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Current Opinion in Physiology

When networks walk a fine line: balance of excitation and inhibition in spinal motor circuits

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Investigations on spinal motor circuits have primarily been related to direct connections to motoneurons and supraspinal input, while the motor pattern generation circuit itself has remained elusive. In the classical half-center model (HCM), motor patterns are generated by feedforward excitation with reciprocal inhibition. However, experiments over the last decade have indicated that inhibition, besides providing reciprocal coordination, may serve additional roles similar to that seen in the brain. Such organization relies on recurrent inhibition to give stability of the spiking activity manifested by simultaneous increases in excitation and inhibition within the network, that is, a 'balanced network'. Here we discuss the theoretical concepts and experimental data for and against this architecture in motor circuits, and suggest how it can be integrated in the conventional HCM.

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Current Opinion in Physiology 2019, **8**:76–83

This review comes from a themed issue on **Motor control systems**

Edited by **Gareth B Miles** and **Claire Wyart**

For a complete overview see the Issue and the Editorial

Available online 5th February 2019

https://doi.org/10.1016/j.cophys.2019.01.006

Introduction

Many biological oscillations are generated by interlinked positive and negative feedback loops, and the presence of these loops is considered a fundamental principle in biology [1]. For rhythm-generating circuits in the spinal cord and the medulla to concur with this principle they should possess negative as well as positive feedback, likely on both the cellular and network level. Nevertheless, the most influential model for spinal motor circuitry, the half-center model (HCM), does not explicitly include negative feedback as a key component of the neuronal circuitry. The HCM, which was introduced by Graham Brown [2], has had a remarkable influence on the spinal research field and its core idea has remained essentially unaltered for more than a century. The components consist of feedforward and recurrent excitation, coupled with reciprocal inhibition between modules [2,3,4], that is, only positive feedback. The activity of each module is a direct reflection of the contraction of a given muscle and this simplicity is both intuitive and appealing. It also entails a clear separation of excitatory from inhibitory input to any neuron in the system. Part of its success is due to the immense evidence of alternating excitation and inhibition to motoneurons, that seems omnipresent across species. Hence, it was unexpected to discover that a portion of spinal neurons received a simultaneous (concurrent) increase in excitation and inhibition (E/I) [5–7]. Previous investigations have reported less clear although similar observations in a smaller number of motoneurons [8,9]. Nonetheless, these observations were noteworthy since they could represent the missing negative feedback, that was otherwise only present as cellular 'fatigue'. The spinal motor network is obviously more complicated than the original HCM, but since extensive overlap in E/I input is widespread in most other parts of the central nervous system, a comparison was imminent: Could spinal networks have more in common with the brain than previously thought? Concurrent E/I tends to reduce the excursion of the membrane potential (V_m) and is often referred to as 'balanced (E/I)' [10,11•]. Balanced E/I has both been observed extensively in experimental investigations of the cerebral cortex and remains a widely appreciated concept in theoretical neuroscience because of the beneficial properties, in particular the negative feedback [10,12,13]. However, since it entails fluctuation-driven spiking and such randomness seemed contradictory to robust motor activity, the resemblance between cortex and the spinal cord was considered 'heterodox' [3]. Nevertheless, a consequent dichotomy between spinal networks and the brain raises new questions: Are spinal networks fundamentally different from other neuronal networks? If so, how? Do spinal motor circuits operate without central negative feedback? Here, we argue that spinal and medullary networks, although unique and adapted for their specific purpose, still share principles with networks in other places, in particular the ubiquitous need for stabilization of reverberating excitation. We review the experimental evidence for and against balanced E/I and discuss how it in fact could be compatible with the HCM.

