Model of Intersegmental Coordination in the Leech Heartbeat Neuronal Network

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We have created a computational model of the timing network that paces the heartbeat of the medicinal leech, Hirudo medicinalis. The rhythmic activity of this network originates from two segmental oscillators located in the third and fourth midbody ganglia. In the intact nerve cord, these segmental oscillators are mutually entrained to the same cycle period. Although experiments have shown that the segmental oscillators are coupled by inhibitory coordinating interneurons, the underlying mechanisms of intersegmental coordination have not yet been elucidated. To help understand this coordination, we have created a simple computational model with two variants: symmetric and asymmetric. In the symmetric model, neurons within each segmental oscillator called oscillator interneurons, inhibit the coordinating interneurons. In contrast, in the asymmetric model only the oscillator interneurons of one segmental oscillator inhibit the coordinating interneurons. In the symmetric model, when two segmental oscillators with different inherent periods are coupled, the faster one leads in phase, and the period of the coupled system is equal to the period of the faster oscillator. This behavior arises because, during each oscillation cycle, the oscillator interneurons of the faster segmental oscillator begin to burst before those of the slower oscillator, thereby terminating spike activity in the coordinating interneurons. Thus there is a brief period of time in each cycle when the oscillator interneurons of the slower segmental oscillator are relieved of inhibition from the coordinating interneurons. This “removal of synaptic inhibition” allows, within certain limits, the slower segmental oscillator to be sped to the period of the faster one. Thus the symmetric model demonstrates a plausible biophysical mechanism by which one segmental oscillator can entrain the other. In general the asymmetric model, in which only one segmental oscillator has the ability to inhibit the coordinating interneurons, behaves similarly, except only one segmental oscillator can control the period of the system. In addition, we simulated physiological experiments in which a “driving” stimulus, consisting of alternating positive and negative current steps, was used to control a single oscillator interneuron and thereby entrain the activity of the entire timing network.

INTRODUCTION

The generation of many rhythmic movements appears to involve the coordination of distributed 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). 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). 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 (Cang and Friesen 2000; Di Prisco et al. 1990).

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 (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).

In this paper, we have used simulations to explore how phase differences may arise between segmental oscillators in the timing network of the leech heartbeat central pattern generator (Peterson 1983a). This network consists of four pairs of identified heart interneurons found in the first four midbody ganglia (G1 to G4; Fig. 1A). Rhythmic oscillations arise from the activity of two segmental oscillators, located in the third and fourth ganglia, which can oscillate independently (Masino and Calabrese 2002a; Peterson 1983a, b). When the segmental oscillators are coupled, a stable phase relationship is established. For example, in Fig. 1B, the phase between the segmental oscillators is about 15% (a positive value indicates that the G4 oscillator interneurons lead the ipsilateral G3 oscillator interneurons). The previous paper in this series showed that, although phase is stable within a single preparation, there is

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variation between preparations from about $-10\%$ to $+20\%$ (Masino and Calabrese 2002a).

To help understand how phase and period are determined at the level of individual interneurons and their synaptic connections, we created two simple models (asymmetric and symmetric) that represent two conceptual simplifications of the timing network (Fig. 1C). We then tested these models in ways that are similar to experiments that can be done in the biological system (Masino and Calabrese 2002a,b). We found that in the symmetric model when two segmental oscillators with different inherent periods were coupled, the faster one led in phase, and that the absolute size of the phase difference was proportional to the period difference. Furthermore, the period of the coupled network was equal to that of the faster oscillator. The ability of the faster segmental oscillator to entrain the slower one arises from a mechanism, which we have called “removal of synaptic inhibition.” The asymmetric model showed similar behavior, except that entrainment only occurred when the inherent period of the G3 segmental oscillator was faster than that of the G4 segmental oscillator. Some of the predictions of these models are tested experimentally in the following paper (Masino and Calabrese 2002b).

**METHODS**

**Computational methods**

Each heart interneuron was represented as a single isopotential compartment with intrinsic and synaptic currents. The dynamics of membrane potential ($V$) of each oscillator interneuron (a neuron originating from the 3rd or 4th ganglion) obey
Two simple models of the timing network

The rationale for the use of the asymmetric and symmetric models is based on the known properties of the timing network (Masino and Calabrese 2002a). At the core of each of the two segmental oscillators is a half-center oscillator, consisting of two heart interneurons, called oscillator interneurons, which form reciprocally inhibitory synapses across the ganglion midline (Fig. 1A) (Masino and Calabrese 2002a). In addition, a segmental oscillator includes the axonal and neuritic processes of heart interneurons called “coordinating interneurons” that originate in the first and second ganglia (G1 and G2). Each coordinating interneuron has two spike initiation sites, located in the third and fourth ganglia (G3 and G4 sites; Fig. 1A). Normally, most spikes arise from the primary initiation sites in the fourth ganglion, which may have slightly higher inherent spike frequencies than the G3 sites (Jezzini et al. 2000; Masino and Calabrese 2002a). Under certain conditions, however, spike initiation may shift to the secondary, G3 sites (Masino and Calabrese 2002a). For example, in a chain consisting of only the first through the third ganglia, spikes are initiated at the G3 sites (Masino and Calabrese 2002a; Peterson 1983a,b). In addition, in the intact timing network, when the G4 oscillator interneurons lead the G3 oscillator interneurons in phase, spike initiation may switch from the fourth ganglion to the third ganglion part way through a coordinating interneuron burst (Masino and Calabrese 2002a; Peterson 1983a,b). Initially, spikes originate from the G4 site; however, when the G4 oscillator interneurons begin to burst, the G4 site becomes inhibited. At this time, the G3 site can begin to spike and continue to spike until the G3 oscillator interneurons burst and inhibit the G3 site. This capacity to switch spike initiation sites results from an asymmetry in the network. The G3 oscillator interneurons inhibit both the G3 and the G4 sites, whereas the G4 oscillator interneurons inhibit only the G4 sites (Fig. 1A).

