Heartbeat Control in the Medicinal Leech: A Model System for Understanding the Origin, Coordination, and Modulation of Rhythmic Motor Patterns

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SUMMARY

We have analyzed in detail the neuronal network that generates heartbeat in the leech. Reciprocally inhibitory pairs of heart interneurons form oscillators that pace the heartbeat rhythm. Other heart interneurons coordinate these oscillators. These coordinating interneurons, along with the oscillator interneurons, form an eight-cell timing oscillator network for heartbeat. Still other interneurons, along with the oscillator interneurons, inhibit heart motor neurons, sculpting their activity into rhythmic bursts. Critical switch interneurons interface between the oscillator interneurons and the other premotor interneurons to produce two alternating coordination states of the motor neurons. The periods of the oscillator interneurons are modulated by endogenous RFamide neuropeptides. We have explored the ionic currents and graded and spike-mediated synaptic transmission that promote oscillation in the oscillator interneurons and have incorporated these data into a conductance-based computer model. This model has been of considerable predictive value and has led to new insights into how reciprocally inhibitory neurons produce oscillation. We are now in a strong position to expand this model upward, to encompass the entire heartbeat network, horizontally, to elucidate the mechanisms of FMRFamide modulation, and downward, to incorporate cellular morphology. By studying the mechanisms of motor pattern formation in the leech, using modeling studies in conjunction with parallel physiological experiments, we can contribute to a deeper understanding of how rhythmic motor acts are generated, coordinated, modulated, and reconfigured at the level of networks, cells, ionic currents, and synapses. © 1995 John Wiley & Sons, Inc.

Keywords: medicinal leech, heartbeat, neuronal network, oscillator interneurons, motor patterns.

INTRODUCTION

To understand how the nervous system controls complex motor acts, it is first necessary to define the neuronal network that controls a particular act. For a few rhythmic behaviors in invertebrates (Selverston and Moulins, 1987; Getting, 1988, 1989a; Friesen, 1989; Benjamin and Elliot, 1989; Satterlie, 1989; Selverston, 1989) all or most of the neural elements that control the behavior have been identified and their synaptic interconnections at least partially defined. We have recently come to realize, however, that a simple connectionist understanding of such pattern-generating networks will not suffice. The morphologic connectivity of these neural networks defines the limits of functional interactions but does not dictate them. A complete understanding of these networks also requires a thorough characterization of the ionic currents and synaptic transfer functions of all the elements. Realistic computer models of these systems can then be formulated, which can lead to deep insights into how these networks function (Mulloney and Perkel, 1988; Getting, 1989b).

Moreover, we now realize that such systems are not static: circuit, synaptic, and intrinsic membrane properties are all modulated (Harris-Warrick

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and Marder, 1991). Alteration of neuronal activity, membrane properties, synaptic interactions, and morphological connectivity can cause anything from a slightly altered pattern of neural activity, corresponding to an adjustment in an ongoing behavior, to a fundamentally different pattern of activity corresponding to the emergence of a new behavior. Examples from the rhythmic motor pattern generators of invertebrates illustrate this spectrum, over which neural circuits can be modulated and reconfigured. In crabs a peptidergic neuron accelerates the cycling of the pattern generator that controls the animal’s pylorus (a foregut chamber) by altering voltage-gated conductances in a pacemaker interneuron (Nusbaum and Marder, 1989). In leeches, the pattern generator controlling the two longitudinal hearts consists of an oscillatory circuit linked to premotor interneurons by a pair of switch interneurons that project bilaterally. Depending on which of these two switch interneurons is active, the left or the right heart will beat peristaltically, while the other beats synchronously (Calabrese and Peterson, 1983; Gramoll et al., 1994). Thus, two alternative configurations of the pattern generator produce mirror image patterns of motor output. In the sea slug Tritonia, a group of mutually excitatory interneurons all excite an inhibitory interneuron that provides strong negative feedback (Getting, 1989a). In this configuration, the excitatory interneurons can act independently to mediate directed reflexive withdrawals to weak sensory input. When another interneuron is activated by strong sensory input, its input reconfigures the functional relationships within the excitatory interneuronal network, by inhibiting the activity of the inhibitory interneuron, and thus drives the excitatory interneurons to fire synchronous bursts that pace escape swimming (Getting, 1989a, 1989b). Thus, common neural elements participate in circuits that program fundamentally different behaviors—defensive withdrawals and escape swimming.

