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NEURONAL MECHANISMS OF FEEDBACK POSTURAL CONTROL

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ABSTRACT

Different species maintain a basic body posture due to the activity of the postural control system. An efficient control of the body orientation, as well as the body configuration, is important for standing and during locomotion. A general goal of the present study was to analyze neuronal feedback mechanisms contributing to stabilization of the trunk orientation in space, as well as those controlling the body configuration. Two animal models of different complexity, the lamprey (a lower vertebrate) and the rabbit (a mammal), were used.

Neuronal mechanisms underlying lateral stability were analyzed in rabbits. The dorsal-side-up trunk orientation in standing quadrupeds is maintained by the postural system driven mainly by somatosensory inputs from the limbs. Postural limb reflexes (PLRs) represent a substantial component of this system. To characterize spinal neurons of the postural networks, in decerebrate rabbit, activity of individual spinal neurons in L4-L6 was recorded during PLRs caused by lateral tilts of the supporting platform. Spinal neurons mediating PLRs have been revealed, and different parameters of their activity were characterized. All neurons were classified into four types according to the combination of tilt-related sensory inputs to a neuron from the ipsi- and contralateral limb (determining the modulation of a neuron). A hypothesis about the role of different types of PLR-related neurons for trunk stabilization in different planes has been proposed.

To reveal contribution of supraspinal influences to modulation of PLR-related neurons, the activity of individual spinal neurons was recorded during stimulation causing PLRs under two conditions: (i) when spinal neurons received supraspinal influences, and (ii) when these influences were temporarily abolished by a cold block of spike propagation in spinal pathways at T12 (“reversible spinalization”). The effects of reversible spinalization on individual neurons were diverse. Neurons, which did not receive supraspinal influences, were located mainly in the dorsal horn, whereas most neurons, receiving excitatory supraspinal influences were located in the intermediate zone and ventral horn. The population of PLR-related neurons presumably responsible for disappearance of muscle tone and PLRs after spinalization was revealed.

The effects of manipulation with the tonic supraspinal drive (by means of binaural galvanic vestibular stimulation, GVS) on the postural system were studied. GVS creates asymmetry in tonic supraspinal drive, resulting in a lateral body sway towards the anode. This new body orientation is actively stabilized. To reveal the underlying mechanisms, spinal neurons were recorded during PLRs with and without GVS. It was found that GVS enhanced PLRs on the cathode side and reduced them on the anode side. It was suggested that GVS changes the set-point of the postural system through the change of the gain in antagonistic PLRs. Two sub-groups of PLR-related neurons presumably mediating the effect of GVS on PLRs were found.

An artificial feedback system was formed in which GVS-caused body sway was used to counteract the lateral body sway resulting from a mechanical perturbation of posture. It was demonstrated that the GVS-based artificial feedback was able to restore the postural function in rabbits with postural deficit. We suggested that such a control system could compensate for the loss of lateral stability of different etiology.

Neuronal mechanisms underlying control of body configuration were analyzed in lampreys. The lamprey is capable of different forms of motor behavior: fast forward swimming (FFS), slow forward swimming (SFS), backward swimming (BS), forward and backward crawling, and lateral turns (LT). The amplitude of the body flexion (characterizing the body configuration) differs in different forms of motor behavior. In the lamprey, signals about the body configuration are provided by intraspinal stretch receptor neurons (SRNs).

To clarify whether the networks generating different forms of motor behavior are located in the spinal cord, in chronic spinal lampreys, electrical stimulation of the spinal cord was performed. It was demonstrated that all forms of motor behavior are generated by the spinal networks.

To study SRN-mediated reflexes and their contribution to the control of body configuration in different motor behaviors, in the *in vitro* preparation we recorded responses of reticulospinal (RS) neurons and motoneurons (MNs) to bending of the spinal cord in different planes and at different rostro-caudal levels during different forms of fictive motor behavior. Bending in the pitch plane during FFS caused SRN-mediated reflexes. MNs on the convex side were activated by pitch bending in the mid-body region. These reflexes will reduce the bend, thus contributing to maintenance of rectilinear body axis in the pitch plane during FFS.

It was found that bending in the yaw plane activated MNs on the convex side during FFS, but on the concave side during different forms of escape behavior (SFS, BS, LT). It was demonstrated that a reversal of reflex responses was due to ipsilateral supraspinal commands causing modifications of the spinal network located in the ipsi-hemicord. A population of RS neurons (residing in the middle rhombencephalic reticular nuclei) presumably transmitting these commands has been revealed. We suggest that modifications of SRN-mediated reflex responses will result in the decrease and increase of the lateral bending amplitude during FFS and escape behaviors, respectively, thus reinforcing movements generated in each specific behavior. Thus in the present study, for the first time, some neuronal mechanisms underlying reflex reversal in vertebrate animals have been revealed.

Key words: postural control, equilibrium, body configuration, reticulospinal neurons, stretch receptor neurons, galvanic vestibular stimulation, artificial feedback, locomotion, spinalization, rabbit, lamprey.

LIST OF PUBLICATIONS

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- I. Zelenin PV, **Hsu L-J**, Lyalka VF, Orlovsky GN, Deliagina TG. (2015) Putative spinal interneurons mediating postural limb reflexes provide basis for postural control in different planes. *Eur J Neurosci* 41:168–181.
- II. Zelenin PV, Lyalka VF, **Hsu L-J**, Orlovsky GN, Deliagina TG (2013) Effects of reversible spinalization on individual spinal neurons. *J Neurosci* 33:18987–18998.
- III. **Hsu L-J**, Zelenin PV, Orlovsky GN, Deliagina TG (2012) Effects of galvanic vestibular stimulation on postural limb reflexes and neurons of spinal postural network. *J Neurophysiol* 108:300–313.
- IV. Zelenin PV, **Hsu L-J**, Orlovsky GN, Deliagina TG (2012) Use of galvanic vestibular feedback to control postural orientation in decerebrate rabbits. *J Neurophysiol* 107:3020–3026.
- V. **Hsu L-J**, Orlovsky GN, Zelenin PV (2014) Different forms of locomotion in the spinal lamprey. *Eur J Neurosci* 39:2037–2049.
- VI. **Hsu L-J**, Zelenin PV, Orlovsky GN, Deliagina TG (2013) Intraspinal stretch receptor neurons mediate different motor responses along the body in lamprey. *J Comp Neurol* 521:3847–3862.
- VII. **Hsu L-J**, Zelenin PV, Orlovsky GN, Deliagina TG. Supraspinal control of spinal reflex responses to body bending during different behaviors in lampreys. *Manuscript*

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Lyalka VF, **Hsu L-J**, Karayannidou A, Zelenin PV, Orlovsky GN, Deliagina TG (2011) Facilitation of postural limb reflexes in spinal rabbits by serotonergic agonist administration, epidural electrical stimulation, and postural training. *J Neurophysiol* 106:1341–1354.

CONTENTS

| | |
|--|-----------|
| INTRODUCTION..... | 1 |
| GENERAL CHARACTERISTICS OF POSTURAL CONTROL | 1 |
| Behavioral goals | 1 |
| Sensory inputs used for postural control..... | 2 |
| Functional organization of postural control system | 3 |
| Animal models..... | 3 |
| <i>Terrestrial quadrupeds</i> | 4 |
| <i>Lampreys</i> | 4 |
| POSTURAL CONTROL IN QUADRUPEDS..... | 5 |
| Maintenance of lateral stability during standing | 5 |
| <i>Postural corrections caused by lateral tilt of the support surface</i> | 6 |
| <i>Functional organization of postural system stabilizing body orientation in the transverse plane</i> | 6 |
| <i>Postural limb reflexes</i> | 7 |
| Postural networks securing lateral stability | 7 |
| <i>Distribution of postural networks in CNS</i> | 7 |
| <i>Contribution of supraspinal systems to control of lateral stability</i> | 8 |
| <i>Effects of elimination of supraspinal influences on postural functions</i> | 9 |
| POSTURAL CONTROL IN A LOWER VERTEBRATE – THE LAMPREY | 10 |
| Stabilization of body orientation..... | 10 |
| Control of body configuration..... | 11 |
| <i>Different forms of motor behavior in lampreys</i> | 11 |
| <i>Locomotor networks</i> | 12 |
| <i>Stretch receptor neurons and their effects on motor output</i> | 13 |
| <i>Modification of reflexes in context of different motor behaviors</i> | 14 |
| AIMS..... | 15 |
| METHODS..... | 17 |
| EXPERIMENTS ON RABBITS | 17 |
| EXPERIMENTS ON LAMPREYS..... | 20 |
| <i>In vivo</i> experiments..... | 20 |
| <i>In vitro</i> experiments..... | 20 |

| | |
|---|-----------|
| RESULTS AND DISCUSSION..... | 23 |
| NEURONAL MECHANISMS FOR STABILIZATION OF TRUNK ORIENTATION IN TRANSVERSE PLANE IN RABBITS | 23 |
| Spinal neurons contributing to generation of postural limb reflexes | 23 |
| <i>Location and activity of PLR-related neurons</i> | <i>23</i> |
| <i>Sensory source of modulation of PLR-related neurons</i> | <i>24</i> |
| <i>Role of supraspinal inputs in modulation of spinal PLR-related neurons</i> | <i>25</i> |
| Effect of galvanic vestibular stimulation on postural networks..... | 27 |
| <i>Effect of GVS on PLRs and PLR-related neurons</i> | <i>27</i> |
| <i>Conceptual model of the trunk stabilization system and effects of GVS.....</i> | <i>28</i> |
| <i>Capacity of GVS-based artificial feedback to restore postural functions</i> | <i>29</i> |
| NEURONAL MECHANISMS FOR CONTROL OF BODY CONFIGURATION DURING DIFFERENT MOTOR BEHAVIORS IN LAMPREYS | 30 |
| Neural mechanisms underlying motor responses to body bending | 30 |
| <i>Sensitivity of stretch receptor neurons to bending in different planes and at different rostro-caudal levels</i> | <i>31</i> |
| <i>Spinal reflexes evoked by natural stimulation of stretch receptor neurons</i> | <i>31</i> |
| Reflex responses to body bending in different forms of motor behavior | 33 |
| <i>Localization of networks generating different forms of motor behavior</i> | <i>33</i> |
| <i>Modifications of SRN-mediated reflexes in different forms of motor behavior.....</i> | <i>34</i> |
| <i>Supraspinal control of SRN-mediated reflexes</i> | <i>34</i> |
| <i>Functional role of SRN-mediated reflexes during different motor behaviors</i> | <i>35</i> |
| CONCLUDING REMARKS AND FUTURE PERSPECTIVES | 37 |
| ACKNOWLEDGEMENTS | 39 |
| REFERENCES..... | 41 |

LIST OF ABBREVIATIONS

| | |
|------------|---|
| AP-5 | (2R)-amino-5-phosphonovaleric acid |
| BC | backward crawling |
| BS | backward swimming |
| CNS | central nervous system |
| CPG | central pattern generator |
| CP-SRN | contralaterally projecting stretch receptor neuron |
| CS | corticospinal |
| dMN | motoneuron innervating the dorsal parts of a myotome |
| EMG | electromyographic |
| FC | forward crawling |
| FFS | fast forward swimming |
| GVS | galvanic vestibular stimulation |
| IP-SRN | ipsilaterally projecting stretch receptor neuron |
| LT | lateral turns |
| ME | microelectrode |
| MN | motoneuron |
| MRRN | middle rhombencephalic reticular nuclei |
| NMDA | N-methyl-D-aspartate |
| PLRs | postural limb reflexes |
| RS | reticulospinal |
| RbS | rubrospinal |
| SCI | spinal cord injury |
| SIF-neuron | strongly inhibited F-neuron |
| SFS | slow forward swimming |
| SRN | stretch receptor neuron |
| vMN | motoneuron innervating the ventral parts of a myotome |
| VS | vestibulospinal |

INTRODUCTION

GENERAL CHARACTERISTICS OF POSTURAL CONTROL

Maintenance of the basic body posture – upright in humans and dorsal-side-up in many other animals (e.g., quadrupeds, lampreys, fishes etc.) – is a vital motor function. Maintenance of this posture is a non-volitional activity based, to a large extent, on the in-born neural mechanisms (Massion, 1998; Vinay et al., 2005). An efficient control of the body posture is equally important for standing and during walking, as well as for providing support of voluntary limb movements (Horak and Macpherson, 1996; Macpherson et al., 1997a). There are two principle modes of operation of the postural control system – the feed forward and feedback ones (Horak and Macpherson, 1996). *The feed forward mode of postural control is a compensation for the destabilizing consequences of voluntary movements or for the expected perturbations of posture caused by external factors. This mode implies a generation of anticipatory postural adjustments. The feedback mode of postural control is a compensation for deviations from the desired posture caused by unexpected external factors. In normal motor behavior these two modes interact. For example, insufficient anticipatory postural adjustments can be compensated by the feedback mechanism (Horak and Macpherson, 1996). The present study was devoted to different aspects of the feedback mode of postural control.*

Behavioral goals

Postural control has two behavioral goals - stabilization of the body orientation in space (equilibrium or balance control) and stabilization of the body configuration (Horak and Macpherson, 1996).

Stabilization of body orientation in space. Different species actively stabilize a definite body orientation in space, that is, in relation to the environmental factors (the gravity vector, position of the support surface, the horizon, etc.). Any deviation from the desired body orientation causes automatic motor response (postural correction), which counteracts the deviation and moves the body back to its initial position.

Stabilization of body configuration. Body configuration is the orientation of body segments in respect to each other. Body configuration can be modified depending on behavioral task (such as standing bipedal or quadrupedal) and depending on the external conditions (such as inclined or narrow support surface under limbs, which require a change of the functional length of the limbs or inter-limb distance). Postural control system is aimed at maintaining the desired body configuration.

These two behavioral goals can be accomplished simultaneously in some postural tasks. For example, when a rabbit is standing on unmovable surface it actively stabilizes the dorsal side-up trunk orientation and a specific configuration of limbs. However, in other postural tasks, the two goals are incompatible. When a rabbit is standing and keeping balance on the sinusoidally tilting platform, to maintain the dorsal side-up trunk orientation it continuously changes the functional length of limbs (limb configuration). In this case, the postural control system sacrifices the goal of stabilization of body configuration in order to stabilize the trunk orientation (Beloozerova et al., 2003).

Sensory inputs used for postural control

The feedback mode of postural control requires sensory information about current body configuration and orientation in space. This information is provided by three major systems.

Somatosensory system in mammals includes afferents from muscle, joint and cutaneous mechanoreceptors. Afferents from load receptors located in the foot sole contribute to providing sensory information about the contact force produced by the limb. It was suggested that these receptors, activated by skin deformation, rapidly respond to postural disturbance and contribute to estimating a direction of the impending body displacement evoked by the disturbance (Ting and Macpherson, 2004). Loss of cutaneous afferents did not affect the latency and EMG pattern of the postural reactions but decreased its amplitude (Honeycutt and Nichols, 2010), suggesting the importance of these afferents for scaling the postural response.

Group Ia and group II afferents from the muscle spindles provide information about biomechanics and perturbation characteristics, which suggests their importance in generating reactions to postural disturbance (Honeycutt et al., 2012). Group Ib afferents from Golgi tendon organs of extensor muscles monitor the load applied to the limb, and together with skin load receptors, provide sensory information about the contact force generated by the limb. It was suggested that Ib afferents of extensor muscles considerably contribute to generation of extensor activity during postural corrections in quadrupeds (Deliagina et al., 2000a) and in humans (Dietz et al., 1992). It was reported that the loss of thick (group I) afferents from limbs resulted in considerable increase of postural response latency resulting in instability, that is difficulty to maintain balance after perturbation (Stapley et al., 2002).

