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REVIEW

Feedback to the future: motor neuron contributions to central pattern generator function

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ABSTRACT

Motor behaviors depend on neural signals in the brain. Regardless of where in the brain behavior patterns arise, the central nervous system sends projections to motor neurons, which in turn project to and control temporally appropriate muscle contractions; thus, motor neurons are traditionally considered the last relay from the central nervous system to muscles. However, in an array of species and motor systems, an accumulating body of evidence supports a more complex role of motor neurons in pattern generation. These studies suggest that motor neurons not only relay motor patterns to the periphery, but directly contribute to pattern generation by providing feedback to upstream circuitry. In spinal and hindbrain circuits in a variety of animals – including flies, worms, leeches, crustaceans, rodents, birds, fish, amphibians and mammals – studies have indicated a crucial role for motor neuron feedback in maintaining normal behavior patterns dictated by the activity of a central pattern generator. Hence, in this Review, we discuss literature examining the role of motor neuron feedback across many taxa and behaviors, and set out to determine the prevalence of motor neuron participation in motor circuits.

KEY WORDS: CPG, Behavior, Circuit, Collateral, Locomotion, Vocalization, Feeding

Introduction

Behaviors are produced by contractions of muscles, which in turn are controlled by the firing of a specific set of motor neurons. What circuits produce stereotyped behavioral patterns? In nearly all rhythmic motor behaviors that have been studied, there is evidence supporting the involvement of central pattern generators (CPGs; see Glossary). These circuits are defined by their ability to generate rhythmic motor patterns in the absence of sensory feedback or other rhythmic inputs (Marder and Bucher, 2007). Often, motor neurons are considered merely the relay from the CPG to the muscles, but what if motor neurons are involved in generating behavioral rhythms as well? In this Review, we describe the mounting evidence that motor neurons contribute to CPG activity across diverse phyla and behaviors. We propose that ongoing research on motor circuits should address the potential role of motor neurons. Toward this aim, we describe how established and emerging technologies can facilitate the discovery of motor neuron involvement in CPG circuits and provide causal relationships between motor neuron function and CPG activity.

Origins of CPG theories

Thomas Graham Brown (1911) performed experiments in which signals to the cat spinal cord – both descending inputs from the brain and sensory inputs from the periphery – were eliminated; these cats remained able to produce rhythmic stepping behavior, suggesting the presence of intrinsic oscillating circuits located in the spinal cord. Brown proposed a 'half-center' model, in which motor neurons and interneurons (see Glossary) generate locomotor rhythms via reciprocal inhibition of the neurons that control flexor and extensor muscles. However, for many decades, Brown's results were largely ignored. Brown's findings were re-explored by Anders Lundberg and his students, leading to work on vertebrate locomotion in a variety of species including cats, rodents and lampreys (for review, see Stuart and Hultborn, 2008). Around the same time, the concept of CPGs was also influenced by the findings of Don Wilson (1961) on locust flight; similar to findings in vertebrates, flight rhythms generated by the nervous system persisted in the absence of sensory input from the periphery and descending inputs from the brain. CPGs are experimentally powerful because they can often be activated and studied in isolated brains, producing 'fictive behaviors' (see Glossary) in which circuit output closely resembles naturally observed behavior patterns (Marder and Calabrese, 1996). Over the next several decades, neurobiologists began studying the CPGs underlying a variety of motor behaviors, and it is now accepted that CPGs underlie most, if not all, rhythmic behaviors (Goulding, 2009; Marder and Bucher, 2007; Marder and Calabrese, 1996).

While it is now well established that CPGs play an important role in the production of motor patterns, misconceptions about the role of motor neurons in these circuits remain. This is evident from textbook descriptions of motor pathways, in which motor neurons are considered to be merely the relay from pattern-generating circuits to muscles (Fig. 1A). In this Review, we describe a variety of network architectures in which motor neurons can provide feedback signals to upstream CPG neurons (Fig. 1B–D), and suggest that motor neurons may be considered to be components of many CPGs (Fig. 1E).

Anatomical evidence of motor neuron connections to premotor cells

Some of the earliest work in neuroscience began by methodically describing the anatomical features of the brains of several vertebrate species. Perhaps the most famous of the scientists performing this work was Santiago Ramón y Cajal, whose results indicated that motor neurons make connections with central neurons, rather than solely to muscles in the periphery. Ramón y Cajal described the presence of motor neuron axon collaterals (see Glossary) – axon branches that remain within the nervous system rather than targeting muscles in the periphery – in the spinal cord of mammals, amphibians, birds and reptiles, and he found that they were particularly prevalent in mammals. He speculated that these branches transmit information from motor neurons to neighboring cells, perhaps functioning to recruit other motor neurons (Ramón y Cajal, 1995). In contrast to those in the spinal cord, Ramón y Cajal

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Glossary

Antidromic stimulation
Electrical stimulation of axons (for example, those found in a motor nerve) that induces axonal action potentials, which propagate back to the neuronal cell body. Such stimulation will also activate axon collaterals (see below), which can then target other neurons.

Axon collateral
A branch of a neuronal axon. In the case of motor neuron collaterals, unlike the main axons that project to and activate muscles, these collaterals make synapses onto other neurons within the nervous system.

Central pattern generators
Neuronal circuits that produce rhythmic motor patterns that persist in the absence of sensory feedback or descending inputs.

