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New channel lineup in spinal circuits governing locomotion

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The locomotor network is a neural circuit capable of generating quite varied and complex patterns of motor activity. While neurotransmission and neuromodulation play a major role in fine-tuning motor function, neuron's firing properties driven by a palette of intrinsic ionic conductances and pump currents, play an equally important role. This review highlights the papers published over the past few years studying ionic conductances in spinal circuits governing locomotion. I discuss how this ensemble of currents underpins the operation of the locomotor network and summarize progress towards identifying the channels that mediate the ionic conductances.

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Introduction

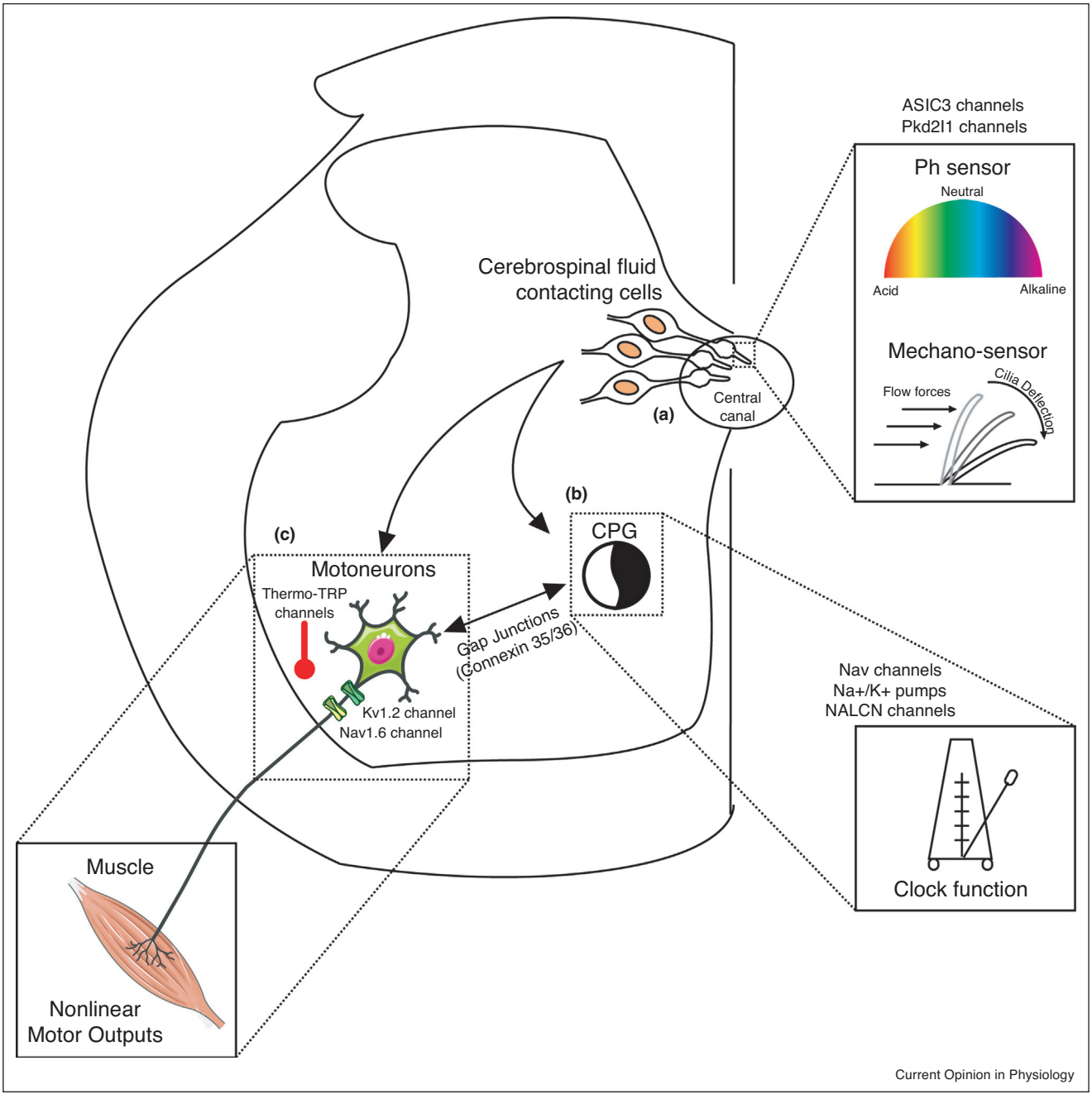
Locomotion is a complex motor behavior that demands a high level of sensorimotor integration at different levels of the central nervous system. Nevertheless, the spinal cord is able to self-sustaining basic locomotor movements in the absence of supraspinal and peripheral inputs [1•]. The spinal circuitry governing locomotion is composed of interconnected interneurons forming the central pattern generators (CPGs) and motoneurons that convey CPG command outputs to the muscles [2•]. To gain insight into the function of the spinal locomotor network, it is important to characterize individual ion channels in locomotor-related neurons, and determine their roles in generating rhythmic and coordinated movements during locomotion. Further to the last reviews on this topic [3–5], I will summarize the most recent advances in assigning distinct motor functions to specific channels and pumps across species.

