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Different forms of locomotion in the spinal lamprey

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Abstract

Forward locomotion has been extensively studied in different vertebrate animals, and the principal role of spinal mechanisms in the generation of this form of locomotion has been demonstrated. Vertebrate animals, however, are capable of other forms of locomotion, such as backward walking and swimming, sideward walking, and crawling. Do the spinal mechanisms play a principal role in the generation of these forms of locomotion? We addressed this question in lampreys, which are capable of five different forms of locomotion – fast forward swimming, slow forward swimming, backward swimming, forward crawling, and backward crawling. To induce locomotion in lampreys spinalised at the second gill level, we used either electrical stimulation of the spinal cord at different rostrocaudal levels, or tactile stimulation of specific cutaneous receptive fields from which a given form of locomotion could be evoked in intact lampreys. We found that any of the five forms of locomotion could be evoked in the spinal lamprey by electrical stimulation of the spinal cord, and some of them by tactile stimulation. These results suggest that spinal mechanisms in the lamprey, in the absence of phasic supraspinal commands, are capable of generating the basic pattern for all five forms of locomotion observed in intact lampreys. In spinal lampreys, the direction of swimming did not depend on the site of spinal cord stimulation, but on the stimulation strength. The direction of crawling strongly depended on the body configuration. The spinal structures presumably activated by spinal cord stimulation and causing different forms of locomotion are discussed.

Introduction

Forward locomotion has been extensively studied in different vertebrate animals. It has been found that the general organisation of the locomotor control system has many features in common. The basic motor pattern underlying locomotion is generated by spinal networks, whereas supraspinal motor centers make this pattern behaviorally relevant: they activate the spinal locomotor networks, regulate the level of their activity, adapt locomotor movements to the external conditions, maintain a specific body configuration during locomotion, keep balance, etc. [for reviews, see Orlovsky *et al.* (1999) and Grillner (1975)].

The principal role of spinal mechanisms in the generation of forward locomotion was revealed in experiments on spinal animals, in which well-coordinated forward locomotor movements could be evoked by stimulation of the spinal cord or by sensory inputs (Brown, 1911; Rovainen, 1976; McClellan & Grillner, 1983; Wallén & Williams, 1984; Rossignol *et al.*, 1989; Ichiyama *et al.*, 2005; Musienko *et al.*, 2007).

Most vertebrates, however, are capable of other forms of locomotion, such as backward walking and swimming, sideward walking, and forward and backward crawling (Buford *et al.*, 1990; Ashley-Ross & Lauder, 1997; Archambault *et al.*, 2001). Do the spinal mechanisms play the principal role in the generation of these forms of locomotion?

To answer this question, we used a lower vertebrate animal, the lamprey, with a relatively simple nervous system and a rich behavioral repertoire. For this animal, five forms of locomotion have been described – fast forward swimming (FFS), slow forward swimming (SFS), backward swimming (BS), forward crawling (FC), and backward crawling (BC). FFS is the main form of undulatory locomotion. During FFS, the waves of body undulations propagate from the head to the tail (Williams *et al.*, 1989). SFS and BS are also undulatory forms of locomotion, with slow backward and forward wave propagation, respectively. They represent a type of struggling behavior. BS and SFS are observed when the lamprey tries to get rid of the continuous tactile stimulation of the rostral or middle part of the body, respectively (Islam *et al.*, 2006; Islam & Zelenin, 2008). Crawling is a form of non-undulatory locomotion, and is used by the lamprey for moving about or out of tight places, where undulatory movements are not possible (Rovainen, 1976). Crawling is produced by a solitary wave of co-contraction of the left and right muscles close to the bent site (Archambault *et al.*, 2001).

It has been shown that two forms of forward locomotion (FFS and FC) can be generated in the spinal lamprey (Rovainen, 1976; McClellan & Grillner, 1983). The aim of our study was to investigate whether the spinal lamprey can produce all other forms of locomotion. With this purpose, we studied the motor effects of electrical stimulation of different sites in the spinal cord, as well as tactile stimulation of different cutaneous receptive fields. We found that all five forms of locomotion could be evoked in the spinal lamprey, and we discuss possible mechanisms for their elicitation.

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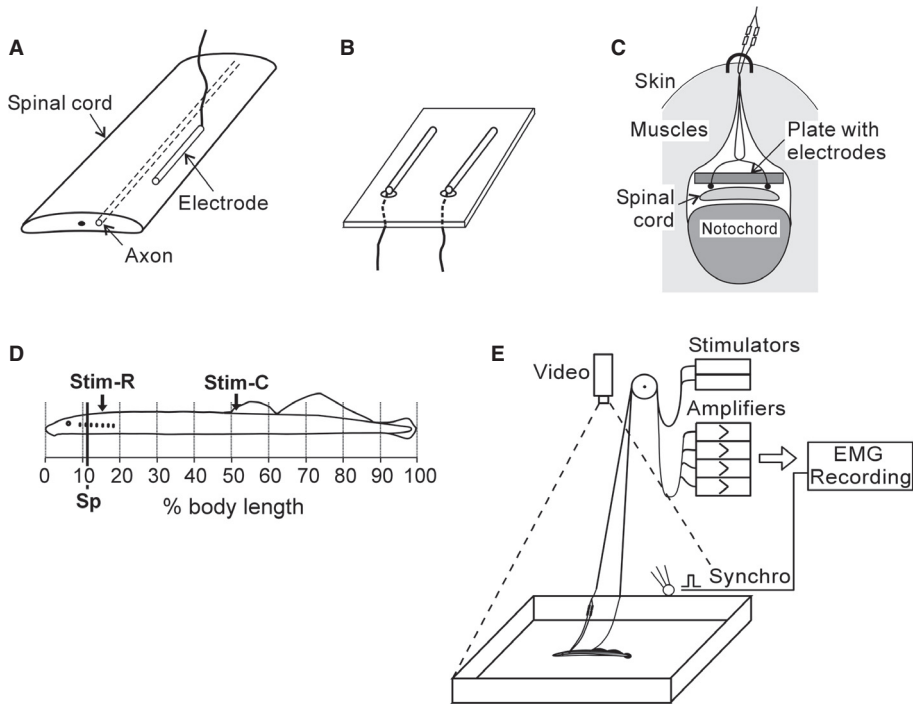

FIG. 1. Experiemental design. (A) Electrical stimulation of the spinal cord was applied through macroelectrodes orientated in parallel with the long spinal axons. (B) Two electrodes were glued to a plastic plate (view from below). (C) The plate was positioned on the dorsal surface of the spinal cord, symmetrically relative to the midline. (D) The electrodes were implanted at different rostrocaudal levels (Stim-R and Stim-C) in lampreys spinalised at the level of the second gill (Sp). (E) Experimental setup for simultaneous recording of kinematic and EMG data. Video and EMG recordings were synchronised by light and electrical pulses recorded simultaneously by both systems (Synchro).

A brief account of parts of this study has been published in abstract form (Hsu *et al.*, 2012).

Materials and methods

Experiments were carried out on 18 adult (length, 25–35 cm) lampreys (*Lampetra fluviatilis*), which were kept in an aerated freshwater aquarium at 7 °C, with a 12-h : 12-h light/dark cycle. All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden).

Electrodes

Stimulation of the spinal cord was performed by means of chronically implanted macroelectrodes similar to those used for recording of reticulospinal (RS) axons in the spinal cord and described previously (Deliagina & Fagerstedt, 2000; Deliagina *et al.*, 2000). In short, the electrodes (silver wires: diameter, 75 μm; length, 2.5 mm) were positioned on the dorsal surface of the spinal cord in parallel with the long spinal axons (Fig. 1A), which are mainly the axons of RS and propriospinal neurons. Two such electrodes (resistance, $<10^3$ Ω) were glued to a plastic plate (length, 3.5 mm; width, 2.5 mm; thickness, 0.25 mm; Fig. 1B). The distance between the electrodes was ~1.5 mm. In some lampreys, a plate with four electrodes separated by 0.5 mm was used, in order to compare the motor effects evoked by stimulation of medial and lateral parts of the spinal cord.

