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Current Opinion in Neurobiology

C. elegans locomotion: small circuits, complex functions

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With 302 neurons in the adult *Caenorhabditis elegans* nervous system, it should be possible to build models of complex behaviors spanning sensory input to motor output. The logic of the motor circuit is an essential component of such models. Advances in physiological, anatomical, and neurogenetic analysis are revealing a surprisingly complex signaling network in the worm's small motor circuit. We are progressing towards a systems level dissection of the network of premotor interneurons, motor neurons, and muscle cells that move the animal forward and backward in its environment.

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Current Opinion in Neurobiology 2015, **33**:117–126

This review comes from a themed issue on **Motor circuits and action**

Edited by **Ole Kiehn** and **Mark Churchland**

http://dx.doi.org/10.1016/j.conb.2015.03.009

Invertebrates have long been fruitful model systems to pursue a computational understanding of rhythmic motor circuits. The small numbers of neurons, defined motor patterns, and physiological accessibility of the stomatogastric nervous system of the crustacean and the locomotion circuit of the leech have driven decades of fundamental experimental and theoretical work on underlying mechanisms [1,2]. By comparison, understanding the principles of rhythm generation during locomotion in *Caenorhabditis elegans*, one of the most fruitful invertebrates for genetic analysis, has lagged behind. Computational models have been proposed based on the known anatomy of the motor circuit but much speculation about signaling properties [3]. New tools for physiological analysis, including genetic sensors for calcium, light-gated regulators for cell activity, electrophysiology, and quantitative behavioral analyses, have begun to allow functional dissection of the molecules, synapses, and cells of the *C. elegans* motor circuit. Here, we present current understanding of the motor circuit that drives backward and forward undulatory movement. We highlight recent advances, remaining questions, and hypotheses about the neuromuscular machinery that drives *C. elegans*' motor behavior.

