Detailed Model of Intersegmental Coordination in the Timing Network of the Leech Heartbeat Central Pattern Generator

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INTRODUCTION

The generation of many rhythmic movements appears to involve the coordination of distributed neural oscillators within the nervous system. For example, the motor patterns that underlie wave-like behaviors, such as undulatory swimming in leech and in lamprey or the beating of crayfish swimmerets, are generated by neuronal networks that can be approximated as chains of coupled segmental oscillators (Friesen and Pearce 1993; Grillner et al. 1993; Sigvardt 1993; Skinner and Mulloney 1998a,b). Each segmental oscillator consists of a local network of neurons that is capable of generating rudimentary rhythmic output (Murchison et al. 1993; Sigvardt 1993). The coordinated output of the entire chain of oscillators often shows phase relationships that are appropriate for the pattern of muscle activation in the intact, behaving animal (e.g., forward swimming) (Wallén and Williams 1984). The appropriate phase relationships between these segmental oscillators arise as an emergent property of the segmental oscillators and the coupling between them. Moreover, in many such systems, intersegmental phase is independent of period, which can vary widely (Mulloney 1997; Mulloney et al. 1998; Wallén and Williams 1984). Although this distributed organization is found in many different animals, there are large differences in terms of the properties of the segmental oscillators, the strength and symmetry of coupling, and the importance of sensory feedback (Hill et al. 2003). Intersegmental coordination results primarily from ascending and descending synaptic connections between the segmental oscillators, although sensory feedback also reinforces and fine tunes the intersegmental phase relationships (Di Prisco et al. 1990; Cang and Friesen 2000).

Narrowly from its beginnings, experimental progress in this field had been inextricably linked with modeling efforts (Cohen et al. 1982; Kopell and Ermentrout 1988). Most models produced to date, however, have been either quite abstract or based on incomplete data about the intrinsic membrane properties or intersegmental connectivity of the network being studied (Bem et al. 2003; Buchanan 1992; Cang and Friesen 2002; Ekeberg and Grillner 1999; Jones et al. 2003; Skinner et al. 1997, 1998b; Williams et al. 1990). These models have nevertheless been very instructive and have constrained the range of mechanisms that can account for phase and period control. Two main hypotheses have been proposed to explain the generation of appropriate phase differences between segmental oscillators. The “asymmetric coupling hypothesis” states that phase differences are generated by asymmetries in the coupling between segmental oscillators (Skinner et al. 1997; Williams et al. 1990). For example, ascending and descending coordinating interneurons may differ in terms of the distances that their axons project, the strength and sign of their synapses, and their postsynaptic targets (Jones et al. 2003; Skinner and Mulloney 1998a,b). In contrast, the “excitability gradient hypothesis” states that phase differences arise from differences in the oscillation periods of the segmental oscillators (Grillner et al. 1993; Ikeda and Wiersma 1964; Matsushima and Grillner 1990, 1992). This difference may be based on either the inherent periods of the segmental oscillators or a gradient of excitation along the nerve or spinal cord (Tunstall and Sillar 1993). A more modern view combines these two hypotheses and recognizes that both neuronal intrinsic membrane properties (excitability) and intersegmental connectivity combine to produce proper phasing between segments (Friesen and Cang 2001; Friesen and Pearce 1993; Ullström et al. 1998).

We have used the leech heartbeat central pattern generator to...
address the problem of intersegmental coordination of oscillatory networks. The timing network of the pattern generator consists of two segmental oscillators, each of which is located in a separate ganglion and is capable of continuous independent oscillation (Fig. 1A). Each oscillator comprises just two mutually inhibitory interneurons that are well characterized in terms of their intrinsic membrane properties and their segmental and intersegmental synaptic interactions (Calabrese et al.).

**FIG. 1.** The timing network of the leech heartbeat central pattern generator: circuit diagram, activity, and the simple symmetric model. A: the timing network contains 4 pairs of bilaterally symmetric interneurons that have cell bodies in the first 4 midbody ganglia. There are 2 segmental oscillators located in the 3rd and 4th ganglia. The coordinating interneurons of the first 2 ganglia are functionally equivalent and are, therefore, combined in representation. Open circles represent cell bodies, open squares represent sites of spike initiation, and small filled circles represent inhibitory synapses. B: the electrical activity of 3 heart interneurons recorded extracellularly from a chain of ganglia (head brain to G4). The heart interneurons are labeled HN and are indexed by body side and midbody ganglion number [e.g., HN(L,3)]. Phase (Φ) of an interneuron X with respect to the G4 oscillator interneuron was calculated, on a cycle by cycle basis, as the difference in the median spike times (ΔtX-4) divided by the G4 cycle period (T4), and then multiplied by 100. A positive phase value indicates that the G4 oscillator leads in phase. C: the timing network can be conceptualized as a simple symmetric network made up of 2 segmental oscillators. D: simulated activity of heart interneurons in the simple symmetric model. The model interneurons are labeled using the same convention as for the living interneurons. The model neurons contain Hodgkin-Huxley style voltage-dependent conductances. The maximal conductance of the hyperpolarization-activated current (g_h) was 5.4 nS in the pair of G4 oscillator interneurons and 4.0 nS in the pair of G3 oscillator interneurons. Symbols above each voltage trace indicate the occurrence of the median spike within each burst. The yellow rectangles show the windows of time in which the coordinating interneurons were active. E: quantification of mutual entrainment in the simple symmetric model. The period of the mutually entrained system (T_C; square symbols) is equal to that of the faster segmental oscillator. When g_h was reduced below the canonical value (4 ns), the period of the G4 segmental oscillator (T4S) was greater than that of the G3 segmental oscillator (T3S). In this range, the period of the coupled system was equal to that of the G3 segmental oscillator. When g_h was increased above the canonical value, the period of the G4 segmental oscillator was less than that of the G3 segmental oscillator. In this range, the period of the coupled system (T_C) closely followed the period of the G4 segmental oscillator. Regardless of which segmental oscillator was faster, the system could only be sped to the half-center oscillator period (T4H or T3H) of the slower oscillator. Figure adapted from Hill et al. (2002).
1995). Detailed models are available for the oscillator interneurons and their interactions with intersegmental coordinating interneurons (Hill et al., 2001; Nadim et al., 1995; Olsen et al., 1995). The simplicity of a neuronal network containing only two oscillators that reside in separate segmental ganglia has made possible the experimental analysis of the oscillators as discrete units that can be uncoupled and then re-coupled (Masino and Calabrese, 2002a–c).

In this paper, we use simulations to explore how phase differences between segmental oscillators and cycle period are determined in the timing network of the leech heartbeat central pattern generator. This work builds on a simple model of the timing network that ignored some details of the firing pattern of the coordinating neurons and their synaptic interactions with the oscillator interneurons (Hill et al., 2002). This earlier model showed behavior that largely matched the behavior of the living system under conditions of mutual entrainment in which the two segmental oscillators feedback on to one another (Masino and Calabrese, 2002a,b). However, this model did not match the living system’s response during driving experiments in which current pulses were used to force one segmental oscillator to oscillate at periods faster and slower than the free-run period (Masino and Calabrese, 2002c). Here we explore the behavior of a model that more realistically represents the coordinating interneurons as multicompartmental cables, which have properties such as spike adaptation and multiple spike initiation sites and receive asymmetric input from the oscillator interneurons of the two segmental oscillators (Masino and Calabrese, 2002a; Peterson, 1983a,b). We show here that these details of the circuit are necessary to capture the properties of the system observed during driving experiments. Our results suggest that a detailed knowledge of both firing patterns and intersegmental connectivity are necessary for understanding how the heartbeat timing network functions. However, we have also found simple mechanisms for phase- and period control that may be easily generalized to other networks with different intrinsic membrane properties and intersegmental connectivity.

Parts of these results have appeared in abstract form (Jezzini et al., 2000).

Methods

Modeling methods

Each oscillator heart interneuron (a neuron originating in the 3rd or 4th ganglion) was represented as a single isopotential compartment with intrinsic and synaptic currents. The coordinating heart interneurons (neurons originating in the 1st or 2nd ganglion) were represented with intrinsic and synaptic currents. The coordinating heart interneurons of the two segmental oscillators (Masino and Calabrese, 1991; Ivanov and Calabrese, 2000), and plastic spike-mediated synapses, which are dependent on the influx of presynaptic Ca2+ through high-threshold Ca2+ channels (Masino and Calabrese, 1991) and are modulated by slow changes in membrane potential (Ivanov and Calabrese, 2003; Nichols and Wallace, 1978a,b). There are also both graded and spike-mediated synapses between the model oscillator interneurons. The equations and parameters of these currents are described in Hill et al. (2001). A mutually inhibitory pair of oscillator interneurons produces alternating bursting activity and is referred to as a half-center oscillator.

Coordinating heart interneurons originate in the first and second ganglia and function to link the half-center oscillators to form the beat timing network. There are no known differences between coordinating interneurons of the first and second ganglia (G1 and G2) with respect to their connectivity and interaction with oscillator interneurons, so ipsilateral interneurons were modeled as a single intersegmental cable (fiber) for computational efficiency (Fig. 2C) and are referred to here as coordinating fibers.