Surgery

Lampreys were operated on under MS-222 (Sandoz, Sigma, St. Louis, MO, USA) anesthesia (100 mg/L). First, they were spinalised at the second gill level, which corresponds to the level of 12% body length (Fig. 1D). Second, implantation of stimulating electrodes was performed as described previously (Deliagina & Fagerstedt, 2000). One or two plates with two or with four electrodes were implanted at different rostrocaudal levels, either rostrally (approximately at the level of 16% of body length), or caudally (approximately at the level of 53% of body length), or at both levels (Fig. 1D). The electrodes faced the dorsal aspect of the spinal cord (Fig. 1C). In some lampreys, bipolar electromyography (EMG) electrodes were implanted (unilaterally or bilaterally) in the body muscles at different rostrocaudal levels. Each lamprey was tested after full recovery from anesthesia (~3 h) and then up to 7 days (usually within 2 days) after surgery. At the end of the experimental series, the lampreys were killed with an overdose of MS-222.

Experimental design

In the experiments with elicitation of different forms of swimming, the lamprey was positioned in an aquarium of size 80 × 80 × 10 cm (Fig. 1E). In the experiments with elicitation of crawling, the lamprey was positioned in a U-shaped plexiglas tunnel (Fig. 9A). The water temperature was maintained at 5–7 °C. The electrodes for stimulation of the spinal cord and for EMG recording were connected via a long flexible cable to outputs of stimulators and inputs of AC amplifiers, respectively.

To evoke different forms of locomotion, two types of stimulation were used: electrical stimulation of the spinal cord, and tactile stimulation of the skin. For the spinal cord electrical stimulation, continuous monopolar stimulation through one or two electrodes was used (frequency, 3–10 Hz; pulse duration, 2 ms; current, 20–1000 μA; train duration, 10–120 s). This efficiently evoked locomotion for up to 7 days after spinalisation (the longest post-surgery time tested). Two types of tactile stimulation were employed: (i) continuous pressure over a large skin area at different rostrocaudal levels was

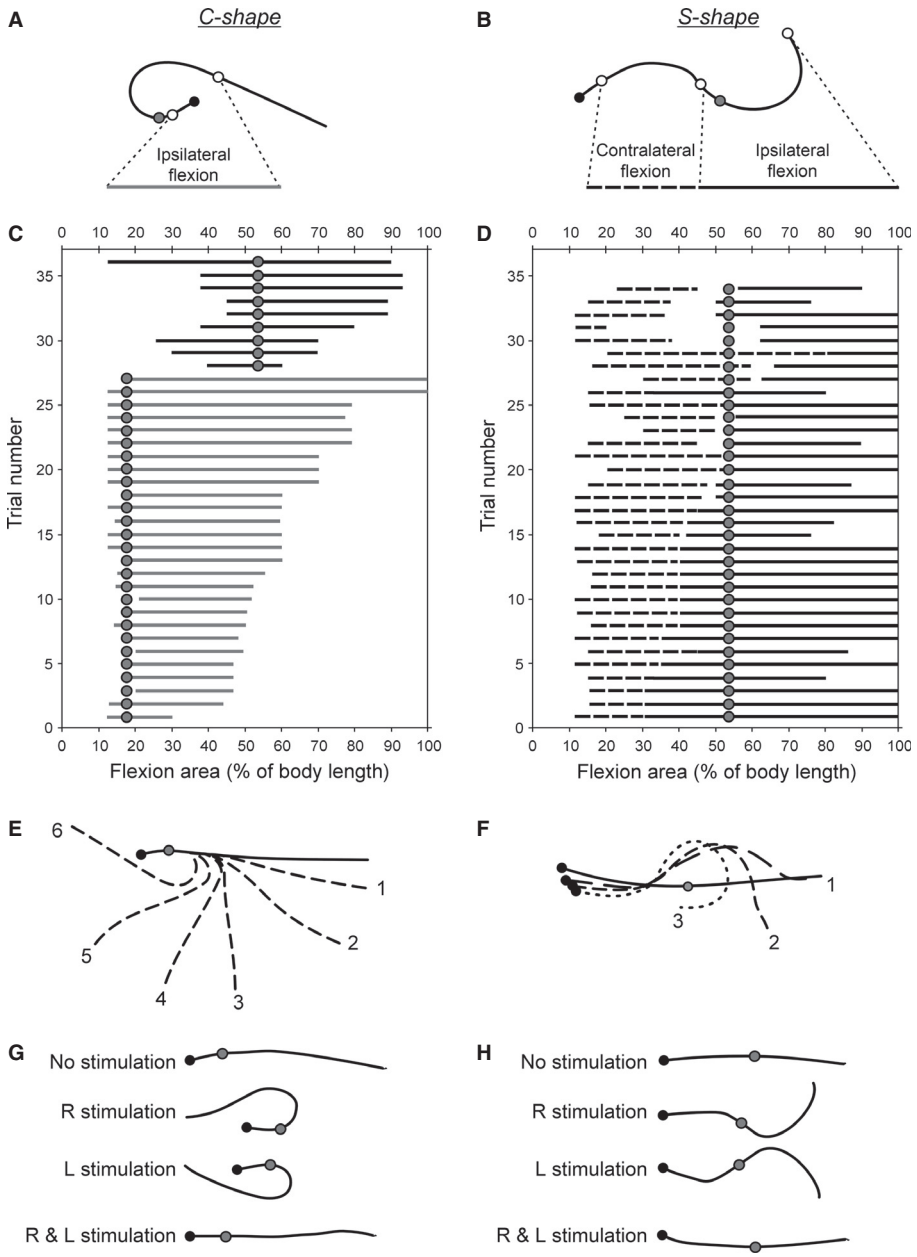

FIG. 2. Effects of unilateral electrical stimulation of the spinal cord. (A and B) Examples of the C-shape (A) and S-shape (B) of the body (estimated according to the body midline) caused by stimulation through the left rostral electrode and through the right caudal electrode, respectively. White circles indicate the reference points (see Materials and methods) used to determine the area of body flexion. Gray circles indicate stimulation sites. The filled black circle at the top of the body midline indicates the position of the sucker mouth. (C and D) The areas of body flexion of C-shape (C) and S-shape (D) in individual trials (number of tested lampreys, $N = 9$). Areas of ipsilateral and contralateral flexion (in relation to the stimulating electrode) are indicated by solid and broken lines, respectively. The trials with rostral and caudal stimulation are presented with light and dark gray lines, respectively. (E and F) Effect of a gradual increase in stimulation intensity through the rostral (E) and caudal (F) left electrodes on body shape. Solid and interrupted lines indicate the body midline shape before and during stimulation, respectively. The numbers next to the broken lines indicate a gradual increase in the stimulation strength. (G and H) Effects on the body shape produced by unilateral (through the left electrode, L stimulation; through the right electrode, R stimulation) and bilateral (R & L stimulation) stimulation of the spinal cord through the rostral (G) and caudal (H) electrodes.

applied by means of a pressure clip (e.g. a hair claw, 5 cm in length); and (ii) local tactile stimulation was applied by means of a pair of forceps. Pauses between sequential tests lasted for at least 3 min. Repetitive stimulation was efficient in evoking locomotion if applied after a rest period of 3–5 min.

Movements of the lamprey were recorded from above with a video camera (25 frames/s), positioned at a distance of 2 m from the aquarium, and later analysed frame by frame. The EMG signals were amplified and stored on a computer. The EMG and video recordings were synchronised by pulses recorded simultaneously with both systems (Fig. 1E).