Chemical synapse
One of two ways that neurons communicate with each other (see 'electrical synapse'). Chemical synapses are formed by the close apposition of membranes from two neurons, in which the presynaptic neuron can release chemical messengers (neurotransmitters) into the intermembrane space, the synaptic cleft. These chemical signals then bind to receptors on the postsynaptic membrane, which triggers changes in electrical signals.

Connectome
Three-dimensional reconstruction of neuronal circuits by serially reconstructing ultra-thin slices of nervous tissue using electron microscopy. 'Connectomics' allows researchers to identify all neurons in a given brain region, as well as map all of their connections.

Electrical synapse
One of two ways that neurons communicate with each other (see 'chemical synapse'). These synapses are formed when membranes of two neurons are in direct contact, with pores formed by gap junction protein complexes that allow electrical signals to freely travel directly between neurons.

Fictive behavior
Because CPGs do not require sensory inputs to function, many can be activated in isolated brains and spinal cords. The output of these CPGs, often measured as recordings of motor nerves, is referred to as fictive behavior, as the patterns of nerve activity closely match those that occur during *in vivo* behavior.

Interneurons
Any neuron that is neither a sensory neuron nor a motor neuron.

Renshaw cells
Spinal neurons that receive excitatory inputs from adjacent motor neurons. Renshaw cells have long been known to provide inhibitory feedback to motor neurons.

Ventral root
The vertebrate spinal cord contains two nerve branches in each segment, a ventral root and a dorsal root. The ventral root contains motor neuron axons that target peripheral muscles, while the dorsal root carries sensory inputs.

suggested that most motor neurons located in the brain lacked collaterals. However, he did identify these branches in some neuronal populations in the brain, such as the nucleus ambiguus and mesencephalic nucleus of the trigeminal nerve. Additional studies have identified motor neuron collaterals in cranial nerve nuclei that Ramón y Cajal reported as lacking collaterals, such as the oculomotor (Evinger et al., 1979) and hypoglossal (Kanjhan et al., 2016) nuclei.

Later anatomical investigations of cat motor neurons supported Ramón y Cajal's findings, showing that many spinal motor pools contain multiple collaterals (Cullheim and Kellerth, 1978). Electron microscopy studies later confirmed that collaterals make synaptic contacts in the central nervous system, by identifying transmitter vesicles in labeled motor collaterals adjacent to postsynaptic structures. For example, Lagerbäck and Ronnevi (1982), identified synaptic contacts between spinal neurons and Renshaw cells (see Glossary). In another study, electron microscopy also positively identified presynaptic structures in collaterals of other spinal motor neurons that control muscle spindle tension (Ulfhake et al., 1986), while yet another study confirmed the existence of motor neuron connections arising from oculomotor collaterals in the brain (Spencer et al., 1982).

Current use of electron microscopy allows for complete mapping of circuits – the resulting connectomes (see Glossary) promise to reveal previously overlooked motor neuron connections to other neurons in the brain. Innovative efforts in the nematode *Caenorhabditis elegans* gave us the first complete wiring diagram of a nervous system, in which motor neurons were shown to form both chemical and electrical synapses (see Glossary) with interneurons (Varshney et al., 2011; White et al., 1986). With recent advances in computational capacity, other large-scale connectomic projects are underway. Ryan et al. (2016) recently generated the connectome of a larval tunicate nervous system, showing that motor neurons make numerous connections (chemical and electrical) with other central nervous system (CNS) neurons. Although connectomes of the entire nervous systems of most species are not expected for years to come, their completion will offer non-biased opportunities to reveal whether central motor neuron synapses are common across animals.

In summary, the anatomical record of motor neurons making synaptic contacts within the CNS – both electrical and chemical – is well established. Evidence of anatomical connectivity, however, even from high-quality connectomes, is insufficient to reveal the mechanisms and functions of motor neuron inputs to CNS circuits. Many physiological studies directly support the notion that motor neuron feedback contributes to CPG function and alters behavior patterns in a wide array of systems. Below, we describe several functional examples of motor neuron involvement in generating three behaviors – locomotion, feeding and vocalization – across several phyla.

Motor neuron activity can alter CPG function across metazoans

Locomotion

Locomotion takes many forms across phyla, depending on the animal's body plan and the substrate through which it moves. Some movements involve propagation of muscle contractions along the length of the body: alternating dorsal–ventral or left–right undulatory contractions underlie behaviors such as swimming in leeches and fish, while bilaterally symmetric peristaltic contractions generate crawling in many animals such as leeches and insect larvae. Limbed animals can produce a range of locomotor patterns with either left–right alternation (e.g. walking in rodents) or bilaterally symmetric movements (e.g. flying in birds).

While the exact details of locomotor patterns vary widely across animals, all locomotor behaviors require precise temporal coordination of multiple muscles. The timing of muscle activation depends on stereotyped (but flexible) rhythm generation in the CNS. Because locomotion is found in most metazoans, and takes many forms, CPGs that control locomotion have likely evolved independently in many cases. Discovering similarities between convergently evolved circuits may point to fundamental properties that promote robust circuit function.

