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Effects of Local Oscillator Frequency on Intersegmental Coordination in the Lamprey Locomotor CPG: Theory and Experiment

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SUMMARY AND CONCLUSIONS

1. Experiments have been performed on in vitro preparations of lamprey spinal cord bathed in D-glutamate, which induces a pattern of activity recorded from ventral roots that is similar to that seen in the intact animal during swimming. The frequency of fictive swimming increases with increasing D-glutamate concentration, but intersegmental phase lag remains unaffected.

2. The effects on intersegmental phase lags of unequal activation of the rostral and caudal halves of a preparation were determined. Unequal activation was produced by placing a diffusion barrier in the middle of the chamber and perfusing the two halves with different concentrations of D-glutamate.

3. Within the rostral compartment, the phase lag increased from control when the rostral D-glutamate concentration was higher than the caudal concentration, and decreased from control when it was lower. By contrast, the phase lags within the caudal compartment did not depend on the ratio of D-glutamate concentration between the two compartments.

4. The frequency of the ventral root activity during differential activation was not significantly different from that of control experiments that had the same concentration as in the rostral compartment.

5. The results are discussed within the context of the mathematical analysis of chains of coupled oscillators by Kopell and Ermentrout and other current theories about the mechanisms of intersegmental coordination in the lamprey.

INTRODUCTION

Rhythmic activity such as locomotion is generated by neuronal circuits that behave as oscillators. Movement in vertebrates involves precise coordination between body segments, and it has been suggested that such coordination results from interactions between unit oscillators (Grillner 1981). The interneurons responsible for these interactions are not known for any vertebrate system. Because of the difficulty of obtaining information about the distribution of axonal lengths, cell connectivity, and relative synaptic strengths in vertebrate nervous systems, a cellular approach to understanding intersegmental coordination has not yet been possible. Therefore we have taken a different approach, using a mathematical theory of coupled oscillators to investigate intersegmental coupling, as applied to the locomotor central pattern generator (CPG) of the lamprey (Williams and Sigvardt 1995).

Locomotion in the lamprey is achieved by the generation of a traveling wave of lateral curvature. Increased swimming speed is produced by an increase in cycle frequency, although the wavelength on the body remains constant at about one body length. The pattern of ventral root activity giving rise to this behavior involves alternating bursts of ventral root impulses on the left and right sides of the spinal cord and a head-to-tail delay of these bursts along the body. A single wavelength is maintained by the scaling of the intersegmental delay along the body with cycle duration. Thus the phase lag between segments (intersegmental time delay divided by cycle duration) is constant along the length of the body and independent of swimming frequency. Because axonal conduction velocity and synaptic delays are constant, these simple mechanisms cannot account for intersegmental delay. Constant phase coupling must arise from more complex interactions among intersegmental coordinating neurons within the central pattern generating network.

As few as two segments taken from anywhere along the cord can produce oscillatory behavior in the lamprey (Grillner et al. 1982), so the networks producing this behavior must be distributed along the cord. Thus the CPG for lamprey locomotion can be described as a chain of coupled oscillators, with the activity recorded from the ventral roots representing its motor output (see Fig. 1*A*). This activity can then be analyzed in the context of the mathematical behavior of such chains (Cohen et al. 1982; Ermentrout and Kopell 1990, 1991; Kopell and Ermentrout 1986; Kopell et al. 1990). This analysis leads to predictions for the behavior of the lamprey spinal cord in vitro in response to experimental manipulation (Williams and Sigvardt 1994; Williams et al. 1990).

In this analysis, each oscillator in the chain is assumed to have its own intrinsic frequency, which it would exhibit if uncoupled from its neighbors. Through coupling, oscillators can speed each other up or slow each other down, depending on the phase lag between them. When the oscillators in a chain have entrained each other, they all operate at the same frequency. In our model, this common frequency is given, for each oscillator, by the sum of its intrinsic frequency and the change in frequency caused by the coupling from its rostral and from its caudal neighbor (see Fig. 1*A*). These are the basic assumptions of the analysis, which are incorporated in the following set of equations (adapted from Kopell and Ermentrout 1988). For the kth oscillator in a chain of n coupled oscillators, all cycling at the same frequency Ω (and thus phase-locked)

$$\Omega = \omega_1 + H_A(\phi_1) \qquad k = 1 \qquad (1)$$

$$\Omega = \omega_k + H_A(\phi_k) + H_D(-\phi_{k-1}) \qquad 1 < k < n \qquad (2)$$

$$\Omega = \omega_n + H_D(-\phi_{n-1}) \qquad k = n \qquad (3)$$

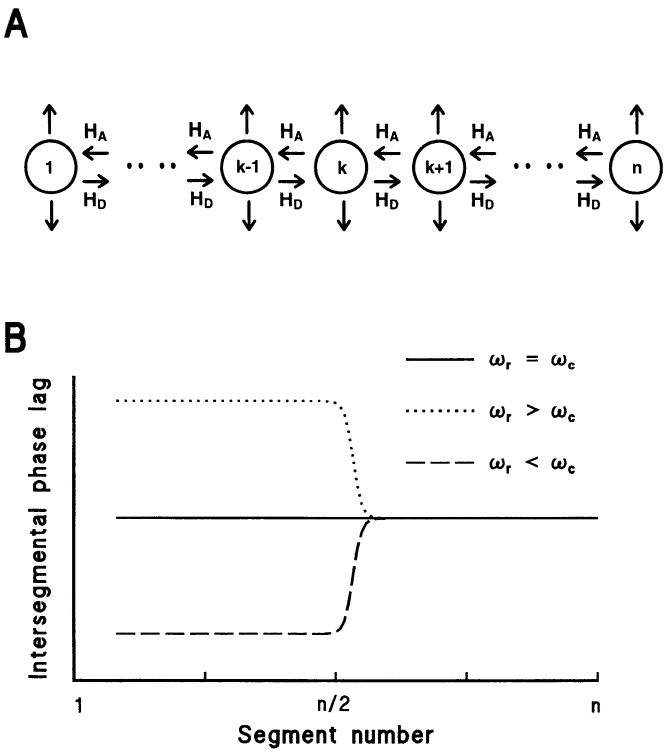

FIG. 1. *A*: chain of coupled oscillators n segments long. Intersegmental coupling mediated by connections labeled H_A (ascending) and H_D (descending). Vertical arrows represent output via motoneurons. *B*: theoretical predictions for the effects on phase lag of a step change in intrinsic frequency midway down a chain with dominant ascending coupling-rostral phase lags change and caudal phase lags do not. Solid line represents the case in which the intrinsic frequencies of the oscillators are the same along the length of the cord; dotted line represents the case in which the intrinsic frequency of the rostral oscillators is greater than the caudal ones, and dashed line when the intrinsic frequency of the caudal ones is higher. Predicted boundary regions on the rostral end of the chain (near segment 1) not shown.

