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Forcing of Coupled Nonlinear Oscillators: Studies of Intersegmental Coordination in the Lamprey Locomotor Central Pattern Generator

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SUMMARY AND CONCLUSIONS

1. This paper reports the results of an investigation of the basic mechanisms underlying intersegmental coordination in lamprey locomotion, by the use of a combined mathematical and biological approach.

2. Mathematically, the lamprey central pattern generator (CPG) is described as a chain of coupled nonlinear oscillators; experimentally, entrainment of fictive locomotion by imposed movement has been investigated. Interpretation of the results in the context of the theory has allowed conclusions to be drawn about the nature of ascending and descending coupling in the lamprey spinal CPG.

3. Theory predicts and data show that *1*) the greater the number of oscillators in the chain, the smaller is the entrainment frequency range and *2*) it is possible to entrain *both* above *and* below the rest frequency at one end but *only* above *or* below at the other end.

4. In the context of the experimental results, the theory indicates the following: *1*) ascending coupling sets the intersegmental phase lags, whereas descending coupling changes the frequency of the coupled oscillators; *2*) there are differences in the ascending and descending coupling other than strength; and it also suggests that *3*) coupling slows down the oscillators.

INTRODUCTION

One approach to the investigation of mechanisms underlying central pattern generation has been to analyze cellular and network properties and to use this information to understand how the system works. This approach has been successful with invertebrate preparations, such as leech (Calabrese and Peterson 1983; Kristan and Weeks 1983), *Tritonia* (Getting 1983), and lobster (Marder and Meyrand 1989; Selverston and Moulin 1986). The cellular approach to studying central pattern generation in vertebrates is, however, much more difficult because of the large number of cells and connections that must be identified. Nonetheless, the cellular approach has been successful in simple vertebrate systems for elucidating the mechanisms of basic rhythm generation and left-right coordination (*Xenopus* embryo, Roberts et al. 1986; lamprey spinal cord, Alford and Williams 1989; Grillner et al. 1988). There is, however, no understanding of the mechanisms underlying intersegmental coordination. Even in simple invertebrate systems, the behavior of the network is not always easily explained on the basis of the properties of the component neurons (Marder 1989). Similarly, in vertebrates, even if the details of the network were known, the complexity might not allow one to understand the emergent properties of the system.

In this paper we present a different approach. We shall use a general mathematical treatment of coupled nonlinear oscillators developed by Kopell and Ermentrout (1986, 1988, 1990). First we will describe the mathematical framework as it relates to what is known about the lamprey spinal cord. Then we will present the results of experiments designed to test and refine the theory. This interaction between mathematics and biology has allowed us to reach conclusions about some properties of intersegmental coordination in the lamprey spinal cord.

Lamprey locomotor CPG can be described as a single chain of coupled nonlinear oscillators

Swimming in the lamprey is characterized by rhythmic bursts of activity in ventral roots, which alternate left and right and have a rostral-caudal intersegmental delay of ~1% of a cycle per segment. The phase lag is independent of cycle frequency (which determines swimming speed). The centrally generated pattern of ventral root activity recorded during fictive locomotion in the isolated spinal cord is the same as that seen in the intact swimming animal (Wallén and Williams 1984). The networks producing this oscillation are distributed along the cord, since as few as two segments taken from anywhere along the cord can produce oscillatory behavior (Grillner et al. 1982b). During stable fictive locomotion, the frequency is uniform along the cord, and the phase relationships between segments are constant; i.e., the oscillators are coupled (Stein 1974). Thus the CPG for lamprey locomotion can be described as a chain of coupled oscillators, with the activity recorded from the ventral roots representing the motor output of the oscillator chain.

Following experimental perturbation of fictive locomotion, the activity resumes its original oscillatory behavior within a few cycles (see, for example, Fig. 2 in McClellan and Sigvardt 1988; Fig. 5 in Rovainen 1985). Such behavior is characteristic of nonlinear oscillators with strongly attracting limit cycles (Hirsch and Smale 1974). Having a limit cycle means that for a given level of activation, there

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is a fixed rhythmic pattern of neural activity; when the oscillator is perturbed the activity may change, but when the perturbation is removed, it returns to its original frequency and pattern. Only a nonlinear oscillator can have a limit cycle; the fact that the pattern goes back to normal within a cycle or two means the limit cycle is strongly attracting.

Our general mathematical framework considers a single chain of coupled oscillators (Kopell and Ermentrout 1986, 1988, 1990). The spinal cord would be more precisely modeled as a double chain of oscillators, corresponding to the left and right sides of the cord. However, since the left-right coordination is strictly antiphasic, the model considers each segment as a single oscillator with an output to both the left and the right sides. The double chain can be shown to have the same behavior as a single chain.

Each oscillator in the chain can be thought of as a network of neurons having three kinds of output: *1*) the motoneuron activity that activates the muscles and that can be recorded from the ventral roots; *2*) the ascending intersegmental coupling signals, whereby one oscillator affects its rostral neighbor; and *3*) the descending coupling signals, which affect the more caudal oscillator in the chain. The observed intersegmental phase lag is a result of the intersegmental coupling. The ventral root output can be measured but not the ascending and descending coupling signals, since the neurons involved have not been identified. In this study we show how a mathematical analysis of the effects of perturbing the system with externally applied movement allows conclusions to be drawn about the ascending and descending coupling.

Each oscillator can be described by a single dependent variable $\Theta(t)$

The output of each oscillator depends on the interactions of all its component neurons. For a particular frequency, ω_k, the kth oscillator has a particular behavior (its limit cycle). A complete description of this behavior could be given by a set of equations describing the properties of all the neural elements as functions of time. All these equations would be periodic, with the same overall frequency, ω_k, corresponding to the swimming cycle period, τ_k. At any time, t, between 0 and τ_k, there will be a set of values for all these properties, representing the state of the oscillator at that time in its progression through the cycle.

Such detailed properties are not known. The power of our general mathematical approach is that it allows us to study some characteristics of the intersegmental coordinating system without knowing the detailed properties of the network. We can replace this ensemble of variables by a single variable $\Theta_k(t)$, representing the state of the kth oscillator at time t and corresponding to its progression through the cycle. Θ_k takes on values from 0 to 2π as t goes from 0 to τ_k, the cycle period.

The instantaneous frequency of the oscillator is given by

$$d\Theta_k/dt = \omega_k$$

Effect of coupling between two oscillators depends on their phase difference

In the mathematical framework developed by Kopell and Ermentrout (1986, 1988, 1990), the kth oscillator can affect the instantaneous frequency of its rostral or caudal neighbor, $d\Theta_{k-1}/dt$ or $d\Theta_{k+1}/dt$. The effect of one oscillator on another depends on how far each has progressed through its cycle, i.e., the effect of oscillator k on oscillator $k + 1$ depends on Θ_k and Θ_{k+1}. Similarly, the effect of oscillator k on oscillator $k - 1$ will depend on Θ_k and Θ_{k-1}.

