Functional Analyses of the Leech Swim Oscillator

W. O. FRIESEN AND C. G. HOCKER
Department of Biology, National Science Foundation Center for Biological Timing, University of Virginia, Charlottesville, Virginia 22903-2477

Received 7 June 2000; accepted in final form 2 April 2001

Friesen, W. O. and C. G. Hocker. Functional analyses of the leech swim oscillator. J Neurophysiol 86: 824–835, 2001. The oscillations that underlie swimming movements in the leech arise from a series of identified concatenated circuits within the ventral nerve cord. In the intact nerve cord, ascending and descending intersegmental interactions via axons within the lateral connectives function both to generate robust oscillations throughout the cord and to establish an anterior-to-posterior phase delay among segmental oscillators. We addressed two questions about this system. First, do the intrasegmental swim circuits in each ganglion function as a single oscillator or do they comprise a pair of coupled oscillators? Second, what are the relative strengths of the ascending and descending intersegmental interactions between the segmental oscillators? Experiments were carried out on semi-intact leeches (Hirudo medicinalis) and on isolated leech nerve cords in which “Z-cut” ganglia were generated by cutting one lateral connective nerve anterior and the contralateral connective nerve posterior to the target ganglion. In these Z-cut ganglia, all rhythmic ascending intersegmental input is conveyed via one lateral connective while rhythmic descending input is conveyed via the contralateral connective. We found that rhythmic bursting recorded from the left and right sides of Z-cut ganglia had identical cycle periods with no phase difference, despite strong intersegmental inputs with differing periods from the two swimming ends of the preparations. We conclude that the swim circuits within individual leech ganglia act as single units. Moreover, we determined through correlation and Fourier spectral analyses, that the functional strengths of ascending and descending intersegmental inputs to Z-cut ganglia located in the middle of the nerve cord are approximately equal.

INTRODUCTION

A fundamental question concerning the nature of the neuronal circuits that generate rhythmic movement, namely whether such circuits lie within the CNS or whether they are critically dependent on phasic sensory information, was already clearly defined at the beginning of the 20th century. Although caveats concerning the importance of sensory feedback for shaping fully expressed movement rhythms are appropriate (Pearson and Ramirez 1990; Pearson et al. 1983), the overwhelming evidence demonstrates that CNS circuits generate the oscillations at the center of all rhythmic animal movements (Delcomyn 1980). For animals in which rhythmic movements involve several appendages or the entire body, such as swimmeret movement of crustaceans or swim undulations of elongated animals (for example lamprey, Xenopus, and leech), a further refinement of the initial research problem is to determine the specific locus of the oscillator circuit. That is, do individual segments in these vertebrates and invertebrates have independent functional circuits or is the rhythm-generating system distributed along the neuroaxis? The answer is that individual segments of the spinal cord in crayfish and leeches (Hocker et al. 2000; Murchison et al. 1993) and a few segments in lamprey have such capability (Buchanan and Grillner 1987).

Another question is whether there are two bilateral oscillators or a single one within each ganglion.

Our initial identification of the swim oscillator circuits in the leech revealed primarily interactions within hemiganglia; except for electrotonic coupling between homologue pairs, only the inhibitory connection of cells 123 and 27 crossed the ganglion midline (Friesen et al. 1978). Given this circuit morphology, it was natural to view the fundamental swim oscillator as consisting of bilateral circuits whose output maintained synchrony via the electrical interactions. Further research led to identification of a midline neuron, cell 208 (Weeks 1982), and its strong chemical and electronic coupling to a bilateral pair of INs, cell 60 (Friesen 1985). More recently, additional connections between the two sides of segmental ganglia that are mediated by the inhibitory motor neurons (MNs) have come to light (Friesen 1989a,b). Given these crossed connections, it seems quite likely that each ganglion contains only one oscillator; that is, left and right sides of the ganglion may not be able to generate oscillations independently. If the circuit in each ganglion indeed functions as a unitary oscillator, we are justified in collapsing the swim circuit diagram of Fig. 1A into the form shown in Fig. 1B, wherein laterality of cells and interactions is lost.

The circuit schematics shown in Fig. 1, together with the finding that individual segments generate at least the rudiments of swimming activity (Brodfuehrer et al. 1995; Hashemzadeh-Gargari and Friesen 1989; Hocker et al. 2000) leads to a model in which the leech swim system is a chain of coupled oscillators (Fig. 2A). In this model, each ganglion comprises two coupled unit oscillators. These oscillators are coupled to many others along the nerve cord not merely to nearest neighbors (Fig. 1C). The experiments we describe here were designed to test and explore the functional strength of interactions between the oscillatory neurons within ganglia. Specifically, we addressed the question “are the neuronal interactions between the two sides (Figs. 1A and 2A) of leech ganglia sufficiently strong to force left and right sides to function as a unit?” Alterna-

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tively, are these crossed connections functionally weak enough to permit the two sides to oscillate independently? Our experimental approach was to subject the two sides of a midbody ganglion to differing rhythmic inputs, with one side driven by the anterior nerve cord and the other by the posterior end (Fig. 2B). We also investigated the functional strengths of intersegmental coupling to determine whether ascending or descending intersegmental connections are dominant. Our results demonstrate that oscillator circuits within individual ganglia act as a unit and that ascending and descending intersegmental interactions between these oscillators are of approximately equal strengths.

