The Journal of Experimental Biology 205, 3707–3716 (2002)
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JEB4433

Changes in locomotor activity parameters with variations in cycle time in larval lamprey

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Accepted 11 September 2002

Summary

In larval lamprey, locomotor activity recorded from whole animals and *in vitro* brain/spinal cord preparations was analyzed to determine how two parameters of locomotor activity, burst proportion (BP; relative duration of motor burst activity) and intersegmental phase lag (ϕ; normalized delay of burst activity along one side of the body), vary with changes in cycle time (*T*). In individual animals, the slopes of BP and ϕ *versus T* were compared using linear regression analysis, followed by statistical analysis of the slopes to determine whether the parameters changed significantly with variations in cycle time.

For locomotor muscle activity in whole animals, the BP values increased significantly with decreases in *T* (i.e. negative slopes), while the slopes for ϕ values *versus T* were not significantly different from zero. For locomotor activity in preparations *in vitro*, the mean slopes for BP values *versus T*, although negative, were not significantly different from zero, and phase lags were also relatively constant with changes in cycle time.

Increases in BP with decreases in cycle time and increases in swimming speed can be expected to generate proportionately more force per cycle, presumably to compensate for the increase in viscous resistance of moving the body more rapidly through water. By contrast, constant intersegmental phase lags will ensure that the relative timing of locomotor burst activity is constant and that an approximately single S-wave along the body is retained during different swimming speeds.

Key words: central pattern generator, coordination, phase lag, oscillator, swimming, lamprey.

Introduction

The basic temporal pattern of locomotor activity (i.e. walking, flying, and swimming) is generated by central pattern generators (CPGs) in the spinal cord that consist of neuronal oscillators as well as motor neurons and coordinating neurons (for reviews, see Stein, 1978; Grillner, 1981; McClellan, 1996). Oscillators project to motor neurons, which directly control contraction of muscles, while coordinating neurons couple the oscillators to control the relative timing of locomotor activity for different parts of the body. The spinal CPGs can produce the basic pattern of locomotor activity without sensory feedback, but sensory inputs are crucial for normal locomotion, particularly in response to environmental perturbations (for a review, see Grillner, 1981). Locomotor activity is initiated by a command system in the brain that activates the spinal CPGs.

Undulatory locomotor behavior (i.e. swimming) in most fish and certain aquatic amphibians is a repetitive motor act that is mediated by two basic features (Gray, 1933a,b, 1936; for reviews, see Grillner and Kashin, 1976; Williams, 1986; Roberts et al., 1997) (Fig. 1). (1) At each segmental level of the body, right–left bending is produced by right–left alternation of burst activity in axial muscles; and (2) rostral-to-caudal propagating body undulations are produced by a rostrocaudal delay of axial muscle burst activity on the same side of the body. These features result in an S-shaped body wave that propagates toward the tail and results in both lateral and backward force vectors (Gray, 1933a,b; Webb, 1984; Bowtell and Williams, 1991). The lateral force vectors in the rostral and caudal body are in opposite directions and cancel, leaving the backward components of the force that push against the water and propel the animal forward. During swimming, there is usually approximately one S-wave along the body during different speeds of swimming (reviewed by Williams, 1986). A single S-wave along the body is a highly efficient manner in which to generate backward force during swimming, since multiple S-waves would require additional muscle contraction forces to produce multiple regions of sharper than normal bending of the body.

For swimming, the CPGs in the spinal cord are thought to consist of a chain of left and right segmental oscillators that are coupled by reciprocal inhibition and that produce left–right alternation (for reviews, see Grillner et al., 1995, 2000; Roberts et al., 1997). Longitudinally in the spinal cord, the oscillators are coupled by a spinal coordinating system that is important

for generating rostrocaudal phase lags (for a review, see McClellan, 1996).

The lamprey, a 'lower' vertebrate, utilizes the 'anguilliform' mode of swimming, in which all or most of the body is 'slender' and flexible, and lateral displacements of the body gradually increase as the undulations propagate toward the tail

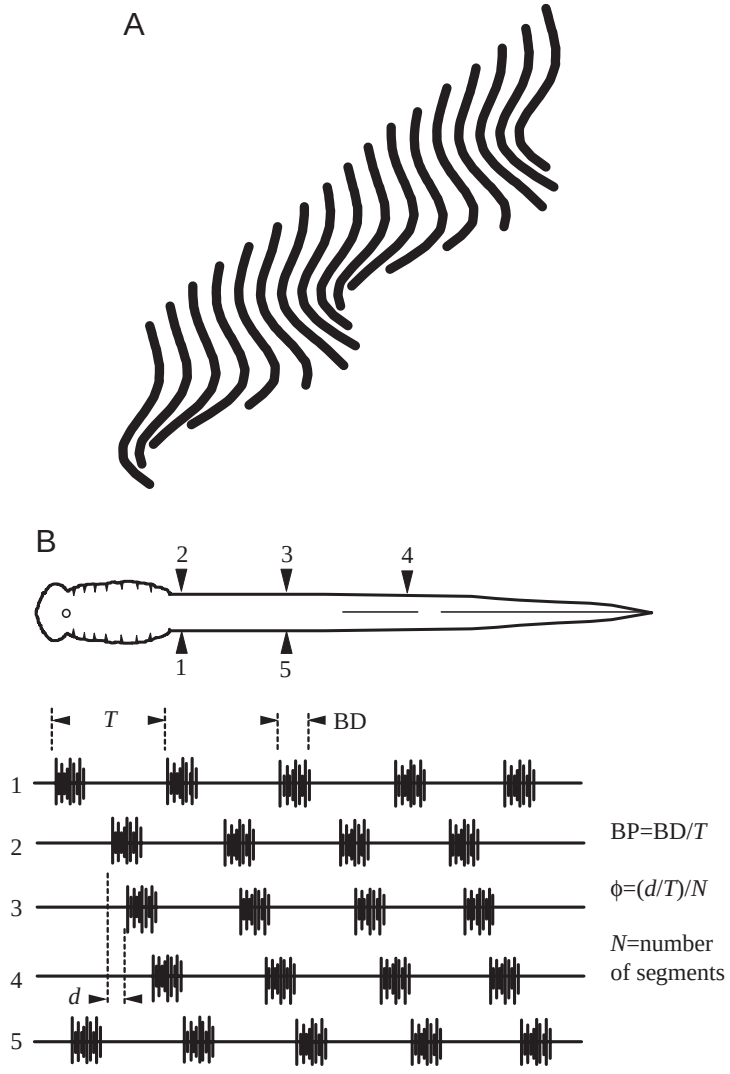

Fig. 1. Swimming in larval lamprey. (A) Idealized diagram illustrating the sequential movements, from left to right, of a lamprey swimming in a forward direction. (B, top) Diagram of a larval lamprey showing the positions of muscle recording electrodes (1–5). (B, bottom) Idealized locomotor activity generated during swimming, characterized by left-right alternation (1↔2 and 3↔5) and a rostrocaudal phase lag (1→5, 2→3 and 3→4). Parameters of locomotor activity: (i) Cycle time (*T*), defined as the time interval between successive cycles or bursts, is inversely related to burst frequency *f* (*f*=1/*T*) and swimming speed. (ii) Burst duration (BD) is defined as the interval between the onsets and offsets of bursts. (iii) Burst proportion (BP) is calculated as the ratio of burst duration and cycle time (BD/*T*). (iv) Burst delay (*d*) is the interval between rostral and caudal bursts in the same cycle on the same side of the body. (v) Intersegmental phase lag (ϕ) is calculated as the ratio of burst delay and cycle time divided by (i.e. normalized to) the number of intervening body segments (*N*) between the recording sites [(*d*/*T*)/*N*].

