

Pattern of Motor Coordination Underlying Backward Swimming in the Lamprey

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Islam, Salma S., Pavel V. Zelenin, Grigori N. Orlovsky, Sten Grillner, and Tatiana G. Deliagina. Pattern of motor coordination underlying backward swimming in the lamprey. *J Neurophysiol* 96: 451–460, 2006; doi:10.1152/jn.01277.2005. The main form of locomotion in the lamprey (a lower vertebrate, cyclostome) is forward swimming (FS) based on periodical waves of lateral body flexion propagating from head to tail. The lamprey is also capable of backward swimming (BS). Here we describe the kinematical and electromyographic (EMG) pattern of BS, as well as the effects on this pattern exerted by different lesions of the spinal cord. The BS was evoked by tactile stimulation of a large area in the anterior part of the body. Swimming was attributed to the waves of lateral body undulations propagating from tail to head. The EMG bursts on the two sides alternated, and the EMG in more caudal segments led in phase the EMG in more rostral segments. Main kinematical characteristics of BS strongly differed from those of FS: the amplitude of undulations was much larger and their frequency lower. Also, the maintenance of the dorsal-side-up body orientation ascribed to vestibular postural reflexes (typical for FS) was not observed during BS. A complete transection of the spinal cord did not abolish the generation of forward-propagating waves rostral to the lesion. After a lateral hemisection of the spinal cord, the BS pattern persisted on both sides rostral to the lesion; caudal to the lesion, it was present on the intact side and reduced or abolished on the lesioned side. The role of the spinal cord in generation of different forms of undulatory locomotion (FS and BS) is discussed.

INTRODUCTION

The object of this study is the neural coordination underlying backward swimming (BS) in the lamprey (a lower vertebrate, cyclostome), although we will first introduce the neural mechanisms for forward swimming (FS) in fish and lamprey. The motor pattern of FS in the lamprey is similar to that in bony fishes: periodical waves of lateral body flexion propagate from the head region toward the tail (Blake 1983; Gray 1968; Grillner and Kashin 1976; Williams et al. 1989).

The neural bases of FS in the lamprey have been characterized in considerable detail. It was found that coordinated locomotor movements can be elicited in the spinal lamprey and the isolated spinal cord itself can produce the motor pattern underlying FS (Wallén and Williams 1984). The intrinsic function of the spinal network is well understood (Grillner 2003; Grillner et al. 1995, 2000). The ability to generate rhythmic activity is distributed along the spinal cord and even a few isolated segments can produce periodical bursts (Cangiano and Grillner 2003, 2005). Both experimental and theoretical data show that the intersegmental coordination can be

produced by a spinal organization viewed as a series of interacting unit burst generators (Grillner 1989; Grillner et al. 1995; Matsushima and Grillner 1992). The spinal network is normally activated by reticulospinal neurons, which in turn are driven by cells in locomotor areas of the mesencephalon and diencephalon (Brocard and Dubuc 2003; Deliagina et al. 2000; El Manira et al. 1997; McClellan and Grillner 1984). It was also found that, during FS, the lamprey actively maintains the dorsal-side-up orientation of its body as the result of vestibular postural reflexes (Deliagina and Fagerstedt 2000; Deliagina et al. 1992; Orlovsky et al. 1992).

The relationships between FS and BS were first investigated in the dogfish. The spinal dogfish exhibits spontaneous locomotor coordination similar to FS, but if the rostral cutaneous innervation field is activated, the coordination is changed to BS, with the caudal segments leading in phase the rostral segments. The ability to reverse the phase coupling is distributed throughout the entire spinal cord (Grillner 1974). Similarly, in the isolated lamprey spinal cord the fictive locomotion, with backward propagating activity, can be generated if the oscillator circuits in the caudal part of the spinal cord have higher excitability than that of more rostral ones (Matsushima and Grillner 1990, 1992).

Backward swimming in intact lampreys has not been investigated systematically because of difficulties in its elicitation. Usually, the lamprey exhibits short episodes of BS when encountering obstacles (McClellan 1989); sometimes the episodes of BS are combined with struggling behavior. In the present study we found a regular way to evoke much longer episodes of BS by tactile stimulation of a large area in the anterior part of the body. This has allowed us to study BS in detail and, by comparing BS and FS, to estimate the similarities and distinctions of the control mechanisms for these two forms of locomotion. First, we investigated kinematical and EMG pattern of BS. Second, we addressed a question if the postural vestibular reflexes (strongly pronounced in FS) operate also during BS, and if the lamprey stabilizes its spatial orientation during BS. Third, we studied the effect on BS produced by lesions to the spinal cord.

A brief account of this study was previously published in abstract form (Islam et al. 2005).

METHODS

Adult lampreys (*Lampetra fluviatilis*, 25–30 cm long, $n = 20$) were used in the experiments. They were kept in an aerated freshwater aquarium at 5°C, with a 12 h:12 h light:dark cycle. The water

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temperature in experimental aquaria was maintained at 5–7°C. All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden).

Surgery

Surgery was performed under MS-222 (Sandoz) anesthesia (100 mg/l). In 17 animals, four bipolar EMG electrodes (flexible wires of 0.15-mm diameter) were inserted in the dorsolateral left and right muscles in the midbody area, at two rostrocaudal levels (a distance between them was 11 to 17 segments). In six of them, after an initial testing, a complete transection of the spinal cord at the level of segments 43–45 ($n = 3$) or segments 62–64 ($n = 3$) was performed. In five animals, after an initial testing, a lateral hemisection of the spinal cord was performed at the level of segments 37–43.

Each animal was tested in 1–2 days after surgery. At the end of the experimental series, the animals were killed with an overdose of MS-222. Post mortem investigation showed that, in all cases, the spinalization was complete. In animals with hemisection, the extent of lesion was verified histologically. In all cases, one half of the spinal cord was completely transected. Rostrocaudal positions of the lesions and of the EMG electrodes were also documented.

Locomotor tests

Backward swimming was evoked by tactile stimulation. For this purpose, a thin elastic “cap” (a finger of a surgical glove, 5 cm in length) was put over the head and the anterior part of the body, to cover about 20% of the body length (Fig. 1A). When released in the water, the lamprey usually swam backward until it could free itself from the cap (≤ 1 min, or even longer). After termination of a period of backward swimming, the lampreys usually swam forward, or attached to the wall or the bottom of the aquarium by their sucker mouth.

In most experiments, the backward swimming was studied in a shallow aquarium (80 × 80 cm, 10-cm depth) (Fig. 1B). Movements of the animal were recorded from above by a video camera (25 frames/s), positioned at the distance of 2 m from the aquarium, and analyzed frame by frame. The EMG electrodes were connected by a long flexible cable to the inputs of AC amplifiers. The EMG signals were amplified, rectified, smoothed (time constant: 50 ms), and then stored on a PC computer. The EMG and videorecordings were synchronized by pulses recorded simultaneously by both systems (Fig. 1B, *Synchro*).

