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VESTIBULAR CONTROL OF BODY ORIENTATION IN LAMPREY

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To my parents Genrietta Ivanovna and Lev Alexeevich

ABSTRACT

Maintenance of body orientation (postural control) is a vital motor function of the brain. The general goal of this project was to understand the organization and operation of neuronal networks responsible for postural control. The lamprey (a lower vertebrate) was used as a model animal. The postural control system in the lamprey, driven by vestibular input, maintains a definite orientation of the longitudinal body axis in relation to horizon (pitch angle) and the dorsal-side-up body orientation (roll angle is 0°). Important elements of the postural network are reticulospinal (RS) neurons, which are driven by vestibular input and transmit commands for postural corrections from the brain to the spinal cord.

In *in vivo* studies, the activity of RS neurons was recorded from their axons in the spinal cord by chronically implanted electrodes. The animals were rotated through 360^0 in the roll or pitch plane in order to stimulate vestibular organs. The activity of individual neurons was separated from the mass activity by a spike-sorting program.

Rotation in the pitch plane revealed two groups of neurons (UP and DOWN), responding to nose-up and nose-down rotation, respectively. These groups presumably mediate opposing vestibular reflexes and cause downward and upward turns of the animal. 22% of the RS neurons responding to pitch tilts also responded to roll tilts. This overlap suggests that the RS pathways are partly shared by the pitch and roll control systems.

A unilateral labyrinthectomy (UL) caused continuous rolling of the lamprey. Testing in the roll plane has shown that UL slightly affected responses on the UL side, whereas those on the opposite side disappeared. This asymmetry is the likely cause for the loss of equilibrium. Eye illumination led to a restoration of responses on the side contraleteral to UL, and to a reduction of responses on the opposite side. The restoration of symmetry of RS responses allows one to explain the behavioral effect of eye illumination – restoration of equilibrium.

When animals were tested in the pitch plane before and after UL, in the group UP responses on the UL side changed only slightly, and on the opposite side they disappeared. In the group DOWN, the UL caused only minor changes in vestibular responses. Thus left and right UP subgroups receive their main input from the contralateral labyrinth. By contrast, neurons of group DOWN receive input from both labyrinths. The UL-induced changes in vestibular responses to pitch tilt will disturb the normal activity of the pitch control system.

Normally, a few weeks after UL lampreys restore postural equilibrium ("vestibular compensation"). When behaviorally compensated animals were tested in the roll plane, it was found that vestibular responses on the side contralateral to UL reappeared, partly restoring symmetry in RS responses. These findings support the hypothesis that the recovery of postural control after UL is due to a restoration of symmetry in the RS motor commands.

In vitro experiments were performed on the brainstem-spinal cord preparation, with intracellular stimulation of individual RS and vestibulospinal (VS) neurons, and recording the responses to these stimuli from spinal motoneurons. Most of the neurons produced effects on motor output, enhancing or suppressing it. The effects of VS neurons on different groups of motoneurons were weaker and less diverse than those of RS neurons. The RS and VS patterns of responses and the extent of neuronal projections suggest that VS neurons are responsible for flexion of the rostral part of the body and turns of the head in different planes, whereas RS neurons are responsible for formation of gross motor synergies.

Keywords: postural control, orientation, vision, vestibular system, visuo-vestibular interaction, vestibular compensation, locomotion, supraspinal control, lamprey

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LIST OF ORIGINAL ARTICLES

This thesis is based on the following papers and manuscript that will be referred to in the text with their roman numbers:

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IV. **Pavlova E.L.**, Popova L.B., Deliagina T.G. Modifications in activity of reticulospinal system during vestibular compensation in lamprey. (*Manuscript*).

V. Zelenin P.V., **Pavlova E.L.**, Grillner S., Orlovsky G.N., Deliagina T.G. (2003) Comparison of the motor effects of individual vestibulo- and reticulospinal neurons on dorsal and ventral myotomes in lamprey. *J. Neurophysol*. 90, 3161-3167.

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LIST OF ABBREVIATIONS

ARRN	anterior mesencephalic reticular nucleus
CNS	central nervous system
co-	contralateral
CPG	central pattern generator
E	eye
EPSP	excitatory postsynaptic potential
i-	ipsilateral
ION	intermediate octavomotor nucleus
KAc	potassium acetate
L	left
MN	motoneuron
MRN	mesencephalic reticular nucleus
MRRN	middle rhombencephalic reticular nucleus
NMDA	N-methyl-D-aspartate
PON	posterior octavomotor nucleus
PRRN	posterior rhombencephalic reticular nucleus
R	right
RS	reticulospinal
RS(R)	reticulospinal on the right side
RS(L)	reticulospinal on the left side
UL	unilateral labyrinthectomy
UL/E	unilateral labyrinthectomy & ablation of ipsi-eye
V	vestibular
VR	ventral root
VS	vestibulospinal

INTRODUCTION

Functional organization of postural system

Different species actively maintain a basic upright, or dorsal side-up, body posture. Maintenance of this posture is a non-volitional activity based on innate neural mechanisms (Massion 1998). Efficient control of the basic posture is important for standing and during walking (Horak & Macpherson 1995; Macpherson et al. 1997; Orlovsky et al. 1999), as well as for providing support for voluntary limb movements (Massion & Dufosse 1988).

Two principal modes of postural activity can be distinguished, the feed-back and feed-forward ones (Horak & Macpherson 1995; Gahery et al. 1980).

The feed-back mode of postural control is a compensation for the deviation from the desired posture. This mode of operation is based on the activity of closed-loop nervous mechanisms. These mechanisms are driven by somatosensory, vestibular, and visual inputs, and compensate for postural disturbances by generating corrective motor responses.

The feed-forward mode of postural control is a generation of anticipatory postural adjustments. When the command for a voluntary movement of the limb, head, or trunk is issued, at the same time (or even earlier) a special command is sent to the postural mechanisms. This command causes a postural response aimed at counteracting the destabilizing consequences of the voluntary movement. Normally, the two modes of postural control operate together. For example, errors in the anticipatory adjustments can be compensated by the closed-loop mechanisms. *The present study is devoted to the feed-back mode of postural control.*

There are two major concepts regarding the functional organization of the postural system operating in the feed-back mode:

1. Non-centralized control. In the classical study by Magnus (1924) performed on quadrupeds, any particular stabilized posture of the animal was considered as a result of interactions between numerous postural reflexes (driven by vestibular, visual, and somatosensory inputs); they either supplement or counteract each other. Experiments by von Holst (1935) on the swimming fish presented additional evidence

in favor of this idea. Finally, results of the recent studies on the mollusc *Clione* (Deliagina et al. 1998, 1999, 2000a,b) and on the lamprey (Deliagina 1997a; Deliagina & Fagerstedt 2000) support the "reflex concept" of postural control.

2. Centralized control. This concept of postural stabilization suggests that a body posture is characterized by a single "regulated variable" (e.g., a position of the center of mass, or an orientation of the longitudinal body axis); a certain value of this variable is stabilized. According to this concept, information about the head and body orientation is delivered by sensory inputs of different modalities (vestibular, visual, and somatosensory). This information is processed and integrated to obtain a current value of the regulated variable. If this value differs from the desired one, a command is sent to motor centers to elicit a corrective movement (Horak & Macpherson 1995; Massion & Dufosse 1988; Ghez 1991; Massion 1994; Massion et al. 1997).

Much less is known about the neuronal organization of the postural system, and a sufficiently detailed description of the postural mechanisms at the network and cellular levels is lacking. Such a description is necessary both for a deep understanding of the postural system *per se*, and as a prerequisite for any attempt to understand the pathological cases: impairment of postural control after damage to different parts of the brain, to sensory organs, etc. *The general goal of this study is to understand the organization and operation of the neuronal networks responsible for stabilization of body orientation in space, and the impairment of these networks in subjects with sensory (vestibular) deficit.*

The principal difficulty for these studies is that the postural control system in higher vertebrates is extremely complex: it includes numerous sensory and motor centers located in different parts of the brain, and the integrity of these parts is necessary for normal function. Moreover, the postural system is a closed-loop system operating on the bases of a complex type of multi-sensorial feedback. Therefore, the usual methods for studying the neuronal networks by employing reduced preparations (brain slices, spinal or decerebrate animals, etc.) can only be used for some aspects of the analysis. Recently, two simpler animal models for studying postural networks were introduced: the mollusc *Clione* and the lamprey (a lower vertebrate). Both models allow *in vitro* and *in vivo* analysis of postural mechanisms (Deliagina 1995, 1997a,b; Deliagina & Fagerstedt 2000; Deliagina et al. 1992a,b, 1993, 1998, 1999,

2000a,b,c; Orlovsky et al. 1992; Panchin et al. 1995; Zelenin et al. 2000, 2003a). One can suggest that such an important function as the maintenance of a basic body posture has many features in common in different species, and principles revealed in simpler animal models apply also to higher vertebrates. In the present study we used the lamprey model, in which some aspects of the postural network activity were previously characterized in our Laboratory (Deliagina & Orlovsky 2001, 2002; Deliagina et al. 2002; Orlovsky et al. 2002).

Lamprey as an experimental model

Lampreys originate from the group of animals that diverged from the main vertebrate line around 450 million years ago (Forey & Janvier 1993). The main advantage of the lamprey as a model animal for studying neuronal networks is that the lamprey has many of the principal parts of the vertebrate brain (the telencephalon, diencephalon, mesencephalon and rhombencephalon), but much fewer neurons than higher vertebrates (Nieuwenhuys 1972; Nieuwenhuys et al. 1998). Lampreys have lateral eyes, dermal light sense, vestibular, somatosensory, olfactory organs, and lateral line system. The lamprey is used in many laboratories to study different aspects of motor control.

Postural control and sensory systems

When swimming, the lamprey actively stabilizes a definite orientation of its body in the transverse (roll) plane – normally, with the dorsal side up (de Burlet & Versteegh 1930; Ullén et al. 1995a; Deliagina 1995, 1997a,b). Any deviation from this orientation evokes a corrective motor response – a change of the plane of locomotor undulations from the horizontal to the oblique one (Zelenin et al. 2003). This motor pattern causes rotation of the lamprey toward the dorsal side-up orientation. The lamprey can swim horizontally and at a definite angle in relation to the horizon, which means that it can stabilize different pitch angles (Ullen et al. 1995a). Any deviation from the stabilized pitch angle evokes a corrective motor response – a flexion of the body in the pitch plane (Ullén et al. 1995a). Since roll and

pitch angles can be controlled independently, the roll and pitch subdivisions of the postural control system can be considered separately.