(Williams, 1986; Davis et al., 1993; also see Grillner and Kashin, 1976). Swimming motor activity in the lamprey and fish can be described by several parameters (Grillner and Kashin, 1976; Wallén and Williams, 1984; McClellan and Hagevik, 1997) (see Fig. 1B). These include (i) cycle time (*T*), which is inversely related to burst frequency (*f*=1/*T*) and swimming speed, (ii) burst duration (BD), (iii) burst proportion (BP=BD/*T*), (iv) burst delay (*d*) and (v) intersegmental phase lag (ϕ=[*d*/*T*]/*N*), where *N* is the number of intervening body segments between the recording sites. For adult lamprey swimming in a 'swim mill', burst proportions (BP) and intersegmental phase lags (ϕ) are relatively constant during changes in cycle time (Wallén and Williams, 1984; for reviews, see Grillner and Kashin, 1976; Williams, 1986), thus:

$$BP = m_1 \times T + b_1 \quad (1)$$

and

$$\phi = m_2 \times T + b_2\,, \quad (2)$$

where *T* is in ms, and the slopes m_1 and m_2 are relatively small (i.e. ≈0), such that BP≈b_1 and ϕ≈b_2. Thus, as cycle times change, the overall relative timing of the locomotor pattern (see Fig. 1B) remains constant, and approximately a single S-wave appears along the body at all times.

Recent evidence from the isolated spinal cord of adult lamprey, in which locomotor activity was evoked by bath-applied pharmacological agents, suggests that intersegmental phase lags might not be constant but appear to increase with decreasing cycle times (Tegnér et al., 1997). However, it is not known if the parameters of locomotor activity are controlled in a similar fashion in larval lamprey. For example, there appear to be some minor qualitative differences in the kinematics of swimming in adult and larval lamprey (A. D. McClellan, unpublished observations). In addition, it has been suggested that the spinal locomotor circuitry in larval lamprey is immature compared to adults, due to a lack or immaturity of some types of cells (Cohen et al., 1990). In larval lamprey, it is important to determine how the parameters of swimming motor activity vary with cycle time, because this information will be necessary for formulating and testing models of spinal CPGs.

In larval lamprey, preliminary results for brain-initiated locomotor activity in brain/spinal cord *in vitro* preparations suggest that the slopes for intersegmental phase lag *versus* cycle time are not significantly different from zero (i.e. ϕ is relatively constant) (McClellan and Hagevik, 1999). In the present study, a much more comprehensive analysis was made using archival locomotor activity from several of our previous studies (see Materials and methods for list of references) to test whether in fact burst proportions (BP) and intersegmental phase lags (ϕ) are constant with changes in cycle time (*T*) in larval lamprey. Spontaneous or sensory-evoked locomotor muscle activity (electromyographs, EMGs) from whole animals as well as brain-initiated *in vitro*

locomotor activity from brain/spinal cord preparations were further analyzed for larval lamprey. For each animal in the whole animal or *in vitro* group, the slopes of the BP and ϕ values *versus T* were determined by regression analysis, and then statistics were used to determine if the composite slope of each parameter *versus* cycle time for the group was significantly different from zero, similar to the methods previously described (Hagevik and McClellan, 1997; McClellan and Hagevik, 1999).

Materials and methods

Source of data

The data analyzed in the present study were derived from larval lamprey *Petromyzon marinus* L. Spontaneous or sensory-evoked locomotor muscle activity (EMGs) in whole animals (Davis et al., 1993; Paggett et al., 1998) and brain-initiated locomotor activity in brain/spinal cord *in vitro* preparations (McClellan, 1994; McClellan and Hagevik, 1999) were further analyzed for the purposes of the present study (see below). Since the methods for the collection of this data are described in detail elsewhere, only the most relevant information will be repeated here.

Animal groups

EMG recordings during locomotion in whole animals

Briefly, pairs of fine copper wires (56 μm diameter) were implanted in contact with body musculature at approximately 20% body length (*BL*), 40% *BL* and 60% *BL* (Fig. 2Ai; electrode configurations were either 1-2-3-4 or 1-2-3-5, see Fig. 1B). Subsequently, locomotor behavior and motor activity occurred either spontaneously or were elicited by brief stimulation of the oral hood or tail. During locomotion, muscle activity (EMGs) was recorded and stored on videotape (Neurodata DR886, 11 kHz sampling rate per channel), and episodes of locomotor activity were selected for analysis during relatively constant velocity swimming along a straight line (Fig. 2Aii).

Brain-initiated locomotor activity in brain/spinal cord in vitro *preparations*

In vitro brain/spinal cord preparations from lamprey were prepared as described in detail elsewhere (McClellan, 1994; Hagevik and McClellan, 1994, 1999; McClellan and Hagevik, 1999). The preparations (Fig. 2Bi) were pinned dorsal side up in a recording chamber containing lamprey Ringer's solution (McClellan, 1990) maintained at 6–9°C. During recordings, the Ringer's solution contained 15 mg l^{-1} D-tubocurarine chloride (Sigma; St Louis, MO, USA) to block possible muscle contractions in any remaining muscle along the notochord. It is unlikely that curare significantly altered the *in vitro* rhythms, since brain-initiated *in vitro* locomotor activity is virtually identical with or without 150 mg l^{-1} curare in the bath, or ten times the concentration used for the present study (N=3; P. Hinton and A. D. McClellan, unpublished data). Suction electrodes were placed in contact with ventral roots in the rostral (approx. 20% *BL*), middle (approx. 40% *BL*) and caudal (approx. 60% *BL*) spinal cord, which correspond approximately to segments 13, 35 and 61, respectively. *In vitro* spinal locomotor activity (Fig. 2Bii) was initiated by chemical

