

1 **A descending pathway facilitates undulatory wave propagation in**  
2 ***Caenorhabditis elegans* through gap junctions**

3

4 Tianqi Xu<sup>1,3</sup>, Jing Huo<sup>1,3</sup>, Shuai Shao<sup>1</sup>, Michelle Po<sup>2</sup>, Taizo Kawano<sup>2</sup>, Yangning Lu<sup>2</sup>, Min  
5 Wu<sup>2</sup>, Mei Zhen<sup>2</sup>, Quan Wen<sup>1,\*</sup>

6

7 1. Chinese Academy of Sciences Key Laboratory of Brain Function and Disease, School  
8 of Life Sciences, Center for Excellence in Brain Science and Intelligence Technology,  
9 University of Science and Technology of China, Hefei, 230027, China

10

11 2. Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital; Department of  
12 Molecular Genetics, Department of Physiology, University of Toronto, Toronto, ON,  
13 Canada, M5G 1X5

14

15 3. These authors contributed equally to this work

16

17 \*Corresponding author: [qwen@ustc.edu.cn](mailto:qwen@ustc.edu.cn)

18

19

1 **Descending signals from the brain play critical roles in controlling and modulating**  
2 **locomotion kinematics. In the *Caenorhabditis elegans* nervous system, descending**  
3 **AVB premotor interneurons exclusively form gap junctions with B-type motor**  
4 **neurons that drive forward locomotion. We combined genetic analysis, optogenetic**  
5 **manipulation, and computational modeling to elucidate the function of AVB-B gap**  
6 **junctions during forward locomotion. First, we found that some B-type motor**  
7 **neurons generated intrinsic rhythmic activity, constituting distributed central**  
8 **pattern generators. Second, AVB premotor interneurons drove bifurcation of B-type**  
9 **motor neuron dynamics, triggering their transition from stationary to oscillatory**  
10 **activity. Third, proprioceptive couplings between neighboring B-type motor neurons**  
11 **entrained the frequency of body oscillators, forcing coherent propagation of bending**  
12 **waves. Despite substantial anatomical differences between the worm motor circuit**  
13 **and those in higher model organisms, we uncovered converging computational**  
14 **principles that govern coordinated locomotion.**

15

16 *Key words:* Descending pathway; motor control; central pattern generator (CPG); *C.*  
17 *elegans*; optogenetics; proprioception; gap junction

## 1 **Introduction**

2

3 Locomotion requires coordinated rhythmic motor activity. In vertebrate and invertebrate  
4 motor systems, oscillatory signals that innervate muscles are generated by dedicated  
5 neurons and neural circuits with intrinsic rhythmic properties, called central pattern  
6 generators (CPGs) [1-6]. Movements, which constitute muscle activities of defined  
7 spatial pattern and temporal sequence, are driven by CPG modules distributed at different  
8 body segments. Coordination of rhythmic movements requires coupling mechanisms, by  
9 which the rhythm of multiple CPGs becomes frequency- and phase-locked [5, 7].

10

11 Although the activity of CPGs is intrinsic and independent of sensory inputs,  
12 proprioceptive or mechanosensitive feedback reshapes the motor dynamics during  
13 movement [8]. In lamprey and leech, for example, activation of specialized  
14 proprioceptive neurons, either by current injection or externally imposed body bending,  
15 entrains the motor CPG activity [9, 10]. In limbed animals, rapid phasic feedback from  
16 mechanoreceptors tunes the rhythmic firing of motor neurons during step cycles [11-13].  
17 Mice lacking Piezo2, a mechanically activated channel expressed in proprioceptive  
18 neurons, exhibit severely uncoordinated body movement [14].

19

20 Descending signals, typically the projections of glutamatergic neurons from the brain to  
21 the spinal or nerve cords, regulate both CPG activity and motor patterns [15]. In fish and  
22 rodent, descending reticulospinal neurons, arising from the brainstem and innervating  
23 excitatory spinal interneurons, can initiate locomotion and modulate speed [16-19];  
24 whereas those that activate inhibitory spinal interneurons can terminate locomotion [20].  
25 Descending interneurons that directly target the forelimb motor neurons are critical for  
26 skillful motor behaviors [21]. In leech, activation of a descending command interneuron,  
27 cell 204, can initiate and maintain swimming behavior [7, 22].

28

29 A deep understanding of the motor circuit must integrate local CPG elements and sensory  
30 feedback, as well as global command signals, to predict behavioral dynamics from  
31 interaction of individual circuit components [23, 24]. With a connectome at synaptic

1 resolution and fully identified cell-types [25, 26], *Caenorhabditis elegans* offers an  
2 opportunity to obtain a complete, system-level understanding of a locomotor circuit.

3

4 The *C. elegans* ventral nerve cord consists of a network of excitatory cholinergic motor  
5 neurons, the A- and B-type that drive backward and forward movement, respectively, and  
6 the inhibitory motor neurons (D-type) that are innervated by the excitatory motor neurons  
7 [27]. The AVB premotor interneurons, which receive numerous inputs from sensory and  
8 other interneurons, extend long processes along the entire ventral nerve cord, and  
9 exclusively form gap junctions with the B-type motor neurons [26, 28, 29] (Figure 1a).  
10 The functions of AVB premotor interneurons and their electrical synapses with B-type  
11 motor neurons, however, are poorly understood.

12

13 Here, we took an integrative approach to defining the circuit logic underlying coordinated  
14 locomotion in *C. elegans*, with an emphasis on how descending signals modulate the  
15 dynamics of the motor circuit. Whereas descending inputs in other systems directly drive  
16 or modulate the activity of locomotor CPGs [16-18], it remains unclear whether similar  
17 CPG elements exist in *C. elegans*. Combining molecular genetics and optical  
18 manipulation of neuronal activity in freely behaving *C. elegans*, we found that mid-body  
19 B-type motor neurons could function as oscillators. To generate coherent body  
20 undulation, the intrinsic activity in B-type motor neurons was entrained by proprioceptive  
21 feedback. To promote forward movement, AVB-B gap junctions drove the bifurcation of  
22 B-type neuron dynamics from stationary to rhythmic activity.

23

24 Previously, we demonstrated the proprioceptive properties in B-type motor neurons:  
25 directional proprioceptive couplings between B-type motor neurons are responsible for  
26 the propagation of rhythmic head bending along the body [30, 31]. Our current  
27 experimental and computational analyses suggest that descending signals from the AVB  
28 premotor interneurons function synergistically with proprioceptive couplings to facilitate  
29 sequential activation of motor neuron activities, allowing body bending to propagate  
30 directionally and efficiently to drive forward locomotion.

31

## 1 **Results**

2

### 3 **Gap junctions between descending AVB premotor interneurons and B-type motor** 4 **neurons facilitate bending wave propagation during forward locomotion**

5

6 The AVB premotor interneurons and B-type motor neurons form heterotypic gap  
7 junctions via the UNC-7 innexin (in AVB) and UNC-9 innexin (in B-type) (Figure 1a)  
8 [28, 29]. To determine whether AVB-B gap junctions play a role in forward locomotion,  
9 we first examined the motor behaviors of *unc-7(hp121)* and *unc-9(fc16)* null mutants  
10 immersed in viscous solution (1 Pa·s viscosity). In these gap junction-deficient mutants,  
11 the intrinsically higher levels of activity in the A-type motor neurons, which control  
12 backward locomotion, prevent animals from generating continuous forward movement  
13 [29]. To reduce interference from motor activity arising from the backward motor circuit,  
14 A-type motor neurons were constitutively silenced by an active K<sup>+</sup> channel [*Punc-*  
15 *4::TWK-18(gf)*] in examined strains and experiments (Supplementary Table).

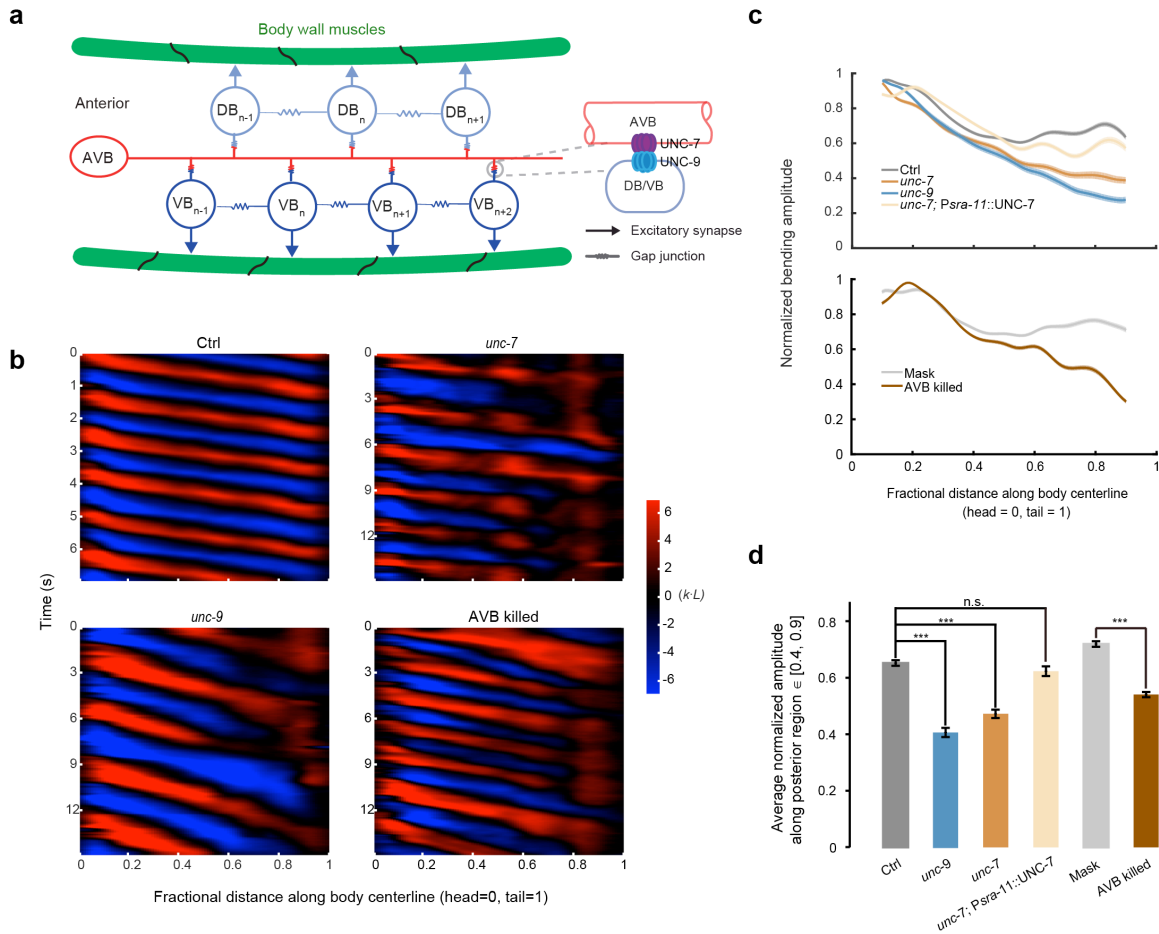
16

17 We visualized and quantified *C. elegans* locomotion kinematics by curvature  
18 kymographs (Figure 1b). During bouts of forward movement, each body segment  
19 alternated between positive (red) and negative (blue) curvature, and the stripes of  
20 curvature propagated from the head to the tail. Coordinates along the worm body were  
21 defined as head = 0 and tail = 1 (Figure 1b). In control animals (wild-type with A-type  
22 motor neurons constitutively silenced), the bending amplitude was highest near the head,  
23 then declined gradually and plateaued at the mid-body (~ 40% of the body length, Figure  
24 1c). Both *unc-7* and *unc-9* mutants exhibited identical phenotypes: their bending  
25 amplitudes diminished monotonically towards the tail (Figure 1b–d and Video 1).

26

27 UNC-7 and UNC-9 are involved in gap junction couplings in many neurons besides AVB  
28 and B-type neurons [28, 32]. We next showed that disruption of AVB-B gap junctions  
29 was responsible for the monotonic decline of bending amplitudes in *unc-7* and *unc-9*  
30 mutants. First, when AVB premotor interneurons were optogenetically ablated using  
31 miniSOG [33] (by either *Plgc-55(B)::miniSOG* or *Psra-11::miniSOG*), we observed the

1 same trend of bending amplitude decline towards the tail (Figure 1b-d and Figure 1 -  
 2 figure supplement 1). Second, restoring innexin UNC-7 in the AVB interneurons (*unc-7*;  
 3 *Psra-11::UNC-7*) in *unc-7* mutants was sufficient to restore the plateau phase of bending  
 4 amplitude (Figure 1c-d and Video 3).



5  
 6  
 7 **Figure 1. AVB-B gap junctions facilitate undulatory wave propagation during forward locomotion**  
 8 **(a)** Schematics of gap junction couplings in the *C. elegans* forward motor circuit. The AVB premotor  
 9 interneurons form gap junctions with all B-type motor neurons, which drive dorsal (DB) and ventral (VB)  
 10 muscle contraction. AVB-B gap junctions require the expression of innexin UNC-7 in AVB interneurons  
 11 and UNC-9 in B-type motor neurons. Neighboring B-type neurons are also gap junction coupled. Other  
 12 types of motor neurons have been omitted from the diagram for simplicity. **(b)** Representative curvature  
 13 kymographs of control (N2; *Punc-4::TWK-18(gf)*), *unc-7* (*unc-7(hp121)*; *Punc-4::TWK-18(gf)*), *unc-9*  
 14 (*unc-9(fc16)*; *Punc-4::TWK-18(gf)*) animals, and AVB-ablated (*Plgc-55::miniSOG*; *Punc-4::TWK-18(gf)*)  
 15 and their control (Mask-ablated) animals. Worms swam in viscous solutions (~ 1 Pa·s viscosity). Body  
 16 curvature was defined as a non-dimensional unit  $k \cdot L$ , where  $L$  is the body length. **(c)** Quantification of the  
 17 bending amplitude along the body for strains described above. Shaded regions represent s.e.m. Amplitude

1 was normalized against the maximum curvature along the body. **(d)** Averaged bending amplitude in the  
2 posterior body  $\in [40, 90]$  for strains described above. \*\*\*  $p < 0.0001$  (Ctrl and *unc-9*  $p = 3.5 \times 10^{-23}$ ; Ctrl and  
3 *unc-7*  $p = 1.9 \times 10^{-20}$ ; Mask and AVB killed  $p = 3.0 \times 10^{-28}$ ), two-sample *t*-test with Bonferroni correction,  
4 n.s.  $p = 0.16$ . Error bars represent s.e.m. Ctrl,  $n = 105$  independent measurements, 13 worms; *unc-7* mutant,  
5  $n = 149$  measurements, 22 worms; *unc-9* mutant,  $n = 93$  measurements, 17 worms; AVB-ablated,  $n = 80$   
6 measurements, 13 worms; Mask (same genotype without ablation),  $n = 96$  measurements, 12 worms.

7

## 8 **Linear proprioceptive coupling model predicts deteriorated undulatory waves in the** 9 **presence of AVB-B gap junctions**

10

11 We next asked why AVB-B gap junction-deficient worms could not efficiently propagate  
12 body bending towards the tail. Previously, we showed that worm undulation during  
13 forward locomotion requires proprioceptive coupling between adjacent body regions  
14 [30]. Curvature change in a mid-body region, induced by a pneumatic microfluidic  
15 device, activates bend-sensitive B-type neurons and defines the curvature of the posterior  
16 neighbor [30]. Consistently, when we trapped the middle body region of a wild-type  
17 worm in a static channel with defined curvature, the unrestrained posterior body region  
18 exhibited bending curvature in the same direction as that imposed by the microfluidic  
19 channel (Figure 2a-b and [30]).