where ω_k is the intrinsic frequency of oscillator k and ϕ_k is the difference in phase between oscillator $k + 1$ and oscillator k. $H_A(\phi_k)$ represents the frequency change produced in oscillator k by the coupling from oscillator $k + 1$, and $H_D(-\phi_{k-1})$ the change produced through descending coupling from oscillator $k - 1$. Evidence indicates that there is no systematic dependence of intrinsic frequency on the rostral-caudal position within the spinal cord (Cohen 1987b). For this reason it is assumed that all the oscillators along the chain have equal intrinsic frequency (equally activated oscillators; all ω_k equal). Using the qualitative theory of differential equations, Kopell and Ermentrout (1988) have shown that if, in addition, ascending and descending coupling are identical, the phase lags cannot be uniform along the chain. To get uniform phase lags (ϕ_k) with uniform activation (ω_k), it is necessary for the coupling to be asymmetric. Either ascending or descending coupling will then "dominate," i.e., will determine the phase lag along most of the chain. To say that the coupling in one direction is "dominant" means that the ability of the coupling signals to change the frequency of the individual oscillators is greater over an effective range of phase lags. It does not necessarily reflect the relative strengths of the synaptic connections made by the ascending and descending fibers.

One of the predictions of the mathematical analysis concerns the effects on the intersegmental phase lag of variations in intrinsic frequency along the chain (Kopell and Ermentrout 1990). In particular, if the intrinsic frequencies of the oscillators in one half of the chain are different from the intrinsic frequencies in the other half, *Eqs. 1–3* can be replaced by

$$\Omega = \omega_r + H_A(\phi_1) \qquad k = 1 \tag{4}$$

$$\Omega = \omega_r + H_A(\phi_k) + H_D(-\phi_{k-1}) \qquad 1 < k < n/2 \tag{5}$$

$$\Omega = \omega_c + H_A(\phi_k) + H_D(-\phi_{k-1}) \qquad n/2 < k < n \tag{6}$$

$$\Omega = \omega_c + H_D(-\phi_{n-1}) \qquad k = n \tag{7}$$

where ω_r is the intrinsic frequency in the rostral half, and ω_c that in the caudal half. For the rostral and caudal halves of the chain to remain entrained, all oscillators must have the same resultant frequency Ω. If the intrinsic frequencies, ω_r and ω_c, are not equal, then the increment or decrement of frequency produced by the coupling $[H_A(\phi_k) + H_D(-\phi_{k-1})]$ must be different in the two halves of the chain. According to these equations, that can only occur if the intersegmental phase lags (ϕ_k) are different in the two halves of the chain.

These equations have a solution in which phase lag changes in one half of the chain but not in the other. Whether the change occurs in the rostral or caudal half depends on the direction of "dominant" coupling. If, for example, ascending coupling is dominant, as has been indicated previously for the lamprey (Williams and Sigvardt 1994; Williams et al. 1990), the intersegmental phase lag in the caudal half of the chain will not change, and the intersegmental phase lag in the rostral half of the chain will change as necessary to make all resultant frequencies equal to Ω (see Fig. 1*B*). If the rostral oscillators have a higher intrinsic frequency than the caudal oscillators, the phase lags in the rostral half of the chain should increase in magnitude; if the intrinsic frequency of the caudal oscillators is greater, the phase lag in the rostral half should decrease in magnitude or even change sign (see Fig. 1*B*). Further analysis of these equations predicts that the resultant frequency of the chain, Ω, should be governed by the dominant coupling. In particular, if ascending coupling is dominant, then the frequency reflects the intrinsic frequency of the caudal oscillators, not the rostral ones. (For a more mathematical analysis of these effects, see Kopell and Ermentrout 1990.)

In this paper we report the results of experiments designed to test the predictions of this analysis. The frequency of fictive swimming in the in vitro lamprey spinal cord increases in a dose-dependent manner with increasing concentration of excitatory amino acid in the solution bathing the preparation (Brodin et al. 1985). Therefore it is reasonable to assume that increasing D-glutamate concentration produces an increase in the intrinsic frequency of the uncoupled oscillators. Here we show the effects on the intersegmental phase lags of bathing the rostral and caudal halves in different concentrations of excitatory amino acid. Preliminary results of this work have been reported (Sigvardt 1993; Sigvardt et al. 1991).

METHODS

Experiments were performed on spinal cords from 12 adult lampreys (*Ichthyomyzon unicuspis*, 22–30 cm long). Animals were

anesthetized in tricaine methylsulphate. Approximately one-half of the spinal cord/notochord (11 preparations were 50 segments long, 1 was 40 segments) was removed from the region caudal to the last gill slit and pinned in a silicone elastomer (Sylgard)–lined preparation dish containing lamprey saline (in mM: 91.0 NaCl, 2.1 KCl, 2.6 $CaCl_2$, 1.8 $MgCl_2$, and 20 $NaHCO_3$), adjusted to pH 7.4 and cooled to 7–9°C. Ventral root activity was recorded en passant with saline-filled glass micropipettes with blunt fire-polished tips. The electrodes were placed on six evenly spaced ventral roots along one side of the spinal cord (see Fig. 2). As the ventral root numbers differed slightly from one preparation to the next, recording positions will be referred to as 1, 2, 3, 4, 5, and 6, starting from the rostral end. Fictive locomotion was induced by the addition of 0.1–1.8 mM D-glutamate to the bathing solution. A Plexiglas partition, with a small notch for the spinal cord/notochord, divided the preparation dish in half (see Fig. 2). The area around the spinal cord/notochord beneath the partition was sealed with petroleum jelly (Vaseline) to allow the two halves of the spinal cord to be bathed with different solutions. Adequacy of the seal between the two compartments was tested in two ways by visual inspection through the dissecting microscope: *1*) after removal of saline from one of the two compartments, and *2*) after the addition of a dark red, opaque sulphorhodamine dye to one compartment. In neither case was there any evidence of movement of fluid past the partition over similar time periods to those used during the experiments. Presumably there would be some diffusion through the tissue directly under the partition. However, because the volume of this tissue is <0.25 ml and the volume of each compartment is ~15 ml, the concentration of D-glutamate over most of the length of the spinal cord should be that of the concentration in the bulk solution in that compartment.