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

**FIG. 1.** Neuronal circuits underlying leech swimming movements. Neurons are depicted as open circles with numbers to identify the cells. Cells 1, 2, 102, and 119 are inhibitory motor neurons (MNs); the others are intersegmental interneurons (INs). **A:** schema for paired segmental oscillators. Both neurons of homologous pairs within individual segments are shown together with the identified interactions between left and right homologs. The resistor symbol at the bottom indicates that all homologs are coupled by electrotonic interactions. Cells DI and VI encompass, respectively, all of the dorsal and ventral inhibitory MNs. **B:** schema for unitary segmental oscillators. Homologous pairs are shown as single units with neurons arranged in columns by their approximate activity phases during swimming (left: 0°, middle: 120°, and right: 240°). **C:** Anterior ganglion and posterior ganglion. Arrows designating intersegmental interactions are meant to include those with distant segments, not merely adjacent ganglia. One fundamental question we address is whether the intersegmental interactions are sufficiently strong to join the two oscillators into a single unit. **B:** schematic of the Z-cut nerve cord. The two posited oscillators in the Z-cut ganglion (M10) receive differing inputs; the right side receives input from anterior ganglia, whereas the left is connected to posterior ganglia. **C:** Z-cut preparation. Individual lateral connectives are severed on opposite ends of a ganglion (M10 in this depiction). We show that the only communication between the two ends of the cord is via the intersegmental interactions within M10. Thus about half of the intersegmental neuronal connections for the 3 ganglia adjacent to the cuts are eliminated. Ganglion 10 is denoted as a “Z-cut” ganglion; such nerve cords are Z-cut preparations.

**METHODS**

**Preparations**

Medicinal leeches, *Hirudo medicinalis*, were obtained from Lecche USA (New York) and were maintained in aquaria at about 20°C.

The leech CNS comprises head and tail ganglia linked by a chain of 21 serially homologous segmental ganglia. Large, paired nerve cord connectives are conduits for the axons of oscillatory intersegmental interneurons (INs). In addition, Faivre’s nerve, a small median nerve, carries, the axons of swim gating neurons. The nomenclature for identifying the ganglia and their associated peripheral nerves was devised by Kristan and coworkers (1974) and modified by O’Gara and Friesen (1995). The number “M1” is assigned to the first segmental (noncerebral) ganglion of the ventral nerve cord. The large, most caudal ganglion, also known as the tail ganglion, is designated by “T.” The remaining ganglia of the nerve cord are numbered consecutively, in a rostrocaudal direction, ganglia M2 through M21.

Dissections were performed as described earlier (Mangan et al. 1994), with the animals immersed in, and hence anesthetized by, cold physiological saline containing (in mmol/l) 115 NaCl, 4 KCl, 1.8 CaCl2, 2 MgCl2, and 10 HEPES buffer, pH 7.4 (Friesen 1981). Following dissection, the preparation was pinned to the bottom of a glass dish to permit cell visualization by dark-field illumination. Preparations were perfused with saline at a rate of 1–2 ml/min. If preparations did not generate swimming activity in normal saline, we added serotonin (50 μM) to the saline to enhance swim expression.
[Recordings from preparations exposed to serotonin are qualitatively identical to those when only saline is used (Willard 1981).] Additionally, for several preparations we perfused with saline containing elevated concentrations of divalent ions to reduce spontaneous neuronal activity: composition (in mmol/l) 91 NaCl, 4 KCl, 10 CaCl$_2$, 10 MgCl$_2$, and 10 HEPES buffer, pH 7.4.

We performed experiments on two types of Hirudo preparations. One consisted of the isolated ventral nerve cord, with the maximum length extending from midbody ganglion 2 (M2) to the tail ganglion (T). Because of the disruption of IN interactions in Z-cut preparations (see following text), it was often difficult to obtain strong swimming in both ends of the isolated nerve cord. To obtain the strong, prolonged swimming activity required for detailed quantitative analyses, we employed another type of preparation: semi-intact leeches (Kristan et al. 1974), in which sensory feedback from the body wall increases the robustness of swimming activity. Semi-intact preparations consisted of nearly intact anterior and posterior ends of the leech, with head ganglia, M1, M20, M21, and tail ganglia either removed or disconnected from the body wall. We removed the body wall in the middle of the animal to expose five nerve-cord ganglia for extracellular recording (diagram, Fig. 4). To generate a Z-cut ganglion in the middle of the animal to expose five nerve-cord ganglia for extracellular recording (diagram, Fig. 4A). To generate a Z-cut ganglion in either preparation, we cut two of the lateral intersegmental connectives; one cut severed the left intersegmental connective adjacent to one end of the designated Z-cut ganglion; the other severed the right lateral intersegmental connective at the other end (Fig. 2C). In most preparations, Faivre’s nerve remained intact following the procedure to cut lateral connectives. The presence or absence of Faivre’s nerve alters the excitability of the preparation but has at most only weak effects on intersegmental coordination (Hocker et al. 2000; Weeks 1981). We found that swimming activity in the two ends of a Z-cut preparation often have differing cycle periods, with the posterior ends exhibiting the shorter period. In some preparations, we enhanced or reversed these period disparities by partitioning the recording chamber and perfusing the two ends of the nerve cord with salines at different temperatures (Pearce and Friesen 1985a). The results presented in the following text are based on 23 Z-cut preparations. In 13 of these preparations, we obtained bilateral recordings from the Z-cut ganglia, in the remainder the recordings were unilateral; the data from 10 (of the 23) preparations were subjected to quantitative analyses.