It has been shown (Sanders and Verhulst 1985) that if the effect of one oscillator on the instantaneous frequency of another is averaged over the entire cycle, then this effect can be expressed as a function of the phase difference between the two oscillators, rather than on the Θs independently. This averaging technique has been shown to be valid if the coupling signals are dispersed around the cycle rather than occurring at only one or two points within the cycle (Ermentrout and Kopell 1990b). For example, the averaging technique can be used if there are as few as three signals during a cycle as long as the signals are evenly spaced around the cycle. This seems a reasonable assumption for vertebrate locomotor CPGs, since the oscillatory activity is likely to be produced by a network of neurons, active in different phases of the cycle. Either these neurons and/or their postsynaptic elements can be expected to participate in the coupling. Furthermore, it has been shown that if the coupling signals are not so dispersed, then the network may not oscillate and can, for example, lock into tonic activity or silence (Ermentrout and Kopell 1990a). Therefore the stability of the oscillation can itself be taken as evidence that the coupling signals are dispersed through the cycle and that the effect of coupling can be expressed as a function of phase difference only.

The frequency of the kth oscillator can thus be represented by

$$d\Theta_k/dt = \omega_k + H^+(\Theta_{k+1} - \Theta_k) + H^-(\Theta_{k-1} - \Theta_k) \qquad (1)$$

where H^+, the ascending coupling function, and H^-, the descending coupling function, depend only on the phase difference between the coupled oscillators (see Fig. 1).

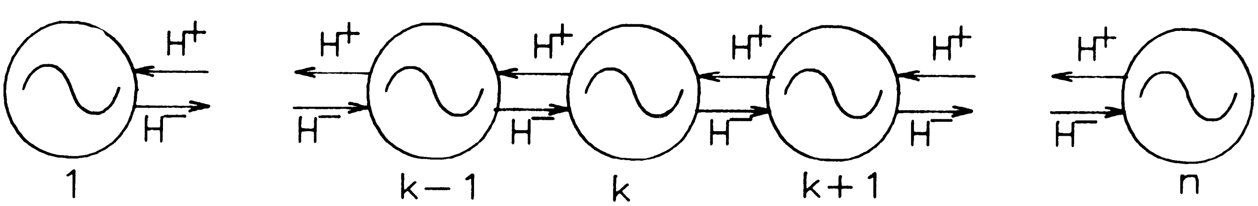

FIG. 1. Lamprey spinal cord represented as a chain of coupled nonlinear oscillators. Each oscillator sends signals to its rostral neighbor via ascending fibers and to its caudal neighbor via descending fibers. These signals are represented by the ascending and descending coupling functions $H^+(\phi)$ and $H^-(-\phi)$, respectively.

The behavior of the chain of coupled oscillators corresponds to the solution of a set of differential equations

During stable swimming all the oscillators in the chain are entrained to the same frequency. In the mathematical formulation this is equivalent to the requirement that $d\Theta_k/dt$ for all k be equal to some ensemble frequency Ω. It is known that in the lamprey there can be substantial experimental variation in ω along the cord, but there is no *systematic* variation (Cohen 1987b). As an approximation we assume that at a given speed of swimming all the oscillators in the chain have the same intrinsic frequency, ω.

The intersegmental phase lag, i.e., the difference in phase between adjacent oscillators, will be represented by $\phi_k = \Theta_{k+1} - \Theta_k$. From this definition it follows that $\phi_{k-1} = \Theta_k - \Theta_{k-1}$ and thus that $\Theta_{k-1} - \Theta_k = -\phi_{k-1}$. Substituting in *Eq. 1*, and adding equations for the first and nth oscillator, the behavior of the network during stable swimming can be described by

$$\Omega = \omega + H^{+}(\phi_1) \tag{2}$$

$$\Omega = \omega + H^{+}(\phi_k) + H^{-}(-\phi_{k-1}) \quad 1 < k < n \tag{3}$$

$$\Omega = \omega + H^{-}(-\phi_{n-1}) \tag{4}$$

For a given value of intrinsic frequency ω, this constitutes n equations in n variables $(\phi_1, \ldots, \phi_{n-1}, \Omega)$.

The most rostral oscillator is affected only by ascending coupling (*Eq. 2*) and the most caudal one only by descending coupling (*Eq. 4*). The minus sign before ϕ_{k-1} in *Eqs. 3* and *4* results from the fact that for both coupling functions, the phase delay is taken (by convention) as the difference between the phase of the oscillator sending the coupling signals and the oscillator receiving them (see *Eq. 1*). Because ϕ_k was defined as the phase advance from an oscillator to its more caudal neighbor, $\phi < 0$ corresponds to a rostral-caudal delay, i.e., activity traveling from head to tail. Thus the mathematical description of stable forward swimming in the lamprey corresponds to steady-state solutions of *Eqs. 2–4* with $\phi_k < 0$.

There are ascending and descending coupling functions that can provide stable phase coupling

Little is known of the mechanisms underlying ascending and descending coupling in the lamprey. However, without specifying particular coupling functions H^+ and H^-, the mathematical framework allows us to investigate the properties of possible coupling functions, i.e., the conditions on H^+ and H^- under which *Eqs. 2–4* have solutions corresponding to the known properties of lamprey locomotion.

Kopell and Ermentrout (1986, 1988, 1990) have shown, both analytically and numerically, that there is a large class of coupling functions for which there are solutions that behave like known biological networks, i.e., solutions with stable, nonzero phase coupling between the oscillators. This class of coupling functions has the following characteristics:

i) There is a range of values of ϕ over which $H^+(\phi)$ is monotonically increasing and $H^-(-\phi)$ is monotonically decreasing.

ii) Within this range there are zero crossings for $H^+(\phi)$ and $H^-(-\phi)$.

These properties lead to solutions of *Eqs. 2–4* with stable phase coupling.

We define the zero-crossing phase values as follows:

ϕ_R = intersegmental phase lag at which the zero crossing of ascending coupling function occurs, i.e., $H^+(\phi_R) = 0$

ϕ_L = the intersegmental phase lag at which the zero crossing of descending coupling function occurs, i.e., $H^-(-\phi_L) = 0$.