20

21 To determine whether the proprioceptive coupling is UNC-7- and UNC-9-dependent, we  
22 examined *unc-7* and *unc-9* mutants constrained by microfluidic channels. Consistent with  
23 a previous finding [30] and observation from control animals, their posterior body  
24 regions still followed the curvature imposed on the anterior body regions (Figure 2b-c  
25 and Figure 2 - figure supplement 1). Therefore, proprioceptive coupling remains largely  
26 intact in the absence of UNC-7- or UNC-9-dependent gap junctions.

27

28 We next took a theoretic approach to probing how AVB-B gap junctions might affect  
29 bending wave propagation. We first adopted a linear proprioceptive coupling model [30],  
30 where we asserted that the undulatory wave started with rhythmic dorsal/ventral bends  
31 near the head (Methods). Directional proprioceptive coupling between adjacent body  
32 regions is fully described by a set of first order differential equations:

1

$$\left\{ \begin{array}{l} C_m \frac{dv_i}{dt} = g_m(-v_i + ck_{i-1}) - g(v_i - v_{AVB}), \\ \tau_h \frac{du_i}{dt} = -u_i + v_i, \\ \tau_\eta \frac{dk_i}{dt} = -k_i + \alpha_{max} * u_i/b. \end{array} \right. \quad i = 2,3, \dots 6 \quad (\text{Equation 1})$$

3

4 We divided the whole worm into several segments, with subscript  $i$  as the segment  
5 number. In the first equation,  $v$  describes relative motor neuron activity: when dorsal B-  
6 type neurons have a higher activity than ventral ones,  $v > 0$ , and vice versa.  $k_i$  is the  
7 curvature of a given segment, and  $ck_{i-1}$  describes the proprioceptive signal from the  
8 neighboring anterior body segment, where  $c > 0$  is the coupling strength.  $C_m$  is the  
9 membrane capacitance and  $g_m$  is the leaky conductance. The term  $-g(v_i - v_{AVB})$   
10 describes the contribution from AVB-B electrical coupling, where  $g$  is the gap junction  
11 conductance. For simplicity, we set  $v_{AVB} = 0$  to eliminate the bias of  $v$ . A more detailed  
12 model that treated dorsal and ventral motor neuron activities separately is described in the  
13 Methods.

14

15 The second equation relates motor neuron activity to muscle torque:  $u$  is a dimensionless  
16 variable and  $\alpha_{max} * u$  is the maximum torque that the muscle cells can generate. The last  
17 equation describes how the segment curvature changes with muscle torque:  $b$  is the  
18 bending modulus of the body and  $\tau_\eta$  is a time constant [30] proportional to the viscosity  
19  $\eta$  of the medium.

20

21 Simulation of the linear coupling model predicted an exponential decay of bending  
22 amplitude towards the tail (Figure 2d). By transforming Equation 1 into a continuous  
23 form (see Methods), we solved this linear model analytically and identified a self-  
24 consistent solution for the spatiotemporal profile of the worm body curvature:

25

$$k(x, t) = k_0 \exp(-x/\xi) \exp\left(i\left(\frac{2\pi x}{\lambda} - \omega t + \phi(x)\right)\right), \quad (\text{Equation 2})$$

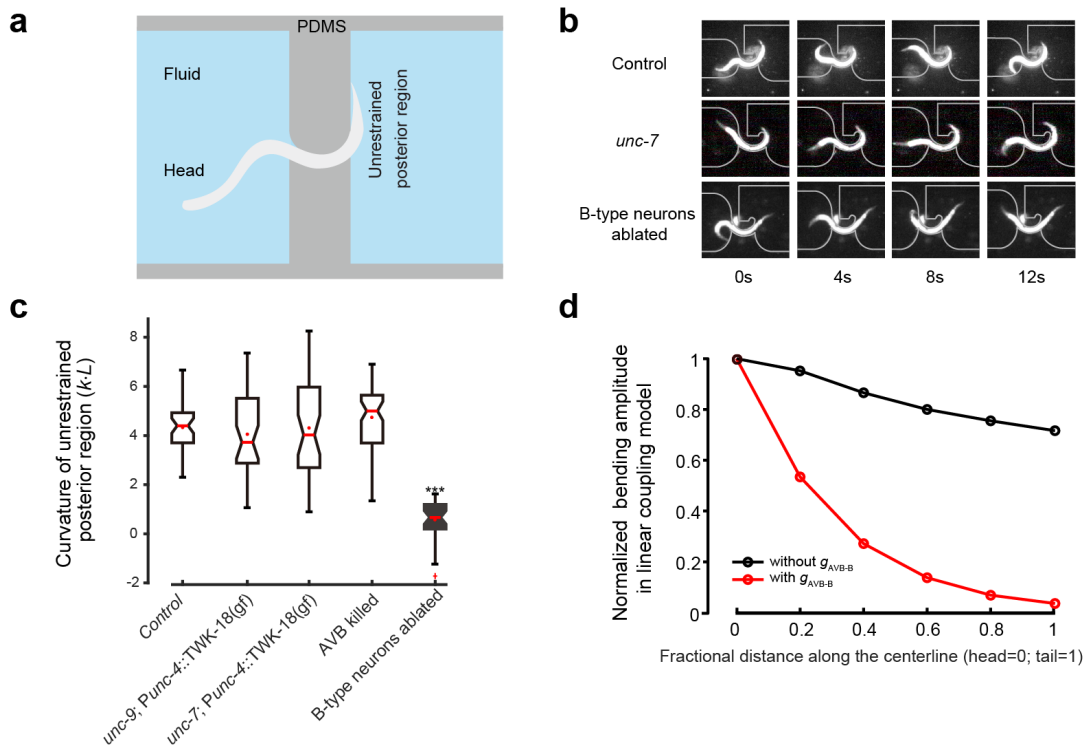
27



1 where  $\omega$  and  $\lambda$  describe the angular frequency and spatial wavelength of undulation,  
 2 respectively;  $\phi(x)$  is a phase lag term, and  $\xi$  is the decay length constant, which, to the  
 3 leading order, is given by:

$$4 \quad \xi \approx \frac{l}{1 - c\alpha_{max}/b}, \quad \text{subject to } \frac{c\alpha_{max}}{b} \leq 1 \quad (\text{Equation 3})$$

6 where  $l$  is the spatial scale for proprioceptive coupling [30].  $\xi \rightarrow +\infty$  leads to identical  
 7 bending amplitude along the body. Incorporation of AVB-B electrical coupling  
 8 effectively changes  $c$  to  $cg_m/(g_m + g)$ . This prefactor further reduces the length  
 9 constant. In a linear coupling model where B-type motor neurons are passive recipients of  
 10 proprioceptive inputs, opposite to our experimental findings, gap junctions between AVB  
 11 and B-type neurons would deteriorate the propagation of bending waves (Figure 2d).  
 12 and B-type neurons would deteriorate the propagation of bending waves (Figure 2d).



15  
16

1 **Figure 2. UNC-7- and UNC-9-dependent gap junctions are not required for proprioceptive couplings**  
2 **in the forward motor circuit. (a)** Schematics of the microfluidic device for constraining a body segment  
3 with defined curvature. **(b)** Time-lapse video images of control (upper panel), *unc-7* (middle panel), and B-  
4 type neuron-ablated (lower panel) worms trapped in the microfluidic device. In both control and *unc-7*  
5 mutant animals, the posterior unrestrained body region followed the curvature of the channel. However,  
6 this was not the case when B-type motor neurons were optogenetically ablated. **(c)** In AVB-B gap junction-  
7 deficient mutants, or AVB-ablated worms, the posterior unrestrained body region also followed the  
8 curvature of the channel. Boxes indicate Q1 to Q3, error bars represent Q1-1.5IQR and Q3+1.5IQR  
9 respectively, notches indicate 95% confidence interval. \*\*\*  $p < 0.0001$  ( $p = 1.3 \times 10^{-6}$ ), compared with  
10 other strains, by Mann-Whitney U test. Control; (N2; *Punc-4::TWK-18(gf)*),  $n = 40$  measurements, 8  
11 worms; *unc-7* (*unc-7*; *Punc-4::TWK-18(gf)*),  $n = 41$  measurements, 12 worms; *unc-9* (*unc-9*; *Punc-*  
12 *4::TWK-18(gf)*),  $n = 38$  measurements, 9 worms; AVB-ablated (*P/gc-55(B)::miniSOG*),  $n = 60$   
13 measurements, 10 worms; B-ablated (*Pacr-5::miniSOG*),  $n = 33$  measurements, 9 worms. **(d)** In the linear  
14 proprioceptive coupling model, incorporating the AVB-B gap junction inputs further deteriorated bending  
15 wave propagation.

16

## 17 **B-type motor neurons are nonlinear functional units**

18

19 The discrepancy between the theoretical model and experimental observations indicates  
20 that some basic assumption in the linear model must be modified. We hypothesized that  
21 proprioceptive signals couple nonlinear functional units within adjacent regions. Intrinsic  
22 oscillation is a common form of nonlinearity. We thus tested whether CPGs exist along  
23 the worm nerve cord.

24

25 Our linear coupling model predicted that abolishing head bending activity would abolish  
26 the undulatory wave; a coupled-CPG model, however, predicts the opposite. To  
27 distinguish these possibilities, we performed spatially selective optogenetic inhibition of  
28 B-type motor neurons in a defined anterior body region (0.1–0.3 along worm coordinates)  
29 of a freely swimming animal (*Pacr-5::Arch*) (Figure 3a–b and Video 2). Upon green light  
30 illumination, we effectively abolished bending near the head region (Figure 3b, curvature  
31 kymograph). The mid-body (~ 50% of the body length), however, continued to generate  
32 rhythmic bending with increased undulation frequency and reduced amplitude (Figure 3b,  
33 3e, Video 2). When a worm swam in 1 Pa·s viscous solution, the undulation frequency  
34 was ~ 0.8 Hz (Figure 3c); when anterior bending activity was optogenetically inhibited,

1 the mid-body undulation frequency doubled ( $\sim 1.6$  Hz, Figure 3c). We observed a similar  
2 phenomenon when anterior undulation was abolished optogenetically, either by inhibiting  
3 both A-type and B-type motor neurons (*Pacr-2::Arch*, Figure 3 - figure supplement 1a),  
4 or by directly inhibiting anterior muscle cells (*Pmyo-3::NpHR*, Figure 3 - figure  
5 supplement 1a).

6

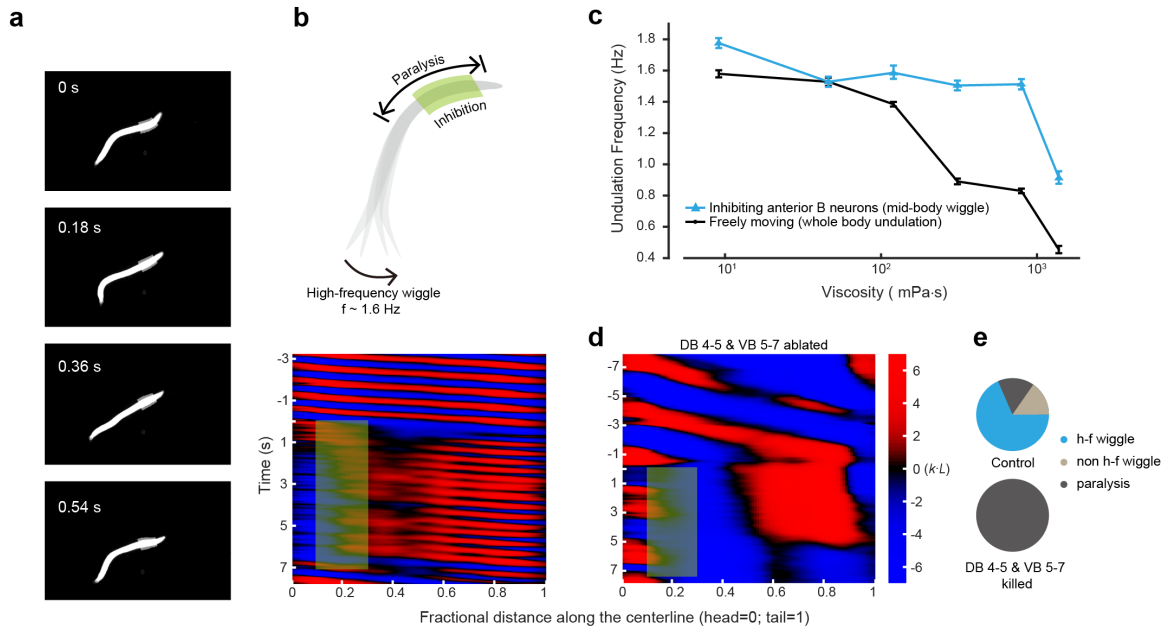
7 These observations favor the possibility that at least some B-type motor neurons are  
8 intrinsic oscillators. Whereas the normal undulation frequency decreased in more viscous  
9 solution (Figure 3c and [34]), the mid-body undulation frequency remained fixed across a  
10 wide range of viscosities when anterior bending activity was abolished (Figure 3c and  
11 Figure 3 - figure supplement 1b). Therefore, the intrinsic frequency of the mid-body  
12 oscillators, which could be entrained by head bending, appeared to be independent of the  
13 mechanical load of the environment.

14

15 To determine whether the B-type motor neurons were responsible for generating intrinsic  
16 mid-body oscillation, we performed systematic ablation of B-type neurons (*Pacr-  
17 5::miniSOG*) in the ventral nerve cord. Ablating a cluster of mid-body B-type motor  
18 neurons (DB4-5 and VB5-7) abolished high-frequency oscillation when anterior bending  
19 activity was abolished (Figure 3d-e). When only a subset of neurons in this cluster was  
20 ablated, the high-frequency mid-body undulation could still be induced (Figure 3 - figure  
21 supplement 1a). In these animals, we occasionally observed independent bending waves  
22 generated by the head and posterior body regions, respectively (Figure 3 - figure  
23 supplement 1c). We also confirmed that D-type inhibitory motor neurons were not  
24 involved in generating intrinsic mid-body undulation (Figure 3 - figure supplement 1a).  
25 Together, these data strongly suggest that B-type motor neurons do not passively respond  
26 to proprioceptive signals from the anterior segment. They instead function as nonlinear  
27 units that resemble distributed CPG modules along the body.

28

29



1  
2  
3 **Figure 3 Mid-body B-type motor neurons generate rhythmic activity independent of proprioceptive**  
4 **coupling.** (a) Time-lapse video images from a recording when B-type motor neurons in an anterior body  
5 region (10–30% along the worm body) were optogenetically inhibited. (b) Upper panel, schematic  
6 illustrates the effect of spatially selective inhibition of B-type motor neurons. Optogenetic inhibition of  
7 anterior B-type motor neurons induced high-frequency undulation in the posterior region; lower panel,  
8 representative curvature kymograph. Green shaded region shows the selected spatiotemporal region for  
9 optogenetic inhibition. (c) *C. elegans* undulation frequency at different viscosity. Black line is undulation  
10 frequency of control animals; blue line is mid-body undulation frequency when anterior bending activity  
11 was abolished. Error bars are s.e.m.;  $n \geq 8$  worms for each data point. (d) Representative curvature  
12 kymograph during optogenetic inhibition of anterior B-type motor neurons, with and without mid-body B-  
13 type neurons (DB 4–5 & VB 5–7). (e) Pie chart summarizes the percentage of locomotor states when  
14 anterior bending activity was abolished. h-f wiggle: mid-body undulation frequency was higher than that  
15 before anterior bending activity was abolished; non-h-f wiggle: mid-body undulation frequency was equal  
16 to or less than that before anterior bending activity was abolished; paralysis: no waves emerged in the mid-  
17 body. Control (*Pacr-5::Arch*),  $n = 241$  measurements, 20 worms; Mid-body B-type neuron-ablated worms  
18 (*Pacr-5::Arch; Pacr-5::miniSOG*),  $n = 77$  measurements, 11 worms.

