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# Responses of Reticulospinal Neurons in the Lamprey to Lateral Turns

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Submitted 28 August 2006; accepted in final form 30 October 2006

**Karayannidou A, Zelenin PV, Orlovsky GN, Deliagina TG.** Responses of reticulospinal neurons in the lamprey to lateral turns. *J Neurophysiol* 97: 512–521, 2007. First published November 1, 2006; doi:10.1152/jn.00912.2006. When swimming, the lamprey maintains a definite orientation of its body in the vertical planes, in relation to the gravity vector, as the result of postural vestibular reflexes. Do the vestibular-driven mechanisms also play a role in the control of the direction of swimming in the horizontal (yaw) plane, in which the gravity cannot be used as a reference direction? In the present study, we addressed this question by recording responses to lateral turns in reticulospinal (RS) neurons mediating vestibulospinal reflexes. In intact lampreys, the activity of axons of RS neurons was recorded in the spinal cord by implanted electrodes. Vestibular stimulation was performed by periodical turns of the animal in the yaw plane (60° peak to peak). It was found that the majority of responding RS neurons were activated by the contralateral turn. By removing one labyrinth, we found that yaw responses in RS neurons were driven mainly by input from the contralateral labyrinth. We suggest that these neurons, when activated by the contralateral turn, will elicit the ipsilateral turn and thus will compensate for perturbations of the rectilinear swimming caused by external factors. It is also known that unilateral eye illumination elicits a contralateral turn in the yaw plane (negative phototaxis). We found that a portion of RS neurons were activated by the contralateral eye illumination. By eliciting an ipsilateral turn, these neurons could mediate the negative phototaxis.

## INTRODUCTION

When swimming, the lamprey (a lower vertebrate, cyclostome) usually maintains a definite orientation of its body in two vertical planes (the roll and the pitch angles) and in a horizontal plane (the yaw angle). In the vertical planes, the orientation is stabilized in relation to the gravity vector by means of two postural control systems driven primarily by vestibular input and controlling the roll and pitch angles (Ullén et al. 1995a). Postural corrections can be caused by changes of the body orientation in relation to gravity, as well as by body accelerations in the corresponding planes. These two types of stimuli are sensed by vestibular organs (Deliagina et al. 1992b; Lowenstein 1970). A detailed analysis of the roll and pitch control systems was done previously (Deliagina 1997a,b; Deliagina and Fagerstedt 2000; Deliagina et al. 1992a,b, 1993, 2000; Pavlova and Deliagina 2002; Zelenin et al. 2000, 2005). It was found that stabilization of the body orientation in each of the planes (roll and pitch) is based on the interaction of two antagonistic postural reflexes and the animal stabilizes the orientation at which the reflexes are equal to each other. In reality, orientation of the animal can be disturbed simultaneously in both planes. Interaction of the two control systems has not been analyzed, however.

In contrast to changes of body orientation in the vertical (roll and pitch) planes, turns in the yaw plane do not affect the

animal's orientation in the gravity field and therefore gravity cannot be used as a reference direction. Do the vestibular reflexes driven by yaw-sensitive vestibular afferents (Lowenstein 1970; Lowenstein et al. 1968) yet play a role in the control of orientation in the yaw plane? In particular, what is their role when the animal's orientation in this plane is changed passively, e.g., under the effect of a water stream or when colliding with obstacles? Also, how do these reflexes interact with active changes of yaw orientation that occur during swimming arising from the periodical locomotor undulations of the head and body (Williams et al. 1989) or during lateral turns when the animal changes the direction of swimming by itself (Ullén et al. 1993, 1995b)? Investigation of the activity of vestibular-driven mechanisms controlling orientation of the lamprey in the yaw plane was the general goal of the present study.

In the lamprey, motor commands are transmitted from the brain to the spinal cord mainly by the reticulospinal (RS) pathways (Brodin et al. 1988; Bussi eres 1994; Nieuwenhuys 1972). The RS neurons receive vestibular input through interneurons of vestibular nuclei (Koyama et al. 1989; Northcutt 1979; Rovainen 1979). They also receive input from other sensory systems, from the forebrain, from different centers in the brain stem, and from the spinal cord (Deliagina et al. 1993; Dubuc et al. 1993; Rovainen 1967, 1979; Viana Di Prisco et al. 1995; Wickelgren 1977). The majority of axons of RS neurons project ipsilaterally and can reach even the most caudal segments of the spinal cord (Bussi eres 1994). They affect segmental interneurons and motoneurons (Buchanan and Cohen 1982; Ohta and Grillner 1989; Rovainen 1974; Wannier et al. 1998). Individual RS neurons exert diverse effects on spinal motor output (Zelenin et al. 2001, 2003). In a recent study by Zelenin et al. (2005), it was shown that individual RS neurons, activated by rotation in a definite plane, elicit the torque counteracting rotation that activated the neuron.

In the study by Zelenin et al. (2005), the population of RS neurons responding to yaw turn was revealed. Most of them were activated by the contralateral turn. However, in that study the vestibular responses of yaw-related RS neurons were not characterized in any detail. Also, the data were obtained on the in vitro preparation (consisting of the brain stem with labyrinths and rostral part of the spinal cord), in which a number of inputs to RS neurons, like those from the forebrain, from the cranial nerves, and from the caudal parts of the spinal cord, were abolished. The first aim of the present study was to examine in intact animals the activity of RS neurons related to the yaw control. For this purpose, we used the method of recording activity of RS neurons from their axons in the spinal cord by means of chronically implanted electrodes (Deliagina and Fagerstedt 2000; Deliagina et al. 2000). Responses of

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individual neurons were extracted from the multielectrode recording of the mass activity, using a spike-sorting procedure (Zelenin 2005).

It was shown earlier that RS neurons of the roll- and pitch-related populations receive their main vestibular input from the contralateral labyrinth (Deliagina and Pavlova 2002; Pavlova and Deliagina 2003). The second aim of the present study was to examine the relative role of the two labyrinths for generation of the yaw-related responses in RS neurons. For this purpose, we compared the RS responses to yaw turn in individual animals before and after a unilateral labyrinthectomy (UL).

It is known that unilateral eye illumination in the lamprey elicits a contralateral turn in the yaw plane (negative phototaxis) (Ullén et al. 1993, 1995b, 1997). The third aim of the present study was to reveal the RS neurons presumably involved in initiation of this response. For this purpose, we studied responses of RS neurons to unilateral eye illumination, as well as interactions of vestibular and visual inputs to these neurons. A brief account of this study was previously published in abstract form (Karayannidou et al. 2005).

## METHODS

Experiments were carried out on eight adult (25–35 cm in length) lampreys (*Lampetra fluviatilis*) that were kept in an aerated freshwater aquarium at 6°C, with a 12 h:12 h light:dark cycle. All experiments were conducted with the approval of the local ethical committee (Norra Djurförsöksetiska Nämnden) in Stockholm.