For testing the capacity for spatial orientation during backward swimming, the lamprey with the stimulating cap was released into a deeper aquarium (110 × 35 cm, 37-cm depth) and videorecorded in the free water, i.e., before the animal contacted the walls or the bottom. The *side view* and the *top view* (by means of a mirror) were recorded simultaneously (Fig. 1C). EMGs were not recorded under these conditions.

Data processing

Characteristics of the swim motor pattern and kinematic analysis of swimming were defined as in the preceding studies (see e.g., Matsu-shima and Grillner 1992; Williams et al. 1989). Briefly, the positions of the points of maximal concave and convex curvature were estimated in video images. The distance between two such neighboring points was taken as a half of the locomotor wavelength; this value was multiplied by 2 and divided by the body length to obtain the wavelength expressed in body lengths. The speed of locomotor waves was calculated by tracking a maximum curvature point along the body; the speed was expressed in body length per second. Tracking positions of one body point (the head tip or the tail tip) provided the trajectory of the point, which allowed calculating the speed along the trajectory. Averaging of the trajectory across the cycle duration provided an

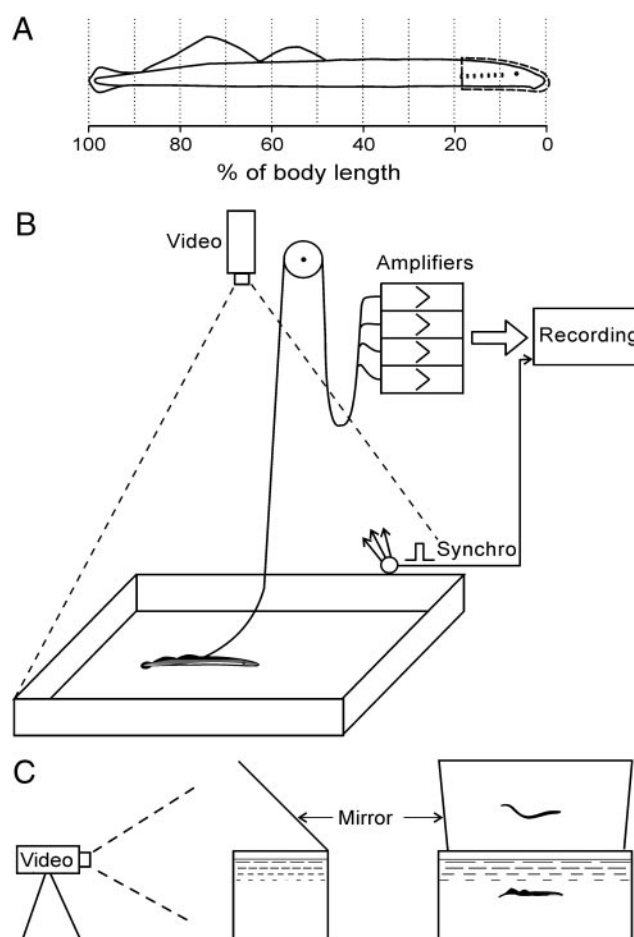


FIG. 1. Experimental arrangement. A: backward swimming (BS) was initiated by tactile stimulation with thin elastic “cap” (a finger of a surgical glove) that covered about 20% of the body length (shown by interrupted line). B: arrangement for parallel recording of kinematical and electromyographic (EMG) data. Video- and EMG recordings were synchronized by light and electrical pulses recorded simultaneously by the 2 systems (*Synchro*). C: arrangement for testing the capacity for spatial orientation. *Side view* and the *top view* (by means of a mirror) were videorecorded simultaneously.

estimate of the whole body (center of mass) progression and allowed calculating the speed of progression.

Onset and termination of an EMG burst were measured, after which the midburst point was calculated. The cycle duration was defined as the time interval between the midpoints of two successive bursts. The burst proportion was defined as the ratio of burst duration and cycle duration. The phase difference between two EMGs was calculated as the ratio of the time interval between the midpoints of the corresponding bursts divided by the cycle duration. The phase lag values were further divided by the number of segments between the two EMG electrodes to obtain the phase lag per segment value. All values are presented as means \pm SD.

RESULTS

Kinematical and EMG characteristics of backward swimming

A representative example of the kinematics of backward swimming in a shallow aquarium is shown in Fig. 2. The head trajectory in three cycles of swimming is shown in Fig. 2A (the time intervals between sequential points, 200 ms). For one of the points, the body configuration is also shown. A character-

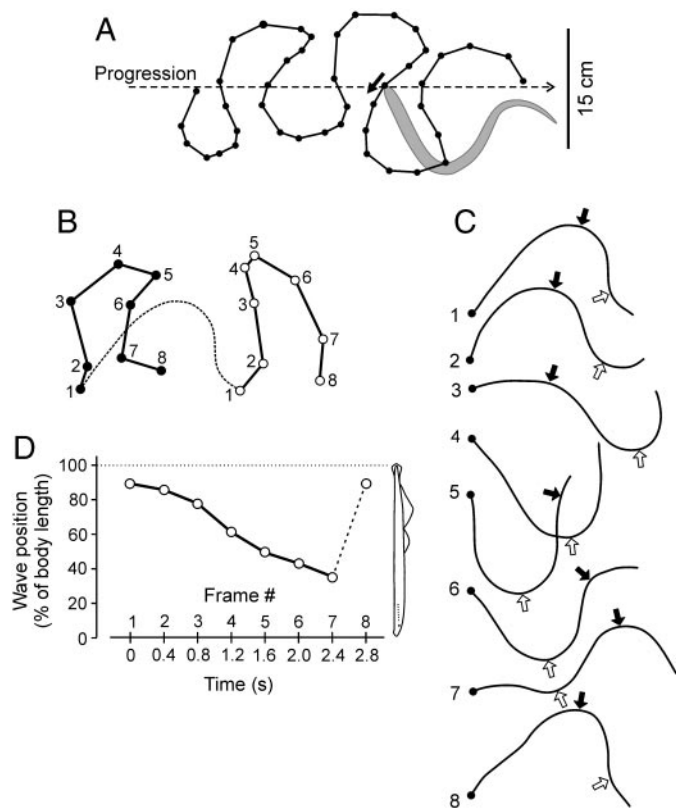


FIG. 2. Representative example of BS in a shallow aquarium. *A*: head trajectory for 3 swim cycles (time intervals between sequential positions, 200 ms; the lamprey was positioned with its dorsal side up). For one of the positions, the body configuration is shown. Arrow indicates the direction of head movement along the trajectory. *B*: head and tail trajectories for one of the swim cycles (time intervals between sequential positions, 400 ms). For position 1, the shape of body midline is shown. *C*: shape of the body midline in eight sequential frames (1–8) of the swim cycle shown in *B*. Points of maximal curvature are indicated, with convexity on the right side (black arrows) and on the left side (white arrows). *D*: a position of maximal left convexity as a function of time (of frame number) for the same swim cycle.

istic feature of BS was the very long duration of a swim cycle: the consecutive cycles in Fig. 2*A* were 3.0, 2.8, and 2.6 s long (in contrast to 0.25–0.5 s during FS under these conditions; see e.g., Fig. 3, *B* and *C*). Another distinctive feature of BS was the large amplitude of the lateral body undulations. The lateral excursions of the head (15–20 cm peak-to-peak, Fig. 2*A*) constituted 50–70% of the body length (30 cm). Figure 2*B* shows both head and tail trajectories in one swim cycle (the time intervals between sequential points, 400 ms), as well as the body midline for frame 1. The lateral undulations of the head and tail were similar in size. Because of these large undulations, the swim trajectory became very curvilinear and the whole body progression represented only a small part of the trajectory length. In the example of Fig. 2*A*, the ratio of the two values was 0.18.