If we are to further our understanding of how neural networks produce adaptive behavior, we must now explore in detail these modulatory and reconfiguring mechanisms and incorporate this knowledge into our computer models. For example, proctolinergic modulation of the LP neuron of the pyloric circuit of the stomatogastric ganglion of the crab involves a voltage-dependent persistent Na⁺ current (Golowasch and Marder, 1992a). A model cell that incorporates all the known voltage-dependent currents of the LP cell (Golowasch and Marder, 1992b), including the proctolin-mediated current, simulates both the unmodulated and the modulated activity of the LP cell in situ (Buchholtz et al., 1992; Golowasch et al., 1992). It is now important to apply such analysis to whole networks.

**OVERVIEW OF THE HEARTBEAT NEURAL CONTROL SYSTEM OF THE LEECH**

Over the past several years, my colleagues and I have used the heartbeat control system of the leech, Hirudo medicinalis (Calabrese and Peterson, 1983; Calabrese and Arbas, 1985; Calabrese et al., 1989) for the study of the mechanisms of motor pattern generation and modulation and have begun to develop realistic computer models that are providing insight into how portions of the network operate (Calabrese and De Schutter, 1992; De Schutter et al., 1993).

The leech is a segmented worm, and the segmental nature of the animal is reflected in its organ systems. The circulatory system is a closed network comprising four longitudinal tubelike vessels—one dorsal, one ventral, and two lateral—that run the length of the animal, communicating in every segment by a series of branch vessels (Mann, 1962). Rhythmic constrictions of the muscular lateral vessels (or hearts) drive circulation in the network (Borofka and Hamp, 1969; Thompson and Stent, 1976a). The heart muscle can generate a myogenic rhythm of electrical and contractile activity, but normally this rhythm is entrained by rhythmic motor outflow driven by the heartbeat central pattern generator (Maranto and Calabrese, 1984a) (Fig. 1). The hearts are coordinated so that one beats with a rear-to-front progression (peristaltically), while the other beats synchronously along its length. Approximately every 20 heartbeats, the two hearts switch coordination states (Calabrese, 1977; Gramoll et al., 1994). The functional implications of these alternating coordination states for efficient circulation have been elucidated (Krahl and Zerbst-Borofka, 1983).

The central nervous system (CNS) of the leech comprises a chain of 21 midbody segmental ganglia linked by bundles of axons called connectives (Payton, 1981). At either end of this chain are fused ganglia called brains. Each segmental ganglion contains some 400 neurons (Macagno, 1980), most of which are bilaterally paired and repeated from ganglion to ganglion. The ganglia are sufficiently
A. 6.

Circuit diagram showing the inhibitory synaptic connections from identified HN interneurons to HE motor neurons. (B) Circuit diagram showing the inhibitory synaptic connections among all the identified HN interneurons. Neurons with the same input and output connections are lumped together. (C) Circuit diagram showing inhibitory synaptic connections among the HN interneurons of the timing oscillator. HN cells of midbody ganglia 1 and 2 are functionally equivalent and are lumped together. The HN(1) and HN(2) neurons receive their synaptic inputs on, and initiate action potentials in, processes located in the third and fourth ganglia (open squares). In all the circuit diagrams, large unfilled circles represent neurons (each identified by the number of its ganglion), lines represent major neurites or axons, and small filled circles represent inhibitory chemical synapses. (D) Simultaneous intracellular recordings showing the normal rhythmic activity of two reciprocally inhibitory (oscillator) HN interneurons and an HE motor neuron postsynaptic to one of them in an isolated nerve cord preparation. Dashed lines indicate a membrane potential of $-50$ mV. Heart interneurons are indexed by body side and midbody ganglion number from cell HN(L,1) to cell HN(R,7). HE motor neurons are similarly indexed.