### Electrodes

The activity of RS neurons was recorded from their axons in the spinal cord by means of chronically implanted macroelectrodes similar to those described in previous papers (Deliagina and Fagerstedt 2000; Deliagina et al. 2000). The electrodes (silver wires, 75  $\mu$ m in diameter and 2 mm in length) were oriented in parallel to the long spinal axons, thus allowing us to record the spike activity from fibers with a conduction velocity of >1.5 m/s.

The electrodes were glued onto a plastic plate [ $7.5 \times 2.5 \times 0.25$  mm (length  $\times$  width  $\times$  thickness)]. For measuring the axon position and conduction velocity (see following text) we used a plate with four electrodes (Fig. 1A). The distance between the left and right electrodes was 1.5 mm, whereas the distance between the corresponding points (rostral or caudal) of the two pairs of electrodes was 3.5 mm (2 mm wire length plus 1.5 mm between the wires). The electrodes had a very low resistance (<10<sup>3</sup>  $\Omega$ ) and a noise level of only a few microvolts (Deliagina et al. 2000).

### Surgery

Implantation of the electrodes was performed in eight animals under MS-222 (Sandoz) anesthesia (100 mg/l). The plate with electrodes was implanted at the level of the third gill, through a longitudinal cut performed along the midline of the dorsal aspect of the body. The plate was positioned so that the electrodes were facing the dorsal aspect of the spinal cord (Fig. 1B). The rostral end of the plate was pushed carefully into the canal between the spinal cord and surrounding tissues to secure the electrode position in relation to the spinal cord. The wound was then closed and sutured so that the connecting wires were tightly fixed between the two sides of the wound. Vestibular and visual responses of RS neurons were tested after full recovery from anesthesia (3–4 h after implantation of the electrodes).

A unilateral labyrinthectomy (UL) was performed in six animals, usually 1 day after implantation of the electrodes as previously

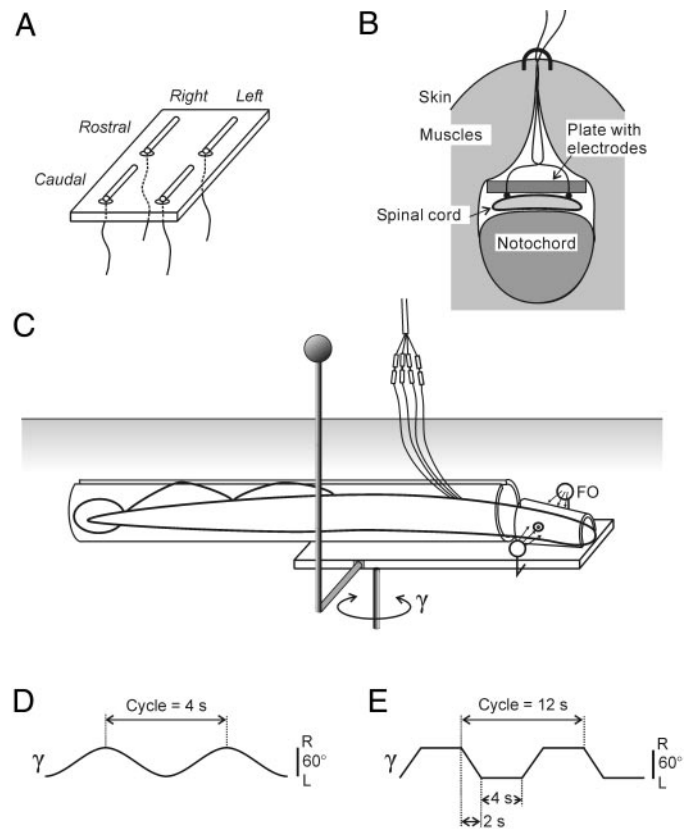


FIG. 1. Methods for recording vestibular and visual responses in reticulospinal (RS) neurons. *A*: design of the electrodes. Four wire electrodes (75  $\mu$ m in diameter and 2 mm in length) were glued on a plastic plate (view from below). *B*: position of the plate with electrodes as seen in the transverse section of the lamprey's body. *C*: experimental device for vestibular and visual stimulation ( $\gamma$ , yaw angle; FO, fiber-optic illumination system). *D* and *E*: characteristics of vestibular stimulation for sinusoidal turns (*D*) and trapezoidal turns (*E*).

described in detail (Deliagina 1997a). In short, the hole was made in the dorsolateral aspect of the vestibular capsule and the labyrinth was removed with a pair of fine forceps under visual control. For a few weeks after UL, the animals were unable to maintain the dorsal side up orientation during swimming and were continuously rolling toward the damaged side (de Burlet and Versteegh 1930; Deliagina 1995, 1997a). Postmortem investigation showed that, in all cases, removal of the vestibular organ was complete. In the six animals used to study the effects of UL, vestibular responses of RS neurons were examined two times: after implantation of the electrodes and after UL. Responses were tested after full recovery from anesthesia.

### Vestibular and visual stimulation

All experiments were carried out at a water temperature of 7–8°C. The lamprey was positioned in a special setup (Fig. 1C) that consisted of a tube fastened to a small platform. After having being positioned into the tube, the lamprey attached to the platform with its sucker mouth. The setup allowed us to rotate the animal in the horizontal (yaw) plane by 60° peak to peak. Two patterns of vestibular stimulation were used. First, the animal was tested during periodic sinusoidal yaw turns, at a period of 4 s (Fig. 1D). Our reason for this was to use vestibular stimuli similar to those caused by periodical head undulations during slow swimming (Islam et al. 2006a,b; Williams et al. 1989). Another stimulation pattern consisted of periodical trapezoidal turns (Fig. 1E), as if the lamprey performed sharp lateral turns when changing the direction of swimming. The transition from one

position to another lasted 2 s, and each position was maintained for 4 s; the cycle duration was 12 s. Illumination of the left or the right eye was produced by two fiber-optic systems (90 W) attached to the turning platform (Fig. 1C). Because normally the resting activity in RS neurons was low or absent (see also Deliagina and Fagerstedt 2000; Pavlova and Deliagina 2002), to reveal not only excitatory but also inhibitory effects, visual stimulation was performed in combination with vestibular stimulation. This also allowed us to characterize the effects of visual input on vestibular responses. Eye illumination was usually turned off before it caused activation of the locomotor system and lateral turn (Deliagina et al. 2000). Typically, the experimental session included 50–100 tilt cycles.

### Processing of data

Signals from the electrodes were amplified by conventional AC amplifiers, digitized with a sampling frequency of 10 kHz, and stored on the hard disk of an IBM AT compatible computer by means of data-acquisition software (Digidata 1200/Axoscope, Axon Instruments, Union City, CA). A representative example of the four-electrode recording of the multiunit responses to trapezoidal turns and to eye illumination is shown in Fig. 2A.

