Responses of Reticulospinal Neurons in Intact Lamprey to Pitch Tilt

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Pavlova, E. L., and T. G. Deliagina. Responses of reticulospinal neurons in intact lamprey to pitch tilt. J Neurophysiol 88: 1136–1146, 2002; 10.1152/jn.00051.2002. In the swimming lamprey, a postural control system maintains a definite orientation of the animal’s longitudinal axis in relation to the horizon (pitch angle). Operation of this system is based on vestibular reflexes. Important elements of the postural network are the reticulospinal (RS) neurons, which are driven by vestibular input and transmit commands for postural corrections from the brain stem to the spinal cord. Here we describe responses to vestibular stimulation (rotation of the animal in the pitch plane) in RS neurons of intact lampreys. The activity of neurons was recorded from their axons in the spinal cord by chronically implanted arrays of macroelectrodes. From the multielectrode recordings of mass activity, discharges in individual axons were extracted by means of a spike-sorting program, and the axon position in the spinal cord and its conduction velocity were determined. Vestibular stimulation was performed by rotating the animal in steps of 45° throughout 360° or by periodical “trapezoid” tilts between the nose-up and -down positions. Typically, the RS neurons exhibited both dynamic responses (activity during movement) and static responses (activity in a new sustained position). The neurons were classified into two groups according to their pattern of response. Group UP neurons responded preferentially to nose-up rotation with maximal activity at 0–135° up. Group DOWN neurons responded preferentially to nose-down rotation with maximal activity at 0–135° down. Neurons of the two groups also differed in the position of their axons in the spinal cord and axonal conduction velocity. An increase in water temperature, which presumably causes a downward turn in swimming lampreys, affected the activity in the UP and DOWN groups differently, so that the ratio UP responses to DOWN responses increased. We suggest that the UP and DOWN groups mediate the opposing vestibular reflexes and cause the downward and upward turns of the animal, respectively. The lamprey will stabilize the orientation in the pitch plane at which the effects of UP and DOWN groups are equal to each other. In addition to the main test (rotation in the pitch plane), the animals were also tested by rotation in the transverse (roll) plane. It was found that 22% of RS neurons responding to pitch tilts also responded to roll tilts. The overlap between the pitch and roll populations suggests that the RS pathways are partly shared by the pitch and roll control systems.

INTRODUCTION

When swimming, the lamprey (a lower vertebrate, cyclostome) maintains a specific body orientation in space. Most often, e.g., during long-distance migrations, the longitudinal body axis is oriented horizontally (0 pitch angle), and the dorsal side is directed upward (0 roll angle). Under certain conditions, however, the pitch angle can differ from zero (swimming “uphill” or “downhill”). In behavioral experiments, it was found that these different pitch angles are maintained due to the activity of a postural control system driven by vestibular input (Ullén et al. 1995a). In the present study, we examine vestibular reflexes participating in the control of pitch angle. This study is an extension of the previous studies devoted mainly to the nervous mechanisms controlling roll angle (Deliagina 1997a,b; Deliagina and Fagerstedt 2000; Deliagina and Pavlova 2002; Deliagina et al. 1992a,b, 1993; Zelenin et al. 2000).

Important elements of the postural network in the lamprey are the reticulospinal (RS) neurons transmitting commands for postural corrections from the brain stem to the spinal cord (Deliagina et al. 1992a; Orlovsky et al. 1992). The RS pathways originate from four reticular nuclei of the brain stem: the mesencephalic reticular nucleus (MRN) as well as anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei (Nieuwenhuys 1972). The RS neurons receive vestibular input through interneurons of the vestibular nuclei (Koyama et al. 1989; Northcutt 1979; Rovainen 1979; Rubinson 1974; Stefanelli and Caravita 1970). They also receive inputs from other sensory systems as well as from the forebrain, from the brain stem centers, and from the spinal cord (Deliagina et al. 1993; Dubuc et al. 1993; Rovainen 1967, 1979; Viana Di Prisco et al. 1995; Wickelgren 1977).