Coordinating fibers [HN(CF)] were modeled as multicompartmental cylindrical cables of realistic dimension (200 × 2 μm) and passive properties (Fig. 2C, Appendix). Coordinating fibers were divided into 150 compartments. In general, each fiber had two spike initiation sites (2-site model) that were each capable of spontaneous activity, one in ganglion 3 (G3 site) and one in ganglion 4 (G4 site). The G3 and G4 initiation sites, each consisting of a group of five compartments, were separated by 50 conduction compartments representing the interganglion portion of the fiber. In some simulations (1-site model), the G3 initiation site was removed by converting its compartments into conduction compartments. Conduction compartments contained only three voltage-dependent currents: IKr, IK1, and IK2. Their maximal conductances were adjusted to produce a realistic action potential propagation delay, similar to that observed in the living system (Masino and Calabrese, 2002a), of ~25 ms between ganglionic initiation sites (Appendix). The ends of the fiber beyond each initiation site consisted of an additional 10 conduction compartments followed by 35 passive compartments (Fig. 2C). The end conduction compartments allowed action potentials to travel unabated through the initiation site, whereas the passive end compartments acted as a sink to absorb axial current and prevented reflection of action potentials. The passive voltage response to current injection at initiation sites was nearly the same as theoretically expected for an infinite cable (Perkel and Mulloney, 1978; Rall, 1977).

The morphology of the coordinating interneurons has precluded direct electrophysiological characterization of the intrinsic currents of the G3 and G4 initiation sites. The small neuritic processes comprising these sites are electrotonically distant from their cell bodies where microelectrode penetration is possible (Masino and Calabrese, 2001a). We assumed that the initiation sites of the model coordinating fibers contain the same currents as measured experimentally in oscillator interneurons (INa, IA, ICaL, ICaS, IK1, IK2, IK3, IK4); Activation parameters of the currents were not altered, but their maximal conductances were varied to tune the spiking activity of each site to the desired characteristics. During normal rhythmic activity in the timing network, the living coordinating interneurons show spike frequency adaptation; within each burst the frequency declines from ~7 to 2 Hz with an average of ~4 Hz (Masino and Calabrese, 2001a). We created two types of model initiation sites: nonadapting and adapting. Non-
adapting initiation sites were made of compartments containing only Na and K currents, and their firing frequency was adjusted by varying the leak reversal potential. These compartments were adjusted so that model coordinating fibers fired tonically with a mean spike frequency of ∼4 Hz when not inhibited. Nonadapting initiation sites were used in only a few model experiments that are noted in the text; otherwise all models contained coordinating fibers with adapting initiation site(s). Adapting initiation sites (i.e., showing spike frequency adaptation) were made of compartments containing the full compliment of active currents. Variable spike properties between different versions of these spike initiation sites were made using different maximal conductances for $I_{Na}$, $I_{CaF}$, $I_{CaS}$, $I_{K}$ (Appendix). All two-site models contained a canonical adapting G4 site that produced bursts in which spike frequency declines from ∼7 to 2 Hz with an average of ∼4 Hz. In two-site models, adapting G3 sites were given a lower intrinsic spike frequency than the G4 site, leading to dominance of the G4 site. Thus the model coordinating neurons were constrained to conform to the observed behaviors of the living neurons (Masino and Calabrese 2001a; Peterson 1983b). For two-site models, we defined a parameter $\Delta f$, the difference in the average frequency of the G4 site and the G3 site ($f_{G4} - f_{G3}$).

In two-site models, synaptic connections were made from G3 oscillator interneurons onto both ipsilateral G3 and G4 initiation sites (all 5 compartments at each site) of the coordinating fibers, whereas G4 oscillator interneurons made synapses only onto the ipsilateral G4 initiation site (all 5 compartments) of the coordinating fibers (Fig. 2C). In one-site models, synaptic connections from the G3 and G4 oscillator interneurons to the coordinating fibers were made only onto the active G4 site (all 5 compartments) of the ipsilateral coordinating fiber (Fig. 2C). In two-site models, synaptic connections were made from the central compartment of each initiation site of a coordinating fiber to the local, ipsilateral G3 and G4 oscillator interneurons. In one-site models, synaptic connections from the coordinating fiber G4 initiation sites to the ipsilateral G4 oscillator interneuron were made by the conduction compartment that corresponded to the previous central compartment of the defunct G3 initiation site. Because one model coordinating fiber represented activity of two coordinating interneurons, each action potential in the single fiber was made to produce two inhibitory postsynaptic potentials (IPSPs) in each of its postsynaptic targets (ipsilateral oscillator interneurons) with the second IPSP occurring with a delay of 135 ms. This delay, which was picked arbitrarily, reduced the synchronous occurrence of two coordinating fiber-mediated IPSPs in an oscillator neuron.

Simulations were done with Genesis, software for Hodgkin-and-Huxley-style models (Bower and Beeman 1998; Hodgkin and Huxley 1952). The exponential Euler integration method was used with a time step of 0.1 ms. When a parameter or experimental perturbation was varied in a series of trials, each simulation in the series began from the same initial conditions and was iterated for 100 s of simulation time before collecting data for an additional 300 s of simulation time for analysis. This procedure allowed the model system to settle down from the perturbing effects of the parameter change.

Physiological methods

Physiological methods were as described by Masino and Calabrese (2001a). Data were digitized using a digitizing board (Digi-Data 1200 Series Interface, Axon Instruments, Foster City, CA) and acquired using pCLAMP software (Axon Instruments) on a personal computer (PC).

Data analysis

A spike train analysis program, written in Matlab (Mathworks, Natick, MA), was used to analyze spike train data from simulations and experiments on a PC. In the subsequent description, we do not differentiate between data from heart interneurons and model heart interneurons. In the analysis program, spikes were detected with a discrimination window. When voltage crossed a threshold value, a spike event was detected. An upper threshold eliminated transient artifacts in the recording. To prevent multiple detection of the same spike, a refractory period (20 ms), during which spikes could not be recognized, was applied after each detected event. Spikes were then grouped into bursts as follows. After an interburst interval (1 s) elapsed without any spikes detected, the next spike event was identified as the first spike of a burst. Subsequent spikes with interspike intervals less than the interburst interval were grouped into that burst. The median spike in each burst was identified by a symbol above the burst.

The analysis program was also used to determine cycle period ($T_X$), duty cycle ($D_X$), and phase ($\phi_{X-4}$) for each interneuron ($X$). The cycle period ($T_X$) was determined for each cell ($X$) by measuring the interval from median spike to median spike of consecutive bursts. The duty cycle ($D_X$) was defined as the fraction of the cycle period occupied by the burst duration ($T_{burst}$, $X$) and expressed as a percentage: $D_X = (T_{burst}, X / T_X) \times 100$. The phase of a given heart interneuron was determined cycle-by-cycle as a percentage based on the formula: $\phi_{X-4} = (T_{burst}, X / T_X - \Delta t_{X-4}) \times 100$, where $\Delta t_{X-4}$ is the difference between the time of the median spike ($t_{4}$) of cell $X$ and the median spike ($t_{4}$) of the phase reference cell (usually the ipsilateral G4 oscillator interneuron), and $T_{4}$ is the cycle period of the reference cell. A phase of 0% indicated a cell with no phase difference relative to the reference cell, whereas a 50% phase difference indicated an anti-phasic relationship. A positive phase difference indicated a phase lag with respect to the reference cell. Stable entrainment was indicated, in both mutual entrainment experiments and driving experiments, if all interneurons in the coupled system were entrained to the same period with a coefficient of variation of ≤5%.

In all graphs values are expressed as means ± SD ($n \geq 12$ consecutive bursts). Likewise numerical values given in the text are in some cases reported as means ± SD.

RESULTS

Modeling strategy

The simulations reported here emulate two types of experiments that were aimed at analyzing the control of period and phase in the heartbeat timing network: mutual entrainment and driving experiments. In the first series of experiments (mutual entrainment experiments), axonal conduction was reversibly blocked with a sucrose solution flowing across the connective between the third and fourth (G3 and G4) segmental ganglia (Masino and Calabrese 2002b). This arrangement allowed us to measure independently the periods of the two segmental oscillators, subnetworks of the heartbeat timing network located in individual segmental ganglia. Because the ganglia were in separate bathing chambers they could be individually treated with agents that either increased or decreased the period of each segmental oscillator. Subsequent to attaining the desired period difference between the independent segmental oscillators, the conduction block was relieved, and the effect of the measured period difference on the period and phase of the complete timing network was assessed. In driving experiments, a single oscillator interneuron was driven with rhythmic current pulses of varying period (50% duty cycle), and entrainment and phase relationships in the timing network were assessed by recording from other oscillator interneurons and in some cases from coordinating interneurons (Masino and Calabrese 2002c). The models that we present here build on our previous model of intersegmental coordination in the timing network.
network in which the coordinating interneurons were modeled as single compartments (Fig. 1C) (Hill et al. 2002).

Model half-center oscillator

In our models, rhythmic bursting arises from two mutually inhibitory oscillator interneurons, which form a half-center oscillator (H). These model oscillator interneurons are not inherently bursting but rather fire tonically in isolation. Recent experiments using extracellular recordings show that the oscillator interneurons burst endogenously when synaptic inhibition is blocked with bicuculline (Cymbalyuk et al. 2002). However, our representation of the system as a half-center oscillator is appropriate because the reciprocal inhibition is strong and controls the period and duty cycle of the oscillator interneurons. These core half-center oscillators cannot be observed experimentally with current methods because even in an isolated ganglion the processes of the coordinating interneurons remain active and functional (Fig. 1A). Nevertheless, it is instructive to assess the characteristics of this oscillatory module in our simulations. To observe the model half-centers in isolation, we simply silenced the activity of the coordinating fibers by removing them from the models. To modify the period of the half-center oscillator, the maximal conductance ($g_h$) of $I_h$ in both oscillator neurons was altered from its canonical value, whereas all other parameters were held constant at canonical values. Period is inversely proportional to ($g_h$) (Hill et al. 2001) (Fig. 1E).