The recorded multiunit spike trains were separated into unitary waveforms, representing the activity of individual axons, by means of data analysis software (Spike2, version 4, CED, Cambridge, UK). Spikes with the same waveform were considered to be generated by the same RS neuron. We extracted single spikes and sorted them using templates (a template is stored as a series of data points). Each point of the template has a width representing the expected error in a signal that matches the template at that point. Spikes match a template if >85% of the points in a spike fall within the template. Building of the templates and extraction of spikes from the mass activity included several steps (Zelenin 2005). 1) One of the channels was used to build

primary templates and extract groups of events that matched them. The minimum percentage of points in a template was 80%, and the width of the template was roughly 20% of the spike amplitude. 2) For each of the groups, corresponding waveforms were extracted from other channels and then reclassified. Thus secondary templates were built for each group of “events” and each neuron had a set of templates (primary and secondary), one template for each channel. After that, we looked through each of the files for a given animal and piled up the templates corresponding to different neurons. In the end, common sets of templates were used to extract the activity of single neurons from all the files.

The inverse proportionality between the spike amplitude and the axon distance from the electrode allowed us to roughly estimate the laterality of individual axons in the spinal cord by comparing the amplitudes of the same spike recorded by the two rostral electrodes (Deliagina et al. 2000). The ratio ( $r$ ) of minimal to maximal spike amplitude was calculated. Axons were divided into the lateral group ( $r \leq 0.4$ ) and medial group ( $r > 0.4$ ). In this paper, the laterality of the axon is used as a morphological characteristic of RS neurons (e.g., “left” neuron is the cell with the axon on the left side of the spinal cord).

To measure the conduction velocity in individual axons, a distance between the corresponding points of the rostral and caudal pairs of electrodes was divided by the time difference between the appearances of the same propagating spike in the two records. Most of the axons (106 of 138) were recorded by both rostral and caudal electrodes; all of them appeared to be descending ones. Their conduction velocity ranged from 1.6 to 4.0 m/s. In the spinal cord of the lamprey, the descending axons with such high conduction velocity belong mainly to RS neurons. The axons of the vestibulospinal neurons and lateral interneurons, which could be recorded in the area of the implanted electrodes, usually have lower conduction velocity (the

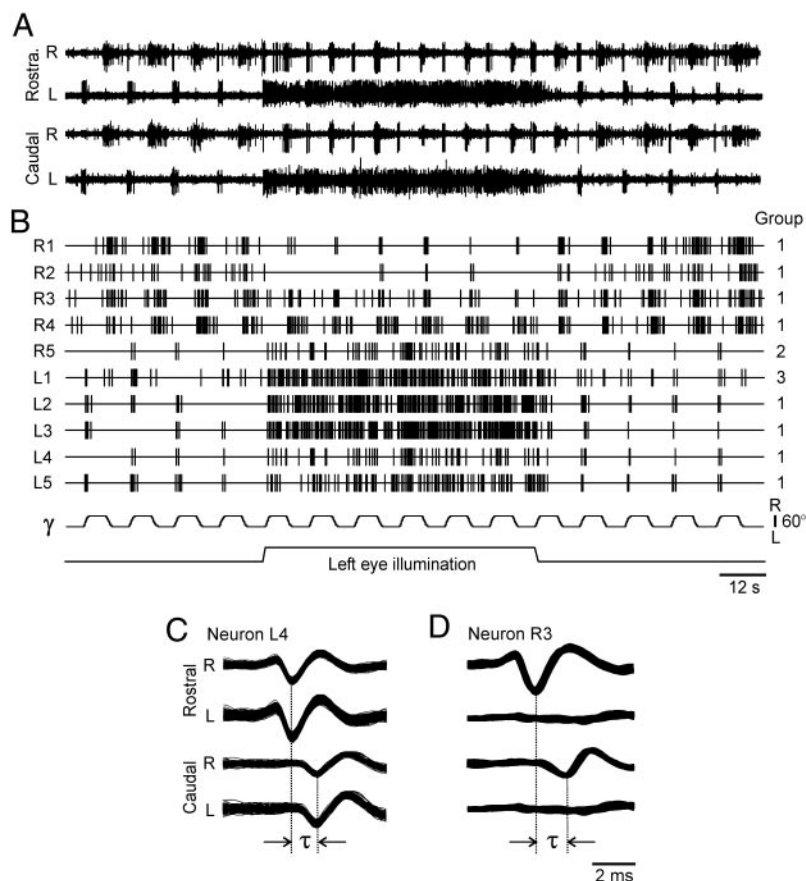


FIG. 2. Representative example of recording and spike sorting. A: mass activity in descending axons elicited by trapezoidal yaw turns ( $\gamma$ ) and left eye illumination, and recorded by 2 pairs of electrodes: Rostral (right, R) and Caudal (left, L). B: activity of 10 individual axons extracted from the mass activity (shown in A) with the spike-sorting program (R and L, axons located on the right side and left side, respectively). C and D: superposition of the activity of 2 individual RS axons (neurons L4 and R3) extracted from the raw data (shown in A) with the spike-sorting program, and recorded by 4 electrodes; the display was synchronized by “event” signals. Responses of these neurons are shown in B.



range of 0.5–1.3 m/s was reported for vestibulospinal neurons by Zelenin et al. 2003; see also Bussi eres and Dubuc 1991; Rovainen 1979).

Figure 2A shows the mass activity in RS pathways caused by trapezoidal turns and recorded by the rostral and caudal electrodes. Five neurons with their axons on the right side of the spinal cord and five neurons with left-side axons were extracted from this record by the spike-sorting program (Fig. 2B). Identification of two of them, L4 (with medial position of the axon,  $r = 0.7$ ) and R3 (with lateral position of the axon,  $r = 0$ ) are illustrated in Fig. 2, C and D, respectively, where the display was synchronized by an “event” signal. One can see that, for each of the neurons, the shape and absolute value of spike waveform in individual electrodes, the ratio between the spike amplitudes in different electrodes, and the time delay between the spikes in the rostral and caudal electrodes were constant.

The activity of a population of RS neurons was presented in a form of histograms. The cycle of sinusoidal turns was divided into ten equal (0.4-s) bins (Fig. 4A) and the frequency of individual neurons was measured for a given bin and then averaged over these bins in sequential cycles and over all neurons. The cycle of trapezoidal turns was divided into six equal (2-s) bins (Fig. 4B) and the frequency of individual neurons was also averaged over sequential cycles and over neurons.

All quantitative data in this study are presented as means  $\pm$  SE. The  $t$ -test was used to characterize the statistical significance when comparing different means; the significance level was set at  $P = 0.05$ .