A major simplification of both the asymmetric and symmetric models is that the coordinating interneurons are represented as having only a single spike initiation site (Fig. 1C). The symmetric network corresponds to a network in which the secondary spike initiation sites in G3 never become active. In this network, both the G3 and G4 oscillator interneurons can prevent the G4 sites from spiking. Thus the model oscillator interneurons of both ganglia inhibit the coordinating interneurons. The asymmetric network corresponds to a network in which the G3 sites become active immediately after the G4 sites become inhibited and fire at the same frequency as the G4 sites. In this network, the synapses from the G4 oscillator interneurons to the coordinating interneurons are ineffective in silencing the coordinating interneurons. Thus only the G3 oscillator interneurons inhibit the coordinating interneurons. A second simplification of both models is that the coordinating interneurons spike tonically during a burst, rather than showing spike adaptation as in the biological system (Fig. 1B) (Masino and Calabrese 2002a).

Inherent period of a model segmental oscillator was varied

In this work, we present results from modeling experiments in which the inherent period of one segmental oscillator was varied while the period of the other one was maintained constant. To vary the period of a segmental oscillator, we changed the maximal conductance of the hyperpolarization-activated current (g_h) in the oscillator interneurons (Fig. 2A). An increase in g_h led to a monotonic decrease in the period of a segmental oscillator (Fig. 2, B and C). According to a model, the activation of the hyperpolarization-activated current (I_h) during the inhibited phase of an oscillator interneuron helps to advance the transition to the burst phase, thereby speeding the oscillations (Hill et al. 2001). A model of a segmental oscillator with a standard set of parameters, called the canonical model, has a value of g_h of 4 nS and a period of 9.7 s. As noted previously
oscillator due to the extra inhibition from the coordinating interneurons (Hill et al. 2001), the period of the segmental oscillator is determined by the period of the oscillator interneurons indicates that the phase relationships of the G4 segmental oscillator (8.8 s), which was the faster oscillator. In a network composed of two segmental oscillators with the canonical value of $g_h$, the phase difference was zero, with little variation from one cycle to the next (Fig. 3, D and E). The period of this network was about 9.7 s, which is equal to the period of both segmental oscillators. When the inherent period of the G4 segmental oscillator was decreased below the canonical value, the G4 segmental oscillator led in phase (Fig. 3, D and F), and the period of the coupled network was equal to the period of the G4 segmental oscillator (8.8 s).

In addition, when we systematically varied $g_h$ in the G4 segmental oscillator, we found that the larger the period difference between the segmental oscillators, the greater the absolute phase difference (Fig. 4, A and B). Furthermore, one-to-one entrainment occurred over a range of phase values from $-25$ to $+20\%$. Outside of this range, phase relationships were not stable and were characterized by relative entrainment; where the interneurons interacted, however, their bursts were not matched one-to-one. For example, in Fig. 4D the G4 segmental oscillator had an inherent period greater than G3 segmental oscillator. The first burst in the G4 oscillator interneuron occurred at roughly the same time as the first burst in the G3 oscillator interneuron. The phase relationships, however, were not stable. With each successive burst the G3 oscillator interneuron led the G4 oscillator interneuron by a larger amount. Eventually, by the fifth burst in G4 oscillator interneuron, there were two bursts in the G3 oscillator interneuron. The periodic nature of the interaction between G3 oscillator interneuron and G4 oscillator interneuron can be seen in an actogram (Fig. 4E). From this point on, one-to-one entrainment will be simply referred to as entrainment.

In addition, we plotted the relationship between the period of the coupled system and the value of $g_h$ in the G4 oscillator interneurons (Fig. 4C). This graph shows a close match between the period of the coupled system ($T_C$) and the period of the faster oscillator, regardless of which segmental oscillator was faster. Furthermore, the range over which one-to-one entrainment occurred was limited by the half-center oscillator period ($T_{H1}$) of the slower segmental oscillator. In other words, the faster segmental oscillator cannot speed the slower segmental oscillator to a period that is less than the slower oscillator’s half-center oscillator period. For example, as the period of the G4 segmental oscillator ($T_{4S}$) was increased above the canonical value, the coupled system showed entrainment as long as the period of the G4 half-center oscillator ($T_{H4}$) was less than that of the G3 segmental oscillator ($T_{3S}$). When $T_{H4}$ was greater than $T_{3S}$ ($g_h$ below 2.4 nS), relative entrainment

In the symmetric network, the faster segmental oscillator leads in phase and determines the coupled period

To test the idea that phase differences in the symmetric network may arise from differences in the inherent periods of the segmental oscillators, we varied the inherent period of the G4 segmental oscillator while maintaining the period of the G3 segmental oscillator constant (Fig. 3A). As demonstrated in the examples given below, this experiment shows that, when two segmental oscillators with different inherent periods are coupled, the faster one leads in phase, and that the period of the coupled system is equal to the period of the faster segmental oscillator.