Cationic channels link locomotion to interoception

Recent studies show that non-selective cationic currents (I_{CaN}) flowing through transient receptor potential (TRP) channels modulate the excitability of vertebrate spinal circuits underlying locomotion and posture [6,7•,8]. In vertebrate spinal cords, polycystic kidney disease 2-like 1 (Pkd2l1) TRP channels are selectively expressed in a single, phylogenetically conserved population population of GABAergic neurons contacting the cerebrospinal fluid along the central canal [9] (Figure 1a). In these neurons referred to as CSF-cNs, Pkd2l1 is sufficient to drive action potential firing, and provides both chemosensation and mechanosensation by transducing pH changes and membrane stretch in I_{CaN}, respectively [10,11••]. Recent investigations in zebrafish investigate how mechanosensory feedback provided by Pkd2l1 shapes active locomotion. These studies demonstrate in mutants for Pkd2l1 that CSF-cNs are less responsive to bending of the tail and animals display a slower swim frequency indicating a role for CSF-cNs in enhancing the speed of locomotion [11••]. Note that the authors also provide evidence implicating CSF-cNs in postural control *in vivo* [12]. The voltage-insensitive cationic acid sensing ion channel 3 (ASIC3) is not a member of the TRP family, but like Pkd2l1, it confers on CSF-cNs the ability to report pH changes of the cerebrospinal fluid within a narrow range from pH 7.4 [10,13••,14••] (Figure 1a). During ongoing fictive locomotion in lampreys, either an increase or a decrease of pH reduces the locomotor burst rate [13••,14••]. Note that ASIC3 channels are also activated during movement through their mechanosensitivity, and similarly reduce the activation of the locomotor network. In sum, CSF-cNs act both as mechanoreceptors and as chemoreceptors through Pkd2l1 and ASIC3 channels and powerfully modulate locomotion and posture by providing feedback to components of the spinal locomotor network [8,13••,15].

I_{CaN} also influences the excitability and the nonlinear firing pattern of lumbar motoneurons in mammals [6,7•] (Figure 1b). Motoneurons of neonatal rats behaves like a four-stroke engine (Figure 2a), such that in response to a brief excitation the I_{NaP}-dependent spiking activity (step 1) triggers Ca^{2+} entry through voltage-gated Ca^{2+} channels (step 2) which initiates a Ca^{2+}-release process (step 3) that ultimately activates a thermosensitive I_{CaN} to promote a plateau potential (step 4). The plateau potential in turn drives a self-sustaining spiking activity and thus relies on I_{CaN} carried by Na^{+}, and is switched off by a temperature below 29 °C (Figure 2a,b). Because rats are poikilotherms at birth, the development of thermoregulatory mechanisms may contribute to the late emergence of posture by regulating the expression of thermosensitive self-sustaining spiking in motoneurons with age (Figure 2b). Such mechanisms may also strongly

