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Activity of individual reticulospinal neurons during different forms of locomotion in the lamprey

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Abstract

Lamprey (a lower vertebrate) can employ different modes of locomotion, i.e. swimming in open water and crawling in tight places. Swimming is due to the periodic waves of lateral undulations with reciprocal activity of right and left muscles. In contrast, crawling (forward and backward) is based on single waves with coactivation of muscles on two sides. Basic mechanisms of swimming and, most likely, crawling reside in the spinal cord, and are activated by supraspinal commands. The main source of these commands is the reticulospinal (RS) system. The goal of the present experiments was to characterize the activity of individual RS neurons during swimming and during crawling in a U-shaped tunnel. The activity was recorded by means of chronically implanted electrodes in freely behaving animals. All recorded RS neurons were active during swimming but silent in quiescent animals. Many of them (61%) showed phasic modulation of their firing rate approximately in phase with the activity of ipsilateral rostral muscles. The majority of the neurons (80%) were also active during crawling. Many of them either increased or decreased their activity during crawling as compared to the background activity. These changes were better correlated with the direction of progression (forward or backward) than with the direction of turning in the tunnel (right or left). No correlation of the activity of RS neurons during locomotion and their sensory inputs was found. The results of this study suggest that different modes of locomotion in lampreys can be caused by considerably overlapping groups of RS neurons.

Introduction

Animals can display different forms of locomotion. An animal can switch between completely different locomotor modes like the bipedal and quadrupedal locomotion of monkey (Nakajima *et al*., 2004) and lizards (Snyder, 1952; Irschick & Jayne, 1999), walking and swimming of salamander (Frolich & Biewener, 1992), sidewinding and concertina crawling in snakes (Jayne, 1988). Also, a usual forward locomotion pattern can be modified to produce backward progression (Thorstensson, 1986; Buford *et al*., 1990; Vilensky & Cook, 2000). Animals can also use a variety of gaits differing in the interlimb coordination (Engberg & Lundberg, 1969).

There is growing evidence that locomotor patterns are produced by the spinal neuronal networks, which are activated by descending (supraspinal) commands (see, e.g. Orlovsky *et al*., 1999). Encoding of these commands by the activity of populations of individual descending neurons is one of the fundamental problems of motor control (see e.g. Deliagina *et al*., 2002). Do the populations of descending neurons, that are active during different types of locomotion, overlap with each other? If they do, what are the firing rates of individual neurons during different forms of locomotion? Do different populations have different sensory inputs?

In the present study, these questions were addressed for the lamprey (a lower vertebrate, Cyclostome). This animal has been intensively used for studying different aspects of motor control. It can display two different modes of locomotion: swimming and crawling. The lamprey swims forward due to lateral undulations of its body that propagate in the rostro-caudal direction (Grillner & Kashin, 1976). These mechanical waves are caused by waves of reciprocal activity of the right and left muscles (Williams *et al*., 1989). Spinal mechanisms generating these movements have been analysed in considerable detail at the network and cellular levels (reviewed in Grillner *et al*., 1995; Grillner *et al*., 2000). When swimming, the lamprey maintains a certain orientation of its body in the transverse, sagittal, and frontal planes. This orientation control system is driven to a large extent by vestibular input (de Burlet & Versteegh, 1930; Ullén *et al*., 1995). Another form of locomotion is crawling in tight places. During crawling, the lamprey adapts its body shape to the configuration of external constraints. Forward or backward progression is caused by a solitary wave of right and left muscles cocontractions located near the body bend (Archambault *et al*., 2001). The spinal mechanisms responsible for generation of this pattern of muscle activity have not yet been investigated. It was suggested that the EMG pattern during steady-state crawling is principally driven by central processes (Archambault *et al*., 2001). An alternative hypothesis assumes a critical importance of the proprioceptive information from stretch receptors in the spinal cord (Grillner *et al*., 1984) that may be able to bind the muscle activity to the current body configuration.

The main route for descending motor commands that activate one or another locomotor pattern in the lamprey is the reticulospinal (RS) pathways (Rovainen, 1967; Nieuwenhuys, 1972; Brodin *et al*., 1988). The bilateral RS pathways originate from approximately 2500 neurons (Ronan, 1989; Bussières, 1994). The majority of the RS neurons project ipsilaterally over long distances, some reaching the most caudal segments, and affect different classes of spinal neurons (Rovainen, 1974; Buchanan & Cohen, 1982; Ohta & Grillner, 1989). Previous experiments have shown that the activity of individual

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RS neurons increased during fictive locomotion *in vitro* (Kasicki & Grillner, 1986; Dubuc & Grillner, 1989) as well as in semi-intact preparation (Brocard & Dubuc, 2003). The *in vivo* experiments showed that episodes of swimming are always preceded and accompanied by massive activation of the RS system (Deliagina & Fagerstedt, 2000; Deliagina *et al*., 2000). However, the activity of individual RS neurons in freely behaving animals during swimming has not been studied in any detail, whereas the activity of RS neurons during crawling was not studied at all.

The analysis of the activity of individual RS neurons during different forms of locomotion – swimming and crawling – was the aim of the present study. The mass activity of RS neurons was recorded in freely behaving animals by means of chronically implanted electrodes, the activity of single RS neurons was extracted from the mass-activity using the spike-sorting procedure, and then the activity of individual RS neurons was correlated with the locomotor activity of the animal. Also, an attempt was made to characterize the RS neurons by their sensory inputs, i.e. their responses to visual, tactile and vestibular stimuli.

The main findings were as follows: first, all recorded neurons were active during swimming, and second, the majority of the neurons were active during crawling. Different neurons were specifically activated or inactivated during crawling in different directions.

A brief account of this study has been published in abstract form (Zelenin *et al*., 2003).

Materials and methods

Experiments were carried out on nine adult (25–35 cm in length) lampreys (*Lampetra fluviatilis*), which were kept in an aerated freshwater aquarium at 5 °C, with a 12-h light : 12-h dark cycle. All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden). Each animal was sequentially tested in different setups, for swimming, crawling, and vestibular stimulation.

Recording and analysis of RS activity

The activity of larger RS axons was recorded by chronically implanted electrodes (for details of the technique see Deliagina *et al*., 2000). In short, the electrodes were made of silver wire (75 μm in diameter, 3 mm in length) and glued to a plastic plate (9 mm long, 2.5 mm wide, and 0.25 mm thick) to be positioned on the dorsal surface of the spinal cord in parallel to RS axons (Fig. 1A). Similar wire electrodes on the opposite side of the plate allowed bipolar recording (Fig. 1B), which led to the reduction of artifacts caused by the electrical activity of the surrounding muscles (electromyogram, EMG). Additional methods to reduce the EMG artifact used in all animals were: (i) denervation of surrounding myotomes bilaterally throughout 5–10 spinal segments, symmetrically in relation to the site of the electrode implantation, and (ii) electrical isolation of the electrodes from the surrounding muscles by wrapping them together with the adjoining segments of the spinal cord in a strip of thin (20 μm) plastic film (Fig. 1C). The electrodes had low resistance ($< 10^3$ Ω) and low noise level (a few microvolts). As shown in previous studies, such electrodes record almost exclusively the spikes of larger RS axons with the conduction velocity of more than 2 m/s (Deliagina & Fagerstedt, 2000; Deliagina *et al*., 2000).