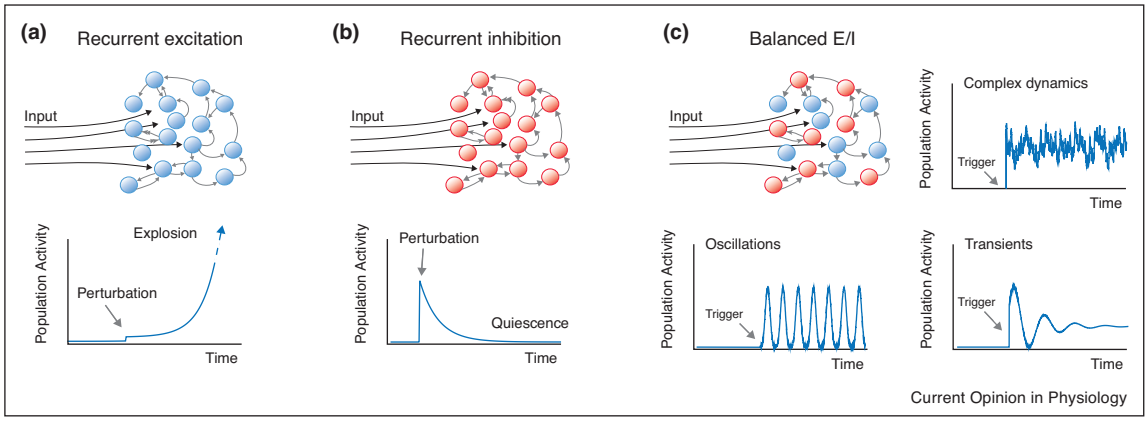
A counter-intuitive principle of neuronal network?

The concept that the activity of inhibitory neurons increases in parallel with that of the excitatory neurons,

is contrary to common sense. Why would the organism use valuable energy on synaptic input that pull V_m in both directions at the same time? Nevertheless, this organisation has been widely observed in brain networks, primarily documented in cortical networks [12,13]. It has been suggested to have important computational properties both on cellular level, for example, modulation of gain [14•], as well as on network level allowing a rich repertoire of behavior and avoiding catastrophic runaway activity [10] that is, associated with recurrent excitatory networks (Figure 1a). Obviously, the runaway will not continue indefinitely, but rather reach saturation, where cellular mechanisms driven by Ca^{2+}-accumulation such as synaptic depression provide a form of negative feedback [15]. Introducing recurrent inhibition stabilizes the activity, but too much inhibition will result in a quiescence and an inability to sustain activity (Figure 1b). A proper mixture of recurrent excitation and inhibition allows not only stable sustained activity, but a rich repertoire of different dynamics, for example, oscillations, transients and more complex behaviors [10,16,17] (Figure 1c). Such a balance is an essential feature of networks with sparse recurrent connections, and this topology is also sufficient to explain the enigmatic chaotic activity even under deterministic conditions, that is, it naturally induces irregular firing pattern without requiring a stochastic component in the model [18]. The balance in cortical circuits is achieved and maintained by plasticity and developmental stage is important, for example, in the auditory cortex [19]. Furthermore, it can be dynamically modified by neuromodulators, and pharmacological manipulation, for example, by blocking the action of inhibition, which effectively removes the negative feedback mechanism and results in epileptic activity [11•]. Similar convulsion appears when GABA and glycine receptors are blocked in the spinal cord [20], suggesting an overall presence of balanced E/I. Spasticity is also associated with a reduced presence of inhibition [21], causing both muscular co-contraction from diminished reciprocal inhibition and spontaneous spasms as a reduction in recurrent inhibition. There are examples of central pattern generators (CPGs) without chemical excitation in invertebrates, for example, the swim CPGs of the sea slugs *Melibe leonina* and *Dendronotus iris* [22]. Here, the positive feedback is mediated by processes within cells and gap-junction mediated excitation between cells.