Data processing

Characteristics of the swim motor pattern were defined as in previous studies (e.g. Williams *et al.*, 1989). The positions of the point of zero curvature were estimated from video images. As reference points, we used the points of zero curvature, the point of spinalisation (12% of

TABLE 1. Stimulation strength used to evoke individual forms of locomotion in the spinal lamprey

Locomotion	Spinal-R	Spinal-C
FFS	76 ± 14 (30–350) (n = 27, N = 7)	79 ± 13 (30–200) (n = 17, N = 8)
BS	292 ± 30 (40–610) (n = 34, N = 8)*	475 ± 43 (20–950) (n = 30, N = 11)*
SFS	254 ± 84 (40–600) (n = 3, N = 3)*	413 ± 80 (40–600) (n = 4, N = 3)*
FC	805 ± 78 (610–1000) (n = 2, N = 2)	325 ± 44 (70–600) (n = 14, N = 4)
BC	884 ± 78 (610–1000) (n = 5, N = 2)	350 ± 51 (100–630) (n = 9, N = 4)

Values are presented as mean ± standard errors (μA), with range in parentheses. n = number of analysed trials; N = number of tested animals. Spinal-R and Spinal-C, locomotion evoked by stimulation of the rostral and caudal parts, respectively, of the spinal cord in spinal lampreys. *Statistically significant difference between the corresponding values during FFS and those during BS and SFS ($P < 0.05$).

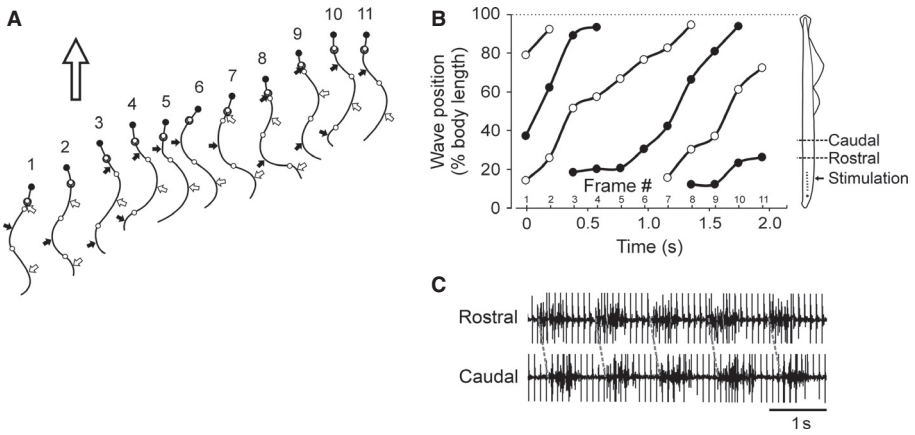

FIG. 3. Examples of FFS evoked by bilateral stimulation of the rostral part of the spinal cord. (A) Shape of the body midline in 11 sequential frames (1–11). The large arrow shows the direction of the whole body progression. Points of curvature are indicated, with convexity on the left side (black arrows) and on the right side (white arrows). The white, gray, and black circles indicate the same characteristic points as in Fig. 2. (B) Positions of left convexity (black circles) and right convexity (white circles) as a function of time (of frame number) for the same swim episode as in A. (C) An example of EMG recordings obtained from the right muscles during FFS. Interrupted lines show rostrocaudal phase lags. The levels of EMG recording (caudal and rostral) and the position of the stimulating electrode (Stim) are shown in B.

body length), and the tip of the tail (100% of body length). A midpoint between two neighboring reference points was taken as a point of either convex or concave curvature. The time between the convex and concave curvature of the same point was taken as half of the cycle duration. The speed of locomotor waves was calculated by tracking a zero curvature point along the body; the speed was expressed as percentage of body length per second. The locomotor wavelength (expressed as body length) was obtained from the speed multiplied by the cycle duration. The phase shift per segment (expressed as percentage of cycle duration) was calculated from the inverse of the wavelength. The tip of the head was tracked for calculation of the amplitude of head excursion. In the episodes of locomotor movements with an immobile head (the head being attached to the bottom with the sucker mouth), the excursion amplitude was calculated for the point at 16% of body length (approximately the position of the rostral stimulating electrode) instead.

All values are presented as means ± standard errors. Student's t-test was used to characterise the statistical significance when different means were compared; the significant level was set at $P < 0.05$. The number of tested animals (N) and the number of trials (n) are presented.

Results

Effects of unilateral stimulation of the spinal cord on body configuration

Unilateral stimulation of the spinal cord (3–10 Hz, 2 ms, 20–100 μA) evoked different patterns of body flexion, depending on the site of stimulation. Stimulation through the rostral electrode caused a C-shape bending of the body (ipsilateral flexion; Fig. 2A, C, E, and G). In most trials (17 of 27 trials, N = 9), the area of body flexion stretched from the site of stimulation (16% of body length) to the mid-body area (45–60% of body length); in nine of 27 trials, this area was extended caudally (up to 70–100% of body length) (Fig. 2C).

In most trials (34 of 43 trials, N = 9), stimulation through the caudal electrode caused an S-shaped bending of the body (contralateral flexion in the rostral part of the body and ipsilateral flexion in its caudal part; Fig. 2B, D, F, and H). In the other nine trials, the C-shape was observed (Fig. 2C, nine upper lines).

An increase in the stimulation strength caused stronger body flexion in both C-shaped and S-shaped patterns (Fig. 2E and F, respectively). In some trials (16 of 70 trials, N = 9), flexion of the body was accompanied by waves of lateral undulations propagating towards the tail.

The body flexion evoked by unilateral stimulation could be reduced and even completely abolished with bilateral stimulation (Fig. 2G and H). Bilateral stimulation was also much more efficient in evoking swimming than unilateral stimulation (see below).

Effects of bilateral stimulation of the spinal cord

In the present study, we were able to evoke, with bilateral electrical stimulation of the spinal cord, all three forms of undulatory locomotion (FFS, BS, and SFS) in the spinal lamprey if the stimulating currents applied through the left and right electrodes were properly