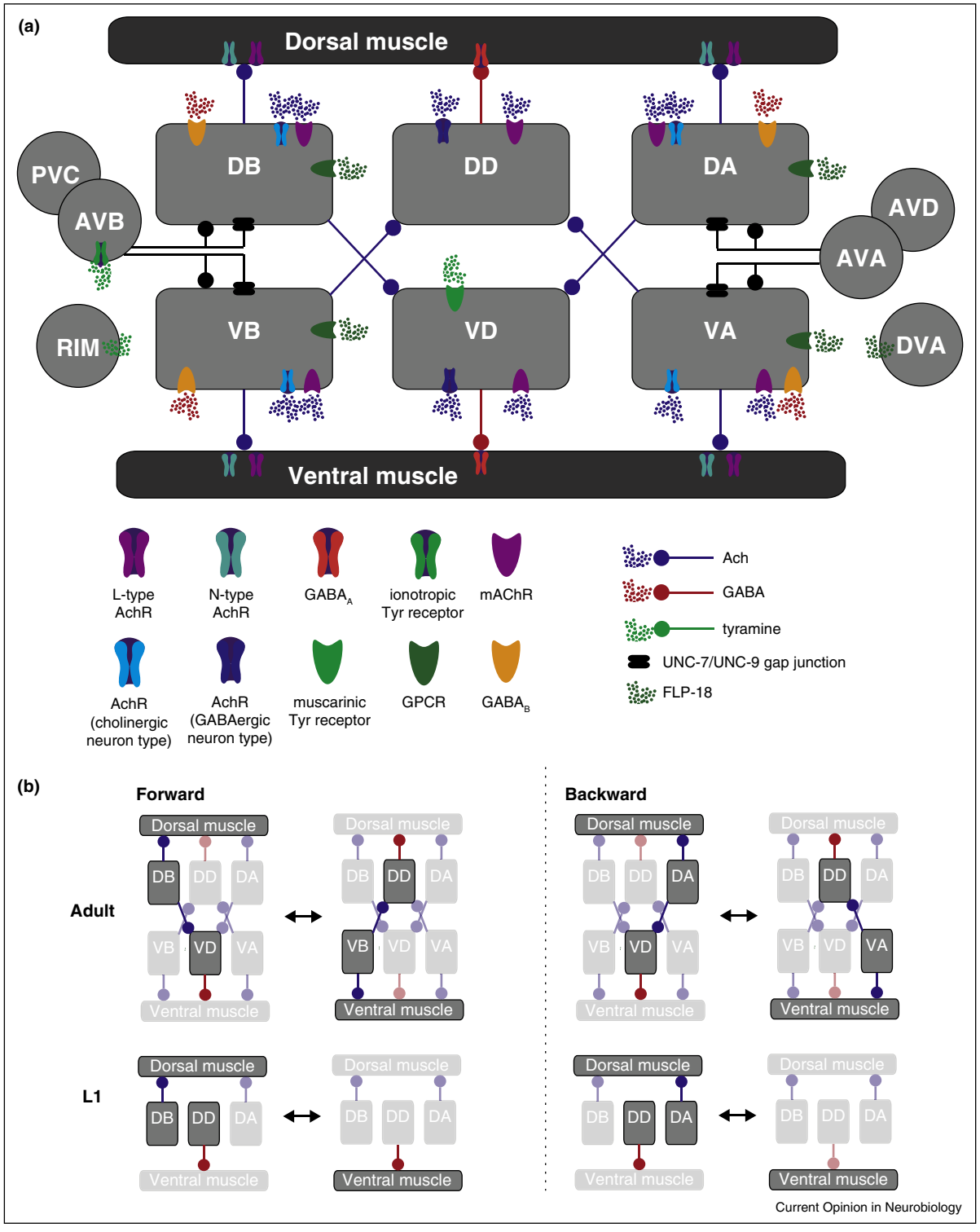
The anatomy and wiring of motor neurons

Most motor neurons that drive undulatory locomotion along the *C. elegans* body — 22 in newly hatched L1 larvae and 75 in subsequent developmental stages — are distributed along the ventral nerve cord (VNC). VNC motor neurons are grouped into five classes, each with different neuron numbers: A (21), B (18), D (19), VC (6), and AS (11). The A, B, and D classes are further divided as the dorsal and ventral muscle innervating subclasses, DA (9), VA (12), DB (7), VB (11), DD (6), and VD (13) [4]. The newly hatched larvae have only the DA, DB and DD class motor neurons (see Figure 1a,b).

Although *C. elegans* is not obviously anatomically segmented, the adult motor circuit exhibits a general pattern: six repeating units of ~12 motor neurons and ~12 muscle cells distributed along the adult body [5,6]. This organization is proposed based on electron microscopy (EM) reconstruction of a few such motifs in adults [5,7]. Motor neurons of the same class are connected to their neighbors by gap junctions. The A and B-type neurons make cholinergic excitatory neuromuscular junctions (NMJs) with muscle cells [8,9]. The D-type neurons make GABAergic inhibitory synapses to muscle cells [10]. The D-type GABAergic neurons are postsynaptic to the cholinergic A and B-type motor neurons that innervate muscles of the opposite side (see Figure 1a,b).

C. elegans was the first animal whose connectome was mapped in near entirety using serial-section EM [11]. The adult wiring diagram was assembled from partially overlapping datasets from five animals. The wiring of the VNC and dorsal nerve cord (DNC), where a majority of NMJs reside, was assembled in the anterior portion using one animal. A posterior portion was assembled from other animals, including data from a male. The original data obtained by John White and coworkers have been reanalyzed [12,13] and revisited through other studies, including an ongoing proofreading project (Steven Cook, David Hall, and Scott Emmons, personal communications). However, these efforts will not compensate for the missing serial sections for 39 neurons, which include

Figure 1



The *C. elegans* motor circuit. **(a)** Complex signaling. A simplified representation of one structural motif of the *C. elegans* motor circuit. It illustrates some connectivity between cholinergic motor neurons (B-type, for forward movement; A-type, for backward movement) and GABAergic neurons (D-type), and muscle cells. Ionotropic and metabotropic cholinergic and GABAergic receptor subunits in the motor neurons and muscle cells that have been identified to play a role in excitatory and inhibitory input balance are depicted (see text). Gap junctions between motor neurons and key premotor interneurons (AVA, AVB) play a role in stabilization and transition between backward and forward movement. Tyraminergic and peptidergic signaling from sensory and interneurons (DVA, RIM) regulate motor neuron activity patterns via GPCRs. **(b)** Developmental remodeling.