Recorded ventral root signals were amplified and stored on analog tape. Data from the tape were digitized at 2 kHz, rectified, and analyzed with the use of RC Electronics hardware and software. Between 42 and 134 (typically 100) bursts of activity were analyzed for each experimental manipulation. For each ventral root, a threshold for spike discrimination was set by visual inspection of the data, and three parameters were chosen for automatically determining the burst onsets and terminations: minimum and maximum allowable burst duration, and a minimum "interrupt" time during which the signal could remain below threshold and still be counted within the ongoing burst of activity. Bursts that were missed by the automatic program but were obvious on visual inspection were entered manually with the use of a cursor control. Intersegmental time delay and cycle duration were calculated from the midpoints between the burst onsets and terminations for each ventral root. (There was no statistically significant difference between time delays and cycle duration calculated from burst onsets and those calculated from burst midpoints.) Cycle duration was defined as the time between midpoints of sequential bursts. The intersegmental phase lag per segment between ipsilateral ventral roots was defined as the difference between the burst midpoints of the two roots divided by the cycle duration of the rostral-most root and by the number of segments between the two roots. Mean values were determined for cycle duration and intersegmental phase lags within the rostral half of the cord (between ventral root positions 1 and 3) and the caudal half (between positions 4 and 6). To allow a comparison with previously published data (Matsushima and Grillner 1990, 1992a), phase lags were also measured between the two compartments (between electrodes 1 and 6).

In a given preparation, several control experiments were done in which the entire spinal cord was bathed in the same concentration of D-glutamate. If the activity was unstable over time, the preparation was discarded. The data from 12 preparations are included in RESULTS. A range of concentrations was used to get a range of frequencies. For each preparation there is a limited range of amino acid concentration within which cycle frequency increases with increasing concentration. Only values within this range were used for the differential activation experiments, in which different concentrations bathed the two halves of the spinal cord. In most preparations, controls were interspersed between the experiments. In each of the 12 preparations, between 2 and 6 controls were analyzed.

RESULTS

Over the range of D-glutamate concentrations used in each preparation, the frequency of fictive swimming increased in a dose-dependent manner (Fig. 3*A;* linear regression shows a statistically significant relationship, $P < 10^{-5}$), as has been shown previously for this and other excitatory amino acid agonists (Brodin et al. 1985). Intersegmental phase lag, on the other hand, does not show such a dose-dependent change (Fig. 3*B;* linear regression not statistically significant, $P = 0.892$) (Wallén and Williams 1984).

In each preparation, the effects on intersegmental phase lag of unequal activation of the two halves were determined. Figure 4 gives the mean data from one set of experiments. Each bar represents the mean ventral root burst as a fraction of the mean cycle duration. In each section of the figure, the top bars are

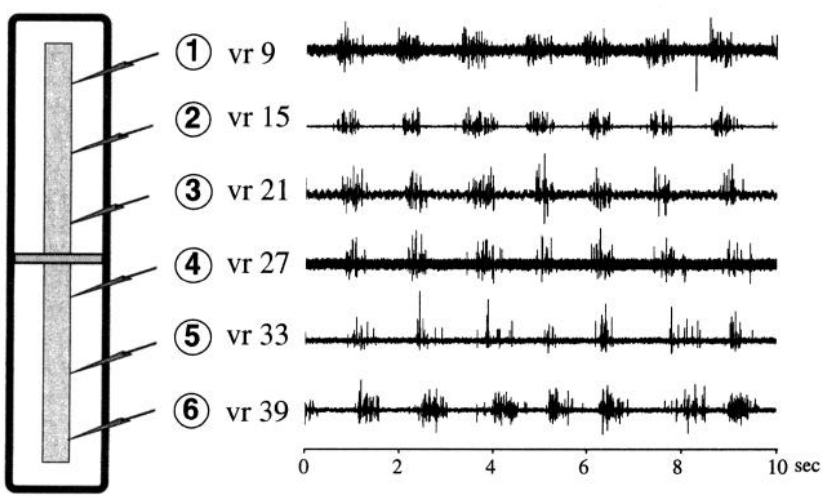

FIG. 2. Lamprey locomotor activity induced by D-glutamate. Ventral root activity recorded extracellularly from in vitro lamprey spinal cord preparation in control conditions. Both compartments of the bath (diagram of setup to the *left*) contain the same concentration of D-glutamate.

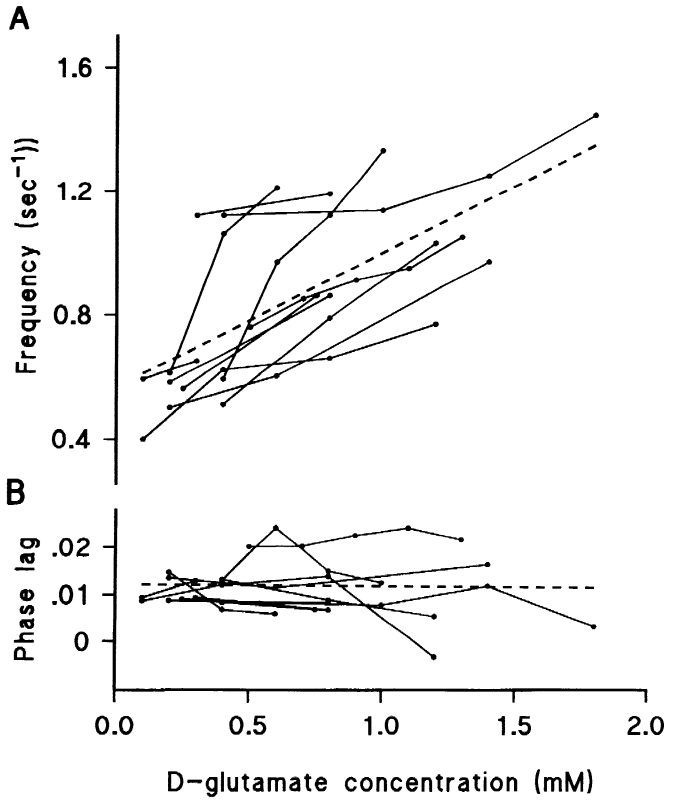

FIG. 3. *A*: cycle frequency as a function of D-glutamate concentration, in control experiments in all preparations. *B*: intersegmental phase lag vs. D-glutamate concentration for same experiments. Solid lines connect data points from individual preparations. Dashed lines are regression lines through all data points.