Annelida

Leeches (*Hirudo*) move by swimming or crawling, depending on whether they are in an aquatic or terrestrial environment. These behaviors are produced by the same groups of muscles, differentially

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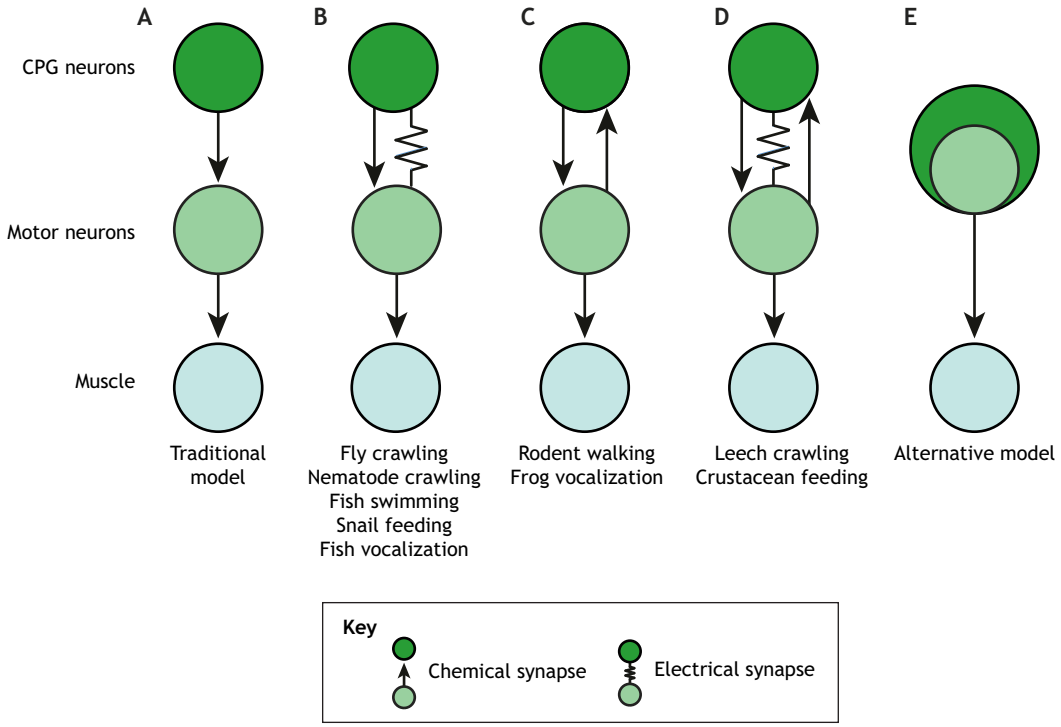

Fig. 1. Diverse motor circuit architectures. (A) Traditional motor circuits are depicted as top-down unidirectional networks, in which central pattern generator (CPG) neurons drive activity in motor neurons; in turn, motor neurons relay these patterns to muscles that produce the behavior. (B–D) Circuits described in this Review, however, represent diverse architectures, in which motor neurons contribute to, or participate in, CPGs. Some circuits possess electrical coupling between motor neurons and premotor rhythm-generating neurons (B). In other circuits, motor neurons make chemical synapses onto CPG interneurons (C), while others have a mixture of both chemical and electrical connections (D). (E) We propose an alternative model in which motor neurons are considered integral components of the CPG. Dark green circles represent pattern-generating interneurons; lighter green circles represent motor neurons; lightest green circles represent muscles. Arrows represent chemical synapses; resistor symbols represent electrical synapses.

activated, and are controlled by largely overlapping circuits that underlie these undulatory and peristaltic movements (e.g. Brodfuehrer et al., 1995; Kristan et al., 2005; Szczupak, 2014). The locomotor CPG in leeches is distributed across 21 segmental ganglia. In each ganglion, motor neurons provide chemical inhibitory inputs onto a pair of non-spiking interneurons (Rodriguez et al., 2012). The voltage of non-spiking interneurons oscillates rhythmically with both fictive swimming and crawling. Modifying the voltage of non-spiking neurons does not affect fictive swimming but it does affect fictive crawling (Rodriguez et al., 2012). Experimentally mimicking inhibitory inputs from motor neurons by hyperpolarizing non-spiking neurons slows the fictive crawling rhythm (Rodriguez et al., 2012). This suggests that the non-spiking interneurons relay an indirect inhibitory feedback signal from motor neurons to upstream CPG neurons. Another study found that inhibiting the motor neurons responsible for body elongation leads to a decrease in crawling period (Rotstein et al., 2017). The authors hypothesized that these motor neurons may be electrically coupled to neurons that generate the elongation pattern, and that the presence of this connection may normally increase the duration of the elongation component of crawling. These studies collectively suggest that, in leeches, motor neurons contribute to the crawling CPG via both chemical and electrical connections (Fig. 1D).

Arthropoda

Crayfish swim using paired ventral abdominal appendages known as swimmerets. The swimmeret pattern is controlled by a CPG in each ganglion, coordinated by a pacemaker CPG in the fifth ganglion (Stein, 1971). In this system, the pairs of antagonistic motor neurons that control swimmeret muscles not only relay CPG patterns to the swimmerets but also participate in rhythm generation (Heitler, 1978). Injection of currents of varying intensity suggests that motor neurons could alter upstream pattern-generating circuitry. During fictive swimming, moderate hyperpolarizing currents (−3 nA) cause an elongation of motor bursts and excitatory post-synaptic potentials onto motor neurons, whereas larger currents (−5 nA) completely eliminate motor patterns (Heitler, 1978). These results suggest that these motor neurons provide positive feedback to reinforce the swimming CPG rhythm; however, the mode of connectivity (chemical or electrical) is not known.

