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Diversity of neurons and circuits controlling the speed and coordination of locomotion

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While often considered to be a simple and stereotyped motor behavior, effective locomotion entails complex changes in vigor, speed, and gait. Studies over several decades in many species have uncovered the basic building blocks of the spinal locomotor CPG. More recently, however, the combination of molecular, electrophysiological, and anatomical tools has begun to uncover a higher degree of neuronal and circuit diversity that endows the locomotor CPG with the necessary flexibility to adjust the vigor, speed, and gait of locomotor movements to internal and external demands. In this review, we summarize some of these recent studies with a focus on how the diversity of neurons in zebrafish generates a modular organization of the locomotor CPG, with individual module components engaged in a task-dependent manner. In addition, we highlight recent evidence on the role of motoneurons as an integral member of the locomotor CPG in vertebrates. Thus, studies of the spinal locomotor networks are beginning to provide direct links between neuronal diversity, their circuit organization and behavioral relevance.

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Introduction

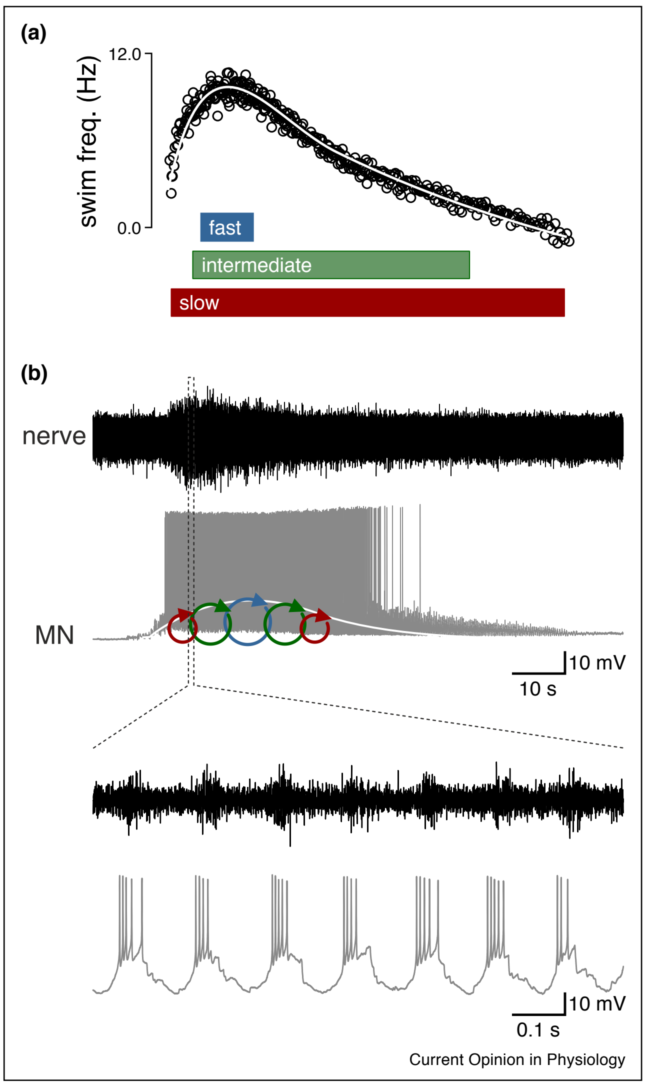
Locomotion, like all behaviors, can be expressed in different forms, gaits, and speeds to match the environmental and organismal demands of an animal and to optimize its survival [1–4,5••,6–8,9••]. The ease by which we move and change our gait and speed masks the true complexity of the neuronal and circuit mechanisms involved. The contribution of specific neuronal populations, defined based on a transcriptional code, in the generation of the locomotor rhythm and its coordination has been assessed both in non-mammalian and mammalian model systems [2–4,5••,6–8,9••,10–12]. However, it is becoming increasingly evident that each transcriptionally defined neuronal class consists of functionally heterogeneous neuronal ensembles. Subtypes within each major spinal neuron class appear to imbedded in different circuits, perform different computations, and are selectively engaged in a task-dependent manner to modify the locomotor speed or gait. The locomotor central pattern generator (CPG), therefore, consists of an ensemble of circuit modules that are deployed alone or in combination according to the needs of an animal. The molecularly defined neuronal populations can no longer be considered as homogeneous classes, instead their functional diversity needs to be unified with a dynamic circuit-level analysis.