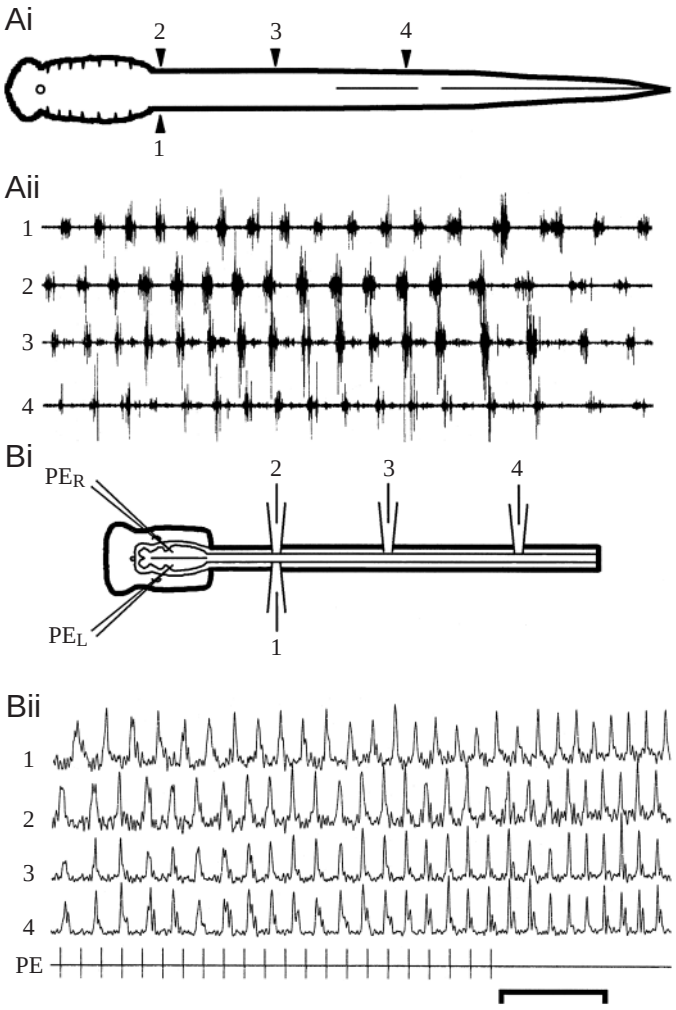

Fig. 2. (A) Muscle activity (electromyographs) during swimming in a larval lamprey (whole animal). (Ai) Diagram of a larval lamprey with EMG recording electrodes (1–4; see Materials and methods for positions of electrodes). (Aii) Episode of spontaneous locomotor muscle activity, characterized by a left–right alternation (1↔2) and a rostrocaudal phase lag (2→3 and 3→4). (B) Locomotor activity in preparations *in vitro*. (Bi) *In vitro* brain/spinal cord preparation from a lamprey in which pharmacological agents (5 mmol l^{-1} D-glutamate/5 mmol l^{-1} D-aspartate) were pressure-ejected through micropipettes (PE$_R$ and PE$_L$) in brain locomotor areas to initiate spinal locomotor activity (see Materials and methods for list of source articles), which was recorded from the ventral roots using suction electrodes (1–4). (Bii) Episode of brain-initiated *in vitro* locomotor activity (1–4; integrated with τ=50 ms) elicited by pressure ejection (PE) in the rostrolateral rhombencephalon and characterized by left–right alternation (1↔2) and a rostrocaudal phase lag (2→3 and 3→4). Note that phase lags for *in vitro* locomotor activity are smaller than those for locomotor muscle activity in whole animals, as previously reported (McClellan, 1994). Time scale, 1 s (A); 5 s (B).

microstimulation in brain locomotor areas, as described previously in detail (McClellan, 1994; Hagevik and McClellan, 1994; McClellan and Hagevik, 1999).

Measurements of the parameters of locomotor activity

Selected episodes of muscle activity during locomotor behavior were played out on a thermal array recorder (Gould TA2000) at 50 mm s^{-1}. Similar procedures were used for *in vitro* locomotor activity, except that this activity was integrated (τ=50 ms) because the signal-to-noise ratio was not always high enough to reliably determine the onsets and offsets of locomotor bursts (see figure 2B,C in McClellan, 1988). The onsets and offsets of locomotor burst activity were digitized with interactive software and a digitizing tablet (GTCO 1117A). Data points were imported into a spreadsheet program (Lotus 1-2-3) for performing calculations of cycle time (in ms), burst proportion and phase lag for each locomotor cycle (see Fig. 1B) as well as for obtaining graphs.

Regression analysis

For each animal, the calculated parameters of locomotor activity were imported into a statistical analysis program (InStat), and linear regression analysis was performed for cycle times *versus* each of the following parameters: BP1, BP2, BP3, BP4, BP5, ϕ_{15}, ϕ_{23} and ϕ_{34} (Fig. 1B; for example, BP1 refers to burst proportions calculated from activity recorded at electrode 1, while ϕ_{15} refers to phase lags calculated from burst activity recorded at electrodes 1 and 5) (see McClellan and Hagevik, 1997). Linear regression analysis was used to determine the slopes and *y*-intercepts of a best-fit line through the data points and whether the points could be sufficiently described by linear analysis. For each locomotor activity parameter (i.e. EMG or *in vitro* activity), the mean slopes for all of the animals within the group were averaged to determine the composite mean ± S.D. for that particular parameter (Table 1).

Statistical analysis

First, for lamprey in the whole animal or *in vitro* group, the slopes of the locomotor activity parameters *versus* cycle time were used to create distribution histograms (Figs 3, 4). These histograms were then evaluated to determine if the slopes were distributed approximately uniformly or if there were other trends (e.g. bimodal distributions). Second, for lamprey in the whole animal or *in vitro* group, the proportion of positive and negative slopes for each locomotor activity parameter *versus* cycle time was analyzed using the Sign test, as previously described (McClellan and Hagevik, 1999), to determine if the composite slope of the parameter for the group was significantly different from zero (i.e. $P \leq 0.05$) (see Table 1).

Table 1. *Analysis of locomotor activity: slopes of burst proportions and phase lags* versus *cycle times*

	EMG locomotor activity	*In vitro* locomotor activity
BP1		
Mean ± S.D.[a]	–2.51E-04±2.98E-04	–0.23E-04±2.14E-04
*N*1/*N*2[b]	5/34***	5/8
*N*3/*N*4[c]	39/2943	13/628
BP2		
	–2.61E-04±2.18E-04	–0.47E-04±1.83E-04
	2/29***	6/7
	31/2629	13/626
BP3		
	–3.43E-04±2.43E-04	–1.40E-04±1.71E-04
	1/38***	3/10
	39/2943	13/614
BP4		
	–5.01E-04±2.66E-04	–1.54E-04±2.51E-04
	0/24***	3/6
	24/1935	9/507
BP5		
	–3.12E-04±1.26E-04	–0.91E-04±1.11E-04
	0/7*	1/3
	7/691	4/113
ϕ_{23}		
	–0.25E-06±1.15E-05	–0.97E-06±3.70E-06
	17/22	11/18
	39/2940	29/1441
ϕ_{34}		
	–0.18E-06±6.39E-06	–2.95E-06±4.53E-06
	11/13	2/7
	24/1935	9/498
ϕ_{15}		
	–1.77E-06±8.08E-06	–1.04E-06±4.19E-06
	2/2	
	15/1001	4/117

[a]Values are means ± S.D. of the overall slope of a given locomotor parameter value *versus* cycle time.

[b]*N*1/*N*2, numbers of animals in which the slope of the locomotor parameter, as determined with regression analysis, was positive (*N*1) or negative (*N*2).

[c]*N*3/*N*4; *N*3, numbers of animals; *N*4, numbers of locomotor cycles.

Sign test: values are significantly different, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.002$.

Results

Distributions of slopes of locomotor activity parameters versus *cycle time*

For muscle activity (EMGs) during locomotion in whole animals, distribution histograms indicated that the slopes for the parameters of locomotor activity *versus* cycle time (range: 130–826 ms) were approximately uniformly distributed (Fig. 3), without obvious gaps or groupings in the distributions, as would be characteristic for bimodal or multimodal distributions. The slopes for burst proportion *versus* cycle time were centered around negative values (Fig. 3A), while the slopes for intersegmental phase lag *versus* cycle time were mostly centered approximately at zero (Fig. 3B).