We define another function $f(\phi)$ as the average of $H^+(\phi)$ and $H^-(-\phi)$, i.e.,

$$f(\phi) = \tfrac{1}{2}[H^{+}(\phi) + H^{-}(-\phi)]$$

We note by $f'(\phi)$, the first derivative of f with respect to ϕ. The class of coupling functions then has the following additional characteristic:

iii) at least one of the following holds

$$f'(\phi_L) < 0 \quad \text{or} \quad f'(\phi_R) > 0$$

This condition again holds for a large class of coupling functions. If the condition is not met, the phase lags along

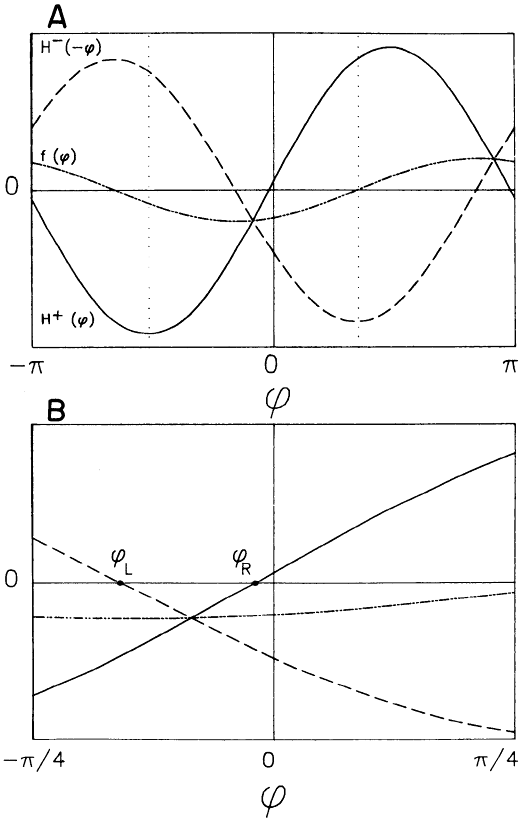

FIG. 2. An example of a pair of ascending and descending coupling functions $H^+(\phi)$ and $H^-(-\phi)$ that have the 5 necessary characteristics (see text). *A*: coupling functions $H^+(\phi)$ and $H^-(-\phi)$ and $f(\phi)$ for ϕ over the entire range from $-\pi$ to $+\pi$. The dotted lines enclose the range of ϕ over which the conditions are met, i.e., the range over which a stable solution to *Eqs. 2–4* can occur. *B*: same coupling functions for ϕ from $-\pi/4$ to $+\pi/4$, showing the zero crossing points, ϕ_L and ϕ_R. Solid line represents $H^+(\phi)$; dashed line, $H^-(-\phi)$; broken line, $f(\phi)$. The coupling functions used are

$$H^{+}(\phi) = 1.1 \sin(\phi + \pi/50) \quad \text{and} \quad H^{-}(-\phi) = -\sin(\phi + \pi/6)$$

the cord could be zero and thus would not match what is seen in the lamprey.

In Fig. 2 is shown one example of a pair of functions $H^+(\phi)$ and $H^-(-\phi)$ that have these three properties.

Swimming frequency and intersegmental phase lag depend on characteristics of ascending and descending coupling

It has been shown mathematically that the intersegmental phase lags (ϕ_k), which occur in the solution to *Eqs. 2-4* are approximately equal for all k except within a boundary layer at the rostral or caudal end (Kopell and Ermentrout 1986, 1990). This boundary layer is quite small if n is large ($\sim$20). In other words, the intersegmental phase delay is constant along the cord, except for a segment or two at one end or the other.

It can be shown that the value taken by the intersegmental phase lag ϕ_k (except for k near 1 or n) is near the zero crossing of either the ascending or descending coupling function. Which value it is near, ϕ_L or ϕ_R, depends on the relative magnitudes of each coupling function at the zero crossing of the other. If $|H^+(\phi_L)| > |H^-(-\phi_R)|$, the value of the intersegmental phase lag ϕ_k becomes approximately ϕ_R, the zero crossing of the ascending coupling. Thus in this case it is the ascending coupling that determines the intersegmental phase lag, and we say that the ascending coupling "dominates." If, on the other hand, $|H^-(-\phi_R)| > |H^+(\phi_L)|$ then descending coupling dominates and the intersegmental phase lag is approximately ϕ_L. If $|H^-(-\phi_R)| = |H^+(\phi_L)|$ then the phase lags need not be uniform down the cord and would be very sensitive to small changes in coupling and local differences in frequency (Kopell and Ermentrout 1990). Within the context of this theory, the uniformity of the intersegmental phase lags along the lamprey spinal cord and their stability indicates that one of the two coupling functions is dominant.

Thus, under our hypotheses, the steady-state chain of oscillators operates with phase lags nearly equal to the zero crossing of the dominant coupling. One of the terms in *Eq. 3* is then very small. If the ascending coupling dominates, for large n, the ensemble frequency then becomes approximately

$$\Omega = \omega + H^-(-\phi_R) \tag{5}$$

If the descending coupling dominates, it becomes approximately

$$\Omega = \omega + H^+(\phi_L) \tag{6}$$

The experiments reported here suggest that ascending coupling dominates in the lamprey spinal CPG.

Entrainment experiments provide information about intersegmental coupling

Movement-generated sensory feedback (Grillner et al. 1982a) may contribute to the control of the relative timing between neural activation and the development of curvature during swimming (Williams et al. 1989). In the in vitro spinal cord, imposed movement can entrain the centrally generated rhythm (Grillner et al. 1981). Such entrainment by mechanosensory feedback occurs locally, at the point where the bending is applied, and the intersegmental coordinating system is responsible for the entrainment of the rest of the spinal cord (McClellan and Sigvardt 1988). Thus it has been possible in the present study to gain information about intersegmental coupling from an investigation of some of the properties of entrainment.

METHODS

Preparation

Experiments were performed on spinal cords from nine adult lampreys (*Ichthyomyzon unicuspis*). Animals were anesthetized in tricaine methylsulphate. The 50 segments of spinal cord/notochord just caudal to the last gill slit were removed and placed in a Sylgard-lined preparation dish in lamprey saline, cooled to 7–9°C. The notochord under segments 5–7, 18–20, 30–32, and 43–45 was pinned firmly to the bottom of the dish (see Fig. 3). Experiments were performed on the entire 50-segment piece, after which the preparation was cut between segments 25 and 26, and experiments performed on both the rostral and the caudal 25-segment piece. In the 50-segment piece, saline-filled glass electrodes were placed on ventral roots 8, 15, 25, 34, and 42. In the 25-segment pieces, an additional electrode was placed on a ventral root between the rostral or caudal two electrodes (see Fig. 3). For each animal, up to six experiments were performed: rostral and then caudal forcing of the 50-segment piece, rostral and caudal forcing of the rostral 25-segment piece, and rostral and caudal forcing of the caudal 25-segment piece.