When the inherent period of the G4 segmental oscillator was increased above the canonical value, the G3 segmental oscillator led in phase (Fig. 3B). Because the long-term stability of phase relationships is difficult to determine from just a few cycles, we created an actogram of the model interneuron bursts (Masino and Calabrese 2002a). In Fig. 3C, symbols that represent the median spike times of each burst form straight, vertical lines, demonstrating that the cycle period and phase relationships were stable. The period of the coupled system was equal to the inherent period of the G3 segmental oscillator (9.7 s), which was the faster oscillator. In a network composed of two segmental oscillators with the canonical value of $g_h$, the phase difference was zero, with little variation from one cycle to the next (Fig. 3, D and E). The period of this network was about 9.7 s, which is equal to the period of both segmental oscillators. When the inherent period of the G4 segmental oscillator was decreased below the canonical value, the G4 segmental oscillator led in phase (Fig. 3, D and F), and the period of the coupled network was equal to the period of the G4 segmental oscillator (8.8 s).

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occurred (Fig. 4, C–E). Likewise, when the period of the G4 segmental oscillator was decreased below the canonical value, entrainment was limited by the G3 half-center oscillator period ($T_{3\text{H}}$; Fig. 4C).

Duty cycle and phase of the coordinating interneurons vary with the phase difference between the segmental oscillators.

One characteristic of the symmetric model is that the oscillator interneurons of either segmental oscillator may terminate...
the bursts in the coordinating interneurons. Thus the coordinating interneurons only spike during the window of time when neither ipsilateral oscillator interneuron is spiking (Fig. 3). Therefore a phase difference between the segmental oscillators led to a reduction in the duty cycle of the coordinating interneurons (Fig. 5A). Furthermore, the minimum duty cycle of the coordinating interneurons was about 20% regardless of which segmental oscillator led (Fig. 5B). In comparison, the duty cycles of the G3 and G4 oscillator interneurons did not vary from 50%.

In addition to the change in duty cycle, the phase of the coordinating interneurons varies with the phase difference between the segmental oscillators. For example, when the phase difference between the segmental oscillators was zero, the phase between the coordinating interneurons and both segmental oscillators was about 53% (Fig. 5A and C). An increase in the phase between the segmental oscillators (\(\phi_3 - \phi_4\)) led to an increase in the phase between the coordinating interneurons and the G4 segmental oscillator (\(\phi_2 - \phi_4\)), and simultaneously, a decrease in the phase between the coordinating interneurons and the G3 segmental oscillator (\(\phi_2 - \phi_3\)). The reason for the increase in the phase between the coordinating interneurons and the G4 segmental oscillator can be seen in Fig. 5A. When the G4 segmental oscillator leads in phase, the oscillator interneurons of the G3 segmental oscillator postpone the onset of bursts in the coordinating interneurons. Thus the
The slopes of the changes in $\phi_2 - \phi_4$ and $\phi_3 - \phi_4$ are 0.5 and $-0.5$, respectively (Fig. 5C). These values reflect the ability of both segmental oscillators to completely inhibit spiking in the model coordinating interneurons. In contrast, in the asymmetric model, the slopes were very different (data not shown). The phase between the coordinating interneurons and the G3 segmental oscillator ($\phi_2 - \phi_3$) was constant ($m = 0$) because the G3 segmental oscillator solely determines the timing of the coordinating interneuron bursts. The relationship between $\phi_2 - \phi_4$ and the phase difference between the segmental oscillators had a slope of 1.0 because the G4 segmental oscillator had no effect on the coordinating interneuron bursts.

**Faster segmental oscillator speeds the slower segmental oscillator by removing inhibition**

We have shown that when two segmental oscillators with different inherent periods are coupled, the oscillator interneurons of the faster segmental oscillator lead in phase. As a result, the oscillator interneurons of the faster segmental oscillator burst before the oscillator interneurons of the slower segmental oscillator and, thereby, terminate the activity of the coordinating interneurons (Fig. 3, B and F). Thus there is a brief period of time when an oscillator interneuron of the slower segmental oscillator only receives inhibition from the contralateral oscillator interneuron. In this manner, the faster segmental oscillator removes coordinating fiber inhibition from the slower one.

To test the idea that removal of the inhibition may be sufficient to explain the ability of the faster segmental oscillator to entrain the slower one, we carried out several different modeling experiments. In the first experiment, we studied the effect of truncating the coordinating interneuron bursts on the interburst interval of the oscillator interneurons. In a series of trials, the bursts of the coordinating interneurons were truncated by injecting negative current at different times (Fig. 6, B and C). Initially, progressively truncating the coordinating interneuron bursts was very effective at decreasing the interburst interval (Fig. 6D, region of curve from point A to B). However, beyond a certain point, further truncation of the bursts had no effect on the interburst interval (Fig. 6D, region of curve around point C). This modeling experiment indicates that inhibition from the coordinating interneurons that occurs late in the inhibited phase is effective at increasing the interburst interval, whereas inhibition that occurs early is not effective. Furthermore, the minimum interburst interval occurred when the duration of the coordinating burst was of intermediate length (Fig. 6, B and D, point B). This result is likely to be due to postinhibitory rebound.

When two segmental oscillators with different inherent periods are coupled, the oscillator interneurons of the slower segmental oscillator lag in phase and thereby postpone the activity of the coordinating interneurons (Fig. 3, B and F). To test whether postponing the onset of the coordinating interneuron bursts has an effect on the interburst interval, we performed a complementary experiment to the one described above. In this experiment, the onset of the coordinating interneuron bursts was postponed by the injection of negative current. Trials, which were begun from one set of initial conditions, show that, up to a critical point, delaying the onset of the coordinating interneuron bursts had little effect on the interburst interval (Fig. 6, E and F). Beyond this point, however, the...
coordinating interneuron bursts were delayed so much that the oscillator interneuron began to spike before the coordinating interneurons, preventing the coordinating interneurons from bursting (Fig. 6G).

