Responses of Reticulospinal Neurons in Intact Lamprey to Vestibular and Visual Inputs

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Deliagina, T. G. and P. Fagerstedt. Responses of reticulospinal neurons in intact lamprey to vestibular and visual inputs. J. Neurophysiol. 83: 864–878, 2000. A lamprey maintains the dorsal-side-up orientation due to the activity of postural control system driven by vestibular input. Visual input can affect the body orientation: illumination of one eye evokes ipsilateral roll tilt. An important element of the postural network is the reticulospinal (RS) neurons transmitting commands from the brain stem to the spinal cord. Here we describe responses to vestibular and visual stimuli in RS neurons of the intact lamprey. We recorded activity from the axons of larger RS neurons with six extracellular electrodes chronically implanted on the surface of the spinal cord. From these 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 around its longitudinal axis in steps of 45° through 360°. Nonpatterned visual stimulation was performed by unilateral eye illumination. All RS neurons were classified into two groups depending on their pattern of response to vestibular and visual stimuli; the groups also differed in the axon position in the spinal cord and its conduction velocity. Each group consisted of two symmetrical, left and right, subgroups. In group 1 neurons, rotation of the animal evoked both dynamic and static responses; these responses were much larger when rotation was directed toward the contralateral labyrinth, and the dynamic responses to stepwise rotation occurred at any initial orientation of the animal, but they were more pronounced within the angular zone of 0–135°. The zone of static responses approximately coincided with the zone of pronounced dynamic responses. The group 1 neurons received excitatory input from the ipsilateral eye and inhibitory input from the contralateral eye. When vestibular stimulation was combined with illumination of the ipsilateral eye, both dynamic and static vestibular responses were augmented. Contralateral eye illumination caused a decrease of both types of responses. Group 2 neurons responded dynamically to rotation in both directions throughout 360°. They received excitatory inputs from both eyes. Axons of the group 2 neurons had higher conduction velocity and were located more medially in the spinal cord as compared with the group 1 neurons. We suggest that the reticulospinal neurons of group 1 constitute an essential part of the postural network in the lamprey. They transmit orientation-dependent command signals to the spinal cord causing postural corrections. The role of these neurons is discussed in relation to the model of the roll control system formulated in our previous studies.

INTRODUCTION

When swimming, the lamprey—a cyclostome—stabilizes a dorsal-side-up orientation of the body in space due to the activity of the postural control system. Any deviation from this orientation (roll tilt) evokes a corrective motor response that may consist of a number of components—a lateral movement of the ventrally flexed tail, a twisting of the body, and a twisting of the dorsal fin (ULLÉN ET AL. 1995a,b). These postural reflexes are driven by vestibular input. After a bilateral labyrinthectomy, the swimming lamprey cannot stabilize any definite orientation and is continuously looping in different planes (DE BURLET AND VERSTEEGH 1930; ULLÉN ET AL. 1995a), whereas after a unilateral labyrinthectomy, the lamprey is continuously rolling around its longitudinal axis (DE BURLET AND VERSTEEGH 1930; DELIAGINA 1995, 1997a,b). In contrast to vestibular input, visual input has only a modulatory function. Both blinded animals and intact animals in darkness swim normally, with their dorsal side up, provided the vestibular input is intact. Illumination of one of the eyes in the intact lamprey evokes a roll tilt toward the source of light (ULLÉN ET AL. 1993, 1995b). This behavior first was described in bony fishes and referred to as the dorsal light response (ORLOVSKY 1991; PLATT 1983; VON HOLST 1935).

In the lamprey, different descending commands, including those for postural corrections, can be transmitted from the brain stem to the spinal cord via three main routes: the reticulospinal (RS), the vestibulospinal, and the propriospinal pathways (FAGERSTEDT ET AL. 1997; ROUSE AND McCLELLAN 1997; ROVAI NEN 1979). Vestibular afferents terminate on the neurons of vestibular nuclei (Bussières and Dubuc 1992; Koyama et al. 1989; Northcutt 1979; RUBINSON 1974). Some of the cells in these nuclei give rise to crossed and uncrossed vestibulospinal tracts, which, however, are poorly developed in the lamprey and reach only the rostral segments of the spinal cord (Bussières 1994; ROVAI NEN 1979; ROVAI NEN ET AL. 1973). Information on the propriospinal pathways in the lamprey is rather limited (see, however, ROUSE AND McCLELLAN 1997). It has been suggested that the main pathway mediating vestibular influences on the spinal mechanisms in the lamprey is the RS pathway (DELIAGINA ET AL. 1993). This pathway is formed by the RS neurons located in four reticulospinal nuclei of the brain stem: the mesencephalic reticular nucleus (MRN), as well as the anterior (ARRN), middle (MRRN), and posterior (PRRN) rhombencephalic reticular nuclei (NIEUWENHUYS 1972). The RS neurons receive vestibular input through the interneurons of the vestibular nuclei (KOYAMA ET AL. 1989; NORTHCU TT 1979; ROVAINEN 1979; RUBINSON 1974; STEFANELLI AND CARAVITA 1970; TRETJAKOFF 1909). 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). The main effect that the RS neurons produce in the spinal cord is an excitation of the interneurons and motoneurons on the ipsilateral side (Brodin et al. 1988; Ohta and Grillner 1989; Rovainen 1967, 1974, 1979; Wannier et al. 1995).