21 motor neurons [7]. It will be important to fill the remaining gaps of the adult motor circuit. It will also be important to obtain wiring diagrams that have yet to be mapped. This includes those of the developing larvae (L1–L4 stage), dauer (a diapautic larval stage induced by unfavorable growth conditions), and males (only a posterior portion of an adult has been mapped) [14].

Distinct motor neuron pools contribute to directional movements

Undulation is largely organized by the A-type and B-type cholinergic neurons and the D-type GABAergic motor neurons. Our initial understanding of their roles was established by laser ablation [15]. In the newly hatched larvae (L1), where only the DA, DB and DD class motor neurons are present, killing a portion of the DA and DB motor neurons disrupted the larvae's ability to move backward and forward, respectively. By contrast, ablating a portion of the DD motor neurons severely disrupted movement in both directions. These results provide the first evidence for separate cholinergic motor neuron pools for forward versus backward movement. While the numerical increase of adult motor neurons discouraged similar laser ablation analyses of the adult motor circuit, a similar role of the same class of cholinergic motor neurons for directional locomotion was substantiated by laser ablation analyses of their input premotor interneurons. Cholinergic A-type and B-type motor neurons are postsynaptic to five pairs of premotor interneurons: AVA, AVB, AVD, AVE, and PVC [11]. AVB and PVC connect mainly to the B-type motor neurons, and their co-ablation led to near complete impairment of forward movement. AVA, AVD, and AVE connect mainly to the A-type motor neurons, and killing them, in particularly AVA, profoundly disrupts backward movement [15,16]. Hence, across development, the A-type and B-type cholinergic motor neurons differentially contribute to backward and forward movements.

The role of the D-type GABAergic motor neurons is less defined and may change during development (Figure 1b). In adults, because the D-type motor neurons are postsynaptic to the A-type and B-type cholinergic motor neurons that innervate muscles on the opposing side, they have been proposed to play a role in contralateral inhibition, similarly to analogous neurons in the nematode *Ascaris* [17], during forward and backward movement [11]. Consistent with this notion, killing VD motor neurons causes ventral biased bending, and killing DD motor neurons causes dorsal biased bending during locomotion [18••]. Optogenetic stimulation or inhibition of DD motor neurons also induces ventral or dorsal bending, respectively [18••]. However, bending is not abolished by ablating either VD or DD [18••], implying a modulatory, not mandatory role for contralateral inhibition in adult movement. In fact, D-type motor neurons are not even necessary for forward locomotion: in the absence of the GABA-synthesizing glutamate-decarboxylase (UNC-25), *unc-25* mutants exhibit fairly normal forward locomotion (albeit being slightly hyper-contracted), and only display dampened backing [10]. This contrasts with the requirement of DD motor neurons for both forward and backward movement in the L1 larvae [15], implying a developmental remodeling.

In vertebrates, GABAergic synapses switch from being excitatory at early stages of development to inhibitory upon increased expression of chloride exporters that reverses the chloride gradient across the neuronal membrane [19]. In *C. elegans*, D-type motor neurons also remodel during early development, but this involves a more complex and not yet understood process. The L1 larvae have six GABAergic D-type motor neurons that persist as the adult DD motor neurons. Partial EM reconstruction at the anterior segment of two L1 larvae revealed ventral NMJs by DD motor neurons, instead of dorsal NMJs that characterize their adult wiring [20]. The ventral NMJs by L1 DDs were confirmed using presynaptic markers [21]. DD motor neurons reverse their axon-dendrite polarity to innervate dorsal muscles in the L2 larval stage and beyond, a process that remains poorly understood [21,22]. The physiological nature of GABAergic signaling in L1 larvae has not yet been confirmed. However, they may be inhibitory as in adults, because acute killing of L1 DD motor neurons by KillerRed induced strongly biased ventral coiling [23]. Surprisingly, the inhibitory nature of GABA signaling may be maintained throughout the L1 stage despite the recent observation that the postsynaptic muscles likely reverse the chloride gradient at mid-L1 [24]. Hence, while the two main cholinergic motor neuron classes (A and B) are dedicated to generate forward and backward movements from birth, the GABAergic D-type motor neurons exhibit substantial developmental plasticity that allow them to modulate locomotion patterns with different mechanisms at different life stages.