For the swim cycle shown in Fig. 2*B*, the shape of the body midline in eight sequential frames (1–8) is represented in Fig. 2*C*. The points of maximal curvature are indicated, with convexity on the right side (black arrows) and on the left side (white arrows). In each frame, there are two pronounced convexities: one on the left side and one on the right side. In sequential frames, the peak of curvature moves along the body from the tail toward the head. Figure 2*D* shows the position of

the maximal left convexity as a function of the frame number (and function of time) for the same swim cycle. In this particular case, the wave of body flexion propagated forward at the speed of nearly 25% of the body length per second.

A representative recording of four EMGs during BS is shown in Fig. 3*A*. The electrodes were positioned bilaterally in the midbody area at two rostrocaudal levels (Fig. 3*D*). A clear-cut periodic bursting pattern is seen in each EMG, with cycle duration of about 2 s and a burst proportion of about 40% of the cycle. At each level, the bursts on the left and right sides alternated. On each side, the bursts of the caudal EMG occurred earlier than those of the rostral EMG (marked by interrupted lines). The phase difference was about 14% of the cycle. Because the distance between the electrodes was 12 segments, the normalized phase lag was 1.2% per segment, a value similar to that of FS (Wallén and Williams 1984) but with the opposite direction of wave propagation.

The episode of BS (Fig. 3*A*) finished when the animal freed itself from the stimulating cap. Immediately afterward, an episode of forward swimming was observed in the same animal (Fig. 3*B*), with a cycle duration of about 0.4 s, that is, five times shorter than during BS (Fig. 3*A*); on each side, the bursts of the rostral EMG led in phase the bursts of the caudal EMG (Fig. 3*C*). One may assume that the excitability of the locomotor networks was similar under these conditions.

We managed to evoke backward swimming (by tactile stimulation of a large area in the anterior part of the body) in all 20 tested lampreys. Swimming episodes consisted of three to 25 cycles. Different characteristics of BS, measured in eight animals, are presented as histograms in Fig. 4. The mean value of each characteristic is indicated (black arrows). In some of the trials, episodes of FS were recorded after BS termination, and the mean value of the corresponding characteristic is also indicated in each graph (white arrows). Table 1 summarizes the mean values (together with SD and range) for each BS and FS characteristic, as well as the FS data from literature.

Figure 4*A* shows a histogram of the swim cycle duration. From this graph and Table 1 one can see that the cycles of BS were on average much longer than the cycles of FS, and their ranges did not overlap. The duration of the EMG burst during BS positively correlated with the cycle duration (Fig. 5*A*), and the burst constituted about 40% of the cycle; this value was similar to that in FS (Fig. 4*B*, Table 1).

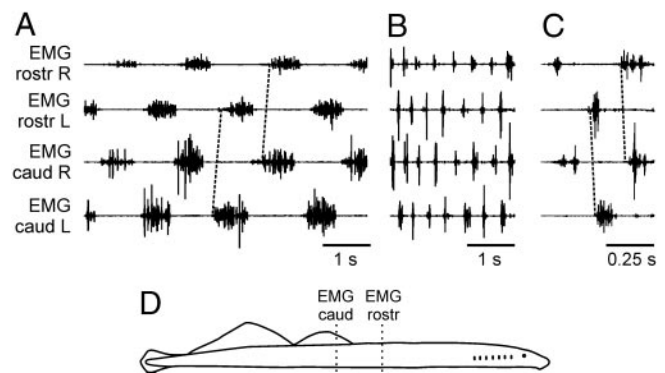


FIG. 3. Representative example of EMG activity during swimming. *A*: BS. *B* and *C*: forward swimming (FS) in the same animal (note different time-scales). EMGs were recorded from the left (L) and right (R) side at 2 rostrocaudal levels (segments 37 and 49) indicated in *D*.

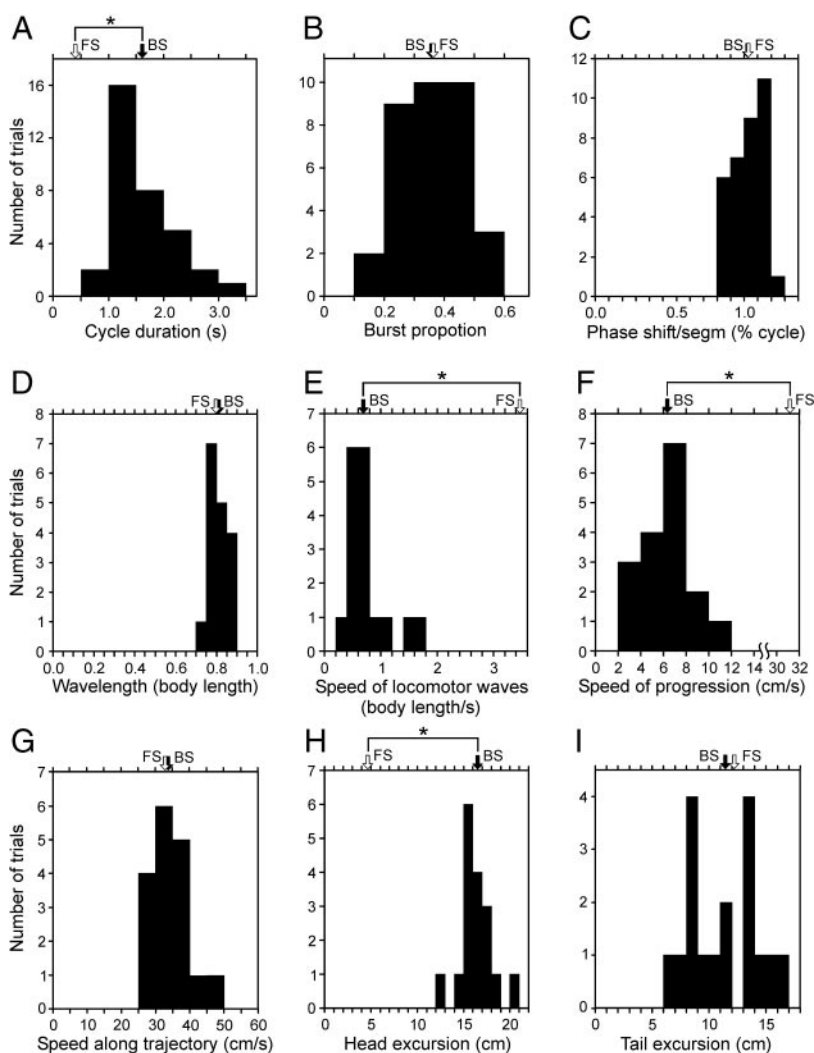


FIG. 4. Characteristics of BS. Each histogram summarizes the values observed in 8 tested animals; each animal was subjected to 3–5 trials. A: swim cycle duration. B: proportion of the EMG burst in the swim cycle. C: phase shift per segment. D: length of locomotor wave. E: speed of locomotor waves. F: speed of progression. G: speed of head movement along trajectory. H: lateral head excursions. I: lateral tail excursions. In each graph, the mean value for BS is indicated (black arrow) along with the corresponding value for FS (white arrow). Difference between the values was statistically significant in A, E, F, and H (t -test, $P < 0.05$).