A detailed study of the responses of RS neurons to natural vestibular stimulation (roll and pitch tilt) was carried out on an in vitro preparation consisting of the brain stem isolated together with the vestibular organs (Deliagina et al. 1992a; Orlovsky et al. 1992). It was found that the majority of RS neurons were activated with the contralateral roll tilt; they exhibited both dynamic and static reactions within specific angular zones (Deliagina et al. 1992a). These responses were caused primarily by excitatory input from specific groups of contralateral vestibular afferents (Deliagina et al. 1992b). A unilateral visual input, produced by tonic electrical stimulation of the optic nerve or illumination of one eye, evoked excitation of the ipsilateral and inhibition of the contralateral RS neurons in MRRN (Deliagina et al. 1993; Ullén et al. 1996). On the basis of these findings, a conceptual model of the roll control system in the lamprey was formulated (Deliagina 1997a; Deliagina et al. 1993) (see Discussion and Fig. 1A).

The characteristics of RS neurons used in the model, however, were obtained in experiments on the in vitro preparation in which a number of inputs to the RS neurons, like those from the forebrain, from the cranial nerves, and from the spinal cord, were abolished. These influences may depend on the behavioral state of the animal and can modify the activity of the postural control system. The aim of the present study was to examine the activity of RS neurons related to the roll control under the natural conditions, that is in intact animals. For this purpose, we used a novel method for chronic recording of the activity of RS neurons from their axons in the spinal cord described in the accompanying paper (Deliagina et al. 2000). Here we further elaborated this method and supplemented it with analysis of the mass activity in the RS pathways by means of a spike-sorting computer program. This allowed us to separately analyze individual RS neurons. Responses of individual RS neurons to roll tilt, to unilateral eye illumination, and to both stimuli applied simultaneously were studied in intact lampreys and then compared with the results of the previous in vitro experiments.

The lamprey has two principal behavioral states—a quiescent state, when the animal is not swimming and attached to a substrate such as the bottom or wall of the aquarium by its sucker mouth, and an active state, when it is swimming. In the present paper, we describe the activity of RS neurons recorded in the quiescent animals. In the accompanying paper (Deliagina et al. 2000), the activity of RS neurons in swimming lampreys is described.

A brief account of this study has been published in an abstract form (Deliagina 1997c).

**METHODS**

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

**Electrodes and their implantation**

The activity of RS neurons was recorded from their axons in the spinal cord by means of chronically implanted macroelectrodes. The method is described in detail in the accompanying paper (Deliagina et al. 2000). In short, the macroelectrodes (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. These have a conduction velocity of >2 m/s. In the lamprey, only RS pathways contain fibers with such a high conduction velocity. The wire electrodes were glued to a plastic plate (6 mm long, 2 mm wide, and 0.25 mm thick). Two different designs of the electrode array were used—with two electrodes (Fig. 1A) and with four electrodes (Fig. 1B). The electrodes had a very low resistance (<10$^3$ Ω) and a noise level of only a few microvolts.

Implantation of the electrodes was performed under MS-222 (San-doz) anesthesia (100 mg/l). The plate with two electrodes was implanted at the level of the third gill, and the plate with four electrodes at the level of the last gill so that the distance between the plates was 20–30 mm. Each of the plates was implanted through a separate 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. 1C) and 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 (Fig. 1D). The wound then was closed and sutured so that the connecting wires were tightly fixed between the two sides of the wound (Fig. 1C).

**Measuring axon position and conduction velocity**

A scheme in Fig. 1F shows a cross-section of the lamprey’s spinal cord with the location of the larger RS axons indicated. The cord is ~1,500 μm wide and 300 μm thick. The sensitivity of the wire electrodes to spikes generated by the larger axons was estimated in preliminary in vitro experiments. A piece of the spinal cord (30 mm in length) was positioned in the chamber filled with a physiological saline. One end of the cord was stimulated by electrical pulses (1 Hz) delivered by a glass suction electrode. The field potentials caused by spike responses in the axons with conduction velocity of 2–4 m/s were recorded by the wire electrode (3 mm in length) positioned close to the other end of the preparation and oriented along its axis. Initially the electrode contacted the cord, and then it was positioned at different distances from the cord surface. It was found that the spike amplitude could reach 100–150 μV when the electrode was positioned on the surface and decreased inversely proportional to the distance when the electrode was moved in saline away from the cord. A typical dependence between the spike amplitude and the distance is shown in Fig. 1E. Even at a distance of 400 μm, the spike amplitude was quite large (~20% of the maximal value or 20–30 μV) and considerably exceeded the noise level. One thus can conclude that spikes in larger axons can be recorded at a distance of ≥400 μm (see also following text and Fig. 3D). Therefore with two electrodes positioned over the midpoints of each half of the cord (Fig. 1G), the activity of most larger spinal axons should be recorded. To record activity of all larger axons, an array with four electrodes located closer to each other was used (Fig. 1H).

The inverse proportionality between amplitude and distance (Fig. 1E) allowed us to estimate the mediolateral position of individual axons in the spinal cord by comparing the amplitudes of the same spike recorded by different electrodes. The array of four electrodes was used for these measurements. We divided the cross-section of the cord into eight equally sized zones (Fig. 1H). Spike amplitudes recorded by the different electrodes were compared. This allowed us to determine (assuming that the electrodes are equally efficient) in which zone the axon coursed. The inequalities that had to be fulfilled to attribute an axon to one of the zones on the right side of the cord...
were as follows: zone 1R, A3 > A2 > A4 > A1; zone 2R, A3 > A4 > A2 > A1; zone 3R, A3 > A1 > A2 > A4; and zone 4R, A1 > A3 > A2 > A4; A1 = 0; where A1, A2, A3, and A4 are the spike amplitudes on the corresponding electrodes. Corresponding inequalities were used for the left side of the spinal cord.