Signaling between motor neurons and muscles balances the excitatory and inhibitory drive

In vertebrates, the interplay between excitation and inhibition that establishes the phasic motor activity patterns occurs at higher levels, for example, between glutamatergic and GABAergic/glycinergic interneurons that

(Figure 1 Legend Continued) During adult locomotion, alternating excitation and inhibition of dorsal and ventral muscles is mediated by B-type and D-type motor neurons during forward movement and is mediated by A-type and D-type motor neurons during backward movement. In L1, only the DA, DB, and DD motor neurons are present, and the DD motor neurons innervate the ventral muscle.

communicate with the cholinergic motor neurons to coordinate muscle contraction [25,26]. The small nervous system of *C. elegan* is compact — only two to three layers of interneurons separate the sensory periphery to motor neurons [27–29]. Although the motor neuron layer contains only a handful of cell types, neurogenetic analyses are revealing a complex molecular signaling network that may augment its computational power.

The *C. elegans* genome contains twenty-nine acetylcholine receptor (AchR) and four GABA receptor subunit-encoding genes [30–32]. The potential for receptor diversity in the motor circuit is enormous, and its significance is only being unraveled. Patch-clamp recordings of body wall muscles allowed direct demonstration of dual excitatory and inhibitory signaling at the neuromuscular system. Specifically, muscles elicit the GABA-mediated and Ach-mediated postsynaptic currents by the inhibitory UNC-49 ionotropic $GABA_A$ receptors and two classes of excitatory, ionotropic acetylcholine receptor complexes with different pharmacological properties (the levamisole L-type and nicotine N-type AchRs) [8,33–36].

A third class of AchRs reside at motor neurons. Neuronal ionotropic acetylcholine receptors (nAchRs) resemble the L-type AchRs in composition, but involve neuron-specific alpha and non-alpha subunits, and are insensitive to levamisole [36,37•,38•,39]. Unexpectedly, nAchRs further diverge in composition and localization: they are dendritic in all motor neuron classes, but are restricted postsynaptically only at GABAergic motor neurons. At least one non-alpha nAChR subunit (ACR-2) is unique for cholinergic motor neurons (A-type and B-type), predicting the existence of additional neuron-subclass AchR subunits and regulators. nAchRs regulate excitatory and inhibitory inputs at the neuromuscular system by directly effecting motor neuron activities [37•,39]. Null mutations for the cholinergic motor neuron-specific subunit ACR-2 lead to not only reduced cholinergic inputs to muscles, but also reduced GABAergic inputs. Gain-of-function *acr-2* mutations increase cholinergic motor neuron inputs, as well as exert an inhibitory effect on GABAergic inputs. All nAchRs contain the ACR-12 alpha subunit, but the loss of ACR-12 from GABAergic motor neurons alone lead to increased excitability at the neuromuscular system due to a reduced inhibitory inputs [38•]. In summary, nAchRs are not only required in excitatory and inhibitory motor neurons to maintain their respective activities, nAchRs in cholinergic motor neurons further function non-cell autonomously to regulate the activity of GABAergic motor neurons.

Through AchRs and GABA receptors that function both at motor neurons and body wall muscles, excitatory and inhibitory signals converge at muscles to affect body bending. A standing puzzle is the surprisingly subtle changes in motor patterns upon even drastic genetic perturbation of the inhibitory versus excitatory input balance (e.g., the complete removal of GABAergic signaling). The diversity of receptors indicates an intricate network of signaling relationships throughout the motor circuit that can be subjected to fine-tuning and modulation (Figure 1a), and may permit similar motor performance to be generated in a variety of mutant backgrounds and environmental conditions. The robustness of the *C. elegans* motor output parallels similar observations of the robust motor performance in other invertebrates, for example, the lobster stomatogastric ganglion [40,41]. Establishing the full network interactions that drive and stabilize the performance of the motor circuit will require a systems level approach.