The postural control system is driven by vestibular input. Bilateral ablation of the labyrinths leads to the loss of postural control in any plane, whereas a unilateral labyrinthectomy (UL) causes rotation around the longitudinal body axis, suggesting a loss of postural control in the roll plane (de Burlet & Versteegh 1930; Ullén et al. 1995a; Deliagina 1997a). By contrast, visual input is not necessary for stabilization of posture – blind animals and animals in darkness swim with their dorsal side up (de Burlet & Versteegh 1930). Visual input can play a modulatory role, however: a unilateral eye illumination evokes a roll tilt towards the source of light – "the dorsal light response" (Ullén et al. 1995b).

Vestibular system

The lamprey vestibular organ consists of two semicircular canals, a single macula area and two ciliated chambers. Semicircular canals correspond to vertical canals of higher vertebrates. Their ampullae have three-armed sensory cristae and can presumably detect accelerations in a horizontal plane, monitored by horizontal canal in higher vertebrates. The base of the labyrinth is occupied by a single macula. It is divided into three regions, an anterior horizontal, a vertical, and a posterior horizontal, which probably are homologous of the utricular, saccular and lagenar maculae in gnatostomes, respectively (Hagelin 1974; Kleerekoper 1972; Lowenstein et al. 1968).

Lowenstein (1970) recorded mass discharges in the lamprey vestibular nerve evoked by rotation of the isolated labyrinth. He concluded that vestibular afferent signals, as judged from mass discharges, carry information about movements in all three planes (horizontal, sagittal, and transverse). Later, responses of individual vestibular afferents to rotation in sagittal and transverse planes were studied (Deliagina et al. 1992b). It was found that canal afferents respond to roll rotation towards the ipsilateral side, while pitch tilt reveals two groups of afferents that respond to nose-up and nose-down rotation, respectively. Otolith afferents, which signal orientation in the gravity field, can be divided into several groups according to their zones of spatial sensitivity in the roll or pitch planes. In the roll plane, most afferents respond maximally at 90º of ipsilateral tilt. There are also smaller groups

with their maximum at the dorsal side-down position, as well as at 90º of contralateral tilt. In the pitch plane, there are groups with the maximal response at 90º nose-up, 90º nose-down, and at 180º. A small group of afferents is active when the brainstem is in a normal position, i.e., horizontal, with the dorsal side up.

Vestibular afferents terminate within the ipsilateral ventral octavolateral nucleus, which is located in the octavolateral area, and can be divided into anterior, intermediate and posterior octavomotor nuclei (Koyama et al. 1989; Rubinson 1974; Gonzales & Anadon 1994; Northcutt 1979a).

Visual system

The general organization of the lateral eyes of the lamprey is similar to that of higher vertebrates. However, it has some peculiarities. In particular, in contrast to higher vertebrates, the lamprey has almost globular lens. Since there are no internal muscles in the eyeball of the lamprey, eye accommodation is achieved by moving the lens by external muscles (Rovainen 1983).

Although all major vertebrate visual projections are present in the lamprey (to the tectum, pretectum, and dorsal thalamus) (Fite 1985; Reperant et al. 1980; Kosareva et al. 1977; Kosareva 1980; Kennedy & Rubinson 1977; Veselkin et al. 1980; De Miguel et al. 1990), most fibers terminate in the optic tectum (Karamian et al. 1975).

Asymmetrical visual input can elicit two different behaviors – the dorsal light response (Ullen 1995b) and the negative phototaxis, that is, horizontal turning movements away from the source of light (Ullen et al. 1993, 1995b). The visual information for these responses is transmitted contralaterally to the pretectum, where it is relayed bilaterally to the reticular formation (Ullen et al. 1997; Zompa & Dubuc 1996, 1998a,b). The dorsal light response is mediated mainly by the crossed pretectal descending pathway, and the negative phototaxis by the uncrossed pathway (Ullen et al. 1997).

Descending control systems

The commands for initiation of locomotion, postural control, steering, etc., need to be transmitted from the brainstem and higher motor centers to the spinal cord. The descending pathways in the lamprey are mainly formed by the reticulospinal (RS) and vestibulospinal (VS) neurons (Rovainen 1979a, 1983; Brodin et al. 1988; Bussiéres & Dubuc 1992; Bussiéres 1994), among which the reticulospinal system is more important (Rovainen 1980, 1983). It contains more neurons, and is the only system which projects up to the caudalmost spinal segments (Bussieres 1994; Swain et al. 1993). On the contrary, the vestibulospinal neurons project only to the rostral segments, up to the end of the gill region (Rovainen 1979a; Rovainen et al. 1973; Bussieres et al. 1999). Sparse projections from the trigeminal system, the tectum, pretectum and the nucleus of longitudinal fasciculus at the mesencephalic-diencephalic border of the basal plate reach only the most rostral spinal cord (Bussieres 1994; Northcutt 1979b; Pombal et al. 1997; Ronan 1989).

Reticulospinal system

The RS pathways originate from four reticular 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; Ronan 1989; Swain et al. 1993). A peculiar feature of the lamprey is the nine pairs of giant RS neurons, the Műller cells, which are located in the MRN, ARRN and MRRN regions and can, together with Mauthner cells, be identified individually between preparations (Rovainen 1983). The RS neurons receive vestibular input through interneurons of the vestibular nuclei (Koyama et al. 1989; Northcutt 1979a; Rovainen 1979a; Rubinson 1974; Stefanelli & Caravita 1970; Tretjakoff 1909). They also receive inputs from other sensory systems (visual, olfactory, somatosensory, and lateral line receptors) as well as from the forebrain, brain stem centers, and spinal cord (Deliagina et al. 1993, 1995; Deliagina and Fagerstedt 2000; Dubuc et al. 1993; Rovainen 1967, 1979a; Viana Di Prisco et al. 1995; Wickelgren 1977). At least some of the RS neurons are the command neurons for the initiation of locomotion. Due to their intrinsic properties (plateau potentials) these neurons are responsible for the

transformation of a brief incoming sensory signal into a long-lasting motor command (Viana Di Prisco et al. 2000).

Most of the RS neurons have ipsilateral projections. In the spinal cord, RS neurons affect motoneurons and different classes of interneurons (Buchanan et al. 1987; Brodin et al. 1988; Ohta and Grillner 1989; Rovainen 1974, 1979b; Wannier et al. 1995). Functional projections of individual RS neurons on the four myotomal segments in the middle and caudal parts of the body (but not in the rostral part) were analyzed using the spike-triggered averaging of the motoneuronal activity. It was found that individual neurons exert a uniform effect on the segmental motor output along the whole extent of their axons. Twenty different patterns, that is, combinations of influences on the segmental motoneuronal pools, were observed (Zelenin et al. 2001).

RS neurons participate in different forms of motor behavior: swimming, steering, postural control. Different sensory stimuli cause activation of RS neurons. Stimulation of the skin photoreceptors (by unilateral tail illumination) causes bilateral activation of RS neurons (Deliagina et al. 1995; Deliagina et al. 2000c). A unilateral eye illumination evokes activation of the ipsilateral RS neurons, whereas water vibration evokes bilateral activation (Deliagina et al. 2000c). Prolonged or intense stimuli of any modality or laterality cause swimming due to the massive bilateral activation of the RS system (Deliagina et al. 2000c). Like in other vertebrates, the RS system in lampreys relays inputs from the mesencephalic locomotor region (MLR) to the spinal locomotor networks (Sirota et al. 2000). Stimulation of the MLR elicits monosynaptic EPSPs of a higher magnitude in the MRRN than in the PRRN. This suggests that RS cells in the MRRN are primarily involved in the initiation and maintenance of low-intensity swimming. At high intensity, RS cells in both the MRRN and the PRRN are recruited (Brocard & Dubuc 2003). During locomotion, RS neurons are rhythmically modulated due to the central feedback from the spinal locomotor CPG (Buchanan & Cohen 1982; Dubuc & Grillner 1989; Kasicki & Grillner 1986; Kasicki et al. 1989; Deliagina et al. 2000c; Zelenin et al. 2003b). Synaptic responses of RS neurons to stimulation of vestibular nerves are modulated during locomotor cycle: responses to stimulation of the ipsilateral nerve are smaller during the ipsilateral burst discharge than during the contralateral activity, whilst

responses to stimulation of the contralateral nerve are minimal during contralateral activity and maximal during ipsilateral activity (Bussieres & Dubuc 1992b).

The RS neurons provide also the commands for lateral turns. During generation of fictive turns in the lamprey brain stem - spinal cord preparation, the activity of most of the RS neurons is correlated with the direction of fictive turns (Fagerstedt et al. 2001). The role of RS neurons in steering was also demonstrated *in vivo*. In the freely behaving intact lamprey, an asymmetry in the mass RS activity on the left and right sides was observed during lateral turns, with 30% prevalence of the ipsilateral side (Deliagina et al. 2000c). *In vitro* experiments have shown that even minor asymmetry in the descending commands results in the activation of the ipsilateral motoneurons, whereas the contralateral motoneurons remain silent due to the system of reciprocal inhibition between the hemi segments (Fagerstedt et al. 2000).

Vestibulospinal system

The vestibulo-spinal tract originates from two nuclei: the intermediate octavomotor nucleus (ION) and the posterior octavomotor nucleus (PON). Both nuclei are the parts of the ventral nucleus of the area octavolateralis in the rhombencephalon. Axons from ION project to the ipsilateral spinal cord, whereas axons from PON, after crossing the midline, give out descending and ascending branches. The descending branch projects to the contralateral spinal cord (Bussieres 1994; Bussieres et al. 1999; Ronan 1989; Rovainen 1979a). Axons of VS neurons are short: only longest of them project up to the 10th to the 15th segments (Bussieres 1994; Bussieres et al. 1999; Rovainen 1979a). VS neurons receive direct sensory input from vestibular afferents (Stefanelly & Caravita 1970; Pflieger & Dubuc 2000). Vestibular afferents from the anterior part of the VIII nerve innervate predominantly VS neurons of the ION, whereas afferents from the posterior part innervate VS neurons in the PON (Pflieger & Dubuc 2000). VS neurons can excite motoneurons in the rostral spinal segments (Rovainen 1979a).

Intracellular recording of the activity of VS neurons during fictive locomotion has shown that the majority of them have rhythmic modulation of their membrane potential, which is correlated with the locomotor discharges in the rostral ventral

roots. Of the rhythmically modulated VS cells, most were maximally depolarized during ipsilateral ventral root discharges and showed a minimum during contralateral activity (Bussieres & Dubuc 1992).

