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Effects of galvanic vestibular stimulation on postural limb reflexes and neurons of spinal postural network

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Hsu LJ, Zelenin PV, Orlovsky GN, Deliagina TG. Effects of galvanic vestibular stimulation on postural limb reflexes and neurons of spinal postural network. *J Neurophysiol* 108: 300–313, 2012. First published April 18, 2012; doi:10.1152/jn.00041.2012.—Quadrupeds maintain the dorsal side up body orientation due to the activity of the postural control system driven by limb mechanoreceptors. Binaural galvanic vestibular stimulation (GVS) causes a lateral body sway toward the anode. Previously, we have shown that this new position is actively stabilized, suggesting that GVS changes a set point in the reflex mechanisms controlling body posture. The aim of the present study was to reveal the underlying neuronal mechanisms. Experiments were performed on decerebrate rabbits. The vertebral column was rigidly fixed, whereas hindlimbs were positioned on a platform. Periodic lateral tilts of the platform caused postural limb reflexes (PLRs): activation of extensors in the loaded and flexing limb and a decrease in extensor activity in the opposite (unloaded and extending) limb. Putative spinal interneurons were recorded in *segments L4–L5* during PLRs, with and without GVS. We have found that GVS enhanced PLRs on the cathode side and reduced them on the anode side. This asymmetry in PLRs can account for changes in the stabilized body orientation observed in normal rabbits subjected to continuous GVS. Responses to platform tilts (frequency modulation) were observed in 106 spinal neurons, suggesting that they can contribute to PLR generation. Two neuron groups were active in opposite phases of the tilt cycle of the ipsi-limb: F-neurons in the flexion phase, and E-neurons in the extension phase. Neurons were driven mainly by afferent input from the ipsi-limb. If one supposes that F- and E-neurons contribute, respectively, to excitation and inhibition of extensor motoneurons, one can expect that the pattern of response to GVS in F-neurons will be similar to that in extensor muscles, whereas E-neurons will have an opposite pattern. We have found that ~40% of all modulated neurons meet this condition, suggesting that they contribute to the generation of PLRs and to the GVS-caused changes in PLRs.

posture; balance; sensory feedback; rabbit

TERRESTRIAL QUADRUPEDS MAINTAIN the dorsal side up trunk orientation during standing, due to activity of the postural system. This closed-loop control system is driven by sensory feedback signals and compensates for deviations from the stabilized orientation by producing corrective motor responses (Beloozerova et al. 2003; Deliagina et al. 2006a; Horak and Macpherson 1996). Postural corrections are generated mainly on the basis of somatosensory information provided by limb mechanoreceptors (Beloozerova et al. 2003; Deliagina et al. 2000, 2006b; Inglis and Macpherson 1995; Stapley and Drew 2009). Visual and vestibular information can also be used in some postural tasks (Horak and Macpherson 1996).

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The postural system stabilizing the trunk also receives input from the vestibular organs that provide information about head orientation and movement. These specific signals are presented as a modulation of the background activity of vestibular afferents [see Wilson and Melvill Jones (1979) for a review]. It has been shown that specific vestibular signals are not essential for the generation of postural corrections in the majority of studied conditions (Beloozerova et al. 2003, Deliagina et al. 2000, 2006b; Inglis and Macpherson 1995). By contrast, unspecific tonic inflow from the 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 level of excitability of extensor motoneurons during standing and therefore, a high tonus in the extensor limb muscles, which is a necessary condition for supporting the body during standing (Duysens et al. 2000). The tonic descending drive can also activate spinal mechanisms for postural corrections (Musienko et al. 2010). The overall goal of the present study was to characterize the effect of tonic vestibular drive on the postural mechanisms, maintaining the lateral stability.

To manipulate the tonic descending drive to spinal postural mechanisms, we used the method of binaural galvanic vestibular stimulation (GVS) (Séverac Cauquil et al. 2000). The GVS is a kind of transcranial direct current stimulation, which 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). These changes in the vestibular input, through brainstem-spinal descending systems [mainly the vestibulospinal tract, as demonstrated in cats by Muto et al. (1995)], exert a strong influence on the subject's posture. The response to binaural GVS in humans includes reciprocal changes in the activity of trunk and limb muscles on the two sides, resulting in a lateral body sway toward the anode (e.g., Séverac Cauquil et al. 2000). A similar reaction to binaural GVS (a lateral sway toward the anode) was observed in intact rabbits (Beloozerova et al. 2003; Gorgiladze 2004). It was also shown that the new orientation of the animal in the transverse plane (caused by a continuous GVS) is actively stabilized. It was suggested that GVS could change a set point of the antagonistic reflex mechanisms controlling the body posture (Beloozerova et al. 2003). The aim of the present study was to test this hypothesis and to reveal the underlying neuronal mechanisms.

A number of studies presented evidence that some important postural mechanisms for trunk stabilization persist in decere-

brate animals (Bard and Macht 1958; Honeycutt et al. 2009; Honeycutt and Nichols 2010; Musienko et al. 2008), and therefore, this preparation can be used for the analysis of these mechanisms. In a recent study on decerebrate rabbits (Musienko et al. 2010), we have characterized postural limb reflexes (PLRs) and suggested that PLRs in intact animals contribute to postural reactions caused by lateral tilts of the support surface. In both cases, the reaction of limb muscles was caused by tilt-related sensory input, mainly from the same limb (Musienko et al. 2010).

In the present study, first, we investigated the effects of continuous GVS on PLRs in decerebrate rabbits. Second, we recorded individual spinal neurons (presumably interneurons) along with PLRs. Correlation of activity of a neuron with PLRs suggested participation of the neuron in the PLR generation. Third, we studied the effects of GVS on the activity of such neurons during PLRs, using binaural GVS of different configurations (with the anode or with the cathode ipsilateral to a neuron). Fourth, to reveal spinal neurons, presumably contributing to the GVS-caused changes of PLRs, the effects of GVS on individual spinal neurons and on PLRs were compared.

We have found that GVS strongly affects PLRs: it enhances PLRs on the cathode side and reduces PLRs on the anode side. This finding supports the hypothesis that continuous GVS affects the stabilized trunk orientation by causing dissimilar changes of gain in the antagonistic PLRs (Beloozerova et al. 2003). Two groups of neurons differing in the phase of their activity during PLRs were found. Comparison of GVS effects on these neurons and on PLRs has shown that ~40% of neurons in each group could potentially contribute to the generation of PLRs and to the GVS-caused regulation of PLR magnitude.

A brief account of part of this study has been published in abstract form (Hsu et al. 2011).

METHODS

Experiments were carried out on 11 adult New Zealand rabbits (weighing 2.5–3.5 kg). All experiments were conducted with approval of the local ethical committee (Norra Djurförsöksetiska Nämden) in Stockholm.

Surgical procedures. The animal was injected with propofol (average dose, 10 mg/kg, administered intravenously) for induction of anesthesia, which was continued on isoflurane (1.5–2.5%), delivered in O_2. The trachea was cannulated. For all subsequent procedures, the animal was positioned in a metal frame, and its head and vertebral column were rigidly fixed (Fig. 1*A*). The spinal cord was exposed by laminectomy at L4 and L5 segments. The dura mater was left intact, except for small holes (~1 mm^2), through which recording electrodes were inserted. Up to eight bipolar electromyographic (EMG) electrodes were implanted into the hindlimb muscles. The EMGs from gastrocnemius lateralis (Gast; ankle extensor), vastus lateralis (Vast; knee extensor), gracilis (Grac; hip adductor acting as hip extensor), and semitendinosus (St; hip extensor and knee flexor) were recorded bilaterally.

The animal was then decerebrated at the precollicular-postmammilary level (Musienko et al. 2008). After decerebration, the anesthesia was discontinued. During the experiment, the rectal temperature and blood pressure were monitored continuously and kept at 37–38°C and >90 mmHg, respectively. Recording was not started <1 h after cessation of anesthesia. The experiments were terminated by a lethal dose of anesthetic (pentobarbital sodium).

Experimental design. The experimental design is shown in Fig. 1*A*. The head and vertebral column were rigidly fixed. The forelimbs were suspended in a hammock. The hindlimbs were hemiflexed and positioned on a horizontal platform consisting of two parts: the right and the left platforms. The limb configuration was similar to that observed in the normal rabbits during standing, with the hip, knee, and ankle angles ~25°, ~60°, and ~50°, respectively (Beloozerova et al. 2003). The distal part of each hindlimb was gently fastened to the platform so that the interfeet distance (11 cm) was similar to that observed in normal rabbits of this size during standing.

The platform, as a whole or the right and left platforms separately, could be tilted periodically by rotation about the medial axis (Fig. 1*A–D*), which led to close-to-vertical displacements of the distal point of the moving limb. Since the position of the hip joint was fixed, tilts of the platform caused loading and flexion of the limb on the platform side moving up and unloading and extension of the opposite limb. These anti-phase flexion/extension (F/E) movements of the two limbs were similar to those observed in the normal rabbits maintaining balance on the tilting platform (Beloozerova et al. 2003).

