Modeling the Leech Heartbeat Elemental Oscillator
II. Exploring the Parameter Space

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Received December 7, 1994; Revised March 9, 1995; Accepted March 17, 1995

Action Editor: T. Williams

Abstract. In the previous paper, we described a model of the elemental heartbeat oscillator in the leech. Here, the parameters of our model are explored around the baseline canonical model. The maximal conductances of the currents and the reversal potential of the leak current are varied to reveal the effects of individual currents and the interaction between synaptic and intrinsic currents in the model. The model produces two distinct modes of oscillation as the parameters are varied, S-mode and G-mode. These two modes are defined, their origin is identified, and the parameter space is mapped into S-mode and G-mode oscillation and no oscillation. Finally, we will make predictions for how the period can be modulated in heart interneurons.

Introduction

In the previous report we described a second-generation model of a two-cell neuronal oscillator controlling the heartbeat in the medicinal leech (Nadim et al., 1995). There are two such oscillators in the timing oscillator that generates the heartbeat pattern. Each oscillator consists of a pair of reciprocally inhibitory interneurons, called HN(L) and HN(R), that oscillate in antiphase when isolated from the other known components of the heartbeat network. Our second-generation model added spike-mediated synaptic inhibition, \( I_{\text{syn}} \), to the graded synaptic inhibition, \( I_{\text{synG}} \), that already existed in a first generation model (Calabrese and De Schutter, 1992). The postsynaptic conductance for the graded transmission depended on the effective presynaptic Ca\(^{++}\) concentration (Angstadt and Calabrese, 1991; De Schutter et al., 1993). Two low-threshold Ca\(^{++}\) currents gave rise to the presynaptic Ca\(^{++}\) concentration: \( I_{\text{CaS}} \) which inactivates rapidly and \( I_{\text{Cas}} \) which inactivates slowly. The plateau is supported by the two Ca\(^{++}\) currents as well as a low-threshold noninactivating Na\(^{+}\) current, \( I_{F} \) (Opdyke and Calabrese, 1994). The membrane potential during the plateau is limited by two K\(^{+}\) currents: \( I_{K1} \) activates rapidly and inactivates and \( I_{K2} \) is slower to activate and is noninactivating (Simon et al., 1992). An A current, \( I_{A} \), is transiently activated at the onset of the plateau (Simon et al., 1992). A fast Na\(^{+}\) current, \( I_{Na} \), generates the action potentials. Spike-mediated synaptic transmission was modeled as postsynaptic conductance changes of fixed time course, triggered by presynaptic spikes (Simon et al., 1994). During the hyperpolarized phase of the oscillatory activity cycle, a hyperpolarization activated inward current, \( I_{A} \), is slowly activated which promotes escape from the synaptic inhibition (Angstadt and Calabrese, 1989). Finally, the model had a passive leak current whose reversal potential is near the midrange of the membrane potential oscillation (Nadim et al., 1995).
Here we report the effects of some parameter changes in the model. These changes allowed us to investigate the interactions between the numerous currents throughout the cycle of oscillation. We were primarily interested in determining how the period of the oscillation is controlled. The heart rate is regulated to match the metabolism of the leech. For example, when the temperature is increased or the leech swims, the heart rate increases (Arbas and Calabrese, 1984). The period of the oscillation is thus an important characteristic of the heartbeat timing oscillator. The endogenous neuropeptide FMRF-NH2 can modulate the period of the heartbeat rhythm (Evans et al., 1991; Kuhlman et al., 1985; Li and Calabrese, 1987). Among the physiological effects of FMRF-NH2 on the heart interneurons are the shift in steady-state activation and inactivation of outward currents to more negative potentials (Simon et al., 1992), and the reduced amplitude and increased duration of spike-mediated IPSCs (Simon et al., 1994). In other oscillatory motor systems modulators control period and simultaneously act to alter the activities of voltage-gated channels or synaptic transmission or both (Golowasch and Marder, 1992, Harris-Warrick and Marder, 1991, Hooper and Marder, 1987).

Other characteristics of the oscillation, such as burst duty cycle, burst amplitude, and spike frequency, were not considered in detail. The spike frequency in the model is strongly linked to the period (see Results) and is hard to dissect out as a single characteristic. The burst duty cycle is nearly constant at 0.5 due to the precipitous onset of the plateau, and the burst amplitude is partly accounted for by discriminating between S-mode and G-mode.

Most of our parameter exploration focused on changing the maximal conductance of the currents. After determining how the period could be changed in the model, we made predictions that can be tested in future experiments. The current model displayed two distinct modes of oscillation, S-mode and G-mode (Nadim et al., 1995). In this paper, the two modes are precisely defined, and the origin of the G-mode oscillation is further investigated.

Methods

The simulations were performed with NeuroLab software (Olsen, 1994). NeuroLab allows the parameters to be set on the command line, and we have written programs that invoke NeuroLab with different parameters. For each run, the 32 state variables of the model stored at every tenth integration step. The state variables and the appropriate set of parameter values sufficient to calculate the conductances and curries when required. The initial condition was identical in all cases and was an arbitrary point on the limit cycle of the canonical model. When the system is started with altered parameters, it will typically display a transient before it settles down on another limit cycle or a fixed point. These transients were never observed to last longer than 5 s. We simulated at least 30 s of the model output and avoided analysis in the transient region.

Subsequent to data generation, various forms of analysis were performed on the data files. The period was calculated as the interval between successive transitions from the hyperpolarized phase to the plateau phase. The instant of the transition was taken to be when the activation, m, of I_{CaS} rose above 0.5 (see Results for further explanation). The limit-cycle period was obtained from the neuron that last generated a plateau. Occasionally, the system entered a bistable state where one cell fired tonically and inhibited the other cell. In this state, the activation of I_{CaS} sometimes crossed 0.5 during each spike and produced incorrect periods. These artifacts were easily eliminated by requiring the period to be longer than 1 s. Some runs started off with a few transient cycles of oscillation before settling in a nonsustained mode. The procedure above calculated the period of the transient cycles, but if the last transition appeared more than a period earlier than the end of the integration, the run was identified as nonsustained. Further, runs with a single transient could possibly oscillate with a period greater than the duration of the integration. Thus the term no oscillation will mean that the parameter configuration was truly nonsustained or that the period was larger than 25 s.