Movements in the rostral spinal segments are important for different forms of motor behavior: locomotion, steering, postural control, etc. No information is available, however, on the effects produced by RS and VS descending systems in this area that they innervate together. *One of the aims of this study was to determine functional projections of individual VS and RS neurons in the rostral spinal segments.*

Mechanisms of postural control in lamprey

A postural control system in the lamprey comprises three main components. Information about the body orientation is delivered by the vestibular afferents. These signals, via the neurons of vestibular nuclei, affect the RS neurons. The RS system processes this information and sends commands for postural corrections to the spinal cord. In response to these commands spinal mechanisms generate postural corrections.

Roll control system

A detailed study of the responses of RS neurons to natural vestibular stimulation (roll 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). Responses to roll tilt were qualitatively similar in all reticular nuclei: contralateral tilt (in relation to the location of the neuron in the brain) caused an activation of RS neurons. However, the angular zones for activation differed between the nuclei. The maximal response was at 45° in MRN, at 90° in MRRN and PRRN, and at 180° in ARRN. Thus, the zones of spatial sensitivity covered the whole range of possible inclinations in the transverse plane (Deliagina et al. 1992a).

The responses of RS neurons to tilt 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).

The main findings of *in vitro* experiments were confirmed in the experiments on intact lampreys (Deliagina and Fagerstedt 2000) – most neurons responded to the contralateral roll tilt, with the maximum at 90º. Theses neurons receive excitatory input from the ipsilateral eye and inhibitory input from the contralateral eye.

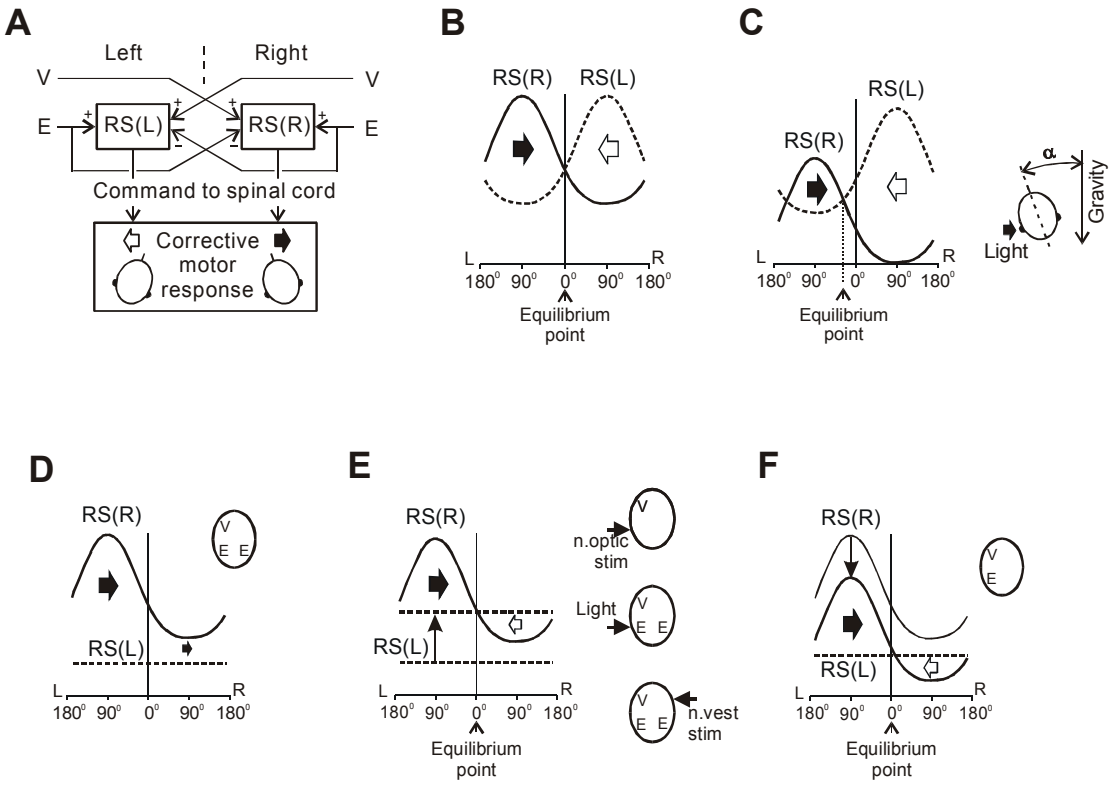

Figure 1. Conceptual model of the roll control system and its operation under different conditions. *A*: conceptual model of the roll control system as proposed earlier (Deliagina & Fagerstedt 2000). The signs (+ and −) indicate the effects on RS neurons produced by sensory inputs. *B-F*: operation of the model under different conditions. The curves represent activity of the left and right groups of RS neurons as a function of roll angle. The presumed directions of rolling caused by RS(R) and RS(L) are indicated by the black and white arrows, respectively. *B*: control (intact lamprey). The two curves intersect, and the system has an equilibrium point at 0° (dorsal-side-up orientation). *C:* left eye illumination leads to the increase of activity in RS(L), and equilibrium point will be shifted to the left (dorsal light response). *D*: the right labyrinth removed. The system has no equilibrium point. *E*: effect of the left eye illumination, stimulation of the left optic nerve or right vestibular nerve. These inputs cause tonic activation of RS(L), and re-creation of the equilibrium point. *F:* ablation of the right eye decreases the activity in RS(R), and the two curves intersect again.

On the basis of these findings, a conceptual model of the roll control system in the lamprey was proposed (Deliagina 1995, 1997a; Deliagina et al. 1993; Deliagina & Fagerstedt 2000). The left and right sub-populations of RS neurons receive their main excitatory input from the contralateral labyrinth (Fig. 1A). Due to this input, the activity of RS neurons is position-dependent, with its pick at 90° of the contralateral roll tilt (Fig. 1B). These two sub-populations also receive an excitatory input from the ipsilateral eye and inhibitory input from the contralateral eye (Fig. 1A). Each of the sub-populations, via spinal mechanisms, elicits ipsilateral rotation of the lamprey. A reason for this suggestion was the observation that the lamprey with a rostral hemisection of the spinal cord is continuously rolling towards the intact side (Zelenin et al. 2003a). The balance between the opposing effects will be achieved when the activities in two sub-populations are equal to each other, this is an equilibrium point of the system (Fig. 1B). This hypothesis about operation of the roll control system was supported in the study with a neuro-mechanical model (Zelenin et al. 2000).

The model of the roll control system (Fig. 1A, B) can explain a number of behavioral phenomena:

(i) *Dorsal light response* (Fig. 1C). Asymmetrical eye illumination increases the RS activity on the illuminated side and decreases this activity on the opposite side. As a result, the intersection of the two activity curves (the equilibrium point) is shifted towards the illuminated side, and the animal will maintain a tilted orientation. Such modifications in the activity of RS neurons under the effect of asymmetrical eye illumination were confirmed in the experiments on intact lampreys (Deliagina and Fagerstedt 2000).

(ii) *Effect of the unilateral labyrinthectomy (UL)* (Fig. 1D). In the lamprey, UL causes rotation of the animal around its longitudinal axis (Deliagina 1997a). According to the model, UL causes inactivation of RS neurons on the contralateral side, the two activity curves no longer intersect, the system has no equilibrium point, and the dominating sub-population of RS neurons causes the main postural deficit – rolling of the lamprey toward the UL side. It was also shown that in the UL lamprey illumination of the ipsilateral eye, or tonic electrical stimulation of the corresponding optic nerve or transected vestibular nerve immediately and reversibly restore equilibrium (Deliagina 1997b). According to the model, all these means cause the

same effect, an increase of activity of the deafferented RS neurons, the two activity curves will intersect, and equilibrium will be restored (Fig. 1E). *One of the aims of the present study was to test if the UL indeed abolishes the activity in the contralateral sub-population of RS neurons, and if restoration of balance is due to re-activation of these neurons.*

Pitch control system

When swimming, the lamprey can stabilize different pitch angles. Like the roll control system, the pitch control system is driven by vestibular input (Ullén et al. 1995a). *In vitro* experiments (Deliagina et al. 1992a; Orlovsky et al. 1992) have shown that responses to pitch tilt were not uniform in RS neurons from different reticular nuclei. Most MRN neurons responded preferentially within the angular zone of 45°-90° nose-up inclinations. The ARRN neurons had their maximal response at the dorsal side-down position (180°). In the MRRN, two major groups responded around 90° nose-down tilt and around 90° nose-up tilt, respectively. Finally, the PRRN neurons were very heterogeneous, with their zones of activity distributed from 0° to 360°. Thus, also in the sagittal plane, the zones of spatial sensitivity in the different reticular nuclei covered the whole range of possible inclinations.

On the basis of these *in vitro* experiments, a hypothesis was proposed that the UP and DOWN groups of RS neurons, driven by the corresponding groups of vestibular afferents (Deliagina et al. 1992b), 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 (Deliagina et al. 1992). This hypothesis, however, was formulated on the basis of *in vitro* experiments, where a number of inputs to RS neurons, like those from the forebrain, from the cranial nerves, and from the caudal spinal cord, were abolished; this may affect vestibular responses in RS neurons. *One of the aims of this study was to examine responses of RS neurons to pitch tilts in intact animals.*

Most often, the lamprey swims horizontally (the pitch angle is 0°), but under certain conditions the pitch angle can differ from 0°. The lamprey actively stabilizes its pitch angle, and usually swims along a linear trajectory in the sagittal plane. In repeated tests, a preferred pitch angle could be maintained over a period of several minutes, even if the initial orientation of the animal was changed considerably (Ullén et al. 1995a). One of the factors presumably affecting the stabilized pitch angle is the water temperature. The lampreys prefer lower temperatures, which are usually associated with deeper water layers (F. Ullén, unpublished observation). *In the present study, the activity of UP and DOWN groups of RS neurons was examined under different temperatures.*

The main motor disorder caused by UL in the swimming lamprey is continuous rolling. *Does the UL-caused impairment of the pitch control system contribute to this symptom?* This question was also addressed in the present study.

Vestibular compensation

In all classes of vertebrates, UL evokes severe motor disorders. They include abnormal eye positions, spontaneous ocular nystagmus, asymmetry in the head and trunk posture, etc. Over time, these disorders gradually diminish. This process is referred to as vestibular compensation, which is one of the best examples of neuronal plasticity (for a review, see Curthoys & Halmagyi 1999; Dieringer 1995; Shaefer & Meyer 1974; Smith & Curthoys 1989; Vidal et al. 1998).