A time profile of platform tilts (and therefore, of foot displacements) was trapezoidal (Fig. 1*E*), which revealed both dynamic and static components of the PLRs. With tilts from 20° left (L) to 20° right (R), the distance between the two extreme (up and down) foot positions was 5 cm (Fig. 1*F*); these positions were symmetrical in relation to the horizontal platform. Each position was maintained for ~2 s, and a transition between them lasted for ~0.5 s. As demonstrated by video recording, these imposed foot displacements caused F/E movement in hip, knee, and ankle joints, with a magnitude of ~10° (Fig. 1*F*). The trapezoid platform tilts were repeated with a period of ~5 s. The tilt angle was monitored by a mechanical sensor, scaled, and recorded as changes in the vertical position of the feet (Fig. 1*E*). The contact forces under the limbs were measured by means of force sensors (Force R and Force L; Fig. 1*B*).

GVS. Binaural configuration of GVS was used; i.e., a constant current was passed between the electrodes (0.5 cm^2 silver/silver-chloride), inserted into the right and left ears. This current activated vestibular fibers on the cathode side and inhibited those on the anode side (Minor and Goldberg 1991). We used two types of stimulation: *1*) the current was switched on, maintained for 15–30 s, and then switched off; *2*) instead of switching the current off, its direction was reversed. The strength of GVS (1–2 mA) was smaller than needed for the maximal motor response (3–4 mA).

Experimental protocol and recordings. Spinal neurons were recorded extracellularly in the L4 and L5 segments, using commercially available varnish-insulated tungsten electrodes (75 μm shaft diameter; FHC, Bowdoin, ME) with an impedance of 4–7 MΩ. We tried to explore evenly the whole cross-section of the gray matter, except for the area of motor nuclei (Portal et al. 1991). The lateral and vertical coordinates of each neuron were marked on the map of the spinal cord cross-section (Shek et al. 1986). Individual neurons were recorded during PLRs caused by periodical tilts of the whole platform, along with EMGs and ground reaction forces. If activity of the neuron was correlated with PLRs (i.e., it was modulated by platform tilts), it was recorded during PLRs 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. The GVS, with a particular configuration, was applied two to four times for 15–30 s. In addition, in a part of the neurons, their responses to separate tilts of the right and left platform were recorded.

Data analysis. Signals from the microelectrode (neuronal activity), the EMG electrodes, as well as the platform position and force sensors were amplified; digitized with sampling frequencies of 30 kHz (microelectrode), 5 kHz (EMGs), and 1 kHz (sensors); displayed on the screen; and saved to a computer disc by means of a data acquisition and analysis system (Power-1401/Spike2; Cambridge Electronic Design, Cambridge, UK). The EMG signals were rectified and smoothed (time constant, 100 ms).

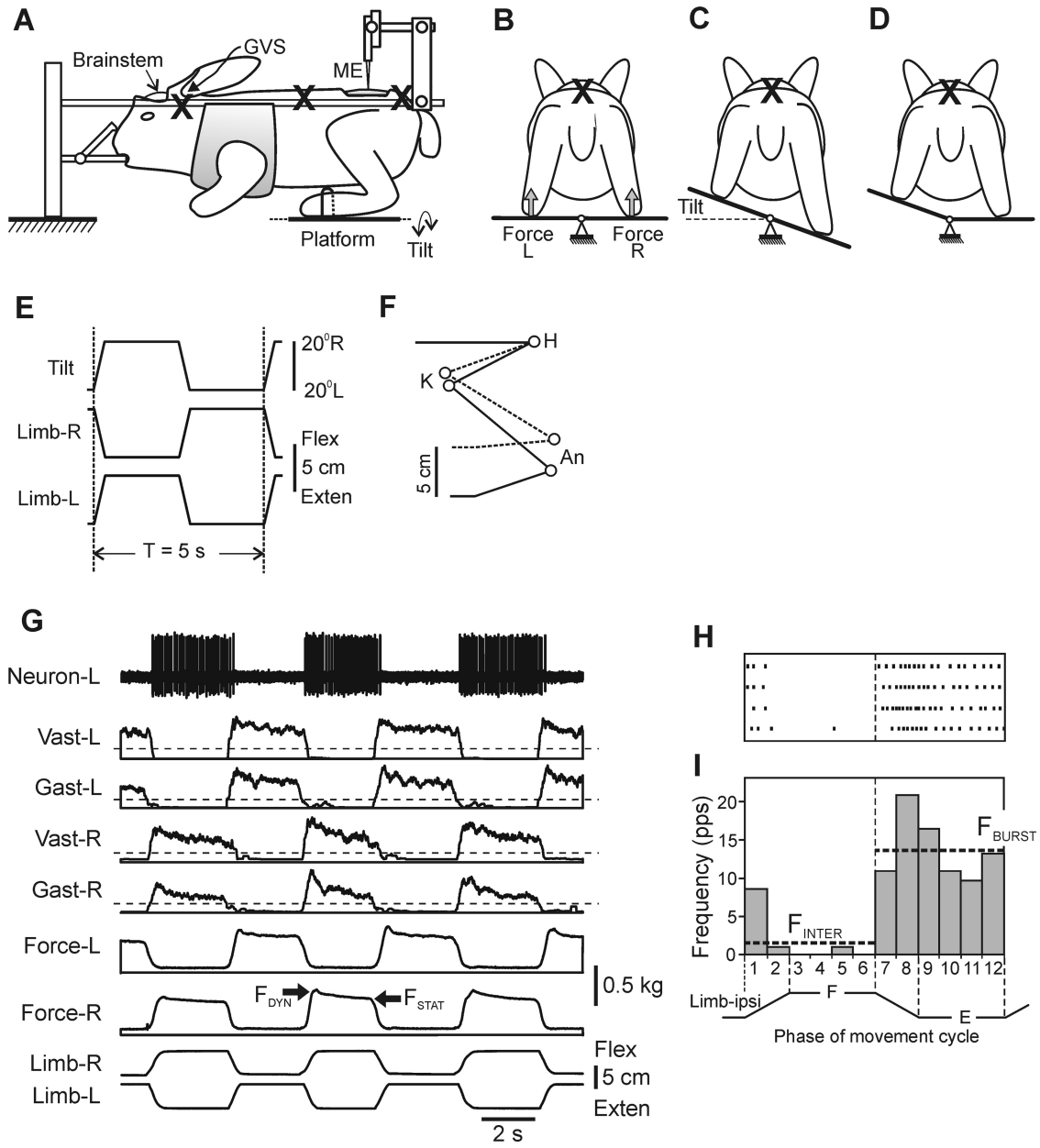

Fig. 1. Experimental design. *A–D*: the decerebrate rabbit was positioned in a rigid frame, and its head and vertebral column were firmly fixed (points of fixation are indicated by X). For testing postural limb reflexes (PLRs), the hindlimbs were positioned on a platform (*B*), which could be tilted periodically in the transverse plane as a whole (*C*) or its right (R) and left (L) parts separately (*D*). Tilt of the whole platform caused flexion of 1 limb and extension of the other limb. The contact forces under the left and right limbs were measured by the force sensors (Force L and Force R). Galvanic vestibular stimulation (GVS) was performed by means of the electrodes positioned in the ears. Activity of spinal neurons from L4–L5 was recorded by means of the microelectrode (ME). *E*: time profile (T) of the flexion (Flex)/extension [Exten; (F/E)] movements of the right and left limb caused by tilts of the whole platform. *F*: limb configuration at the 2 extreme platform positions (H, K, and An: hip, knee, and ankle joint, respectively). *G*: an example of recording the neuronal activity during PRLs. Extensor electromyographic (EMG) and force responses were caused by anti-phase F/E movements of the 2 limbs. The responses usually consisted of the dynamic and static components, which are indicated on the Force R trace (F_{DYN} was measured at the peak; F_{STAT} at the onset of extension, respectively). The EMGs of gastrocnemius lateralis (Gast) and vastus lateralis (Vast) of the left and right limbs were recorded. The levels of EMG activity in these muscles, after the limbs were positioned on the horizontal platform, are shown by dashed lines. The neuron was located on the left side of the spinal cord and was activated during flexion of the right limb. *H*: a raster of responses of the neuron in 4 sequential movement cycles of the left limb. *I*: a histogram of spike activity of the neuron in different parts (1–12) of the cycle. Two-level approximation of the neuronal activity during the movement cycle is shown; burst frequency (F_{BURST}) and interburst frequency (F_{INTER}) are the mean frequencies during limb extension (*bins 7–12*) and limb flexion (*bins 1–6*), respectively. pps, pulses/s.

The waveform analysis of neuronal activity was used to discriminate and identify the spikes of a single neuron by using the Spike2 waveform-matching algorithm. Only neurons with a stable response and spike shape were used for analysis.