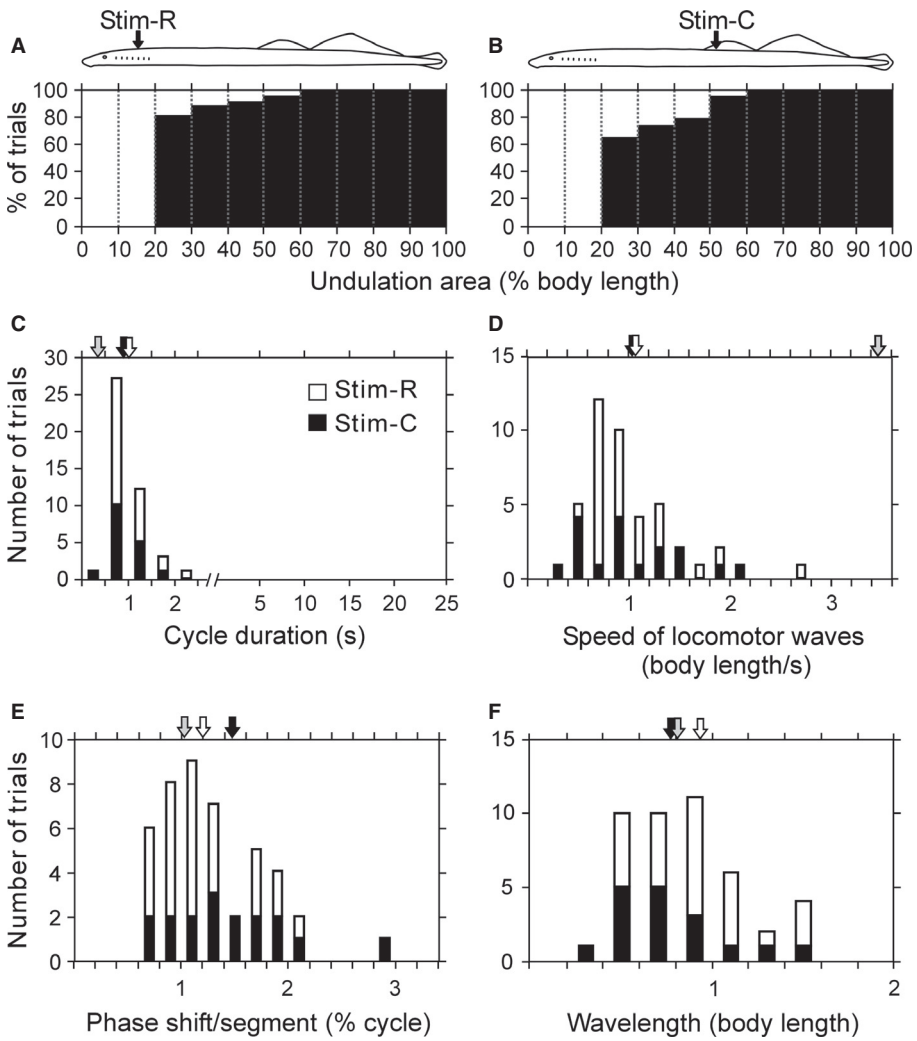

FIG. 4. FFS in spinal lampreys. (A and B) Histograms of relative numbers of trials with different undulation areas during FFS caused by stimulation of the rostral (A) and caudal (B) sites of the spinal cord. (C–F) Summary of kinematic characteristics of FFS in spinal lampreys. (C) Swim cycle duration. (D) Speed of locomotor waves. (E) Phase shift per segment. (F) Length of locomotor wave. The mean values for FFS evoked by rostral (Stim-R) and caudal (Stim-C) stimulation in spinal lampreys, and the corresponding values for intact lampreys (Islam *et al.*, 2006), are indicated by white, black and gray arrows, respectively.

adjusted (for the range of effective currents, see Table 1). A slight deviation from the optimal current values resulted in the appearance of right–left asymmetry in the swimming pattern.

FFS evoked by bilateral stimulation of the spinal cord

In the spinal lamprey, FFS could be evoked by bilateral stimulation of the spinal cord in both rostral and caudal areas. The observed pattern of FFS was basically similar to that in the intact lamprey. An example of FFS evoked by stimulation through the rostral electrode is shown in Fig. 3A. In this trial, the sucker was detached from the bottom of the aquarium, and the whole animal moved forwards, as indicated by a large arrow. Figure 3B shows the position of the left and right convexity as a function of time/frame number for the swim episode shown in Fig. 3A. The wave of body flexion propagated backward from the point at ~20% of body length to the point at 100% of body length, at a speed of ~55% body length/s. An example of an EMG recording obtained during FFS is shown in Fig. 3C. The EMG electrodes were positioned in the middle body area at two rostrocaudal levels on the same side (inset in Fig. 3B). A clear periodic bursting pattern was observed in each EMG recording. The bursts of rostral EMG activity occurred earlier than those of caudal EMG activity.

To evoke FFS, bilateral stimulation was used. On average, the effective current was 76 μA for rostral stimulation and 79 μA for caudal stimulation (Table 1). An increase in the stimulation strength resulted in increases in EMG burst amplitude and frequency, as well as an increase in FFS speed (not illustrated). In some trials (20%), FFS could outlast the stimulation, with a gradual decrease in undulation amplitude. We found no difference between FFS evoked by stimulation through the medial electrode and that evoked by stimulation through the lateral electrode.

Figure 4A and B shows the undulation area during FFS evoked by stimulation at the rostral or caudal sites. In the majority (81%) of FFS episodes evoked by rostral stimulation, the wave propagated from 20% to 100% body length (Fig. 4A). In the trials with caudal stimulation, there were fewer (66%) episodes of FFS with the same undulation area (Fig. 4B).

Figure 4C–F shows the distributions of different kinematic characteristics of FFS evoked by rostral and caudal stimulation. The

TABLE 2. Main characteristics of FFS and BS in spinal and intact lampreys

	FFS			BS		
Characteristics of swimming	Spinal-R	Spinal-C	Intact	Spinal-R	Spinal-C	Intact
Cycle duration (s)	1.01 ± 0.09* (0.52–2.12)	0.89 ± 0.09* (0.40–1.60)	0.38 ± 0.09 (0.19–0.88)	7.74 ± 0.54* (1.34–16.12)	11.06 ± 1.09* (2.00–22.76)	1.62 ± 0.10 (0.89–3.62)
Speed of wave (body length/s)	1.01 ± 0.09* (0.50–2.69)	1.02 ± 0.13* (0.33–2.14)	3.47 ± 0.64 (2.57–5.30)	0.12 ± 0.02* (0.04–0.57)	0.07 ± 0.01* (0.02–0.23)	0.68 ± 0.07 (0.30–1.80)
Phase shift per segment (% cycle)	1.20 ± 0.08 (0.65–2.05)	1.45 ± 0.13 (0.69–2.89)	1.03 ± 0.03 (0.96–1.19)	1.51 ± 0.09* (0.59–2.50)	1.90 ± 0.10* (1.25–3.24)	1.03 ± 0.02 (0.81–1.20)
Wavelength (body length)	0.92 ± 0.05 (0.49–1.54)	0.78 ± 0.07 (0.35–1.45)	0.81 ± 0.03 (0.72–0.86)	0.76 ± 0.06 (0.40–1.69)	0.56 ± 0.03* (0.31–0.80)	0.80 ± 0.01 (0.75–0.87)

Values are presented as means ± standard errors, with range in parentheses. Spinal-R and Spinal-C, locomotion evoked by stimulation of the rostral and caudal parts, respectively, of the spinal cord in spinal lampreys. *Statistically significant difference between the corresponding values in spinal and intact lampreys. Data for FFS intact and BS intact have been published previously (Islam *et al.*, 2006).

distributions were generally similar. The mean values of each parameter under the two conditions were close to each other. Comparison of these values with those in intact lampreys (Fig. 4C–F) showed that, in spinal lampreys, the cycle was 2.5 times longer and the speed of locomotor waves was 3.5 times slower than in intact lampreys. The phase shift per segment and the wavelength in spinal lampreys varied over a wide range, but, on average, were similar to those in lampreys. Mean values of the kinematic characteristics of FFS under different conditions are shown in Table 2.

BS evoked by bilateral stimulation of the spinal cord

In intact lampreys, BS is characterised by undulations of large amplitude, with the waves slowly propagating from the tail towards the head (Islam *et al.*, 2006; Islam & Zelenin, 2008). In the spinal lamprey, BS could be evoked by bilateral stimulation of the same sites of the spinal cord (in both rostral and caudal areas) that were stimulated to evoke FFS, but with a stronger current. An example of BS evoked by rostral stimulation is shown in Fig. 5A. The right and left convexity moved in the caudorostral direction (from 100% to ~20% of body length), which is clearly seen in Fig. 5B. The speed of wave propagation was not constant along the body. Different waves could propagate faster or slower in different parts of the body (Fig. 5B). Figure 5C shows examples of EMG recordings, with the electrodes positioned in the caudal body area at two rostrocaudal levels on the same side (inset in Fig. 5B). A clear periodic bursting pattern was observed in each EMG recording. The bursts of caudal EMG activity occurred earlier than those of the rostral EMG.

The stimulation strength required to evoke BS was, on average, four to six times larger than that required to evoke FFS (Table 1). BS stopped as soon as the stimulation was switched off. We found no systematic difference between BS evoked by stimulation of the medial spinal cord and that evoked by stimulation of lateral areas of the spinal cord.

The area of locomotor undulations during BS was considerably smaller than that during FFS. In only 25% of BS episodes evoked by rostral stimulation did the wave reach the point of 20% of body length (Fig. 6A). With caudal stimulation, the wave never reached this point, and in 97% of episodes it propagated no further than 50% of body length (Fig. 6B). An example of such 'caudal body BS' is shown in Fig. 7. In this example, the waves of contractions propagated up to 40% of body length, whereas, more rostrally, no consistent wave propagation could be seen (Fig. 7B). In 64% of cases of 'caudal body BS' evoked by caudal stimulation, reciprocal right–left contractions were observed in the rostral part of the body (in one-third of these trials, a wave of contractions propagated in the rostrocaudal direction). In episodes of BS evoked by rostral stimulation, rostrocaudally propagating waves were never observed, and in only 23% of trials were non-propagating right–left contractions seen in the rostral part of the body.

