Activity of Reticulospinal Neurons During Locomotion in the Freely Behaving Lamprey

T. G. DELIAGINA,[1,2] P. V. ZELENIN,[1,2] P. FAGERSTEDT,[1] S. GRILLNER,[1] AND G. N. ORLOVSKY[1]
[1]*The Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institute, SE-171 77 Stockholm, Sweden; and* [2]*A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119 899, Russia*

Deliagina, T. G., P. V. Zelenin, P. Fagerstedt, S. Grillner, and G. N. Orlovsky. Activity of reticulospinal neurons during locomotion in the freely behaving lamprey. *J. Neurophysiol.* 83: 853–863, 2000. The reticulospinal (RS) system is the main descending system transmitting commands from the brain to the spinal cord in the lamprey. It is responsible for initiation of locomotion, steering, and equilibrium control. In the present study, we characterize the commands that are sent by the brain to the spinal cord in intact animals via the reticulospinal pathways during locomotion. We have developed a method for recording the activity of larger RS axons in the spinal cord in freely behaving lampreys by means of chronically implanted macroelectrodes. In this paper, the mass activity in the right and left RS pathways is described and the correlations of this activity with different aspects of locomotion are discussed. In quiescent animals, the RS neurons had a low level of activity. A mild activation of RS neurons occurred in response to different sensory stimuli. Unilateral eye illumination evoked activation of the ipsilateral RS neurons. Unilateral illumination of the tail dermal photoreceptors evoked bilateral activation of RS neurons. Water vibration also evoked bilateral activation of RS neurons. Roll tilt evoked activation of the contralateral RS neurons. With longer or more intense sensory stimulation of any modality and laterality, a sharp, massive bilateral activation of the RS system occurred, and the animal started to swim. This high activity of RS neurons and swimming could last for many seconds after termination of the stimulus. There was a positive correlation between the level of activity of RS system and the intensity of locomotion. An asymmetry in the mass activity on the left and right sides occurred during lateral turns with a 30% prevalence (on average) for the ipsilateral side. Rhythmic modulation of the activity in RS pathways, related to the locomotor cycle, often was observed, with its peak coinciding with the electromyographic (EMG) burst in the ipsilateral rostral myotomes. The pattern of vestibular response of RS neurons observed in the quiescent state, that is, activation with contralateral roll tilt, was preserved during locomotion. In addition, an inhibition of their activity with ipsilateral tilt was clearly seen. In the cases when the activity of individual neurons could be traced during swimming, it was found that rhythmic modulation of their firing rate was superimposed on their tonic firing or on their vestibular responses. In conclusion, different aspects of locomotor activity—initiation and termination, vigor of locomotion, steering and equilibrium control—are well reflected in the mass activity of the larger RS neurons.

INTRODUCTION

The reticulospinal (RS) system plays a predominant role in the control of posture and locomotion in all vertebrates. This paper describes the activity of the RS system during locomotion in the lamprey, a phylogenetically ancient vertebrate. The lamprey is used extensively as an animal model for studying basic neural mechanisms controlling motor behavior (see e.g., Grillner et al. 1995; Wallén et al. 1992). In the lamprey, the RS system is the main descending system projecting from the brain stem to all parts of the spinal cord. The vestibulospinal system is much less developed, projecting only to the anterior part of the spinal cord (Ronan 1989; Rovainen 1979). In principle, signals from the brain to the spinal cord also can be transmitted in part by propriospinal systems (see e.g., Shik 1993) but the knowledge on these systems in the lamprey is rather limited (see, however, Rouse and McClellan 1997).

The RS system in the lamprey is composed of several hundred neurons located in the following four bilateral reticular nuclei of the brain stem: the mesencephalic reticular nucleus (MRN) and the anterior, middle, and posterior rhombencephalic reticular nuclei (ARRN, MRRN, and PRRN, respectively) (Bussiéres 1994; Nieuwenhuys 1972; Ronan 1989; Swain et al. 1993). The majority of the RS neurons are glutamatergic (Brodin et al. 1988; Ohta and Grillner 1989). They exert an excitatory action on their targets throughout the whole extent of the spinal cord and thus activate the segmental locomotor networks to initiate swimming. These studies also suggest that the intensity of locomotion is determined by the degree of activation of RS neurons (Grillner et al. 1995; McClellan and Grillner 1984; Ohta and Grillner 1989).

When swimming, the lamprey performs numerous maneuvers related to steering and equilibrium control. These modifications of the locomotor pattern are most likely caused by the corresponding modifications of the activity of RS system. All sensory inputs (vestibular, visual, somatosensory), affecting postural orientation and steering in the swimming lamprey, strongly modify the activity of RS neurons (Deliagina et al. 1992, 1993, 1995; Ullén et al. 1996, 1998). In addition, many RS neurons exhibit rhythmical modulation in response to "efference copy" signals arriving from the spinal cord during swimming (Kasicki et al. 1989; Vinay and Grillner 1992) and therefore are involved in an internal feedback loop. The RS system thus can be considered as a multifunctional system, affecting different aspects of the locomotor activity of the lamprey.

Information on the activity of RS system in the lamprey has been obtained mainly in in vitro experiments, using preparation of the isolated brain stem, with or without the spinal cord, in which many inputs to RS neurons were eliminated. A goal of the present study was to investigate the activity of RS system in the behaving intact lamprey.

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When studying the activity of central neurons in intact mammals, the bony skull usually is used as a rigid platform for securing the recording microelectrode (see e.g., Drew et al. 1986). In the lamprey, however, this method is difficult to apply because the cartilage tissue surrounding the brain is comparatively soft. To overcome these difficulties, we have developed a novel method for recording activity of RS neurons from their axons by means of macroelectrodes implanted in close proximity to the spinal cord.

The following problems are considered in the present paper: *1*) correlations between the mass activity of RS system and the locomotor activity elicited by different sensory inputs. It is known that swimming in the lamprey can be evoked by a number of physiological stimuli—by tactile stimulation of different parts of the body (McClellan 1984, 1988; McClellan and Grillner 1983), by eye illumination (Ullén et al. 1993b), by illumination of the tail region where dermal photoreceptors are located (Ullén et al. 1993b), by water vibration (Currie 1991), and by vestibular stimulation, that is, inclination of the animal (Orlovsky et al. 1992). These different types of sensory stimuli were employed in the present study. *2*) Modifications of the mass RS activity related to changes in the intensity of locomotion and to the changes in the animal's orientation. *3*) Reflection of different aspects of locomotor control in the activity of individual RS neurons. Activity of individual RS neurons in the intact nonswimming lamprey is considered in the accompanying paper (Deliagina and Fagerstedt 2000).

Brief accounts of this study have been published in abstract form (Deliagina et al. 1997; Orlovsky et al. 1997).

METHODS

Experiments were carried out on 10 adult (25–35 cm in length) intact lampreys (*Lampetra fluviatilis*), which were kept in an aerated freshwater aquarium at 7°C, with a 12 h:12 h light:dark cycle.