To measure the conduction velocity in individual axons, the distance between the rostral and caudal electrodes was divided by the time difference between the appearance of the corresponding spikes in the two records.

Vestibular and visual stimulation

Vestibular and visual responses were investigated on the next day after implantation of the electrodes, during the light period of the 12 h:12 h light:dark cycle. The lamprey was positioned in a special setup (Fig. 2A) 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. The setup allowed us to rotate the animal around its longitudinal axis through 360°. Rotation was performed manually by means of a handle and cogged wheels. The roll tilt angle (α) 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 in the whole range of angles. The contacts of the implanted wires with the input cable of the amplifiers always occurred above the water surface. All the experiments were carried out at a water temperature of 7–8°C.

Two patterns of vestibular stimulation were used. First, the animal was tested by two full turns; the rotation in the first and in the second turn were performed in the opposite directions. Our reason for this was that the vestibular reactions in the lamprey strongly depend on the direction of rotation, as has been shown in in vitro experiments (Orlovsky et al. 1992). Figure 2B shows how the roll angle was changed. The initial orientation of the animal was with the dorsal side down (180°). Rotation was performed in 45° steps. The transition from one position to the next lasted ~1 s, and each position was maintained for ~3 s. The response of neurons was measured separately for three intervals of each step (1, 2, and 3, see inset in Fig. 2B). The response in the interval 1 (during movement) will be termed the dynamic response; responses in the intervals 2 and 3 (when a new position was maintained) will be termed provisionally the early and late static responses.

A different pattern of stimulation was the periodic trapezoid tilting with alternating tilts to the left and to the right in relation to the normal orientation. Two values of tilt angles were used, 45 and 90° (Fig. 2C and D). The transition from one position to another position lasted ~1 s, and each position was maintained for ~3 s. Again, we divided the period of stimulus application into six intervals (Fig. 8) and measured responses of a neuron separately for each interval. Illumination of the left or right eye was performed by two fiber optic systems (90 W) attached to the rotating platform (Fig. 2A). Visual stimulation was performed either separately or in combination with vestibular stimulation.

Processing of data

Signals from the implanted 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 (Digidata 1200/Axoscope, Axon Instruments, Foster City, CA). Figure 3A shows an example of recordings obtained by the four-electrode array (Fig. 1H).

The recorded multiunit spike trains were separated into unitary
waveforms representing the activity of individual axons using data analysis software (Datapac III, Run Technologies, Laguna Hills, CA). The analysis included the following steps: 1) all spike-like unitary waveforms in each channel (electrode), with amplitude greater than $\pm 10 \mu V$ and duration between 1.5 and 2.5 ms, were selected for the “event” analysis. 2) The unitary waveforms occurring in only one channel, as well as the unitary waveforms occurring simultaneously in two or more channels, were considered to originate from one action potential in a single axon and to represent an event. A time interval of 60.5 ms was used as a criterion of coincidence when the analogous parts of the waveform (e.g., a negative deflection) were used for mutual comparison. This interval was increased to 61 ms if the positive wave in one channel was compared with the negative wave in the other channel. 3) In each test, selection of spikes generated by a single axon was based on the proximity of corresponding events in a plane where the amplitude of the waveforms (positive, negative, or peak-to-peak) in two selected channels was represented along the x and y axes. Figure 3C shows a two-dimensional plot of measurements obtained from one of the experiments in the animal Ch23 (illustrated in Fig. 3A). In this plot, 2,125 events were characterized by the peak-to-peak amplitude of the waveforms in two channels, 1 and 4. This plot allowed us to separate five clusters of units; rectangular frames in Fig. 3C show the limits of variability established empirically by an experimenter for each of the clusters (R1, R7, L1, L2, L10). By using other two-dimensional plots (amplitude 1 against amplitude 2, 2 against 3, positive amplitude against negative amplitude, etc.) usually more clusters of units could be separated. In this particular experiment, many events, which were located near the origin of E1–E4 axes, appeared located much farther from the axes origin when presented in a different coordinate system. Also the events located between the clusters in one coordinate system, might occur clustered in another system. 4) Clustering errors were corrected interactively. For this purpose, all waveforms in a given cluster were superimposed. This is illustrated in Fig. 3D for the cluster R7; the display was synchronized by the event signal. By using such a plot, units that did not meet some additional criteria (e.g., a similarity in the amplitude or shape in any of the channels) could be removed. 5) The localization of the axon in the spinal cord was performed by comparing the spike amplitude in different channels. For example, the spike in R7 axon had the largest amplitude on the most lateral right electrode E4 (Fig. 3D), which indicated its lateral, right-side position. For accurate measuring the axon position, it is important that the electrodes were equally efficient and were located properly in relation to the spinal cord. That the electrodes were similar in efficacy and located symmetrically in relation to the spinal cord was proved by the observation that group 1 axons, located close to the spinal cord midline (see RESULTS), induced similar signals in the left and right electrodes both in the rostral and in the caudal electrode arrays in all experiments. 6) Activity of individual neurons was plotted in time. Figure 3B shows the activity of 11 individual neurons obtained from the recording of the mass activity presented in Fig. 3A. 7) The criteria for identification of unitary spikes, obtained on the basis of measurements in one test of a given animal, then were used in other tests of the same animal. And 8) conduction velocity was measured from plots of signals recorded by the rostral and caudal electrodes. Figure 3E shows the plot of superimposed waveforms from the cluster R7 (see Fig. 3C) on the rostral (E6) and caudal (E4) electrodes. Such plots allowed measuring...
the time delay between the appearance of corresponding spikes in the two electrodes.