Processing of neuronal activity is illustrated in Fig. 1, *G–I*, for a neuron recorded on the left side of the spinal cord and tested by tilting of the whole platform. In this test, we considered the activity of all individual neurons in the movement cycle of the ipsilateral limb; the onset of limb flexion was taken as the cycle onset. With tilting of only the ipsilateral platform, the cycle started at the onset of flexion of the ipsilateral limb. With tilting of only the contralateral platform, the cycle started at the onset of extension of the contralateral limb. For

the neuron shown in Fig. 1*G*, the raster of the spike activity in sequential cycles was obtained (Fig. 1*H*). The cycle was divided into 12 bins (Fig. 1*I*). *Bins 1–2* corresponded to flexion of the ipsilateral limb; *bins 3–6*, to maintenance of the flexed position; *bins 7–8*, to extension of the limb; and *bins 9–12*, to maintenance of the extended position. The firing frequency in each bin was calculated and averaged over the identical bins in all cycles at a given condition, and the phase histogram was generated (Fig. 1*I*). The mean frequency during flexion of ipsi-limb (*bins 1–6*) and that during extension (*bins 7–12*) was compared. The larger and the smaller values were named the burst frequency (F_{BURST}) and the interburst frequency (F_{INTER}), respectively. The neuron was considered as modulated by tilts if the difference between the mean F_{BURST} and mean F_{INTER} at control condition were statistically significant. For these neurons, we calculated the coefficient of frequency modulation: $K_{MOD} = 1 - F_{INTER}/F_{BURST}$. All quantitative data in this study are presented as the mean ± SE. Student's *t*-test was used to characterize the statistical significance when comparing different means; the significance level was set at $P = 0.05$.

RESULTS

Effects of GVS on PLRs. Prior to tilting, all tested rabbits ($n = 11$) had extensor activity in both hindlimbs and developed contact forces of 0.3–0.5 kg [for a description of the general motor status of these decerebrate animals, see Musienko et al. (2008)]. Tilts of the platform caused PLRs with a pattern similar to that described earlier (Musienko et al. 2010). As illustrated in Fig. 1*G*, flexion of each of the limbs was accompanied by activation of its extensor muscles, Gast and Vast, as well as by an increase in the force developed by the limb. By contrast, extension of the limb caused an inactivation of its extensors and a decrease in the force value. As shown in our previous study (Musienko et al. 2010), PLRs are caused mainly by tilt-related somatosensory information from the ipsilateral limb. This information can originate from muscle spindles, Golgi tendon organs, load receptors of the foot sole, and others [for discussion, see Musienko et al. (2008, 2010)]. The responses during limb flexion contained both dynamic and static components (see also Fig. 5*A*). The average values of the dynamic and static components of the force response were 0.69 ± 0.06 kg and 0.57 ± 0.05 kg, respectively (Fig. 2*F*). They were similar to those observed in the previous study (Musienko et al. 2010).

Effects of GVS with different polarity on the PLRs are shown in Fig. 2. In Fig. 2*A*, we repetitively changed the direction of injected current so that the anode was on the left side, and cathode was on the right side during *time periods 1*, *3*, and *5*; the position of anode and cathode was opposite to that during *periods 2* and *4*. One can see that a change of the current direction strongly affected the magnitude of PLRs generated by the limbs—the EMG and force responses were much larger with the ipsilateral cathode than with the ipsilateral anode. The effects were similar in all recorded extensor muscles (Grac, Vast, and Gast), as well as in a hip extensor-knee flexor St. These effects are seen in more detail after averaging the EMGs (Fig. 2, *B–E*).

We also compared the PLR values with GVS with those without GVS (control). It was found that the cathodal GVS caused an increase of PLRs compared with control, whereas the anodal GVS caused their decrease (see e.g., Fig. 5*A*). Similar results were obtained in all tested rabbits. Figure 2*F* shows the effects of GVS on the force responses averaged over all rabbits. Both dynamic and static components of the response (indicated in Fig. 1*G*) were larger than in control when the cathode was ipsilateral to the limb, and they were smaller than in control when the anode was ipsilateral to the limb. A relative difference between the forces under the two GVS conditions was ~40%. The GVS produced no significant effect on the force generated at the extended configuration of the limb.

Activity of spinal neurons during PLRs. Putative spinal interneurons were recorded in L4 and L5 segments from the area outside of the motor nuclei (Portal et al. 1991) in six rabbits. All neurons were recorded during periodical tilts of the whole platform, which caused passive F/E limb movements, resulting in generation of PLRs. Activity of a neuron was considered modulated (i.e., correlated with PLRs) if its mean firing frequencies during flexion and during extension of the ipsilateral limb were statistically different (e.g., Figs. 1*G* and 5*A*). The nonmodulated neurons constituted 25% of all recorded neurons. One should note, however, that the proportion of nonmodulated neurons was underestimated, since we were focused on recording the modulated neurons, and the neurons, whose discharge frequencies seemed nonmodulated when listening on the sound monitor, were not recorded. Only modulated neurons were subjected to analysis in the present study. Positions of these neurons on the cross-section of the spinal cord at L4 and L5 levels are shown in Fig. 3, *A* and *B*.

Altogether, 106 modulated neurons were recorded. According to the phase of response in a tilt cycle of the ipsilateral limb, we divided these neurons into two groups: F-neurons had a higher firing frequency in the flexion phase of the ipsilateral limb than in its extension phase (as the neuron in Fig. 5*A*), whereas E-neurons had a higher frequency in the extension phase of the ipsilateral limb than in its flexion phase (as the neuron in Fig. 1*G*). The neurons had opposite phases of responses in the movement cycle of the contralateral limb.

F-neurons and E-neurons constituted 54% and 46% of all modulated neurons, respectively. Figure 3, *C* and *D*, shows the averaged response to tilts for all F-neurons and all E-neurons, respectively. On the average, the F_{BURST} was 17.9 ± 2.0 pulses/s (pps) in the F-group and 13.8 ± 1.3 pps in the E-group, whereas the F_{INTER} frequency was 5.8 ± 1.1 pps and 4.3 ± 0.8 pps, respectively. In each group, the depth of modulation in different neurons was rather diverse (Fig. 3, *E* and *F*), but the majority of neurons was deeply modulated ($K_{MOD} > 60\%$).

The distribution of F-neurons and E-neurons on the cross-section of the spinal cord is shown in Fig. 3, *A* and *B*. To characterize the distribution, we divided the cross-section into three zones (*1–3*) and calculated the relative number of F-neurons and E-neurons in each of the zones. As shown in Fig. 3*G*, F-neurons prevailed in *zones 1* and *2*, whereas E-neurons prevailed in *zone 3*. However, our sample of neurons was not sufficient for the statistical analysis of these regional differences.

To estimate contribution of sensory inputs from the ipsilateral and contralateral limbs to tilt-related modulation of neurons, in a part of modulated neurons (48 out of 106), we recorded their responses, not only to tilts of the whole platform but also to separate tilts of its right and left parts (ipsi- and contralateral platforms in relation to a neuron). We observed four different patterns of responses in these three tests, which