Experimental protocol

In each experiment the arm of a Gould servocontrolled pen motor was attached to one of the free ends of the particular spinal cord/notochord piece under study. Fictive locomotion was induced by the addition of 0.5 mM D-glutamate to the bathing solution. The end of the preparation attached to the motor arm was moved side to side by driving the motor with a sinusoidal electric signal. During such imposed movement, ventral root activity and the output of the servomotor representing the movement were recorded. For each experiment, the highest and lowest frequencies were determined for which one-to-one entrainment could be maintained for at least 30 cycles, and these frequencies were taken as the limits of the entrainment range. Before each experiment, control activity (no movement) was recorded, and the rest rate determined. Approximately 100 cycles of activity for each experimental condition were analyzed by the use of either Cambridge Electronic Devices or RC Electronics software. Cycle periods and phase lags from movement to the midpoint of the ventral root bursts were determined.

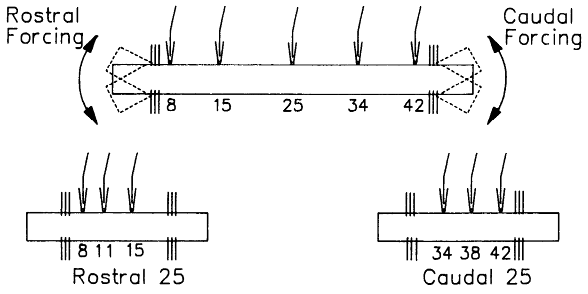

FIG. 3. Diagram of the in vitro lamprey spinal cord preparation. The activity in several ventral roots from a 50-segment piece of spinal cord was recorded while either the rostral or caudal free end of the preparation was moved from side to side. After measurements of the entrainment ranges, the 50-segment piece of cord was cut in the middle, and the experiments repeated on both the rostral and caudal 25 segments.

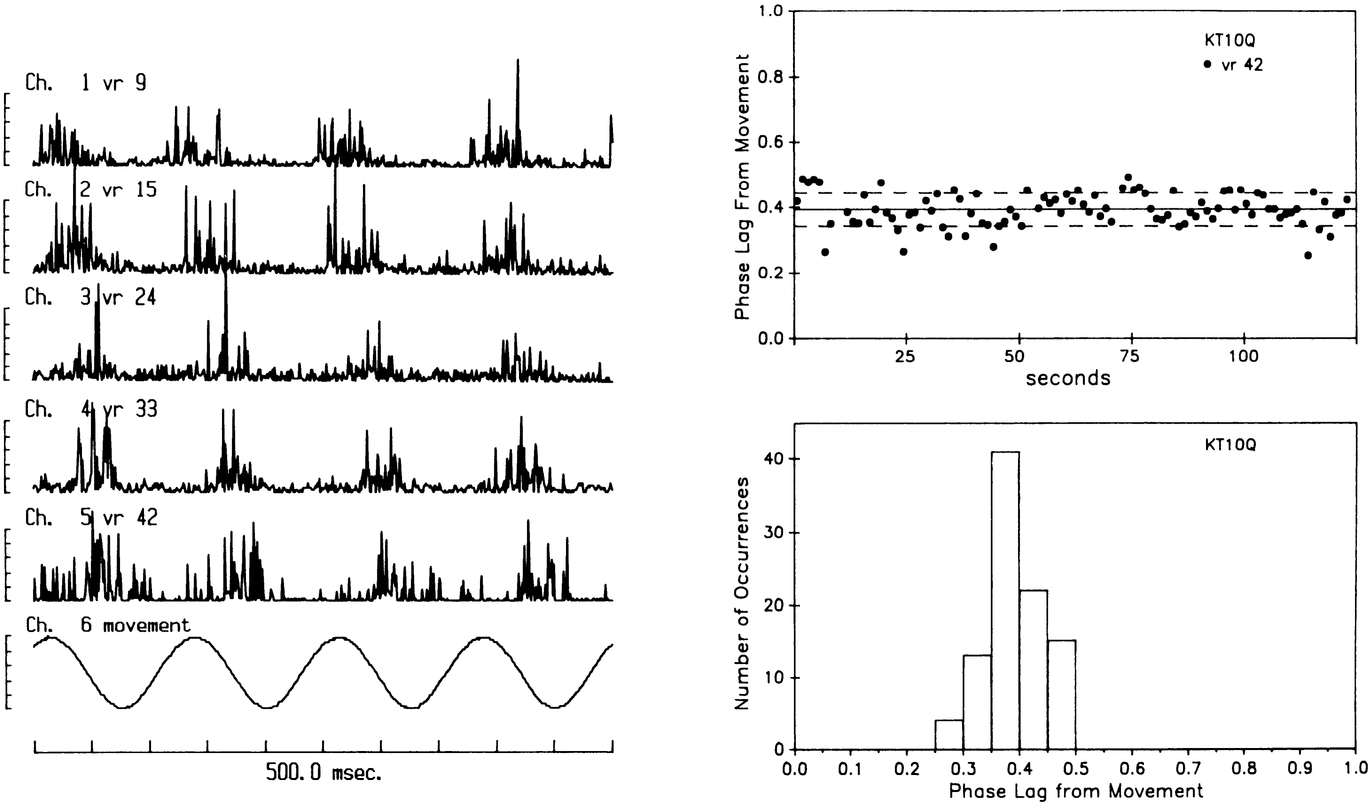

FIG. 4. Entrainment of ventral root activity of a 50-segment piece of spinal cord entrained by side-to-side movements of the caudal end of the spinal cord/notochord at 0.8 Hz. The rest rate for this spinal cord preparation was 0.7 Hz. *Left*: activity recorded from 5 ventral roots (vr) along the cord is entrained one to one with the movement. *Top right*: phase lag from movement to the midpoint of the ventral root burst in vr 42 is ~0.4 throughout the 100 cycles of recorded activity. Solid line represents the mean phase lag; dashed line, one standard deviation about the mean. *Bottom right*: frequency histogram shows the preferred phase lag is between 0.35 and 0.40.

RESULTS

Figure 4 illustrates data collected during an experiment with a 50-segment piece on which bending was imposed at segment 45. It can be seen that the activity recorded from each of the five ventral roots is entrained and that the rostral-caudal delay characteristic of locomotion is preserved. The phase lag from the movement to the ventral root burst remains relatively constant over the 100 cycles of activity as shown plotted against time and in a phase histogram in Fig. 4. Similar phase plots were obtained for all frequencies in all experiments reported.