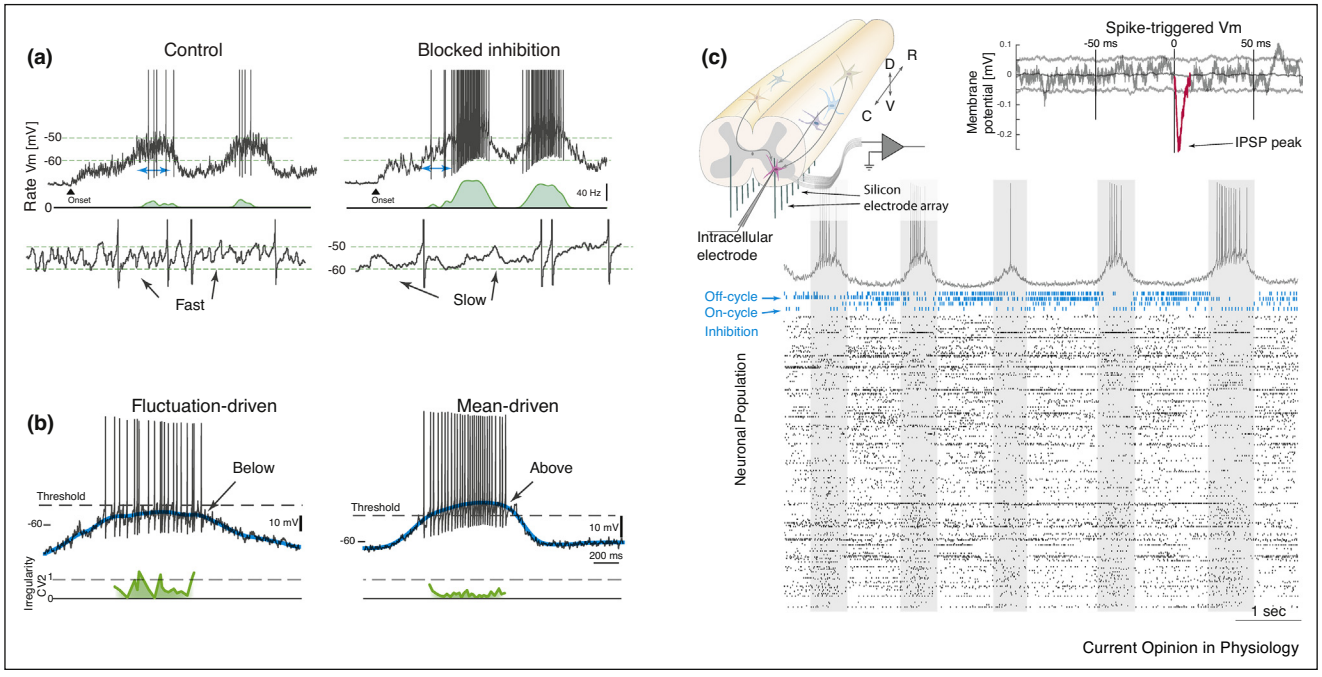
In the cortex, the enigmatic irregular spiking activity was successfully explained by balanced E/I, which is now considered an essential network property [12]. Similar explanation for the irregular spiking in spinal networks was suggested, since spiking irregularity vanish and the V_m transients become slower when inhibition is blocked (Figure 2a). The slower V_m-dynamics is due to the reduced synaptic conductance, that is, an increase in membrane resistance (R_m) and time-constant ($\tau = R_m C_m$, where C_m is the constant membrane capacitance). Generally, irregularity in spiking and V_m fluctuations are hallmarks of concurrent E/I [16]. Another consequence of balanced E/I is the increase in membrane conductance, which can lead to a high-conductance state [24]. Such indirect indicators are valuable, since a direct measurement of the synaptic input is non-trivial. Methods of estimation of synaptic input have been proposed, which essentially explores the membrane conductance and potential, for example, estimated via R_m [6]. Other less quantitative approaches include application of pharmacology and optogenetics.