## RESULTS

### Responses of individual RS neurons to vestibular stimuli

In quiescent animals, the activity of RS neurons was very low or absent. Vestibular stimulation activated RS neurons. In eight animals, data on the activity of 138 neurons (five to 27 neurons in individual animals) were extracted from the mass activity. Based on their pattern of response to vestibular stimulation, the RS neurons were divided into four groups. Examples of vestibular responses of neurons from different groups are shown in Fig. 3A and the relative number of neurons in different groups is given in Fig. 3B. *Group 1* neurons were activated by the contralateral turn ( $n = 98$ , 71%); *group 2* neurons, by the ipsilateral turn ( $n = 13$ , 9%); *group 3* neurons, by both contralateral and ipsilateral turns ( $n = 16$ , 12%); and *group 4* neurons showed no phasic response to turns ( $n = 11$ ,

TABLE 1. Summary of data in individual animals

Animal	$n$	Group, %				Value of Peak, Hz ( $\pm$ SD)	Phase of Peak
		1	2	3	4		
1	19	80	5	10	5	$3.16 \pm 1.99$	0.75
2	17	76	0	12	12	$2.91 \pm 2.47$	0.75
3	14	64	7	7	22	$3.65 \pm 2.76$	0.75
4	16	81	7	12	0	$1.92 \pm 0.82$	0.85
5	17	76	12	6	6	$4.74 \pm 3.09$	0.75
6	14	79	0	7	14	$3.99 \pm 3.66$	0.65
7	5	40	60	0	0	$4.02 \pm 2.69$	0.75
8	8	88	12	0	0	$5.62 \pm 3.55$	0.85

For individual animals there are shown: total number of neurons ( $n$ ), relative size of different neuron groups, and the value  $\pm$  SD and phase of the peak response of group 1 neurons in the sinusoidal test (see Fig. 4A).

8%). Of these four groups, only the neurons of *group 1* and *group 2* had directionally specific vestibular responses and therefore could participate in the stabilization of orientation in the yaw plane.

The overwhelming majority of neurons belonged to *group 1*, both in the whole population and in individual animals (Table 1). Only in animal #7, was *group 2* larger than *group 1*, although the number of recorded cells in this animal was very small. *Group 1* was analyzed in detail. Figure 4A shows the summary of responses of all *group 1* neurons to sinusoidal turns and Fig. 4B, to trapezoidal turns. In both tests, the responses were well pronounced. In tests with sinusoidal turns, the mean value of activity during contralateral rotation (phases 0.5–1) was  $2.2 \pm 0.2$  versus  $0.4 \pm 0.1$  Hz during ipsilateral rotation (phases 0–0.5). In tests with trapezoidal turns, the mean value of activity in the contralateral turn (phases 0.5–1) was  $1.4 \pm 0.1$  versus  $0.2 \pm 0.1$  Hz in the ipsilateral turn (phases 0–0.5). The difference in both cases was statistically significant (paired  $t$ -test). In trapezoidal tests, the responses elicited by the contralateral turn decayed when a new position was maintained (Fig. 4B).

By comparing the spike amplitude recorded by the left and right rostral electrodes, we estimated the laterality of axons of *group 1* neurons (see METHODS and Fig. 2, C and D). Figure 5A shows the percentage of axons with different ratios ( $r$ ) of the minimal to the maximal amplitude. Most neurons (90%) belonged to the lateral group ( $r \leq 0.4$ ).

By measuring the time difference between the occurrence of a spike in the rostral and caudal electrodes (see METHODS and Fig. 2, C and D) the conduction velocity was determined for 77 neurons. The distribution of conduction velocities for these axons is shown in Fig. 5B. The velocity ranged from 1.6 to 4.0 m/s, but for 80% of axons the velocity was  $>2$  m/s. In the spinal cord of the lamprey, the only descending axons with such a high conduction velocity are the axons of larger RS neurons (see METHODS). From Fig. 5B one can also see that there was no clear correlation between the conduction velocity and axon position in *group 1* neurons. For *group 2* neurons, the conduction velocity ranged from 2.6 to 3.6 m/s and the majority of them (80%) belonged to the medial group ( $r > 0.4$ ).

In a part of the neurons, the activity was present on only one pair of electrodes; as a rule, this was the rostral pair. Responses of these neurons did not differ from those in the units identified as RS neurons and therefore they were considered together. The absence of activity of the neuron on the caudal electrodes

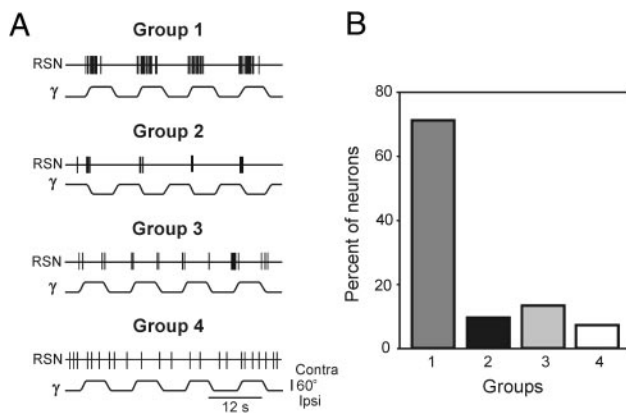


FIG. 3. Classification of RS neurons according to their pattern of vestibular response. A: examples of responses to trapezoidal yaw turns in 4 groups of neurons. *Group 1* responded to contra turn, *group 2* responded to ipsi turn, *group 3* responded to both turns, and *group 4* did not respond to turns. B: relative number of neurons in different groups ( $n = 138$ ).

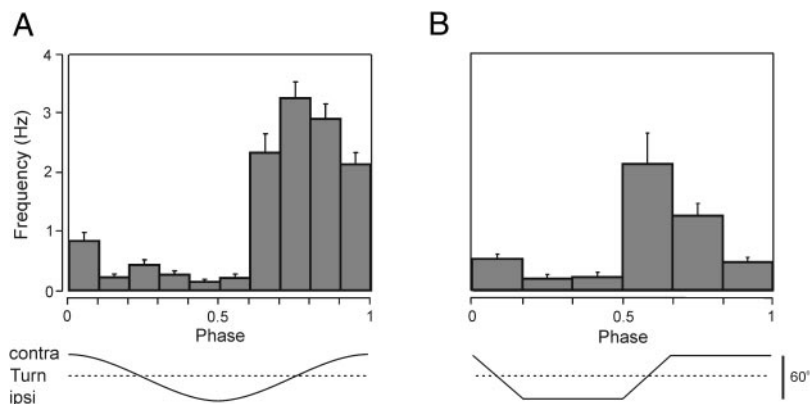


FIG. 4. Summary of responses of *group 1* neurons to sinusoidal (A) and trapezoidal (B) yaw turns. Histograms show the mean frequency ( $\pm$ SE) in different phases of the rotation cycle. Rotation was performed toward the ipsilateral labyrinth (ipsi) in the first half of the cycle (phases 0–0.5) and toward the contralateral labyrinth (contra) in the second half (phases 0.5–1) ( $n = 82$  in A and  $n = 80$  in B).

was most likely caused by a damage of the axon by the implanted array.

#### Effect of unilateral labyrinthectomy on vestibular responses of *group 1* neurons

Because RS neurons were normally silent before vestibular stimulation, the effect of stimulation could be roughly evaluated by simply counting the number of activated neurons. In six animals, we counted the number of active *group 1* neurons both before and after UL (Fig. 6A). The UL caused almost threefold reduction in the number of responding neurons on the side contralateral to UL (from 46 neurons in control to 16 neurons). On the side ipsilateral to UL, the number of responding *group 1* neurons decreased only slightly (from 32 to 25).