This result was confirmed when the experiment was repeated in six sets of trials, each set beginning from different initial conditions (Fig. 6H). Using different initial conditions was necessary because the response of the system to postponing the onset of the coordinating interneuron bursts was quite variable. The averaged data show that over a large range delaying the bursts of the coordinating interneurons did not change the interburst interval. Beyond a critical point, however, the oscillator interneuron began to burst before the coordinating interneurons, causing a precipitous decrease in the interburst interval.

These modeling experiments demonstrate that coordinating interneuron inhibition that occurs late in the inhibited phase is very effective at increasing the interburst interval, whereas inhibition that occurs early in the inhibited phase has relatively little effect. Therefore the faster segmental oscillator, which leads in phase, can speed the system, whereas the slower segmental oscillator, which lags in phase, cannot affect the period of the system.

The effectiveness of coordinating interneuron inhibition...
changes within the inhibited phase of an oscillator interneuron for several reasons. According to a modeling study, at the beginning of the inhibited phase, inhibition from the contralateral oscillator interneuron is strong, consisting of both spike-mediated and graded transmission (Hill et al. 2001). Thus the total inhibitory conductance is sufficiently large to hold the oscillator interneuron at a hyperpolarized potential without extra inhibition from the coordinating interneurons. In contrast, by the end of the inhibited phase, the graded conductance has completely waned, the hyperpolarization-activated current ($I_h$) has become activated, and removal of inactivation of Ca$^{2+}$ currents has occurred. At this point, the inhibited interneuron is primed to begin the next burst, but it is held in the inhibited state by the spike-mediated inhibition from the contralateral oscillator interneuron. The exact timing of the transition to the burst phase is dependent on a delicate balance between the rate of decline in spike frequency of the contralateral oscillator interneuron and the level of $I_h$ conductance (Hill et al. 2001). Thus extra spike-mediated inhibition from the coordinating interneurons is very effective at delaying the onset of the next burst.

The modeling experiments shown above do not demonstrate whether the removal of inhibition can account for the observation that the slower segmental oscillator can be sped to its half-center oscillator period. To answer this question, we measured the effect of changing the duration of the coordinating interneuron inhibition on the cycle period of a segmental oscillator. In this experiment, the synapses from the coordinating interneurons to the oscillator interneurons were deleted and replaced with square-wave conductances (Fig. 7). The reversal potential of this conductance was equal to that of an inhibitory synapse ($-62.5 \text{ mV}$). Each cycle, the onset of the conductance was triggered from the first spike in the burst of the ipsilateral G2 coordinating interneuron (not shown). The amplitude of this conductance was adjusted so that when the duration of the conductance was equal to the duration of a normal coordinating interneuron burst the period of the system was equal to that of the canonical segmental oscillator (Fig. 7C). As the duration of the conductance was decreased, there was a nearly linear decrease in the period of the system from the canonical segmental oscillator period to the canonical half-center oscillator period (Fig. 7D, region of the curve between point C and a 2 s duration). Eventually, however, as the duration of the conductance was reduced to very short values (below 2 s), there was no further decrease in period. This result demonstrates that removal of inhibition can speed a segmental oscillator to the period of its half-center oscillator, but not beyond. In the opposite direction, as the duration of the conductance was extended beyond the duration of a coordinating interneuron burst, there was also no change in the period (Fig. 7D, points to the right of point C). This result indicates that after an oscillator neuron has begun to burst, further inhibition has little effect.

**Canonical symmetric network can only be driven faster than its mutually entrained period**

In the biological system, driving experiments have been done in which the period of the entire timing network was controlled by a stimulus applied to a single heart interneuron. In these experiments, alternating positive and negative current steps were injected into an oscillator interneuron, thereby controlling its oscillation period (Peterson 1983b; Peterson and Calabrese 1982). Furthermore, due to the strong inhibitory synapses between oscillator interneurons, the activity of the contralateral oscillator interneuron was controlled, and, within limits, the period of the entire heartbeat network could be controlled (Peterson 1983b; Peterson and Calabrese 1982). These experiments are fundamentally different from mutual entrainment experiments because they are open loop in nature. Information flows from the driven oscillator to the follower oscillator, but not in the other direction. Although the synapses from the coordinating interneurons to the driven segmental oscillator are still present, they are rendered ineffective if the driving stimulus is strong.
We replicated these driving experiments in the model with essentially the same methods as in the biological system. However, because the model often shows spike failure in response to depolarizing current, the model was driven by changes in conductance rather than current. A single oscillator interneuron received a 20-nS conductance that alternated between a reversal potential of -40 and -55 mV with a duty cycle of 50%, causing the interneuron to alternate between spiking and silent phases. As in the biological system, driving one oscillator interneuron also controlled the contralateral interneuron. Unlike the mutual entrainment experiments in which phase was plotted against the period difference between the segmental oscillators, we plotted phase against the period difference between the driven segmental oscillator and the mutually entrained system (Fig. 8B). This convention follows that used in biological experiments in which the period of the mutually entrained system is more easily measured than the inherent periods of the segmental oscillators.