Figure 1



Neuronal network topologies and their dynamics. **(a)** Recurrent excitation has a susceptibility to instability. Small perturbations from afferent input will likely result in an internal activity that quickly growths catastrophically ('explosion'). Hence, this topology is unsuitable for generation of motor activity. **(b)** A topology of pure recurrent inhibition lacks the ability to sustain internal activity, and quickly becomes quiescent. Thus, this topology is also not suitable for generation of motor activity. **(c)** A mixture of the two, gives a balanced network, which has the potential for a plethora of activity, such as oscillations, transients or more complex dynamics depending the synaptic weights and the initial conditions set by afferent input [10,16,17].

Figure 2



Heterogeneity in pattern of synaptic input during rhythmic motor activity. **(a)** Motoneuron receiving balanced E/I (left) during rhythmic motor activity. Synaptic input is 'unbalanced' by blocking inhibition (right). As a result the V_m is released causing a depolarization and increase in spike rate (green). Another consequence is a dramatic slow-down of fluctuations (cf. bottom right vs. left, blow-up at blue arrows). Spikes clipped. **(b)** A neuron receiving balanced E/I with mean (blue line) below threshold (broken line) causes irregular fluctuation-driven spiking (left), whereas another neuron receive mean-driven (e.g. excitation only, with mean above threshold) causes AHP-dominated regular spiking (right). **(c)** Combined intracellular and multi-electrode recordings (top left), allows identification of inhibitory connections via inhibitory post-synaptic potentials (IPSP) in the spike-triggered V_m (top right). Bottom: Spike times of 4 inhibitory interneurons were identified (blue raster), 3 have mostly off-cycle spiking (alternating with excitation), whereas one has on-cycle spiking (i.e. concurrent with excitation). Spike times of the rest of the recorded population shown below in black raster ($n = 249$). Adapted with permission: (a) [14•], (b) [11•] and (c) [41•].

Balanced E/I in respiratory system

Early indicators of balanced E/I were found in the respiratory system where phrenic and hypoglossal motoneurons received simultaneous E/I [25–27]. The balance of E/I was suggested to be important for controlling the hypoglossal motoneuronal excitability [27]. Regarding a balance within the respiratory network itself, the role of inhibitory neurons remains controversial. Some experiments indicate that inhibitory neurons are not essential in the rhythm generating circuits since the breathing rhythm continued after blocking inhibition [28], although this approach may have engaged compensatory mechanisms [29]. About half of the neurons in the preBötzinger complex are inhibitory and blocking their activity in the anaesthetized mouse caused a strong increase in the burst amplitude of the glutamatergic neurons during the respiratory cycle [30••]. Based on these observations investigators have proposed that the rhythmogenic network consists of a population of bursting glutamatergic neurons, which are balanced by inhibition in a sparse connectivity within the preBötzinger complex [29,31••].

Evidence in spinal circuits

For circuits in the spinal cord, balanced E/I was observed during rhythmic hindlimb movements of the spinalized turtle performing scratching [5,7,32]. This conclusion was deduced from conductance measurements combined with pharmacological verification and quantification of irregularity of spiking (Figure 2]), and the investigation was inspired by previously observed high conductance [33]. Nevertheless, the generalization of these observations has been controversial. Testing in mammals using the neonatal mouse performing fictive locomotion indicated reciprocal E/I [34]. Another preliminary study in the neonatal mouse, used wide-field calcium imaging of genetically tagged neurons (En1-derived V1 neurons) and found many inhibitory neurons that were active in-phase with the nearby motoneuron pool, which suggested concurrent inhibition to the excitatory phase [35].

A more recent investigation in turtles reported reciprocal E/I input to motoneurons in opposition to balanced input using whole-cell recordings [36]. The authors argued that the previously observed high-conductance [5,32,33], was