In the lamprey (a lower vertebrate), muscles moving the body do not contain proprioceptors, and the information signaling body configuration is provided by intraspinal stretch receptor neurons (SRNs, also known as edge cells; Grillner et al., 1982; 1984), located at the margins of the spinal cord.

Vestibular system comprises afferents from mechanoreceptors of the otolith organ and the semicircular canals located in the inner ear (labyrinth). They signal about the head orientation in the gravity field and about the head movement, respectively. These specific signals are presented as a modulation of the background activity of vestibular afferents (Fernandez and Goldberg, 1976). In mammals, it has been shown that specific vestibular signals are not essential for the generation of postural corrections when the subject is standing on the solid surface (Beloozerova et al., 2003; Inglis and Macpherson, 1995). However, they are important for the maintenance of the body orientation on the soft surface, i.e., when somatosensory input is not reliable (Black et al., 1988). Unspecific tonic inflow from continuously firing vestibular afferents to the vestibular nuclei is highly important for the normal functioning of postural mechanisms (Deliagina et al., 1997; Wilson and Melvill Jones, 1979). Activated by this tonic inflow, the descending systems (vestibulospinal, reticulospinal, etc.) provide tonic, bilaterally symmetrical influences on different spinal mechanisms. The vestibulospinal drive determines a high tonus in the extensor muscles, which is a necessary condition for supporting the body during standing (Duysens et al., 2000).

In contrast to mammals, specific vestibular signals play a crucial role for the postural control in a lower vertebrate, the lamprey. A specific vestibular information about deviation of the body orientation in a particular plane from the desirable one evokes postural corrections (Deliagina et al., 1992; Deliagina and Fagerstedt, 2000; Deliagina et al., 2014).

Visual system provides information about the head position and the head movement in relation to the environment. In higher vertebrates, the lack of visual input produces a little effect

on the stabilization of trunk orientation during standing on a firm surface (Deliagina et al., 2000a; Beloozerova et al., 2003). However, visual input is important when other two sensory systems, i.e., the vestibular and the somatosensory ones, cannot provide sufficient information about the body orientation and position (Black et al., 1988).

In a lower vertebrate, the lamprey, visual input plays a modulatory role. It affects the stabilized body orientation: asymmetrical illumination of eyes evokes a roll tilt towards the brighter light, and the animal stabilizes this new orientation of the body in space (Ullén et al., 1995a).

Functional organization of postural control system

There are two major concepts about functional organization of the postural system when it operates in the feedback mode:

Centralized control of posture. This widely accepted concept of postural stabilization is based on the notions of the control theory (Levine, 1996). It suggests that a specific body posture is characterized by a ‘regulated variable’ (e.g., the position of the center of mass or the orientation of the longitudinal body axis) and that a specific value of this variable is stabilized. According to this ‘central concept’ of postural control, 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 generalized characteristic of body posture (e.g., the position of the center of mass or the orientation of the body axis). If this variable differs from its desired value, a command is sent to motor centers to elicit a corrective movement (Horak and Macpherson, 1996; Ghez, 1991; Massion, 1994; Massion et al., 1997).

Non-centralized control of posture. This concept was formulated in the classical study by Magnus (1924), who suggested that any particular stabilized posture of the animal results from interactions between numerous reflexes (driven by vestibular, visual and somatosensory inputs), and that the reflexes either supplement or counteract each other. The studies on “simpler” animals – the mollusc *Clione* (Deliagina et al., 1998; 1999), the lamprey (Deliagina and Fagerstedt, 2000; Deliagina and Palova, 2002; Palova and Deliagina, 2002) and the fish (von Holst, 1935) support the ‘reflex’ concept of postural control.

It seems possible that postural control in higher vertebrates is based on both principles depending on environmental conditions. It was suggested that the centralized control is used in a “simple” environments (e.g., when orientation of the head and trunk is disturbed in a particular plane) (Ghez, 1991; Lacquaniti et al., 1984; Massion, 1998). In such environments the postural system operates as a unit (e.g., postural corrections of the head, as well as in anterior and posterior part of the trunk are generated simultaneously). The non-centralized control is used in more complex environments (e.g., when the dorsal side-up orientation of only posterior part of the trunk is disturbed in the transverse plane).

Animal models

The majority of studies devoted to postural control were performed on humans. These studies were focused on the characterization of reactions (kinematics, forces and EMGs) caused by different postural perturbations in intact subjects and in patients with damages to different parts of CNS or to different sensory systems. These studies have revealed a number of important aspects of the functional organization of postural control system. They tell, however, much less about organization and operation of corresponding neuronal mechanisms.

Due to a wide spectrum of experimental methods, which can be used, animal models of different complexity present much more opportunities for analytical studies of neuronal networks underlying the control of posture. Control of posture is an evolutionary old motor function. When the comparative approach is used, the hope is that such basic problem as control of posture has similar solutions in the nervous systems of different species, and thus the results obtained in animals may have significance for understanding the mechanisms of postural control in humans.

In the present study, two animal models of different complexity were used: a higher vertebrate animal (the rabbit, a terrestrial quadruped) and a lower vertebrate animal (the lamprey).

Terrestrial quadrupeds

Despite differences in stance configuration and biomechanics, there are a number of similarities between terrestrial quadrupeds and humans:

1. Their body consists of a number of segments, which can be stabilized in space independently of each other. For example, quadrupeds and humans can stabilize the body orientation in space and, at the same time, move the head, or maintain different head positions.
2. The same types of sensory information (somatosensory, visual, and vestibular) are used for control of posture. Moreover, both human and terrestrial quadrupeds rely mainly on somatosensory information from limbs (skin pressure receptors, muscle spindles and Golgi tendon organs) for stabilization of the trunk orientation when standing on solid surface.
3. Two modes of operation of the postural control system (feed-forward and feedback) are observed in both humans and quadrupeds.
4. The CNS of humans and quadrupeds contains similar structures and pathways.

Due to “even temper”, the rabbit is capable (without training) of keeping the basic body configuration for a long time, and responds to postural perturbations without any additional movements. This capability makes the rabbit a unique animal model for behavioral studies devoted to functional organization of postural control system. While the functional organization of the postural control system responsible for stabilization of the body orientation in transverse plane was analyzed in considerable detail (Beloozerova et al., 2003), the knowledge about the underlying neuronal mechanisms is scarce. *In the present study, the rabbit was used for investigation of the neuronal mechanisms responsible for lateral stability during standing.*

Lampreys

The lamprey (cyclostome) represents the most primitive group of presently living vertebrates that diverged from the main vertebrate line around 450 million years ago (Forey and Janvier, 1993). The lamprey has many of the principle parts of the vertebrate CNS (the spinal cord, rhombencephalon, mesencephalon, diencephalon and telencephalon), but much fewer neurons than higher vertebrates (Kappers, 1936; Rovainen, 1979; Niewenhuys et al., 1998). It has a number of sensory systems similar to those in higher vertebrates (lateral eyes, vestibular organs, olfactory organs), as well as a number of sensory systems (lateral line system, dermal light sense, intraspinal SRNs) observed only in lower vertebrates.

The lamprey is an aquatic animal, whose motor behavior includes different forms of locomotion based on propagation of lateral undulations along the body. During fast forward swimming, the lamprey actively maintains a definite orientation of the body in space, which is stabilized as one segment. The lamprey presents good opportunities for analytical studies of the neuronal networks controlling different forms of motor behavior. This is because, *first*, the lamprey has orders of magnitude fewer nerve cells of each type than higher vertebrates. *Second*, an *in vitro* preparation of the brainstem and spinal cord has been developed, which can remain active for relatively long time (up to several days). *Third*, the motor patterns underlying different types of motor behaviors (different forms of locomotion, turning, postural reflexes, etc.) can be elicited in this isolated nervous system. These features of the lamprey allow performing analysis of motor functions not only at the behavioral, but also at the network and cellular levels. The lamprey has been used extensively as an experimental animal model for studying the organization and operation of the neuronal networks underlying generation of the basic locomotor pattern (Grillner et al., 1995; Grillner, 1997; 2003), stabilization of the body orientation in space (Deliagina and Fagerstedt, 2000; Pavlova and Deliagina, 2002; Karayannidou et al., 2007), as well as neuronal mechanisms underlying supraspinal control of different motor behaviors (Zelenin et al., 2007; Zelenin, 2005; 2011; Stephenson-Jones et al., 2013; Grillner et al., 2013; Kardamakis et al., 2015). *In the present study, the lamprey was used to investigate the neuronal mechanisms contributing to the control of the body configuration during different motor behaviors.*

POSTURAL CONTROL IN QUADRUPEDS

Maintenance of lateral stability during standing

During standing, terrestrial quadrupeds (cat, rabbit, etc.) maintain the dorsal side-up orientation of their trunk due to the activity of the postural system. This system stabilizes the body orientation in transverse plane, and thus secures lateral stability of the animal. This task is the most demanding postural task for terrestrial quadrupeds since the transverse dimension of the base of their support is narrower than its longitudinal dimension. Any deviation from the desirable body orientation elicits a corrective motor response. The pattern of this response

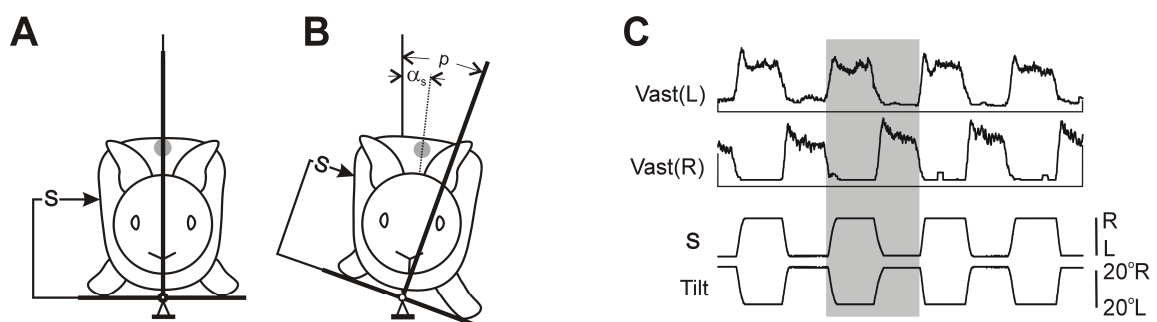


Figure 1. Maintenance of body orientation in the transverse plane in the standing rabbit. **A,B.** Experimental design for testing postural responses to lateral tilts of the support surface. **C.** Motor and EMG responses to trapezoidal tilts. Vast (L) and Vast(R) are left and right m. vastus lateralis, respectively. See text for explanations.

depends on the types of postural perturbation. *In the present study, the lateral tilt of the supporting surface was employed for postural perturbation.*

Postural corrections caused by lateral tilt of the support surface

Cats and rabbits can easily maintain their dorsal side-up body orientation when standing on a surface periodically tilted in the frontal plane (Beloozerova et al., 2003; Deliagina et al., 2006). The lateral tilt of the supporting platform (p in Fig. 1B) evokes deviation of the dorso-ventral axis of the trunk from the vertical and a displacement of the center of mass towards the lateral border of the base of support, which results in loading of the limbs on the side moving down and unloading of the opposite limbs. Tilt-related sensory signals evoke extension of the limbs on the side tilted down and flexion of the limbs on the opposite side (Fig. 1B). These limb movements are caused by activation of extensor muscles in the limbs ipsilateral to the side of the platform moving down and reduction of the extensor activity in the opposite limbs (Fig. 1C, Beloozerova et al., 2003; Deliagina et al., 2006). These compensatory limb movements cause displacement of the trunk in relation to the platform in direction opposite to the direction of the tilt (the trunk corrective movement measured by mechanical sensor S in Fig. 1) and move the dorso-ventral trunk axis (α_s in Fig. 1B) toward the vertical. Simultaneously one can observe movement of the dorso-ventral axis of the head toward the vertical. However, the postural corrections do not compensate fully for the platform tilt and after their execution the dorso-ventral axis of the head and trunk remain slightly deviated from the vertical (Deliagina et al., 2000a).

Functional organization of postural system stabilizing body orientation in the transverse plane

The postural control system usually operates as a functional unit and stabilizes both head and trunk orientation. Under certain conditions, however, the system clearly dissociates into the subsystems controlling independently the head and the trunk (Barberini and Macpherson, 1998; Beloozerova et al., 2005, Berthoz and Pozzo, 1988, Boyle, 2001; Deliagina et al., 2000a). These subsystems are driven by sensory signals of different modalities: the head orientation is stabilized mainly on the basis of vestibular and visual information; for trunk stabilization, somatosensory inputs from limbs are most important (Beloozerova et al., 2003; Deliagina et al., 2000a).

Further analysis of the system for trunk stabilization demonstrated that under certain environmental conditions it dissociates into two relatively independent sub-systems responsible for stabilization of the anterior and posterior parts of the trunk, respectively (Fig. 2). They are driven by somatosensory inputs from the corresponding limbs (Beloozerova et al., 2003; Deliagina et al., 2006). Coordination between these sub-systems is primarily based on influences of the anterior sub-system on the posterior one (the green line in Fig. 2; Deliagina et al., 2006). Each sub-system contains two reflex mechanisms – limb controllers for the right and left limbs, generating a part of the corrective limb movement in response to sensory input from the same limb (the red line in Fig. 2); another part is formed on the basis of sensory influences from the contralateral limb (the blue line in Fig. 2; Deliagina et al., 2006). It was suggested that a control system consisting of semi-autonomous sub-systems better adapts to complicated environmental conditions (Orlovsky et al., 1999).

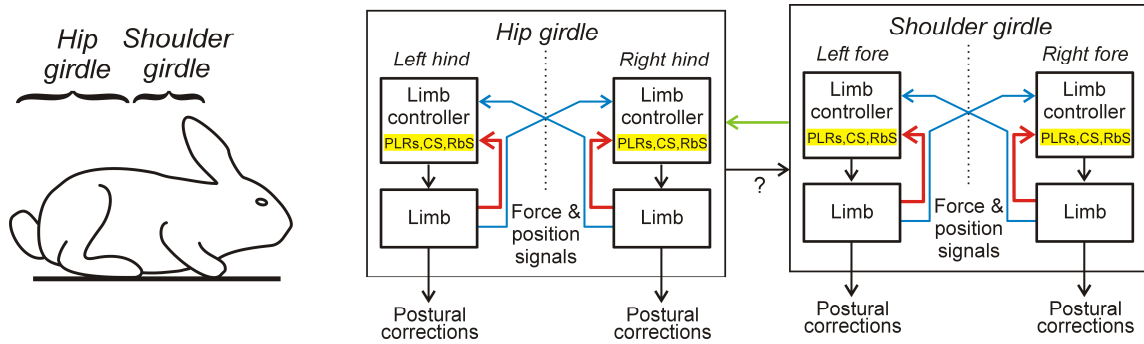


Figure 2. Functional model of the postural system stabilizing the trunk orientation in the transverse plane. Lateral stability of the anterior and posterior parts of the body (shoulder and hip girdles) is maintained by two relatively independent sub-systems. Postural limb reflexes (PLRs), corticospinal (CS), and rubrospinal (RbS) neurons are parts of this mechanism. See text for explanations.

