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Modifications of Locomotor Pattern Underlying Escape Behavior in the Lamprey

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Islam SS, Zelenin PV. Modifications of locomotor pattern underlying escape behavior in the lamprey. *J Neurophysiol* 99: 297–307, 2008. First published November 14, 2007; doi:10.1152/jn.00903.2007. Two forms of undulatory locomotion in the lamprey (a lower vertebrate) have been described earlier: fast forward swimming (FFS) used for long distance migrations and slow backward swimming (SBS) used for escape from adverse tactile stimuli. In the present study, we describe another form of escape behavior: slow forward swimming (SFS). We characterize the kinematic and electromyographic patterns of SFS and compare them with SBS and FFS. The most striking feature of SFS is nonuniformity of shape and speed of the locomotor waves propagating along the body: close to the site of stimulation, the waves slow down and the body curvature increases several-fold due to enhanced muscle activity. Lesions of afferents showed that sensory information critical for elicitation of SFS is transmitted through the dorsal roots. In contrast, sensory signals that induce SBS are transmitted through the dorsal roots, lateral line nerves, and trigeminal nerves. Persistence of SFS and SBS after different lesions of the spinal cord suggests that the ascending and descending pathways, necessary for induction of SBS and SFS, are dispersed over the cross section of the spinal cord. As shown previously, during FFS (but not SBS) the lamprey maintains the dorsal-side-up body orientation due to vestibular postural reflexes. In this study we have found that the orientation control is absent during SFS. The role of the spinal cord and the brain stem in generation of different forms of undulatory locomotion is discussed.

INTRODUCTION

In this study we describe slow forward swimming (SFS) in the lamprey (a lower vertebrate, cyclostome). This type of undulatory locomotor movements has not been studied previously. It strongly differs from the previously described types of undulatory locomotion: fast forward swimming (FFS) and slow backward swimming (SBS). It also differs from crawling in tight places where undulatory movements are not possible (Archambault et al. 2001). The goal of the present study was to compare these three forms of undulatory locomotion (SFS, SBS, and FFS) and to assess similarities and distinctions in their control mechanisms.

The main form of locomotion in the lamprey is FFS. Its motor pattern 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 motor pattern underlying FFS in the lamprey is generated by the neuronal network (central pattern generator) residing in the spinal cord (Wallén and Williams 1984). This network has been analyzed in detail (see e.g., Grillner 2003; Grillner et al. 1995, 2000). It was found that the ability to generate rhythmic oscillations is distributed along the spinal cord, and that even a few isolated segments can produce periodical bursts (Cangiano and Grillner 2003, 2005). Both experimental studies and theoretical analyses 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 for FFS is normally activated by reticulospinal neurons, which in turn are driven by cells in locomotor areas of mesencephalon and diencephalon (Brocard and Dubuc 2003; Brodin et al. 1988; Deliagina et al. 2000; El Manira et al. 1997; McClellan and Grillner 1984; Sirota et al. 2000). It has also been found that, during FFS, the lamprey actively maintains the dorsal-side-up orientation of its body due to vestibular postural reflexes (Deliagina and Fagerstedt 2000; Deliagina et al. 1992; Orlovsky et al. 1992).

Backward swimming in the lamprey can be observed less often. The lamprey exhibits short episodes of SBS when encountering obstacles (McClellan 1989). Sometimes, the episodes of SBS are combined with struggling behavior. Recently, we found a regular way to evoke long episodes of SBS by tactile stimulation of a large area in the anterior part of the body, which allowed us to study SBS in detail (Islam et al. 2006a). We found that SBS strongly differs from FFS: the direction of wave propagation is reversed, the body progression is slower, the cycle duration is longer, the amplitude of oscillations is larger, and the spatial orientation during swimming is not maintained. We suggested that SBS is a component of escape behavior initiated by extensive tactile stimulation of the head or gill region.

In the present study we applied extensive tactile stimulation more caudally, in the middle of the body. Such a stimulus imitates a natural situation when an animal is grasped by a predator and attempts to escape, or is stuck in a tight place and maneuvers itself free. This method of stimulation evoked a different mode of locomotion: SFS. Four types of experiments were performed. First, we investigated kinematic and EMG patterns of SFS and compared them with those of SBS and FFS. Second, we partially denervated the receptive fields for SFS and SBS to reveal the afferents involved in induction of these two forms of slow swimming. Third, we studied the effects on SFS and SBS produced by lesions of the spinal cord, to localize the ascending and descending pathways involved in initiation of slow swimming. Fourth, we addressed the question of whether the body orientation during SFS is stabilized by

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vestibular reflexes, as has been demonstrated for FFS (but not for SBS). The present study is important for understanding the mechanisms of slow rhythm generation by spinal cord neuronal networks, as well as for elucidating the control of these networks by supraspinal structures.

A brief account of this study has been published in abstract form (Islam et al. 2006b).

METHODS

Adult lampreys (*Lampetra fluviatilis*, 25–30 cm long, $n = 46$) 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 temperature in the experimental aquaria was maintained at 5–7°C. All experiments were approved by the local ethics committee (Norra Djurförsöksetiska Nämden).

Surgery

Surgery was performed under MS-222 (Sandoz) anesthesia (100 mg/l). In 12 animals, up to 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. In 4 other animals, the EMG electrodes were inserted on the same side at different rostrocaudal levels.

In 14 animals, the receptive fields for SFS and SBS were denervated (Fig. 8, *A* and *B*; for details, see RESULTS). In the other 12 animals, the following three types of transection of the spinal pathways were performed at the level of the third gill (Fig. 8*C*): transection of the medial spinal cord, bilateral transection of the lateral columns, or lateral hemisection of the spinal cord. Four animals were completely spinalized at the level of the first gill. We used the lesion methods reported by Ullén et al. (1997): the spinal canal was opened and the spinal cord was exposed; after the lesion, the wound was sutured.

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. The extent of the spinal cord lesions was verified histologically. In the animals with medial lesions, at least one third of the spinal cord was transected. In the animals with lateral lesions, at least one third of the spinal cord was transected on each side. In all animals with hemisections, one half of the spinal cord was completely transected. The rostrocaudal positions of the lesions and of the EMG electrodes were also documented.

Locomotor tests

Slow swimming was evoked by tactile stimulation. For this purpose, a thin elastic ring (a piece of toe protector, 8 mm in diameter and 5–35 mm in length; Scholl) was put over the body or the head (Fig. 1*A*). Longer rings produced qualitatively the same but slightly stronger effects including larger amplitude of body movements and longer episodes of swimming. However, these differences were not studied systematically. Tactile stimulation produced by the ring depended on the stretch of the ring, which in turn was proportional to the difference between the circumference of the ring (25 mm in a loose state) and the body. Along the rostral half of the body where the stimulation was applied, the body circumference varied between 60 and 70 mm, suggesting the corresponding changes in the pressure exerted by the ring and in the intensity of stimulation. We estimate the ratio of the minimal and maximal pressure to be about $(60 - 25)/(70 - 25) \cong 0.75$. We think that such variability is not significant.

