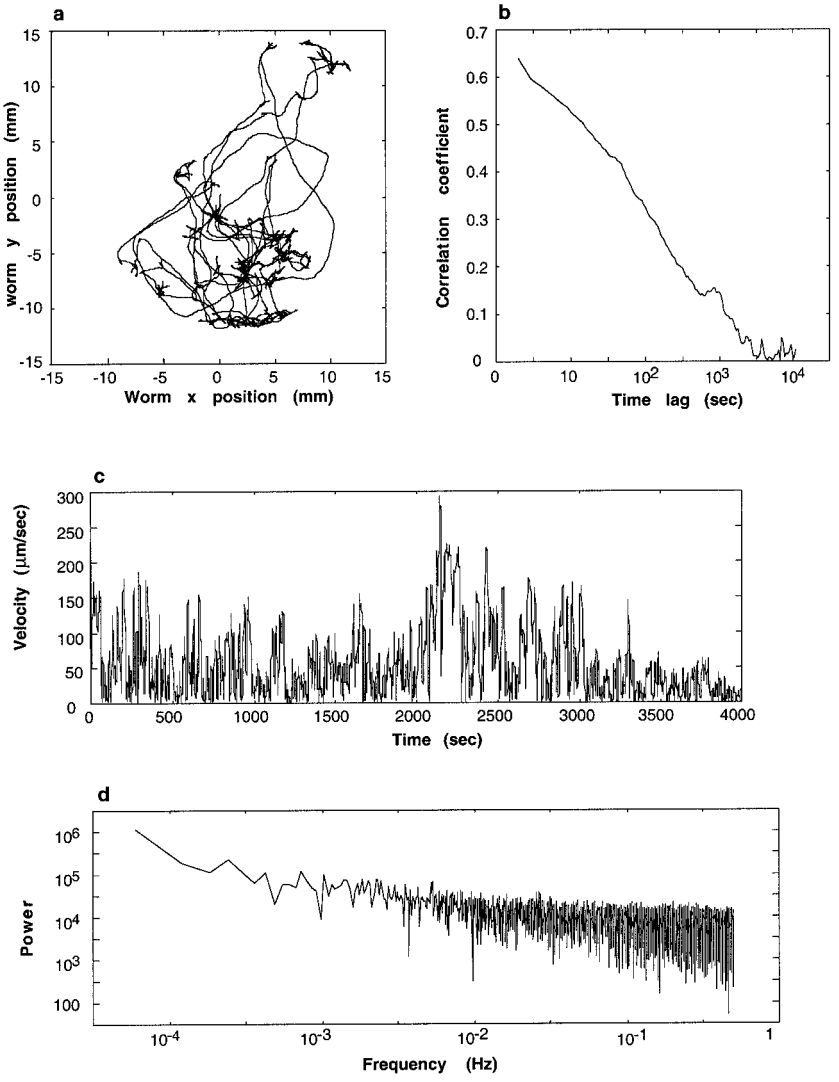

Figure 1 Temporal pattern of velocity. (a) Path of animal. Shown is the plot of the x- and y-position of one animal over a 6-h time interval, recorded by the tracking computer. (b) Velocity autocorrelation. Shown is the correlation coefficient between velocities separated by a given time lag, averaged over all wild-type data. Individual traces have a similar profile. (c) Velocity of animal. A representative trace of the velocity, averaged over 10-s intervals, of a single wild-type worm. The mean velocity is 15.9 μm/s. Regions of increased and diminished activity are visible, as expected from the autocorrelation, but no obvious periodicity is present. (d) Power spectrum of velocity. The magnitude of the Fourier transform of the velocity taken from a representative recording of a wild-type worm. No significant peaks are present, indicating the lack of periodicity in velocity.

velocity was a random process involving multiple behavioral states.

We also analyzed the pattern of directional changes in wild-type animals. Both reversals (switches from forward to backward movement) and other large turns represent an important feature of locomotory behavior in *C. elegans*, and have been shown to underlie behavioral responses to a number of sensory stimuli (Chiba and Rankin, 1990; Pierce-Shimomura et al., 1999; Zheng et al., 1999). To obtain a quantitative description of the pattern of reversals and turns, we used the positional data collected by our system to measure the timing of large changes in direction for wild-type animals in a bacterial lawn [Fig. 2(a)]. Analysis of these data indicated that in the presence of food, nearly all directional changes greater than 120 degrees detected by our system were the result of a reversal rather than a large turn (see Methods). When we analyzed the timing of these events, we found that successive intervals between directional changes were virtually uncorrelated (as were intervals at other spacings), suggesting that reversal intervals model as an

