Mechanisms of Modulation of a Neural Network

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Neural networks form the basis for the generation and control of various patterns of behavior. Such networks are subjected to modulatory systems that influence their operation and, thereby, the behavior. In the lamprey locomotor network, analysis on the ion channel, synaptic, and cellular levels has given new insights into the organization of such modulatory systems.

How does the nervous system generate the different types of behavior that animals, including man, may display? A complete answer to this question requires identification of the neural circuits responsible, the different neural components involved, their connectivity, transmitters, the types of channels they possess, and their modulation via various receptors. With regard to vertebrate locomotion, it is well established that the neural network in the spinal cord can operate in the absence of sensory feedback. When present, however, it can profoundly shape the ongoing network activity. Spinal network activity is normally initiated by descending inputs from the brain stem in the intact animal (6) or by pharmacological activation of excitatory amino acid receptors in in vitro preparations. The neuronal components and their synaptic interactions have been characterized in spinal networks of relatively simple vertebrates; that is, in the lamprey and the Xenopus tadpole (2, 5). In the lamprey, the basic circuitry responsible for generating the undulatory swimming activity consists of excitatory glutamatergic and inhibitory glycineergic neurons (Fig. 1A) (5). The alternation between left and right side activity is due to reciprocal inhibition between the two sides of the locomotor network (Fig. 1A). The locomotor activity results from a wave of body undulations that, during normal locomotion, is propagated from head to tail with a phase delay between each segment, which is controlled by intersegmental coordinating mechanisms. The animal can also reverse the phase coupling from tail to head in backward swimming. The spinal locomotor network is subject to influence from various modulators via specific receptors, which act on specific ion channels and modulate synaptic transmission. Thereby, the operation of the locomotor network can be fine-tuned, and the coupling between the different segments can also be regulated. This results in a very flexible network organization.

In this review, we will first describe some pertinent ion channels present in the lamprey spinal cord neurons, in particular calcium channels and calcium-dependent potassium channels, and their role in the function of the spinal circuitry during generation of the basic locomotor activity. We will then summarize how various neurotransmitters that operate on ion channels modulate this activity, including presynaptic and postsynaptic modulatory mechanisms involved in fine-tuning the network. Finally, we will briefly illustrate the usefulness of detailed mathematical modeling of the different neuron types and of the locomotor network in revealing the mechanisms underlying the operation of the network and its modulation.

Calcium and calcium-dependent potassium channels

The lamprey spinal cord neurons possess both low-voltage-activated (LVA) and high-voltage-activated (HVA) calcium channels (3, 9). Several types of HVA calcium channels have been characterized in lamprey spinal cord neurons. The HVA component of the total calcium current is largely (~70%) mediated by calcium influx through N-type channels, whereas the currents through L- and P/Q-type channels represent ~15% and ~5%, respectively (3). Calcium influx through N-type channels plays a major role in mediating synaptic transmission in the locomotor network, and blockade of these channels completely disrupts the locomotor rhythm. N- and P/Q-type, but not L-type, calcium channels are coupled to activation of calcium-dependent potassium (KCa) channels (Fig. 1, B and C) (15). Apamin-sensitive KCa channels underlie the late afterhyperpolarization (AHP) following the action potential, which is the main determinant of the spike frequency regulation in single neurons at a given level of excitatory drive (Fig. 1D). The summation of the AHP is of importance for spike frequency adaptation, which acts as a burst-terminating factor during locomotor activity, allowing the switch of the activity from one side to the other. The KCa channels mediating the AHP are blocked by the specific toxin apamin, which also affects the frequency and stability of the locomotor rhythm (5).

LVA channels inactivate and underlie a transient inward current, and they are found only in some neurons. Calcium entry through these channels is responsible for the postinhibitory rebound occurring after a period of hyperpolarization of the membrane potential. This may boost the membrane depolarization to enable it to reach the threshold for firing an action potential. The importance of the LVA channels in the operation of the locomotor network has been addressed using mathematical modeling (12). Blockade of these channels changes the locomotor rhythm from a strict alternation between left and right sides to a more irregular activity, indicating that LVA channels underlying postinhibitory rebound contribute to the stability of locomotor burst activity.
Dynamic fluctuations of intracellular calcium during network activation

The influx of calcium in different neurons, and the concomitant activation of $K_{Ca}$ channels, gives rise to a variety of different actions that are involved in the control and modulation of network operation. Achieving a better insight into the dynamics of calcium influx in different types of neuron and during different forms of neuronal activation therefore appears crucial for understanding the mechanisms that underlie the locomotor behavior and its modulation. Fast-scanning confocal microscopy, following labeling with a calcium fluorophore, has been used to study calcium dynamics in lamprey spinal motoneurons (Fig. 2) (1). A localized calcium increase occurred postsynaptically in distal dendrites of the motoneuron upon synaptic excitation evoked by stimulation of reticulospinal axons. These axons form monosynaptic, glutamatergic connections with motoneurons. If the motoneuron was synaptically excited at subthreshold strength, there was still a clear calcium influx, although no postsynaptic spike was evoked, only an excitatory postsynaptic potential (EPSP; Fig. 2B). This influx could be partially blocked by the N-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovaleric acid (APV). This suggests the involvement of LVA-type calcium channels in the postsynaptic dendritic membrane, in addition to calcium entry through NMDA channels and, possibly, through calcium-permeable $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionete (AMPA) channels.