The runs were segregated into three classes: no oscillation, G-mode, and S-mode oscillation, as identified in Nadim et al. (1995). It was possible to determine the mode automatically by either considering the inactivation, h, of I_{CaS} at the point of the last transition, or calculating the duty cycle of the graded synaptic transmission. The time of the transition and the corresponding state variables were found by interpolation between successive integration steps. The duty cycle of the graded synaptic transmission was calculated as the fraction of the period during which the conductance of the graded transmission was above 1 nS. This level was chosen as the point where the graded transmission was comparable to the other conductances operating in the cell. For comparison, the mean value of
variables of the model are ion step. The state variables of parameter values are conductances and currents. Simulation was identical in point on the limit cycle of the system is started with a display a transient limit cycle or a fixed never observed to last at least 30 s of the model the transient region. Various forms of a data file. The period between successive transient phase to the plateau was taken to be 1 s above 0.5 (see ). The limit-cycle period that last generated but was identified as a bistable and inhibited the dynamics of I_{CAS} sometimes produced incorrectly eliminated by re- than 1 s. Some runs of oscillation began. The procedure transient cycles, but than a period earlier he run was identified with a single transition period greater than the term no oscillation configuration was truly was larger than 25 s. Three classes: no oscillation, as identified possible to determine considering the initial transition of the graded synaptic transition and the found by interpolation. The duty cycle was calculated which the conductances above 1 nS. This the graded trans- conduction op the mean value of the spike-mediated transmission during the hyperpolarized phase was 7 nS.

The sensitivity coefficients used in Fig. 9 were calculated as fractional change in period over fractional change in the parameter, that is,

\[ s_p = \frac{(T(p + \Delta p) - T(p - \Delta p))/T(p)}{2\Delta p/p} \]

where \( T(p) \) is the period as function of the parameter \( p \). The change in the parameter, \( \Delta p \), was made small to stay within an approximately linear region of \( T(p) \) but kept large enough to produce an appreciable change in \( T \). In practice, a change of 5 to 10% in a parameter was found to be appropriate.

Results

The membrane potential oscillation in the canonical model is shown in Fig. 1. The period of this oscillation was 7.8 s. The model proved to oscillate over a wide range of parameters. The conductances of individual currents were varied from 0 to twice the canonical value, and the ionic reversal potentials were changed around the canonical values. In most configurations, the model produced stable oscillation; only in a few cases did the oscillation cease. The period of oscillation ranged from 6 s to 25 s for these parameter sweeps.

The model continued to oscillate when either of the two synaptic mechanisms was blocked (Fig. 1). During the parameter sweeps, the model oscillated in one of two distinct modes, S-mode and G-mode (Nadim et al., 1995). In G-mode, the synaptic inhibition was dominated by the graded component. Blocking the spike-mediated transmission thus produced a “pure” G-mode. In S-mode, the spike-mediated transmission dominated, and blocking the graded component provided a “pure” S-mode (Fig. 1). We can now proceed to define S-mode and G-mode more precisely, even for cases where both the graded and spike-mediated synaptic inhibition were present.

Two Modes of Oscillation: S-mode and G-mode

The transition from the hyperpolarized phase to the plateau phase occurs as the hyperpolarization of the inhibited cell activates the \( h \)-current, \( I_h \). This inward current builds up slowly and helps overcome the outward synaptic currents. At the same time the spike-mediated synaptic current decreases due to a decrease in the spike frequency of the active cell. Eventually the net current becomes inward, thus depolarizing the cell to the point where the persistent Na\(^+\) current and the low-threshold Ca\(^{2+}\) currents are activated and become regenerative, producing a depolarizing plateau. The point of transition was defined to be the instant the activation variable, \( m \), of \( I_{CAS} \) rose through \( m = 0.5 \). This definition seems appropriate because the time when \( m \) reached 0.5 was always close to the instant when the inward currents became regenerative. The transitions are marked with circles in Fig. 3.

A switch from the canonical S-mode to “pure” G-mode and back to S-mode is demonstrated in Fig. 2. The initial cycles show the canonical model oscillating in S-mode. Approximately 1 s prior to the expected transition to a plateau in the HN(L) model cell, the spike-mediated synaptic transmission, \( I_{syn} \), was blocked in both cells. The abrupt removal of \( I_{syn} \) released the HN(L) model cell from synaptic inhibition, and it instantaneously produced a plateau. It is thus evident that in S-mode, the transition to the plateau was delayed by the spike-mediated IPSPs. During this delay in the canonical model, the inhibited cell depolarized slowly due to the activation of \( I_h \). In the range of membrane potential occurring during this time, the slow Ca\(^{2+}\) current, \( I_{CAS} \), slowly inactivated with a time constant of approximately 1 s.

Because the HN(L) model cell rose to its plateau 1 s earlier than in S-mode, the \( I_{CAS} \) inactivated to a lesser extent than normal so that \( g_{CAS} \) expressed during the plateau was higher. The larger \( g_{CAS} \) produced a higher plateau potential. The peak \( I_{CAS} \) (time averaged over a few spikes) was 20% higher than in the canonical model, while the peak \( g_{CAS} \) was 27% higher.

The graded synaptic current, \( I_{syn} \), in the opposite (HN(R)) model cell increased two-fold due to the increased Ca\(^{2+}\) currents in the HN(L) model cell. The large \( I_{syn} \) in HN(R) model cell hyperpolarized the cell nearly to the synaptic reversal potential (−62.5 mV). The \( h \)-current was activated more by the larger hyperpolarization (\( m \) of \( I_h \) peaked during the hyperpolarized phase at 10% above the canonical level), and the graded synaptic current was not strong enough to keep the cell hyperpolarized. The duration of this half cycle was shorter than a half cycle in either S-mode or the G-mode that occurred soon afterwards. At this point, the HN(R) model cell rose to its plateau and a stable limit cycle in “pure” G-mode had been achieved. The transient induced by removing \( I_{syn} \) lasted only a half cycle. The peak \( g_{CAS} \) during the "pure" G-mode oscillation was 50% higher than in the canonical model and 18% higher than during the transient plateau in the HN(L)
Fig. 1. At the top, the membrane potential for one cell in the canonical model is shown through two cycles of oscillation. The cppt model cell oscillated in antiphase to the cell shown. The canonical model is described in Nadim et al. (1995) and closely approximates activity of heart interneurons. Changes in parameters refer to this model as the baseline. The graded synaptic transfer and the spike-mediated synaptic transfer can alone produce oscillation in the model. When blocking the spike-mediated transmission ($\bar{g}_{\text{Syn}} = 0$), the graded role was enhanced compared to the canonical model, and the model oscillated with strong graded inhibition during the hyperpolarized phase. When blocking the graded transmission ($\bar{g}_{\text{Grd}} = 0$), the model produced an oscillation that resembled that of the canonical model.
Fig. 2. The canonical model entered G-mode when the spike-mediated synaptic transmission was removed from both cells and returned to S-mode when it was reintroduced. The interval during which the spike-mediated transmission was blocked is labeled with the horizontal bar. The sudden removal of spike-mediated synaptic inhibition enabled the HN(L) model cell to immediately generate a plateau. The slow Ca\textsuperscript{++} conductance, g_{CaS}, supporting the plateau increased over the next two cycles. The increase in g_{CaS} was found to be due to less inactivation during the hyperpolarized phase. In G-mode, the 50% increase in g_{CaS} produced a four fold increase in g_{Syn} of the opposite cell. When the spike-mediated transmission was reintroduced, the next plateau transition was postponed (in HN(L)). The inhibition caused the HN(L) membrane potential to remain for a longer time in the potential range where g_{CaS} inactivated. During the subsequent plateau phase, g_{CaS} was reduced by 19% while the graded conductance in the opposite cell reduced by 56%. The conductances of the slow Ca\textsuperscript{++} current and the graded synaptic current continued to decrease during the next 10 to 15 s when the oscillation returned to the canonical oscillation.
model cell. The resulting $g_{Syn}$ was 20 times higher than in the canonical model, and the period of oscillation was reduced from 7.8 s in the canonical mode to 6.4 s in the "pure" G-mode.