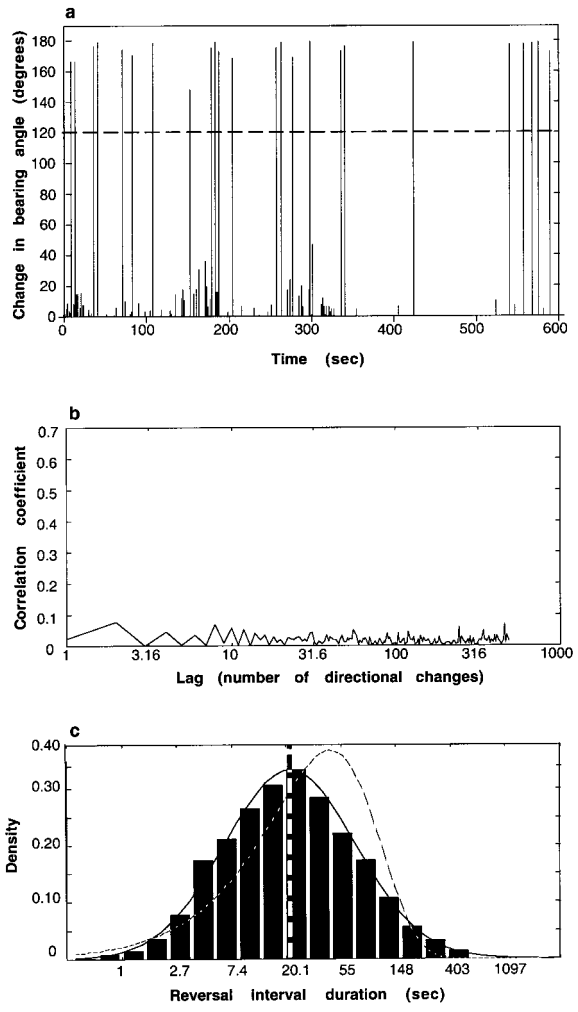

Figure 2 Temporal pattern of directional changes. (a) Angle of bearing over time. Shown is the change in bearing angle over a 45-mm segment of worm travel. The data are from a representative wild-type animal. Manual observation of videotapes indicated that under our assay conditions, angle changes of greater than 120 degrees (dashed line) nearly always (124/125 events) corresponded to switches between backward and forward movement (reversals). (b) Reversal interval autocorrelation. Shown is the correlation coefficient between reversal intervals (i.e., intervals between successive 120° directional changes) separated by the indicated lag, averaged over all wild-type data. Successive intervals are virtually uncorrelated, as are intervals at other spacings, suggesting that reversal intervals may model as an independent random variable. (c) Reversal frequency. Shown is a histogram of the reversal interval times of wild-type worms plotted on a log scale (black bars). The best fit of an ideal lognormal distribution (solid curve) appears as a Gaussian on a log scale, demonstrating that the distribution of reversal intervals can be modeled as a random process with a lognormal probability density. A lognormal random variable has two parameters: the logarithmic mean and logarithmic standard deviation. The best fit to the data shown is 17.8 s ± 1.20 between reversals (thick dashed line). The data do not model well as an idealized Poisson process (thin dashed curve).

independent random variable [Fig. 2(b)]. Furthermore, the distribution of intervals between directional changes indicated a temporal pattern that could be modeled as a stochastic process of a specific type: a random process with a lognormal probability density [Fig. 2(c)]. A lognormal random variable has two parameters: the logarithmic mean (μ) and logarithmic standard deviation (σ). These two parameters can be straightforwardly estimated from real data; thus, the reversal pattern of a given nematode strain appeared to be effectively describable in terms of these two parameters (Table 1).

Temporal Correlation between Locomotion and Egg-Laying

Because egg-laying events were temporally clustered but spatially dispersed, we reasoned that egg-laying might be temporally coordinated with movement. Therefore, we investigated whether the patterns of reversals and velocity fluctuations changed during periods of active egg-laying. By synchronizing the time codes of the tracking computer (which recorded body position) and the videotape recorder (used to identify egg-laying events), we were able to examine the velocity pattern before and after each egg-laying event [Fig. 3(a)]. Surprisingly, we observed that in the 30 s immediately prior to egg-laying events, the animal's average velocity was significantly elevated, with the peak velocity occurring approximately 15 s before the egg was laid. Direct observation of video recordings indicated that these velocity bursts corresponded to periods of uninterrupted forward movement at relatively high speeds. Not every egg-laying event was preceded by a velocity burst, and the magnitude of the velocity increase varied substantially from animal to animal [Fig. 3(b)]. Thus, the increase in velocity did not appear to be an integral component of the egg-laying motor program; rather, the initiation of the egg-laying motor program appeared to increase the probability of forward movement, or vice versa. Together, these data indicated that a marked increase in locomotor activity occurred during periods of active egg-laying, an increase that may account for the relative spatial dispersal of eggs laid during an egg-laying burst.