19

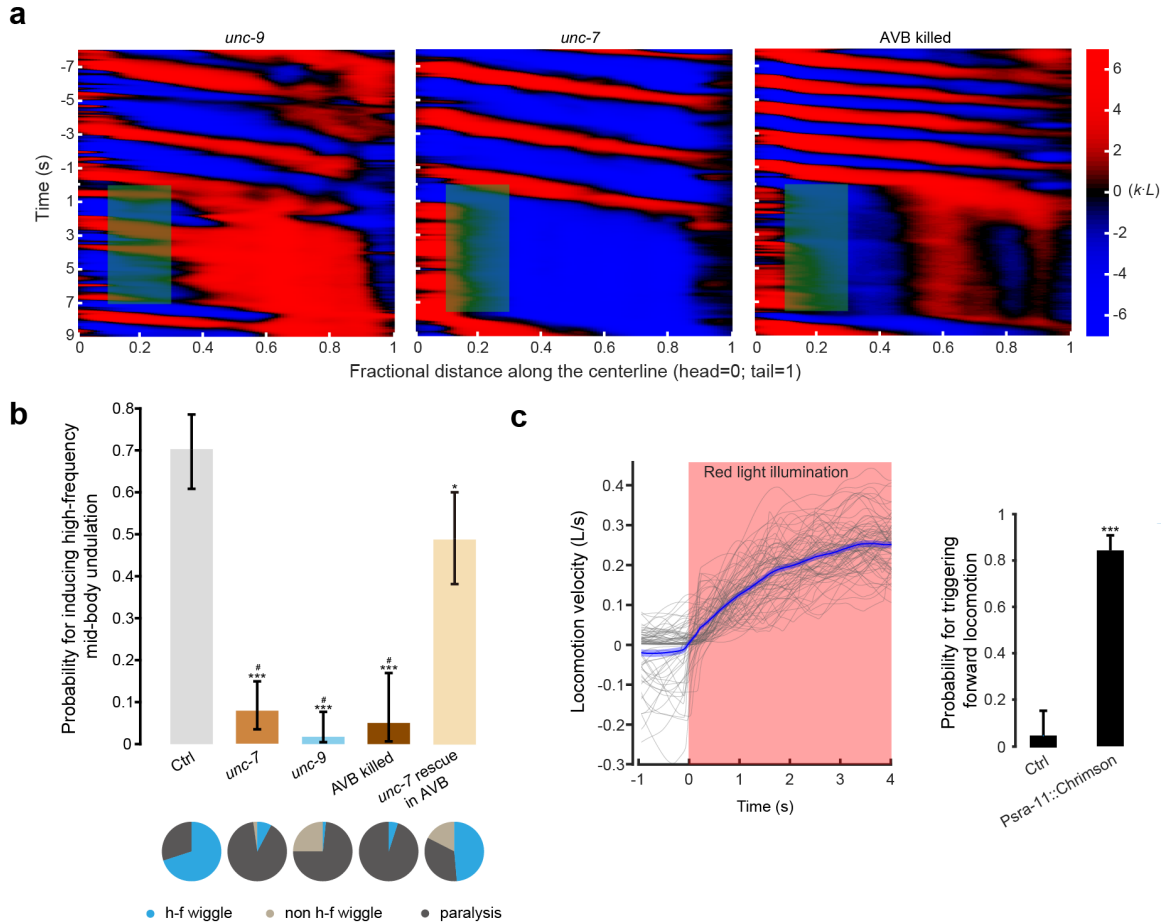
## 1 **AVB-B gap junctions drive the bifurcation of B-type motor neuron dynamics**

2

3 We next asked whether AVB-B gap junction inputs played a role in high-frequency mid-  
4 body undulation when anterior bending activity was abolished. Indeed, in AVB-B gap  
5 junction-deficient mutants (*unc-7* and *unc-9*), as well as AVB-ablated animals,  
6 optogenetic inhibition of anterior bending no longer induced mid-body undulation  
7 (Figure 4a-b). Instead, optogenetic inhibition of either anterior B-type motor neurons or  
8 anterior body wall muscles led to paralysis of the whole-body (Figure 4a-b and Figure 4 -  
9 figure supplement 1). Restoring UNC-7 innexin expression in AVB interneurons (*unc-7*;  
10 *Psra-11::UNC-7*; *Punc-4::TWK-18(gf)*) significantly increased the efficacy for inducing  
11 high frequency mid-body undulation (Figure 4b and Video 3). These results strongly  
12 suggest that AVB interneurons and their gap junction couplings with B-type motor  
13 neurons are required for inducing mid-body high frequency undulation. Consistently,  
14 optogenetic activation of the AVB interneurons (*Psra-11::Chrimson*) reliably triggered  
15 forward locomotion from either the pause state or the backward movement (Figure 4c,  
16 Video 7).

17

18 AVB interneurons exhibit elevated yet *non-oscillatory* calcium activity during forward  
19 locomotion [29, 35, 36]. Our data thus suggest that AVB-B gap junctions drive a  
20 bifurcation of B-type neuron dynamics, leading to a transition from the stationary to  
21 oscillatory state. In the presence of AVB-B gap junctions, time-varying proprioceptive  
22 signals from the anterior body may easily induce changes in motor neuron membrane  
23 potential, thus facilitating bending wave propagation.



1  
2 **Figure 4. AVB-B electrical coupling drives the bifurcation in the B-type neuron dynamics. (a)**  
3 Representative curvature kymographs in AVB-B gap junction-deficient or AVB-ablated worms when  
4 anterior bending activity was abolished. **(b)** Probability for inducing high-frequency mid-body undulation  
5 when anterior bending activity was abolished. Pie chart summarizes the percentage of worm locomotor  
6 states. Error bars indicate 95% binomial proportion confidence interval. \*\*\*  $p < 0.0001$ , \*  $p = 0.002$ , #  $p <$   
7  $0.0001$  (\* compared with Ctrl, # compared with UNC-7 rescued in AVB. Ctrl and *unc-7*  $p = 1.8 \times 10^{-20}$ ; Ctrl  
8 and *unc-9*  $p = 1.4 \times 10^{-26}$ ; Ctrl and AVB killed  $p = 5.0 \times 10^{-12}$ ; Ctrl and *unc-7* rescue in AVB  $p = 0.002$ ; *unc-7*  
9 rescue in AVB and *unc-7*  $p = 2.3 \times 10^{-10}$ ; *unc-7* rescue in AVB and *unc-9*  $p = 4.2 \times 10^{-15}$ ; *unc-7* rescue in  
10 AVB and AVB killed  $p = 4.1 \times 10^{-6}$ ) Chi-square test. Strains for comparisons are: control [*Pacr-5::Arch*;  
11 *Punc-4::TWK-18(gf)*],  $n = 113$  measurements, 13 worms; *unc-7* [*unc-7(hp121)*; *Pacr-5::Arch*; *Punc-*  
12 *4::TWK-18(gf)*]  $n = 102$  measurements, 21 worms; *unc-9* [*unc-9(fc16)*; *Pacr-5::Arch*; *Punc-4::TWK-*  
13 *18(gf)*]  $n = 116$  measurements, 26 worms; AVB-ablated [*Plgc-55(B)::miniSOG*, *Pacr-5::Arch*]  $n = 40$   
14 measurements, 11 worms; UNC-7 rescued in AVB [*unc-7(hp121)*; *Pacr-5::Arch*; *Punc-4::TWK-18(gf)*;  
15 *Psra-11::UNC-7*]  $n = 86$  measurements, 12 worms. **(c)** Optogenetic activation of AVB premotor  
16 interneurons could trigger forward locomotion. Left panel, optogenetic activation (*Psra-11::Chrimson*,  $n =$   
17 133 measurements, 11 worms), showing locomotion velocity with time, red light (635 nm, 12 mW/mm<sup>2</sup>)  
18 was turned on at  $t = 0$ . Gray traces represent individual trials, and shaded region represents s.e.m. Right,

1 probability for triggering forward locomotion. Ctrl represents worms without feeding all-*trans* retinal. Error  
 2 bars indicate 95% binomial proportion confidence interval, \*\*\* $p < 0.0001$  ( $p = 2.6 \times 10^{-7}$ ), Chi-square test.

3

4 **Nonlinear proprioceptive coupling model recapitulates AVB-B gap junction-**  
 5 **dependent bifurcation of B-type motor neuron dynamics**

6

7 We next sought theoretical verification of our functional model. For simplicity, we  
 8 developed a phenomenological model based on active  $\text{Ca}^{2+}$  and  $\text{K}^+$  conductance [37], in  
 9 which the dynamics of dorsal and ventral motor neurons within a given body segment are  
 10 governed by the following equations:

11

$$12 \left\{ \begin{array}{l} C_m \frac{dV_{di}}{dt} = -g_L(V_{di} - E_L) - g_{Ca}m_\infty(V_{di}) * (V_{di} - E_{Ca}) - g_Kn_d * (V_{di} - E_K) + c'k_{i-1} + g(V_{AVB} - V_{di}) \\ \tau_n \frac{dn_d}{dt} = -n_d + n_\infty(V_{di}) \\ C_m \frac{dV_{vi}}{dt} = -g_L(V_{vi} - E_L) - g_{Ca}m_\infty(V_{vi}) * (V_{vi} - E_{Ca}) - g_Kn_v * (V_{vi} - E_K) - c'k_{i-1} + g(V_{AVB} - V_{vi}) \\ \tau_n \frac{dn_v}{dt} = -n_v + n_\infty(V_{vi}) \end{array} \right.$$

13

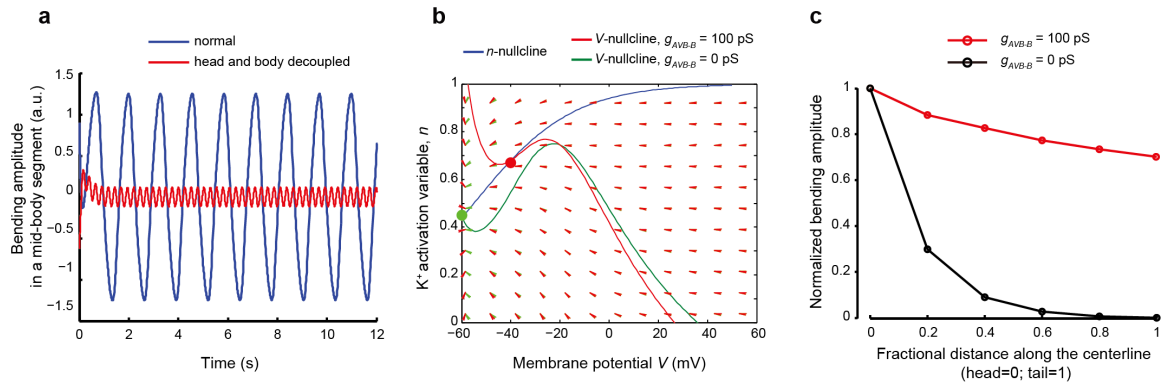
(Equation 4)

14

15 In Equation 4,  $V$  is motor neuron membrane potential; subscripts  $d$  and  $v$  denote dorsal  
 16 and ventral sides, respectively;  $m$  and  $n$  are voltage-dependent  $\text{Ca}^{2+}$  and  $\text{K}^+$  activation  
 17 variables, respectively. Parameter values in this equation were chosen (Methods) to  
 18 accommodate the following experimental findings. First, when the head and body  
 19 undulations were decoupled ( $c'k_1 \approx 0$ ), gap junctions between AVB interneurons and B-  
 20 type motor neurons induced high frequency motor neuron oscillation (Figure 5a). Second,  
 21 proprioceptive signals arising from head bending entrained posterior motor neuron  
 22 oscillation (Figure 5a). The nullcline analysis, which allows for visualization of the  
 23 dynamical variables on a vector field, provided useful information on the transition from  
 24 the resting state to the limit cycle in the presence of AVB-B gap junctions (Figure 5b).  
 25 Furthermore, simulation recapitulated the experimental finding that AVB-B gap junctions  
 26 helped equalize bending amplitude along the body (Figure 5c).

27

28



1

2

3 **Figure 5. Simulation of a nonlinear proprioceptive coupling model. (a)** Bending activity of a mid-body

4 segment. Blue, bending amplitude during normal locomotion; red, when the head and body undulation was

5 decoupled, oscillation with higher frequency and lower amplitude emerged. **(b)** Nullcline analysis of motor

6 neuron dynamics. The  $n$ - and  $V$ -nullclines were defined as  $\frac{dn}{dt} = 0$ ,  $\frac{dV}{dt} = 0$ , respectively. In the absence of

7 proprioceptive coupling and AVB-B gap junctions ( $g_{AVB-B} = 0$ ), the fixed point of the membrane potential

8 dynamics (green) corresponds to a steady state; in the presence of AVB-B gap junctions, that is,  $g_{AVB-B} =$

9  $100 \text{ pS}$ , the system undergoes bifurcation, and the new fixed point becomes unstable (red), leading to

10 periodic oscillation. **(c)** A comparison of bending amplitude along the body, with and without AVB-B gap

11 junctions. All parameters used in the simulation are described in the Methods.



1 **Electrical couplings between motor neurons and AVB-B gap junctions permit rapid**  
2 **and reciprocal interactions between head and body motor activities**

3

4 Having considered the functions of the AVB-B gap junctions, we next asked whether  
5 local gap junctions between B-type motor neurons (Figure 1a), identified by anatomical  
6 wiring [25, 26], played additional roles in forward locomotion.