The UL affected not only the number of responding neurons on the contralateral side, but also their frequencies. Figure 6, B and C shows frequency curves of *group 1* neurons recorded on the side contralateral to UL in control (B) and after UL (C). The mean frequency of response, that is, the activity during the contralateral turn (phases 0.5–1), was significantly lower than that before UL ( $1.4 \pm 0.3$  vs.  $2.5 \pm 0.3$  Hz in control) ( $t$ -test). On the side ipsilateral to UL, the responses before and after UL were similar (Fig. 6, D and E) and the mean frequency of response in both conditions ( $1.8 \pm 0.3$  Hz in control vs.  $1.8 \pm 0.4$  Hz after UL) did not differ significantly ( $t$ -test).

The main effects of UL—that is, a considerable reduction in the number of responding *group 1* neurons (on the side contralateral to UL) and a decrease in their frequencies—were

robust and observed in each of the six animals both in sinusoidal and trapezoidal tests.

#### Effect of unilateral eye illumination

The effect of unilateral eye illumination was studied in eight animals. Figure 2B illustrates modifications of the vestibular responses of individual RS neurons caused by left eye illumination in animal A16. The effects in individual neurons were different. The eye illumination caused an increase of activity in four left (L2–L5) and in one right (R4) *group 1* neurons; in one right *group 2* neuron (R5); in one left *group 3* neuron (L1); and a decrease of activity in three right *group 1* neurons (R1–R3). After switching off the illumination, the activity of neurons returned to the initial level.

**IPISILATERAL EYE ILLUMINATION.** As shown in Fig. 7A, most of the RS neurons ( $n = 68$ , 89%) were activated by ipsilateral eye illumination, and only eight neurons (11%) were inhibited. From Fig. 7A one can also see that the majority of activated neurons ( $n = 51$ , 75%) and all inhibited neurons belonged to *group 1*. Ipsilateral eye illumination also activated seven neurons (10%) of *group 2*, three neurons (5%) of *group 3*, and seven neurons (10%) of *group 4*.

Figure 7, B–E shows separately the summary of responses to sinusoidal turns (frequency curves) in two populations of *group 1* neurons that were activated (B and C) and inhibited (D and E) by ipsi-eye illumination. In both populations, vestibular responses persisted during eye illumination (Fig. 7, C and E).

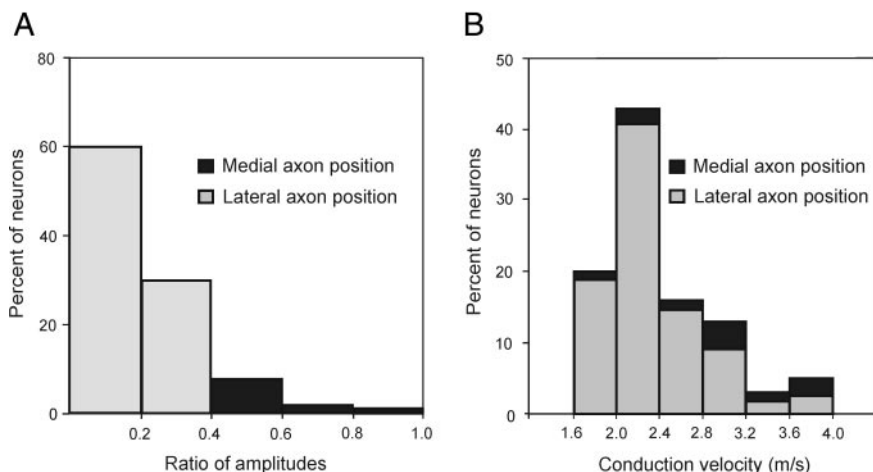


FIG. 5. Axon position and conduction velocity for *group 1* neurons. A: relative number of neurons with different ratios ( $r$ ) of minimal to maximal spike amplitude recorded by 2 rostral electrodes. Axons were arbitrarily divided into the lateral group ( $r \leq 0.4$ ) and medial group ( $r > 0.4$ ). B: distribution of conduction velocities ( $n = 98$  in A and  $n = 77$  in B).

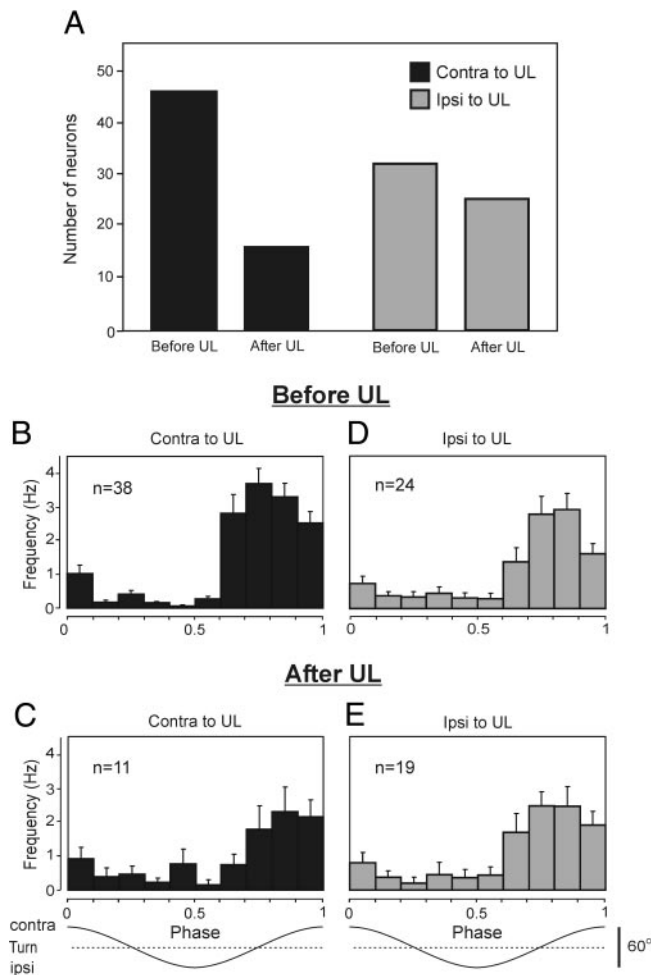


FIG. 6. Effect of unilateral labyrinthectomy (UL) on the activity of *group 1* neurons (summary over 6 animals). *A*: number of neurons activated during yaw turns before and after UL. Data are presented separately for the neurons located on the side contralateral and ipsilateral to UL. *B–E*: summary of responses to sinusoidal turns in 2 subgroups of *group 1* neurons: contralateral to UL (*B* and *C*) and ipsilateral to UL (*D* and *E*). Responses were characterized by the average discharge frequency ( $\pm$ SE) of the active neurons as a function of the yaw angle. *B* and *D*: responses before UL. *C* and *E*: responses after UL. In each animal, UL was performed on the side where the number of RS neurons recorded in control was the smallest.