When $I_{Syn}$ was subsequently turned back on, the model regained normal S-mode oscillation following a transient region lasting 10 to 15 s (Fig. 2). The spike-mediated transmission was switched on while the HN(R) model cell was on a plateau. The spikes in the right cell produced IPSPs in the left cell that postponed the onset of the following HN(L) plateau by 0.7 s. During these 0.7 s, the HN(L) membrane potential was kept in a range that inactivated $I_{Cas}$, and the following plateau had a peak $g_{Cas}$ reduced to 87% of G-mode. The reduced HN(L) Ca$^{++}$ current during the plateau led to a graded conductance in the HN(R) model cell that was reduced to 44% of G-mode. The presence of spike-mediated synaptic inhibition therefore prevented the production of strong graded transmission and enabled the oscillation to reenter the canonical S-mode.

A distinguishing characteristic between S-mode and G-mode oscillation appeared in the inactivation of the slow Ca$^{++}$ current, $I_{Cas}$. Figure 3 shows the dynamics of $I_{Cas}$ in S-mode and G-mode. The S-mode trace is from the canonical model, and the G-mode trace was obtained by setting the conductance of the h-current, $\tilde{g}_h$, to 10.5 nS, that is, 50% above its canonical value of 7 nS. This oscillation was considered a general G-mode oscillation, since both the spike-mediated and the graded inhibition were present. In both cases, $I_{Cas}$ was rapidly activated at the onset of the plateau ($m$ approached 1) and rapidly deactivated at the onset of the hyperpolarized phase ($m$ fell to 0). Thus the activation, $m$, is close to 1 during the plateau and approximately 0 during the hyperpolarized phase.

In S-mode, $I_{Cas}$ deinactivated ($h$ increased) to $h \approx 0.85$ during the early part of the hyperpolarized phase and then inactivated ($h$ decreased) as the point of transition was approached. At the point of transition, $h$ had a value of $h \approx 0.55$, i.e. the slow Ca$^{++}$ conductance attained 55% of its maximal value during the plateau. During the plateau, the current inactivated further to $h \approx 0.25$. Note that $g_{Cas}$ was fully activated ($m \approx 1$) during the plateau in both S-mode and G-mode, and thus the value of $g_{Cas}$ attained was only limited by the amount of inactivation.

In G-mode, $I_{Cas}$ deinactivated during the hyperpolarized phase to a maximum value of $h \approx 0.9$, slightly higher than in S-mode and inactivated toward the transition to $h \approx 0.7$. Thus, 70% of the maximum possible $g_{Cas}$ was expressed during the plateau.

The slow Ca$^{++}$ conductance at the point of transition was 27% higher in G-mode than in S-mode, and the source of this increase was reduced inactivation of $I_{Cas}$ at the onset of the plateau. All G-mode oscillations produced during the parameter sweeps proved to have higher values of $n_{Cas}$ at the transition than S-mode oscillations (Fig. 4A). In the 387 variations of the model that oscillated, some in S-mode and some in G-mode, the inactivation of $I_{Cas}$ segregated into two classes, either above or below a criterion value of $h = 0.58$ (Fig. 4A). The runs with $h > 0.58$ were confirmed to be in G-mode and the runs with $h < 0.58$ were confirmed to be in S-mode.

In the previous report we demonstrated that the amplitude and the duty cycle of the graded synaptic transfer was higher in G-mode than in S-mode (Nadim et al., 1995). The duty cycle of the graded transfer also segregated into two populations that correspond to G-mode and S-mode oscillation, on either side of a criterion value of $0.33$ (Fig. 4B). Any given oscillation was categorized into the same mode by both the inactivation of $I_{Cas}$ and the duty cycle of $I_{Syn}$ (Fig. 4C).

Figure 5 shows that S-mode and G-mode are significantly different. The clear and consistent separation of the two modes suggests that both the inactivation of $I_{Cas}$ and the duty cycle of the graded synapse were reliable methods for quantitatively discriminating between S-mode and G-mode. The definition of S-mode and G-mode can now be phrased quantitatively, given discriminating levels of inactivation of $I_{Cas}$ at 0.58 or the duty cycle of graded synaptic transfer at 0.33.

**Period Sensitivity to Intrinsic and Synaptic Currents**

**The Synaptic Conductances $g_{Syn}$ and $g_{SynG}$.** Increasing the maximal conductance of the spike-mediated synapse, $g_{Syn}$, above the canonical value increased the period of oscillation of the model (Fig. 6). Increasing $g_{Syn}$ required more $h$-current to activate in order to cancel the increased synaptic inhibition and to take the cell to the plateau transition. Since $I_h$ built up slowly, the point when the net current became inward was delayed as $g_{Syn}$ was increased, and the period increased. Lowering $g_{Syn}$ sufficiently below the canonical value provoked the onset of G-mode. The G-mode oscillation at 75% of canonical $g_{Syn}$ is shown in Fig. 6. Lowering the spike-mediated inhibition allowed the hyperpolarized cell to spend less time in the region where $I_{Cas}$ inactivated prior to the plateau transition. The resulting stronger and longer lasting graded transmission then led to G-mode oscillation.
t the point of transition in S-mode, and led to cyclic inactivation of G-mode oscillations, which proved to have a less than S-mode oscillations of the model. In some G-mode, the graded transfer was confirmed to be by the inactivation of $I_{\text{Syn}}$, (Fig. 4C). G-mode are significantly separated with the inactivation and synaptic functions that correspond to the overall function of $I_{\text{Cas}}$ at 0.58 or transfer at 0.33.

**Synaptic Currents**

and $\tilde{\xi}_{\text{Syn}}$. Increasing the spike-mediated current increased the (Fig. 6). Increasing activate in order to allow and to take the $I_h$ built up slowly, the same inward was decreased period increased.