Neuromodulation by metabotropic signaling and feedback inhibition

Neurotransmitter-activated, extrasynaptic G-protein coupled receptors (GPCRs) further extend the signaling capabilities of motor neurons by mediating feedback inhibition [42]. The *C. elegans* genome contains three muscarinic acetylcholine receptors (mAChR) and two $GABA_B$ receptor subunits [31]. The GAR2 mAChR is expressed in both cholinergic and GABAergic motor neurons, and contributes to a feedback inhibition of the cholinergic motor neuron activity when acetylcholine levels are elevated [42]. The GBB-1/GBB-2 heterodimeric $GABA_B$ receptors are specifically expressed by cholinergic motor neurons. Cholinergic activation of the GABAergic motor neurons stimulates GABA release, which, through these $GABA_B$ receptors, provides an alternative feedback inhibition on cholinergic motor neurons [42,43].

In addition to neurotransmitters, neuropeptides can regulate the excitatory and inhibitory input balance at the neuromuscular system [44]. The gain-of-function mutations in ACR-2, which amplify excitatory inputs and reduce inhibitory inputs to muscles [37•,44], exhibit periodic convulsions due to synchronization of cholinergic motor neuron activities [45]. Such a convulsion is partially offset by an increased production of FMRFamide neuropeptides, FLP-1 and FLP-18, from cholinergic motor neurons. This convulsion is also partially mitigated by increasing the expression of GPCRs — NPR-1 in neurons and NPR-5 in muscles. The loss of a common processing enzyme for most neuropeptides EGL-3 also partially mitigates convulsion [44]; hence additional neuropeptides may contribute to homeostatic regulation.

Neuromodulation has long been known to profoundly affect the invertebrate motor systems. In *C. elegans*, specific monoamines and neuropeptides have recently been shown to modify motor circuit output. For example, the stretch-activated mechanosensory neuron DVA regulates bending amplitude during locomotion [46]. This modulation

involves peptidergic modulation of cholinergic motor neurons: DVA releases the NLP-12 neuropeptide, which potentiates cholinergic NMJs via the CKR-2 GPCR [47••]. This points to a mechanosensory gain control mechanism in the motor circuit. Perhaps the most profound example of neuromodulation in the motor circuit is that a motor step in the escape response depends on monoaminergic modulation [18••]. Tyramine, released from the RIM premotor neurons following the anterior touch, augments the amplitude of the ventral bend to reorient *C. elegans* away from the stimulus. The SER-2 GPCR tyramine receptor is specifically expressed in the VD GABAergic motor neurons; its activation inhibits GABAergic inputs to ventral muscles, allowing deep bending.

Different locomotion patterns require a homeostatic and dynamic modulation between excitatory and inhibitory inputs to muscles. The large inventory of signaling molecules and receptors in the motor circuit, and particularly the feedback loops that connect them, poses both challenges and opportunities in modeling dynamic motor behaviors. Realistic computational models will need defined dynamics of signaling relationships throughout the motor circuit. To define those relationships, we need methods to measure motor neuron activities in moving animals with comparable sensitivity to electrophysiological recordings from muscle cells [8,48••]. New ultrafast genetically encoded probes for calcium dynamics as well as for membrane potential are promising, but still need to be improved for systematic dissection of motor circuit dynamics [49–54].

Reciprocal activation of the A-class and B-class motor neurons by premotor interneurons to transit between directional movements

The first successful *in vivo* calcium imaging in intact animals was achieved using *C. elegans* and a ratiometric genetic calcium sensor cameleon [55]. The relatively low signal-to-noise ratio of early versions of genetic calcium sensors required animals to be immobilized. But small signals precluded reliable characterization of the motor circuit, where recording movement is essential to interpreting correlation and causality between the motor and circuit activities. With steady improvement on calcium sensors for increased signal/noise ratio and kinetics, ratiometric techniques for motion artifact correction [49,56], and comparison of results from different imaging setups and analysis algorithms, it has become feasible to examine physiologically relevant motor circuit patterns by calcium imaging [57•,58•,59,60••,61•,62,63].