Figure 6C–F shows a distribution of different kinematic characteristics of BS evoked by rostral and caudal stimulation. The mean values of each characteristic under the two conditions (indicated by black and white arrows) were similar to each other. Comparison of these values with those in intact lampreys (gray arrows in Fig. 6C–F) showed that, in spinal lampreys, the cycle duration was five to seven times longer, and the speed of locomotor waves was 6–10 times slower, than in intact lampreys. The phase shift per segment in spinal lampreys was 1.5–2 times larger. The wavelength was similar to that in intact lampreys during rostral stimulation, and much shorter during caudal stimulation. Mean values of the kinematic characteristics of BS under different conditions are shown in Table 2.

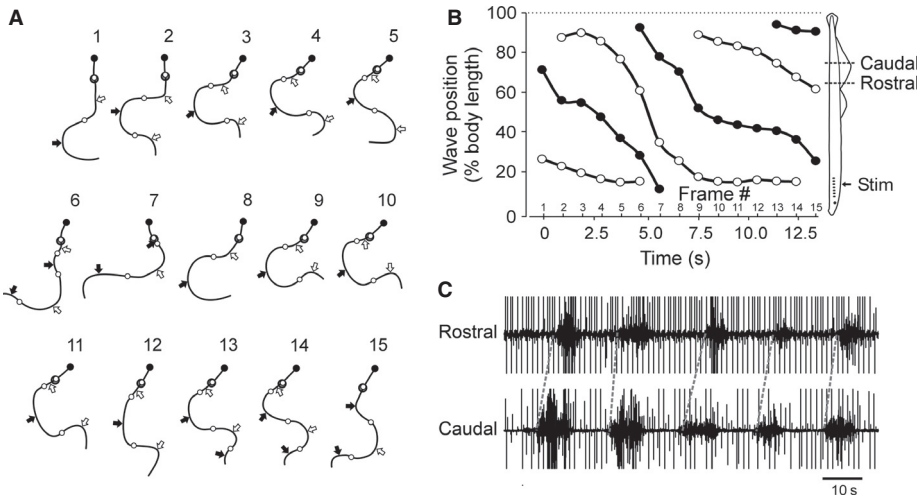

FIG. 5. Examples of BS evoked by bilateral stimulation of the rostral part of the spinal cord. (A) Shape of the body midline in 15 sequential frames (1–15). During this episode of BS, backward body progression was absent, because the lamprey was attached to the bottom of the aquarium by its sucker mouth. The circles and arrows indicate the same characteristic points as in Fig. 3A. (B) Positions of left convexity (black circles) and right convexity (white circles) as a function of time (of frame number) for the same swim cycles. (C) An example of EMG recordings obtained during BS. Interrupted lines show caudorostral phase lags. The levels of EMG recording (rostral and caudal) and the position of stimulating electrode (Stim) are shown in B.

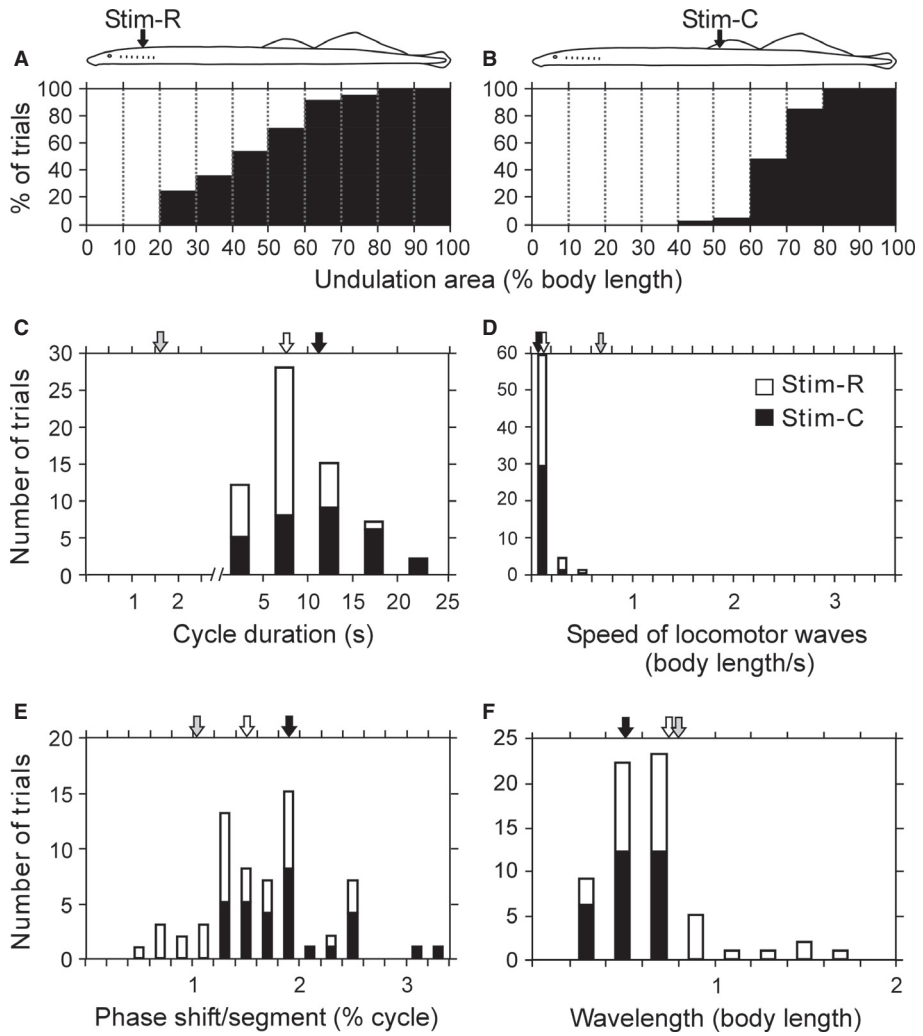

FIG. 6. BS in spinal lampreys. (A and B) Histograms of relative numbers of trials with different undulation areas during BS caused by stimulation of the rostral (A) and caudal (B) sites of the spinal cord. (C–F) Summary of kinematic characteristics of BS in spinal lampreys. The white, gray, and black arrows indicate the same characteristic points as in Fig. 4C–F.

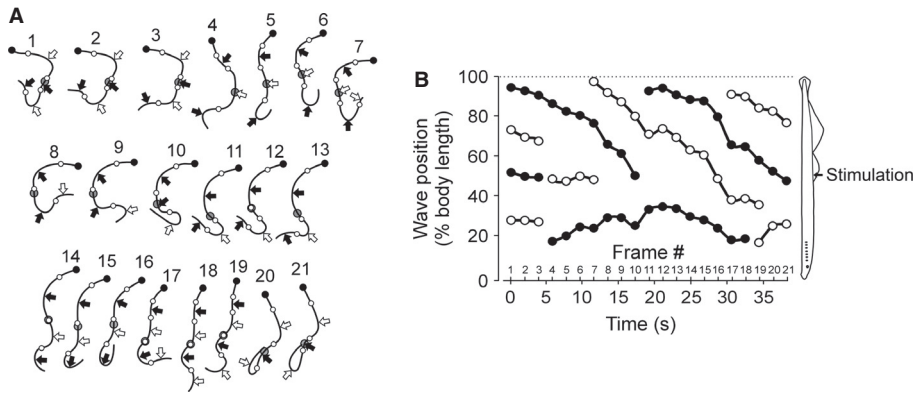

FIG. 7. Example of BS evoked by bilateral stimulation of the caudal part of the spinal cord. (A) Shape of the body midline in 21 sequential frames (1–21). During this episode of BS, the lamprey was not attached to the bottom of the aquarium by its sucker mouth. The circles and arrows indicate the same characteristic points as in Fig. 3A. (B) Positions of left convexity (black circles) and right convexity (white circles) as a function of time (of frame number) for the same swim cycles.