The canonical value for G-mode oscillation in Fig. 6. Lowering the hyperpolarization region where $I_{\text{Cas}}$ increases theCas. The resulting d transmission then

![Graph](image)

**Fig. 3.** G-mode has more slow Ca$^{2+}$ current available at the point of transition. A and B: The activation ($m$) and inactivation ($h$) of $I_{\text{Cas}}$ are shown through a cycle of oscillation in A: S-mode (canonical parameters, that is, $\tilde{\xi}_h = 7 \text{ nS}$) and B: G-mode ($\tilde{\xi}_h = 10.5 \text{ nS}$). The transition was defined as the point where $m$ rose through 0.5 (marked by circles). The inactivation at the transition is marked by squares. In both modes, the membrane potential prior to the transition to the plateau was in a range that inactivated $I_{\text{Cas}}$. In the canonical model, $h$ had reduced to 0.55 at the point of transition, while in G-mode, $h$ decreased to 0.7. Consequently, only 55% of $\tilde{\xi}_{\text{Syn}}$ was expressed during the plateau in S-mode, while 70% of $\tilde{\xi}_{\text{Cas}}$ was expressed in G-mode. (The conductance of $I_{\text{Cas}}$ is $\tilde{\xi}_{\text{Cas}} = I_{\text{Cas}} m^3 h$).

Figure 7 shows the variation in the period in the model as the conductances of the currents are increased from 0 to twice the canonical value. The period was relatively insensitive to $\tilde{\xi}_{\text{Syn}}$ while it was very sensitive to $\tilde{\xi}_{\text{Cas}}$ (Fig. 7A). From 80% of the canonical $\tilde{\xi}_{\text{Syn}}$ and upward, the period increased nearly linearly with $\tilde{\xi}_{\text{Syn}}$, ranging from 6.5 s at 80% to 12.5 s at 200%. The G-mode oscillations below 80% of the canonical value of $\tilde{\xi}_{\text{Syn}}$ had an approximately constant period of 6.5 s, indicating that in G-mode, the synaptic currents were dominated by $I_{\text{Syn}}$.

The two synaptic conductances were comparable during the initial third of the hyperpolarized phase, while only the spike-mediated inhibition was effective during the late hyperpolarized phase since the graded inhibition had decayed (Nadim et al., 1995). The effect
Fig. 4. The inactivation of $I_{Cas}$ and the graded transmission duty cycle (as a fraction of the period) for all parameter configurations that were found to oscillate. Both measures fall into one of two categories that are clearly separated. The run numbers were assigned as single conductances were increased from 0 to twice the canonical value in fixed increments and as ionic reversal potentials were made more negative in fixed increments. For clarity, the point style was changed each time a new parameter was changed. A: The inactivation of $I_{Cas}$ at the point of transition segregates into two categories. The suggested criterion level is indicated by the dashed line at $h_{Cas} = 0.58$. B: The duty cycle of the graded synaptic transmission segregates into two categories. The suggested criterion level is indicated by the dashed lines at a duty cycle of 0.33. C: The two measures agree in the classification of all oscillatory runs.
A. The inactivation of the slow Ca\(^{2+}\) current, \(h_{\text{Ca}}\), differs in S-mode and G-mode. Error bars indicate the standard deviation (\(P < 0.001\) in both cases).

B. The duty cycle of the graded inhibition differs in S-mode and G-mode.

Fig. 5. S-mode and G-mode are different. A: The inactivation of the slow Ca\(^{2+}\) current, \(h_{\text{Ca}}\), differs in S-mode and G-mode. B: The duty cycle of the graded inhibition differs in S-mode and G-mode. Error bars indicate the standard deviation (\(P < 0.001\) in both cases).

The inhibition during the initial part of the hyperpolarized phase was a slight decrease in the period, as seen in the plot of period versus \(g_{\text{syn}}\). The extra graded inhibition caused the plateau currents to deactivate faster, thereby allowing a quicker transition to the inhibited phase. The inhibited phase would be more hyperpolarized, which would activate more \(h\)-current and depolarize the cell faster to the next plateau.

The time-averaged spike-mediated transmission, \(I_{\text{syn}}\), provided inhibition of similar magnitude to \(I_{\text{syn}}\) during the first third of the hyperpolarized phase. The effects of increasing \(g_{\text{syn}}\) therefore included one component that postponed the plateau transition and one component that provided a stronger deactivation of the plateau in the opposite cell similar to the graded transmission. These effects contribute toward slowing the oscillation down and accelerating the oscillation, respectively. The effect during the latter part of the hyperpolarized phase was dominant, since the net effect of increasing \(g_{\text{syn}}\) was to increase the period (Fig. 7A).

\textbf{The \(h\)-Current Conductance \(\tilde{g}_h\).} Reducing \(I_h\) from its canonical value caused a steep increase in the period (Fig. 7B). At \(\tilde{g}_h = 0\), the period was nearly 19 s, more than twice the period of the canonical model (Fig. 8). The membrane potential through one cycle of oscillation with \(\tilde{g}_h = 0\), the canonical value, and twice the canonical value is shown in Fig. 8. In the absence of \(I_h\), the transition occurred due to the fall in the presynaptic spike rate. The fall in the spike rate slowly released the postsynaptic cell from the spike-mediated inhibition. Since there was no current to promote escape, this oscillation seems to operate purely in release mode. This
Fig. 6. Membrane potential oscillation at low, canonical, and high $g_{\text{syn}}$. Increasing the spike-mediated synaptic transmission slowed the oscillation down. The oscillation switched from S-mode to G-mode at low $g_{\text{syn}}$. The amplitude of the oscillation increased as the synaptic inhibition was decreased. The dashed lines indicate $-50$ mV.
mechanism may not operate in the heart interneurons because their oscillation is disrupted when $I_h$ is blocked with external Cs$^+$ (Angstadt and Calabrese, 1989).

As $g_N$ was increased, $I_h$ produced a net inward current earlier in the inhibited cell and provoked the transition to a plateau, thus speeding up the oscillator. At $g_N$ 30% above the canonical value, the oscillation went into G-mode (Fig. 7B). At this point, the membrane potential approached the point of transition fast enough so that there was no significant inactivation of $I_{Ca}$ during the hyperpolarized phase, and G-mode thus ensued. The oscillation in G-mode with $g_N$ at 50% above the canonical value is shown in Fig. 8, where the period was 6 s.

The Persistent Na$^+$ Conductance $g_P$. The period ranged from 6 s at $g_P = 0$ to 9.5 s at $g_P$ twice its canonical value (Fig. 7B). Strengthening $I_P$ prolonged the cycle period by contributing to inward currents underlying the plateau phase, causing increased spike rate and spike-mediated synaptic inhibition. Therefore, increasing $g_P$ prolonged the hyperpolarized phase by increasing the spike-mediated transmission.