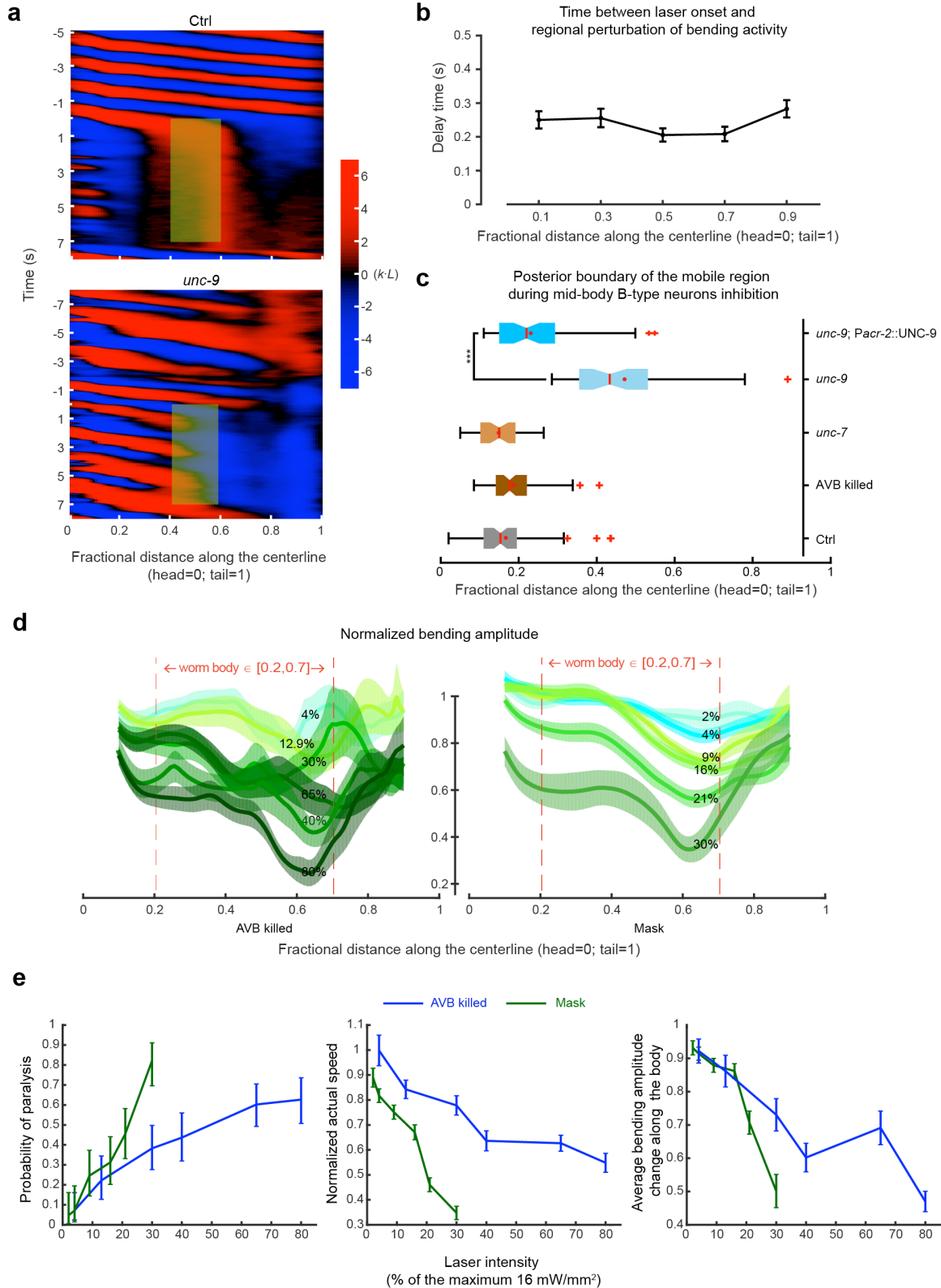
7

8 This question arises from our observation that inhibition of B-type motor neurons in  
9 middle or posterior body regions, unlike the anterior body region, induced rapid (within ~  
10 300 ms) paralysis of the whole worm (Figure 6a–b, Figure 6 - figure supplement 1, and  
11 Video 4). Electrical coupling is the best candidate for rapid redistribution of a  
12 hyperpolarization current among B-type motor neurons (*Pacr-5::Arch*). In both *unc-7*  
13 mutants and AVB-ablated worms, whole body paralysis could still be induced upon  
14 silencing mid-body and posterior B-type motor neurons (Figure 6c) using the maximum  
15 laser intensity, suggesting that the hyperpolarization signal could still be transduced in  
16 the absence of AVB-B gap junctions. In *unc-9* mutants, however, rhythmic bending in the  
17 anterior body persisted when mid-body B-type motor neurons were silenced (Figure 6a,  
18 6c and Video 5). Restoring UNC-9 expression in cholinergic motor neurons, including  
19 the B- and A-type motor neurons (*Pacr-2::UNC-9*), restored the ability to optogenetically  
20 induce whole body paralysis (Figure 6c). Therefore, electrical couplings among motor  
21 neurons contributed to the paralytic effect.

22

23 The probability for optogenetically inducing whole-body paralysis, however, was dose-  
24 dependent. When the mid-body B-type motor neurons were inactivated at lower laser  
25 intensity, which likely reduced the degree of hyperpolarization, forward locomotion was  
26 not abolished. Instead, both undulation amplitude and locomotor speed were reduced  
27 (Figure 6d–e). Interestingly, in the absence of AVB interneurons, a higher laser intensity  
28 was required to modify the locomotor kinematics and to induce whole worm paralysis  
29 (Figure 6d-e). These results suggest a potential mechanism by which gap junctions  
30 between motor neurons and AVB-B gap junctions function together to retrogradely

- 1 regulate head bending activity, and hence reconfigure the dynamics of worm body
- 2 undulation.



3

1

2 **Figure 6** Electrical coupling among motor neurons and AVB-B gap junctions allows for rapid and  
3 reciprocal interactions between head and body motor activities. **(a)** Comparison of representative curvature  
4 kymographs of a control [*Pacr-5::Arch; Punc-4::TWK-18(gf)*] and *unc-9* mutant [*unc-9(fc16); Pacr-*  
5 *5::Arch; Punc-4::TWK-18(gf)*] worm during optogenetic inhibition of mid-body B-type motor neurons.  
6 Green shaded regions are selected regions (0.4–0.6 fractional distance) subjected to 0–7 s stimulation by  
7 the green laser. **(b)** Delay time between the onset of green laser and changes in bending. Error bars are  
8 s.e.m., also see Figure 6 - figure supplement 1. **(c)** Quantification of the whole-body paralytic effect during  
9 optogenetic inhibition of mid-body B-type motor neurons. Red crosses are outliers, red lines are medians,  
10 red dots are means, error bars represent Q1-1.5IQR and Q3+1.5IQR, respectively, and notches indicate  
11 95% confidence interval. In *unc-9* mutants, the boundary shifted towards the mid-body, reflecting that the  
12 anterior body could still undulate. \*\*\*  $p < 0.0001$  ( $p = 2.4 \times 10^{-11}$ ), Mann-Whitney U test. Control [*Pacr-*  
13 *5::Arch; Punc-4::TWK-18(gf)*],  $n = 70$  measurements, 17 worms; *unc-7* [*unc-7(hp121); Pacr-5::Arch;*  
14 *Punc-4::TWK-18(gf)*]  $n = 43$  measurements, 11 worms; *unc-9* [*unc-9(fc16); Pacr-5::Arch; Punc-4::TWK-*  
15 *18(gf)*]  $n = 59$  measurements, 17 worms; AVB-ablated (*Plgc-55::miniSOG, Pacr-5::Arch*)  $n = 65$   
16 measurements, 18 worms; UNC-9 rescued in the B-type and A-type motor neurons [*unc-9(fc16); Pacr-*  
17 *5::Arch; Pacr-2::UNC-9*]  $n = 48$  measurements, 9 worms. **(d)** Laser intensity-dependent effects on the  
18 bending amplitude along the body. Error bars are s.e.m. **(e)** Laser intensity-dependent effects on efficacy  
19 for inducing whole-body paralysis, locomotion speed and mean amplitude decreases along the body [0.2,  
20 0.7]. Error bars (speed and amplitude) are s.e.m., error bars (paralysis) are 95% binomial proportion  
21 confidence intervals.  $n \geq 42$  measurements,  $\geq 6$  worms for each data point. Maximum laser intensity: 16  
22 mW/mm<sup>2</sup>.

23

## 24 Discussion

25

26 Electrical synapses are prevalent in invertebrate motor circuits, as well as in developing  
27 and mature vertebrate spinal cords [38-40]. The functional implications of gap junctions  
28 in the context of motor circuits and behavior can be counterintuitive, confounding, and  
29 are often underestimated [41, 42]. For example, in the mature zebrafish spinal cord,  
30 electrical couplings between motor neurons and premotor interneurons point to a nimble  
31 feedback mechanism for modulating rhythmic activity [43]. Experiments and modeling  
32 of crustacean stomatogastric ganglions suggest that the interplay between electrical and  
33 chemical synapses provides degenerate circuit mechanisms for switching between fast  
34 and slow oscillatory behaviors [44]. In the reversal motor circuit of *C. elegans*, the  
35 electrical coupling between AVA premotor interneurons and A-type motor neurons

1 provides state-dependent effects. At rest, they exhibit a shunting effect, by which the  
2 activity level in the backward locomotion circuit is suppressed so that a bias towards  
3 forward movement can be established [29]. Upon stimulation, these gap junctions allow  
4 antidromic-rectification of A-type motor neuron activity that prolongs reversal movement  
5 [45].

6  
7 In the motor circuit that drives *C. elegans* forward movement, we discovered that AVB-B  
8 electrical couplings induced intrinsic oscillations in mid-body B-type motor neurons, and  
9 facilitated undulatory wave propagation. To better understand how descending inputs  
10 drove the bifurcation of motor neuron dynamics, we developed a nonlinear dynamic  
11 model based on voltage dependent conductance. Detailed characterization of the intrinsic  
12 membrane conductance and experimental test of our model required electrophysiological  
13 recordings in motor neurons, a difficult technique that has only recently become possible  
14 [45]. Intriguingly, a complementary study of the *C. elegans* reversal motor circuit found  
15 that voltage-dependent  $\text{Ca}^{2+}$  channels were required for generating intrinsic rhythmic  
16 activity in the A-type motor neurons (Shangbang Gao and Mei Zhen, personal  
17 communication, May 2017). Whether the same channels drive B-type motor neuron  
18 oscillation will be addressed in future studies.

19  
20 Coordinated movement in larger animals involves networks of CPGs that are coupled  
21 through mechanosensory feedback [5]. In leech, stretch-sensitive feedback can alter the  
22 intersegmental phase lags between CPGs [10, 46]. During stick insect walking,  
23 proprioceptive feedback can entrain the motor patterns that drive individual joints,  
24 leading to interjoint coordination [13]. Specific classes of mechanosensory neurons in fly  
25 larvae are required for the propagation of peristaltic waves [47, 48].

26  
27 Here, our data indicate that distributed CPGs, operated by at least some B-type motor  
28 neurons, exist in the *C. elegans* ventral nerve cord. Because worms must adapt to extreme  
29 ranges of external mechanical loads imposed by a changing environment [34], directional  
30 proprioceptive signals, also transduced by B-type motor neurons [30], are essential for  
31 entraining the rhythm of body oscillators, and for propagating coherent bending waves

1 from the head to the tail (Figure 7). In a linear coupling model where motor neurons  
2 passively respond to proprioceptive inputs, bending amplitude would suffer an  
3 exponential decay towards the tail. Our experimental finding and theoretical analysis thus  
4 provide new insight into the interplay between sensory feedback and intrinsic oscillators  
5 within the motor circuit.

6

7 We also found a previously unknown role of electrical couplings between motor neurons,  
8 which permits rapid and reciprocal interaction between the head and body motor  
9 activities (Figures 6–7). Over-expression of UNC-9 in the B-type motor neurons led to  
10 constitutive paralysis (Video 6); we suspect that strong electrical couplings between  
11 motor neurons tend to synchronize motor activity along the whole body, thus  
12 deteriorating bending wave propagation. Weak electrical couplings between motor  
13 neurons, on the other hand, might help mediate head bending activity and augment the  
14 excitability of motor neurons along the body. Direct testing of the functional contribution  
15 of local electrical couplings requires the elimination of electrical synapses between motor  
16 neurons, while sparing the AVB-B ones. Current genetic tools for manipulating gap  
17 junction expression with defined wiring specificity remain to be developed because both  
18 types of gap junctions require UNC-9 innexin.

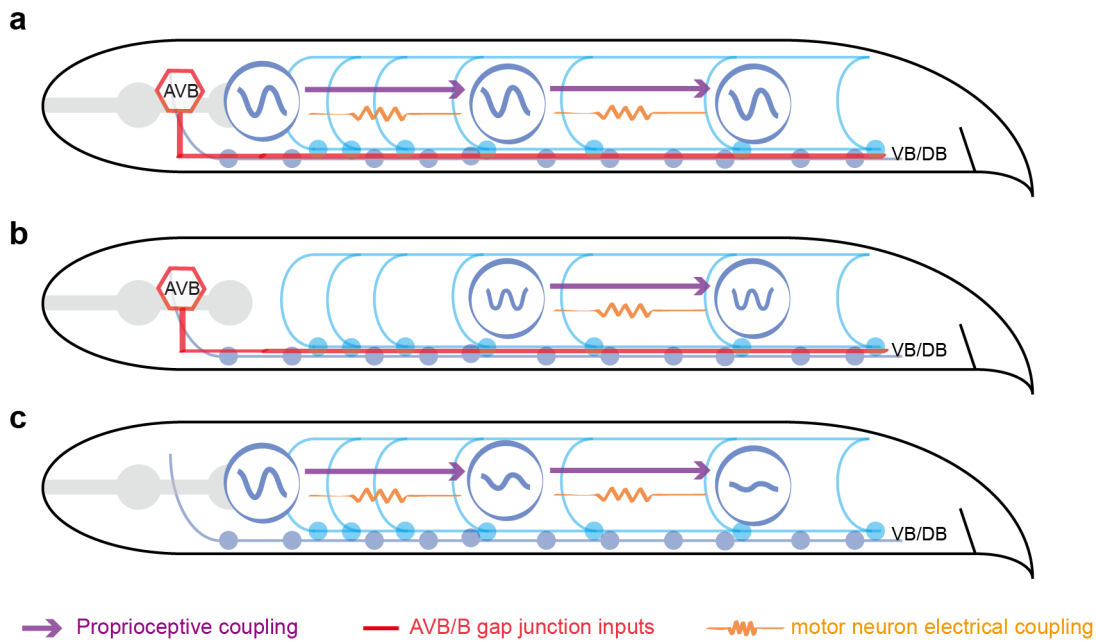
19

20 UNC-9 hemichannels are also expressed by the body wall muscle cells [49]. Could  
21 electrical couplings between muscle cells and those between motor neurons have similar  
22 functions? We (and others) found that optogenetic inhibition of mid-body muscle cells  
23 did not affect bending activity in the anterior body region, but did abolish bending  
24 activity in the posterior body region [50], consistent with our proprioceptive coupling  
25 model (Figure 7). Furthermore, in *unc-13* mutants, where all chemical synaptic inputs  
26 from motor neurons to muscles are eliminated, targeted optogenetic activation of ventral  
27 or dorsal muscle cells induces local body bending, not bending in neighboring regions  
28 [30]. These data suggest that in *C. elegans* any electrical coupling between body wall  
29 muscle cells only plays a restricted role in local bending activity.

30

1 Despite substantial anatomical differences between the worm motor circuit and those in  
2 higher organisms, we identified converging principles that govern coordinated  
3 locomotion. By integrating global descending signals, biomechanical feedback, and  
4 neuromuscular dynamics, our findings and models represent a key step towards a full  
5 system model of animal locomotion.

6



8

9 **Figure 7** Model schematics of coordinating forward locomotion in *C. elegans*. **(a)** Proprioceptive coupling  
10 between B-type motor neurons, AVB-B gap junction coupling, and weak electrical coupling between motor  
11 neurons work synergistically to drive and propagate a coordinated undulatory wave from the head to the  
12 tail. **(b)** When a strong and time-varying proprioceptive signal from an anterior body region is absent,  
13 AVB-B gap junction coupling induces mid-body high frequency undulation. **(c)** In the absence of AVB-B  
14 gap junction inputs, proprioceptive couplings are less effective in propagating bending waves, leading to  
15 rapidly decaying bending amplitude towards the tail.

16

## 1 **Methods**

### 2 **Worm strains and cultivation**

3 Wild-type (N2), mutant, and transgenic worms were cultivated using standard methods  
4 [51]. Strain information can be found in the Supplementary Experimental Procedures.  
5 Transgenic worms used in all optogenetic experiments were cultivated in the dark at 20–  
6 25°C on NGM plates with *Escherichia coli* OP50 and all-*trans* retinal (ATR). We  
7 performed all experiments using young adult hermaphrodites.

8

### 9 **Microfluidic device**

10 Custom microfluidic devices were fabricated in polydimethylsiloxane (PDMS) using soft  
11 lithography. We loaded each microfluidic channel with dextran solution [ $\sim$ 25% dextran in  
12 M9 buffer (1 Pa·s viscosity)]. An individual worm was transferred into the inlet of each  
13 microfluidic channel and worm position within each channel was manually controlled by  
14 syringes connected to polyethylene tubing.