In earlier studies (Deliagina et al. 1992a; Orlovsky et al. 1992), responses of RS neurons to tilts in different planes were investigated in vitro in a preparation consisting of the brain stem isolated together with vestibular organs. It was found that RS neurons responded to rotation in the sagittal (pitch) plane; their responses consisted of the “dynamic” component (activity during movement) and the “static” component (activity in a sustained new position). The neurons exhibited directional sensitivity; that is, they responded more strongly to rotation in one direction than in the other. Most recorded neurons belonged to one of the two groups, i.e., those responding to the nose-up rotation (group UP) with their maximal response at 45–90° up and those responding to the nose-down rotation (group DOWN) with their maximal response at 45–90° down. In addition, some neurons exhibited their maximal activity at the dorsal-side-down position.

By using the same preparation, responses of vestibular afferents to pitch tilt were also investigated (Deliagina et al. 1992b). The canal afferents responded only “dynamically” to rotation either in nose-up or -down direction, whereas the otolith afferents responded both dynamically and “statically” to
a change in position; some of them exhibited directional sensitivity. A few groups of otolith afferents differing in their spatial zones of sensitivity in the pitch plane could be distinguished. One can suggest that responses to pitch tilts in RS neurons are caused by inputs from specific groups of otolith and canal afferents converging on the neurons.

On the basis of these in vitro experiments, a hypothesis was advanced that the UP and DOWN groups of RS neurons, driven by the corresponding groups of vestibular afferents, constitute an essential part of the pitch control system (Deliagina et al. 1992a). It was suggested that they mediate two opposing vestibular reflexes, i.e., group UP causes a downward turn of the lamprey, whereas group DOWN causes an upward turn. This control system stabilizes the pitch angle at which the motor effects of the two groups are equal to each other.

The data on vestibular responses of RS neurons considered in the preceding text, however, were obtained on the in vitro preparations in which a number of inputs to RS neurons, like those from the forebrain, from the cranial nerves, and from the spinal cord, were abolished; this may affect vestibular responses in RS neurons. The first aim of the present study was to examine the activity of RS neurons, related to the pitch control, in intact animals. We used the recently developed method of recording the activity of RS neurons from their axons in the spinal cord by means of chronically implanted electrodes (Deliagina and Fagerstedt 2000; Deliagina et al. 2000b). Responses of individual neurons were then extracted from the multielectrode recording of the mass activity by means of a spike-sorting program (Deliagina and Fagerstedt 2000). The main result of this study is that the UP and DOWN groups of RS neurons are present in the intact animals as well.

In vitro experiments (Deliagina et al. 1992a) have shown that group UP of RS neurons is rather inconsistent, i.e., in some of experiments the UP neurons were not found, whereas in other experiments, the UP neurons could spontaneously change their pattern of response and even become silent. It was suggested that these changes in the group UP activity reflected the changes in the “set-point” of the pitch control system. The second aim of the present study was to test this hypothesis and to examine the activity of UP and DOWN groups under the conditions that may influence the pitch angle. One of these is the water temperature: the lampreys prefer lower temperatures, which are usually associated with deeper water layers (unpublished observation). In the present study, we examined vestibular responses of RS neurons under different temperatures and found that an increase in temperature (which presumably causes a downward turn of the swimming animal) resulted in an increase of the ratio of the activity in the UP group to that in the DOWN group.

From behavioral experiments it is known that the motor patterns for changing the body orientation in the roll and pitch planes strongly differ from each other (Ullén et al. 1995a): changes in the pitch angle are caused by body flexion in the sagittal plane, whereas roll tilt can be caused by a number of motor patterns including body twisting, tail bending, and fin deviation. Because all commands for postural corrections are transmitted by RS pathways, a question arises: to what extent do the populations of RS neurons, transmitting roll and pitch commands, overlap? In vitro experiments (Orlovsky et al. 1992) have shown that most RS neurons responded to both roll and pitch tilts. The third aim of the present study was to address this issue in intact animals. We examined responses of individual RS neurons to both roll and pitch tilts and found that the populations of RS neurons responding to roll tilt and to pitch tilt partly overlap.

Brief accounts of this study have been published (Pavlova and Deliagina 2000, 2001).

METHODS

Experiments were carried out on 11 adult (25–35 cm in length) intact lampreys (Lampetra fluviatilis), which were kept in an aerated freshwater aquarium at 6°C, with a 12 h:12 h light:dark cycle. Responses to pitch and roll tilts were examined in eight animals; in two of them and in three other animals, the effect of temperature on the pitch-evoked responses was studied.