The Leak Conductance $g_l$. The period decreased from 10.3 s at no leak current, $g_l = 0$, to 5.5 s when the leak conductance was twice the canonical value. The period was reduced as $g_l$ increased since during both the inhibited phase and the plateau phase, the leak current provided a repolarizing current toward the midrange of the membrane potential oscillation (Fig. 7B).

The Ca$^{++}$ Conductances $g_{CaF}$ and $g_{CaS}$. The period was reduced from 8 s to 7 s as $g_{CaF}$ was increased from 0 to 200% of its canonical value (Fig. 7C). This was contrary to the expectation that an inward...
current during the plateau that also enhanced the inhibition of the opposite cell would prolong the period. The fast Ca$^{2+}$ current, $I_{\text{Ca}^{2+}}$, is a brief, transient current activated at the onset of the plateau phase (Nadim et al., 1995). Its contribution to the overall plateau potential appeared to be minimal, although it may be important in the process of regenerative onset of the plateau. However, the fast Ca$^{2+}$ current contributed significantly to the graded synaptic conductance (De Schutter et al., 1993). The period change for a sweep of $g_{\text{SynG}}$ was similar to that of $g_{\text{Ca}^{2+}}$ (Fig. 7A), and suggested that $I_{\text{Ca}^{2+}}$, indirectly through $I_{\text{SynG}}$, reduced the period by providing strong inhibition in the opposite cell. That cell entered the hyperpolarized phase faster and $I_h$ started earlier to promote escape.
Increasing \( \tilde{g}_{\text{CAS}} \) generally increased the period (Fig. 7C). The canonical model was positioned at a peak such that either an increase of up to 10% or a decrease of \( \tilde{g}_{\text{CAS}} \) decreased the period. Thus, the canonical value of \( \tilde{g}_{\text{CAS}} \) was such that the period was the longest possible while remaining in S-mode. An increase of more than 10% caused the model to enter G-mode oscillation, where the period steeply increased with \( \tilde{g}_{\text{CAS}} \). The duty cycle of \( I_{\text{Syn}} \) in G-mode was 0.5, that is, 100% of the inhibited phase (Fig. 4), so \( I_{\text{Syn}} \) was active during the late stage of the inhibited phase. Contrary to S-mode, where \( I_{\text{Syn}} \) was transiently active only at the early part of the hyperpolarized phase, in G-mode \( I_{\text{Syn}} \) can delay the onset of the plateau and decrease the period (Fig. 7C). Raising \( \tilde{g}_{\text{CAS}} \) not only increased the graded inhibition, it also enhanced the plateau and thus increased the spike rate and the spike-mediated synaptic inhibition. We devote a subsequent section to further describe the effects of \( I_{\text{CAS}} \) on the oscillation.

The \( K^+ \) Conductances \( \tilde{g}_{K1}, \tilde{g}_{K2} \). Increasing the conductance of the two outward currents, \( I_{K1} \) and \( I_{K2} \), above the canonical values reduced the period (Fig. 7D). An increase in \( \tilde{g}_{K1} \) provided more postspike hyperpolarization and reduced the spike rate, thus reducing the spike-mediated inhibition and the period. An increase in \( \tilde{g}_{K2} \) led primarily to lower plateau potentials, thus reducing the spike rate and the period.

At low values of either \( K^+ \) conductance the model entered G-mode oscillation. At low \( \tilde{g}_{K1} \) (below 40%), the fast \( Na^+ \) current, \( I_{Na} \), inactivated during the plateau phase since \( I_{K1} \) did not provide the postspike hyperpolarization required to deactivate \( I_{Na} \) after a spike. Thus, at the plateau transition the spike-mediated inhibition was effectively zero and a G-mode oscillation occurred as for \( \tilde{g}_{\text{Syn}} = 0 \). The oscillation was thus similar to the slow oscillation described in the previous paper (Nadim et al., 1995), with a burst of two to three spikes at the onset of the plateau.

The model also entered G-mode when \( \tilde{g}_{K2} \) was reduced below 30% of the canonical value. The oscillation was like the low \( \tilde{g}_{K1} \) oscillation but with a longer burst of spikes lasting approximately 600 ms at the onset of the plateau.

Sensitivity Analysis. It is instructive to compare each current's relative contribution to the period in the three modes of oscillation: S-mode, G-mode, and the slow oscillations (Fig. 9). The S-mode was represented by the canonical model, the G-mode was produced by setting \( \tilde{g}_{h} = 14 \) nS, twice its canonical value, and the slow oscillation was produced by reduced reversal potentials of the \( Na^+ \) currents and the \( h \)-current (\( E_{Na} = -12 \) mV and \( E_{h} = -46 \) mV) (Nadim et al., 1995). The value of \( \tilde{g}_{h} \) for the G-mode oscillation was higher than in Fig. 3 to ensure the parameter changes did not bring it back into S-mode.

In the canonical model, the period was most sensitive to \( \tilde{g}_{K1} \) and \( \tilde{g}_{\text{Syn}} \). The spike frequency was reduced by increasing \( \tilde{g}_{K1} \), thus reducing the spike-mediated synaptic inhibition and reducing the period. Similarly, decreasing \( \tilde{g}_{\text{Syn}} \) reduced the period.

Comparing the sensitivity of \( \tilde{g}_{h} \) across the three modes of oscillation, \( \tilde{g}_{h} \) most effectively modulated the period in S-mode. The escape mechanism due to \( I_{h} \), had a relatively small effect in G-mode and the slow oscillation, which are dominated by the graded synaptic inhibition. The sensitivity to the leak conductance, \( g_l \), was also highest in S-mode and reduced by more than 50% in G-mode and slow mode.

The sensitivity to an increase in \( \tilde{g}_{\text{Syn}} \) switched from decreasing the period in S-mode to increasing the period in G-mode and the slow oscillation. This switch occurred as the graded inhibition changed from only complementing the dominant \( I_{\text{Syn}} \) in S-mode to providing the main source of inhibition in G-mode.

The Slow \( Ca^{++} \) Current and the Period

It was difficult to dissect out the direct role of the slow \( Ca^{++} \) current on the period. Varying \( \tilde{g}_{\text{CAS}} \) alone not only changed \( I_{\text{CAS}} \) but also the graded synaptic conductance, \( g_{\text{Syn}} \). As \( \tilde{g}_{\text{CAS}} \) was increased, the period increased, but this could have been due to either a more depolarized plateau phase and therefore increased spike rate and increased spike-mediated inhibition, or it could have been due to stronger graded inhibition.