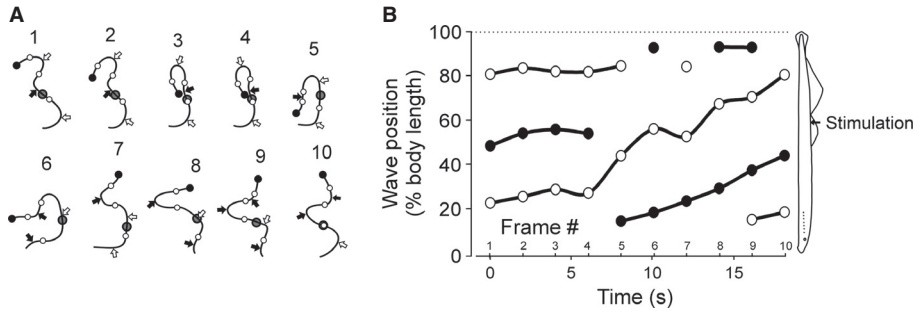

FIG. 8. An example of SFS evoked by bilateral stimulation of the caudal site of the spinal cord. (A) Shape of the body midline in 10 sequential frames (1–10). During this episode of SFS, the lamprey was not attached to the bottom of the aquarium by its sucker mouth. The circles and arrows indicate the same characteristic points as in Fig. 3A. (B) Positions of left convexity (black circles) and right convexity (white circles) as a function of time (frame number) for the same swim cycles.

SFS evoked by bilateral stimulation of the spinal cord

SFS in intact animals is characterised by head and body undulations of large amplitude, with the locomotor waves slowly propagating from the head towards the tail (Islam & Zelenin, 2008). In the present study, such large-amplitude slow rostrocaudally propagating waves were observed during bilateral stimulation of the spinal cord. An example of SFS is shown in Fig. 8A. The right and left convexity moved along the body from the head to the mid-body area. The amplitude of undulation was much larger than that during FFS (compare Fig. 3A and Fig. 8A). Figure 8B shows the positions of the left and right convexities as a function of time (frame number) for the swim episode shown in Fig. 8A. The wave of body flexion propagated at a speed of 7.6% body length/s, which is much slower than that during FFS. We managed to evoke SFS in only seven trials. The stimulation strength for elicitation of SFS was larger than that for FFS, but smaller (although insignificantly) than that for BS (Table 1). SFS stopped as soon as the stimulation was switched off.

Mean values of the kinematic characteristics of SFS in spinal lampreys under different conditions are shown in Table 3. The amplitude of head excursion was dramatically larger during SFS than during FFS: the minimum observed during SFS was approximately two times larger than the maximum observed during FFS. In the episodes of locomotor movements when a lamprey was attached to the bottom with its sucker mouth, excursion of the point at 16% of body length was calculated instead of the head excursion. The ranges of this parameter observed during SFS and FFS did not overlap, and the average during SFS was four to five times larger than during FFS. On average, the SFS cycle duration was four to seven times longer and the speed of locomotor waves was approximately two times slower than the corresponding values during FFS (Table 3). During SFS in spinal lampreys, the amplitude of head excursion was similar to that in intact lampreys, the cycle duration was 3–5.5 times longer, and the wave propagation was approximately two times slower.

Effects of spinal cord stimulation in the lamprey positioned in the tunnel

In a U-shaped tunnel (Fig. 9A), the intact lamprey can crawl in both directions (Archambault *et al.*, 2001). In the spinal lamprey, both FC and BC could be evoked by unilateral stimulation of the spinal cord. Similar strengths of stimulation of the same site of the spinal cord were used to evoke FC and BC (Table 1).

The direction of evoked crawling depended on the distance of the bending site from the head (*X* in Fig. 9A). Stimulation on the outer side of the body (which contacted the outer wall) in most trials (nine of 11, $N = 5$) evoked BC if *X* was between 15% and 35% of body length, and evoked FC in the other two trials with larger *X*-values (Fig. 9B and D). This was observed during both rostral and caudal stimulation. In most trials (11 of 13, $N = 5$), stimulation on the inner body side evoked FC (Fig. 9C and E). In some trials of inner side stimulation, the lamprey changed the direction of crawling

TABLE 3. Main characteristics of SFS in spinal and intact lampreys

	SFS			FFS		
Characteristics of swimming	Spinal-R	Spinal-C	Intact	Spinal-R	Spinal-C	Intact
Cycle duration (s)	3.95 ± 2.23*† (1.34–8.40)	7.36 ± 2.22*† (1.6–12.36)	1.32 ± 0.10 (0.52–3.12)	1.01 ± 0.09 (0.52–2.12)	0.89 ± 0.09 (0.40–1.60)	0.38 ± 0.09 (0.19–0.88)
Speed of wave (body length/s)	0.44 ± 0.18 (0.09–0.67)	0.52 ± 0.42 (0.07–1.79)	0.88 ± 0.08 (0.55–1.73)	1.01 ± 0.09 (0.50–2.69)	1.02 ± 0.13 (0.33–2.14)	3.47 ± 0.64 (2.57–5.30)
Wavelength (body length)	0.98 ± 0.22 (0.76–1.41)	1.37 ± 0.50† (0.76–2.86)	1.01 ± 0.04 (0.83–1.61)	0.92 ± 0.05 (0.49–1.54)	0.78 ± 0.07 (0.35–1.45)	0.81 ± 0.03 (0.72–0.86)
Head excursion (cm)	NA	17.91 ± 3.65 (14.26–21.57)	13.00 ± 0.67 (8.00–20.80)	4.20 ± 0.48 (1.60–7.87)	3.3 ± 0.46 (1.79–4.57)	4.80 ± 0.90 (2.70–6.20)
Body excursion with sucking mouth (cm)	5.94 ± 1.81 (2.98–9.23)	6.42 ± 2.81 (3.61–9.23)	NA	1.15 ± 0.17 (0.68–1.68)	1.54 ± 0.17 (0.49–2.58)	NA

Values are presented as means ± standard errors, with range in parentheses. Spinal-R and Spinal-C, locomotion evoked by stimulation of the rostral and caudal parts, respectively, of the spinal cord in spinal lampreys. Amplitude of excursion with the sucking mouth was calculated by tracking the point of 16% of body length (approximately the position of the rostral electrode). NA indicates that there were no trials of this type. *Statistically significant difference between the corresponding values in spinal and intact lampreys †Statistically significant difference between the corresponding values during SFS and FFS in spinal lampreys. Data for intact lampreys have been published previously (Islam & Zelenin, 2008). Number of locomotor episodes with the immobile head: SFS during rostral stimulation, $n = 3$; SFS during caudal stimulation, $n = 2$; FFS during rostral stimulation, $n = 10$; FFS during caudal stimulation, $n = 11$.

several times. Crawling always stopped as soon as the stimulation was switched off. The velocity of crawling in the spinal lamprey (0.12–23.3 cm/s) was much more diverse than in the intact lamprey (6–16 cm/s) (Archambault *et al.*, 2001).

Effects of tactile stimulation

In the spinal lamprey, a brief pinch of the tail evoked S-shape bending of the body, whereas a continuous pinch evoked FFS ($N = 5$, $n = 20$). Continuous pressure applied over a large area (between 70% and 90% of body length) evoked SFS ($N = 5$, $n = 20$). Tactile stimulation evoked BS in only one of the tested lampreys, in which continuous pressure applied in the area between 20% and 40% of body length evoked a few cycles of BS with a slow wave propagation.

In the spinal lamprey positioned in the U-shaped tunnel, FC could be evoked by pinching the tail or the second dorsal fin. In contrast, BC was never evoked by tactile stimulation of any tested region ($N = 5$, $n = 20$).