When released in the water, the lamprey usually swam until the ring slid off ($\leq$1 min). In all episodes chosen for quantitative analysis of body movements and EMG patterns during SFS and SBS, the stimulus did not move in relation to the body. When the stimulus was positioned in the gill region, it could cover all gills. However, the animal's behavior was the same regardless of whether the gills were covered partially or completely, and the episodes were therefore analyzed together.

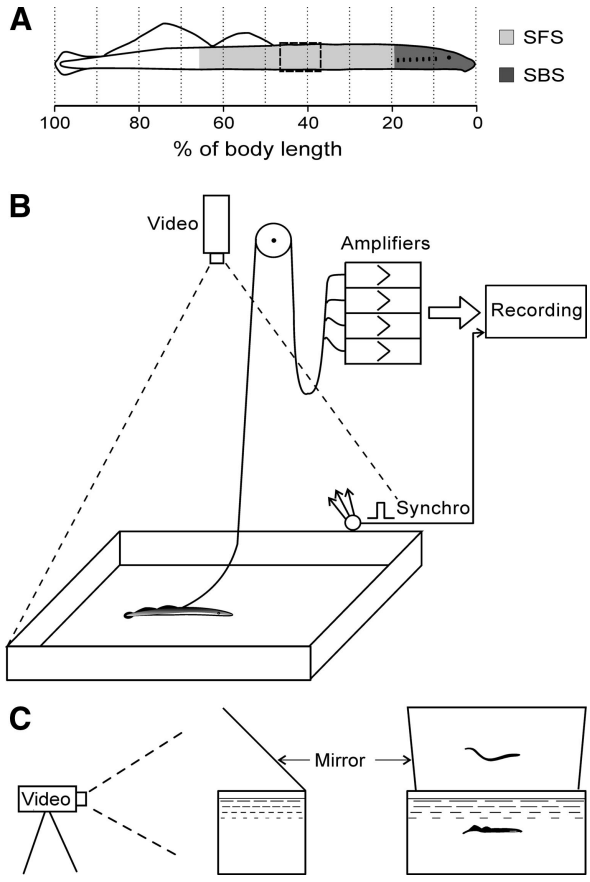

FIG. 1. Experimental arrangement. *A*: slow forward swimming (SFS) and slow backward swimming (SBS) were initiated by tactile stimulation with a thin elastic "ring" that covered 1.5–10% of the body length (shown by the dashed rectangle). Light gray and dark gray areas indicate the receptive fields for SFS and SBS, correspondingly. *B*: arrangement for parallel recording of kinematic and electromyographic (EMG) data. The 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. The *side* and the *top views* (by means of a mirror) were video recorded simultaneously.

In most experiments, swimming was studied in a shallow aquarium (80 × 80 cm, 10-cm depth) (Fig. 1*B*). Movements of the animal were recorded from above by a video camera (25 frames/s) positioned at a distance of 2 m from the aquarium, and analyzed frame by frame. The EMG electrodes were connected, via 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 video recordings were synchronized by pulses recorded simultaneously by both systems (Fig. 1*B*, Synchro).

For testing the capacity for spatial orientation during SFS, the lamprey with the stimulating ring was released into a deeper aquarium (110 × 35 cm, 37-cm depth) and video recorded 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. 1*C*). EMGs were not recorded under these conditions.

Data processing

The characteristics of the swim motor pattern were defined as in the earlier studies (see e.g., Matsushima and Grillner 1992; Williams et al.

1989). The cycle duration was calculated from the video recording. The speed of locomotor waves was calculated by tracking a zero curvature point along the body; the speed was expressed in body length per second. The wavelength was estimated by a product of the cycle duration and the locomotor wave speed, and expressed in body lengths. Tracking positions of one body point (the head tip or the tail tip) provided the trajectory of this point, which allowed calculation of the speed along the trajectory and of the amplitude of head and tail excursions. Averaging the trajectory across the cycle duration provided an estimate of the whole body progression and allowed calculation of the speed of progression. The burst proportion was defined as the ratio of the EMG burst duration to the cycle duration. The body curvature in a given point of the body midline was defined as $C = 1/R$, where R is the radius of the circle drawn through the point (P in Fig. 2*E*) and through two points (P_1 and P_2 in Fig. 2*E*) located at the distance of 0.05 body length rostrally and caudally to P. The radius was expressed in body lengths; thus the curvature was expressed in inverse body lengths, $(\text{b.l.})^{-1}$. The maximal curvature observed in a given point during a locomotor cycle was taken as the curvature amplitude in this point. All values are presented as means ± SD.

RESULTS

Kinematics of slow forward swimming

When a lamprey was released in the aquarium with a ring on its body, its behavior depended mainly on the stimulus position. If the stimulus was placed in the anterior part of the body (area from 0 to 20% of body length; Fig. 1*A*), the animal exhibited the SBS pattern. The characteristics of SBS obtained in the present study did not differ from those observed earlier (Islam et al. 2006a) (Table 1).

If the stimulus was placed caudally to the gills, two different modes of locomotion could be evoked. The stimulus between the gills and the dorsal fins (area from 20 to 50% of the body length; Fig. 1*A*) most often evoked SFS (83 of 85 episodes) and very rarely FFS (2 of 85 episodes). If the stimulus was placed at the level of the caudal dorsal fin, vigorous FFS with high frequency (2–4 Hz) and large amplitude of undulations was observed (10 episodes). Stimuli applied at the level of the rostral dorsal fin (area from 50 to 65% of the body length; Fig. 1*A*) usually evoked SFS (12 of 15 episodes) and seldom FFS (3 of 15 episodes). In 3 episodes, we observed spontaneous switching between SFS and FFS; these cases were not included in the analysis.

Kinematics of SFS was quantitatively analyzed for 41 episodes in 10 animals (each episode included ≥10 swimming cycles). The data are summarized in Table 1. During SFS, the animals often (in 28 of 41 episodes) attached themselves to the bottom of the aquarium with their sucker mouth, but continued undulatory movements. All characteristics (except for the speed of progression and the amplitude of head excursions) in the attached animals were similar to those in the swimming