Forming a model-8-cell timing network by coupling segmental oscillators

The smallest subnetwork that can be assessed experimentally is that in an isolated third or fourth ganglion, a segmental oscillator (S). This subnetwork consists of a mutually inhibitory pair of oscillator interneurons (half-center oscillator) and the local axonal and neuritic processes of the coordinating interneurons and their associated synaptic connections (Fig. 1A) (Masino and Calabrese 2002a). Even after isolation from their cell bodies, the coordinating interneurons continue to function and interact synthetically with the oscillator interneurons (Peterson 1983a).

The 8-cell model timing network was formed from two half-center oscillators (G3 and G4) and two coordinating fibers (1- or 2-site) and all their associated synaptic connections (Fig. 1); essentially we coupled two model segmental oscillators. In our model, a single coordinating fiber represents two coordinating interneurons on the same side of the body (see METHODS). Two different configurations of the timing network were modeled (Fig. 2C). One model contains coordinating fibers with one spike initiation site in G4 (1-site model) and the other model contains coordinating fibers with spike initiation sites in both G3 and G4 (2-site model). We refer to these 8-cell models of the timing network as the “coupled system” (C).

To assess the effects of period differences between the segmental oscillators on the phase and period of the coupled system, it was necessary to observe the segmental oscillators independently. In the one-site model, one segmental oscillator could be observed in isolation by simply removing the pair of oscillator interneurons in the other ganglion (Fig. 2C). Because of the functional symmetry inherent in such one-site models, only one segmental oscillator need be observed and manipulated (G4) and the other could be assumed to act identically. In the two-site model, an isolated G3 or G4 segmental oscillator could be observed by removing the other pair of oscillator interneurons and their associated pair of coordinating fiber initiation sites (Fig. 2C). Because each segmental oscillator in such two-site models is associated with a different pair of coordinating fiber initiation sites, each segmental oscillator (G3 and G4) had to be independently manipulated and assessed. To create period differences between the segmental oscillators, $g_h$ was altered in one pair of oscillator interneurons as described in the preceding text for the half-center oscillators.

Simulated experiments

Mutual entrainment. Our experimental paradigm can be summarized as follows: create the appropriate segmental oscillators, alter the period of one of them (usually the G4 oscillator) by varying $g_h$ in both oscillator interneurons [An increase in $g_h$ leads to faster oscillations (Hill et al. 2001)], and couple the segmental oscillators and observe the period and phase relationships of the complete timing network. Mutual entrainment in the coupled system was indicated if all interneurons were entrained to the same period (see METHODS).

This paradigm is illustrated for our previous simple symmetric model in which coordinating interneurons were represented with single compartments (Fig. 1C) (Hill et al. 2002). When a period difference is created between the segmental oscillators by accelerating the G4 segmental oscillator ($g_h$ is increased above its canonical value of 4 nS), then the G4 oscillator leads in phase (Fig. 1D). In this simple model, the period of the coupled system is equal to the period of the faster segmental oscillator (Fig. 1E). When $g_h$ was reduced below the canonical value (4 nS), the period of the G4 segmental oscillator ($T_{4g}$) was greater than that of the G3 segmental oscillator ($T_{3g}$). This range, the period of the coupled system was equal to that of the G3 segmental oscillator. The slower oscillator was observed with $g_h$ increased above the canonical value, the period of the G4 segmental oscillator was less than that of the G3 segmental oscillator. In this range, the period of the coupled system ($T_{c}$) closely followed the period of the G4 segmental oscillator. Note that the range of mutual entrainment extends from the intersection of the G3 half-center oscillator period (horizontal small-dotted line, $T_{3h}$) and the $T_{4s}$ dashed line to the point where the G4 half-center oscillator period (dotted and dashed curve $T_{4h}$) intersects the horizontal $T_{3s}$ dotted line. The system works by removal of inhibition. Phase differences between the oscillators allow the leading oscillator interneurons to truncate the coordinating interneuron bursts and thus remove inhibition that would normally fall late in the inhibited phase of the oscillator interneurons of the slower oscillator (Fig. 1D). The slower oscillator can be accelerated in this way to, at the limit, its half-center oscillator period (Hill et al. 2002). The type of analysis embodied in Fig. 1E is used repeatedly in the modeling experiments on mutual entrainment.

Driving. Simulated driving experiments were performed on the 8-cell-coupled system. Pulses of inhibitory conductance with a duty cycle of 50% were used as external input to drive one segmental oscillator (either G3 or G4). Two square-waves, 180° out of phase with each other, each with an amplitude of 70 nS and a reversal potential that alternated between −55 and
–40 mV, were used to control the spiking activity of the two oscillator interneurons in a segmental oscillator. In this way, a pair of oscillator interneurons was driven to oscillate at a series of specified cycle periods different from the free run period of the coupled system. Successful driving of the entire coupled network was indicated by the same criteria as for mutual entrainment (see METHODS).

**Coordinating fiber model, HN(CF)**

We created a coordinating fiber model with two spike-initiation sites (G3 and G4) and tested its properties. In the living system, coordinating fibers do not burst endogenously (Cymbalyuk et al. 2002), but rather they rebound after receiving inhibitory input from oscillator interneurons with a tran-
sient burst of spikes that declines in frequency (Masino and Calabrese 2002a). Normally, ~75% of the spikes in coordinating neuron arise at the G4 site, and in most preparations, the inherent spike frequency of the G3 site is less than that at the G4 site. We aimed to model the rebound firing with spike frequency adaptation of the coordinating interneurons and the dominance of the G4 initiation site. We hypothesized that the dominance of the G4 site was due to its greater inherent spike frequency. To test this idea, we adjusted the G3 site to spike at a constant frequency of 2.0 Hz and the G4 site to spike at a constant frequency of 3.8 Hz when not inhibited (data not shown). Both sites were silenced with injected current and then released simultaneously. Spikes arose in the fiber at a constant 3.8 Hz, and all spikes could be shown by latency measurements to arise at the G4 site. The G4 site was then again silenced with injected current; now spikes arose at a constant 2.0 Hz and all spikes could be shown by latency measurements to have arisen at the G3 site. Even with a frequency difference of as little as 0.5 Hz, the faster initiation site suppressed the slower site within one to three spikes, and silencing the faster site with injected current allowed the suppressed site to resume firing after a period only slightly longer than its normal interspike interval.

We next added spike frequency adaptation and established canonical G3 and G4 initiation sites in our coordinating fiber model. See Appendix for properties of the canonical and modified G3 and G4 sites. The spike frequency within a burst of the canonical G4 site decreased from ~7 to 2 Hz with an average of 3.6 Hz when it was released from hyperpolarization. In all models reported here, the G4 site retained its canonical properties. The canonical G3 site also showed rebound and adaptation but had an average spike frequency in a burst of 1.9 Hz. Thus the difference in the average frequency of the two canonical sites was 1.7 Hz; we defined this difference as $\Delta f$. $\Delta f$ was varied in some model experiments to observe its effects on coupled system properties; in each case, this change in $\Delta f$ was accomplished by changing the average frequency of the G3 site (see Appendix). Figure 2A shows the firing pattern of these canonical sites after release from hyperpolarizing current injection in one-site coordinating fiber models containing each of the two canonical sites. In each case, the site was allowed to reach its steady-state firing frequency. Figure 2B shows the interaction between the two canonical sites in a two-site coordinating fiber model as the two sites are released from or silenced with hyperpolarizing current. The G4 site is dominant whenever it is not silenced with current, but the G3 site becomes active quickly after the G4 site is silenced with current (Fig. 2B, inset). Adaptation continues to build at each site whenever that site is not hyperpolarized by injected current (or by synaptic inhibition). Adaptation occurs as the result of the inactivation of $I_{Ca}$ (slowly inactivating low-threshold Ca current) after release from hyperpolarization and is independent of spike activity. Rebound at these sites occurs because hyperpolarization removes this inactivation and thus relieves adaptation.

Period of a segmental oscillator varies with coordinating fiber input

Coordinating fiber inhibition acts to slow the period of a segmental oscillator. A canonical segmental oscillator constructed by adding two canonical G4 One-site (mean spike frequency of 3.6 Hz) coordinating fibers to a canonical half-center oscillator had a period ($T_{3H}$) of 9.3 $\pm$ 0.1 s (Fig. 2D). As the mean spike frequency of the coordinating fiber was reduced from the canonical value to zero, the period became shorter, eventually ending at the period of the canonical half-center oscillator ($T_{4H} = 8.6 \pm 0.2$ s). A similar effect of coordinating fiber inhibition is seen if the period of the half-center oscillator is increased to 10.1 $\pm$ 0.2 s by reducing $g_h$ in both oscillator interneurons to 2 nS (Fig. 2D). Therefore the slowing effect of coordinating fiber inhibition is general and does not depend on the specific values such as $g_h$ in the model.

One-site model with spike frequency adaptation

A canonical coupled system with one-site coordinating fibers showing spike frequency adaptation, hence referred to as a one-site model, has the same functional symmetry as our previous simple symmetrical model (Hill et al. 2002). The G3 and G4 oscillator interneurons equally inhibit the sole G4 spike initiation site of each coordinating fiber (cf. Figs. 1C and 2C). Therefore the one-site model allows us to assess the role of spike frequency adaptation in the coupled system without any other substantive changes from our previous simple symmetrical model. The canonical one-site model had a period of 9.4 s, slightly longer that the period of the segmental oscillators ($T_{3H}$ and $T_{4H} = 9.3$ s) from which it was constructed (Fig. 3C). This increase in period was due to increased rebound firing of the coordinating fibers at the G4 site because of inhibition by both the G3 and G4 oscillator interneurons. There was no phase difference between the G3 and G4 oscillators ($\Phi_3 - \Phi_4 = 0$%); Fig. 3C). The firing of the coordinating neurons filled the inhibited phase of both (G3 and G4) ipsilateral oscillator interneurons, and the high-frequency portion of the coordinating neuron bursts fell early in the inhibited phases of these oscillator interneuron (data not shown). Our previous work with a simple symmetrical model of the timing network (Hill et al. 2002) showed that if coordinating neuron inhibition falls early in the inhibited phase of oscillator interneuron activity, it is ineffective, whereas late inhibition delays the oncoming burst phase and slows the period of the oscillator interneurons.