Mechanical entrainment of the lamprey spinal cord in vitro corresponds mathematically to a forced chain of oscillators

We now add a forcing function $H_F(\phi_M)$ to our equations, where ϕ_M is the phase lag between the imposed movement and the local oscillator. This function is added to the first

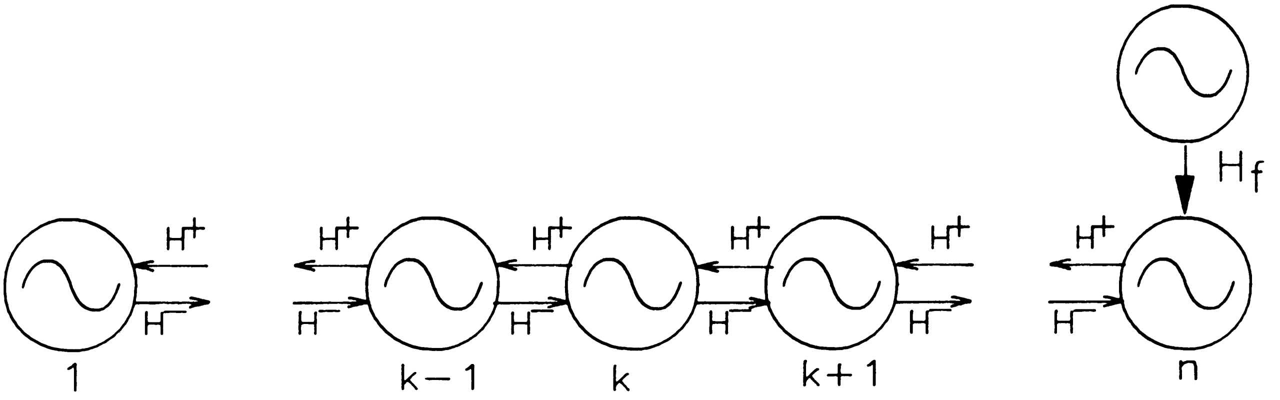

FIG. 5. The entrainment of lamprey spinal cord activity by movement at the caudal end as represented by a chain of oscillators, the last oscillator of which is forced by a periodic forcing function, H_F.

equation, for forcing at the rostral end, or to the *n*th equation, for forcing at the caudal end (Fig. 5).

$$
\begin{array}{ll}
\text{Rostral forcing} & \text{Caudal forcing} \\
\Omega = \omega + H^{+}(\phi_1) + H_{\mathrm{F}}(\phi_{\mathrm{M}}) & \Omega = \omega + H^{+}(\phi_1) \\
\Omega = \omega + H^{+}(\phi_2) + H^{-}(-\phi_1) & \Omega = \omega + H^{+}(\phi_2) + H^{-}(-\phi_1) \\
\cdot & \cdot \\
\Omega = \omega + H^{+}(\phi_k) + H^{-}(-\phi_{k-1}) & \Omega = \omega + H^{+}(\phi_k) + H^{-}(-\phi_{k-1}) \\
\cdot & \cdot \\
\Omega = \omega + H^{+}(\phi_{n-1}) + H^{-}(-\phi_{n-2}) & \Omega = \omega + H^{+}(\phi_{n-1}) + H^{-}(-\phi_{n-2}) \\
\Omega = \omega + H^{-}(-\phi_{n-1}) & \Omega = \omega + H^{-}(-\phi_{n-1}) + H_{\mathrm{F}}(\phi_{\mathrm{M}})
\end{array}
$$

As in the unforced case, we have n equations in n variables. We have introduced an extra variable, ϕ_{M}, the phase lag between the movement and the local oscillator. However, the ensemble frequency Ω is no longer unknown; it is set by the frequency of the applied movement.

Compared with the equations for the unforced chain (*Eqs. 2–4*), it is easier to see how the equations for the forced chain are satisfied for given values of the intrinsic frequency ω and the imposed frequency Ω. For example—for caudal forcing—if we are within the frequency range for which entrainment can occur (i.e., where the equations have a solution), we can understand the steady-state solution as follows.

The first equation (for caudal forcing) has only one variable ϕ_1, which will take on the value for which $H^{+}(\phi) = \Omega - \omega$. This value of ϕ_1 then determines the value of $H^{-}(-\phi_1)$ in *Eq. 2* and, therefore, the value required for $H^{+}(\phi_2)$ and of ϕ_2. The values of ϕ_k up to ϕ_{n-1} are determined similarly. This leaves the nth equation, which now determines the value of ϕ_{M}.

The above description applies to the steady state, and not to the "on transient." When the motor is turned on, the effect of forcing is felt first at the segment forced, and the effects work their way along the chain until the steady state is reached.

Theory predicts and data show that there is a range of frequencies over which fictive locomotion can be entrained by imposed movement

Expansion of the mathematical framework to allow for forcing of chains of oscillators requires that the coupling functions have two additional characteristics (Kopell et al. 1990a).

We note by $f''(\phi)$, the second derivative of ϕ.

iv) In the range in which $H^{+}(\phi)$ is monotonically increasing and $H^{-}(-\phi)$ is monotonically decreasing (e.g., Fig. 2), $f''(\phi) \neq 0$.

This is a condition of nonlinearity. Because $H^{+}(\phi)$, $H^{-}(-\phi)$, and $f(\phi)$ are periodic, they must be nonlinear. Condition *iv)* specifies that the sign of the dominant nonlinearity does not change over the relevant interval. This is true for a large class of coupling functions.

v) Within the range over which $H^{+}(\phi)$ is monotonically increasing and $H^{-}(-\phi)$ is monotonically decreasing, there must be two values of ϕ for which $f(\phi) = f(\phi_{\mathrm{L}})$ and two for which $f(\phi) = f(\phi_{\mathrm{R}})$.