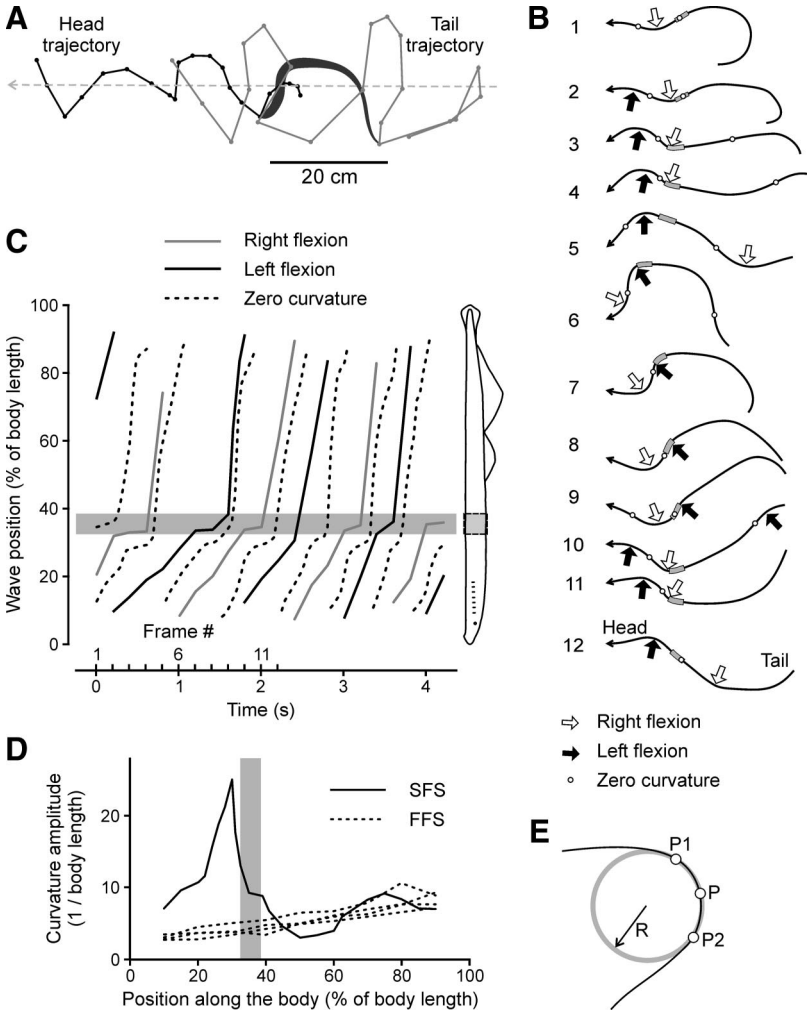

FIG. 2. Representative example of SFS in a shallow aquarium. *A*: head and tail trajectories for 3 swim cycles. Time intervals between sequential positions were 200 ms. The lamprey was positioned with its dorsal side up. For one of the positions, the body configuration is shown. *B*: shape of the body midline in 12 sequential frames (*1–12*). The body outline shown in *A* corresponds to frame *6*. The position of the stimulus at 35% of body length is indicated with a gray rectangle. The points of maximal and zero curvature are indicated: maximal right flexion (white arrows), maximal left flexion (black arrows), and zero curvature (open circles). *C*: positions of maximal right curvature (gray lines), maximal left curvature (black lines), and zero curvature (dashed black lines) as a function of time (and of frame number in *B*). The stimulus position is indicated with a gray band. *D*: amplitude of curvature as a function of position along the body during the same episode of SFS. The stimulus position is indicated with a gray band. The dashed lines show the amplitude of curvature in 4 episodes of fast forward swimming (FFS) for comparison. *E*: method of curvature measurement. See METHODS for details. The curvature of the body midline was defined as $C = 1/R$, where R is a radius of the circle drawn through the point of measurement (P) and 2 adjacent points (P1 and P2).

TABLE 1. *Main characteristics of different modes of swimming*

Characteristics of swimming	SFS	SBS	FFS
Cycle duration, s	1.32 ± 0.62* [0.52–3.12] (n = 41)	1.62 ± 0.61 [0.89–3.62] (n = 34)	0.38 ± 0.21 [0.19–0.88] (n = 5)
Speed of progression, cm/s	19.3 ± 9.4* [3.3–40.1] (n = 13)	6.3 ± 2.5 [2.5–11.9] (n = 17)	31.2 ± 12.0 [19.2–43.0] (n = 4)
Head speed along trajectory, cm/s	39.8 ± 11.4 [19.7–54.7] (n = 13)	34.1 ± 6.4 [23.8–50.0] (n = 17)	32.5 ± 11.6 [20.8–44.0] (n = 4)
Head excursions, cm	13.0 ± 4.3* [8.0–20.8] (n = 41)	16.5 ± 1.9 [12.5–20.9] (n = 17)	4.8 ± 1.8 [2.7–6.2] (n = 4)
Tail excursions, cm	28.4 ± 5.1* [19.3–40.0] (n = 41)	11.4 ± 2.9 [6.7–16.9] (n = 17)	12.2 ± 3.8 [7.1–16.5] (n = 4)
Burst proportion	0.46 ± 0.08* [0.30–0.65] (n = 25)	0.36 ± 0.10 [0.15–0.53] (n = 34)	0.36 ± 0.08 [0.27–0.48] (n = 5)

Values are means ± SD for each cell of SFS, SBS, and FFS columns; range [ ]; n, number of analyzed trials. Means for SFS significantly different from those for FFS are indicated with * (t-test, $P < 0.05$). Data for SBS and FFS were previously published (Islam et al. 2006).

animals. These characteristics for swimming and nonswimming animals were pooled and presented together.

Stimuli placed at the border between the receptive fields for SBS and SFS did not produce any consistent effect. The animal could sporadically display SBS, SFS, or noncoordinated undulations; the movements usually ceased within 1–10 s. Such cases were not included in the analysis.

An example of SFS is shown in Fig. 2. The head and the tail trajectories in three cycles of swimming are shown in Fig. 2*A* (the time interval between sequential points is 200 ms). For one pair of points, the body configuration is also shown. The cycle duration was 0.67 s, that is, much longer than the cycle duration of 0.25–0.50 s typical for FFS in the same conditions. The average cycle duration for all SFS episodes was about 3.5 times longer than the average cycle duration for FFS, although the ranges for SFS and FFS slightly overlapped (Table 1). The cycle duration of SFS was close to that of SBS. Another feature of SFS similar to SBS and different from FFS was the large amplitude of the lateral body undulations (Fig. 2*A*, Table 1). The lateral undulations of both the head and the tail were about 2.5-fold larger for SFS than for FFS. Due to these large undulations, the swim trajectory was very curvilinear, and the whole body progression represented only about 50% of the trajectory length (Table 1).

A striking feature of SFS was nonuniformity of propagation of the locomotor waves along the body. The shape of the body midline in 12 sequential frames (*1–12*) is presented in Fig. 2*B*. The points of maximal flexion to the right and to the left are shown by white and black arrows, respectively, and the points with zero curvature, by white circles. Figure 2*C* shows the position of the maximal right and left flexion, as well as the position of the zero curvature as a function of time (frame number); the stimulus position is indicated with a gray band. From Fig. 2*C* it is evident that the speed of the wave propagation changed considerably along the body. Rostral to the stimulus site, the speed was about 0.4 b.l./s (body length per second). This value was larger (0.3–1.8 b.l./s) during SBS and even larger (2.6–5.3 b.l./s) during FFS (Islam et al. 2006a). Near the stimulus site, the waves slowed down to about 0.1 b.l./s. Caudally to the stimulus site, the waves propagated with a high speed, ≤2.0 b.l./s. The degree of body flexion during SFS was also very different from that observed during FFS. This is illustrated in Fig. 2*D*. FFS is characterized by moderate flexions, with the curvature amplitude gradually increasing from 3–4 $(\text{b.l.})^{-1}$ near the head to 8–10 $(\text{b.l.})^{-1}$ near the tail (dashed lines in Fig. 2*D*). During SFS, the curvature amplitude was much larger in the rostral part, with a sharp peak of about 25 $(\text{b.l.})^{-1}$ in front of the stimulus site. Caudally to the stimulus site, the curvature dropped to about 3 $(\text{b.l.})^{-1}$ and, closer to the tail, it increased again to about 8 $(\text{b.l.})^{-1}$ (solid line in Fig. 2*D*).