Ultimately, any increase in the speed of locomotion is mediated by the sequential recruitment of different motor units organized as slow (slow fatigue resistant; SR), intermediate (fast fatigue resistant; FR) and fast (fast fatigable; FF) groups [13–15]. At slow, less vigorous movements only the slow motor units are active, while at fast, more vigorous movements all motor units from slow to fast are engaged. Therefore, the organization of the locomotor CPG circuits should be aligned to the diversity of motor units to allow their recruitment individually or in combination. A detailed circuit analysis in juvenile/adult zebrafish has revealed that the locomotor CPG consists of three circuit modules that channel the excitatory drive selectively to the different muscle types [16,17]. This modular organization, which extends to the premotor interneuron network that drives motoneuron activity, enables a flexible control of the locomotor speed and gait. This review will, thus, summarize some of these recent data and discuss their relevance for the organization of locomotor circuits.

Modular circuit organization

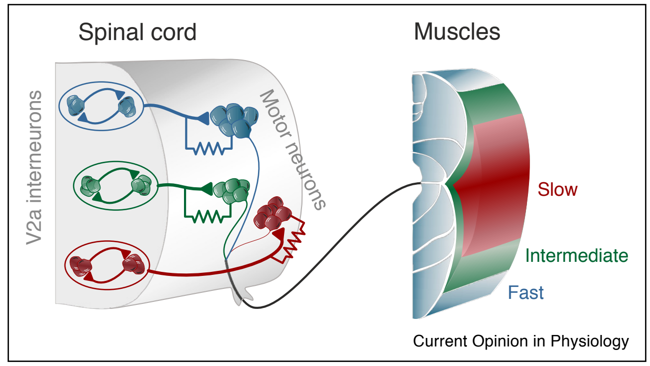
An important task of the locomotor CPG is to generate a complex sequence of activity to control different muscles, and hence coordinates the movement of different joints in limbed animals and the rostro-caudal propagation of alternating axial muscle contractions in non-limbed animals [1–3,7,8,9••,10,18,19]. In addition, the CPG needs to integrate commands from the brain to calibrate the vigor, speed, and coordination of locomotor movements in a context-dependent manner. The excitatory V2a interneurons are required for locomotor rhythm generation — their ablation in larval zebrafish impairs the locomotor activity while their optogenetic activation induces coordinated locomotion [20,21]. In adult/juvenile zebrafish, this interneuron population is functionally heterogeneous and comprises three subtypes characterized by their recruitment order at slow (<4 Hz), intermediate (4–8 Hz) or fast (>8 Hz) swimming speeds [17,22] matching that of motoneurons innervating slow, intermediate and fast muscle fibers [16,23] (Figure 1). A detailed circuit-level analysis in adult/juvenile zebrafish has shown that V2a interneurons and motoneurons form three circuit

Figure 1



Speed change during locomotion.
(a) The speed of swimming varies continuously by activating different motor neuron pools from slow (red), to intermediate (green) and fast (blue) in the adult zebrafish. **(b)** The swimming activity is monitored from both motor nerves and identified motor neurons.

Figure 2



Modular organization of the locomotor CPG.
The locomotor circuits are endowed with a modular organization involving three separate microcircuits. Each microcircuit comprises a subtype of V2a interneurons that are selectively connected to a specific motor neuron pool. These modules are successively recruited from slow to intermediate and fast to increase the speed of locomotion. In addition, motor neurons retrogradely influence the locomotor CPG function via gap junctions.

modules with stronger and more frequent connections within modules compared to between modules [22,24••]. The different circuit modules are sequentially engaged from slow to intermediate and then to fast to increase the speed of swimming. This modular organization enables a more flexible adjustment of the speed of movements engaging the different circuit modules either separately to produce slow swimming, or fast swimming following escape, or combined sequentially to increase the speed of swimming movements (Figure 2).

