Mechanisms of Oscillation in Dynamic Clamp Constructed Two-Cell Half-Center Circuits

ANDREW A. SHARP, FRANCES K. SKINNER, AND EVE MARDER
Volcan Center for Complex Systems, Brandeis University, Waltham, Massachusetts 02254-9110

SUMMARY AND CONCLUSIONS
1. The dynamic clamp was used to create reciprocally inhibitory two-cell circuits from pairs of pharmacologically isolated gastric mill neurons of the stomatogastric ganglion of the crab, Cancer borealis.

2. We used this system to study how systematic alterations in intrinsic and synaptic parameters affected the network behavior. This has previously been possible in purely computational systems.

3. In the absence of additional hyperpolarization-activated inward current (Ih), stable half-center oscillatory behavior was not observed. In the presence of additional Ih, a variety of circuit dynamics, including stable half-center oscillatory activity, was produced.

4. Stable half-center behavior requires that the synaptic threshold lie within the voltage envelope of the slow wave oscillation.

5. Changes in the synaptic threshold produce dramatic changes in half-center period. As predicted by previous theoretical work, when the synaptic threshold is depolarized, the period first increases and then decreases in a characteristic inverted U-shaped relationship. Analysis of the currents responsible for the transition between the active and inhibited neurons shows that the mechanism of oscillation changes as the synaptic threshold is varied.

6. Increasing the time constant and the conductance of the inhibitory synaptic current increased the period of the half-center oscillator.

7. Increasing the conductance of Ih or changing the voltage dependence of Ih can either increase or decrease network period, depending on the initial mode of network oscillation. A depolarization of the activation curve causes the network to respond in a similar fashion as increasing the conductance of Ih.

8. Many neuromodulatory substances are known to alter synaptic strength and the conductance and voltage dependence of Ih, parameters we studied with the dynamic clamp. To understand the response of the network to modulation of a single parameter, it is necessary to understand the nature of the altered conductance and how it interacts with the other conductances in the system.

INTRODUCTION
Rhythmic motor behaviors are produced by central pattern generators (Cohen et al. 1988; Harris-Warrick and Marder 1991; Jacklet 1989; Marder and Calabrese 1996; Pearson and Ramirez 1992) whose output depends on the interaction of synaptic and voltage-dependent conductances of the neurons within the circuit. These circuits are also richly modulated (Katz et al. 1994; Marder and Weimann 1992) by substances that may alter synaptic strengths and/or the intrinsic properties of the neurons (Kaczmarek and Levitan 1987). Additionally, temporal dynamics of presynaptic activity may alter synaptic strength by processes such as facilitation (Hawkins et al. 1983) or long-term potentiation (Bliss and Lomo 1973).

A common feature in central pattern generator circuits is reciprocal inhibition between functional antagonists (Getting 1989; Harris-Warrick and Marder 1991; Marder and Calabrese 1996). In some cases the rhythmic motor patterns are directly produced by “half-center oscillators” (Brown 1911). In these systems, including leech heartbeat and tadpole and Chione swimming (Arshavsky et al. 1993; Calabrese and DeSchutter 1992; Marder and Calabrese 1996; Satterlie 1985), the timing of the motor pattern is critically controlled by pairs of reciprocally inhibitory neurons or neuronal groups. In other neural circuits, reciprocal inhibition is a circuit component and contributes to the final integrative output of the network (Getting 1989; Miller and Selverston 1982a,b; Rowat and Selverston 1993). The ubiquitous presence of half-center oscillators makes it critical to understand what cellular and synaptic properties contribute to the frequency and maintenance of rhythms produced by reciprocally inhibitory neurons.

Early theoretical studies on half-center oscillators recognized that two neurons connected by inhibitory connections could produce rhythmic alternating bursts even when neither neuron was itself capable of bursting in isolation (Perkel and Molloney 1974), provided that there was some time-dependent process that contributed to the ability of the inhibited neuron to overcome the inhibition or that caused the active neuron to lose its ability to inhibit the silent neuron (Perkel and Molloney 1974; Wilson and Waldron 1968).

Wang and Rinzel (1992) provided the first detailed explanation of different mechanisms that can create oscillations in two-cell reciprocally inhibitory networks. Their model consisted of a leak conductance, a conductance that can generate postinhibitory rebound excitation (based on a T-type calcium current), and a synaptic current with a sigmoidal relationship to the presynaptic voltage. They identified two mechanisms, “release” and “escape,” that can generate antiphase oscillations. The release mechanism is characterized by the presynaptic cell falling below its synaptic threshold (the threshold for a cell’s release of transmitter), thereby allowing the inhibited cell to rebound above threshold. An escape mechanism is when the inhibited cell depolarizes above its synaptic threshold, thus terminating the activity of its partner. Wang and Rinzel (1992) found that the network frequency was very sensitive to the duration of inhibition in the release case, but relatively insensitive to this in the escape regime. This approach allowed them to determine numerically the mechanisms of oscillation and to attribute them to the specific properties of a real biological conductance.
Skinner et al. (1994b) extended the approach taken by Wang and Rinzel (1992) with the use of Morris-Lecar-type cells (Morris and Lecar 1981) reciprocally coupled by inhibitory synapses. The presynaptic voltage dependence of the activation variable for these synapses was steplike. Skinner et al. (1994b) studied the properties of this model with the use of parameters in which the neurons were of the relaxation type (i.e., some processes occur on a much faster time scale than others) and defined four mechanisms (synaptic escape, synaptic release, intrinsic escape, and intrinsic release) for the generation of oscillations and frequency control in a two-cell reciprocally inhibitory network. For the intrinsic mechanisms [those in which the uncoupled neurons were intrinsically bursting or in which the burst termination (intrinsic release) or burst initiation (intrinsic escape) was determined by the intrinsic properties of the neurons], the network frequency is insensitive to the synaptic threshold. In the other two cases (synaptic release and synaptic escape), the network frequency is strongly determined by the threshold voltage of the synaptic connections. Interestingly, the network period increases in the synaptic escape and decreases in the synaptic release mode when the synaptic threshold is depolarized. Skinner et al. (1994b) noted that the sharp distinction between the four classes of transition becomes increasingly blurred as the model neurons move away from the relaxation mode and as the steepness of the synaptic activation curve is reduced.