Implantation of the electrodes was performed under tricaine methane sulphonate anaesthesia (MS-222, 100 mg/L; Sigma, St. Louis, MO). In six animals, only one plate with electrodes was implanted at the level of the 30–35th spinal segment. In the other three animals, two plates with electrodes were implanted, one at the level of the 19–21st segment and the other, in front of the dorsal fin (35–37th segment) so that the distance between the plates was 40–55 mm. Recording with one plate did not allow measuring of the axon conduction velocity, but the motor patterns were presumably less distorted by denervation of the body musculature. Each of the plates was implanted through a separate longitudinal cut performed along the midline of the dorsal aspect of the body. The wounds were then closed and sutured so that the connecting wires were tightly fixed between the sides of the wounds.

Two bipolar EMG electrodes were implanted bilaterally in the muscles at approximately the level of the 25th spinal segment.

The implanted electrodes for recording the activity in the RS pathways and the EMGs were connected, via a long flexible cable, to the inputs of AC amplifiers. Signals from the electrodes were band-pass filtered (300–500 Hz), amplified, digitized with a sampling frequency of 10 kHz, and recorded to the disk of an IBM compatible computer by means of data acquisition software (Digidata 1200/ Axoscope, Axon Instruments, Foster City, CA). The recorded multiunit spike trains were separated into unitary waveforms representing the activity of individual axons using the spike-sorting procedure in conventional data analysis software (Spike2, version 4, CED, Cambridge, UK). The spikes with the same waveform were supposedly generated by the same RS neuron in all tests (swimming, crawling, sensory stimulation). The spike extraction included several steps. (i) One of the channels was used to build primary templates and extract groups of events that fitted them. Only the spikes with amplitude higher than 50 μV (typically, approximately 100 μV) were taken into account. The template width varied in the range from 10 to 25 μV but was always less than 20% of the spike amplitude. (ii) For each of the groups, the corresponding waveforms from the other channels were extracted and then reclassified, that is secondary templates were built for each of the groups. Thus for each RS neuron, there was a set of templates (primary plus secondary), one template for each channel. An event was taken as a spike generated by an RS neuron if all its spike-like waveforms extracted from all channels fitted the corresponding templates. It did not matter which of the channels (right, left, rostral or caudal) was used to build the primary templates, the final set of the templates was practically the same. In the beginning of our analysis of the data recorded for a given animal, we looked through all files and piled up templates corresponding to different neurons. After that, this common set of templates was used to extract activity of single neurons from all these files. Thus we analysed activity of the same neurons in all conditions (rest, swimming, crawling, sensory stimulations). Approximately 2–5% of high-amplitude events were discarded as not fitting any of the templates. Probably, these were the spike waveforms distorted by summation during intense activity.

Swimming

The lamprey swam freely in an aquarium (50 × 30 × 15 cm) for 20–200-s periods, randomly changing the speed and direction of swimming. The locomotion appeared either spontaneously or was evoked by different sensory stimuli, that is, pinching the head or the tail, illumination of the eye or the tail (Ullén *et al*., 1993; Deliagina *et al*., 1995).

For the neurons modulated in the locomotor rhythm (Rayleigh test for directionality, $P < 0.05$), relations of the RS activity to the ongoing swimming rhythm were analysed (all analysis was performed with custom-written Spike2 scripts). The maxima of the rectified and low pass filtered (50 Hz) EMG of the ipsilateral muscles was taken as the

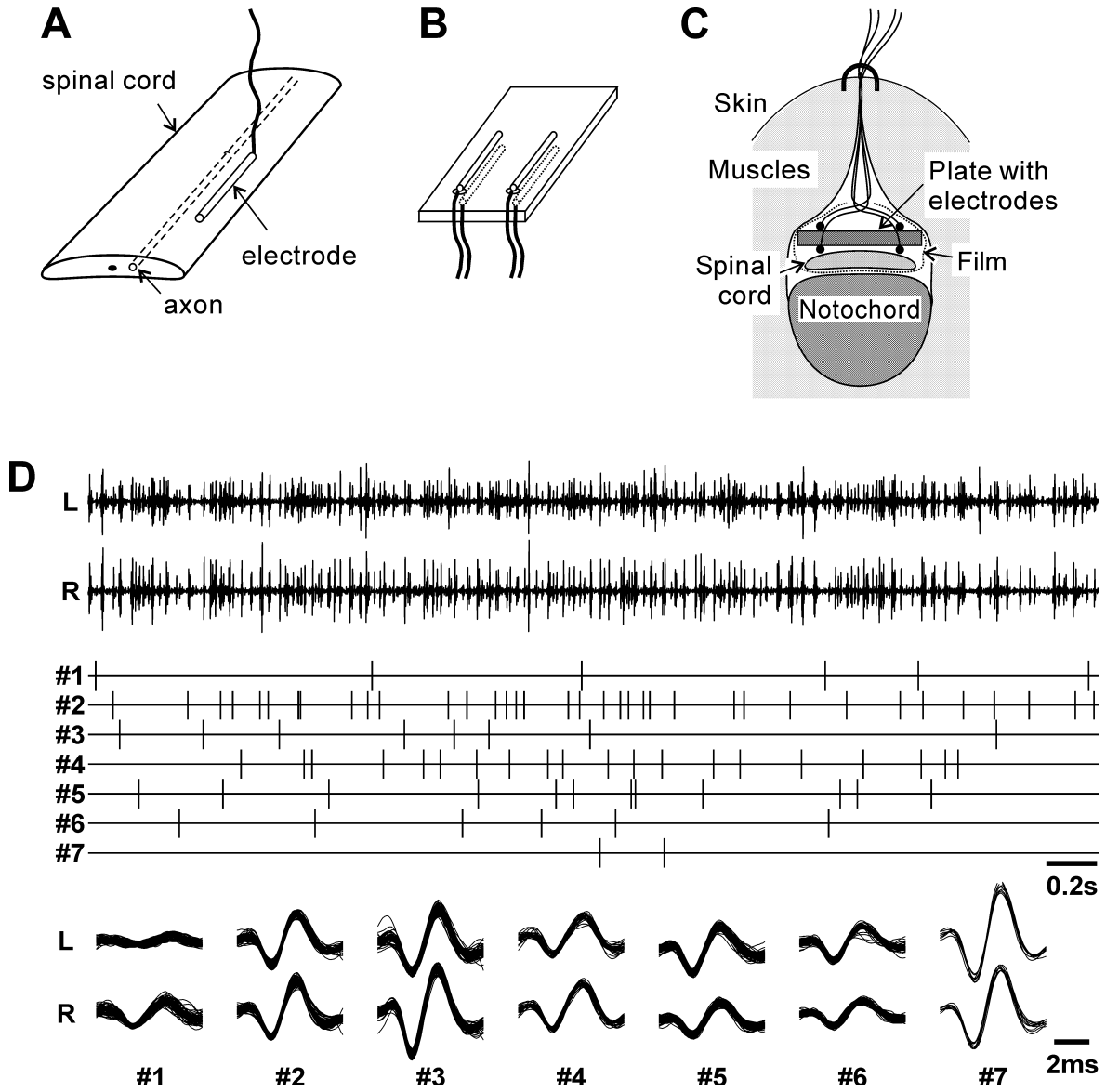

FIG. 1. Recording of activity of single RS neurons in intact lampreys. (A) Activity of larger RS neurons was recorded from their axons in the spinal cord with macroelectrodes orientated in parallel to the long spinal axons. (B) The electrodes were glued to both surfaces of a plastic plate. (C) The plate was positioned on the dorsal surface of the spinal cord. The plate with the electrodes, together with the adjoining segments of the spinal cord, were wrapped in a strip of thin plastic film to reduce the artifacts caused by the muscle activity. (D) Activity of individual RS axons (#1–7) was extracted from the mass activity recorded by the right (R) and left (L) electrodes. Superimposed spikes of the RS neurons extracted from a two minute episode are shown.

starts of the swim cycles. With respect to these cycles, the phase dependence of the RS spiking frequency was calculated, that is, how the RS spikes are distributed with respect to the swimming cycle. Usually, the phase dependence had a sine-like shape with one peak per cycle. To characterize this dependence, its Fourier image was calculated:

$$f(\varphi) = f_0 + f_1 cos(\varphi - \varphi_1) + f_2 cos(2\varphi - \varphi_2) + f_3 cos(3\varphi - \varphi_3) + \ldots = = f_0 + f_1 cos(\varphi - \varphi_1) + r(\varphi).$$

The constant component f_0 of the image provides the average frequency over the whole swimming episode. The first harmonic $f_1 cos(\varphi - \varphi_1)$ is a sine approximation of a one-peak modulation with the preferred phase of firing φ_1, and $r(\varphi)$ is the remainder after the first two terms of the series. This method of calculation of the preferred phase is by definition identical to circular statistics (Zar, 1974). The coefficient of modulation was estimated: $M = 2f_1/(f_0 + f_1) \times 100\%$.