Diversity of V2a interneurons and their modular connectivity

A defining factor for the gradual increase of the vigor (speed and force) of locomotor movements is the successive recruitment of the three speed modules and hence the gradual activation of muscle units in an order from slow, to intermediate and then fast. We recently showed that these modules do not represent reiterations of a canonical circuit obeying the same connectivity rules, but rather they are selectively tailored to ensure the primacy of recruitment of the slow module over the fast one [24••]. In this study, we showed that V2a interneurons within each speed module comprise two types with distinct firing patterns, morphological features and synaptic integration modes that preferentially target either slow or fast motoneurons. A bursting-type of V2a interneurons, with unidirectional axons, predominantly targets the dendrites of slow motoneurons to mediate strong excitatory drive relying on powerful non-linear synaptic potentiation involving the activation of NMDA receptors. Conversely a second, non-bursting V2a type, with bidirectional axons target the somata of fast motoneurons to provide weaker, NMDA-independent non-potentiating synaptic excitation. This circuit-level analysis has revealed a more comprehensive interplay between the diversity of V2a interneurons, their assembly into modular circuits, and their cellular and synaptic properties, which are tuned synergistically in a module-specific manner according to the vigor of the locomotor movement they control. The module encoding slow, less vigorous locomotor movements relies on a circuit design in which the convergence of a small number of bursting-type V2a interneurons is sufficient to recruit slow motoneurons. This module is supplemented with a mechanism for amplification of the excitatory drive, resulting in short-term potentiation that enhances the excitation and consequently scales down the number of converging V2a interneurons necessary to recruit slow motoneurons. In contrast, the module encoding fast, more vigorous locomotor movements relies on a much larger number of non-bursting-type V2a interneurons converging onto fast motoneurons, with less involvement of NMDA-dependent non-linearity. The recruitment of this module, thus, requires synchronized inputs from a large number of converging non-bursting V2a's to recruit fast motoneurons This reserves the fast module for highly salient stimuli, such as a predatory strike, that would provide the coincident inputs necessary to trigger a rapid escape response dependent on the activation of fast muscle. This is a clear example of how neuronal diversity is selectively captured and transformed to instruct CPG speed modules to perform different computations in a task-dependent manner.

In mice, V2 interneurons also display diverse firing patterns and can be recruited with increased locomotor rhythm frequencies [25–29]. Furthermore, a recent study has reported the existence of two types of V2a interneurons based on their postnatal expression levels of the transcription factor Chx10 [30••]. Type I interneurons retain a high level of expression of Chx10 and project only locally in the spinal cord, whereas type II downregulate Chx10 expression and project supraspinally into the brain. While the two types of V2a interneurons are distributed along the rostro-caudal axis of the spinal cord, the number of type II neurons decreases gradually in the thoracic and lumbar regions.

These findings demonstrate a higher degree of diversity in V2a interneurons than originally appreciated in both zebrafish and mice. In addition, circuit analysis in zebrafish has linked the diversity within this interneuron class to their selective circuit connectivity and dynamics within each of the speed modules.

Adaptation of circuit organization during zebrafish development

The musculature in zebrafish, and the spinal circuit driving these swimming muscles, reflects the behavioral needs of the animal at a given stage of development. In zebrafish larvae, mostly fast muscle and a thin layer of embryonic slow muscles are developed [31–33]. Similarly, at larval stages, zebrafish swim in a burst-and-glide pattern with episodic high frequency swimming (~20–60 Hz) alternating with quiescent periods [18]. At juvenile and adult stages the pattern switches to longer, continuous swimming bouts which coincides with the well-established development of adult musculature [32,34,35]. At this stage the swimming speeds range from <1 Hz to 21 Hz while higher frequencies are reached during escape [16,36,37], which momentarily suppresses ongoing swimming via a specific circuit mechanism supplemented with endocannabinoid modulation [37]. All muscles are well developed in juvenile/adult stages and are organized into layers of slow, intermediate and fast muscles and three pools of motoneurons innervating the slow, intermediate and fast muscles can be clearly distinguished based on their position in the spinal column and their intrinsic properties in juvenile/adult zebrafish [16,23]. While the separate organization of three speed-tuned circuit modules is not yet fully formed in larvae, electrophysiological analysis has suggested that the excitatory inputs to primary motoneurons innervating dorsal versus ventral muscles receive segregated excitatory inputs and hence are embedded in different circuit modules [38–41].