merely due to excitation balanced by a voltage-activated intrinsic K^+-current. Nevertheless, these findings left a number of issues open. First, the outward-current occurred above −30 mV, far above the V_m that previous high conductance was observed [32]. Second, their interpretation was inconsistent with the observation that when blocking chloride-based inhibition (glycinergic) the conductance was clearly reduced and resulting in excessive spiking (Figure 2a) [5,14•]. Further, the spiking after-hyperpolarization (AHP) was shunted when synaptic input appeared while keeping depolarization constant with current injection [5]. When blocking both E/I, the V_m conductance was substantially reduced, and forcing the V_m to the same depolarization with electrode current had no effect on the conductance in this range [6]. Third, NMDA-bursting motoneurons in absence of synaptic input also did not exhibit high-conductance [32], although the voltage should activate same intrinsic conductances. Together these observations are incompatible with the proposition that the high conductance is primarily due to intrinsic conductance instead of concurrent E/I. Shortly thereafter another report was published, which was more compatible with previous observations: While the majority of the recorded neurons received reciprocal E/I, a 'substantial overlap between excitation and inhibition' was observed in a subset of cells [37]. Hence, it appears there is a diversity within the population, where some cells receive balanced E/I and others receive reciprocal E/I. This is akin to the diversity observed among hypoglossal motoneurons, where some received balanced E/I and others received for example excitation-only [27]. The quantitive difference of the reported number of cells belonging to either of these categories could be explained by a method-based selection bias, when using sharp- versus whole-cell electrodes.

Other investigations have demonstrated a role of balanced E/I to control force in conjunction with the reciprocal input. The motoneurons received a baseline of balanced E/I, which created a potential for dis-inhibition and therefore a paradoxical effect of a larger force [38]. Investigations using zebrafish fictive swimming has indicated a systematic shift in the balanced between E/I to motoneuron pool depending on the speed of locomotion [39], whereas pharmacologically induced swimming has clear separation of E/I [40]. In conclusion, there is a great diversity in experimental observations that cannot easily be explained by prevailing models.