Electrodes

The activity of RS neurons was recorded from their axons in the spinal cord by means of chronically implanted macroelectrodes as described in detail in the previous papers (Deliagina and Fagerstedt 2000; Deliagina et al. 2000b). In short, the electrodes (silver wires 75 µm in diameter and 3 mm in length) were oriented in parallel to the long spinal axons. They allowed an almost exclusive recording of the spike activity from larger fibers, which have a conduction velocity of more than 2 m/s. In the lamprey, only RS pathways contain fibers with such a high conduction velocity. The electrodes were glued to a plastic plate (5 mm long, 2.2 mm wide, and 0.25 mm thick). Two different designs of the electrode array were used, with four electrodes and with two electrodes (Deliagina and Fagerstedt 2000) (see also inset in Fig. 2A).

Surgery

Animals were operated under MS-222 (Sandoz) anesthesia (100 mg/l). Implantation of electrodes was performed as described in detail earlier (Deliagina and Fagerstedt 2000). The plates with two electrodes and with four electrodes were implanted at the levels of the third and the last gill, respectively, so that the distance between the plates was 20–25 mm. The electrodes faced the dorsal aspect of the spinal cord.

Experimental protocol

Responses of RS neurons to rotation in the sagittal (pitch) plane were examined in all 11 animals on the next day after implantation of the electrodes. To rotate the lamprey, it was positioned in an apparatus (Fig. 1A) that consisted of a tube fastened to a small platform. After having been positioned into the tube, the lamprey usually attached to the platform with its sucker mouth. From behavioral experiments, it is known that when changing the pitch angle, the lamprey normally moves along an arc with a radius approximately equal to a half body length (Ullén et al. 1995a). In the present study, to have vestibular stimuli similar to natural ones, we rotated the lamprey around the axis situated in the mid-body area. Rotation was performed manually by means of a handle passing through the front wall of the aquarium. The pitch tilt angle (θ) was measured by a potentiometric transducer. The level of water in the aquarium with the setup was high enough to completely cover the animal during rotation within the angular range used. The contacts of the implanted wires with the input cable of the amplifiers always occurred above the water surface.

Two types of vestibular stimulation were used. First, the animal was tested by two full turns in the sagittal (pitch) plane; the rotation in the turn a and in the turn b were performed in the opposite directions to reveal a directional sensitivity of responses. Figure 1B shows how the pitch angle was changed. The initial orientation of the animal was with its dorsal side down (180°). Rotation was performed in 45° steps. The transition from one position to the next lasted
approximately 1 s, and each position was maintained for approximately 3 s. Second, the animal was tested by periodic trapezoid tilting with alternating tilts from the nose-up position to the nose-down position and the reverse (Fig. 1C). The transition from one position to the other lasted approximately 1 s, and each position was maintained for approximately 3 s. Normally, the experiments were carried out at a water temperature of 5–6°C. In the experiments aimed at examining the effect of temperature, however, the animals were sequentially tested in the water with a temperature of 4 and 14°C.

In addition to the main test, that is, rotation in the pitch plane, the animals were also tested by rotation in the transverse (roll) plane around the animal’s longitudinal axis. A setup for these experiments was described in detail in the previous paper (Deliagina and Fagerstedt 2000). For these tests, each animal was transferred from the setup for pitch rotation to the one for roll rotation.

Data processing

Signals from the electrodes were amplified by conventional AC amplifiers, digitized with a sampling frequency of 10 kHz, and recorded to the disk of an IBM AT compatible computer by means of the data-acquisition software (Digitida 1200/Axoscope, Axon Instruments, Foster City, CA). The recorded multiunit spike trains were separated into unitary waveforms, representing the activity of individual axons, by means of data analysis software (Datapac III, Run Technologies, Laguna Hills, CA). The analysis was described in detail in the previous papers (Deliagina and Fagerstedt 2000; Deliagina and Pavlova 2002).

To determine the angular zones of sensitivity of individual RS neurons in the tests with full-turn rotation, their vestibular responses were characterized quantitatively. For this purpose, each step of rotation was divided into three intervals (1–3, see inset in Fig. 1B), and the firing frequency of a neuron was measured separately for each of the intervals in each step. The activity in the interval 1 (during movement) will be termed the dynamic response; the activity in the intervals 2 and 3 (when a new position was maintained) will be termed the early and late static responses, respectively.