The development of optogenetic tools – in which light-sensitive ion channels can be used to activate or silence genetically identified neurons – has greatly expanded the experimental opportunities for testing the role of motor neurons in pattern-generating circuits. Recent work in *Drosophila* larvae examined motor neurons involved in generating peristaltic locomotor patterns. In the isolated ventral nerve cord, motor neurons in different segments play distinct roles in regulating locomotion (Matsunaga et al., 2017). When motor neurons in segments A4, A5 and A6 were optogenetically silenced, the locomotor frequency decreased, whereas when motor neurons in segment A6 or A7 were optogenetically activated, the frequency increased. The involvement of motor neurons in locomotor rhythms was shown to be dependent on electrical coupling via gap junctions, because animals with mutated gap junction genes or preparations treated with a gap junction blocker did not show an effect of optogenetic manipulation. This suggests that motor neuron feedback is conveyed via electrical connections between motor neurons and other locomotor CPG neurons (Fig. 1B).

Nemotoda

In *C. elegans*, the ventral nerve cord contains two types of excitatory motor neurons, A-type and B-type, that control backward and forward movement, respectively. Premotor neurons (AVB interneurons) project extensively along the ventral nerve cord and connect via gap junctions to B-type motor neurons, which are capable of functioning as oscillators. Electrical coupling with AVB interneurons facilitates coordination of motor neuron activity that generates forward locomotion (Xu et al., 2018). A-type motor neurons, required for backward locomotion, receive inputs from a different class of premotor neurons – AVA interneurons – through mixed electrical and chemical synapses. These A-type motor neurons generate intrinsic oscillations that rely on voltage-dependent calcium channels (Gao et al., 2018). When premotor interneurons and the other motor neuron types are ablated, intrinsic A-type motor neuron activity alone is sufficient to drive backward movement.

Whereas A- and B-type motor neurons function in pairs to coordinate locomotor undulations, AS motor neurons function independently without a bilateral partner; thus, they innervate muscles asymmetrically. AS motor neurons are involved in forward and backward locomotion and oscillate in phase with body bend angle during both behaviors. Like A- and B-type motor neurons, these motor neurons are connected to premotor interneurons with gap junctions, as well as receiving chemical synaptic inputs from several types of premotor neurons. Research suggests that AS motor neurons play a role in the coordination of dorso-ventral and antero-posterior undulation, and feedback is key in the coordination of backwards movement. When AS motor neurons are stimulated, AVA, but not AVB, interneurons depolarize in response. This supports functional feedback specifically to AVA interneurons and suggests a role in the regulation of backward locomotion (Tolstenkov et al., 2018). Taken together, these studies suggest an important role of motor neurons in generating multiple forms of nematode locomotion, with motor neurons influencing premotor neuron activity via electrical synapses (Fig. 1B).

Chordata

In many vertebrate species, motor neurons participate in rhythm generation in locomotor circuits. Research in tadpole (Perrins and Roberts, 1995), zebrafish (Song et al., 2016), chick (Wenner and O'Donovan, 1999, 2001) and rodent (Mentis et al., 2005) spinal circuits suggests that motor neurons may influence and participate in the control of motor behaviors. Birdsey Renshaw (1941) discovered some of the earliest functional evidence in vertebrates that motor neurons provide feedback to neurons in the spinal cord. He found that when the motor nerve is antidromically stimulated (see Glossary), interneurons (which we now call Renshaw cells) begin to fire and motor neurons receive delayed inhibition (Renshaw, 1941). John Eccles later found that inputs to Renshaw cells depend on acetylcholine, further supporting the idea that the interneuron firing is a result of direct inputs from motor neurons (Eccles et al., 1954).

Although motor neurons release acetylcholine at the neuromuscular junction, recent studies have shown that motor neurons can release both acetylcholine and glutamate from central synapses. Excitatory inputs onto Renshaw cells following ventral root (see Glossary; i.e. the motor nerve) stimulation in neonatal mice are blocked only after the combined application of both cholinergic and glutamatergic antagonists (Mentis et al., 2005; Nishimaru et al., 2005). Dual recordings from pairs of Renshaw cells and motor neurons show that Renshaw cells make many inhibitory synaptic contacts with a single motor neuron and, as a result, stimulating a single Renshaw cell can effectively silence motor neurons (Bhumbra et al., 2014). Could this be important for pattern generation? The inhibitory feedback signal to motor neurons from Renshaw cells does not appear to represent a pathway that allows motor neurons to modulate upstream pattern-generating circuits. One proposed alternative is that Renshaw cells function as a variable gain regulator of motor output (Hultborn and Pierrot-Deseilligny, 1979). However, Renshaw cells may not only provide feedback to motor neurons. Instead, they may also provide inputs to interneurons and receive inputs from descending neurons, suggesting a role in circuit dynamics. For instance, Renshaw cells project to each other, to 1a inhibitory interneurons and to ventral spinocerebellar neurons (Jankowska and Hammar, 2013). Thus, it is possible that motor neurons can modulate locomotor CPG cells indirectly via Renshaw cell activation.