One-site model

MUTUAL ENTRAINMENT. Creating a period difference between the segmental oscillators leads to phase differences in the coupled system with the faster oscillator leading (Fig. 3A). We created a period difference by varying $g_h$ in the G4 oscillator interneurons. Because of the functional symmetry of the one-site model, equivalent results were obtained by varying $g_h$ in the G3 oscillator interneurons (data not shown). Phase has a near linear dependence on the period difference of the segmental oscillators and on the underlying period difference of the half-center oscillators (Fig. 3, B and C). The period of the coupled system follows the period of the faster segmental oscillator more closely than that of the slower segmental oscillator, but it is always slightly slower than the period of the faster segmental oscillator (Fig. 3D). The range of mutual entrainment lies between that of the unvaried G3 half-center oscillator period [$T_{3H}$: biggest G4 phase lead; highest value of HN(4) $g_h$] and the point where the period of the coupled system meets the period of the varied G4 half-center oscillator period.
When the G4 segmental oscillator is the faster oscillator, the change in period of the coupled system is similar to the change in period of the G4 segmental oscillator ($T_{4S}$), but when the G4 oscillator is the slower oscillator, the coupled system experiences little change in period ($T_{C}$).

As in the simple symmetric model, the faster segmental oscillator cannot accelerate the slower oscillator faster than the half-center oscillator period of the slower segmental oscillator.
However, unlike the simple symmetric model, in the one-site model, the slower oscillator clearly influences the period of the coupled system (cf. Figs. 1E and 3D). For example, when $\theta_3$ is varied in the G4 oscillator, the limits of mutual entrainment extend beyond the intersection between the half-center oscillator period ($T_{H3}$) of the slower oscillator and the period of the faster segmental oscillator (in Fig. 3D this equality occurs where $T_{H4} = T_{3S}$ and where $T_{H4} = T_{4S}$). The reason the slower oscillator can slow the faster oscillator is well illustrated in Fig. 3A. The slower G3 oscillator lags behind the faster G4 oscillator, delaying the onset of the coordinating fiber burst. Therefore the high-frequency portion of the coordinating neuron burst falls later in the inhibited phase of the leading G4 oscillator thus slowing that oscillator. A corresponding slowing mechanism is seen (data not shown) when the G3 oscillator leads. In contrast to the simple symmetric model, which only allowed entrainment to occur based on the acceleration of the slower oscillator by the faster oscillator, in the one-site Model, due to spike frequency adaptation in the coordinating fibers, the phase difference between the oscillators creates both accelerating and slowing effects that permit mutual entrainment.

To determine whether the period of the coupled system was mainly determined by the faster or slower segmental oscillator and to facilitate comparison with data from the living system, we replotted the model data in a different manner (Masino and Calabrese 2002b). For each trial, the faster and slower of the two segmental oscillators was determined, and their periods were plotted separately against the period of the coupled system. This plot confirms that although $T_C$ is longer than the period of the faster segmental oscillator, it is closer to the period of the faster segmental oscillator than the period of the slower segmental oscillator (Fig. 4C), demonstrating that the primary mechanism for coordination in the coupled system is acceleration of the slower oscillator.

As in the simple symmetric model, in the one-site model, the coordinating fibers fire in the window of time between the end of the burst of the trailing ipsilateral oscillator interneuron and the beginning of the burst of the leading ipsilateral oscillator interneuron (cf. Figs. 1D and 3A). Because the G4 and G3 oscillator interneurons equally inhibit the coordinating fibers, the coordinating fiber duty cycle decreases symmetrically as the absolute value of $\Phi_3 - \Phi_4$ increases (Fig. 4A), and the phase of the coordinating fiber bursts relative to the oscillator interneurons ($\Phi_{CF} - \Phi_4$ and $\Phi_{CF} - \Phi_3$) varies symmetrically with the phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$); the absolute values of slopes of the regression lines for these relationships are nearly equal; slopes 0.50 and $-0.48$, respectively (Fig. 4B). This symmetric relationship arises from the equal ability of the G3 and G4 oscillator interneurons to inhibit the activity of the coordinating fibers. This symmetry in the phasing of the coordinating fiber bursts is not observed in the living system, where the slopes of the change in $\Phi_{CF} - \Phi_4$ and $\Phi_{CF} - \Phi_3$ versus $\Phi_3 - \Phi_4$ are 0.7 and $-0.3$ (Masino and Calabrese 2002a), reflecting a dominance of the G3 oscillator.

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**FIG. 4.** Mutual entrainment in the one-site model. A: the coordinating fiber duty cycle decreases symmetrically as the absolute value of the phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$) increases. The duty cycle of the oscillator interneurons does not vary because of their half-center configuration. B: the phase of the coordinating fiber bursts relative to the oscillator interneurons ($\Phi_{CF} - \Phi_4$ and $\Phi_{CF} - \Phi_3$) varies symmetrically with the phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$); the slopes of the regression lines for these relationships are 0.50 and $-0.48$, respectively. C: the period of the coupled system ($T_C$) is closer to the faster segmental oscillator period ($T_S$) than to the slower segmental oscillator period, although $T_C$ is generally longer than the period of the faster segmental oscillator. Phase differences were created between the G3 and G4 oscillators by systematically varying $\theta_3$ in the G4 pair of model oscillator interneurons.
in controlling the phase of the coordinating fibers in the living system.

**Driving.** During driving experiments in the simple symmetrical model, the undriven segmental oscillator could not be entrained to periods greater than its segmental oscillator period because the only available mechanism for oscillator interaction was removal of coordinating fiber inhibition (Hill et al. 2002). In other words, in contrast to the living system (Masino and Calabrese 2002c), the driven segmental oscillator may never slow the system by lagging in phase. In the one-site model, which has the same functional symmetry as the simple symmetric model, the slowing mechanism based on spike frequency adaptation described in the preceding text permits the driven oscillator to lag in phase and the system to be driven slower than the segmental oscillator period of the undriven oscillator (Fig. 5C). When one segmental oscillator is driven slower than the period of the other, it shifts the high-frequency portion of coordinating fiber burst to very late in the inhibited phase of the leading undriven oscillator, effectively slowing the undriven oscillator (Fig. 5B). Driving to faster periods is accomplished by removal of late coordinating fiber inhibition (Fig. 5A). Driving produces entrainment over a broad, nearly symmetric range of periods (±10%) and a similarly broad range of phases (±20%). The phase difference between the oscillators (Φ3 − Φ4) is nearly linearly related to the period difference between the driven oscillator (TDriven) and the free run period of the coupled system (TUndriven, Fig. 5C).

The functional symmetry of the one-site model makes the G3 and G4 oscillators perform identically during driving (data not shown). This equivalence of the G3 and G4 oscillators is not observed in the living system, where during driving the G3 oscillator entrains the coupled system over a broader, more symmetric period range than the G4 oscillator (Masino and Calabrese 2002c). Therefore the addition of spike frequency adaptation in the coordinating fibers corrected one flaw in our previous simple symmetrical model (Hill et al. 2002) but did not capture the asymmetries observed when driving the living coupled system (Masino and Calabrese 2002c).

### Two-site model with spike frequency adaptation

In an attempt to capture the asymmetries observed in the heartbeat timing network, we implemented the two-site model with spike frequency adaptation, henceforth referred to as the two-site model (Fig. 2C). This model is functionally asymmetric because, as in the living system, the G3 oscillator interneurons inhibit both coordinating fiber initiation sites, whereas the G4 oscillator interneurons inhibit only the G4 site. For a canonical two-site model, we chose a difference in spike frequency between the G4 and G3 sites (Δf = 1.7 Hz) that reflects measurements made in the living system (Masino and Calabrese 2002a).

The canonical two-site model had a period (T_C = 9.4 s), approximately equal to the period of the G4 segmental oscillator (T_4S = 9.3 s), but longer than the period of the G3 segmental oscillator (T_3S = 8.9 s) (Figs. 7C and 8C). Even though the canonical two-site model the G3 and G4 half-center oscillators have identical periods, the corresponding segmental oscillators do not. The G3 segmental oscillator has a shorter period than the G4 segmental oscillator because the G3 initiation sites of the coordinating fibers have a lower average firing frequency than the G4 sites. In the coupled system, the dominance of the G4 sites of the coordinating fibers causes the G3 oscillator to slow near the period of the G4 oscillator. In this canonical coupled system, the G4 oscillator has a slight phase lead (3 ± 2%; data not shown). The phase lead is due to coordinating fiber spikes that arise at the G3 site just after the onset of the G4 burst. These spikes have no effect on the G4 burst, which has already begun, but slightly delay the imminent G3 burst. Spikes that arise at the G3 site and overlap with the G4 bursts are often observed in the living system (Masino and Calabrese 2002a).