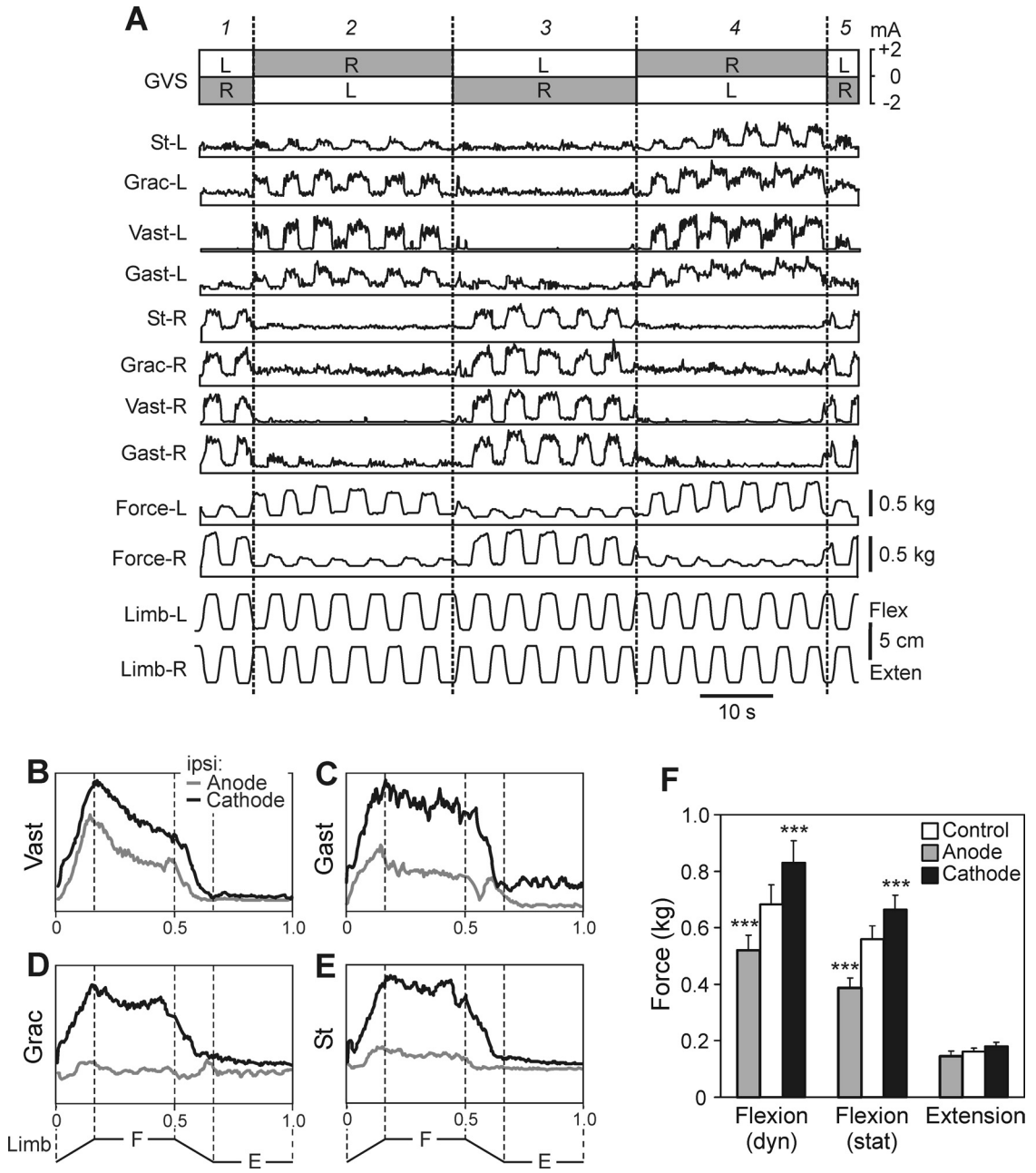

Fig. 2. Effects of GVS on PLRs. *A*: a representative example of GVS effects on PLRs. The configuration of GVS was changed repetitively so that the anode was on the left side and cathode was on the right side during *time periods 1*, *3*, and *5*; the position of anode and cathode was opposite during *periods 2* and *4*. The reflex responses of the limb (the EMG value in 8 tested muscles and the force magnitude) were much larger when the cathode was ipsilateral to the limb than when the anode was ipsilateral to this limb. St, semitendinosus; Grac, gracilis. *B–E*: comparison of averaged responses ($n = 5$) in Vast (*B*), Gast (*C*), Grac (*D*), and St (*E*) caused by GVS with either anode or cathode ipsilateral to the limb. *F*: comparison of force magnitude (mean ± SE) produced by a limb during flexion and extension at different conditions: without GVS (Control) and during GVS with anode or cathode ipsilateral to the limb ($N = 11$; $n = 29$). F_{DYN} and F_{STAT} components of the force response are shown separately. Indication of significance level: $***P < 0.001$.

are illustrated in Fig. 4, *A–D*. The neuron in Fig. 4*A* had the same phase and amplitude of response when the whole platform and only the ipsi-platform were tilted. The neuron did not respond to tilts of the contra-platform. Thus its modulation was determined by tilt-related somatosensory input coming from only the ipsi-limb. This can be an excitatory input coming when the limb is extended or inhibitory input (coming to a tonically active neuron) when the limb is flexed. Similar results were obtained for the majority (54%) of tested neurons (Fig. 4*E*). In 15% of neurons (Fig. 4*E*), modulation was determined by sensory signals from only the contra-limb, as in the neuron shown in Fig. 4*D*. In 33% of neurons, tilt-related sensory inputs from both limbs were revealed. Figure 4*B* shows a neuron that responded to flexion of the ipsi-limb during tilts of the ipsi-platform and to extension of the contra-limb during tilts of the contra-platform. Thus during tilts of the whole platform, this neuron received complementary sensory inputs from both limbs, which contributed to activation of the neuron during flexion of the ipsi-limb. Six neurons (13%) with complementary inputs from both limbs were found (Fig. 4*E*); in

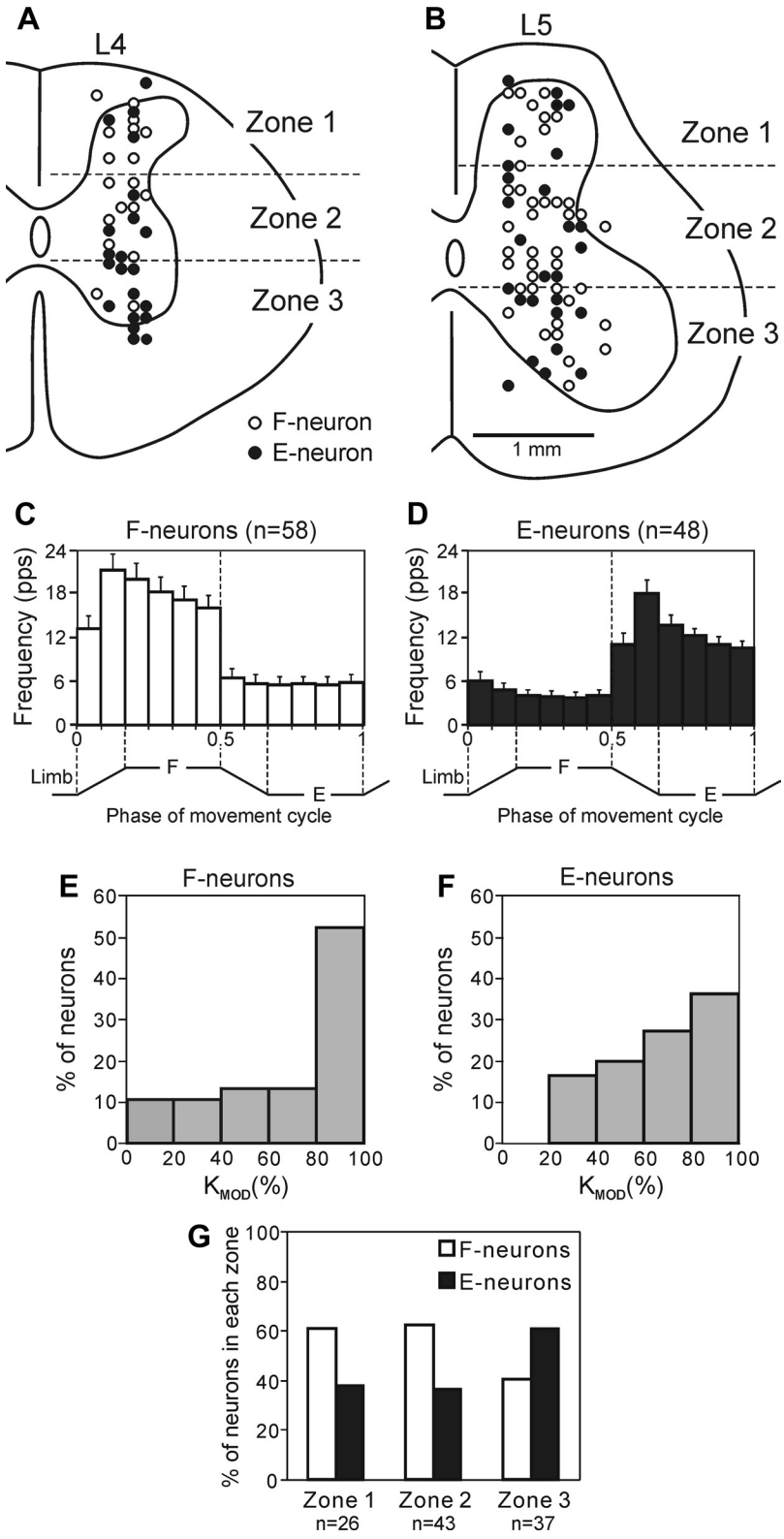

Fig. 3. Population characteristics of 2 groups of spinal neurons (F and E) modulated by tilts. *A* and *B*: positions of neurons on the cross-section of the spinal cord in the *segments L4* (*A*) and *L5* (*B*). Three zones of gray matter are indicated: the dorsal (*1*), intermediate (*2*), and ventral (*3*) ones. *C* and *D*: averaged response frequency in different phases of movement cycle of the ipsilateral limb (mean ± SE) for F-neurons (*C*) and E-neurons (*D*). *E* and *F*: distribution of the coefficient of modulation (K_{MOD}) for F-neurons (*E*) and for E-neurons (*F*). *G*: relative number of F-neurons and E-neurons in each of the 3 zones of gray matter.

four of them (8%), input from the ipsi-limb was stronger than input from the contra-limb, and in two neurons (4%), input from the contra-limb prevailed over input from the ipsi-limb. The neuron shown in Fig. 4*C* received opposite tilt-related sensory inputs from the ipsi- and contra-limb. It responded to flexion of the ipsi-limb during tilts of the ipsi-platform and to flexion of the contra-limb during tilts on the contra-platform. However, during tilts of the whole platform, the neuron was activated during flexion of the ipsi-limb, suggesting that the phase of its modulation was determined by tilt-related sensory input from the ipsi-limb. Similar results were obtained for 18% of neurons (Fig. 4*E*).