Studies on mammals have shown that compensation of the UL-induced motor deficits is associated with a restoration of the "central symmetry", which includes the recovery of tonic activity in the deafferented vestibular nuclei (Precht 1974; Smith & Curthoys 1988a,b; Hamann & Lannou 1988; Chan et al. 1999; Ris et al. 1997). It is generally accepted that there is causal relation between the restoration of spontaneous resting activity in the vestibular nuclear complex on the UL side and the reduction of motor disorders (Precht et al. 1966; Ris et al. 1995). It was also found that plastic changes occur in other parts of CNS as well, e.g., in the spinal cord (Dieringer 1995), suggesting that the changes underlying vestibular compensation are not limited to only the brainstem.

Vibert et al. (1999) proposed a hypothesis that vestibular compensation initially relies on the external cues given by the intact sensory systems. After this, an internal reorganization of the vestibular-related networks takes place. Finally, changes in the intrinsic properties of vestibular neurons themselves occur, including an increase of pacemaker activity (Ris et al. 2001). The intrinsic mechanism hypothesis was proposed earlier by Darlington & Smith (1996). The change in the intrinsic excitability was shown in one type of the neurons in the medial vestibular nucleus in rats during "vestibular compensation" (Him & Dutia 2001). Different mechanisms seem to be responsible for the different stages of vestibular compensation (Curthoys 2000; Curthoys & Halmagyi 1999). In particular, NMDA receptors are important for the initiation, but not for the maintenance of vestibular compensation (Smith & Darlington 1997; King et al. 2002). An increase of synaptic release because of the plastic changes in the postural network of the spinal cord during vestibular compensation might also play a role (Dieringer 1995). Reactive synaptogenesis during compensation was also reported (see Dieringer 1995; Curthoys & Halmagyi 1999 for a review).

In the lamprey, the UL dramatically affects postural control: it causes continuous rolling towards the damaged side. Normally, a recovery of postural equilibrium (vestibular compensation) takes a few weeks. However, lampreys can maintain equilibrium immediately after surgery if UL was combined with removal of the ipsilateral eye (Deliagina et al. 1997a). According to the model of the roll control system (Fig. 1A,F), elimination of the excitatory drive (from visual input) to the RS neurons ipsilateral to UL causes a downward translation of their activity curve and re-creation of the equilibrium point in the system. Though these animals maintain equilibrium in light, compensatory processes in their postural network, nevertheless, take place. It was found that ablation of the remaining eye in 1-3 days after UL causes rolling, whereas ablation in 50 days does not (Deliagina et al. 1997b). *One of the aims of the present study was to characterize long-term modifications in reticulospinal commands following unilateral labyrinthectomy in the two groups of animals (UL alone, and UL combined with ablation of the ipsilateral eye).*

Specific aims of the study

1. To characterize commands for postural corrections in the pitch plane transmitted to the spinal cord by individual reticulospinal neurons in the intact lamprey. *(Paper I)*

2. To reveal changes in reticulospinal commands for postural corrections in the roll and pitch planes caused by a unilateral labyrinthectomy. *(Papers II and III)*

3. To characterize long-term modifications in reticulospinal commands following unilateral labyrinthectomy. *(Paper IV)*

4. To study functional projections of vestibulospinal and reticulospinal neurons to the rostral part of the spinal cord. *(Paper V)*

METHODS

All experiments in this study were performed on adult lampreys. *In vivo* recordings of reticulospinal activity were carried out on river lampreys (*Lampetra fluviatilis*) (*Papers I–IV*). They were obtained from Alvkarleby (Sweden) and Karleby (Finland). *In vitro* experiments were performed on brainstem–spinal cord preparation dissected from the silver lamprey (*Ichthyomyzon unicuspis*) (*Paper V*). They were obtained from Iowa (USA). Animals were kept in an aerated freshwater aquarium at 7°C, with a 12 h:12 h light:dark cycle. All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden).

In vivo experiments

Electrodes

The activity of RS neurons was recorded from their axons in the spinal cord by means of chronically implanted electrodes (Deliagina et al. 2000c; Deliagina & Fagerstedt 2000). The macroelectrodes (75 μm silver wires coated with a Teflon insulation, except for their active part) were glued to a plastic plate. During surgery, the plate was positioned on the spinal cord so that the electrodes were oriented in parallel to the long spinal axons. The length of the active part was chosen to be 3 mm. This is close to the longitudinal extent of the axonal membrane excited by the propagating action potential: with a conduction velocity in large RS axons ranging from 2 to 4 m/s, and a spike duration being ~1 ms, the length of the excited axonal segment will be 2-4 mm. Thus, at the moment when the excited membrane area opposes the electrode, the whole electrode occurs positioned in approximately equi-potential points. No current will flow along the electrode, and it will record the extra-axonal potential with the same amplitude, as would do a microelectrode positioned at the same distance from the axon. An advantage of the wire electrode is its mechanical stability, a very low resistance ($<10^3$ Ω), and a low noise level (a few μV). In thinner axons, the excited part of the fiber will be shorter, and the electrode will be positioned along the points with different potentials. As a result, currents flowing along the electrode will cause a considerable shunting effect. In addition, thinner axons provide

smaller membrane currents as compared to thicker axons. Thus the wire electrode can serve as a filter for recording spikes almost exclusively from the larger fibers with a conduction velocity >2 m/s situated in parallel with the electrode. In lampreys, only the RS pathways contain fibers with such a high conduction velocity (Ohta & Grillner 1989; Rovainen 1978).

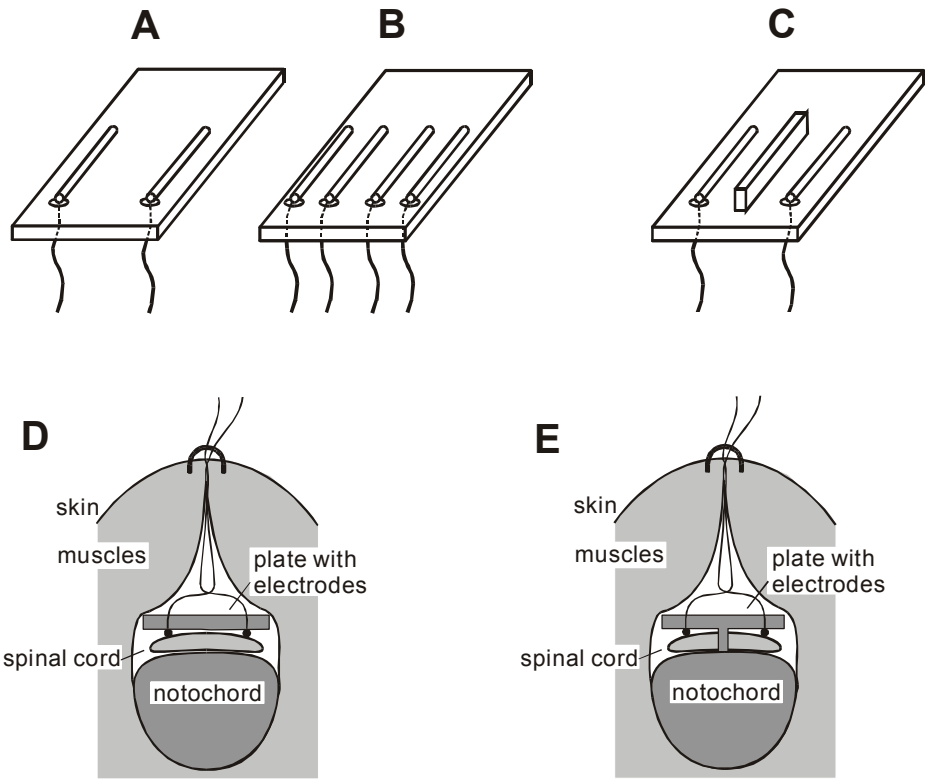

Figure 2. Macroelectrodes for recording activity of reticulospinal (RS) axons. *A-C*: three different designs of electrodes – 2-electrode array (*A*), 4-electrode array (*B*), and 2-electrode array with a longitudinal wall (*C*), view from below. Silver wires (75 µm in diameter and 3 mm in length) were glued to a plastic plate. *D* and *E*: position of the implanted plate, with or without the longitudinal wall (*E* and *D*, respectively) as seen in the transversal section of the lamprey body.

In special *in vitro* experiments (Deliagina and Fagerstedt 2000), field potentials were recorded by the wire electrode in an isolated part of the spinal cord. It was shown that the electrode can record activity in the larger axons at a distance of ~ 400 µm. With a width of the spinal cord of ~1500 µm, one can suggest that each of the electrodes will record activity of larger RS axons over almost the whole cross-section of the ipsilateral half of the spinal cord.

Implantation of electrodes

Implantation of electrodes was performed under MS-222 (Sandoz) anesthesia (100 mg/l). The animal was positioned in the bath with the anesthetic solution that covered the gills whereas the surgical field was above the water level. Three different arrangements of electrodes were used: two-electrode array, four-electrode array, and two electrodes separated by an isolating wall (Fig. 2A-C). In most of experiments, the plate with two electrodes was positioned at the level of the third gill and plate with four electrodes – at the level of the last gill, so that the distance between the two plates was 20-30 mm. This allowed comparing the spike occurrence at the rostral and caudal electrodes in order to determine the direction of spike propagation and the conduction velocity (Deliagina & Fagerstedt 2000). The four-electrode array was also used for estimating the medio-lateral position of the axon in the spinal cord. The plates with electrodes were implanted through a longitudinal cut performed along the midline of the dorsal aspect of the body so that the spinal cord was exposed (Fig. 2D,E). The layer of fat covering the spinal cord was removed, and the plate was positioned on the dorsal surface of the spinal cord. For implantation of the plate with two electrodes separated by the wall, the midline split of the spinal cord extending for ~5 mm was performed. The wall was then positioned in an incision between the two halves of the spinal cord to record separately from the left and right RS pathways (Fig. 2E). The wound was then closed and sutured so that the flexible connective wires were fixed tightly between the two sides of the wound.

Unilateral labyrinthectomy

Operation was carried out under MS-222 anesthesia. To remove the labyrinth, a hole was made on the dorsolateral aspect of the vestibular capsule. Through this hole, the labyrinth was completely removed by means of a pair of fine forceps under visual control. After removal, the intact medial wall of the vestibular capsule and a stump of the 8th nerve could be seen. *Post mortem* investigation showed that, in all cases, removal of the vestibular organ was complete and that the medial wall of the capsule was undamaged.