Egg-laying, like locomotion, occurs in a specific temporal pattern. Specifically, egg-laying events are clustered in bursts, or active phases, which are separated by long inactive phases during which eggs are retained. Both the onset of the active phase and egg-laying within the active phase are aperiodic and model as Poisson processes with distinct rate constants. In principle, the velocity burst might correlate specifically with the onset of the active egg-laying phase;

Table 1 Velocity and Reversal Parameters for Wild-Type, Mutant, and Ablated Animals

Animal Type	Mean Velocity (μm/s)	Mean Reversal Interval (s)	Reversal Parameters: μ_1: Logarithmic Mean Reversal Interval (s)	Reversal Parameters: σ_1: Variance (log s)
N2	15.9 ± 26.7	37.9 ± 76.85	17.8	1.19
egl-1 (n986)	17.4 ± 26.9	45.47 ± 92.24	22.6	1.13
tph-1 (mg280)	4.92 ± 8.4	93.41 ± 336.45	25.3	1.69
cat-4 (e1114)	8.13 ± 19.3	64.89 ± 179.16	18.5	1.54
ASH^-	16.9 ± 29.0	40.15 ± 102.96	19.3	1.17
AVF^-	85.4 ± 15.5	64.01 ± 131.34	23.6	1.44
glr-1::ICE (AVB^-)	86.2 ± 17.0	172.70 ± 995.97	19.1	1.86
nmr-1::ICE (AVB^+)	74.1 ± 11.9	198.29 ± 527.21	42.52	1.75

alternatively, it might precede all egg-laying events. To investigate this question, we compared velocities around different types of egg-laying events: the first egg laid in a cluster (i.e., a group of eggs each no more than 2 min apart), the middle eggs in cluster, and the last egg in a cluster [Fig. 3(c)]. We observed that all egg-laying events were preceded by significant increases in velocity, although the first egg in a cluster did exhibit the largest velocity peak. Thus, it appeared that the transient change in locomotor behavior correlated temporally with all egg-laying events and not merely the onset of the active egg-laying phase.

We also investigated whether egg-laying affected the likelihood of reversal events. To address this question we measured the frequency of directional changes during the time immediately before and after the occurrence of an egg-laying event. If egg-laying increased the probability of reversals, we would expect a peak in this trace around the time of egg-laying events; conversely, if egg-laying decreased the probability of reversals, this trace would be expected to show a dip. In fact, we observed a marked increase in the frequency of directional changes from the time of egg-laying until about 30 s subsequent to the egg-laying event [Fig. 3(d)]. Inspection of video recordings confirmed that these changes in direction that followed shortly after egg-laying events corresponded to reversals. Thus, execution of the egg-laying motor program appeared to correlate with two changes in locomotive behavior: an increased propensity for forward locomotion before egg-laying, and an increased propensity for directional changes, in particular reversals, during and after an egg-laying event.

Coordination of Egg-Laying and Locomotion Requires the HSN Motorneurons

What is the neural mechanism that coordinates egg-laying and locomotion? To address this question, we examined the effects of neurons that regulate egg-laying behavior on the control of locomotion. We first assayed the role of the HSNs, a pair of serotonergic motorneurons that have synaptic output to a number of brain interneurons and have also been shown to control the onset of the active phase of egg-laying. To assess the effects of the HSNs on locomotion, we recorded the movements of animals carrying a mutation in the gene *egl-1*, which specifically eliminates the HSNs by causing inappropriate cell death (Conradt and Horvitz, 1998). When we analyzed the locomotive pattern of *egl-1* mutants, we observed that the overall movement velocity and directional change frequency were normal (Table 1). However, in contrast to wild-type animals, they did not display the velocity peak prior to egg-laying [Fig. 4(a) and (c)], nor did they exhibit the reversal frequency peak after egg-laying [Fig. 4(d)]. Thus, the HSNs were specifically required for both the velocity peak and the reversal frequency peak coordinating egg-laying and locomotion.