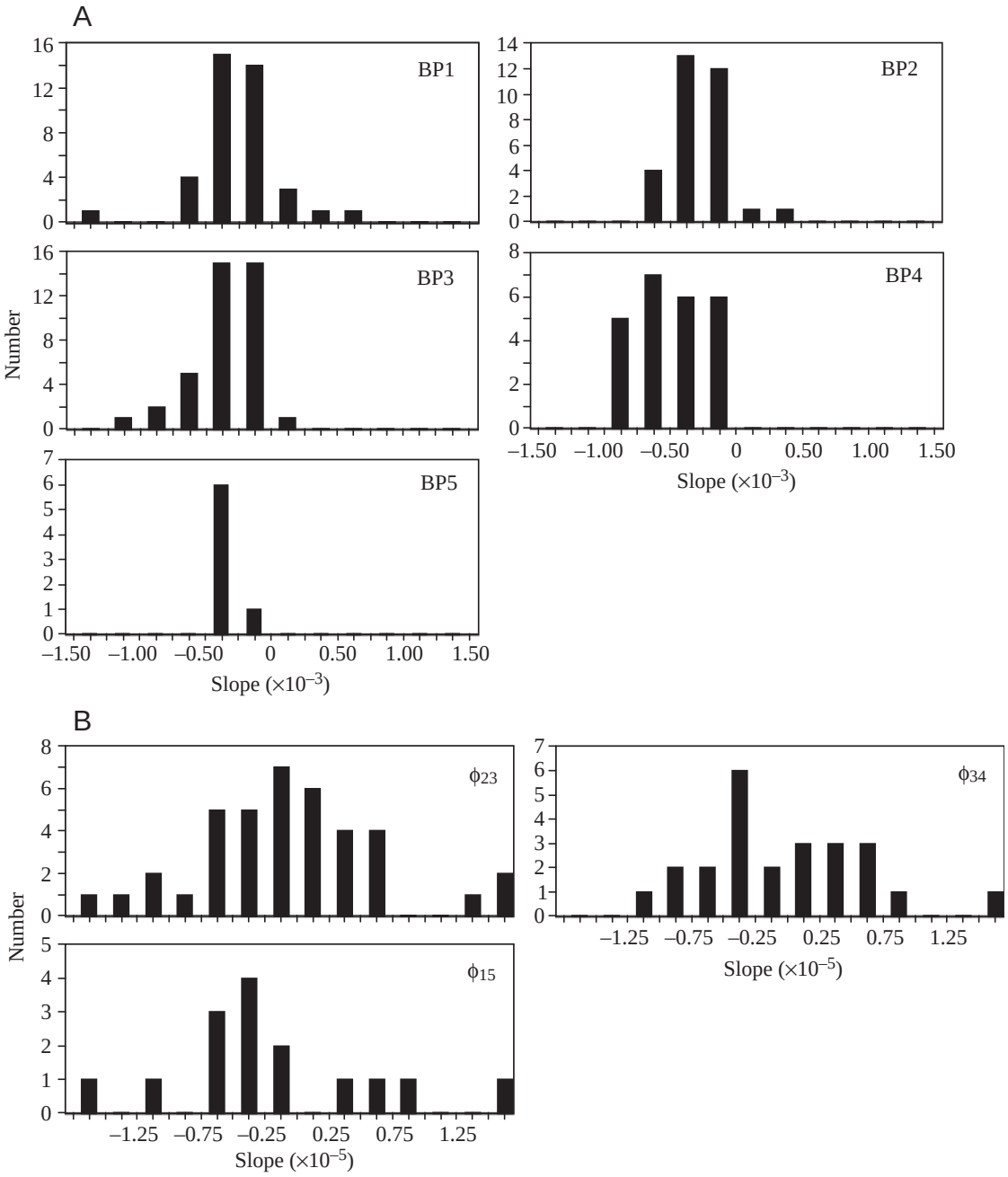

Fig. 3. Analysis of locomotor muscle activity (EMGs). Distribution histograms of the slopes for (A) BP1–BP5 and (B) ϕ_{23}, ϕ_{34} and ϕ_{15} *versus* cycle time (see Fig. 1B) (*N*=7–39 animals; see Table 1, left). The slopes of these parameters of locomotor activity *versus* cycle time were, to a first approximation, normally distributed, which validated use of the Sign test to determine if the composite mean slopes were significantly different from zero (see Materials and methods).

As was the case for muscle activity from whole animals, for *in vitro* locomotor activity, the distribution histograms of the slopes for the parameters of locomotor activity *versus* cycle time (range: 409–3416 ms) were approximately uniformly distributed (Fig. 4). In addition, the distributions of slopes for burst proportion *versus* cycle time were centered around negative values (Fig. 4A), while those for intersegmental phase lag *versus* cycle time were mostly centered approximately around zero (Fig. 4B).

Since the distribution histograms for the slopes of locomotor activity parameters *versus* cycle time were approximately uniformly distributed, use of the Sign test to evaluate this data was justified (see Materials and methods). Thus, for all lamprey in the whole animal or *in vitro* group, the Sign test was applied to the proportion of positive and negative slopes for each locomotor activity parameter *versus* cycle time to determine if the composite slope was significantly different than zero. In contrast, had the distribution histograms been

multimodal, with groupings of slope values, the Sign test probably would have been inappropriate. For example, if a given distribution histogram for a parameter had been bimodal, with half of the slopes centered around a positive value and half centered around a negative value, the Sign test would indicate that the slopes are not significantly different from zero, even though this obviously is not true.

Statistical test of the slopes for the parameters of locomotor activity

Locomotor muscle activity

In individual whole animals, analysis of locomotor muscle activity (i.e. EMGs) indicated that there was a clear tendency for burst proportions (range of mean values for all animals ≈0.12–0.44) to be larger for shorter cycle times than for longer cycle times (see Fig. 5 and legend). For all whole animals, regression analysis (lines in Fig. 6Ai,Bi) indicated that most of the slopes for burst proportion *versus* cycle time had values that were negative (Fig. 6Ci). Furthermore, the mean slopes of BP *versus T* for each parameter (i.e. BP1–BP5) were negative and ranged from -2.5×10^{-4} to -5.0×10^{-4} (Table 1). Analysis of the proportion of positive and negative slopes using the Sign test indicated that the slopes for all of the BP values *versus* cycle time were significantly less than zero ($P\leq0.05$, Table 1). Thus, for EMGs in larval whole animals, burst proportions appear to