RESULT S

Axon position and conduction velocity

Activity in spinal pathways was recorded by chronically implanted electrodes in seven animals. By analyzing these files, the activity of 56 individual axons (≤11 axons in individual animals) was separated from the mass activity. By comparing the spike amplitude recorded by different electrodes, the lateral position of each of these axons was estimated. The distribution of the absolute values of laterality over the mediolateral zones (see METHODS and Fig. 1H) is shown in Fig. 4A. The axons were unevenly distributed over the cross-section of the cord with a maximal density in zone 3.

By measuring the time difference between the occurrence of a spike in the rostral and caudal electrodes (see METHODS and Fig. 3E), the direction of spike propagation and the conduction velocity were determined for 29 units that were recorded by both electrode arrays. In all these cases, the spikes propagated in the caudal direction. The distribution of the conduction velocities for these axons is shown in Fig. 4B. The velocity ranged from 2.6 to 4.6 m/s with two peaks—around 3.2 and 4.2 m/s. In the spinal cord of the lamprey, the only descending axons with such a high conduction velocities are the axons of larger RS neurons.

Responses of individual RS neurons to vestibular and visual stimuli

In the absence of sensory stimulation, the resting activity of RS neurons in nonswimming lampreys was low or absent. Vestibular and visual stimulation activated the RS neurons. We
will use data from the *animal Ch23* to illustrate the responses of individual RS neurons to vestibular and visual stimulation (Figs. 3, A and B, 5 and 6). This example also will show that the RS neurons can be divided into two distinct groups (each consisting of 2 symmetrical, left and right subgroups) differing in their pattern of response to sensory stimuli. They also differ in the axon location and its conduction velocity (see *Quantitative characteristics of group 1 and Quantitative characteristics of group 2*).

As shown in Fig. 5A, the neurons of subgroup 1R (R7–R9), with their axons located on the right side of the spinal cord, and the neurons of subgroup 1L on the left side (L4, L5, L6, L10, and L11), exhibited an excitatory response to contralateral tilt when tested by trapezoid inclinations with an amplitude of 45 or 90° (for methods see Fig. 2, A, C, and D). The response contained both a dynamic component (activity during movement) and a static component (discharge when a new position was maintained). Ipsilateral tilt resulted in an inactivation of subgroup 1R and 1L neurons.

Activity of the same neurons during two full turns performed in the opposite directions is shown in Fig. 5B (for methods see Fig. 2, A and B). In the first turn (rotation toward the right labyrinth), most subgroup 1L neurons exhibited a dynamic response with any change of position. In addition, a static response was observed within the zone of angles 45°–135°R. In
the second turn (rotation toward the left labyrinth), subgroup 1L neurons exhibited only weak dynamic responses to a change of position. In addition, neurons L6 and L11 exhibited a weak static response at 135 and 90°R. Activity of the subgroup 1R neurons was a “mirror image” of the activity of subgroup 1L neurons: they responded in the second turn, both statically (in the zone 45–135°L) and dynamically. In the first turn, activity of the subgroup 1R neurons was much weaker than in the second turn. Thus testing by two successive turns revealed a very pronounced directional sensitivity of the group 1 neurons: both dynamic and static responses were much stronger when the contralateral side was moving downwards.

Group 1 neurons received excitatory input from the ipsilateral eye. Illumination of the left eye (see METHODS and Fig. 2A) evoked activation of the subgroup 1L neurons (Fig. 6A). In contrast, illumination of the right eye evoked activation of the subgroup 1R neurons (Fig. 6B).

Figure 3B shows the effect of vestibular stimulation combined with unilateral visual stimulation. Illumination of the left eye caused an increase of the responses to vestibular input in subgroup 1L neurons and a decrease of response in subgroup 1R neurons.

To summarize, the group 1 neurons had zones of maximal sensitivity at 45–135° of contralateral roll tilt when tested by rotation toward the contralateral labyrinth; they received an excitatory input from the ipsilateral eye, and their vestibular responses were increased during the ipsilateral eye illumination and decreased during the contralateral eye illumination.

Neurons of subgroup 2R (R1 and R3), with their axons located on the right side of the cord, and one neuron of subgroup 2L (L2), with its axon on the left side, exhibited rather weak vestibular reactions. They responded mainly dynamically to both left and right roll tilt (Fig. 5A), to rotation in both directions throughout 360° (Fig. 5B), and received excitatory input from both eyes (Fig. 6, A and B). In neurons of group 2, the ipsilateral and contralateral excitatory visual inputs could be comparable in their effect, as in the neurons R3 and L2 or one of the inputs could be stronger than the other as in R1. Vestibular responses in the group 2 neurons could be enhanced by illuminating any of the two eyes. Figure 3B
illuminates an increase of vestibular responses caused by illumination of the left eye.

To summarize, in contrast to group 1, the group 2 neurons responded dynamically to both ipsilateral and contralateral rotatory movements, they received excitatory input from both eyes, and their vestibular responses were strengthened by illuminating any of the eyes.

Quantitative characteristics of group 1

Of 56 RS neurons recorded in individual animals, 48 neurons (86%) were classified as group 1 neurons according to their pattern of response to vestibular and visual stimuli. In individual experiments, from 3 to 11 neurons of this group were found. Their axons were distributed over all four zones of the ipsilateral half of the cord, but the maximal density was observed in the zones 2 and 3 (see Fig. 4A, □). For the axons that were recorded by both the rostral and caudal electrodes (n = 24), the conduction velocity was calculated. In most cases, its value was within the range from 2.6 to 3.8 m/s; two axons, however, had a higher velocity, 4.0–4.6 m/s (Fig. 4B, □).