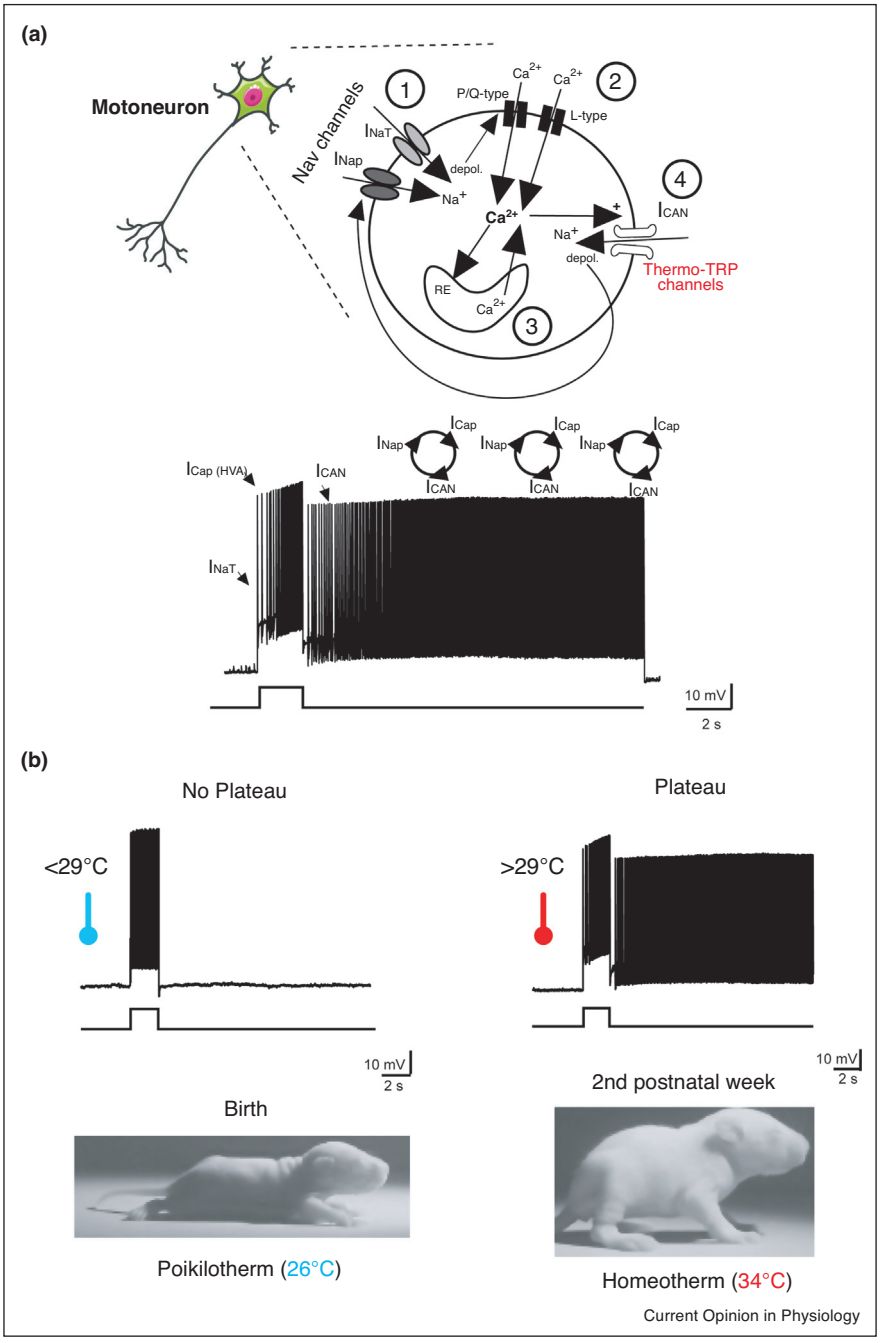
Figure 1



Individual ion channels in locomotor-related neurons, and their role in the operation of the spinal locomotor network. **(a)** At the level of the central canal, cerebrospinal fluid contacting cells (CSF-cNs) act both as mechanoreceptors and as chemoreceptors through Pkd2l1 and ASIC3 channels and modulate locomotion and posture by providing feedback to components of the spinal locomotor network. **(b)** At the locomotor CPG level, NALCN channels, Na^+/K^+ pumps and the persistent sodium current mediated by sodium channels regulate the rhythm-generating network. **(c)** At the motoneuronal level, thermo-TRP, Nav1.6 and Kv1.2 channels promote nonlinear firing properties for powering up motor outputs. Through Gap junctions, motoneurons regulate the spiking activity of their upstream partners involved in rhythm generation and pattern formation.

