Oscillation in motor pattern-generating networks

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Oscillation in motor pattern generators is driven either by pacemaker neurons with inherent bursting properties or through network interactions. In a few examples in invertebrates and lower vertebrates, the mechanisms by which reciprocal inhibition combines with inherent membrane properties to produce network oscillation are beginning to emerge. A recently developed theoretical framework provides a context for understanding and comparing these findings.

Current Opinion in Neurobiology 1995, 5:816–823

Introduction

Networks of central neurons contribute to the patterning of almost all rhythmic movements in animals. Oscillation within these motor pattern-generating networks results from the combination of intrinsic electrical properties of the component neurons and their synaptic interactions [1–4]. In this review, I will summarize recent advances, both experimental and theoretical, that have contributed to our understanding of the origins of oscillation in pattern-generating networks. Particular emphasis will be given to those networks where reciprocal inhibition plays an important role in producing oscillation. It has been emphasized repeatedly that the membrane and synaptic properties that produce oscillation are subject to extensive neuromodulation [5–7], but these considerations are beyond the scope of this review.

Oscillators with intrinsic bursters

In some pattern-generating networks, a neuron or a group of neurons with intrinsic or conditional bursting properties serve as a source of the rhythm; synaptic interactions with other network elements can modify this rhythm, but the inherent activity of the pacemaker neuron(s) remains dominant. The R15 neuron of Aplysia has served as a useful experimental system for studying the membrane properties that support rhythmic bursting [8]. Such neurons have been successfully modeled [9,10], and the theoretical framework for understanding their activity is well developed [11].

The best characterized pattern-generating network paced by bursting neurons is the pyloric rhythm generator of the crustacean stomatogastric nervous system, which has been extensively reviewed (see e.g. [2,12]).

A group of three electrically coupled neurons—the AB cell, which is usually considered an intrinsic burster, and the PD cells, which are considered conditional bursters—can act as pacemakers for the rhythm. According to the modulatory state of the ganglion, either the AB cell, the PD cells, or both, pace the pyloric rhythm. These pacemaker neurons are embedded in a network of inhibitory synaptic connections with other pyloric neurons that themselves have complex intrinsic membrane properties, including the ability to produce plateau potentials. The interactions of the pacemaker neurons with other pyloric neurons strongly influence the final pyloric pattern that is produced, in terms of period and phase relations among the participating neurons. Thus, even in a system that has been considered dominated by bursting pacemakers, network interaction plays a defining role. Recent experimental and modeling studies have emphasized the dynamic regulation of bursting properties in this system [13,14**].

Intrinsic membrane properties and reciprocal inhibition

In many pattern-generating networks, synaptic interactions among neurons and intrinsic membrane properties both contribute to rhythmicity. No single neuron alone can be classified as a timing oscillator or pacemaker. Such networks include those that control leech swimming [15] and heartbeat [16], Lymnea feeding [17] and respiration [18], the crustacean gastric mill [19], and swimming in Clione [20], Tritonia [6], lamprey [21] and larval Xenopus [20]. In all of these networks, reciprocal inhibitory synaptic interactions between neurons or groups of neurons are found, and oscillations derive from the interplay of reciprocal inhibition with membrane

Abbreviations

3,4-DAP—3,4-diaminopyridine; HN interneurons—heart interneurons; 1A—fast transient K⁺ current; 1CaF—rapidly inactivating low-threshold Ca²⁺ current; 1CaS—slowly inactivating low-threshold Ca²⁺ current; 1K1—inactivating delayed rectifier-like K⁺ current; 1K2—persistent delayed rectifier-like K⁺ current; 1L—leakage current; 1NaL—low-threshold persistent Na⁺ current; 1V—voltage-gated post-inhibitory rebound current; IPSP—Inhibitory postsynaptic potential; 1H—hyperpolarization-activated inward current; NMDA—N-methyl-D-aspartate; TEA—tetraethylammonium.
properties such as plateau potentials, sag potentials and post-inhibitory rebound.