During activity of the spinal locomotor network, the motoneurons receive a barrage of rhythmic synaptic input from excitatory and inhibitory network interneurons at the segmental level. Does calcium influx into motoneuron dendrites also occur under these conditions? Figure 2A shows a portion of a distal motoneuron dendrite in which rhythmic fluctuations of calcium fluorescence could be detected, although that neuron did not fire action potentials. These fluctuations were time locked to the bursting activity of the ventral roots, i.e., the rhythm of the locomotor network (Fig. 2C).

These findings thus demonstrate postsynaptic, localized influx of calcium in distal motoneuron dendrites phasically driven by the changing synaptic input received by the motoneuron during each locomotor cycle.

Modulation of ionic channels and locomotor activity

A large number of aminergic and peptidergic modulatory systems and metabotropic $\gamma$-aminobutyric acid (GABA)$_{\beta}$ and glutamate receptors are present in the lamprey central nervous system. These systems tune the activity of the locomotor network to meet varying external and internal demands. Immunohistochemical studies have shown cells below the central canal that are immunoreactive to 5-HT, dopamine, and tachykinins. They give rise to a dense ventromedial plexus in which the dendrites of locomotor network neurons are distributed. In this plexus, modulators are released in a paracrinic fashion because these cells do not form conventional synaptic contacts with dendrites of spinal neurons. The frequency of the locomotor bursts is reduced by blocking the reuptake of either 5-HT or dopamine during fictive locomotion (Fig. 2D) (5, 11). Similarly, exogenous application of these modulators also reduces the locomotor frequency.
Through activation of 5-HT1A-like receptors, 5-HT blocks KCa channels mediating the AHP (Fig. 2D) (5, 14). This reduces the spike frequency adaptation in single neurons, which, in turn, delays the burst termination. The increased ventral root burst duration then leads to a decrease of the locomotor frequency. Likewise, dopamine inhibits calcium channels via activation of D2 receptors, thereby indirectly reducing the amplitude of the AHP (11). Thus the costored 5-HT and dopamine exert synergistic effects to modulate the frequency of the locomotor rhythm.

The locomotor network is also modulated by activation of GABA receptors. In the lamprey spinal cord, there are three types of GABA immunoreactive neurons (5). Bipolar GABAergic neurons in the dorsal horn colocalize neuropeptide Y (NPY) and form close appositions with axons of sensory neurons (4), and they also mediate presynaptic inhibition (see below). Small neurons surround the central canal, whereas multipolar neurons are found in the lateral grey column. The multipolar neurons make both axo-axonic and axo-dendritic synaptic contacts with network interneurons. There is an endogenous release of GABA in the spinal cord, which acts on both GABA_A and GABA_B receptors to modulate locomotor activity (13). The mechanisms underlying GABAergic modulation have been analyzed in detail; GABA_A receptors appear to act both presynaptically by depressing transmitter release from network interneurons via presynaptic inhibition (4) and postsynaptically by hyperpolarizing neurons in the spinal cord. GABA_B receptors inhibit HVA calcium channels activated during the action potential, as well as LVA channels responsible for the postinhibitory rebound (9). As a result, GABA will reduce the spike frequency adaptation in single neurons and delay the termination of the locomotor burst. Furthermore, the inhibition of LVA calcium channels by GABA_B receptor activation will also influence the intersegmental coordination during locomotion in the lamprey spinal cord (13). Activation of group I metabotropic glutamate receptors (mGluRs) increases the locomotor burst frequency and causes a stabilization of the motor pattern. These effects are, at least partially, mediated by a depolarization of network activity.
neurons and a potentiation of NMDA-induced depolarizations (8). Group III mGluR activation depresses synaptic transmission in the spinal cord through presynaptic inhibition and reduces the locomotor burst frequency.

Application of tachykinins causes a prolonged increase in locomotor burst frequency, lasting for 24 h or more (10). The initiation of this effect is mediated through activation of protein kinase C that leads to potentiation of NMDA-mediated synaptic transmission. The prolonged maintenance of an increased burst frequency requires protein synthesis (10).