Electrodes

The activity of RS neurons was recorded from their axons in the spinal cord by means of chronically implanted electrodes. The idea underlying this novel method was to use not micro- but macroelectrodes (thin wires) oriented in parallel to the long spinal axons (Fig. 1*A*). If the length of the electrode is close to the longitudinal extent of the axonal membrane excited by the propagating action potential, the whole electrode occurs positioned in approximately equipotential points in the moment when the excited membrane area opposes the electrode (Fig. 1*B*). No current will flow along the electrode, and it will record the extraaxonal potential with the same amplitude as a microelectrode positioned at the same distance from the axon. An advantage of the wire electrode is its mechanical stability, a very low resistance ($<10^3\Omega$), and a low noise level (a few microvolts). In a thinner axon (Fig. 1*C*), the excited part of the fiber will be shorter, the electrode will be positioned along points with different potentials, and a considerable shunting effect will be caused by currents flowing along the electrode. In addition to this effect, thinner axons provide smaller membrane currents as compared with the thicker axons. Thus the wire electrode can serve as a filter for recording spikes almost exclusively from the larger fibers situated parallel with the electrode.

The large fibers in the lamprey spinal cord are the RS axons of the Mauthner cells and Müller cells from MRN, ARRN, and MRRN; the middle-size fibers are the RS axons originating from MRRN and PRRN (Fig. 1*D*) (Ohta and Grillner 1989; Rovainen 1982). Most of these fibers have conduction velocity ranging from 2 to 5 m/s (Ohta and Grillner 1989; Rovainen 1978). With a spike duration in these

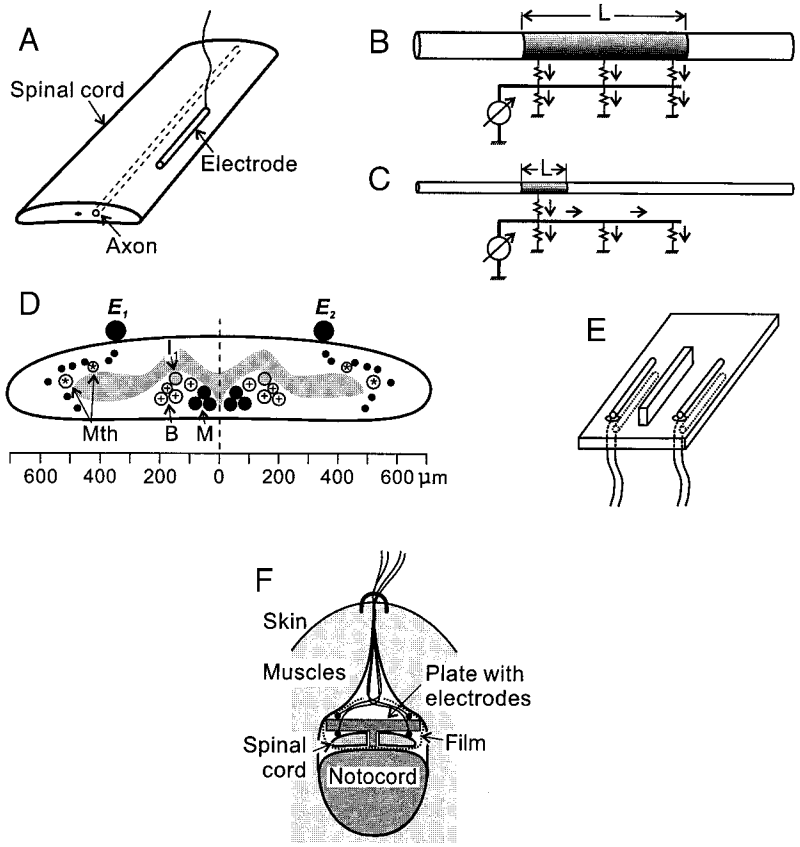

FIG. 1. Macroelectrodes for recording activity of reticulospinal (RS) axons. *A*: orientation of the an electrode on the dorsal surface of the spinal cord in relation to the position of the RS axon. *B* and *C*: wire electrode serves as a filter for recording spikes almost exclusively from the larger fibers. Length of the electrode (3 mm) was approximately equal to the longitudinal extent of the axonal membrane excited by the propagating spike (L) in the thicker RS axons (*B*). For the thinner axons, L was smaller and the efficiency of recording was reduced because of shunting (*C*). Arrows in *B* and *C* indicate extraaxonal currents. *D*: position of the electrodes (E_1 and E_2) in relation to the spinal cord. Location of the large-size RS axons of Mauthner cells (Mth), Müller cells (B, M, and I types), and middle-size axons (small black circles) on the cross-section of the spinal cord is shown schematically. *E*: design of electrodes, view from below. Four silver wires (75 μm diam and 3 mm length) were glued to a plastic plate with a longitudinal wall. *F*: position of the implanted plate with electrodes as seen on the transversal section of the lamprey's body. Plate with the electrodes together with the adjoining segments of the spinal cord were isolated electrically from the surrounding muscles by a strip of thin (20 μm) plastic film.

axons equal to ~1 ms, the length of the excited axonal segment will be 2–4 mm. To record from these axons, we used electrodes with a length of 3 mm. They were made of 75-μm silver wire coated with a Teflon insulation, except for at their 3-mm-long active part. This part was glued to a plastic plate, 6 mm long and 0.25 mm thick (Fig. 1*E*). The width of the plate was equal to or slightly larger than that of the spinal cord (1.7–2 mm). Similar wires were glued to the opposite side of the plate. This allowed us, by using a bipolar recording technique and differential amplifiers, to substantially reduce the artifacts caused by the electrical activity of the surrounding muscles. The thin silver wires were then soldered to thicker (0.25 mm), flexible, Teflon-coated copper wires that conveyed signals to the amplifier input cable.

The distance between the left and right electrodes was chosen so that each of them occurred positioned above the center of the corresponding half of the spinal cord (Fig. 1*D*). Experiments described in the accompanying paper (Deliagina and Fagerstedt 2000) have shown that the electrodes can record activity in the larger axons at a distance of ≤400 μm. With a width of the spinal cord of ~1,500 μm (Fig. 1*D*), one can suggest that each of the electrodes will record activity of the larger axons over almost the whole cross-section of the ipsilateral half of the spinal cord.

To record separately from the left and the right RS pathways, a longitudinal plastic wall was glued to the plate with the electrodes (Fig. 1*E*). This wall then was positioned in the split made between the two halves of the spinal cord, and thus the left and right RS pathways were isolated electrically from each other in the area of the electrode. In most experiments, this isolation was practically complete, and each electrode recorded exclusively the ipsilateral RS activity. In some experiments, however, the isolation was incomplete and secured only a 5- to 10-fold reduction of the amplitude of the contralateral spikes (see e.g., Fig. 9*A*).