In the population of activated neurons (*B* and *C*), the mean value of activity during contralateral rotation (phases 0.5–1) was  $3.4 \pm 0.3$  versus  $1.9 \pm 0.2$  Hz during ipsilateral rotation (phases 0–0.5). In the population of inhibited neurons (*D* and *E*), the mean value of activity during contralateral rotation (phases 0.5–1) was  $1.5 \pm 0.3$  versus  $0.4 \pm 0.2$  Hz during ipsilateral rotation (phases 0–0.5). The difference in both populations was statistically significant (paired *t*-test).

In the population of activated neurons, the activity during both ipsi- and contrarotation increased when the eye was illuminated (compare *B* and *C* in Fig. 7). The mean value of activity during contralateral rotation (phases 0.5–1) was  $3.4 \pm 0.3$  versus  $1.8 \pm 0.4$  Hz in control. The mean value of activity during ipsilateral rotation (phases 0–0.5) was  $1.9 \pm 0.2$  versus  $0.2 \pm 0.1$  Hz in control. The difference in both cases was statistically significant (paired *t*-test).

In the population of inhibited neurons, the activity during contrarotation decreased when the eye was illuminated (com-

pare *D* and *E* in Fig. 7). The mean value of activity during contralateral rotation (phases 0.5–1) was  $1.5 \pm 0.3$  versus  $3.2 \pm 0.5$  Hz in control. The difference was statistically significant (paired *t*-test). During ipsilateral rotation (phases 0–0.5) the activities during eye illumination and in control ( $0.4 \pm 0.2$  and  $0.8 \pm 0.2$  Hz, respectively) did not differ significantly.

**CONTRALATERAL EYE ILLUMINATION.** The contralateral eye illumination caused inhibition in the majority of RS neurons ( $n = 37$ , 59%), excitation in 25 neurons (39%), and did not affect one neuron (2%) (Fig. 8*A*). From the inhibited neurons, the majority ( $n = 34$ , 92%) belonged to *group 1*, one neuron (3%) to *group 2*, and two neurons (5%) to *group 3*. From the activated neurons, 13 neurons (52%) belonged to *group 1*, five neurons (20%) to *group 2*, five neurons to *group 3* (20%), and two neurons (8%) to *group 4*. Figure 8, *B–E* shows frequency curves for two populations of *group 1* neurons that were activated (*B* and *C*) and inhibited (*D* and *E*) by contra-eye illumination. In both populations, vestibular responses per-

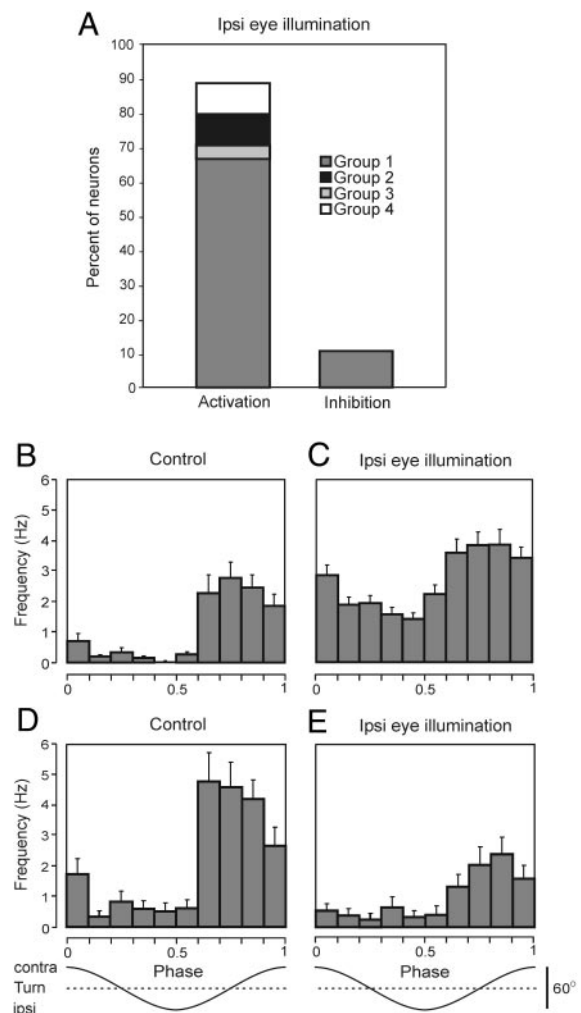


FIG. 7. Effect of ipsilateral eye illumination on the activity of RS neurons. *A*: effect on the neurons of different groups ( $N = 8$ ,  $n = 76$ ). *B–E*: summary of responses to sinusoidal turns in *group 1* neurons under 2 conditions: before eye illumination (*B* and *D*) and during eye illumination (*C* and *E*). Responses were characterized by the average discharge frequency ( $\pm$ SE) of active neurons as a function of the yaw angle. Data are presented separately for the neurons that were excited (*B* and *C*,  $n = 51$ ) and inhibited (*D* and *E*,  $n = 8$ ).



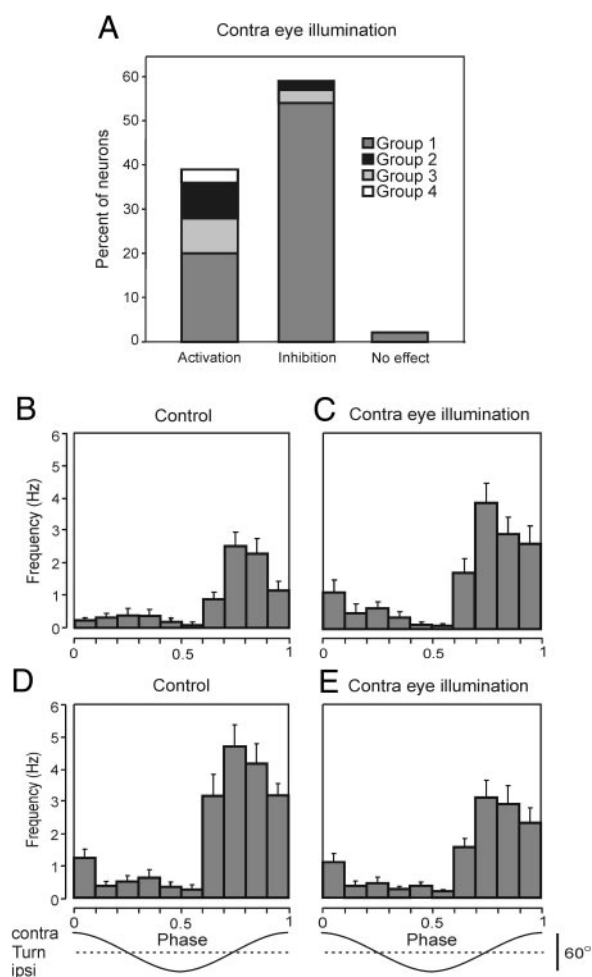


FIG. 8. Effect of contralateral eye illumination on the activity of RS neurons. A: effect on neurons of different groups ( $N = 8$ ,  $n = 63$ ). B–E: summary of responses to sinusoidal turns in *group 1* neurons under 2 conditions: before eye illumination (B and D) and during eye illumination (C and E). Responses were characterized by the average discharge frequency ( $\pm$ SE) of active neurons as a function of the yaw angle. Data are presented separately for the neurons that were excited (B and C,  $n = 13$ ) and inhibited (D and E,  $n = 34$ ).

sisted during eye illumination (Fig. 8, C and E). In the population of activated neurons, the mean value of activity during contralateral rotation (phases 0.5–1) was  $2.2 \pm 0.3$  versus  $0.5 \pm 0.2$  Hz during ipsilateral rotation (phases 0–0.5). In the population of inhibited neurons, the mean value of activity during contralateral rotation (phases 0.5–1) was  $2.0 \pm 0.3$  versus  $0.5 \pm 0.1$  Hz during ipsilateral rotation (phases 0–0.5). The difference in both populations was statistically significant (paired *t*-test).