The HSNs contain multiple neurotransmitters, each of which could in principle be responsible for its effects on movement. One of these molecules, serotonin, appears to function specifically to facilitate the onset of the active egg-laying phase. To determine whether serotonin release by the HSNs could also be involved in egg-laying and locomotion, we analyzed the egg-laying and velocity data for strains carrying loss-of-function mutations in two genes required for serotonin biosynthesis: *tph-1* (encoding tryptophan hydroxylase; Sze et al., 2000) and *cat-4* (a gene required for AA-decarboxylase activity; Loer and Kenyon, 1993). We observed that all three serotonin-deficient mutants lacked the velocity burst prior to egg-laying as well as the peak in directional change probability following egg-laying [Fig. 4(b) and (c)]. Unlike the HSN-deficient animals, all serotonin-deficient mutants also showed a significant decrease in overall locomotive velocity (Table 1). These results

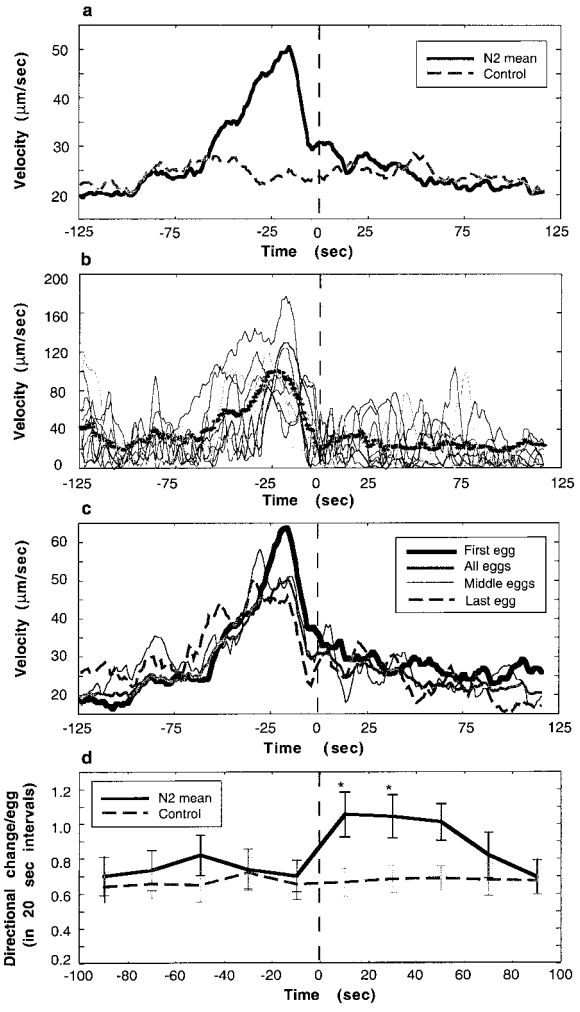

Figure 3 Velocity and reversal frequency surrounding egg-laying events. (a) Mean velocity pattern surrounding egg-laying events. Shown is a plot of the mean wild-type velocity surrounding egg-laying events (dotted vertical line at $t = 0$), determined by synchronizing the time codes of the tracking computer (which records body position) to the videotape recorder (used to identify egg-laying events). The highest peak occurs at fifteen seconds before egg-laying. Shown is the mean data of 10 worms, 61 h, 186 egg-laying events. Shown also is a plot of control data, the velocities around a list of times chosen uniformly at random over the length of the recordings. (b) Velocity pattern surrounding individual egg-laying events. Shown is a plot of every velocity trace around the egg-laying events of one recording, with the mean velocity in bold. (c) Velocity pattern surrounding egg-laying events categorized by order within a cluster. Shown are the plots of the velocities around three different egg-laying events: the first egg laid in a cluster (i.e., a group of eggs each no more than 2 min apart), the middle eggs in a cluster, and the last egg in a cluster. (The mean is also shown for comparison). All egg-laying events were preceded by statistically significant increases in velocity ($p < .001$), and the velocity burst of the first eggs was statistically significant from the velocity burst of the last eggs ($p < 0.05$), according to the Mann-Whitney rank sum test. (d) Reversal frequency surrounding egg-laying events. Shown is a plot of the mean frequency of large directional changes surrounding egg-laying events (dotted vertical line) in wild-type *C. elegans*, over the course of 10 recordings, 61 h, and 186 egg-laying events. Inspection of videotapes indicated that nearly all large directional changes around egg-laying events corresponded to reversals. After egg-laying occurs, there is a statistically significant increase ($p < .05$) in the frequency of directional changes, according to the Mann-Whitney rank sum test. Shown also is a plot of control data, the frequencies around a list of imaginary egg-laying times chosen uniformly at random over the length of the recordings; this plot does not show any significant differences from the mean frequency.

suggested that serotonin functions generally to promote forward locomotion and is required specifically for the changes in locomotion pattern during periods of active egg-laying.