Implantation of electrodes

Implantation of the electrodes was performed under MS-222 (Sandoz) anesthesia (100 mg/l). The animal was positioned in the bath with the anesthetic solution that covered the gills whereas the surgical field was above the water level. The plate with electrodes was implanted at the level of the last gill through a longitudinal cut performed along the midline of the dorsal aspect of the body so that the spinal cord was exposed. The layer of fat covering the spinal cord was removed, and the midline split of the spinal cord extending for ~5 mm was performed. The plate was positioned on the dorsal surface of the spinal cord, with the longitudinal wall inserted into the split (Fig. 1*F*). In addition to the differential recording technique (see preceding text), two more methods were used to reduce the artifacts caused by the electrical activity of the muscles surrounding the electrodes. First, in all experiments, before implanting the electrodes, the dorsal and ventral roots were cut bilaterally throughout 10–15 spinal segments, symmetrically in relation to the site of electrode implantation, and the corresponding myotomes thus were denervated. We did not observe any significant changes of locomotor performance after such surgery. Second, in five experiments, the implanted plate with the electrodes, together with the adjoining segments of the spinal cord, were wrapped in a strip of thin (20 μm) plastic film (Fig. 1*F*) to isolate the electrodes electrically from the surrounding muscles. An indication that there was no electromyographic (EMG) contamination was the absence of any voltage when recording, during vigorous swimming, between the left and right electrodes facing dorsally (Fig. 1*F*). The wound was then closed and sutured so that the flexible copper wires were fixed tightly between the two sides of the wound (Fig. 1*F*).

In a few experiments, an additional plate with electrodes was implanted at the level of the third gill, so that the distance between the two plates was 20–30 mm. This allowed us, by comparing the moment in time of spike occurrence in the two electrodes, to determine the direction of spike propagation in the axons and to calculate their conduction velocity (for details, see Deliagina and Fagerstedt 2000).

Experimental setup

In most experiments, the lamprey was freely moving in a shallow aquarium (80 × 80 or 50 × 30 cm) (Fig. 2*A*). Movements of the animal were videorecorded (25 frames/s). The implanted electrodes for recording the activity in the RS pathways were connected, via a long flexible cable, to the inputs of AC amplifiers. Via the same cable, two bipolar EMG electrodes (flexible wires, 150 μm diam, implanted bilaterally in the myotomes of the gill region) also were connected to the amplifier inputs. The electrophysiological and video recordings were synchronized by pulses (1 Hz) recorded simultaneously by both systems (Fig. 2*A*, *Synchro*). Different sensory stimuli were used to evoke locomotion—pinching the head or the tail, illumination of the eye or tail by means of a fiber optic system (90 W), or water vibration (50 Hz) produced in a close proximity to the head of lamprey.

In some experiments, a special setup was used that allowed us to apply vestibular stimuli during the locomotor-like activity of the lamprey (Fig. 2*B*). The animal was mounted on a platform so that a part of its body (~20% of the total length), including the denervated segments, was fixed gently between the two plates of the holder. This part of the body thus was immobilized, whereas the anterior and posterior parts could move. The locomotor-like activity appeared either spontaneously or it could be evoked by tactile stimulation. During this activity, the anterior and posterior parts of the body performed periodical lateral undulations characteristic of swimming,

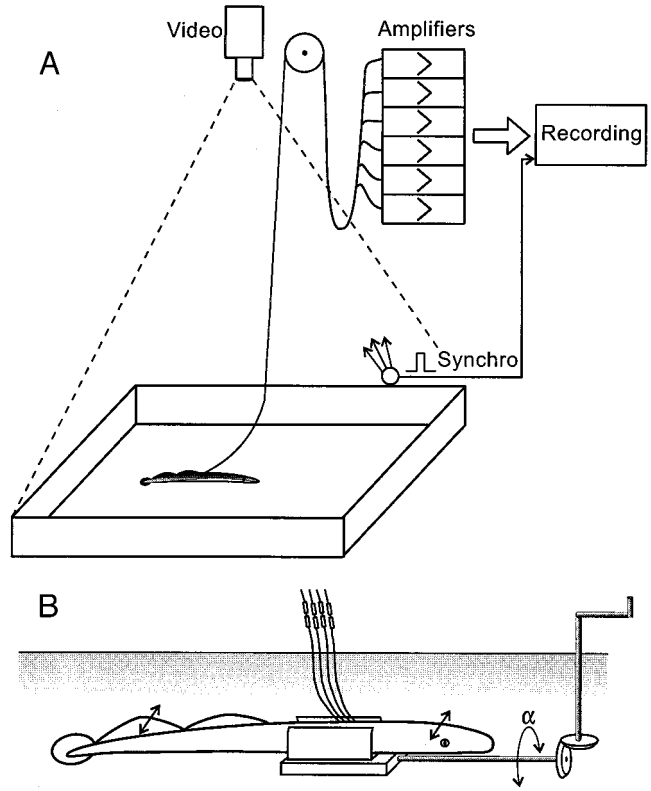

FIG. 2. Experimental arrangements. *A*: arrangement for simultaneous recordings of neuronal activity, electromyograms (EMGs), and movements in the freely behaving animal. Electrophysiological recordings and the video recordings were synchronized by pulses (1 Hz) recorded simultaneously by both systems (*Synchro*). *B*: arrangement for recording vestibular responses in RS neurons during swimming. Central, denervated region of the body was fixed in the experimental devise. Lamprey could perform locomotor-like undulatory movements by its anterior and posterior body parts (indicated by arrows). Device could be rotated manually (α, roll tilt angle).

as shown by the bilateral arrows in Fig. 2*B*. Because of the fixation of the midbody area, the lamprey was not able to produce a roll tilt by itself, but the tilt could be imposed by rotating the platform manually. Usually alternating 90° tilts to the right and to the left were performed. In animals exhibiting locomotor-like activity, the body was rigid enough so that an imposed tilt caused an inclination in all parts of the body. In quiescent animals, additional mechanical restraints, connected to the rotating platform, allowed a transmission of rotation to all parts of the body (see also Deliagina and Fagerstedt 2000).

Processing of data

Signals from the implanted electrodes were amplified by conventional AC amplifies, 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 mass RS activity (multiunit spike trains) then was presented in the form of temporal histograms (100, 200, or 500 ms binwidth) using software analysis (Datapac III, Run Technologies, Laguna Hills, CA). Small waveforms were excluded from the analysis by adjusting the threshold to a level slightly above the noise level.

Videorecordings were analyzed frame by frame, and the behavioral state of the animal was classified into one of the three categories: attached to a substrate, lateral turn (left or right), and rectilinear swimming. By using the synchronizing markers, electrophysiological recordings were aligned with data obtained from videorecordings.