In an experiment to test the role of \( I_{\text{CAS}} \) on the plateau phase alone, we added another \( Ca^{++} \) current, \( I_{Ca^{++}} \), that had exactly the same dynamics as \( I_{\text{CAS}} \). However, it was not linked to the graded synapse. Altering \( \tilde{g}_{\text{CAS}} \), the total \( Ca^{++} \) current in the presynaptic cell was varied while the graded inhibition in the opposite cell, produced by \( I_{\text{CAS}} \) alone, was unchanged.

Keeping \( \tilde{g}_{\text{CAS}} \) at the canonical value while varying \( \tilde{g}_{\text{CAS}} \) between \(-2\) and \(+2\) nS maintained the synaptic inhibition as in the canonical model. Increasing the extra \( Ca^{++} \) conductance, \( \tilde{g}_{\text{CAS}} \), increased the period through a mechanism similar to that for \( I_{h} \) (Fig. 10).

This test was repeated after the oscillation was brought into G-mode by adding 1 nS of \( \tilde{g}_{\text{CAS}} \) to the
canonical model. The period increased somewhat as $g_{Ca}$ was increased, but the increase was not as steep as when increasing $g_{CaS}$ (Fig. 10). Thus in G-mode, both the effects of $g_{CaS}$ on the plateau phase and on the graded synapse amount to increasing the period.

An inhibited cell depolarized slowly toward the transition through activation of the $h$-current by hyperpolarization and a decrease in the spike-mediated inhibition. The decrease in $I_{syn}$ was associated with a fall in the presynaptic spike rate, from a peak spike frequency of 23 Hz at the onset of the plateau to 15 Hz at the transition in the canonical model, caused by inactivation of $I_{Cas}$. These two mechanisms have been generalized as escape and release, respectively (Wang and Rinzel, 1992). We have shown that escape is not necessary for oscillation in the canonical model (Fig. 8.)
although the period of the canonical model is sensitive to $\tilde{g}_h$ (Fig. 7B). In order to determine whether some release is necessary for oscillation in the canonical model, we had to remove the inactivation of $I_{\text{Ca}^{2+}}$ during the plateau without changing the graded synaptic current.

In Fig. 11, the HN(L) model cell went through a normal transition to a plateau. At the moment $g_{\text{Ca}^{2+}}$ reached its maximum value, we added an extra $\text{Ca}^{2+}$ conductance that started out at zero and slowly increased in a mirror image fashion as $g_{\text{Ca}^{2+}}$ decreased due to inactivation.

The extra $\text{Ca}^{2+}$ current, $I_{\text{Ca}^{2+}}$, had identical activation dynamics to $I_{\text{Ca}^{2+}}$, but differed from $I_{\text{Ca}^{2+}}$ in that its inactivation variable, $h^*$, was $h^* = 1 - h$, where $h$ was the inactivation in $I_{\text{Ca}^{2+}}$, and in that it was not linked to the graded synapse. The current $I_{\text{Ca}^{2+}}$, represented the part of the normal $\text{Ca}^{2+}$ current, $I_{\text{Ca}^{2+}}$, that was inactivated at any given time. The total slow $\text{Ca}^{2+}$ current, $I_{\text{Ca}^{2+}} + I_{\text{Ca}^{2+}}$, had no net inactivation. At the peak of $g_{\text{Ca}^{2+}}$, the activation of $I_{\text{Ca}^{2+}}$ was $m = 0.99$ and the inactivation was $h = 0.48$, thus the peak slow $\text{Ca}^{2+}$ conductance was inactivated to half of the canonical maximal conductance, $\tilde{g}_{\text{Ca}^{2+}}$. Simply inserting $I_{\text{Ca}^{2+}}$ would double the total slow $\text{Ca}^{2+}$ current. To remove this offset conductance we inserted a passive $\text{Ca}^{2+}$ conductance with a negative value that produced no the step in $\text{Ca}^{2+}$ conductance as the two extra currents were introduced. The net conductance of these two extra $\text{Ca}^{2+}$ conductances increased at the same rate as the original $I_{\text{Ca}^{2+}}$ was inactivated, thus keeping the total $\text{Ca}^{2+}$ conductance in the presynaptic cell at the level of the peak $g_{\text{Ca}^{2+}}$ in the canonical model (Fig. 11). In practice, the passive $\text{Ca}^{2+}$ conductance had to be more negative than the inactivated part of $I_{\text{Ca}^{2+}}$ since $I_{\text{Ca}^{2+}}$ activated further beyond the peak in $g_{\text{Ca}^{2+}}$, even in the canonical model. The peak occurred when the rate of inactivation became larger than the rate of activation. For this study, however, it proved to be sufficient to keep the total $\text{Ca}^{2+}$ conductance at a level just below the peak $g_{\text{Ca}^{2+}}$.

In the resulting model, the presynaptic cell fired at a constant rate of 23 Hz on the plateau. The $h$-current, $I_h$, in the inhibited cell activated slowly but was not able to depolarize the membrane potential to a point where the inward currents were activated sufficiently to become regenerative. In comparison, the spike rate in the canonical model immediately prior to the transition was 15 Hz. This result shows that some fall in the presynaptic spike rate was required for oscillation in the canonical model. The heart interneurons do not show appreciable spike frequency adaptation during their burst (Peterson, 1983). Moreover, as pointed out in the previous paper (Nadir et al., 1995), heart interneurons rarely, if ever, approach spike frequencies of 20 Hz; they usually fire at around 10 Hz. Thus it is unclear that the type of adaptation seen in the model applies to the oscillation in the heart interneurons.

**Interaction Between $E_I$ and $I_h$**

The leak reversal potential, $E_I$, proved to be an important parameter in establishing the canonical model (Nadir et al., 1995). Here, we investigated its interaction with the hyperpolarization activated inward current, $I_h$. Varying $E_I$ and $g_h$ simultaneously, the model displayed S-mode and G-mode oscillations as well as no oscillation.

G-mode occurred at $E_I$, 2 mV above the canonical value, and raising the value of $E_I$ required less $I_h$ to produce G-mode oscillation (Fig. 12A). Lowering $E_I$ below the canonical value had other effects than simply activating more $I_h$. When both $E_I$ and $g_h$ were
Fig. 11. A fall in spike frequency is required for oscillation in the canonical model. An extra current was added to compensate for the inactivation of the slow Ca$^{++}$ current on the plateau. The graded synaptic release depended only on the original Ca$^{++}$ current. See text for further details.

sufficiently reduced from the canonical value, the oscillation ceased.