This formula allows avoiding ambiguity in determining the peak and the trough in a 'noisy' phase dependence. On the other hand, in the case of pure sine shape of phase-activity dependence, it is equivalent to the commonly used assessment of the modulation coefficient: $(f_{max} - f_{min})/f_{max} \times 100\%$, where f_{max} and f_{min} are the peak and the trough of the activity in the cycle.

Crawling

For studying of the RS activity during crawling, a U-shaped tunnel was used, 1.8-cm wide, with one limb 29-cm long and the other 17-cm long, distance between the limbs being 1.5 cm. Initially, the lamprey was positioned with its dorsal side up in the long limb with its head

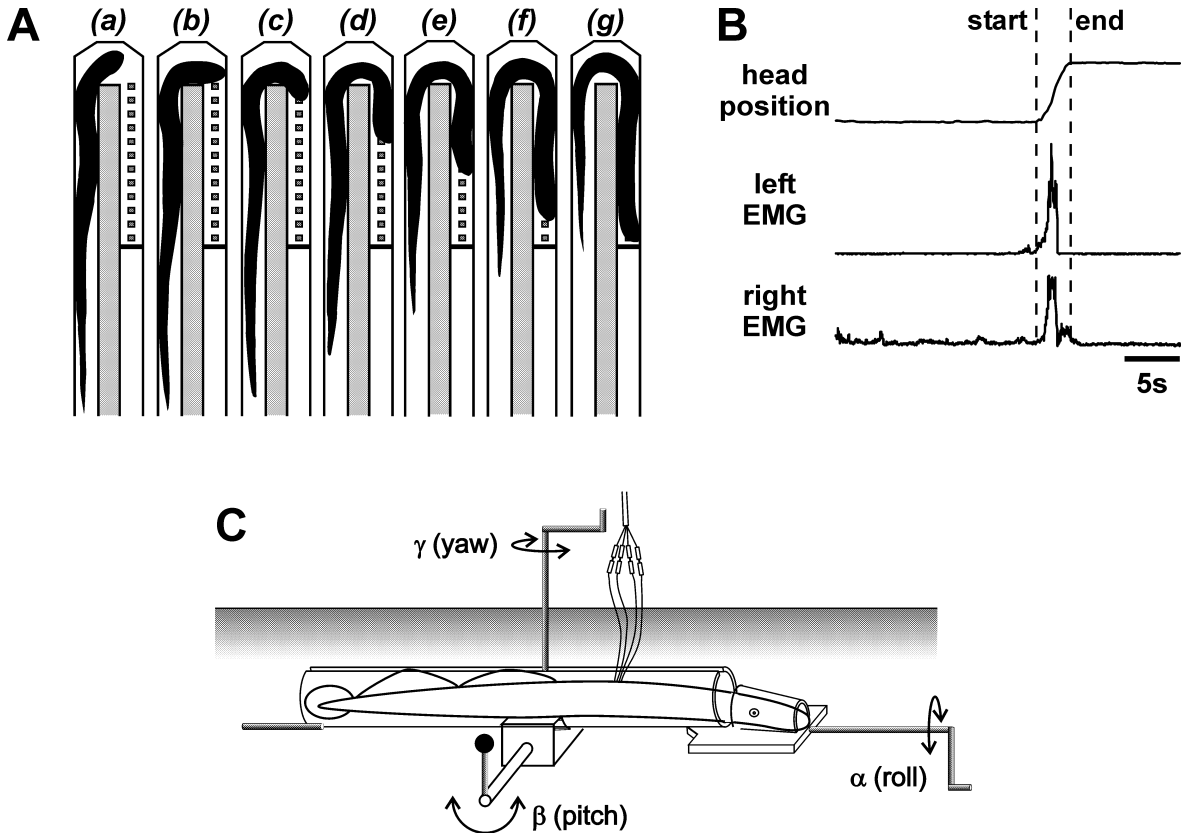

FIG. 2. (A) Experimental design for studying crawling movements. Frames (*a–h*) show the lamprey performing a forward right turn (time between frames 320 ms). The series of squares in the short limb of the tunnel represents the photo-sensors used to measure the head position (modified from Archambault *et al*., 2001). (B) An example of muscle activity during crawling. (C) Experimental design for studying responses of RS neurons to rotation in different planes.

facing the turn. From this position, it crawled forward until its nose bumped the end of the short limb of the tunnel (Fig. 2A, positions *a–g*). The next locomotor episode was the backward crawling until the tail occurred in the end of the long limb of the tunnel. To evoke forward or backward progression, the tail or the head was briefly mechanically stimulated. The stimulation initiated exploratory behaviour that could be followed by either an episode of crawling or relaxation of the animal. The crawling never started sooner than 3 s after the end of the stimulation. Each animal was tested at least three times for each direction of crawling: forward right, forward left, backward right, backward left (right and left refer to the concave side of the animal during crawling). For right and left crawling, the limbs of the tunnel were reconfigured.

The head position in the short limb of the tunnel was monitored by an array of photocells placed under the aquarium. The output of the photocell array was minimal when the animal's head was positioned in front of the entrance into the short limb, and maximal when the animal reached the end of the short limb (Fig. 2A and B). Below, these two positions will be called the start and the end of the crawling for the forward crawling, and the end and the start for the backward crawling. The period of crawling also included the time when the head of the animal was between the limbs of the tunnel. However, this time was much shorter than the time when the head moved in the short limb of the tunnel.

For each crawling direction, the starts and ends of the crawling periods were aligned. The instantaneous frequency for each RS neuron was averaged across the trials. The data are presented in the form of a histogram for average instantaneous frequency or in the form of 'rasters' with normalized timescale.

Tactile, visual, and vestibular stimulation

Responses of RS neurons to tactile, visual, and vestibular stimuli were studied in quiescent animals attached to a horizontal surface with their sucker mouth.

Tactile responses of RS neurons were induced by light rubbing and tapping the skin in different parts of the body with a plastic rod. To induce visual responses, the right or left eye, or the tail photoreceptors were illuminated through a fibre optic system.

To study vestibular responses, the lamprey was positioned in a tube that could be rotated around the longitudinal axis through 360° (roll tilts), or transverse axis through 360° (pitch tilts), or vertical axis through 120° (yaw turns; Fig. 2C). Two patterns of rotation were used. For roll and pitch tilts, the animal was tested by two full turns; the rotation in the first and in the second turn was performed in the opposite directions. The initial orientation of the animal was with the dorsal side down (180°). Rotation was performed in 45° steps. The transition from one position to the next lasted approximately 1 s, and each position was maintained for approximately 2 s. For yaw turns, the stimulation pattern was the asymmetric trapezoid 120° rotation to the left and to the right. Transition from one position to another lasted 0.5–1 s for fast rotation and 2.5–4 s for slow rotation, and each position was maintained for 1–4 s until the discharge induced by the preceding rotation stopped. With each type of rotation, each animal

was tested at least three times. All average data are presented as mean ± SD.