**Electrophysiological recordings**

The neuronal oscillations underlying swimming movements are detectable as MN impulse bursts in extracellular recordings from peripheral nerves. The most useful recording site is the dorsal posterior (DP) nerve, which exhibits bursts from a single large axon, that of the dorsal excitatory MN (cell DE-3). In each segment, the median impulse of DP nerve bursts provides a convenient phase reference point for swim oscillations (Kristan et al. 1974). Motor neuron output is a reliable indicator of the period and phase of individual segmental oscillators (Friesen et al. 1978; Poon et al. 1978).

For intracellular recordings, MNs were identified on the basis of their positions within the ganglion, inherent electrical properties and activity, and synaptic connections with other MNs. Glass capillary microelectrodes were filled with 3 M potassium chloride; electrode resistances ranged from 20 to 40 MΩ. Neuronal signals were visualized on an oscilloscope (Tektronix 5111), and recorded with an FM tape recorder (Vetter Model A) or a PCM recorder (Vetter 3000). Transcription from tape to paper was via a penwriter (Gould 2400).

**Data analyses**

Extracellular records were digitized at a sampling rate of 1 or 5 kHz per channel using a DAS-8 (Metrabyte) A/D converter. Digitized records were sampled for the occurrence of cell DE-3 impulses with a simple threshold routine and converted to files listing the temporal occurrence of each impulse. These temporal files were then converted to instantaneous frequency by calculating the interspike intervals, at 10-ms intervals. We performed linear unbiased autocorrelations and cross-correlations on the frequency files to find the period of the bursting and examine phase relationships. We used discrete Fourier analyses to calculate the power spectrum of the frequency data. Some of the analyses were carried out with the rhythm analysis system [RAS, a MatLab (MathWorks) version of our LAS analysis system] (Friesen 1989a) to digitize extracellular records, to detect nerve impulses, and to compute cycle periods. Numerical values in the text are given as means ± SE.

**RESULTS**

Our conceptual model for the system of neuronal circuits that control swimming movements in the leech (Fig. 2A) rests on the fact that swim-related INs have extensive interactions both within and between segmental ganglia. This model requires that some of the interneuronal interactions within ganglia cross the midline but that intersegmental interactions are uncrossed. Previous experiments (Friesen 1989c) demonstrated that there are numerous intrasegmental interactions between contralateral INs; hence the first requirement is met (Fig. 1A). We show in the following text that the second requirement also is met.

**Oscillator INs do not cross the midline**

Except for cell 208, the soma of oscillatory INs lie within one of the lateral packets of nerve cord ganglia. Neurites from these INs either lie ipsilateral to the soma and give rise to an axon in the ipsilateral intersegmental connective nerve (e.g., cell 33) (Friesen et al. 1978) or cross the midline within ganglia to give rise to an axon contralateral to the cell body (e.g., cell 115) (Friesen 1989b). We tested whether the axons of the oscillatory INs cross the midline in a series of experiments in which we cut one lateral connective and the median Faivre’s nerve anterior to a nerve cord ganglion and the contralateral connective and Faivre’s nerve posterior to that ganglion, generating a Z-cut preparation (Fig. 3). We then positioned a stimulating suction electrode, which stimulated axons in all three connectives, and recorded activity in all three via a suction electrode at several positions along this preparation. First, we positioned the stimulating electrode between the ganglion and the lesion and recorded impulse activity at the end of the nerve cord. With this configuration, we recorded a barrage of impulses with a short and constant latency, as well as individual delayed impulses with no fixed latency (Fig. 3A). The short-latency impulses are those conveyed by the uninterrupted axons of one lateral nerve cord. Next we relocated the stimulating electrode to the end of the nerve cord beyond the lesion (Fig. 3B). Now, although all three connectives were again stimulated, the right lesion interrupted impulses traveling through one lateral connective and Faivre’s nerve. At the same time the second lesion, to the left of the ganglion, interrupted all impulses in the contralateral connective. Because no constant-latency impulses appear at the recording electrode with this configuration, it is clear that no axons cross from one side to the other between the two lesioned sites. More specifically, no intersegmental axons cross within ganglion M11. Finally, as a control, we relocated the recording electrode to a point right of the anterior lesion, between the right lesion and the ganglion (Fig. 3C). Once again, we detected a constant-latency volley of
impulses after each stimulus, demonstrating that both lateral connectives were undamaged and capable of conveying impulses.

We performed similar experiments with both long and short chains of ganglia between the lesions and in preparations bathed either in normal saline or in saline with elevated divalent cations. In all experiments, the double lesions interrupted short-latency impulse traffic between the ends of the nerve cord. We conclude that intersegmental axons of INs within the lateral connectives do not cross the midline to continue in the contralateral connective nerve. Thus any interactions between the two ends of a Z-cut preparation (as modeled in Fig. 2B) must be via chemical or electronic synaptic interactions within the Z-cut ganglion.