influence the temperature-dependency of motor behaviors in cold-blooded animals. The nature of the channel at the origin of thermosensitive Na^+-mediated I_{CaN} remains unknown. Some lines of evidence suggest a contribution of thermo-TRP channels [7•] such as TRPV1 and TRPM8 that contribute to the excitation and inhibition of spinal locomotor CPGs, respectively [16] (Figure 1b). Still, it should be stressed that additional

Figure 2



Thermosensitive nonlinear firing properties in motoneurons. **(a)** Ca^{2+} entry via voltage-dependent Ca^{2+} current occurs during the train of action potentials. The resulting increase in intracellular Ca^{2+} triggers a voltage-independent cation current that depolarizes the membrane to maintain the spiking discharge. The repetitive spiking activity will then induce Ca^{2+} entry via voltage-dependent Ca^{2+} current and so forth. **(b)** The CPG for locomotion is functional at birth but rodents can only walk later, during the 2nd postnatal week. The posture is the main limiting factor for locomotion. The first postnatal week is a time for rapid changes in firing properties of lumbar motoneurons. Plateau potentials are thermosensitive and emerge only at temperatures >29 °C. Because rats are poikilotherms at birth (26 °C), the development of thermoregulatory mechanisms may contribute to the late emergence of posture by regulating the expression of thermosensitive plateaus with age.

studies will be necessary to define the involvement of other thermos-TRPs channels such as TRPM4 and TRPM5 for which the activation at warm temperature and the selective permeability to Na^+ are compatible with I_{CaN} identified in lumbar motoneurons. Finally, out of the physiological context, the activation of I_{CaN} by inflammatory pain mediators sensitizes motoneurons by increasing their excitability and promoting plateau potentials [6].

In sum, in addition to their traditional role in transducing sensations from the periphery, TRP channels link locomotion to interoceptive information. This endows the spinal locomotor network the ability to adjust motor behaviors to the internal state of the body.

Gap junction channels link motoneurons to the locomotor-rhythm generator of aquatic vertebrates

Vertebrate CPGs are often viewed as operating in a top-down manner in which premotor interneurons drive motoneurons. Among premotor interneurons, V2a interneurons represent a source of excitation that imparts on the spinal circuit the capacity to generate locomotion [17•]. A recent study in zebrafish shows the existence of electrical coupling linking motoneurons to V2a interneurons via connexin 35/36 channels [18••] (Figure 1). This gap junctional network allows motoneurons to regulate the spiking activity of their upstream partners involved in rhythm generation and pattern formation. Specifically, optogenetic inhibition of motoneurons decreases the frequency of locomotion by reducing the activity of V2a interneurons via gap junctions. These results reveal a prominent feedback control of motoneurons onto components of the rhythm generator circuit and suggest that motoneurons are an intregral part of the locomotor CPG. In mammals, the pharmacological blockade of gap junctions in the spinal cord reveals the importance of electrical coupling to synchronize bursting cells in order to generate rhythmic activity [19]. However, the existence of V2a-motoneuron coupling remains unclear in mammals [20•] while the motoneuron feedback to regulate the locomotor CPG in mice occur independently of electrical connections [21••].