Results

Activity of 71 axons was recorded in the spinal cord. In all 20 axons that were recorded at two different rostro-caudal levels, the spikes propagated in the caudal direction with conduction velocities ranged from 2.5 to 4.7 m/s (average 3.7 ± 0.6 m/s). These findings strongly suggest that these were the axons of larger RS neurons (Deliagina & Fagerstedt, 2000; Deliagina *et al*., 2000). Based on the relative amplitude of their spikes recorded by the right and left electrodes, 39 axons were located on the right side of the spinal cord, and 32 on the left side.

Activity of RS neurons during swimming

When an animal was attached to a wall of the aquarium with its sucker mouth, RS neurons were not active. Different sensory stimuli could activate the animal and it started swimming, as monitored by alternating EMG bursts on the right and left sides (Fig. 3A). Prior to locomotion, many RS neurons started firing. During swimming, 71 RS neurons were active. The mean firing frequencies ranged from 0.3 Hz to 28 Hz, with the average over all neurons of 4.9 ± 7.6 Hz (Fig. 3B). It is noteworthy that the neurons active during crawling or activated by sensory stimuli (see below), all belonged to the population active during swimming.

Of the 71 neurons active during swimming, 43 neurons (61%) were modulated in the locomotor rhythm and had a preferred phase of firing. Figure 4A shows an example of locomotor cycle-related modulation. This RS neuron was less active in the first half of the cycle, and more active in the second half, with the preferred phase 234°. The peak activity of the neuron preceded the peak of the ipsilateral EMG in segment 25 (that was used to determine the swimming cycle). Figure 4B shows characteristics of all modulated neurons. Each vector represents one RS neuron, the direction of the vector showing the preferred phase, and the vector length showing the modulation coefficient. For the majority of the neurons, their preferred phases preceded the peak of ipsilateral EMG by approximately 90°, the average for the whole population being 265° ± 43° (Fig. 4B). The locomotor EMG wave propagates in the caudal direction with constant speed and its length is approximately equal to the body length that consists of 100 segments (Williams *et al*., 1989). Hence, the majority of the RS neurons were active in phase with the ipsilateral muscles of the most rostral segments (the presumed phase of activity of these muscles is indicated with a dashed arc in Fig. 4B). The modulation coefficients were in a range from 20% to 100% (61% ± 22%).

Activity of RS neurons during crawling

Being positioned in the long limb of the U shaped tunnel (as in Fig. 2A, position *a*) and having detached from the bottom after mechanical stimulation of its tail, the lamprey was rather active; it flexed the anterior part of its body in search for an opening, or

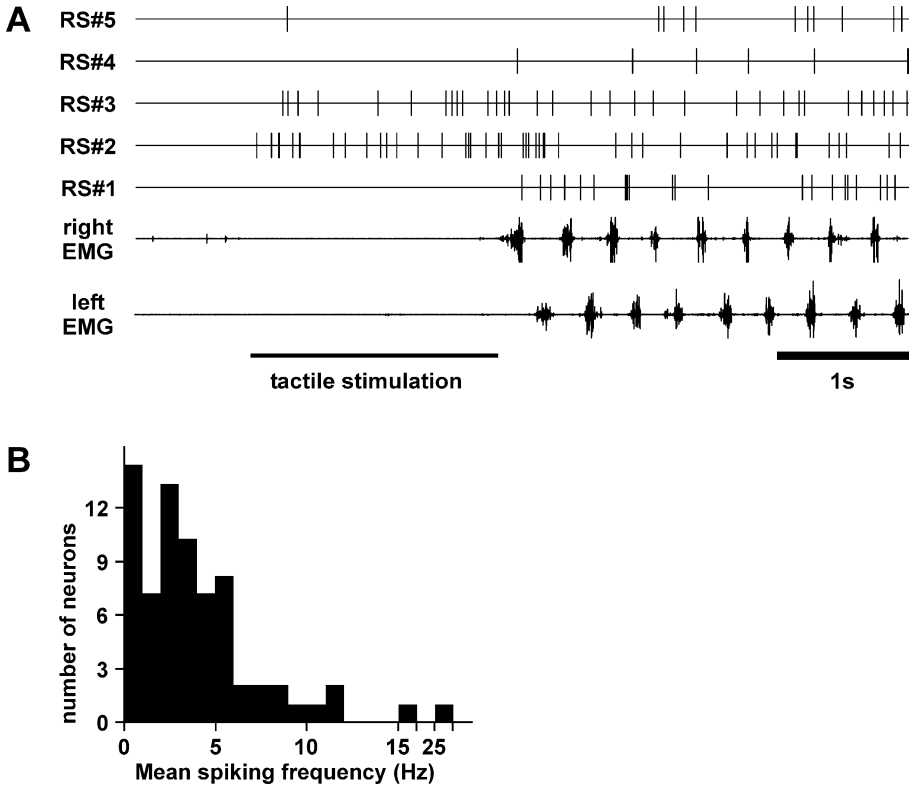

FIG. 3. (A) An example of activity of RS neurons during the start of swimming. In a lamprey sucking to the bottom of the aquarium, all RS neurons were silent. During tactile stimulation of the tail, some of the neurons were activated. With the start of swimming (monitored by rhythmic EMG), all RS neurons became active. Axons RS#1 and RS#2 were from the left side while the others were from the right side. (B) Distribution of mean frequencies of RS neurons during swimming. Note that the *x*-axis scale is linear but different below 15 Hz and above 15 Hz.

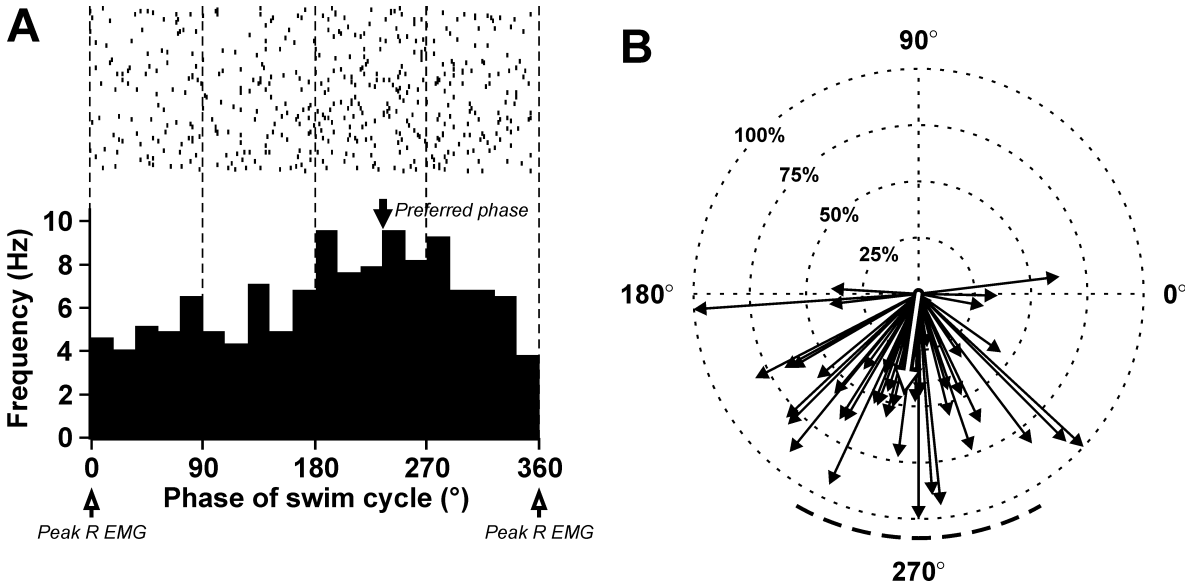

FIG. 4. (A) An example of modulation of activity of an RS neuron during swimming. A phase histogram and raster of activity of a right RS neuron are shown (0° corresponds to the peak of right EMG). Preferred phase was 234°, modulation coefficient was 58%. (B) Distribution of preferred phases and modulation coefficients of RS neurons over the locomotor cycle. Each vector represents one neuron. The direction of the vector represents the preferred phase, and the vector length indicates the modulation coefficient. The peak of the EMG in ipsilateral muscles of segment 25 was taken as the cycle onset. The dashed arc indicates the estimated position of the EMG burst in the ipsilateral muscles of segment 1. The white vector is the average for the whole population.