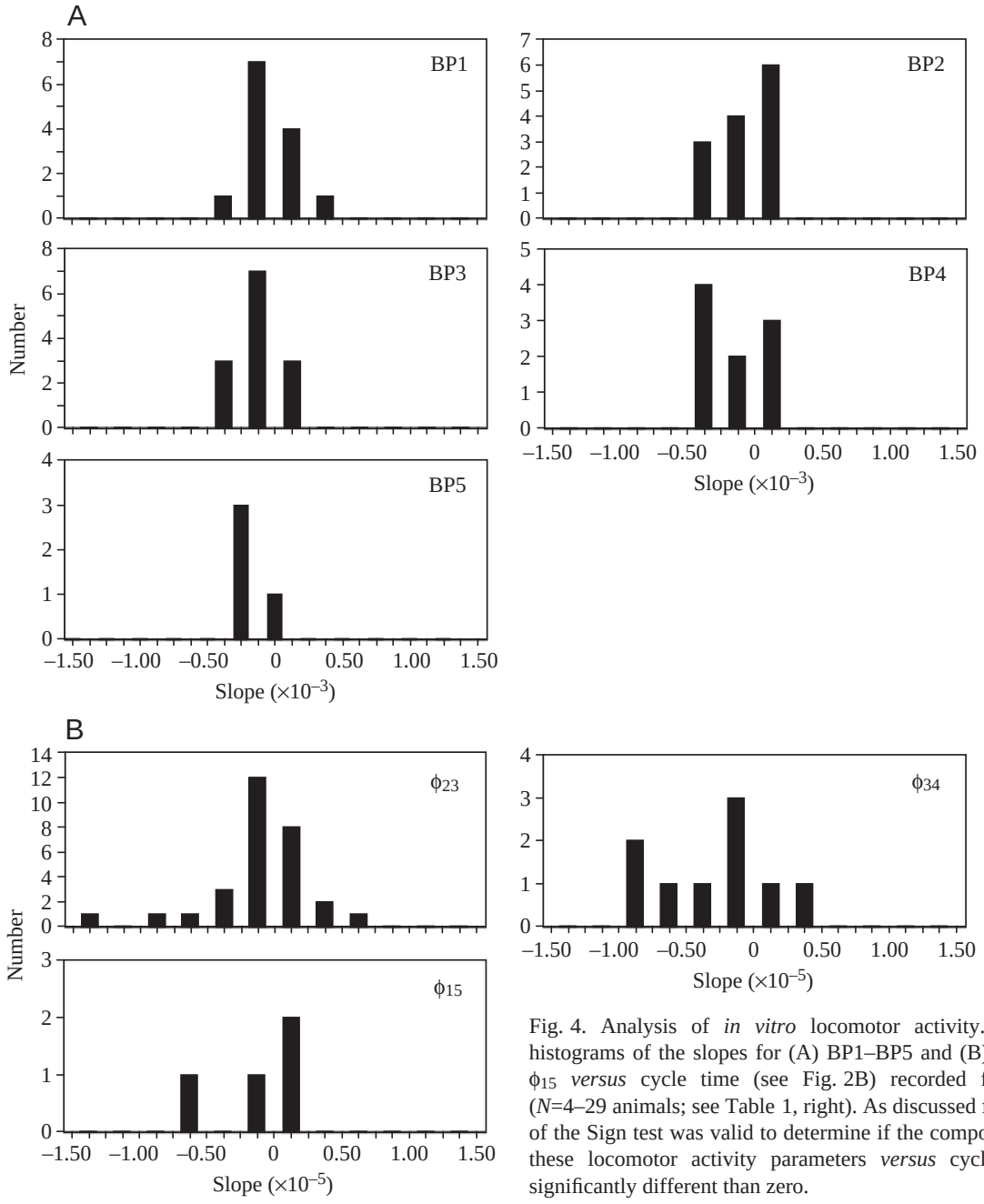

Fig. 4. Analysis of *in vitro* locomotor activity. Distribution histograms of the slopes for (A) BP1–BP5 and (B) ϕ_{23}, ϕ_{34} and ϕ_{15} *versus* cycle time (see Fig. 2B) recorded from animals (N=4–29 animals; see Table 1, right). As discussed for Fig. 3, use of the Sign test was valid to determine if the composite slopes of these locomotor activity parameters *versus* cycle time were significantly different than zero.

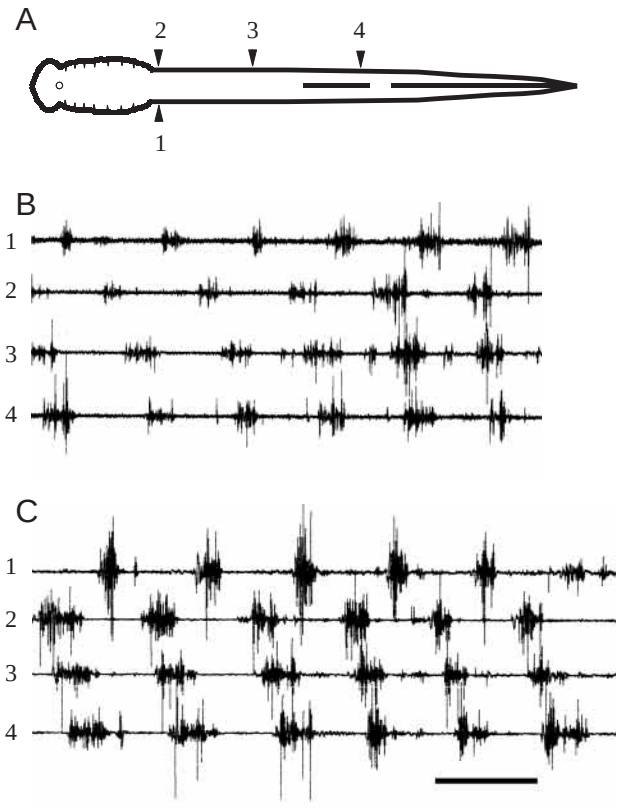

Fig. 5. (A) Larval lamprey (length=122 mm) with EMG recording electrodes at 20% *BL* (1,2), 40% *BL* (3) and 60% *BL* (4). (B,C) Muscle activity recorded during (B) moderately slow swimming (*T*=436±42 ms) and (C) faster swimming (*T*=225±13 ms). Note that the 'chart speed' was adjusted so that the cycle times of the two episodes would appear similar in the figure (see time scale below). (B) During slow swimming, the mean burst proportions (BP1, BP2, BP3, BP4) were 0.23, 0.31, 0.39 and 0.31, and mean phase lags (ϕ_{23}, ϕ_{34}) were 0.0086 and 0.0047, respectively. (C) During the faster swimming episode, in which the cycle times were approximately 200 ms shorter than in B, the mean values for these parameters were (BP1, BP2, BP3, BP4) 0.28, 0.37, 0.42 and 0.45, and (ϕ_{23}, ϕ_{34}) 0.0066 and 0.0063, respectively. Time scale, 500 ms (A); 250 ms (B).

increase significantly with decreasing cycle times (i.e. a negative slope).

In individual whole animals, intersegmental phase lags (range of mean values for all animals ≈0.0039–0.0110) for locomotor muscle activity did not appear to vary appreciably with changes in cycle time (see Fig. 5 and legend). For all whole animals, regression analysis (lines in Fig. 6Aii,Bii) indicated that the individual slopes for phase lag *versus* cycle time could be positive or negative (Fig. 6Cii). The mean slopes of ϕ *versus T* for each parameter (i.e. ϕ_{23}, ϕ_{34} and ϕ_{15}) ranged from -0.17×10^{-6} to -1.8×10^{-6} (Table 1), and analysis of the proportion of positive and negative slopes using the Sign test indicated that these slopes were not significantly less than zero ($P>0.05$, Table 1). Thus, phase lags for muscle activity in larval whole animals do not appear to change significantly with variations in cycle time.

In vitro *locomotor activity*

For brain-initiated locomotor activity recorded from *in vitro* brain/spinal cord preparations, the composite mean slopes for burst proportion (range of mean values for all animals ≈0.28–0.52) *versus* cycle time had negative values that ranged from -0.23×10^{-4} to -1.5×10^{-4} (Table 1). However, application of the Sign test to the proportion of positive and negative slopes for burst proportions *versus* cycle time (i.e. BP1–BP5) indicated that these slopes were not significantly different from zero (Table 1).

For intersegmental phase lags (range of mean values for all animals ≈0.0013–0.0055), the composite mean slopes *versus* cycle time ranged from -0.97×10^{-6} to -2.9×10^{-6} (Table 1). Applying the Sign test to the proportion of positive and negative slopes for ϕ *versus T* indicated that these slopes were not significantly different from zero (Table 1), similar to our previously reported preliminary results (McClellan and Hagevik, 1999).

Discussion

Slopes of locomotor activity parameters versus *cycle time in larval lamprey*

Burst proportion

For locomotor muscle activity in larval lamprey, the composite mean slopes for burst proportion *versus* cycle time were negative (Fig. 4A), and the Sign test indicated that these slopes were significantly less than zero (Table 1). In *in vitro* preparations, although the mean slopes of burst proportion *versus* cycle time were also negative (Fig. 4A), they were not significantly less than zero (Table 1).