The rhythmic activity pattern of the heart motor neurons derives from the cyclic inhibition they receive from this heartbeat central pattern generator (Thompson and Stent, 1976b; Calabrese, 1979) (Fig. 1). When these inhibitory inputs to the heart motor neurons are blocked with bicuculline, the motor neurons fire at a steady rate (Schmidt and Calabrese, 1992). The pattern generator comprises seven bilateral pairs of identified heart (HN) interneurons that occur in the first seven segmental ganglia (Thompson and Stent, 1976b, 1976c; Calabrese, 1977; Peterson and Calabrese, 1982; Calabrese and Peterson, 1983). The heart interneurons are connected to one another by inhibitory synapses, and the pairs in the third, fourth, sixth, and seventh ganglia inhibit the heart motor neurons (Thompson and Stent, 1976a; Calabrese, 1977) (Fig. 1(A)).

The first four pairs of heart interneurons can reset and entrain the rhythm of the entire pattern-generating network of interneurons [Fig. 1(B)]. The other three pairs of HN neurons are followers of these anterior pairs (Peterson and Calabrese, 1982). Two foci of oscillation in this network have been identified in the third and fourth ganglia, where the oscillation is dominated by the reciprocal interactions of the third and fourth pair of HN interneurons, respectively (Peterson, 1983a). Reciprocally inhibitory synapses between the bilateral
pairs of HN neurons in these ganglia [Fig. 1(C,D)], combined with an ability of these interneurons to escape from inhibition and begin firing, pace the oscillation (Peterson, 1983a, 1983b). Thus, each of these two reciprocally inhibitory heart 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, thus forming the beat timing oscillator for the system (Peterson, 1983b) [Fig. 1(C)].

The beat timing oscillator network projects by inhibitory synapses (from the ipsilateral heart interneurons of the third and fourth ganglion) to switch heart interneurons in the fifth ganglion [Fig. 1(B)]. These switch interneurons project bilateral synapses to the heart interneurons of the sixth and seventh ganglia (Calabrese, 1977). Only one of the switch interneurons produces impulse activity at any given time; the other is silent, although it receives rhythmic inhibition from the beat timing oscillator [Fig. 2(B)] (Calabrese, 1977; Gramoll et al., 1994). With a period approximately 20 times longer than the period (10 s) of the heartbeat cycle, the switch interneurons change roles. Since the switch interneurons link the timing oscillator to the rear premotor heart interneurons of the sixth and seventh ganglia, this asymmetry in the activity of the switch interneurons [Fig. 2(B)] leads to an asymmetry in the activity phases of the heart interneurons and hence in the coordination of heart motor neurons on the two sides of the nerve cord [Fig. 2(A)].

The activity states of the switch interneurons are controlled by an autonomous switch oscillator. Manipulation of the activity state of one switch interneuron does not influence the activity state of the other, but switches in activity state are always reciprocal (Calabrese, 1977; Calabrese and Peterson, 1983). This oscillator remains unidentified. Recent voltage-clamp studies have revealed the mechanism by which the switch oscillator controls the activity state of the switch interneurons (Gramoll et al., 1994). In the off state, switch neurons have a persistent outward current that is not voltage sensitive and reverses around −60 mV. A switch to the active state is caused by turning off this current. Thus, in the inactive state, a switch interneuron is tonically inhibited by the switch oscillator via a persistent leak current. We have used Dynamic clamp (Sharp et al., 1993a, 1993b) to simulate the turn-on and turn-off of such a leak and
have found that it is sufficient to change the activity state of the manipulated switch interneuron.

The heartbeat neural control system can be conceptualized as being made up of two oscillators: a beat timing oscillator comprising the first four pairs of heart interneurons (two elemental oscillator pairs and two coordinating fiber pairs) [Fig. 1(C)], and a switch timing oscillator, which governs the activity of the switch interneurons. The two oscillators converge on the switch interneurons, and, together with the rear premotor heart interneurons, they make up the heartbeat central pattern generator [Fig. 1(B)]. The output of the central pattern generator is configured into two alternating coordination states of heart motor neurons by the alternating activity states of the two switch interneurons [Fig. 2(B)].