The determination of theoretical mechanisms (e.g., in Skinner et al. 1994b) to understand how oscillations are generated and controlled in half-centers does not necessarily imply that such mechanisms would operate within the parameter regimes in which complex biological neurons and networks function. In addition, model observations such as the network period changing in opposite directions as the synaptic threshold is modified (Skinner et al. 1994b) may not necessarily occur in a network of real neurons if the model does not reliably capture the dynamics of real neurons. We now use the dynamic clamp to construct a half-center oscillator from biological neurons to determine whether the results of our earlier modeling experiments (Skinner et al. 1994b) are seen within physiologically relevant parameter regimes, and with a half-center based on different biophysiological properties.

We use the dynamic clamp to form reciprocally inhibitory synapses between pairs of real stomatogastric neurons, the gastric mill (GM) cells. The synapses are of a non-impulse-mediated type typical of many neural networks (Marder and Calabrese 1996), including the stomatogastric nervous system (Graubard et al. 1983; Johnson et al. 1995). The dynamic clamp is additionally used to provide each neuron with an I_h conductance. I_h is a hyperpolarization-activated inward current found in many neurons in a variety of nervous systems. It contributes to recovery from synaptic inhibition and plays crucial roles in bursting and plateau phenomena (Angstadt and Calabrese 1989; DiFrancesco and Noble 1989; Golowasch and Marder 1992; Kiehn and Harris-Warrick 1992a,b; McCormick and Pape 1990a) and its conductance and voltage dependence are modulated by amines such as serotonin in both vertebrate and invertebrate systems (Kiehn 1991; Kiehn and Harris-Warrick 1992a,b; McCormick and Pape 1990b). Because the synaptic currents and I_h are computer controlled, this method allows us to adjust their conductance, time course, and voltage dependence. We use this method to explore the mechanisms of oscillation in this network and to determine how modulation of a parameter controlling I_h or synaptic currents affects network behavior. Preliminary experiments with a similar half-center system have been performed in leeches (Skinner et al. 1994a) and crab (Skinner et al. 1993a). This work has been presented in abstract form (Sharp et al. 1994; Skinner et al. 1994c).

**Methods**

**Physiology**

Crabs (Cancer borealis) were obtained from local fishermen and the stomatogastric nervous system was dissected as previously described (Hooper et al. 1986; Weimann et al. 1991). The effects of descending modulatory inputs on the stomatogastric ganglion were reduced by local application of the ganglion with that superior esophageal nerves connect to the stomatogastric nervous. The four GM neurons were identified by their activity on the dorsal ventricular nerve and/or the dorsal gastric nerve. Inhibitory synapses within the stomatogastric ganglion were blocked by superfusion with 10^{-8} M picROTOXIN in physiological saline. In some experiments, 10^{-7} M tetrodotoxin was added to block sodium conductances.

Intracellular recordings of two GM neurons were performed with thin-walled borosilicate electrodes filled with 0.6 M KSO_{4} and 20 mM KC1 (tip resistance 15−20 MΩ). Neurons were selected to have similar membrane impedance and spikes thresholds. Recordings were made with Axoclamp-2A amplifiers (Axon Instruments) in the discontinuous current-clamp mode. Sampling rates were 2.5-5 kHz and the filtering circuit of the second Axoclamp was slowed to the first.

**Dynamic clamp**

Artificial synaptic and I_h conductances were added with the use of the dynamic clamp (Sharp et al. 1993a,b) (Fig. 1). The membrane potential was digitized by a Labmaster TL-1 Interface (Scientific Solutions) and passed to a 50-MHz 486 PC (Zeos). The artificial currents were calculated with the use of Delcamp software (DynaQuest Technologies) and injected into the neurons' cell bodies. The update rate for this loop was 3−5 kHz.

The synaptic current is described by simple first-order kinetics (e.g., see Destexhe et al. 1994)

\[ I_{syn} = v_{max}S(V_{m} - V_{syn}) \]

\[ (1 - S)\frac{dS}{dt} = (S - S) \]

where

\[ S(V_{m}) = \begin{cases} \tanh \left( \frac{(V_{m} - V_{ns})}{V_{w}} \right) & \text{if } V_{m} > V_{ns} \\ 0 & \text{otherwise} \end{cases} \]

\( v_{max} \) is the maximal synaptic conductance; \( S \) is the instantaneous synaptic activation; \( S_{ns} \) is the steady-state synaptic activation; \( V_{ns} \) is the synaptic reversal potential; \( V_{w} \) and \( V_{ns} \) are the presynaptic and postsynaptic voltages, respectively; \( v_{ns} \) is the time constant for the synaptic decay; \( V_{ns} \) is the synaptic threshold voltage; and \( V_{w} \) is the synaptic slope voltage. Note that the \( (1 - S_{ns}) \) term occurs in Eq. 1 because the time constant, \( v_{ns} \), refers only to synaptic decay and not to a combination of synaptic rise and decay times. The steady-state synaptic activation is shown in Fig. 1B for two synaptic thresholds.
with little change in membrane properties. For these experiments, the GM neurons were isolated pharmacologically from synaptic and modulatory inputs. Under these conditions, they have relatively simple membrane properties. The resting potential for these neurons is typically between –70 and –50 mV, and they are silent in the absence of modulatory and synaptic inputs. The neurons fire action potentials tonically when depolarized (Fig. 2A), with little spike frequency adaptation. The GMs have little or no I_h and have fairly linear current-voltage relationships.