We first drove the left G3 oscillator interneuron of the canonical symmetric model (Fig. 8A). In this model, the G3 and G4 segmental oscillators are identical. Thus in the mutually entrained network there was no phase difference and the period was 9.7 s. Entrainment only occurred when the G3 segmental oscillator led in phase (Fig. 8, B, D, and E), and when system was driven to periods between the G4 half-center oscillator period and the G4 segmental oscillator period (Fig. 8C). Outside of this range, relative entrainment occurred. In the canonical symmetric model, the G4 segmental oscillator period is equal to the mutually entrained period (Fig. 8C). Thus the system can only be driven faster than the mutually entrained period.

The mechanism by which the driven segmental oscillator entrains the network is essentially the same as the mechanism by which the faster segmental oscillator determines the period of the mutually entrained system. The oscillator interneurons of the driven segmental oscillator remove inhibition from the

**FIG. 8.** The canonical symmetric network can only be driven faster than its mutually entrained period. **A:** a driving stimulus was applied to HN(L,3). **B:** entrainment only occurred when the driven oscillator led in phase. **C:** this network may be driven between the G4 half-center oscillator and the G4 segmental oscillator period, which in this network is equal to the mutually entrained period (\(T_{\text{mutual}}\)). **D:** when the HN(L,3) interneuron was driven faster than \(T_{\text{mutual}}\), it led in phase. The bar beneath the voltage record on HN(L,3) indicates when the driving conductance was excitatory (-40 mV). **E:** when the HN(L,3) interneuron was driven at a period equal to \(T_{\text{mutual}}\) its phase lead was very small.
oscillator interneurons of the follower segmental oscillator (Fig. 8D). This mechanism can only work when the driven oscillator leads in phase. Thus the driven period must be faster than the inherent segmental oscillator period of the follower oscillator. Also, similar to the mutually entrained network, the lower limit to which the system can be driven is the half-center oscillator period of the follower oscillator. Because the network is symmetric, corresponding results were obtained by driving the G4 oscillator interneurons.

Symmetric network can be driven slower than its mutually entrained period if the driven segmental oscillator originally leads in phase

In the biological system, a phase difference often exists between the segmental oscillators of the coupled network, with the G4 segmental oscillator leading in most cases (Masino and Calabrese 2002a). To simulate driving experiments under these situations, we created two symmetric networks: one in which the G3 segmental oscillator led in phase (Fig. 9, A–C) and one in which the G4 segmental oscillator led in phase (Fig. 9, D–F).

To make the G3 segmental oscillator lead in phase, the period of the G4 segmental oscillator was increased (Fig. 9A). In the mutually entrained network, the period was 9.7 s (Fig. 9C), equal to that of the G3 segmental oscillator, and the phase was about $-11\%$ (Fig. 9B). As in the canonical symmetric network, a driving stimulus could entrain the network to a period between the G4 half-center oscillator period and the G4 segmental oscillator period (Fig. 9C). However, in this network, the mutually entrained period was faster than the G4 segmental oscillator period (cf. Figs. 8C and 9C). Thus the network could be driven slower than the mutually entrained period.

**FIG. 9.** A symmetric network can be entrained by a driving stimulus to periods between the half-center and segmental oscillator periods of the follower ganglion. A: $g_h$ was reduced in the G4 oscillator interneurons. B: in the mutually entrained system, the G3 oscillator interneurons led in phase. When the system was driven faster and slower than the mutually entrained period, the G3 oscillator interneurons continued to lead in phase. C: the system could be driven both faster and slower than the mutually entrained period. D: $g_h$ was reduced in the G3 oscillator interneurons. E: in the mutually entrained system, the G4 oscillator interneurons led in phase. When the system was driven, the G3 oscillator interneurons led in phase. F: the period of the mutually entrained system was equal to that of the G4 segmental oscillator, therefore the system could only be driven faster than the mutually entrained period.
The mechanism by which the system can be driven slower than the mutually entrained period is essentially the same as the “removal of inhibition” mechanism. In the mutually entrained network, the G3 segmental oscillator leads in phase. Thus when the network is driven slower than the mutually entrained period, the phase lead of the G3 segmental oscillator decreases (Fig. 9B), causing an increase in the duration of the coordinating interneurons bursts. This extra late inhibition from the coordinating interneurons slows the G4 segmental oscillator to the driven period. Thus the driving stimulus can increase the period of the system up to, but not beyond, the period of the G4 segmental oscillator.

To create a symmetric network in which the G4 segmental oscillator led in phase, the period of the G3 segmental oscillator was increased (Fig. 9D). The period of the mutually entrained system was 9.7 s (Fig. 9F) and the phase was 11% (Fig. 9E). As with the examples described above, the system could be driven to periods between the G4 half-center oscillator period and the G4 segmental oscillator period (Fig. 9D). Thus this network could be driven faster than its mutually entrained period, but not slower. In this case, a driving stimulus caused an abrupt shift in phase. In the mutually entrained system, the G4 segmental oscillator led in phase. Entrainment only occurred, however, when the G3 segmental oscillator led in phase (Fig. 9E). Because the network is symmetric, corresponding results were obtained by driving the G4 oscillator interneurons.

In the mutually entrained asymmetric network, entrainment only occurs when the G3 segmental oscillator leads in phase.