Similar kinematic patterns were observed in all episodes of SFS. As in the example presented in Fig. 2, the extent of flexion always increased as the wave propagated from the head to the stimulus site, reached its maximum near the stimulus site, and decreased afterward. Figure 3 shows the maximal body curvature in different episodes of SFS plotted versus position of the points with maximal curvature in relation to the body (Fig. 3*A*) and to the stimuli (Fig. 3*B*). The maximal curvatures during SFS were much larger than the curvatures during FFS (dashed lines in Fig. 3*A*). Almost all points of maximal curvature were clustered in front of the stimulus site (Fig. 3*B*).

The locomotor wave propagation always slowed down and the curvature increased near the site of stimulation. We tracked the position of the point of zero curvature during propagation of locomotor waves. Figure 4 shows the relation between the posi-

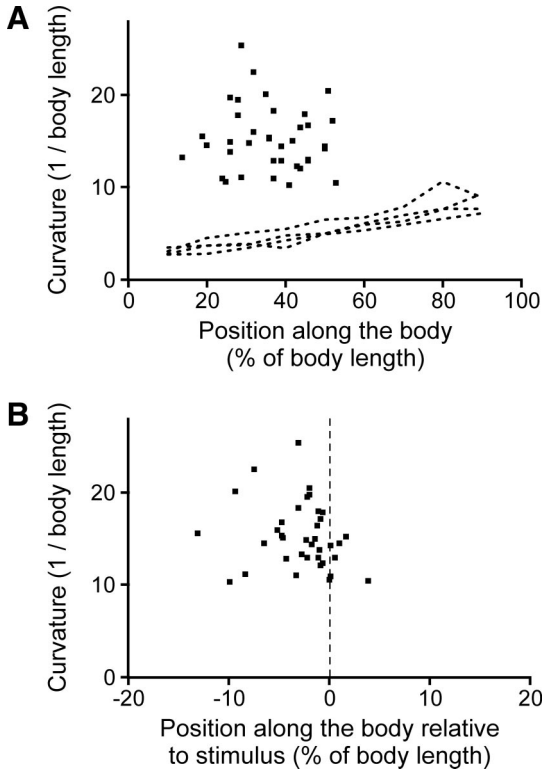

FIG. 3. The value of the maximal curvature in different episodes of SFS. *A*: curvature in the points of its maximum along the body (for comparison, the dashed lines show the amplitude of curvature in 4 episodes of FFS). *B*: position of the points with maximal curvature in relation to the stimulus position. Note that the points in *B* are clustered in front of the site of stimulation. Number of episodes n = 37.

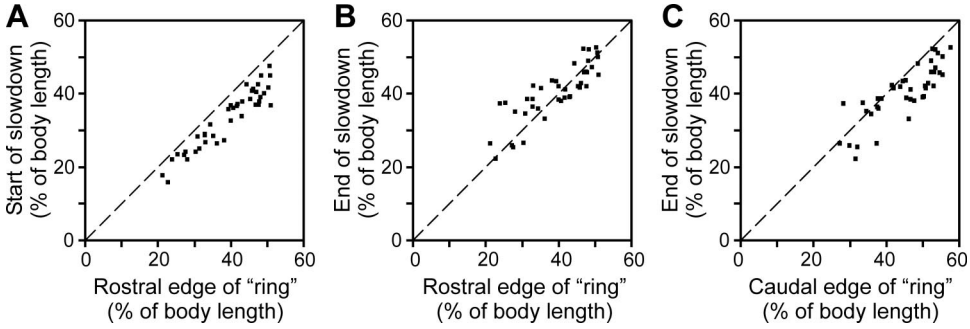

FIG. 4. Correlation of position of the stimulus and position of the start/end of the locomotor wave slowdown during SFS. The zero curvature point was tracked. *A*: start of slowdown vs. the rostral edge of the stimulus. *B*: end of slowdown vs. the rostral edge of the stimulus. *C*: end of slowdown vs. the caudal edge of the stimulus.

tion of the stimulus (its caudal and rostral edges) and the points of the body where the wave decelerated and accelerated. The wave slowed down near the rostral edge of the stimulus (Fig. 4*A*), that is, when the point of maximal flexion appeared close to the stimulus site. The wave then sped up when the zero curvature point arrived at the site of stimulation (Fig. 4, *B* and *C*), that is, when the point of maximal flexion had passed the stimulus site. Figure 5, *A–C* presents distributions of the wave speeds in different episodes before, during, and after slowdown. Rostrally to the stimulus site, the wave propagated with the average speed similar to that seen during SBS and much lower than that during FFS (Fig. 5*A*). During the slowdown near the stimulus, the wave speed decreased even more, twofold on average (Fig. 5*B*). After the slowdown, the wave speed increased several-fold (Fig. 5*C*). The distributions of the calculated wavelengths (the product of the wave speed × the cycle duration) for the three speed categories are presented in Fig. 5, *D–F*. Before the slowdown, the average wavelength (shown by the gray arrow) was approximately equal to the body length, like during FFS and SBS (shown by white and black arrows, respectively; Fig. 5*D*). During the slowdown, the wavelength decreased twofold on average, sometimes being as short as 5% of body length (Fig. 5*E*). After the slowdown, the wavelength was very variable; on average it was about twofold longer than that during FFS and SBS.

The ranges of the cycle duration for SFS and FFS slightly overlapped (Table 1). However, the average cycle duration during SFS was about threefold longer than that during FFS. Thus in this study, a movement pattern was called SFS only if: *1*) the cycle duration was >0.5 s (a typical cycle duration for FFS), and *2*) the propagation of the locomotor wave along the body was uneven, with a slowdown of the wave and a marked increase of body curvature near the stimulus.

Muscle activity underlying slow forward swimming

The EMG activity during SFS was closely correlated with the kinematics. During an episode illustrated in Fig. 6, the stimulus was sliding along the body. The EMGs recorded in