In whole animals, a negative slope for burst proportion *versus* cycle time that is relatively constant (e.g. $\approx-2.0\times10^{-4}$) over a modest range of cycle times indicates that as cycle times decrease, for example from 1000 ms to 500 ms, and swimming speed increases, burst proportion (i.e. the relative burst duration) will increase by about 0.1 (e.g. from 0.3 to 0.4; see Fig. 6Ci). Thus, for faster swimming speeds, locomotor bursts will occupy a larger fraction of the cycle and generate proportionately more force per cycle. For faster swimming speeds, this increase in force generation may be necessary to overcome the additional viscous resistance of moving the body rapidly through water.

In whole animals, a negative slope for burst proportion *versus* cycle time could be due, in part, to sensory feedback. However, this may also be a property of the spinal CPGs, since in *in vitro* preparations, the composite mean slopes for burst proportion *versus* cycle time, although not significantly different from zero, were centered around negative values (Fig. 4A). For example, during locomotor activity in the lamprey, spinal motoneurons receive alternating depolarizing and hyperpolarizing synaptic potentials in a quasi-sinusoidal fashion (Russell and Wallén, 1983; Wallén et al., 1985). As cycle times become shorter, the amplitude of this quasi-sinusoidal synaptic drive to motoneurons would be expected to increase in order to recruit additional neurons (Davis and

Murphey, 1969; see figure 4 in Grillner and Kashin, 1976). If the thresholds for action potentials are constant, at shorter cycle times an increase in the amplitude of the sinusoidal synaptic input to motoneurons could contribute, in part, to an increase in burst proportion.

Why have changes in burst proportion with variations in cycle time not been observed in previous studies in the lamprey? First, in our previous studies with larval lamprey, variations of the parameters of locomotor activity with changes in cycle time were not analyzed statistically. In addition, in these previous studies (e.g. McClellan 1990), the data for all animals were pooled together, and this tends to obscure possible changes *versus* cycle time because each animal has a slightly different mean BP and mean *T*. Second, in contrast to the present study that examined 'free swimming' in larval lamprey, in a previous study that involved adult lamprey swimming in a 'swim-mill', changes in BP with *T* were not seen (Wallén and Williams, 1984). These differences might be due to minor kinematic differences of swimming in larval and adult animals. However, there may be some differences between swimming in a swim-mill, in which water is forced to flow past a stationary animal, and 'free swimming', in which an animal must actively generate forces against the water to mediate forward progression. For example, running on a treadmill and running over ground, although qualitatively similar, exhibit several kinematic (Schache et al., 2001; Frishberg, 1983) and muscle activity differences (Murray et al., 1985; Wank et al., 1998).

In larval lamprey, during the initial phase of burrowing, when an animal attempts to penetrate the viscous substrate (e.g. sand), mean cycle times are significantly shorter and burst proportions are significantly larger than during swimming (Paggett et al., 1998). Thus, in the present study, for whole animals that swam at the shortest mean cycle times (approximately 130–250 ms), some of the increase in burst proportions could be in response to the resistance encountered during rapid swimming through the water. However, even for animals that swam at longer mean cycle times (e.g. 400–750 ms), most of the slopes for burst proportion *versus* cycle time were negative (e.g. Fig. 6Ci).

For fish swimming in a 'swim mill', there is some evidence to suggest that burst proportions can increase with decreasing cycle times. For example, during swimming in trout (figure 4A,C in Grillner and Kashin, 1976), for a mean cycle time of approx. 300 ms the mean burst proportion was approx. 0.320, while for a mean cycle time of approx. 135 ms during faster swimming, the mean burst proportion increased to approx. 0.490.

Intersegmental phase lags

For locomotor muscle activity and *in vitro* locomotor

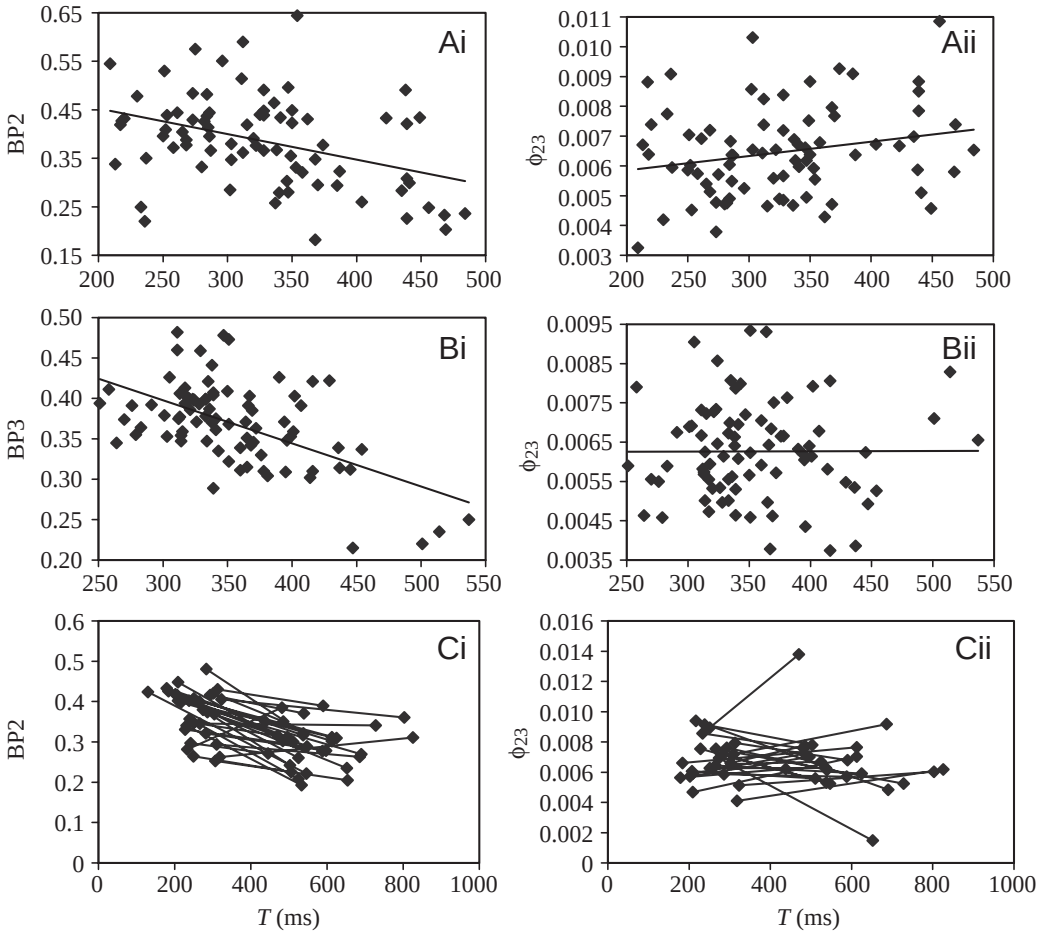

Fig. 6. (A,B) Analysis of locomotor muscle activity from two whole animals (A and B), showing individual data points for burst proportion (BP2 in Ai; BP3 in Bi) and intersegmental phase lag (ϕ_{23} in both Aii and Bii) plotted against cycle time *T*. Lines are derived from linear regression analysis (see Materials and methods). (C) Linear regression lines for all animals for (Ci) burst proportion, BP2, and (Cii) phase lag, ϕ_{23}. Note that the regression line for each animal is plotted over the range of cycle times that occurred for that given animal.

activity in larval lamprey, the composite slopes for intersegmental phase lag *versus* cycle time were centered around zero (Figs 3B,4B), and none of these composite slopes were significantly different from zero (Table 1). Thus, it appears that intersegmental phase lags do not change significantly with variations in cycle time, similar to our previous results (McClellan and Hagevik, 1999).