We investigated mutual entrainment in the asymmetric model in a manner similar to our study of the symmetric model. The period of the G4 segmental oscillator was varied by changing $g_h$ in the G4 oscillator interneurons, while maintaining the period of the G3 segmental oscillator constant (Fig. 10A). In the asymmetric network, mutual entrainment occurred only when the inherent period of the G3 segmental oscillator was less than that of the G4 segmental oscillator (Fig. 10B). When the G4 segmental oscillator was faster, entrainment did not occur because the G4 segmental oscillator could not speed the G3 segmental oscillator. As in the symmetric network, the period of the mutually entrained system was equal to that of the faster segmental oscillator, which in this case could only be the G3 segmental oscillator (Fig. 10C).

Unlike the symmetric network, the system could be entrained to periods shorter than the half-center oscillator period of the slower oscillator (Fig. 10F). Thus this network could be driven faster than its mutually entrained period, but not slower. In this case, a driving stimulus caused an abrupt shift in phase. In the mutually entrained system, the G4 segmental oscillator led in phase. Entrainment only occurred, however, when the G3 segmental oscillator led in phase (Fig. 9E). Because the network is symmetric, corresponding results were obtained by driving the G4 oscillator interneurons.

**FIG. 10.** Mutual entrainment in the asymmetric network. A: $g_h$ in the G4 oscillator interneurons was varied. B: entrainment only occurred when the G3 oscillator interneurons led in phase. The G4 segmental oscillator period was used to plot this data rather than the G4 half-center oscillator period because if the G4 segmental oscillator were isolated, it would consist of both oscillator interneurons and coordinating interneurons. C: unlike the symmetric network, the system could be entrained to periods shorter than the half-center oscillator period of the slower oscillator. D: the bursts of the coordinating interneurons overlapped with the trailing ends of the bursts of the G4 oscillator interneurons.
lator interneuron, allowing the contralateral oscillator interneuron to escape earlier, resulting in faster oscillations.

In the asymmetric network, the G3 segmental oscillator can drive the G4 segmental oscillator faster than its half-center oscillator period.

In the asymmetric network, the G4 oscillator interneurons do not inhibit the coordinating neurons. Therefore experiments in which G4 oscillator interneurons were driven did not result in entrainment (data not shown). When a G3 oscillator interneuron was driven, the results were generally similar to those found with the symmetric network. The asymmetric network can be driven faster, but not slower, than the inherent period of the follower segmental oscillator, and the driven segmental oscillator leads in phase (Fig. 11, A–C). Unlike the symmetric network, however, the asymmetric network can be driven faster than the half-center oscillator period of the G4 segmental oscillator (Fig. 11C). If both segmental oscillators had the same inherent period, entrainment occurred only when the system was driven to periods shorter than the period of the mutually entrained system (Fig. 11C). In contrast, in an asymmetric network in which the G4 segmental oscillator lagged in phase (Fig. 11, D and E), the period of the mutually entrained system was shorter than the G4 segmental oscillator period (Fig. 11F). Thus the network could be driven to periods that were either longer or shorter than that of the mutually entrained system.

Driving a coordinating interneuron can speed the symmetric network.

In the biological system, driving a coordinating interneuron can entrain the entire heartbeat network (Peterson and Calabrese 1982). In these experiments, single coordinating interneurons were driven by injecting current into their cell bodies. We did a similar experiment with the symmetric model. The

![Diagram of asymmetric network](image)

**Fig. 11.** The asymmetric network could be driven faster than the G4 half-center oscillator period. **A:** $g_h$ was 4.0 nS in both the G3 and G4 oscillator interneurons. **B:** entrainment occurred when the G3 oscillator interneurons led in phase. **C:** the system could only be driven faster than the period of the mutually entrained system because the periods of the G4 segmental oscillator and the mutually entrained system were equal. **D:** $g_h$ was reduced in the G4 oscillator interneurons. **E:** entrainment occurred when the G3 oscillator interneurons led in phase. **F:** the system could be driven slower than the period of the mutually entrained system.
left G2 coordinating interneuron was driven by a 20-nS conductance that alternated between a reversal potential of −40 and −55 mV. Spike frequencies were similar to those in unperturbed model coordinating interneurons (Fig. 12, B and C). In this modeling experiment, the synapses from the G3 and G4 oscillator interneurons to the driven G2 coordinating interneuron were deleted to prevent the bursts in the oscillator interneurons from inhibiting the driven G2 coordinating interneuron (Fig. 12A). Removing these synapses reflects the inability of the oscillator interneurons to affect the firing of a coordinating interneuron that is stimulated at its soma.

We tested a symmetric network in which the G3 segmental oscillator led the G4 segmental oscillator by about 15%. This system could be driven faster, but not slower than the period of the mutually entrained network (Fig. 12D). The mechanisms by which the system was driven faster were removal of late inhibition and overlap between the bursts of the driven coordinating interneuron and the trailing ends of the bursts of the oscillator interneurons (Fig. 12, B and C). The network could not be driven slower than the period of the mutually entrained system because the inhibition that the leading segmental oscillator received was no stronger than that which it would normally receive during mutual entrainment. Entrainment to slower periods only occurs when the spike frequency of the driven coordinating interneuron is higher than normal (data not shown).