RESULTS

Activity of spinal axons in nonswimming and swimming animals

Most of the time the lampreys can be found in a quiescent state, attached to the bottom or walls of the aquarium by their sucker mouth. The activity in spinal axons, recorded in the attached animals, was rather low (Fig. 3*A*, beginning of the recording). Sensory stimuli could evoke an increase of the axonal activity. Illumination of one of the eyes evoked activation of spinal axons on the ipsilateral side (*middle part* of Fig. 3*A*, see also Fig. 4). The spikes recorded by the rostral electrode were followed by the spikes recorded by the caudal electrode with a delay of a few milliseconds (Fig. 3*B*), demonstrating that the spikes were generated by descending axons with a conduction velocity of a few meters per second. The overwhelming majority of axons recorded in the present study and in a related study (Deliagina and Fagerstedt 2000) had conduction velocities from 2.5 to 5 m/s, which is characteristic of larger RS neurons—Müller cells and Mauthner cells from MRN, ARRN and MRRN, as well as of midsize RS neurons from MRRN and PRRN (Ohta and Grillner 1989; Rovainen 1978). These descending axons have a conduction velocity exceeding that of propriospinal axons and thus must originate in the brain stem reticular nuclei. The frequency of discharge in individual descending axons, caused by visual stimuli, usually did not exceed 3–5 Hz (see also Deliagina and Fagerstedt 2000).

Illumination of the eye (Fig. 3*A*) elicited in the attached lamprey a roll tilt toward the light source. This movement is termed an attached-state dorsal light response; it is caused by asymmetrical contraction of muscles around the mouth (Ullén et al. 1993a). With prolonged stimulation, an "explosive" increase of axonal activity usually occurred, followed by a detachment of the lamprey from the substrate and swimming (*swim* in Fig. 3*A*). During swimming, the axonal activity was so high that individual spikes were difficult to identify. In a few cases, grouping of spikes into bursts was observed in some parts of recording, as illustrated in Fig. 3*C;* the bursts in the caudal electrode were delayed by a few milliseconds in relation to the bursts in the rostral electrode. These delays were similar to those observed in the attached state (Fig. 3*B*), suggesting that the high level of activity recorded during swimming was caused, at least partly, by the same group of larger RS neurons. In a few cases, we managed to trace the activity of the same individual neurons both in the quiescent state and during swimming. Their firing frequency increased considerably during swimming, sometimes ≤10–12 Hz (see Fig. 9).

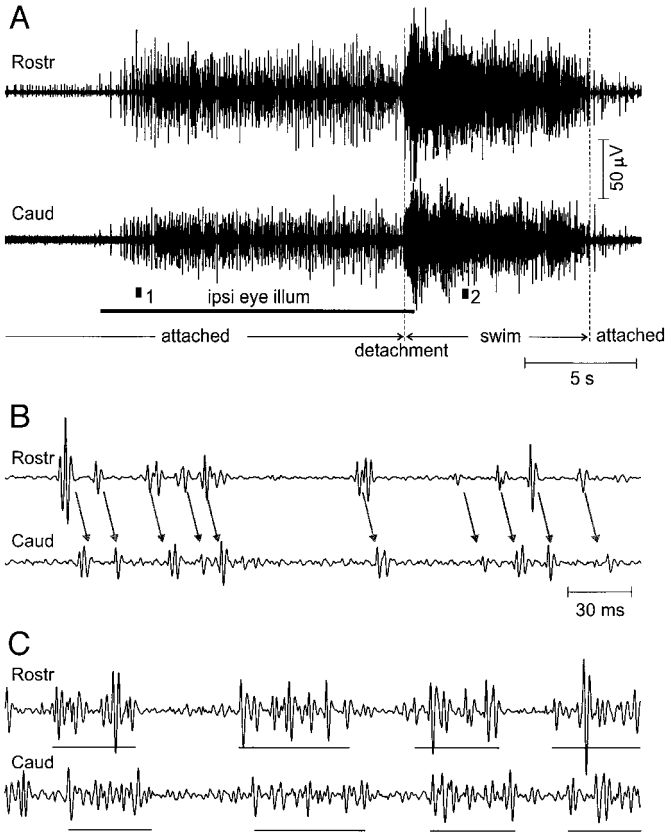

FIG. 3. *A*: activity of RS neurons at rest, during ipsilateral eye illumination, and during swimming. Activity was recorded by 2 electrodes implanted at different rostrocaudal levels. Parts of the recording indicated by bars 1 and 2 are shown with higher time resolution in *B* and *C*, respectively. In *B*, the sequence of the spike occurrence on the rostral and caudal electrodes is indicated by arrows. In *C*, horizontal bars indicate bursts of axonal activity; these bursts in the caudal electrode are delayed by a few milliseconds in relation to those in the rostral electrode.

Further evidence that the high level of mass activity, recorded by the implanted electrodes, was caused by discharges in descending reticulospinal axons rather than in the ascending axons, was obtained in the experiment of Fig. 4*A*. It shows an activation of the spinal axons during swimming evoked by eye illumination. The lamprey subsequently was spinalized at five segments rostral to the recording electrodes, and locomotor activity then could be evoked caudal to the lesion by pinching the tail (Fig. 4*B*). Despite intense locomotor movements, no axonal activity comparable in size to that of Fig. 4*A* was recorded in the spinalized preparation.

