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Cite this article: Wen Q, Gao S, Zhen M. 2018 *Caenorhabditis elegans* excitatory ventral cord motor neurons derive rhythm for body undulation. *Phil. Trans. R. Soc. B* **373**: 20170370.
http://dx.doi.org/10.1098/rstb.2017.0370

Accepted: 13 August 2018

One contribution of 15 to a discussion meeting issue 'Connectome to behaviour: modelling *C. elegans* at cellular resolution'.

Subject Areas:
neuroscience

Keywords:
central pattern generators, motor neuron, projection-premotor interneuron, proprioception, functional compression, neuromechanical model

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Caenorhabditis elegans excitatory ventral cord motor neurons derive rhythm for body undulation

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The intrinsic oscillatory activity of central pattern generators underlies motor rhythm. We review and discuss recent findings that address the origin of *Caenorhabditis elegans* motor rhythm. These studies propose that the A- and mid-body B-class excitatory motor neurons at the ventral cord function as non-bursting intrinsic oscillators to underlie body undulation during reversal and forward movements, respectively. Proprioception entrains their intrinsic activities, allows phase-coupling between members of the same class motor neurons, and thereby facilitates directional propagation of undulations. Distinct pools of premotor interneurons project along the ventral nerve cord to innervate all members of the A- and B-class motor neurons, modulating their oscillations, as well as promoting their bi-directional coupling. The two motor sub-circuits, which consist of oscillators and descending inputs with distinct properties, form the structural base of dynamic rhythmicity and flexible partition of the forward and backward motor states. These results contribute to a continuous effort to establish a mechanistic and dynamic model of the *C. elegans* sensorimotor system. *C. elegans* exhibits rich sensorimotor functions despite a small neuron number. These findings implicate a circuit-level functional compression. By integrating the role of rhythm generation and proprioception into motor neurons, and the role of descending regulation of oscillators into premotor interneurons, this numerically simple nervous system can achieve a circuit infrastructure analogous to that of anatomically complex systems. *C. elegans* has manifested itself as a compact model to search for general principles of sensorimotor behaviours.

This article is part of a discussion meeting issue 'Connectome to behaviour: modelling *C. elegans* at cellular resolution'.

1. Origin and regulation of motor rhythms

Neurons and neural circuits with intrinsic oscillatory activities underlie rhythmicity of motor behaviours, from respiration, heartbeat, gastric motility to locomotion [1–5]. In the absence of rhythmic inputs from the descending neural networks or sensory organs, isolated vertebrate spinal cords, and their invertebrate counterpart, the ventral nerve cords, retain a capacity to generate rhythmic and patterned motor neuron activity or fictive locomotion [6–10]. Hence oscillators for locomotory activities reside within the spinal or ventral nerve cords, where premotor interneurons form local circuits with excitatory motor neurons to orchestrate motor outputs [1,2,11].

Selective recruitment, modulation and coordination of locomotory oscillators constitute the form and transit between different motor patterns. This process is regulated at multiple levels. Within the spinal cords of rodents and fish, distinct

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pools of spinal premotor interneurons play dedicated roles in rhythm generation and pattern coordination. Different modes of movements correlate with selective recruitment of excitatory and inhibitory premotor interneuron groups [1,12]. Mechanosensory interneurons provide local proprioceptive and sensory feedbacks to the spinal premotor interneurons [13–17]. While excitatory premotor interneurons are generally considered to originate the rhythm, motor neurons are integral components of the oscillatory output. In the crayfish and leech swimmerets, the *Caenorhabditis elegans* and *Drosophila* larva motor circuit, as well as the zebrafish spinal cord, motor neurons that receive mixed electric and chemical synaptic inputs can retrogradely affect the activity of premotor interneurons [18–22]. Lastly, the oscillatory activities of local central pattern generators (CPGs) are regulated by descending inputs from the brain, via synaptic and neuromodulatory pathways, for initiation, reconfiguration and coordination. For example, in the lamprey and rodents, pools of interneurons of the retinospinal region or the brainstem project into the spinal cord, and their activities can either turn on or shut down different motor patterns [23–26].

2. CPGs for body bending reside in the *C. elegans* ventral nerve cord

Despite a deep understanding of anatomy [27–31] and theoretical studies [32–34], direct experimental evidence that addresses the potential existence and identity of *C. elegans* CPGs has been lacking. Obtaining a mechanistic understanding of the *C. elegans* motor rhythm has been difficult. Our progress has been hindered by the technical difficulty of *in situ C. elegans* electrophysiology experiments [35], and mystified by the membrane physiology of *C. elegans* neurons, which lack voltage-gated sodium currents, and with an exception [36], do not appear to fire action potentials [37]. Its anatomical organization of the nervous system also seems to be unconventional: unlike most spinal and ventral nerve cords, where premotor interneurons and motor neurons co-reside, only the soma of motor neurons, of both the excitatory and inhibitory classes, occupy the *C. elegans* ventral cord. Among them, the A- and some B-class excitatory motor neurons are main executors of body undulation during backward and forward movement, respectively. Recent studies reveal that these motor neurons function as the respective CPGs for backward and forward movements [38–40].

The A-class motor neurons exhibited intrinsic oscillatory activities (figure 1*a*) that were sufficient to execute slow backward movements without premotor interneurons (figure 1*b*). After the removal of all premotor interneuron inputs, the A-class excitatory motor neurons continued to generate oscillatory and phase-coupled activities (figure 1*b*) that triggered rhythmic action potential bursts in body wall muscles (figure 1*a*). Robust calcium oscillation maintained in posterior A-class motor neurons even when chemical synaptic transmission was further eliminated from the entire nervous system in this preparation (figure 1*a*). Selective ablation of a subgroup of the A-class motor neurons led to disruption of local bending, but did not inhibit rhythmic bending in neighbouring body segments [39] (figure 2*a*). These results suggest that the A-class motor neurons can function as non-bursting oscillators to drive body bending and to generate backward movements (figure 1*b*).