To describe the overall activity of group 1 neurons, we used two characteristics: the percent of simultaneously active neurons and the frequency curve, that is, the average discharge frequency as a function of the roll angle. These values were calculated separately for each of the three intervals (1, 2, and 3) of each step (see inset in Fig. 2B), and then averaged over all group 1 neurons recorded in all seven animals. This allowed us to characterize separately the dynamic response (activity in the interval 1) and the early and late static responses (activity in the intervals 2 and 3).

A histogram of the relative number (percent) of simultaneously active group 1 neurons is shown in Fig. 7A. In this graph, responses in both subgroups, 1R and 1L, were summarized. Along the horizontal axis, the successive angles of roll tilt reached during two turns (a and b) performed in opposite directions in relation to the recorded neuron, are indicated. The angle of 0° corresponds to a normal orientation of the animal (dorsal side up). Designations i and co below the graph indicate whether the ipsilateral or contralateral labyrinth in relation to the recorded neuron was pointing downward (cf. Fig. 2B).

During turn a, that is, rotation toward the contralateral labyrinth (Fig. 7A, left), any change of orientation evoked a dynamic response in most RS neurons. Especially efficient were rotations 45°-0°, 0°-45° co, 45° co-90° co, and 90° co-135° co, which activated 75–95% of neurons. During turn b, that is, rotation toward the ipsilateral labyrinth (Fig. 7A, right), the dynamic reactions were several times weaker than in turn a.

The majority of group 1 neurons exhibited not only a dynamic response but also a static response in a certain angular zone. As shown in Fig. 7A, the static responses were mostly pronounced in the turn a, in the positions 45° co and 90° co, where ≤69% of neurons were activated. When the same positions were reached by rotation in the opposite direction (turn b), only a small proportion of the neurons was activated.

A frequency curve for the group 1 neurons (Fig. 7D) has the same essential features as the histogram of the number of active neurons (Fig. 7A): the neurons responded dynamically throughout turn a, with larger responses in the zone 0°–180° of the contralateral tilts; they responded statically in the zone 45° co-135° co; and responses during turn b were much smaller that in turn a. Additional information that comes from the frequency curve is that the dynamic responses were much stronger than the static responses. Within the zone of maximal activity of the group 1 neurons (turn a, 45° co-135° co), the frequency in the dynamic responses was >7 Hz, whereas that in the static responses was only 1–2.5 Hz.

Responses of group 1 neurons to trapezoid tilts (oscillations between 45° co and 45° co) are shown in Fig. 8A. The whole cycle of oscillations was divided into six intervals, and the frequency of individual neurons was measured for each interval and then averaged over all neurons. This graph, like that in Fig. 7D, shows a predominance of the responses to the contralateral tilt over those to the ipsilateral tilt. A comparison of Figs. 7D and 8A also shows that the response in the same final position (45° co) is very different when this position was reached by rotation from 0° (Fig. 7D, turn a) and from 45° co (Fig. 8A). In the latter case, responses in the intervals 2 and 3 (early and late static responses) were two to three times larger than in the former case. A possible explanation of this difference could be that the vestibular responses depend on the angular speed of rotation, which was two times higher in the trapezoid movements.

Illumination of the ipsilateral eye evoked an excitatory response in all neurons of group 1. In all neurons of this group, vestibular responses were augmented considerably under the effect of the simultaneously applied ipsilateral visual input and reduced under the effect of contralateral input. Quantitative characteristics of these excitatory and inhibitory effects are presented in Figs. 7 and 8. Illumination of the ipsilateral eye resulted in an increase of the number of active neurons within their normal angular zone of activity, and slightly widened the zone (compare Fig. 7, A and B). The value of the static and dynamic responses also increased (compare Fig. 7, D and E). Illumination of the contralateral eye resulted in a dramatic reduction of the number of group 1 neurons exhibiting a static response (Fig. 7C); their frequency curve shows that the static activity was practically absent (Fig. 7F).

Similar effects of visual stimulation could be observed when vestibular stimulation was caused by trapezoid movements (Fig. 8). The ipsilateral visual input caused a twofold increase of the static responses (compare A and B), whereas the contralateral input caused an even stronger inhibition of both dynamic and static responses (compare A and C).

Quantitative characteristics of group 2

Eight RS neurons (14%) were classified as group 2 neurons. They were found only in three of seven animals. Seven of these neurons had an axon in zone 1, and one neuron in zone 2 (see Fig. 4A, ■). Five axons that were recorded by both rostral and caudal electrodes had a conduction velocity between 3.8–4.6 m/s (Fig. 4B, ■).

A histogram of the number of active neurons (Fig. 9A) and their frequency curve (Fig. 9D) show that neurons of this group responded dynamically in both turns, in contrast to group 1 neurons that responded only in turn a (Fig. 7A). Maximal responses were observed in the zone 90°, 0° in turn a and in the zone 90° co-0° in turn b.
All group 2 neurons exhibited an excitatory response to illumination of any of the two eyes (as illustrated in Fig. 6 for the animal Ch23). Illumination of any eye, combined with vestibular stimulation, enhanced vestibular responses in the group 2 neurons in both turn a and turn b (Fig. 9, B and C and E and F), in contrast to group 1 where vestibular responses increased only under the effect of ipsilateral visual input (Figs. 7, B and E, and 8B).