AVF Interneurons Are Necessary for the Velocity Peak Prior to Egg-Laying

How might serotonin released from the HSNs stimulate locomotion? Although the HSNs make neuromuscular junctions only with the vulval muscles, they also make synaptic connections with several interneurons in the head. Among the most prominent synaptic outputs of the HSNs are to a pair of interneurons called AVFs. AVF's synaptic output is primarily directed to AVB, a so-called command interneuron implicated in promoting forward locomotion, and to AVJ, which also directs its synaptic output to AVB. Thus, a reasonable hypothesis to explain the HSN's effects on locomotion was that the HSNs might modulate the activity of AVF, which in turn, could activate the forward command interneurons and promote forward movement.

To test this hypothesis, we examined the effects of ablating the AVF neurons on the animal's locomotor and egg-laying behavior. When we ablated the AVFs, we observed an effect similar to that seen in the serotonin-deficient mutants: the velocity burst prior to egg-laying was reduced if not eliminated, and the average velocity overall was significantly reduced [Fig. 5(a) and (b);. In addition, the peak in directional change probability after egg-laying was eliminated {Fig. 5(c)]. In contrast, the effect of AVF ablation on egg-laying was quite distinct from that of HSN ablation or serotonin deficiency. Whereas HSN-ablations and serotonin deficient mutations slowed the rate of egg-laying and increased the duration of the inactive egg-laying phase (Waggoner et al., 1998), AVF ablations did not (Fig. 5 legend). Together, these results

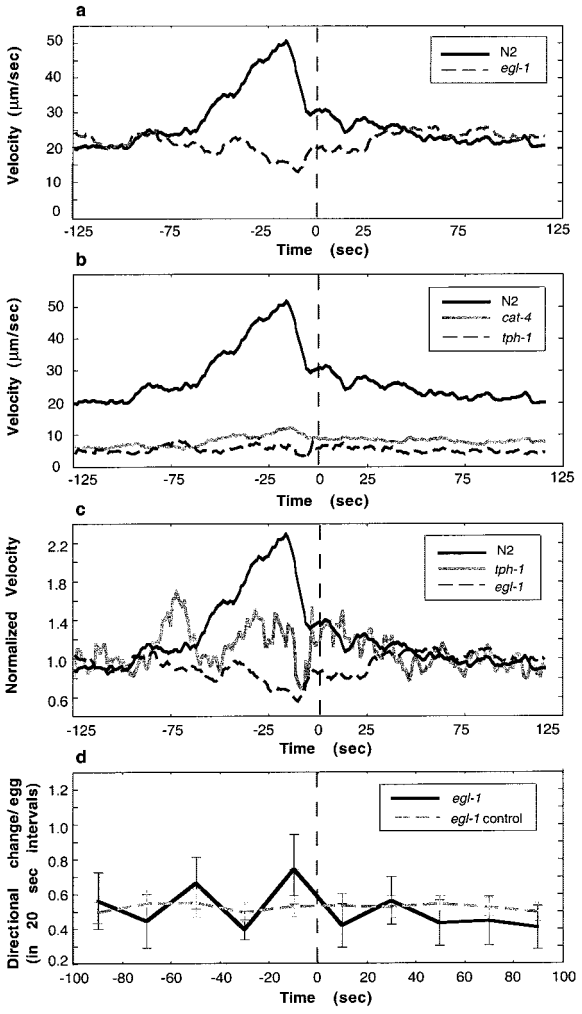

Figure 4 Effect of *egl-1* mutation and serotonin deficiency on the velocity burst and reversal frequency. (a) Effect of *egl-1* mutation on the velocity burst. Shown are the plots of mean velocity surrounding egg-laying events for wild-type (10 recordings, 61 h, 186 egg events) and *egl-1* mutants (8 recordings, 61 h, 63 egg events). Although the mean velocity of *egl-1* mutants is similar to wild-type, *egl-1* mutants show no significant increase in velocity prior to egg-laying. (b) Effect of serotonin deficient mutants *tph-1* and *cat-4* on the velocity burst. Shown are the plots of mean velocity surrounding egg-laying events for wild-type, *cat-4* mutants (10 recordings, 55 hours, 139 egg events), and *tph-1* mutants (6 recordings, 27 h, 56 egg events). The mean velocity of these mutants is significantly reduced, and there is a statistically significant difference between the velocity of wild-type and the mutants. (c) Normalized effect of *tph-1* and *egl-1* on the velocity burst. Shown are the plots of the normalized velocity around egg-laying events, where normalized velocity equals velocity divided by the mean velocity of the period without the velocity burst, to get a mean normalized velocity of approximately 1.0 for each strain. Here, it is possible to visualize that the velocity burst in the *tph-1* or *egl-1* mutants is dramatically reduced, although there is more noise in the *tph-1* mutants. (d) Effect of *egl-1* on reversal frequency surrounding egg-laying. Shown is a plot of mean frequency of directional changes surrounding egg-laying events. *egl-1* mutants do not exhibit the reversal peak following an egg-laying event.

suggested that the AVFs might mediate the effects of the HSNs on locomotion, but did not mediate the HSNs' effects on egg-laying per se.