from ventral roots in the rostral half of the preparation, and the bottom three bars from roots in the caudal half. In control experiments the entire preparation was bathed in either 0.2 or 0.8 mM D-glutamate (Fig. 4, *A* and *B*). In two further experiments on the same preparation, the two halves were bathed in different concentrations: either the rostral half was bathed in the lower of the two control concentrations and the caudal half in the higher one (Fig. 4*C*), or the rostral concentration was higher and the caudal lower (Fig. 4*D*). The phase lags in each half of the preparation are illustrated by the slopes of the regression lines through the midpoints of the bursts. Within the rostral compartment, the phase lag was approximately the same in the two control experiments (Fig. 4, *A* and *B*) but decreased, even changing sign in some experiments, when the caudal concentration was higher (Fig. 4*C*) and increased when the rostral concentration was higher (Fig. 4*D*). In the caudal compartment, by contrast, the phase lag in the experimental regimes (Fig. 4, *C* and *D*) was similar to that in the control experiments (Fig. 4, *A* and *B*).

In both control experiments the rostral phase lag was less than the caudal phase lag in this particular preparation. In other preparations the rostral phase lag was greater than or more nearly equal to the caudal phase lag, as can be seen in Table 1, which gives the results of all experiments. These data illustrate the large variability seen in measurements of intersegmental phase lag from one preparation to the next. For this reason we performed a number of experiments and based our conclusions on statistical analysis.

The effects of differential activation on the phase lag within each compartment are shown in Fig. 5 for the data from all experiments shown in Table 1. Each dot represents an experiment in which the D-glutamate concentration was different in the two halves of the preparation. Change in phase lag from control is plotted against the ratio of the two control frequencies. These control frequencies are the frequencies that were shown by the whole cord when it was bathed in the two concentrations of D-glutamate used in the experimental trial. This ratio can thus be taken as a measure of the ratio of the intrinsic frequencies during the experimental trial. It can be seen that within the rostral compartment (Fig. 5*A*) the experimental phase lag increases from control when the rostral frequency is higher than the caudal one, and decreases when it is lower. By contrast, the phase lags within the caudal compartment (Fig. 5*B*) show no dependence on the ratio of frequencies.

It can also be seen in Fig. 5 that for both rostral and caudal phase lags, there is considerable scatter in the data. Therefore a multiple analysis of variance (MANOVA) was

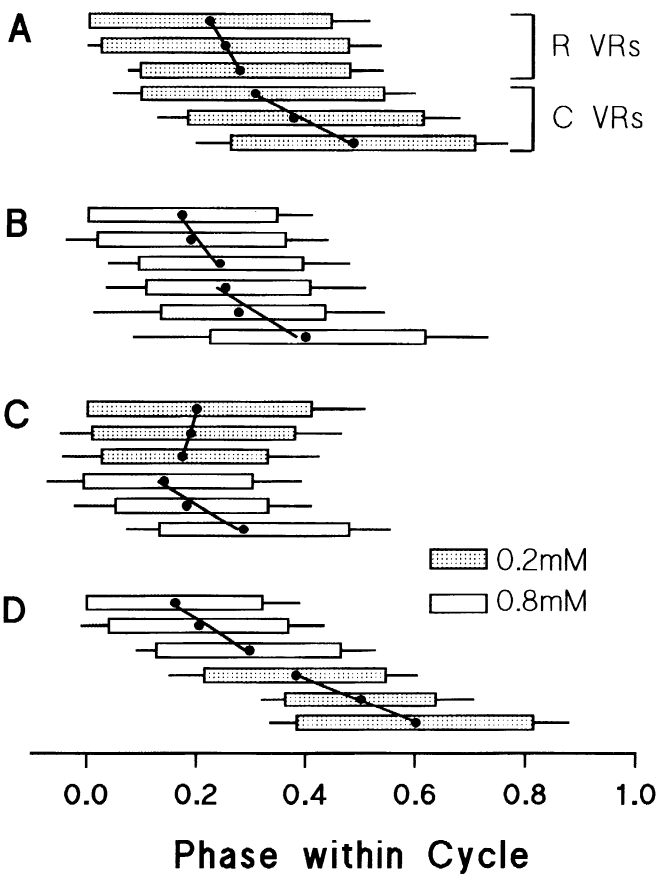

FIG. 4. Mean data from 1 preparation in which the effects of ventral root activity under control conditions are compared with those under differential activation. *A*: 0.2 mM D-glutamate in both compartments. *B*: 0.8 mM D-glutamate in both compartments. *C*: 0.2 mM D-glutamate rostral, 0.8 mM D-glutamate caudal. *D*: 0.8 mM D-glutamate rostral, 0.2 mM D-glutamate caudal. Recordings from ventral roots 9, 15, 21 (Rostral VRs) and 27, 33, 39 (Caudal VRs). Bars represent the mean times of onset and termination of the burst activity recorded in each ventral root. Filled circles represent the burst midpoint. All times are measured from the onset of activity in the most anterior ventral root recorded (vr 9) and plotted as phase within the cycle, by dividing by the cycle duration. Error bars represent standard error of the mean.