Under some conditions it is possible to generate oscillations by placing simple, inhibitory synapses between a pair of nonoscillatory model cells (e.g., Skinner et al. 1993b). Therefore we placed artificial inhibitory synapses between a pair of GM neurons with the dynamic clamp, in attempts to generate oscillatory behavior (Fig. 2). We found that we were unable to obtain stable half-center behavior by coupling GM neurons, regardless of the parameters controlling the synapses or the level of constant current injection. However, if we added I_h with the use of the dynamic clamp, we were then able to generate stable half-center bursting. Figure 2A shows the two neurons in the uncoupled state in the absence of I_h. Figure 2B shows that when these neurons were coupled, one neuron tonically fired, permanently inhibiting the second, and transitions between activity in the two neurons did not occur. Figure 2C shows that the neurons fired more rapidly and depolarized after the addition of I_h in the uncoupled state. When these neurons were synthetically coupled they produced stable half-center oscillations (Fig. 2D).

Stable half-center oscillations were not the only patterns produced by two GM neurons coupled in reciprocally inhibitory circuits. Figure 3 illustrates some of the circuit outputs that we observed as we varied parameters (see Fig. 3 legend). Figure 3A shows single spikes that are virtually synchronous; Fig. 3B shows a pattern in which one cell fires both in synchrony and in antiphase with the other cell. In Fig. 3C the two neurons were firing synchronously in bursts. In Fig. 3D the neurons switched between several states spontaneously. Figure 3E is stable half-center behavior, and Fig. 3F shows rapid antiphase spiking.

In this paper we focus on the effects of 1) synaptic threshold, 2) synaptic time constant, 3) maximal synaptic conductance, 4) maximal I_h conductance, and 5) voltage dependence of I_h activation on the frequency and mechanisms of oscillations. As a result, we are able to build a dynamical oscillator from biological parameters. The stomatogastric ganglion has four GM neurons with large cell bodies that can be recorded from for several hours.
stable half-center oscillations in two-cell reciprocally inhibitory circuits such as that seen in Fig. 3E.

**Alterations of synaptic threshold**

The theoretical work of Skinner et al. (1994b) showed that modifications of synaptic threshold could cause profound changes in network period, depending on the underlying mechanism by which oscillation was produced. Particularly, an inverted U-shaped plot of period as a function of synaptic threshold was obtained. The left portion of the curve (more hyperpolarized synaptic thresholds), in which the period increases with increasing synaptic threshold, was produced by a synaptic escape mechanism (the inhibited cell depolarizes past its synaptic threshold and starts to inhibit its partner), and the right portion (more depolarized synaptic thresholds), in which the period decreases with increasing synaptic threshold, was produced by a synaptic release mechanism (the inhibited cell is released as its partner falls below its synaptic threshold). The central region was a result of either intrinsic mechanisms (the theoretical intrinsic release or intrinsic escape mechanisms) or a combination of the intrinsic and synaptic mechanisms (Skinner et al. 1994b). Therefore we first studied the effect of synaptic threshold on period, with the aim of also understanding how transitions between the two alternating neurons occur.

In a typical experiment, we depolarized both neurons above their threshold for action potential generation such that their basal potentials and spike frequencies were similar. This level of constant current was maintained throughout a given experiment. In this depolarized state, the behavior exhibited by the uncoupled neurons represents the free or uninhibited cell referred to in Skinner et al. (1994b). We then added artificial synapses and I_p to each neuron. Figure 4 shows the effect of changing the synaptic threshold on the network period in one such experiment. The recordings show the network behavior at three different synaptic thresholds, -49 mV (Fig. 4A), -45 mV (Fig. 4B), and -42 mV (Fig. 4C), and a full plot of the period as a function of synaptic threshold (Fig. 4D). Note that as the synaptic threshold was increased, the period first increased and then decreased, in an inverted U-shaped curve. (The difference in burst duration between the cells is a result of the fact that the 2 neurons were not perfectly symmetrical with respect to their intrinsic properties.) The results shown in Fig. 4 were obtained from one pair of GM neurons. This type of period versus synaptic
threshold relationship was obtained with 13 pairs of GM neurons, for >60 different sets of parameters.

Although the period is markedly changed by alterations in the synaptic threshold, the slow envelope of membrane potential oscillations [from the top of the slow depolarization on which the spikes are riding to the bottom of the inhibitory postsynaptic potentials (IPSPs)] is not appreciably altered. This is important because stable half-center oscillations are found when the synaptic threshold is between these two boundary membrane potentials. When the threshold is more hyperpolarized, behaviors such as those seen in Fig. 3, A-D, occur. Additionally, both cells may be silent, or they can be stuck in the state with one cell on and one cell off (Fig. 2B). When the synaptic threshold is more depolarized than the top of the depolarized envelope, antiphase spiking activity is seen (Fig. 3F).

The other striking difference between oscillations obtained at different threshold values is the appearance of the IPSPs. For the more negative threshold values, the IPSPs are very smooth (Fig. 4A). As the threshold voltage is depolarized, the IPSPs become increasingly jagged (Fig. 4, B and C). For the more negative threshold value, the synaptic activation is nearly saturated by the base potential of the burst. As the threshold is depolarized, the base potential obtained by the burst is insufficient to saturate the activation and the action potentials start to play a more important role in determining the synaptic potential.

Oscillatory mechanisms

To determine what mechanisms of oscillation were occurring in different regions of the experimentally obtained inverted U-shaped plot of period versus synaptic threshold (e.g., Fig. 4), we exploited the dynamic clamp to analyze \( I_{h} \) and synaptic currents separately during half-center operation. To examine the current generated by one of these conductances, the command voltage generated by Dclamp must be decompressed (see METHODS), because the total dynamic clamp current is a sum of \( I_{h} \) and synaptic currents. An example of the individual currents is shown in Fig. 5. In each panel we show the membrane potentials of the two neurons \( V_{1} \) and \( V_{2} \), \( I_{h} \) in the two neurons, \( I_{syn} \), the synaptic current produced in cell 1 because of the action of cell 2, and \( I_{syn2} \), the synaptic current produced in cell 2 because of the action of cell 1. The convention we use for dynamic clamp currents is that inward currents are positive-going and outward currents are negative (Sharp et al. 1993a,b).