Results from optical neurophysiology of the motor circuit are strongly dependent on recording conditions. In an early preparation in which the anterior half of the animal was glued, the activity of DB, VB and VA motor neurons in the fixed portion was correlated with the residual motion of the unglued posterior. In this preparation, B-type neurons and VA neurons were active during bouts of anticipated forward and backward movement, respectively. However, the rhythmic activity expected to accompany wave propagation and alternating DB and VB activity expected to accompany alternating ventral and dorsal bending were not observed [64]. In more recent setups where animals were allowed to crawl on agar pads under coverslips, the alternating DB/VB and DA/VA activity pattern change could be observed, as well as a simultaneous increase and decrease of the A and B-type motor neuron activity when animals switch from forward to backward movement, and a reciprocal change when switching from backward to forward movement [60••].

The cholinergic A-type and B-type motor neurons are postsynaptic to premotor interneurons [11]. At the premotor interneuron level, an increase of AVA (connected to the A-class motor neurons) and AVB (connected to the B-class motor neurons) activity coincides with the onset of backward and forward movement, respectively, consistent with their inferred role for driving backward and forward movement [15,27,57•,59]. Calcium levels in premotor interneurons however do not oscillate, even in animals moving throughout undulation cycles. Therefore, unlike in vertebrates, the central pattern generators for movement may not be coded in interneurons [65]. Rather, these premotor interneurons establish and shift between stable forward and backward motor states.

It has long been appreciated that the motor circuit network harbors substantial gap junction connectivity between premotor interneurons and motor neurons [11,66•]. Recent studies reveal that these gap junctions drive the switch between forward and backward states [60••]. During forward locomotion, the B-type motor neurons exhibit higher levels of activity than the A-type motor neurons, and vice versa during backward movement. This imbalance requires the UNC-7–UNC-9 heterotypic gap junctions between the AVA premotor neurons and A-type motor neurons, with a minor involvement between the AVB and B-type motor neurons [60••,66•]. Forward and backward promoting premotor interneurons exhibit anti-correlated activity changes, even when animals were restricted for limited movement, indicating an intrinsic cross-inhibition between these neurons. Information flows directionally from premotor interneurons to motor neurons, allowing the premotor interneurons to shift the motor circuit states. Gap junctions between AVA and A further function as current shunts to reduce AVA excitability at rest, biasing the circuit state for forward movement [60••].

Proprioceptive feedback to propagate bending waves during forward movement

It has long been appreciated that the motor circuit of *C. elegans* has a mechanism for proprioceptive feedback.

The cholinergic motor neurons have long neurites devoid of synaptic specializations. Byerly and Russell first suggested that these neurites might serve a stretch-sensing role. Several computational models of the motor circuit predicted the requirement for stretch-sensitive feedback to generate robust traveling waves with proper adaptation to external load [67–70]. Optogenetic and genetic manipulation of different motor neuron classes, combined with calcium imaging and microfluidic devices that allowed control of body bending, showed that proprioceptive feedback occurs within the motor circuit itself, localized to the B-type cholinergic motor neurons during forward locomotion [71••]. When B-type motor neurons sense the bending of anterior segments, they drive local bending. When the dynamics of proprioceptive coupling are combined with *C. elegans* biomechanics, a viscosity-dependent change in undulatory wavelength can be predicted to closely fit experimental observation [72•].