#### Two-site model

**Mutual entrainment.** The asymmetries in the two-site model mean that the G3 and G4 oscillators are no longer functionally equivalent. Therefore to study the effects of changes in segmental oscillator period on phase relations and

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**FIG. 5.** Driving in the 1-site model. A: the G4 oscillator was driven faster than the period of the coupled system and in phase. B: the G4 oscillator was driven slower than the period of the coupled system and lagged in phase. C: the phase difference between the G3 and G4 oscillators (Φ3 − Φ4) varied with the percent change in period ([TDriven − TUndriven]/TUndriven) between the driven oscillator (TDriven) and the undriven coupled system (TUndriven). Electrodcs in the icon to the right indicate that the G4 oscillator that was driven by square-wave conductance changes. All model parameters were canonical.
period of the coupled systems, we varied \( g_h \) separately in the G4 (Fig. 6A and Fig. 7) and G3 (Fig. 6B and Fig. 8) oscillator interneurons. Like in the one-site model, the alterations of \( g_h \) in one of the half-center oscillators led to phase differences between the segmental oscillators in the coupled system (Fig. 6). Unlike in the one-site model, these phase differences did not lead to truncation of the coordinating fiber bursts; the coordinating fibers never fired during the ipsilateral G3 oscillator interneuron bursts but did fire at lower frequency during the G4 oscillator interneuron bursts whenever the ipsilateral G3 oscillator interneuron was silent. This phenomenon occurs because spike initiation switches to the G3 site whenever the G4 site is inhibited and the G3 oscillator interneurons are silent. These switches in spike initiation site can be seen as kinks and nonmonotonicity in the instantaneous spike frequency plots for the coordinating fibers (Fig. 6). Because the G3 sites fire at a lower frequency than the G4 sites, the net effect of the firing of a G4 oscillator interneuron is to reduce coordinating fiber activity; whereas in the one-site model, the effect of G4 oscillator interneuron firing is to silence coordinating activity completely.

The relationship of phase difference (\( \Phi_3 - \Phi_4 \)) to period difference between the G3 and G4 half-center oscillators (\( T_{3H} - T_{4H} \)) is similar whether \( g_h \) is varied in the G4 (Fig. 7A) or the

FIG. 6. Mutual entrainment in the two-site model. Phase differences were created between the G3 and G4 oscillators by adjusting \( g_h \) in 1 pair of model oscillator interneurons as indicated above each panel (the canonical value of \( g_h \) is 4 nS), to create period differences between the G3 and G4 segmental oscillators. When the G3 and G4 oscillators are equivalent, the G4 oscillator has a small phase lead. A: the G4 oscillator leads (\( g_h \) is increased above the canonical value) and terminates spiking activity in the G4 sites, but spikes begin to originate from the G3 sites. B: the G3 oscillator leads (\( g_h \) is increased above the canonical value) and releases the G3 sites before the end of the G4 oscillator interneuron bursts. The same change in \( g_h \) in each oscillator causes a phase difference of the markedly different absolute magnitude because the 2-site model is functionally asymmetric.

FIG. 7. Mutual entrainment in the two-site model: varying the period of the G4 segmental oscillator. A and B: the phase difference between the G3 and G4 oscillators (\( \Phi_3 - \Phi_4 \)) in the coupled system varies with the period difference between the G3 and G4 half-center oscillators (\( A \)). C: the period of the coupled system decreases slightly as the period of the G4 oscillator becomes shorter than its canonical value (high \( g_h \)). D: the change in period of the coupled system from the canonical period is close to the change in period of the faster segmental oscillator from its canonical period. Period differences were created between the G3 and G4 segmental and half-center oscillators by adjusting \( g_h \) in the pair of G4 oscillator interneurons as indicated above each panel (the canonical value of \( g_h \) is 4 nS).
G3 oscillator (Fig. 8A). Moreover this relationship is similar to that observed in the one-site model. Thus intrinsic differences in the membrane properties between the oscillator interneurons of the two ganglia still determine the phase difference between the segmental oscillators, and when there is no such intrinsic difference, there is no phase difference. The situation is more complex when one considers the relationship of phase difference, there is no phase difference. The period of the coupled system stays parallel to, but is always longer than, the period of the faster segmental oscillator (Figs. 7C and 8C). The curves are shifted to the left because when the half-center oscillators are identical, the G3 segmental oscillator will be faster because it receives lower frequency inhibition from the G3 site of the coordinating fibers than the G4 segmental oscillator receives from the G4 site of the coordinating fibers.

There are differences in the control of period between the one- and two-site models. In the two-site model, the period of the coupled system no longer appears to follow the period of the faster oscillator, especially when $g_{h}$ is varied in the G4 oscillator; the period is always at least slightly shorter than the period of the faster segmental oscillator (Figs. 7C and 8C). The same mechanisms for accelerating and slowing are available in the two-site model as in the one-site model, but for the G4 oscillator, the accelerating mechanism is reduced compared with the G3 oscillator because the G4 oscillator can reduce but cannot remove coordinating fiber inhibition from the G3 oscillator (cf. Figs. 3D and 7C), whereas the G3 oscillator can completely remove coordinating fiber inhibition from the G4 oscillator.

When $g_{h}$ is varied in the G4 oscillator, the limits of mutual entrainment lie between that of the (unvaried) G3 segmental oscillator period ($T_{3S}$; biggest G4 phase lead; highest value of HN(4) $g_{h}$, and extends well beyond (as $g_{h}$ is reduced) the point where the period of the coupled system meets the period of the varied G4 half-center oscillator period ($T_{4H}$; biggest G4 phase lag; lowest value of HN(4) $g_{h}$). When the G4 oscillator leads, it can never accelerate the coupled system beyond the G3 segmental oscillator period because it can only reduce coordinating fiber inhibition to the G3 oscillator to the level it would receive as an independent segmental oscillator receiving input from the G3 site of the coordinating fibers (Fig. 6A). When the G4 oscillator lags, mutual entrainment extends to periods shorter than that of the G4 half-center oscillator ($T_{4H}$) because a new accelerating mechanism is available to the G3 oscillator that is not seen in the one-site model during mutual entrainment. When the leading G3 oscillator interneuron stops firing, the G3 sites of the coordinating fiber are released from inhibition and provide inhibition at the end of the ongoing burst of the lagging G4 oscillator (Fig. 6B). This inhibition reduces the spike frequency of the ipsilateral G4 oscillator interneuron, allowing the contralateral oscillator interneuron to escape from inhibition and thus terminate the burst of the ipsilateral oscillator interneuron early. We call this acceleratory mechanism “early burst termination.” When $g_{h}$ in the G4 oscillator is decreased from the canonical value (4 nS), the period of the coupled system ($T_{C}$) stays parallel to, but is always longer than, the period of the of the G3 segmental oscillator ($T_{3S}$; Fig. 7C). The period, which is $\sim 9.5$ s, is greater than $T_{3S}$ because the leading G3 oscillator receives coordinating fiber inhibition from the high-frequency G4 site. When the data are plotted as a change in period, it is clear that there is little change in period of the coupled system ($\Delta T_{C}$) except when the G4 segmental oscillator is accelerated by increasing $g_{h}$ above its canonical value (Fig. 7D). Thus the G4 oscillator controls the period of the mutually entrained system only when it leads the G3 oscillator and at the limit it can only accelerate the G3 oscillator to $T_{3S}$. When the G4 oscillator is inherently slower than the G3 oscillator, it is very ineffective at slowing the coupled system because the G3 oscillator accelerates it through two
mechanisms, the removal of inhibition and early burst termination (Fig. 6B).

When $g_{3h}$ is varied in the G3 oscillator, the range of mutual entrainment of the G3 and G4 oscillators lies between a point considerably faster than the period of the unvaried G4 half-center oscillator period [$T_{3HB}$; biggest G3 phase lead; highest value of $HN(3) g_{3h}$] and extends well beyond [at slower coupled system periods ($T_C$)] the unvaried G4 segmental oscillator period ($T_{4S}$) [biggest G3 phase lag; lowest value of $HN(3) g_{3h}$]. As described earlier, when the G3 oscillator leads, it can accelerate the G4 oscillator beyond its (unvaried) half-center oscillator period ($T_{4H}$; Fig. 8C) by early burst termination (Fig. 6B). When the G3 oscillator lags, it moves the high-frequency inhibition from the coordinating fibers to late in the inhibited phase of the G4 oscillator activity cycle thus creating a strong delaying effect (Fig. 6A); therefore mutual entrainment can extend to periods slower than the period of the (unvaried) G4 segmental oscillator ($T_{4S}$; Fig. 8C). When $g_{3h}$ is varied in the G3 oscillator, the period of the coupled system ($T_C$) runs parallel to but is always longer than the period of the (varied) G3 segmental oscillator because of the increase in coordinating fiber inhibition, which results from the higher spike frequency of the G4 sites with respect to the G3 sites. Plotted as a change in period, it is clear that the period change of the coupled system ($\Delta T_C$) closely follows that of the G3 segmental oscillator ($\Delta T_{3S}$; Fig. 8D). Therefore the G3 oscillator exerts strong control over the period in the coupled system during mutual entrainment when it leads the G4 oscillator and also to a more limited extent when it lags (Figs. 8, C and D). Entrainment occurs over only a very limited range when the G3 segmental oscillator is slower than the G4 segmental oscillator. Because the G4 oscillator cannot inhibit the G3 sites of the coordinating fibers, the coupled system can go no faster than the G3 segmental oscillator period ($T_{3S}$), the period expressed with coordinating fiber inhibition from the lower frequency G3 sites. This condition differs considerably from that in the one-site model (Figs. 3, D and E), where the G4 oscillator could very effectively accelerate a slower G3 oscillator.