Particular attention has been paid to plateau potentials in reciprocally inhibitory networks (e.g. [22–24]). Plateau formation upon release from hyperpolarization may account for the post-inhibitory rebound often observed in these networks [25]. Several different types of conductance mechanisms can account for plateau formation, including T-type Ca2+ conductance and NMDA-mediated conductance. An important role for sag potentials—slowly developing depolarizations elicited by hyperpolarizations, such as those produced by synaptic inhibition—in pacing oscillation is frequently seen. A hyperpolarization-activated inward current (Ih), which is slow to activate and deactivate, produces the sag potentials. It allows the inhibited cell(s) of a reciprocally inhibitory network to escape that inhibition and terminate activity in the opposite cell(s), thus forcing the transition necessary for oscillation [26, 27]. Because of its relatively slow deactivation in some systems, Ih itself may also contribute to the initial phase of post-inhibitory rebound.

The importance of plateau potentials and Ih in generating oscillations is emphasized by experimental and modeling studies of thalamocortical neurons, which produce oscillations corresponding to slow states in mammals [28, 29] (Fig. 1). These neurons possess inactivating, low-threshold (T-like) Ca2+ currents that produce plateau-like potentials when the neurons are released from hyperpolarized levels and also have a well-developed Ih. Exposure to transmitters associated with sleep states hyperpolarizes the neurons by activating a K+ leak current, so they are brought into a range where the Ca2+ currents are deinactivated and Ih is activated. Ih rapidly depolarizes the neuron to a level where a Ca2+ plateau is generated, producing a burst of spikes. The plateau deinactivates Ih but relatively quickly inactivates, and membrane potential is again driven to the hyperpolarized levels that reinitiate the oscillatory cycle by the K+ leak current (Fig. 1c). In this system, the hyperpolarizing drive necessary to activate Ih for it to act as a pacemaker current, and to deinactivate low-threshold Ca2+ currents, is provided by the K+ leak current, whereas in reciprocally inhibitory networks this hyperpolarizing drive is provided by synaptic inhibition (cf. Fig. 3).

Oscillators based on reciprocal inhibition

I will now focus on three pattern-generating networks where recent progress has been made in understanding the role that reciprocal inhibitory synaptic interactions and membrane properties play in generating oscillations, and will attempt to place them in a general theoretical framework. The pattern generators controlling swimming in Clione and Xenopus use neurons with pacemaking or bursting properties: in Xenopus, these properties are regularly modulated on and off, whereas in leech heartbeat, the pattern generator employs neurons that do not normally burst in isolation.

It is important to note that considerable attention has been given to exploring the role of reciprocal inhibition, plateau potentials and post-inhibitory rebound in the segmental pattern-generating networks controlling swimming in leech [15, 30, 31] and lamprey [21, 32], crustacean gastric mill [19], and snail respiration [18, 33].

Clione and Xenopus swimming

In the pedal ganglia of the pelagic mollusk, Clione, two antagonistic populations of electrically coupled interneurons form reciprocal inhibitory synapses and drive activity in antagonistic pools of motor neurons to produce a swimming motor pattern [20, 34, 35]. These
interneuronal populations have different electrical and synaptic properties, but produce similar alternating single plateau-like potentials of up to 150 ms in duration, which drive the elevator and depressor motor neuron bursts. All of the interneurons show strong post-inhibitory rebound [34,35], and at least some of them can produce regular trains of the plateau-like potentials when isolated from the ganglion [36]. Removal of one of these populations from the circuit by strong hyperpolarization of a single neuron has been reported to halt oscillation in the network [34], but other reports indicate that oscillation can persist with such hyperpolarization [20,35].

In a recent study, Panchin et al. [37] blocked inhibitory transmission from the ventral phase interneurons to the dorsal phase interneurons using atropine. Under these conditions, both populations continue to alternate albeit at a somewhat lower frequency. These authors have interpreted this finding as a strong indication that endogenous pacemaking by the interneurons is the dominant mechanism for rhythm generation. Nevertheless, reciprocal inhibition and post-inhibitory rebound shape the final alternating pattern, and cycle period is largely determined by the duration of the reciprocal inhibitory synaptic potentials [20]. In Clione, these inhibitory postsynaptic potentials (IPSPs) are long (100 ms), which is at least in part due to the long duration of the 'spikes' in the swim interneurons.