TABLE 1. *Effects of differential activation on intersegmental phase lags*

	[D-Glutamate]		Rostral Phase Lag		Caudal Phase Lag		1–6 Phase Lag	
Animal	[Rostral]	[Caudal]	Control	Experimental	Control	Experimental	Control	Experimental
			Rostral D-glutamate concentration higher than caudal concentration					
1	0.40	0.10	0.0069	0.0054	0.0079	0.0096	0.0112	0.0126
1	0.80	0.10	0.0069	0.0089	0.0079	0.0067	0.0112	0.0131
4	0.80	0.30	0.0062	0.0072	0.0114	0.0184	0.0082	0.0129
5	0.80	0.20	0.0056	0.0114	0.0137	0.0182	0.0086	0.0146
12	0.75	0.25	0.0063	0.0049	0.0103	0.0187	0.0080	0.0261
20	0.70	0.50	0.0184	0.0193	0.0254	0.0161	0.0191	0.0202
20	0.90	0.50	0.0184	0.0183	0.0254	0.0149	0.0191	0.0197
20	1.10	0.50	0.0184	0.0186	0.0254	0.0164	0.0191	0.0243
20	1.30	0.50	0.0184	0.0174	0.0254	0.0313	0.0191	0.0123
21	0.30	0.10	0.0052	0.0142	0.0165	0.0140	0.0116	0.0139
23	1.00	0.40	0.0078	0.0142	0.0013	−0.0005	0.0088	0.0116
23	1.40	0.40	0.0078	0.0111	0.0013	−0.0002	0.0088	0.0122
23	1.80	0.40	0.0078	0.0077	0.0013	0.0014	0.0088	0.0129
25	0.80	0.40	0.0042	0.0103	0.0141	0.0192	0.0084	0.0167
25	1.20	0.40	0.0042	0.0090	0.0141	0.0126	0.0084	0.0164
28	0.60	0.20	0.0126	0.0160	0.0113	0.0136	0.0134	0.0153
30	0.60	0.40	0.0122	0.0116	0.0206	0.0154	0.0148	0.0127
30	0.80	0.40	0.0122	0.0118	0.0206	0.0184	0.0148	0.0161
30	1.00	0.40	0.0122	0.0161	0.0206	0.0044	0.0148	0.0155
			Caudal D-glutamate concentration higher than rostral concentration					
1	0.10	0.40	0.0069	0.0045	0.0079	0.0099	0.0112	0.0103
1	0.10	0.80	0.0069	0.0083	0.0079	0.0120	0.0112	0.0131
4	0.30	0.80	0.0062	−0.0012	0.0114	0.0064	0.0082	0.0004
5	0.20	0.80	0.0056	−0.0021	0.0137	0.0126	0.0086	−0.0004
12	0.25	0.75	0.0063	−0.0026	0.0103	0.0059	0.0080	0.0033
25	0.40	0.80	0.0042	0.0053	0.0141	0.0208	0.0084	0.0089
25	0.40	1.20	0.0042	0.0035	0.0141	0.0111	0.0084	0.0070
28	0.20	0.60	0.0126	0.0102	0.0113	0.0135	0.0134	0.0118
28	0.20	1.40	0.0126	−0.0018	0.0113	0.0163	0.0134	0.0092
29	0.40	0.80	0.0074	0.0029	0.0130	0.0164	0.0095	0.0097
29	0.40	1.20	0.0074	−0.0047	0.0130	0.0195	0.0095	0.0055
30	0.40	0.60	0.0122	0.0036	0.0206	0.0188	0.0148	0.0103
30	0.40	0.80	0.0122	0.0041	0.0206	0.0193	0.0148	0.0110
30	0.40	1.00	0.0122	0.0063	0.0206	0.0229	0.0148	0.0128
31	0.20	0.40	0.0065	0.0115	0.0063	0.0115	0.0059	0.0102
31	0.20	0.60	0.0065	0.0036	0.0063	0.0022	0.0059	0.0013

Mean intersegmental phase lag data from all experiments. R > C: rostral concentration higher than caudal concentration. C > R: caudal concentration higher than rostral concentration. Control values for phase lag are mean values for a given preparation for all control trials in that preparation, because intersegmental phase lag is independent of D-glutamate concentration.

performed on all the data (35 experimental trials and 39 control experiments), with intersegmental phase lag as the dependent variable and three independent categories: *1*) different animals, *2*) rostral versus caudal phase lags, and *3*) the activation conditions of *1*) control, *2*) rostral concentration higher than caudal, or *3*) caudal concentration higher than rostral. The analysis indicated significant differences in intersegmental phase lag between the rostral and caudal compartments ($F = 22.1$, $P < 0.001$) and a significant interaction between this effect and the effect of the activation condition ($F = 5.23$, $P < 0.01$).

Subsequent separate MANOVA of only the rostral phase lags indicated a significant dependence on the activation condition ($F = 17.5$, $P < 0.001$). In this case (Fig. 6*A*), paired *t*-tests showed that when the caudal concentration was higher than rostral, the rostral phase lags were significantly less than control ($P < 0.001$), whereas when the rostral concentration was higher, the rostral phase lags were significantly greater than control ($P < 0.004$). By contrast, MANOVA of the caudal phase lags indicated that they were not significantly different for the three activation conditions ($F = 0.20$, $P > 0.82$). Paired *t*-tests confirmed that the caudal phase lags during differential activation did not differ significantly from controls ($P > 0.1$) for either rostral concentration higher or caudal concentration higher (Fig. 6*B*). In the context of the theory, the observed changes in rostral phase lags and the lack of change in the caudal phase lags indicate that ascending coupling is dominant in the control of intersegmental phase lags.

The values of the rostral and caudal phase lags under control conditions (○ in Fig. 6, *A* and *B*) indicate that there may be differences between the rostral and caudal phase lags in control conditions. However, paired *t*-tests comparing rostral with caudal phase lags gave a *P* value of only 0.015, which means that the chance of this being a statistically significant difference is small. That the phase lags are not statistically different along the length of the cord has been reported previously (Wallén and Williams 1984).