The B-class motor neurons also exhibit oscillatory activities, but with more restricted capacities. The mid-body B-class motor neurons exhibited oscillation when activated by the electric synaptic inputs from premotor interneurons projecting along the ventral nerve cord [40]. The oscillation of mid-body B-class motor neurons was observed when chemical synaptic transmission of the entire nervous system was eliminated (figure 3*a*).

A plethora of sodium, calcium and potassium channels underlie a CPG's intrinsic membrane potential oscillations [42,43]. UNC-2, the *C. elegans* orthologue of the P/Q/N-type high-voltage-activated calcium channel, is one of the components [39]. The reduction and gain of UNC-2 conductance in the A-class motor neurons led to a decrease and increase, respectively, of the amplitude and frequency of muscle rPSC bursting (figure 4*a*) and their calcium oscillation (figure 4*b*). These effects were observed in the absence of premotor interneurons (figure 4*a*,*b*).

Whether UNC-2-mediated calcium conductance also underlies the B-class motor neuron oscillation has not been addressed, but indirect evidence supports this notion. Animals with reduced UNC-2 activity exhibited reduced velocity during both forward and backward movements (figure 5*a*). In the absence of A-class motor neurons, animals with increased UNC-2 activity continued to exhibit increased forward velocity (figure 5*b*). High-voltage-activated calcium conductance probably represents a conserved, endogenous constituent of the membrane intrinsic oscillation. In isolated lamprey spinal neuron soma, the N-type calcium currents prominently potentiate bursting, and are coupled with the burst-terminating calcium-activated potassium currents [44,45]; the intrinsic, high-frequency gamma band oscillation in the rat pedunculopontine nucleus requires the N- and/or P/Q-type calcium currents [46–48].

The complete channel composition of *C. elegans* oscillators awaits further dissection. Future work should also address mechanisms that underlie the difference between the high and low intrinsic activities of the A- and B-class motor neurons. The involvement of other ventral cord motor neurons in rhythm generation requires further investigation. The D-class GABAergic motor neurons modulate, but are not necessary for, body bending [39,40,49], arguing against them being CPGs. The perturbation of AS, another class cholinergic and excitatory motor neurons, affects both forward and backward movement; whether they harbour intrinsic oscillatory activity is unknown [50].

3. Proprioception entrains and coordinates CPG-driven body undulation

Proprioception is a prominent modulator of CPG's oscillatory properties [14,51]. Mechanisms must be in place to coordinate CPGs' activities distributed along the *C. elegans* body to form a cohesive propagating bending wave [34].

During forward movements in microfluidic devices, local and directional proprioceptive coupling between the adjacent body regions plays a critical role in the propagation of undulation [40,41]. When the mid-body region was trapped in a microfluidic channel with a defined curvature, the unrestrained posterior body region exhibited the same curvature in the same direction (figure 3*b*). When dynamic curvature change was imposed with a pneumatic microfluidic device,

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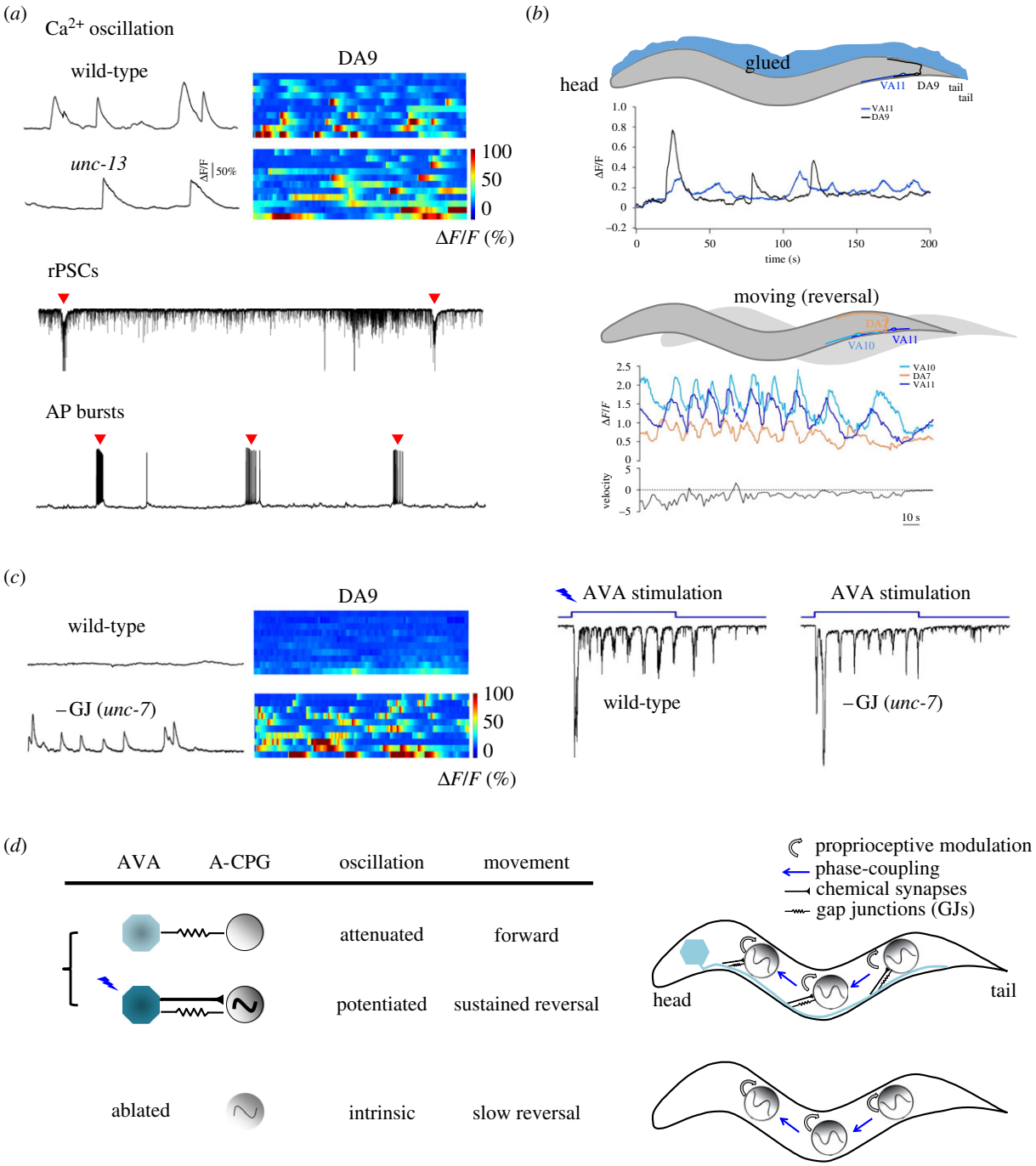