15

### 16 **Behavioral quantification and optogenetic manipulation**

17 Experiments were performed on a Nikon inverted microscope (Ti-U, Japan) under 10×  
18 magnification with dark field illumination. Worms were immersed in viscous solution  
19 ( $\sim$ 25% dextran in M9 buffer in most cases), sandwiched between two glass slides, and  
20 retained within the field of view of an imaging objective by a custom tracking system.  
21 Video sequences were taken by a Basler CMOS camera (aca2000-340km, Germany), and  
22 the worm body centerline was extracted in real time. We used MATLAB custom software  
23 (MathWorks, Inc. Natick, MA, USA) for post-processing behavioral data. We used the  
24 CoLBeRT system [50] to perform spatially-selective optogenetic manipulation for  
25 different motor circuit components. For optogenetic inhibition, we used a 561-nm solid-  
26 state laser with maximum intensity at 16 mW/mm<sup>2</sup>.

27

28 In the relevant figure legends, the  $n$  numbers, in most cases, denote the numbers of  
29 measurements on indicated numbers of different animals for the experiments, and  
30 represent both independent biological replication and technical replication.

31

## 1 **Optogenetic ablation**

2 Optogenetic ablation was carried out using transgenic strains, in which mitochondrially  
3 targeted miniSOG (mini singlet oxygen generator) was specifically expressed in *C.*  
4 *elegans* neurons. Upon blue light illumination, mito-miniSOG caused rapid cell death in a  
5 cell-autonomous manner [33]. To ablate AVB neurons [*Plgc-55(B)::miniSOG* or *Psra-*  
6 *II::miniSOG*], L3/early L4 worms cultivated on OP50 were transplanted to an unseeded  
7 NGM plate, restricted within a 1.7 cm<sup>2</sup> area via filter paper with a hole in the center and  
8 soaked with 100 μM CUCL<sub>2</sub>. Worms were illuminated with blue LED light (M470L3-C5,  
9 Thorlabs, Inc., USA) with an intensity of 80.2 mW/cm<sup>2</sup>, measured by a power meter  
10 (PM16-130, Thorlabs, Inc., USA). The temporal sequence was 0.5/1.5 s on/off pulses for  
11 30 min. After illumination, worms were transplanted to newly OP50-seeded NGM plates  
12 with/without ATR for behavioral experiments. Mask ablations were performed via green  
13 light illumination using the same genotype under otherwise the same experimental  
14 conditions.

15  
16 For selective B-type motor neuron ablation (*Pacr-5::miniSOG*), single L3/early L4  
17 worms were transferred from the OP50-seeded NGM plates to 3% agarose (wt/vol)  
18 coated glass slides. The worms were covered by a cover glass to remain stationary.  
19 Spatially selective illumination patterns were generated by a digital micromirror device  
20 (DLI4130 0.7 XGA, Digital Light Innovations, TX, USA) to target individual neurons  
21 through a 20× objective mounted on a Nikon inverted microscope (Ti-U, Japan). Neurons  
22 were identified using mCherry fluorescence signals. We used a 473-nm blue laser with an  
23 intensity of 29 mW/mm<sup>2</sup>. The temporal sequence was 0.5/1.5 s on/off pulses for 15 min.  
24 After illumination, the worms were recovered by 2 μl of M9 buffer, and then transferred  
25 to OP50-seeded NGM plates with/without ATR for behavioral experiments.

26

## 27 **Linear proprioceptive coupling model for undulatory wave propagation**

28 We first developed a linear coupling model to simulate wave propagation along the body  
29 of *C. elegans*. In this model, the head motor circuit contains an oscillator that dominates  
30 the rhythmic motion of the whole body. Other segments cannot oscillate by themselves,  
31 but oscillate according to the proprioceptive input from the anterior segment. This simple



1 phenomenological model does not simulate the detailed dynamics of individual neurons,  
2 but instead aims to elucidate several key properties of wave propagation based on linear  
3 proprioceptive coupling.

4

5 The dynamics of the head oscillator are governed by the following equations:

6

$$7 \quad \left\{ \begin{array}{l} \tau_s \frac{dv}{dt} = v - \beta v^3 - ak \\ \tau_h \frac{du}{dt} = -u + v \\ \tau_\eta \frac{dk}{dt} = -k + \alpha_{max} * u/b, \end{array} \right. \quad \text{(Equation 5)}$$

8

9 where  $v$  describes the relative neural activity of the head motor neurons, a variable that  
10 represents the dorsal-ventral bias of neural activity. When dorsal head motor neurons  
11 have stronger neural activity than ventral ones,  $v > 0$ , and vice versa. The nonlinear  
12 term,  $v - \beta v^3$ , as well as the negative sensory feedback term  $-ak$  are critical for  
13 generating head oscillation. The second equation relates neural activity and muscle torque  
14 and the third equation relates muscle torque and body shape change in a defined segment.  
15 Their functional forms are identical to those in Equation 1. According to earlier work [30,  
16 34], the time constant:

17

$$18 \quad \tau_\eta = \frac{C_N}{b} \left( \frac{2\pi}{\lambda} \right)^4 \approx \frac{30\eta}{b} \left( \frac{2\pi}{\lambda} \right)^4, \quad \text{(Equation 6)}$$

19

20 where  $C_N$  is the drag coefficient perpendicular to the worm body, a parameter that is  
21 proportional to the viscosity of medium  $\eta$ . In the regime of large viscosity,  $\tau_\eta \gg \tau_h, \tau_\eta \gg$   
22  $\tau_s$ , and the period of the oscillator is largely determined by  $\tau_\eta$ .

23

24 Equation 5 allows for the generation of rhythmic head bending activity, and the  
25 oscillation frequency ( $f \sim 1/\tau_\eta$ ) decreases with viscosity, a key phenomenon consistent  
26 with experimental observations (Figure 3c and [34]). Apart from the head, we divided the

1 worm body into five segments (the number of segments was not essential for our  
 2 theoretical argument), each with different dynamics, as described in Equation 1. The  
 3 combination of Equations 5 and 1 allows the propagation of the head bending activity  
 4 along the whole worm body. Equations 5 and 1 arise naturally from a biologically more  
 5 realistic model, where dorsal and ventral activities are treated separately. In this case, we  
 6 have:

7

$$8 \quad \left\{ \begin{array}{l} \tau_s \frac{dv_d}{dt} = v_d - \beta v_d^3 - ak \\ \tau_h \frac{du_d}{dt} = -u_d + v_d \\ \tau_s \frac{dv_v}{dt} = v_v - \beta v_v^3 + ak \\ \tau_h \frac{du_v}{dt} = -u_v + v_v \\ \tau_\eta \frac{dk}{dt} = -k + \alpha_{max} * (u_d - u_v)/b \end{array} \right. \quad \text{Head} \quad (\text{Equation 7})$$

$$9 \quad \left\{ \begin{array}{l} C_m \frac{dv_{di}}{dt} = g_m(-v_{di} + ck_{i-1}) - g(v_{di} - v_{AVB}) \\ \tau_h \frac{du_{di}}{dt} = -u_{di} + v_{di} \\ C_m \frac{dv_{vi}}{dt} = g_m(-v_{vi} - ck_{i-1}) - g(v_{vi} - v_{AVB}) \\ \tau_h \frac{du_{vi}}{dt} = -u_{vi} + v_{vi} \\ \tau_\eta \frac{dk_i}{dt} = -k_i + \alpha_{max} * (u_{di} - u_{vi})/b \end{array} \right. \quad \text{body, } i = 2, 3, \dots \quad (\text{Equation 8})$$

10

11 Subscripts  $d$  and  $v$  denote dorsal and ventral sides, respectively. If we subtract “dorsal”  
 12 equations from “ventral” equations, Equations 1 and 5 will be derived, provided we  
 13 consider that dorsal and ventral motor activities are anti-phased, namely  $v_d = -v_i$  and  
 14  $v_{di} = -v_{vi}$ . Simulation using Equations 7 and 8 did not show any essential difference  
 15 compared with simulation using Equations 5 and 1.

16

17 The linear proprioceptive coupling model makes two predictions that can be tested  
 18 experimentally. First, abolishing head motor activity should eliminate undulatory wave

1 propagation. However, when the head and body were decoupled in our experiments, we  
2 observed high frequency undulation emerging from the mid-body. Second, while  
3 simulation suggested AVB-B gap junction coupling would deteriorate the propagation of  
4 the bending amplitude (Figure 2d), our experimental data revealed the opposite.  
5 Therefore, we concluded that the linear coupling model was oversimplified.

6

7 Parameters used in simulation are:

8 
$$a = 0.385 \text{ mm}, \alpha_{max}/b = 2 \text{ mm}^{-1}, \beta = 1.$$

9 
$$C_m = 4.5 \text{ nF}, g_m = 100 \text{ pS}, c = 0.6 \text{ mm}, \tau_h = 75 \text{ ms}, \tau_s = 75 \text{ ms}$$

10 
$$\tau_\eta = \frac{C_N}{b} \left( \frac{2\pi}{\lambda} \right)^4 \approx \frac{30\eta}{b} \left( \frac{2\pi}{\lambda} \right)^4, \quad b = 9.5 \times 10^{-14} \text{ N} \cdot \text{m}^2, \lambda = 1 \text{ mm}.$$

11 
$$\eta = 1 \text{ Pa} \cdot \text{s}.$$

12

### 13 Analytical solution to linear proprioceptive coupling model

14 To gain a deeper insight into the bending amplitude decay during wave propagation  
15 (Figure 2d), we developed an analytical understanding for the linear coupling model by  
16 transforming Equation 1 into a continuous form.

17

18 The dynamic equations for bending wave propagation can now be rewritten by the  
19 following equations:

20

21 
$$\begin{cases} C_m \frac{\partial v}{\partial t} = g_m(-v + ck(x-l)). \\ \tau_\eta \frac{\partial k}{\partial t} = -k + \alpha_{max}v/b. \end{cases} \quad 0 < x < L \quad (\text{Equation 9})$$

22

23 In the first equation,  $ck(x-l)$  describes how proprioceptive couplings drive the change  
24 in neural activity in motor neurons.  $l$  is the characteristic length of proprioceptive  
25 coupling from an anterior body region, and  $L$  is the length of the worm. The second  
26 equation relates the change of body shape with neural activity. For simplicity, we dropped  
27 the equation that relates neural activity and muscle torque in Equation 1. The delay time

1 between neural activity and muscle torque, characterized by  $\tau_h$ , was absorbed by the time  
2 constant  $\tau_\eta$  in Equation 9.

3

4 We assert a harmonic motion to describe the motor activity of the head. Thus,  $v(x)$   
5 and  $k(x)$  can be written as:

6

$$7 \quad \begin{cases} v = v_0 \exp\left(i\left(\frac{2\pi x}{\lambda} - \omega t\right)\right). \\ k = k_0 \exp\left(i\left(\frac{2\pi x}{\lambda} - \omega t\right)\right). \end{cases} \quad x \leq 0 \quad \text{(Equation 10)}$$

8

9 The neural activity and curvature are not in-phase. Thus, the phase factors are absorbed  
10 into coefficients  $v_0$  and  $k_0$ , which are all complex numbers.

11

12 The self-consistent solution for the motor activity along the body is sinusoidal, namely

13

$$14 \quad \begin{cases} v = V(x) \exp\left(i\left(\frac{2\pi x}{\lambda} - \omega t\right)\right). \\ k = K(x) \exp\left(i\left(\frac{2\pi x}{\lambda} - \omega t\right)\right). \end{cases} \quad 0 < x < L \quad \text{(Equation 11)}$$

15

16 Substituting Equation 11 into Equation 9 and defining  $\tau_m = C_m/g_m$ , we have

17

$$18 \quad -i\omega\tau_m V(x) = -V(x) + c \exp\left(-i\frac{2\pi l}{\lambda}\right) K(x-l). \quad \text{(Equation 12)}$$

$$19 \quad -i\omega\tau_\eta K(x) = -K(x) + \alpha_{max} V(x)/b. \quad \text{(Equation 13)}$$

20

21 Replacing  $K(x-l)$  with  $K(x) - K'(x)l + K''(x)l^2/2$ , and using  $l \ll \lambda$ , we find

22

$$23 \quad \frac{cl^2}{2} K''(x) - clK'(x) + \left((i\omega\tau_m - 1)(1 - i\omega\tau_\eta)b/\alpha_{max} + c\right) K(x) = 0$$

24

$$\text{(Equation 14)}$$

1

2 The solution is

$$3 \quad K(x) = k_0 \exp\left(-\frac{B + \sqrt{B^2 - 4AC}}{2A}x\right), \quad (\text{Equation 15})$$

4 where

$$5 \quad A = \frac{cl^2}{2}, \quad B = -cl, \quad C = (i\omega\tau_m - 1)(1 - i\omega\tau_\eta)b/\alpha_{max} + c$$

6

7 Thus, the decaying length constant satisfies

8

$$9 \quad 1/\xi = \text{Re} \frac{B + \sqrt{B^2 - 4AC}}{2A}. \quad (\text{Equation 16})$$

10

11 Expanding it to power series of  $\omega$  and only preserving lower order terms, we have

12

$$13 \quad \xi = \frac{l}{1 - \frac{c\alpha_{max}}{b} + \omega^2 \left( \frac{c\alpha_{max}(\tau_c^2 + \tau_\eta^2)}{2b} + \left( \frac{c\alpha_{max}}{b} - 1 \right) \tau_m \tau_\eta \right)}. \quad (\text{Equation 17})$$

14

15 The time constant  $\tau_\eta$  increases with viscosity of medium  $\eta$ , and the decay length constant  
16  $\xi$  decreases with  $\eta$ .

17

18 To the leading order, the decay length constant can be approximated as

19

$$20 \quad \xi \approx \frac{l}{1 - \frac{c\alpha_{max}}{b}}. \quad (\text{Equation 18})$$

21

22 When we incorporate electrical couplings between AVB interneurons and B-type neurons  
23 into this model, the equations become

24

$$\begin{cases} \tau_m \frac{\partial v}{\partial t} = -v - gv/g_m + ck(x-l). \\ \tau_\eta \frac{\partial k}{\partial t} = -k + \alpha_{max}v/b. \end{cases} \quad 0 < x < L, \quad (\text{Equation 19})$$

where  $g$  is the gap junction conductance. In this case, the decay length constant reduces to:

$$\xi \approx \frac{l}{1 - \frac{g_m c \alpha_{max}}{(g + g_m) b}}, \quad (\text{Equation 20})$$

which further deteriorates the propagation of the bending amplitude. This prediction is inconsistent with our experimental observations.

### 10 **Nonlinear proprioceptive coupling model**

11 The discrepancy between our theoretical and experimental observations forced us to  
 12 develop a modified nonlinear coupling model, with the head oscillator governed by the  
 13 same dynamics as that in the linear coupling model:

$$\begin{cases} \tau_s \frac{dv_d}{dt} = v_d - \beta v_d^3 - ak \\ \tau_h \frac{dn_d}{dt} = -n_d + v_d \\ \tau_s \frac{dv_v}{dt} = v_v - \beta v_v^3 + ak \\ \tau_h \frac{dn_v}{dt} = -n_v + v_v \\ \tau_\eta \frac{dk}{dt} = -k + \alpha_{max} * (n_d - n_v)/b \end{cases} \quad (\text{Equation 21})$$

16  
 17 For all other segments, our model incorporated the experimental observations that B-type  
 18 motor neurons generate intrinsic oscillations when the head and body are decoupled and  
 19 when AVB interneurons and B-type motor neurons are electrically coupled ( $g > 0$ ). The  
 20 intrinsic membrane properties in the B-type motor neurons remain unknown. For

1 simplicity, we developed a phenomenological model based on active  $\text{Ca}^{2+}$  and  $\text{K}^+$   
 2 conductances [37].