We also examined the effect of ablating the ASH neurons, another set of neurons that receive synaptic input from the HSNs and have synaptic output to the command interneurons. In contrast to the results of AVF ablation experiment, ablation of the ASH neurons did not cause a significant change in either the general pattern of locomotion (Table 1) or the velocity and reversal bursts around egg-laying [Fig. 5(a) and (b)]. These results suggest that unlike AVF, ASH is not critical for coordinating egg-laying and locomotion.

We also investigated the roles of the command interneurons themselves, in particular AVB, in the temporal coupling of egg-laying and locomotion. We analyzed the locomotion behavior of two strains in which specific command interneurons undergo inappropriate cell death due to misexpression of the ICE protease under a cell-type-specific promoter. In one strain, all the command interneurons (i.e., AVA, AVB, AVC, and PVC, as well as AVJ) were absent; in the other AVA, AVC, and PVC were killed but AVB and AVJ were spared. Consistent with previously published analyses of these strains, we observed that both showed abnormal locomotion patterns: their average velocity was significantly reduced, and the intervals between directional changes was significantly increased. However, perhaps surprisingly, both strains exhibited a significant velocity burst prior to egg-laying [Fig. 5(d)]. Thus, these data indicate that the coordination of egg-laying and movement does not require the command interneurons, and may involve direct modulation of motorneurons by AVF or other decision-making interneurons (Fig. 6).

DISCUSSION

Stochastic Modeling of Locomotor Events

Locomotion involves some of the most intricate and complex motor patterns displayed by *C. elegans* and represents a critical aspect of the worm's behavioral repertoire; yet, the long-term temporal patterns of locomotion in *C. elegans* are not well understood. We have shown here that two key aspects of nematode