Figure 1. An integrative model for backward locomotion: local reversal oscillators are phase-coupled via proprioception, and dually regulated by descending inputs. (*a*) The A-class motor neurons exhibit intrinsic, oscillatory activities that are sufficient to drive backward movement. (Top panel) Calcium oscillation in the posterior A motor neuron was observed in animals where chemical synaptic transmission and all premotor interneurons were removed from the nervous system. Left, sample traces; right, raster plot of recording from multiple animals. (Bottom panel) A dissected ventral cord muscle preparation from an animal where all premotor interneurons were removed exhibited anterior A-class motor-dependent rhythmic postsynaptic currents (rPSCs) and action potential (AP) bursts, both denoted by red arrowheads. (*b*) The A-class motor neurons may use intrinsic proprioceptive properties to self-organize phase-coupling during backward movement. (Top panels) A comparison of calcium activities exhibited by posterior A-class motor neurons in an immobilized (left) and a freely moving (reversal) animal (right), where all premotor interneurons were removed from the nervous system. Movements strengthened both calcium oscillation of and phase-coupling among A-class motor neurons. (*c*) The AVA premotor interneurons provide descending inputs that dually regulate the A-class motor neuron's oscillation through a mixed gap junction and chemical synapse configuration. Gap-junction-mediated coupling between AVA and A-class motor neurons shunts their intrinsic oscillation, whereas chemical synapses allow optogenetically activated AVA to potentiate their oscillation. (*d*) A model: backward movement is driven by oscillation from a chain of distributed CPGs (the A-class motor neurons), phase-coupled by proprioceptive feedback and regulated by descending inputs. Figure panels adapted from [39,53].

rapid curvature changes and bending waves followed in the posterior body [41] (figure 3*b*).

Whereas no dedicated local sensory neurons exist in the ventral nerve cord, the B-class motor neurons are found to transduce the bend-sensitive signals during forward movements. When a body segment was compelled to bend towards the dorsal side, the dorsal muscle-innervating B-class motor neuron sustained a higher level of intracellular calcium activity than the ventral muscle-innervating B-class, and vice versa during ventral bending [41]. The bend-sensitivity of B-class motor neurons allows the curvature change in an anterior body to define the curvature of the

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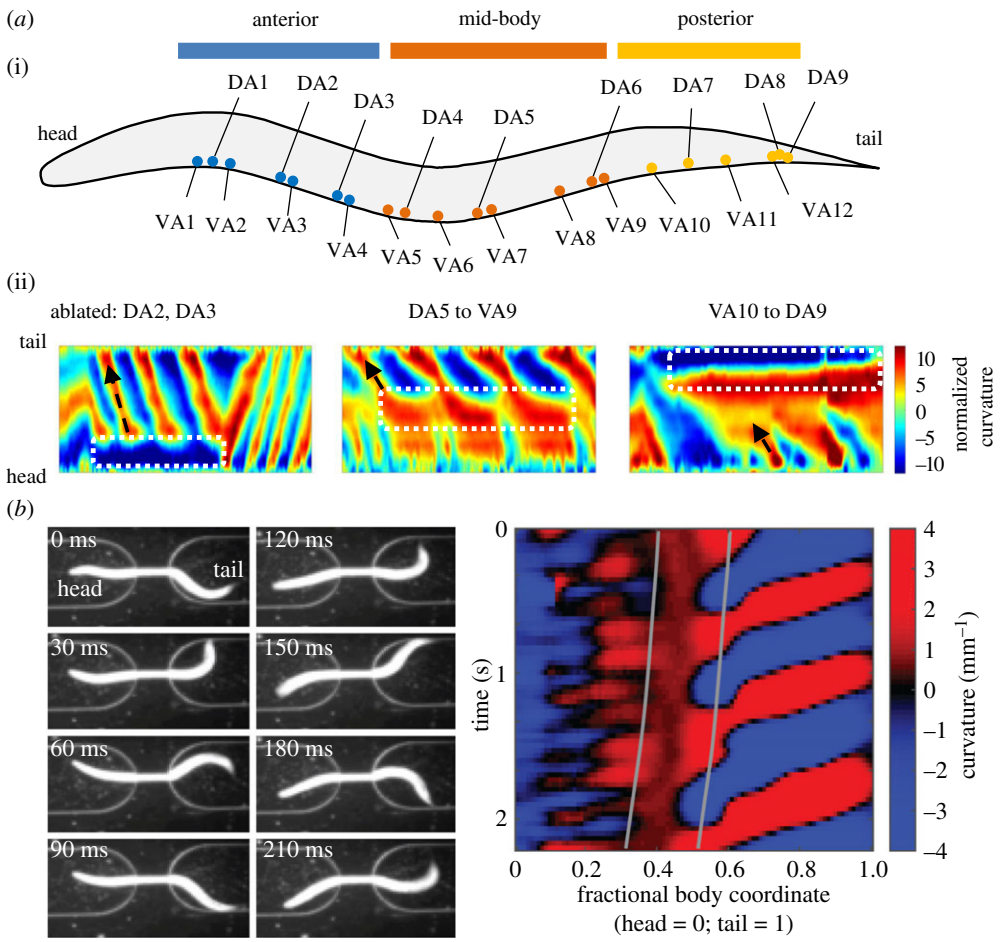

Figure 2. Multiple A-class motor neurons function as oscillators during backward movements. (*a*) Ablation of A-class motor neurons that reside in a restricted body segments did not prevent bending wave propagation at adjacent regions. (i) Schematics of the soma position of the A-class motor neurons, arbitrarily separated into the anterior, mid- and posterior segments. (ii) Representative kymographs of bending curvature along the body of animals missing A-class motor neurons in the anterior (left), mid- (centre) and posterior (right) body segments. (*b*) An animal with its mid-body trapped inside a microfluidic device continued to generate anteriorly propagating bending waves in the unrestrained anterior and posterior segment with different frequencies, as shown in a series of video frames (left), and by kymograph of time-varying curvature along the body (right) where vertical lines mark the anterior and posterior limits of the straight channel. Panel (*a*) adapted from [39]. See Materials and Methods.

posterior neighbour, facilitating the propagation of bending waves from head to tail.