Furthermore, a detailed volume EM reconstruction of larval spinal cord has recently revealed that the displaced, fast ipsilateral excitatory V2a interneurons are the only one displaying a specificity in their connectivity with large, presumably primary motoneurons [42••]. The rest of the excitatory interneurons appear to indiscriminately target both large and medium size motoneurons [42••]. While already born, the small motoneurons receive virtually no synaptic inputs and appear not yet to be integrated in the spinal circuit — this presumably occurs later during development and these motoneurons are likely to correspond to the slow pools in juvenile/adult zebrafish. Thus, developmental changes occur as zebrafish develop from larval towards adult stages ranging from changes in swimming pattern and frequency, the integration of slow motoneurons, as well as the modular circuit organization. Indeed, as zebrafish develop towards juvenile/adult stages the circuits are not merely scaled up, but undergo specific organizational modifications that contribute towards changes in prey capture strategies and social behaviors [43,44]. This serves to highlight the zebrafish as an important model organism for studying the developmental changes of locomotor circuits and present numerous opportunities for the future.

Speed-dependent subtypes of V0 interneurons

The commissural V0 interneurons are a major spinal neuron class that broadly consists of excitatory V0v and the inhibitory V0d interneurons [45,46]. In mice, the two major types of V0 interneurons play complementary roles in controlling the left–right alternation in a speed-dependent manner. The predominantly inhibitory V0d interneurons selectively control the coordination at slow speeds which involve a locomotor gait requiring left–right alternation between limbs. In contrast at high locomotor speeds, when the gait changes from limb alternation to synchrony associated with gallop or bound, the predominantly excitatory V0v interneurons become engaged and their ablation compromises the coordination [47,48]. The predominant engagement of V0v during high speed locomotion is supported by findings in juvenile/adult zebrafish where these interneurons are mostly active at higher speeds, whilst a small proportion of V0v interneurons can also be active at intermediate or slow swimming frequencies [49]. At larval stages, less is known about the recruitment patterns of the V0v population. However, a subtype of V0v interneuron (MCoDs) is only active during slow swimming and become inhibited at higher speeds [50,51]. These neurons are also inhibited by sensory inputs from CSFc neurons that sense strong bends associated with fast swimming movements [52]. The two types of V0 – like V2a – interneurons seem to be recruited in a task-dependent manner to ensure the coordination of locomotion. In addition, at least for a specific subtype (MCoDs), V0v in zebrafish seem to shift their activation range from being predominately active during slow swimming in larvae to fast swimming at more mature juvenile/adult stages. The activation pattern of V0d interneurons in zebrafish is still unknown although the available data suggest that they are predominantly active during fast swimming in the larva. Furthermore, these neurons have been described as having more homogeneous morphological features relative to V0v interneurons at this stage [53]. While these findings have revealed many important functional features of V0 interneurons and their diversity, the connectivity and dynamics of their synaptic interactions with other CPG interneurons and motoneurons is not yet fully resolved.

When motoneurons look back and integrate the CPG

Motoneurons are the output channel through which the CNS executes and scales behaviors by coordinating the sequential activation of muscles around many joints. While it is well established that motoneurons can activate Renshaw cells via collaterals, this circuit has been mainly implicated in limiting motoneuron excitation without any effect on the locomotor CPG. Motoneurons have largely been considered as passive recipients of motor programs elaborated by upstream networks of interneurons, without any influential contribution to the generation of these programs. Recent evidence in zebrafish and mouse has now shown that this prevailing view is invalid and that motoneurons exert a more influential control on the locomotor CPG to the extent that they should be considered as bone fide components of the CPG.