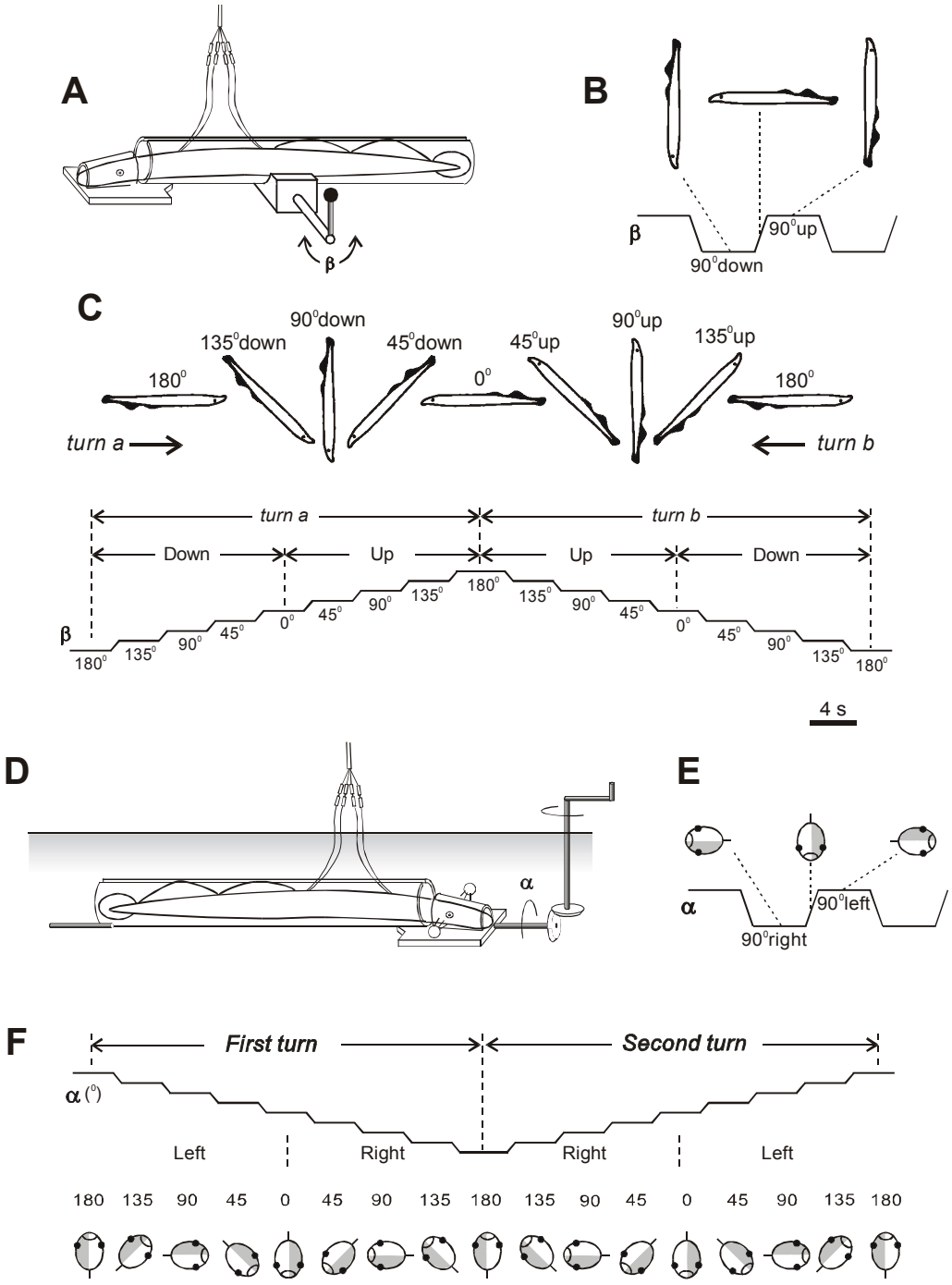

Figure 3. Arrangement for recording vestibular and visual responses in RS neurons. *A*: experimental device for rotation in the pitch plane (β, pitch angle). Activity of RS axons was recorded by implanted electrodes. *B*-*C*: methods of vestibular stimulation. *B*: trapezoid tilting. *C:* Two sequential full turns in opposite directions (*a* and *b*) were performed in steps. Successive positions of the animal in each step are shown for turn *a* (→) and for turn *b* (←). *D*: experimental device for rotation in the roll plane (α, roll angle). *E* and *F*: methods of vestibular stimulation. *E*: trapezoid tilting. *F*: two sequential full turns in opposite directions (*a* and *b*) were performed in steps. Successive positions of the animal in each step are shown. Right half of the lamprey body is shaded.

Experimental design

For recording vestibular and visual responses of RS neurons, the lamprey was positioned in a setup located in the aquarium. The level of water in the aquarium was high enough to cover the animal during rotation in the whole range of angles. All the experiments were carried out at a water temperature of ~7°C. Each setup consisted of a tube fastened to a small platform. During experiments, the lamprey was attached to the platform with its sucker mouth. Two setups were used differing in the plane of rotation (Fig. 3A,D). The "roll" setup (Fig. 3D) allowed rotation of the animal in the transverse plane (*Papers I, II, IV*), whereas the "pitch" setup (Fig. 3A) – in the sagittal plane (*Papers I, III*). Rotation was performed manually. Two patterns of rotation were used in both transverse and sagittal planes. *First,* the animal was tested during two full turns performed in 45° steps (Fig. 3C,F). Rotation in the first and second turn was performed in opposite directions to examine a directional specificity of vestibular responses (Orlovsky et al. 1992; Deliagina & Fagerstedt 2000). A transition from one position to the next lasted ~ 1 s, and each position was maintained for ~ 3 s. The responses during movement were termed dynamic responses, and those during maintenance of a given orientation – static responses. *Second*, we performed periodic trapezoid tilting (Fig. 3B,E), with alternating 90° tilts in relation to the normal orientation. Again, the transition from one position to the next lasted ~ 1 s, and each position was maintained for ~ 3 s.

Eye illumination was performed by two fiber optic systems (90 W) attached to the rotating platform (Fig. 3D). The implanted electrodes for recording the activity in RS pathways were connected, via a flexible cable, to inputs of AC amplifiers.

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). The tilt angle was measured by potentiometric transducer.

The recorded multiunit spike trains were separated into unitary waveforms (representing the activity of individual axons) using date analysis software (Datapac III, Run Technologies). All spike-like unitary waveforms in each channel (electrode), with the amplitude greater than a certain threshold, were selected for the "event" analysis. The unitary waveforms occurring in only one channel, as well as unitary waveforms occurring simultaneously in several channels, were considered to originate from one action potential in a single axon and to represent an event.

Grouping of spikes generated by a single axon was done using cluster analysis (Deliagina & Fagerstedt 2000). It was based on similarities of the amplitudes of different events on the two channels. Since delineation of clusters was done manually, errors could take place. The events in one cluster were superimposed and the waveforms that differed in shape on any of the channels were removed. However, modification of the waveforms shape could also be due to summation of spikes. Rejection of such waveforms would lead to underestimation error. Medio-lateral position of the axon and its conduction velocity were used as additional criteria for identification of spikes generated by a single axon. The medio-lateral position of the axon was estimated by comparing the amplitudes of the spike recorded by the electrodes of the four-electrode array. The conduction velocity was calculated from the time delay between the appearances of corresponding spikes in the rostral and caudal electrodes. These would minimize the number of foreign events taken into the cluster. Thus, the main error in spike sorting was underestimation of the total number of spikes generated by a neuron. However, this did not affect the conclusions of the study, because they were based not on absolute but relative values of spike frequencies.

In vitro experiments

To study functional projections of RS and VS neurons in the rostral spinal segments, *in vitro* experiments were performed on a brainstem-spinal cord preparation, using the previously developed technique (Zelenin et al. 2001). The brainstem was isolated with 15-35 segments of the spinal cord. The dorsal and ventral branches of the ventral roots were exposed. The preparation was kept in a chamber

filled with Ringer solution (Orlovsky et al. 1992). To induce fictive locomotion (Grillner et al. 1981, 1995) D-glutamate (0.5-1 mM) was applied. The fictive locomotion provided a background activity of motoneurons, which could be affected by the spikes of individual RS or VS neurons. The activity of motoneurons (MNs) was recorded extracellulary by suction electrodes either from the analogous branches of ventral roots (VR) along the spinal cord, or from four branches in one of the segments. Individual RS axons were recorded intracellulary in the first segment of the spinal cord, and individual VS neurons (located in ION) were recorded from their cell bodies by means of KAc-filled microelectrodes with a tip resistance of 10-30 MΩ. Single spikes in the RS axon or in the VS neuron were evoked by positive current pulses (7-15 ms pulse duration, pulse period 100 ms, current up to 20 nA) passed through the microelectrode. Propagation of the spike along the axon was monitored by a suction electrode placed on the spinal cord surface. Each VS neuron was identified by the orthodromic spike recorded in the spinal cord and by the monosynaptic response to ipsilateral vestibular nerve stimulation performed by another suction electrode. For each individual RS and VS neuron, a post-spike histogram was generated for the spikes of MNs recorded in each VR branch. The moment of the RS and VS spike occurrence in the cell body was taken as the origin of the time axis on the histogram. Typically, responses to a few thousands of RS or VS spikes (up to 20 min of stimulation at 10 Hz) were used for generation of a histogram.

RESULTS AND DISCUSSION

Pitch control system

The pitch control system was investigated in *Paper I.* For this purpose, the responses of RS neurons to rotation in the pitch plane were recorded in intact lampreys using chronically implanted macroelectrodes. Rotation in steps evoked dynamic responses and much weaker static responses. Both types of responses depended on the direction of rotation and on the tilt angle.

Two groups of neurons were classified depending on 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.

The two groups partly differed in their axon position in the spinal cord and axonal conduction velocity. A considerable part of group DOWN neurons had axons with a high conduction velocity positioned centrally in the spinal cord. This is characteristic of Müller cells. Other neurons of this group and the majority of group UP neurons had axons with a lower conduction velocity and/or more lateral position in the spinal cord, which is characteristic of the middle-size PRRN and MRRN neurons.

To explain the origin of dynamic and static responses in RS neurons, and their directional and spatial characteristics, one can suggest that both canal and otolith afferents contribute to the activation of RS neurons during pitch tilts. It seems likely that DOWN and UP groups of canal afferents, as well as P1 (maximum at 45-90° down) and P2 (maximum at 45-90° up) groups of otolith afferents (Deliagina et al 1992b) are responsible for activation of DOWN and UP groups of RS neurons, respectively.