The phase shift per segment during BS practically did not depend on the cycle duration (Fig. 5B). Both in BS and FS, the phase shift was about 1% per segment (Fig. 4C, Table 1). These data, obtained from the EMG analysis, corresponded well with the data on the wavelength (the value reciprocal to the phase shift) obtained from videorecording (Fig. 4D, Table 1).

The speed of locomotor waves propagating along the body had an average value of 0.68 ± 0.27 body length/s. The speed of waves during FS was much faster: 3.47 ± 1.26 body length/s (Fig. 4E, Table 1).

Because of curvilinear trajectory, progression during BS was very slow (Fig. 4F, Table 1), with an average value of 6.3 ± 2.5 cm/s, despite the fact that the head speed along the trajectory was high (35.2 ± 7.2 cm/s, Fig. 4G, Table 1). During FS, because of small head oscillations, the speed of progression practically did not differ from the head speed along the trajectory (32.5 ± 11.6 cm/s). During FS, lateral excursions of the head were much smaller than those of the tail, whereas during BS both head and tail excursions were of a large amplitude (Fig. 4, H and I, Table 1).

Relationships between muscle activity and body flexion

A synchronized recording of body shape and EMGs (see METHODS) allowed us to correlate the body kinematics with the

muscular activity. Figure 6A shows two cycles of EMG activity at two locations along the body, and Fig. 6B shows the body configuration (midline) for 17 sequential video frames taken during this period. The frame numbers in Fig. 6B correspond to the numbers of time points (abscissa) in Fig. 6A. The EMGs were recorded bilaterally at two rostrocaudal levels indicated in Fig. 6B by filled circles (rostral point) and open circles (caudal point). For each frame (1–17), we calculated the curvature of the body midline at the two sites of recording. The curvature was defined as $C = 1/R$, where R is the radius (arbitrary units) of the circle drawn through the point of recording (P in Fig. 6C) and through two points located at the distance of 0.1 body length rostrally and caudally to P (for methods, see Zelenin et al. 2003).

In Fig. 6A, the body midline curvature (in two sites along the body) is presented as a function of time (frame number), along with the EMGs recorded from the same sites. In each site, the EMG burst lasted approximately for the period of transition from the maximal curvature with ipsilateral convexity to that with contralateral convexity. Thus the active muscles initially caused a progressive decrease of the contralateral body flexion to subsequently increase the ipsilateral body flexion. Electrical and mechanical events in the rostral site were both delayed in relation to those in the caudal site (note a shift of the shaded

TABLE 1. *Main characteristics of swimming*

Characteristics of Swimming	BS	FS	FS (Previously Published Data)
Cycle duration, s	1.62 ± 0.61* [0.89–3.62] (n = 34)	0.38 ± 0.21 [0.19–0.88] (n = 5)	[0.13–0.66] ¹
Burst proportion	0.36 ± 0.10 [0.15–0.53] (n = 34)	0.36 ± 0.08 [0.27–0.48] (n = 5)	0.352 ± 0.038 ¹
Phase shift per segment, % cycle	1.03 ± 0.12 [0.81–1.20] (n = 34)	1.03 ± 0.06 [0.96–1.19] (n = 5)	0.97 ± 0.13 ¹
Wavelength, body length	0.81 ± 0.04 [0.75–0.87] (n = 17)	0.80 ± 0.06 [0.72–0.86] (n = 4)	0.72 ± 0.07 ²
Speed of wave, body length/s	0.68 ± 0.27* [0.30–1.80] (n = 17)	3.47 ± 1.27 [2.57–5.30] (n = 4)	
Speed of progression, cm/s	6.3 ± 2.5* [2.5–11.9] (n = 17)	31.2 ± 12.0 [19.2–43.0] (n = 4)	[16–260] ²
Head speed along trajectory, cm/s	34.1 ± 6.4 [23.8–50.0] (n = 17)	32.5 ± 11.6 [20.8–44.0] (n = 4)	
Head excursions, cm	16.5 ± 1.9* [12.5–20.9] (n = 17)	4.8 ± 1.8 [2.7–6.2] (n = 4)	
Tail excursions, cm	11.4 ± 2.9 [6.7–16.9] (n = 17)	12.2 ± 3.8 [7.1–16.5] (n = 4)	

Values for each cell of backward swimming (BS) and forward swimming (FS) columns contain means ± SD, range [], n = number of analyzed trials. Significantly different means of BS and FS (*t*-test, *P* < 0.05) are indicated by asterisks. Previously published data are from ¹Wallén and Williams (1984) and ²Williams et al. (1989).

areas that delineate analogous events in the two points of recording).

A similar analysis was done for forward swimming. We selected an episode of slow FS with a cycle duration (0.9 s) comparable to that of BS (1.5 s in Fig. 6). Figure 7*A* shows two cycles of EMG activity during FS; Fig. 7*B* shows the body midline for 27 sequential video frames taken during this period. The EMG burst lasted approximately for the time of increasing and then decreasing curvature with ipsilateral convexity; that is, the muscles initially decelerated the contralateral flexion and then caused the ipsilateral flexion (Fig. 7*A*). Thus the active muscles initially caused a cessation of the contralateral body flexion and afterward the ipsilateral body flexion. Both electrical and mechanical events in the caudal site were delayed in relation to the rostral site (note a shift of the shaded area that delineates analogous events in the two points of recording).