At the lower synaptic threshold (−60 mV) shown in Fig.
5A, the following process is observed. When cell 1 starts to fire, cell 2 is inhibited below its synaptic threshold (horizontal line on voltage recordings). $I_h$ in cell 2 activates, and this starts to depolarize the cell. As cell 2 crosses its synaptic threshold, it starts to inhibit cell 1, as can be seen in the recording of $I_{syn}$. Note that before the transition actually occurs, there is a period of time during which both cells inhibit each other. Finally, cell 1 is hyperpolarized below its synaptic threshold, and the transition is complete, because cell 2 now fires freely. This is a synaptic escape mechanism, because the transition is determined by the ability of the inhibited cell to depolarize over its synaptic threshold. Also, the uninhibited cell is not restricted to be silent or an endogenous burster (Skinner et al. 1994b).

At the more depolarized threshold ($-46$ mV) shown in Fig. 5C, a different process occurs. The transition seems to be more closely associated with the decay of the non-impulse-mediated slow component of the synaptic current (the envelope on which the spike-mediated IPSPs are summed). The decay of the synaptic current in cell 1 due to cell 2 ($I_{syn}$) is produced by even modest changes in the membrane potential of the active cell 2. Therefore, as $I_h$ in cell 2 turns off, the modest hyperpolarization has a strong effect on the decay of the synaptic current, allowing the inhibited cell 1 to fire. The amplitude of the persistent synaptic current is smaller at this depolarized threshold value because the depolarized plateau of the active cell is not far above the synaptic threshold. Thus the transition is governed by the release of the inhibited cell from the waning synaptic inhibition and by the buildup of $I_h$ in the inhibited cell. This process can be considered as a mixture of the intrinsic escape and synaptic release mechanisms where the transition occurs, because the inhibited cell can be released from inhibition because there is a decay in the synaptic current.

To identify exactly which mechanism is determining an oscillatory transition requires examination of the underlying currents and membrane potentials. However, the period of oscillation represents the interaction of components that are responsible for the different mechanisms. The inverted U-shaped relationship represents a continuum of mechanistic interactions weighted by the different mechanisms: synaptic escape on the left, intrinsic escape in the middle and synaptic release on the right. Asymmetric oscillation (e.g., Fig. 5) can result from the two cells making transitions with slightly different balances in these interactions.
The plot of period versus synaptic threshold in Fig. 5D illustrates another interesting observation. In some experiments a discontinuity in the period versus synaptic threshold plot was obtained. When we set up the half-center, and varied the synaptic threshold, we obtained two regions of stable half-center activity, one at low synaptic thresholds and a second at high synaptic thresholds, with a break at intermediate values of synaptic thresholds. Figure 5F shows that at a threshold of −52 mV, one cell was ‘stuck’ continuously firing, and the second cell was stuck continuously inhibited. Examination of the currents shows that this occurred because the inhibited neuron did not have adequate I_h to depolarize across its synaptic threshold (it was unable to escape) and was too hyperpolarized to generate action potentials. Moreover, the decay of I_h in the active neuron was insufficient to cause the active neuron to fall below its spike or synaptic thresholds.

Spike removal

The recordings in Fig. 6, A–C, show a biological half-center that has a break in the range of synaptic thresholds that gives stable half-center activity (Fig. 6G, open squares). When the preparation was placed in tetrodotoxin to block the spikes (Fig. 6, D–F), stable half-center activity was still seen, although the range was significantly reduced to a range dominated by synaptic escape (more negative threshold potentials) and one threshold potential (−33 mV) dominated by synaptic release. Note that the slow wave envelopes in the spiking and nonspiking mechanisms were almost superimposable for the synaptic threshold of −52 mV, but that the waveforms at depolarized levels are somewhat different.

In Fig. 6H we used different parameters for both I_h and the synaptic conductance to produce stable half-center activity throughout the synaptic threshold range in control saline.
When tetrodotoxin was added, oscillations were only produced in the synaptic escape regime, over a very restricted region of the synaptic threshold.

**Effect of changing the synaptic time constant**

Figure 7 shows that the network period increased as the synaptic time constant was increased from 1 to 500 ms. The number of action potentials per burst increased as the burst duration was increased. As the bursts elongated the underlying oscillation became more rounded and the spike frequency adaptation that occurred as $I_h$ deactivated became increasingly evident. As the neurons fired more action potentials on the rising wave of the oscillation, a small increase in spike frequency was observed. Notice that the amount of time that both neurons were concurrently above the synaptic threshold increased as the time constant was increased. In some cases, both neurons fired action potentials briefly at the same time during transitions between the active and inhibited states for large values of the synaptic time constant (Fig. 7E).

Figure 7 illustrates that an increase in the synaptic time constant increases the period of oscillation when the network is in a synaptic escape regime. Figure 8 shows that this relationship holds across the range of synthetic thresholds that generate stable half-center activity and therefore is not dependent on the mode of oscillation.

The effects observed as a result of changing the synaptic time constant can be explained as follows. When the synaptic time constant is increased, the synaptic current follows the membrane potential of the presynaptic neuron more slowly. Therefore, when the inhibited neuron reaches the synaptic threshold, the effects on the more depolarized neuron build up more slowly. This results in a slower hyperpolarization
of the more depolarized neuron. This in turn results in a slower decrease in the more depolarized neuron’s effects on the more hyperpolarized neuron. Even after a neuron falls below the synaptic threshold, the other neuron continues to receive inhibition for some time. This scenario results in a more rounded waveform and an increase in the time spent making the transition between states.

The above explanation for the period increase and rounding of the waveform does not explain why the rapid IPSP component seems relatively unaltered by the change in synaptic time constant (Fig. 7, A–C). The explanation for this lies in the voltage dependence of the synaptic activation variable (see Eq. 1). The activation rate is smaller for presynaptic potentials close to the synaptic threshold (So is small) and becomes almost instantaneous when the synapse is saturated (So approaches 1). Because the action potentials saturate the activation function, the synaptic current generated by them is almost always occurring at a very high rate.

**Effect of changing the maximal synaptic conductance**

Figure 9 shows the effect of increasing the amplitude of the synaptic conductance in the GM half-center network when it is operating in a synaptic escape mode. As the synaptic conductance is increased,

1. the network period increases,
2. the duration of bursting and the number of action potentials per burst increases,
3. the amplitude of the underlying slow oscillation increases,
4. the initial spike rate during a burst increases, and
5. the variability in period increases.