Over the range of entrainment, the phase relationship between the segmental oscillators was quite stable (Fig. 12D), indicating that the driven coordinating interneuron had a similar speeding effect on both segmental oscillators. Unlike the systems explored above, the duty cycle of the oscillator interneurons varied considerably from 50%. For example, as the network was driven faster the duty cycle of left G3 oscillator interneuron decreased due to overlap between its bursts and the bursts of the driven G2 coordinating interneuron (Fig. 12, B, C, and E). In contrast, the duty cycle of the left G4 oscillator interneuron was below 40% at all entrained periods. The G4 segmental oscillator was inherently slower than the G3 segmental oscillator; therefore the bursts of the driven G2 coordinating interneuron always overlapped with those of the left

![FIG. 12.](http://jn.physiology.org/)

The symmetric network could be driven faster than the mutually entrained period by a coordinating interneuron. A: a driving stimulus was applied to HN(L,2). \( \bar{g}_H \) was reduced in the G4 oscillator interneurons. B: the system could be driven faster than the mutually entrained system. C: the system could be driven to a period equal to that of the mutually entrained system. D: the phase between the G3 and G4 oscillator interneurons did not change very much when the system was driven to different periods. E: the duty cycle of the HN(L,3) interneuron decreased as the driven period decreased, whereas the duty cycle of the HN(L,4) interneuron was always short.

*J Neurophysiol* • VOL 87 • MARCH 2002 • www.jn.org
G4 oscillator interneuron (Fig. 12, B, C, and E). The decrease in an oscillator interneuron’s duty cycle below 50% was matched by a reciprocal increase in the duty cycle of its contralateral partner.

**Periodic synaptic inhibition can slow or speed a half-center oscillator**

Based on the modeling experiments we presented above, the effect of coordinating interneuron inhibition on the period of a half-center oscillator appears to depend on its phase with respect to the oscillator interneurons. To test this idea directly, we added cyclical barrages of inhibition to a half-center oscillator (Fig. 13, A and B). In a series of trials, we varied the cycle period of the inhibitory input and, after the system settled into a stable phase relationship, determined the phase between the input and the oscillator interneurons. We found that when the period of the inhibitory input was near that of the half-center oscillator, the inhibitory bursts had a phase of about 40% (Fig. 13C). At this phase, the barrages fall near the beginning of the inhibited phase of the oscillator interneurons, where the inhibition neither speeds nor slows the oscillations (not shown). When the period of the input was less than that of the half-center oscillator, the phase decreased (Fig. 13C), and the barrages overlapped with the trailing ends of the bursts in the oscillator interneurons (Fig. 13A). With longer periods, the phase of the input was greater, demonstrating that entrainment to longer periods requires late inhibition (Fig. 13, B and C). The upper limit of entrainment occurred when the period was near that of the canonical segmental oscillator (Fig. 13C). This experiment confirms that the phase of inhibitory input determines its effect on period.

**DISCUSSION**

We have tested two models (asymmetric and symmetric) that represent two simple conceptualizations of the heartbeat timing neuronal network. These models help us understand how the system may work in two ways. First, they demonstrate plausible biophysical mechanisms that could underlie intersegmental coordination. And second, they make predictions that can be tested against the behavior of the biological system. The next paper in this series (Masino and Calabrese 2002b) compares the behavior of the biological system to these two models.

**Difference in the inherent periods of the segmental oscillators leads to a phase difference**

In the symmetric model, when two segmental oscillators with different inherent periods are mutually entrained, the faster one leads in phase, regardless of whether it is the G3 or G4 segmental oscillator. In addition, the absolute size of the phase difference depends on the size of the period difference: the larger the period difference, the larger the absolute phase difference. Also, the model shows stable phase relationships over a large range of phase values from about −25 to +20%. This wide range of possible phase values corresponds well with the range of values observed in the biological system, which are between −10 and +20% (Masino and Calabrese 2002a). In contrast, in the asymmetric network, entrainment only occurs when the G3 segmental oscillator leads the G4 segmental oscillator (negative phase values). Thus the symmetric model corresponds better to the biological system than the asymmetric model in terms of mutual entrainment.

**Removal of inhibition accounts for the ability of the faster segmental oscillator to entrain the slower segmental oscillator**

In the symmetric network, when two segmental oscillators with different inherent periods are coupled, the period of the mutually entrained system is equal to that of the faster segmental oscillator. This property arises because, cycle by cycle, the oscillator interneurons of the faster segmental oscillator burst before the oscillator interneurons of the slower segmental oscillator. The faster oscillator interneurons thereby inhibit the coordinating interneurons, and there is a brief period of time...
when the slower oscillator interneurons are relieved of coordinating interneuron inhibition. This removal of inhibition causes the slower oscillator interneurons to be sped to the period of the faster oscillator interneurons. The limit to which the faster segmental oscillator can speed the system is the half-center oscillator period of the slower oscillator.

In the asymmetric network, there is an additional mechanism by which the G3 segmental oscillator can speed the G4 segmental oscillator. In this network the bursts of the coordinating interneurons can overlap temporally with the trailing ends of the bursts in the ipsilateral G4 oscillator interneuron. This overlap causes a decrease in the spike frequency, allowing the contralateral G4 oscillator interneuron to advance to its burst phase earlier. Cycle by cycle, this early transition to the burst phase allows the G4 segmental oscillator to be sped faster than its half-center period.

**Phase difference between the segmental oscillators can lead to a shift in phase of the coordinating interneurons**

In both the symmetric and asymmetric models the phase of the coordinating interneurons shifts as the phase changes between the segmental oscillators. In the mutually entrained symmetric network, a change in the phase between the oscillator interneurons \((\phi_3 - \phi_4)\) leads to a shift in the phase of the coordinating interneurons with respect to both the G3 and the G4 oscillator interneurons. For example, if \(\phi_3 - \phi_4\) increases, \(\phi_2 - \phi_4\) increases with a slope of 0.5, and \(\phi_0 - \phi_3\) decreases with a slope of \(-0.5\). The fact that the absolute values of these slopes are equal reflects the ability of both the G3 and G4 oscillator interneurons to inhibit the coordinating interneurons. In contrast, in the asymmetric network, as \(\phi_3 - \phi_4\) increases, \(\phi_2 - \phi_4\) increases with a slope of 1.0, and \(\phi_0 - \phi_3\) stays constant (a slope of 0), reflecting the ability of the G3 oscillator interneurons alone to inhibit the coordinating interneurons.