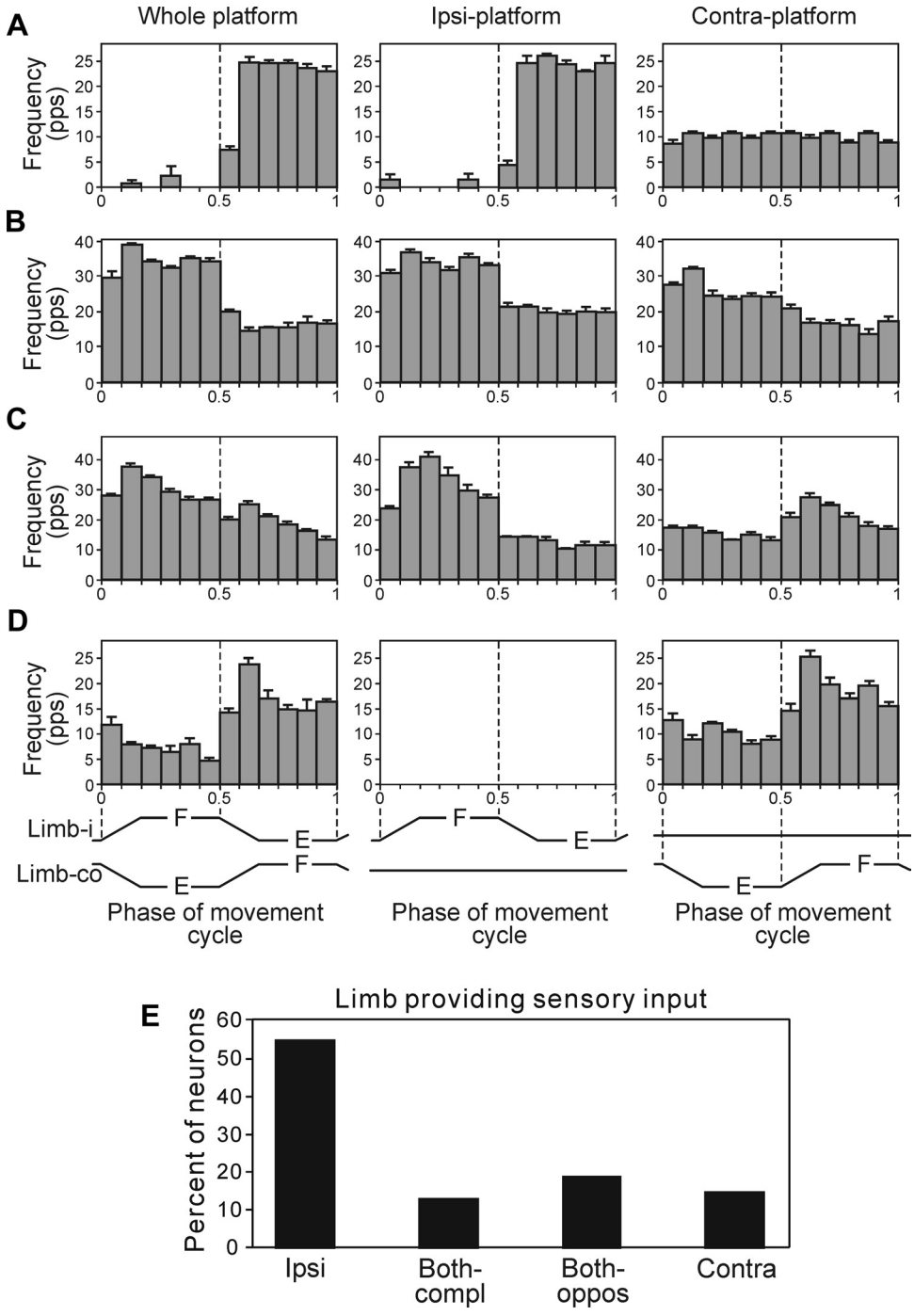

Fig. 4. Different types of convergence of tilt-related sensory inputs from different limbs on spinal neurons. *A–D*: examples of 4 neurons with different patterns of responses to tilts of the whole platform, ipsi-platform, and contra-platform. *A*: the neuron has identical responses to tilts of the whole platform and ipsi-platform; it has no response to tilts of the contra-platform. *B*: the neuron has complementary responses (compl) to tilts of the ipsi-platform and of the contra-platform. *C*: the neuron has opposite responses (oppos) to tilts of the ipsi-platform and of the contra-platform. *D*: the neuron has identical responses to tilts of the whole platform and contra-platform; it has no response to tilts of the ipsi-platform. *E*: the proportion of neurons with different sources of modulation.

To summarize, in the majority of neurons (81%), the phase of response to tilts of the whole platform was determined by tilt-related somatosensory input from the ipsilateral limb. Such neurons were equally represented in F- and E-groups (81% and 82%, respectively).

Effects of GVS on modulated spinal neurons. We found that GVS affected 61% of modulated neurons. A representative example of GVS effects is shown in Fig. 5*A* for a neuron recorded on the right side of the spinal cord. Without GVS (control; *period 1* of the recording), this neuron was active during flexion of the right limb and classified as an F-neuron. When the anode was on the right side (*period 2*), GVS caused a decrease in the activity of the neuron compared with control. When the polarity was reversed, i.e., the cathode was on the right side (*period 3*), GVS caused an increase in the activity of the neuron compared with control. Thus GVS allowed us to regulate (up and down) the neuronal responses to tilts. In Fig. 5*A*, one can also see that the effects of GVS on this F-neuron (activation with the ipsilateral cathode and inactivation with the ipsilateral anode) were the same as the effects of GVS on the extensor EMGs and contact force of the ipsilateral limb.

To characterize the effects of GVS on individual neurons, we compared (by using a *t*-test, with $P < 0.05$) the mean F_{BURST} of each neuron under two conditions: with the anode or the cathode ipsilateral to a neuron. In the graphs for F-neurons (Fig. 5*B*) and for E-neurons (Fig. 5*C*), each neuron is presented

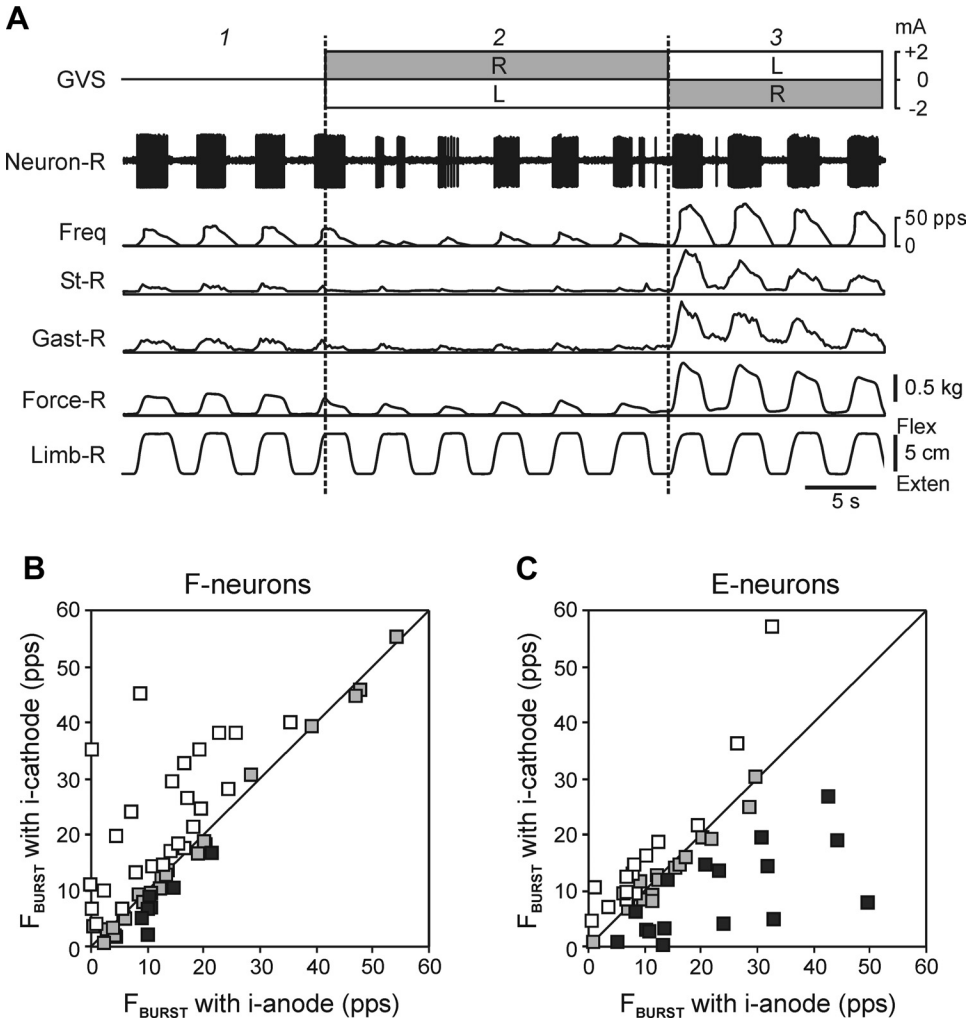

Fig. 5. Effects of GVS on F- and E-groups of spinal neurons. *A*: a representative example of GVS effects on activity of F-neuron recorded on the right side of the spinal cord. This neuron was activated with flexion of the right limb, and its activity was strongly correlated with the force and EMG responses in this limb. The firing frequency of the neuron averaged over an interval of 100 ms is shown (Freq). *B* and *C*: mean F_{BURST} of individual neurons under 2 conditions: with the anode (abscissa) and with the cathode (ordinate) ipsilateral to a neuron. White and black squares indicate the neurons whose F_{BURST} was significantly higher (*t*-test; $P < 0.05$) with ipsilateral cathode or with ipsilateral anode, respectively. Gray squares indicate neurons with no significant difference between the 2 frequencies.

by a small square, with its abscissa and ordinate representing the mean F_{BURST} under these two conditions. The neurons, which were more active (i.e., had higher F_{BURST}) with ipsilateral cathode, more active with ipsilateral anode, and those with no difference in activity, are indicated by different symbols.