The mediolateral position of individual axons in the spinal cord was estimated by comparing the amplitudes of the same spike recorded by different electrodes of the four-electrode array (electrodes 1–4 in Fig. 2A1). The conduction velocity in individual axons was evaluated by measuring the time delay between the appearance of the same spike in the rostral electrodes (5 and 6 in Fig. 2A1) and caudal electrodes (1–4 in Fig. 2A1) (for details, see Deliagina and Fagerstedt 2000).

All the analytical procedures and possible sources of errors during the spike sorting have been described in detail in the previous papers (Deliagina and Fagerstedt 2000; Deliagina and Pavlova 2002) and are briefly summarized in DISCUSSION. Besides the possible errors introduced by spike sorting, an additional possible source of errors in the present study could have been a change in the recording conditions caused by displacement of the electrode arrays while carrying the animal from the pitch setup to the roll setup. However, there were no marked changes in spike waveforms following a transfer of the animal from one setup to the other in any of eight experiments, as illustrated for one of the animals in Fig. 2. Figure 2A1 shows spikes of one of the RS neurons recorded by the rostral and caudal electrodes (5 and 6 and 1–4, see Fig. 2A1, inset) when the animal was positioned in the pitch setup and stimulated by rotation in the sagittal plane, as in Fig. 2B2 (the display was synchronized by the “event” signal) (see Deliagina and Fagerstedt 2000). Then the animal was moved to the roll setup and stimulated by rotation in the transverse plane, as in Fig. 2B2. 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 practically unchanged after moving the animal (compare Fig. 2, A1 and B1).

As was revealed in the experiments with full-turn pitch rotation followed by spike-sorting (see RESULTS), the overwhelming majority of RS neurons belonged either to one or the other of the two groups (UP or DOWN) and responded preferentially either to upward or to
downward pitch tilt. We took advantage of this finding, and when examining the effect of temperature on the vestibular responses, we used only trapezoid tests and compared the mass activity evoked by an upward tilt with that evoked by a downward tilt. For this purpose, each cycle of tilting was divided into two intervals (UP and DOWN, see Fig. 6, inset), and the number of spikes generated by all RS neurons was measured separately for each of the intervals. This method allowed us to avoid difficulties with spike sorting caused by changes in the spike amplitude and duration under different temperatures.

RESULTS

Characteristics of up and down groups

Normally, the resting activity in RS neurons was low or absent. Vestibular stimulation activated RS neurons. Figure 3 shows the responses of nine RS neurons to full-turn rotation recorded in the animal P8. All these neurons could be divided into two groups (UP or DOWN) according to the pattern of their response to vestibular stimuli. Neurons of group UP (5-9 in Fig. 3) responded preferentially to the nose-up rotation (turn a). The response contained both dynamic and static components. Both components were position-dependent, with their maximum within the zone of 0–135° up. Neurons of group DOWN (1–4) responded preferentially to the nose-down rotation (turn b), with the maximal response within the zone of 0–135° down.

Altogether, 114 RS neurons exhibited specific responses to vestibular stimulation in the tests with full-turn rotation. Of these, 90 neurons belonged to group UP, and 24 neurons to group DOWN.

When tested by trapezoid tilts, all the neurons of group UP, revealed in the full-turn tests, were also preferentially active during the nose-up tilts, and all neurons of group DOWN were active during the nose-down tilts. In addition, many new neurons appeared in the trapezoid tests that were not active in the full-turn tests. This was due to much stronger vestibular stimulation, that is rotation by 180 against 45°. Of these new neurons, 52 neurons were classified as group UP neurons, and 15 as group DOWN neurons. Thus on the basis of the two tests, 142 of 181 neurons (78%) were classified as group UP neurons, and 39 (22%) as group DOWN neurons. In each group, almost an equal number of neurons were recorded on the left and right side of the spinal cord. Besides UP and DOWN neurons, a few neurons exhibited the activity with no obvious correlation to the direction of rotation and to a specific angular zone; they were excluded from further analysis.

The population activity of groups UP and DOWN was described by using two characteristics: the percent of simultaneously active neurons as a function of the pitch angle and the frequency curve, that is the mean discharge frequency of active neurons as a function of the pitch angle (Deliagina and Fagerstedt 2000). A histogram of the relative number of active neurons of group UP is shown in Fig. 4A1. The neurons responded preferentially to the nose-up rotation (turn a). In this turn, any change of orientation evoked a dynamic response in many RS neurons; most neurons were activated within the zone of 0–135° up.