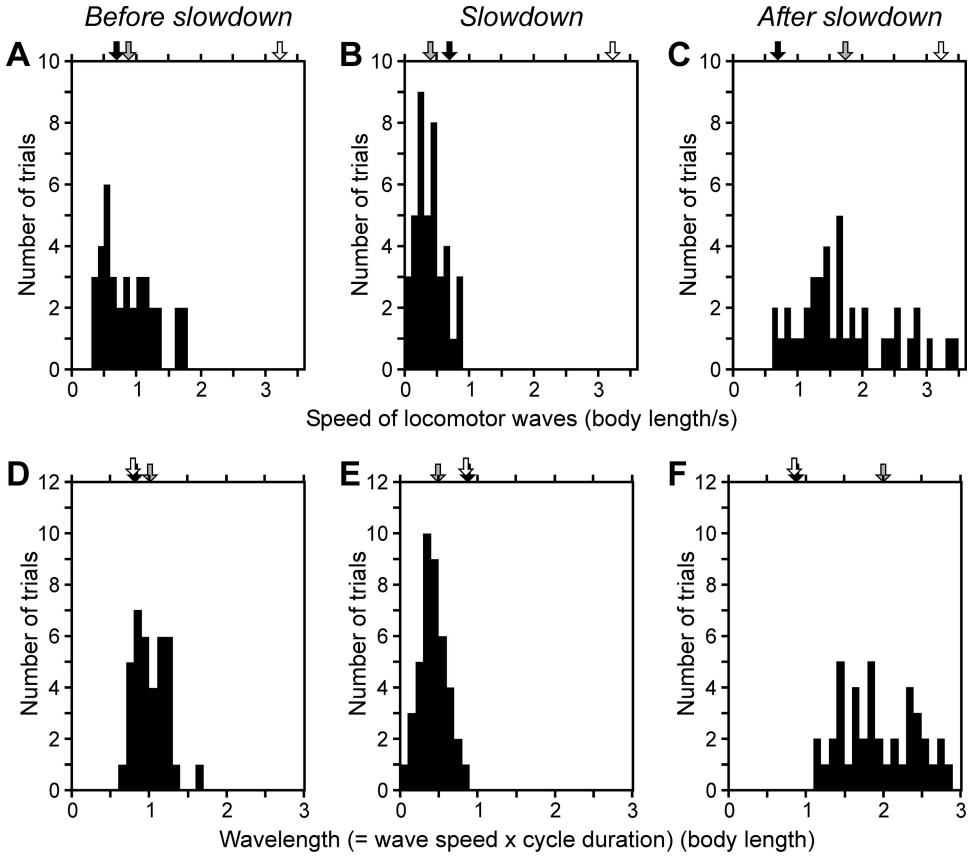

FIG. 5. Speed of locomotor waves (*A–C*) and calculated length of the locomotor waves (*D–F*) in different parts of the body: before the wave slowdown (*A*, *D*), during the slowdown (*B*, *E*), and after the slowdown (*C*, *F*). In each graph, the mean values for FFS, SFS, and SBS are indicated, respectively, with white, gray, and black arrows.

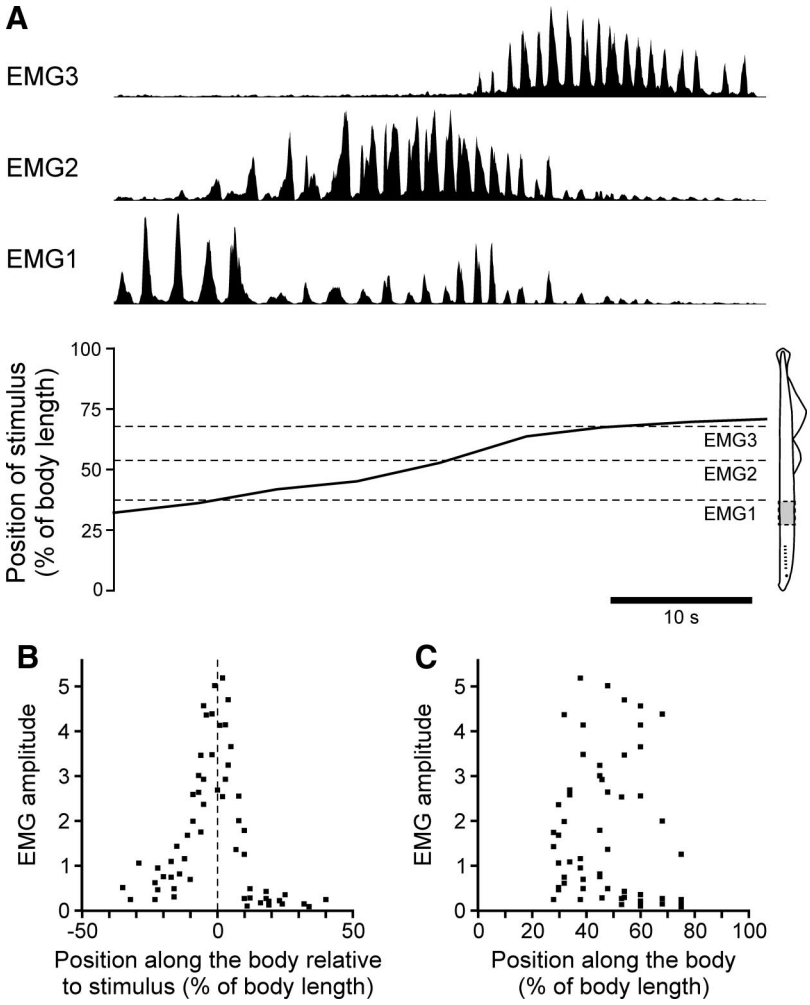

FIG. 6. *A*: correlation of EMG amplitude and the stimulus position. The EMGs were recorded on one side of the body at 3 rostrocaudal levels (EMG1, EMG2, EMG3). The stimulating ring (shown by a gray rectangle on the schematic drawing of a lamprey) was slowly sliding along the body during the swim episode. The current position of the middle of the ring and the EMG signals are presented as a function of time. Note that the amplitude of the EMG is high when the stimulus is close to the site of recording but low or even absent when the stimulus is far. *B* and *C*: EMG amplitude during SFS normalized to EMG amplitude during FFS at the same recording sites, plotted against the recording site position. In *B*, the recording site position is measured in relation to the stimulus. In *C*, the absolute position of the recording site along the body is used. Note that EMG amplitude close to the stimulus site is higher than when it is farther away.

three sites along the body (EMG1, EMG2, and EMG3) showed a considerable increase in amplitude when the stimulus was near the site of recording and a decrease when the stimulus was farther away. Such increase in muscle activity near the stimulus site and decrease as it moved farther away was seen in all 16 episodes of SFS in four animals in which EMGs were recorded at three to four different rostrocaudal levels and the stimulus was positioned near different recording sites. Figure 6*B* shows the EMG amplitude in these episodes during SFS normalized to the EMG amplitude during FFS recorded at the same sites. The electrode position was measured relative to the stimulus position. The EMG amplitude was very high within 10% of body length from the stimulus (2.95 $\pm$ 1.28, mean $\pm$ SD), it was lower rostral to this region (0.78 $\pm$ 0.42), and much lower caudal to it (0.23 $\pm$ 0.12; all three means were significantly different, *t*-test with $P < 0.01$). The difference in the EMG amplitudes was not due to a systematic difference in the quality of recording at the sites because the amplitude at the same site varied widely depending on the stimulus position. This is illustrated in Fig. 6*C* where the EMG amplitude is plotted against the absolute position along the body instead of the position relative to the stimulus. Because of this apparent predominance of the muscle activity near the stimulus site, we focused on the correlation of the EMGs and movements in this region. A representative recording of four EMGs during SFS is shown in Fig. 7. The electrodes were positioned bilaterally in the midbody area at two rostrocaudal levels. A periodic bursting pattern is seen in each EMG, with a 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. The body curvature (at two sites along the body) is presented along with the EMGs recorded from the same sites. At each site, the EMG burst lasted throughout the transition from the maximal contralateral flexion to the maximal ipsilateral flexion, as well as during the initial part of the period of ipsilateral flexion. Thus the active muscles initially flexed the body ipsilaterally and then kept it in the flexed state. The same relation between body flexion and the EMGs was seen in all episodes of SFS. This relation was similar to that observed during SBS (Islam et al. 2006a).