In the two-site model, unlike in the one-site model where duty cycle of the coordinating fibers is controlled by the phase difference between the G3 and G4 oscillators ($\Phi_3 - \Phi_4$; cf. Figs. 3A and 9A), the coordinating fiber bursts fill the time between ipsilateral G3 oscillator interneuron bursts (inhibited phase) so their duty cycle varied little with the phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$; Fig. 9A). The system acts in this way because only G3 oscillator interneurons can inhibit both coordinating fiber initiation sites. In the living system, the duty cycle of the coordinating fibers is likewise not related to the phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$) but is highly variable and is often smaller than in the two-site model (Masino and Calabrese 2001a). In contrast to the one-site model (Fig. 4B) and similar to the living system, the absolute values of the slopes of the regression lines of the phases between the coordinating fibers and the ipsilateral G3 and G4 oscillator interneurons ($\Phi_{CF} - \Phi_4$ and $\Phi_{CF} - \Phi_3$) were different; slopes 0.68 and $-0.30$, respectively, in the two-site model (Fig. 9B) and 0.7 and $-0.3$, respectively, in the living system (Masino and Calabrese 2002a). Because of the G3 oscillator’s ability to silence the coordinating fibers, the phase of the coordinating fiber bursts moves more closely hews to the G3 oscillator than to the G4 oscillator. For comparison, in a completely asymmetric model in which only the G3 oscillator inhibits the activity of the coordinating fibers and thus controls them completely, the phase $\Phi_{CF} - \Phi_3$ would remain constant ($m = 0$) and the phase $\Phi_{CF} - \Phi_4$ would change ($m = 1$) (Hill et al. 2001). In the living system, the corresponding slopes are 0.7 and $-0.3$, respectively (Masino and Calabrese 2001a), matching closely the values observed in the two-site model (Fig. 9B). The period of the coupled system ($T_C$) is generally closer to that of the faster segmental oscillator than the slower segmental oscillator (Fig. 10A). In the living system, the period of the coupled system is close to that of the faster segmental oscillator (Fig. 10B) (Masino and Calabrese 2001a).

**DRIVING.** As discussed in the preceding text for the one-site model, the addition of spike frequency adaptation to the coordinating fibers makes it possible for the driven oscillator to

**FIG. 9.** Mutual entrainment in the two-site model. A: the coordinating fiber duty cycle, like that of the oscillator interneurons, does not vary with phase difference between the G4 and G3 oscillators ($\Phi_3 - \Phi_4$). B: the phase of the coordinating fiber bursts relative to the oscillator interneurons ($\Phi_{CF} - \Phi_4$ and $\Phi_{CF} - \Phi_3$) varies asymmetrically with the phase difference between the G4 and G3 oscillators ($\Phi_4 - \Phi_3$); the slopes of the regression lines for these relationships are 0.68 and $-0.30$, respectively. Phase differences were created between the G3 and G4 oscillators by adjusting $g_{3h}$ in 1 pair of model oscillator interneurons while the other pair was held at its canonical value ($g_{3h} = 4\, \text{mS}$ is canonical).
entrain the coupled system to periods longer than the segmental oscillator period of the undriven oscillator (cf. simple symmetrical model) (Hill et al. 2001). The two-site model also includes spike frequency adaptation; therefore it should also have this ability. However, the asymmetries of the two-site model make it likely that the G3 and G4 oscillators would differ in their ability to entrain the coupled system in driving experiments. Therefore we independently tested the ability of the G4 (Fig. 11A, 1 and 2) and of the G3 (B, 1 and 2) oscillator to entrain the coupled system.

Both oscillators are able to drive the coupled systems to faster and slower periods. Driving produces entrainment over a more restricted range of faster and slower periods than in the one-site model (±10%), and the range is different for the two oscillators (driven G3 oscillator: −11 to +5%; driven G4 oscillator: −5 to +7%; Fig. 12B). Likewise, the phase range for entrainment observed is different for the two oscillators (driven G3 oscillator: −18 to +23%; driven G4 oscillator: −28 to +20%). The phase difference between the oscillators (Φs − Φu) is nearly linearly related to the period difference between the driven oscillator (T_{Drived}) and the undriven coupled system (T_{Undriven}; Fig. 12B). The phase difference between the oscillators creates the accelerating and slowing effects that permit entrainment during driving. The G4 oscillator is slightly better at entraining the coupled system to longer periods than is the G3 oscillator (+7 vs. +5%). When the G4 oscillator lags in phase, it shifts high-frequency coordinating fiber inhibition to late in the inhibited phase of the ipsilateral G3 oscillator interneuron. This inhibition is particularly effective because it falls immediately after the end of ongoing low frequency inhibition from the G3 site (Fig. 11A2). When the G3 oscillator is driven to slower periods, it too can disinhibit the higher spike frequency G4 site of the coordinating fiber late in the inhibited phase of the other (G4 in this case) oscillator but in the absence of ongoing inhibition G3 site (Fig. 11B2).

The functional asymmetry of the 2-site model makes the G3 and G4 oscillators perform differently during driving experiments (Fig. 12B). The G3 oscillator is substantially better than the G4 oscillator at entraining the coupled system to shorter periods (−11 vs. −5%; Fig. 12B). As in mutual entrainment, the G3 oscillator is capable of accelerating the G4 oscillator by two mechanisms: removal of inhibition and early burst termination (Fig. 11B1). The G4 oscillator is less effective at driving the coupled system to shorter periods than the G3 oscillator because it can only accelerate the G3 oscillator to its segmental oscillator period (Fig. 11A1). Similarly in driving experiments in the living system, the G4 oscillator is particularly weak at entraining the coupled system to periods faster than the free run period (Fig. 13C and D) (Masino and Calabrese 2002c). Therefore the addition of a second spike-initiation site to the coordinating fibers that is solely controlled by the G3 oscillator corrected a remaining flaw in the one-site model, the functional equivalence of the G3 and G4 oscillators in driving experiments (Fig. 5).

The effect of Δf. The analysis described in the preceding text was done with a canonical two-site model with an average spike frequency difference (Δf) between the G3 and G4 initiation sites of the coordinating fibers of 1.7 Hz. In the living system, the firing frequency of these spike initiation sites of the coordinating interneurons differs substantially among preparations. In some preparations, there is barely any difference in the average spike frequency of the two sites, whereas in others, the average spike frequency of the G4 sites is much higher (Masino and Calabrese 2002a). We reasoned that such variability should have an effect on the symmetry of entrainment by the two oscillators in driving experiments. In the model, Δf was varied by changing the average spike frequency of the G3 initiation site while maintaining the spike frequency of the G4 site constant (see APPENDIX). In principle, if Δf is large, the

FIG. 10. Mutual entrainment in the two-site model: comparing the two-site model with the heartbeat timing network (living system). A: in the two-site model, the period of the coupled system (Tc) is closer to the period of the faster segmental oscillator than to that of the slower segmental oscillator but is always longer than the period of the faster segmental oscillator. B: in the living system, the period of the coupled system (Tc) is very close to the period of the faster segmental oscillator. Data replotted from Masino et al. (2002b).
influence of the G3 sites of the coordinating fibers should be slight, and the model should be more symmetric. In the limit of a maximal Δf (i.e., the G3 site fires at 0 Hz), the model should be perfectly symmetric because it reduces to the one-site model (Fig. 5C). Conversely, if Δf is small, then the influence of the G3 sites should be great, and the model should be more asymmetric. If Δf ≥ 0 Hz (i.e., the G3 sites fire at an average frequency equal to or greater than that of the G4 sites), the G4 oscillator would not be able to entrain the G3 oscillator at all (see simple asymmetric model) (Hill et al. 2002). In the simulations, varying Δf had the expected influence on the entrainment symmetry (Fig. 12). Increasing Δf to 2.1 Hz (Fig. 12C) made the two oscillators more equivalent and decreasing Δf to 1.0 Hz (Fig. 12A) made them less equivalent in driving experiments. A low Δf (1.0 Hz Fig. 12A) restricted the ability of the G3 oscillator to slow the system because inhibition from the G3 site occurs after the ipsilateral G4 oscillator interneurons has begun to burst (e.g., Fig. 11B2). In the canonical model (Δf = 1.7 Hz), the spike frequency of the G3 sites is quite low; therefore this inhibition has very little effect on the G4 oscillator interneuron bursts. However, if the spike frequency of the G3 sites is high, inhibition falling after the start of the G4 oscillator bursts is disruptive and increases the coefficient of variation of the bursts, one of our criteria for stable entrainment (see METHODS). A low Δf also limited the ability of the G4 oscillator to entrain the system to shorter periods compared with the canonical model (cf. Fig. 12, A and B) because the ability of the G4 oscillator to accelerate the G3 oscillator is reduced (T_{3S} is close to T_{4S}). Natural variation in Δf may account in part for the observed differences in the range of entrainment observed experimentally among living preparations (Fig. 13, C and D).

The effect of an initial phase difference. In the living system for a given preparation, the intersegmental phase difference of the mutually entrained system is stable for the duration of an experiment; however, the phase value may lie anywhere between −10 and +20% (Fig. 13, C and D) (Masino and Calabrese 2002a). This variation among preparations reflects differences in the intrinsic periods of the segmental oscillators (Masino and Calabrese 2002b). Simulations (simple symmetric model) have shown these initial phase differences substantially influence the period range in mutual entrainment and driving experiments because the range of entrainment is determined by the intrinsic properties of the oscillators such as their half-center and segmental oscillator periods (Hill et al. 2002). The influence of these inherent periods in mutual entrainment experiments in the one- and two-site models is apparent in the analyses of Figs. 3, 7, and 8. In the simulations of the two-site model described in the preceding text, the model networks all began with an initial phase difference of zero. To further assess the ability of the two-site models to capture the activity of the living system, we simulated driving experiments using model networks with different initial phase differences by varying g_{ih} in the oscillator interneurons of one segmental oscillator (Fig. 13, A and B).