3

4 The motor activity in a given body segment is now governed by the following equations:

$$\left\{ \begin{array}{l}
 C_m \frac{dV_{di}}{dt} = -g_L(V_{di} - E_L) - g_{Ca}m_{\infty}(V_{di}) * (V_{di} - E_{Ca}) - g_Kn_{di} * (V_{di} - E_K) + c'k_{i-1} + g(V_{AVB} - V_{di}) \\
 \tau_n \frac{dn_{di}}{dt} = -n_{di} + n_{\infty}(V_{di}) \\
 C_m \frac{dV_{vi}}{dt} = -g_L(V_{vi} - E_L) - g_{Ca}m(V_{vi}) * (V_{vi} - E_{Ca}) - g_Kn_{vi} * (V_{vi} - E_K) - c'k_{i-1} + g(V_{AVB} - V_{vi}) \\
 \tau_n \frac{dn_{vi}}{dt} = -n_{vi} + n_{\infty}(V_{vi}) \\
 \tau_u \frac{dM_{di}}{dt} = -M_{di} + M(V_{di}) - M(V_{vi}) \\
 \tau_u \frac{dM_{vi}}{dt} = -M_{vi} + M(V_{vi}) - M(V_{di}) \\
 \tau_{\eta} \frac{dk_i}{dt} = -k_i + \alpha'_{max}/b * (M_{di} - M_{vi})
 \end{array} \right. .$$

6

(Equation 22)

7

8 In Equation 22,  $m, n$  are voltage-dependent gating variables;  $m_{\infty}(V), n_{\infty}(V)$  are steady-  
 9 state activation functions. We assume that the time constant for the gating variable  $m$  is  
 10 small and  $m$  follows instantaneously with the voltage.  $M(V)$  relates cell membrane  
 11 potential and muscle torque. We assume they all have sigmoidal functional forms:

12

$$13 \quad m_{\infty}(V) = \frac{1}{1 + \exp\left(\frac{V - V_m}{\theta_m}\right)}, \quad n_{\infty}(V) = \frac{1}{1 + \exp\left(\frac{V - V_n}{\theta_n}\right)}, \quad M(V) = \frac{1}{1 + \exp\left(\frac{V - V_{mus}}{\theta_{mus}}\right)}.$$

14

(Equation 23)

15

16  $g(V_{AVB} - V)$  is the current flowing through the gap junction between AVB interneurons  
 17 and B-type neurons, where  $V_{AVB}$  is the membrane potential of the AVB interneurons, held  
 18 at a constant value.

19

20 The terms  $-M(V_v)$  and  $-M(V_d)$  in Equation 22 reflect contralateral inhibition from the  
 21 D-type GABAergic motor neurons: dorsal D neurons can be activated by ventral B  
 22 neurons and thereby inhibit dorsal muscle activity, and vice versa.

1

2 We simulated six segments (including the head), similar to that in the linear coupling  
3 model. The segment number was roughly in accordance with the number of DB-type  
4 motor neurons. The parameter values are listed below.

5

6 
$$\tau_s = 50.0 \text{ nF}, \tau_h = 200 \text{ ms}, C_m = 3.0 \text{ nF}, \tau_n = 30 \text{ ms}, \tau_u = 85 \text{ ms}.$$

7 
$$\tau_\eta = \frac{C_N}{b} \left(\frac{2\pi}{\lambda}\right)^4 \approx \frac{30\eta}{b} \left(\frac{2\pi}{\lambda}\right)^4, \quad b = 9.5 \times 10^{-14} \text{ N} \cdot \text{m}^2, \lambda = 1 \text{ mm}.$$

8 
$$g_m = 100 \text{ pS}, g_L = 100 \text{ pS}, g_{Ca} = 400 \text{ pS}, g_K = 500 \text{ pS}.$$

9 
$$a = 0.385 \text{ mm}, \beta = 1, c' = \frac{5 \text{ mA}}{\text{mm}}, \frac{\alpha_{max}}{b} = 10 \text{ mm}^{-1}, \alpha'_{max}/b = 2.1 \text{ mm}^{-1}.$$

10 
$$E_L = -60 \text{ mV}, E_{Ca} = 60 \text{ mV}, E_K = -70 \text{ mV}, E_o = -60 \text{ mV}, V_{AVB} = -20 \text{ mV}.$$

11 
$$V_m = -29 \text{ mV}, V_n = -55 \text{ mV}, V_{mus} = -45 \text{ mV}.$$

12 
$$\theta_m = 10.25 \text{ mV}, \theta_n = 20 \text{ mV}, \theta_{mus} = 10 \text{ mV}.$$

13 
$$\eta = 1 \text{ Pa} \cdot \text{s}.$$

14

15 Comparing the case  $g = 0$  and  $g = 100 \text{ pS}$ , we found that gap junction coupling  
16 between AVB interneurons and B-type neurons could equalize the bending amplitude  
17 (Figure 5c). High frequency oscillation within middle segments could also be generated  
18 when the head and body were decoupled (Figure 5a).

19

## 20 **Acknowledgments**

21 The authors thank Christopher Fang-Yen and Anthony Fouad for helpful discussion. This  
22 work was funded by the CAS Hundred Talents Plan and the National Science Foundation  
23 of China (NSFC-31471051 and NSFC-91632102 to Q. Wen) and the Canadian Institute  
24 of Health Research (CIHR-MOP93619, MOP123250 to M. Zhen).

25

## 26 **Competing Interests**

27 The authors declare no competing financial interests.

28

29



## 1 References

- 2 1. Brown, T.G., *The Intrinsic Factors in the Act of Progression in the Mammal*.  
3 Proceedings of the Royal Society of London Series B-Biological Sciences, 1911.  
4 **84**(572): p. 308-319.
- 5 2. Delcomyn, F., *Neural basis of rhythmic behavior in animals*. Science, 1980.  
6 **210**(4469): p. 492-8.
- 7 3. Grillner, S., *The motor infrastructure: from ion channels to neuronal networks*.  
8 Nat Rev Neurosci, 2003. **4**(7): p. 573-86.
- 9 4. Kiehn, O., *Locomotor circuits in the mammalian spinal cord*. Annu Rev Neurosci,  
10 2006. **29**: p. 279-306.
- 11 5. Marder, E., et al., *Invertebrate central pattern generation moves along*. Curr Biol,  
12 2005. **15**(17): p. R685-99.
- 13 6. Marder, E. and R.L. Calabrese, *Principles of rhythmic motor pattern generation*.  
14 Physiological Reviews, 1996. **76**(3): p. 687-717.
- 15 7. Mullins, O.J., et al., *Neuronal control of swimming behavior: comparison of*  
16 *vertebrate and invertebrate model systems*. Prog Neurobiol, 2011. **93**(2): p. 244-  
17 69.
- 18 8. Pearson, K.G., *Proprioceptive regulation of locomotion*. Curr Opin Neurobiol,  
19 1995. **5**(6): p. 786-91.
- 20 9. McClellan, A.D. and W. Jang, *Mechanosensory inputs to the central pattern*  
21 *generators for locomotion in the lamprey spinal cord: resetting, entrainment, and*  
22 *computer modeling*. J Neurophysiol, 1993. **70**(6): p. 2442-54.
- 23 10. Yu, X. and W.O. Friesen, *Entrainment of leech swimming activity by the ventral*  
24 *stretch receptor*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 2004.  
25 **190**(11): p. 939-49.
- 26 11. Pearson, K.G., *Generating the walking gait: role of sensory feedback*. Prog Brain  
27 Res, 2004. **143**: p. 123-9.
- 28 12. Akay, T., et al., *Degradation of mouse locomotor pattern in the absence of*  
29 *proprioceptive sensory feedback*. Proc Natl Acad Sci U S A, 2014. **111**(47): p.  
30 16877-82.
- 31 13. Hess, D. and A. Buschges, *Role of proprioceptive signals from an insect femur-*  
32 *tibia joint in patterning motoneuronal activity of an adjacent leg joint*. J  
33 Neurophysiol, 1999. **81**(4): p. 1856-65.
- 34 14. Woo, S.H., et al., *Piezo2 is the principal mechanotransduction channel for*  
35 *proprioception*. Nature Neuroscience, 2015. **18**(12): p. 1756-1762.
- 36 15. Lemon, R.N., *Descending pathways in motor control*. Annu Rev Neurosci, 2008.  
37 **31**: p. 195-218.
- 38 16. Hagglund, M., et al., *Activation of groups of excitatory neurons in the mammalian*  
39 *spinal cord or hindbrain evokes locomotion*. Nat Neurosci, 2010. **13**(2): p. 246-  
40 52.
- 41 17. Thiele, T.R., J.C. Donovan, and H. Baier, *Descending control of swim posture by*  
42 *a midbrain nucleus in zebrafish*. Neuron, 2014. **83**(3): p. 679-91.
- 43 18. Severi, K.E., et al., *Neural control and modulation of swimming speed in the*  
44 *larval zebrafish*. Neuron, 2014. **83**(3): p. 692-707.
- 45 19. Buchanan, J.T., et al., *Reticulospinal Neurons Activate Excitatory Amino-Acid*  
46 *Receptors*. Brain Research, 1987. **408**(1-2): p. 321-325.

- 1 20. Bouvier, J., et al., *Descending Command Neurons in the Brainstem that Halt*  
2 *Locomotion*. Cell, 2015. **163**(5): p. 1191-203.
- 3 21. Esposito, M.S., P. Capelli, and S. Arber, *Brainstem nucleus MdV mediates skilled*  
4 *forelimb motor tasks*. Nature, 2014. **508**(7496): p. 351-6.
- 5 22. Weeks, J.C. and W.B. Kristan, *Initiation, Maintenance and Modulation of*  
6 *Swimming in the Medicinal Leech by the Activity of a Single Neuron*. Journal of  
7 Experimental Biology, 1978. **77**(Dec): p. 71-88.
- 8 23. Friesen, W.O. and W.B. Kristan, *Leech locomotion: swimming, crawling, and*  
9 *decisions*. Curr Opin Neurobiol, 2007. **17**(6): p. 704-11.
- 10 24. Dickinson, M.H., et al., *How animals move: An integrative view*. Science, 2000.  
11 **288**(5463): p. 100-106.
- 12 25. Chen, B.L., D.H. Hall, and D.B. Chklovskii, *Wiring optimization can relate*  
13 *neuronal structure and function*. Proceedings of the National Academy of  
14 Sciences of the United States of America, 2006. **103**(12): p. 4723-4728.
- 15 26. White, J.G., et al., *The Structure of the Nervous-System of the Nematode*  
16 *Caenorhabditis-Elegans*. Philosophical Transactions of the Royal Society of  
17 London Series B-Biological Sciences, 1986. **314**(1165): p. 1-340.
- 18 27. White, J.G., et al., *The structure of the ventral nerve cord of Caenorhabditis*  
19 *elegans*. Philos Trans R Soc Lond B Biol Sci, 1976. **275**(938): p. 327-48.
- 20 28. Starich, T.A., et al., *Interactions between innexins UNC-7 and UNC-9 mediate*  
21 *electrical synapse specificity in the Caenorhabditis elegans locomotory nervous*  
22 *system*. Neural Development, 2009. **4**.
- 23 29. Kawano, T., et al., *An Imbalancing Act: Gap Junctions Reduce the Backward*  
24 *Motor Circuit Activity to Bias C. elegans for Forward Locomotion*. Neuron, 2011.  
25 **72**(4): p. 572-86.
- 26 30. Wen, Q., et al., *Proprioceptive coupling within motor neurons drives C. elegans*  
27 *forward locomotion*. Neuron, 2012. **76**(4): p. 750-61.
- 28 31. Zhen, M. and A.D. Samuel, *C. elegans locomotion: small circuits, complex*  
29 *functions*. Curr Opin Neurobiol, 2015. **33**: p. 117-26.
- 30 32. Altun, Z.F., et al., *High resolution map of caenorhabditis elegans gap junction*  
31 *proteins*. Dev Dyn, 2015. **244**(7): p. 903.
- 32 33. Qi, Y.B., et al., *Photo-inducible cell ablation in Caenorhabditis elegans using the*  
33 *genetically encoded singlet oxygen generating protein miniSOG*. Proc Natl Acad  
34 Sci U S A, 2012. **109**(19): p. 7499-504.
- 35 34. Fang-Yen, C., et al., *Biomechanical analysis of gait adaptation in the nematode*  
36 *Caenorhabditis elegans*. Proc Natl Acad Sci U S A, 2010. **107**(47): p. 20323-8.
- 37 35. Faumont, S., et al., *An image-free opto-mechanical system for creating virtual*  
38 *environments and imaging neuronal activity in freely moving Caenorhabditis*  
39 *elegans*. PLoS One, 2011. **6**(9): p. e24666.
- 40 36. Laurent, P., et al., *Decoding a neural circuit controlling global animal state in C.*  
41 *elegans*. Elife, 2015. **4**.
- 42 37. Izhikevich, E.M., *Dynamical systems in neuroscience : the geometry of*  
43 *excitability and bursting*. Computational neuroscience. 2007, Cambridge, Mass.:  
44 MIT Press. xvi, 441 p.
- 45 38. Connors, B.W. and M.A. Long, *Electrical synapses in the mammalian brain*.  
46 Annu Rev Neurosci, 2004. **27**: p. 393-418.

- 1 39. Kiehn, O. and M.C. Tresch, *Gap junctions and motor behavior*. Trends Neurosci, 2002. **25**(2): p. 108-15.
- 2
- 3 40. Szczupak, L., *Functional contributions of electrical synapses in sensory and*
- 4 *motor networks*. Curr Opin Neurobiol, 2016. **41**: p. 99-105.
- 5 41. Manor, Y., et al., *Low-amplitude oscillations in the inferior olive: a model based*
- 6 *on electrical coupling of neurons with heterogeneous channel densities*. J
- 7 Neurophysiol, 1997. **77**(5): p. 2736-52.
- 8 42. Marder, E., *Electrical synapses: beyond speed and synchrony to computation*.
- 9 Curr Biol, 1998. **8**(22): p. R795-7.
- 10 43. Song, J., et al., *Motor neurons control locomotor circuit function retrogradely via*
- 11 *gap junctions*. Nature, 2016. **529**(7586): p. 399-402.
- 12 44. Gutierrez, G.J., T. O'Leary, and E. Marder, *Multiple mechanisms switch an*
- 13 *electrically coupled, synaptically inhibited neuron between competing rhythmic*
- 14 *oscillators*. Neuron, 2013. **77**(5): p. 845-58.
- 15 45. Liu, P., et al., *Antidromic-rectifying gap junctions amplify chemical transmission*
- 16 *at functionally mixed electrical-chemical synapses*. Nat Commun, 2017. **8**: p.
- 17 14818.
- 18 46. Yu, X.T., B. Nguyen, and W.O. Friesen, *Sensory feedback can coordinate the*
- 19 *swimming activity of the leech*. Journal of Neuroscience, 1999. **19**(11): p. 4634-
- 20 4643.
- 21 47. Cheng, L.E., et al., *The role of the TRP channel NompC in Drosophila larval and*
- 22 *adult locomotion*. Neuron, 2010. **67**(3): p. 373-80.
- 23 48. Guo, Y., et al., *Transmembrane channel-like (tmc) gene regulates Drosophila*
- 24 *larval locomotion*. Proc Natl Acad Sci U S A, 2016. **113**(26): p. 7243-8.
- 25 49. Liu, Q., et al., *Low conductance gap junctions mediate specific electrical coupling*
- 26 *in body-wall muscle cells of Caenorhabditis elegans*. J Biol Chem, 2006. **281**(12):
- 27 p. 7881-9.
- 28 50. Leifer, A.M., et al., *Optogenetic manipulation of neural activity in freely moving*
- 29 *Caenorhabditis elegans*. Nature Methods, 2011. **in press**.
- 30 51. Brenner, S., *The genetics of Caenorhabditis elegans*. Genetics, 1974. **77**(1): p. 71-
- 31 94.
- 32

1 **Videos**

2

3 **Video 1** | An *unc-7* mutant animal swam freely in solution at ~ 1 Pa·s viscosity. Despite  
4 anterior body undulation, the animal exhibited dragging in the tail.