The central pattern generator is subject to neuromodulation; a variety of identified sensory, neurosecretory, and motor pathways modulate the period of the beat timing (Arbas and Calabrese, 1984, 1990). A family of endogenous RFamide neuropeptides (Evans et al., 1991) also modulate beat timing (Kuhlman et al., 1985b) and these peptides have been localized to neurons with similar modulatory effects (Arbas and Calabrese, 1984; Kuhlman et al., 1985a). The target of this central modulation must be the oscillator heart interneurons of the third and fourth ganglia.

We have focused on modulation of heartbeat rhythm by the endogenous neuropeptide FMRFamide. At lower doses FMRFamide speeds the heartbeat rhythm but at higher concentrations it disrupts the rhythm (Kuhlman et al., 1985b; Simon et al., 1992). As determined in voltage-clamp studies, FMRFamide has two separable effects on the oscillator heart interneurons, which pace the heartbeat rhythm: (1) It shifts the steady-state activation and inactivation of Iq1 (Simon et al., 1992); and (2) it modulates spike-mediated synaptic transmission by reducing the amplitude but increasing the duration of inhibitory postsynaptic currents (Simon et al., 1994). This effect on synaptic transmission is presynaptic, as indicated by quantal analysis and lack of effect of FMRFamide on the response to focally applied inhibitory transmitter (Simon et al., 1994). At present, it is not clear whether these biophysical effects can account for the acceleratory and disruptive effects of FMRFamide on the heartbeat rhythm. Only with a detailed model of a heart interneuron elemental oscillator will the mechanisms of oscillation and its modulation be understood.

**PROGRESS TOWARD UNDERSTANDING THE MECHANISMS OF OSCILLATION IN OSCILLATOR (RECIROCALLY INHIBITORY) HEART INTERNEURONS**

The remainder of this review will be devoted to presenting biophysical and recent modeling studies (most of which are as yet unpublished and will be presented as work in progress) that begin to explain the mechanism of oscillation of a heart interneuron elemental oscillator.

Several ionic currents have been identified in single electrode voltage-clamp studies that contribute to the activity of oscillator heart interneurons. These include, in addition to the fast Na+ current that mediates spikes, two low-threshold Ca2+ currents (Angstadt and Calabrese, 1991) [one rapidly inactivating (I_{Ca1}) and one slowly inactivating (I_{CaS})], three outward currents (Simon et al., 1992) [a fast transient K+ current (I_k) and two delayed rectifier-like K+ currents, one inactivating (I_{K1}), and one persistent (I_{Kp})], a hyperpolarization-activated inward current (I_h) (DiFrancesco and Noble, 1989)—(mixed Na/K, \(E_{rev} = -20 \text{ mV}\)) (Angstadt and Calabrese, 1989)—and a recently discovered low-threshold persistent Na+ current (I_p) (Opdyke and Calabrese, 1994). The inhibition between oscillator interneurons consists of a graded component that is associated with the low-threshold Ca2+ currents (Angstadt and Calabrese, 1991) and a spike-mediated component that appears to be mediated by an undescribed high-threshold Ca2+ current (Simon et al., 1994). Spike-mediated transmission is sustained even at the high spike frequency observed during normal bursting (Nicholls and Wallace, 1978a, 1978b), whereas graded transmission wanes during a burst owing to the inactivation of low-threshold Ca2+ currents (Angstadt and Calabrese, 1991). Blockade of synaptic transmission with bicuculline leads to tonic activity in oscillator heart interneurons, and Cs+, which specifically blocks I_h, disrupts normal bursting. In reduced Na+ salines, spikes are blocked and oscillations, based solely on graded synaptic transmission occur (Arbas and Calabrese, 1987a, 1987b). Dynamic clamp (Sharp et al., 1993a, 1993b) studies in which reciprocal inhibition was artificially restored in bicuculline-treated oscillator interneuron pairs showed that even nonfatiguing inhibition sustains oscillation (Skinner et al., 1994a).