performed small undulatory body movements, tail movements, etc. Usually, in a few seconds, the animal was able to find the entrance to the other limb of the tunnel and produced a single forward crawling movement from the long limb into the short one. The backward movement was carried out in a similar fashion but the anterior part of the animal was first positioned in the short limb of the tunnel (as in Fig. 2A, position *g*). Before the backward crawling, the animal tried to find the way out by moving its head in different directions; then these movements abruptly stopped and the backward crawling occurred. After testing the crawling with the right turn (as in Fig. 2A), the crawling with the left turn was tested. The crawling proceeded at relatively constant speed and could take from 1.5 to 5.9 s for forward crawling (3.4 ± 1.2 s) and from 2.1 to 5.4 s for backward crawling (3.7 ± 1.0 s).

Of all 71 recorded neurons, 14 were not active during crawling episodes as well as before and after them. They were excluded from the analysis. The mean frequencies of firing during crawling for the other 57 neurons were spread over a range from 0.3 Hz to 37 Hz, with the population average being 4.1 ± 5.8 Hz. The distributions of frequencies during crawling in four different directions are shown in Fig. 5. They did not differ significantly from each other ($P > 0.5$, Kolmogorov–Smirnov test). Similarly, for each of the four crawling directions, the distribution of firing frequencies during crawling did not differ from the distributions of the background firing frequencies before and after crawling ($P > 0.5$, Kolmogorov–Smirnov test). The background frequencies ranged from 0 Hz to 30 Hz, with the population average 4.1 ± 5.1 Hz. These data suggest that the average level of activity of the population of RS neurons can not be used for encoding the direction of crawling, and the activity of individual RS neurons must be analysed.

Comparison of firing frequencies of individual RS neurons during crawling in different directions was hampered by a large variation of the activity among trials (up to fourfold, see for example, Fig. 6A). On the other hand, the changes of the activity in relation to the background were very consistent. Figure 6 shows two representative examples of the activity changes during crawling. In Fig. 6A, an RS

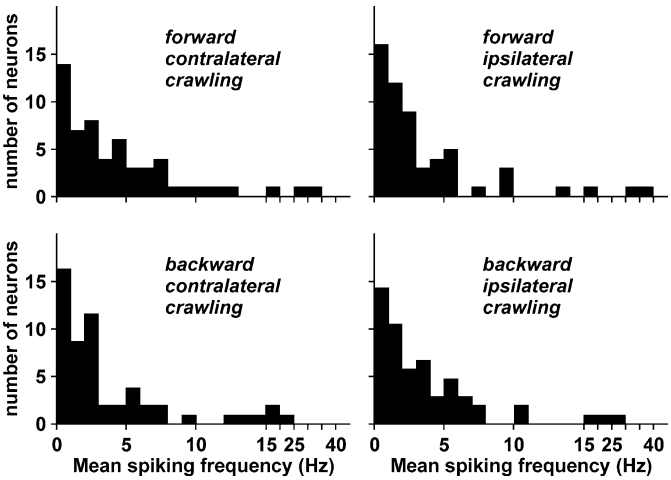

FIG. 5. Distribution of mean frequencies of RS neurons during crawling in four directions, i.e. forward ipsilateral to the neuron, forward contralateral, backward ipsilateral, backward contralateral. There was no statistically significant difference between any two of the distributions.

neuron fired at the frequency of approximately 1 Hz before and after crawling. At the beginning of crawling, the firing frequency sharply increased and remained high (from 2 to 8 Hz in different trials) during the whole episode. In another example, presented in Fig. 6B, an RS neuron was active in all five trials before crawling and in three trials after crawling. During crawling, it was not active. The mean frequency during crawling was compared with that during adjacent 2-s intervals ('background' activity). A statistically significant ($P < 0.01$, paired *t*-test) increase of activity during crawling in relation to the background was observed in 75 cases out of 228 (57 neurons × 4 directions of crawling = 228 cases all together), and a decrease was seen in 60 cases. Of all 57 neurons, 45 neurons changed their activity during crawling in at least one direction. The increase averaged

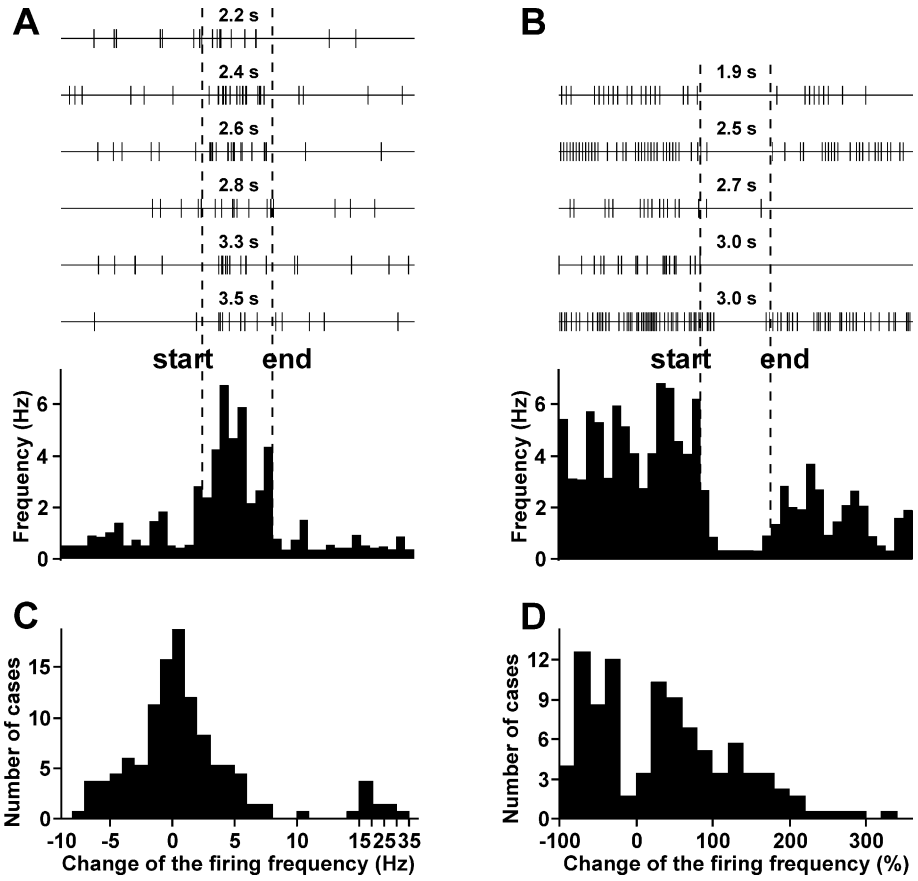

FIG. 6. (A) An example of the RS neuron, which was more active during forward left crawling than before and after it. Each raster represents activity of the neuron during one trial. The histogram gives the firing frequency averaged across all these trials. Time scales are normalized to the duration of the crawling episodes. The actual duration of each episode is indicated above the corresponding raster. Starts and ends of crawling are indicated with dashed vertical lines. (B) An example of the RS neuron, which was inhibited during forward left crawling. (C and D) Absolute (C) and relative (D) changes of the firing frequency of RS neurons during crawling in relation to the average activity before and after crawling.