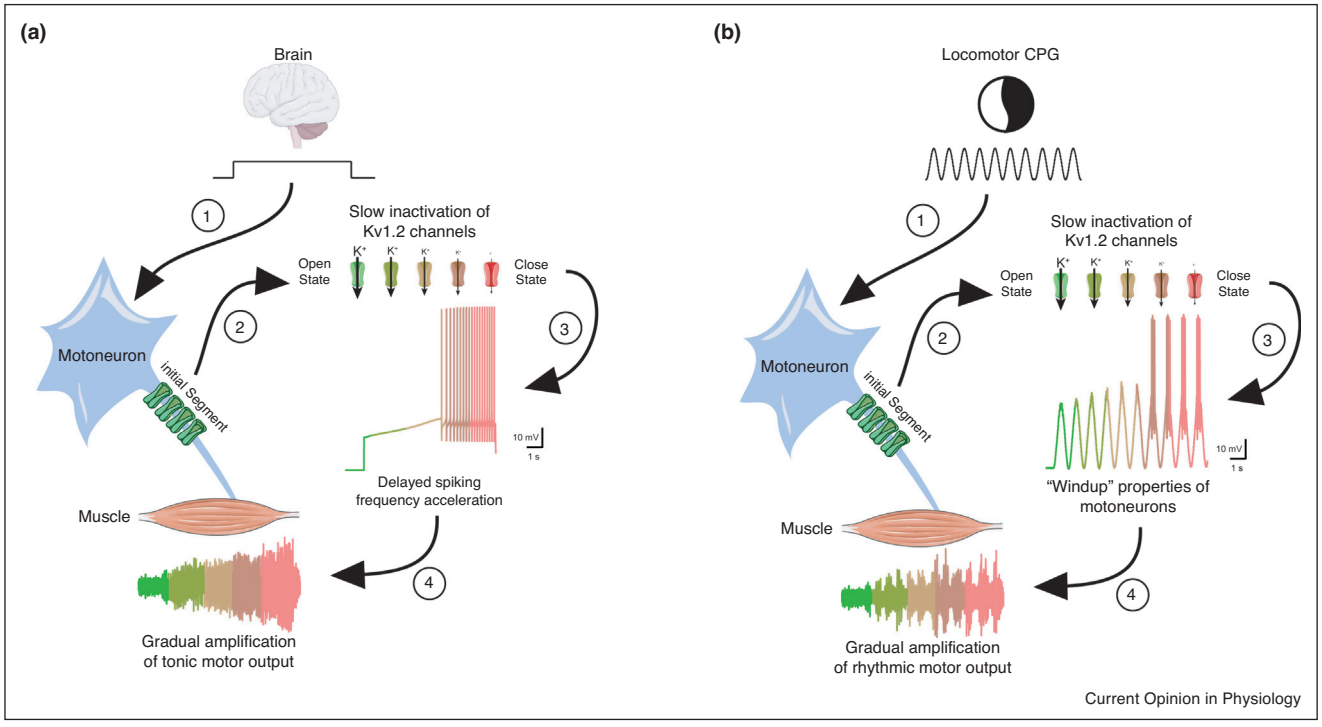
Motoneuronal Kv1.2 channels gates locomotor transition

Spinal motoneurons are not simply the final common pathway for movement but possess a number of important nonlinear properties that can shape motor outputs. The most distinctive nonlinear firing property is the generation of self-sustained tonic spiking evoked by a brief excitation that arises from the plateau potential mediated by a thermosensitive I_{CaN} (see above). The voltage transition to the plateau is constantly initiated by a subthreshold slow membrane depolarization. For decades, this 'launch pad' of the plateau potential, has been attributed to the activation of nifedipine-sensitive L-type Ca^{2+} channels. A recent study combining *in vitro* and *in vivo* motoneuron recordings with computational modeling provides evidence that the voltage transition to a plateau does not involve activation of Ca^{2+}channels, but instead results from the slow inactivation of nifedipine-sensitive Kv1.2 channels highly expressed at the initial segments of motoneurons [22••] (Figures 1b and 3). After an initial hyperpolarization induced by the recruitment of Kv1.2 channels, the very slow inactivation of Kv1.2 initiates a slow voltage transition to a delayed spike-frequency acceleration reported in large motoneurons [23,24] (Figure 3a). Thus, during tonic motor commands from the brain, Kv1.2 channels may help motoneurons to reach their 'preferred firing range' for a smooth control of muscle output. Another important feature of Kv1.2 channels is their slow recovery from inactivation that provides motoneurons with a memory trace of their previous activity. As a result, successive brief excitations in motoneurons become more efficient in reaching the firing level due to a cumulative depolarization of the membrane potential (Figure 3b). This Kv1.2-mediated phenomenon, commonly referred as a 'wind up', provides a potent gain control mechanism in mammalian spinal motoneurons and has a behavioral role in enhancing locomotor drive from the CPG during the transition from immobility to steady-state locomotion. In sum, Kv1.2 channels play a fundamental role in the dynamics of locomotor circuits by switching motoneurons between gating and amplifying modes.