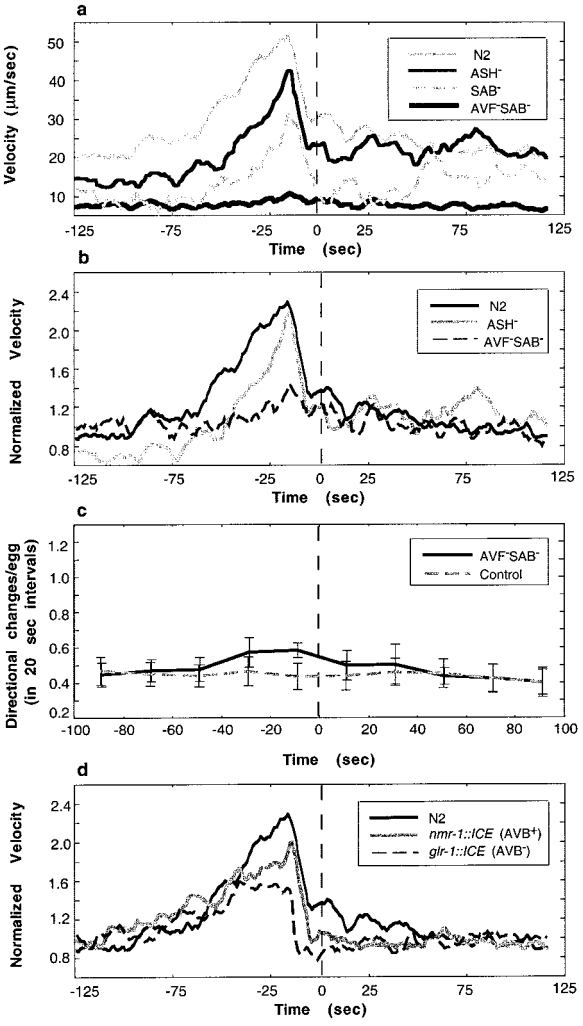

Figure 5 Effect of decision and command interneuron ablations on the velocity burst and reversal frequency. (a) Effect of decision interneuron ablation on the velocity burst. Shown are the plots of mean velocity surrounding egg-laying events for wild-type, ASH-ablated animals (5 recordings, 34 h, 85 egg events) and AVF-ablated animals (7 recordings, 42 h, 174 egg events). For AVF-ablated animals, both the mean velocity and the velocity peak are significantly reduced (*$p < .01$). Unlike HSN ablation, AVF ablation did not shorten the intercluster egg-laying interval; intercluster and intracluster time constants for AVF- animals were 1285 ± 216 s and 30.6 ± 20 s, compared to 1728 ± 470 s and 100 ± 27 s for control animals (3 recordings, 18 h, 35 intervals). (b) Normalized effect of decision interneuron ablation on the velocity burst. When the velocities are normalized, there is no statistically significant difference in the velocity peak for ASH-ablated animals. (c) Effect of AVF ablation on reversal burst. Shown is a plot of mean frequency of directional changes surrounding egg-laying events. Ablated animals do not exhibit the reversal peak following an egg-laying event. (d) Effect of killing command interneurons on the velocity burst. Shown are the plots of normalized velocity surrounding the egg-laying events for wild-type, *nmr-1::ICE* mutants (8 recordings, 65 h, 226 egg events), and *glr-1::ICE* mutants (10 recordings, 75 h, 159 egg events). Although the average velocity was significantly reduced for both strains (data not shown), both also exhibit a significant velocity burst prior to egg-laying.

locomotion, translational velocity and reversal frequency, can be modeled as stochastic processes. The pattern of reversals models closely as a lognormal random variable, while the velocity fluctuates in a more complex random pattern. The stochastic nature of both these features of locomotive behavior implies that the switch between forward and backward movement is arrhythmic and may involve random fluctuations in the functional states of neuronal circuits dedicated to locomotion.

Both velocity changes and directional changes have been shown to play a key role in the responses of nematodes to sensory stimuli. For example, an animal's velocity has been shown to decrease significantly when the animal enters a lawn of its bacterial food source (Sawin et al., 2000). Likewise, a nonlocalized mechanical stimulus (i.e., "tap") causes an animal to change its direction of movement, either from forward to backward or vice versa, while a localized touch to the head or tail causes the animal to change its direction of movement away from the stimulus (Chiba and Rankin, 1990). Finally, changes in the occurrence of long periods of uninterrupted forward movement ("runs") and bouts of frequent sharp turns ("pirouettes") have been shown to mediate navigation in a chemoattractive gradient (Pierce-Shimomura et al., 1999). The behavioral models described here make it possible to quantitatively describe the temporal pattern of reversals in terms of two specific parameters of a lognormal random variable: the logarithmic mean and logarithmic variance. By estimating these parameters from behavioral mutants or animals carrying specific neuronal ablations, it will be possible to gain more detailed insight into how individual genes and neurons influence the timing of these locomotory events.

Influence of Egg-Laying Behavioral States on Locomotion

Interestingly, we observed that both directional reversals and velocity fluctuations were temporally correlated with egg-laying behavior. Specifically, egg-laying events tended to be preceded by an increase in locomotor velocity and followed by an increase in reversal frequency. Previous studies of the timing of egg-laying events indicated that animals fluctuate be-

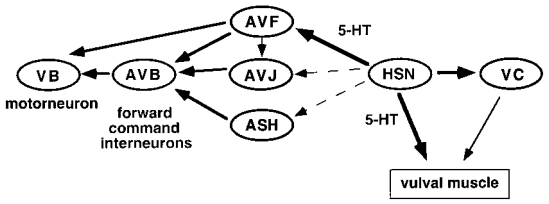

Figure 6 Neural model of the coordination of egg-laying and locomotion.

tween inactive and active egg-laying phases; eggs are retained during the inactive phase and laid in bursts during the active phase (Waggoner et al., 1998). The results described here indicate that the active egg-laying phase also involves changes in locomotor behavior, such that animals have an increased propensity for directional changes and rapid body movement between clustered egg-laying events. The primary consequence of these changes in locomotor pattern is that egg-laying events are spatially dispersed despite being temporally clustered. It should be noted that the temporal link between egg-laying and locomotory behavior is not deterministic; the extent and duration of the velocity burst varies widely from egg-laying event to egg-laying event, and some events are not preceded by a velocity burst at all. Thus, egg-laying appears to influence locomotion stochastically by modifying the probability of executing behavioral events associated with forward locomotion.