Effects of denervation and spinal lesions on slow forward and backward swimming

We used transections of different afferent nerves and partial lesions of the spinal cord to determine the neural pathways critical for initiation of SBS and SFS. We focused on the ability of an animal with lesions to display slow swimming when the relevant receptive field was stimulated. The criterion of SBS was propagation of the locomotor wave from the tail to the head. The criteria of SFS were low frequency of the locomotor movements (in the range observed for intact ani-

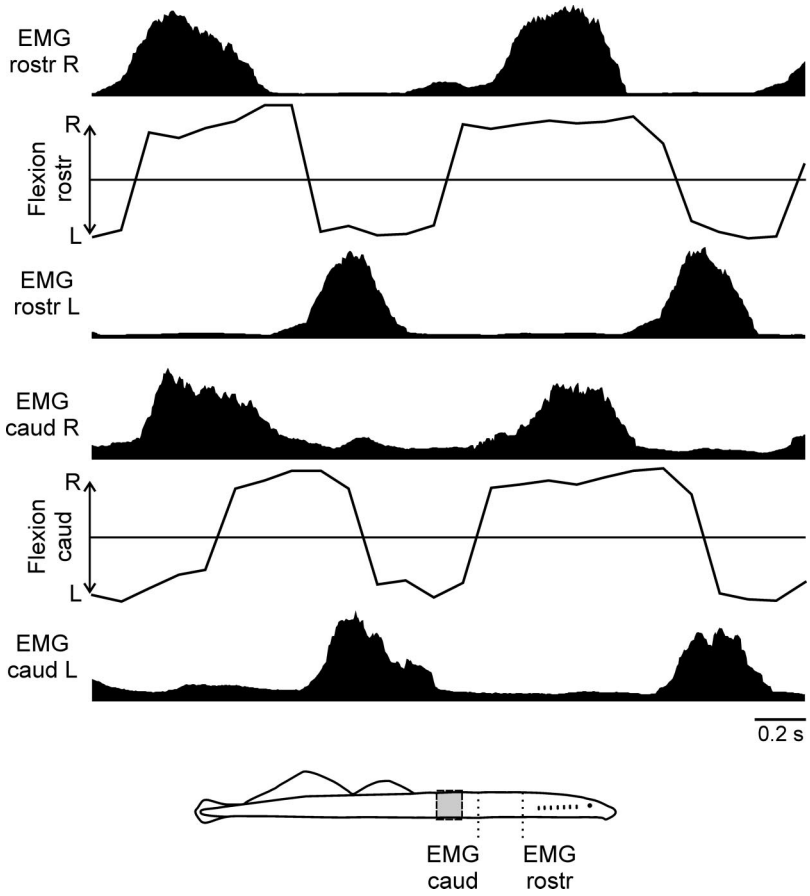

FIG. 7. Correlation of body kinematics and muscular activity during SFS. The EMGs were recorded during a period of about 2 cycles, from the left (L) and right (R) sides at the rostral (EMG rost, segment 20) and caudal (EMG caud, segment 30) levels (indicated in the schematic drawing of a lamprey; the stimulus position at 40% of body length is shown with a gray rectangle). For the same period, a body midline curvature (arbitrary units) at the sites of EMG recording is presented as a function of time.

mals) and uneven propagation of the locomotor wave along the body, with slowdown near the site of stimulation. The quantitative differences in kinematics between intact and lesion animals were ignored.

In three animals, the dorsal and ventral roots on both sides of the body were transected rostral to the dorsal fins, so that the denervated region was 3.5–4 cm long (Fig. 8*A*). In these animals, the tactile stimulus placed in the denervated region did not evoke SFS. Swimming of these animals did not differ from the normal FFS. On the basis of these experiments we suggest that the tactile information transmitted through the dorsal roots is critical for the induction of SFS. An alternative hypothesis is that, because the ventral roots were also lesioned in these experiments, the SFS motor pattern was prevented from being expressed. In other words, movements of the stimulated part of the body are suggested to be of critical importance for all characteristics of SFS: long cycle duration, uneven wave propagation, and strongly modified EMG pattern in the other, still innervated, parts of the body. This hypothesis seems less plausible.

In four animals, transection of dorsal and ventral roots was done bilaterally in the gill region (Fig. 8*B*). In these animals, tactile stimuli applied in this region evoked SBS as in intact animals. However, when the spinal root transection was combined with bilateral transection of the posterior branch of the lateral line nerve (at the first gill level; Fig. 8*B*) SBS could not be evoked ($n = 4$). Transection of the lateral line nerves alone did not prevent SBS ($n = 3$).

In another set of experiments ($n = 3$), the trigeminal nerves were cut bilaterally near the brain stem. Continuous tactile stimulation of the head by a ring placed on the head in these animals did not evoke SBS. The same stimulation applied before transection of the trigeminal nerves evoked SBS. The ability to swim backward was not impaired by the transection, however, since tactile stimulation of the gill region evoked normal SBS. Taken together, these experiments have shown that tactile information necessary for induction of SBS comes via a number of routes: the dorsal roots, the posterior branches of the lateral line nerves, and the trigeminal nerves. They also show that sensory information comes to the brain stem, which, in turn, sends the command for SBS to the spinal cord.

In four animals, the spinal cord was transected at the level of the first gill. The tactile stimuli were applied to the SBS and SFS receptive fields but did not evoke any movements or EMG activity. In those situations, we tried to provide tonic activation to the spinal networks and to facilitate the SBS and SFS by pinching the tail, although without success. It is likely that ascending sensory signals to and descending activation commands from the brain are necessary for elicitation of SFS and SBS.

To localize spinal pathways critical for SFS and SBS, partial transections of the spinal cord were done at the level of the second gill. The lesions were limited to the medial pathways ($n = 5$), lateral pathways on both sides ($n = 4$), or all pathways on one side of the spinal cord ($n = 7$) (Fig. 8*C*). None of these lesions abolished the ability of the animals for slow swimming. Tactile stimulation of the corresponding receptive fields evoked SFS or SBS, with the pattern similar to that in intact animals (in terms of the cycle duration, the speed of progression, the amplitude of head and tail excursions, and the speed