The segmental motor pattern that underlies swimming in early stage Xenopus tadpoles bears remarkable similarities to the Clione swim pattern, and the pattern-generating networks that give rise to these patterns are organized along parallel lines [20]; although, as the larva matures, the pattern becomes considerably more complex. In each spinal segment, motor neurons innervating axial muscles on each side of the body alternate spike-like activity that produces the alternating lateral undulations of the body associated with swimming. In each spinal hemi-segment, inhibitory pre-motor interneurons, which form reciprocal inhibitory connections across the midline, produce alternating spike-like potentials that bear a remarkable similarity to those produced by the swim pattern-generating interneurons of Clione.

Unfortunately, these interneurons have not been well characterized. It is assumed that, like the motor neurons, they fire only once during prolonged depolarization, and they show strong post-inhibitory rebound when they are depolarized [20]. Rebound is thought to be important in pacing rhythmic activity, and simulated spinal networks of these interneurons generate a robust rhythm based on reciprocal inhibition and rebound [38]. However, several mechanisms certainly contribute to rhythmicity in the network.

In recent experiments, K+ currents were blocked with TEA or 3,4 DAP, and caused stereotypical disruptions in the rhythm, indicating their importance in rhythm generation [39]. Moreover, hemisected spinal cord preparations can generate a swim-like rhythm; this rhythm is thought to arise from recurrent inhibition of swim interneurons by ipsilateral glycineergic axons and resultant post-inhibitory rebound [20]. Moreover, in such hemisected preparations, some rhythmic neuronal discharge in motor neurons can be evoked by sensory stimulation even when glycineergic inhibition is blocked with strychnine, providing some evidence for inherent oscillatory neuronal properties [20].

Interesting contrasts and similarities between the Xenopus and Clione swim pattern generators highlight principles of organization. In both systems, at least under some conditions, inherent rhythmic activity alone sustains rhythmicity. Post-inhibitory rebound is pronounced in both systems, reciprocal inhibition produces alternation, and cycle period is determined largely by the duration of the reciprocal inhibitory synaptic potentials [20]. In contrast to the Clione swim generator, which operates nearly continuously, that of Xenopus is geared to episodic activity. The excitation necessary to maintain the prolonged depolarization necessary for a swimming bout in Xenopus is provided by activation, through sensory input, of excitatory interneurons that use long dual component (NMDA and non-NMDA) synaptic potentials; mutual excitation within the excitatory interneuron pool sustains activity and leads to a summated steady depolarization of the swim interneurons [20]. Modeling studies indicate that while non-NMDA-mediated mutual excitation can sustain oscillation, NMDA-mediated mutual excitation, which enhances post-inhibitory rebound, acts to stabilize swimming activity and extend its lower frequency range. It also steepens the dependence of frequency on synaptic drive [40]. Excitation within the Clione swim generator is sustained by electrical coupling within the reciprocally inhibitory interneuron pools [20,35].

Leech heartbeat

A network of seven bilateral pairs of heart (HN) interneurons produces rhythmic activity that paces the segmental heart motor neurons [16] (Fig. 2c). The synaptic connections among the interneurons (Fig. 2a) and from the interneurons to the motor neurons are inhibitory. The first four pairs of HN interneurons control the timing of the network (Fig. 2b). The timing oscillation is dominated by the reciprocal interactions of the third and fourth pair of HN interneurons, respectively [16]. The oscillation is paced by reciprocally inhibitory synapses between the bilateral pairs of HN neurons in these ganglia (Fig. 2 b,c), combined with an ability of these interneurons to escape from inhibition and begin firing [16]. Thus, of these two reciprocally inhibitory HN interneuron pairs can each be considered an elemental oscillator. The HN interneurons of the first and second ganglia act as coordinating fibers, serving to link these two elemental oscillators (Fig. 2b).