In accordance with these three types of GVS effects, we divided F- and E-groups of neurons into three subgroups each. Representative examples of the effects of GVS on the neuronal responses to platform tilts are shown in Fig. 6*A* for three subgroups of the F-group (F1, F2, F3) and three subgroups of the E-group (E1, E2, E3). One can see that F1- and E1-neurons have a stronger response to tilts during GVS with ipsilateral cathode than with ipsilateral anode. F2- and E2-neurons have stronger response with ipsilateral anode than with ipsilateral cathode. Finally, F3- and E3-neurons were not affected by GVS. The relative number of neurons in each subgroup is given in Table 1. One can see that E-neurons were distributed almost evenly among the E1-, E2-, and E3-subgroup. By contrast, the vast majority of F-neurons belonged to the F1- and F3-subgroup.

Population characteristics of neuronal activity in different subgroups are presented in Fig. 6, *B–E*. There are shown the mean F_{BURST} (Fig. 6, *B* and *C*) and F_{INTER} (Fig. 6, *D* and *E*) during GVS at two different configurations of GVS: with ipsilateral anode and with ipsilateral cathode, averaged over all neurons in each subgroup. One can see that GVS has a qualitatively similar effect on the F_{BURST} and F_{INTER} in different subgroups. The effect of GVS on the F1- and E2-subgroup was stronger than on the F2- and E1-subgroup.

The distribution of neurons of different subgroups on the cross-section of the spinal cord is shown in Fig. 7, *A* and *B* (for F-group), and Fig. 7, *C* and *D* (for E-group). As in Fig. 3, *A* and *B*, we divided the cross-section into three zones and calculated the relative number of neurons in each of the subgroups in each zone. For the F-group, the F1- and F3-subgroup were more numerous than the F2-subgroup in each of the zones (Fig. 7*E*). For the E-group, the E1- and E3-neurons prevailed over the E2-neurons in *zones 1* and *2*, whereas E2-neurons were most numerous in *zone 3* (Fig. 7*F*). From Fig. 7, *A–D*, one can also see that the proportion of neurons without GVS influences (*subgroups F3* and *E3*) was almost two times larger in *segment L4* than in *segment L5* (60% against 32%). However, our sample of neurons was not sufficient for the statistical analysis of these regional differences.

DISCUSSION

Effects of GVS on PLRs. PLRs in decerebrate and spinal rabbits were characterized in our previous studies (Lyalka et al. 2011; Musienko et al. 2008, 2010). Similar methods for elic-

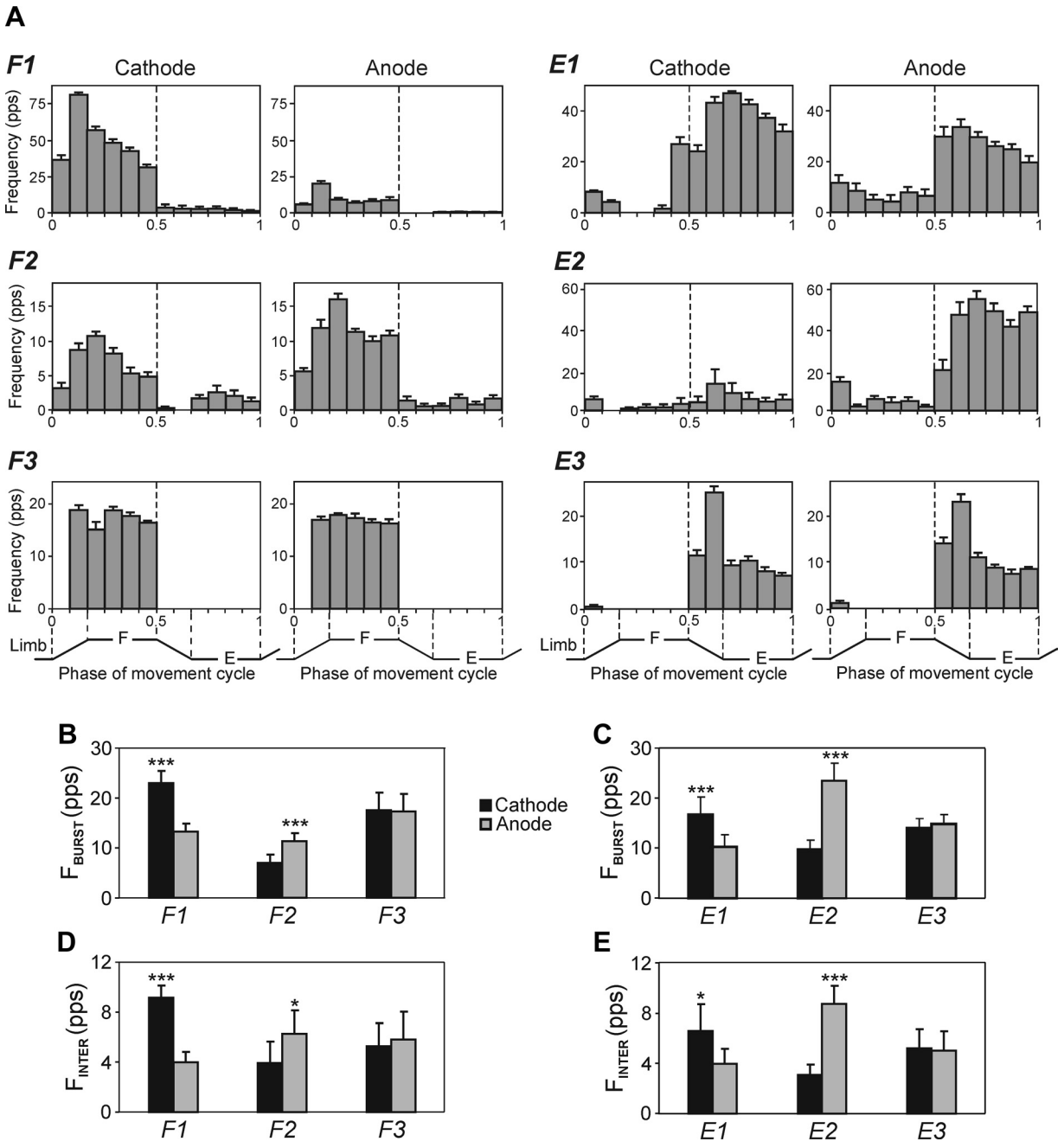

Fig. 6. Classification of spinal neurons based on their modulation patterns and on GVS effects. *A*: examples of the neurons from all 6 subgroups. For each neuron, the histogram of its activity in the F/E cycle of the ipsilateral limb was obtained under 2 conditions, with ipsilateral cathode and with ipsilateral anode. The neurons from F1- to F3-subgroups were activated during limb flexion and those from E1- to E3-subgroups during limb extension. In F1- and E2-neurons, the activity was significantly higher with ipsilateral cathode than with ipsilateral anode. In F2- and E1-neurons, the activity was significantly higher with ipsilateral anode than with ipsilateral cathode. GVS had no effects on F3- and E3-neurons. *B–E*: the effect of GVS on the mean F_{BURST} (*B* and *C*) and mean F_{INTER} (*D* and *E*) under 2 conditions: with ipsilateral anode (gray bars) and with ipsilateral cathode (black bars). The averaging was done over all neurons in each of the subgroups. Indication of significance level: $*P < 0.05$; $***P < 0.001$.

itation of PLRs were used in the present study (Fig. 1, *A–D*). In the decerebrate rabbit with a rigidly fixed pelvis and vertebral column, PLRs were evoked by periodic F/E movements of the hindlimbs, which were caused by tilts of the supporting platform. Essential characteristics of these movements (limb configuration, F/E amplitude, anti-phase relationships between the left and right limbs; Fig. 1, *E* and *F*) were similar to those observed in intact rabbits maintaining balance on a tilting platform (Beloozerova et al. 2003). One could expect that due to this similarity, the sensory inflow to the spinal cord from limb afferents during PLRs did not differ considerably from the inflow during normal postural reactions.

The motor pattern of PLRs [Figs. 1*G*, 2, and 5*A*; see also Musienko et al. (2010)] included activation of extensors during limb flexion, which resulted in an increase of the force produced by the limb. During limb extension, the extensor activity and force decreased. A similar pattern of responses to tilts was observed in the unrestrained decerebate rabbit (Musienko et al.