Leech midbody ganglia comprise a single swim oscillator

When intact, the full-length isolated nerve cord acts as a unitary oscillator for swimming, with left-right symmetry and with motor output that is phase-delayed along the nerve cord. Although there exists some variation in the intersegmental phase lags, segmental swim circuits in the intact cord express the same period of MN activity (Fig. 4A). Because of bilateral symmetry in the oscillator circuits (Fig. 1A), the left and right intersegmental connectives carry identical information. However, the oscillator circuits are not functionally identical in all parts of the nerve cord. Instead, cycle periods generated by anterior ganglia in isolated nerve cords are usually longer than those generated by more posterior ganglia (Pearce and Friesen 1985a,b). Our approach for testing the hypothesis that individual ganglia comprise a unit swim oscillator was to subject the left and right sides of segmental ganglia to rhythmic inputs with differing cycle periods either in isolated nerve cord or in semi-intact preparations. In either case, our preparations consisted of long chains of ganglia with a lateral connective cut on one side of a targeted “Z-cut” ganglion and the contralateral connective cut on the other side (Fig. 2C). The Z-cut ganglion thus receives unilateral input from rostral ganglia on one side and bilateral input from caudal ganglia on the other side.

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and unilateral input from caudal ganglia on the contralateral side. Because of the nonuniformity of cycle periods in the nerve cord, the cycle period of ascending inputs to Z-cut ganglia was usually longer than the descending ones. (It is important to note that the cuts to the lateral connectives allow descending axons in the intact anterior connective nerve and ascending INs from the contralateral posterior nerve cord to impinge without diminution onto neurons of the Z-cut ganglion.)

BURSTING IN Z-CUT PREPARATIONS IS NOT PHASE-LOCKED. The physiological consequences of the Z-cut procedure are illustrated in Fig. 4. In the control preparation, prior to lesioning, the entire preparation generates a unified swimming pattern with a single cycle period (0.64 ± 0.02 s) as shown in the extracellular records (Fig. 4A) and observed in movements of the innervated ends. When the two cuts are made (Fig. 4B), the unity of the system is disrupted. Now the phase-locked swimming rhythm that existed before the lesions were imposed is divided into two rhythms. The cycle period in the anterior nerve cord as measured in DP(R,7) bursts is 1.24 ± 0.07 s, whereas the period of the swim oscillations in the posterior end of the cord, recorded from DP(L,9), is 0.69 ± 0.04 s (Fig. 4B). The coupling between anterior and posterior ends of the cord, via synaptic interactions within Z-cut ganglion M8, is too weak to phase-lock oscillations with the two very different periods.

Neuronal oscillations within the Z-cut ganglion result from the summed actions of its intrinsic (relatively weak) oscillator circuit and the relatively strong rhythmic inputs from the two ends of the nerve cord. Consequently, the MN activity in the Z-cut ganglion reflects both the long-period oscillations of the anterior nerve cord sector, the shorter period bursting in the posterior end of the preparation, and the intrinsic rhythmicty of the Z-cut ganglion. Thus it is useful to view the Z-cut preparation as comprising three oscillators: one in each end of the preparation and a third within the Z-cut ganglion. Quantitative analyses of such oscillations in a long swim episode are described in the following text.

LEFT/RIGHT RECORDINGS FROM Z-CUT GANGLIA SHOW SYNCHRONOUS BURSTS. To determine whether the differing periods of two ends of the nerve cord can drive the left and right sides of the Z-cut ganglion at two frequencies, we recorded MN activity on both sides of Z-cut ganglia. In the sample swim episode illustrated (Fig. 5A), the differences in cycle period between the two ends of the preparation were small (anterior, 0.81 ± 0.02 s; posterior, 0.72 ± 0.03 s), and hence the phase relationship between the oscillations in the anterior and posterior ends changed gradually. These phase changes cause variations in the intensity of bursts (impulse frequency) recorded from the Z-cut ganglion [DP(R,10) and DP(L,10)]. These bursts are strong at the beginning and end of the record but weak in the middle as the phase of the posterior ganglia first lags, then leads, and finally again lags that of the anterior one. Despite these changing phase relationships between the two ends of the preparation, the two DP records from M10 were synchronous throughout the episode.

To assess phase locking and intrasegmental phase relationships between left and right sides of Z-cut ganglia, we digitized swimming bursts from both sides of Z-cut ganglia in seven preparations. We converted these digitized records of impulse activity into instantaneous frequencies and then performed auto- and cross-correlations. We observed a peak at 0 time lag in three of the preparations (Fig. 5B) and small shifts from 0 time lag in the remaining four. Converted to phase differences, these seven preparations had a mean phase difference of 2.8 ± 5.5° (mean ± SD), with largest difference 22.5°. This mean difference in phase between left and right sides was not significant (t = 1.33; 6 df, P > 0.2).

BURSTING IN Z-CUT GANGLIA IS MODULATED BY ACTIVITY IN THE ANTERIOR AND POSTERIOR NERVE CORDS. The bursting activity in Z-cut ganglia results from the mixing of rhythmic drives from anterior and posterior sectors of the nerve cord. Perhaps not surprisingly, when the periods of these two inputs differ slightly beating is observed (isolated CNS preparation, Fig. 6A; anterior period, 0.69 ± 0.01 s, posterior period, 0.76 ± 0.02 s). Figure 6A illustrates clearly this beating phenomenon as the waxing and waning in the intensity of the impulse bursts in the DP recordings obtained from the Z-cut ganglion (M10). At the beginning of the illustrated swim episode, posterior bursts follow the anterior ones with a nearly appropriate phase lag, leading to strong constructive interference during the first three bursts. As the swim bursts in the posterior ends become more phase delayed because of their longer cycle period, destructive interference causes the successive weakening and finally cessation of bursting in the Z-cut ganglion (similar to data shown in Fig. 5). Near the end of this