TABLE 1. *Summary of entrainment ranges*

			Entrainment Rates	
Expt. No.	Forcing	Rest Rate, Hz	Low	High
KT2				
Whole cord	Rostral forcing	0.56	0.58	0.70
	Caudal forcing	0.50	0.48	0.58
Rostral cord	Rostral forcing	0.80	0.72	0.84
Caudal cord	Caudal forcing	0.75	0.70	0.98
KT3				
Whole cord	Caudal forcing	0.80	0.78	0.89
Rostral cord	Caudal forcing	0.80	0.94	1.02
Caudal cord	Rostral forcing	0.80	0.74	1.02
	Caudal forcing	0.70	0.82	1.00
KT4				
Whole cord	Rostral forcing	0.55	0.55	0.64
	Caudal forcing	0.60	0.45	0.80
Rostral cord	Rostral forcing	0.55	0.56	0.66
	Caudal forcing	0.40	0.20	0.34
Caudal cord	Rostral forcing	0.60	0.56	0.64
	Caudal forcing	0.60	0.34	0.86
KT5				
Whole cord	Rostral forcing	0.65	0.56	0.80
Rostral cord	Rostral forcing	0.60	0.86	1.00
	Caudal forcing	0.55	0.38	0.66
Caudal cord	Rostral forcing	0.85	0.86	1.00
	Caudal forcing	0.85	0.84	1.08
KT7				
Whole cord	Rostral forcing	0.80	0.82	0.90
	Caudal forcing	0.85	0.76	0.86
Rostral cord	Rostral forcing	0.60	0.68	0.72
	Caudal forcing	0.65	0.34	0.94
Caudal cord	Rostral forcing	0.65	0.54	0.76
	Caudal forcing	0.65	0.64	0.94
KT8				
Whole cord	Rostral forcing	0.30	0.26	0.38
	Caudal forcing	0.33	0.32	0.50
Rostral cord	Rostral forcing	0.50	0.46	0.54
	Caudal forcing	0.45	0.34	0.48
Caudal cord	Rostral forcing	0.38	0.50	0.70
KT9				
Whole cord	Rostral forcing	0.55	0.65	0.72
	Caudal forcing	0.55	0.57	0.68
Rostral cord	Rostral forcing	0.80	0.75	0.86
	Caudal forcing	0.70	0.35	1.00
Caudal cord	Rostral forcing	0.75	0.61	0.98
	Caudal forcing	0.75	0.56	1.20
KT10				
Whole cord	Rostral forcing	0.60	0.66	0.80
	Caudal forcing	0.70	0.70	0.90
Rostral cord	Rostral forcing	0.90	0.95	1.15
	Caudal forcing	1.00	0.65	1.65
Caudal cord	Rostral forcing	0.90	0.86	1.35
	Caudal forcing	0.90	0.84	1.50
KT11				
Whole cord	Rostral forcing	0.85	0.92	1.00
	Caudal forcing	0.85	0.87	1.15
Caudal cord	Rostral forcing	1.00	0.99	1.30
	Caudal forcing	1.00	0.82	1.70

As shown by Kopell, Ermentrout, and Williams (1990a), this condition is necessary to allow the chain of oscillators to be forced over the large frequency ranges seen in the present experiments. If *v)* is not met and the chain is long, the range of entrainment is vanishingly small for forcing at one of the ends. These conditions hold for the functions illustrated in Fig. 2.

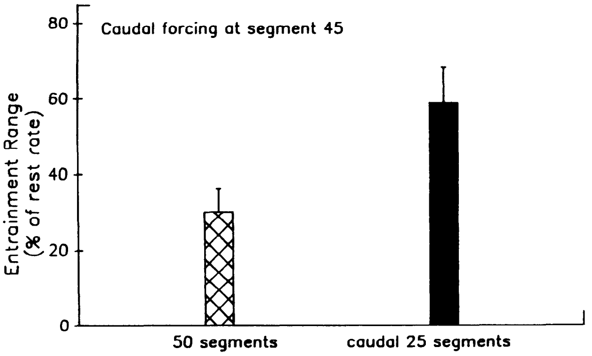

FIG. 6. Dependence of size of entrainment range on length of cord. The 50-segment cord is compared with the 25-segment cord in which bending was applied at the same place, segment 45. Averaged data from experiments listed in Table 1. $n = 8$.

It should be noted that the equations can be solved for individual coupling functions that do not satisfy the above conditions. It has been shown, however, that such coupling functions need not in general lead to uniform phase lags along the cord (Kopell et al. 1990a).

In the mathematical description of forced chains of oscillators (Kopell et al. 1990a), the limits of the entrainment frequency range depend on the number of oscillators in the chain (n), whether ascending or descending coupling dominates, and on whether $f''(\phi)$ is greater or less than zero [see hypothesis *iv*)].

In Table 1 is shown the entrainment ranges for all experiments.

It can be shown mathematically that the greater the number of oscillators in the chain, the smaller is the entrainment frequency range. This prediction has been confirmed experimentally, as shown in Fig. 6.

According to the mathematics, it is possible to entrain *both* above *and* below the rest frequency at one end but *only* above *or* below at the other end (depending on whether f'' is greater or less than zero; see hypothesis iv). The data confirm this: with rostral forcing it is not possible to entrain at frequencies below the rest frequency. With caudal forcing, it is possible to entrain both above and below the rest rate. This is illustrated in Fig. 7. Statistical analysis of the data listed in Table 1 reveals that for rostral forcing, the lower limit of the entrainment is not significantly different from the rest rate ($t = 0.24$, $P = 0.81$) but that for caudal forcing, the lower limit is different from the rest rate ($t = 3.41$, $P = 0.0025$).

The mathematics show that if the ascending coupling dominates, it is possible to entrain both above and below the rest frequency when forcing at the caudal end, but not at the rostral end. Thus, in the context of this theory, the data imply that the ascending coupling dominates.

Theory and results predict sign of nonlinearity in coupling

According to the theory, if $f'' > 0$, the rest rate provides a *lower* limit for entrainment at one or the other end of the cord (depending on which coupling dominates). For example, if ascending coupling dominates, it is possible to entrain *above* but not *below* the rest rate at the rostral end. In contrast, if $f'' < 0$, the rest rate would provide an *upper* limit for entrainment.

Since the data of Fig. 7 show that it is possible to entrain above but not below the unforced frequency at the rostral end, the theory suggests that $f'' > 0$. For all but some special classes of coupling functions, this nonlinearity indicates that the coupling of the oscillators slows down the oscillators, i.e., that the intrinsic frequency of each local oscillator (ω) is higher than the ensemble frequency of the chain (Ω). However, there are examples in which this nonlinearity is consistent with having the intrinsic frequency lower than the ensemble frequency (Ermentrout and Kopell, unpublished observations).