A constant intersegmental phase lag indicates that as cycle times decrease and swimming speeds increase, the rostral-to-caudal delay of ipsilateral burst activity will decrease proportionally to the duration of the cycle (see Grillner and Kashin, 1976; Williams, 1986). Therefore, as cycle times change, the relative timing of burst activity will remain approximately constant, and a single S-wave along the body will be retained, which is a highly efficient manner for swimming.

In adult lamprey, intersegmental phase lags of locomotor activity have been reported to be relatively constant with changes in cycle time for both whole animals swimming in a 'swim mill' and isolated spinal cords (Wallén and Williams, 1984). In contrast, in a more recent study with isolated spinal cords from adult lamprey, in which locomotor activity was evoked by bath-applied pharmacological agents, data suggest that intersegmental phase lags might not be constant but appear to increase with decreasing cycle times (Tegnér et al., 1997). There are at least two possible explanations for the opposing results found in the present study in larval lamprey in which phase lags were relatively constant. First, perhaps there are some minor differences in the operation of spinal locomotor networks in larval and adult lamprey (see Cohen et al., 1990). Second, for whole animal and *in vitro* brain/spinal cord preparations in the present study, spinal locomotor networks were activated by descending systems in the brain. By contrast, it is not known if application of pharmacological agents to the isolated spinal cord activates locomotor networks with the full complement of mechanisms that occur during descending activation from the brain.

In theory, in the lamprey, rostrocaudal phase lags could be due to at least three mechanisms (for a review, see McClellan, 1996): (a) short distance coupling between oscillators in adjacent regions of the spinal cord; (b) long distance coupling between oscillators in more separated regions of cord; and (c) gradients in oscillator frequency along the spinal cord. Several lines of evidence suggest that short distance coupling is much stronger in the descending direction than ascending coupling (Hagevik and McClellan, 1994; reviewed in McClellan, 1996) and is the main mechanism that contributes to rostrocaudal phase lags (McClellan and Hagevik, 1999; Hagevik and McClellan, 1999; Matsushima and Grillner, 1992; also see Mellon et al., 1995; Buchanan et al., 1995).

Related studies in other animals

In late embryonic *Xenopus laevis*, swimming is produced by single spikes per cycle in motoneurons in each segment (for a review, see Roberts et al., 1997). During changes in cycle time, there is a constant delay, instead of a constant phase lag, between ipsilateral rostral and caudal spikes (Tunstall and Roberts, 1991). Just a short time later, in young tadpoles, the single spikes per cycle are replaced by bursts of action potentials, and the coordinating system then maintains a constant intersegmental phase lag during changes in cycle time (for a review, see Tunstall and Sillar, 1993). It has been suggested that a head-to-tail gradient of excitation and inhibition within the spinal locomotor networks determines the normal anterior-to-posterior propagation of swimming motor activity (Tunstall and Sillar, 1993; Tunstall and Roberts, 1994; also see Skinner and Mulloney, 1998).

In crayfish, the paired swimmerets make coordinated periodic power stroke/return stroke movements, and each pair appears to be controlled by a separate oscillator (Ikeda and Wiersma, 1964). The rostrocaudal phase lag between the movements of each pair of appendages is approximately 25%. Coupling between the swimmeret oscillators is thought to be asymmetrical and dominated by ascending coupling, and the posterior segment is thought to lead each cycle of activity (Braun and Mulloney, 1995; Mulloney, 1997; for a review, see Skinner and Mulloney, 1998).

In the leech, swimming is produced by alternating contractions of ventral and dorsal musculature in each body segment. The oscillators in specific ganglia that generate the swimming pattern are thought to be coupled symmetrically (Friesen and Hocker, 2001) with a U-shaped excitability gradient along the ventral nerve cord (Hocker et al., 2000; for a review, see Friesen and Cang, 2001). Furthermore, unlike the lamprey spinal locomotor networks, in the leech both short-distance and long-distance coupling appear to be important for coordination of swimming because the phase lags for swimming activity in the isolated nerve cord are dependent on the number of segments (Pearce and Friesen, 1985). Finally, phase lags for swimming in the leech appear to be partially dependent on sensory inputs (Cang and Friesen, 2000), unlike that in the lamprey (Wallén and Williams, 1984).

Conclusions

For swimming motor activity in larval lamprey, the slopes for burst proportion *versus* cycle time were negative, and in whole animals, these slopes were significantly less than zero. These results suggest that, as cycle times decrease and swimming speed increases, the portion of the cycle occupied by a burst will increase, presumably to generate additional force to compensate for the increase in viscous resistance of moving the body rapidly through water. By contrast, intersegmental phase lags were relatively constant during changes in cycle time, presumably to retain the ergonomically efficient S-shaped body wave during different speeds of swimming.

We thank Dr Richard Madsen for consultation about the statistics. Supported by NIH grant NS29043, NSF grant IBN 9817905, and American Paralysis Association grant MA1-9605 awarded to A.D.M. Support for M.R.B. was provided in part by the LS UROP at the University of Missouri.

References

Bowtell, G. and Williams, T. (1991). Anguilliform body dynamics: modeling the interaction between muscle activation and body curvature. *Phil. Trans. R. Soc. Lond. B* **334**, 385-390.

Braun, G. and Mulloney, B. (1995). Coordination in the crayfish swimmeret system: differential excitation causes changes in intersegmental phase. *J. Neurophysiol.* **73**, 880-885.

Buchanan, J. T., Kasicki, S. and Romorowski, D. (1995). Swim oscillator coupling signals in the lamprey spinal cord. *Soc. Neurosci. Abst.* **21**, 687.

Cang, J. H. and Friesen, W. O. (2000). Sensory modification of leech swimming: Rhythmic activity of ventral stretch receptors can change intersegmental phase relationships. *J. Neurosci.* **20**, 7822-7829.

Cohen, A. H., Dobrov T. A., Li, G., Kiemel, T. and Baker, M. T. (1990). The development of the lamprey pattern generator for locomotion. *J. Neurobiol.* **21**, 958-969.

Davis, G. R., Troxel, M. T., Kohler, V. J., Grossmann, E. R. and McClellan, A. D. (1993). Time course of locomotor recovery and functional regeneration in spinal-transected lampreys: Kinematics and electromyography. *Exp. Brain Res.* **97**, 83-95.

Davis, W. J. and Murphey, R. K. (1969). Discharge patterns of swimmeret motoneurones in the lobster, simulated with a digital computer. *J. Exp. Biol.* **50**, 119-128.

Friesen, W. O. and Cang, J. (2001). Sensory and central mechanisms control intersegmental coordination. *Curr. Opin. Neurobiol.* **11**, 678-683.

Friesen, W. O. and Hocker, C. G. (2001). Functional analyses of the leech swim oscillator. *J. Neurophysiol.* **86**, 824-835.

Frishberg, B. A. (1983). An analysis of overground and treadmill sprinting. *Med. Sci. Sports Exerc.* **15**, 478-485.

Gray, J. (1933a). Studies in animal locomotion. I. The movement of fish with special reference to the eel. *J. Exp. Biol.* **10**, 88-104.

Gray, J. (1933b). Studies in animal locomotion. II. The relationship between waves of muscular contraction and the propulsive movements of the eel. *J. Exp. Biol.* **10**, 386-400.