Much of this biophysical data was incorporated
The membrane potential $V$ of each cell in the model is given by the capacitative equation

$$-C \frac{dV}{dt} = \left( \sum_{\text{ion}} I_{\text{ion}} \right) + I_{\text{SynG}} + I_{\text{SynS}}.$$  

The value chosen for the membrane capacitance of the cell is $C = 500 \text{ pF}$. The ionic currents $I_{\text{ion}}$ are given by the Hodgkin-Huxley rate equations

$$I_{\text{ion}} = \tilde{g}_{\text{ion}} m^p h^q (V - E_{\text{ion}})$$
$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m$$
$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h.$$  

Table 1 shows the rate constants ($\alpha$ or $\beta$) given by the function

$$\frac{c_1 + c_2 V + c_3 \exp \left( \frac{c_4 + V}{c_5} \right)}{c_6 + \exp \left( \frac{c_7 + V}{c_8} \right)}.$$

The graded synaptic current is

$$I_{\text{SynG}} = \frac{g_{\text{SynG}} P^3}{K + [P]^3} (V - E_{\text{Syn}})$$
$$\frac{d[P]}{dt} = I_{\text{Ca}} - B(V)P \quad (I \text{ in nA})$$
$$I_{\text{Ca}} = \max(0, -I_{\text{Ca}}^\text{in} - I_{\text{Ca}}^\text{syn} - A),$$

where $K = 10^8$,

$$B(V) = 0.003 + 0.017/(1 + \exp(0.21(V + 43.6))),$$

and $A$ is given by

$$\tau_A(V) \frac{dA}{dt} = A_\infty(V) - A$$

with

$$\tau_A(V) = 1000/(1 + \exp(0.3(V + 37)) + \exp(-(V + 45)))$$
$$A_\infty(V) = 0.1 + 0.2/(1 + \exp(-0.4(V + 37))).$$

The spike-mediated synaptic current is given by

$$I_{\text{SynS}} = \tilde{g}_{\text{SynS}} \left( 1 - e^{-t/\tau_{\text{rise}}} \right) e^{-t/\tau_{\text{fall}}} (V - E_{\text{Syn}})$$

where $\tau_{\text{rise}} = 2.5 \text{ ms}$ and $\tau_{\text{fall}} = 11 \text{ ms}$ are, respectively, the rising and falling time constant of the spike-mediated post-synaptic current.

**Figure 3** Model of a leech heartbeat elemental oscillator.

into a first generation, conductance-based model of an elemental (two cell) oscillator, using standard Hodgkin-Huxley (Hodgkin and Huxley, 1952) representations of each voltage-gated current and 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 (Calabrese and De Schutter, 1992; De Schutter et al., 1993). Because graded synaptic transfer was dependent on presynaptic Ca$^{2+}$ currents, which inactivate, the postsynaptic response to a voltage-clamp step in the presynaptic cells waned with time in the model as in the real neurons (Angstadt and Calabrese, 1991). The first-generation model simulated the essence of the observed oscillation and showed the importance of $I_h$ in regulating the oscillation period through escape from inhibition. It had two major
flaws, however: (1) There was no specific formulation for spike-mediated transmission, so that there were no discrete inhibitory postsynaptic potentials in the model. This finding led us to explore the possibility that separate mechanisms (Ca\(^{2+}\) currents) exist for spike-mediated and graded synaptic transmission (Simon et al., 1994); (2) low-threshold Ca\(^{2+}\) currents were turned very high (large \(g_s\)). This was necessary for two reasons: to provide enough inhibition (through graded synaptic transfer) to silence the other cell effectively for a sustained period, and to depolarize the cell sufficiently to sustain spiking during the burst phase. The former led to the conviction that spike-mediated transmission plays a crucial role in silencing activity in the other cell, and the latter caused us to pursue other sustained inward currents that support spiking and led to the discovery of \(I_p\). Thus, experiments led to a realistic model, which in turn led to further experiments.