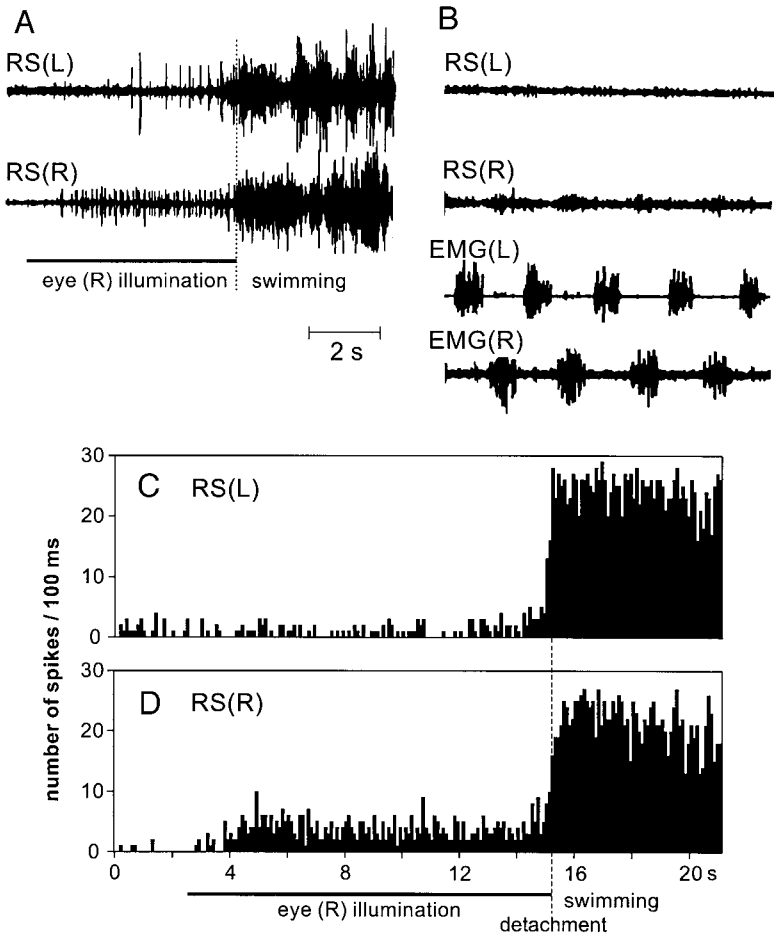

FIG. 4. Mass activity of the RS system increases during locomotion evoked by unilateral eye illumination. *A*: in the intact lamprey, illumination of the right eye led to massive activation of the left and right RS pathways, RS(L) and RS(R), and to swimming. *B*: same animal was spinalized rostral to the recording electrodes and then swimming was evoked by pinching the tail. In this situation, locomotor movements (monitored by bilateral EMG bursts) were not accompanied by any substantial activity in the spinal axons. *C* and *D*: quantitative characterization of the locomotor-related RS activity evoked by the right eye illumination. Temporal histograms show the number of spikes per bin (100 ms) in the left (*C*) and right (*D*) RS pathways.

A sharp activation of RS neurons at the onset of locomotion was always bilateral. This bilateral pattern of activity was observed with all types of stimuli eliciting locomotion, that is with unilateral eye illumination (Fig. 4, *A, C,* and *D*), tactile stimulation of the head (Fig. 5), vibration of the water (Fig. 7), illumination of the tail dermal photoreceptors (Fig. 8*A*), and lateral tilt of the animal (not illustrated), or when locomotion started spontaneously (not illustrated).

Correlation between RS activity and locomotor activity

We tried to find correlations between the level of the mass RS activity and different characteristics of locomotion. One of these is the moment when the animal detached from the substrate and started swimming. For the trial illustrated in Fig. 5, *A* and *B*, the level of RS activity on the two sides at the moment of detachment is indicated by arrows. In repeated tests in the

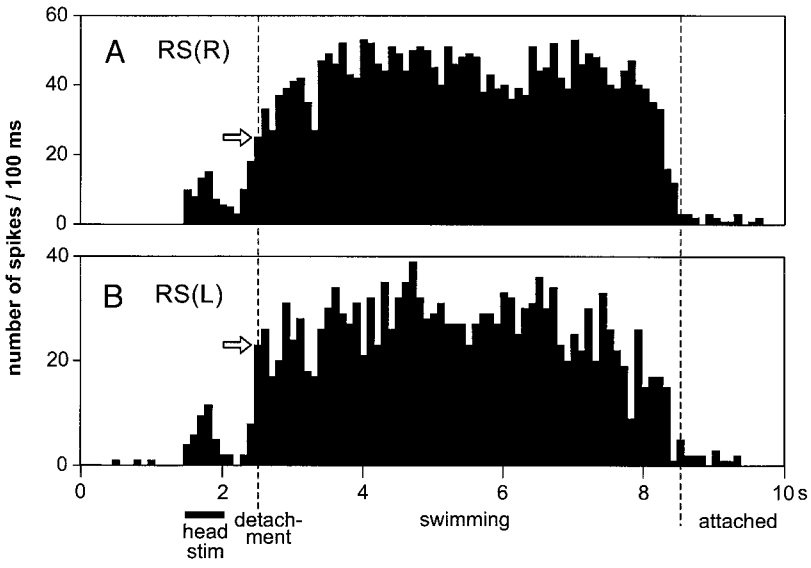

FIG. 5. Massive activation of the right (*A*) and left (*B*) RS pathways evoked by tactile stimulation of the head. Moments of the detachment and attachment are indicated.

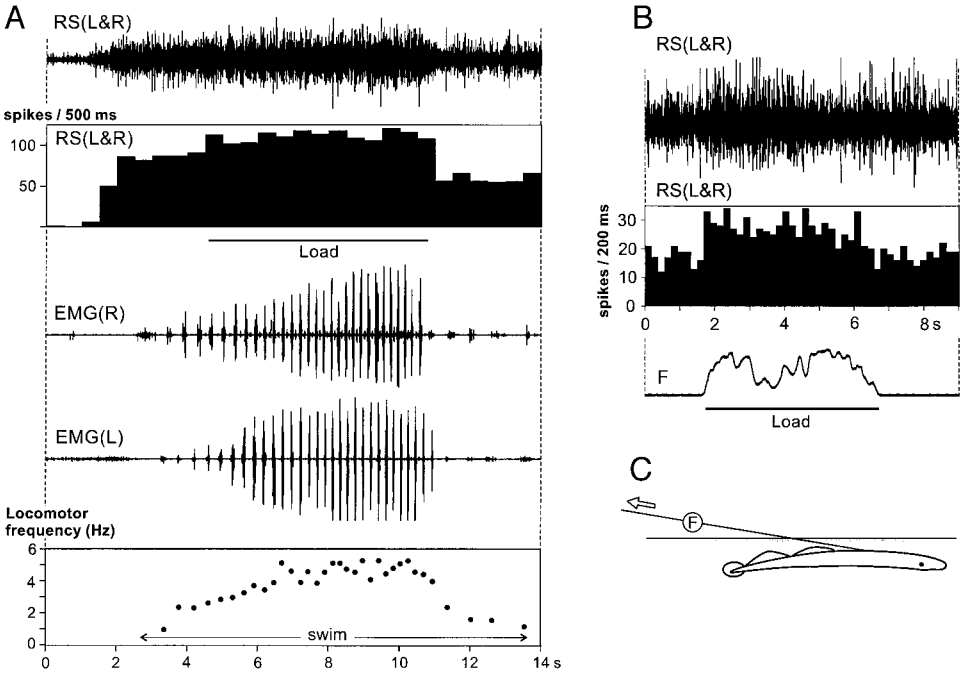

FIG. 6. Correlation between the summated activity in the left and right RS pathways and the vigor of locomotion. *A*: different characteristics of locomotion were recorded along with the summated mass activity in the left and right RS pathways, RS(L&R). This activity is presented as row data, that is summated signals from the left and right electrodes (*top*) and as a time histogram (*2nd* from *top*). Vigor of locomotion was characterized by the value of bilateral EMG bursts and by the locomotor frequency. *B*: summated mass activity in the left and right RS pathways recorded along with the force (*F*) applied to the swimming lamprey when the lead attached to the animal was stretched. Method of loading the lamprey is shown in *C* (*F*, force-to-voltage transducer).

same animal or in different animals, the detachment always occurred at the rising phase of RS activity, when this activity reached 53 ± 26% (mean ± SD; $n = 30$) of the plateau level. At the end of the swim episode, the lamprey usually attached itself to the substrate when the level of RS activity was very low, as illustrated in Fig. 5.