In zebrafish, the rhythm generating excitatory V2a interneurons drive the activity of motoneurons via mixed electrical and chemical synapses. While the chemical synapses and their dynamics provide the excitation necessary for sequential recruitment of motoneurons (see above) [22,24••], the electrical synapses enable motoneurons to exert a retrograde influence on the activity of the V2a interneurons [54••]. These electrical synapses allow any change in the membrane potential of motoneurons to propagate retrogradely in an analog fashion and influence the firing and synaptic transmission from the V2a interneurons. In V2a–MN pairs connected with mixed electrical and chemical synapses, tonic depolarization of the motoneuron propagates from the dendrites to the presynaptic terminals of V2a interneurons to locally increase the amount of transmitter released. This depolarization can also propagate all the way to the soma of V2a interneurons, which is electrically compact, to influence their firing threshold and frequency. Conversely, tonic hyperpolarization of the motoneuron decreased the firing frequency of the V2a interneurons and their synaptic strength. During locomotion, motoneurons are continuously undergoing rhythmic fluctuations in their membrane potential due to in-phase excitation and mid-cycle inhibition. During the excitatory phase, the gradual depolarization of the membrane potential of motoneurons is propagated online retrogradely to the excitatory CPG interneurons to potentiate their transmitter release, decrease their recruitment threshold and increase their firing. In contrast, as the membrane potential of motoneurons begins repolarizing during the onset of mid-cycle inhibition the synaptic drive weakens as does the firing of the excitatory CPG interneurons. There is, thus, a continuous retrograde influence of motoneurons via gap junctions that can strongly impact the function of the locomotor CPG. This is supported by the fact that optogenetic silencing of motoneurons during ongoing swimming activity drastically decreases the burst frequency and duration of the swim bout.

In newborn mice, optogenetic manipulation of the activity of motoneurons during ongoing drug-induced locomotor activity also altered the frequency, phase and the stability of the rhythm [55••]. Like in zebrafish, optogenetic inhibition of motoneurons slowed down the frequency of the locomotor rhythm while activation of motoneurons accelerated the rhythm. These effects persisted after partial pharmacological blockade of gap junctions and were mediated, in part, via activation of AMPA receptors. These results suggest that motoneurons in newborn mice also influence the locomotor CPG via chemical synaptic transmission likely by release of glutamate onto excitatory interneurons [55••,56••].

Indeed, previous evidence has highlighted the potential of motoneurons to provide a positive feedback reinforcement of the locomotor CPG and influence its function.

For example, retrograde signaling via endocannabinoids endows motoneurons with the capacity to influence the strength of synaptic input they receive from CPG interneurons and hence influence the locomotor activity [57–61]. Several converging lines of evidence are now arguing strongly for a direct contribution of motoneurons to the elaboration of the locomotor rhythm, and hence that they should be considered integral members of the locomotor CPG.

Concluding remarks and perspectives

The use of diverse model organisms across different developmental stages, in combination with the recent development of powerful experimental tools, has greatly advanced our knowledge of the organization and function of the CPG circuit controlling locomotion. At the core of the locomotor CPG are networks of interconnected excitatory and inhibitory interneurons that generate the locomotor rhythm and its embedded coordination. However, there is a growing body of work showing that each molecularly defined interneuron class in fact consists of a diverse population of neurons which can be grouped according to their anatomy, intrinsic properties and ultimately function. Analysis in adult zebrafish has revealed that the locomotor CPG comprises at least three circuit modules, akin to a three-gear engine with automatic transmission, that are sequentially engaged as the speed and force of locomotion increases. Furthermore, recent evidence demonstrating electrical feedback from motoneurons to the pre-motor network is shifting the long-held view of motoneurons as passive conveyors of excitatory drive towards a consideration that they are bone fide members of the locomotor CPG.

Recent studies on the locomotor CPG across species are beginning to link neuronal diversity to circuit organization and their behavioral relevance. However, whilst a necessary step in the right direction, it is imperative in the future that we continue to link these circuits to the sensory apparatus, and to understand how external and internal perturbations are processed in a context-dependent manner [62••,63••,64••]. In addition, we need to develop tools and strategies to monitor and interfere with the abundance of diverse modulatory neurons, and their downstream pathways, to tackle the complex and powerful roles that neuromodulation plays in adapting the activity of the locomotor CPG both in the short-term and long-term. The sheer complexity of neuromodulation is a valuable lesson already learnt from many keys studies in invertebrates [65,66••,67••].

Conflict of interest statement

Nothing declared.

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