Table 1. *Classification of spinal interneurons and extensor motoneurons based on 2 criteria: the tilt-caused modulation and the GVS effects*

Subgroup of Neurons	Percent of All Neurons	Activation with	GVS Ipsi-Cathode	GVS Ipsi-Anode
F1	25	Flexion	+	−
F2	7	Flexion	−	+
F3	23	Flexion	0	0
E1	14	Extension	+	−
E2	15	Extension	−	+
E3	16	Extension	0	0
Extensor MNs*		Flexion	+	−

+, activation by galvanic vestibular stimulation (GVS); −, inhibition by GVS; 0, no effect of GVS; F1–F3, flexion subgroups; E1–E3, extension subroups. *Characterized on the basis of extensor electromyograms. MNs, motoneurons.

2008), suggesting that its postural reactions were based on the PLR mechanisms. It was also found that PLRs in each limb were caused mainly by tilt-related afferent signals from the same limb (Musienko et al. 2010). Similar results were obtained for intact animals, in which postural reactions to tilts in each limb were also caused mainly by afferent signals from the same limb (Deliagina et al. 2006b). These findings suggest that mechanisms of PLRs can contribute to the maintenance of lateral stability in intact subjects.

In the present study, it was found that continuous GVS strongly affected the magnitude of PLRs: the extensor EMGs and the force, developed during limb flexion, considerably increased when the cathode was ipsilateral to the limb and decreased when the anode was ipsilateral to the limb (Fig. 2). These findings suggest that a tonic vestibulospinal drive (caused by continuous GVS) can increase and decrease the gain in postural reflex pathways. We have also found that effects of GVS are similar in the extensor muscles of all main joints of the limb (Fig. 2, *A–E*), suggesting that the activity of a gross extensor synergy, observed in different postural tasks [see e.g., Chvatal et al. (2011); Ting and Macpherson (2005)], can be regulated as a whole by means of GVS.

Two chains of antagonistic PLRs, as well as the effects of GVS on these chains, are schematically shown in Fig. 8*A*. This scheme also reflects an important finding (Grillner and Hongo 1972)—that the vestibulospinal tract can excite the extensor motoneurons, both directly and indirectly, through spinal interneurons, which integrate descending and afferent information. Candidate interneurons with such integrative function (*subgroups F1* and *E2* in Fig. 8*A*) were found in the present study (see below).

In the standing, intact rabbit, continuous binaural GVS elicited a lateral body sway toward the anode, and this new body orientation was actively stabilized (Beloozerova et al. 2003). To explain these findings, it was suggested that GVS affects a set point of the antagonistic reflexes controlling body orientation in the transverse plane (Beloozerova et al. 2003). This hypothesis was supported by the results of the present study. It was found that continuous GVS produced opposite

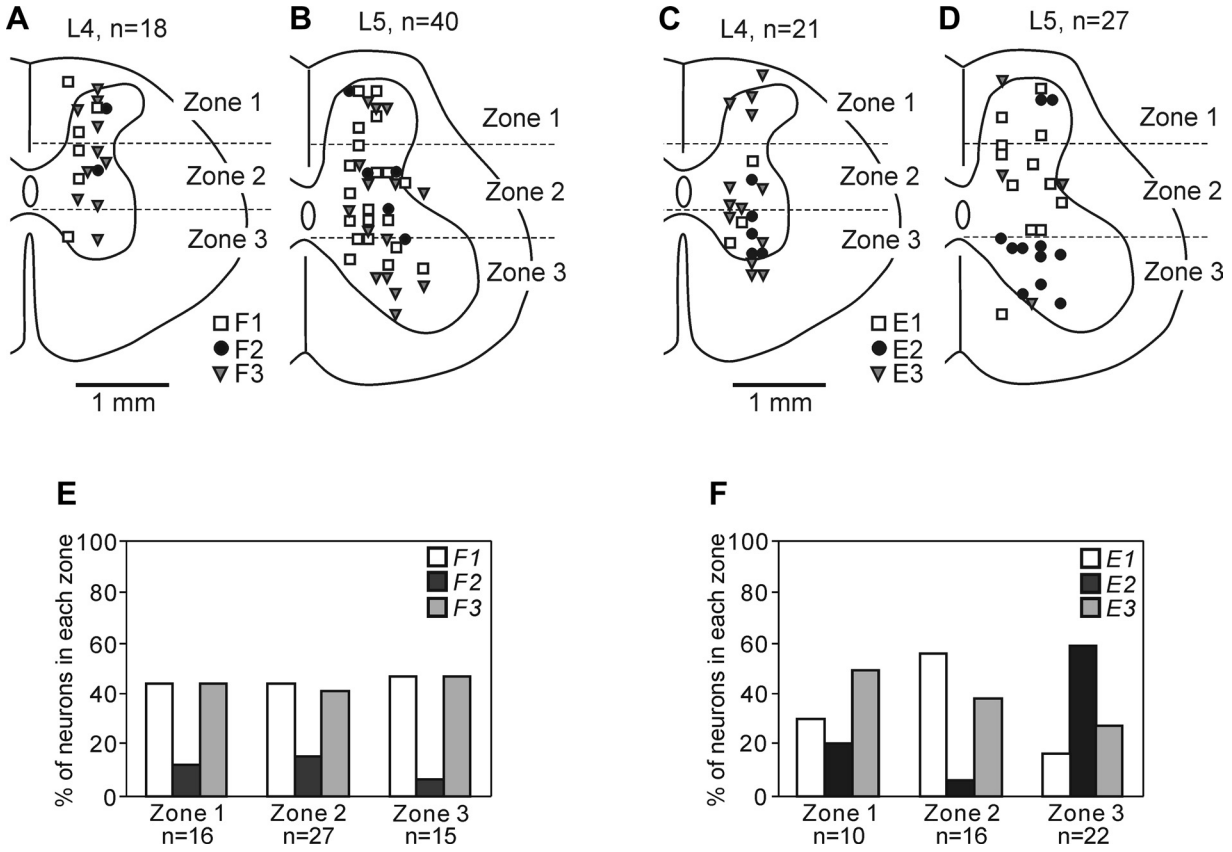

Fig. 7. Distribution of neurons of different subgroups in the spinal cord. *A–D*: positions of individual neurons on the cross-section of the spinal cord in *segment L4* (*A* for F-group; *C* for E-group) and *L5* (*B* for F-group; *D* for E-group). Three zones of gray matter are indicated: dorsal (*1*), intermediate (*2*), and ventral (*3*). *E* and *F*: relative numbers of neurons in different subgroups in each of the 3 zones (*E* for F-group; *F* for E-group).