The network period increased when the synaptic conductance was increased at ranges of synaptic threshold that spanned the range of oscillatory mechanisms (data not shown), much as seen for the synaptic time constant in Fig. 8.

The effects observed as a result of an increase in the synaptic current can be described as follows. When the synaptic conductance is increased, it causes a larger synaptic
current to be generated at a given presynaptic potential. This results in a larger hyperpolarization in the postsynaptic cell. It takes a longer time for the $I_{\text{IH}}$ current to activate in the postsynaptic cell and to overcome this hyperpolarization and cross the synaptic threshold. The increase in $I_{\text{IH}}$ also generates a larger postinhibitory rebound and the neuron spikes more rapidly following the initiation of the burst.

Close examination of the membrane potential with the synaptic threshold in Fig. 9C indicates that the slow wave of the postsynaptic neuron does not reach the synaptic threshold as a result of the increase in $I_{\text{IH}}$ (seen as the slow depolarization in the postsynaptic neuron), but falls a few millivolts short. This indicates that the network is no longer operating in the synaptic escape mode, but rather in the intrinsic escape mode. The postsynaptic neuron must now fire an action potential to escape inhibition. This change in mechanisms is reflected in the increased variability of the period (Fig. 9D). At this potential the probability of firing an action potential is low and this decreases the probability that an action potential will initiate the transition at a given time. If the synaptic conductance is increased sufficiently, the postsynaptic neuron is hyperpolarized below the spiking threshold and oscillations cease.

**Effect of changing the maximal $I_{\text{IH}}$ conductance**

Figure 10 shows the effect of altering the maximal conductance of $I_{\text{IH}}$, $g_{\text{IH}}$, on the GM network. Notice that as $g_{\text{IH}}$ is increased, the network period and burst duration decrease (Fig. 10, $A-D$). However, despite the change in burst duration, the rate of firing remains fairly stable. The relationship of the threshold potential to the envelope of the slow wave is altered by the conductance of $I_{\text{IH}}$. As $g_{\text{IH}}$ increases, the envelope of the slow wave becomes more depolarized. The general depolarization is sufficient to alter network period, but it is not large enough to induce a significant change in firing rate.

Starting with a high value of $g_{\text{IH}}$, the interactions that occur because of a change in $g_{\text{IH}}$ can be described as follows. In Fig. 10C ($g_{\text{IH}} = 80$ nS), the network is oscillating rapidly and operating in the synaptic escape mode. When $g_{\text{IH}}$ is decreased to 50 nS (Fig. 10B), the period increases. This occurs because the equivalent activation state of $I_{\text{IH}}$ now generates less current and therefore it takes longer to reach the current level (higher $I_{\text{IH}}$ activation state) necessary to cross the synaptic threshold. The increase in burst duration that accompanies the period increase results in a lower $I_{\text{IH}}$ activation state when a neuron makes the transition to the off state. This lower level of $I_{\text{IH}}$ current also causes the neurons to be more hyperpolarized when they first make the transition to the off state.

When $g_{\text{IH}}$ is decreased further, such as in Fig. 10A (30 nS), the network starts to oscillate more slowly and the variability of the period increases. The network is now operating in an intrinsic escape mode. The inhibited neuron is not able to generate enough current with $I_{\text{IH}}$ to cross the synaptic threshold. However, in this case the neuron is able to still generate an action potential and escape inhibition by means of an intrinsic escape mechanism. If $g_{\text{IH}}$ is decreased sufficiently, half-center oscillations will cease entirely.

Figure 11 shows the effect of changes in $g_{\text{IH}}$ across the entire range of synaptic thresholds that produce half-center oscillations. Increasing $g_{\text{IH}}$ on the depolarized side of the inverted U-shaped relationship results in an increase in network period, in contrast to the decrease seen on the more hyperpolarized side (where the recordings in Fig. 10 are taken). This results from the different role that $I_{\text{IH}}$ plays in timing the synaptic release mechanism than that which it plays in timing the synaptic escape mechanism. In the synaptic release mechanism, if $g_{\text{IH}}$ is increased, there is more current generated by $I_{\text{IH}}$ at any activation state. This results in the plateau being somewhat higher and generating more synaptic current. This in turn causes greater hyperpolarization in the postsynaptic neuron and a greater level of $I_{\text{IH}}$. The end result is that the increased time required for $I_{\text{IH}}$ to deactivate causes the period to increase.

**Effect of changing the voltage dependence of $I_{\text{IH}}$ activation**

Figure 12 shows the effect of changing the voltage dependence of the activation of $I_{\text{IH}}$ on the GM network. For this set of parameter values ($V_{\text{b}} = -48$ mV), network period decreases as the half-activation potential is changed in a depolarizing direction (Fig. 12, $A-D$). The potential of the slow wave is also depolarized when $V_{1/2}$ is depolarized (Fig. 12, $A-C$). Close inspection of Fig. 12, $A-C$, reveals that the network undergoes a change in operational mode from intrinsic escape to synaptic escape when $V_{1/2}$ is depolarized. Mechanistically, these changes can be described in the following manner. For very hyperpolarized values of $V_{1/2}$ the neuron in the off state is not capable of activating enough $I_{\text{IH}}$ to bring the neurons to spiking threshold and the network is not able to sustain oscillations. As $V_{1/2}$ is depolarized, the $I_{\text{IH}}$ activation state is increased and the hyperpolarized neuron is able to reach spiking threshold and initiate an intrinsic
escape (Fig. 12A). Further increase in $V_{1/2}$ allows the neuron to cross the synaptic threshold before firing an action potential and effect a synaptic escape (Fig. 12B). Further depolarization of $V_{1/2}$ increases the activation state of $I_A$ and this depolarizes the neurons to such a point that both neurons are constantly above the synaptic threshold at all times and oscillations become very rapid (Fig. 12C). With more depolarization of $V_{1/2}$ the neuron's membrane potential becomes too depolarized with respect to the synaptic threshold and oscillations cease.