In the population of activated neurons, the activity during contrarotation increased when the eye was illuminated (compare B and C in Fig. 8). The mean value of activity during contralateral rotation (phases 0.5–1) was  $2.2 \pm 0.3$  versus  $1.4 \pm 0.2$  Hz in control. The difference was statistically significant (paired *t*-test).

In the population of inhibited neurons, the activity during contrarotation decreased when the eye was illuminated (compare D and E in Fig. 8). The mean value of activity during contralateral rotation (phases 0.5–1) was  $2.0 \pm 0.3$  versus  $3.0 \pm 0.4$  Hz in control. The difference was statistically significant (paired *t*-test).

The population of neurons ( $n = 25$ ) activated by contralateral eye illumination could initiate negative phototaxis. Their conduction velocity ranged from 1.8 to 4 m/s; for 23 of them their axons were located laterally in the spinal cord.

## DISCUSSION

### *Yaw-related RS neurons*

The RS neurons recorded in the present study responded to rotation in the yaw plane. To simulate head movement during undulatory swimming and sharp lateral turn, we used sinusoidal and trapezoidal turn trajectories, respectively. The RS neurons responded to both types of stimulation. All recorded neurons were classified into four groups according to their pattern of response to vestibular stimulation. The overwhelming majority of them (*group 1*) were activated by the contralateral yaw turn (Fig. 3). It was recently shown that the main effect of RS neurons activated by vestibular input during the contralateral yaw turn is the ipsilateral body flexion (Zelenin et al. 2005). Taken together, these findings suggest that *group 1* neurons participate in the corrections of orientation in the yaw plane, by causing a turn of the animal in the direction opposite to the initial deviation (see following text).

The majority of *group 1* neurons had an axonal conduction velocity of  $>2$  m/s (Fig. 5B), which is characteristic for the large- and middle-size RS cells (Ohta and Grillner 1989; Rovainen 1967, 1978). Most of their axons were located in the lateral parts of the spinal cord (Fig. 5A). A similar distribution was found for the RS neurons responding to roll tilt (Deliagina and Fagerstedt 2000). In the distribution of pitch-related neurons, two peaks were observed: in the medial and lateral areas of the spinal cord (Pavlova and Deliagina 2002).

A small proportion of neurons activated by the ipsilateral yaw turn (*group 2*) could also participate in the control of yaw angle (see following text). By contrast, neurons of *groups 3* and *4* did not discriminate the direction of rotation and could not directly participate in yaw control. However, the presence of tonic activity in these neurons suggests that they might affect the level of excitability in the spinal networks.

It has to be noted that the method used in the present study allowed us to record from the large- and middle-size RS neurons. These cells, however, constitute a small proportion (roughly 10%) of the whole RS population, which consists mainly of small neurons (Bussi eres 1994); vestibular responses of these small neurons remain unknown.

### *Origin of vestibular responses in group 1 neurons*

In lampreys, a turn of the head in the yaw plane results in activation of the vestibular afferents projecting to the brain stem through the anterior branch of the contralateral vestibular nerve and through the posterior branch of the ipsilateral nerve (Lowenstein 1970; Lowenstein et al. 1968). Using electrical stimulation of individual branches, Pflieger and Dubuc (2004) showed that RS neurons are excited by the branches activated by the contralateral yaw turn and are inhibited by the branches activated by the ipsilateral yaw turn. In the present study we found that natural stimulation of vestibular organs by turns in the yaw plane results in activation of the contralateral RS neurons, confirming the functional role of vestibular projections to RS neurons revealed by Pflieger and Dubuc (2004).



To find out the relative contribution of afferents from the two labyrinths to the origin of yaw-responses in RS neurons, experiments with unilateral labyrinthectomy were performed. It was found that UL caused a significant reduction in the activity of RS neurons on the contralateral side: both the number of responding neurons and their frequency decreased. On the ipsilateral side, the number of responding neurons decreased only slightly, whereas their frequency was similar to that in control (Fig. 6). Thus for the majority of yaw-related neurons, the main source of excitatory vestibular influences was the contralateral labyrinth. These influences seem to be transmitted by the afferents in the posterior branch of the contralateral vestibular nerve (Pflieger and Dubuc 2004). Earlier it was shown that the RS neurons responding to tilts in the roll plane and to nose-up tilts in the pitch plane also receive their main vestibular input from the contralateral labyrinth (Deliagina and Pavlova 2002; Pavlova and Deliagina 2003).

Persistence of weaker vestibular responses in some RS neurons on the side contralateral to UL suggests that the excitatory input to RS neurons from the ipsilateral labyrinth supplements the main input from the contralateral labyrinth. These influences seem to be transmitted by the afferents in the anterior branch of the ipsilateral vestibular nerve (Pflieger and Dubuc 2004). Elimination of this weak ipsilateral input caused by UL is the likely reason for a slight reduction in the number of responding neurons on the side ipsilateral to UL (Fig. 6A).

#### Functional role of group 1 neurons

Figure 9 shows a conceptual model of the yaw control system based on the two major findings. 1) In the present study it was shown that two subpopulations of RS neurons—the right, RS(R) and the left, RS(L)—are driven by vestibular inputs (mainly from the contralateral labyrinth), so that they are activated with the contralateral yaw turn. 2) It was recently shown that the RS neurons, activated by a contralateral yaw turn, send commands to the spinal cord, which cause an ipsilateral yaw turn of the animal, that is, rotation opposing the initial turn (Zelenin et al. 2005). For example, if an external force causes passive turning of the lamprey to the left, the right subpopulation is activated by the vestibular input. This subpopulation will elicit a corrective turn of the animal to the right by the spinal mechanisms and restoration of the initial orientation in the yaw plane. Thus the yaw control system, based on vestibular reflexes, counteracts any deviations from the rectilinear swimming caused by external factors.

It should be noted that, in the control mechanisms for the pitch and roll planes, the corresponding RS neurons receive vestibular signals about orientation in the gravity field, thus allowing the system to maintain definite angles in these planes (Deliagina et al. 2006). By contrast, turns in the horizontal plane do not affect the animal's orientation in relation to the gravity vector. For that reason, the yaw control mechanism receives vestibular signals only about dynamic changes of the yaw orientation, but not about the orientation itself. As a result, this mechanism can reduce rapid but not slow deviations from the swim trajectory. Thus maintenance of a certain direction of swimming in the yaw plane requires participation of other sensory inputs (e.g., visual) signaling the absolute value of the yaw angle.