4.4 ± 6.6 Hz (115 ± 69% in relation to the background), while the decrease averaged 2.8 ± 2.1 Hz (54 ± 22%; Fig. 6C and D).

These changes in activity of RS neurons during crawling were specific for different crawling directions. Figure 7 provides an example of the RS neuron that was almost silent during right and left forward crawling, and increased its activity during right and left backward crawling. Each recorded neuron was characterized by a qualitative pattern, that is a combination of signs of changes of its activity during crawling in different directions. Figure 8 summarizes these patterns for all 57 neurons. Altogether, 16 different patterns were observed. These patterns were grouped according to their ability to discriminate different crawling directions.

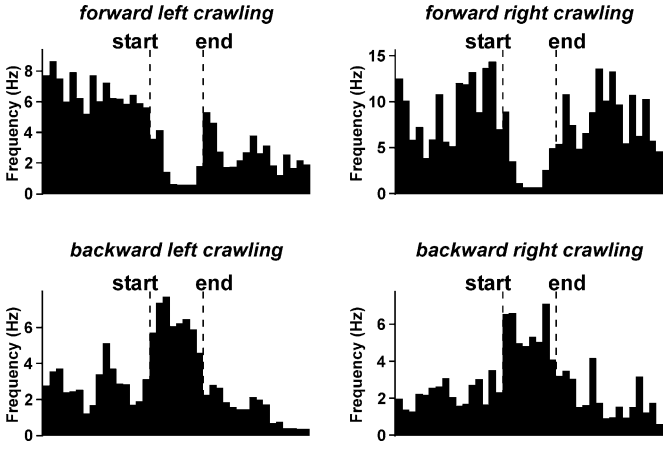

FIG. 7. An example of activity of an RS neuron during turns in four different directions. This neuron was activated during backward crawling and inhibited during forward crawling.

Neurons of group A ($n = 21$) did not discriminate crawling directions. This group includes the neurons that were activated, inhibited, or not affected during crawling in any direction.

Neurons of group B ($n = 24$) did not discriminate the direction of turn during crawling but discriminated forward/backward direction of crawling. Subgroup B1 includes the neurons that were activated during forward crawling and not affected or inhibited during backward crawling. Subgroup B2 includes the neurons that were activated during backward crawling, inhibited during forward crawling, or displayed both excitation during backward crawling and inhibition during forward crawling.

Finally, neurons of group C ($n = 12$) discriminated not only the forward/backward direction of crawling but also the direction of turn. The neurons of subgroup C1 were activated during forward crawling with ipsilateral turn. The neurons of subgroup C2 were activated during backward crawling with ipsilateral turn and inhibited during crawling in all other directions. Subgroup C3 includes six neurons with diverse patterns, but with a common feature i.e. activation during backward crawling with contralateral turn.

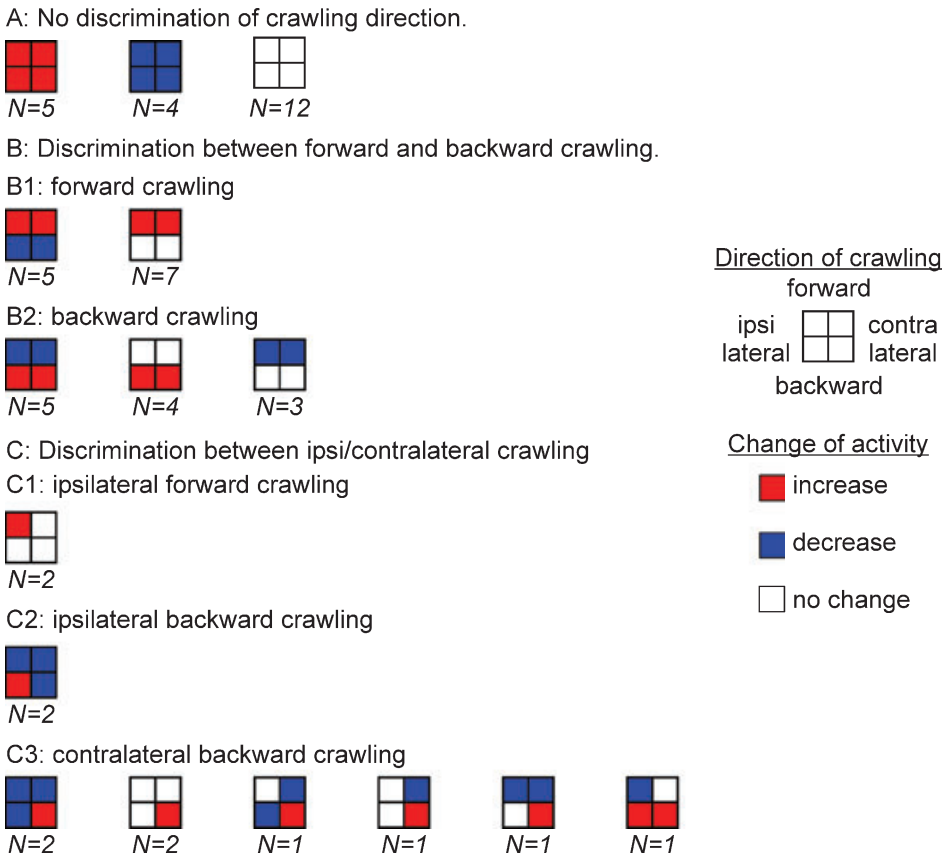

FIG. 8. Changes of activity of RS neurons during crawling. Each neuron was characterized by a qualitative pattern, that is a combination of signs of changes of its activity (increase, decrease, no change) during turns in different directions. The patterns were classified into three groups (A–C) according to their ability to discriminate between different crawling directions. Numbers below each diagram show the numbers of recorded RS neurons with a given pattern.

Responses of RS neurons to sensory stimuli

The majority of RS neurons (53 out of 71) were excited by tactile stimuli like neurons RS#2 and RS#3 in Fig. 3A. Most of them (45 out of 53) responded to stimuli applied to any part of the body. Only three neurons responded to stimulation of the head and did not respond to stimulation of the rest of the body. Five neurons did not respond to stimulation of the head but responded to stimulation of the other parts of the body.

Thirty-two neurons responded to illumination of eyes. Fifty-three neurons responded to illumination of tail photoreceptors. Twenty-four of them responded both to the eyes and the tail illumination.