5

6 **Video 2** | Optogenetic inhibition of the anterior region of a freely swimming wild type  
7 worm induced high frequency undulation in the posterior body region.

8

9 **Video 3** | A worm with UNC-7 expression restored in AVB and other neurons (*Psra-11::UNC-7*)  
10 swam in solution at ~ 1 Pa·s viscosity. Both bending amplitude and high-  
11 frequency undulation in the posterior body region were restored.

12

13 **Video 4** | Optogenetic inhibition of the mid-body B-type motor neurons in a wild type  
14 worm resulted in whole body paralysis.

15

16 **Video 5** | Optogenetic inhibition of the mid-body B-type motor neurons in an *unc-9*  
17 mutant worm led to paralysis in the posterior region, whereas the anterior body  
18 undulation persisted.

19

20 **Video 6** | Over-expression of UNC-9 innexin in B-type motor neurons (*Pacr-5::UNC-9*)  
21 caused whole body paralysis. Worms were incapable of propagating body undulations  
22 along their bodies.

23

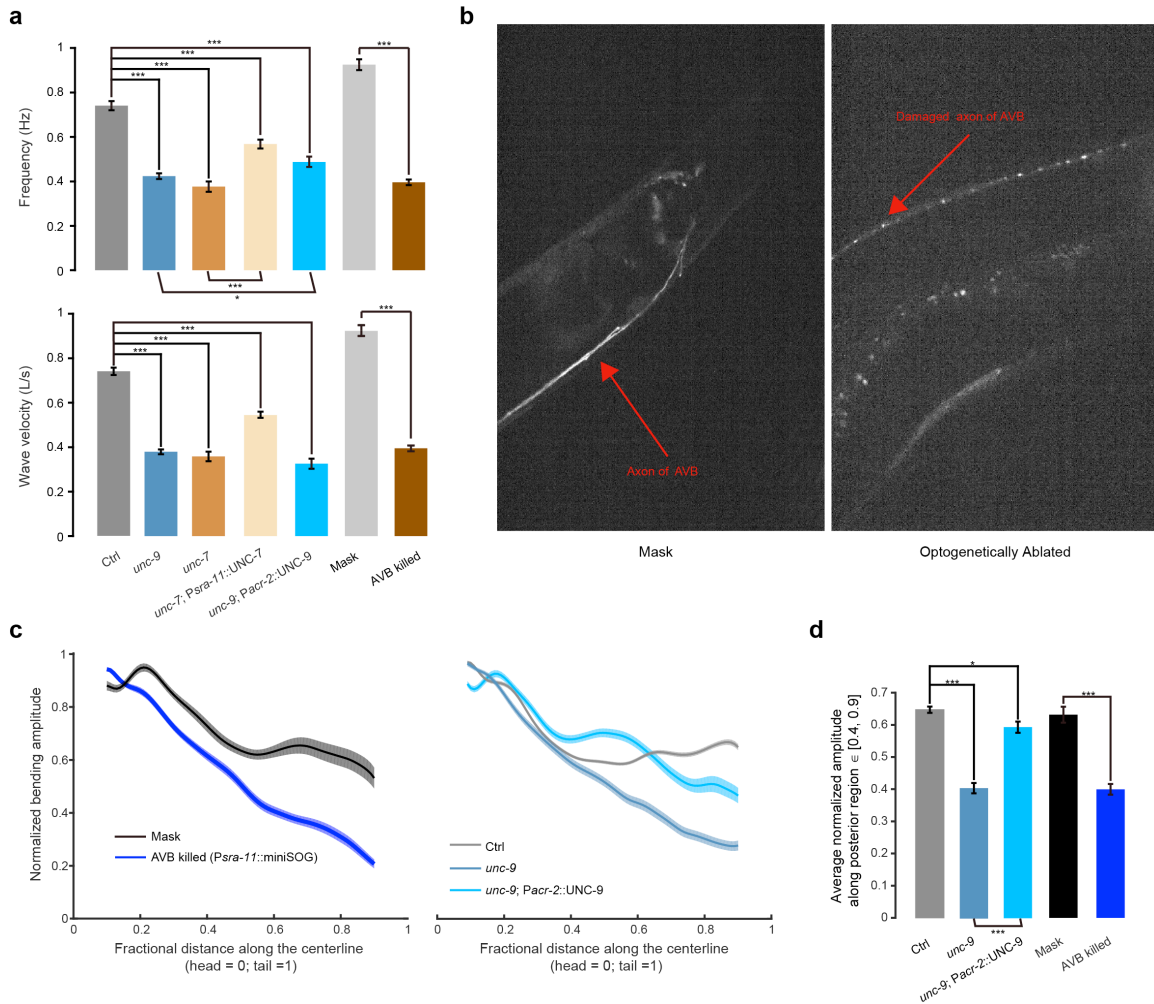
24 **Video 7** | Optogenetic activation of AVB premotor interneurons (*Psra-11::Chrimson*)  
25 could trigger forward locomotion from the pause state.

26

27

28

1 **Figure 1 – supplement figure 1 | AVB-B electrical couplings facilitate undulatory**  
 2 **wave propagation during forward locomotion**



3  
 4  
 5  
 6 **(a)** Locomotion kinematics of tested strains. Upper panel shows the comparison of  
 7 undulation frequency in viscous medium (~ 1 Pa·s). Lower panel shows the comparison  
 8 of wave velocity, calculated in worm body length per second (*L/s*). Error bars show  
 9 s.e.m. \*\*\*  $p < 0.0001$ , \*  $p = 0.01$  ( Up: Ctrl and *unc-9*  $p = 4.1 \times 10^{-28}$ ; Ctrl and *unc-7*  $p =$   
 10  $1.7 \times 10^{-24}$ ; Ctrl and *unc-7;Psra-11::UNC-7*  $p = 8.8 \times 10^{-9}$ ; Ctrl and *unc-9; Pacr-2::UNC-9*  
 11  $p = 1.13 \times 10^{-13}$ ; *unc-7;Psra-11::UNC-7* and *unc-7*  $p = 2.8 \times 10^{-9}$ ; Mask and AVB killed  $p =$   
 12  $2.4 \times 10^{-39}$ . Bottom: Ctrl and *unc-9*  $p = 2.2 \times 10^{-33}$ ; Ctrl and *unc-7*  $p = 3.1 \times 10^{-30}$ ; Ctrl and  
 13 *unc-7;Psra-11::UNC-7*  $p = 1.9 \times 10^{-16}$ ; Ctrl and *unc-9;Pacr-2::UNC-9*  $p = 2.7 \times 10^{-28}$ ; *unc-*  
 14 *7;Psra-11::UNC-7* and *unc-7*  $p = 3.0 \times 10^{-10}$ ; *unc-9;Pacr-2::UNC-9* and *unc-9*  $p = 0.01$ ;  
 15 Mask and AVB killed  $p = 5.6 \times 10^{-41}$ ), two-sample *t*-test with Bonferroni correction. Ctrl

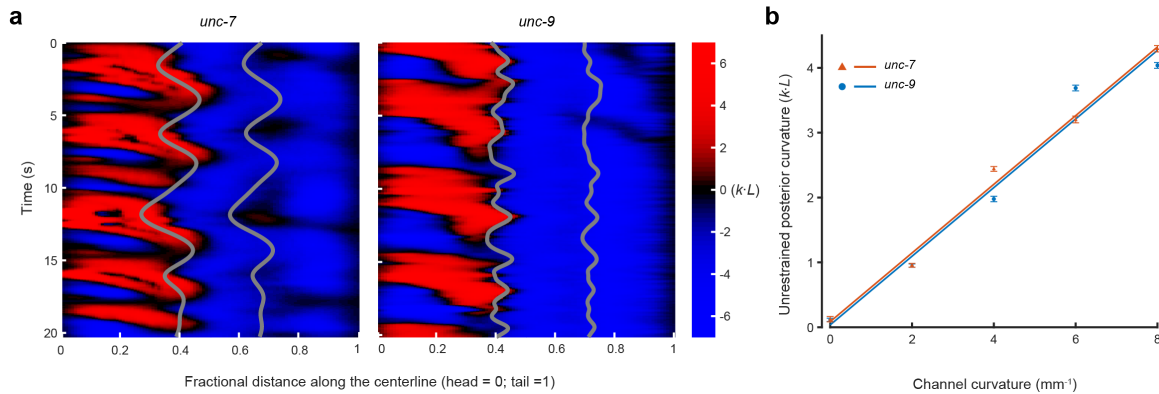
1 [N2; *Pacr-5::Arch*; *Punc-4::TWK-18(gf)*],  $n = 107$  measurements, 13 worms; *unc-7*  
2 mutant, [*unc-7(hp121)*; *Pacr-5::Arch*; *Punc-4::TWK-18(gf)*],  $n = 99$  measurements, 22  
3 worms; *unc-9* mutant [*unc-9(fc16)*; *Pacr-5::Arch*; *Punc-4::TWK-18(gf)*],  $n = 144$   
4 measurements, 17 worms; AVB ablated worm [*Pg/c-*  
5 *55(B)::tom20::miniSOG::UrSL::wCherry*],  $n = 80$  measurements, 13 worms; Mask,  $n =$   
6 96 measurements, 12 worms. **(b)** Comparison of AVB axon fluorescence signals in mask  
7 and AVB-ablated worms. Arrows indicate intact and damaged axons. **(c)** Left:  
8 Comparison of normalized bending amplitude along the worm body in mask and AVB-  
9 ablated worms. We used a different transgenic strain (*Psra-11::miniSOG*) to drive the  
10 expression of miniSOG in AVB neurons. Right: Comparison of normalized bending  
11 amplitude along the worm body in CTRL, *unc-9*, and UNC-9 rescue in B-type and A-  
12 type motor neurons [*unc-9(fc16)*; *Pacr-2::UNC-9*; *Pacr-5::arch*]. Rescue of UNC-9 in B-  
13 type and A-type motor neurons largely restored the posterior bending amplitude. Shaded  
14 regions show s.e.m. **(d)** Bending amplitude averaged over the posterior region  $\in [40, 90]$   
15 of the worm body. \*\*\* $p < 0.0001$ , \* $p = 0.003$  (Ctrl and *unc-9*  $p = 3.5 \times 10^{-23}$ ; *unc-9*; *Pacr-*  
16 *2::UNC-9* and *unc-9*  $p = 1.7 \times 10^{-12}$ ; Mask and AVB killed  $p = 2.4 \times 10^{-10}$ ), two-sample  $t$ -  
17 test with Bonferroni correction. Error bars represent s.e.m. Ctrl,  $n = 105$  measurements,  
18 13 worms; *unc-9* mutant,  $n = 93$  measurements, 17 worms; UNC-9 rescue in motor  
19 neurons,  $n = 64$  measurements, 9 worms. Mask,  $n = 28$  measurements, 5 worms; AVB-  
20 ablated worms (*Psra-11::tom20::miniSOG*)  $n = 100$  measurements, 10 worms.

21

22

1 **Figure 2 – supplement figure 1 | AVB-B electrical couplings are not necessary for**  
2 **transducing proprioceptive signals.**

3



4

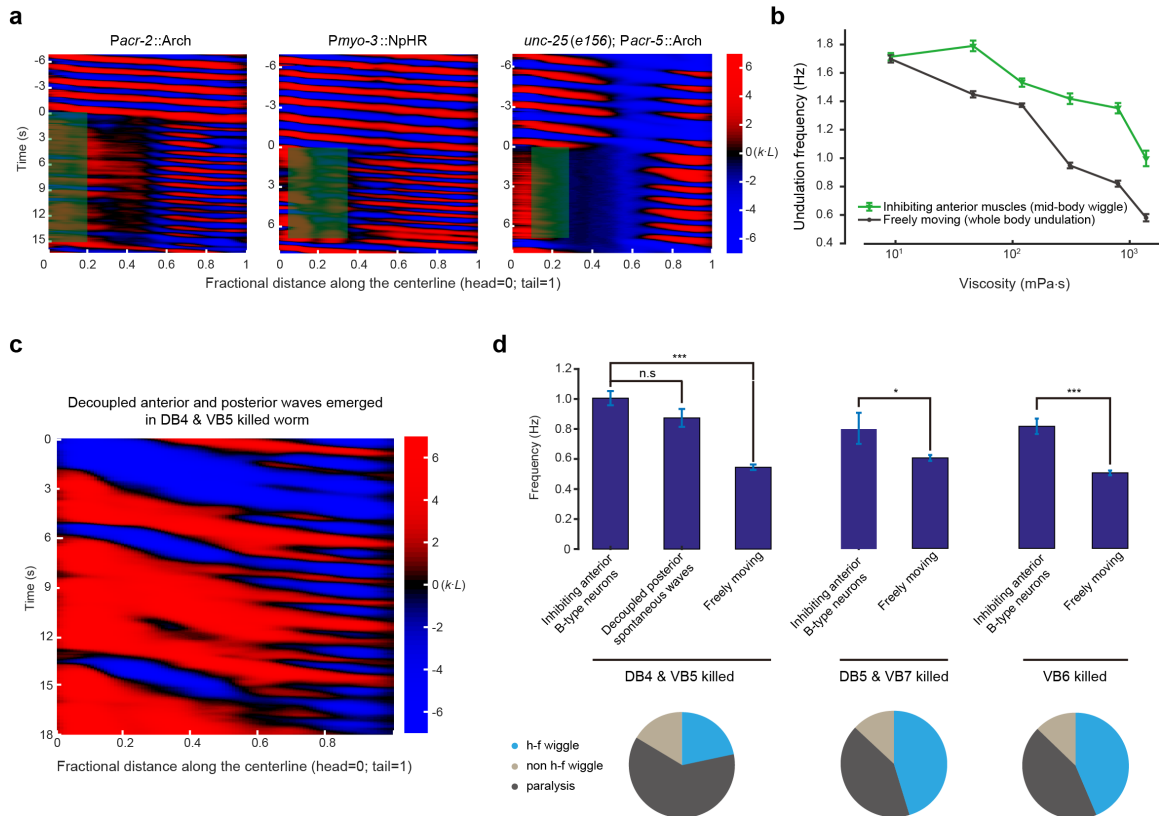
5

6 **(a)** Representative kymographs showing an *unc-7* mutant [*unc-7*(hp121); *Punc-4::TWK-*  
7 *18*(gf)] and an *unc-9* mutant worm [*unc-9*(fc16); *Punc-4::TWK-18*(gf)], whose mid-  
8 bodies were constrained in a microfluidic channel with defined curvature (6 mm<sup>-1</sup>). Gray  
9 lines indicate anterior and posterior boundaries of the channel. **(b)** Unconstrained  
10 posterior body curvature as a function of channel curvature. Each data point:  $n = 5$   
11 worms, error bars represent s.e.m. Blue and red lines are linear fit ( $R^2 > 0.97$ ).