As proposed by Magnus (1924) in a reflex concept of postural control, any 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, both in the initial study on the brainstem-labyrinths preparation

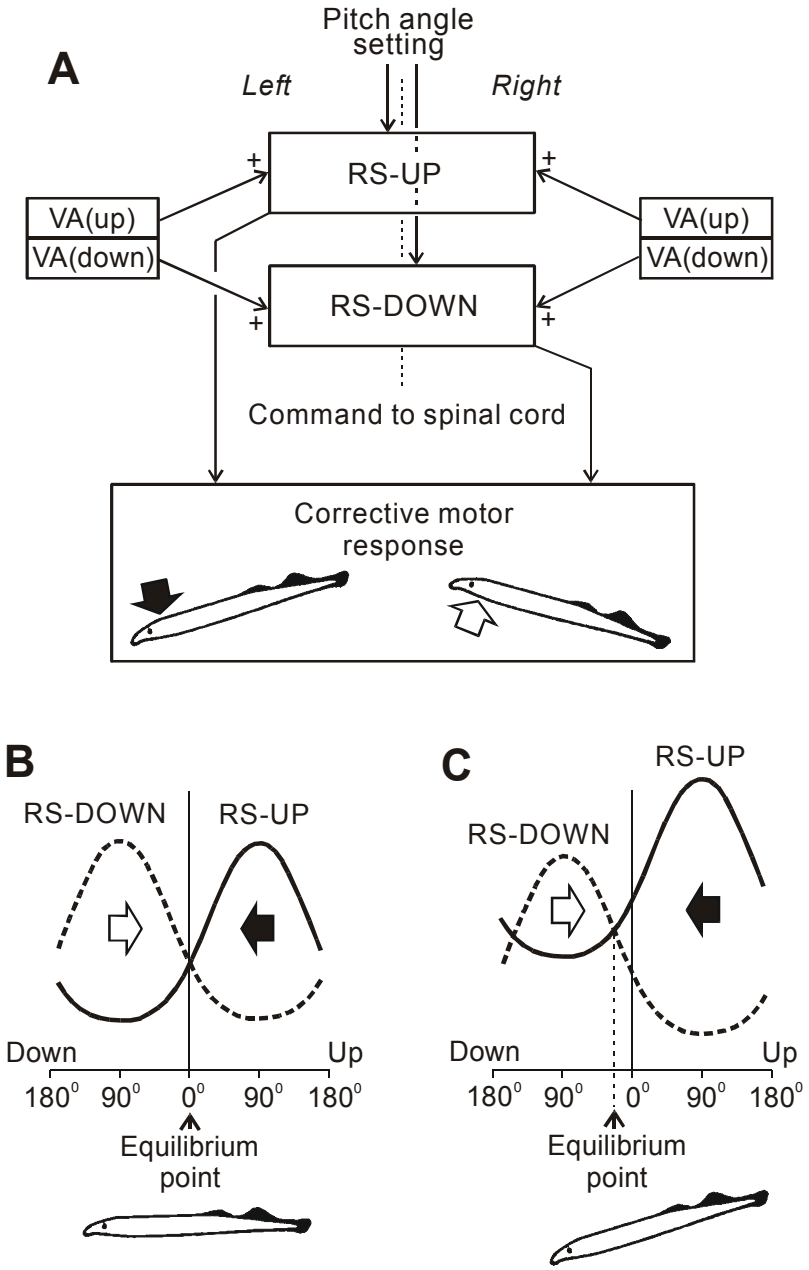

Figure 4. Presumed role of group UP and DOWN neurons. *A*: conceptual model of the pitch control system. Two groups of RS neurons (UP and DOWN) are driven by vestibular afferents responding to nose-up and nose-down tilts, respectively. The UP and DOWN groups affect spinal network and cause downward and upward turning of the lamprey, respectively (black and white arrows). *B*: operation of the system during horizontal swimming. Curves represent the activity of UP and DOWN groups of RS neurons (arbitrary units) and their motor effect as a function of pitch angle. Vestibular input causes activation of the groups with upward and downward tilt, respectively. Directions of turning caused by UP and DOWN groups are indicated by black and white arrows, respectively. System has an equilibrium point at 0° (horizontal orientation). *C*: operation of the system when the activity of group UP increased in relation to that of group DOWN due to the specific tonic drive (*Pitch angle setting*). The equilibrium point is displaced toward the down pitch angles.

(Deliagina et al. 1992a) and in the present study on intact animals (*Paper I*) allowed to apply this general idea to the pitch control system of this animal. In both studies, two groups of RS neurons, the UP and DOWN ones, have been revealed. We suggest 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. 4A. The UP and DOWN groups are driven by VA(up) and VA(down) vestibular afferents responding to the nose-up and nose-down tilt, respectively. Due to these vestibular inputs, the activities of UP and DOWN groups are orientation-dependent (Fig. 4B). We also suggest that group UP causes a downward turn of the lamprey, whereas group DOWN causes an upward turn. The system will stabilize the orientation at which the effects produced by the two groups of RS neurons are equal to each other. Normally this occurs at the zero pitch angle (an equilibrium point in Fig. 4B), and the animal stabilizes a horizontal orientation of its body. Any deviation from this orientation will evoke a compensatory movement aimed to restore the initial position.

An indirect support for the hypothesis about the role of UP and DOWN groups was obtained in *Paper I*. We have found that the factor, which most likely 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. 4C). Because of the increase in the UP/DOWN ratio, an intersection of the two activity curves will be displaced from 0° toward the downward tilt angles (Fig. 4C). 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 UP/DOWN ratio and, consequently, a change of the stabilized pitch angle is shown schematically in Fig. 4A (*Pitch angle setting*).

Interaction between roll and pitch control systems

The swimming lamprey can stabilize its orientation in the roll plane and in the pitch plane (Ullén et al. 1995a). For simplicity, here the roll and pitch control systems are considered separately. In reality, however, these two systems constituting a postural control system are active together. 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, in *Paper I* we examined responses in individual RS neurons not only to pitch tilts but also to roll tilts. It was found that 22% of RS neurons responding to pitch tilts also responded to roll tilts. This result was obtained in quiescent animals. In swimming animals, when RS neurons are more active (Deliagina et al. 2000c), this value could be even greater. The overlap between the pitch and roll populations suggests that the RS pathways are partly shared by the pitch and roll control systems. Thus, the same neurons can participate in different forms of motor behavior. The different motor patterns (locomotion, turns in the roll, pitch or yaw plane, etc.) can be produced due to combinations of the effects of neurons with different projections to the spinal cord (Zelenin et al. 2001).

Operation of roll control system after unilateral labyrinthectomy

In the lamprey, UL severely damages the roll control system, which results in continuous rotation of the animal around its longitudinal axis during swimming (de Burlet & Versteagh 1930; Deliagina 1997a). In order to assess operation of the roll control system under such pathological conditions (unilateral vestibular loss), in *Paper II* the responses of individual RS neurons to roll tilt were investigated; the same neurons were recorded both before and after UL. From behavior experiments it is also known that, in the continuously rolling UL lamprey, illumination of the eye contralateral to UL leads to immediate and reversible restoration of equilibrium (Deliagina 1997a). To address this issue, in *Paper II* we also investigated the responses of RS neurons in UL animals to vestibular stimulation combined with eye illumination.

The model of the roll control system proposed earlier (Fig. 1A, Deliagina 1997a) implies that UL causes inactivation of RS neurons on the contralateral side, (Deliagina 1997a) as illustrated for the right-side labyrinthectomy in Fig. 1D. Because of inactivation of RS(L) neurons, the two activity curves no longer intersect, the system has no equilibrium point, and the dominating RS(R) group will cause the main

postural deficit – rolling of the lamprey to the right. These predictions of the model were confirmed in *Paper II*. It was found that vestibular responses on the UL-side changed only slightly, whereas the responses on the opposite side disappeared almost completely. The two activity curves, which intersected in intact animals (Fig. 1B), did not intersect after UL (Fig. 1D). The asymmetry in the bilateral activity of RS neurons, and disappearance of the equilibrium point in the roll control system, is the most likely cause for the loss of equilibrium in UL animals.

When studying the effects of asymmetrical visual input, we have found that illumination of the eye contralateral to UL resulted in a restoration of vestibular responses in the neurons inactivated by UL, and in an appearance of vestibular responses in some other neurons that did not respond to vestibular input before UL. All these responses had directional sensitivity and zones of spatial sensitivity similar to those observed before UL, as illustrated schematically for the right-side UL in Fig 6B. However, their magnitude was smaller than before UL. Evidently, the responses to tilts in RS(L) neurons were caused by the input from the intact (left) labyrinth. This input became efficient due to an increase of RS(L) excitability caused by a tonic excitatory drive from the illuminated eye.

In addition to activation of the silent (contralateral to UL) population of RS neurons, eye illumination caused some reduction of the magnitude of vestibular responses in the RS neurons on the UL side due to an inhibitory input to RS neurons from the contralateral eye. These modifications of vestibular responses in the ipsilateral and contralateral groups of RS neurons will lead to a restoration of symmetry in bilateral activity of RS neurons, to a re-creation of the equilibrium point in the system, and to a termination of rolling. The equilibrium point, however, may occur not at 0° but shifted toward the UL side, and the animal will maintain the correspondingly tilted position.

The main effect of eye illumination, that is an appearance of activity in the silent group of RS neurons, was predicted by the model of the roll control system (Fig.1E). However, the fact that this activity is roll-dependent (Fig. 6B) was not anticipated. This roll-dependent modulation was due to the ipsilateral vestibular input. This input is now incorporated into the model (interrupted lines in Fig. 6D). Experiments were performed to estimate a relative contribution of the ipsi- and

contralateral vestibular inputs to the roll-dependent activity of RS neurons. In the UL-lamprey, the RS neurons, either on UL-side or on the opposite side, were tonically activated by illuminating the ipsilateral eye, and then a 90° tilt was performed. The responses were normalized to the background-firing rate induced in the neurons by eye illumination. It was found that the ipsi- and contralateral vestibular inputs to RS neurons supplement each other: they evoke activation of neurons with the contralateral tilt and reduce their activity with the ipsilateral tilt. However, the contralateral excitatory input is a dominating one.

Operation of pitch control system after unilateral labyrinthectomy

In *Paper III*, changes in the operation of the pitch control system caused by UL were investigated. Responses of RS neurons to pitch tilts before and after UL were examined *in vivo*. It was found that UL produced different effects on the UP and DOWN groups of RS neurons. In the group UP neurons, the responses remained almost unchanged on the side ipsilateral to UL and were dramatically reduced on the opposite side. This finding indicates that the group UP neurons receive their excitatory input predominantly from the contralateral labyrinth, whereas the input from the ipsilateral labyrinth is very weak. In the group DOWN neurons, the effects of UL on both sides were similar: the number of responding neurons increased, although their discharge frequency decreased. This finding indicates that the group DOWN neurons receive similar inputs from both labyrinths. The inputs seem to contain both excitatory and inhibitory components.