The theory and results predict differences between ascending and descending coupling other than strength

The ascending coupling $H^+(\phi)$ and the descending coupling $H^-(\phi)$ may be considered as two separate coupling systems, which in principle can be entirely unrelated. Each alone would produce a traveling wave; $H^+(\phi)$ would produce a wave with phase lag ϕ_R, and $H^-(\phi)$ one with phase lag ϕ_L. If it were the case that ascending and descending coupling differed only in strength, i.e., $H^+(\phi)$ were a constant times $H^-(\phi)$, it would follow from the definitions of ϕ_L and ϕ_R that $\phi_L = -\phi_R$. That is, the waves produced by either coupling alone have the same wavelength but travel

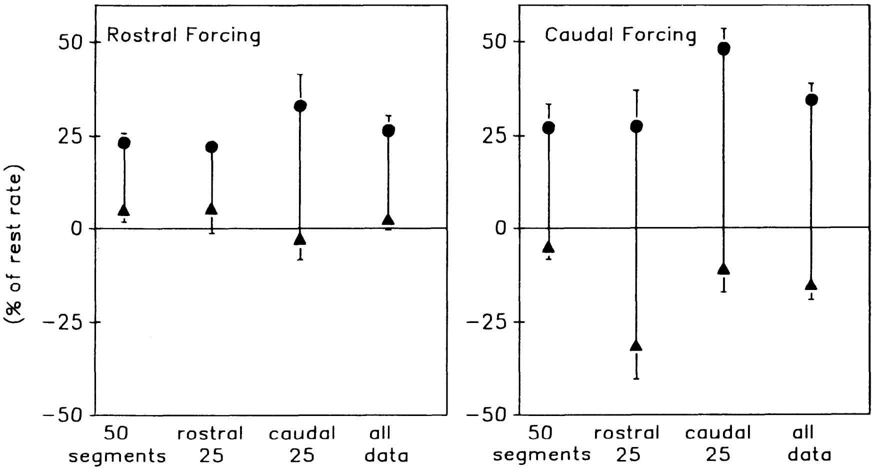

FIG. 7. Natural frequency provides the lower bound of the entrainment range for rostral forcing, whereas with caudal forcing entrainment can occur both above and below the natural frequency. Data from experiments listed in Table 1. Natural frequency has been subtracted from the upper and lower limits of the entrainment range and divided by the rest rate. Hence zero represents the natural frequency. Each symbol represents the mean and standard error of the mean for each condition. "All data" represent the average of the 3 conditions for either rostral or caudal forcing. Filled circles, upper end of entrainment range; filled triangles, lower end. The difference between the lower limit of the entrainment range for rostral versus caudal forcing is statistically significant ($t = 3.35$, $P = 0.002$).

in opposite directions. This is true because the two one-way coupling functions exert their effects in opposite directions.

The data described in this paper are not compatible with $H^+(\phi)$ a multiple of $H^-(\phi)$, by the following reasoning: if the ascending coupling function dominates, the wave is determined by ϕ_R. Because the wave in the lamprey travels rostral to caudal, we conclude that $\phi_R < 0$. But it follows from Kopell and Ermentrout (1990) that if $H^+(\phi)$ is a multiple of $H^-(\phi)$ and $f''(\phi) > 0$, then $\phi_L < \phi_R$; this contradicts $\phi_L = -\phi_R$ if $\phi_R < 0$. Therefore $H^+(\phi)$ in our system cannot be a multiple of $H^-(\phi)$, i.e., they must differ in some way other than strength alone.

The functions used in Fig. 2 are consistent with the data. Note that $H^-(-\phi)$ has a zero crossing ϕ_L that is also negative, with $\phi_L < \phi_R$. Such a function $H^-(-\phi)$, alone, would produce a wave rostral to caudal, i.e., in the same direction as that induced by $H^+(\phi)$, not the opposite direction.

DISCUSSION

Interaction of theory and experiment

In this paper we have shown that a general mathematical framework for coupled oscillators can be tailored by experimental evidence to create a good model of the lamprey spinal locomotor CPG. Each constraint of the model within the mathematical framework is supported by the anatomy and/or behavior of the spinal cord. The model has allowed conclusions to be drawn about intersegmental coordination in the lamprey spinal cord, without the necessity for detailed knowledge of the cells and their interactions.

Experiments such as those reported here yield quantitative data and are relatively easy to perform but without a theoretical framework, it is difficult to see what such data may reveal about the underlying neural network. For example, the experiments clearly show that for rostral forcing the natural frequency of the cord provides the lower limit of the entrainment frequency range. This result tells us nothing without the mathematical framework, but within the framework it tells us that the ascending coupling is dominant and sets the intersegmental phase lag.

The mathematics also places in a more formal context results that may appear intuitively reasonable, for example, that the greater the number of oscillators in the chain, the smaller is the entrainment frequency range.

In the theoretical framework, the lamprey CPG has been modeled as a chain of discrete oscillators. In the vertebrate spinal cord, however, there is no anatomic evidence for the existence of unit segmental oscillators (as in the leech, for example). The ventral roots exit the cord regularly, reflecting the segmentation of the musculature, but this does not reflect segmentation of motoneurons or interneurons within the cord. The cells exist instead as rostral-caudal columns. Current theoretical work investigates the conditions under which an unsegmented array of oscillators can behave as a discrete chain (Ermentrout and Kopell 1990a).

In this study we have also assumed that there is only nearest-neighbor coupling; this is probably not the case in the lamprey (Cohen 1987a; Rovainen 1985). For an unforced chain, inclusion in the mathematical framework of multiple coupling over several segments has been shown not to change the basic conclusions of the theory (Kopell et al. 1990b). The corresponding work remains to be done for forced chains. It is also important to discover the effects of long-range coupling, i.e., coupling at a distance that is a significant fraction of body length.

In the theory there is a requirement for the coupling functions to be monotonically increasing within the operating range of ϕ. [A monotonic decrease in $H^-(-\phi)$ over a range ϕ_1 to ϕ_2 is equivalent to a monotonic increase in $H^-(\phi)$ over the range $-\phi_2$ to $-\phi_1$.] Such a condition also exists in phase-response curve (PRC) theory (Perkel et al. 1964), which deals with one-way coupling only and is closely related to a special case of the general mathematical framework considered here. PRC theory has been successfully applied to the metachronal waves of crayfish swimmerets, for example (Stein 1976). PRC theory cannot be used in the lamprey because it is clear that the coupling is both ascending and descending. Because it is known that mechanical entrainment occurs locally (McClellan and Sigvardt 1988), the ability to entrain the entire spinal cord by mechanical forcing at either end implies that both ascending and descending coupling exist. Further indirect evidence of the bidirectionality of the coupling has been reported (Cohen 1987a; Dale 1986; Rovainen 1985).

The general model of intersegmental coordination presented here should be useful for gaining insights into the mechanisms underlying intersegmental coordination in other systems that can be modeled as chains of oscillators such as the crayfish swimmeret system (Stein 1976), leech heartbeat (Calabrese and Peterson 1983), leech swimming (Pearce and Friesen 1988), and the oscillators coordinating the hip, knee, and ankle during vertebrate locomotion (Grillner 1985).