12

13

1 **Figure 3 – supplement figure 1 | Mid-body B motor neurons intrinsically generate**  
 2 **rhythmic activity.**



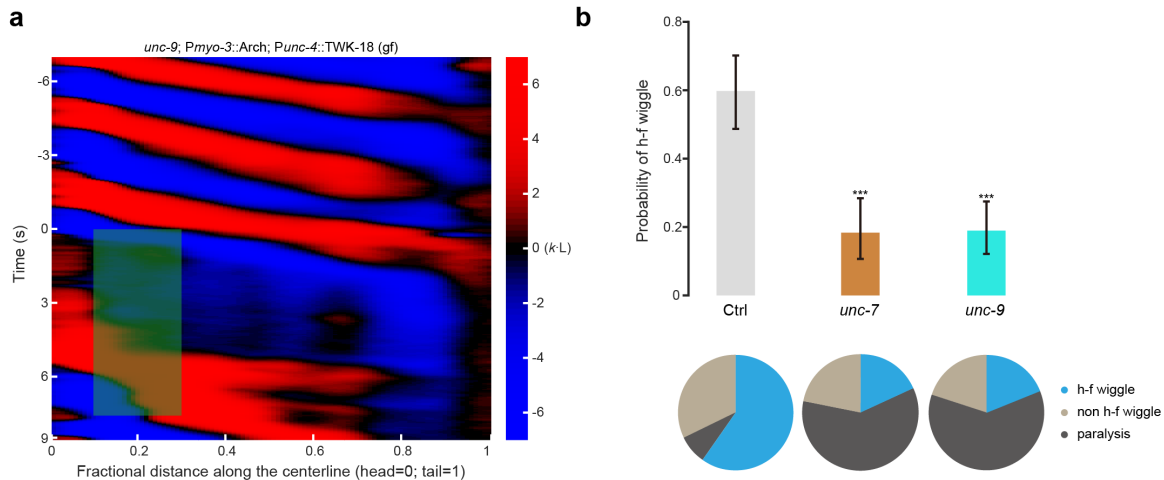
3  
 4  
 5  
 6 **(a)** Representative kymographs demonstrate that fast mid/posterior body undulatory  
 7 waves could be induced by inhibiting anterior A- & B-type motor neurons (*Pacr-2::Arch*,  
 8 11 out of 25 measurements) by inhibiting anterior muscle cells (*Pmyo-3::NpHR*, 52 out of  
 9 87 measurements) or by inhibiting anterior B-type motor neurons in GABA  
 10 neurotransmitter-deficient worms [*unc-25(e156); Pacr-5::Arch*, 33 out of 45  
 11 measurements]. **(b)** Undulation frequency with medium viscosity. Black, normal  
 12 swimming worm; green, mid-body undulation when the head and body were decoupled  
 13 by inhibiting anterior muscle cells. Each data point represents mean and s.e.m from  $n \geq 4$   
 14 worms. **(c)** Representative kymographs of a freely swimming worm in which DB4 &  
 15 VB5 motor neurons were optogenetically ablated (*Pacr-5::tomm20::miniSOG*).  
 16 Decoupled spontaneous waves emerged from anterior and posterior body regions. **(d)** Up,  
 17 summary of undulation frequencies after selectively ablating a subset of B-type motor



1 neurons in the mid-body. Three behavioral outcomes were compared. (1) Fast mid-body  
2 undulation induced by optogenetically inhibiting anterior B-type motor neurons. (2)  
3 Spontaneous posterior bending waves in DB4&VB5-ablated worms. (3) Freely  
4 swimming worms. Bottom, pie chart summarizes worm locomotor states across trials  
5 during optogenetic inhibition of anterior B-type motor neurons. State definitions can be  
6 found in the main text. Error bars show s.e.m., \*\*\*  $p < 0.0001$ , n.s.  $p = 0.15$  (DB4 & VB5  
7 killed  $p = 6.1 \times 10^{-13}$ ; DB5 & VB7 killed  $p = 0.01$ ; VB6 killed  $p = 5.8 \times 10^{-6}$ ), two-sample  $t$ -  
8 test. DB4 & VB5 killed  $n = 11$  worms, DB5 & VB7 killed  $n = 8$  worms, VB6 killed  $n = 9$   
9 worms.  
10  
11

1 **Figure 4 – supplement figure 1 | AVB-B electrical couplings drive a bifurcation in B-**  
2 **type neuron dynamics**

3



4

5

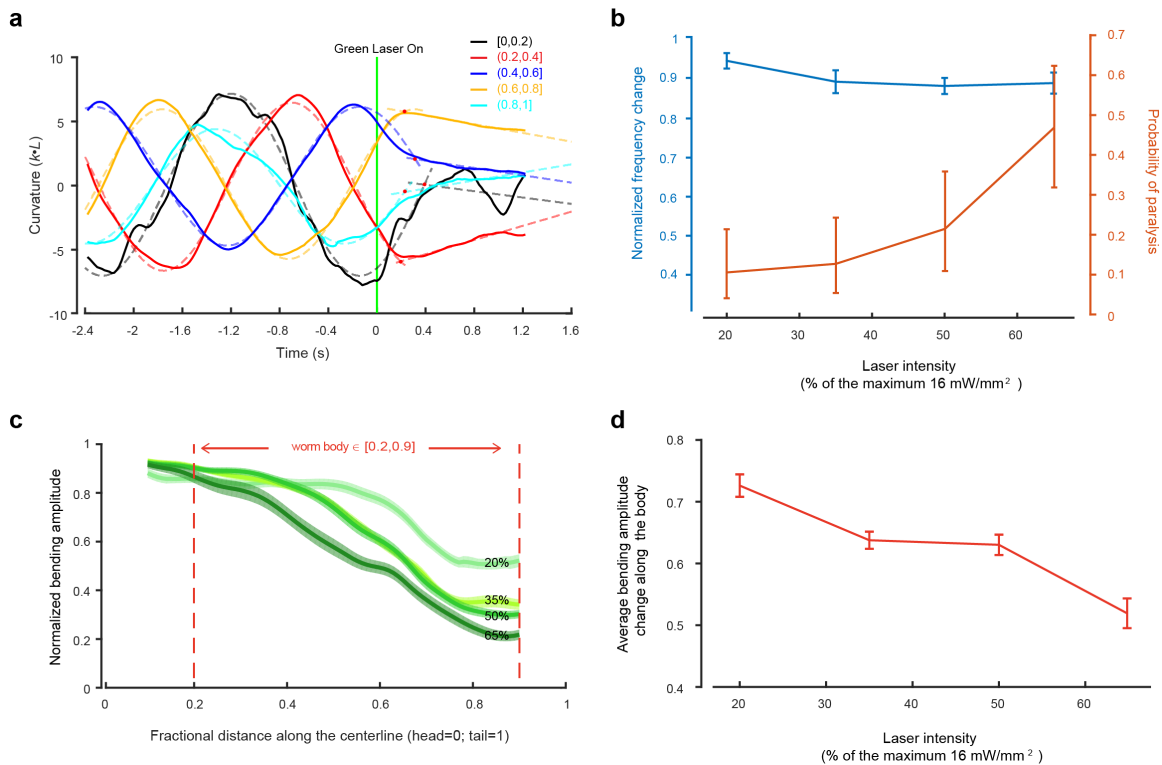
6 **(a)** Representative curvature kymographs of head-body-decoupled experiments in AVB-  
7 B gap junction-deficient *unc-9* mutants [*unc-9*; *Pmyo-3::Arch*; *Punc-4::TWK-18(gf)*].  
8 Anterior muscle cells were optogenetically inhibited to abolish the proprioceptive signals  
9 arising from head bending activity. **(b)** Probability for inducing high frequency mid-body  
10 undulation when the head and body were decoupled by inhibiting anterior muscle cells.  
11 Pie chart summarizes worm locomotor states across trials. Error bars indicate 95%  
12 binomial proportion confidence interval. \*\*\*  $p < 0.0001$  ( Ctrl and *unc-7*  $p = 3.6 \times 10^{-8}$ ;  
13 Ctrl and *unc-9*  $p = 3.3 \times 10^{-9}$ ), Chi-Square test. Ctrl [*Pmyo-3::NpHR*, *Punc-4::TWK-*  
14 *18(gf)*],  $n = 87$  measurements, 10 worms. *unc-7* mutant [*unc-7(hp121)*; *Pmyo-3::Arch*;  
15 *Punc-4::TWK-18(gf)*],  $n = 82$  measurements, 18 worms. *unc-9* mutant [*unc-9(fc16)*;  
16 *Pmyo-3::Arch*; *Punc-4::TWK-18(gf)*],  $n = 111$  measurements, 21 worms.

17

18

1 **Figure 6 – supplement figure 1 | Electrical couplings between motor neurons allow**  
 2 **for rapid and reciprocal interactions between head and body motor activities.**

3



4

5

6 **(a)** Estimation of reaction time when mid-body B-type motor neurons were  
 7 optogenetically inhibited. First, we fit the regional curvature with a sinusoidal function  
 8 right before the green laser was turned on. Second, we performed a linear fit of regional  
 9 curvature during the optogenetic inhibition. The crossover point (red dot) represents the  
 10 wiggle-to-paralysis transition for each segment. Curvatures were averaged over a defined  
 11 color-coded region, represented by a fractional distance along the worm body coordinates  
 12 (head = 0, tail = 1). **(b)** Dose-dependent effect on the undulation frequency and efficacy  
 13 for inducing whole-body paralysis when B-type motor neurons within a posterior body  
 14 region ( $\in [0.7, 0.9]$ ) were optogenetically inhibited. Error bars (blue), s.e.m; error bars  
 15 (orange), 95% binomial proportion confidence interval.  $n \geq 5$  worms for each data point.  
 16 **(c)** Dose dependent effect on the bending amplitude along the worm body when B-type  
 17 motor neurons within a posterior body region ( $\in [0.7, 0.9]$ ) were optogenetically

1 inhibited. Shaded regions are s.e.m. **(d)** Average bending amplitude change along the  
2 body  $\in [0.2, 0.9]$ . Error bars are s.e.m. Maximum laser intensity was  $16 \text{ mW/mm}^2$ .  
3  
4

1 **Supplementary Table | Strain information**  
 2  
 3

Strain Name	Experiment	Genotype	Construct (plasmid no.); injection concentrations
WEN0315	B motor neuron inhibition in WT	<i>wenIs0001</i> ; <i>wenEx0315</i>	<i>Pacr-5::Arch::UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001) <i>Punc-4::TWK::18(gf)::UrSL::wCherry</i> (pJH2108) 30 ng/μL; <i>Plin-14::gfp</i> 20 ng/μL; <i>Pmyo-3::wCherry</i> (pJH1774) 2.5 ng/μL (wenEx0315)
WEN0317	B motor neuron inhibition in <i>unc-7</i> mutants; Microfluidic experiments	<i>wenIs0001</i> ; <i>wenEx0317</i> ; <i>unc-7(hp121)</i>	<i>Pacr-5::Arch:: UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (WenIs0001) <i>Punc-4::TWK::18(gf)::UrSL::wCherry</i> (pJH2108) 30 ng/μL; <i>Plin-14::gfp</i> 20 ng/μL; <i>Pmyo-3::wCherry</i> (pJH1774) 2.5 ng/μL (wenEx0317)
WEN0304	B motor neuron inhibition in <i>unc-9</i> mutants; Microfluidic experiments	<i>wenEx0304</i> ; <i>hpEx2088</i> ; <i>unc-9(fc16)</i>	<i>Pacr-5::Arch:: UrSL::wCherry</i> (pJH2918) 30 ng/μL; <i>Plin-14::gfp</i> 20 ng/μL (Wen Ex0304) <i>Punc-4::TWK::18(gf)::UrSL::wCherry</i> (pJH2108) ; <i>Podr-1::gfp</i> (hpEx2088)
ZM7297	AVB ablation	<i>hpls331</i> ;	<i>Plgc-55(B)::tomm-20::miniSOG::UrSL::wCherry</i> (pJH2890); <i>lin-15</i>
WEN0318	B motor neuron inhibition upon AVB ablation; Microfluidic experiments	<i>wenIs0001</i> ; <i>hpls331</i> ;	<i>Pacr-5::Arch::UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001) <i>Plgc-55(B)::tomm-20::miniSOG::UrSL::wCherry</i> (pJH2890) (hpls331)
WEN0335	B motor neuron inhibition in <i>unc-7</i> mutants, with UNC-7 expression restored in AVB	<i>wenEx0335</i> ; <i>wenIs0001</i> ; <i>unc-7(hp121)</i>	<i>Punc-4::TWK::18(gf)::UrSL::wCherry</i> (pJH2108) 30 ng/μL; <i>Psra-11::UNC-7(cDNA)::wCherry</i> (pJH2037) 20 ng/μL; <i>Plin-14::gfp</i> 20ng/μL; <i>Pmyo-3::wCherry</i> 2.5 ng/μL (wenEx0335) <i>Pacr-5::Arch:: UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001)
WEN0314	B motor neuron ablation; Microfluidic experiments	<i>wenIs0001</i> ; <i>hpls374</i> ;	<i>Pacr-5::Arch:: UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (hpls374) <i>Pacr-5::tomm-20::miniSOG::UrSL::wCherry</i> (pJH2842) (wenIs0001)
WEN0001	B motor neuron inhibition	<i>wenIs0001</i>	<i>Pacr-5::Arch::UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001)
WEN0320	B motor neuron inhibition upon AVB ablation	<i>wenIs0001</i> ; <i>wenEx0320</i>	<i>Pacr-5::Arch::UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001) <i>Psra-11::tomm-20::miniSOG::UrSL::wCherry</i> (quan0065) 50 ng/μL; <i>Pmyo-3::wCherry</i> 2.5 ng/μL (wenEx0320)
ZM9275	A & B motor neuron inhibition	<i>hpls615</i>	<i>Pacr-2(s)::Arch::wCherry</i> (pJH3717) (hpls615)
ZX444.6	Muscle inhibition	<i>zxEx29</i> ; <i>lin-15(n765ts)</i>	<i>Pmyo-3::NpHR::ECFP</i> ; <i>lin-15+</i> (zxEx29)
WEN0307	B motor neuron inhibition in GABA deficient mutants	<i>wenIs0001</i> ; <i>unc-25(e156)</i>	<i>Pacr-5::Arch::UrSL::wCherry</i> (pJH2918); <i>Podr-1::gfp</i> (wenIs0001)
ZM7796	B motor neuron ablation	<i>hpls374</i>	<i>Pacr-5::tomm-20::miniSOG::UrSL::wCherry</i> (pJH2842) (hpls374)
WEN0372	AVB activation	<i>wenEx0372</i>	<i>Psra-11::Chrimson::wCherry</i> (quan0164) 30 ng/μl; <i>Plin-24::GFP</i> (wenEx0372)
WEN0363	Muscle	<i>wenEX0363</i> ;	<i>Pmyo-3::Arch::UrSL::wCherry</i> (quan0124) 30 ng/μl;

	inhibition in <i>unc-7</i> mutants	<i>unc-7(hp121)</i>	<i>Punc-4</i> ::TWK-18(gf)::UrSL::wCherry (pJH2108) 30 ng/μl (wenEx0363)
WEN0323	Muscle inhibition in <i>unc-9</i> mutants	<i>wenEx0323</i> ; <i>unc-9(fc16)</i>	<i>Pmyo-3</i> ::Arch::UrSL::wCherry (quan0124) 30 ng/μl; <i>Punc-4</i> ::TWK-18(gf)::UrSL::wCherry (pJH2108) 30 ng/μl (wenEx0323)
WEN0337	<i>unc-9</i> mutants with restored expression in A and B motor neurons	<i>wenEx0337</i> ; <i>unc-9(fc16)</i>	<i>Pacr-2</i> ::UNC-9::wCherry (pJH1897) 2 ng/μl; <i>Pacr-5</i> ::Arch::UrSL::wCherry (pJH2918) 30 ng/μl (wenEx0337)
WEN0366	Overexpression of UNC-9 in B motor neurons	<i>wenEx0366</i>	<i>Pacr-5</i> ::Arch::UrSL::wCherry (pJH2918) 30 ng/μl; <i>Pacr-5</i> ::UNC-9 (pJH2140) 30 ng/μl (wenEx0366)

1