The correlation between the mass RS activity and the vigor of swimming was studied in experiments by loading the lamprey (Fig. 6*C*). The experimenter could hamper the forward progression of the animal by pulling a lead attached to the lamprey's body. In a part of these experiments, the applied force was measured by the transducer (F in Fig. 6*B*). The loading resulted in an increase of the mass RS activity, whereas unloading resulted in a decrease of this activity (Fig. 6, *A* and *B*). Two major values characterizing the vigor of locomotion—the amplitude and the frequency of locomotor EMG bursts—increased markedly when the load was applied (Fig. 6*A*). A strong positive correlation between the vigor of locomotion and the mass RS activity was observed in all tested animals ($n = 5$). Thus the lamprey tries to continue the forward swimming and to overcome the load.

In some cases, locomotion started with a lateral turn followed by a rectilinear swimming. A characteristic feature of the turn was a prolonged unilateral muscle activity (Fig. 7*A*, *bottom 2 traces*). The turn usually was associated with some prevalence of the mass RS activity on the ipsilateral (to the turn) side over the activity on the contralateral side (Fig. 7*A*, histograms). We calculated the degree of asymmetry in the left and right RS activity during a turn. For this purpose, the activity on each side during the turn was normalized to the mean level of activity during the rectilinear swimming that followed the turn. The ratio (*asymmetry* in Fig. 7*B*) of the activity on the ipsilateral and the contralateral side then was averaged over the duration of turn. Seven turns in three animals were used; the turning angles were between 90 and 135°. In six cases, the ipsilateral RS activity during the turn was larger than the contralateral one (asymmetry >1, Fig. 7*B*). The asymmetry averaged over all the turns was 1.3 ± 0.3 (Fig. 7*C*).

During swimming, rhythmic modulation of the mass RS activity was observed in all experiments. The degree of modulation strongly differed between the experiments, however; it also could change spontaneously during a trial or even completely disappear to reappear some cycles later. The modulation is well seen in Figs. 4*A*, 8, and 9 but not in the histograms probably because the binwidth (100 or 200 ms) was comparable with the locomotor cycle duration (200–300 ms, Fig. 6*A*). The activity on a given side usually had a peak coinciding with the burst of EMG activity in the ipsilateral muscles in the anterior part of the body, as indicated by bilateral arrows in Fig. 8*A*. The rhythm of modulation fluctuated along with the rhythm of locomotor body undulations (Fig. 8*B*).

In most cases, individual neurons could not be distinguished in the mass RS activity occurring during locomotion. In three experiments, however, activity of individual neurons could be traced, as illustrated in Fig. 9, where two larger units [*unit 1* (*right*) and *unit 2* (*left*)] are clearly seen both before (*A*) and during swimming (*B*). In both trials, vestibular reactions were elicited in the RS neurons by tilting the animal (for methods, see Fig. 2*B*). At rest, there was no activity in either neuron (beginning of the recording in Fig. 9*A*). Both neurons exhibited vestibular reactions and were activated with contralateral tilt. During locomotion, the activity of both neurons increased markedly, and their firing rate became rhythmically modulated (beginning of the recording in Fig. 9*B*). The two RS neurons on the right and left side tended to be active in the same phase of the locomotor cycle. In most cases, however, RS neurons in opposite sides exhibited anti-phase modulation. The pattern of vestibular responses, that is activation with contralateral tilt, was preserved during locomotion (Fig. 9*B*). In addition, a depression of activity with ipsilateral tilt can be clearly seen. Rhythmic modulation of the activity of these RS neurons was superimposed on their vestibular responses. Qualitatively sim-

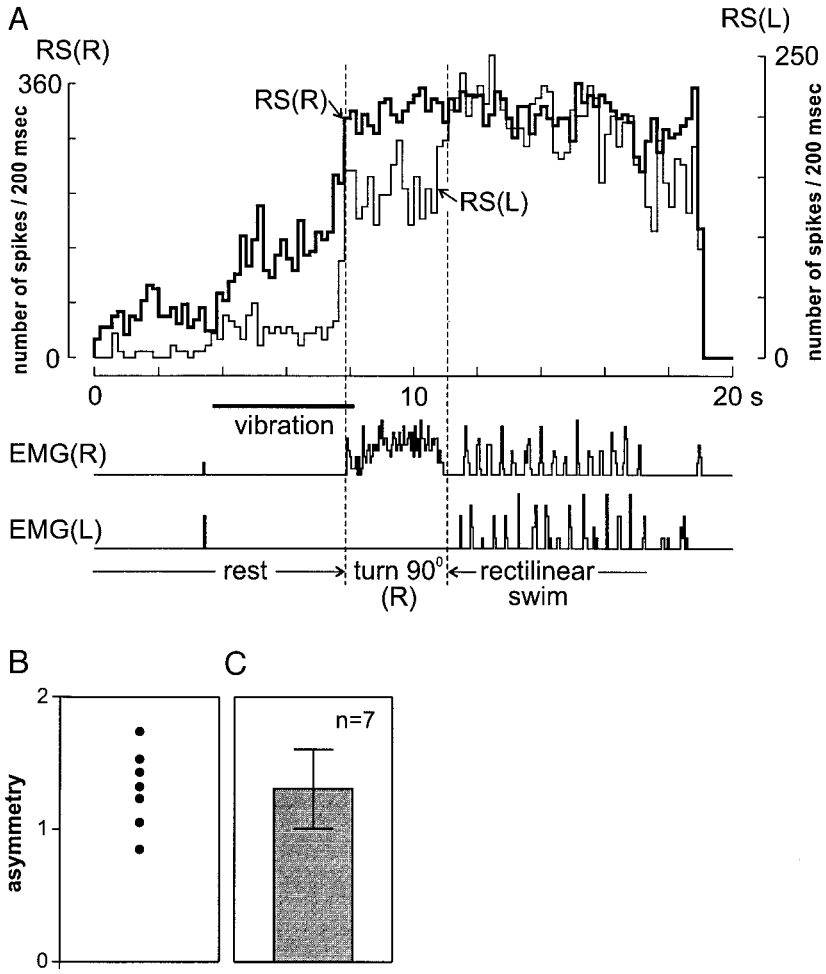

FIG. 7. Mass activity in the right and left RS pathways during a lateral turn followed by a rectilinear swimming. *A*: temporal histograms of RS(L) and RS(R) activity, and bilateral EMGs. Locomotion was evoked by water vibration. *B* and *C*: degree of asymmetry, that is the ratio of the normalized activities on the ipsilateral and contralateral sides, averaged over the duration of turn for 7 individual turns (*B*) and over all the turns (±SD; *C*).

ilar results have been obtained in all three experiments with vestibular stimulation during locomotion.

DISCUSSION

The macroelectrodes used in the present study allowed us to record activity almost exclusively from the large and midsize spinal axons that have a conduction velocity >2 m/s (see also Deliagina and Fagerstedt 2000). We suggest that "filtration" of the larger axons was caused by two factors: a shunting effect of the wire electrode for slowly propagating spikes and smaller extraaxonal currents in smaller axons than in larger axons.