The persistent sodium current: a prevalent rhythmogenic conductance for locomotion

At the core of the rhythm-generating network for locomotion are sets of ventrally positioned glutamatergic interneurons with ipsilateral axons to drive motoneurons. A series of modelling and experimental studies have demonstrated that some of them have the intrinsic ability to oscillate in a frequency range similar to stepping rhythms [19]. The search for the biophysical basis for rhythmogenesis has identified the persistent sodium current (I_{NaP}) as contributing to rhythmic bursting in locomotor-related interneurons [25,26]. The immediate conclusion was that the locomotor rhythm generation could emerge from excitatory circuits incorporating I_{NaP} as a 'pacemaker' current. In line with this, the pharmacological blockade of I_{NaP} abolishes locomotor-like activity in rats [19,27,28••], mice [29]) or salamanders [30] and severely affects locomotor behaviors in zebrafishes [31•] and Xenopus laevis tadpoles [32]. Furthermore, as a consequence of activity-dependent changes in extracellular calcium ($[Ca^{2+}]_o$) and potassium ($[K^+]_o$) concentrations during locomotion, a large number of CPG interneurons are converted from regular-spiking to bursting mode mediated by I_{NaP} [28••] (Figure 4). Altogether, a picture emerges that the locomotor rhythm arises from a dynamic interplay between circuit-based activity and cellular processes with a critical role for I_{NaP}. Beyond its rhythmogenic role, the I_{NaP} also

Figure 3



Roles of motoneuronal Kv1.2 channels in the dynamics of spinal locomotor circuits. **(a)** In motor tasks that involve a tonic recruitment of motoneurons such as during posture, the slow inactivation of Kv1.2 initiates a voltage transition to a delayed nonlinear spiking activity. Thus, Kv1.2 channels may be useful in generating a smooth gradation of muscle force **(b)** In motor tasks that involve repetitive movements such as during locomotion, the slow recovery from inactivation of Kv1.2 channels elicits a temporal facilitation of muscles activities upon the initiation of locomotion.

contributes to the self-sustaining spiking activity of motoneurons triggered by a brief excitation [7•]). Therefore, apart from its role in the locomotor rhythm generation, the I_{NaP} might be important in the maintenance of postural tone (Figures 1,2).