Discussion

Spinal mechanisms generating different forms of locomotion

A fundamental question in neuroscience is how different forms of behavior are generated by the central nervous system. Most vertebrates are capable of different forms of locomotion. For example, cats can perform forward, backward and sideward locomotion Buford *et al.*, 1990; Musienko *et al.*, 2012), and zebrafishes and *Xenopus* tadpoles can perform forward swimming, and BS, called 'struggling' (Kahn & Roberts, 1982; Kahn *et al.*, 1982; Budick & O'Malley, 2000; Liao & Fetcho, 2008). In the lamprey, five forms of locomotion have been described: FFS, SFS, BS, FC, and BC (Williams *et al.*, 1989; Archambault *et al.*, 2001; Islam *et al.*, 2006; Islam & Zelenin, 2008). It has been established that, in the lamprey, the basic motor pattern of FFS and FC can be generated by spinal mechanisms alone, as FFS and FC were observed in spinal animals (Rovainen, 1976; McClellan & Grillner, 1983). The aim of this study was to determine: (i) whether the other forms of locomotion (BS, SFS, and BC) can also be generated by spinal mechanisms; and (ii) how they can be selectively activated.

To address these issues, we developed a technique for electrical stimulation of the spinal cord in chronic spinal lampreys. The main finding of the present study is that any form of lamprey locomotion can be evoked by spinal cord stimulation (Figs 3, 5, 8, and 9), provided that a proper site and a proper strength of stimulation are used. One can thus conclude that the spinal cord of the lamprey contains the neuronal networks that generate the whole repertoire of locomotion used by this animal in daily life. These results complement earlier studies (Grillner, 1974; Dale & Roberts, 1984; Soffe, 1991) demonstrating that, in the spinal dogfish and tadpole, both forward swimming and BS can be evoked.

The kinematic parameters of locomotion evoked by electrical stimulation were not the same as in the intact lamprey. On average, the locomotion frequency and the speed of the wave propagation during FFS and BS in spinal lampreys were several times lower than in intact lampreys. Similarly, forward swimming evoked in spinalised zebrafish larvae had a lower frequency than that observed in intact animals (McDearmid & Drapeau, 2006). In contrast, the phase lag and the wavelength in spinal lampreys were similar to those in intact lampreys (except for BS evoked by caudal stimulation). These findings suggest that the phase lag is mostly determined by the

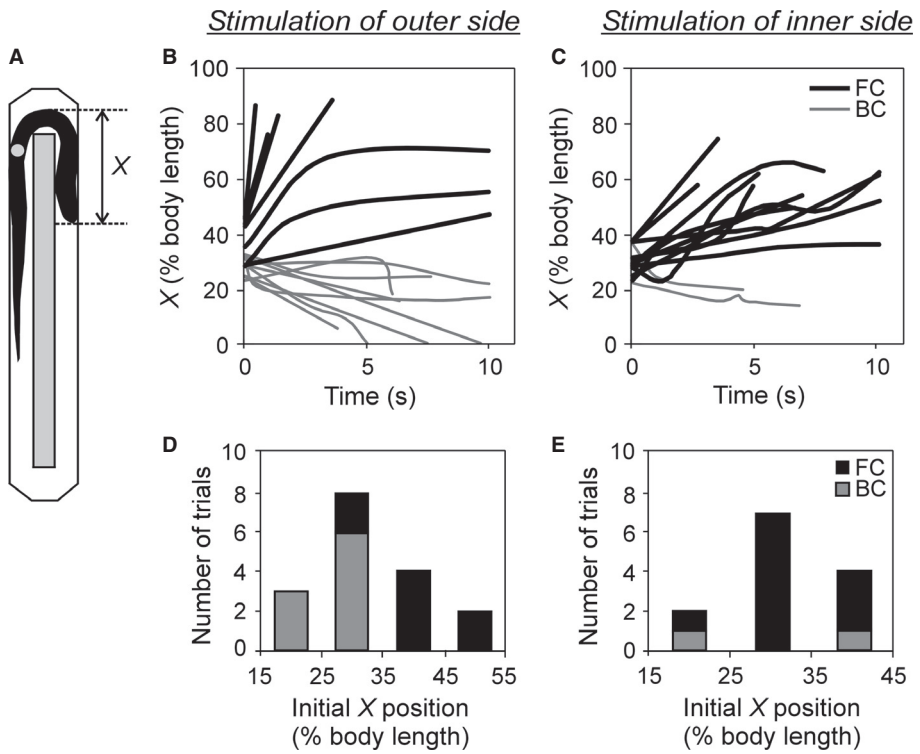

FIG. 9. Effects of spinal cord stimulation in the lamprey positioned in the U-shaped tunnel. (A) A spinal lamprey positioned in the tunnel. *X*, the distance from the bending site to the head. (B–E) Effects of unilateral spinal cord stimulation through the electrode positioned on the outer side (B and D) or the inner side (C and E) of the spinal cord in relation to body bending. (B and C) Head progression as a function of time. (D and E) Number of trials with FC and BC as a function of *X*-value.

spinal network properties. On the other hand, in intact lampreys, the cycle duration and, correspondingly, the speed of wave propagation can be regulated by descending commands (as demonstrated for FFS) (Sirota *et al.*, 2000; Zelenin, 2011), which is not possible in spinal animals.

The motor pattern of BS in the spinal lamprey was much less regular than that of FFS. The wave propagation was not smooth, and could stop in the mid-body area. Strong stimulation (necessary to evoke BS) could activate not only the mechanisms responsible for BS but also those evoking FFS or other motor patterns. This concurrent activation of different mechanisms could explain distortions of the wave propagation. It also explains the extremely long cycle duration of BS in spinal lampreys.

The neuronal organisation of spinal networks generating SFS, BS, FC and BC in the lamprey is unknown. Recording of individual spinal interneurons during forward swimming and BS in the zebrafish revealed two groups of neurons (Liao & Fetcho, 2008). It was suggested that one group (active during both forward and backward locomotion) constitutes the core of the swimming rhythm generator, and another group (active during backward locomotion only) inverts the direction of the locomotor wave propagation from the rostrocaudal to the caudorostral direction. In contrast, in *Xenopus* tadpoles, two separate groups of interneurons with different physiological properties were suggested to be core generators for forward swimming and BS (Li *et al.*, 2007).

Structures mediating locomotor effects of electrical stimulation

Electrical stimulation could potentially activate different types of neurons. These could be the axons of neurons of descending tracts, mainly the RS ones (Tretjakoff, 1909; Rovainen, 1967a, 1974). Stimulation could also activate ascending neurons with long axons (giant interneurons) located in the caudal spinal cord (Rovainen, 1967b; Rovainen *et al.*, 1973). It could activate the propriospinal neurons as well, including the lateral interneurons, whose axons project to the tail region. We think, however, that the RS axons are the most probable candidates for mediating the effects of stimulation, for several reasons: (i) many of the RS axons are thick, and thus have low activation thresholds; (ii) the RS neurons are numerous, and many of them project along the entire extent of the spinal cord (Rovainen, 1967a; Nieuwenhuys, 1972; Brodin *et al.*, 1988); (iii) they are involved in the control of a variety of behaviors, including different forms of locomotion (Deliagina *et al.*, 2000; Zelenin, 2005, 2011); and (iv) they can strongly affect the motor output of the locomotor networks (Zelenin *et al.*, 2001). We usually tested the lampreys within 2 days after spinalisation, so most of the RS axons were still alive (Roederer *et al.*, 1983; Zhang *et al.*, 2005). Therefore, we assume that the effects of electrical stimulation are produced through RS axons, and below we consider our experimental data in the light of this assumption.