In the spinal cord of the lamprey, there are ~20 larger RS axons (10–50 μm diam) on each side; they have a conduction velocity of 2–5 m/s. These axons belong to the large cells—Müller cells and Mauthner cells from MRN, ARRN, and MRRN—and to the middle-size cells from MRRN and PRRN (Fig. 1*D*) (Ohta and Grillner 1989; Rovainen 1978, 1982). Most RS neurons are much smaller and have a very low conduction velocity in their axons (Bussiéres 1994; Kasicki et al. 1989; Ronan 1989). Most likely they did not contribute significantly to the sample of neurons recorded in this study. Thus all the results and conclusions of this paper relate to a small subpopulation of larger RS neurons with comparatively high conduction velocity.

Correlation between the level of RS activity and the vigor of locomotion

The main result of the present study is that initiation of locomotion is always associated with a strong bilateral activation of the RS system. This bilateral activation occurred irrespective of the modality and laterality of the applied sensory stimulus. The RS activity considerably outlasted the duration of the sensory stimulus, and swimming continued as long as a high level of RS activity was present. A similar bilateral activation of RS neurons in response to a unilateral sensory input (illumination of tail dermal photoreceptors) was observed earlier in a semi-intact preparation of the larval lamprey (Deliagina et al. 1995).

A question of fundamental interest is where and how the unilateral sensory signals of different modalities are transformed into a bilateral, long-lasting activity. One possibility would be that this occurs within the RS system itself. There is no evidence to suggest interconnections between the left and right subdivisions of the RS system, however. Furthermore in this and previous studies, it has been shown that some sensory inputs, such as vestibular and visual signals, can elicit a selective unilateral excitation of RS neurons without exciting their contralateral partners (Deliagina et al. 1992, 1993; Orlovsky et al. 1992). This applies to both nonswimming and swimming lampreys (see Fig. 9). The transformation from a unilateral to a bilateral signal therefore probably will take place at a prere-

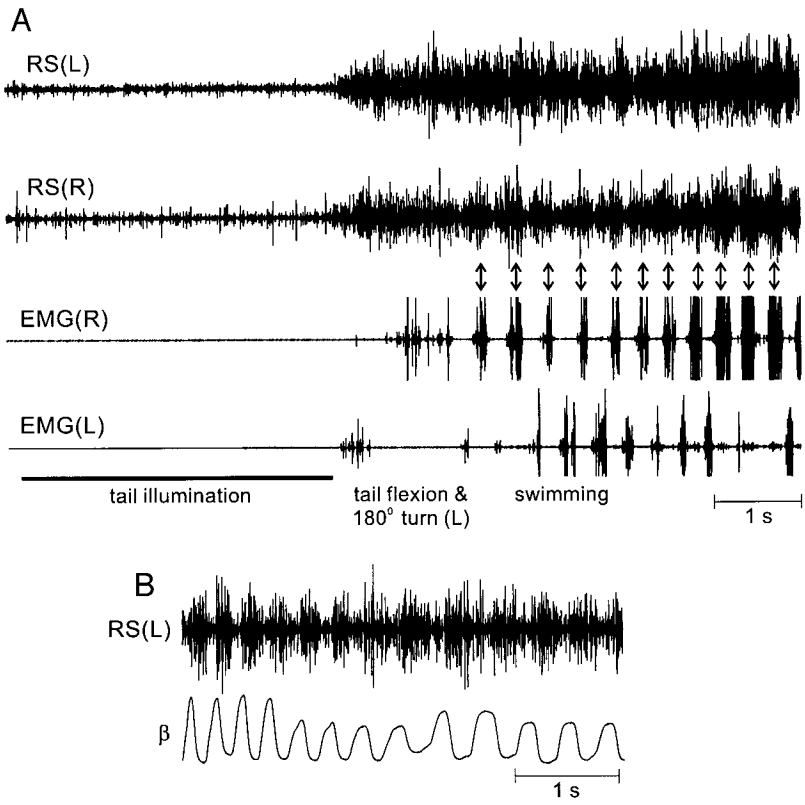

FIG. 8. Locomotor cycle-related modulation of the mass RS activity during swimming. *A*: activity in the left and right RS pathways recorded along with bilateral EMG. Locomotion was evoked by tail illumination. Bilateral arrows indicate the correlation between the peak of modulation and EMG burst. *B*: activity in the left RS pathway recorded along with the lateral body undulations. (Setup shown in Fig. 2*B*; lateral deviations β were measured by a mechano-electrical transducer).

ticular level. One of the sites of this transformation is the mesencephalic locomotor region located in the brain stem between pons and mesencephalon in higher vertebrates (Jordan 1986, 1991; Shik et al. 1966); this region has bilateral projections to RS neurons and evokes symmetrical locomotion in response to a unilateral stimulation (Orlovsky 1970a,b). In the lamprey, an analogous region has been described (Sirota et al. 1995). A second area found in the lamprey is located in diencephalon (El Manira et al. 1997). In the transformation from a short stimulus to the long-lasting response of RS neurons, plateau potentials generated by some RS neurons in response to sensory stimuli (Viana Di Prisco et al. 1997) and possibly also other brain stem neurons may play a role.

In the present study it also was found that the level of mass RS activity strongly correlated to the vigor of locomotion as characterized by the frequency of body undulations, the amplitude of EMG bursts and the propulsive force developed by the animal (Fig. 6). This finding suggests that the RS system in the lamprey and other vertebrates is responsible not only for the central initiation of locomotion but also for the regulation

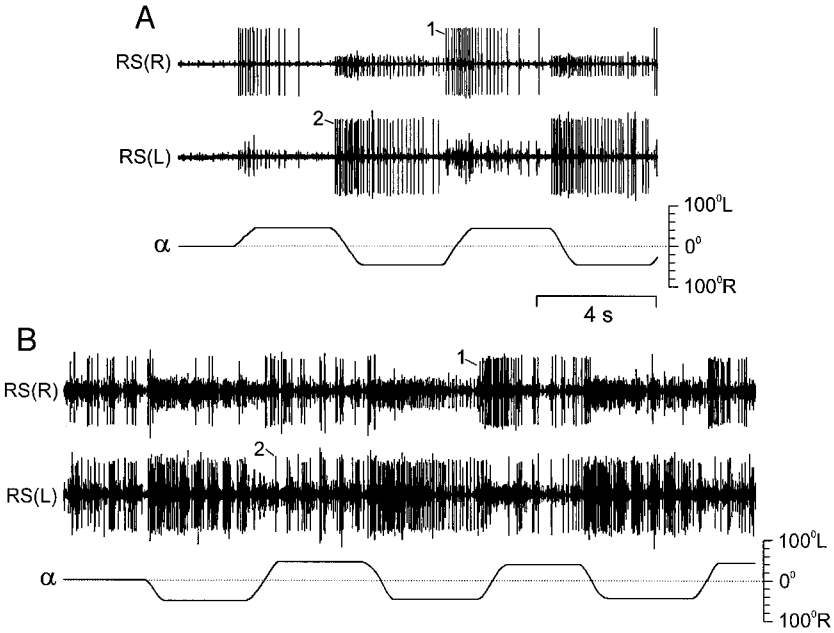

FIG. 9. Responses in the left and right RS pathways to roll tilt in the nonswimming (*A*) and swimming (*B*) lamprey (α, roll tilt angle). Two neurons (1 and 2) could be traced both under nonswimming and swimming conditions.

of the frequency and amplitude of oscillations generated by the spinal locomotor network (Drew et al. 1986; Jordan 1991; McClellan 1988; McClellan and Grillner 1984; Ohta and Grillner 1989; Orlovsky 1970b).