**FIG. 7.** Summary of responses to roll tilt in the whole group 1 neurons. A–C: relative number of active neurons as a function of roll angle. D–F: average discharge frequency of neurons as a function of roll angle. Lighting conditions: no eye illumination (A and D); ipsilateral eye illumination (B and E); contralateral eye illumination (C and F). In A–C, each step of rotation was divided into 3 intervals (see Fig. 2, inset), and the number of active neurons was calculated for each of the intervals and then divided by the total number of recorded cells (n = 48, 38, and 28 for A and B, C and D, and E and F, respectively). In D–F, the average frequency was calculated as the number of spikes generated by all recorded neurons in each of the intervals of the step and then divided by the number of neurons. Angle of 0° corresponds to the dorsal-side-up orientation of the lamprey. Angular zones where the ipsilateral (i) or contralateral (co) labyrinth is facing downward are indicated. Rotation was performed toward the ipsilateral labyrinth in the turn a and toward the contralateral one in the turn b. In each of the angular steps, the dynamic responses (activity during rotation) are shown by black bars, the early and late static responses—by 2 successive shaded bars.
Possible errors caused by spike-sorting procedure

Delineation of clusters is an empirical step in spike sorting; it may cause two types of errors—misidentification and loss of spikes. Some aspects of this problem were considered by Camp and Pinsker (1979). It was shown that establishing lesser limits of variability for a cluster leads to elimination of “foreign” spikes but also to a reduction of the number of spikes of a given neuron. The Datapac III program allowed us to considerably reduce clustering errors by removing manually foreign spikes without loss of “true” spikes (see Methods). An estimate for errors in clustering 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 <20%.

As far as misidentification is concerned, it is important to note that different clusters of units, separated in the present study by the spike-sorting procedure, differed also in their conduction velocity (see results). This was a direct and independent indication that the spike-sorting program operated effectively. We took into consideration all spikes that formed a cluster in at least one of the tests (vestibular, visual, or combined simulation) even in the cases when they were less active and did not form clear-cut clusters in other tests. With this method, we avoided a loss of neurons weakly activated by stimuli. Thus the main error in spike sorting was that in the total number of spikes generated by a neuron during trial and, therefore, in its firing frequency; this error did not exceed 20%, however. The error in mean frequency caused by underestimation of the cluster size did not affect any principal conclusions of the present study, that is classification of neurons into two groups, position of their zones of activity, and prevalence of the dynamic response over the static one.

Characterization of group 1 and 2 axons

For recording the activity of reticulospinal (RS) neurons, we used a new type of electrode. Theoretical considerations and experimental evidence presented in the accompanying paper (Deliagina et al. 2000) suggest that these electrodes can record selectively the activity from the larger RS axons with a conduction velocity of >2 m/s. This is supported further in the present study: all recorded RS axons had a high conduction velocity (2.6–4.6 m/s, Fig. 4B).

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. Their positions on the transversal section of the spinal cord are schematically shown in Fig. 1F. 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. At least some of these cells can be identified individually. Most of the Müller axons are located in the middle area of the spinal cord. The middle size axons (10–30 μm diam) belong to the middle size cells located in the MRRN and PRRN. The latter are sometimes termed V cells. The majority of the middle size axons are located in the lateral part of the spinal cord. The conduction velocity of the middle 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. It was found that group 2 neurons had a high conduction velocity (~4.2 m/s) and a central position of the axon in the spinal cord (Fig. 4, A and B). This is characteristic of the M, B, and I1 Müller cells (Fig. 1F). One can therefore suggest that group 2 neurons belong to this neuron population. Most neurons of group 1 had a lower conduction velocity (2.6–3.8 m/s) than the group 2 cells (Fig. 4B). Their axons were located more laterally (zones 2 and 3, Fig. 4A). This is characteristic of the middle size axons originating from the PRRN and MRRN. A few neurons of group 1, however, had characteristics similar to those of group 2 neurons—a central position of the axon and a high conduction velocity (see Fig. 4, A and B).

Vestibular responses in group 1 and 2 neurons and their possible origin

All neurons recorded in the present study were classified into two groups differing in a number of characteristics, namely, in their responses to vestibular and visual stimulation, in the position of their axons in the spinal cord and in the axonal conduction velocity. The patterns of vestibular responses in these two groups will be considered first and then compared...
with the available data on the vestibular input to the brain stem postural network, that is, the activity of vestibular afferents.

*Group 1* neurons exhibited pronounced responses to both types of vestibular stimuli, that is, rotation about longitudinal axis through 360° in a stepwise manner and trapezoid lateral tilts. We divided the response in each step of movement into three parts that were provisionally termed the dynamic response (activity during movement) and the early and late static responses (activity after termination of movement). Both dynamic and static responses were directionally specific—they occurred during rotation in one direction (toward the contralateral labyrinth) and practically did not occur during rotation in the opposite direction (Figs. 7, A and D). The zone of static responses approximately coincided with the zone of larger dynamic responses (Fig. 7, A and D).

Vestibular responses in *group 2* neurons strongly differed from those in *group 1*: they had no directional specificity, and contained mainly a dynamic component (Fig. 9, A and D).

Responses of vestibular afferents to natural vestibular stimulation of the same kind as in the present study were earlier investigated in a preparation of the lamprey brain stem isolated
with the vestibular organs intact (Deliagina et al. 1992b). It was found that canal afferents responded dynamically to rotation toward the ipsilateral labyrinth applied at any initial orientation of the animal (0–360°). Otolith afferents had both dynamic and static responses in different angular zones. The largest group of these afferents (R1) responded in the zone of 0–135°, with the ipsilateral labyrinth facing down. The otolith afferents had practically no directional sensitivity.