These inputs were incorporated into the model of the pitch control system (Fig. 5A). The left and right subgroups of the group UP neurons receive their main inputs from the contralateral labyrinths, and much weaker inputs from the ipsilateral labyrinths. Due to these inputs, they are activated with the nose-up tilt. The left and right subgroups of the group DOWN neurons receive their inputs from both labyrinths, and they are activated with the nose-down tilt.

According to the model, the UL-induced changes in vestibular responses to pitch tilt will disturb the normal activity of the postural control system, as illustrated for the right-side UL in Fig. 5B. Any nose-up tilt will evoke a much weaker activation

of the left UP subgroup due to the removal of the main (right) vestibular input to this subgroup. By contrast, a nose-down tilt will still evoke symmetrical activation of the left and right DOWN subgroups since they receive approximately equal inputs from the remaining (left) labyrinth.

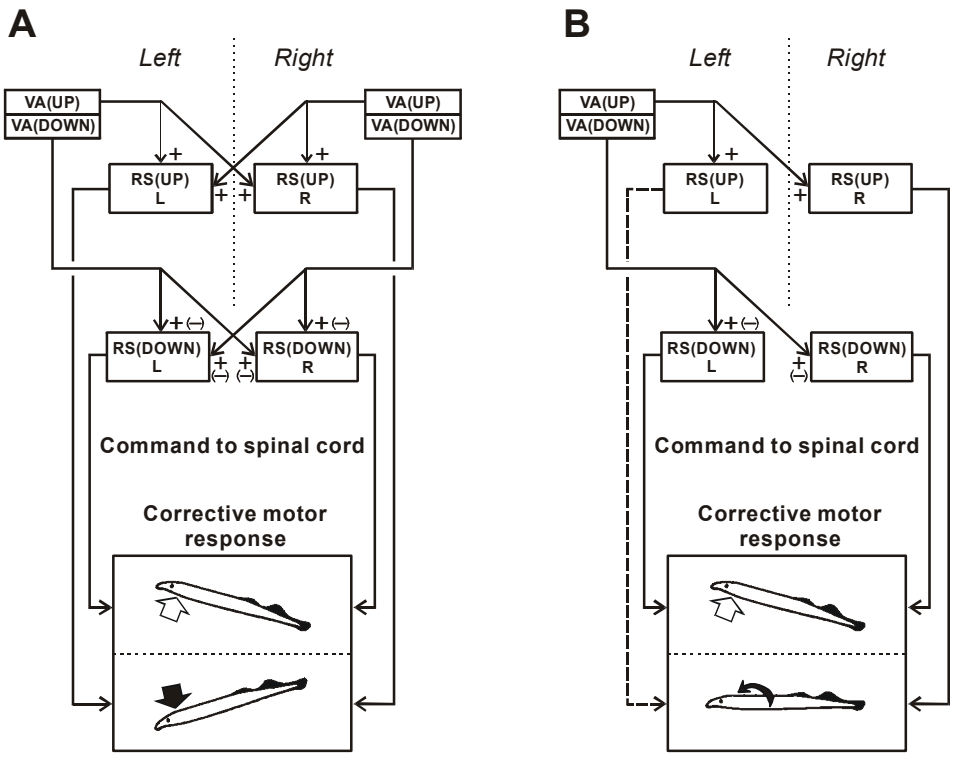

Figure 5. Impairment of the pitch control system caused by UL. *A*: a conceptual model of the pitch control system proposed in *Paper I*, with modifications based on the results of *Paper II*. *B*: impairment of the system caused by right UL. Large black and white arrows indicate the motor responses elicited by the groups DOWN and UP, respectively. (See legend to Fig. 4.)

It seems likely that any nose-up pitch tilt in UL animals, because of incorrect functioning of the pitch control system, will evoke rolling. Indeed, in the study of the roll control system we found that UL evokes an asymmetry in the left/right RS commands, which causes a continuous rolling. In the pitch control system, UL also evokes an asymmetry in the left/right RS commands. Since the populations of RS neurons transmitting the roll and pitch commands partly overlap (*Paper I*), one can expect that the effects of asymmetry in the two systems will be similar. Thus the

motor deficits caused by the impaired roll and pitch control systems will summate, enhancing the main UL symptom, the rolling.

Recovery of roll control during vestibular compensation

In *Paper II* it was shown that UL severely damaged the roll control system. The vestibular responses on the side contralateral to UL disappeared, whereas the responses on the side ipsilateral to UL remained unchanged as illustrated schematically in Fig. 1D. It was suggested that this asymmetry in the activity of RS neurons is a cause for the loss of equilibrium, and a restoration of symmetry is a necessary condition for the recovery of equilibrium control. The lampreys subjected to UL are capable of spontaneous recovery of the equilibrium control, which is a part of the process of vestibular compensation (Deliagina 1997a). The goal of *Paper IV* was to elucidate if the recovery of equilibrium control in these animals is associated with a restoration of symmetry in the bilateral RS activity. We used two experimental models of vestibular compensation (Deliagina 1997a,b): (i) The lampreys subjected to UL alone (UL animals). These animals reach a compensated state in a few weeks after UL. (ii) The lampreys subjected to UL combined with ablation of the ipsilateral eye (UL/E animals). Initially, they are able to maintain equilibrium in light but not in darkness, but in a few weeks they reach a compensated state and become able to maintain equilibrium both in light and in darkness. In compensated UL animals, as well as in non-compensated and compensated UL/E animals, vestibular responses in RS neurons (elicited by rotation of the animal in the roll plane) were recorded in light and in darkness. It was found that, in both UL and UL/E animals, their ability to maintain equilibrium was associated with a partial restoration of symmetry in the RS activity.

In the compensated UL animals, the vestibular responses in RS neurons on the two sides (shown schematically in Fig. 6B) were qualitatively similar to each other; they were also similar to those in the intact lamprey (Deliagina & Fagerstedt 2000; Deliagina & Pavlova 2002). The neurons responded preferentially to rotation toward the contralateral side. However, the vestibular responses in the co-UL group differed, to some extent, from the normal ones. First, the magnitude of the responses was

reduced. Second, the population of these neurons was less homogenous in respect to the response pattern than the i-UL group. Despite these abnormalities, it seems likely that the i-UL and co-UL activity curves in the compensated animals intersect, and the roll control system has an equilibrium point.

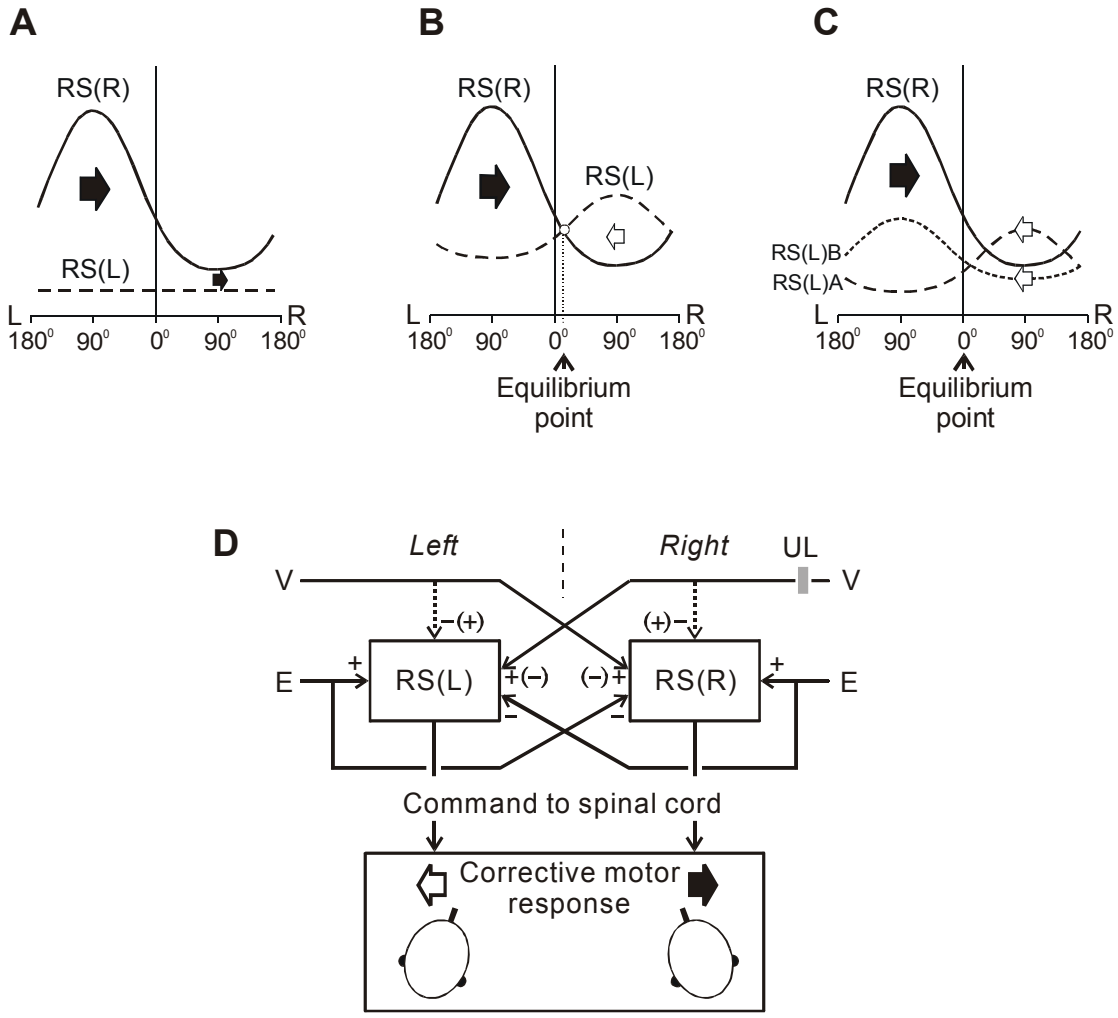

Figure 6. Impairment of the roll control system caused by UL and restoration of postural function. *A*-*C*: schematic representation of experimental data on RS activity under different conditions (*Papers II* and *IV*). *A*: effects of the right side UL or UL/E. *B*: restoration of vestibular responses due to eye illumination in the non-compensated UL and UL/E animals, or spontaneous recovery of these responses in the compensated UL animals. *C*: spontaneous recovery of vestibular responses in the compensated UL/E animals. *D*: conceptual model of the roll control system as proposed earlier (Deliagina & Fagerstedt 2000), and its modifications based on the data of *Papers 2* and *4* (interrupted lines). The signs (+ and −) indicate the major effects on RS neurons produced by sensory inputs; the signs in brackets indicate the minor effects. (See legend to Fig. 1.)