Postural limb reflexes

An essential part of the corrective movements of the limb caused by the tilts of the supporting platform is postural limb reflexes (PLRs, Musienko et al., 2010; Deliagina et al., 2014). These reflexes were studied in decerebrate rabbits, whose CNS contains basic postural networks (see below). PLRs are driven by tilt-related sensory inputs from stretch and load receptors of the limbs, and the pattern of their EMG activity is similar to that observed in intact animals balancing on the tilting platform. It was demonstrated that PLRs are generated in response to tilt-related sensory information from the same limb suggesting that they are part of the limb controller generating a substantial part of a corrective movement of the limb on the basis of sensory information from the same limb (Fig. 2).

Postural networks securing lateral stability

Distribution of postural networks in CNS

It has been known for almost a century that terrestrial quadrupeds decerebrated at precollicular-premamillary level can maintain equilibrium during standing, and during locomotion (Magnus, 1924; Bard and Macht, 1958). Later it was demonstrated that decerebrate rabbit produces postural corrections in response to push and to lateral tilts of the supporting platform (Musienko et al., 2008). The EMG pattern of these corrections was similar to that observed in intact animals, though the magnitude of corrections was reduced as compared to intact animals (Musienko et al., 2008; Honeycutt et al., 2009; Honeycutt and Nichols, 2010). These findings indicate that basic postural networks reside in the brainstem, cerebellum, and spinal cord, and thus decerebrate preparation could be used for their analysis. However, the value of postural corrections in decerebrate animals is reduced, suggesting that input from the forebrain increases excitability of the basic postural networks (Musienko et al., 2008).

Both spinal and supraspinal mechanisms participate in the control of body posture. Presumed interactions between the spinal and supraspinal levels of the postural system stabilizing trunk orientation in the transverse plane are shown in Fig. 3. For each of the girdles (shoulder and hip), there are two closed-loop nervous mechanisms (shown for the hindlimbs in Fig. 3). One of the mechanisms (Short loop in Fig. 3) resides in the spinal cord. It is driven by input from limb mechanoreceptors and contributes to generation of postural corrections in

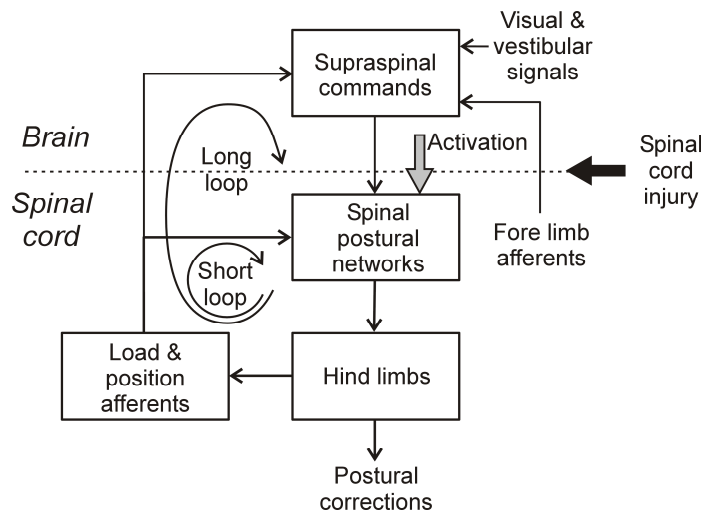


Figure 3. Main components of the postural system in quadrupeds. Two closed-loop control systems (the short and the long loop) participate in the postural control.

response to postural disturbances. The existence of this mechanism was demonstrated in experiments, in which EMG pattern of PLRs was evoked by tonic stimulation of the spinal cord in the spinal rabbit (Musienko et al., 2010). This result also suggests that, in intact animals, the spinal postural networks are activated by tonic supraspinal drive (Activation in Fig. 3). It was shown that one of the sources of this drive is the ventral tegmental field (Musienko et al., 2008).

The other mechanism (Long loop in Fig. 3) includes the brainstem, cerebellum, and motor cortex. This mechanism is also driven by input from limb mechanoreceptors, but in addition it receives vestibular and visual information. It generates supraspinal commands for postural corrections, supplementing those generated by the short-loop spinal mechanism. These commands are sent from the forebrain and brainstem motor centers to the spinal cord via different descending pathways (reticulospinal, vestibulospinal, rubrospinal, corticospinal).

One of the aims of the present study was to characterize spinal elements of the postural networks, that is to characterize spinal interneurons contributing to generation of PLRs.

Contribution of supraspinal systems to control of lateral stability

Two descending systems (corticospinal, CS, and rubrospinal, RbS) were investigated in intact cats during postural corrections caused by tilts (Karayannidou et al., 2008; Zelenin et al., 2010). Activity of RbS and CS neurons correlated with postural reactions, suggesting their contribution to generation of these reactions. In the majority of these neurons, modulation of their activity was primarily determined by the tilt-related somatosensory input from the projection (contralateral) limb. One can suggest that, CS and RbS neurons belong to the limb controller (Fig. 2), and thus they are primarily involved in the intra-limb postural coordination.

Activity of the reticulospinal (RS) neurons was studied in the cat during postural reactions to drop of support (Stapley and Drew, 2009). It was suggested that they participate in initiation of corrective reactions, since the majority of them responded to perturbation with a short latency and discharged before the initial change in EMGs. Only about 10% of neurons responded to drop of only one of the limbs, suggesting that they encode a command contributing to initiation of only one specific postural reaction. The majority of individual RS neurons responded to drop of any or two or three limbs, thus contributing to generation of

different specific postural correction patterns. One can suggest that the motor response to postural command transmitted by these neurons depends on the current state of spinal networks affected by specific supraspinal and somatosensory inputs. Another possibility is that muscle groups activated by these RS neurons contribute to different specific postural correction patterns. Such mechanism was found in a lower vertebrate, the lamprey (Zelenin et al., 2007).

Vestibulospinal (VS) neurons, along with RS neurons, transmit the tonic supraspinal drive to the spinal cord, which is caused by the unspecific inflow from the continuously firing vestibular afferents. As a result, these descending systems provide tonic, bilaterally symmetrical influences on different spinal mechanisms, including postural ones. The effects of manipulation with the tonic supraspinal drive (by means of binaural galvanic vestibular stimulation, GVS) on the postural system were studied. GVS excites and inhibits vestibular afferents on the side of the negative (cathode) and positive (anode) electrode, respectively (Goldberg et al., 1984; Minor and Goldberg, 1991). Thus the left/right asymmetry in tonic supraspinal drive is created, which results in a lateral body sway toward the anode observed in all studied species including humans (e.g., Séverac Cauquil et al., 2000; Beloozerova et al., 2003; Gorgiladze, 2004). In the standing rabbit, the GVS-caused new body orientation is actively stabilized (Beloozerova et al., 2003). It was suggested that GVS could change a set point in the antagonistic reflex mechanisms controlling the body posture. *One of the aims of the present study was to test this hypothesis and to reveal the underlying neuronal mechanisms by examining the GVS effect on PLRs and on the spinal interneurons contributing to their generation.*

Different diseases and traumatic injuries of CNS result in impairment of the system controlling body posture and balance (see e.g., Maynard et al., 1990; Sommerfeld et al., 2004). In a number of these cases, the spinal pathways transmitting supraspinal commands for postural corrections remain mostly undamaged (Tator et al., 1993), and a possible reason for postural deficits is an insufficient value and/or incorrect timing of these commands addressed to the spinal cord. These commands could possibly be improved by means of an artificial feedback based on GVS affecting the brainstem-spinal descending systems. *One of the aims of the present study was to test the efficacy of an artificial feedback based on GVS to improve the lateral stability in subjects with impaired postural functions.*

Effects of elimination of supraspinal influences on postural functions

Spinalization, which deprives spinal networks of all supraspinal influences, causes a severe impairment of the postural system. Spinal animals are not able to maintain normal (dorsal-side-up) trunk orientation and their postural control (including PLRs) does not recover with time (Macpherson et al., 1997b; Lyalka et al., 2008, 2009, 2011).

In all studied mammals, including humans, the immediate reaction to an extensive spinal cord injury (SCI) is “spinal shock,” characterized by a dramatic reduction of extensor tone and most spinal reflexes, including PLRs (Ditunno et al., 2004; Lyalka et al., 2011). It was suggested that abolition of spinal reflexes after SCI is primarily caused by a large reduction in the excitability of spinal motoneurons (MNs) (Barnes et al., 1962; Walmsley and Tracey, 1983; Frigon and Rossignol, 2006). Changes in transmission of afferent signals to MNs were also demonstrated (Bennet et al., 2004). However, there have been very few studies devoted to the effect of SCI on the activity of individual interneurons mediating different spinal reflexes, and these studies were focused on the effect of partial damage of the spinal cord upon spinal interneurons responding to load receptors (Miller et al., 1995; Chen et al., 2001). *One of the*

aims of the present study was to determine immediate effect of spinalization on activity of individual spinal interneurons, in particular those contributing to generation of PLRs.

POSTURAL CONTROL IN A LOWER VERTEBRATE – THE LAMPREY

The lamprey has two main behavioral states, quiescent and active (Rovainen, 1979). In the quiescent state, the animal attaches to a substratum by its sucker mouth, while its trunk muscles are almost completely relaxed. In the active state, it detaches from the substratum and exhibits different forms of locomotion.

Stabilization of body orientation

The lamprey actively stabilizes the basic body orientation in space (horizontal, dorsal side-up) only during one form of locomotion, i.e., fast forward swimming (FFS), which enables it to migrate for a long distance (Islam et al., 2006; Islam and Zelenin, 2008). Deviation in any plane from this orientation evokes a specific postural correction, which moves the body to its original orientation. The deviation causes activation of a specific population of vestibular afferents (Deliagina et al., 1992), which in turn activate a specific population of reticulospinal (RS) neurons. They transform sensory inputs into the motor effects that counteract postural perturbation. A strong correlation was found between (i) vestibular inputs to an individual RS neuron, and (ii) the effect of motor output exerted by the same RS neuron (Zelenin et al., 2007). As a rule, if a neuron is activated by a tilt in a given plane, its motor effect resulted in a torque counteracting the tilt.

On the basis of experimental data (Deliagina and Fagerstedt, 2000; Pavlova and Deliagina, 2002; Karayannidou et al., 2007), functional models of postural control systems responsible for stabilization of body orientation in the roll, pitch and yaw planes were formulated (Deliagina et al., 2014). The system for body stabilization in each particular plane includes two chains of antagonistic vestibular reflexes mediated by two groups of RS neurons, which cause movement of the animal in the opposite directions (Fig. 4A,B). The system has an equilibrium point: it stabilizes the body orientation with equal activities of the two antagonistic groups of RS neurons (Fig. 4B). However, some environmental factors can shift the equilibrium point by affecting differently the gain of the two reflex chains. For example, normally the roll system stabilized at dorsal-side-up body orientation (Fig. 4B). However, illumination at the left eye increases the activity of ipsilateral RS neurons (Fig. 4C) and shifts upward its activity curve. As a result, the equilibrium point is shifted to the left, and the system will stabilize the new body orientation with some left roll tilt (Deliagina et al., 1993; Ullén et al., 1996; Deliagina and Fagerstedt 2000).

Operation of the postural system for stabilizing body orientation in the lamprey is very similar to that in the mollusk *Clione*, in which the vertical head-up body orientation is also stabilized by two antagonistic chains of reflexes (Deliagina et al., 1998). The stabilized body orientation of *Clione* can also be affected by changing the gain in one of the reflex chains. Thus

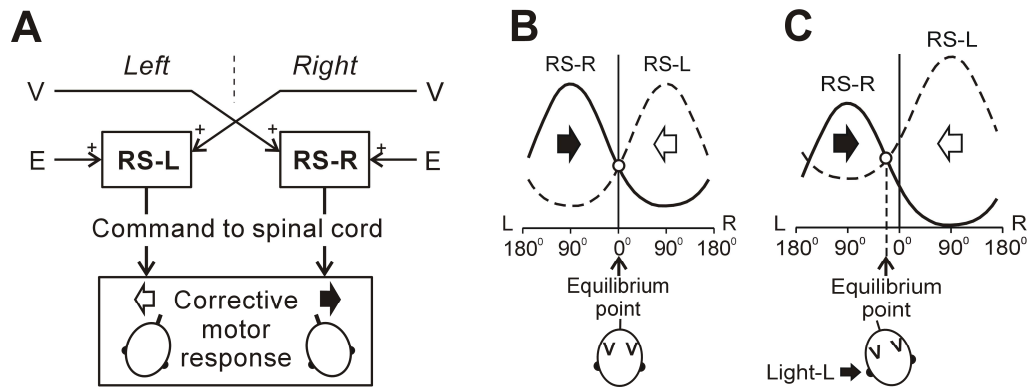


Figure 4. Conceptual model of postural system controlling orientation of the lamprey in the roll plane. **A.** Two groups of RS neurons (RS-L and RS-R) receive inputs from the labyrinths (V) and eyes (E); they affect the spinal networks to evoke rolling of the lamprey. **B.** Operation of the system when driven only by vestibular inputs. The abscissa shows a deviation of the dorso-ventral axis from the vertical (L - left tilt, R - right tilt); the ordinate shows the activity of RS-R and RS-L. Vestibular input causes activation of RS-R and RS-L with the contralateral tilt. Direction of rolling caused by RS-R and RS-L are indicated by the black and white arrows, respectively. The 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 left and the corresponding tilt of the animal.

principles of operation of postural networks underlying control of the antigravity behavior are similar in evolutionary remote species.

Control of body configuration

Different forms of motor behavior observed in the lamprey are achieved due to propagation of a single or multiple waves of lateral flexion along the body. The amplitude of the body flexion (the body configuration) is different in different forms of motor behavior. In the spinal cord of lampreys, the stretch receptor neurons (SRNs, also known as edge cells) were found (Grillner et al., 1984). These neurons are activated by lateral body bending (Grillner et al., 1982). Potentially, signals from SRNs could contribute to the control of body configuration during different forms of motor behavior, as well as to evoke the corrective movements in response to disturbance of body configuration caused by external factors. *One of the aims of the present study was to investigate the neuronal mechanisms underlying control of body configuration during different motor behaviors in lampreys.*

Different forms of motor behavior in lampreys

Lampreys are capable of two different modes of locomotion, swimming and crawling. The fast forward swimming (FFS), the main form of lamprey locomotion, is used for long-distance migrations. The lamprey swims forward due to the body undulations in the yaw plane that propagate from the head to the tail (Grillner and Kashin, 1976). During FFS, the animal usually keeps a linear trajectory in the pitch plane (Ullén et al., 1995b). Slow forward swimming (SFS) and backward swimming (BS) are also undulatory forms of locomotion, with slow head-to-tail and tail-to-head wave propagation, respectively (Islam et al., 2006; Islam and Zelenin, 2008). Crawling is a form of non-undulatory locomotion, and is used by the lamprey for moving about or out of tight places, where undulatory movements are not possible (Rovainen, 1976). Crawling is produced by a solitary wave of co-contraction of the left and

right muscles close to the bent site (Archambault et al., 2001). The lamprey can crawl forward or backward.