Optogenetic experiments reveal that proprioception entrains the B-class motor neuron's oscillation. When proprioceptive signal from the most anterior body region was eliminated by either optogenetically inhibiting the head muscles or anterior B-type motor neurons, the mid-body generated rhythmic bending activity, with a higher frequency than normal undulation [38,40] (figure 3*c*). Hence, the B-class motor neurons situated in the anterior and posterior body region may operate at different intrinsic frequencies, but directional proprioceptive coupling entrains their activities to generate coherent body undulation (figure 3*c*,*d*).

Whether and how proprioception regulates backward movements requires further examination. Several lines of indirect evidence support the notion of proprioceptive entrainment and coupling of reversal CPGs. When all premotor interneurons and B-class motor neurons were removed, animals continued to exhibit slow but organized, anteriorly propagating body undulation, as well as phase-coupled calcium oscillation of multiple A-class motor neurons [39] (figure 1*b*). This contrasted the case when these animals were glued down, in which the A-class motor neurons exhibited less robust or coordinated calcium oscillation [39] (figure 1*b*). Sparse ablation of a small number of the A-class motor neurons only blocked local body bending ([39] (figure 2*a*). When the mid-body was constrained by a straight microfluidic channel, the unconstrained anterior and posterior body regions continued to exhibit undulation at different frequencies (figure 2*b*). These observations imply that the A-class motor neurons are also proprioceptive, and use proprioception for self-organized phase-coupling (figure 1*d*).

Proprioceptive coupling probably plays a critical role for phase-locked coherent bending during both directional movements, but the forward and reversal circuit may incorporate such an ingredient differently. Most B-class motor neurons, with low intrinsic oscillatory activity, may rely strongly on proprioception for sequential activation during propagation. Multiple A-class motor neurons, with higher intrinsically oscillatory activities, may mainly use their proprioceptive property to self-organize a cohesive wave during propagation.

4. Descending pathways by the projection-premotor interneurons control movements

C. elegans ventral cord excitatory motor neurons integrate roles of rhythm generation and proprioception to organize and execute body undulations. Consistent with the A- and B-class motor neurons exhibiting differences in their intrinsic

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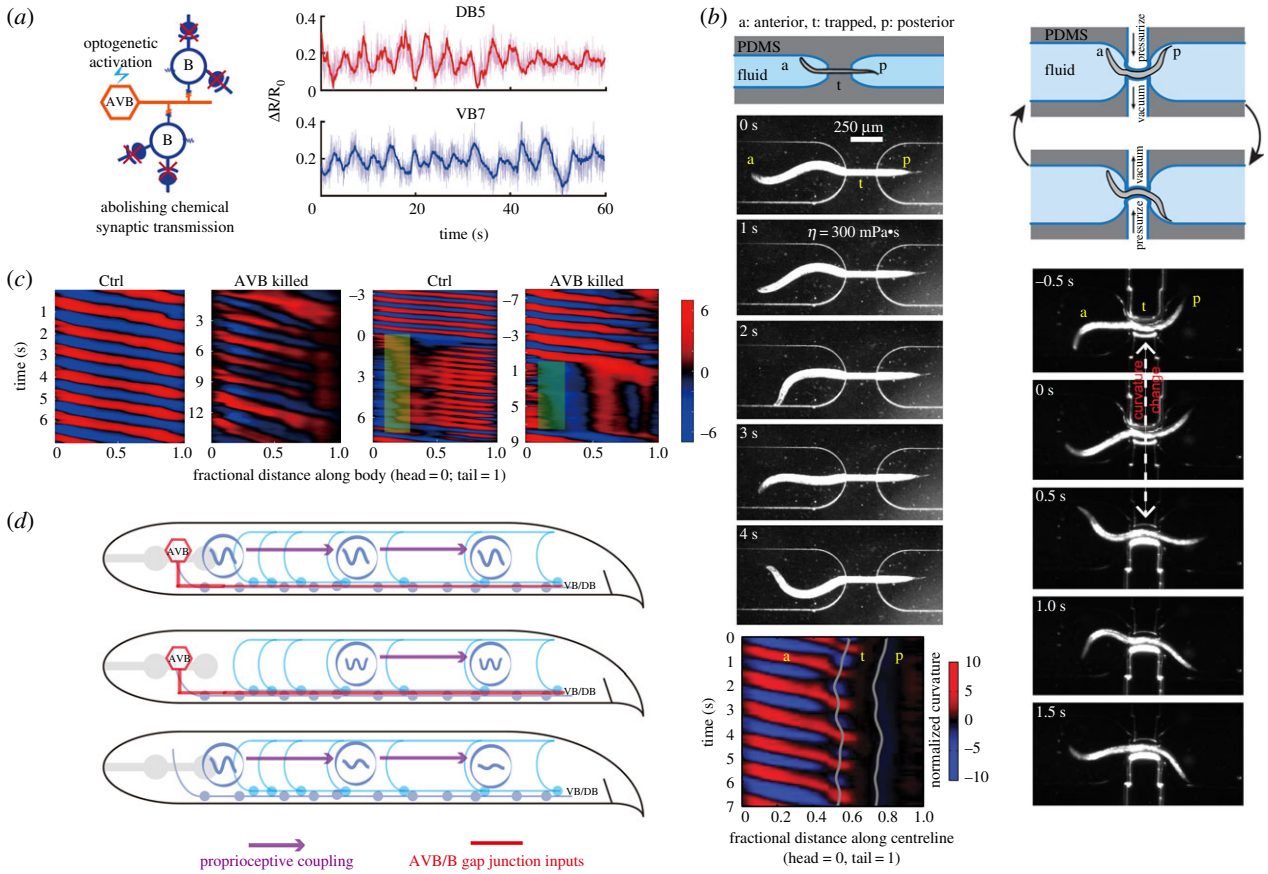