Lack of spatial orientation during backward swimming

During FS, the lamprey is typically oriented with its dorsal side up due to the vestibular-driven postural system (see e.g., Deliagina 1998; Deliagina et al. 1992; Ullén et al. 1995). The situation is different during BS. When released in a shallow aquarium with a cap on its head (Fig. 1*B*), the lamprey sank to the bottom with either the dorsal or ventral side facing upward. In a subsequent episode of BS, the lamprey did not attempt to change this initial orientation, and swam along the bottom with either the dorsal side up (as in Fig. 2*A*) or the ventral side up (as in Fig. 6*B*). Further evidence for the absence of any specific spatial orientation was obtained in the experiments with back-

ward swimming in free water (*n* = 3), in which the lamprey (with a head cap on) was released in a deep aquarium and videorecorded (from *top* and *side*, see Fig. 1*C*) before it made contact with the side or bottom of the aquarium (about 10 s). By analyzing this recording (a part of the recording is shown in Fig. 8*A*), we confirmed that BS took place, that is, the position of the points of maximal flexion moved forward in the sequential frames (as in Fig. 8*B*, where two cycles of BS are represented). By combining the two projections (Fig. 8*A*) it was possible to evaluate the roll tilt angle of the animal. A representative example of the tilt angle versus time trajectory is shown in Fig. 8*C* for the same period as shown in Fig. 8*B*. During two cycles of BS in the free water, the roll angle was continuously changing, and no preferred orientation in the roll plane was observed. This result suggests that the two orientations (dorsal or ventral side up), observed when the lamprey was swimming backward along the bottom of the shallow aquarium, were not stabilized but rather determined by mechanical reasons, i.e., by alignment of the plane of undulations with the plane of the bottom. Also, deprivation of vision (caused by the head cap) was not a reason for the lack of orientation: similar results were obtained in experiments (*n* = 3) with the cap not covering the eyes.

Effect of spinal lesions on backward swimming

To elucidate whether the forward-propagating waves underlying BS can be initiated from different levels of the spinal cord, we performed a complete transection of the spinal cord in the midbody area, and recorded the EMGs at two levels rostral to the lesion (*n* = 6). A normal EMG pattern of BS was

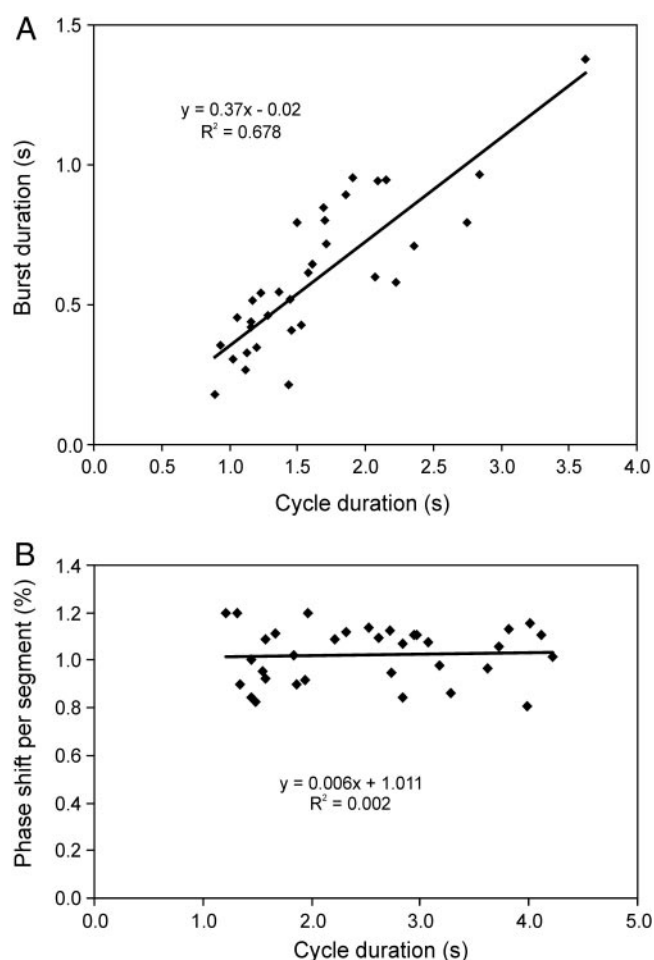


FIG. 5. Correlation of the burst duration and the phase lag with the cycle duration during BS. *A*: burst duration as a function of the cycle duration. *B*: phase shift per segment as a function of the cycle duration. Linear regression lines are shown.

observed, with the EMG bursts in the caudal site leading those in the rostral site (as in Fig. 9*A*) in animals ($n = 3$) with more rostral lesions (segments 43–45, S1 in Fig. 9*B*) and in animals ($n = 3$) with more caudal lesions (segments 62–64, S2 in Fig. 9*B*).

In another series of experiments ($n = 5$) we investigated the changes in the BS pattern caused by a hemisection in the midbody area (segments 37–43). EMGs from two sites—rostral and caudal to the hemisection (Fig. 10*C*)—were recorded. Figure 10*A* shows the EMG activity recorded before the lesion, with alternating left and right EMGs at each level, and with the caudal EMGs leading the rostral EMGs. After a right hemisection, the EMG activity in the site rostral to the lesion persisted on both sides, whereas in the site caudal to the lesion the EMG activity persisted on only the intact side but was substantially reduced or abolished on the damaged side (Fig. 10*B*). Similar results were obtained in all five animals.

DISCUSSION

Comparison of forward and backward swimming

The lamprey has two forms of undulatory locomotion: forward swimming and backward swimming. For migrating over

long distances, the lamprey uses forward swimming. The basic motor pattern of FS in the lamprey does not differ significantly from that described for fish (Gray 1968; Grillner 1974; Grillner and Kashin 1976; Williams et al. 1989): the propulsive force is generated by periodical waves of lateral body flexion propagating along the body from head to tail (Williams et al. 1989). The spinal and supraspinal nervous mechanisms of FS in the lamprey have been analyzed in considerable detail (for review see Grillner et al. 1995, 2000).

Backward swimming is not a commonly observed form of locomotion in the lamprey. Short episodes of BS can appear when encountering obstacles, or when the animal's head occurs in tight split, or as a component of struggling behavior. Because of difficulties in eliciting BS experimentally, it has not previously been investigated systematically. Much longer episodes of BS have now been generated by tactile stimulation of the anterior part of the body, allowing us to study BS in detail and to compare the motor patterns of BS and FS.

As was first shown by McClellan (1989), BS in lampreys is based on the same principle as FS, i.e., the waves of periodic lateral body flexion propagate along the body but in the opposite direction (as in Fig. 2*C*). The traveling waves generate a propulsive force moving the animal backward. Interestingly, during both BS and FS, the length of the mechanical waves traveling along the body was approximately equal to the body length. Also, the phase shift per segment was similar at different swim frequencies (Fig. 4, *C* and *D*, Table 1; see also Williams et al. 1989).

BS differs from FS not only in the direction of locomotor waves. On average, the cycle duration during BS was nearly fourfold longer than during FS under these conditions (Fig. 4*A* and Table 1; see also Wallén and Williams 1984; Williams et al. 1989). The lateral head undulations during BS (about 50% of the body length, peak to peak, Fig. 6) were much larger than during FS (about 15% of the body length, Fig. 7; see also Williams et al. 1989). Because the undulations were so large, the swim trajectory became very curvilinear (Fig. 2*A*) and the speed of progression of the whole body constituted only a small proportion (typically, 0.2) of the speed along the trajectory (Fig. 4, *F* and *G*).