Vestibular compensation in the lamprey includes two stages. At the initial stage, visual input plays an important role and is necessary for the maintenance of equilibrium. In fully compensated animals, this input becomes less important and can be even abolished (Deliagina 1997b). Correspondingly, we have found that the vestibular responses on the co-UL side in the compensated animals were present both in light and in darkness.

The finding that the co-UL activity is close to the normal one in the compensated UL animals strongly supports the hypothesis (Deliagina 1997a,b; *Paper II*) that a restoration of bilateral symmetry in the RS commands underlies a recovery of equilibrium control. These data also support a more general assumption that restoration of the "central symmetry" in UL animals (i.e., the symmetry in the activity in vestibular nuclei and their targets) constitutes an essential component of vestibular compensation (Deliagina et al. 1997; Vidal et al. 1998; Smith & Curthoys 1989; Curthoys & Halmagyi 1999).

In the non-compensated UL/E animals, vestibular responses in RS neurons on the co-UL side were crucially dependent on the lighting conditions: the responses were present in light but absent in darkness. This finding well corresponds to the behavior of these animals: they are able to maintain equilibrium in light but not in darkness. The patterns of responses in co-UL and i-UL groups in light and in darkness were similar to those observed in the non-compensated UL animals during contralateral eye illumination and in darkness (*Paper II*), respectively. These findings indicate that input from the eye contralateral to UL enhances the responses to a week input from the ipsilateral labyrinth in both groups of animals. The data on the non-compensated UL/E animals present the control to be compared with the data on the compensated animals.

In the compensated UL/E animals, vestibular responses in RS neurons on the co-UL side were not dependent on the lighting conditions: the responses were present both in light and in darkness. This finding well corresponds to the behavior of these animals: they are able to maintain equilibrium both in light and in darkness. Though the co-UL neurons were active in the compensated UL/E animals, their response characteristics noticeably differed from the normal ones. The population of these neurons was not homogeneous, but rather included three sub-groups with their peaks

of activity positioned in different angular zones; they also had different directional sensitivity. The responses in the two larger co-UL sub-groups, A and B, are schematically shown in Fig. 6C for the right-side UL. The activity curve for the left sub-group A was similar to the normal one - it has its peak in the zone of contralateral (right) tilts. By contrast, the left sub-group B has its peak in the zone of ipsilateral (left) tilts, i.e., this sub-group is activated along with the antagonistic, RS(R) group. A possible function of the sub-group B is a reduction of the effects of the antagonistic group RS(R). Due to this reduction, the "weak" sub-group A will be able to counteract the "strong" group RS(R). This hypothesis is supported by the fact that the activity of the sub-groups depended differently on lighting conditions: activity in the sub-group B is decreased in light in parallel with the activity in the antagonistic group RS(R). By contrast, the activity of the sub-group A is increased in light.

The appearance of position- and movement-dependent responses in the co-UL neurons in the compensated animals was caused by the input from the remaining labyrinth. Due to this input, the responses of RS neurons occur both in the normal angular zone and in the abnormal ones. Responses in the normal zone, evoked by a weak but pre-existing vestibular input, can appear because of a number of reasons, including an increase of the excitability of RS neurons, increase of synaptic release, plastic changes in other parts of the postural network, etc. (see INTRODUCTION for discussing this issue).

The responses of RS neurons in the abnormal angular zones cannot be explained by changes in the efficacy of pre-existing vestibular input to these neurons, but rather by the appearance of new functional connections to RS neurons from the vestibular afferents with the corresponding zones of spatial sensitivity.

Functional projections of reticulospinal and vestibulospinal neurons in rostral spinal segments

In contrast to the middle and caudal parts of the spinal cord receiving supraspinal commands exclusively via RS pathways, the rostral spinal segments (neck area) are innervated by both RS and VS pathways (Rovainen 1979a; Bussiéres 1994). In *Paper V,* we investigated functional projections of individual RS and VS neurons

in the rostral spinal segments, that is their effects on the activity of spinal motoneurons. An *in vitro* brainstem-spinal cord preparation was used, and RS- or VS-spike-triggered averaging technique was employed to reveal small effects of individual descending neurons.

In the study by Zelenin et al. (2001) functional projections of individual RS neurons in the middle and caudal parts of the spinal cord were investigated. It was found that they exert very diverse effects on the segmental motor output, but these effects were uniform in all segments. In the present study we have found that this conclusion can be extended to the neck region as well, that is the RS neurons exert uniform effects upon the segmental motor output along the whole extent of their axons. By producing a uniform effect on motor output in numerous spinal segments, individual RS neurons will elicit flexion in a specific plane in a considerable part of the body. It seems thus likely that the group of larger RS neurons is responsible for formation of gross motor synergies that include the head, rostral part of the body, and its more caudal parts, like synergies for locomotion and turns of the whole animal in different planes.

When studying the effects of VS neurons (located in the intermediate octavomotor nucleus and projecting ipsilaterally) we found that they projected only to a few rostral segments. Like the RS neurons, the individual VS neurons exerted uniform effects upon the segmental motor output along the extent of their axons. However, their effects were much weaker than those of RS neurons. When segmental projections of VS neurons were tested, it was found that they exerted much less diverse effects than RS neurons. Only the patterns with excitatory effects on one or both ipsilateral branches were found. Usually no effects were observed on the contralateral ventral root branches (only in three cases the inhibitory effects were revealed).

The VS system, projecting to rostral segments only, is capable of eliciting head movements without affecting the middle and caudal body parts. By activating VS neurons with different patterns of influences or their combinations, the CNS can elicit head movement in the horizontal, vertical, or oblique planes.

The RS and VS descending systems may receive common inputs and thus operate together. Both systems receive vestibular inputs and can thus participate in

the control of body orientation (Deliagina and Fagerstedt 2000; *Paper II*). Larger RS neurons receive numerous sensory inputs and inputs from other motor centers, and are involved in initiation of different forms of motor behavior (Deliagina et al. 1995; Deliagina and Fagerstedt 2000; Deliagina et al. 2000c; Fagerstedt et al. 2001; Deliagina et al. 2002).

The revealed characteristics of VS and RS projections strongly suggest that these two neuron groups play different roles in controlling body movements. The VS neurons may be responsible for the fine control of head position, whereas the RS neurons – for turns of the whole body in different planes.

CONCLUDING REMARKS

Different species actively maintain a basic upright, or dorsal side-up, body posture. Efficient control of the basic posture is important for standing and during walking, as well as for providing support for voluntary limb movements. Nervous mechanisms for postural control in higher vertebrates are very complex, and their analysis is extremely difficult. In the present study, we investigated the organization and operation of neuronal networks responsible for postural control in the lamprey. This animal is a relatively simple and convenient model for investigation of different aspects of motor control. The main conclusions of this study are as follows:

In *Paper I*, nervous mechanisms controlling body orientation in the sagittal (pitch) plane were analysed. We found two groups of command (reticulospinal, RS) neurons with opposite patterns of vestibular responses: the UP and DOWN groups. This allowed us to explain operation of the pitch control system in the framework of the model with two opposing vestibulo-reticulospinal reflexes proposed earlier for the system controlling body orientation in the transverse (roll) plane (Deliagina 1997a). According to the model, a condition for the maintenance of a definite body orientation is equality of the two opposing reflexes. In the roll plane, these reflexes are mediated by two symmetrical, LEFT and RIGHT groups of RS neurons. In the pitch plane, the opposing reflexes are mediated by UP and DOWN groups of RS neurons. The LEFT and RIGHT groups partly overlap with the UP and DOWN groups, suggesting that the command pathways are partly shared by the roll and pitch control systems. This finding is important for understanding general principles of encoding the supraspinal motor commands. To further address this issue, examining the overlap between the roll and pitch command systems on one hand, and the system controlling body orientation in the horizontal plane on the other hand, appears a promising approach.

In the present study, a classical “reflex concept” of postural control (Magnus 1924) has found a further support. We have identified the groups of RS neurons mediating postural reflexes. These neurons are also known to integrate vestibular and visual sensory inputs, the function previously ascribed to a hypothetical postural center (a “central concept” of postural control, Horak and Macpherson 1995).

In *Papers II and III*, we investigated the effects on the postural neuronal network exerted by a removal of one labyrinth (a unilateral labyrinthectomy, UL). The UL is known to result in a loss of equilibrium in the lamprey. We found that UL caused a lateral asymmetry in the activity of RS neurons, in both roll and pitch control systems. These findings strongly suggest that the asymmetry in descending motor commands is the cause for the loss of equilibrium after UL. A further support for this idea was obtained in experiments with eye illumination: this factor restores both symmetry and equilibrium.

In *Paper IV*, we investigated the neuronal correlates of the process of vestibular compensation, that is, the spontaneous recovery of equilibrium control following UL. The vestibular compensation is observed in all vertebrates, and the analysis of its mechanisms in a simpler animal model is very important. We have found that an UL-caused asymmetry in the bilateral activity of RS neurons, observed in the non-compensated animals, is considerably reduced in the compensated ones. These findings strongly support the hypothesis (Smith and Curthoys 1989) that the restoration of symmetry in the activity of vestibular-driven central neurons is of a primary importance for vestibular compensation. Two factors were found to contribute to the restoration of vestibular responses in RS neurons: enhancement of the already existing input from the remaining labyrinth, and formation of a new input driven by the vestibular afferents that normally do not affect the RS neurons. In further studies, a promising approach to the problem of vestibular compensation would be a search for the factors promoting restoration of bilateral symmetry in RS commands.

In *Paper V*, we investigated the motor effects produced by individual neurons of descending pathways, the reticulospinal and vestibulospinal ones. These data are necessary for understanding of how the supraspinal commands evoke postural corrections. We have found a dramatic difference between the RS and VS functional spinal projections. The larger RS neurons projected to a considerable part of the spinal cord and exerted very diverse effects on the segmental motor output. By contrast, the VS neurons projected to only the neck segments, and their effects were weaker and less diverse. These data suggest different functional roles of the two systems: the VS neurons are responsible for flexion of the rostral part of the body and head movement,

whereas the larger RS neurons are responsible for formation of gross motor synergies like the whole body turn for correcting the posture. In further studies, a promising approach to the problem of encoding and decoding the descending motor commands would be examining the correlation between the postural disturbances, the responses of individual RS neurons, and their motor effects.

In conclusion, due to employment of a simpler animal model, the lamprey, and sophisticated techniques we managed to analyze different aspects of a very complex motor behavior, the postural control, at the network level.

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