Figure 3. A biophysical model for forward locomotion that integrates local oscillators, proprioceptive couplings and descending inputs from premotor interneurons. (*a*) The B-class motor neurons in the mid-body exhibited rhythmic calcium activities upon optogenetic activation of premotor interneuron AVBs. Note that imaging experiments were carried out in mutants *unc-13* (*e51*) where chemical synaptic transmission in the whole nervous system was largely abolished. (*b*) Local and directional (anterior to posterior) proprioceptive couplings propagate body undulations. When a mid-body region of a worm was constrained in a straight microfluidic channel, the posterior body region emerged from the channel would remain still and straight. Curvature kymograph showed that the bending waves could only propagate to the anterior limit of the channel. When dynamic curvature change in the worm mid-body was imposed by a pneumatic microfluidic device, rapid curvature changes and bending waves followed in the posterior body. (*c*) Descending inputs from AVB interneurons are required for mid-body oscillations. Curvature kymographs show that, in an AVB-ablated worm, the bending amplitude decayed monotonically towards the tail during forward locomotion. When an anterior body region of a wild-type worm was immobilized via optogenetic inhibition of B-class motor neurons or muscle cells, higher frequency and low-amplitude bending waves emerged from the mid-body. Ablating AVB premotor interneurons would abolish the mid-body bending waves. (*d*) Local body oscillators, proprioceptive coupling between B-class motor neurons and AVB-B gap junction coupling work synergistically to drive and propagate a coordinated undulatory wave from the head to the tail. When a strong and time-varying proprioceptive signal from an anterior body region is absent, AVB-B gap junction coupling induces mid-body high-frequency undulation. In the absence of AVB-B gap junction inputs, proprioceptive couplings are less effective in propagating bending waves, leading to rapidly decaying bending amplitude towards the tail. Figure panels adapted from [40,41].

activities, the projection-premotor interneurons use different strategies to modulate their activities.

The B-class motor neurons exhibit a low level of intrinsic oscillatory activities compared with the A-class motor neurons [39]. The AVB premotor interneurons project along the ventral cord to form gap junctions with all B-class motor neurons. These electrical couplings allow depolarized AVB to trigger the bifurcation of mid-body B-class motor neurons' oscillatory activities to promote forward movement [40] (figure 3*c*). The A-class motor neurons, by contrast, have intrinsically high level of activities. The premotor interneurons AVA project along the ventral nerve cord to form both gap junctions and chemical synapses with all A-class motor neurons [28,52]. Their electrical couplings shunt the A-class motor neurons' intrinsic oscillation, reducing the propensity of spontaneous reversals [39,53] (figure 1*c*,*d*). However, when AVA were stimulated to overcome the shunting effect, depolarized AVAs would potentiate the A-class motor neuron oscillation through chemical and electrical synapses to sustain long reversals [39,53,54] (figure 1*c*,*d*).

The interplay between the two descending pathways partly explains how *C. elegans* executes the two distinct motor programmes (figures 1*d* and 3*d*) with an inherent bias for forward movement [40,53] (figure 6). By shunting the reversal CPGs through electric coupling with the AVA premotor interneurons, animals favour forward movement by default. Upon depolarization, the AVA premotor interneurons potentiate A-class motor neurons to sustain backward movements. Upon hyperpolarization, they use electrical synapses to facilitate efficient inhibition of all A-class motor neuron activities [39,53]. Similarly, the depolarization and hyperpolarization of the AVB premotor interneurons could potentiate and halt forward locomotion, respectively, through their electrical coupling with the B-class motor neurons [40].

Bi-directional couplings between the descending pathways to and between excitatory motor neurons can have additional physiological implications. The ablation of the projection-premotor interneurons and, specifically, genetic ablation of their gap junction input to excitatory motor neurons lead to *kink*, a motor state in which the forward and backward circuit