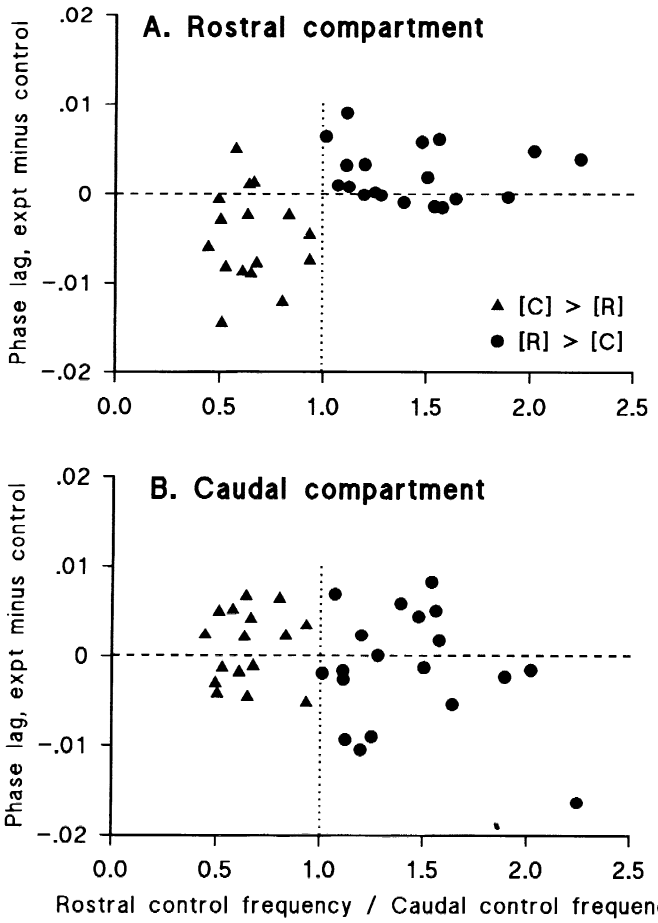

FIG. 5. Effect of differential activation on phase lag. *A*: phase lags measured within the rostral compartment. *B*: phase lags measured within the caudal compartment. Abscissa: ratio between the rostral and the caudal control frequencies. These control frequencies are the frequencies that were shown by the whole cord when it was bathed in each of the 2 concentrations of D-glutamate used in the experimental trial. Values <1 represent higher D-glutamate concentration in the caudal compartment (▲), and values >1 represent higher concentration in the rostral compartment (●). Ordinate: change in phase lag from control.

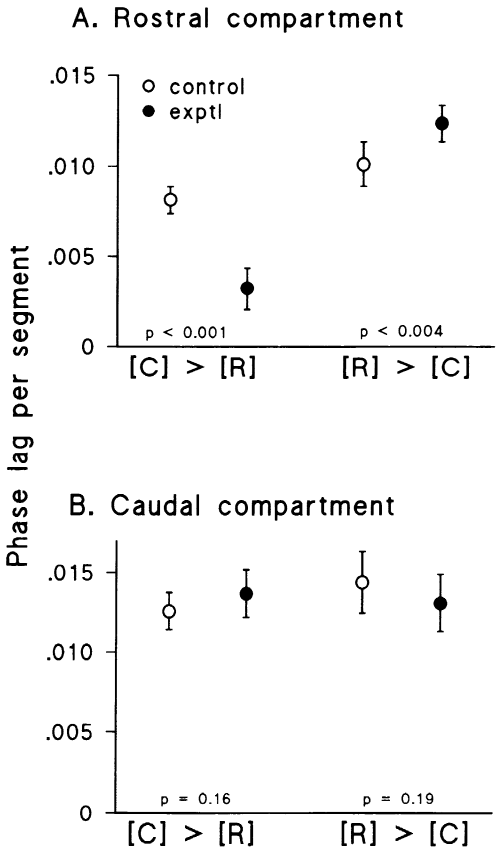

FIG. 6. Average intersegmental phase lags within each compartment, under control conditions (○) and with the 2 halves of the preparation bathed in different amino acid concentrations (●). Points represent overall mean values from all experiments. Error bars: standard error of the mean; *P* values are from 1-tailed paired *t*-tests performed on the mean values from individual experiments, compared with the control values for the respective preparations. *A*: phase lags measured within rostral compartment. *B*: phase lags measured within caudal compartment.

We have also measured the intersegmental phase lags across the boundary between the two halves of the cord (between positions 1 and 6), and our results are similar to those reported by Matsushima and Grillner (1990, 1992a). These results are shown in Fig. 7. In experiments in which the caudal concentration was higher than the rostral concentration, the phase lag measured across the boundary decreased significantly from control values, ($P < 0.005$, 1-tailed paired *t*-test). When the rostral concentration was higher, the phase lag across the boundary increased significantly ($P < 0.005$).

Frequency during differential activation

In contrast to intersegmental phase lag, the average frequency must be the same in the rostral and caudal halves of the cord, if the two are to remain entrained together. Figure 8 compares the frequency seen during differential activation with those of control experiments with the same concentration as that in either the rostral or the caudal compartment (see also Table 2). Paired *t*-tests showed that the frequency of the ventral root activity during differential activation was not significantly different from that of control experiments that had the same concentration as in the rostral compartment. This was true both when the caudal concentration was higher than the rostral ($P = 0.17$) and when the rostral concentration was higher ($P = 0.14$). However, the frequen-

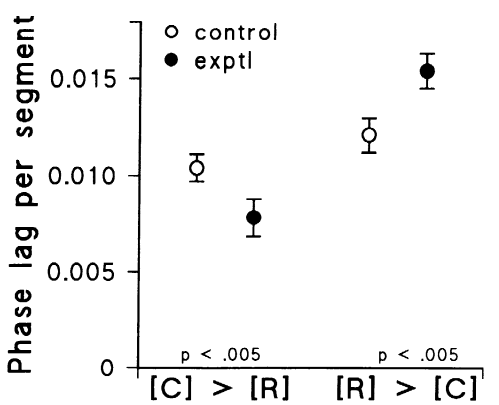

FIG. 7. Average intersegmental phase lags between compartments, under control conditions (○) and with the 2 halves of the preparation bathed in different amino acid concentrations (●). Phase lag measured between ventral root 1 and ventral root 6. For details see legend of Fig. 6.