Clues from the ensemble activity

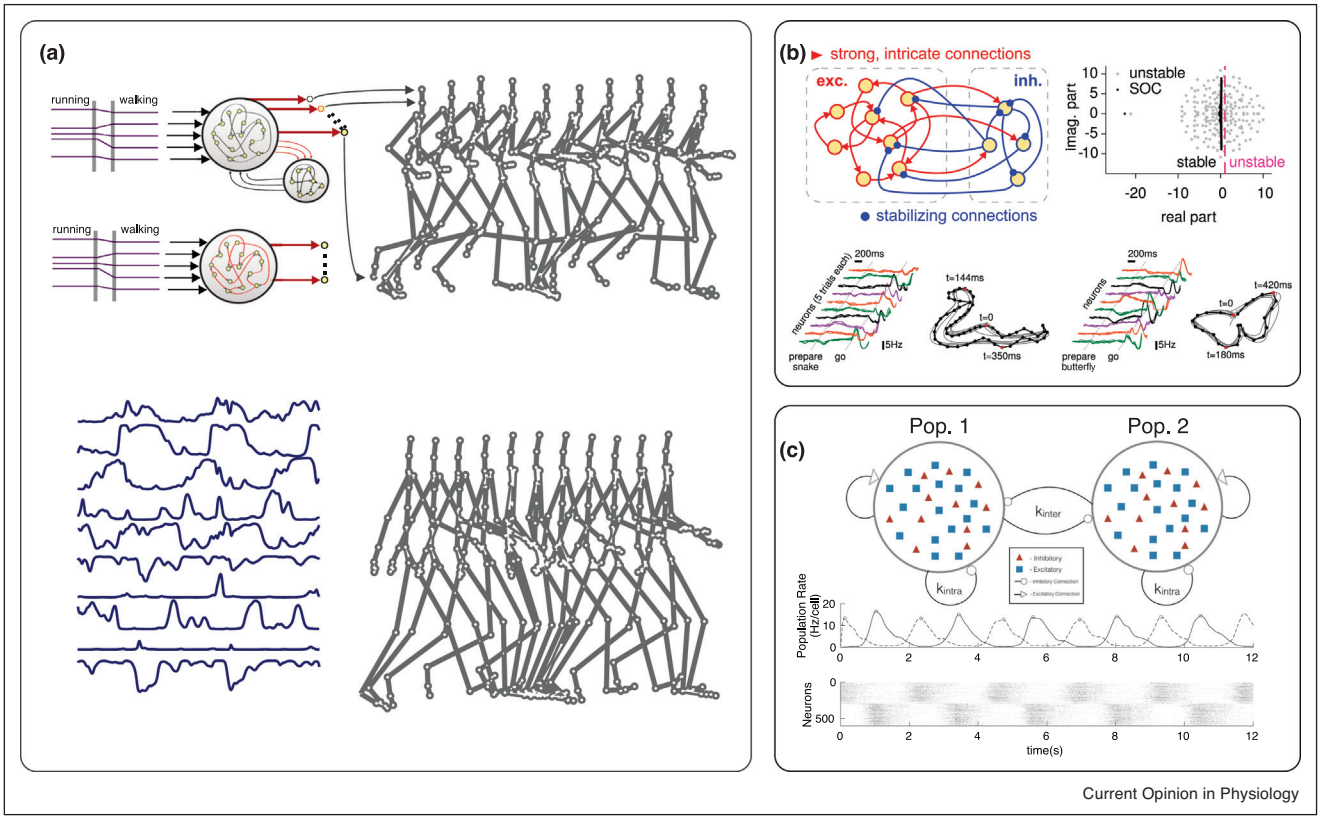
An alternative indicator of cellular diversity is the irregularity of spiking. If a neuron receives balanced E/I the V_m will most often linger below threshold and randomly elicit action potentials (Figure 2b, left). This is known as fluctuation-driven spiking [23]. The opposite case is where pure excitatory input drives the mean V_m across threshold and the inter-spike intervals are determined by AHP. This causes regular spiking and is known as mean-driven discharge (Figure 2b, right). A recent study utilized this discrepancy to characterize the population spiking using multi-electrode arrays in the spinal cord [11•]. Here, the prevalence of irregular spiking was significantly higher than that reported using only patch electrodes [37]. Combining multi-electrode extracellular recordings with intracellular monitoring, it was possible to verify a direct excitatory or inhibitory connection. The network connectivity was remarkably sparse [41•]. In these experiments, it was possible to tease apart a small number of inhibitory connections to a single neuron during motor activity. The timing of spike patterns of these inhibitory neurons in relation to the motor cycle is an illustration of the heterogeneity. Three presynaptic neurons spiked primarily out of phase, that is, reciprocal inhibition, one primarily in-phase, that is, balanced E/I, all with some overlap (Figure 2c).

The diversity in experimental data suggest a more complex mode of operation. Some of the neurons receive strongly tied E/I, as one would expect from a balanced network. Yet, others have clear reciprocal organization, as would be expected from the Ia-interneurons and commissural fibers [2,3]. Yet others, have less clear separation. Could it be that spinal circuits both have local balanced components and reciprocal inhibition? One certainly does not exclude the other [7]. When measuring the population spiking activity during rhythmic motor activity, the diversity become more apparent. The amount of irregular spiking is an indicator of the concurrent E/I, and this metric illustrated that many neurons do not spike either regularly or irregularly. Rather, they can change between mean-driven and fluctuation-driven spiking, throughout the trials [11•]. A composite population metric showed at least 50% of the neurons spend more than half of their time in the fluctuation-driven regime, indicating a general presence of balanced E/I [11•].

Wind of change from computational neuroscience: recurrent connectivity

Neuronal networks with recurrent excitation that is, finely balanced by inhibition has the capacity to possess a plethora of different dynamic patterns, that can be turned-on given the appropriate activation [16,17]. Recent work demonstrated that models of recurrent random networks can be trained to generate patterns with close resemblance to various forms of human locomotion and controlled in a truly multifunctional fashion (Figure 3a,b) [42]. Such computational models are interesting because they illustrate multiple important and relevant issues. First, circuits are not required to have a modular and reciprocal organisation to induce motor patterns. Therefore these models represent a fundamentally different alternative to the HCM. Second, the neurons in these networks do not possess any special properties, rather the behavioral output emerges out of the