Correlation between the asymmetry in RS activity and the lateral turns

Lateral turns in the lamprey are caused by a strong and prolonged contraction of body musculature on the side of the turn. The wave of contraction subsequently propagates along the body toward the tail (McClellan and Hagevik 1997; Ullén et al. 1998; Zelenin et al. 1997). In the present study, we have found that lateral turns were associated with an asymmetry in the mass RS activity on the left and right sides (Fig. 7). This asymmetry was comparatively small (1.3 ± 0.3), however, in contrast to the asymmetry in motor output—during the turn, the EMG activity was only present on the ipsilateral side of the body. A possible explanation for this discrepancy could be that the turns are generated by the spinal locomotor network that generates rectilinear swimming and that this network is sensitive to any asymmetry in descending commands. Two lines of evidence support this suggestion. First, the symmetry of motor output during fictive swimming generated by the spinal cord can be strongly affected by stimulation of a single RS neuron (Buchanan and Cohen 1982). Second, a small asymmetry in the mass activity of the left and right RS pathways, caused by electrical stimulation, can result in a generation of a pure unilateral motor output (Zelenin et al. 1997). In both cases, the "amplification" of the asymmetry occurred in the spinal cord, and it is most likely based on the operation of the system of reciprocal inhibition between the two hemi-segments (Cohen and Harris-Warrick 1984; Grillner and Wallén 1980; Wallén et al. 1993).

Correlation between vestibular responses in RS neurons and postural corrections

Behavioral experiments (Ullén et al. 1995a,b) have shown that the swimming lamprey maintains its dorsal-side-up orientation due to the activity of the postural control system driven by vestibular input. A deviation from the normal orientation elicits a set of corrective motor responses including a lateral body flexion and a twisting of the body and dorsal fins. These motor responses are caused by the RS system. The present study demonstrated that lateral tilt of the lamprey during locomotor-like activity (Fig. 2*B*) evokes activation of contralateral RS neurons (Fig. 9*B*). This pattern of response was similar to that observed in the nonswimming lamprey (Fig. 9*A*) (see also Deliagina and Fagerstedt 2000) and in an in vitro preparation consisting of the brain stem and labyrinths (Deliagina et al. 1992). The activation of the contralateral RS neurons caused by lateral tilt presumably presents the commands addressed to the spinal cord where they evoke postural corrections. It remains unclear, however, how these commands affect the spinal network. That these commands are sufficient for postural stabilization was indicated by model experiments (Zelenin et al. 1998), using a technique of artificial feedback as originally developed for *Clione* by Deliagina et al. (1998). In these experiments, the activity of RS neurons, recorded by implanted electrodes in the intact lamprey during its locomotor-like activity, was used to control an electromechanical robot rotating the animal in the roll plane. This "hybrid" system was able to stabilize the dorsal-side-up orientation of the lamprey.

Rhythmic modulation of RS neurons

In most experiments, we observed a periodical modulation of the mass activity in RS pathways linked to the locomotor cycle with the peak activity coinciding with the burst of EMG in the ipsilateral rostral myotomes (Fig. 8). The lack of modulation observed in a part of recordings could be related to the fact that individual RS neurons may discharge in different phases of the swim cycle. This modulation was most likely caused by efference copy signals coming to the brain stem from the spinal locomotor CPG because a similar pattern of modulation was observed also during fictive swimming (Dubuc and Grillner 1989; Kasicki and Grillner 1986; Kasicki et al. 1989). Rhythmical modulation of the activity in RS pathways also was observed in walking cats (Drew et al. 1986; Orlovsky 1970b). The functional role of this modulation is not clear, however, primarily because the spinal targets of RS neurons are not well characterized. It was suggested that the modulation can perform a gating function, linking the supraspinal commands to a specific phase of the locomotor cycle (Arshavsky et al. 1986). Evidence in favor of this suggestion was obtained in the present study. As shown in Fig. 9*B*, signals about lateral tilt are transmitted to the spinal network by the RS neurons 1 and 2 only in a certain phase of the locomotor cycle and are not transmitted in the opposite phase.

Multifunctional role of RS neurons

A question of fundamental interest concerns the distribution of functions between different subdivisions of RS system. Are different functions—initiation of locomotion, steering, and postural stabilization—controlled by different groups of RS neurons or does each neuron participate in the control of different functions? There is overwhelming evidence that most RS neurons are multifunctional. First, a multitude of inputs from the cranial nerves, from the spinal cord afferent pathways and from the locomotor CPG, converge on RS neurons (see e.g., Deliagina et al. 1992, 1993; Dubuc et al. 1992; Kasicki et al. 1989; Vinay et al. 1998). The present study has shown (see Fig. 9*B*) that one and the same RS neuron responds faithfully to lateral tilt, efference copy signals from the spinal CPG, and is activated during the initiation of locomotion. Second, stimulation of individual RS neurons during fictive swimming affected both the locomotor frequency and the symmetry of segmental motor output (Buchanan and Cohen 1982), suggesting that they could be involved both in the initiation of swimming and in the generation of postural corrections. Experiments with microstimulation of the brain stem reticular formation during fictive swimming (Wannier et al. 1998) also have shown that most of the stimulated sites evoke asymmetry in the segmental motor output, and a half of them also affect the locomotory rhythm. No sites affecting only the frequency of the locomotory rhythm have been found.

It seems thus most likely that the brain stem-spinal cord interaction does not depend on "private labeled lines" for each type of motor behavior (locomotion, steering, postural control); this interaction rather depends on shared communication lines.

Decoding of the commands transmitted by the RS system takes place in the spinal cord. The operation of the decoding mechanisms is not clear. One can suggest that decoding is based on the comparison of signals delivered via different subdivisions of the RS system. In particular, for extracting information about lateral tilts, the activities in the left and right RS pathways have to be compared (Deliagina 1997; Deliagina et al. 1993); the summated activity in these pathways may carry information on the intensity of locomotion.

The polyfunctional organization of RS system discussed in the preceding text presumably accounts for the great difficulty that investigators have had in understanding this system while investigating anesthetized preparations or only one type of behavior.

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