Several ionic currents that contribute to the activity of oscillator HN interneurons have been identified in single electrode voltage clamp studies. These include,
in addition to the fast Na\(^+\) current that mediates spikes, two low-threshold Ca\(^{2+}\) currents (one rapidly inactivating [INaF] and one slowly inactivating [INaS]) [41], three outward currents (a fast transient K\(^+\) current [IK; and two delayed rectifier-like K\(^+\) currents, one inactivating [IK1], and one persistent [IK2]) [42], a hyperpolarization-activated inward current (ILH; mixed Na\(^+\)/K\(^+\); \(E_{rev} = -20\) mV) [27], a low-threshold persistent Na\(^+\) current (IP) [43], and a leakage current (IL; \(E_{rev} = -52\) mV).

The inhibition between oscillator interneurons consists of a graded component that is associated with the low-threshold Ca\(^{2+}\) currents [41] and a spike-mediated component that appears to be mediated by an uncharacterized high-threshold Ca\(^{2+}\) current [44]. Spike-mediated transmission is sustained even at the high spike frequency observed during normal bursting [45], whereas graded transmission wanes during a burst owing to the inactivation of low-threshold Ca\(^{2+}\) currents [41]. Blockade of synaptic transmission with bicuculline leads to tonic activity in oscillator HN interneurons [46]; Cs\(^+\), which specifically blocks INa, leads to tonic activity or sporadic bursting [27]. In reduced Na\(^+\)-salines, spikes are blocked, and oscillations based solely on graded synaptic transmission occur [22,26]. Dynamic clamp [47] studies in which reciprocal inhibition was artificially restored in bicuculline-treated oscillator interneuron pairs, showed that even non-fatiguing inhibition sustains oscillation [48].

Much of this biophysical data was incorporated into a first generation conductance-based model of an elemental (two cell) oscillator, using standard Hodgkin-Huxley representations of each voltage-gated current and a synaptic transfer function, which related transmitter release to presynaptic Ca\(^{2+}\) build-up and decline, via low-threshold Ca\(^{2+}\) currents and a Ca\(^{2+}\) removal mechanism, respectively [49,50]. The first generation model simulated the essence of the observed oscillation...
and showed the importance of $I_h$ in regulating oscillation period through escape from inhibition. This model had several flaws, however; most importantly, there was no specific formulation for spike-mediated transmission and discrete IPSPs were not observed.

A second generation model has been recently formulated by Nadim, Olsen and colleagues [51**,52**]. This model adds spike-mediated synaptic transmission, and addresses several other minor flaws of the older model with new experimental data. Free parameters in the model were the maximal conductance ($\text{max} g_{\text{ion}}$) for each current (voltage-gated or synaptic). The $\text{max} g_{\text{ions}}$ were adjusted to be close to the average observed experimentally. The reversal potential, $E_{\text{ion}}$, for each current, except $I_h$, was determined experimentally and considered fixed. Final selection of parameters to form a canonical model was dictated by model behavior under control conditions, passive response of the model to hyperpolarizing current pulses, and reaction of the model to current perturbations. The model cells were also required to fire tonically when all inhibition between them was blocked, because the real neurons fire tonically in bicuculline [46].

The canonical model generates activity that closely approximates that observed for an elemental oscillator (Fig. 2d). Analysis of current flows during this activity (Fig. 3) indicates that graded transmission occurs only at the beginning of the inhibitory period, acting to turn off the opposite neuron; sustained inhibition of the opposite neuron is all spike-mediated. The inward currents in the model neurons act to overcome this inhibition and force a transition to burst phase of oscillation. $I_h$ always acts to drive the membrane toward its reversal potential (~52 mV), $I_h$ is slowly activated by the hyperpolarization-associated inhibition adding a delayed inward current that drives the activation of $I_p$ and eventually the low-threshold Ca$^{2+}$ currents ($I_{\text{CaS}}$ and $I_{\text{CaF}}$). These regenerative currents form a plateau that supports burst formation. As $I_p$ does not inactivate, it provides steady depolarization to sustain spiking, whereas the low-threshold Ca$^{2+}$ currents help force the transition to the burst phase and provide graded inhibition to silence the opposite neuron, but inactivate as the burst proceeds. Outward currents also play important roles, especially the $I_K$. Namely, $I_{K2}$, which activates and deactivates relatively slowly and does not inactivate, regulates the amplitude of the depolarized plateau that sustains the burst, whereas $I_{K1}$, which activates and deactivates relatively quickly and inactivates, controls spike frequency.