![FIG. 5. Swimming activity in semi-intact Z-cut ganglia. A: preparation (top) with lateral connectives cut (arrows) to create a Z-cut midbody ganglion at M10. In this preparation, the cycle period of swimming activity in the anterior nerve cord was slightly longer (0.81 ± 0.02 s) than in the posterior sector (0.72 ± 0.03 s). Thus the intersegmental inputs to the left and right sides of the Z-cut ganglion (M10) are asymmetrical. Nevertheless, records from the right and left aspects [DP(R,10) and DP(L,10)] of M10 show simultaneous bursts that are not tightly phase-locked with bursting in either the anterior or posterior records. Vertical lines are meant as an aid in observing phase relationships in the 4 traces. B: cross-correlation of activity in an M8 Z-cut ganglion in another semi-intact preparation. The peak of the correlation occurs at 0 time lag, demonstrating that the left and right sides of M8 are oscillating in phase. The 95% confidence limit for the central peak is ±0.035.](http://jn.physiology.org/10.22036.33.6)
episode, the phase relationship between the anterior and posterior oscillators once more has the appropriate delay and strong bursting resumes in the Z-cut ganglion. Please note that when strong bursts occur in the Z-cut ganglion, the phase of this activity is between that of the anterior oscillators, strongly implying that activity in the Z-cut ganglion is the normal “swim-like” bursting of the system rather than simply activity driven by intersegmental input. Also note that bursts on the two sides of the Z-cut ganglion are synchronized; the two sides do not independently follow activity in the anterior and posterior chains. Thus similar phase-locked beating rhythms occur in isolated CNS and semi-intact Z-cut preparations.

BURSTING IN Z-CUT GANGLIA DEPENDS ON THE LENGTHS OF THE ANTERIOR AND POSTERIOR SECTORS. In the preparation illustrated in Fig. 6A, there are eight midbody ganglia anterior to the Z-cut ganglion and 11 at the posterior end. Thus two nearly equal long chains of oscillators are imparting their input to the Z-cut ganglion. We examined the effects of changing the inputs to the Z-cut ganglion by progressively shortening the anterior chain of ganglia both to reduce the robustness of swimming in the anterior oscillator and to decrease the number of INs interacting with M10. The consequence was that the cycle period of the anterior oscillator increased until, with only two ganglia in the chain, the cycle period of the anterior chain nearly doubled ( anterior period, 1.43 ± 0.12 s, Fig. 6B). With swim activity in the anterior cord thus weakened, activity in the two ends of the cords assumed a 1-to-2 relative coordination during most of the episode (posterior period, 0.76 ± 0.05 s). Bursts in the Z-cut ganglion were phase-locked to activity in the posterior cord and much more regular than when anterior and posterior chains were of equal lengths (cf. Fig. 6, A and B). Nevertheless, these bursts were strongest during alternate posterior bursts [in DP(R,15)] when these bursts were preceded by ones in the anterior sector of the preparation [in DP(L,9)]. Thus the posterior chain comprising 11 midbody ganglia clearly had a much greater influence on bursting in the Z-cut ganglion than the two-ganglion anterior chain.

PHASE RELATIONSHIP BETWEEN LEFT AND RIGHT SIDES OF Z-CUT GANGLIA. We digitized records from the preparation used in Fig. 6 to examine the phase relationship between bursting on the two sides of Z-cut ganglia. We found that MN activities on the two sides of Z-cut ganglia are always very nearly in phase, at 0°. As illustrated in Fig. 7, there are small differences in the characteristics of bursts on the two sides. Often the two sides will undergo small modulations in burst duration and frequency, which reflect their differing inputs from the anterior and posterior nerve cord. Such small differences can be seen in Fig. 7A, 2 and 4. In addition, the phase relationships between the two sides sometimes reveal small deviations from 0°. These deviations are not greater than the normal variance in ganglia that have not been Z-cut. We observed no large fluctuations in phase that would be present if there were two oscillators were cycling at differing periods (Fig. 7B1) in the Z-cut ganglia.

We performed quantitative analyses with circular statistics (Fisher 1995; Yu et al. 1999) on five preparations to determine left-right phase relationships in Z-cut preparations. These phase differences ranged from 2.9 to 13.2° (values were: 2.9, 3.3, 2.9, 7.3, and 13.2°). These left-right phase deviations from zero were statistically significant at the 0.05 level in only one of these five preparations. The mean of phase values from all five experiments was 6.3 ± 4.23° (mean ± SD). Although small, the average phase difference was significant (t = 3.34, 4 df, P < 0.05). In all preparations, with either anterior or posterior nerve-cord sectors having the shorter cycle period, we always found that swimming activity on two sides of Z-cut ganglia had identical cycle periods. We conclude that synaptic interactions across the midline of nerve cord ganglia are sufficiently strong to prevent the two sides from expressing differing cycle periods even when driven strongly by INs with different periods from the two ends of the nerve cord. Such differing drives act primarily to differentially modulate the amplitude of the oscillations on the two sides. Thus segmental ganglion comprise one, rather than two, functional oscillator circuit. The synaptic interactions within segmental ganglia (Fig. 1A) are evidently sufficiently strong to form a unified oscillator.

