

Information theory rules out the reflex-chain model of *C. elegans* locomotion

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1 **Despite decades of research, whether the *C. elegans* traveling-wave**
2 **sinusoidal body pattern during locomotion is produced (a) by the**
3 **undulations of the head followed by wave propagation down the**
4 **body, or (b) via centrally coordinated posture control along the body,**
5 **is still under debate. By studying relationships between the time**
6 **series of postural angles along the body extracted from videos of**
7 **moving worms, we find that the reflex-chain model can be refuted, in**
8 **both forward and backward locomotion as well as during swimming**
9 **and crawling behaviors. We show that information theory applied to**
10 **animal behavior can yield insights into the neural control of behavior.**

C. elegans | information theory | locomotion | motor control

1 **H**ow the nematode *C. elegans* moves in a well-executed
2 serpentine fashion is still unknown despite a detailed
3 anatomical knowledge, connectome and genetic access to each
4 of its 302 neurons (1, 2). The body motor system of *C.*
5 *elegans* consists of overlapping 95 body wall muscle cells that
6 ring the body and 75 body motor neurons grouped into 12
7 similar neuromuscular units running down the body (3). Two
8 main models exist for *C. elegans* locomotion: a reflex-chain
9 model where the dorsoventral undulations of the head set
10 up an oscillatory pattern that propagates down the body
11 via connections between adjacent neuromuscular units and
12 biomechanical linkage, and an alternative active posture model
13 where the sinusoidal body posture along the entire body is
14 effected by active neural control not solely deriving from lateral
15 neuromuscular signaling from the head to tail (Fig 1a). The
16 earliest computer simulations of *C. elegans* movement were
17 based on a reflex-chain model, and more recent simulations
18 based on proprioceptive reflex chains recapitulate aspects of
19 *C. elegans* movement (4–7). Worms crawl on their side with a
20 smoothly propagating sinusoidal undulation with little body
21 slippage outside of the sinusoidal path they trace out on their
22 crawling surface; we surmise that the appearance of a smooth
23 and consistent traveling wave inspired the reflex-chain model.
24 An alternative model, which we term the active posture
25 model, posits that worm motion is driven by multiple sites of
26 centrally coordinated neural signals along the body. (Fig. 1a).
27 These signals may be produced by a pattern generator (CPG)
28 consisting of one more cells. Recently, rhythmically active
29 groups of neurons for forward and backward locomotion have
30 been identified (8–12), but whether these groups of neurons
31 represent autonomous CPGs is still to be resolved.

Results

33 **Cross-correlation of postural angle time series reveals non-**
34 **monotonic noise accumulation down the body.** To generate
35 quantitative worm movement data, we recorded high reso-
36 lution videos of worms crawling on an agar surface using a

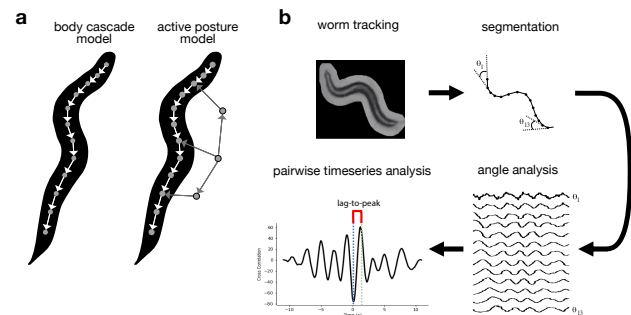


Fig. 1. Locomotion models and analysis overview. a. Schematics of the reflex-chain model (left) versus the active posture model (right). b. Overview of analysis of worm movement. Sustained bouts of forward locomotion were tracked, worm skeletons were segmented, and the worm tangent angles were plotted versus time for each segment. Finally, pairwise measures – time lag to peak cross-correlation, peak absolute cross-correlation, and mutual information were calculated for all joint angle pairs.