We have improved our first-generation model of a heart interneuron elemental oscillator (that is, a reciprocally inhibitory heart interneuron pair) in several ways (Fig. 3; Table 1):

1. We added \(I_p\).
2. We added spike-mediated transmission, so that each presynaptic spike elicits a postsynaptic conductance described by an alpha function. The alpha function was derived by fitting average rise times and fall times of spike-mediated inhibitory postsynaptic currents (postsynaptic voltage clamp) in oscillator interneuron pairs (Simon et al., 1994).
3. We modified the graded synaptic transmission model so that the effect of presynaptic Ca\(^{2+}\) build-up on transmitter release saturates.
4. We thoroughly refitted the Hodgkin-Huxley equations for the voltage-gated currents to

Table 1  Rate Parameters for the Ionic Currents

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<th>(E_{ion})</th>
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<th>(c_3)</th>
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</tr>
<tr>
<td>(I_{h})</td>
<td>5</td>
<td>-21</td>
<td>(m_2)</td>
<td>m</td>
<td>(\alpha)</td>
<td>-0.00082</td>
<td>-0.00002</td>
<td>0</td>
<td>44.6</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(\beta)</td>
<td>0.00042</td>
<td>0</td>
<td>0</td>
<td>52.8</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

Conductances in nS and potentials in mV.
published data and new data from voltage-clamp experiments. Once the rate constants of the equations (that is, the alphas and betas) were determined by the data they were not further modified in the model.

5. We constructed I-V curves (using slow voltage ramps) to determine leak conductance more accurately (gₗ) and leak reversal potential (Eₗ). Because Eₗ is so difficult to pinpoint experimentally, it is a free parameter in the model.

6. We moved the model from a Macintosh environment, Nodus software (De Schutter, 1989), to the UNIX environment. All simulations are run on a Sun SPARC Station LX under Solaris 2.2 or PC clone under LINUX. The model was developed using Phaseplane software (Rinzel and Ermentrout, 1989) and then implemented with Neurolab (Olsen, 1994). Neurolab interacts directly with the UNIX shell so that programs can be written to control it, such as to automate parameter searches. The model is accessible through our WWW site.

7. We adjusted the free parameters—in this case, the maximal conductance (gₓₓₓₓ) for each current (voltage-gated or synaptic)—to be close to the average observed experimentally. The reversal potential, Eₓₓₓₓ, for each current, except Iₜ₂, was determined experimentally and was 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. We also required that the model cells fire tonically when all inhibition between them was blocked, because the real neurons fire tonically in bicuculline.

The canonical model generates activity that closely approximates that observed for an elemental oscillator (Fig. 4). Analysis of current flows during this activity (Fig. 5) 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. Iₜ₁ always acts to drive the membrane toward its reversal potential (-52.5 mV). Iₜ₂ is slowly activated by the hyperpolarization-associated inhibition, adding a delayed inward current that drives the activation of Iₜ₉ and eventually the low-threshold Ca²⁺ currents (Iₜ₅₆ and Iₜ₅₇). These regenerative currents form a plateau that supports burst formation. Iₜ₉, because it does not inactivate, provides steady depolarization to sustain spiking, whereas the low-threshold Ca²⁺ 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 Iₖ currents. Iₖ₂, which activates and deactivates relatively slowly and does not inactivate, regulates the amplitude of the depolarized plateau that underlies the burst, whereas Iₖ₁, which activates and deactivates relatively quickly and inactivates, controls spike frequency.

Neurolab allows automated parameter searches, and we are systematically exploring the effects of varying free parameters. Figure 6 shows a series of model voltage traces in which the gₛₜ₅₆ (the maximal spike-mediated synaptic conductance) was varied both above and below the canonical value.
Increasing \( g_{\text{synS}} \) slows the oscillation, whereas reducing \( g_{\text{synS}} \) speeds the oscillation. Reducing \( g_{\text{synS}} \) to zero produces a fast oscillation in which 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 deinactivation of these currents is not effective during the inhibitory period, when \( g_{\text{synS}} \) is near canonical levels or higher. In the canonical model cells, as in the real cells, prolonged and intense graded transmission occurs on rebound from a hyperpolarizing pulse (Fig. 7).