To escape from threats or to avoid obstacles, during FFS the lamprey turns in the yaw or in the pitch plane and thus changes the direction of swimming. During turning, the locomotor EMG burst and the cycle are longer than those during ordinary swimming, which results in a large bending of the body (Fagerstedt and Ullén, 2001). BS and SFS, in which the amplitude of body undulation is larger than that in FFS, are used when the lamprey tries to get rid of the continuous tactile stimulation of the rostral (including the head) or middle part of the body, respectively (Islam et al., 2006; Islam and Zelenin, 2008).

Locomotor networks

The network for generating FFS was studied in considerable detail (Grillner, 2003). The general organization of the locomotor network for FFS in lampreys (Fig. 5) has many features in common with that for forward locomotion in mammals (Orlovsky et al., 1999). In both lampreys and mammals, the basic locomotor pattern is generated by the spinal network, which is known as the central pattern generator (CPG). Spinal locomotor CPG can operate in the absence of sensory feedback. It is activated by commands transmitted from locomotor regions (e.g., the mesencephalic locomotor region, MLR) via RS system (for mammals: Shik et al., 1966; Garcia-Rill and Skinner, 1987a,b; Steeves and Jordan, 1980; for lampreys: Sirota et al., 2000; Brocard and Dubuc, 2003).

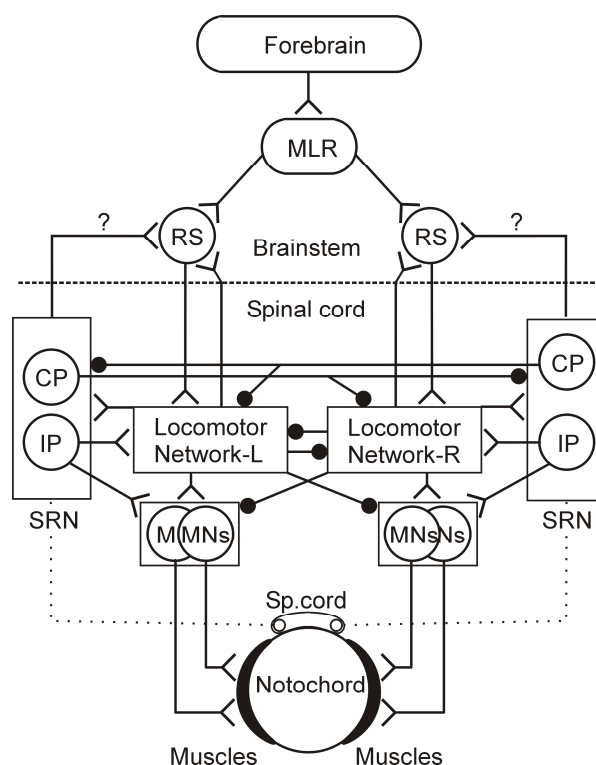


Figure 5. General organization of the system controlling FFS in the lamprey. For simplicity, only one of the bilateral MLRs was illustrated in the scheme. The connection between SRNs and RS neurons was not known. IP and CP are ipsi- and contra-laterally projecting SRNs, respectively.

It was demonstrated that the network for generating forward crawling is also located in the spinal cord, since this mode of locomotion can be evoked in the spinal lamprey (Rovainen, 1976). *One of the aims of the present study was to investigate whether the spinal cord can produce all other forms of locomotion (i.e., SFS, BS, backward crawling).*

During locomotion in mammals, supraspinal centers are affected by two types of signals from the spinal cord – those coming from CPG (the “efference copy”), and those coming from limb mechanoreceptors (afferent feedback) (Orlovsky et al., 1999). In lampreys, the input from locomotor CPG to RS neurons has been demonstrated (Fig. 5; Kasicki et al., 1989). *One of the aims of the present study was to clarify if RS neurons in the lamprey receive sensory feedback from the spinal SRNs.*

Stretch receptor neurons and their effects on motor output

In the lamprey, there are no muscle spindles in the body muscles (Fessard and Sand, 1937), and the proprioceptive information is provided by intraspinal stretch receptor neurons (SRNs). SRNs (also known as edge cells) are located in the margin of the spinal cord, and are activated by the longitudinal stretch of this area (Grillner et al., 1984). Since the spinal cord is situated on the notochord, bending of the body, which causes bending of the notochord, affects the activity of SRNs.

Bending in the yaw plane activates SRNs on the stretched (convex) side, and inactivates those on the compressed (concave) side (Grillner et al., 1982). Two types of SRNs have been identified - those with ipsilateral projections (IP-SRNs) and those with contralateral projections (CP-SRNs). The IP-SRNs excite ipsilateral motoneurons (MNs) and CPG interneurons, while CP-SRNs inhibit contralateral MNs, interneurons, and SRNs (Viana Di Prisco et al., 1990; Fig. 5). Thus, body bending in the yaw plane performed at rest causes SRNs mediated intraspinal reflexes: activation and inactivation of MNs on convex and concave side, respectively (Viana Di Prisco et al., 1990).

Due to connections with the neurons of the CPG, SRNs can affect the locomotor rhythm (Grillner et al., 1981; Viana Di Prisco et al., 1990). It has been shown that rhythmical bending of the isolated spinal cord/notochord preparation in the yaw plane can entrain the rhythm of “fictive swimming” (Grillner et al., 1981; McClellan and Jang, 1993). The entrainment phenomenon was also observed in legged animals, such as cats (Andersson et al., 1978; Andersson and Grillner, 1983; Kriellaars et al., 1994) and stick insects (Akay et al., 2007; Borgmann et al., 2009). In cats, rhythmic limb movements can entrain the activity of the CPGs, even when the sensory input is limited mainly to the hip joint. In stick insects, sensory feedback induced by front-leg stepping entrains the activity of CPGs in caudal segments of the walking system (Borgmann et al., 2009). In lampreys, it was shown that bending of the rostral and caudal segments produced different effects on the locomotor rhythm (Tytell and Cohen, 2008), suggesting that processing of SRNs signals is different at different rostro-caudal levels of the spinal cord, and thus spinal reflexes mediated by SRNs might be different along the extent of the spinal cord. *One of the aims of the present study was to test this hypothesis by analyzing the responses of SRNs and MNs to bending at different sites along the lamprey body.*

From simple anatomical considerations it is evident that stretching the marginal area of the spinal cord (necessary for SRNs activation) can occur not only with the notochord bending in the yaw plane but also with bending in the pitch plane. One can therefore expect that SRNs will be activated with notochord bending in the pitch plane as well, and they may elicit reflex motor responses to this bending. These reflexes may potentially contribute to the maintenance

of rectilinear projection of body axis in the pitch plane observed during FFS in intact lampreys, as well as to upward and downward maneuvers caused by body flexion in this plane (Ullén et al., 1995b). However, these reflexes were not investigated previously. *One of the aims of the present study was to analyze responses of SRNs and MNs to body bending in the pitch plane.*

Modification of reflexes in context of different motor behaviors

In different species, both vertebrate and invertebrate, sensory input from the mechanoreceptors of locomotor organs (limbs in bipeds and quadrupeds, trunk in fish, etc.) is important for generation of various types of movements and for their adaptation to environmental conditions (Forsberg et al., 1977; Orlovsky et al., 1999; Pearson, 2008; Gervasio et al., 2013). At rest, these signals can evoke a number of spinal reflexes, which can be substantially modified (up to a complete reversal) with the initiation of a particular motor behavior (Pearson and Collins, 1993; Büschges and El Manira, 1998; Hellekes et al., 2012). For example, in cats, inputs from Ib afferents from extensor muscles inhibit the homonymous MNs at rest, but excite the same MNs during forward walking (Pearson and Collins, 1993). In arthropods, similar resistant reflex responses evoked by inputs from load receptors of the leg at rest were modified to enhance the motor output of network for forward walking (Büschges and El Manira, 1998). Such reflex reversal was not observed during backward walking in stick insects (Akay et al., 2007; Hellekes et al., 2012), suggesting that the reflex modification is task-specific.

Different motor behaviors in vertebrates are selected by supraspinal centers (Armstrong, 1986; Deliagina et al., 2000b; Orlovsky et al., 1999; Zelenin, 2005; 2011). Do these centers also participate in the modification of spinal reflexes observed in different motor behaviors? *One of the aims of the present study was to clarify if the spinal reflex responses mediated by SRNs in lampreys are modified to fit different motor behaviors, and to reveal RS neurons that modify these responses.*

AIMS

1. To characterize spinal neurons of postural networks in rabbits. (*Paper I*)
2. To characterize the immediate effect of elimination of supraspinal drive on individual spinal neurons of postural networks. (*Paper II*)
3. To characterize the effect of GVS-caused changes of tonic supraspinal drive on spinal neurons of postural networks, and to test the efficacy of the GVS-based artificial feedback for restoration of the postural control in rabbits with postural deficit. (*Papers III, IV*)
4. To determine location of networks generating different forms of locomotion in lampreys. (*Paper V*)
5. To study neuronal mechanisms controlling the body configuration during different motor behaviors in lampreys (*Papers VI, VII*)

METHODS

Four major types of experiments in this study were performed on two animal models – the rabbit and the lamprey.

1. The experiments with *in vivo* recording of spinal neurons were carried out on decerebrate rabbits (*Papers I-III*).
2. The study of the efficacy of GVS-based artificial feedback for restoration of postural functions was carried out on decerebrate rabbits (*Paper IV*).
3. The *in vivo* study devoted to localization of networks generating different forms of locomotion was carried out on spinal lampreys (*Paper V*).
4. The *in vitro* studies of reflex responses to body bending were carried out on lampreys (*Papers VI, VII*).

All experiments were approved by the local ethical committee (Norra Djurförsöksetiska Nämnden) in Stockholm.

This section contains a brief description of the main methodological approaches. Details of experimental procedures are given in the corresponding papers.

EXPERIMENTS ON RABBITS

In these experiments, we studied (i) the general characteristics of spinal neurons contributing to the generation of the postural limb reflexes (PLRs), (ii) the effects of elimination of supraspinal influences on these neurons, (iii) the effects of galvanic vestibular stimulation (GVS) on PLRs, as well as on PLRs-related neurons, and (iv) the efficacy of GVS-based artificial feedback for restoration of postural functions. All experiments were performed on adult New Zealand rabbits.

Surgical procedures. Surgery was performed under isoflurane anesthesia. The trachea was cannulated and the animal was decerebrated at the precollicular-postmammillary level. In experiments with recording of spinal neurons, the spinal cord was exposed by laminectomy at L4-L6 segments for insertion of the microelectrode. In experiments with reversible spinalization (that is a temporal elimination of supraspinal influences by means of cold block), laminectomy was also performed at T11-L1 for mounting the cooling element. Bipolar EMG electrodes were inserted bilaterally into the selected limb muscles. Recordings were started not earlier than in 1 h after cessation of anesthesia.

Experimental design. The main experimental design is shown in Fig. 6A. The head of the decerebrate rabbit was rigidly fixed in a metal frame, and the forequarters were suspended in a hammock. In experiments with recording of spinal neurons, the vertebrate column and pelvis were also fixed. The hindlimbs of the rabbit were positioned on the horizontal platform (Fig. 6B), with limb configuration and the inter-feet distance similar to that observed in freely standing rabbit. The platform as a whole, or its right or left parts separately, could be tilted periodically (Fig. 6C-E). Because the vertebrate column and pelvis were fixed, tilts of the platform led to flexion/extension movements at the hindlimbs. The tilt trajectory was

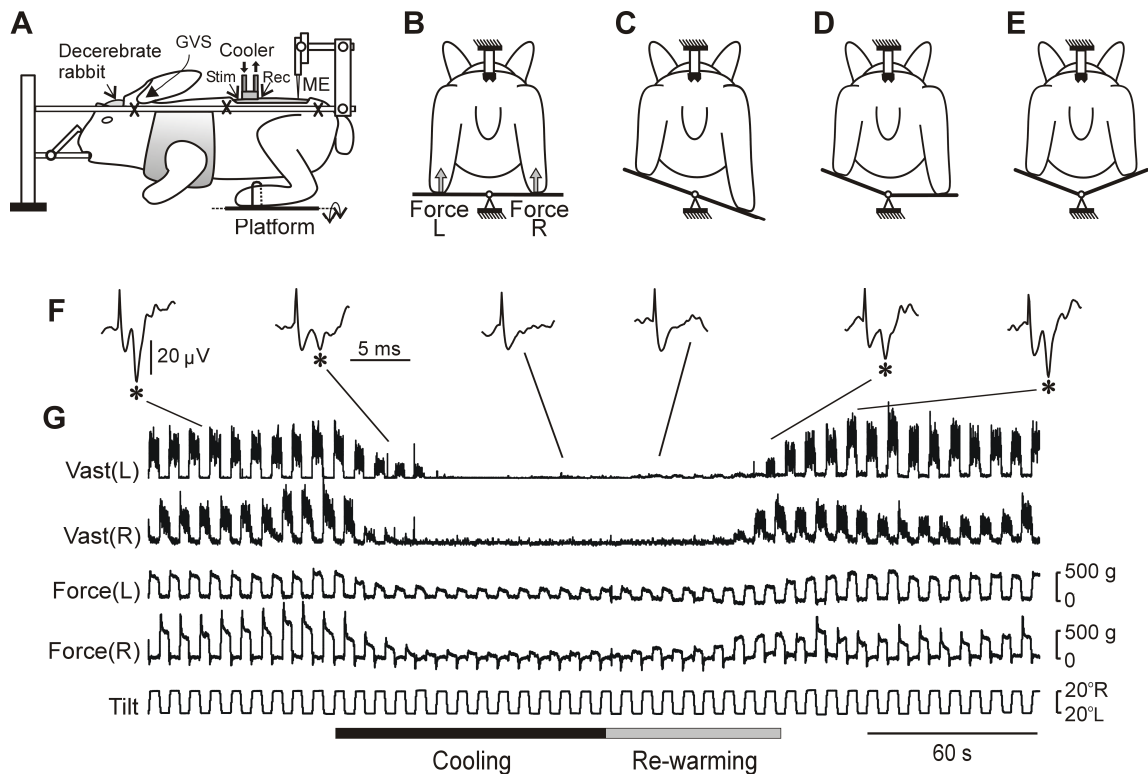


Figure 6. A-E. Experimental designs for the experiments with recording of spinal neurons. See text for explanation. F,G. Effect of reversible spinalization on the signal transmission in ventral spinal pathways (F) and on PLRs (G). To test the efficacy of signal transmission in spinal pathways, stimulating (Stim in A) and recording (Rec in A) electrodes were inserted into the ventral funiculi rostral and caudal to the cooler (in segments T11 and L1). F. The response at L1 (marked by asterisk) to stimulation of T11 performed at different time points (shown in G). Note that disappearance of responses during cooling correlated with disappearance of the force and EMG responses to tilts, while re-appearance of responses during re-warming correlated with restoration of these responses. Abbreviations: Vast(L), Vast(R) and Force(L), Force(R), activity of the left and right m. vastus lateralis, and contact force under the left and right hindlimb, respectively.

trapezoidal. It was recorded by mechanical sensors (Fig. 6G, Tilt). The contact forces under the limbs were measured by means of force sensors (Fig. 6, Force).

Postural limb reflexes (PLRs). With the experimental design shown in Fig. 6A, the tilt-related somatosensory stimulation was caused by loading and flexion of the limb on the platform side moving up and simultaneous unloading and extension of the opposite limb. This stimulation evoked PLRs, which included activation of extensors in the flexing limb and increase of its contact force, as well as inactivation of extensors in the extending limb and decrease of its contact force (see Fig. 6G before cooling). A separate tilt of the left (Fig. 6D) or right platform evoked PLRs mainly in the ipsilateral limb (Musienko et al., 2010).