MEMBRANE POTENTIAL OSCILLATIONS IN Z-CUT GANGLIA ARE SYNCHRONOUS. To investigate whether synchronous activity recorded from excitatory MN on the two sides of Z-cut ganglion accurately reflects that of deeper levels, we penetrated dorsal inhibitor cells (DI-1) on both sides of a Z-cut ganglion. We found that just as at the level of excitatory MNs, bilateral...
inhibitor homologs remained phase-locked when the subjected to inputs of differing periods from the anterior (period, 1.11 ± 0.7 s) and posterior (period, 0.94 ± 0.08 s) ganglion chains (Fig. 8). We conclude that also at the level of the inhibitory MNs, which participate in generating the swim rhythm, Z-cut ganglia comprise a unitary oscillator.

Relative strengths of ascending and descending input to the Z-cut ganglion

PROLONGED SWIM EPISODE FOR QUANTITATIVE ANALYSES. With the unity of segmental swim oscillators established, we were interested in assessing the relative strengths of ascending and descending interactions among the segmental swim circuits. We know that intersegmental interactions play a critical role in generating not only the progressive phase delays of bursting along the nerve cord but also in generating the cord-wide rhythm itself. Our recent findings (Hocker et al. 2000) demonstrate that there is a greater heterogeneity in the ability of nerve cord ganglia to generate stable swim-like oscillations than we had previously understood. In fact, only ganglia in the middle of the nerve cord are individually capable of generating stable, constant-period oscillations. Oscillations generated by the anterior ganglia are weak and irregular; ganglia posterior to ganglion M13 appear incapable of individually generating any swim-like oscillations without sensory feedback from the body wall. To evaluate the functional strength of ascending and descending intersegmental interactions between ganglia, we again employed Z-cut preparations. We describe here in some detail the quantitative analyses of one particularly prolonged swim episode. Similar analyses were performed on nine other preparations that exhibited briefer swim episodes.

Our approach was to generate swim episodes in semi-intact Z-cut preparations while recording from DP nerves of anterior, posterior, and Z-cut ganglia. We then digitized these records, converted the spike trains to instantaneous impulse frequencies for quantitative analyses of the periodic behavior of the records. The specific preparation for one experiment and a 30-s record of one prolonged swim episode are depicted in Fig. 9. The record of Fig. 9 shows a strong swim episode in which the anterior and posterior body sectors underwent body-wall undulations and the DP nerves yielded strong MN impulse bursts. Because the cycle period of the anterior nerve cord is greater than that of the anterior, the phase relationship is not fixed. Rather it varies over the entire 360° range. B2: phase relationship between the two sides of the Z-cut ganglion [DP(R,10) vs. DP(L,10) bursts]. Please note that the phase relationship between left and right sides remains near 0° (average difference is 4.7° and not statistically significant—95% interval is 5.2°). Moreover, the number of impulses and the duration of bursts covary in the two records. Nevertheless the two sides do show small differences in burst characteristics and undergo minor phase shifts.
software) (Friesen 1989a; Hocker, unpublished data) and then analyzed those temporal files to detect the occurrence of cell 3 impulses with a simple threshold algorithm. Further data reduction included the conversion of the temporal data into instantaneous frequency by taking the reciprocals of the interimpulse intervals and plotting the resulting values at 10-ms intervals. This conversion generates graphs in which the impulse bursts of extracellular recordings are represented as bouts of high-frequency activity with low-frequency interburst intervals (Fig. 10).

CORRELATION ANALYSIS. To determine the average period of the impulse bursts in the prolonged swim episodes, we performed autocorrelation analyses on records of the instantaneous impulse frequencies. The correlation graph for the anterior sector has large, symmetrical peaks that repeat with at multiples of a 1.37 s time lag (Fig. 11, top). Thus the cycle period in the anterior cord is long and constant. The corresponding cycle frequency is 0.73 Hz. Similarly, the correlation diagram for the posterior sector (Fig. 11, bottom) exhibits repeated peaks at multiples of 0.85 s (corresponding to 1.18 Hz). The lower amplitude of these peaks, together with their decreasing amplitude at greater time lags, indicates that the swim oscillations in this sector are less constant than in the anterior sector of the preparation. The occurrence of peaks at both 1.53 and 0.96 s time lags in the autocorrelation graph for the Z-cut ganglion (Fig. 11, middle) is most interesting. These peaks occur at period values corresponding closely to those in the anterior and posterior sectors. Although both anterior and posterior sectors of the nerve cord strongly influence the oscillations in the Z-cut ganglion, the effect of the anterior sector, which gives rise to the larger peak (at 1.53 s), is clearly stronger. We observed clear double peaks in autocorrelations of impulse bursts recorded from Z-cut ganglia in two other preparations. Thus ascending and descending inputs are both functionally important—each is effective in setting the frequency of the oscillations in Z-cut ganglia.

POWER SPECTRAL ANALYSIS. We applied a second metric to these swim data, power spectral density analyses, to learn more about the relative strengths of the ascending and descending inputs to Z-cut ganglia. For these analyses, we determined the power spectral densities in the frequency records (Fig. 12). Plotted on a linear scale, we found peak values at 0.74 Hz (anterior sector) and 1.08 Hz (posterior sector) and two peaks at very similar frequency values, 0.72 and 1.11 Hz, in the Z-cut ganglion. Thus by this metric as well, anterior and posterior sectors of the nerve cord provide functionally significant input to Z-cut ganglia.