**Presynaptic modulation of signal transmission**

One powerful mechanism to modulate neural network function is presynaptic inhibition that depresses transmission at different synapses. In the lamprey spinal cord, presynaptic inhibition occurs at different levels, i.e., at sensory, interneuronal, and descending sites of synaptic transmission. Both excitatory and inhibitory network interneurons receive phasic GABAergic presynaptic modulation, which results in a gating of the synaptic transmission in phase with the ipsilateral ventral root activity (4). This presynaptic modulation is mediated by simultaneous activation of both GABA_α_ and GABA_β_ receptors. The latter receptors act via activation of G proteins and inhibit calcium entry during action potentials in presynaptic axons, thereby depressing synaptic transmission (4).

Locomotor-related presynaptic inhibition also occurs in sensory neurons that carry cutaneous information (Fig. 3A). Intracellular recordings from intraspinal cutaneous afferent dorsal cells show phasic depolarizations that originate in the axon and occur in phase with the ipsilateral ventral root burst in the same segment (4). As a consequence, the amplitude of the postsynaptic EPSPs evoked by sensory neurons in second-order relay interneurons is modulated in a phase-dependent manner. The efficacy of the synaptic transmission from the afferents will thus vary in each locomotor cycle and be the smallest during the ipsilateral burst. Such presynaptic locomotor-related modulation of sensory transmission represents a mechanism for phasic gating of sensory input.

Axons of these sensory dorsal cells receive synaptic inputs from bipolar interneurons colocalizing both GABA and NPY.
Segmental networks may be selectively and locally controlled. Consequently, the level of descending drive impinging on the spinal transmission can vary along the spinal cord, and this suggests that the degree of presynaptic modulation of reticulospinal synaptic transmission from reticulospinal axons may be activated by 5-HT and dopamine release from the ventromedial plexus that surrounds reticulospinal axons. Presynaptic 5-HT and dopamine receptors (4, 5) may underlie these effects through processes independent of calcium influx (Fig. 3A). Activation of GABAergic and NPY receptors depresses synaptic transmission from the sensory neurons (5). It is likely that GABA is released at low levels of activity of the bipolar interneurons, whereas NPY is released when the level of activity is higher. The synaptic transmission from sensory cutaneous neurons is also subject to presynaptic inhibition by 5-HT, which mediates its effect through inhibition of calcium channels (4). 5-HT-immunoreactive (ir) fibers originating from small sensory neurons in the dorsal root ganglia make close contacts with the axons of cutaneous dorsal cells (Fig. 3A). These 5-HT-ir fibers may carry nociceptive information and interact with large sensory afferents to provide presynaptic inhibition under specific circumstances. Moreover, tachykinin-ir sensory fibers also form close appositions with sensory cutaneous neurons, and activation of tachykinin receptors by substance P causes a potentiation of transmission from dorsal column axons due to presynaptic facilitatory mechanisms (5).

Synaptic transmission from the large reticulospinal axons is subject to presynaptic modulation by group II and group III mGluRs, which are colocalized on the same reticulospinal axon (Fig. 3B) (7). These receptors mediate presynaptic inhibition through mechanisms independent of calcium influx through HVA channels. The presynaptic mGluRs could serve as glutamatergic autoreceptors, limiting the extent of reticulospinal-mediated excitation of spinal neurons, perhaps during overflow of glutamate during high levels of activity (7). Reticulospinal synaptic transmission is also modulated by presynaptic 5-HT and dopamine receptors (4, 5). These receptors may be activated by 5-HT and dopamine release from the ventromedial plexus that surrounds reticulospinal axons. Synaptic transmission from reticulospinal axons may thus be controlled locally by the amount of glutamate release at the segmental level, which can activate presynaptic mGluRs, and/or by local activation of the 5-HT/dopamine plexus. This suggests that the degree of presynaptic modulation of reticulospinal transmission can vary along the spinal cord, and consequently, the level of descending drive impinging on the segmental networks may be selectively and locally controlled.

**Computer simulation analysis of network operation and modulation**

Given this detailed information about mechanisms revealed at the cellular and ion channel levels, how does one assess the functional significance of the various mechanisms for the operation of the network? Likewise, when investigating the mechanisms of action of the different modulators, how does one find out which consequences a particular effect on an ion channel will have on the overall network behavior? A strategy that has proven very fruitful in answering these essential questions is to utilize mathematical modeling and computer simulations of the activity of the experimentally defined network and its component neurons. In this way, properties of individual nerve cells have been faithfully simulated using “semirealistic” modeling with five compartments (one for the soma, three for the dendritic tree, and one for the initial segment) and with each compartment having sodium, potassium, calcium (LVA, HVA), and KCa channels (5). The model neuron (Fig. 4A) fires action potentials with an early and a late AHP and shows spike frequency adaptation. Also, the synaptic contacts have been modeled, with excitatory (NMDA and AMPA) or inhibitory postsynaptic potentials. In addition, when investigating the functional significance of the various mechanisms for the network, how does one find out which consequences a particular effect on an ion channel will have on the overall network behavior? A strategy that has proven very fruitful in answering these essential questions is to utilize mathematical modeling and computer simulations of the activity of the experimentally defined network and its component neurons.