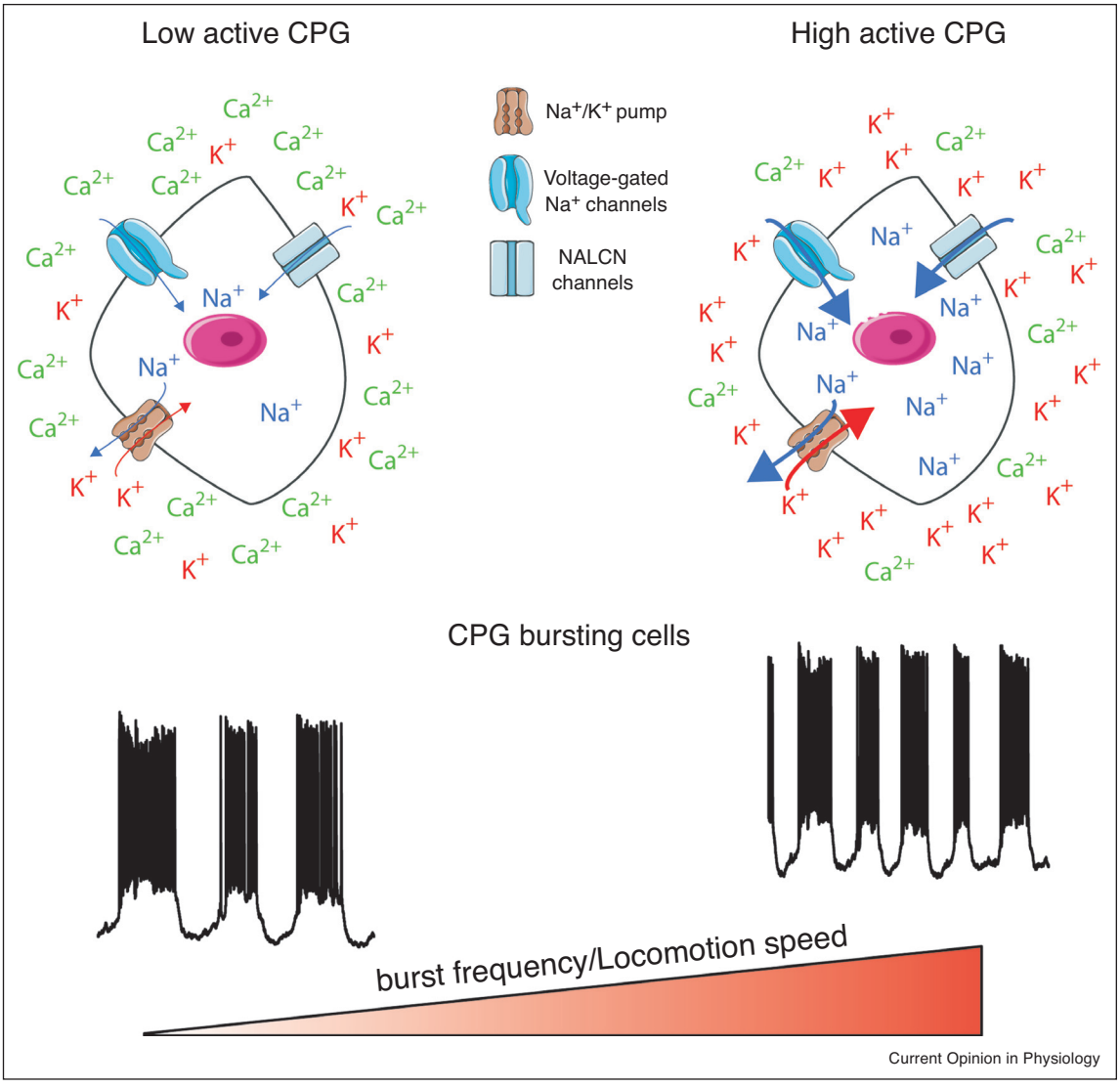
An important yet unanswered question is whether I_{NaP} in the spinal locomotor network arises from different sets of sodium channels or whether I_{NaP} results from a unique channel type. Although the expression profile of sodium channels at the CPG level is unknown, the distribution of sodium channels within the axon initial segments of lumbar motoneurons has been detailed [33] (Figure 1b). It appears that Nav1.1 expression is restricted to a narrow proximal domain in contact with the soma, segregated from the dominant isoform Nav1.6 uniformly expressed along the initial segment. The I_{NaP} might thus be a composite product of several sodium channels with a substantial contribution of Nav1.6, at least in motoneurons. Furthermore, a causal relationship between the proteolytic cleavage of Nav1.6 channels by calpain and the upregulation of I_{NaP} has been found in motoneurons after a spinal injury [34••].

Na^+/K^+-pump as a dynamic regulator of spinal locomotor networks

The $[K^+]_o$ increases to a steady-state level of 6 mM when the locomotor network is active [28••] (Figure 4), but the roles of different cellular mechanisms involved in $[K^+]_o$ homeostasis during neuronal activity *in situ* are poorly understood. The electrogenic Na^+/K^+ pump commonly increases its activity during intense neuronal firing to limit both the rise of $[K^+]_o$ and the intracellular Na^+ accumulation (Figure 4). Interestingly, a series of recent investigations in *Xenopus* tadpoles and neonatal mice demonstrate a central role of Na^+/K^+ pumps in regulating active spinal locomotor networks [35,36•,37•,38•]. Briefly, the pharmacological blockade of Na^+/K^+ pumps with low concentrations of ouabain significantly increases the frequency and duration of rhythmic locomotor bouts. This effect involves the block of a dynamic activity-dependent pump hyperpolarization in a subset of premotor neurons [35] (Figure 1).