In addition to excitatory inputs onto Renshaw cells, motor neurons also project to other motor neurons (Bhumbra and Beato, 2018; Nishimaru et al., 2005). Motor neurons in 2 week old postnatal mice make exclusively glutamatergic contacts with other motor neurons in the spinal cord to provide excitatory recurrent feedback (Bhumbra and Beato, 2018; Nishimaru et al., 2005). Like contacts with Renshaw cells, these connections also cannot directly alter CPG function, but they can modulate the strength and precise timing of motor patterns.

Additionally, motor neurons of immature rats synapse onto other, as yet poorly described, interneurons (Machacek and Hochman, 2006). These contacts appear to be part of an excitatory recurrent feedback system. Ventral root stimulation in disinhibited rat spinal cords (i.e. those treated with GABA and glycine receptor antagonists) induces locomotor bursting. In addition, ventral root stimulation in spinal cords treated with noradrenaline induces delayed locomotor-like bursting. Machacek and Hochman (2006) also made whole-cell recordings from non-Renshaw spinal interneurons that receive excitatory inputs following nerve stimulation in the presence of noradrenaline. Ventral root stimulation has also been shown to induce locomotor-like activity in non-mammalian vertebrates. For example, ventral root stimulation in embryonic chick triggers bursts of activity in the locomotor circuit through a hypothesized avian Renshaw cell homolog (Wenner and O'Donovan, 2001).

While ventral root stimulation experiments may support the presence of motor neuron connectivity to neurons in the locomotor CPG, they do not mimic the naturally occurring behavior, nor do they identify the mechanisms underlying motor neuron contributions. Because motor neurons provide recurrent feedback inhibition and excitation to other motor neurons, ventral root stimulation alone fails to confirm whether the resulting output from stimulation is due to direct action on motor neurons or to recruitment of CPG neurons. Therefore, it is crucial to determine whether motor neurons directly target CPG neurons.

A more recent set of experiments described excitatory motor neuron connectivity to one class of non-Renshaw interneuron. Chopek et al. (2018) found that motor neurons in postnatal mice activate a population of ipsilateral V3 interneurons via glutamatergic synapses; V3 interneurons, in turn, project bilaterally to neurons in the locomotor CPG. When V3 interneuron signaling is suppressed, locomotor patterns become more variable and left–right alternation is disrupted, suggesting that V3 interneurons maintain robust, bilaterally symmetric locomotor rhythms (Zhang et al., 2008). Thus, glutamatergic inputs to V3 interneurons represent a possible route for motor neuron contributions to pattern generation in mice (Fig. 1C).

A recent study in neonatal mice used optogenetics to activate and silence choline acetyltransferase-expressing (ChAT+) neurons and Islet1-expressing (Isl+) neurons (Falgairolle et al., 2017). These two

cell types were of interest because they represent two overlapping groups of neurons primarily made up of motor neurons. The researchers found that silencing ChAT+ or Isl+ neurons led to a decreased locomotor frequency, an altered phase and slower motor neuron firing. After neuronal silencing ceased, there was a transient increase in the frequency of locomotor activity and motor neuron firing. Optogenetic activation of these neurons led to increased motor neuron firing and locomotor frequency. These effects persisted in the presence of cholinergic receptor antagonists, suggesting that the effects are independent of acetylcholine. Instead, the researchers found these effects depended on glutamate receptors. This work strongly supports the possibility that motor neurons can regulate locomotor rhythms via glutamatergic feedback to the CPG circuit.

Most of the mammalian spinal studies described above involve neonatal and postnatal rodents; however, locomotor CPG activity can be observed even in embryonic stages. In the spinal cords of embryonic mice, waves of spontaneous activity can be observed, and are believed to be necessary for the proper development of locomotor circuits. Motor neuron collaterals in the embryonic spinal cord may be responsible for acetylcholine-dependent spontaneous activity (Hanson and Landmesser, 2003). These waves of spontaneous activity appear to be supported via a connection from motor neurons to excitatory glycinergic and GABAergic interneurons (Hanson and Landmesser, 2003). The question remains whether this spontaneous activity is required to form a normally functioning locomotor circuit. This was investigated in embryonic mouse mutants that lack ChAT. These animals lack spontaneous locomotor activity at embryonic day (E)12.5 (Myers et al., 2005). By E18.5, they produce spontaneous activity, but the left–right and extensor–flexor coordination is abnormal. Application of dopamine, serotonin and *N*-methyl aspartate (a glutamate receptor agonist) evokes fictive locomotion in E18.5 wild-type mice. In ChAT mutant mice, however, fictive locomotor bursting duration and period are elongated, and left–right and extensor–flexor coordination is abnormal. Applying cholinergic antagonists to wild-type preparations also results in longer burst durations and periods, but does not shift left–right or extensor–flexor phase relationships. These findings suggest that cholinergic activity is required during development to successfully organize and activate the locomotor circuitry. Thus, it is possible that some CPGs do not involve motor neuron inputs in adult animals, but motor neuron involvement may still be important for proper circuit development.