Gray, J. (1936). Studies in animal locomotion. IV. The neuromuscular mechanism of swimming in the eel. *J. Exp. Biol.* **13**, 170-180.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods and fish. In *Handbook of Physiology, Motor Control* (ed. V. Brooks), pp. 1179-1236. Bethesda: American Physiological Society.

Grillner, S., Cangiano, L., Hu, G., Thompson, R., Hill, R. and Wallén, P. (2000). The intrinsic function of a motor system – from ion channels to networks and behavior. *Brain Res.* **886**, 224-236.

Grillner, S., Deliagina, T., Ekeberg, O., El Manira, A., Hill, R. H., Lansner, A., Orlovsky, G. N. and Wallén, P. (1995). Neural networks that co-ordinate locomotion and body orientation in lamprey. *Trends Neurosci.* **18**, 270-279.

Grillner, S. and Kashin, S. (1976). On the generation and performance of swimming in fish. In *Neural Control of Locomotion*, vol. 18 (ed. R. M. Herman, S. Grillner, P. S. G. Stein and D. G. Stuart), pp. 181-202. New York: Plenum.

Hagevik, A. and McClellan, A. D. (1994). Coupling of spinal locomotor networks in larval lamprey revealed by receptor blockers for inhibitory amino acids: Neurophysiology and computer modeling. *J. Neurophysiol.* **72**, 1810-1829.

Hagevik, A. and McClellan, A. D. (1997). Coordination of locomotor activity in the lamprey spinal cord: Mechanisms for long distance coupling. *Soc. Neurosci. Abst.* **23**, 205.

Hagevik, A. and McClellan, A. D. (1999). Coordination of locomotor activity in the lamprey: Role of descending drive to oscillators along the spinal cord. *Exp. Brain Res.* **128**, 481-490.

Hocker, C. G., Yu, X. and Friesen, W. O. (2000). Functionally heterogeneous segmental oscillators generate swimming in the medical leech. *J. Comp. Physiol.* **186**, 871-883.

Ikeda, K. and Wiersma, C. A. G. (1964). Autogenic rhythmicity in the abdominal ganglion of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol. A* **12**, 107-115.

Matsushima, T. and Grillner, S. (1992). Neural mechanisms of intersegmental coordination in lamprey: Local excitability changes modify the phase coupling along the spinal cord. *J. Neurophysiol.* **67**, 373-388.

McClellan, A. D. (1988). Functional regeneration of descending brainstem command pathways for locomotion demonstrated in the in vitro lamprey CNS. *Brain Res.* **448**, 339-345.

McClellan, A. D. (1990). Locomotor recovery in spinal-transected lamprey: Role of functional regeneration of descending axons from brainstem locomotor command neurons. *Neuroscience* **37**, 781-798.

McClellan, A. D. (1994). Time course of locomotor recovery and functional regeneration in spinal cord-transected lamprey: In vitro preparations. *J. Neurophysiol.* **72**, 847-859.

McClellan, A. D. (1996). Organization of spinal locomotor networks: Contributions from model systems. *Comm. Theor. Biol.* **4**, 63-91.

McClellan, A. D. and Hagevik, A. (1997). Descending control of turning locomotor activity in larval lamprey: Neurophysiology and computer modeling. *J. Neurophysiol.* **78**, 214-228.

McClellan, A. D. and Hagevik, A. (1999). Coordination of spinal locomotor activity in the lamprey: Long distance coupling of spinal oscillators. *Exp. Brain Res.* **126**, 93-108.

Mellon, N., Kiemel, T. and Cohen, A. H. (1995). Correlational analysis of fictive swimming in the lamprey reveals strong functional intersegmental coupling. *J. Neurophysiol.* **73**, 1020-1030.

Mulloney, B. (1997). A test of the excitability-gradient hypothesis in the swimmeret system of crayfish. *J. Neurosci.* **17**, 1860-1868.

Murray, M. P., Spurr, G. B., Sepic, S. B., Gardner, G. M. and Mollinger, L. A. (1985). Treadmill versus floor walking: Kinematics, electromyogram, and heart rate. *J. Appl. Physiol.* **59**, 87-91.

Paggett, K., Gupta, V. and McClellan, A. D. (1998). Adaptive variations of undulatory behaviors in larval lamprey: Comparison of swimming and burrowing. *Exp. Brain Res.* **119**, 213-223.

Pearce, R. A. and Friesen, W. O. (1985). Intersegmental coordination of the leech swimming rhythm. II Comparison of long and short chains of ganglia. *J. Neurophysiol.* **54**, 1460-1472.

Roberts, A., Soffe, S. R. and Perrins, R. (1997). Spinal networks controlling swimming in hatchling *Xenopus* tadpoles. In *Neurons, Networks, and Motor Behavior* (ed. P. S. G. Stein, S. Grillner, A. I. Selverston and D. G. Stuart), pp. 83-89. Cambridge, MA, USA: MIT Press.

Russell, D. F. and Wallén, P. (1983). On the control of myotomal motoneurones during 'fictive swimming' in the lamprey spinal cord in vitro. *Acta Physiol. Scand.* **117**, 161-170.

Schache, A. G., Blanch, P. D., Rath, D. A., Wrigley, T. V., Starr, R. and Bennell, K. L. (2001). A comparison of overground and treadmill running for measuring the three-dimensional kinematics of the lumbo-pelvic-hip complex. *Clin. Biomech.* **16**, 667-680.

Skinner, F. K. and Mulloney, B. (1998). Intersegmental coordination in invertebrates and vertebrates. *Curr. Opin. Neurobiol.* **8**, 725-732.

Stein, P. S. G. (1978). Motor systems, with special reference to locomotion. *Annu. Rev. Neurosci.* **1**, 61-81.

Tegnér, J., Krieger, P. and Grillner, S. (1997). Is the intersegmental phase lag in the isolated spinal cord independent of the swimming frequency? *Soc. Neurosci. Abst.* **23**, 205.

Tunstall, M. J. and Roberts, A. (1991). Longitudinal coordination of motor output during swimming in *Xenopus* embryos. *Proc. R. Soc. Lond. B* **244**, 27-32.

Tunstall, M. J. and Roberts, A. (1994). A longitudinal gradient of synaptic drive in the spinal cord of *Xenopus* embryos and its role in co-ordination of swimming. *J. Physiol. Lond.* **474**, 393-405.

Tunstall, M. J. and Sillar, K. T. (1993). Physiological and developmental aspects of intersegmental coordination in *Xenopus* embryos and tadpoles. *Sem. Neurosci.* **5**, 29-40.

Wallén, P., Grillner, S., Feldman, J. L. and Bergelt, S. (1985). Dorsal and ventral myotome motoneurons and their input during fictive locomotion in lamprey. *J. Neurosci.* **5**, 654-661.

Wallén, P. and Williams, T. L. (1984). Fictive locomotion in the lamprey spinal cord *in vitro* compared with swimming in the intact and spinal animal. *J. Physiol.* **347**, 225-239.

Wank, V., Frick, U. and Schmidtbleicher, D. (1998). Kinematics and electromyography of lower limb muscles in overground and treadmill running. *Int. J. Sports Med.* **19**, 455-461.

Webb, P. W. (1984). Form and function in fish swimming. *Sci. Amer.* **251**, 72-82.

Williams, T. L. (1986). Mechanical and neural patterns underlying swimming by lateral undulations: A review of studies on fish, amphibia, and lamprey. In *Neurobiology of Vertebrate Locomotion* (ed. S. Grillner, P. S. G. Stein, D. G. Stuart, H. Forssberg and R. M. Herman), pp. 141-155. Houndsmills, UK: The Macmillan Press Ltd.