When the synaptic threshold potential is depolarized well above the spiking threshold, changes in the half-activation potential of $I_A$ have different effects on network period at different synaptic thresholds (Fig. 12E). The right portion of the plot in Fig. 12E represents a transition from synaptic to intrinsic escape as $V_{1/2}$ is hyperpolarized, similar to the situation in Fig. 12D. The decrease in network period seen in the left portion of the plot in Fig. 12E as $V_{1/2}$ is hyperpolarized further results from the higher synaptic threshold and subsequent lower synaptic current allowing a synaptic release mechanism to be utilized.

We also studied the effect of altering the half-activation potential of $I_A$ on the network period over the entire range of synaptic thresholds over which half-center behavior was observed (data not shown). The inverted U-shaped relationship moves in the same direction as the change in $V_{1/2}$. This is due to the shift in the slow wave potential when $V_{1/2}$ is altered. Therefore a change in $V_{1/2}$ can cause either an increase or decrease in network period depending on the initial mode of oscillation.

**Discussion**

**Modeling with the dynamic clamp**

This paper is the first to use the dynamic clamp (Sharp et al. 1993a,b) as a modeling tool with which to explore the dynamics of small circuits. Therefore it is useful to consider the strengths and weaknesses of this method, in contrast to conventional modeling techniques.

In conventional simulations the modeler makes an explicit choice about the complexity of the model neurons and synaptic connections. Often relatively simple neuronal and synaptic models are used (e.g., Skinner et al. 1994b; Wang and Rinzel
1992) because these are more amenable to analysis, and it is hoped that general principles will be easily extracted. However, these simple models may not accurately capture the properties of real neurons and real synapses. For example, in the paper by Skinner et al. (1994b) that influenced the design of the experiments reported here, the clear distinction between the different mechanisms of oscillation held for the cases in which the neurons were relaxation oscillators and the synaptic release function was very steep. One of the aims of this work was to determine whether the essential conclusions of Skinner et al. (1994b) would hold in more biologically realistic cases. Before the use of the dynamic clamp, the only way to do this would be to build more detailed conductance-based models. Indeed, in the few cases in which the essential biophysical measurements have been made from biological systems (e.g., Nadim et al. 1995; Olsen et al. 1995) this approach has been quite successful. However, all conventional models, no matter how detailed, are by their very nature oversimplifications. In the dynamic clamp experiments the model neuron is not oversimplified, because the model neuron is a real neuron. The advantage of this is that the dynamic clamp modeler does not have to specify the parameters for all the conductances of the cell (in our experiments we did not have to make up the properties of the Na⁺ and K⁺ currents in the action potential, but knew that they were correct, because they were intrinsic to the cell). The disadvantage of this method is that the dynamic clamp modeler does not have direct access to all of the parameters in the model, but only to those being added in the experiments described here we do not have any way of knowing the parameters of the Na⁺ and K⁺ currents in these cells but do have access to those of \(I_{\text{Na}}\) and \(I_{\text{K}}\) (Fig. 5). Obviously, because all biological neurons are not identical, it is no more likely that the results of dynamic clamp simulations will necessarily generalize to all neurons any more than the results of any other simulations that also depend on specific choices of model. For this reason, we do not expect dynamic clamp simulations, such as those carried out here, to replace either the use of simple neuronal caricatures (e.g., Rowat and Seiverston 1993; Skinner et al. 1994b; Wang and Rinzel 1992) or detailed, conductance-based models (Nadim et al. 1995; Olsen et al. 1995) but to provide an alternative method that ensures that the investigator can use biologically realistic parameters for currents and cellular processes that may not have been measured or modeled.
Reciprocal inhibition: alternation or synchrony?

It is often assumed that reciprocally inhibited neurons will always fire in alternation. However, theoretical studies have shown that reciprocal inhibition strongly synchronizes neurons when the inhibition is slow relative to the spikes (Van Vreeswijk et al. 1994; Wang and Rinzel 1992).

In this paper we show that stable half-center oscillation requires that the synaptic threshold be properly positioned, or ‘tuned,’ to the voltage dependences of the other properties of the neurons and synapses. Half-center operation requires that the synaptic threshold be within the peak and trough of the slow wave envelope of the membrane potential oscillations. Because this is also true in the theoretical work of Skinner et al. (1994b), which did not incorporate \( I_h \), it seems likely that this result would hold in other systems that do not require \( I_h \) to generate oscillations. When it is either too hyperpolarized or too depolarized, other modes of coupling, including in-phase spiking, out-of-phase spiking, etc. (Fig. 3), result. Therefore a modulatory substance that alters the effective threshold for synaptic release might move two reciprocally inhibitory neurons from firing in stable half-center alternation to synchrony or another pattern of activity.

Modulation of synaptic threshold

Skinner et al. (1994b) found that the period of a half-center that depends on synaptic properties is very sensitive to the synaptic threshold, and that this relationship has an inverted U shape (e.g., Fig. 4). Thus a modulator that increases the synaptic threshold (Johnson et al. 1995) can either increase or decrease the network period, depending on whether the network is initially operating in the synaptic escape mode (at more hyperpolarized synaptic thresholds) or in the synaptic release mode (at more depolarized synaptic thresholds), respectively.

In the stomatogastric nervous system and in many other rhythm pattern generators, graded synaptic transmission plays a critical role in pattern generation (Angstadt and Calabrese 1991; Burrows and Siegler 1978; DiCaprio 1989; Graubard 1978; Graubard et al. 1983; Johnson and Harris-Warrick 1990; Johnson et al. 1994, 1995; Pearson and Fourtner 1975). In these systems transmitter release is a graded function of presynaptic membrane potential, with a threshold for transmitter release that can be as hyperpolarized as \(-60\) mV (Graubard 1978; Johnson and Harris-Warrick 1990; Johnson et al. 1995). We chose the range of membrane potentials over which the synaptic threshold was varied to reflect values in the literature for stomatogastric ganglion neurons. Moreover, amine modulation of graded synaptic release in the stomatogastric ganglion changes the apparent threshold for graded release on the order of 3–10 mV (Johnson and Harris-Warrick 1990; Johnson et al. 1994, 1995). Changes as small as 5 mV in the synaptic threshold moved the half-center from the synaptic release to the synaptic escape mode of operation (e.g., Figs. 4, 8, and 11). This indicates that modulatory substances in the stomatogastric ganglion are capable of moving the network through the full inverted U-shaped plot of period versus synaptic threshold.