Increasing $\text{max} g_{\text{synS}}$ (the maximal spike-mediated synaptic conductance) in the model, slows the oscillation, whereas reducing $\text{max} g_{\text{synS}}$ speeds the oscillation. Reducing $\text{max} g_{\text{synS}}$ to zero leads to a fast oscillation.
where graded transmission is very intense and lasts for the entire inhibitory period, unlike the much shorter duration of graded transmission in the canonical model. Thus under canonical conditions graded transmission is suppressed. Analysis of state variables (m and h) for low-threshold Ca$^{2+}$ currents indicates that deactivation of these currents is not effective during the inhibitory period, when max$g_{mNS}$ is near canonical levels or higher. In the canonical model cells, as in real cells, the potential for prolonged and intense graded transmission is revealed upon rebound from a hyperpolarizing pulse.

Two fundamentally different modes of oscillation thus exist in the model: one dominated by spike-mediated transmission, S-mode, as in the canonical model, and one dominated by graded synaptic transmission, G-mode, as when max$g_{mNS}$ is reduced from canonical levels. Apparently, such G-mode oscillations correspond to the oscillations seen in reduced Na$^{+}$ salines [26], in which spikes (and thus spike-mediated transmission) are blocked. The period of the oscillation is sensitive to the level of max$g_{m}$, as would be predicted from its key role in forcing the transition from the inhibitory phase to the burst phase. Decreasing max$g_{m}$ from canonical levels slows the oscillation proportionately, while increasing it first speeds the oscillations but at higher levels brings on G-mode oscillations.

Theoretical framework
A theoretical framework for understanding how reciprocally inhibitory neurons oscillate has been developed by Wang and Rinzel [53]. Their model neurons are minimal. Each contains a synaptic conductance which is a sigmoidal function of presynaptic membrane potential with a set threshold and instantaneous kinetics, a constant leak conductance, and a voltage-gated post-inhibitory rebound current, I$_{pr}$. This current was originally envisioned to be a T-like Ca$^{2+}$ current (low-threshold, inactivating), but its expression in the model can also accommodate an h current. Two different modes of oscillation appear in the model, ‘release’ and ‘escape’ [53]. For the release mode to occur, the synaptic threshold must be above the steady-state voltage of the neurons when uninhibited. In the release mode, inactivation of I$_{pr}$ erodes the depolarized or active phase of a neuron so that it falls below threshold for synaptic transmission. Consequently its partner is released from inhibition and rebounds into the active depolarized state. For the escape mode to occur, the synaptic threshold must be below the steady-state voltage of the neurons when uninhibited. This condition can be accomplished simply by increasing $g_{pr}$. In the escape mode, once I$_{pr}$ becomes deinactivated by the hyperpolarization associated with inhibition, it activates and overcomes the maintained synaptic current so that the neuron escapes into the active phase and thus inhibits its partner.

Skinner et al. [54••] have extended this analysis using similar model neurons based on the Morris–Lecar equations (low-threshold, non-inactivating inward current and delayed rectifier current) with a synaptic conductance, which is a steep sigmoidal function of presynaptic membrane potential with a set threshold and instantaneous kinetics. Such model neurons, like the Wang and Rinzel neurons, oscillate between a depolarized plateau and a sustained inhibitory trough. Four modes of oscillation can be differentiated depending on two conditions: whether the release is due to cessation of synaptic transmission (i.e. crossing synaptic threshold), called synaptic release, or termination (i.e. deactivation of the inward current, activation of the delayed rectifier, or both) of the depolarized plateau, called intrinsic release; or whether the escape is due to the commencement of synaptic transmission (i.e. crossing synaptic threshold), called synaptic escape, or expression of the depolarized phase (i.e. crossing plateau threshold), called intrinsic escape. Varying the synaptic threshold causes transitions between the modes.