Vestibular stimuli produced responses in 53 neurons. Figure 9 illustrates vestibular responses of an RS neuron that was activated by turns in all three planes. The neuron responded preferentially to left (contralateral) roll tilts (Fig. 9A), nose-up pitch tilts (Fig. 9B), and left (contralateral) yaw turns (Fig. 9C). Besides the neurons that responded to turns in all three planes, there were neurons that responded to turns in only one plane, or to turns in two planes. The proportions of the RS neurons that responded to different combinations of turns are presented in Fig. 10A. Each grey circle represents the neurons that responded to turns in one plane. The intersections of two circles represent those neurons that responded to turns in two planes. The intersection of all three circles represents the neurons that responded to turns in all three planes. The majority of neurons (68%) responded to yaw turns. However, many of them responded equally well to the right and left turns. The neurons that stabilize the animal's orientation in a certain plane should have a unidirectional response, for example they should respond preferentially to nose-up pitch tilt but not to nose-down tilt. The proportions of the neurons that responded in such a unidirectional way are presented in Fig. 10B. Such neurons were rather equally distributed among the groups. The unidirectional pitch responses were the most frequent (55% of all neurons), while unidirectional roll and yaw responses were seen in 37% and 29% of all neurons, correspondingly.

No correlation between tactile, visual and vestibular inputs of RS neurons and their activity during crawling in different directions was found. Of the three neurons that responded to tactile stimulation of the head and did not respond to tactile stimulation of the tail, two belonged to the crawling group A, and one to the subgroup B1. Of the five neurons that responded to tail stimuli and did not respond to head stimuli, one neuron belonged to the group A, two to the subgroup B1, and the other two to the subgroup C3. In a similar fashion, the neurons that responded preferentially to visual and vestibular stimuli had representatives in all crawling subgroups and no bias was seen.

Discussion

Functional role of RS neurons during swimming

In the present study, the activity of individual RS neurons during locomotion of intact lampreys has been characterized for the first time. This study has shown that many RS neurons are active during two forms of locomotion – swimming and crawling.

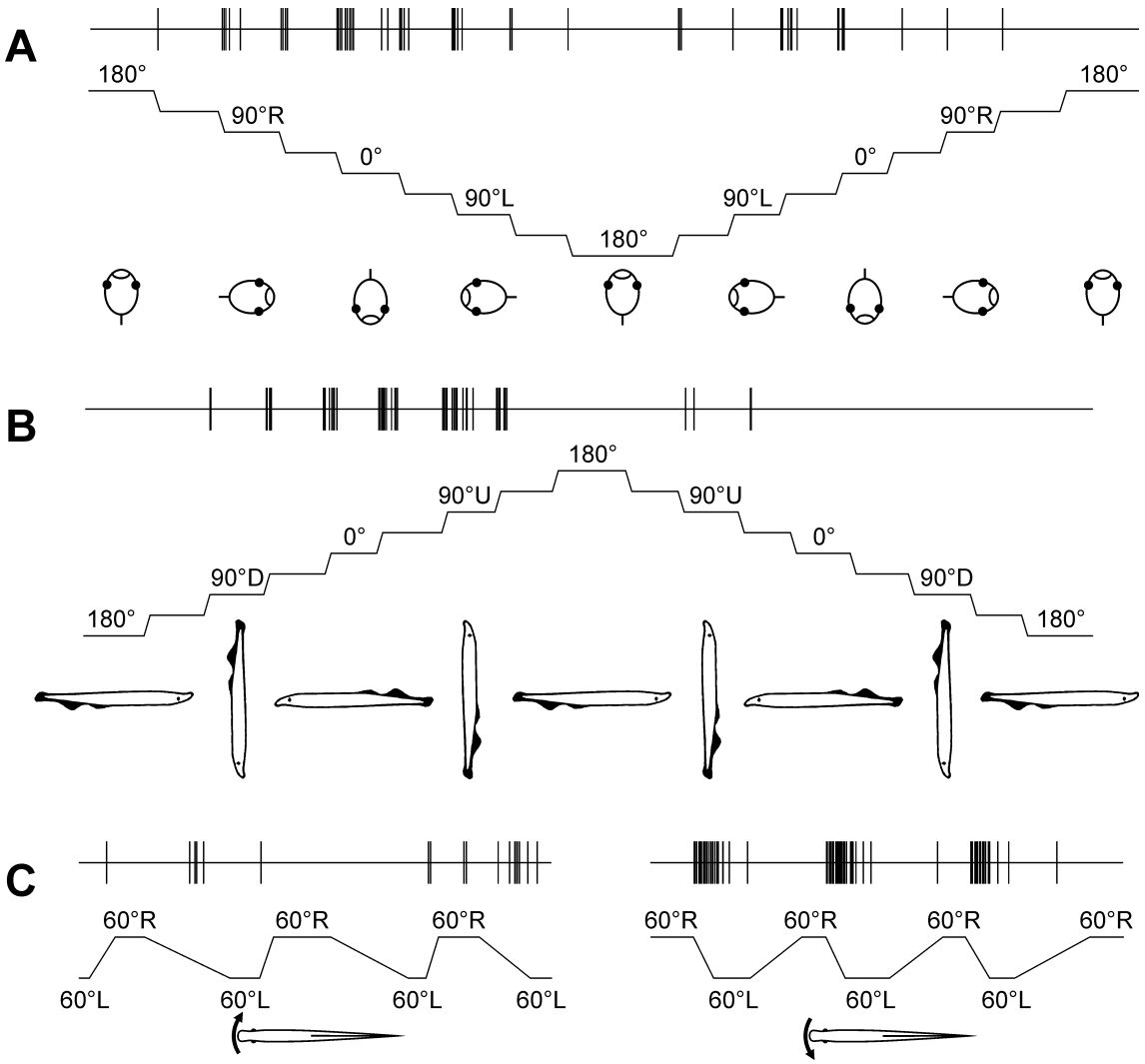

FIG. 9. An example of a right RS neuron that responds to turns in the roll (A), pitch (B) and yaw (C) planes. Two full turns in opposite directions were performed in the roll and pitch planes. The yaw stimuli were right and left turns with the peak-to-peak value of 120°. In one yaw test the right turns were fast, while left ones were slow. In the other yaw test the fast left turns and slow right turns were used. The tilt angles are indicated (R, right; L, left; U, up; D, down). The angle of 0° corresponds to the normal orientation (dorsal side up). Successive positions in 90° steps in relation to the direction of gravity force are schematically shown in A and B. The curved arrows in C indicate the direction of the fast yaw rotation.

During forward swimming, all recorded RS neurons were activated. This fact can be interpreted in two different ways. First, it may mean that each activated RS neuron contributes to the activation of the spinal networks responsible for swimming. Second, it is possible that only a part of the neurons participate in the activation of swimming while the activity of the other neurons is increased to occur within their 'working range' during swimming. The increased firing frequency of these RS neurons may be necessary to produce noticeable effects upon the spinal networks, and to have a possibility for both up- and down-regulation of these effects. Indirect evidence for this second alternative is the high percentage (66%) of the neurons observed in the present study that displayed direction-sensitive vestibular responses. Such neurons produce specific motor effects that counteract the changes of the animal's posture (Zelenin *et al*., 2004). These RS neurons might not be necessary for activation of locomotion, but their role in stabilization of the body orientation presumes their activation during swimming. A possible way to clarify the functional role of the RS neurons during swimming is to correlate their activity with changes of the ongoing swimming movements, for example, changes of speed, dorsal and ventral turns, lateral turns to the right or left, roll turns. The search for such correlations will be the goal of our future studies.

During swimming, the firing rate of RS neurons was relatively low (approximately 5 Hz on average). Similar firing frequencies of RS neurons were observed in the lamprey during swimming evoked by stimulation of the mesencephalic locomotor region in semi-intact preparation (Sirota *et al*., 2000; Brocard & Dubuc, 2003). These frequencies are much lower than those observed in RS neurons in higher vertebrates (Drew *et al*., 1986; Buford & Davidson, 2004). These low firing rates in lampreys seem to be compensated for by stronger influences of individual neurons upon the spinal networks. It was shown that one spike of an RS neuron can evoke large synaptic responses in spinal neurons (Rovainen, 1974; Buchanan & Cohen, 1982; Ohta & Grillner, 1989) and increase or decrease the mean activity of motoneurons by more than 10% (Zelenin *et al*., 2001). In contrast, in mammals, the motor effects of single RS neurons are so small that they have not yet been detected.