Because the impact of these inherent periods in determining mutual entrainment is complex especially in the two-site model, we did not fully analyze their role in these driving experiments, but their importance can be clearly seen in the model data of Fig. 13, A and B. For example, when the model G4 oscillator has a large initial phase lead, the driven G4 oscillator can only entrain the coupled system to periods slower than the free run period (red curve, Fig. 13A). This result makes sense based on the modeling results given in the pre-
ceeding text. In a mutually entrained system in which the G4 oscillator has a large phase lead, it weakly accelerates the coupled system, reducing the free run period (Fig. 7D). Given the weak ability of the driven G4 oscillator to entrain the system to faster periods, it is not surprising that from this initial point the G4 oscillator cannot entrain the system to even faster periods. On the other hand, the driven G4 oscillator can entrain the system to slower periods than in the canonical model (green curve, Fig. 13A) because the period of the G3 segmental oscillator is slower relative to the free run period than in the canonical model. Likewise, in physiological experiments, the G4 oscillator was not particularly good at entraining to shorter periods (the sole exception is the red curve, Fig. 13C). Conversely, in the simulations when the G4 oscillator has a large phase lead, driving the G3 oscillator can only entrain the coupled system to periods faster than the free run period (red curve, Fig. 13B). When the G3 oscillator lags in phase, its ability to drive the system to even slower periods is constrained because beyond a maximum phase difference of ~23%, the window of time in which coordinating fiber inhibition falls is too small to provide sufficient inhibition to slow the G4 oscillator (Fig. 11B). In living preparations with large G4 phase leads similar results were obtained (red and gold curves, Fig. 13D).

Although the two-site model was remarkably good at capturing both the period range and the phase range for entrainment when driving either the G3 or the G4 oscillator, the slopes of the entrainment curves are steeper in the model than in the living system (Fig. 13). This difference suggests that coordinating fiber inhibition in the living system is stronger than in the two-site model because in the living system a small phase difference (i.e., a small change in coordinating fiber inhibition) yields a large change in period.

**DISCUSSION**

It has become a truism that network activity emerges through the interplay of network connectivity and intrinsic cellular membrane properties. How this interplay gives rise to intersegmental phase relations in a segmentally distributed network has been the subject of physiological and modeling analyses in several systems (e.g., Cang and Friesen 2000, 2002; Ekeberg and Grillner 1999; Jones et al. 2003; Skinner and Mulloney 1998a,b; Skinner et al. 1997; Walle´n et al. 1992). Our own modeling efforts in this regard have focused on the timing network of the leech heartbeat central pattern generator (CPG). This network, which is composed of two segmental oscillators, is at the core of a CPG that controls motor neurons that innervate the muscular walls of two tube-like hearts (Calabrese 1977; Thompson and Stent 1976a–c; Wenning et al. 2002a,b).

We have proceeded in a stepwise fashion, gradually expanding the scope of our network model while simultaneously adding cellular and synaptic details as they become available. In previous papers, we explored the basis of oscillations in a pair of reciprocally inhibitory oscillator interneurons within a single segmental ganglion (Calabrese et al. 1995; Nadim et al. 1995; Olsen et al. 1995). We then formed a model of the entire timing network by adding the interactions between these oscillator interneurons and coordinating interneurons (Hill et al. 2001, 2002). In our first generation model of the timing net-
work, the simple symmetric model, we ignored the details of the firing properties of the coordinating neurons and their connectivity with the oscillator interneurons (Fig. 1C). In this model, the coordinating interneurons were represented as single compartments that fired tonically and were inhibited in a symmetric manner by the G3 and G4 oscillator interneurons (Fig. 1, C and D). This model was nonetheless able to account for many of our experimental results concerning phase and period control. In the mutually entrained network, phase differences arise due to period differences between the segmental oscillators, and the faster oscillator leads in phase and controls the cycle period (Masino and Calabrese 2002a,b). The simple symmetric model replicated these results and demonstrated a biologically plausible mechanism by which the faster oscillator could accelerate the slower oscillator. Cycle-by-cycle the faster oscillator interneurons relieve the slower oscillator interneurons of coordinating interneuron inhibition, at a crucial time, late in their inhibited phase (Fig. 1D) (Hill et al. 2002). In this way, a given period difference between the segmental oscillators is accommodated by a phase difference that results in removal of enough of inhibition to accelerate the slower oscillator to the period of the faster oscillator.

This simple symmetric model, however, cannot explain observed asymmetries in behavior of the timing network during mutual entrainment and driving experiments. Although in mutual entrainment experiments, the system behaves primarily in a symmetric fashion, a subtle asymmetry exists in the phase relationship between the coordinating interneurons and the oscillator interneurons. As the intersegmental phase increases, the phase of the coordinating interneurons relative to the G3 oscillator interneurons changes more slowly than their phase relative to the G4 oscillator interneurons, indicating that the G3 oscillator interneurons control the firing of the coordinating interneurons more strongly than the G4 oscillator interneurons do (Masino and Calabrese 2002a,b). The asymmetric nature of the timing network is even more apparent in driving experiments in which rhythmic current pulses are used to drive one segmental oscillator and thereby entrain the entire coupled system to periods faster and slower than the mutually entrained period (Masino and Calabrese 2002c). These experiments are fundamentally different from mutual entrainment experiments in that they are open loop; the driving stimulus imposed on the system does not receive feedback from the biological system. These driving experiments revealed two important characteristics of the coupled network that could not be replicated in the simple symmetric model. First, although the coupled system can be entrained to longer periods equally well by either the G3 or the G4 oscillator, the G3 oscillator shows a stronger ability to entrain the system to shorter periods (Fig. 13, C and D) (Masino and Calabrese 2002c). Second, the driven oscillator may slow the coupled system by lagging in phase relative to the undriven oscillator (Masino and Calabrese 2002c). In contrast, in the simple symmetric model, the driven G3 and G4 oscillators are equal in their ability to entrain coupled system. Also, in the simple symmetric model, the driven oscillator cannot lag in phase because the only mechanism of entrainment is removal of inhibition, which can accelerate but cannot slow the undriven oscillator (Hill et al. 2002). As a consequence, a driving stimulus cannot entrain the

FIG. 13. Driving in the two-site model: comparing the two-site model with the heartbeat timing network (living system). A and B: the two-site model was started with different initial phase differences as indicated by the colored bars on the right y axis border that correspond to the different entrainment curves. Phase differences were created between the G3 and G4 segmental oscillators by adjusting $g_{\text{h}}$ in 1 pair of model oscillator interneurons while the other pair was held at its canonical value (the canonical value of $g_{\text{h}}$ is 4 nS). The model G4 oscillator [HN(4)] interneurons and the model G3 oscillator [HN(3)] interneurons were driven by square-wave conductance changes. All model parameters were canonical. C and D: the living system showed different initial phase differences under condition of mutual entrainment in different preparations. The initial phases are indicated by the colored bars on the right y axis border that correspond to the different entrainment curves. G4 oscillator [HN(4)] interneurons and G3 oscillator [HN(3)] interneurons were driven by current injection. In both the 2-site model (A and B) and the living system (C and D), the phase difference between the G3 and G4 oscillators ($\Phi_3 - \Phi_4$) varied with the percent change in period ($\Delta T_{\text{Driven}} - T_{\text{Undriven}}/T_{\text{Undriven}}$) between the driven oscillator ($T_{\text{Driven}}$) and the undriven coupled system ($T_{\text{Undriven}}$). Data in C and D replotted from Masino et al. (2002c). In each graph of this figure, the color of the entrainment curve, hot to cool, indicates the relative order of the initial phase difference from the most positive (G4 oscillator leads in phase, red) to the most negative (G4 oscillator lags in phase, blue).
coupled system to periods longer than the segmental oscillator period of the undriven oscillator (e.g., in a model with an initial phase of 0%, the mutually entrained period).

Here we explored a model in which the coordinating interneurons were represented as multicompartmental cables, allowing them to receive synaptic input from the oscillator interneurons in the same pattern as in the living system (cf. Figs. 1A and 2C). In addition, the spiking properties of the coordinating interneurons were matched to those of the real coordinating interneurons; the model coordinating interneurons rebounded from inhibition and showed spike frequency adaptation (cf. Figs. 1B and 2B). We first tested the effects of spike frequency adaptation with a one-site model, which has a pattern of synaptic connectivity that is functionally equivalent to that of the simple symmetric model (cf. Figs. 1C and 2C).

We found that spike frequency adaptation allows the driven oscillator to lag in phase and entrain the coupled system to periods slower than the segmental oscillator period of the undriven oscillator (Fig. 5). In this model, when the driven oscillator lags in phase, it delays the onset of the coordinating fiber bursts and thereby shifts the high-frequency portion of the coordinating fiber bursts to late in the inhibited phase of the undriven oscillator. This additional late inhibition has a strong slowing effect on the undriven oscillator, allowing it to adopt the period of the driven oscillator (Fig. 5B).