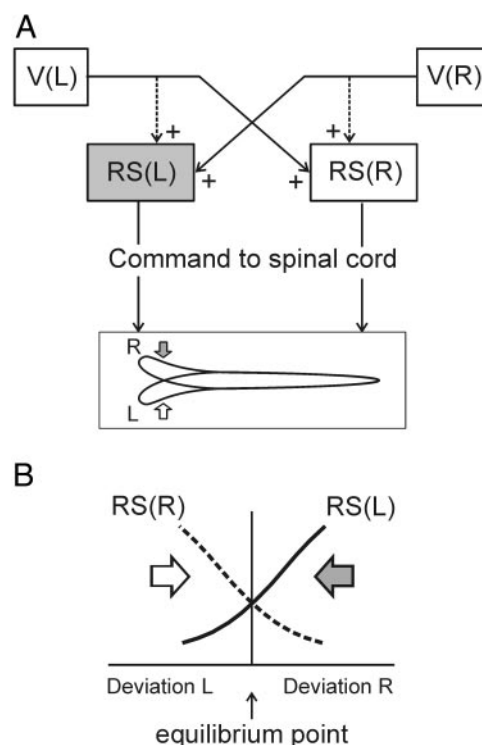


FIG. 9. Presumed function of group 1 neurons. A: a conceptual model of the yaw control system in the lamprey. Two subpopulations of RS neurons, RS(R) and RS(L), are driven by vestibular afferents from the left, V(L) and right, V(R) vestibular organs. As a result of these inputs, RS(R) and RS(L) respond to the left and right yaw turn, respectively. RS neurons affect the spinal network and cause right and left turning of the lamprey, respectively (white and gray arrows). Solid lines indicate the major effects on RS neurons produced by vestibular organs; interrupted lines indicate the minor effects. B: operation of the system during swimming. Two curves represent the activity of RS(R) and RS(L) subpopulations caused by a dynamic deviation of the head movement from the rectilinear one. Motor effect of each RS subpopulation is proportional to its activity. Direction of turning caused by RS(R) and RS(L) are indicated by the gray and white arrows, respectively. System has an equilibrium point, where the effects of RS(R) and RS(L) are equal to each other.

In the swimming lamprey, the propulsive force is generated as a result of the periodical locomotor undulations of the head and body in the yaw plane (Wallén and Williams 1984; Williams et al. 1989). One can expect that vestibular reflexes caused by external factors and by undulatory head movements interact. Vestibular reflexes can also be rhythmically modulated by the spinal locomotor CPG by two routes: 1) the RS neurons themselves are modulated by efference copy signals (Kasicki et al. 1989) and 2) the efficacy of vestibular input to these neurons is rhythmically gated (Bussi eres and Dubuc 1992; Pflieger and Dubuc 2004). To study this problem, recording of the activity of RS neurons during swimming and correlation of this activity with active and passive head movements should be carried out.

Group 2 neurons, responding to the ipsi-turn, could supplement group 1 by activating contralateral myotomes, although these effects have not been demonstrated (Zelenin et al. 2006).

#### Effects of unilateral visual input

Nonpatterned visual stimuli (unilateral eye illumination) in the lamprey evoke two types of motor behavior: “negative phototaxis” and “dorsal light response.” In the swimming

lamprey, the negative phototaxis is a lateral turn away from the source of light (Ullén et al. 1993, 1995b, 1997). The negative phototaxis can also be observed in the lamprey attached to the bottom by its sucker mouth; it includes detachment from the substrate and a lateral turn away from the source of light, followed by the rectilinear swimming (Ullén et al. 1993, 1995b, 1997). For this type of response, the RS neurons causing lateral turn should be activated. Another type of reaction to asymmetrical eye illumination—the dorsal light response—consists of the roll tilt toward the source of light (Ullén et al. 1993, 1995b, 1997). It requires activation of RS neurons causing rolling, i.e., rotation about the longitudinal body axis.

In the present study we have identified a population of RS neurons (*group 1*) sensitive to turns in the yaw plane and presumably involved in the control of orientation in this plane. Can these neurons (or a part of them) be involved in the control of the negative phototaxis? It is known that visual input activates RS neurons through the neurons in the contralateral pretectum (Zompa and Dubuc 1996). They project bilaterally to RS neurons and the noncrossed projection seems to cause the negative phototaxis (Ullén et al. 1997). Thus for performing this particular function, the yaw-related RS neurons (which are known to cause the ipsilateral yaw turn; Zelenin et al. 2005) should be activated by illumination of the contralateral eye.

However, our experiments have shown that most of the *group 1* neurons were excited by illumination of the ipsilateral eye and inhibited by illumination of the contralateral eye (Figs. 7 and 8). The net effect of the whole RS population would thus be not the negative but the positive phototaxis. Only a small proportion of *group 1* neurons (27%) was excited by illumination of the contralateral eye, and thus they constitute the candidate neurons for mediating the negative phototaxis.

This controversy can be explained in different ways. First, one can suggest that visual input to RS neurons, observed in resting animals, is subjected to substantial modifications when the animal is in the active state, with enhancement of the connections that underlay the negative phototaxis and with inhibition of those preventing this response. In this respect it is interesting to note that, when repeatedly eliciting the responses to unilateral eye illumination, the turn away from the light was the most often observed reaction, but the turn toward the light sometimes also occurred (Ullén et al. 1995b, 1997). Second, one can suggest that there are other RS neurons, without specific reactions to yaw rotation, but nevertheless causing turns in the yaw plane. We found that some neurons activated by contralateral eye illumination had no specific responses to yaw turns (Fig. 8). They could participate in initiation of the negative phototaxis.

In the present study we argued that the majority of yaw-related RS neurons (*group 1*), as a consequence of specific relationships between their sensory inputs and motor effects, counteract lateral deviations of the head (caused by external destabilizing factors) and thus promote maintenance of a rectilinear swim trajectory. Does this stabilizing system also operate in the cases when the lateral turns are initiated by the lamprey itself as, say, during the negative phototaxis? Our experiments with eye illumination have shown that unilateral visual input did not change the basic pattern of responses of RS neurons to yaw turns: they were activated during the contralateral rotation (Figs. 7 and 8). These findings suggest that the

yaw-stabilizing system counteracts not only passive but also active turns of the head. One can suppose that the commands for active turn, addressed to RS neurons, overcome the head-stabilizing effects of vestibular reflexes.

#### ACKNOWLEDGMENTS

The authors are grateful to Dr. R. Hill for valuable comments on the manuscript.

#### GRANTS

This research was supported by the Swedish Research Council, Section of Medicine Grant 11554 and Section of Natural Science and Technology, and Gösta Fraenckels Foundation.

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