Reversible spinalization. For blocking the spike propagation in spinal pathways by means of cooling (reversible spinalization), the cooler was positioned on the dorsal aspect of T12 (Fig. 6A). By pumping a cooling agent through the cooler, we decreased the temperature of adjacent tissues to below the threshold for spike propagation, which led to abolition of signal transmission in the spinal pathways under the cooler (Fig. 6F) resulting in disappearance of PLRs (Fig. 6G). Thus the effect of reversible spinalization, that is disappearance of PLRs was

similar to that observed after surgical spinalization (Musienko et al., 2010). Re-warming of the spinal cord led to restoration of spike propagation (Fig. 6F) leading to restoration of PLRs (Fig. 6G).

Galvanic vestibular stimulation (GVS). GVS was caused by a constant current passing between the electrodes, inserted into the right and left ears (GVS in Fig. 6A). This current activated vestibular fibers on the cathode side and inhibited those on the anode side (Minor and Goldberg, 1991).

Recordings of neurons. Individual neurons (presumed interneurons) were recorded extracellularly from the spinal segment L4-L6 by means of commercially available varnish-insulated tungsten electrodes (ME in Fig. 6A). We tended to explore systematically the whole cross-section of the gray matter except for the area of motor nuclei. The lateral and vertical coordinates of each neuron were marked on the map of the spinal cord cross-section. In the end of experiment, reference electrolytic lesions were made in the spinal cord. After histological procedure, positions of recording sites were estimated in relation to the lesions.

In experiments devoted to the general characteristics of PLRs-related neurons, activity of individual neurons from L5-L6 was recorded during PLRs evoked by lateral tilts of the whole platform (Fig. 6C) along with EMGs and ground reaction forces. In addition, the neurons were tested by tilts of the platform under only the ipsilateral (Fig. 6D) or only the contralateral limb, as well as during in-phase tilts of the platforms under both limbs (Fig. 6E). In experiments with reversible spinalization, each individual neuron was recorded under three conditions: (1) before cooling (control), (2) during cooling, and (3) during rewarming. In experiments with GVS, each individual neuron was also recorded under three conditions: (1) without GVS (control); (2) during GVS, with the anode ipsilateral to a neuron; (3) during GVS, with the cathode ipsilateral to a neuron.

We suggested that if activity of the spinal neuron correlated with PLRs, it contributed to their generation. For details of the analysis of neuronal activity see Methods sections in *Papers I-III*.

GVS-based artificial feedback. In these experiments, the head of the animal was rigidly fixed and the forequarters were suspended in a hammock. The hindlimbs were positioned on a tilting platform; they provided support for the posterior part of the body and could perform corrective movements in response to tilts of the platform. However, the amplitude of the corrective movement in the caudally decerebrated rabbit was not enough to counteract the body sway caused by the tilts. To restore the lateral stability, GVS-caused body sway was used to counteract the lateral body sway resulting from mechanical perturbation of posture. To make the GVS value dependent on the postural perturbation (i.e., on the lateral body sway caused by tilt of the platform), an artificial feedback loop was formed in the following ways: (i) Information about body sway was provided by a mechanosensor; (ii) The GVS current was applied when the sway exceeded a threshold value; the polarity of the current was determined by the sway direction.

EXPERIMENTS ON LAMPREYS

In vivo experiments

In the *in vivo* experiments, we investigated if different forms of locomotion can be evoked in adult spinal lampreys (*Lampetra fluviatilis*). The animals were spinalized at the second gill level. One or two plates with stimulating electrodes (Fig. 7A) were implanted on the dorsal surface of the spinal cord (Fig. 7B) at different rostro-caudal levels (Fig. 7C). Bipolar EMG electrodes were implanted in the body muscles at different rostro-caudal levels. For elicitation of different forms of undulatory locomotion, the lamprey was positioned in an aquarium of 80×80×10 cm. For elicitation of crawling, the animal was positioned in a U-shaped plexiglas tunnel. To evoke different forms of locomotion, two types of stimulation were used: continuous monopolar electrical stimulation of the spinal cord and tactile stimulation of the skin. Movements of the lamprey caused by stimulation were recorded by video camera along with EMGs.

In vitro experiments

In *in vitro* experiments, intraspinal SRNs-mediated reflexes were studied. Two types of *in vitro* preparations dissected from adult lampreys (*Ichthyomyzon unicuspis* and *Petromyzon marinus*) were used. To study motor responses to bending in different planes and at different rostro-caudal levels, the preparation of the spinal cord isolated together with the notochord was used (Fig. 7D). To study the reflex responses to bending during different motor behaviors, as well as their supraspinal control, the preparation consisting of the brainstem and the spinal cord

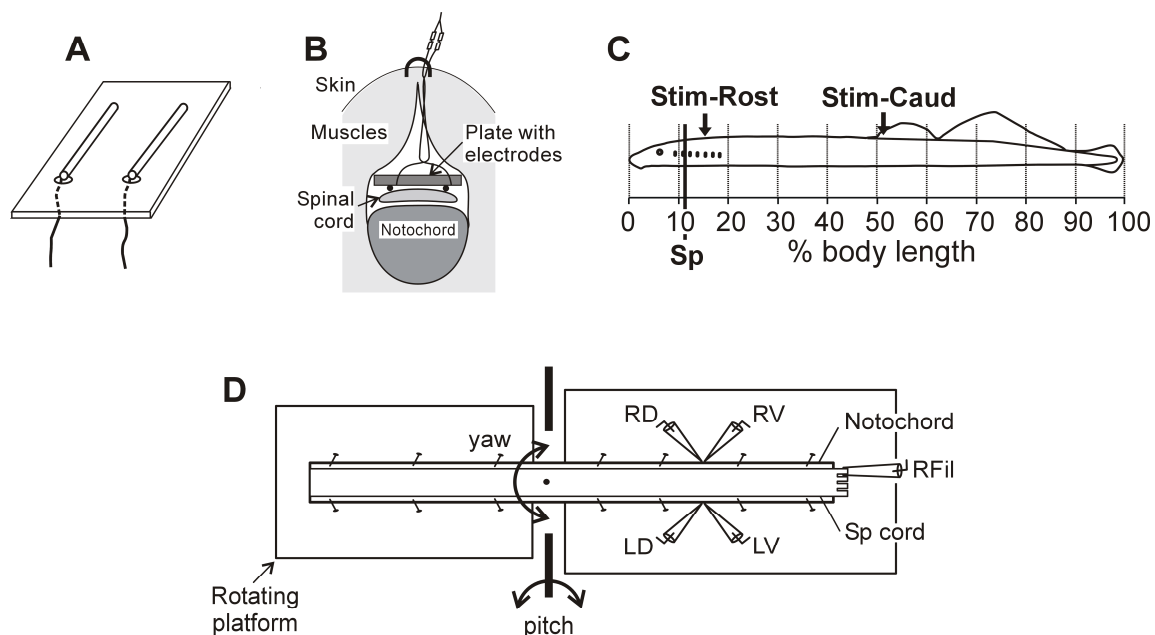


Figure 7. **A.** Design of the stimulating electrodes. **B.** Position of the plate with electrodes as seen in the transverse section of the lamprey's body. **C.** The electrodes were implanted at different rostrocaudal levels (Stim-Rost and Stim-Caud) in lampreys spinalized at the level of the second gill (Sp). **D.** A piece of the spinal cord was isolated together with the notochord and attached to two platforms. One platform was stationary and the other could be rotated in the yaw (horizontal) and pitch (sagittal) planes, causing bending of the preparation. Responses of MNs (innervating dorsal and ventral parts of a myotome) to bending were recorded from the dorsal (D) and ventral (V) branches of the right (R) and left (L) ventral roots in one of the segments by means of suction electrodes. The stump of the spinal cord was split into small filaments to record axons of SRNs in Ca^{2+} -free solution by means of a suction electrode (recording from the right filament, RFil, is shown).

isolated together with the cranium and the notochord was used. In some experiments, the spinal cord was spited along the midline.

Each of the preparations was attached to the two platforms positioned in the experimental chamber. In experiments with the spinal cord preparation, either the rostral or the caudal part of the preparation was pinned to a stationary platform, and the other part to a small movable platform that could be rotated in both yaw and pitch planes, thus bending the preparation in the corresponding plane (Fig. 7D). In experiments with the brainstem-spinal cord preparation, the rostral part of the preparation was pinned to the stationary platform, and the caudal part, to the movable one. The bending of the spinal cord was performed using a trapezoidal temporal pattern of the platform rotation.

In the spinal cord preparation, fictive FFS was evoked by D-glutamate (0.5 to 2.0 mM). In the brainstem-spinal cord preparation, different forms of fictive locomotion were evoked. FFS was elicited either by electrical stimulation of the MLR or by application of D-glutamate to the spinal cord. To evoke different forms of escape behavior (SFS, BS, and lateral turns), different sites of the trigeminal nerve were electrically stimulated with different parameters.

Motor responses to bending were monitored by recording the activity of MNs either from the ventral roots or from the dorsal and ventral branches of ventral roots by means of suction electrodes (Fig. 7D). Activity of SRNs was recorded by means of the suction electrode from their axons in thin filaments dissected from the stump of the spinal cord (Fig. 7D). Synaptic transmission was blocked by reducing the Ca^{2+} to zero and increasing Mg^{2+} to 4 mM in the Ringer's solution. The axons in the filament that were activated by bending the preparation in a Ca^{2+} -free solution were considered to be SRNs axons. RS neurons in different reticular nuclei were recorded intracellularly with sharp glass microelectrodes (3M K-acetate, 20-70 M Ω).

RESULTS AND DISCUSSION

NEURONAL MECHANISMS FOR STABILIZATION OF TRUNK ORIENTATION IN TRANSVERSE PLANE IN RABBITS

Spinal neurons contributing to generation of postural limb reflexes

Postural limb reflexes (PLRs) represent a substantial component of the postural system responsible for stabilization of dorsal-side-up orientation in quadrupeds. Spinal neurons contributing to the generation of PLRs were revealed, and different aspects of their activity were characterized in *Papers I, II and III*.

Location and activity of PLR-related neurons

To reveal neurons related to the generation of PLRs, individual spinal neurons in L4-L6 were recorded during PLRs. The activity of the majority (>70%) of recorded neurons correlated with PLRs, suggesting that they contribute to the PLRs generation. A proportion of non-modulated neurons was small. One possible explanation of this result is that supraspinal drive in decerebrate animals preferably activated the posture-related population of spinal neurons, while other neurons, for instance the locomotion-related ones (see e.g., Orlovsky et al., 1999), were not active and therefore were not recorded in our experiments. The overwhelming majority of neurons was recorded outside of the motor nuclei (Fig. 8A, dotted line) and was considered as putative interneurons.

According to the phase of their activity, all PLRs-related neurons were divided into two groups: F-neurons were excited in-phase with extensors of the ipsilateral limb (as the neuron in Fig. 8B), while E-neurons – in anti-phase (as the neuron in Fig. 8C). F- and E-neurons were intermingled and scattered across the whole cross-section of the gray matter (Fig. 8A). The latter finding was not surprising since tilt-related somatosensory signals are most likely transmitted by group I and II afferents from the limb muscles, and spinal interneurons receiving inputs from these afferents are located in different areas of the gray matter (Jankowska et al., 2002; 2008; 2009; Edgley et al., 2003; Bannatyne et al., 2009). F-neurons were slightly more numerous in each of the three zones (Fig. 8A) of the gray matter.

The analysis of spatial distribution of neurons with different activity characteristics (*Paper I*) has shown that the mean frequency of F- and E-neurons located in different areas of the gray matter was similar, suggesting that spinal circuits located in these areas are similarly activated. The depth of modulation (i.e., the difference between the mean frequency during flexion and extension of the ipsilateral limb) decreased in the dorso-ventral direction. We found neither any clear latero-medial changes in the distribution of different parameters, nor any clear peaks or troughs in this distribution.

F- and E-groups are not homogeneous. They could contain segmental and propriospinal interneurons, as well as the ascending tract neurons, some of which may be implicated in supraspinal postural feedback loops, while others may be involved in sensory perception. The ascending neurons may include, e.g., spinocerebellar tract neurons, which receive inputs from group I and II afferents (Jankowska and Puczyńska, 2008; Jankowska and Hammar, 2013). One can assume that at least some of the recorded F- and E-neurons are pre-motor interneurons that activate and inhibit extensor motoneurons (MNs), respectively. Such pre-motor

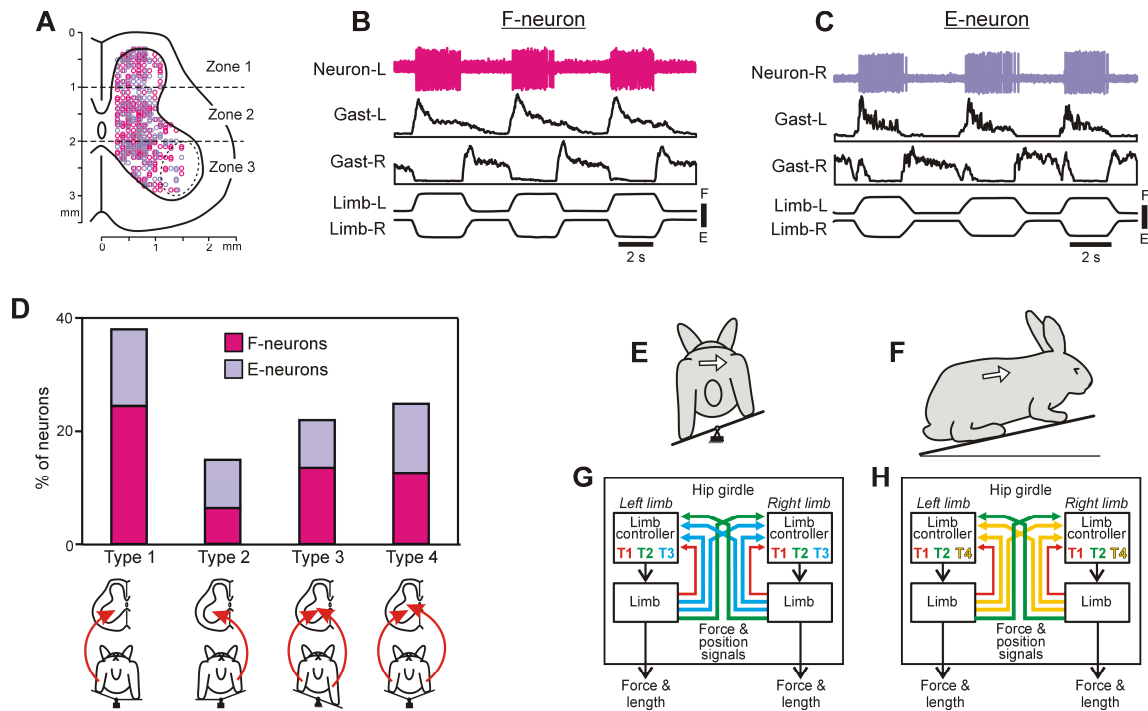


Figure 8. **A.** Position of recorded F- and E-neurons on the cross-section of the spinal cord. The area of motor nuclei is indicated by a dotted line. Three zones of the gray matter are shown: the dorsal (1), intermediate (2) and ventral (3) ones. **B,C.** Examples of the activity of F-neuron (**B**) and E-neuron (**C**) during PLRs caused by the whole platform tilts. Abbreviations: Limb-L and Limb-R, flexion-extension movements of the left and right limbs, respectively; Gast-L and Gast-R, activity of the left and right m. gastrocnemius lateralis, respectively. **D.** Relative number of different types of F- and E-neurons. **E,F.** Postural corrections caused by tilts of the support surface in the transverse plane (**E**) and in the sagittal plane (**F**). An arrow shows the direction of postural corrections caused by tilt. **G,H.** Orientation of hindquarters in the transverse plane (**G**) and in the sagittal plane (**H**) is stabilized by many parallel reflex chains that involve neurons of different types. These types (T1-T4) are indicated by the same color as their sensory inputs from the hindlimbs.

interneurons, with inputs from group I and II afferents, have been found in the lumbosacral enlargement of the spinal cord (Bannatyne *et al.*, 2006, 2009; Cavallari *et al.*, 1987; Jankowska *et al.*, 2005).