All of the mechanisms of motor neuron involvement in vertebrate locomotion discussed in this section have involved chemical synapses. However, gap junctions are also prevalent in vertebrate motor circuits, both during development and in adults. In adult zebrafish, recent work has supported the possibility of motor neuron feedback via gap junctions (Song et al., 2016). Specifically, motor neurons are electrically coupled to excitatory V2a interneurons in the locomotor CPG. When motor neurons are experimentally hyperpolarized, V2a firing decreases; when motor neurons are depolarized, V2a firing increases. When motor neurons are inhibited during fictive locomotion, V2a recruitment is disrupted and the locomotor rhythm slows. This suggests that motor neurons and V2a interneurons function as electrically coupled ensembles that influence locomotor rhythms (Fig. 1B).

Feeding and digestion

For multicellular heterotrophs, feeding is a vital function. In most cases, there are dedicated structures tasked with obtaining food, breaking it down and absorbing nutrients. Body plans vary widely between species, necessitating an equal diversity in the organs involved in feeding and digestion. Like locomotion, effective feeding and digestion movements must be temporally coordinated. Because of the diversity of these systems, the CPGs underlying their control are also undoubtedly distinct. Traits that are shared between these independently evolved circuits may represent effective solutions for reliably generating these movements.

Mollusca

The pond snail *Lymnaea stagnalis* has a three-phase feeding cycle (protraction, rasp and swallow) controlled by buccal, glandular and gut muscles that, in turn, are controlled by a feeding CPG; this CPG activates distinct groups of motor neurons during each phase of eating. Buccal, but not glandular or gut, motor neurons are electrically coupled to interneurons in the CPG (Fig. 1B). Injecting positive or negative current into buccal motor neurons during fictive feeding leads to resetting of the motor pattern. In a subset of the motor neurons, injecting positive current increases the frequency of the fictive behavioral rhythm, whereas negative current decreases the frequency (Staras et al., 1998).

There is also evidence that the *Aplysia* feeding CPG has motor neurons that participate in rhythm generation. Feeding in *Aplysia* uses two body parts: the lips and the radula. These are controlled by separate CPGs, which interact to coordinate behaviors. Motor neurons controlling the lips are located in the cerebral ganglion, whereas motor neurons that control the buccal muscles – which control the radula – are located in the buccal ganglion. Stimulating one of the cerebral motor neurons can activate the CPG that controls lip movement (Perrins and Weiss, 1996). Experimentally hyperpolarizing this motor neuron eliminates synaptic inputs coming from unidentified CPG neurons, suggesting that the motor neuron may be electrically coupled to upstream neurons, though this has not been directly tested (Fig. 1B).

Motor neurons in the feeding CPG of *Tritonia* (a group of marine gastropods) are also able to drive motor rhythms (Willows, 1980). A widespread mechanism that contributes to rhythm generation in CPGs is post-inhibitory rebound, in which a neuron will reliably spike following inhibitory input from another neuron (or group of neurons) in the circuit (Marder and Bucher, 2007). Variation in the strength and timing of the post-inhibitory rebound can control motor rhythm frequency or alternating firing of opposing muscles. In *Tritonia diomedea*, a pair of buccal ganglion motor neurons appear to generate post-inhibitory rebound spikes following both intrinsic inhibitory inputs and experimentally induced hyperpolarization (Willows, 1980). The motor neurons also generate rhythmic bursting in response to tonic excitation, and induce feeding-like patterns in other buccal motor neurons. Further, sustained experimental inhibition of these neurons blocks spontaneous fictive feeding rhythms. Thus, these buccal motor neurons may play a central role in generating feeding motor patterns, though the exact mechanisms and connectivity are not known.

Arthropoda

In the stomatogastric ganglion (STG) of lobsters, crabs and other crustaceans, motor neurons not only control the behavior but also make up the majority of the CPG (Marder and Bucher, 2007). Twenty-three of the approximately 30 neurons that control the gastric mill and pyloric rhythms (which are involved in food processing) are motor neurons and directly participate in the CPG (Marder, 1976). For example, the core pacemaker of the pyloric rhythm consists of an endogenously bursting pacemaker neuron (AB) electrically coupled to two pyloric dilator motor neurons (PD; Marder and Eisen, 1984). While these neurons are synchronized by

their strong electrical coupling, neuromodulators have distinct effects on each cell type (Marder and Eisen, 1984). In turn, differential modulation of AB and PD neurons can alter their inputs to other STG neurons, resulting in a shift of phase relationships of the muscles driven by the pyloric circuit. A more recent study suggested that differences in PD neuron membrane properties could affect motor output. One finding of this study was that expression of an inactivating potassium channel gene in PD neurons was positively correlated with pyloric period (Goaillard et al., 2009). Thus, the PD motor neurons have a central role in setting rhythmic activity of the pyloric CPG, which is mediated via both electrical and synaptic connections (Fig. 1D).

Vocalization

Like locomotion and feeding, communication is a nearly universal feature of animal species. Animals produce signals to communicate a variety of information including caller identity, mating status, aggression and alarm. These communication signals use a variety of sensory modalities, including light, chemical and sound. While olfactory and visual communication typically involve limited motor sequences (such as sniffing or visual saccades), acoustic communication – in particular, sound production – often requires temporally precise and energetically costly control of muscles. In most cases, vertebrate vocal behaviors appear to have largely co-opted peripheral structures and central circuits originally evolved to control respiratory movements (Bass et al., 2008). The sound-producing structures, and the muscles that control them, vary across species. For example, bird songs are generated by the syrinx, whereas frogs and mammals use their larynges to generate sound. Other sound-producing structures can be found in vertebrates, such as the swim bladder in teleost fishes, discussed below. Regardless of the vocal organs used, vocal patterns of many species appear to be generated by CPGs in the brain.