A frequency curve for group UP (Fig. 4A2) has the same
essential features as the histogram of the number of active neurons (Fig. 4A1). The neurons responded dynamically throughout turn a, with larger responses within the zone 0–135° up. The static responses were much weaker than the dynamic ones (0.3 Hz against 3–4 Hz); they usually contained only “early” component. Responses during turn b were almost absent.

The population activity of group DOWN mirrored that of group UP as reflected both in the histogram of the number of active neurons (Fig. 4B1) and in the frequency curve (Fig. 4B2). Most neurons responded preferentially to the nose-down rotation (turn b), with the maximal response within the zone 0–135° down. In this zone, about 30% of the neurons exhibited weak static responses.

The position of the axon in the spinal cord was determined for 130 of 181 recorded RS neurons of groups UP and DOWN (see METHODS). Figure 5A shows the mediolateral distribution of RS axons in the spinal cord. There were two peaks of the axon density—in the medial zone (1) and in the lateral zone (4). The group DOWN axons were located preferentially in the medial zone, and group UP axons—in the medial and lateral zones. In all cases when the conduction velocity was measured (n = 116), the spikes propagated in the caudal direction. The conduction velocities ranged from 1.6 to 5.6 m/s (Fig. 5B). In the spinal cord of the lamprey, the only descending axons with such a high conduction velocity are the axons of RS neurons. Group DOWN neurons were almost evenly distributed over the range of 2–5.6 m/s, whereas most neurons of group UP had velocities ranging from 2 to 3.6 m/s.

Effect of temperature on UP and DOWN groups

The effect of temperature was studied by using trapezoid tilts for vestibular stimulation (see METHODS). We calculated two values: the UP response, that is, the total number of spikes generated by all neurons during the upward tilt and in the up position and the DOWN response, that is, the total number of spikes generated during the downward tilt and in the down position (see Fig. 6, inset). An UP/DOWN ratio was then calculated for each cycle and averaged for each animal over all tilt cycles at a given temperature. In most animals, RS neurons of group UP were more numerous than those of group DOWN (see preceding text), UP responses prevailed over DOWN responses, and their ratio was more than unity. Figure 6 shows the UP/DOWN ratio for five individual animals at two temperatures, 4 and 14°C. In all five cases, the ratio increased at the higher temperature, this increase was statistically significant in four animals (t-test, P < 0.05, marked by ● in Fig. 6).

Comparison of responses to pitch and roll tilt

In addition to the main test, that is a full-turn rotation in the pitch plane, all animals were also tested by a full-turn rotation in the transverse (roll) plane. The results of this test did not differ from those described earlier (Deliagina and Fagerstedt 2000). All neurons responding to roll tilt (n = 103) could be divided into two groups according to their pattern of response. Group 1 neurons (n = 76) were activated by rotation toward the contralateral labyrinth, whereas group 2 neurons (n = 27) responded to rotation in both directions. The populations of neurons activated by pitch and roll tilts partly overlapped, as illustrated by in Fig. 7A. It was found that 39 of 181 neurons (22%), responding to pitch tilts, also responded to roll tilts. Of these 39 neurons, 35 neurons belonged to group UP and 4 to group DOWN. When tested by roll tilt, most of these neurons were classified as group 1 neurons (29 neurons from group UP and 3 neurons from group DOWN). In group 2, six neurons belonged to group UP and 1 neuron to group DOWN.

The population of neurons classified as group UP in the pitch tests and as group 1 in the roll tests were numerous enough that frequency curves (mean ± SE) for both tests could be produced (Fig. 7, B and C). The curve for pitch test (Fig. 7B) was similar to that obtained in the present study for the whole group UP (Fig. 4A2), whereas the curve for roll test (Fig. 7C) was similar to that obtained in the previous study for group 1 (see
Fig. 7D in Deliagina and Fagerstedt 2000). However, the maximal frequencies in the pitch test were slightly lower than in the roll test (compare Figs. 7, B and C).