Sensory source of modulation of PLR-related neurons

To reveal the sources of modulation of PLR-related neurons (the ipsilateral or/and contralateral hindlimb), responses of individual F- and E-neurons to tilts of only one of the platforms (either ipsi- or contralateral, Fig. 6D), as well as during in-phase (Fig. 6E) and during anti-phase (Fig. 6C) tilts of the platforms under both limbs were recorded (*Paper I*).

According to tilt-related inputs from the ipsilateral and contralateral limbs, all PLR-related neurons were divided into four types (Fig. 8D). In *Type 1* (T1) neurons (38%), modulation was determined by sensory input from the ipsi-limb only, suggesting their involvement in the intra-limb coordination, i.e., in the generation of corrective limb movements in response to sensory inputs from the same limb (Fig. 8G,H). In intact animals, a substantial part of corrective limb movements is generated by this mechanism (Deliagina *et al.*, 2006).

In *Type 2* (T2) neurons (15%), modulation was determined by sensory input from the contra-limb only (Fig. 8D). They may include commissural interneurons (with sensory input from the contra-limb) involved in the intra-limb coordination (together with T1-neurons), and

ipsilaterally projecting neurons (with sensory input from the contra-limb) involved in the inter-limb coordination (Fig. 8 *G,H*). In the intact animal, somatosensory signals from the contra-limb contribute to the generation of corrective limb movements (Deliagina *et al.*, 2006). In decerebrate animals, signals transmitted by T2-neurons with contra-input could be sub-threshold, resulting in the weakness of PLRs during contra-limb tilts.

Type 3 (T3) and *Type 4* (T4) neurons received tilt-related sensory input from both limbs. T3 neurons (22%) received complementary inputs from the limbs: these neurons responded to flexion of the ipsilateral limb and extension of the contralateral limb, or to extension of the ipsilateral limb and flexion of the contralateral limb (Fig. 8*D*). T4 neurons (25%) received opposing inputs from the two limbs: these neurons responded to flexion of the ipsilateral limb and flexion of the contralateral limb, or to extension of the ipsilateral limb and extension of the contralateral limb (Fig. 8*D*). In the majority of T3 and T4 neurons, the input from the ipsilateral limb was stronger, and thus in T4 neurons this input determined the response to the whole platform tilt. We found that summation of tilt-related sensory inputs from the two hindlimbs was almost linear. T3 and T4 neurons are most likely involved in the inter-limb coordination during postural corrections (Fig. 8*G,H*).

F- and E-neurons with ipsi- and contralateral inputs were intermingled and scattered across the gray matter. In most F-neurons, the contra-input was much weaker than the ipsi-input. In E-neurons, the contra-input was stronger and the ipsi-input was slightly weaker than those in F-neurons. One possible explanation for this finding could be a lesser activation of limb afferents signaling limb extension than that signaling limb flexion, due to a hemi-flexed limb configuration in both phases of the tilt cycle. Previously, interneurons with ipsi-inputs from the group I and II afferents and ipsilateral projections were found in different areas of the gray matter (Bannatyne *et al.*, 2006; 2009). The commissural neurons with ipsi-inputs from group I and II afferents, and with terminals in different areas of the contralateral gray matter (which could mediate input from the contra-limb) were also described (Bannatyne *et al.*, 2006; 2009; Jankowska *et al.*, 2009).

T1-T4 neurons are presumably involved in population coding of commands for postural corrections. Roughly, corrections in the transverse plane (arrow in Fig. 8*E*), which require reciprocal movements of the left and right limbs, are caused by T1-T3 neurons (Fig. 8*G*). Corrections in the sagittal plane (arrow in Fig. 8*F*), which require in-phase movements of the left and right limbs, are caused by T1-, T2-, and T4-neurons (Fig. 8*H*).

One can suggest that the directional tuning of each individual PLR-related neuron to sensory inputs (signaling about the postural disturbers in a particular plane) matches the motor effects that this neuron produces, i.e., the motor effects of each individual neuron counteract the postural disturbances that activate the neuron. Thus, PLR-related neurons are the key elements of the feedback loops participating in the stabilization of body orientation in a number of planes. Such matching has been previously demonstrated for the reticulospinal neurons eliciting postural reactions in the lamprey (Zelenin *et al.*, 2007), as well as for the nociceptive withdrawal reflex in the rat (Schouenborg, 2008).

Role of supraspinal inputs in modulation of spinal PLR-related neurons

To study the contribution of supraspinal influences to the activity of individual F- and E-neurons, activity of individual interneurons was recorded during stimulation causing PLRs under two conditions: (1) when neurons received supraspinal influences and (2) when these

influences were temporarily abolished by a cold block of spike propagation in spinal pathways at T12 (“reversible spinalization”) (*Paper II*).

It was found that elimination of supraspinal commands produced diverse but mostly inhibitory effects on F- and E-neurons. A small proportion of neurons was activated during cooling, suggesting a relative weakness of inhibitory supraspinal influences on these neurons as compared to excitatory ones. In the overwhelming majority of neurons, cooling did not affect their phase of response, suggesting that these neurons belong to the networks generating the spinal component of PLRs, and that supraspinal postural commands strongly affect these neurons. In 19% of neurons non-modulated before cooling, the modulation appeared during cooling, suggesting that supraspinal influences reduce activity in the reflex arcs transmitting somatosensory information to these neurons, and thus affected processing of sensory information in the spinal cord. These neurons could be responsible for the incorrectly phased EMG responses to tilts that appeared after spinalization (Musienko et al., 2010). The proportion of F-neurons inactivated during cooling was significantly larger than found in E-neurons (79% vs. 48%), suggesting that excitatory supraspinal drive to F-neurons is considerably stronger than to E-neurons, which can explain an increase in extensor activity and enhancement of PLRs. The neurons differently affected by cooling were specifically distributed across the spinal cord: the relative number of F- and E-neurons unaffected by cooling was the largest in dorsal area, and the amount of inactivated neurons was the largest in intermediate and ventral parts of the gray matter. In the activated and inactivated F- and E-neurons, cooling affected both mean burst frequency and mean interburst frequencies, suggesting that most neurons received, respectively, inhibitory and excitatory supraspinal drive during both phases of the tilt cycle.

A population of F-neurons residing in the ventromedial part of the gray matter was revealed, which exhibited a dramatic (>80%) decrease in their activity during cooling. It was shown that some neurons in this area produce excitation of extensor MNs (Jankowska, 1992). It was suggested that strongly inactivated F-neurons (SIF-neurons) are the premotor neurons,

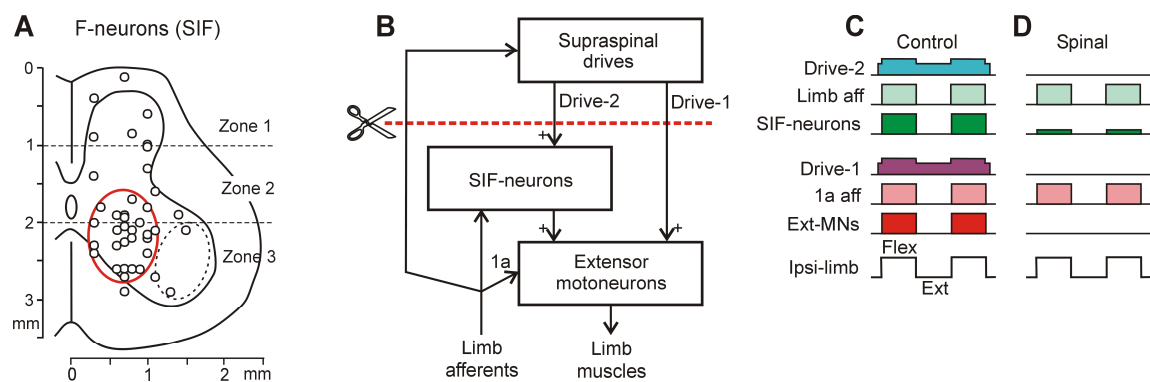


Figure 9. **A.** Position of SIF-neurons on the cross-section of the spinal cord. **B-D.** Presumed neuronal mechanisms underlying disappearance of PLRs after spinalization. **B.** Principal components of PLR mechanisms. **C,D.** Activity of these components in the nonspinalized (**C**) and spinalized (**D**) animals subjected to periodical platform tilts causing flexion/extension limb movements. Extensor motoneurons receive a direct excitatory drive from supraspinal structures (Drive-1). Another drive is mediated by SIF-neurons (Drive-2). Extensor motoneurons are modulated by sensory input from limb afferents either directly (by Ia afferents) through strongly inactivated F-neurons (SIF-neurons), or due to the long spinosupraspinal loop. Spinalization (red interrupted line in **B**) deprives the extensor motoneurons of both supraspinal drives, which results in disappearance of the extensor tone and PLRs (**D**).

which activate the extensor MNs, and thus, elimination of the excitatory supraspinal drive to these neurons is responsible for disappearance of extensor tone and PLRs after spinalization. Presumed neuronal mechanisms underlying the disappearance of PLRs after spinalization are shown in Fig. 9.

Effect of galvanic vestibular stimulation on postural networks

Binaural galvanic vestibular stimulation (GVS) causes a lateral body sway toward the anode (Séverac Cauquil et al., 2000; Beloozerova et al., 2003; Gorgiladze, 2004). Earlier it was shown that in standing rabbits this new body orientation is actively stabilized, suggesting that GVS changes a set-point in the reflex mechanisms controlling body posture (Beloozerova et al., 2003). The aims of the present study were (i) to reveal the underlying neuronal mechanisms by studying the GVS effect on PLRs and on the PLR-related neurons (*Paper III*), and (ii) to test the efficacy of the GVS-based artificial feedback for restoration of postural functions (*Paper IV*).

Effect of GVS on PLRs and PLR-related neurons

It was found that continuous GVS strongly affected the magnitude of PLRs: the extensor EMGs and the force developed during limb flexion were considerably increased when the cathode was ipsilateral to the limb, and decreased when the anode was ipsilateral to the limb. These findings suggest that a tonic supraspinal (including vestibulospinal) drive (caused by continuous GVS) can increase and decrease the gain in postural reflex pathways.

According to the effect of GVS on individual PLR-related neurons, three subgroups of F-neurons and three subgroups of E-neurons were distinguished: F1- and E1-neurons had a stronger response to tilts during GVS with ipsilateral cathode than with ipsilateral anode. F2- and E2-neurons had stronger response with ipsilateral anode than with ipsilateral cathode. Finally, F3- and E3-neurons were not affected by GVS. The majority of modulated neurons (61%; subgroups F1, F2, E1, and E2) responded to GVS, suggesting that these spinal neurons participated in the integration of descending and afferent information. The minority of modulated neurons (39%; subgroups F3 and E3) did not respond to GVS and therefore, did not mediate vestibulospinal influences.

In the F1-subgroup (25% of all modulated neurons), the pattern of activity caused by tilts, as well as the GVS effects on this activity, were both similar to those in extensor MNs. One can suggest that the F1-neurons are excitatory interneurons contributing to activation of extensor MNs during PLRs, as well as mediating the effects of GVS on extensor MNs. The F1-neurons are included in the conceptual model of the trunk stabilization system (Fig 10A). The majority of F1-neurons was located in the intermediate area and in the ventral horn, i.e., in the areas of termination of the vestibulospinal tract (Nyberg-Hansen and Mascitti, 1964; Petras, 1967), and thus could receive direct vestibulospinal influences.

In the E2-subgroup (15% of all modulated neurons), the pattern of activity caused by tilts, as well as GVS effects on this activity, were opposite to those in extensor MNs. It is possible that, to generate PLRs, the E2-group was inhibited by supraspinal drive caused by GVS, which in turn disinhibited extensor MNs. The E2-neurons are included in the conceptual model of the trunk stabilization system (Fig 10A). Neurons of this subgroup are located mainly in the ventral horn; they could include interneurons mediating the reciprocal inhibition (Hultborn et al., 1976).

The proportion of neurons without GVS influences (subgroups F3 and E3) was almost two times larger in the segment L4 than in the segment L5. This finding suggests stronger vestibulospinal influences on L5 than on L4. In the cat, more intense vestibulospinal projections to the central segments of the lumbo-sacral enlargement have been reported (Petras, 1967).

Conceptual model of the trunk stabilization system and effects of GVS

Two chains of antagonistic PLRs, as well as the effects of GVS on these chains, are schematically shown in Fig. 10A. This scheme reflects also a finding (Grillner and Hongo, 1972) that the vestibulospinal tract can excite extensor MNs both directly and indirectly, through the spinal interneurons (presumably subgroups F1 and E2) that integrate descending and afferent information.