Analog to digital conversion at the NMJ

A dynamical model of the motor circuit is not sufficient. We also need to know the nature of the transformation of motor neuron activity into muscle activity at the NMJ. Recent advances in electrophysiology have produced a detailed understanding of this transformation. Because of the absence of voltage-gated sodium channels in its genome [31], it had been thought that worms lack classical action potentials. Steadily improving electrophysiological preparations, however, have revealed regenerative potentials with different molecular mechanisms [8,48••,73–78]. Calcium-dependent, regenerative plateau potentials have been observed in some neurons in the head ganglion [76]. Calcium-dependent, all-or-none action potentials have been observed in the body wall muscles of dissected worms, and are driven by the L-type voltage-gated calcium channel and the Kv1 voltage-gated and Ca^{2+}/Cl^{-}-gated potassium channels [48••,79••]. *C. elegans* muscle action potentials are longer in duration than those of the mammalian skeletal muscles. The resting potential of body wall muscles (∼−25 mV), significantly higher than that of vertebrate muscles, is intermediate between the reversal potentials of ionotropic acetylcholine and GABA receptors under the recording condition, allowing bidirectional modulation by excitatory and inhibitory neurotransmitters. Cholinergic and GABAergic inputs potentiate and reduce action potential frequency, respectively, and in turn correlate with muscle contraction and relaxation.

Whereas muscle cells respond in a digital manner, motor neuron outputs appear tonic and graded [80•]. This was demonstrated by electrophysiological recording of muscle cells upon optogenetic manipulation of motor neurons: tonic neurotransmitter release is lowered by hyperpolarizing cholinergic motor neurons and raised by depolarizing cholinergic motor neurons in proportion to the strength of stimulation. The NMJs exhibit short-term plasticity. Cholinergic synapses are depressed by high-frequency stimulation whereas GABAergic synapses may facilitate before depression. How information is processed by the *C. elegans* nervous system likely requires years to become fully resolved, but if its muscular system has the ability to convert graded inputs into digital outputs, such a property should contribute to the neural network's ability to generate stable motor functions.

The biomechanics of undulatory movement

Motor circuit signaling organizes muscle activity to trigger movements within the physical constraints of the body and its contact with the environment. Therefore, modeling motor circuit outputs should include biomechanics. *C. elegans* can swim, burrow and crawl. Thus, its motor circuit is extraordinarily adaptable to the physics of the environment. In the laboratory, worms typically swim in water or crawl on agar surfaces, circumstances that already reveal considerable gait adaptation. The undulation wavelength of a swimming worm is roughly twice the body length, generating alternating C-shapes with each ventral or dorsal muscle contraction. When crawling on a wet surface, they endure forces that are ∼10,000-fold larger than water viscosity due to surface tension and friction. Under such a dramatic increase in external load, they adapt gait by reducing both undulation frequency (from ∼2 Hz to ∼0.2 Hz) and wavelength (from ∼2 to ∼1/2 of the overall length) [72•,81,82].

How can *C. elegans* respond to such an extreme load variation with such a modest gait change? To put this feat in perspective, a person would have to swim with the same motion in molasses as in water. Biomechanical measurements of the worm body provide some insight. The stiffness of the body is due to both high elastic modulus of its cuticle (the Young Modulus, E = 10–400 MPa, is comparable to rubber) and internal viscous shear [72•,83,84]. Using measured viscoelasticity, it is possible to estimate the muscle power that is required to bend the body itself or to push the body against surroundings. In environments that pose little resistance (e.g., water), most muscle power is used to drive the bending of a stiff body. Only when the viscosity of surroundings increases by ∼100-fold does the muscle power needed to push against the environment begin to compare with the power needed to bend the body. For over a ∼10,000-fold increase in environmental viscosity, the muscle power varies by less than twofold [72•]. Hence, the *C. elegans* motor circuit operates in low gear, pushing the animal through high resistance environments, and exhibiting little acceleration in low resistance environments.

One of the unique advantages of studying *C. elegans* is the opportunity to compare the circuits across the developmental time course, and across the different body plans of the hermaphrodites, male or dauer larva. Current motor circuit analysis has mostly been performed in young adult hermaphrodites. To compare and contrast the motor circuits and movements of different forms of the same

animal should yield deeper understanding. Laying the groundwork, biomechanics has been carefully measured across development, from the L1 larva to the adult [85•]. This was made possible by a versatile micromechanical device that measures internal viscoelasticity at all size scales. The worm was found to be self-similar, exhibiting consistent biomechanical properties throughout development. This general property of self-similarity was recently shown to be able to predict the dynamics of undulatory waves in the male, by simply applying a biomechanical model developed for the hermaphrodite to the male's more slender body [71••,86]. An open question is how the physiology and wiring of the motor circuit might also adapt to the dramatic increase in body size as animals grow from the L1 larva to the adult, or whether the motor circuits adapt to behavioral difference between the hermaphrodite, male, and dauer larvae. Mapping more wiring diagrams will help to answer such questions.