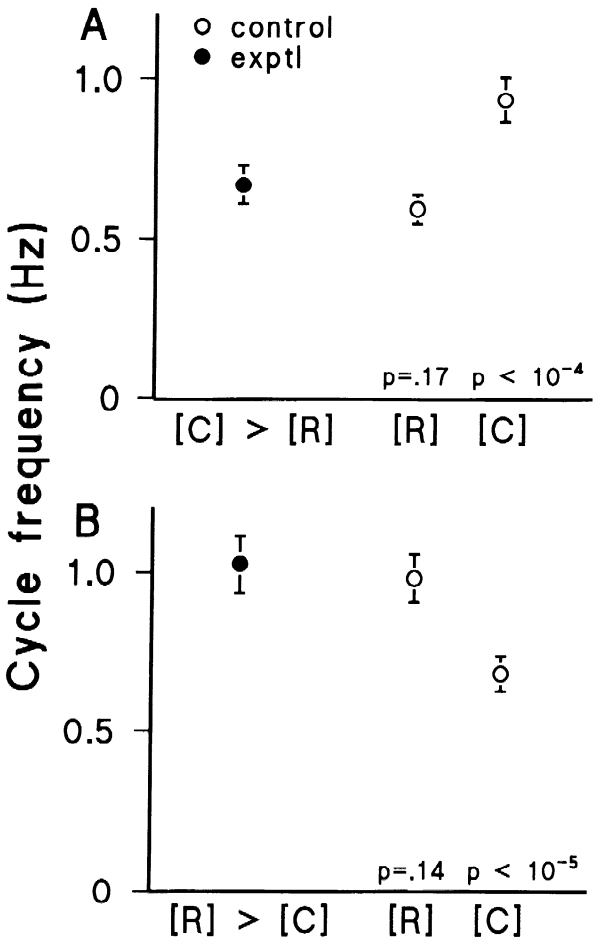

FIG. 8. Frequency of ventral root bursts, averaged over all experiments. *A*: caudal concentration of D-glutamate greater than rostral. *B*: rostral concentration greater than caudal. Filled circles: frequency with differential activation. Open circles: control frequency for the same concentration as in the rostral compartment [R] or in the caudal compartment [C].

cies were significantly different from the control for the caudal compartment ($P < 10^{-4}$ for caudal higher than rostral; $P < 10^{-5}$ for rostral higher). Thus the frequency of the differentially activated preparation appears to reflect the intrinsic frequency of the rostral segments, rather than the caudal ones. This is opposite to the mathematical prediction for a chain with only nearest-neighbor coupling, dominant in the ascending direction (Kopell and Ermentrout 1990).

DISCUSSION

Effects of differential activation

The experiments reported in this paper were designed to test predictions of a model of the lamprey spinal cord as a chain of coupled oscillators (Kopell and Ermentrout 1990). A basic assumption of this study is that D-glutamate changes the intrinsic frequencies of the local oscillators along the spinal cord.

From a biological point of view, we cannot rule out the possibility that D-glutamate also affects the strength of the intersegmental coupling. Changes in intersegmental coupling strength would not, however, change the predictions of the mathematics, as long as two criteria are met: the dominant coupling must remain dominant, and the characteristic phase lag for the dominant coupling [the value of ϕ for which the ascending coupling function $H_A(\omega)$ is equal to 0] must not change (see Williams and Sigvardt 1995). The fact that phase lag does not change when the whole cord is bathed in different concentrations of D-glutamate indicates that both these assumptions are valid, and that it is legitimate to treat the effects of D-glutamate as being on the intrinsic frequencies alone.

Evidence for asymmetric coupling in the lamprey

In a chain of phase-coupled oscillators, phase lags can be produced either by nonuniformity of the intrinsic frequency of the individual oscillators in the chain (Cohen et al. 1982; Matsushima and Grillner 1990) or by asymmetry in the intersegmental coupling (Kopell and Ermentrout 1986, 1988, 1990). Such considerations raise two separate questions: first whether there is asymmetry in the intersegmental coupling, and second, if such asymmetry does exist, which direction dominates in determining the intersegmental phase lag and the frequency of oscillation.

In response to the first question, the results reported here indicate that intersegmental coupling in the lamprey spinal cord is asymmetric in the rostral and caudal directions. There are now four quite separate lines of evidence for such asymmetry. *1*) Activity generated by in vitro preparations can be entrained over a greater range of frequencies by imposed movement applied to the caudal end than to the rostral end (Williams et al. 1990). *2*) Intersegmental phase lags change significantly near the rostral but not the caudal end of in vitro preparations (Williams and Sigvardt 1994). *3*) Phase lags change significantly within the rostral but not the caudal compartments of differentially activated preparations (the current study). *4*) The frequency of differentially activated preparations is dominated by the rostral compartment, whether the rostral or the caudal compartment has the higher intrinsic frequency (the current study).

In the context of the model, the first three of these results indicate that ascending coupling is dominant in determining the intersegmental phase lag along the cord (Kopell and Ermentrout 1990; Kopell et al. 1990). In contrast, the fourth result indicates that descending coupling somehow dominates in determining the frequency.

No configuration of the current model (*Eqs. 4–7*), which assumes only nearest-neighbor coupling, is consistent with this full set of results. Accounting for the present results may require the introduction into the model of another type of coupling that affects oscillator frequency independently of phase lag. There is both anatomic and physiological evidence that multisegmental propriospinal neurons play a role in intersegmental coupling (Cohen 1987a; Mellen et al. 1995; Miller et al. 1995; Rovainen 1985). Simulations are underway to explore the possible functional role of combinations of short- and long-range coupling (Cohen and Kiemel 1993; Mellen et al. 1995; Miller and Sigvardt 1995; Williams and Sigvardt 1993). However, further progress awaits more quantitative data on the distribution and relative strength of the long-coupling.

Among the identified classes of interneurons that are candidates for providing intersegmental coordinating signals, most have axons that extend either predominantly in the

TABLE 2. *Effects of differential activation on cycle frequency*

Animal	[D-Glutamate]		Cycle Frequency		
	[Rostral]	[Caudal]	Experimental	R-control	C-control
	Rostral D-glutamate concentration higher than caudal concentration				
1	0.40	0.10	0.600	0.623	0.395
1	0.80	0.10	0.809	0.595	0.395
4	0.80	0.30	1.364	1.193	1.115
5	0.80	0.20	0.758	0.864	0.585
12	0.75	0.25	0.782	0.861	0.560
20	0.70	0.50	0.740	0.853	0.759
20	0.90	0.50	0.798	0.908	0.759
20	1.10	0.50	0.913	0.949	0.759
20	1.30	0.50	1.043	1.055	0.759
21	0.30	0.10	0.735	0.654	0.587
23	1.00	0.40	1.244	1.136	1.124
23	1.40	0.40	1.410	1.248	1.124
23	1.80	0.40	1.653	1.439	1.124
25	0.80	0.40	0.770	0.795	0.510
25	1.20	0.40	0.946	1.029	0.510
28	0.60	0.20	0.805	0.596	0.497
30	0.60	0.40	0.845	0.973	0.593
30	0.80	0.40	1.212	1.122	0.593
30	1.00	0.40	1.401	1.330	0.593
	Caudal D-glutamate concentration higher than rostral concentration				
1	0.10	0.40	0.668	0.395	0.623
1	0.10	0.80	0.431	0.395	0.595
4	0.30	0.80	1.126	1.115	1.193
5	0.20	0.80	0.826	0.585	0.864
12	0.25	0.75	0.524	0.560	0.861
25	0.40	0.80	0.452	0.510	0.795
25	0.40	1.20	0.407	0.510	1.029
28	0.20	0.60	0.394	0.497	0.596
28	0.20	1.40	0.471	0.497	0.970
29	0.40	0.80	0.597	0.619	0.663
29	0.40	1.20	0.680	0.619	0.771
30	0.40	0.60	0.550	0.593	0.973
30	0.40	0.80	0.574	0.593	1.122
30	0.40	1.00	0.633	0.593	1.330
31	0.20	0.40	0.569	0.611	1.058
31	0.20	0.60	0.904	0.611	1.211