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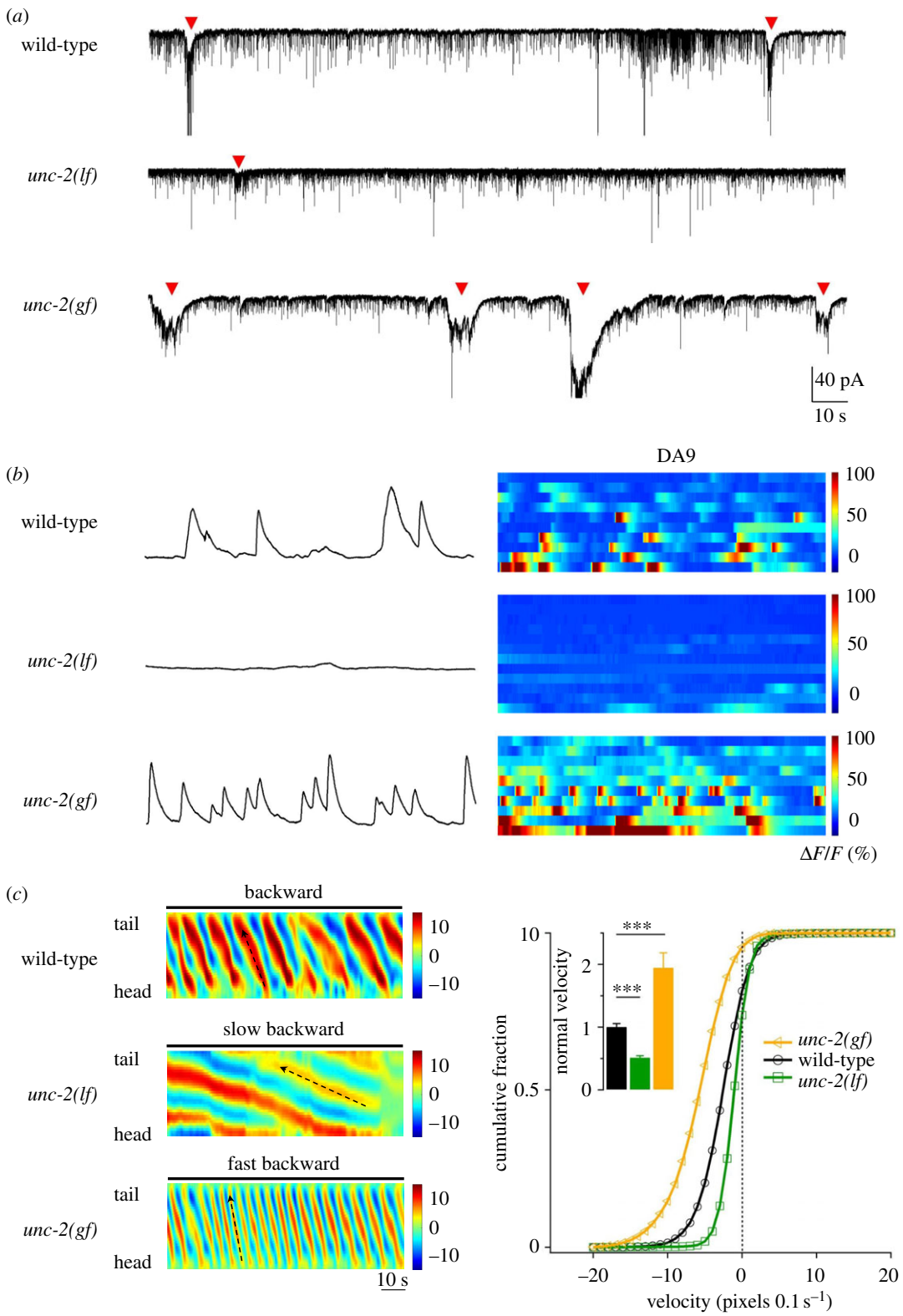

Figure 4. A high-voltage-gated calcium current UNC-2 underlies intrinsic membrane oscillation for oscillators for backward movement. (*a,b*) The decrease (lf) and increase (gf) of UNC-2 currents in A-class motor neurons led to decreased and increased amplitude and frequency of anterior A-motor neuron-dependent rhythmic rPSCs in dissected ventral cord muscle preparations, denoted by arrowheads (*a*), and reduced and increased frequency of calcium oscillation in a posterior A-class motor neuron DA9 (*b*). (*c*) These animals exhibited decreased and increased velocity during backward movement, as shown by representative bending curvature kymographs and the distribution of the instantaneous velocity of wild-type and *unc-2* mutant animals. Note that all premotor interneurons and B-class motor neurons were removed from the nervous system in these animals, reiterating the sufficiency of an intrinsic activity of A-class motor neurons to drive cohesive anterior bending propagation and organized backward movement. Figure panels adapted from [39].

fails to establish the usual imbalance in their activity output [39,53]. The weakly rectifying electrical couplings between the AVA premotor interneurons and A-class motor neurons [55] may allow activated motor neurons to antidromically amplify the excitatory chemical synaptic inputs from AVA, prolonging evoked reversals [55]. Gap junction coupling between the AVB interneurons and B-class motor neurons [28,52] may similarly enable retrograde regulation of the dynamics of AVB, subsequently modulating the coupling of multiple B-motor neurons [40].

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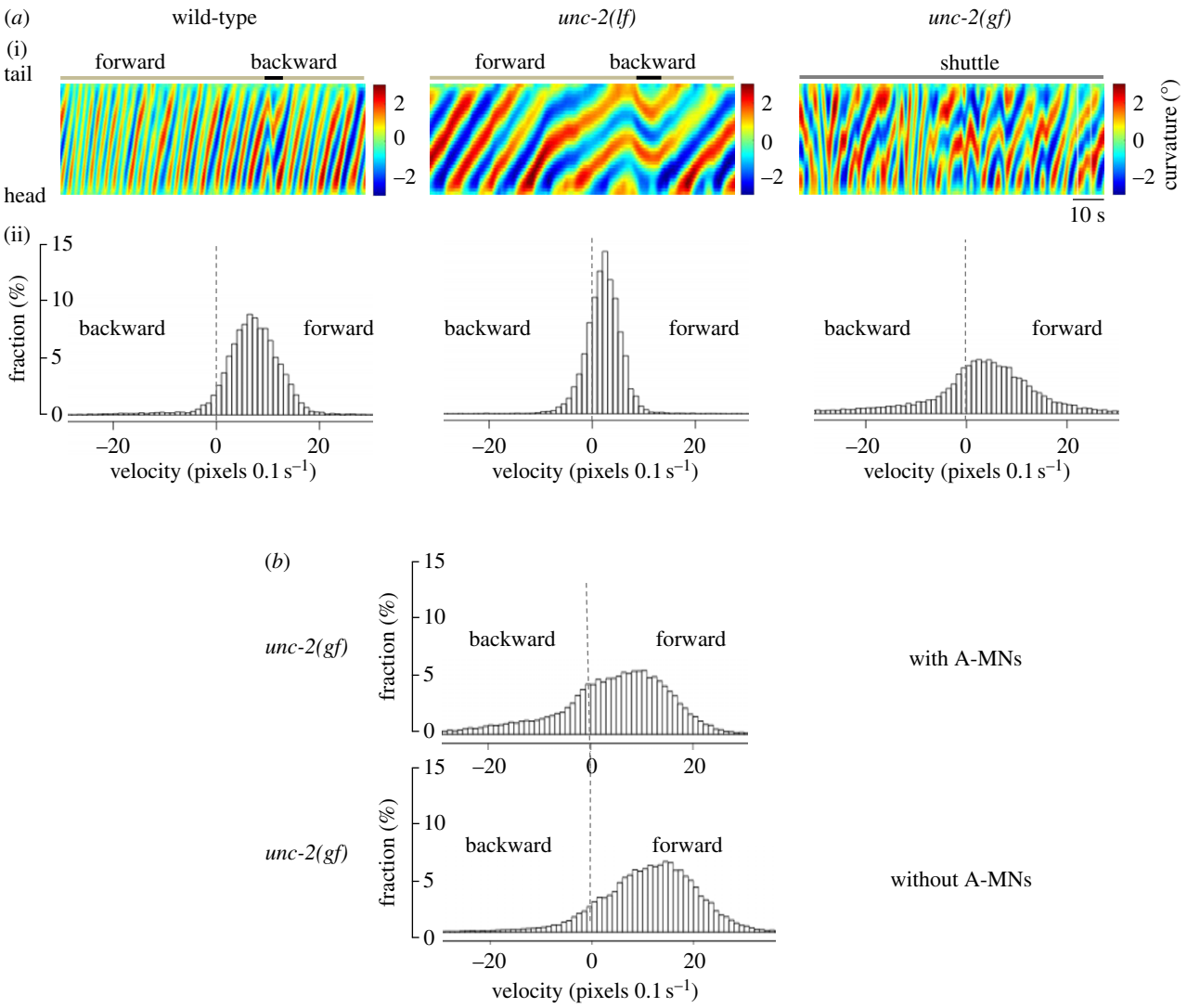