In “pure” S-mode ($\bar{g}_{\text{Syn}} = 0$), the model can clearly not produce G-mode oscillation. At $\bar{g}_h$ above 4 nS and $E_l$ around $-50$ mV, such a model maintained its S-mode oscillation in a region where the canonical model entered G-mode (Figs. 12A and B). The spike-mediated synaptic transmission alone was capable of producing oscillation in that region, but the graded transmission in the canonical model provided extra inhibition that activated more $h$-current, and consequently reduced the inactivation of $I_{\text{Cas}}$ and G-mode ensued. Just below the island of no oscillation in Fig. 12A (at $E_l$ around $-47.5$ mV and at low $\bar{g}_h$), the graded transmission allowed the canonical model to maintain S-mode oscillation where the
Fig. 12. The modes of oscillation in the ($\bar{g}_h, E_l$) plane. A: Parameter variation of the canonical model resulted in S-mode and G-mode oscillation, and no oscillation. B: "Pure" S-mode has no graded inhibition ($\bar{g}_h g_M G = 0$) and could therefore not produce G-mode oscillation. The region of S-mode oscillation extended into the G-mode region of the canonical model at high $\bar{g}_h$. At low $\bar{g}_h$ and $E_l$ around $-48$ mV, the graded inhibition in the canonical model enabled it to oscillate in S-mode where the "pure" S-mode model stopped oscillating. C: The modes of a single cell. A single cell fires tonically above $E_l = -56.75$ mV and becomes an endogenous burster at low $E_l$ and high $\bar{g}_h$. (The lines separating the various modes have errors of 0.25 mV for $E_l$ and 0.2 nS for $\bar{g}_h$. The asterisks label the canonical parameters).

model with only spike-mediated synaptic transmission did not oscillate.

An isolated model cell fired tonically in the region of the canonical values (Fig. 12C). In the region above $E_l = -56.75$ mV, which included the canonical $E_l$, the leak current helped to depolarize the cell (by activating $I_{lp}$) above the threshold for action potentials.

The two-cell canonical oscillator settled into three states of no oscillation in the area of the ($\bar{g}_h, E_l$) plane investigated here (Fig. 12A). At the island of
no oscillation, the cells produced irregular firing patterns where both cells were active without phase locking. The area of no oscillation at low $E_l$ and $g_h$, the canonical model displayed two behaviors: above $E_l = -56.75 \text{ mV}$, the model assumed a bistable state where one cell fired and inhibited the other through spike-mediated IPSPs, and below $E_l = -56.75 \text{ mV}$, both cells went silent. This behavior is reminiscent of the disrupted activity observed when $I_h$ is blocked in external $\text{Cs}^+$ in heart interneurons (Angstadt and Calabrese 1989) and suggests that $E_l$ may be more negative in the interneurons than in the canonical model neurons.

Below $E_l = -56.75 \text{ mV}$, the single cell either went silent or started bursting. The bursting occurred as the plateau terminated predominantly through inactivation of $I_Ca_{\text{S}}$ and the leak current hyperpolarized the model cell sufficiently to activate $I_h$ strongly. The $h$-current then depolarized the model cell to threshold for the next plateau. At such low $E_l$, the leak current was outward even during the hyperpolarized phase and counteracted $I_h$. At low $g_h$, the $h$-current was not strong enough to counteract $I_l$ and bring the model cell to threshold for a plateau. To produce bursting as $E_l$ was lowered, more $g_h$ was required to produce a net inward current that could initiate the plateau transition. The boundary between silence and bursting therefore sloped toward higher $g_h$ as $E_l$ was lowered (Fig. 12C).

The area of the parameter space where the single cell became a burster was included in the area of S-mode oscillations in Fig. 12A. In this region, the spike-mediated synaptic transmission was sufficient to phase lock the two model single-cell oscillators in antiphase.

The period of the oscillation was much more sensitive to $g_h$ and $E_l$ in S-mode than in G-mode (Fig. 13). The two modes were separated by a sharp change in the slope of the period versus parameters. In both modes the period generally increased as $E_l$ was lowered and $g_h$ was reduced.

**Discussion**

In the parameter sweeps, we focused on changing the maximal conductance of one current at a time. This procedure changes the relative strength of a single current with respect to the other currents and revealed the effect of that current on the nature of the model oscillation. In particular, it showed how effective the current was in determining the period of the oscillation. As the conductances were increased from the canonical values, only some currents significantly changed the
period: the two K+ currents, \( I_{K1} \) and \( I_{K2} \), the h-current, \( I_h \), and the leak current, \( I_l \), accelerated the oscillation; the persistent Na+ current, \( I_p \), and the spike-mediated synaptic transmission, \( I_{Syn} \), slowed the oscillation down. The other currents appear to be less effective in changing the period.

One can understand how the currents control the period in the model by considering the point of transition from the inhibited phase to the plateau phase. As the membrane potential approaches the transition, the h-current slowly activates and eventually cancels the spike-mediated synaptic inhibition. The build up of \( I_h \) is complemented by a fall in the spike rate and the spike-mediated inhibition due to inactivation of the slow Ca\(^{2+}\) current. At the point of transition, the membrane potential has reached a level where the inward currents \( I_p \), \( I_{Ca} \), and \( I_{Ca} \) become regenerative. The net inward current depolarizes the cell that activates inward currents more. This regenerative process stops when the inward currents are fully activated. The net depolarization at the onset of the plateau is approximately 10 mV. During the plateau the inactivation of \( I_{Ca} \) causes a slow decrease of the plateau and a drop in the spike rate.

Increasing the spike-mediated synaptic inhibition requires more h-current to depolarize the membrane potential to the point of transition. The activation of \( I_h \) is slow, so the transition is delayed with respect to the canonical model and the oscillation is slowed down. Likewise, increasing \( g_h \) produces an earlier transition and an acceleration of the oscillation.

Three currents, \( I_{K1} \), \( I_{K2} \), and \( I_p \), all appear to control the period mainly through modifying the spike rate. Higher spike rates increase the spike-mediated synaptic inhibition and prolong the period.

The leak current constrains the membrane potential excursions both during the plateau phase and during the hyperpolarized phase. The reversal potential of \( I_l \) is such that the leak current is inward during the hyperpolarized phase and outward during the plateau. Increasing \( g_l \) reduces the plateau potential and the IPSPs and accelerates the oscillation in both phases. We were able to dissect out the effects of \( I_l \) on the two phases. By raising \( E_l \) to a more positive potential, the driving force of \( I_l \) was reduced during the plateau while the driving force during the hyperpolarized phase was increased. If the effect on the plateau was greater than that on the hyperpolarized phase, we should have seen an increase in the period as \( I_l \) would provide less outward current to help terminate the plateau. However, the period decreased as \( E_l \) was raised (Fig. 13A), thus suggesting that \( I_l \) is more effective in pulling the hyperpolarized cell up from inhibition than in terminating the plateau.