During FS the lamprey stabilizes the dorsal-side-up orientation of its body in the gravity field as a result of vestibular postural reflexes (Ullén et al. 1995). In contrast, during BS the lamprey did not maintain any specific orientation in space (Fig. 8). The absence of any stabilized spatial body orientation during BS and a very slow speed of BS suggest that BS is not used for locomotion over long distances but only for withdrawal when forward progression is hampered.

The waves of lateral body flexion are caused by the waves of muscle contraction that, in turn, are caused by the waves of excitation of motoneurons propagating along the spinal cord (Wallén and Williams 1984; Williams et al. 1989). The relationships between the muscle activity (EMG) and the body flexion during BS were determined in the present study. The EMG bursts during BS lasted for about 40% of the swim cycle (Fig. 4*B*), a value similar to that of FS (Wallén and Williams 1984; Williams et al. 1989). During BS, the EMG bursts on the two sides alternated and the EMG in more caudal points led in phase the EMG in more rostral points. The active muscles (as judged by their EMGs) initially caused a progressive decrease

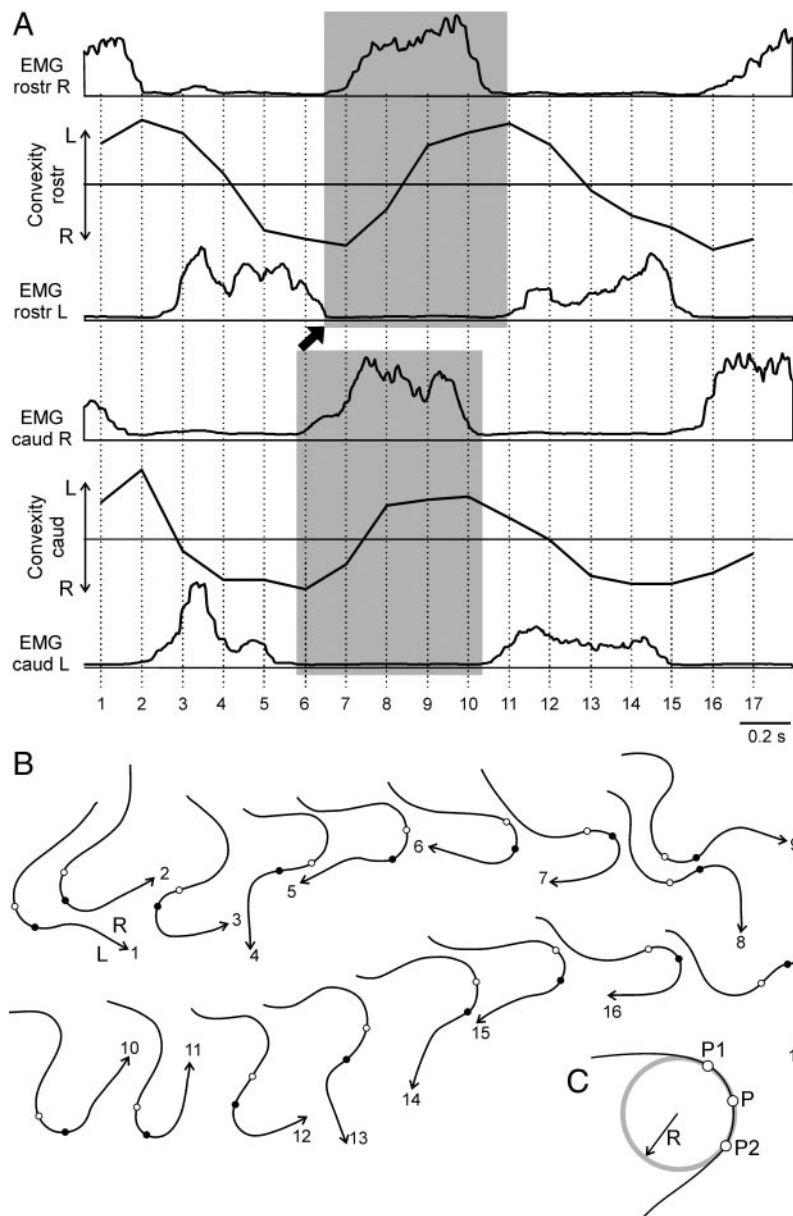


FIG. 6. Correlation of body kinematics and muscular activity during BS. A: EMGs were recorded, during a period of roughly 2 cycles, from the left (L) and right (R) sides at the rostral (Rost, segment 36) and caudal (Caud, segment 46) levels (indicated in B). For the same period, a body midline curvature (arbitrary units) in the sites of EMG recording is presented as a function of time. Shaded areas delineate the analogous parts of trajectories in the 2 points of recording. B: calculation of body curvature. Body midline in 17 sequential video frames taken during the period shown in A. In this swim episode, the lamprey was positioned with its ventral side up. Frame numbers in B correspond to the numbers of time points in A. Rostral and caudal sites of recording are indicated by filled and empty circles, respectively. C: measuring of curvature. For each frame, the curvature of the body midline at the 2 sites of recording was defined as $Cur = 1/R$, where R is a radius of the circle drawn through the point of recording (P) and 2 adjacent points (P1 and P2).

of the contralateral body flexion and then they caused an increasing ipsilateral body flexion (Fig. 6). The phase relationships between the EMGs and the body flexion in FS were slightly different: the active muscle initially decelerates the contralateral flexion and then caused a decrease of the contralateral body flexion followed by the ipsilateral flexion (Fig. 7; see also Wallin and Williams 1984). This difference in timing of body flexion and EMG bursts for BS and FS may be caused by higher velocities of body bending necessary for the faster FS rhythm.

Generation of the pattern of backward swimming

The spinal locomotor central pattern generator (CPG) in the lamprey is usually considered as a distributed system, that is, a chain of unitary oscillators activated by a population of glutamatergic reticulospinal neurons (see e.g., Brodin et al. 1988;

Grillner et al. 1981, 1995, 2000). Each oscillator is capable of generating the rhythm of swimming, whereas the propagating locomotor waves arise from the interactions between the oscillators.

Two different views have been formulated to explain propagation of locomotor waves. The "trailing oscillator" hypothesis (Matsushima and Grillner 1990, 1992) implies that intrinsic frequencies of the oscillators are not equal to each other and that the difference between activity levels of oscillators is the primary factor determining the direction of propagation of the waves (see also Cohen 1987; Rand et al. 1988). This hypothesis suggests that the "fastest" oscillator imposes its rhythm on the whole chain and determines the direction of wave propagation. Therefore to elicit FS, the oscillators in the rostral spinal segments should be made the fastest through additional excitatory drive, whereas for BS the caudal segments should receive additional excitation.