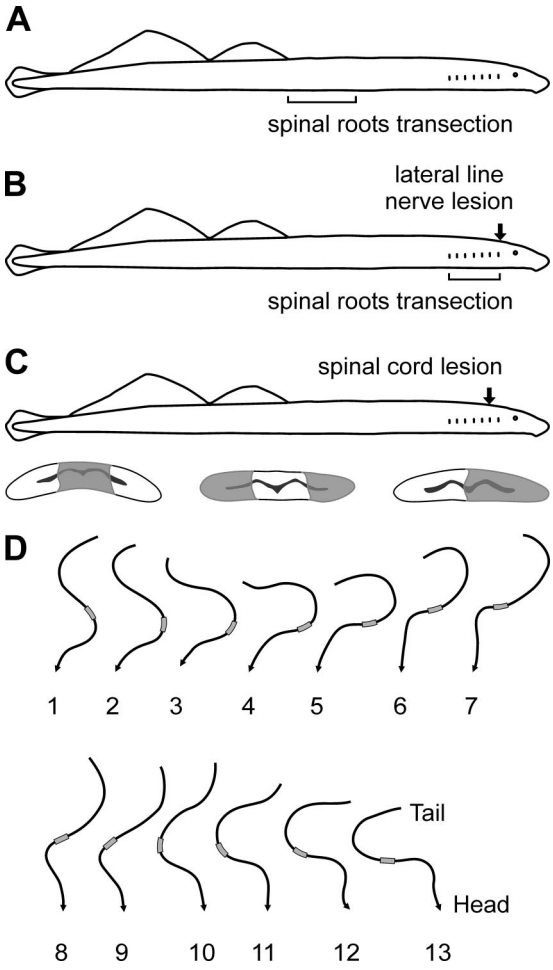

FIG. 8. Lesion experiments. *A*: area of the spinal roots transection (in the region of receptive field for SFS). *B*: the site of the lateral line nerve transection, and the area of the spinal roots transection (in the area of receptive field for SBS). *C*: the site of the spinal cord lesions and minimal across all tested animals extent of the lesions: medial lesion, bilateral lesion, unilateral hemisection. Gray matter is indicated. Lesioned area is shaded. *D*: an example of SFS after the left hemisection of the spinal cord. Thirteen sequential frames are shown (200-ms time intervals). An arrowhead indicates the head position. The stimulus position at 40% of the body length is shown with a gray rectangle. Note the similarity of the SFS movements after the hemisection and those in the intact animal (Fig. 2*B*).

of the wave propagation along the body). An example of SFS in the animal with the left hemisection of the spinal cord is shown in Fig. 8*D*. The cycle duration was about 1.0 s. The head and tail excursions were large. Note also the slowdown of the locomotor wave and strong flexion near the site of stimulation (Fig. 8*D*, *frames 6–10*). These movements look very similar to those of intact animals (Fig. 2*B*). The ability of the animals with spinal cord lesions to display both SFS and SBS suggests that the pathways involved in both types of slow swimming are dispersed across the spinal cord.

Lack of spatial orientation during slow forward swimming

During FFS, the lamprey is typically oriented with its dorsal side up due to the vestibular-driven postural system (see e.g., Deliagina 1997; Deliagina et al. 1992; Ullén et al. 1995). During SBS, the lamprey displays no control of its orientation (Islam et al. 2006a). Similarly, no control of spatial orientation was observed during SFS. In a shallow aquarium (Fig. 1*B*), the lamprey swam with either the dorsal side up or the ventral side up and did not try to change this orientation. In a deep aquarium (Fig. 1*C*), when the lamprey swam without touching the walls, it continuously and chaotically changed its orientation. An example of SFS in deep water is shown in Fig. 9. The video recording of the side and top views of the animal (Fig. 9*A*, the same period is indicated in Fig. 9, *B* and *C* with a black bar) allowed us to estimate the position of the maximal body flexion (Fig. 9*B*) and the roll tilt angle of the animal (Fig. 9*C*). We found that the characteristics of swimming were typical for SFS: the cycle duration was long, the body curvature was large (Fig. 9*A*), and the waves of body flexion slowed down near the site of stimulation (indicated in Fig. 9*B* by the gray band). During this episode of SFS, the roll angle was continuously changing, and no preferred orientation in the roll plane was observed (Fig. 9*C*).

Altogether we tested the ability to stabilize spatial orientation during SFS in three animals (32 trials). The stimulus was positioned at different rostrocaudal levels within the SFS receptive field. In 27 trials, the animals could not keep themselves afloat and sank to the bottom within 2–3 s. Of the 5 episodes when the animal stayed in the middle of water without touching the aquarium walls for ≥4 s (that corresponded to about three swimming cycles), the stimulus was either between the dorsal fins (2 trials; one of the longest episodes is illustrated in Fig. 9) or in front of the dorsal fins (3 trials). In all 32 episodes, the animal's orientation was constantly changing and no preferred orientation was observed. It did not matter whether the animals were in the middle of water or touching the bottom.

DISCUSSION

Comparison of different forms of undulatory locomotion in the lamprey

Herein, we describe one of the forms of undulatory movements observed in the lamprey. When these movements are performed in free water, they allow the animal to move slowly forward. This is why we term this motor behavior "slow forward swimming" (SFS), although the lamprey can generate the same motor pattern when it is attached to the bottom with its sucker mouth.

We have found that SFS has some important features in common with the slow backward swimming described earlier (Islam et al. 2006a). In both forms of undulatory swimming, the SFS and SBS, the cycle duration was similar and, on average, was much longer than the cycle duration of FFS (Table 1). In addition, both SFS and SBS had a lower speed of progression and larger amplitude of head excursions compared with those of FFS. Also, SFS had two- to threefold larger amplitude of tail excursions. Another characteristic feature of both modes of slow swimming was the lack of stabilization of the body orientation in space, which contrasted to the very efficient stabilization of orientation during FFS (Ullén et al. 1995; Zelenin et al. 2003).

Taken together, these characteristic features of SFS and SBS suggest that the two forms of motor behavior are not effective for long-distance migrations. Instead, they can be used for escape and withdrawal behavior, respectively. This corre-

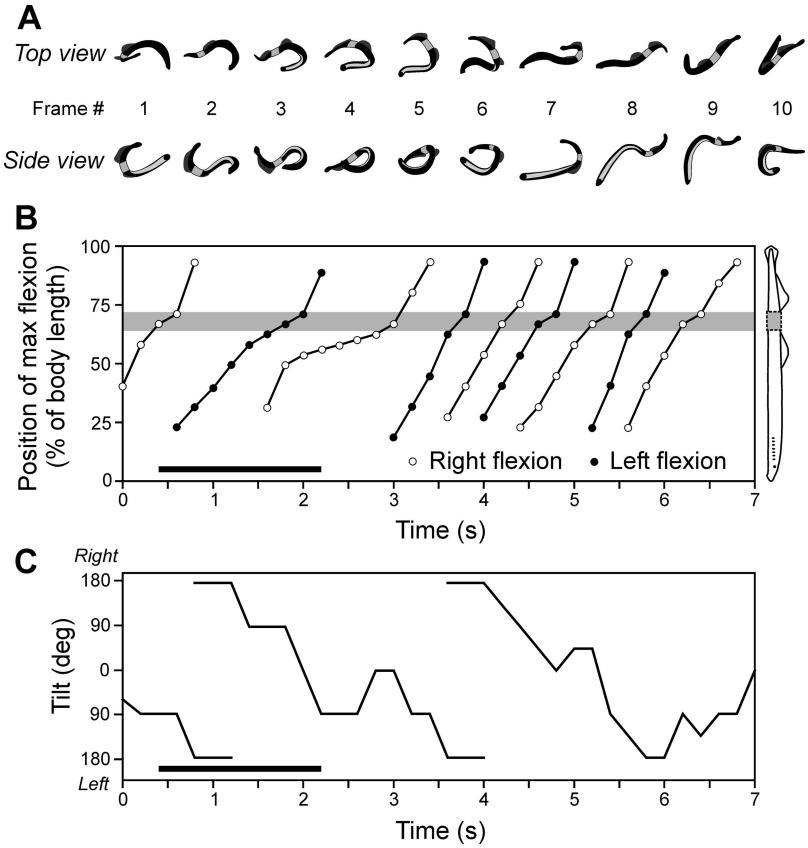

FIG. 9. Absence of spatial orientation during backward swimming revealed in a deep aquarium. *A*: body configuration in 10 sequential frames during a period of 2 s (*top* and *side views*). *B*: position of the maximal lateral flexion in 35 sequential frames. Time intervals between the frames are 200 ms. *C*: roll tilt angle in these frames. In *B* and *C*, a horizontal line indicates the period shown in *A*. Stimulus position at 65% of body length is indicated with the gray rectangle.

sponds well to the location of the receptive fields from which one can evoke SBS or SFS. The lamprey exhibits SBS if something threatening is encountered during forward progression, or if the rostral part of the body (the head and gill region) is trapped in some way. If the animal were held in the caudal part, where the body tapers off (in the area of the caudal dorsal fin and tail), just an increase in intensity of ordinary forward swimming (FFS) would be effective to get out. In contrast, if the animal is held in the middle part of the body (between the gill region and the caudal dorsal fin), the SBS and FFS may be ineffective, and a special motor pattern, SFS, would likely be used.