Figure 5. UNC-2 may also underlie the activity of oscillators for forward movements. (*a*) The decrease (*lf*) and increase (*gf*) of UNC-2 activity led to velocity decrease and increase in both forward and backward movements. (i) Representative curvature kymographs in respective genetic background. Wild-type animal exhibits active movements consisting of anterior to posterior bending wave propagation, with occasional and short reverse movements. (ii) Histograms of instantaneous velocity distribution by animals of respective genotypes. Positive and negative values refer to forward and backward movements, respectively. (*b*) Forward velocity continued to exhibit an increase after the A-class motor neurons were ablated in *unc-2(gf)* animals, shown as the histogram of velocity distribution. Both lines of evidence support the idea that UNC-2 activity directly affects the forward circuit.

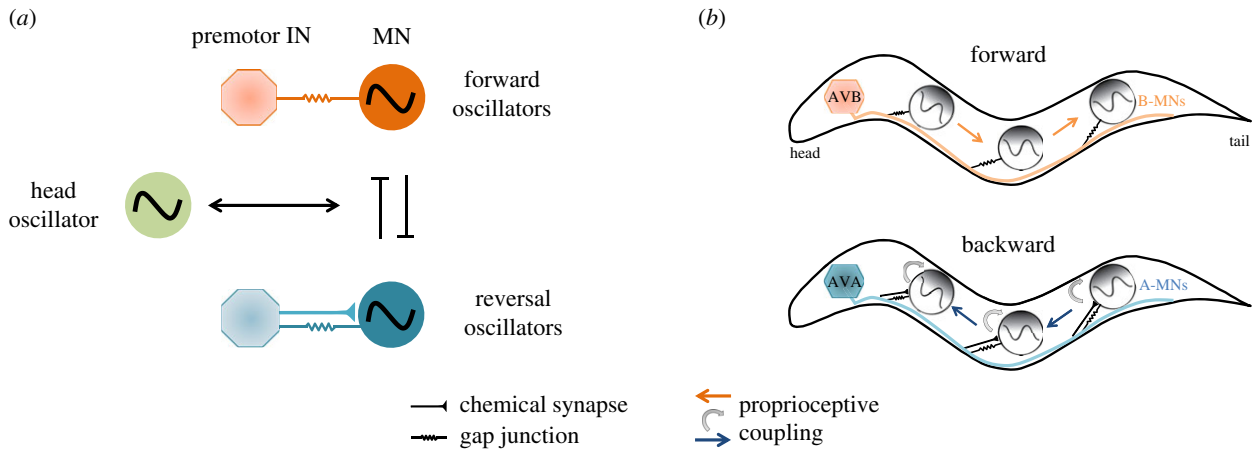

Figure 6. Schematics of a model of *C. elegans* locomotion as dynamic coupling of multiple motor states. (*a*) Head oscillation and body undulation are separately controlled. Descending inputs and directional phase-couplings allow distributed local oscillators to drive body undulation during forward and backward locomotion, respectively. A mutually inhibitory motif is introduced to flexibly control the two motor programme sub-circuits. Head–body undulation can be bi-directionally coupled with the forward or backward body undulation to generate different motor programmes. (*b*) The spatial layout of descending projection-premotor interneurons, local motor neuron CPGs and proprioceptive couplings between motor neurons for body undulation that drive forward and backward movements.

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In fish and rodents, dedicated reticulospinal neurons project from the brain stem to the spinal cord, where they innervate either excitatory or inhibitory spinal premotor interneurons. Activating those that innervate excitatory spinal premotor interneurons can initiate locomotion [23,25,56,57], whereas activating those that innervate inhibitory spinal premotor interneurons can terminate locomotion [24]. In the *C. elegans* motor circuit, premotor neurons project along the ventral nerve cord to innervate excitatory ventral cord motor neurons, and can both facilitate and halt movements. Hence, functionally, *C. elegans* premotor interneurons more closely resemble the projection neurons in more motor circuits.

5. Towards a full mechanistic and computational model of *C. elegans* locomotion

There have been continuous efforts to model *C. elegans* locomotion [33,34,58–61]. Cohen and colleagues [32,62,63] developed proprioceptive coupling models for bending wave undulation, providing insights into gait adaptation [64–66]. Recently, Olivares and co-workers [32] constructed a CPG model based on local network motifs along the ventral nerve cord; however, proposed biophysical mechanisms only partly agree with available experimental data; caveats remain that the direction of proposed CPG coupling is opposite to what has been discussed above.

With a mechanistic dissection of the oscillators and their regulation for forward and backward locomotion, we can aim for a phenomenological model with minimum assumptions and parameters that recapitulates *in vivo* neuromuscular activities and locomotory behaviours in a changing environment. Models may be developed at different levels of abstraction, guided by the principle of Occam's razor. New modelling ingredients are only added when they are required to explain experimental observations and to generate testable predictions.