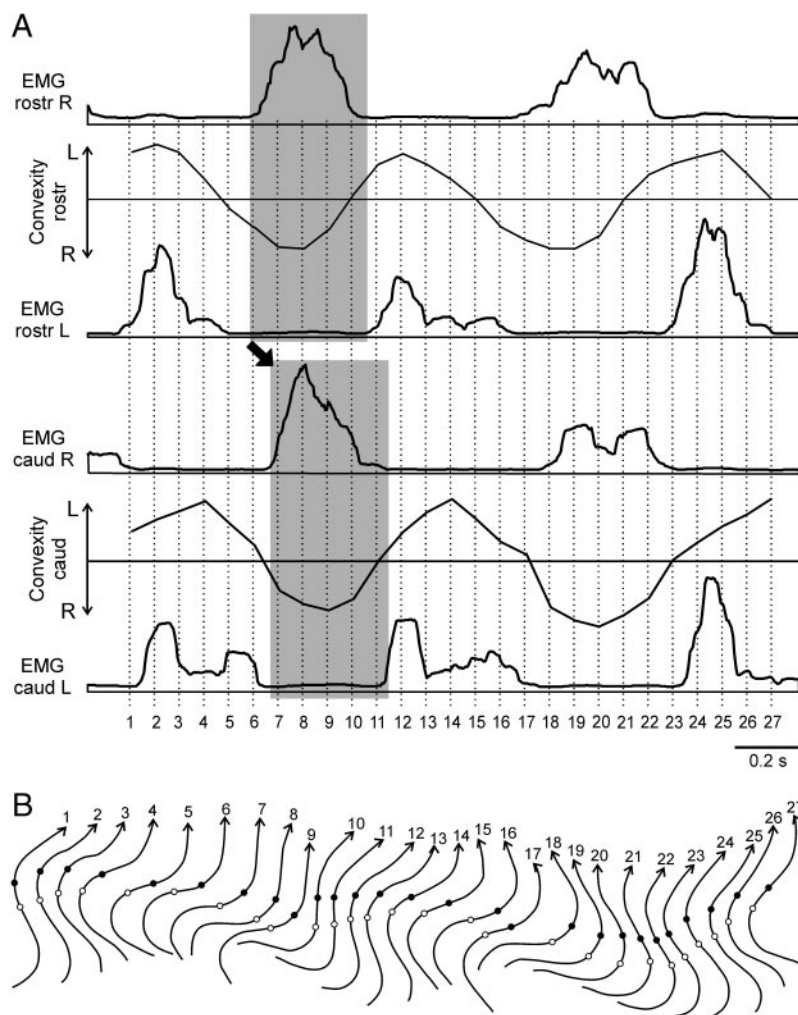


FIG. 7. Correlation of body kinematics and muscular activity during FS. *A*: EMGs were recorded during a period of approximately 2 cycles, from the left (L) and right (R) sides at the rostral (Rost) and caudal (Caud) levels (indicated in *B*). For the same period, a body midline curvature (arbitrary units) in the sites of EMG recording is presented as a function of time. *B*: body midline in 27 sequential video frames taken during the period shown in *A*. In this swim episode, the lamprey was positioned with its dorsal side up. See legend to Fig. 6 for explanations.

The alternative view (Kopell and Ermentrout 1986, 1988), which we will refer to as the “network reconfiguration” hypothesis, suggests that all the unitary oscillators in the spinal cord are equivalent to each other and the direction of propagation of the waves is determined by asymmetry of connections of each oscillator with its rostral and caudal neighbors. According to this idea, two types of connections between the oscillators—one for FS with prevailing rostrocaudal influences and another one for BS with prevailing caudorostral influences—could determine the direction of the wave propagation.

The two hypotheses can explain different directions of wave propagation in FS and BS in the following way. In the framework of the “trailing oscillator” hypothesis, one can suggest that tactile stimulation of the head and neck area leads to activation of a special “BS group” of reticulospinal neurons (not identified at present). These neurons activate differently the unitary oscillators along the spinal cord, thus creating a gradient of their intrinsic frequency, so that any oscillator along the cord has higher intrinsic frequency than that of its rostral neighbor. The frequency gradient could also explain our finding that, after a complete transection of the spinal cord at any level, BS could be elicited rostral to the lesion (Fig. 9). It is noteworthy that the sensory input from the rostral region could also directly influence the pattern generator in this part of the spinal cord to lower its excitability, which appears to be the

case in the spinal dogfish (Grillner 1974). By contrast, the “FS group” of reticulospinal neurons will create a rostrocaudal gradient of the intrinsic frequency of unitary oscillators and thus cause backward-propagating locomotor waves.

In the framework of the “network reconfiguration” hypothesis, one can suggest that both the “FS group” and the “BS group” of reticulospinal neurons activate the corresponding system of connections between the unitary oscillators along the whole extent of the spinal cord, necessary for FS and BS, respectively. This hypothesis also explains the persistence of BS (rostral to the lesion) after a transection of the spinal cord.

Another distinctive feature of BS is the very large amplitude of body undulations and a low frequency of these undulations compared with those of FS. To explain these differences, each of the two hypothesis should be supplemented by a suggestion that the “BS group” of reticulospinal neurons not only activates the unitary oscillators, but also modifies their intrinsic properties (compared with the FS mode), and thus causes the oscillations with low frequency and large amplitude. The latter suggestion was supported by recent findings that two different, distinct modes of rhythmic activity could be evoked in the spinal cord by application of *N*-methyl-D-aspartate, one mode with a fast rhythm and the other with a slow rhythm, which are similar to the rhythms of FS and BS, correspondingly (Cangiano and Grillner 2003). Different neuromodulators could also

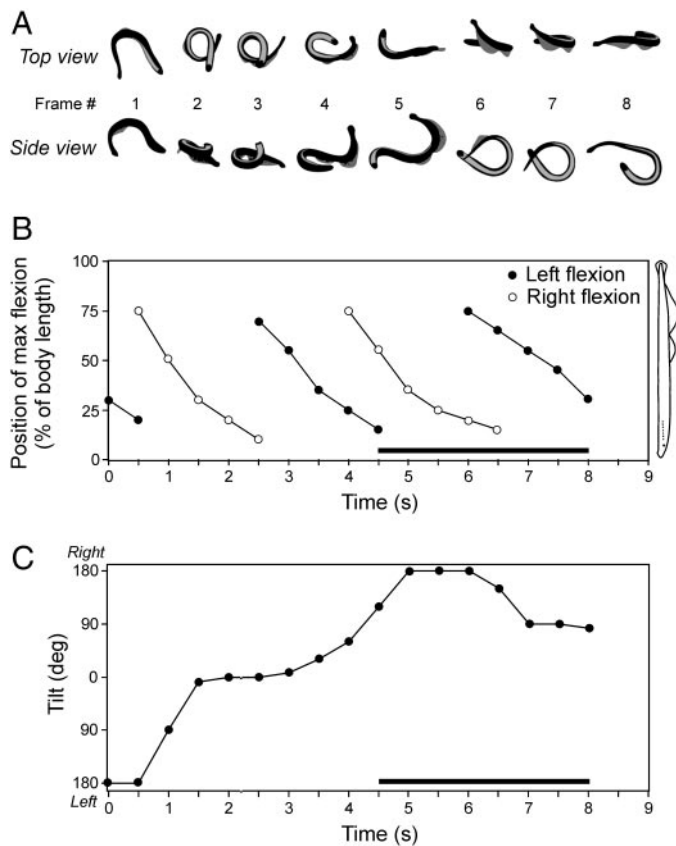


FIG. 8. Absence of spatial orientation during BS revealed in a deep aquarium. *A*: body configuration in 8 sequential frames during a period of 3.5 s (*top* and *side* views). *B*: position of the maximal lateral flexion in 17 sequential frames. *C*: roll tilt angle in these frames. In *B* and *C*, a horizontal line indicates the period shown in *A*.

be responsible for the modifications of properties of the CPG network during BS; in particular, 5-HT (serotonin) is known to cause more intense bursts with much longer burst duration (Harris-Warrick and Cohen 1985; Wallén et al. 1989).