It appears that the HN interneuron oscillator operates in the escape mode. Whenever I$_{h}$ is sufficiently activated to overcome the waning synaptic current, a transition from the inactive state to the active state occurs [51••, 52•]. It is not clear, however, whether this escape should be considered intrinsic or synaptic, because the gradual transition from the active to inactive states and vice versa, and the gradual dependence of synaptic transfer on presynaptic potential, preclude any discrimination. By contrast, the Cline swim oscillator appears to operate in the intrinsic release mode. ‘Spike’ (active-state) termination terminates inhibition and allows the opposite cell to rebound into the active state. Perhaps this mode is more suited to the operational frequency range of this oscillator, which is some 10 times faster that the leech heartbeat oscillator.

Conclusions
Since 1914, when Brown [55] first postulated that reciprocal inhibition could produce oscillation in spinal motor networks, we have been endeavoring to understand how such half-center oscillators might work. Detailed physiological and modeling studies in invertebrates and lower vertebrates are beginning to shed light on the underlying mechanisms. Plateau potentials and I$_{h}$ current play important roles in producing oscillation. Moreover, a general theoretical framework is now available that can be used to compare results from different preparations. This two-pronged approach of experimental and theoretical analyses, with computer simulation providing a bridge between the two, promises further advances in the understanding of other and more complex neuronal networks.

Acknowledgements
Research in the author’s lab was supported by National Institutes of Health grants NS24072 and NS34975.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

15. Crab stomatogastric ganglion neurones in primary culture undergo a progressive change from being relatively inexcitable, to producing large action potentials upon depolarization with injected current, to spontaneous (or inducible with current injection), bursting over the course of 4 days. Several ionic currents were identified in these neurones using two electrode voltage clamps. The assumption of the bursting phenotype is associated with an increase in inward current densities and a decrease in outward current densities, and modeling studies indicate that such changes in current densities are sufficient to explain the changes in cellular properties. These results indicate that ionic conductances are dynamically regulated in neurones, and this regulation may be associated with the activity state of neurones in situ.


In Cline, atropine was used to block inhibitory transmission from the ventral phase swim interneurons to the dorsal phase interneurons. The period of inhibition in the experiments of the authors continued to alternate albeit at somewhat lower frequency. The authors have interpreted this finding as a strong indication that endogenous pacemaker by the interneurons is the dominant mechanism for rhythm generation. While these experiments do reveal endogenous pacemaker capabilities, the period change associated with blockade of one half of the reciprocal inhibitory interaction suggests that normally the synaptic interaction dominates in pacing the rhythm.


In this modeling study, the authors investigate the importance of the voltage dependency of NMDA-mediated positive feedback in sustaining rhythm generation in the epididymal swimming central pattern generator of larval Xenopus. Their results indicate that NMDA voltage dependency enhances post-inhibitory rebound (through plateau formation) and thus stabilizes the pattern generator and extends its frequency range. NMDA-mediated conductance is important in generating such plateau properties in lamprey swim circuit neurons (see also [21]).


An elemental neuronal oscillator consisting of two reciprocally inhibitory neurons was modeled using biophysical data from leech heart interneurons. Standard Hodgkin-Huxley equations were used to describe ionic currents and both spike-mediated and graded synaptic interactions were included. Criteria were established for selecting parameters for establishing a canonical model that closely approximates the activity of heart interneurons. The model reveals the subtle interplay between synaptic and ionic currents that promote oscillation.


The canonical elemental oscillator model established in the previous paper [51] was explored by systematically varying the maximum conductance parameter for all ionic and synaptic currents. These studies reveal two important roles for Ih and spike-mediated synaptic transmission: in controlling the oscillation period. They also demonstrate that although graded transmission is largely suppressed during canonical oscillations, it can support oscillation when spike-mediated transmission is weakened or absent.


This modeling study elaborates on and extends the theoretical framework developed by Wang and Rinzel [53] for understanding how reciprocally inhibitory neurons can produce oscillation. It explores both escape and release mechanisms for producing oscillation and differentiates between synaptic and intrinsic modes within each of these mechanisms, depending on whether a neuron enters its depolarized or hyperpolarized phase before or after crossing synaptic threshold. It then explores the importance of the synaptic threshold for controlling period within each mechanism. It concludes that period is relatively insensitive to synaptic threshold for the intrinsic mechanisms but varies predictably with synaptic threshold for the intrinsic mechanisms.