DISCUSSION

Possible errors caused by spike sorting and by instability of recording conditions

A detailed analysis of possible errors caused by the spike sorting procedure was given in the previous paper (Deliagina and Fagerstedt 2000). In brief, discharges in individual axons were separated (clustered) on the basis of multiple criteria: the simultaneous occurrence of a spike in all electrodes of the array, the constancy of the spike waveform in each electrode, the constancy of the axon position in the spinal cord, and the constancy of the axonal conduction velocity. Due to the multitude of criteria, both possible types of errors in clustering, i.e., misidentification and loss of spikes, were reduced considerably. An estimate for these errors was obtained when the same cluster of units was separated on the basis of inputs from
different combinations of electrodes and even the electrodes from the rostral and caudal arrays. It was found that the difference in the number of spikes in a cluster was always less than 20%. These errors might lead to the corresponding errors in the mean firing frequency of RS neurons. However, such small errors in frequency could not affect the principal conclusion of the present study, that is, the existence of UP and DOWN groups of RS neurons (see Results).

As judged from minor changes of the spike waveform in the RS neurons identified initially in the pitch setup and then in the roll setup (Fig. 2), the transfer of the animal had practically no effect on the recording conditions. This allowed us to compare quantitatively the “pitch” and “roll” populations of RS neurons.

Characterization of group UP and DOWN axons

In the present study, we used a new type of electrode, which can record selectively the activity from the larger axons (Deliagina and Fagerstedt 2000; Deliagina et al. 2000b). All axons recorded in this study had a conduction velocity ranging from 1.6 to 5.6 m/s. In the lamprey spinal cord, only RS neurons have axons with such a high conduction velocity.

According to morphological data (Rovainen 1982), there are several groups of the middle- and large-size RS axons in the spinal cord of the lamprey. The largest of them (30–50 μm diam) belong to different groups of Müller cells (M, I, and B types located in the MRN, ARRN, and MRRN, respectively) and Mauthner cells. Most of the Müller axons are located in the middle area of the spinal cord. The medium-size axons (10–30 μm diam) belong to the medium-size cells located in the MRRN and PRRN. The latter are sometimes termed V cells. The majority of the medium-size axons are located in the lateral part of the spinal cord. The conduction velocity of the medium- and large-size axons is in the range from 1 to 5 m/s (Ohta and Grillner 1989; Rovainen 1978).

These data can be compared with the results of the present study summarized in Fig. 5. It was found that the majority of group UP axons were located in the lateral zones of the spinal cord and had a conduction velocity ranged from 2 to 3.6 m/s. This is characteristic of the medium-size axons originating from the PRRN and MRRN.

A considerable part of group DOWN neurons had axons with a high conduction velocity (>3.6 m/s) positioned centrally in the spinal cord. This is characteristic of Müller cells. Other neurons of this group had lower conduction velocities and/or more lateral position of the axon, which is characteristic of the middle-size PRRN and MRRN neurons.

Vestibular responses in group UP and DOWN neurons and their possible origin

When tested by vestibular stimuli, RS neurons exhibited both dynamic responses (activity during movement) and static responses (activity in a new sustained position). The dynamic response was much stronger than the static one. According to characteristics of vestibular responses, most recorded neurons were classified into two groups, UP and DOWN (Figs. 3 and 4). In both groups, the responses were directionally specific, that is, they occurred mostly during rotation in one direction (nose-up in group UP and nose-down in group DOWN). Both dynamic and static components of the response were position dependent: they were larger within a specific angular zone (0°–135° up in group UP and 0–135° down in group DOWN).

As in higher vertebrates, the labyrinths in the lamprey contain both semicircular canals and otolith organs (Lowenstein et al. 1968). The canal afferents respond dynamically to rotation, whereas the otolith afferents respond both dynamically and statically to a change of position. It seems likely that both types of afferents contribute to the activation of RS neurons during tilts. When examining responses of vestibular afferents to pitch tilt, two groups of canal afferents, UP and DOWN, responding to rotation in the nose-up or -down direction, respectively, as well
as a few groups of otolith afferents differing in their spatial zones of sensitivity, were identified (Deliagina et al. 1992b). Among the otolith afferents, group P1 had the maximal response in the zone 45–90° down, and group P2 in the zone 45–90° up. One can suggest that down and up groups of canal afferents, as well as P1 and P2 groups of otolith afferents contribute to the activation of down and up groups of RS neurons, respectively.

Both dynamic and static components of vestibular responses in RS neurons are directionally specific. The directional specificity of the dynamic responses in up and down groups of RS neurons could be caused by the excitatory inputs from the up and down groups of canal afferents, respectively. A dependence of the value of the dynamic response on animal position might be caused by a summation of the input from otolith afferents exhibiting the position-dependent dynamic component of response, and the input from canal afferents (Deliagina et al. 1992b).