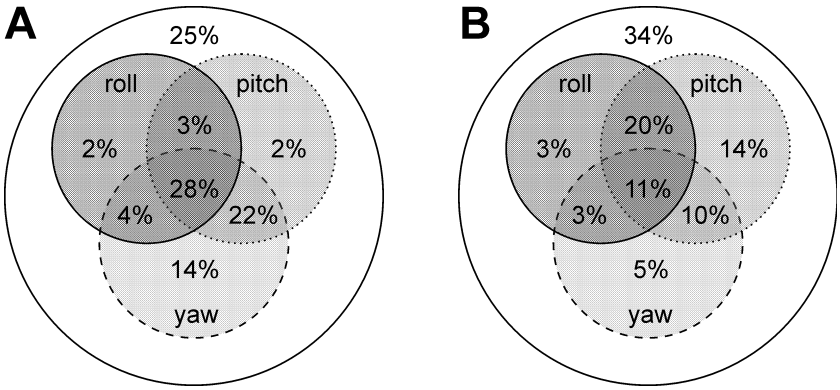

FIG. 10. Proportions of RS neurons responding to rotation in different planes. Three grey circles demarcated by the solid, dotted and dashed lines represent the populations of RS neurons that respond to roll, pitch and yaw, respectively, while overlapping parts of these circles represent RS neurons that respond to turns in two or three different planes. The white circle represents all recorded neurons, while its part that lies outside the grey circles represents the neurons that did not respond to vestibular stimuli. (A) Proportions of the neurons with any kind of response to the vestibular stimuli. (B) Proportions of the neurons, with only unidirectional responses taken into account, e.g. a neuron responds to right roll tilt but does not respond to left roll tilt. Such neurons are presumably involved in stabilization of the body posture and steering.

The results of the present study have confirmed previous observations that for the majority of RS neurons, their activity is modulated by the locomotor rhythm both *in vitro* (Kasicki *et al*., 1989) and *in vivo* (Deliagina *et al*., 2000). The modulation pattern has one peak per cycle. The preferred phases of firing were clustered around the peak of activity of ipsilateral muscles in the first spinal segments. A similar position of the preferred phase was observed during fictive swimming. It has been proposed that the phasic modulation of RS neurons coordinates the commands transmitted by these neurons with the ongoing locomotor movements (Kasicki *et al*., 1989). However, it may be noteworthy that only two of 43 modulated neurons completely stopped firing between bursts, the others displayed some 'background' activity throughout the swimming cycle. This persistence of the activity in the majority of RS neurons leaves a possibility not only to up-regulate but also to down-regulate the RS commands in any phase of the locomotor cycle.

Role of the RS neurons in crawling and possible mechanisms of crawling

The other form of locomotion examined in the present study was the nonundulatory crawling in a narrow tunnel. This mode of locomotion is probably used in natural habitats for moving about or out of tight places. It is typically performed as a sharp right or left lateral turn around a support (Fig. 2A). In contrast to the reciprocal activity of muscles in any point along the body during swimming, the motor pattern used for crawling is characterized by (i) muscle activity localized near the curved part of the body, and (ii) coactivation of the right and left muscles (Archambault *et al*., 2001). In the present work, it was found that the activity of many RS neurons increased or decreased during crawling episodes. This suggests that a tonic 'crawl'-command is sent to all parts of the body, while the local spinal networks execute this command depending on the local body curvature. Such a mechanism would also explain why the activity of many RS neurons does not depend on the direction (right or left) of the crawling turn. It may be that the command for crawling is just 'crawl forward' or 'crawl backward' while the turn direction is determined by the local body curvature and will be different if several supports are provided to the animal in different parts of its body.

As shown in previous work, the body configuration can vary between different crawling episodes and it can be different in different areas along the body, yet the EMG patterns are similar across trials and at different segmental levels (Archambault *et al*., 2001). For example, the bent part of the body can contact with the inner wall, or with the outer wall, or with neither of these walls. Such changes in body configuration exert a strong effect upon the tactile input, but they do not change the EMG pattern. These observations make unlikely the hypothesis that tactile information is used for localization of the bent part of the body. Alternatively, the proprioceptive information may be used; it can be provided by the stretch receptor cells located in the lateral margins of the lamprey spinal cord (Grillner *et al*., 1984). These cells are activated when the lateral part of the spinal cord is stretched due to lateral bending of the body. In quiescent spinal cord, the stretch receptors elicit monosynaptic excitation in ipsilateral spinal neurons and monosynaptic inhibition in contralateral ones (Viana Di Prisco *et al*., 1990). However, their functional connections during crawling remain to be explored. The stretch receptors are probably unable to sense dorsal and ventral body flexions. If so, this may explain the fact that the crawling movements were lateral and never included dorsal or ventral flexions.

There is no definite answer to the question whether the same or different RS neurons are responsible for activation of swimming and crawling. Of the population of RS neurons that were active during swimming, some were activated and others were inactivated during crawling. One may speculate that the RS neurons activated during crawling transmit specifically (or predominantly) the crawling command while those inhibited during crawling are responsible for the activation of swimming and do not help or even interfere with the activation of crawling.

An important difference between swimming and crawling motor patterns is the reciprocal activity of right and left muscle during swimming and their coactivation during crawling. One can suppose that the RS neurons involved in activation of crawling, inhibit the inhibitory spinal interneurons that ensure reciprocal activity of the right and left parts of the spinal network (Buchanan, 1982; Cohen & Harris-Warrick, 1984; Ohta *et al*., 1991; Buchanan & McPherson, 1995) while the 'swimming' RS neurons excite these interneurons. Another possible target for the 'crawling' RS neurons are the stretch receptors. If these receptors are important for crawling, one can expect that the synapses from them to the other spinal neurons are potentiated by the 'crawling' RS neurons.

Role of sensory inputs to RS neurons

Forward and backward crawling can be used to avoid unwanted tactile and visual stimuli presented to the caudal and rostral parts of the body, correspondingly. Therefore, it was reasonable to expect that the RS neurons that are activated during forward crawling, respond to tactile and visual stimulation of the tail while those activated during backward crawling, respond to tactile stimulation of the head and illumination of the eyes. However, no such relationship was observed. It may be that the RS neurons that activate the spinal networks responsible for crawling, respond specifically only to strong tactile or even painful stimuli that were not used in this study.

Approximately two-thirds of the RS neurons recorded in this study had vestibular inputs that suggest their involvement in the control of posture and steering. The majority of the neurons, tested by rotation in the roll, pitch, and yaw planes, responded to turns in more than one plane. This could be due to a complex pattern of responses of vestibular afferents to turns in different planes (Deliagina *et al*., 1992) and to convergence of different vestibular inputs upon RS neurons. This also corresponds well to the functional projections of RS neurons observed in the previous study; the majority of the neurons had projection patterns that produced a combined turn in more than one plane (Zelenin *et al*., 2001).

There was no correlation between vestibular inputs of RS neurons and their activity during crawling. This may be because the postural corrections based on vestibular information are not necessary during crawling in tight places. One can expect that during crawling the vestibular input to the RS neurons is suppressed, and the spinal networks, used for postural corrections during swimming, may be not active during crawling so that the influences of 'postural' RS neurons are not effective or produce different effects.

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Abbreviations

EMG, electromyogram; RS, reticulospinal.

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