The power spectral density peak in the Z-cut record is higher at 0.72 Hz than at 1.11 Hz (20,000 vs. 9,500), a ratio for ascending versus descending effects of 2.1. Corresponding ratios in two other preparations in which we recorded from the two sides of Z-cut ganglia were 1.41 and 2.25 for the two sides,
respectively, in one and 2.04 and 1.53 for the two sides, respectively, of the other. One might be tempted to conclude that the ascending IN connections therefore are functionally stronger than the descending connections. However, the power peak in the anterior record was considerably greater (80,000) than that of the posterior one (23,000) for the preparation shown in Fig. 12 as well as for the two other preparations. Thus the peak-height disparity in the Z-cut record might simply reflect a stronger, more coherent source of ascending rhythmic input rather than a difference in the actual strength of synaptic connections. To compensate for the larger anterior power spectrum peak, we normalized the peaks in the Z-cut DP records by the values of the peaks in the anterior and posterior sectors. Dividing 20,000 by 80,000 (to obtain the relative strength of the effect of the anterior sector on the Z-cut ganglion) and 9,500 by 23,000 (to obtain the same metric for the effect of the posterior sector) generates normalized peaks of 0.25 for ascending and 0.41 for descending inputs for the spectral power transferred to the Z-cut ganglion. For the two additional preparations in which these detailed calculations were feasible, the ratios for the ascending connections were 0.25 and 0.20, for the two preparations, respectively, and for the descending connections the ratios were 0.38 and 0.25, for the two preparations, respectively. These differences in the normalized ratios for connections strengths in these three preparations are not significant (ascending mean ± SD are 0.29 ± 0.11; descending, 0.30 ± 0.07). By either measure, we can conclude that both descending and ascending IN interactions are functionally important and that the strengths of these interactions do not differ greatly.

DISCUSSION

Our aim in performing these experiments was to determine two system-level properties of the leech swim oscillator circuits. First, our results demonstrate that the segmental circuits of bilateral INs and MNs function as a single oscillator rather than as two independent units. We also showed that ascending or descending interactions between these segmental oscillators are of approximately equal strength. This latter conclusion is based on our observation that oscillations in Z-cut ganglia exhibit strong periodicities from both the anterior and posterior sectors of the nerve cord. The strengths of the periodicity conveyed depend on the specific configuration of the preparation—a very short chain of ganglia conveys very little period...
The span of intersegmental projections

Individual ganglia from M2 through M12 include oscillatory circuits that can generate the rudiments of swimming activity (Hashemzadeh-Gargari and Friesen 1989; Hocker et al. 2000; Weeks 1981). The complete set of circuits that generate swimming activity in the leech is complex; that is, the segmental circuits are highly interconnected. Not only are there numerous interactions within segmental ganglia (Fig. 1, A and B), but all of these INs project also to more anterior or more posterior ganglia (Fig. 1C). The span of the projections for some of these IN is at least five segments (Poon et al. 1978; Weeks 1982). In experiments not presented in detail here (Friesen, unpublished data), we examined the projections of cells 115 and 28. The maximal extent of the intersegmental projections, measured physiologically via detection of extracellularly recorded impulses in intersegmental connective nerves, was no more than seven segments. In the absence of precise data for the span of every oscillatory IN in all ganglia, we employed this new information about cells 115 and 28 to make the approximation that the projection span of an inhibitory IN is five or six segments but not more than seven segments. In a topological sense, then, the functional oscillator unit in the middle segments of the nerve cord comprises about 12 segments and more than 150 oscillatory INs (13 INs per ganglion × 12 ganglia).

How many oscillators per segment?

Experiments in other preparations show that individual CNS segments may comprise one or two oscillators. In the lamprey, for example, the swim circuits appear to function as a single bilateral unit. Intracellular recordings from lamprey interneurons by Buchanan and coworkers (Buchanan 1982; Buchanan and Cohen 1982) revealed reciprocal inhibitory synaptic interactions between contralateral interneurons, the CCIN. Extensive modeling experiments carried out on the lamprey swim circuit, which rely on these data, are based explicitly on the assumption that reciprocal inhibition between contralateral inhibitory neurons contributes to the generation of swim oscillations (Kotaleski et al. 1999; Ullström 1998; Wadden et al. 1997). The experiments by Cohen and Harris-Warrick (1984) do suggest that lamprey spinal segments may comprise two oscillators. In their study, crossed inhibitory synaptic connections in lamprey spinal cords were blocked with strychnine, revealing that rhythmic activity can occur independently on the two sides of the cord. However, motor neuron output on the two sides of the cords was nearly synchronous rather than antiphasic, hence the oscillations observed probably were not generated by the same mechanisms as the normal antiphasic swim rhythm. In vertebrate studies on the generation of rhythmic movements, the evidence points to separate left-right oscillators. Thus in low spinal cats, the two hindlimbs could walk with differing periods (Forssberg et al. 1980). Experiments on turtles similarly indicate that left and right sides of the spinal cord have some independence in generating scratch reflexes (Stein et al. 1995, 1998a,b). In this latter context, it is interesting to note that the authors stress repeatedly that the central pattern generator is bilaterally distributed—as it is in the leech swim oscillator.