Figure 3



Contemporary models of motor pattern generation. **(a)** A proof of principle: Multiple patterns of human locomotion can be generated by a random network model with recurrent connectivity. Adapted with permission [42]. **(b)** Multiple both simple and complex movements, such as drawing a snake or a butterfly, can be generated by a random balanced network when the circuit has been optimized for stability by inhibition. Adapted with permission [16]. **(c)** Two random balanced networks with reciprocal connections can induce rhythmic breathing patterns. Adapted with permission [31••].

network — hence the behavior is not determined by cellular properties, but is a network phenomenon [23,43]. The rhythm generation has often been suggested to originate from a 'core kernel' of cells [3] with properties that make their V_m oscillate. Nevertheless, such pacemaker cells have remained elusive. The issue is an active topic of discussion especially in the respiratory field [28,29], but there is a consensus that network properties are crucial. Hence, the demonstration that networks without cellular specialization are able to not only generate rhythmic output [17,42], but also multiple forms of patterns where the frequency and amplitude can be modulated independently, is interesting as a principle. Other modelling studies perform a comprehensive inclusion of experimental observations (see e.g. [44]).

A model that incorporated the balanced network architecture into the HCM (Figure 3c) was recently developed for the respiratory system [31••]. This consisted of a hybrid between the classical HCM and balanced local networks. This is also an important demonstration, that balanced networks and the half-center organization are not mutually exclusive. The diversity in synaptic input, for example reciprocal versus balanced E/I input, could be explained by a similar hybrid organization. Some neurons are more integrated into the local balanced circuitry, whereas others have more dominant reciprocal connections. This configuration may also be dependent on the level of activity, that is, required force and running speed as well as the particular task. A distinct behavior may activate a different circuit, thus engage more balanced input to the neurons previously receiving reciprocal input and vice versa. The key feature that would allow this type of flexibility is recurrent connectivity, both among excitatory, but also among and between inhibitory interneurons.

Connectome, cell types and recurrent inhibition

To properly elucidate the issue of connectivity would require a comprehensive reconstruction of the spinal connectome. Such a painstaking mapping of the wiring could be accomplished with a novel technique, the serial block-face electron microscopy [45]. This technique is currently able to resolve up to a cubic millimeter of tissue.

The data acquisition takes several years and generates approximately 1 petabyte of data [45]. This would constitute an unfathomable amount of details and complexity, which represent a major computational challenge to process. This approach is more feasible in insects and smaller vertebrates like larval zebrafish [46••], since a cubic mm is far from large enough to cover the network in for example mammals. In mice for instance, retrograde tracing using rabies virus indicated widely distributed premotor interneuron pools extending up to 8 spinal segments [47]. Here, a cubic mm would only cover a tiny fraction.