Here, we propose to integrate local oscillators, proprioceptive feedback, descending inputs and neuromuscular dynamics to define the computational algorithms for generation of undulations, and their posterior- and anterior-directed propagation during backward and forward movements, respectively (figures 1*d*, 3*d* and 6*b*). Roles of other motor neurons, such as the GABAergic D-class motor neurons, which facilitate contralateral inhibition, and the AS-class motor neurons, should be incorporated in the model. Some progress has been made to characterize the forward movements [40,41], but much work remains, and no efforts have been made for the backward movements. While multiple CPG models can serve as the base to model A- and B-class motor neuron oscillations, we must first obtain further experimental dissection of their intrinsic membrane conductance, through a combination of calcium- and voltage-imaging, electrophysiological recordings and molecular genetic analyses of candidate channel mutants.

Models for other motor primitives, including head oscillation, head casting and body turning [58,67–70] may be similarly constructed. With an understanding of individual modules, we are better positioned to use the connectome [28] as a road map to model how they are coordinated, such as the interaction between head oscillation and body undulation during forward and backward movements (figure 6*a*).

During *C. elegans* sensorimotor transformation, a rapid segregation from sensory to motor representation may occur in or after the first-layer interneurons [68,71–76]. Recurrent connections between local interneurons and projection (premotor) interneurons are prominent in the *C. elegans* connectome [28]. These connections underlie the rich and organized dynamics that dictate selection and transition between different motor states.

Deciphering the computation that interneurons collectively perform remains a major task. Tackling this problem requires identifying function motifs between interneurons, defining the input–output function of each neuron and probing the dynamics of synaptic plasticity of their connections through experimental and computational approaches. Dynamical systems theory may provide insights into constructing experimentally testable models. The premise is that such models can be developed with rigour and completeness, ultimately connecting the motor control algorithms and their implementation through ion channels, synapses and neurons.

6. Towards a comparative approach for general principles for circuit architecture

Previous studies have revealed cellular and molecular features that seem to place *C. elegans* at odds with other animal models. In most locomotory networks, premotor interneurons and motor neurons exhibit rhythmic action potential bursts, but most *C. elegans* neurons, premotor interneurons and motor neurons included, do not fire classic action potentials [37,55,71,77,78].

While the *C. elegans* motor neurons exhibit the property of non-bursting oscillators, the *C. elegans* body wall muscles generate L-type voltage-gated calcium-current-dependent action potentials [79–82]. Optogenetic activation of either premotor interneurons or A-class motor neurons triggered rhythmic action potential bursts in body wall muscles [39,54]. These results indicate that the *C. elegans* locomotory network can achieve motor rhythmicity through a combined oscillatory and bursting property of motor neurons and muscles. In the absence of voltage-activated sodium channels, high-voltage-activated calcium channels take on the deterministic roles in the rhythmic output of neural circuits.

These results unveil a simplified, but functionally homologous motor circuit infrastructure for *C. elegans*: the body wall muscles convert graded synaptic transmission to digital signalling through their bursting property; the ventral cord motor neurons integrate the role of rhythm generation and proprioception; the premotor interneurons play the role of projection interneurons to activate, halt or reconfigure CPGs.

Taken together, functional compression occurs at the numerically constrained *C. elegans* motor circuit: a single neuron or neuron class assumes the role of multiple layers of the microcircuit of the spinal and ventral nerve cords. Such a property allows a small nervous system to serve as compact models to dissect organizational logic of neural circuits, exemplified by the example of intricate roles of the conserved, mix synapse configuration at the invertebrate nerve and vertebrate spinal cords.

7. Materials and Methods

Figure 2*b*. A young wildtype (N2) adult *C. elegans* was loaded into a microfluidic device filled with the NGM media. The mid-body of the worm was trapped by a straight channel (around $\sim$ 200 μm). This animal attempted spontaneous forward and backward locomotion that was distinguished by the bending waves exhibited by its head and tail. Body

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curvature of the worm was analysed and visualized with custom-written MATLAB script as described in [41].

Figure 5(*a*). We recorded 1-day old wildtype (N2), unc-2(lf) (CB55 unc-2(eff)) and unc-2(gf) (ZM9040 unc-2(hp647)) adults during spontaneous and free crawling on thinly seeded OP50 plates, and quantified their bending curvature and velocities. Recordings and analysis were carried out as described in [39,53]. (*b*) Locomotor behaviours of unc-2(gf) animals with and without the A-class motor neurons were recorded using ZM7819 (unc-2(hp647); hpIs366) 1-day old adults. To ablate the A-class motor neurons, ZM7819 L1 stage larva cultured on standard NGM plates were exposed to the blue LED light for 40 minutes with lids open. Animals were allowed to recover in darkness and grew to 24 h post-L4 stages before recording. After recording, each adult were mounted to a wide-field compound fluorescent microscope to examine whether all A-class motor neurons had been ablated. Only data obtained from animals where all A-class motor neurons were ablated were used for quantification. Non-LED light exposed ZM7819 L1 larva were cultured and imaged in parallel. They were considered the 'wildtype' control. hpIs366 is an integrated transgenic array that expresses mitochondria-targetted miniSOG in the A-class (and a few other) neurons. Detailed description of hpIs366 construction, neuron ablation, behaviral recording, and velocity analyses were described in [39].

Data accessibility. This article has no additional data.

Competing interests. Authors have no competing interests.

Funding. This work was funded by the CAS Hundred Talents Plan and the National Science Foundation of China (NSFC-31471051 and NSFC-91632102 to Q.W.), the Strategic Priority Research Program of the Chinese Academy of Sciences (Pilot study, grant XDPB10 to Q.W.), the National Science Foundation of China (NSFC-31671052 to S.G.), the Natural Science foundation of Hubei (2018CFA039 to S.G.), Wuhan Morning Light Plan of Youth Science and Technology (2017050304010295 to S.G.), the Junior Thousand Talents Program of China (to S.G.) and the Canadian Institute of Health Research (CIHR-MOP93619, MOP123250 to M.Z.).

Acknowledgements. We are grateful for discussions with our colleagues, most prominently Christopher Fang-Yen and Alexander Gottschalk.

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