Although the model for transmitter release is quite simple, and directly relates the strength of the synaptic conductance to the presynaptic membrane potential without computing the local intracellular calcium concentration, it is an adequate first approximation of the input-output relationship of the biological synaptic release function in the stomatogastric nervous system, in which there is a steep relationship between presynaptic membrane potential and release in the \(-50\) to \(-30\) mV range of membrane potential (Graubard 1978; Johnson and Harris-Warrick 1990; Johnson et al. 1994).

Spikes and mechanisms

The theoretical model (Skinner et al. 1994b) did not include spikes. Spikes provide the neuron with additional intrinsic properties that are important for the generation of network oscillations via intrinsic mechanisms. In our experiments, an intrinsic escape mechanism can occur when \( I_h \) depolarizes the inhibited neuron up to spiking threshold so that a burst is initiated to cause the transition. An intrinsic release mechanism can occur when \( I_h \) deactivates so that the neuron hyperpolarizes past spiking threshold, terminating the burst to cause the transition. If action potentials are not present in the individual neurons, then the range of synaptic thresholds for which network oscillations occur is significantly reduced. We have shown this to be the case by removing spikes from the biological neurons in the dynamic clamp experiments (Fig. 6). This was also confirmed by constructing models that included spikes or not (data not shown). In particular, the application of tetrodotoxin to the biological system resulted in a gap in the plot of the period as a function of synaptic threshold as the intrinsic escape regime was attenuated or lost. In other words, the spikes and/or other inward currents in the biological system allow the circuit to slide through an intrinsic escape regime.

In Figs. 5D and 11, a gap in the plot of period versus synaptic threshold was seen in some parameter regimes. This
occurred because the inhibited neuron did not have enough $I_h$ to depolarize across its synaptic threshold to effect a synaptic escape and was too hyperpolarized to generate action potentials and effect an intrinsic escape. Additionally, the deactivation of $I_h$ in the active neuron was insufficient to allow the neuron to fall below its spike or synaptic thresholds to effect an intrinsic or synaptic release mechanism respectively. Thus the presence of spikes works to extend the range of synaptic thresholds over which oscillations can occur by allowing the intrinsic mechanisms to play a greater role in the generation of oscillatory behavior.

**Mixing of mechanisms**

The four theoretical mechanisms defined in Skinner et al. (1994b) were only distinct if the model neurons were of a relaxation type and the synaptic activation function was steplike. Otherwise, there was a blurring of the distinction between the four classes of transition (Skinner et al. 1994b). This implies that in some parameter regions one might expect to obtain network oscillations that use a mixture of the different mechanisms. This is precisely what we found in several of the dynamic clamp experiments. In particular, we showed that the oscillations obtained in Fig. 5C were obtained via a combination of intrinsic escape and synaptic release mechanisms.

In the detailed model of the leech heartbeat system (Nadim et al. 1995; Olsen et al. 1995) the oscillations were obtained with the use of both intrinsic escape and synaptic release mechanisms. The intrinsic escape was due to $I_h$, which was activated in the inhibited phase, and by a persistent sodium conductance that has a low activation threshold. The synaptic release mode was promoted by the decay of the synaptic currents. The graded component decayed in amplitude and the spike-mediated component decayed in frequency.

**Synaptic time constant**

In one set of experiments we asked how half-center period is influenced by changes in the synaptic time constant. We spanned a range of values (from 1 to 500 ms) relevant to the operation of the stomatogastric ganglion and large enough to allow workers in vertebrate preparations who are interested
in comparing the effects of fast γ-aminobutyric acid-A synapses with those of slower γ-aminobutyric acid-B synapses in reciprocally inhibitory networks to obtain a sense of the significance of those different time course actions on network dynamics.

In the dynamic clamp experiments, we found that increasing the synaptic time constant resulted in an increase in network period regardless of the underlying mechanism in operation (Figs. 7 and 8). However, we found that it was possible to obtain a decrease in network period as the synaptic time constant was increased, with a model half-center network that used simple Morris-Lecar (Morris and Lecar 1981) representations for the individual neurons (data not shown). This decrease was achieved only if the timing of the oscillations was dictated by the intrinsic properties of the neuron, i.e., if an intrinsic mechanism was in operation. This suggests that networks of neurons with different intrinsic properties could have opposite changes in network period when the synaptic time constant is changed, if intrinsic mechanisms are being used to generate the oscillations.

**Synaptic conductance**

Increasing the synaptic conductance caused an increase in the period regardless of the network’s oscillatory mode. We also showed how changes in synaptic strength could alter the mode of oscillation for the network. Changes in synaptic strength of 20–30% could cause a change of up to 100% in network period. Also, if the synaptic strength is increased too much relative to the intrinsic properties of the neurons, the network stops oscillating (data not shown). As described earlier, this is because the larger synaptic current prevents the inhibited neuron from reaching either spiking or synaptic threshold to elicit an escape. This creates a discontinuity in the smooth transitions between mechanisms as the synaptic threshold is altered. Taken together, these results indicate that even moderate changes in synaptic strength that can be generated by long-term potentiation or facilitation or through the agency of a neuromodulator can alter the mode of oscillation and/or cause very large changes in the period of the network.

$I_{N}$ and oscillations

This particular half-center depends on $I_{N}$ to oscillate. Voltage clamp measurements of $I_{N}$ show that it requires hyperpolarization for activation and that the rate is slow and nonmonotonic, exhibiting complex kinetics (e.g., DiFrancesco and Noble 1989; McCormick and Pape 1990a). Destexhe et al. (1993) found it necessary to include these complex kinetics into simulations of single thalamocortical neurons to obtain spindle-like or waxing and waning oscillations. The model we have employed for $I_{N}$ is taken from Buchholtz et al. (1992) and does not take into account the nonmonotonic rate constant. However, the experiments were performed in voltage regimes in which the $I_{N}$ relaxation rate was not changing, so we do not expect the complex kinetics of the $I_{N}$ relaxation rate to make a significant difference in the mechanisms described here.