Complex patterns of vestibular responses in RS neurons, observed in the present study, can be explained by the convergence of signals from different groups of vestibular afferents. Group 2 neurons had only dynamic responses in the whole range of angles (0–360°) without a directional specificity. The simplest explanation for this pattern could be a convergence of excitatory inputs from the canal afferents of the ipsilateral and contralateral labyrinths. Group 1 neurons had both dynamic and static responses. The directionally specific dynamic responses could be caused by excitatory input from the canal afferents of the contralateral labyrinth. The static responses could be caused by excitatory input from the group R1 otolith afferents of the contralateral labyrinth. Evidence in favor of this suggestion was obtained in earlier experiments with unilateral labyrinthectionomy which resulted in elimination of static responses in contralateral RS neurons (Deliagina et al. 1992b). The directionality of the static responses of group 1 neurons, observed in the present study, may be caused by the interaction of orientation-dependent excitatory input from otolith afferents with two directionally sensitive inputs—excitatory input from the contralateral canal afferents (Deliagina et al. 1992b; Orlovsky et al. 1992) and inhibitory input from the ipsilateral ones (Orlovsky et al. 1992). Such an interaction also could explain the increase of the static response observed in the same position when this position was reached by a faster movement (see RESULTS).

Comparison of vestibular responses of RS neurons observed in different behavioral states of intact animal and in reduced preparation

The activity of RS neurons strongly depends on the general activity of the animal—it is very low in nonlocomoting animals, and dramatically increases with the onset of locomotion (Deliagina et al. 2000). The RS neurons, which were recorded in the present study from nonlocomoting animals and responded to visual and vestibular stimulation, seem to belong to the most excitable subdivision of the RS system. A comparison of vestibular responses of these neurons before and during locomotion (Deliagina et al. 2000) has shown that the main characteristics of the responses were the same under both conditions. During swimming, presumed group 1 neurons responded to the contralateral roll tilt, and the responses had both dynamic and static components. However, there were some differences in the response patterns under the two conditions. During locomotion, the vestibular responses had a higher discharge frequency, and the locomotory rhythm was superimposed on these responses. Because of the high level of RS activity, individual spikes were difficult to separate during locomotion. However, the similarities of the vestibular responses in the swimming and nonswimming animals allowed us to analyze the operation of the postural network in the lamprey under simpler conditions, that is, in the nonswimming animals.

A large body of data on vestibular responses in RS neurons was obtained in the previous studies carried out on the in vitro preparation, using the same kind of vestibular stimulation as in the present study (Deliagina et al. 1992a,b; Orlovsky et al. 1992). Comparison of these data with the data obtained in the present study demonstrates their essential similarities. In the in vitro preparation, the majority of RS neurons responded both dynamically and statically to contralateral roll tilt within the angular zone 0–135°, with the contralateral labyrinth facing down. In this respect they were similar to group 1 neurons in intact animals. There are also some differences between the two preparations, however. 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. An additional difference between the two preparations was that group 2 neurons were not found in the in vitro experiments. This difference may be related to the abolishing of most tonic sensory inputs to RS neurons in the in vitro preparation. We also have not found in the intact lamprey the neurons corresponding to a small group (found in the reduced preparation) with the maximal response in the dorsal-side-down position.

Modification of vestibular responses in RS neurons caused by visual input

Neurons of groups 1 and 2 strongly differed in their response to a unilateral eye illumination. Group 1 neurons were excited by illumination of the ipsilateral eye. Vestibular responses in these neurons were considerably enlarged under the effect of ipsilateral eye illumination (Fig. 7, B and E) and decreased under the effect of contralateral eye illumination (Fig. 7, C and F). In the in vitro preparation, neurons with similar characteristics were found in the MRRN (Deliagina et al. 1993; Ullén et al. 1996); other reticular nuclei were not examined in this respect, however. These neurons were activated with ipsilateral eye illumination and inhibited with contralateral eye illumination. Because of a convergence of visual and vestibular inputs, vestibular responses in RS neurons were potentiated with the ipsilateral eye illumination and reduced with the contralateral eye illumination. In group 2 neurons, in contrast to group 1, illumination of either eye evoked an excitatory response and considerably enhanced the vestibular responses (Fig. 9, B and E and C and F). Corresponding neurons were not found in in vitro experiments.

Central pathways for visual influences on the postural orientation were investigated in lesion experiments (Ullén et al. 1997). It was found that the visual input reaches the RS neurons through the contralateral pretectal area. Neurons in this area, presumably mediating visual influences on RS neurons, were described by Zompa and Dubuc (1996, 1998a,b).

Functional role of group 1 neurons

Previous studies on the in vitro preparation led to formulation of a conceptual model of the roll control system in the lamprey (Fig. 10A) (Deliagina 1992a; Deliagina et al. 1992a,b, 1993). The key elements of the model are the two subpopulations of RS neurons, the left [RS(L)] and the right [RS(R)]. The
main input to these neurons is excitatory from the contralateral labyrinth. Because of this input, the activity of RS neurons is orientation dependent, with its peak at ~90° of contralateral roll tilt (Fig. 10B). The two subpopulations also receive an excitatory input from the ipsilateral eye and an inhibitory input from the contralateral eye. Each of the subpopulations, via spinal mechanisms, elicits ipsilateral rotation of the lamprey (Fig. 10B, arrows). The system will stabilize an orientation in space with equal activities of RS(L) and RS(R), that is, the dorsal-side-up position (equilibrium point in Fig. 10B). The model also could explain the origin of the dorsal light response, that is, a roll tilt toward the illuminated eye (Fig. 10C, inset). Illumination of an eye causes an additional excitation of the ipsilateral RS neurons and inhibition of the contralateral ones; this will result in a shift of the equilibrium point of the system toward the illuminated eye (Fig. 10C).