Chordata

Vocal motor neurons that control swimbladder drumming in toadfishes are located in the hypoglossal nucleus homolog (Albersheim-Carter et al., 2015). In one species of toadfish, *Porichthys notatus*, the motor neurons are coupled by gap junctions to pacemaker and pre-pacemaker neurons, as shown by electron microscopy and transneuronal labeling following biocytin filling of the vocal nerve (Bass and Marchaterre, 1989; Bass et al., 1994). Electrical coupling between motor neurons has been demonstrated by collision tests in which intracellularly evoked action potentials do not eliminate depolarizations following antidromic stimulation of the vocal nerve (Chagnaud et al., 2012). In another toadfish, *Opsanus tau*, the presence of gap junctions is supported by extensive co-labeling of neurobiotin-positive soma with an antibody that labels gap junctions (Cx35/36) in the vocal nuclei (Rosner et al., 2018). While the anatomical connections of vocal motor neurons within the CPG are well established in these fish (Fig. 1B), the functional implications of these electrical connections remain unclear.

In the vocal system of the frog *Xenopus laevis*, vocal motor neurons are located in the nucleus ambiguus, and drive contractions of the larynx (Albersheim-Carter et al., 2015; Zornik and Kelley, 2008). Recent work in *X. laevis* has indicated that motor neuron input to the CPG is required for normal vocal behavior (Lawton et al., 2017). The vocal CPG of *Xenopus* includes the parabrachial nucleus, where premotor neurons control call duration and sound pulse rate (Barkan et al., 2017, 2018; Zornik and Yamaguchi, 2012). These premotor neurons project directly to motor neurons in the nucleus ambiguus, where laryngeal motor neurons reside. Interneurons in the nucleus ambiguus also project back to the premotor nucleus (Zornik and Kelley, 2007; Zornik and Yamaguchi, 2012). When motor neurons are silenced with an intracellular sodium channel blocker (by backfilling axons in the motor nerve), premotor neurons cease to code sound pulse rate and, instead, spike much faster than during normal vocal production (Lawton et al., 2017). This suggests that output from motor neurons onto other CPG neurons is required for generating vocal patterns. What synaptic connections mediate this pathway? When a nicotinic acetylcholine receptor antagonist was applied to the motor nucleus, vocal patterns were disrupted. When the vocal nerve was stimulated, short-latency inhibitory signals were recorded in premotor neurons; this inhibition was blocked by application of nicotinic antagonists (Lawton et al., 2017). These experiments suggest that motor neurons provide a polysynaptic inhibitory input onto premotor vocal interneurons that is mediated by acetylcholine (Fig. 1C). Because vocal rhythms are disrupted when this pathway is blocked, motor neurons appear to serve as an essential component of the vocal CPG.

In summary, vocal hindbrain CPGs in both teleost fish and frogs have been shown to involve motor neurons. Unlike the other examples of motor neuron involvement in vertebrate CPGs, which are all found in the spinal cord, these studies show that motor neuron feedback can play a role in regulating CPGs located in the brain.

Conclusions and moving forward: how widespread is motor neuron regulation of CPGs?

In this Review, we have described evidence for motor neuron involvement in regulating CPG function across many motor circuits and phyla (Fig. 2). One potential explanation for the widespread role of motor neurons in CPGs is that this trait was present in the common ancestor of bilaterians. This hypothesis is supported by the nature of motor circuits in Cnidaria, the sister group to Bilateria (Simion et al., 2017). For example, electron microscopy of perioral tissue in *Hydra* revealed that the two neuron types – sensory cells and ganglion cells – are highly interconnected (Westfall and Kinnamon, 1984). Reciprocal chemical synapses are found both within and between cell types, and both sensory and ganglion cells make synaptic contacts with muscle cells (Westfall and Kinnamon, 1984; Fig. 2). In the jellyfish *Aequorea aequorea*, many motor neurons that control swimming muscles are electrically coupled via gap junctions, and can generate bursting patterns when synaptic signaling is blocked (Satterlie, 1985). Because these swim motor neurons are the only cells active in phase with swimming, this suggests that the CPG may be solely composed of these motor neurons (Satterlie, 1985; Fig. 2). Thus, cnidarian cells that activate muscles are highly interconnected with each other and other cell types, supporting the possibility that motor neurons played a central role in motor pattern generation in the common ancestor to both cnidarians and bilaterians.