A wait of several 10 s is necessary to reproduce a locomotor episode similar to the previous one. This post-locomotor depression results from a Na^+/K^+ pump-mediated

Figure 4



Ionic channels involved in the generation and the modulation of the locomotor rhythm. Activity-dependent changes in extracellular calcium $[Ca^{2+}]_o$ and potassium $[K^+]_o$ concentration reveal pacemaker neurons in the locomotor CPG neurons. TTX-sensitive persistent Na+ channels and NALCN channels are upregulated by the $[Ca^{2+}]_o$ decrease. The persistent sodium current contributes to the intrinsic burst generation while NALCN increases the frequency of bursts by depolarizing cells. The increased $[K^+]_o$ also provides an additional depolarization of pacemaker cells via the reduction of the voltage-gated potassium and leak currents, which also increases the frequency of oscillations. Finally, dynamic activity-dependent Na+/K+ pumps regulate the locomotor rhythm by hyperpolarizing premotor and motor neurons and clamping down the $[K^+]_o$ increase.

ultraslow membrane hyperpolarization that, in addition to shifting the membrane potential away from the spiking threshold, releases the *A*-type K^+ current from inactivation, delaying the activation of neurons when activity resumed [38•,39]. Interestingly, the Na^+/K^+ pump acts as a short-term memory mechanism of the motor network performance as the post-locomotor depression correlates with the intensity of the previous locomotor episode [37•,38•]. At the molecular level, the Na^+/K^+ pumps containing the α3-subunit are thought to be responsible for reducing the excitability of the CPG network. This subunit is broadly expressed in locomotor-related interneurons such as Pitx2 [36•] but appears absent from α-motoneurons [40]. Thus, the nature of the Na^+/K^+ pump at the origin of the ultraslow membrane hyperpolarization in motoneurons [36•] likely differs from that of CPG interneurons and remains to be identified. Dynamics of the Na^+/K^+ pump current along with those of the hyperpolarization-activated current (Ih) has been also reported to shape the bursting activity in CPG neurons

of leeches [41], and tadpoles [42]. In sum, Na^+/K^+-pump interactions with *A and H*-currents may endow spinal locomotor networks with the capacity to self-regulate rhythmic motor output in an activity-dependent manner.

NALCN (sodium leak channel non-selective) gates transition from slow to fast locomotor movements

The tetrodotoxin (TTX)-insensitive and voltage-independent NALCN channel mediates a background Na^+ leak current that helps increase neuronal excitability by setting the resting potential above the equilibrium potential for K^+. Recent studies imply a requirement of this channel in neural networks generating rhythmic locomotor behaviors [43,44•,45]. Specifically, in *Caenorhabditis elegans*, mutations in NALCN lead to a unique motor deficit characterized by abrupt and frequent stops during swimming, while the ability to move slowly by crawling is still conserved [43]. Molecular genetics combined with electrophysiological recordings indicate that a reduced premotor interneuron network activity, instead of a motoneuron dysfunction, is the primary cause of the motor deficits [45]. Thus, NALCN appears to regulate the premotor interneuron network to switch from a slower to a faster form of locomotion (Figure 1), possibly by depolarizing pacemaker neurons such as observed within the *Lymnaea Stagnalis* CPG [46] (Figure 4). Of special interest, the respiratory rhythm requires NALCN in mice [47], and a NALCN leak current can be activated by lowering $[Ca^{2+}]_o$ [46]. Even if pacemakers emerge from a $[Ca^{2+}]_o$ decrease within murine locomotor CPGs [28••] (Figure 4), the involvement of NALCN in regulating the locomotion rate in mammals remains an open question.

Conclusion

In the last few years, the role of ion currents in the spinal locomotor network has received substantial attention, the identification of the channels at the molecular level is sill in its infancy, although some of the main players, such as TRP gene-encoded or sodium channels, are beginning to emerge. Research on the functional role of ion channels faces technical constraints, in particular the lack of drugs able to block specifically one of the hundreds of subunits belonging to the same family. The use of mutagenesis is also difficult insofar as functional compensations among ion channels are common. To overcome these limitations, future developments leading to the photocontrol of native ion channels with photoswitchable conditional subunit techniques are promising [48]. Whatever the difficulties, continuing efforts in understanding the role of ion channels in the spinal cord will not only advance our basic understanding into the operation of the locomotor network but should have direct effects in the treatment of channelopathies at the origin for numerous human disorders [49].

Conflict of interest statement

Nothing declared.

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