Changing the conductance of $I_{N}$ had a very different effect on the network than did the changes in the synaptic properties. Increasing $g_{N}$ caused a depolarizing shift in the range of synaptic threshold potentials that would generate half-center oscillations. We found that changing $g_{N}$ produced opposite effects on the period of the network depending on the mode of oscillation. Increasing or decreasing $g_{N}$ effectively moves the position of the slow wave with respect to the synaptic threshold and therefore “sweeps” through the modes of oscillation until the synaptic threshold lies outside the slow wave and network oscillations cease. It is also possible that oscillations cannot occur when the synaptic threshold lies within the range of the slow wave. This occurs for small values of $I_{N}$ when the network is in an intrinsic escape mode. The network period is fairly sensitive to small changes in $g_{N}$, but perhaps not as sensitive as it is to changes in synaptic strength.

Changing the half-activation potential of $I_{N}$ produced results very similar to those produced by changing $g_{N}$. Changes of the half-activation potential in a depolarizing direction caused a depolarizing shift in the inverted U-shaped relationship of period as a function of synaptic threshold much like that produced by increasing $g_{N}$. Not only does this mean that changing the half-activation potential can cause different changes in network period for different oscillatory modes, but that changes in the activation curve can sweep the network through oscillatory modes and both increase and decrease network period at a given synaptic threshold. The network is so sensitive to changes in the half-activation potential that a 5-mV change can result in a very large change in network frequency or mode of oscillation.

Biogenic amines have been reported to alter both the conductance and voltage dependence of $I_{N}$ in vertebrates and invertebrates (Kiehn and Harris-Warrick 1992b; McCormick and Pape 1990b). The range of parameter values used in this study easily falls within the range of aminergic effects reported.

**Spike-mediated transmission**

The synaptic transmission produced via the dynamic clamp technique was not of a spike-mediated type. The inclusion of spike-mediated transmission would add another level of complexity requiring separate analysis. The synapses represent a first-order approximation to the input-output relationship of the biological synaptic release function in the stomatogastric nervous system. However, because these neurons spike, and if the synaptic threshold is depolarized enough such that the action potentials produce most of the synaptic interactions, (e.g., very jagged IPSPs in Fig. 4C), transmission occurs mostly due to the spikes, but with the use of a graded synaptic model. Comparison with the theoretical mechanisms suggests that the underlying mechanism when the synaptic threshold is at this depolarized value is the synaptic release mechanism. As shown in Fig. 6G, when the spikes are removed with the use of tetrodotoxin, it is still possible to obtain oscillations in this regime, i.e., via a synaptic release mechanism, but oscillations occur for a very narrow range of synaptic thresholds when they occur. As described above, the presence of action potentials in neurons acts to extend the range of synaptic thresholds over which oscillations can occur.

In a recent detailed biophysical model of the leech heart-
beat system (Nadim et al. 1995), both graded and spike-mediated transmission were modeled. It was found that the model could produce two distinct modes of oscillation that they termed S mode, for oscillations dominated by spike-mediated inhibition, and G mode, for those dominated by graded synaptic inhibition. The difference is a consequence of spike-mediated synaptic transfer and not of spiking, which is present in both modes. Although it is not known to what extent spike-mediated or graded transmission is dominant during the oscillations of the leech heart interneurons, S mode oscillations appear to produce activity closer to that observed in the real neurons.

Olsen et al. (1995) found that changing the conductance of the graded synaptic transmission did little to affect the network period, whereas increasing the conductance of the spike-mediated transmission produced an almost linear increase in period. Their model of graded synaptic transmission is a complicated nonlinear function of presynaptic calcium and thus cannot be directly compared with the results we obtained with changing synaptic conductance. Their spike-mediated synaptic current is described by an alpha function. It is interesting to note that their network period increased with increasing the conductance of spike-mediated transmission, similar to our result in which the period increased with increasing synaptic conductance. Olsen et al. obtained an increase in period as g_s was decreased, with the network operating in S mode. We also found that the network period in our constructed half-center increased with decreasing g_s if the oscillations were being generated with the use of synaptic escape and/or intrinsic escape mechanisms, but not synaptic release.

Conclusion

With the use of dynamic clamp constructed half-centers, we have directly demonstrated the theoretical predictions of Skinner et al. (1994b). Despite differences in the biophysical details of the model cells (in Skinner et al. 1994b) and the real neurons used here, the network period first increased and then decreased when the synaptic threshold was increased, producing an inverted U-shaped relationship of period versus synaptic threshold. These changes in period were due to changes in the oscillatory mechanism as predicted in Skinner et al. (1994b).

One interesting result of this study is that the characteristics of synaptic and intrinsic conductances (synaptic strength, magnitude of intrinsic conductances, voltage dependence of activation, etc.) must be carefully balanced to maintain smooth transitions between modes of oscillation during modulation of these properties. It may even be necessary to invoke multiple forms of modulation either sequentially or concurrently to maintain smooth transitions in network period. It might also be possible that the nervous system could utilize a mismatch in synaptic and intrinsic properties to create distinct phase spaces whereby different components could be brought in and out of register.

In summary, in this paper we have investigated the mechanisms of half-center behavior and determined how modulation of the synaptic or intrinsic conductances can alter the oscillatory mode and output of the circuit. The dynamic clamp has allowed us a level of control over the parameters that are critical in the production of half-center behavior in a biological situation that was previously not possible to achieve. We have shown that neural networks can be very sensitive to small changes in their synaptic and intrinsic conductances and that these changes can either increase or decrease the network period. We have also demonstrated that modulation of these elements can have effects that are highly dependent on the nature of conductances that are intrinsic to the neurons. The response of the network to modulation is a result of the interaction of synaptic and intrinsic conductances with each other. This illustrates that if one wishes to study how a network is modulated by a given substance it is critical to identify the components of the system that are most important in generating the output of the network and how these elements interact.

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