The directional specificity of the static response in RS neurons could be caused, first, by a directional specificity observed in a part of otolith afferents (Deliagina et al. 1992b). Second, it could be due to the interaction of effects on RS neurons exerted by the otolith input and the directionally specific canal input. It was shown that the static response in RS neurons increases when the preceding dynamic response is stronger (Deliagina and Fagerstedt 2000). This gives an explanation of why the static response at a given tilt angle depends on the direction of rotation.

**Functional role of group up and down neurons**

Magnus (1924) considered a stabilized body posture as a net effect of a number of counteracting reflexes of vestibular, visual, and somatosensory origin. The data obtained in the experiments on the lamprey brain-stem-labyrinths preparation (Deliagina et al. 1992b) allowed to apply this general idea to the roll and pitch control systems of this animal. In particular, it was suggested that the pitch control system operates on the basis of two antagonistic vestibular reflexes mediated by the up and down groups of RS neurons, as illustrated in Fig. 8a. The up and down groups are driven by vestibular afferents responding to the nose-up tilt [VA(up)] and nose-down tilt [VA(down)], respectively. Due to these vestibular inputs, the activities of up and down groups and their motor effects are orientation-dependent (Fig. 8b). It was suggested that group up causes a downward turn of the lamprey, whereas group down causes an upward turn. These motor effects are shown by the black and white arrows in Fig. 8, A and B. The system will stabilize the orientation at which the effects produced by the two groups of RS neurons are equal to each other. One can suggest that normally this occurs at the zero pitch angle (an equilibrium point in Fig. 8b), and the animal will stabilize a horizontal orientation of the body. Any deviation from this orientation will evoke a compensatory movement aimed to restore the initial position.

A further support for this hypothesis was obtained in the present study. First, it was found that the up and down groups are present in intact animals as well (Fig. 4). Second, it was found that the factor which presumably causes a downward turn of the animal (higher temperature), affects the vestibular responses in up and down groups differently. This results in an
increase in the ratio of UP group activity to DOWN group activity (Fig. 6). Because of the increase in the \( \text{UP/Down} \) ratio, an intersection of the two activity curves will be displaced from 0° toward the downward tilt angles (Fig. 8C). This new pitch angle (equilibrium point) will be stabilized by the vestibular-driven postural control system. A tonic input to the UP and DOWN groups, causing a change in the \( \text{UP/Down} \) ratio and, consequently, a change of the stabilized angle is shown schematically in Fig. 8A (Pitch angle setting). The equilibrium point is displaced toward the down pitch angles.

In the present study, the number of neurons in the UP group appeared much larger than in the DOWN group. A possible explanation for this finding could be that the motor effects of the DOWN neurons are stronger than those of the UP neurons. Another explanation could be that, under our experimental conditions (the lamprey was attached to the substrate with its sucker mouth), the pitch control system was tuned for downward turning.

In the previous papers (Deliagina and Fagerstedt 2000; Zelenin and al. 2000), it was shown that a change of postural orientation of the lamprey in the roll plane caused by visual input (dorsal light response) (Ullén et al. 1995b) is based on a similar principle of differential tonic influences on the two antagonistic groups of RS neurons. A change of postural orientation in the mollusk Clione is also based on a differential regulation of the two opposing postural gravitational reflexes (Deliagina et al. 2000a). These similar results, obtained on different species, suggest a generality in the revealed mechanisms of postural modifications.

**Relationships between pitch and roll control systems**

As shown in behavioral experiments, the swimming lamprey can stabilize its orientation in the roll plane and in the pitch plane (Ullén et al. 1995a). Because all commands for postural corrections are transmitted from the brain stem to the spinal cord by RS pathways, a question arises whether the commands for corrections in the roll and pitch planes are transmitted by the same or different populations of RS neurons. To answer this question, we examined responses to pitch and roll tilts in individual RS neurons. It was found that the populations of RS neurons, responding to pitch and roll tilts, partly overlapped (Fig. 7A). The overlapping subpopulation constituted 22% of the whole pitch population and included neurons from both UP and DOWN pitch groups. When tested by roll tilts, these neurons occurred mainly in the roll group 1, that is they were activated by the contralateral roll tilt. However, the responses to roll tilts were stronger than the responses of the same neurons to pitch tilts (compare Fig. 7, B and C).