Previously, it was shown that RS neurons in the intact lamprey are activated bilaterally during FFS and BS in free water (Deliagina *et al.*, 2000; Zelenin, 2011). They are also symmetrically activated in the semi-intact preparation during FFS evoked by stimulation of the mesencephalic locomotor region (Brocard *et al.*, 2010). Any asymmetry in the excitatory drive to the left and right spinal hemisegments can be further amplified by reciprocal inhibition between these hemi-segments (Fagerstedt *et al.*, 2000), resulting in an asymmetrical motor pattern, which is required during turning (Deliagina *et al.*, 2000). These data fit with our present finding that fine-tuning of the stimulation strengths of left and right electrodes was required to cause symmetrical swimming, and a slight deviation resulted in

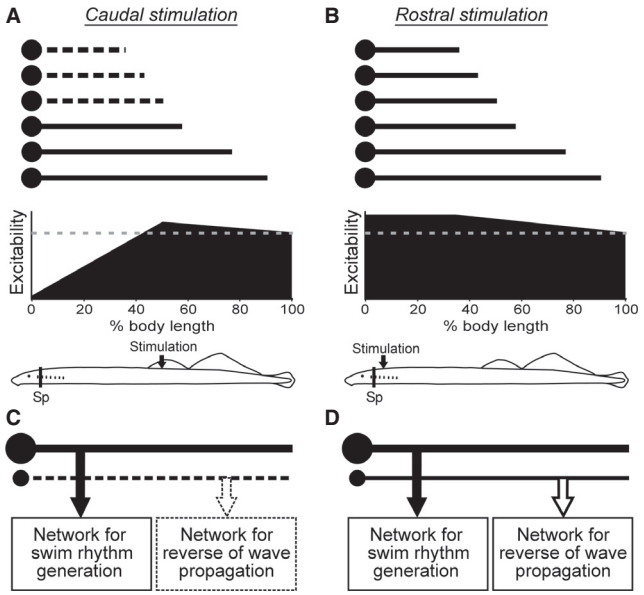

FIG. 10. Possible roles of different RS neurons in elicitation of swimming. (A and B) The excitability of locomotor networks along the spinal cord can be increased by activation of different groups of RS neurons. (A) Schematic of RS neurons with different axon lengths. Axons that are activated by stimulation are solid. Shorter axons that are not activated by the stimulation are dashed. Caudal stimulation activates only long-projecting RS neurons, which cannot increase the excitability in rostral segments as much as is necessary for elicitation of locomotor oscillations (the threshold required for swimming is indicated by the gray broken line). (B) Rostral stimulation activates both long-projecting and short-projecting neurons, which together make the excitability of spinal networks sufficient for elicitation of locomotor oscillations along the whole extent of the spinal cord. (C and D) Mode of locomotion depends on the stimulation strength. (C) Weak stimulation activates RS neurons with thicker axons, which activate the network for swim rhythm generation underlying FFS. (D) Stronger stimulation activates not only thick RS axons but also thin ones. The latter activate the network for the reversal of wave propagation, which results in BS. Sp, site of spinalisation.

considerable asymmetry. Unilateral electrical stimulation usually evoked static C-shaped or S-shaped body flexion. In only a few trials did the unilateral stimulation evoke undulatory movements that were superimposed on the static bends (C-shaped or S-shaped), suggesting that unilateral RS commands could activate the segmental oscillators to generate undulation. The static contractions evoked by unilateral electrical stimulation may constitute a natural behavior analogous to the startle response seen in the lamprey and the teleost fish, which is initiated through descending pathways (Eaton *et al.*, 1977, 2001; McClellan, 1984; Currie & Carlsen, 1987; Fetcho, 1991).

The undulation area during swimming (both FFS and BS) depended on the site of stimulation – rostral stimulation evoked swimming with a larger undulation area than caudal stimulation, and this difference was more evident during BS (Fig. 6A and B). Caudal stimulation activates only long RS axons (Fig. 10A), whereas rostral stimulation activates both long and short RS axons (Fig. 10B). Therefore, it is possible that the activity in long RS axons is not sufficient to increase excitability in the rostral spinal locomotor networks. If this is so, short RS axons provide the necessary extra excitation to the rostral segments so that they can generate locomotor oscillations.

The groups of RS neurons involved in the control of FFS and BS are partly different. In freely behaving lampreys, 62% of RS axons are activated during FFS, and only 6% during BS; the BS-specific axons are smaller (Zelenin, 2011). In the present study, FFS in the caudal part of the body could be evoked by caudal stimulation. Thus, activation of the long low-threshold (thick) RS axons seems to be sufficient to activate the locomotor networks, which generate FFS (Fig. 10C). Stimulation at the same site with increased strength could evoke BS. This finding suggests that long RS axons with higher activation thresholds cause a reversal of the direction of locomotor waves, thus producing BS (Fig. 10D). Most RS neurons with axonal projections to the caudal part of the body (>60% of body length) are of medium size, and their proportion is larger in the middle and posterior rhombencephalic reticular nuclei (Bussières, 1994). These medium-size and long-projecting RS neurons are good candidates for the transmission of commands for BS.

Neither in FFS nor in BS did we find systematic differences between locomotion evoked by stimulation of the medial area of the spinal cord and that evoked by stimulation of the lateral area of the spinal cord. Previously, it was found that lesions of either the medial or lateral area do not interfere with the initiation of FFS and BS in freely behaving lampreys (McClellan & Grillner, 1983; Islam *et al.*, 2006). Together, these findings suggest that the axons transmitting commands for FFS and BS are spread over a wide area of the spinal cord cross-section.

Two groups of RS axons have been found, which are activated or inhibited during crawling, suggesting that a tonic 'crawl' command is sent to the spinal cord (Zelenin, 2005). This view is supported by our present finding that spinal cord stimulation (imitating a tonic supraspinal command) could evoke both FC and BC. However, in the spinal lamprey, the direction of crawling was determined by the site of body bending. This suggests that: (i) our electrical stimulation activated both RS axons that transmit commands for FC and those that transmit commands for BC; and that (ii) somatosensory information plays an important role in crawling.

Role of sensory information in different forms of locomotion

Somatosensory information is important for the initiation of different forms of locomotion in intact lampreys. As shown previously (Rovainen, 1976; McClellan & Grillner, 1983), two forms of locomotion (FFS and FC) could be evoked in the spinal lamprey by tactile stimulation of a definite area (tail pinching). In the present study, we confirmed these results, and also found that pressure applied to the caudal part of the body evoked SFS. Thus, although the spinal lamprey can produce all five forms of locomotion (as revealed by electrical stimulation of the spinal cord), tactile stimulation is sufficient to reliably evoke only some of them (FFS, SFS, and FC). It is possible that, in the lamprey, the descending commands for backward locomotion play a more important role, in contrast to, for example, the dogfish or the tadpole, in which the tactile input is sufficient to evoke both forward and backward locomotion in the spinal animal (Grillner, 1974; Soffe, 1991).

There is one more sensory input that may be essential for crawling (both FC and BC): the signals coming from the intraspinal stretch receptor neurons, also known as edge cells (Grillner *et al.*, 1984). Intact lampreys crawl to move in or out of tight places. The motor pattern of crawling is a solitary wave of co-contraction of the left and right muscles close to the bending site (Archambault *et al.*, 2001). Thus, it has to be adapted to the configuration of external constraints. In the present study, we found that the direction of crawling of the spinal lamprey was determined by the position of the bent site along the body: FC was usually seen if the body was bent within its rostral third, and BC was seen if the body was bent

more caudally. Recently, we studied the motor responses in the isolated spinal cord caused by the stretch receptor neurons. We found that the responses have opposite signs in the rostral third of the body and the caudal two-thirds (Hsu *et al.*, 2013). The similar position of the reversal point found in these two different studies strongly suggests a significant contribution of edge cells to the generation of the motor pattern of crawling.

To conclude, the present study has shown that all five forms of locomotion observed in intact lampreys (FFS, SFS, BS, FC, and BC) are generated by the spinal networks. These networks could be selectively activated by electrical stimulation of the spinal cord, suggesting that, in intact lampreys, they are activated by specific tonic excitatory supraspinal drive. In addition, some of these spinal networks (generating FFS, SFS, and FC) could be activated by somatosensory input from the specific areas of the body. Finally, it was demonstrated that sensory signals concerning body configuration can influence the direction of crawling. Future studies will shed further light on the organisation and operation of these spinal networks, as well as on the location of supraspinal neurons that selectively activate the spinal network, generating a specific form of locomotion.

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Abbreviations

BC, backward crawling; BS, backward swimming; EMG, electromyography; FC, forward crawling; FFS, fast forward swimming; RS, reticulospinal; SFS, slow forward swimming.

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