What is the biological purpose for this coordination of egg-laying and movement? Clearly, there is no mechanical requirement for increased motility during egg-laying, because the processes can be uncoupled (e.g., in an AVF-ablated animal) without impairing normal egg-laying behavior. Moreover, an opposite correlation between egg-laying and movement has been reported for another species of nematode; in *Prionchulus punctatus*, egg-laying occurs only during periods of periods of inactivity (Maartens, 1975). This variation in the nature of the correlation between egg-laying and movement may reflect different mating or foraging strategies utilized by different nematode species or subspecies. For example, it was recently demonstrated that wild subspecies of *C. elegans* exhibit one of two distinct foraging strategies; "social" strains cluster together on food, whereas "solitary" strains disperse across a food source (de Bono and Bargmann, 1998). An increase in locomotion during the active egg-laying phase could perhaps facilitate a solitary foraging strategy by scattering an animal's progeny across the food source. A survey of the locomotor patterns of social and solitary wild isolates of *C. elegans* might provide insight into this possibility.

Effects of Interneurons and Motorneurons on Behavioral Decision Making

By analyzing the locomotion patterns of animals carrying cell-specific neuronal ablations, we identified specific roles for several classes of neurons in coordinating egg-laying and movement. Together, these data provide an outline of the neural circuitry that controls locomotory behavioral decision making, and mediates the temporal coordination of egg-laying with movement. Specific roles in these processes were identified most strikingly for two classes of neurons: the AVF decision-making interneurons and the HSN egg-laying motorneurons.

The AVF neurons appear to play a specialized role in controlling the onset of reversals and velocity fluctuations. Ablation of AVF caused a marked decrease in the mean translational velocity as well as a nearly complete elimination of both the velocity burst prior to egg-laying and the reversal burst following egg-laying. Thus, the AVFs appear to function as decision-making interneurons, because they do not affect the ability to generate the reversals or velocity fluctuations but strongly influence the frequency and timing of these events. The AVFs direct most of their synaptic output to AVB, a forward command interneuron thought to be a key component of the central pattern generator for forward locomotion. Therefore, it is reasonable to hypothesize that AVF's ability to increase the likelihood of sustained forward movement is mediated at least in part through modulation of AVB. However, because animals lacking all the command interneurons still exhibit the velocity burst, AVF may also promote forward movement through an AVB-independent neural mechanism. The AVFs direct some synaptic output to the VB motorneurons, which have been shown to directly generate the forward locomotion pattern (Chalfie et al., 1985). Thus, it is possible that the coordination of egg-laying and movement could also involve direct modulation of the VBs by AVF (Fig. 6).

Another class of neurons that were required to coordinate egg-laying and movement were the HSNs, a pair of egg-laying motorneurons. Although the HSNs are important for promoting egg-laying, they are not essential; HSN-ablated animals still lay eggs, and these egg-laying events are still clustered in active phases (Waggoner et al., 1998). However, animals carrying an ablation of the HSNs completely failed to undergo the changes in both velocity and reversal frequency that normally accompany periods of active egg-laying, indicating that these neurons were essential for coupling the temporal patterns of egg-laying and movement. Yet in contrast to the AVF interneurons, the HSNs had little

effect on the frequency of reversals or the pattern of velocity fluctuations except during periods of active egg-laying. Thus, the HSNs appear to specifically affect locomotory behavior during the active egg-laying phase, probably through modulation of decision-making neurons such as AVF. Because mutants deficient in serotonin, a neuromodulator released from the HSNs, also showed an uncoupling of egg-laying and locomotor patterns, it appears likely that the modulation of locomotory interneurons by the HSNs probably involves serotonergic neurotransmission.

Interestingly, serotonin deficiency, unlike HSN ablation, had a significant effect on the pattern of locomotion during inactive egg-laying periods. In fact, the locomotion phenotype of *tph-1* and *cat-4* mutants was remarkably similar to that of the AVF-ablated animals. These observations suggest that serotonin may function more generally as a modulator of AVF activity, and that other neurons in addition to HSN may promote increased locomotor activity through serotonin release. The evidence that serotonin is a modulator that promotes locomotor activity was unexpected, as exogenous serotonin has been shown previously to inhibit movement (Segalat et al., 1995). The inhibitory action of serotonin on movement appears to involve direct inhibition of neurotransmitter release from ventral cord motorneurons. Thus, the seemingly paradoxical actions of serotonin as both an activator and an inhibitor of might be explained as reflecting two distinct cellular targets for serotonin within the neural circuitry (i.e., the AVF interneurons and the ventral cord motorneurons, respectively). Alternatively, these differences might reflect concentration-dependent differences in the action of serotonin on targets in locomotory pathways. The application of quantitative analytical techniques such as those described here should make it possible to dissect apart the distinct features of locomotory behavior affected by serotonin and other modulators, and hence identify the specific neural mechanisms that underlie these distinct modulatory actions.

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