The phase relationships in the biological system are intermediate between those of the two models. In the biological system, an increase in \(\phi_3 - \phi_4\) leads to an increase in \(\phi_2 - \phi_4\) with a slope of 0.7 and a decrease in \(\phi_0 - \phi_3\) with a slope of \(-0.3\) (Masino and Calabrese 2002a). This result may reflect the observation that the G3 oscillator interneurons inhibit both spike initiation sites of the coordinating interneurons, whereas the G4 oscillator interneurons inhibit only their G4 sites (Masino and Calabrese 2002a; Peterson 1983b).

**In driving experiments, entrainment only occurs when the driven segmental oscillator leads in phase**

We did modeling experiments that replicate biological experiments in which a driving stimulus, consisting of repetitive current pulses applied to a single heart interneuron, can entrain the entire heartbeat network (Peterson 1983b; Peterson and Calabrese 1982). A major difference between the two models is that in the symmetric model both the G3 and G4 oscillators are equal in their ability to drive the system, while in the asymmetric model only the G3 oscillator can drive the system. In other respects, the two models are quite similar. In both models, in order for entrainment to occur, the driven oscillator must lead in phase and therefore must have a faster inherent period than the follower segmental oscillator. The mechanisms of entrainment are identical to those of the mutually entrained system. Thus in the symmetric network, the driven oscillator can speed the follower oscillator to its half-center oscillator period. While in the asymmetric network, the driven G3 oscillator can entrain the G4 oscillator to shorter periods than its half-center oscillator period.

In both models, the system can only be driven slower than the mutually entrained period if the driven oscillator originally leads in phase. This result is consistent with the removal of inhibition mechanism. When the leading oscillator is driven slower, the phase difference between the segmental oscillators becomes smaller. This decrease in phase causes less inhibition to be removed from the slower, follower oscillator, leading to an increase in period. The limit to which the system can be slowed is the segmental oscillator period of follower oscillator.

A major finding of these driving experiments is that the driven oscillator could not entrain the system to periods greater than the segmental oscillator period of the follower oscillator. While the driven oscillator can remove inhibition from the follower oscillator and, thereby, speed the system, there is no mechanism by which the driven oscillator can add inhibition to the system to slow it.

**Both the symmetric and the asymmetric model conform to the excitability-gradient hypothesis**

In both models, a phase difference is generated if there is a difference in the inherent periods of the two segmental oscillators. This result is consistent with the excitability-gradient hypothesis, which has been proposed as a mechanism underlying intersegmental phase differences in a number of systems (Grillner et al. 1993; Ikeda and Wiersma 1964; Matsushima and Grillner 1990, 1992). Furthermore, a phase difference can only be generated by a period difference in either model. For example, even in the asymmetric network, the G3 segmental oscillator must have a shorter inherent period than the G4 segmental oscillator for a phase difference to arise.

**Comparison with other cellular level models of intersegmental coordination**

Models of intersegmental coordination at the level of individual neurons and their interconnections have been created for a number of systems. For example, Skinner and Mulloney (1998b) used simulations to study the neuronal network that underlies swimmeret beating in the crayfish. They tested a number of models of intersegmental coordination suggested by experimental data as well as the predictions of a phase-coupled oscillator model (Skinner et al. 1997) and found that only one behaved similarly to the biological system. The architecture of this model is very different from that of the models we have presented here. In the crayfish model, the coordinating interneurons do not contribute to rhythm generation in their home ganglion. Also, the ascending and descending connections are asymmetric: they have different postsynaptic targets, and their signs are not symmetric.

Cellular level models have also been created of the neuronal network that generates the motor pattern for swimming in the lamprey. This network is thought to consist of interneurons and motor neurons that can be divided into specific types based on their physiological properties and their connectivity to other neurons of the pattern generator (Grillner et al. 1998). A
simulation of the segmental circuit could account for the observed phase relationships between cell types and the motor output of a single segment (Wallén et al. 1992). Intersegmental coordination was modeled by adding connections between these classes of neurons in different segments. For example, models have been created in which excitatory interneurons make excitatory ipsilateral connections over an equal number of ascending and descending segments, while inhibitory interneurons make contralateral inhibitory connections that extend further in the descending direction (Hellgren Kotalaski et al. 1999; Wadden et al. 1997). These models show that this type of asymmetry can lead to a rostrocaudal phase lag, as occurs in forward swimming. However, unlike the biological network, the phase lag increases with a decrease in cycle period (Wadden et al. 1997). A characteristic feature of the lamprey swim circuit is that interneurons that participate in rhythm generation within their home segment also act as coordinating interneurons. This feature is similar to the leech heartbeat timing network. An important difference is that the coordinating interneurons in the leech have multiple spike initiation zones.

In summary, the models we have presented demonstrate plausible mechanisms that may underlie coordination of segmental oscillators in the leech heartbeat timing network. These models differ markedly in architecture from those proposed for other systems, suggesting that perhaps intersegmental coordination can be accomplished by a number of very different network configurations.

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