Leech swimming, like lamprey swimming, results from a rostral-caudal wave of activation along the length of the cord. However, unlike lamprey swimming, the phase lags are not constant along the cord. Pearce and Friesen (1988) have recently developed a model for the production of intersegmental coordination during swimming in the leech. Although the model is a numerical simulation and, therefore, not directly comparable to the one presented here, it incorporates some of the same features: *1*) the oscillators are described in terms of frequency and phase; *2*) both ascending and descending coupling signals are incorporated; *3*) the assumption used by Pearce and Friesen (1988) of multiple coupling pulses active during a particular portion of the cycle is equivalent to the method of averaging used in our model; *4*) models of experiments involving changes in coupling strength result in phase transitions similar to those found both in our experimental and theoretical work (Sigvardt and Kopell, unpublished observations).

The coordination of the rear-to-front peristaltic form of the leech heartbeat relies at least in part on inhibitory postsynaptic potentials (IPSPs) from unidentified heart interneurons [HN(X)] cells that arrive in rostral-caudal order onto the excitatory neurons to the heart muscle (Calabrese and Peterson 1983). However, neither quantitative analysis of phasing nor modeling of how this phasing may result from known connections has been done.

Coupling functions govern intersegmental delay and ensemble frequency

Indirect evidence has been provided for ascending and descending propriospinal interneurons capable of inducing or entraining oscillations at a distance (Cohen 1987a; Dale 1986; McClellan and Sigvardt 1988; Rovainen 1985). However, there is otherwise no experimental data concerning mechanisms for intersegmental coupling in the lamprey. In the mathematical framework the constraints on the coupling functions are those that result in activity with stable, nonzero phase coupling between oscillators; i.e., stable lamprey swimming. These constraints then tell us how the intersegmental coordinating system could be designed to produce stable swimming.

The observation that the spinal cord can be entrained within a substantial range of frequencies by forcing at *either* the rostral or the caudal end provides a significant mathematical constraint on the system. Not all chains of oscillators behave in this way. In particular, if hypothesis v) is violated, the range of entrainment is vanishingly small for forcing at one of the ends of the cord. Examples of systems in which v) is violated may be constructed using coupling in which $H^+(\phi)$ and $H^-(\phi)$ are "tuned" to have $(\phi_L) = (\phi_R)$, so that both $H^+(\phi)$ and $H^-(\phi)$ give the same traveling wave (Kopell et al. 1990a). Systems satisfying our hypotheses can be entrained over a substantial range of frequencies by forcing at either end.

In the absence of forcing, the system operates at a phase lag near the zero crossing of the dominant coupling function. "Dominance" of ascending coupling does not imply that the coupling signals in the ascending direction are stronger. Indeed, the functions $H^+(\phi)$ and $H^-(\phi)$ need not be related at all, so we cannot speak of their relative amplitudes. [In the special case that $H^+(\phi)$ is a multiple of $H^-(\phi)$, the coupling with the larger amplitude is dominant. We have argued above that this special case is not relevant.] However, it was shown by Kopell, Ermentrout, and Williams (1990a) that there is a simple characterization of dominance in terms of the effect of the coupling on the emergent frequency of the network, as we shall now describe.

Since the effect of the dominant coupling on the ensemble frequency is near zero at the resultant phase lag, the ensemble frequency is approximately equal to the intrinsic frequency plus the effect of the nondominant coupling at that phase lag (see *Eqs.* 5 and 6). Using $H^+(\phi)$ and $H^-(\phi)$ as possible dominant couplings, we then get two possible ensemble frequencies. The actual dominant coupling turns out to be the one for which the ensemble frequency is the closer of the two possibilities to the intrinsic frequency. It then follows that the dominant coupling sets the intersegmental phase lag, whereas the nondominant coupling determines how much the ensemble frequency differs from the intrinsic frequency.

The dominance of ascending intersegmental coupling may reflect a greater need for mechanical feedback near the caudal end. In the lamprey, the mechanical wave arises near the head and travels toward the tail so that errors in the relative timing between activation and movement would grow progressively worse in the more caudal regions in the absence of feedback. By this reasoning, it is more important for feedback from caudal regions to have an entrainment range that goes both above and below the rest rate. The dominance of ascending over descending coupling provides this.

To have a traveling wave, the phase lag as set by the ascending coupling function must not be zero. According to the theory, therefore, the zero crossing (ϕ_R), must not be at the origin. This property can, therefore, be considered a constraint on evolution for the production of nonsynchronous coupling by neural networks.

The lamprey can swim over a range of swimming speeds (corresponding to a range of frequencies). The rostral-caudal delay in activation increases in proportion to the cycle length so that the intersegmental phase lag is independent of frequency. In the mathematical framework the coupling functions were assumed to be dependent only on phase difference (ϕ), independent of either the intrinsic frequency ω, or the ensemble frequency Ω. In consequence, the intersegmental phase lags are also independent of frequency, as in the swimming lamprey. This contrasts with phase lags produced by synaptic or conduction delays that do not scale with frequency. For phase constant swimming, the ascending and descending interneuronal coupling systems must therefore be designed to produce this frequency independence.

Strategies for modeling neural systems

Traditional neurophysiological techniques, such as single-cell or multiple-cell recordings and anatomic and pharmacologic studies, can provide detailed information about the properties of cells and their connections. However, for multicellular networks, the amount of detail and its normal variability are so great that the detail can obscure the essential features of the system that underlie its function. To deduce the relationship between the structure and the function of the particular neural network, a theoretical framework is essential. Such a theoretical framework or model allows one to organize the large quantities of information and separate the patterns of structure that are essential to the network's function from those that may vary without functional significance.

Such modeling activities can be approached in many complementary ways, none of which alone is wholly adequate. One method is to build "realistic" models, which incorporate as much detail as is known and to guess other parameters. One drawback of this data-dominated strategy is that the equations can then approach in complexity the original system. Furthermore, the result need not yield much insight into the mechanisms of the emergent behavior. At the other extreme are very abstract methodologies that begin with many simplifying and/or generalizing assumptions. The resulting models are removed from the original biological setting to render them mathematically pliant. As a result it is often hard to compare the predictions with experiment. Although such models may offer insight into how the function *might* be performed, it is usually impossible to assert that this is how it *is* performed in the biological system.

We have adopted an intermediate strategy: investigate a large collection of possible models that taken together form a theoretical framework. Within this general framework, we have tried to make only those simplifications that can be justified on biological grounds and have then deduced, by analytical means, which details are important in generating the known biological behavior. Such interplay between theory and experiment is bidirectional: data collection can be restricted to parameters that have been shown to be functionally relevant, and theoretical analysis can be directed toward revealing experimental methods for testing alternative assumptions. When theory and experiment are related in this interactive, mutually constraining way, those features of the neural network that are vital to its function may be more easily revealed.

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