Remaining gaps in basic motor circuit dynamics

Key questions remain in how motor circuit dynamics is encoded. Muscles receive excitatory and inhibitory inputs from motor neurons and convert them to orchestrated muscle bending via digital output of electrical signals (action potentials). Multiple AchR and GABAergic receptors of different properties reside and function from both classes of motor neurons to muscles (Figure 1a). The enormous diversity of receptor subunits could potentially endow an intricate network of signaling relationships throughout the motor circuit, but the physiological property difference among different receptor clusters remains to be defined. How excitatory and inhibitory signaling at muscles is regulated temporally and spatially to define the various bending patterns should be better addressed.

Synaptic interconnections between premotor interneurons likely help the system select and maintain each movement direction. But AVA/AVE and AVB do not exhibit obvious reciprocal wiring and how cross-inhibition is achieved is unresolved. A minimalistic theoretical analysis of the wiring of the motor circuit, based on the effects of systematic laser ablation of the premotor interneurons on the velocity and duration of forward and backward movements, proposed that most signaling relationships among premotor interneurons should be inhibitory [87]. This theory can be tested experimentally. Current studies suggest that the interplay between premotor interneurons is likely much more complex. Cross-inhibition may involve additional premotor interneurons, such as RIM, that make direct synaptic connections with both backward promoting (AVA) and forward promoting (AVB) premotor interneurons. Upon activation, RIM can activate AVA via gap junctions, and release tyramine to inhibit AVB through a tyramine-gated chloride channel LGC-55 [61•,88]. It may also involve inputs from upper layers such as interneuron AIB [61•] and sensory neuron ASH [75].

Another main gap is identifying the rhythmic generator (CPGs) for either forward or backward movement. The progressively decreased anterior–posterior bending wave led to the prediction of oscillators at the head for both forward and backward movements, but their identities are unknown [70]. In the small nervous system of *C. elegans*, CPGs might be encoded in single neurons, using networks of molecular interactions to auto-regulate membrane potentials. In theory, it is possible to generate rhythmic activities by endowing neurons with a combination of stretch-sensitivity and bi-stability, for example, by triggering membrane potential reversals with each phase of dorsal and ventral bending [68,69]. Stretch-sensitivity has been shown to contribute to propagation of undulatory waves in *C. elegans* [71••]. Emerging methods in high-speed pan-neuronal imaging of the *C. elegans* nervous system may help to resolve these issues [89••,90••]. Simultaneous imaging of many neurons may help to locate the source of rhythmic activities that are translated into propagating undulatory movements, and uncover the switch of the neural network states that accompanies transitions between the motor states.

Conclusions

Much theoretical and experimental work have focused on how the higher level signal processing allows the chemosensory, thermosensory, and mechanosensory inputs to trigger motor responses (e.g., [91–95,96•,97,98•,99•]). Understanding the logic of how the motor circuit generates and transits between the basic motor patterns in its repertoire should lay an essential framework for understanding how complex behaviors such as tactic navigation or the escape response are organized. An improved toolbox is in place to dissect motor circuit activity and behavior in *C. elegans*. The worm may yet become the animal that allows full modeling of complex behaviors from perception to motion without gaps.

Conflict of interest statement

Nothing declared.

Acknowledgements

We appreciate thoughtful comments from Andrew Chisholm. We have narrowly focused on recent literature on the motor circuit for undulation and we apologize to authors who have done seminal work in other areas of *C. elegans* neurobiology that has facilitated the progress on the motor circuit. ADTS was supported by the NIH, NSF, and HFSP. MZ was supported by the CIHR, NSERC, and HFSP.

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- •• of outstanding interest

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