Increasing the graded synaptic conductance (\( g_{Syn} \)) leads to a slightly faster oscillation. Our results suggest that the stronger graded inhibition of the opposite cell by the plateau transition in the "escaping" cell, accelerated the deactivation of the currents underlying the waxing plateau of that cell, the attainment of the hyperpolarized phase, and thus the activation of \( I_h \). The delay intermitting the plateau when graded transmission is weak or absent can be seen in the trace of Fig. 1 (compare, for example, the canonical trace with that where \( g_{Syn} = 0 \)) and this delay leads in turn to a delayed activation of \( I_h \) and a longer period. Increasing \( g_{Ca} \) and small increases in \( g_{Ca} \) has the same effect as increasing \( g_{Syn} \) in that they increase the graded transmission and more rapidly terminate the plateau of the opposite cell and activate its \( I_h \).

While the graded transmission speeds the oscillation up, the spike-mediated transmission slows the oscillation down. These results indicate that synaptic inhibition in a pair of reciprocally inhibitory cells has two functions. At the onset of synaptic inhibition, the inhibition deactivates the plateau currents in the bursting cell, terminates the plateau, and initiates the hyperpolarized phase. In our model, this early inhibition is generated by both the graded and the spike-mediated synapse. Later on during the hyperpolarized phase, the synaptic inhibition enters a maintenance phase where its function is to oppose building inward currents and thus delay the transition to the plateau phase. This inhibition is represented by the spike-mediated transmission in our model. Increasing the early inhibition decreases the period whereas increasing the late inhibition increases the period.

Marder (1991) generalized action potentials and plateau potentials as regenerative potentials. Action potentials and plateau potentials both involve a process where a depolarization of the membrane potential produces the activation of an inward current. The inward current depolarizes the membrane more, which activates more of the inward current. The depolarization of the membrane potential is thus a regenerative process. Action potentials and plateau potentials differ only in the voltage range and time scale over which they operate on. Note that in this model, both \( I_{K1} \) and the synaptic inhibition provide repolarization after a regenerative potential, that is, an action potential and a plateau potential, respectively.

Oscillations in reciprocally inhibitory model neurons have been observed to result from two basic
mechanisms, escape and release (Wang and Rinzel, 1992). Each of these two mechanisms have subsequently been subclassed into intrinsic and synaptic types (Skinner et al., 1994). When the synaptic transfer function, as a function of voltage, has a clear threshold, the synaptic escape and synaptic release modes can be identified by changing the synaptic threshold (Skinner et al., 1994). The synaptic escape mode was identified as the region where the period increased as the synaptic threshold was raised, and the synaptic release mode was characterized by a decrease in the period as the threshold was raised. Intrinsically escape occurred in neurons that were endogenous bursters, and intrinsic release occurred when the plateaus self-terminated. In our model there is no single parameter that corresponds to a synaptic threshold. The maximal conductance of the slow calcium current, $g_{CaS}$, is to some extent analogous to a threshold of graded synaptic transmission, where increasing $g_{CaS}$ corresponds to lowering the synaptic threshold. The period both increases and decreases as $g_{CaS}$ is varied around the canonical value, but since the graded synaptic inhibition is present only during the early inhibited phase in S-mode, these regions correspond to neither synaptic escape nor synaptic release. Skinner et al., (1994) did not include spike-mediated inhibition in their analysis. In the canonical model of the HN cells the spike-mediated inhibition dominates the inhibition and is controlled by the presynaptic spike frequency. Both a release mechanism and an escape mechanism exist in the canonical model. The release is caused by a fall in the spike frequency due to inactivation of $g_{CaS}$. The escape mechanism is produced by the activation of the $h$-current. Skinner et al., (1994) realized that it was difficult to determine the oscillation mode when the synaptic transfer function was smooth and did not have a well-defined threshold. In our canonical model, where the spike frequency of a single model cell increases smoothly with depolarization, threshold is likewise ill-defined and the synaptic transfer smooth.

The escape and the release mechanisms were selectively removed from our model. When removing the escape mechanism ($g_{h} = 0$), the release caused by inactivation of the slow Ca$^{2+}$ current was sufficient to sustain oscillation (Fig. 8). The period increased and the spike frequency at the plateau transition dropped to 6 Hz. When removing the release mechanism, that is, the inactivation of $I_{Na}$, the $h$-current with canonical $g_{h}$ was incapable of depolarizing the inhibited cell to a point where the inward currents became regenerative (Fig. 11). In that test, the depolarized cell fired at a constant frequency of 23 Hz. The spike frequency in the canonical model starts at 23 Hz but declines to 15 Hz at the transition point, well above the frequency at which release occurs when $g_{h} = 0$ (6 Hz). These results show that there is release in the form of spike adaptation in the canonical model, and that the $h$-current enables the inhibited cell to generate plateaus earlier and at higher presynaptic spike frequencies than in the absence of $I_{Na}$. Thus, the model produces an oscillation that has elements of both escape and release.

The heart interneurons oscillate with a period of approximately 8 to 10 s under normal experimental conditions. The period can be modulated by several means. The oscillation accelerated with temperature from a period of 60 s at 4°C to nearly 4.5 s at 26°C (Arbas and Calabrese, 1984). When partially dissected preparations exhibited body movements, like beading, shortening, and swimming, the rate of the heartbeat rhythm was observed to increase. In these cases, modulation of the period seems to be centrally mediated. Increasing the spike rate in mechanosensory cells, serotonergic Retzius cells, and Leydig cells all accelerate the heartbeat oscillator (Arbas and Calabrese, 1984, 1990). Bath application of the endogenous peptide FMRFamide has been shown to speed up the oscillation of the HN cells (Evans et al., 1991; Simon et al., 1992). The known physiological effects of FMRFamide include a reduction of the amplitude and an increase in the duration of the spike-mediated IPSPs (Simon et al., 1994) and shifts of steady state activation and inactivation of K$^{+}$ currents to more negative potentials (Simon et al., 1992).

In the model, four currents appear to dominate the control of the period: the $h$-current, the K$^{+}$ currents, the spike-mediated transmission, and the leak current. The period is sensitive to $g_{h}$, but there is no physiological evidence that the $h$-current is modulated by FMRF-amide in the heart interneurons (Angstadt and Calabrese, 1989). Both the K$^{+}$ currents and the spike-mediated transmission are known to change physiologically in the heart interneurons in the presence of FMRF-amide and may thus modulate the period in the heart interneurons. The fourth candidate for modulating the period in the model is the leak current. The period of the model is sensitive to both the maximal conductance and the reversal potential, but it is unknown whether the leak current is modulated in the heart interneurons.

The work reported here has raised questions that we will attempt to answer with new experiments and further modeling. We are currently doing experiments to
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