Mean frequency data from all experiments including controls, R > C and C > R. R, rostral; C, caudal.

descending or ascending direction and therefore provide synaptic output that is asymmetrically distributed in the cord (reviewed in Grillner et al. 1991). In addition, it has been shown that when inhibitory drive is blocked by strychnine, excitatory drive of motoneurons extends further caudally than rostrally (Dale 1986; Hagevik and McClellan 1994). This is further evidence for asymmetry in the coupling, but in the context of the mathematical model, length does not imply dominance, so this work gives no information about whether ascending or descending intersegmental coupling is dominant in determining phase lag or frequency.

Alternative hypothesis for intersegmental coordination: the "trailing oscillator" hypothesis

Matsushima and Grillner (1990) have suggested that the intersegmental phase lags in the lamprey are due to an increase in the intrinsic frequency of the most rostral oscillator, as first explored mathematically by Cohen et al. (1982). According to this hypothesis, the most rostral oscillator (the "leading" oscillator) has higher excitability and will entrain the remaining ("trailing") oscillators to the same frequency. The leading segment determines the overall frequency, whereas the excitability difference between the leading segment and the other oscillators will determine the phase lag between each segment along the spinal cord (Grillner et al. 1993, 1995; Matsushima and Grillner 1990, 1992a,b).

Evidence taken in support of this hypothesis (Matsushima and Grillner 1990, 1992a,b; Tegnér et al. 1993) is primarily from experiments similar to those reported here but in which phase lags were measured between ventral roots in differentially activated compartments, rather than within the two compartments. Our results (Fig. 7) confirm their findings. However, these results are compatible with both hypotheses. In the context of the mathematical theory, measurements of phase lag between the two compartments cannot distinguish between asymmetric activation and asymmetric coupling. An experiment that does provide a critical test is to measure changes in the intersegmental phase lags within each compartment. In our hands, such experiments (see Figs. 4–6) have shown significant changes in phase lag in the rostral

half of the preparation but not the caudal half. Matsushima and Grillner (1992a) have also presented results from two individual experiments in which intersegmental phase lags were measured within compartments (Figs. 5 and 6 in Matsushima and Grillner 1992a). In these two experiments, changes occurred in the phase lags in the caudal as well as the rostral half of the preparation. We have observed similar changes in phase lag within the caudal compartment in individual experiments, as can be seen in Table 1. However, the mean values for all experiments ($n = 35$) were not significantly different from the controls, in contrast to the significant change seen in the rostral compartment.

Matsushima and Grillner (1990, 1992a) have also measured frequency changes in preparations partitioned into three compartments and differentially activated. They report that the frequency was nearest to that pertaining to the compartment with the highest concentration of amino acid, whether that compartment was rostral, caudal, or middle. This conclusion (which is in contrast to our findings) is based on an unspecified number of experiments, for which no statistics are reported. Reinterpreting our experiments in terms of which compartment has the higher amino acid concentration, it can be seen from the data in Table 2 that in 17 of the 35 experiments, the frequency was indeed closer to that of the compartment with the higher D-glutamate concentration, as reported by Matsushima and Grillner. However, in 31 of the 35 experiments, the frequency was nearer to that corresponding to the rostral half of the preparation, whether it had a higher or lower D-glutamate concentration than the caudal half. Furthermore, statistical analysis supports this conclusion.

Another explanation for the difference between the results of Matsushima and Grillner (1990, 1992a) and the ones reported here may be related to the existence of more extensive long-range coupling in our preparations, which contained 50 segments. In contrast, the preparations used by Matsushima and Grillner were <24 segments long, 16–18 segments long in most cases. Thus the difference in the reported results may be related to the recent finding that there are significant changes in certain characteristics of the fictive locomotor pattern produced by pieces of cord <20 segments long, compared with those up to 50 segments (Miller et al. 1995).

Levels of analysis and modeling

Intersegmental coordination underlying swimming has been studied in the leech (for review see Friesen and Pearce 1993) and the *Xenopus* embryo (Tunstall and Roberts 1994) and tadpole (Tunstall and Sillar 1993). Like the lamprey, the leech and *Xenopus* tadpole have constant phase lags. In the *Xenopus* embryo, intersegmental delay is constant, rather than scaling with cycle duration. In *Xenopus* embryo there is evidence for gradients in synaptic strengths. In both leech and *Xenopus* nervous systems, more detail is available than in the lamprey of the numbers and types of neurons and their connections, and both have been modeled in some detail at the cellular level. To construct a similarly realistic model of neurons and synapses representing the CPG in the lamprey, detailed anatomic and electrophysiological knowledge of the connections between neurons, the lengths of axons, and the strengths and distribution of synapses is required. The large number of neurons in the lamprey spinal cord has made this impossible.

Broadly defined classes of neurons in the lamprey spinal cord have been described, and paired-cell recordings have elucidated some of the connections between them (Buchanan 1982, 1993; Buchanan and Cohen 1982; Buchanan et al. 1989; Rovainen 1974). Simulations of the lamprey CPG have been done on the basis of these broad general classes of neurons with postulated intersegmental connections that give rise to a rostral-to-caudal delay (Buchanan 1992; Grillner et al. 1995; Williams 1992a,b). Those of Grillner and colleagues incorporate the greatest level of biophysical detail (reviewed in Grillner et al. 1995).

Because detailed knowledge of the lamprey circuitry is not available, we have modeled the lamprey spinal cord with an approach that does not require such detail. This approach has provided insights into the general organization of the spinal cord circuitry underlying locomotion. Further development of the model and integration with experimental work is continuing.

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