37 custom-built motorized-stage microscope and image-based
38 tracking software system (13) and performed video analysis
39 (14) to silhouette and segment the worms. We then extracted
40 time series of the postural tangent angles between each of
41 13 body segments (Fig. 1b). As expected, these time series
42 resembled a series of phase-lagged noisy sinusoids. Performing
43 analysis with a finer discretization of body segments did not
44 change the key findings.

45 The sinusoidal appearance of the signals suggested that
46 cross-correlation analysis would be revealing. The cross-
47 correlation of two closely related sinusoidal signals in the
48 presence of noise consists of a set of peaks of decaying mag-
49 nitude (Fig. 1b). The x-coordinate of the peak of the cross-
50 correlation provides an estimate of the time lag of the signals.
51 The maximum absolute value of the cross-correlation provides
52 a scalar estimate of the relatedness of the signals measured
53 at the most favorable relative time delay, and it is reduced
54 by the amount of noise present in the transformation between
55 the signals. To simulate the undulations of forward locomotion
56 under the reflex-chain model, we created a sine wave to
57 represent head postural angle time series, added noise and a
58 phase delay to the signal to generate the posteriorly adjacent
59 postural angle time series, and iterated this procedure down
60 the body.

61 We computed the cross-correlation between each body joint
62 angle with respect to the anterior-most (head) joint angle

JW wrote the code for the project and performed the experiments. JW and SK worked together on all other aspects of the project, including conceptualization, data analysis, and writing.

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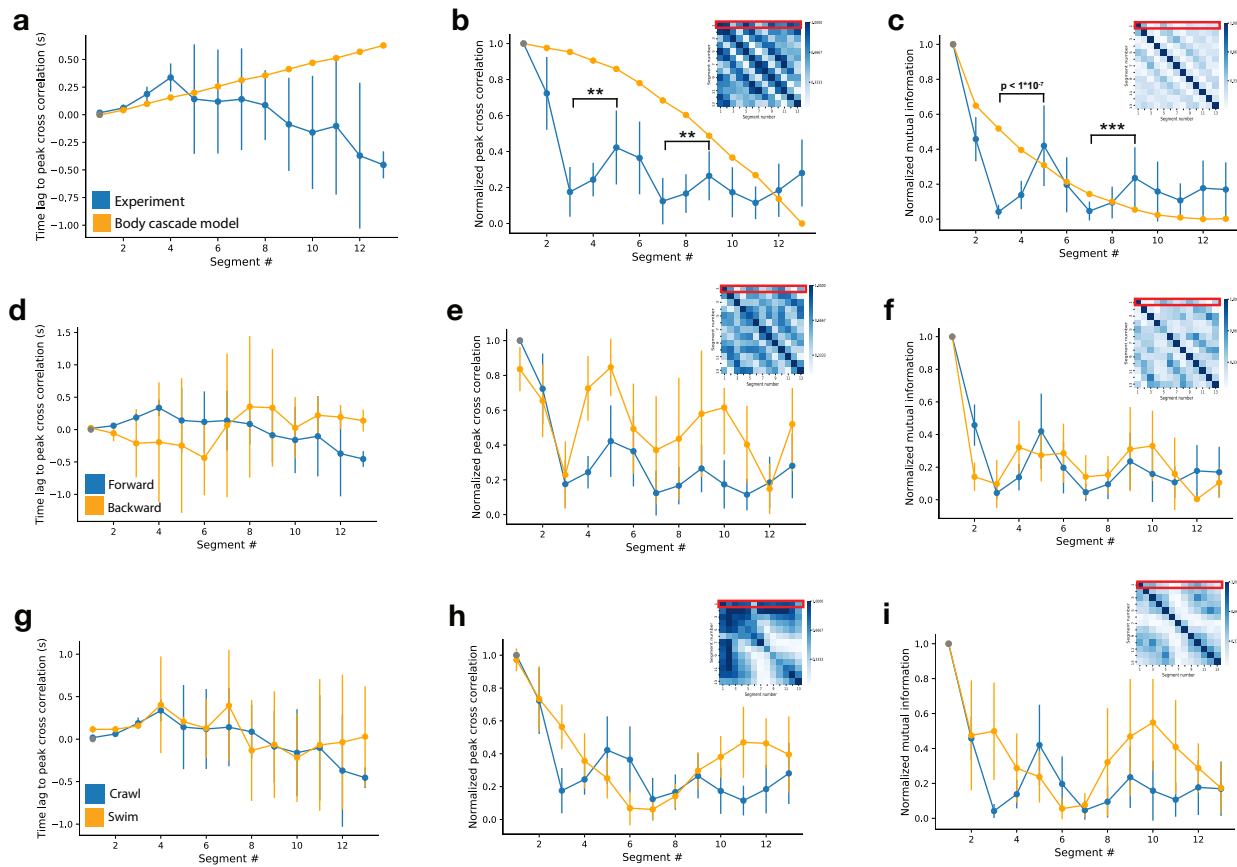