Nonetheless, a full network reconstruction may not be necessary to get an overview of the architecture and verify the presence of balanced networks. Tissue clearing methods and 3D-microscopy of cells expressing genetically targeted fluorophores [48] could assist in partial reconstruction of circuit motifs. Motifs responsible for balanced E/I, are recurrent inhibition and feedforward inhibition. Recurrent excitation is also required, but this is a given due to sustained motor activity (Figure 1). The extent to which recurrent inhibition is an integral part of spinal motor networks is poorly elucidated. Certainly, recurrent inhibition is present in the form of Renshaw cells (RCs). RCs belong to a broad class of cells, the V1-population, within the five cardinal classes of inhibitory interneurons in the spinal cord [49,50]. They inhibit the local motoneurons, from which they receive direct collateral input. Nevertheless, the RCs also receive input from the other parts of the locomotor network [51,52], where they could deliver feedforward inhibition. Targeting RCs vesicular transporter spurred compensatory adaptation [53••], which is an indication of the capacity for plastic changes. The V1-population primarily has ipsi-lateral connections, including the reciprocal Ia flexor-extensor inhibition [49,50]. It is composed of a myriad of sub-populations as demonstrated by a Bayesian regression analysis of their transcription factors, that identified at least 50 functionally distinct V1 sub-populations [54••]. Some of these could provide local recurrent inhibition in the central pattern generator network, thus establish local balance between E/I. The V1-neurons possess a diversity of biophysical properties [55••], and ablation can lead to hyperflexion [56]. Optical imaging in neonatal mice indicated that many of the En1-derived V1 neurons were active in-phase with the nearby motoneurons, which suggests concurrent E/I input to these [35]. Imaging the same neuronal type in zebrafish larvae also indicated concurrent activity although the temporal resolution prevented distinction between phases [57]. Another class of ipsilaterally projecting inhibitory cells is the V2b-population [58,59] in which some could also provide recurrent inhibition. Ablation of these led to exaggerated hindlimb extension [56]. The V2a population is excitatory (glutamatergic) and some of these may be involved in feedforward inhibition: Mouse locomotion was halted by activating brainstem V2a neurons [60••], which indicates indirect inhibition from supraspinal center on local locomotor circuits in the lumbar region.

Conclusions and outlook

One of the major concerns of the traditional half-center model, and variants thereof, is the absence of negative feedback on network level and the instability that it entails. Modules with pure recurrent excitation would quickly spin out of control on timescales 10-100 ms, much faster than general motor activity. The reciprocal inhibition, which is the dominant inhibition in the HCM, does not provide negative feedback. Rather, it exerts its effect on the antagonist module, which is already essentially silent. Furthermore, reciprocal inhibition is mutual and thus causes dis-inhibition, which is effectively positive feedback. Hence, the inhibition in the HCM provides no help in stabilization. The observation of balanced E/I presented in this review may be a clue to the inner workings of spinal motor networks and how they remain stable. We suggest that part of the role of recurrent inhibition — as well as feedforward inhibition — is to provide balance in the network as an essential part of generation of motor patterns. The motoneurons that receive balanced E/I, for reasons yet to be elucidated, could be more integrated into the balanced network architecture than the motoneurons that receive pure reciprocal E/I. The role of Renshaw cells has remained puzzling [51,53••]. These cells may represent just one type within a larger group of interneurons providing recurrent inhibition. Since RCs are directly connected to motoneurons they have been easy to identify, and have therefore likely received more attention than other types of recurrent inhibitory cells. There are many other types of inhibitory interneurons, that have remained uncharacterized, because their circuit motifs are more synaptic layers removed from motoneurons. We suggest that many of these participate in providing the missing negative circuit feedback.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by The Independent Research Fund Denmark (RB and HL), European Research Council (Mobilex, HL) and the Lundbeck Foundation (RB).

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- •• of outstanding interest

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Using a combination of modeling of balanced networks with hybrid half-center organization and experiments, the investigators demonstrate that inhibition affects synchrony, period variability, and overall frequency of the preBötzinger neurons and coupled rhythmogenic networks. This work expands our understanding of ubiquitous rhythmic motor networks.

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This study combines pairwise intracellular recordings with multi-electrode arrays to elucidate the architecture of the spinal CPG network. The expected pairwise correlation from a common source CPG is absent, indicating either a sparse convergent connectivity or active desynchronization by recurrent inhibition.

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Bayesian sparse regression analysis is performed and 50 functionally distinct V1 subpopulations are identified.

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The authors investigate the V1 subpopulation based on the expression of 32 transcription factors. They develop antibodies for 19 transcription factors, and demonstrate that these V1 subpopulations are spatially segregated along the dorsoventral and mediolateral axes. Electrophysiological recordings also show that the 19 diverse subpopulations express different biophysical properties.

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The investigators identify genetically defined excitatory neurons in the medulla that have strong control on locomotor activity. Unexpectedly, these excitatory neurons cause locomotor arrest, which suggests indirect inhibition of local locomotor circuits.