Dynamic characteristics of the vestibular responses in RS neurons were not taken into account when formulating the model of the roll control system (Fig. 10, A and B) because they were weakly pronounced in the in vitro preparation. The present study has shown, however, that in the intact lamprey the pure static, that is, only orientation dependent, vestibular responses in RS neurons are rather weak, whereas the responses to a movement are strong. In group 1 neurons, these dynamic responses are directionally specific, they are present in the whole range of angles (0–360°) with their maximum within the zone 0–135° of the contralateral tilts (Fig. 7D). Here we argue that responses of this type in group 1 neurons, under certain conditions, are sufficient for stabilization of the lamprey’s orientation in space even in the absence of pure static responses. Operation of the roll control system based on the dynamic reactions in RS neurons is illustrated in Fig. 10D. In this graph, the magnitude of the dynamic response in group 1 neurons is presented as a function of the roll angle separately for the two subgroups, 1R and 1L (based on Fig. 7D); the arrows below the graph indicate a direction of rotation necessary for activating the subgroup 1R (black arrow) and 1L (white arrow). As in the original model (Fig. 10A), we assume that the subgroups 1R and 1L evoke rotation of the lamprey in opposite directions (indicated in Fig. 10C by the white and black short arrows, respectively).

From Fig. 10D one can see that dynamic responses to the vestibular stimulus of a given absolute value (45° tilt) differ in magnitude between the subgroups 1R and 1L in all orientations of the animal except for the normal one, and the RS neurons causing rotation toward the normal orientation display a stronger vestibular response than the opposing neurons. One thus can assume that the system will compensate for any initial deviation of the lamprey from the normal orientation provided the initial tilting, and the subsequent corrective motor responses are rapid enough to evoke dynamic reactions in RS neurons.

Illumination of one of the eyes differentially affects the dynamic responses in the ipsilateral and contralateral subgroups of the group 1 neurons. Figure 10E shows the effect of left eye illumination (based on Fig. 7, E and F). Now the responses of equal magnitude in the subgroups 1R and 1L (intersection of the 2 curves) are observed not at 0° (as in Fig. 10D) but between 45 and 90° of the left tilt, suggesting that this orientation will be stabilized. Another site of intersection of the two curves (~135°) represents a point of unstable equilibrium. From this point, under the effect of the prevailing subgroup 1L, the system will restore equilibrium by rotating to the left.

A postural system, based exclusively on the dynamic reactions in RS neurons, cannot compensate for slow deviations from the normal orientation. There are, however, three additional factors that contribute to postural stabilization in the lamprey and allow compensation for slow deviations from the normal orientation. First, as shown in the present study, vestibular reactions in RS neurons considerably outlast the applied vestibular stimuli. These delayed responses strongly depend on the animal’s orientation and thus can be considered as static responses to tilt, though they are sensitive to the direction of rotation as well. These static responses are presented in Fig. 10F as a function of roll tilt separately for the right and left subgroups of group 1 neurons. These static reactions, though they are rather small, could evoke corrective motor responses to slow postural disturbances. Second, as shown in the accompanying paper (Deliagina et al. 2000), the static component of the vestibular response in RS neurons increases during swimming. Third, in the swimming lamprey, periodical oscillations of the head (~1 Hz), caused by locomotor body undulations, are always present (Williams 1989) and superimposed on any sustained postural orientation. These vestibular stimuli may elicit additional dynamic responses in RS neurons, which will contribute to the postural stabilization.

Direct evidence that postural stabilization in the lamprey can be based on dynamic vestibular reactions in RS neurons was obtained in model experiments (Zelenin et al. 1998) using the technique of artificial feedback (Deliagina et al. 1998). In these experiments, biological signals, that is, activity of RS neurons of group 1 were recorded in the intact immobilized lamprey by implanted electrodes and 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 and exhibited the dorsal light response when one of the eyes was illuminated.

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**FIG. 10.** Presumed role of group 1 neurons in postural control. A: conceptual model of the postural control system in the lamprey (modified from Deliagina 1997a) (see text for explanations). Two subpopulations of RS neurons, RS(R) and RS(L), receive inputs from the labyrinths (V) and eyes (E); they affect the spinal networks to evoke rolling of the lamprey. B: operation of the system when driven only by vestibular inputs. Curves represent activity in RS(R) and RS(L) as a function of roll angle (L, left tilt; R, right tilt). Vestibular input causes activation of RS(L) and RS(R) with the contralateral tilt. Directions of rolling caused by RS(R) and RS(L) are indicated by the black and white arrows respectively. System has an equilibrium point at 0° (dorsal-side-up orientation). C: operation of the system when the left eye is illuminated. This visual input causes a shift of the equilibrium point to the right. D and E: a dynamic component of the vestibular response in the subgroup 1R and 1L neurons. F and G: static component of the vestibular response in the subgroup 1R and 1L neurons. Lighting conditions: no eye illumination (D and F), left eye illumination (E and G). Long white and black arrows below D and E show the direction of rotation necessary for activation of the subgroups 1L and 1R respectively. Short white and black arrows in D and F show the presumed direction of rolling caused by the subgroups 1L and 1R, respectively. Graphs D–G are based on Fig. 7, D–F.
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