Fig. 2. a-c: Postural angle time series relationships during forward locomotion. a. Time lag to the peak cross correlation vs angle # calculated for the reflex-chain model (orange) and wild-type worms (blue) for forward locomotion, $n=10$. b. Heat map shows peak abs. cross-correlations computed between all angle pairs; first row is shown in the larger plot. Note that the experimental data is non-monotonic, in contrast to the model. (Segment 5 – segment 3 and segment 9 – segment 7, random sampling with replacement, $**p<0.01$) c. Mutual information plotted between all angles in the inset heatmap, with the mutual information relative to the head angle plotted. (Segment 5 – segment 3 and segment 9 – segment 7, random sampling with replacement, $***p<0.001$) **d-f: Forward versus backward crawling.** d. Time lag to peak cross-correlation for forward (blue) and backward (orange) locomotion (compared to the head angle for forward and tail angle for backward), $n=9$ worms. e. Peak abs. cross-correlation normalized to the head angle for forward locomotion (blue) and tail angle for backward locomotion (orange). f. The peak mutual information normalized to the head angle for forward (blue) and tail angle segment for backward (orange). **g-i: Forward crawling versus swimming.** g. Time lag to peak cross-correlation for crawling (blue) and swimming (orange) locomotion compared to the anterior-most segment. $n=10$ worms for each group. h. Peak abs. cross-correlation for crawling (blue) and swimming (orange) locomotion normalized to the anterior-most segment. i. The mutual information for crawling (blue) and swimming (orange) locomotion normalized to the anterior-most segment.

63 during this simulated pattern of forward locomotion (Fig.
 64 1b). As expected, in the reflex-chain model simulation, the
 65 time lag to peak cross-correlation with respect to the first
 66 segment time series increased monotonically, and the peak
 67 absolute cross-correlation with respect to the first segment
 68 time series decreased monotonically with increasing segment
 69 number (Fig. 2a,b). We then performed the same analysis of our
 70 experimental data. For this analysis, we selected contiguous
 71 time series sections when the animal was crawling forward
 72 and not turning. In our experimental worms, we did not
 73 observe a stably increasing time lag to peak cross-correlation
 74 (Fig. 2a), and strikingly, we observed a strong breaking of
 75 monotonicity in the peak absolute cross-correlation (Fig. 2b).
 76 There were local minima in the peak absolute cross-correlation
 77 of angle pairs (1,5) and (1,9). This deviation from monotonicity
 78 suggests that the reflex-chain model is a poor fit to experiment.
 79 However, there was trial-to-trial variability in the pattern of
 80 peak correlations and time lag; thus, we sought a more robust

measure of information transmission.

81

Mutual information suggests centrally coordinated posture control.