It perhaps should not be surprising that motor neurons contribute to CPG function. Recent evolutionary developmental biology (‘evo-devo’) studies have revealed that motor neurons share developmentally important markers across phyla, suggesting a common origin of many motor neuron populations (Thor and Thomas, 2002; Vergara et al., 2017). Given that genetic expression profiles influence phenotypes such as axon guidance and synaptic targets (Hobert and Kratsios, 2019), shared developmental profiles likely confer the same tendency of motor neurons to establish central synapses. The persistence of these trends may indicate adaptive advantages of motor neuron involvement in CPG function over feedforward circuit architecture. One possibility is that circuits incorporating motor neuron feedback are more functionally robust

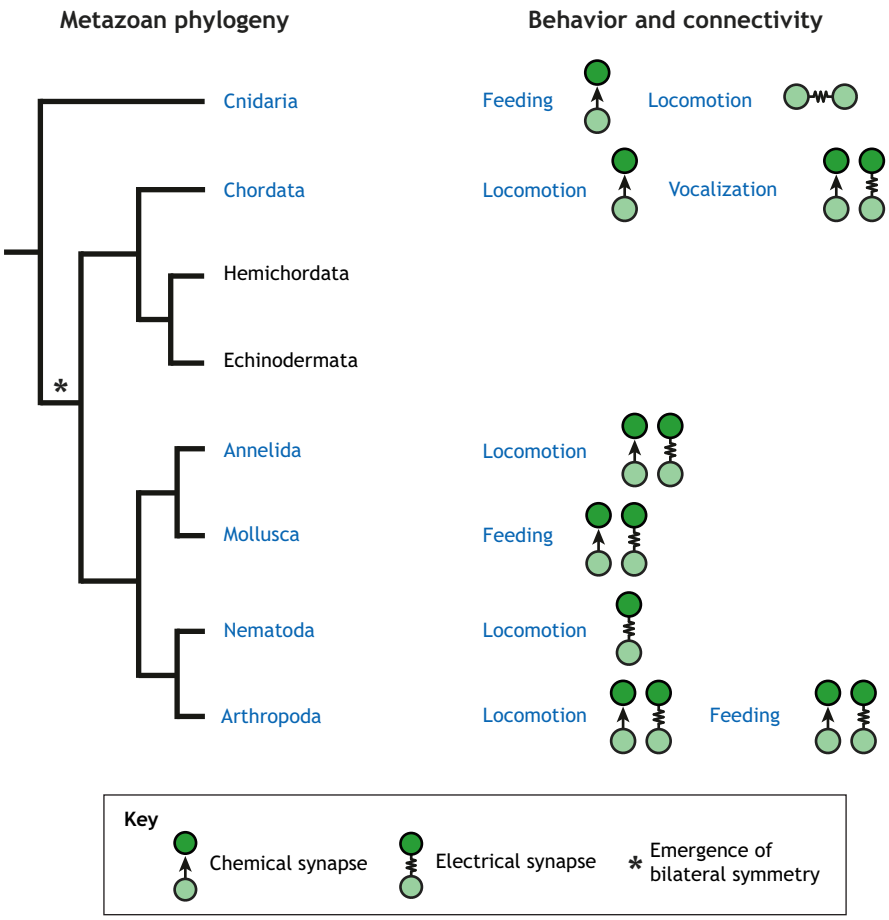

Fig. 2. Motor neuron involvement in central pattern-generating circuits across phyla. Evolutionary relationships of major bilaterian phyla (adapted from Ramos-Vicente et al., 2018). In this Review, we present examples from several species in which motor neurons provide inputs to CPGs through chemical, electrical or mixed synapses. Blue text denotes phyla and behaviors described in this Review. Motor circuits in the sister to Bilateria, Cnidaria, also have examples of motor neuron involvement in generating motor patterns. This suggests that the common ancestor of both cnidarians and bilaterians may also have possessed this bottom-up circuit architecture. Dark green circles represent pattern-generating interneurons; light green circles represent motor neurons. Arrows represent chemical synapses; resistor symbols represent electrical synapses. In the cnidarian locomotor system described in this Review, the entire CPG appears to comprise only electrically coupled motor neurons.

in the face of external and internal perturbations than those lacking such feedback, ensuring their persistence throughout evolution.

Questions remain as to whether motor neuron contributions to CPG function are relatively rare, both within and across species, or whether they are the rule rather than the exception. The best paths toward answering these questions will incorporate unbiased examination of neuronal and circuit properties. A promising approach is electron microscopy-mediated connectomes. As discussed above, two existing connectomes (those of *C. elegans* and *Ciona intestinalis*) revealed motor neuron connections to CNS neurons (Ryan et al., 2016; Varshney et al., 2011; White et al., 1986). Generating connectomes built on ultrastructural data (using electron microscopy) across bilaterians, though not yet practical, may ultimately reveal neuronal connections that might be overlooked or missed using targeted approaches, including electrophysiology and optogenetics.

In the meantime, ongoing investigations of all motor circuits should consider exploring the possible contributions of motor neurons. Classical electrophysiological methods are generally sufficient for determining whether connections exist between motor neurons and other CPG neurons, so the first step of identifying these connections is widely available for virtually all organisms and behaviors. Determining the causal role of motor neurons in CPG function is more challenging. In this regard, optogenetics is currently the best approach, as it can allow researchers to both activate and inhibit entire groups of motor neurons. Because such approaches rely on the availability of transgenic lines in which transgenes are selectively expressed in some or all motor neurons, most of these studies are being carried out in traditional laboratory organisms such as mice, zebrafish, fruit flies and nematodes. As genome editing becomes more widely available in less intensively studied species, these questions can be rigorously tested across taxa. Finally, computational models that incorporate newly discovered motor neuron feedback pathways can help to verify experimental results, and computational experiments perturbing motor neuron connections can help generate new hypotheses regarding the magnitude of motor neuron influences in these circuits. Given the evidence presented in this Review, we hypothesize that such efforts will reveal widespread and critical functions of motor neurons in regulating the majority of behaviors in most, if not all, species.

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