Presumed effects of these two antagonistic reflex chains in the unrestrained standing rabbit are illustrated in Fig. 10B-E. The effects without GVS are shown in Fig. 10B. Any deviation of the dorso-ventral body axis from the vertical (lateral sway) causes opposite changes in PLR-R and PLR-L (solid and interrupted lines, respectively). In turn, PLR-R and PLR-L produce opposite motor effects - they cause body sway in opposite directions as indicated by black and white arrows, respectively. With symmetrical PLRs, the two curves

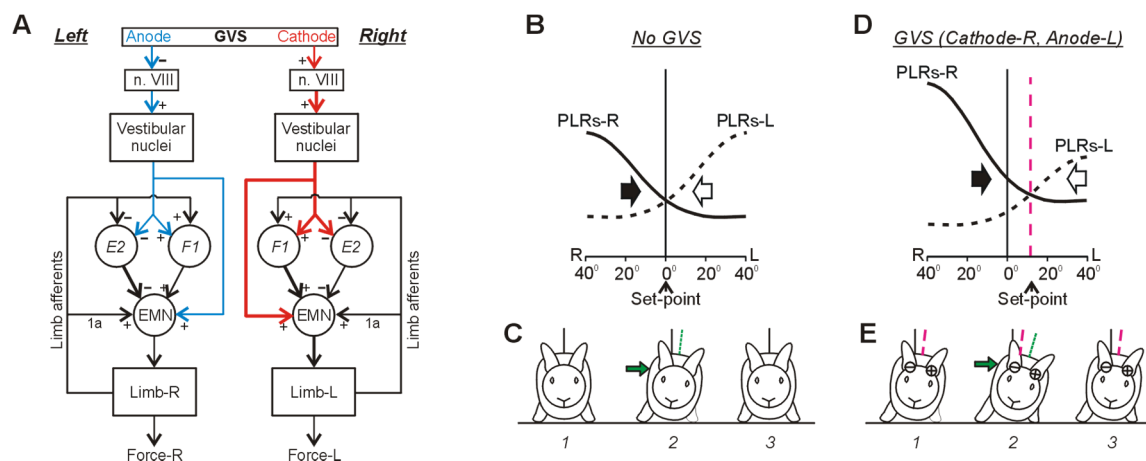


Figure 10. Conceptual model of the trunk stabilization system and effects of galvanic vestibular stimulation. **A.** Schematic representation of two chains of PLRs (Left and Right), as well as the effects of GVS on these chains. In each chain, flexion of the limb activates afferents of this limb. They cause excitation of extensor motoneurons (EMN) through monosynaptic pathways (group Ia afferents) and through polysynaptic pathways mediated by spinal interneurons (groups F1 and E2). Extensor motoneurons activate extensor muscles, which counteract limb flexion. The GVS causes asymmetry of the two chains (indicated by different size and thickness of the corresponding red and blue arrows). With cathode on the right side, GVS activates vestibular afferents in the right VIII nerve (n. VIII), which activate neurons of the right vestibular nuclei. These neurons, through the right vestibulospinal tract, affect the spinal postural reflexes on the right side (for simplicity, crossed-effects are not considered). Due to descending drive, excitability of extensor motoneurons and F1-interneurons is increased, and excitability of E2-interneurons decreased (as compared to the left side). **B-E.** Presumed effects of the two antagonistic reflex chains in the unrestrained standing rabbit, without GVS (**B,C**) and during GVS with cathode-R and anode-L (**D,E**). **B,D.** The abscissa shows a deviation of the dorso-ventral body axis from the vertical (lateral sway); the ordinate shows the value of PLR-R and PLR-L (solid and interrupted line, respectively). Black and white arrows indicate the motor effect (lateral sway) caused by PLR-R and PLR-L, respectively. **C,E.** The stabilized orientation (1), effect of the lateral push (2), and the restored orientation (3). The stabilized body orientation and the body orientation immediately after the push are indicated by the pink and green interrupted lines, correspondingly. (See Text for details).

intersect at 0° (no lateral sway; Fig. 10B). This orientation (Fig. 10C, 1) is stabilized, i.e., the rabbit will return to this orientation after any deflection caused, for example, by the lateral push (Fig. 10C, 2 and 3).

Continuous GVS (e.g., with cathode-R, anode-L) causes an increase in the gain of PLR-R and a decrease in those of PLR-L (Fig. 10D). Now, the two curves intersect not at 0° but at some angle of the left sway. This tilted orientation (Fig. 10E, 1) will be stabilized; i.e., the rabbit will return to this orientation after any deflection from it (Fig. 10E, 2 and 3). Thus GVS, by creating asymmetry in the tonic left and right supraspinal drive, changes the set-point of the postural system through the change of the gain in antagonistic PLRs. A similar principle of balance control was also found in simpler animals—a mollusk (*Clione*) and a lower vertebrate (lamprey; see INTRODUCTION) (Deliagina and Fagerstedt, 2000; Deliagina et al., 1998; Deliagina et al., 2014).

Capacity of GVS-based artificial feedback to restore postural functions

The rabbit decerebrated at the postmammillary level cannot maintain the dorsal-side-up body posture and balance on a tilting platform, due to a decreased value of reflex responses to tilts, as well as due to abnormal phasing of these responses (Musienko et al., 2008; 2010). The goal of the present study was the restoration of normal postural control in the hindquarters of postmammillary rabbit (*Paper IV*), first by increasing the gain of postural reflexes, and second by proper phasing of these reflexes in relation to postural perturbations. For this purpose, we supplemented a part of the control system (the sensory feedback) with an artificial feedback (Fig. 11A).

We took advantage of the fact that the motor response to GVS is a lateral body sway and used this response to counteract the sway caused by a postural perturbation, i.e., by lateral tilt of the support surface. The idea of using the GVS-caused motor response for postural corrections was first proposed by Scinicariello et al. (2001), and later used by Orlov et al., (2008). These authors have demonstrated that in standing healthy humans a properly timed

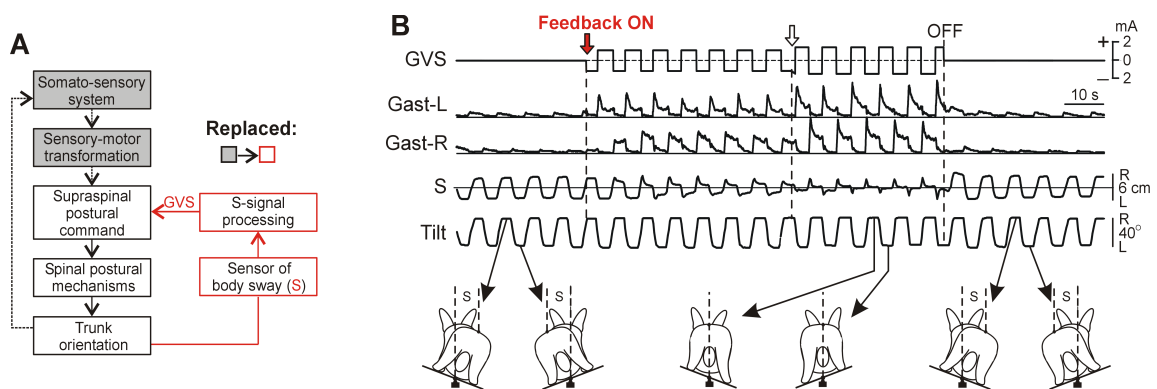


Figure 11. A. “Hybrid” system for postural control in the decerebrate rabbit. Some components of the native postural system constituting the sensory feedback loop (indicated by interrupted lines) were supplemented by their synthetic homologues (red lines). See text for explanations. B. The GVS-based feedback improves postural control. Without feedback, periodical tilts of the platform caused large lateral oscillations of the body (see S trace). The polarity of GVS is indicated for the right electrode (for the left electrode the polarity was opposite). After the feedback loop was closed (ON) the body oscillations were reduced considerably. When GVS current was increased (arrow), the oscillations became even smaller. When the feedback loop was opened (OFF), the oscillations became large again. Gast-L and Gast-R, activity of the left and right m. gastrocnemius lateralis, respectively.

GVS can reduce the body sway caused by postural perturbations. The artificial feedback loop was formed in the following way. First, the lateral body sway was taken as a regulated variable of the control system. This signal was provided by a mechanical sensor (S in Fig. 11A,B). Second, the signal S was used to determine characteristics of the injected current (S-signal processing in Fig. 11A) according to the following algorithm: the current was turned on when S reached the right or the left threshold level (S_R or S_L), the polarity of current being opposite for S_R and S_L . The value of injected current was constant and did not depend on the magnitude and speed of the body sway.

This system was examined by tilting the platform under the rabbit. We have found that the system can cause (i) a significant increase in EMG responses to tilts in limb extensors, and (ii) a significant decrease in the lateral oscillations of the trunk (Fig. 11B). The phasing of all EMGs in relation to postural perturbations was correct, i.e., similar to that in intact rabbits (Beloozerova et al., 2003). The reduction of lateral trunk oscillations demonstrates that the GVS-based system is capable to maintain the dorsal-side-up trunk orientation. Thus, an important postural function, i.e., maintenance of the dorsal-side-up body orientation, which was considerably impaired in postmammillary rabbits, can be restored by means of the GVS-based external feedback. We believe that the control system with the artificial GVS-based feedback can also compensate for other postural perturbations (e.g., lateral push applied to the body, lateral translation of the support surface), provided these perturbations result in a lateral body sway.

We suggest that the control system, with the artificial GVS-based feedback, can compensate for the loss of postural orientation of different etiology, including the loss caused by an incomplete spinal cord injury (SCI) in humans. It was shown that normal reaction to GVS (the lateral body sway) persisted in a part of SCI patients (Iles et al., 2004; Liechti et al., 2008; Wydenkeller et al., 2006). This finding implies that the spinal pathways responsible for the effect of GVS (which descend in the ventral part of the spinal cord, Muto et al., 1995) were not damaged in these patients. We suggest that the noninvasive technique developed for the rabbit and described in this paper can be transferred onto the patients with incomplete SCI to improve their postural control. Thus, the hybrid model of postural system may have not only theoretical importance but also clinical applications.

NEURONAL MECHANISMS FOR CONTROL OF BODY CONFIGURATION DURING DIFFERENT MOTOR BEHAVIORS IN LAMPREYS

Neural mechanisms underlying motor responses to body bending

In vertebrates, proprioceptive inputs provide sensory information about body configuration that is important for the control of different movements. In the lamprey, muscle spindles were not found in trunk muscles, and proprioceptive inputs are provided by intraspinal mechanoreceptors – the stretch receptor neurons (SRNs) (Grillner et al., 1984). The aim of the present study (*Paper VI*) was to analyze responses of SRNs and MNs to body bending in different planes and at different rostral-caudal levels. For this purpose, *in vitro* preparation of the spinal cord isolated together with notochord was used, and responses to bending were recorded from SRNs, as well as from MNs innervating the dorsal (dMNs) and ventral (vMNs) parts of a myotome.

Sensitivity of stretch receptor neurons to bending in different planes and at different rostro-caudal levels

The activity of SRNs was recorded from their axons in a filament of the spinal cord, while the synaptic transmission was blocked to separate the cells with mechanical sensation (Grillner et al., 1982). We have found that SRNs are sensitive to body bending in the pitch plane. They were activated by ventral bending, and can thus provide sensory input for reflex motor responses in this plane. In the yaw plane, two types of SRN axons with different responses to bending were found in a filament: those responding to ipsilateral bending and those responding to contralateral bending. Since splitting the spinal cord along the midline abolished ipsilateral responses, while the contralateral responses were preserved, one can suggest that axons responding to contralateral bending belong to ipsilaterally projecting SRNs (IP-SRNs), and axons responding to ipsilateral bending belong to contralaterally projecting SRNs (CP-SRNs) (Viana Di Prisco et al., 1990). It was found that individual IP-SRNs can convey information about body bending at the distance of up to 15 segments, which is an estimated maximal length of their axons. A similar estimate for the maximal length of axons (20 segments) was obtained in a morphological study (Tang and Selzer, 1979). We have also found that the basic pattern of SRN responses to yaw and pitch bending was the same when bending was performed at different rostro-caudal levels.

One can thus conclude that the population of SRNs can provide information about body bending at different rostro-caudal levels and in different planes.

Spinal reflexes evoked by natural stimulation of stretch receptor neurons

It was found that responses of MNs to bending depended on the plane of bending and on the rostro-caudal level of bending.

Bending in the yaw plane could evoke two types of responses. Bending in the mid-body area (segments 35-50) caused activation of MNs projecting to the convex side (“convex response”), and bending in the rostral area (segments 10-30) caused activation of those projecting to the concave side (“concave response”). In both cases, similar patterns were usually observed in the dMNs and vMNs, suggesting that reflex motor responses to body bending in yaw plane would occur in the same plane, either increasing or decreasing the value of bending.

Generation of convex motor responses caused by bending in the yaw plane can be explained on the basis of known connections between SRNs and neurons of the spinal networks shown in Fig. 12A (Viana Di Prisco et al., 1990; Vinay et al., 1996; see Figure legend for explanations). However, generation of concave motor responses cannot be explained on the basis of known connections.

Bending in the pitch plane could evoke numerous patterns of motor responses with different combinations of activity of the four groups of MNs. The most consistent were the responses in the mid-body region elicited by bending in segment 40: ventral and dorsal bending caused activation of dMNs and vMNs, respectively. These responses cannot be explained on the basis of known network connections (Fig. 12A). One possible change in this network, which would allow generating these responses, could be selective inhibition (either mono- or polysynaptic) of contralateral vMNs caused by CP-SRNs.

We found that splitting the spinal cord along the midline resulted in uniform responses of MNs to pitch and yaw bending along the spinal cord: MNs responded only to contralateral

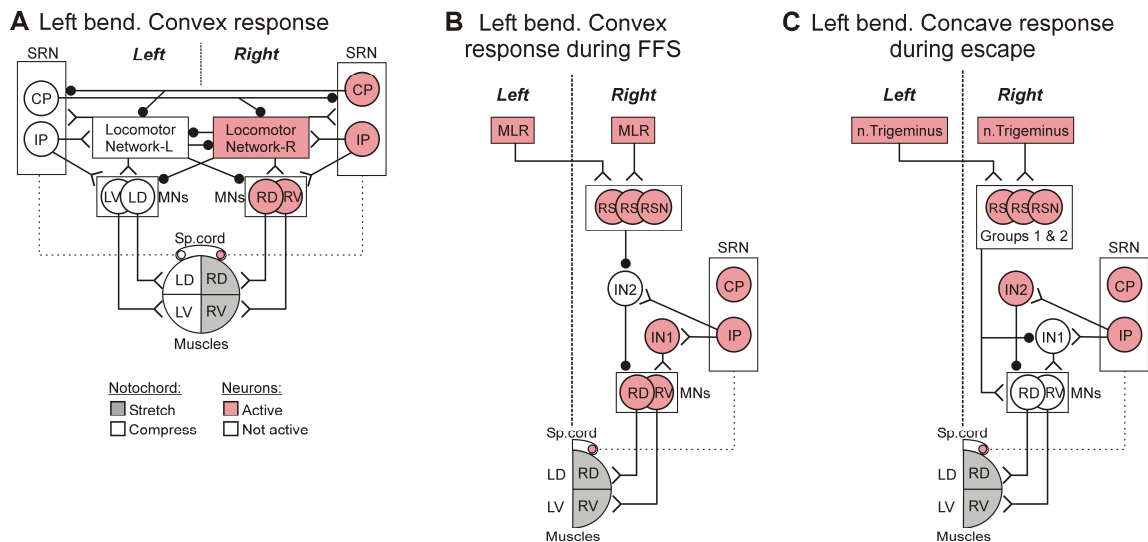


Figure 12. A. Spinal circuitry (based on known connections of SRNs) explains convex response to lateral bending in isolated spinal cord. Bending to the left causes stretching of notochord and spinal cord tissue on the opposite (right) side, resulting in activation of IP-SRN (IPs) and CP-SRN (CPs) on that side. The IPs excite MNs innervating the dorsal and ventral parts of the myotome (RD and RV, respectively). This monosynaptic route is supplemented by the polysynaptic route through the right locomotor network. At the same time, CPs on the stretched right side inhibit several groups of neurons on the opposite side, including IPs and CPs. B,C. Changes in a hypothetical circuitry underlying reflex responses to bending during FFS (B) and during escape behavior (C). Two groups of spinal interneurons, IN1s and IN2s, receive excitatory inputs from the ipsilateral IPs, and excite or inhibit ipsi-MNs (RD and RV), respectively. A part of IN1s are interneurons of the CPG for FFS (Viana Di Prisco et al., 1990). B. Stimulation of mesencephalic locomotor region (MLR), which evokes FFS, activates a specific population of RS neurons (RSNs). They inhibit IN2s (at least disynaptically), and now bending to the left, which activates right IPs and CPs, results in activation of ipsi-MNs (convex response). C. Stimulation of trigeminal nerves, which evokes escape behavior, activates Group 1 and Group 2 RSNs. They inhibit IN1s (at least disynaptically) and activate ipsi-MNs. Now bending to the left results in inhibition of ipsi-MNs and bending to the right – in their disinhibition (concave response). Abbreviations: open triangle, excitatory connection; small filled circle, inhibitory connection.

bending in the yaw plane and only to ventral bending in the pitch plane. This result suggests the importance of contralateral influences in processing of SRNs signals for the generation of SRN-mediated reflexes in the isolated spinal cord.