A central theorem of information theory is the *data processing inequality*: a propagating signal can only lose, and not gain information from transmission from point to point, due to the accumulation of noise (15). If a worm moved according to the reflex-chain model, the mutual information between the head joint angle and each successive body angle would monotonically decrease (Fig. 2b, c). However, we found a strong experimental deviation from monotonic information loss. The two local maxima of the mutual information relative to angle 1 occur at the same angle numbers (5 and 9) as the two local maxima of the peak absolute cross-correlation, suggesting that active postural control may be transmitted to the periphery through two specific points. We also measured the mutual information between all angle pairs (Fig. 2c, inset).

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97 **Forward crawling, backward crawling, and swimming are**
98 **under centrally coordinated postural control.** We extended
99 our analysis to backwards locomotion, in this case using the
100 posterior-most (tail) angle as angle 1. *C. elegans* backwards loco-
101 motion is shorter in bout duration and crawling length than
102 forwards locomotion, so we employed shorter time windows
103 than those used for forward locomotion. Similar to forward
104 locomotion, we found a non-monotonic peak absolute cross-
105 correlation and non-monotonic mutual information (Fig. 2e,
106 f). The peak absolute cross-correlation has local maxima for
107 angle pairs (1,5) and (1,10) (Fig. 2e, f). A reflex-chain model
108 can thus be rejected for both directions of crawling, and both
109 appear to coordinate control at two points along the body.

110 We then analyzed worm swimming. It has been argued
111 that *C. elegans* swimming and crawling represent distinct
112 neural control patterns (16) rather than solely the result of
113 biomechanical influence of a changing physical substrate. We
114 found the time-lag to absolute cross-correlation to be non-
115 monotonic but, in contrast to the crawling state, the peak
116 absolute cross-correlation has only one, rather than two local
117 maxima, suggesting a different mode of central coordination.
118 (Fig. 2g, h). The reflex-chain model can be rejected for
119 swimming worms as well as crawling worms.

120 Discussion

121 We claim that the reflex-chain model of worm movement
122 is inconsistent with fine analyses of behavioral data. Our
123 data suggests there are two body locations where central
124 coordination reaches the periphery. With higher resolution
125 video recordings, detailed anatomical registration of neural and
126 neuron-to-muscle connectivity data from the worm connectome
127 could suggest particular neurons and connections responsible
128 for centrally coordinated posture control.

129 Our data is consistent with recent loss-of-function studies.
130 One study showed that forward-rhythm undulations persist in
131 posterior body segments even when anterior body segments
132 are paralyzed (8). Another study found that when anterior A
133 motor neurons were ablated, it did not prevent the propagation
134 of reversal waves in posterior body segments (9). In addition
135 to recent studies suggesting the presence of neural oscillators,
136 there is also evidence for lateral information transmission be-
137 tween adjacent neuromuscular units (17). If there are multiple
138 CPG groups driving locomotion, our data suggest that they are
139 strongly coupled. We hypothesize that coordinated oscillatory
140 postural control signals reach the neuromuscular periphery
141 at two specific points along the body, bypassing intervening
142 neuromuscular units. These signals are shaped into a spatially
143 smooth traveling body waveform by lateral neuromuscular
144 signal transmission and further smoothed by biomechanical
145 linkage.

146 We assume that there is not severe segment-to-segment
147 heterogeneity in the noise accumulated during the local biome-
148chanical transformation from muscle to body bend angle; if
149 this transformation noise were both strong and wildly differ-
150ent along the body, it could undermine our interpretation of
151the non-monotonicity of our measures. However, we find this
152unlikely given the robustness of the results and lack of an
153intuition as to how such heterogeneity might occur.

Materials and Methods

154 We recorded videos of wild-type (N2) worms using a custom track-
155ing microscope and TierpsyTracker software (13, 14). We manu-
156ally identified bouts of forward crawling, backward crawling, and
157swimming. Analysis code is available at [https://github.com/focolab/](https://github.com/focolab/worm-locomotion-control)
158worm-locomotion-control and was written in python.
159

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