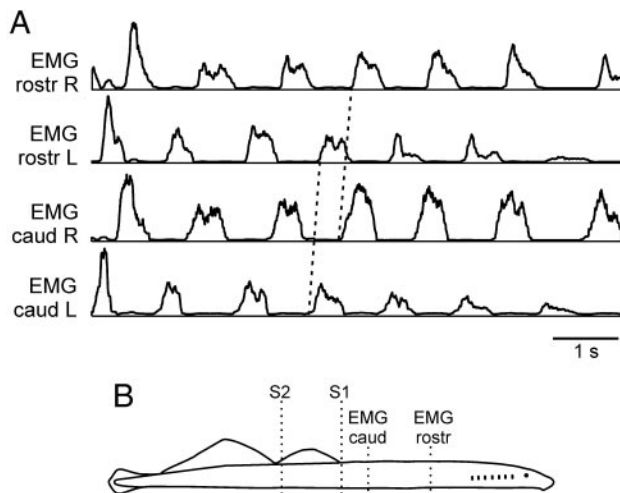


FIG. 9. Effect of transection of the spinal cord on the EMG pattern during BS. *A*: EMGs were recorded from the left (L) and right (R) sides at 2 rostrocaudal levels (segments 22 and 38) after a complete transection of the spinal cord at segment 45. *B*: levels of recording (EMG Rostr and EMG Caud) and the level of the spinal cord transection in this experiment (S1) and in the other ones (S2).

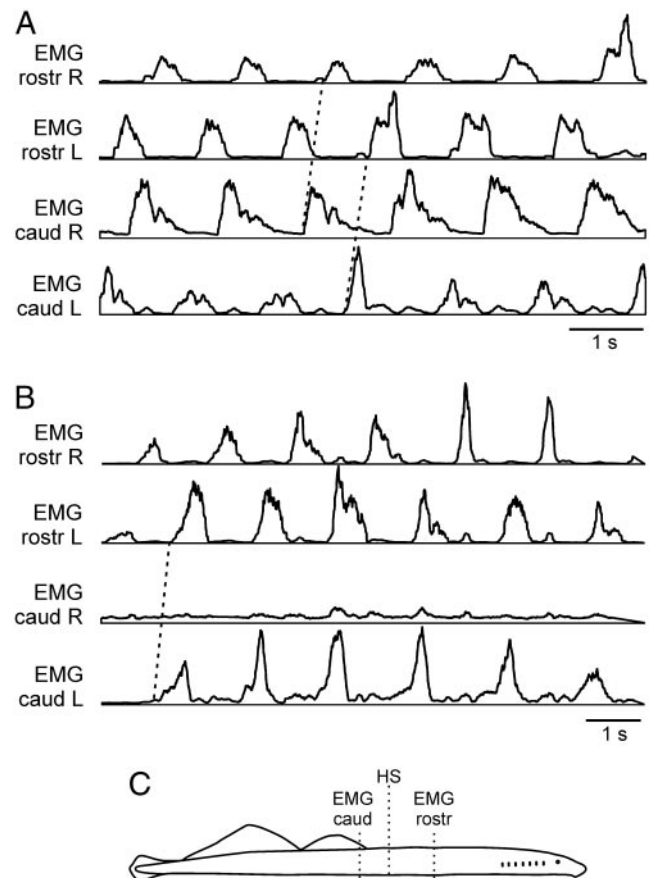


FIG. 10. Effect of hemisection of the spinal cord on the EMG pattern during BS. *A*: EMG activity before hemisection at segment 40. *B*: EMG activity after hemisection. EMGs were recorded from the left (L) and right (R) sides at 2 rostrocaudal levels (segments 29 and 46). *C*: levels of recording (EMG Rostr and EMG Caud) and the level of the spinal cord hemisection (HS).

Each of the unitary oscillators in the spinal cord of adult lampreys consists of two symmetrical (left and right) parts with mutual inhibitory connections (Grillner et al. 1995, 2000). Experiments with longitudinal split of the spinal cord have shown that both fast and slow rhythms can be generated by an isolated part of the cord (hemicord) (Cangiano and Grillner 2003, 2005). These findings strongly suggest that each half of the unitary oscillator is capable of rhythm generation, and their mutual inhibitory influences secure their antiphase activity. An additional support for this hypothesis, concerning specifically BS, was obtained in the present study. Our experiments with the hemisection of the spinal cord have shown that the rhythm of BS persisted below the lesion, but only (or mainly) on the intact side (Fig. 10). One possible interpretation of this finding is that the hemicord is able to generate long bursts characteristic for the BS rhythm. Another interpretation is that the interneurons of both left and right parts of the unitary oscillators remain active after hemisection and generate alternating BS bursts, although the excitability of motoneurons caudal to the hemisection is so much reduced (in the absence of supraspinal excitatory drive) that they do not respond to the signals from the rhythm-generating interneurons.

During FS, the propulsive system based on the body undulations and the system for spatial orientation based on the vestibular reflexes closely interact with each other and consti-

tute a gross motor synergy, which allows the animal to stabilize its spatial orientation when swimming (Ullén et al. 1995; Zelenin et al. 2005). Both the commands for activation of the spinal locomotor networks and the commands for postural corrections are transmitted by the reticulospinal system, and vestibular responses contribute to the activity of reticulospinal neurons (Deliagina and Fagerstedt 2000; Deliagina et al. 2000). By contrast, during BS the lamprey does not stabilize its spatial orientation. An intriguing question is whether the “BS group” of reticulospinal neurons receives vestibular input and transmits position-dependent signals to the spinal cord during BS or vestibular input to this group is absent or inhibited during BS.

The undulatory forward and backward swimming are not the only forms of locomotion in the lamprey. For moving on the bottom and in tight places, the lamprey uses the mechanisms of crawling (forward or backward). When crawling, the lamprey adapts its body configuration to the position of the external constraints and moves as the result of a single wave of lateral flexion propagating along the body (Archambault et al. 2001; Zelenin 2005). It is not known how this mode of the spinal network activity is controlled by the reticulospinal system. In particular, it is not clear to what extent the groups of reticulospinal neurons, responsible for initiation of the two types of swimming and two types of crawling, overlap with each other.

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