It was found that the responses of MNs to bending in yaw and pitch planes could be recorded at the distance of up to 40 segments from the site of bending, which is much longer than the presumed maximal length of SRNs axons (15 segments). These results imply involvement of the propriospinal neurons with long axons (e.g., lateral interneurons, Viana Di Prisco et al., 1990) in the transmission of SRN signals along the spinal cord, as well as in the formation of large functional synergies comprising dozens of segments. Thus, MNs in a given segment could respond to yaw bending in rostral and mid-body segments with concave and convex responses, respectively. We have demonstrated also that individual MNs change the phase of their response depending on the site of bending. These results suggest that the organization of the spinal networks processing the signals from the rostral and mid-body SRNs is different.

Reflex responses to body bending in different forms of motor behavior

Lampreys are capable of different forms of motor behavior: fast forward swimming (FFS), slow forward swimming (SFS), backward swimming (BS), forward (FC) and backward crawling (BC), lateral turns. Localization of networks generating different forms of motor behavior was done in *Paper V*. The amplitude of the body flexion (characterizing the body configuration) differs in different forms of motor behavior. Since SRNs are signaling the body bending, SRN-mediated reflexes could contribute to control of body configuration during different motor behaviors. The analysis of SRN-mediated reflexes during different motor behaviors, as well as their supraspinal control was studied in *Paper VII*.

Localization of networks generating different forms of motor behavior

To clarify, whether all forms of locomotion can be generated by spinal mechanisms, we developed a technique for electrical stimulation of the spinal cord in chronic spinal lamprey (see Methods).

The main result of the present study is that all forms of locomotion (FFS, SFS, BS, FC, BC) can be evoked by spinal cord stimulation, provided a proper site and strength of stimulation are used. One can thus conclude that the spinal cord of the lamprey contains the neuronal networks generating the whole repertoire of locomotion used by this animal in daily life. These results complement earlier studies demonstrated that in the spinal lamprey FFS and FC can be evoked (Rovainen, 1976; McClellan and Grillner, 1983), and in the spinal dogfish and tadpole both forward and backward swimming can be evoked (Dale and Roberts, 1984; Soffe, 1991).

Some kinematic parameters of locomotion evoked in the spinal lamprey differed from those in intact animal. The locomotion frequency and the speed of the wave propagation during FFS and BS in spinal lampreys were several times lower than in intact lampreys. Similar results were obtained in spinalized zebrafish larvae (McDermid and Drapeau, 2006). These could be explained by absence of descending commands in spinal animal, which in intact animal control the speed of wave propagation (as demonstrated for FFS, Sirota et al., 2000; Zelenin, 2011). In contrast, the phase lag and the wavelength in spinal lampreys were similar to those in intact lampreys. These findings suggest that the phase lag is mostly determined by the spinal network properties.

A number of evidences suggest that the effects of electrical stimulation of the spinal cord are mediated by RS axons. *First*, RS neurons are numerous, and many of them project along the entire extent of the spinal cord (Rovainen, 1967; Nieuwenhuys, 1972; Brodin et al., 1988). Many of RS axons are thick and thus have low activation thresholds. *Second*, they are involved in the control of different forms of locomotion (Deliagina et al., 2000b; Zelenin, 2005, 2011). *Third*, we tested the animals within 2 days after spinalization. Thus, most of RS axons were still alive (Roederer et al., 1983; Zhang et al., 2005).

We found that the undulation area during swimming (both FFS and BS) depended on the site of stimulation – rostral stimulation evoked swimming with a larger undulation area than caudal stimulation. Thus, one can suggest that activation of only long RS axons is sufficient to activate caudal spinal locomotor networks, but it is not sufficient to increase excitability in the rostral ones.

We found that stimulation of the same site of the spinal cord evoked either FFS or BS in the caudal part of the body depending on the strength of stimulation. A weaker stimulation evoked FFS, and the stronger one – BS. This finding suggests that activation of the long low-threshold (thick) RS axons seems to be sufficient to activate the network, which generate FFS,

and that long thin RS axons with higher activation thresholds cause a reversal of the direction of locomotor waves, thus producing BS. It was shown that groups of RS neurons involved in control of FFS and BS are partly different, and BS-specific axons are thinner (Zelenin, 2011).

We found that the direction of crawling of the spinal lamprey was determined by position of the bent site along the body: FC was usually observed if the body was bent within its rostral third, BC was seen if the body was bent more caudally. Thus, signals coming from SRNs may be important for control of crawling. In *Paper VI* we found that motor responses caused by SRNs have opposite signs in the rostral third of the body and in the caudal two-thirds. The similar position of the reversal point found in these two different studies strongly suggests a significant contribution of SRNs to the generation of the motor pattern of crawling.

Modifications of SRN-mediated reflexes in different forms of motor behavior

To reveal modifications of SRN-mediated reflexes during different motor behaviors, motor responses to bending applied during different fictive behaviors (evoked by supraspinal commands in *in vitro* preparation) have been analyzed (*Paper VII*).

We found that during MLR-evoked FFS, the convex response to lateral bending was observed in MNs at all rostro-caudal levels. However, during FFS evoked by application of D-glutamate to the spinal cord, concave response was observed in MNs of rostral segments. Similar result was obtained in the isolated spinal cord (*Paper VI*). This finding suggests that, first, networks underlying concave response to bending in the rostral segments could be selectively activated and thus contribute to the behaviors requiring large amplitude bending in the rostral part of the body like steering during FFS (Kozlov et al., 2014; Saitoh et al., 2007; Fagerstedt and Ullén, 2001). Second, the spinal networks, activated by spinal cord stimulation and by MLR-stimulation, differ to some extent. Similar conclusion was recently formulated for mammals (Musienko et al., 2012).

We found that during escape behavior (SFS, BS, lateral turns) caused by stimulation of different sites of the trigeminal nerve, SRN-mediated reflex was reversed, i.e., the concave response to bending was observed in MNs at all rostro-caudal levels. State-dependent reflex reversal was observed previously in both vertebrates (Pearson and Collins, 1993; Forsberg et al., 1977) and invertebrates (Akay et al., 2007; Hellekes et al., 2012).

Supraspinal control of SRN-mediated reflexes

We found that even stimulation of sites of the trigeminal nerve, which did not evoke any type of escape behavior, caused reversal of the reflex response to bending, i.e., the response was convex and concave before and during the stimulation, respectively. These results suggest the existence of a specific population of supraspinal neurons transmitting commands for reflex reversal, which could be activated separately or in combination with other populations of supraspinal neurons initiating a specific type of escape behavior. Co-activation of RS neurons with different functions during FFS was demonstrated earlier (Zelenin, 2011).

Reticulospinal (RS) system is the main descending system in the lamprey (Rovainen, 1979). We have found two groups of RS neurons activated by trigeminal nerve stimulation causing reflex reversal. Group 1 neurons were activated by stimulation of both ipsi- and contralateral trigeminal nerves, and Group 2 neurons – by stimulation of contralateral trigeminal nerve only. The majority of Group 1 and 2 neurons were located in MRRN: Group 1 neurons were more numerous in the rostral part, and Group 2 neurons – in the caudal part of MRRN. We have shown that electrical stimulation of most sites within MRRN evoked reflex

reversal. Group 1 and Group 2 neurons were also activated during different forms of escape behavior. These results allow us to suggest that Group 1 and Group 2 neurons transmit commands for reversal of the SRN-mediated spinal reflexes during escape behavior.

We found that NMDA receptors contribute to formation of supraspinal commands causing reflex reversal, since blocking NMDA receptors by bath-application of AP-5 to the brainstem or by local application to MRRN either abolished or strongly weakened reflex reversal. NMDA receptors can mediate the synaptic transmission from trigeminal afferents to RS neurons at different locations - either at the sensory relay neurons or at the RS neurons (Viana Di Prisco et al., 1995; 1997; 2005).

Reflex reversal caused by trigeminal nerve stimulation persisted after splitting the spinal cord along its midline, suggesting that modifications in the unilateral spinal network cause the reflex reversal. Figure 12B,C shows a hypothetical circuit underlying the reversal of SRN-mediated reflexes (see Figure legend for explanation). In *Paper VI*, we have shown that concave responses to bending in rostral MNs (observed in *in vitro* spinal cord preparation during FFS evoked by D-glutamate) were reversed to convex responses after longitudinal splitting the spinal cord. Thus, modifications in the unilateral spinal network (underlying generation of concave responses in rostral MNs to bending) could also be caused by signals transmitted from the contralateral hemicord.

We have also found that RS neurons in all reticular nuclei receive sensory feedback from SRNs signaling body configuration. Similar feedback from limb afferents to RS neurons exists also in mammals (Orlovsky et al., 1999), suggesting similarities in functional organization of motor control in lower and higher vertebrates.

About half of Group 1 and Group 2 RS neurons received SRN-inputs. During reflex reversal, activity of some of these neurons was not affected by signals from SRNs, suggesting that their function was a tonic activation of the reflex pathways underlying the reversal. Activity of some Group 1 and Group 2 neurons was still modulated by signals from SRNs during reflex reversal, suggesting that these neurons modulate the efficacy of reversed reflexes in accordance with the current body configuration. We never found RS neurons receiving SRN-input only during reflex reversal, suggesting that RS neurons do not receive feedback from the reflex pathways responsible for the reversal (e.g., inputs from IN2 in Fig. 12C).

Functional role of SRN-mediated reflexes during different motor behaviors

Lampreys use FFS for long-distance migrations. For the high velocity body progression during FFS, MNs on the convex side of undulating body are activated (Islam et al., 2006) in order to slow down the current body bending, and to initiate the bending on the other side. We have found that passive bending in yaw plane evoked convex response during FFS, suggesting that the SRN-mediated reflex responses amplify the motor outputs, and thus promote the generation of undulations. SRN-mediated reflexes caused by bending the mid-body region in the pitch plane (i.e., activation of dMNs by ventral bending, and vMNs – by dorsal bending) reduce the bend, thus contributing to maintenance of rectilinear body axis in this plane during FFS.

Escape behavior is characterized by large body undulations, which are less efficient in body progression but advantageous in getting rid of threats. To generate large body undulations, MNs on the concave side of the body are activated (Islam et al., 2006; 2008). We have found that passive bending evoked concave response during escape behavior (SFS, BS, and lateral turns), suggesting that the SRN-mediated reflex responses contribute to

augmentation of the body undulations amplitude during escape. Thus, reversal of SRN-mediated reflex response to body bending is aimed at reinforcement of movements generated in each specific behavior.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Maintenance of body orientation in space and control of body configuration (postural control) are vital motor functions. While the functional organization of the postural system was analyzed in quite detail, the knowledge about the underlying neuronal mechanisms is quite scarce. In the present study, neuronal mechanisms contributing to the lateral stability in the rabbit and to the control of body configuration in the lamprey were investigated.

In the rabbit we have characterized spinal neurons (presumed interneurons) contributing to generation of postural limb reflexes (PLRs), which represent a substantial component of the postural system securing lateral stability. PLR-related neurons were classified into several groups according to the phase of their activity during PLRs and according to the source of sensory inputs determining this phase. Different parameters of their activity have been characterized. A hypothesis about the role of different types and groups of PLR-related neurons for the trunk stabilization in different planes has been proposed. It was suggested that motor effects of each individual PLR-related neuron counteracts postural disturbances that activate the neuron. To test this hypothesis, in future studies one needs to correlate the sensory input to the PLR-related neuron with its motor effect.

We have demonstrated that elimination of supraspinal drive causes a dramatic reduction in the activity of spinal neurons, which correlates with disappearance of PLRs. This finding suggests the crucial role of supraspinal signals in PLRs generation. The neurons generating the spinal component of PLRs, as well as the population of neurons presumably responsible for disappearance of PLRs after spinalization have been revealed.

We have shown that asymmetry in tonic supraspinal drive caused by continuous GVS can (depending on GVS polarity) increase the gain in postural reflex pathways of one limb and simultaneously decrease the gain in postural reflex pathways of the contralateral limb. Two groups of PLR-related neurons, presumably mediating the effect of GVS on PLRs, have been revealed. It was suggested that lateral stability is maintained due to interaction of two antagonistic PLRs, and GVS causes a shift of the set-point of the postural control system through the change of the gain in antagonistic reflex chains resulting in stabilization of the orientation with a lateral sway. A similar principle of balance control, as well as a similar mechanism underlying a change of stabilized orientation were found in simpler animals—a mollusk *Clione* and a lower vertebrate, the lamprey (Deliagina et al., 1998; Deliagina and Fagerstedt, 2000).

We have demonstrated that a GVS-based artificial feedback can restore postural function (balance control) in rabbits with postural deficit. We suggest that such a control system can compensate for the loss of lateral stability of various etiologies.

PLR-related spinal neurons characterized in the present study do not represent a homogeneous group, and most likely, contain different types of segmental and propriospinal interneurons, as well as neurons of ascending pathways. To reveal the identity (Ia interneurons, Renshaw cells, etc.) of different groups and types of the PLR-related neurons characterized in the present study is an important goal of future investigations. Another important goal is the analysis of supraspinal postural commands, whose critical importance for generation of functional PLRs has been demonstrated in the present study. The analysis of GVS effects on these commands transmitted by different descending systems is also important for the selection

of patients for whom the GVS-based artificial feedback could be beneficial to improve the postural functions. Testing the efficacy of the GVS-based artificial feedback to improve the postural functions in animal models with postural deficits of different etiologies is also an important direction of future studies.

In lampreys, we analyzed spinal reflexes mediated by stretch receptor neurons (SRNs) during different forms of motor behavior, which are characterized by different amplitude of body flexion (body configuration). We have demonstrated that SRN-mediated reflexes observed during fast forward swimming (FFS) are reversed during different forms of escape behavior. We suggested that modifications of SRN-mediated reflex responses will result in the decrease and increase of the lateral bending amplitude during FFS and escape behaviors, respectively, thus reinforcing movements generated in each specific behavior. Such task-specific reflex reversal was also described in other species (Pearson and Collins, 1993; Hellekes et al., 2012).

We have found that the SRN-mediated reflex reversal is evoked by a unilateral supraspinal command causing modifications in the spinal network processing SRN-signals, which is located in the ipsilateral hemicord. A population of reticulospinal (RS) neurons (located in the middle rhombencephalic reticular nuclei) presumably transmitting this supraspinal command has been revealed. We have shown that as in mammals (Orlovsky et al., 1999), RS neurons in lampreys receive sensory information about body configuration (signals from SRNs). However, this sensory feedback is not critical for SRN-mediated reflex reversal.

We have demonstrated that not only FFS (Rovainen, 1976; McClellan and Grillner, 1983), but also all forms of escape behavior are generated by spinal networks. To reveal organization of spinal networks processing the SRN-signals in context of a particular motor behavior, as well as interaction of these networks with those generating the basic pattern of this behavior, is the goal of future studies. Another important question for future studies is to clarify if the population of RS neurons controlling SRN-mediated reflexes during a particular motor behavior, and the population of RS neurons initiating this behavior, are separate populations, or they partly overlap.

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