Recent work on the swimmeret system in the crayfish demonstrates that the beating movements of each swimmeret can be generated within hemiganglia of the nerve cord. In these experiments, the abdominal ganglia of the nerve cord were cut down the midline or interactions were blocked with TTX. Such preparations were sometimes capable of generating weak, antiphasic oscillations between MN antagonists (Murchison et al. 1993). Because of the massive disruption of intrasegmental interactions, these experiments do not answer the functional question of whether the swimmeret pairs operate as a unit in the intact system. [Similar bisections of leech ganglia destroy all rhythmic activity in the cut ganglia (Friesen, unpublished data).]

Our experiments demonstrate that swim circuits within midbody ganglia functionally comprise a single, unitary oscillator.
Even very disparate intersegmental inputs from anterior versus posterior ganglia cannot drive the two sides at differing periods nor cause significant differences, except for one preparation, in left-right phases in swim bursts (Fig. 5B). Although the strong intrasegmental interactions across the midline of leech ganglia (Fig. 1A) were strongly suggestive of this unity, only a functional test could provide the conclusive answer to the question posed in the introduction. It is interesting to note that the neuronal circuits generating the rhythmic contractions of the bilateral heart tubes in leeches also comprise a single unit within individual ganglia. In this system, as in the lamprey and leech swim circuits, there are strong inhibitory synaptic interactions across the midline (Calabrese and Arbas 1989).

What is the relative strength of ascending and descending interactions?

Two important questions regarding intersegmental coordination are whether the ascending or descending interactions between segmental oscillators are symmetric and whether one of them dominates to generate normal phase relationships in multisegmental motor systems (Skinner and Mulloney 1998b). The ascending and descending interactions are asymmetric in three diverse species: leeches (Friesen 1989c), lamprey (Buchanan 1982, 1996), and crayfish (Braun and Mulloney 1995; Skinner and Mulloney 1998a), suggesting that such asymmetry is ubiquitous. These interactions, together with the intrinsic cycle periods of segmental circuits, determine the intersegmental phase relationships. In crayfish, where there appears to be no gradient in cycle period along the swimmeret neuroaxis (Mulloney 1997), the functional intersegmental interaction strength appears to be strongest in the posterior direction (Braun and Mulloney 1995). The issue of whether intersegmental interaction strengths are functionally polarized in the lamprey is still unresolved (Hagevik and McClellan 1999). One possibility is that ascending coupling dominates for entrainment and setting intersegmental phase lags, and descending coupling dominates for setting the cycle period (Sigvardt and Williams 1996).

In leeches, the INs that generate the swimming rhythm have either long rostral or caudal intersegmental projections. In the currently identified set, there are five neurons that project caudally—two cells 115, two cells 123, and cell 208, and eight that project in the rostral directions—two each of cells 27, 28, 33, and 60. The experiments presented here now provide one quantitative measure of the effectiveness of these INs and of any that remain undiscovered. At least in the middle of the nerve cord, where the differences in the amplitudes of correlation peaks (Fig. 11) and power spectral peaks (Fig. 12) in Z-cut ganglia are small, neither the rostral nor the caudal projections dominate. It should be noted that in our experiments to determine the relative strength of intersegmental coupling, the length of the intact anterior cord comprised either six or seven ganglia, whereas the posterior cord comprised between 10 and 14 ganglia. Because the span of intersegmental interactions in the leech is only about six segments (Pearce and Friesen 1985b), equal numbers of segments provided input to the Z-cut ganglion from both anterior and posterior ends of the cord. We propose that intersegmental phase lags in the leech nerve cord result from the specificity of the interconnections—that is from the specific postsynaptic target neurons and sign (inhibitory or excitatory) of the synaptic interactions—not from differences in synaptic strength. These currently identified intersegmental interactions between INs can account qualitatively for the anterior to posterior phase lags (Friesen and Pearce 1993).

Overview of the leech swim oscillator system

Leech swim oscillator INs are, to the extent examined—M2–M18—found in all ganglia of the leech ventral cord. However, we now view the system as a concatenation of functionally dissimilar units. Ganglia in the middle third of the nerve cord, M7–M12, can individually generate moderately strong, swim-like oscillations with a cycle period of about 1.0 s. The anterior ganglia generate at best weak, irregular swim-like bursts. Those posterior to M12 are incapable of oscillations in isolation (Hocker et al. 2000; Pearce 1985a). Coupling between swim IN is strong, not only within ganglia as we have demonstrated here, but also between ganglia (Friesen et al. 1978). Thus despite segmental heterogeneity, the intact leech nerve cord is a functional unit, generating a common cycle period with progressive rostrocaudal phase lags when the animal, or reduced preparation, is swimming. We conclude that the leech swim circuit cannot be viewed as a chain of weakly coupled robust segmental oscillators. Rather it is a system of individually weak unit oscillators that are strongly coupled into a single functional unit by powerful intra- and intersegmental interactions.

We gratefully acknowledge Dr. Martin Straume, Center for Biological Timing, for expert assistance with data analyses. Thanks to Dr. Gisele Oda, X. Yu, and J. Cang for stimulating discussions and K. Dame for editorial assistance.

This work was supported by National Science Foundation Grants IBN9410779, IBN97-23320, and DIR99-20162.

Present address of C. G. Hocker: Div. of Neuroscience S-603, HHMI—Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030-3498.

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