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 the other dominated by graded synaptic transmission (G-mode), as when \( g_{\text{synS}} \) is reduced from canonical levels. Apparently, such G-mode oscillations correspond to the oscillations seen in reduced Na\(^+\) salines (Arbas and Calabrese, 1987b), in which spikes (and thus spike-mediated transmission) are blocked. G-mode oscillations can be derived from the canonical model by varying parameters beside \( g_{\text{synS}} \). Increasing \( g_{n} \) is a particularly effective way of speeding the oscillation, as would be predicted from its key role in forcing the transition from the inhibitory phase to the burst phase, thus bringing on G mode. Increasing \( g_{n} \) reduces the inactivation of low-threshold Ca\(^{2+}\) currents during the inhibitory trough to bring on...
Figure 7  Perturbation of the one of the cells in a real elemental oscillator (CELLS) or a model elemental oscillator (MODEL) with a hyperpolarizing pulse of current (-0.2 nA) resets the oscillation to a new phase. On releasing the perturbed neuron from the hyperpolarizing pulse, it produces a robust depolarized plateau along with a pronounced and prolonged graded inhibition of the other cell. Analysis of current flows and state variables in the model during the simulation shows that the hyperpolarizing pulse removes inactivation of low-threshold Ca^{2+} currents. In the canonical model, a steady level of inactivation of these currents prevents strong graded inhibition, but the potential for such inhibition is revealed by the deinactivation accompanying the hyperpolarizing pulse.

G-mode oscillations. Increasing either $E_i$ or $g_i$ speeds the oscillation by providing increased depolarizing drive during the inhibitory phase. Increasing $g_{K}$ speeds the oscillation by reducing spike frequency, thus reducing the effectiveness of spike-mediated transmission.

A theoretical framework for understanding how reciprocally inhibitory neurons oscillate was developed by Wang and Rinzel (1992). Their model neurons are minimal. Each model neuron contains a synaptic conductance that is a sigmoidal function of presynaptic membrane potential with a set threshold and instantaneous kinetics, a constant leak conductance, and a voltage-gated postinhibi-
tory rebound current, I_{pr}. Two different modes of oscillation appear in the model: release and escape (Wang and Rinzel, 1992). 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, the 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. (1994b) have extended this analysis using similar model neurons based on the Morris-Lecar (Morris and Lecar, 1981) equations (low-threshold noninactivating 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: (1) synaptic release, due to cessation of synaptic transmission (going below synaptic threshold); (2) intrinsic release, due to the termination of the depolarized plateau (caused by deactivation of the inward current, activation of the delayed rectifier, or both); (3) synaptic escape, due to the commencement of synaptic transmission (going above synaptic threshold); and (4) intrinsic escape, due to the expression of the depolarized plateau (going above plateau threshold). Varying the synaptic threshold causes transitions between the modes.

We are exploring the parameter space of our elemental oscillator to determine whether the analysis of Skinner et al. can encompass our realistic model. So far, it appears that the G-mode grades between synaptic release (low g_n, low g_{syn} in which the waning of graded synaptic inhibition, due to inactivation of presynaptic low-threshold Ca^{2+} currents, predominates at the transition to activity of the inhibited cell) and intrinsic escape (high g_n in which the build-up of I_p during the inhibitory phase predominates at the transition to activity of the inhibited cell), whereas the S-mode corresponds to intrinsic escape (low to canonical g_n, canonical to high g_{syn}). The correspondence is intriguing but should not be carried too far. For instance, in the G-mode, where the correspondence appears strong, both in the model and in the real cells, the graded synaptic transmission smoothly wanes through the inhibitory period, making the distinction between escape and release a judgment call. Also, in the S-mode, which we think corresponds to the normal state of an elemental oscillator, intrinsic escape is not pure, because spike frequency adapts during a burst, thus reducing the effective spike-mediated synaptic inhibition.

Our modeling study has demonstrated the complexity of real neuronal oscillators and has pointed out that fundamentally different modes of oscillation occur in different regions of parameter space, perhaps corresponding to different states of modulation. We are now in a position to explore different modulatory states and to expand our model to encompass the entire heartbeat control neuronal network and the morphological complexity of the heart interneurons. We believe that such detailed modeling studies can illuminate general principles applicable to other motor pattern generators.

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REFERENCES


control heartbeat in the medicinal leech. J. Neurosci. 7:3953-3960.