We next tested the effects of adding coordinating fibers that have multiple spike initiation sites and that receive asymmetric synaptic connections from the oscillator interneurons to our model. In the living system, each of the coordinating fibers has two independent spike initiation sites, one in the third ganglion and one in the fourth ganglion (Fig. 1A) (Masino and Calabrese 2002a; Peterson 1983b). These initiation sites are not equivalent; the G4 initiation sites fire more consistently and generally with a higher average frequency than the G3 sites (Masino and Calabrese 2002a). In addition, the G3 oscillator can silence both the G3 and G4 the initiation sites, whereas the G4 oscillator can only silence the G4 sites (Masino and Calabrese 2002a; Peterson 1983b). We first modeled a single coordinating fiber to understand the behavior of a single axon with two initiation sites with different inherent spike frequencies. In this model, spikes arose at the high-frequency site, but when the high-frequency site was inhibited, spike initiation rapidly shifted to the low-frequency site (Fig. 2B). When we tested our two-site model, which incorporates coordinating fibers with two initiation sites and the asymmetric synaptic coupling, we found that this model was able to reproduce the asymmetries seen in mutual entrainment and driving experiments (Masino and Calabrese 2002a,c) (Figs. 4B and 13).

In the simulated mutual entrainment experiments, the phase relationships between the coordinating fibers and the G3 and G4 oscillator interneurons were nearly identical to those seen in the living system. This asymmetric behavior arises because the G3 oscillator interneurons completely silence the firing of the coordinating interneurons, whereas the G4 oscillator interneurons only reduced the firing frequency (Figs. 6 and 9B). In simulated driving experiments, the ability of the G3 oscillator to accelerate the coupled system to shorter periods was stronger than that of the G4 oscillator (Fig. 13, C and D) because when the G3 oscillator leads in phase, in addition to accelerating the G4 oscillator by the removal of coordinating fiber inhibition, the G3 oscillator accelerates the G4 oscillator through early burst termination. Immediately after the end of a burst of a G3 oscillator interneuron, spikes arise from the low-frequency G3 sites of the coordinating fibers and overlap with the trailing end of the G4 oscillator interneuron bursts, thereby terminating the G4 oscillator bursts early (Fig. 11B1). In contrast, the ability of the G4 oscillator to drive the system faster is limited because it can reduce but cannot eliminate coordinating fiber inhibition to the G3 oscillator (Fig. 11A1). The G4 oscillator interneurons decrease inhibition to the G3 oscillator interneurons by inhibiting the high-frequency G4 site and thereby shifting spike initiation to the low-frequency G3 site.

What have we learned from the bottom-up approach?

In contrast to the “top down” approach in which theoretical methods are used to discover general principles of intersegmental coordination (Sigvardt and Miller 1998), our modeling strategy has been “bottom up.” Arguably, a flaw of the bottom-up approach is that it is idiiosyncratic to the neuronal circuit studied and therefore does not lend itself to generalization. We would argue, however, that in creating a detailed model a network, we have been able to identify simple, biologically plausible mechanisms of coordination that could be involved in the control of intersegmental coordination in other networks.

One general lesson that we have learned is that the details of a system can be of fundamental importance. Using our simple symmetric model, we discovered that the primary mechanism of coordination in this neuronal network is removal of inhibition. However, not until we incorporated more realistic representations of the coordinating interneurons was our model able to match the rich dynamical behavior of the living system. Thus there is an inherent risk in deciding a priori which details of a system are important for its function.

We also found that a network may behave differently when studied under different conditions (open loop versus closed loop). Under conditions of mutual entrainment (closed loop), the timing network behaves in a largely symmetrical fashion and intersegmental phase differences are based on differences in the intrinsic oscillation frequencies of the two segmental oscillators (Masino and Calabrese 2002b). However, during driving experiments (open loop), the timing network behaves asymmetrically; the G3 oscillator has a stronger capacity to control the period of the coupled system than the G4 oscillator (Masino and Calabrese 2002c). In the study of the lamprey swim network, there is a controversy regarding the extent to which intersegmental coordination is governed by an excitability gradient or asymmetric coupling (Grillner et al. 1993; Sigvardt 1993). Forcing experiments (open loop) have revealed strong asymmetries in intersegmental coupling (Sigvardt and Williams 1996; Williams et al. 1990) that are not seen under conditions of mutual entrainment (Matsushima and Grillner 1992). Perhaps the experimental paradigm biases the results; driving experiments may emphasize asymmetric features of a network, whereas mutual entrainments experiments may emphasize symmetric features.

Moreover, we have found that synaptic inhibition can flexibly couple oscillatory neuronal networks. In the lamprey and tadpole swim networks, the side-to-side oscillations produced by a segmental oscillator are based on reciprocal inhibition between interneurons (Grillner et al. 1993; Roberts and Tunsell 1990). Likewise, in the crayfish swimmeret system he-
missegmental oscillations that control a single swimmeret appears to be based on reciprocal inhibition (Skriner and Mul- loney 1998b). Here, we have identified mechanisms of intersegmental coordination in the leech heartbeat timing net- work that could be of significance in other networks with strong reciprocal inhibition such as these. In our models, inhibi- tion falling early in the inhibited phase of an interneuron seems to have little effect on the interneuron; it neither accel- erates nor slows the interneuron (Hill et al. 2002). In contrast, late inhibition slows the interneuron in a phase dependent manner; as the phase increases the slowing effect increases in a near linear manner (Hill et al. 2002). This late inhibition may be truncated (removal of inhibition), accelerating the interneu- ron, or it may be strengthened (addition of inhibition), slowing the interneuron. In a mechanism that at first glance seems paradoxical, spike frequency adaptation in the inhibitory coor- dinating fibers provides the ability to increase this late inhibi- tion because phase differences between the oscillators shift the high-frequency portion of the coordinating fiber burst to late in the leading oscillator’s inhibited phase. Furthermore, very strong acceleration of an interneuron can be achieved by inhibi- tion that falls during the trailing end of the burst phase (early burst termination). Thus changes in the strength and phase of inhibition may accelerate or slow an interneuron, allowing for a stable phase relationship to be formed between segmental oscillators. Similar inhibitory effects may be involved in inter- segmental coordination in the swim network of the tadpole. In a model, inhibition falling early in the inhibited phase of a neuron causes a phase advance, whereas inhibition falling late in the inhibited phase causes a phase delay (Tunstall et al. 2002). Unlike in the leech where the acceleratory effect is based on either removal of inhibition or early burst termina- tion, in the tadpole, acceleration is produce by postinhibitory rebound (Merrywest et al. 2003; Roberts and Tunstall 1990).

Most importantly we emphasize that intrinsic period differ- ences and connectivity work hand-in-hand to produce phase differences between coupled oscillators and that a bottom-up approach in understanding systems of coupled oscillators is a necessary compliment to more abstract approaches (e.g., Cohen et al. 1982; Kopell and Ermentrout 1988).

**APPENDIX: COMPARTMENTAL PARAMETER VALUES FOR COORDINATING FIBERS**

Parameters shared by all compartments (canonical)

Passive properties are: $R_m = \text{Ohm-m}^2$, $R_A = \text{Ohm-m}$, $C_M = 0.01 \text{F/m}^2$, length $= 2 \times 10^{-4} \text{m}$, diameter $= 2 \times 10^{-7} \text{m}$.

Reversal potentials are: $E_{Na} = 0.045 \text{V}$, $E_{Cs} = 0.135 \text{V}$, $E_K = -0.07 \text{V}$, $E_L = -0.021 \text{V}$, $E_{syn} = -0.0625 \text{V}$, $E_{I_L} = 0.04 \text{V}$.

Passive compartments (canonical)

Shared passive properties and $E_L = 0.04 \text{V}$, only.

Conduction compartments (canonical)

Maximal conductances ($g_{om}$) are: $g_{Na} = 10 \times 10^{-9} \text{S}$, $g_{K1} = 6 \times 10^{-9} \text{S}$, $g_{K2} = 4 \times 10^{-9} \text{S}$.

G4 initiation site compartments (canonical)

Maximal conductances ($g_{om}$) are: $g_{Na} = 10 \times 10^{-9} \text{S}$, $g_{P} = 5.5 \times 10^{-10} \text{S}$, $g_{CaF} = 2.5 \times 10^{-10} \text{S}$, $g_{CaS} = 1.5 \times 10^{-11} \text{S}$, $g_{K1} = 6 \times 10^{-9} \text{S}$, $g_{K2} = 4 \times 10^{-9} \text{S}$, $g_{KA} = 4 \times 10^{-9} \text{S}$, $g_{Kh} = 2 \times 10^{-10} \text{S}$.

G3 initiation site compartments $\Delta f = 1.7$ (canonical)

Maximal conductances ($g_{om}$) are as in canonical G4 initiation site compartments except: $g_{P} = 5.3 \times 10^{-10} \text{S}$, $g_{CaF} = 2.6 \times 10^{-10} \text{S}$, $g_{CaS} = 5 \times 10^{-12} \text{S}$.

G3 initiation site compartments $\Delta f = 1.0$

Maximal conductances ($g_{om}$) are as in canonical G4 initiation site compartments except: $g_{P} = 5.4 \times 10^{-10} \text{S}$, $g_{CaF} = 2.6 \times 10^{-10} \text{S}$, $g_{CaS} = 5 \times 10^{-12} \text{S}$.

G3 initiation site compartments $\Delta f = 2.1$

Maximal conductances ($g_{om}$) are as in canonical G4 initiation site compartments except: $g_{P} = 5.3 \times 10^{-10} \text{S}$, $g_{CaF} = 2.1 \times 10^{-10} \text{S}$, $g_{CaS} = 1 \times 10^{-12} \text{S}$.

Constant frequency G4 and G3 site compartments (Fig. 3A)

Maximal conductances ($g_{om}$) are as in canonical G4 initiation site compartments except: $g_{CaF} = 0 \text{S}$, $g_{CaS} = 0 \text{S}$, and $g_{P}$ was adjusted to give various spike frequencies.

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