The most prominent features of SFS—that is, the slowdown of the locomotor waves and sharp bending of the body near the rostral edge of the stimulus site—can be interpreted as an attempt to use the stimulating (holding) object as a support for pulling the body out of the hold, using the rostral part of the body as a lever. If the attempt with flexion to one side fails, the next SFS wave with flexion to the other side is generated.

Should the pulling be effective and the body can slide forward in relation to the stimulus, the stimulus would then slide backward along the body. In this case the site of the body, where the characteristics of locomotor pattern are modified, must follow the stimulus. This can be done by a command from the brain addressed specifically to the stimulated body region. Alternatively, the descending command could be addressed to the whole spinal cord. This command could then modify properties of the spinal networks so that they respond specifically to the local tactile stimulation, with a local decrease of the wave speed and an increase of muscle activity, resulting in a strong local body flexion.

SFS local modifications of the motor pattern near the stimulus may be mediated by the 5-HT system. This system in the spinal cord is known to cause intense efferent bursts during fictive locomotion, with much longer burst duration, lower frequency of bursts, and with a reduced wavelength (Harris-Warrick and Cohen 1985; Wallén et al. 1989). 5-HT fibers in the dorsal roots of the lamprey spinal cord have been revealed immunohistochemically (Harris-Warrick et al. 1985; Van Dongen et al. 1985). These supposedly sensory 5-HT neurons are good candidates for elicitation of slow swimming.

Loss of spatial orientation control during slow swimming

During both forms of slow swimming, SBS and SFS, the lampreys did not stabilize their spatial orientation. This contrasts to the very efficient stabilization of the dorsal-side-up orientation during FFS. The orientation control during FFS seems important because it helps to camouflage an animal swimming in the water: contrast is minimized with the dark back of the animal against the dark bottom background and the pale belly against the sky background. The slow swimming is likely used to get out of tight places or to escape if an animal has been caught. Neither of the two conditions requires control of spatial orientation for successfully maneuvering free. One can also speculate that continuous chaotic changes of the orientation may help the animal to escape.

During FFS, the lamprey maintains its orientation due to vestibular postural reflexes. The vestibular information about deviations from the preferred orientation is transmitted to the reticulospinal neurons that send commands to the spinal cord for modifications of the basic locomotor pattern. These modi-

fied movements lead to torques rotating the body back to the preferred orientation. We do not know yet the reasons for the loss of the orientation control during SBS and SFS. One simple explanation may be purely mechanical. During SBS and SFS, the body movements are much slower and the torques generated by the body movements are too weak to return it to the preferred orientation. Another explanation implies changes in neural mechanisms of the orientation control. *1*) The transmission of the vestibular information to the reticulospinal command neurons can be blocked or modified. *2*) The reticulospinal neurons responsible for the spatial control may be inactivated. *3*) The postural commands may be ineffective or misinterpreted by the executing spinal networks when they are generating the SBS or SFS patterns. Any of these mechanisms alone or in combination with the others would theoretically disrupt the spatial orientation control. There is also another interesting possibility: activity of the reticulospinal neurons responsible for orientation may be chaotic during SBS and SFS due to some intrinsic brain stem generator of random commands for postural corrections with a purpose of producing movements in multiple planes to more quickly rid the animal of the stimulus.

Possible role of spinal and supraspinal mechanisms in two forms of slow swimming

In the spinal dogfish it has been shown that locomotor coordination similar to forward swimming is changed to backward swimming if the rostral cutaneous innervation field is stimulated (Grillner 1974). In the lamprey, one cannot exclude that tactile afferents directly activate the SBS generator. However, it seems more likely that this form of locomotion is evoked by specific commands coming from the brain. This is evident when SBS is evoked by stimulation of the head receptive field. Besides that, information about tactile stimuli in the gill region is transmitted through the lateral line nerves and is sufficient to evoke SBS even in a situation when the dorsal roots are cut and no sensory signals can directly reach the spinal CPG.

In SFS, the spinal mechanisms apparently play a crucial role. The most prominent modifications of the locomotor pattern are localized close to the site of stimulation no matter where the stimulus is applied. If the stimulus moves along the body during a trial, the site of the modified motor activity moves together with it. With no tactile stimulus applied, the wave speed, the wavelength, the body curvature, and the EMG intensity were always uniform along the body, and no distortions of the uniformity (typical for SFS) were observed. However, we think that the spinal reflexes cannot solely account for the whole pattern of SFS. First, the change of the cycle duration is difficult to explain by the spinal reflexes. Second, spontaneous switching between SFS and FFS, with unchanged stimulation, suggests that the stimulus alone does not completely determine the mode of locomotion. One may suppose that SFS is evoked by direct afferent input to the spinal networks, whereas the FFS is evoked by the descending command from the brain stem that overrides all other influences on CPG. However, a more plausible hypothesis is that tactile information from the SFS receptive field is sent to the brain, where a descending command is generated. This command could affect the spinal network and induce SFS with long cycle duration; it may also modify the sensitivity of the neuronal networks to tactile inputs so that the wave speed, wavelength, and the efferent activity are strongly changed locally in the vicinity of the stimulus site.

We do not yet know which groups of descending neurons are responsible for elicitation of locomotion. We performed partial lesions of the spinal cord (bilateral, medial, and unilateral) in an attempt to localize the pathways responsible for different modes of locomotion. However, these lesions did not prevent SBS or SFS. This means that the pathways are dispersed over the cross section of the spinal cord. In the lamprey, the main descending system transmitting commands to all regions of the spinal cord is the reticulospinal (RS) system. One may expect that specific populations of RS neurons are responsible for elicitation of FFS, SFS, and SBS. Do these populations overlap, and to what extent? Answers to these questions constitute the aim of future studies.

To conclude, the slow forward swimming in the lamprey differs from other forms of undulatory swimming in that the locomotor waves during SFS propagate unevenly along the body. This phenomenon seems to result from the local changes in the spinal network induced by external stimuli. Such modifications of the locomotor pattern allow the lamprey to better adapt to environmental conditions. This work complements a previous one (Islam et al. 2006a) and provides us with the data necessary for the analysis of reconfigurations of the spinal generator network underlying different locomotor patterns, as well as for revealing the specific commands responsible for initiation of these patterns.

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