The degree of overlap between the pitch and roll populations of RS neurons found in the present study (22%) seems to be underestimated. All experiments in the present study were performed on quiescent animals in which the activity of RS neurons is low, and only a small proportion of them (the most excitable ones) respond to vestibular stimuli. In the swimming lamprey, the RS activity is much higher (Deliagina et al. 2000b), and one can expect that additional neurons will respond to both roll and pitch tilts. In the walking intact cat, it was also shown that many RS neurons modify their activity during both roll and pitch tilts (Matsuyama and Drew 2000), suggesting their involvement in the postural control in the two planes.

The overlap between the pitch and roll populations of RS neurons suggests that the RS pathways are partly shared by the pitch and roll control systems and that special mechanisms are needed for decoding the mixed commands arriving via these pathways to the spinal cord. The task of decoding is simplified by the fact that the pitch commands are symmetrical, that is, the number of RS neurons activated by pitch tilt on the left and right sides is approximately the same (see RESULTS), whereas the roll commands are asymmetrical. Therefore decoding of the pitch commands can be based on the summation of the RS activities on the two sides, whereas decoding of the roll commands—on the subtraction of these activities. This idea was confirmed in the experiments on a neuro-mechanical model (Zelenin et al. 2001). One can also suggest that the overlapping and non-overlapping parts of the pitch and roll populations may differ in their spinal projections (Zelenin et al. 2001).
Comparison of vestibular responses of RS neurons in intact animal and in reduced preparation

In the previous studies (Deliagina et al. 1992a,b; Orlovsky et al. 1992) vestibular responses in RS neurons to pitch tilt were examined in the in vitro brain-stem-labyrinths preparation. Comparison of these data with the data obtained in the present study demonstrates their essential similarities. Two groups of neurons, identified in the in vitro experiments, were similar to the groups up and down revealed in the intact lamprey. These neurons exhibited both dynamic and static responses to rotation in the pitch plane, and their maximal responses were observed either at 0–135° up or at 0–135° down. There were, however, some differences between the two preparations. In intact animals, the static responses were weaker, the dynamic responses stronger, and the directional sensitivity of the responses was much more pronounced than in the in vitro preparation. Also, in the intact lamprey, only a small proportion of RS neurons discharged during both pitch and roll tilt, in contrast to the in vitro preparation where most neurons responded to both stimuli (Orlovsky et al. 1992). We also have not found in the intact lamprey the neurons corresponding to a small group (found in reduced preparation) with the maximal response in the up-side-down position. One of the possible causes for these differences could be the difference in a position of the axis of rotation. In the in vitro experiments, it was close to the head, whereas in intact animals the axis was situated in the mid-body area. This cause seems unlikely, however, because the same differences between the two preparations were observed in the studies of the roll control system, when the preparation was rotated around the same (longitudinal) axis. In the intact lamprey, the dynamic response was stronger, the static response was weaker, the directional sensitivity was more pronounced, and the up-side-down group of neurons was absent (Deliagina and Fagerstedt 2000; Deliagina et al. 1992a). It is also unlikely that the observed differences were caused by different neuronal populations sampled in the two preparations because in both cases the sample was biased toward the larger neurons. It seems most likely that differences between the results obtained in the two preparations were caused by abolishment of most tonic afferent inputs to RS neurons in the in vitro preparation. A part of these inputs are inhibitory, e.g., the input from some trigeminal afferents (Deliagina, unpublished data). Elimination of these inputs may lead to an increase of excitability of RS neurons. By contrast, the excitability of RS neurons in the quiescent intact lamprey attached to the substrate with its sucker mouth, is low (Deliagina et al. 2000b). One can suggest that, with an increase in their excitability (e.g., during locomotion) (Deliagina et al. 2000b), the RS neurons would respond to the vestibular input that was below the threshold in the attached state.

Also, in intact animals the ratio of the number of neurons between the up and down groups was 3:5:1, whereas in the in vitro preparations this ratio was close to 1:1 (Deliagina et al. 1992a). This difference may also be related to the abolishment of inputs from the spinal cord, forebrain, and the majority of cranial nerves to RS neurons in the in vitro preparation. Without these inputs, the down group becomes larger, and one can suggest that the pitch control system is tuned to stabilize the horizontal orientation of the body in the sagittal plane. By contrast, in the intact lamprey attached to the substrate, these inputs might evoke a bias in the excitability of up and down groups that results in tuning the pitch control system to stabilize the nose-down pitch tilt (see section Functional role of group up and down neurons).

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