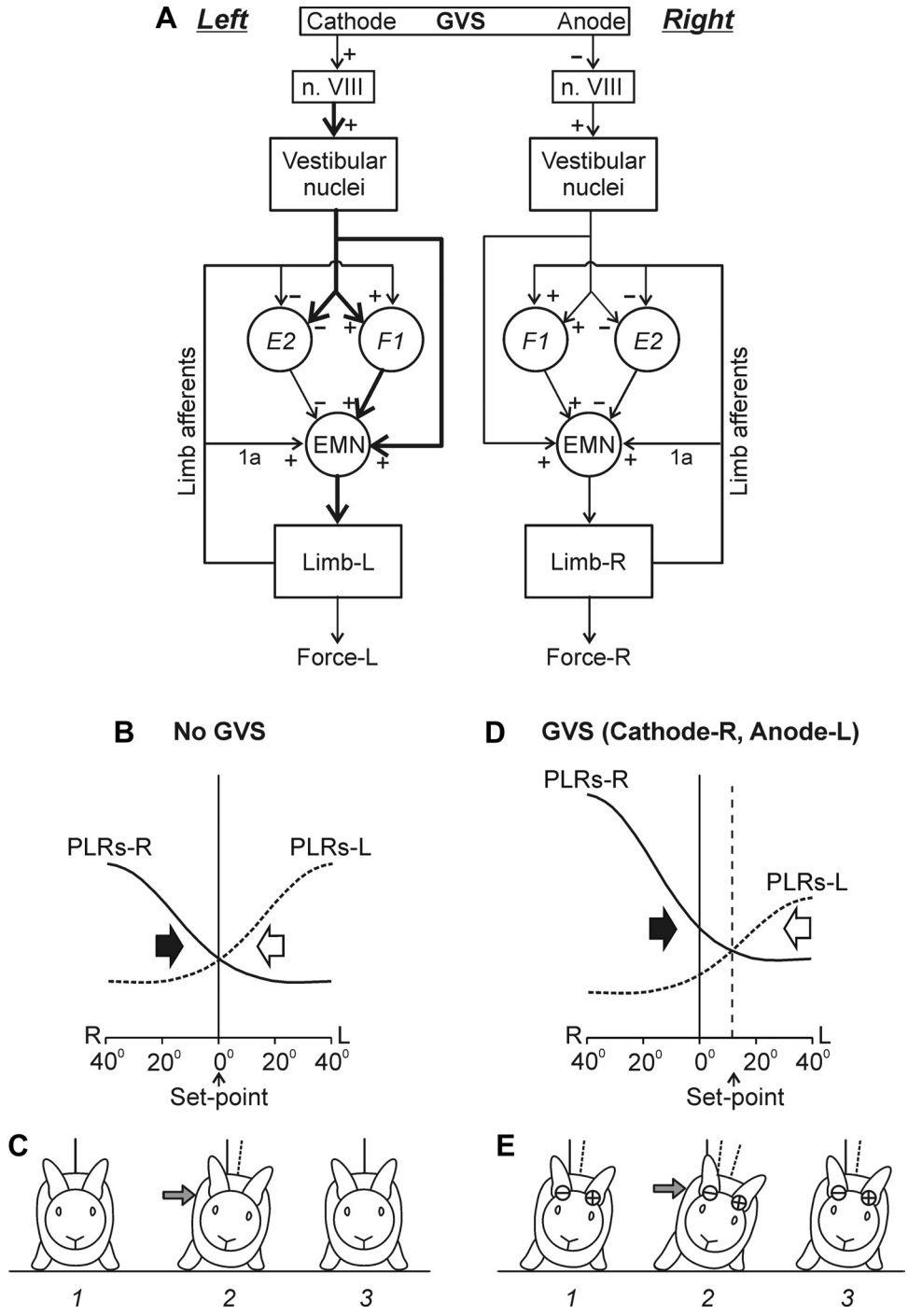

Fig. 8. Conceptual model of the trunk stabilization system and effects of GVS. *A*: schematic representation of 2 chains of PLRs (Left and Right), as well as the effects of GVS on these chains. In each chain, flexion of the limb activates afferents of this limb. They cause excitation of extensor motoneurons (EMN) through monosynaptic pathways (*group 1a* 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 2 chains (indicated by different size and thickness of the corresponding arrows). With cathode on the left side, GVS activates vestibular afferents in the left VIII nerve (n. VIII), which activates neurons of the left vestibular nuclei. These neurons, through the left vestibulospinal tract, affect the spinal postural reflexes on the left 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 with the right side). *B–E*: presumed effects of the 2 antagonistic reflex chains in the unrestrained standing rabbit, without GVS (*B* and *C*) and during GVS with cathode-R and anode-L (*D* and *E*). *B* and *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 dashed lines, respectively). Black and white arrows indicate the motor effect (lateral sway) caused by PLR-R and PLR-L, respectively. *C* and *E*: the stabilized orientation (*1*), effect of the lateral push (*2*), and the restored orientation (*3*; see DISCUSSION for details).

effects on the antagonistic reflexes—PLRs on the cathode side were facilitated, whereas those on the anode side were suppressed (Fig. 2*A*). Figure 8, *B–E*, illustrates presumed effects of the two antagonistic reflex chains in the unrestrained, standing rabbit. The effects without GVS are shown in Fig. 8*B*. Any deviation of the dorsoventral body axis from the vertical (lateral sway) causes opposite changes in PLR-R and PLR-L. In turn, PLR-R and PLR-L produce opposite motor effects—they cause body sway in opposite directions. With symmetrical PLRs (as in Fig. 8*B*), the two curves intersect at 0° (no lateral sway). This orientation (Fig. 8*C*, 1) will be stabilized, i.e., the rabbit will return to this orientation after any deflection (caused, e.g., by the lateral push; Fig. 8*C*, 2 and 3).

Continuous GVS (e.g., with cathode-R, anode-L) causes an increase in PLR-R and a decrease in PLR-L (Fig. 8*D*). Now, the two curves intersect, not at 0° but at some angle of the left sway. This tilted orientation (Fig. 8*E*, 1) will be stabilized; i.e., the rabbit will return to this orientation after any deflection from it (caused, e.g., by lateral push; Fig. 8*E*, 2 and 3). Thus GVS allows changing the set point in the control system.

A similar principle of balance control was also found in simpler animals—a mollusk (Clione) and a lower vertebrate (lamprey) (Deliagina and Fagerstedt 2000; Deliagina et al.

1998; Deliagina et al. 2006a). In the lamprey, antagonistic postural reflexes are mediated by two populations of reticulospinal neurons, and different factors producing asymmetry in their activity affect the stabilized body orientation. However, in contrast to terrestrial quadrupeds, in simpler aquatic animals, postural reflexes are generated in response to vestibular signals.

Supraspinal influences are very important for the generation of PLRs. Our previous studies (Musienko et al. 2010) have shown that PLRs disappeared after spinalization, but they could be partly restored by means of electrical stimulation of the spinal cord. These findings suggest that the spinal cord contains neuronal networks mediating PLRs, and these networks are activated by the tonic supraspinal drive. This drive can be substituted by an artificial drive induced by stimulation of the spinal cord. Another possible method for substitution of the natural supraspinal drive, i.e., activation of spinal postural networks by GVS, was demonstrated in the present study. One should note that supraspinal systems provide not only the tonic excitatory drive for activation of spinal postural mechanisms but also phasic postural commands, as was demonstrated for the cortico- and rubrospinal systems (Beloozerova et al. 2005; Zelenin et al. 2010).

Responses of spinal neurons to tilts. A rigid fixation of the pelvis and vertebral column (Fig. 1, *A–D*) allowed us to record spinal neurons during large-scale movements of both hindlimbs. We recorded the activity of neurons (in *segments L4–L5*) from the area outside of the motor nuclei and on this ground, considered them as interneurons. The neurons were recorded during PLRs, evoked by periodical tilts of the platform. Modulation of the neuronal discharge frequency, which correlated with PLRs, was considered as the indication of possible involvement of the neuron in the generation of PLRs.

We have found that the majority (~75%) of recorded neurons was modulated, whereas the proportion of nonmodulated neurons was small. One possible explanation for this result is that the supraspinal drive in decerebrate animals preferably activated the posture-related population of spinal neurons, whereas 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. However, the proportion of nonmodulated neurons in the present study could be underestimated (see RESULTS).

This study has shown that in the majority of modulated neurons (81%), the phase of their responses to tilts of the whole platform was determined by the tilt-related somatosensory input from the ipsilateral limb [see also Deliagina et al. (2009)]. In our previous study, it was also found that PLRs in a given limb are generated on the basis of afferent signals coming mainly from the same limb (Musienko et al. 2010). These were our reasons to classify all modulated neurons in accordance with the phase of their activity in the cycle of F/E movements of the ipsilateral limb. All of these neurons were divided into two groups: F-neurons were more active during flexion of the ipsilateral limb, and E-neurons were more active during its extension. Therefore, F-neurons were modulated in-phase with extensor motoneurons, whereas E-neurons were modulated in anti-phase to extensor motoneurons. The majority of neurons in each group was deeply modulated ($K_{MOD} \geq 60\%$; Fig. 3, *E* and *F*). F-neurons and E-neurons constituted 54% and 46% of all modulated neurons, respectively. A small prevalence of the neuronal group, active in-phase with extensors over those active in anti-phase, could be caused by a dominance of extensor activity during PLRs. Taking into account that PLRs of each limb are generated mainly in response to the tilt-related somatosensory input from the same limb (Musienko et al. 2010), one can suggest that modulation in the majority of neurons was determined by the same input.

Modulated neurons of the F-group and E-group were found in all areas of the gray matter but with a different proportion of the two groups in each area (Fig. 3, *A*, *B*, and *G*). This finding suggests that the modulated neurons within each group do not represent a homogeneous population. Some of these neurons can be involved in the processing of afferent signals eliciting PLRs (cells in *zone 1*). Other neurons (from *zones 2* and *3*) can participate in generation of the motor pattern of PLRs by exciting (F-neurons) or inhibiting (E-neurons) the extensor motoneurons of the ipsilateral limb. Modulated neurons can also project to the opposite side of the spinal cord and contribute to PLRs in the contralateral limb. Some of the neurons can give rise to the ascending pathways, e.g., the dorsal and ventral spinocerebellar tracts, whose cell bodies are located in the L4–L5 segments [see e.g., Arshavsky et al. (1972); Jankowska and Puczynska (2008)]. Tilt-related somatosensory information transmitted by these pathways is important for the formation of supraspinal commands for postural corrections [see e.g., Beloozerova et al. (2005)].

Effects of GVS on tilt-evoked responses of spinal neurons. According to the effect of GVS on a neuron, three subgroups of F-neurons and three subgroups of E-neurons could be distinguished (Table 1). 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. These neurons could belong to different identified types of spinal interneurons, which are known to receive input from vestibulospinal neurons, including *group Ia*-activated inhibitory interneurons (Hultborn et al. 1976), *group II*-activated interneurons (Davies and Edgley 1994), commissural interneurons (Jankowska et al. 2005), and neurons of the ventral spinocerebellar tract (Baldissera and Roberts 1975).

The minority of modulated neurons (39%; *subgroups F3* and *E3*) did not respond to GVS and therefore, did not mediate vestibulospinal influences. Since the activity of F3-neurons and E3-neurons was well modulated by platform tilts (Fig. 6*A*), these neurons (together with F1-, F2-, E1-, and E2-neurons) could participate in the control of extensor motoneurons during PLRs.

In the F1-subgroup (25% of all modulated neurons), the pattern of activity was similar to that in extensor motoneurons (monitored by recording the extensor EMGs). Periodic tilts of the platform caused modulation of F1-neurons, with a higher level of activity during flexion of the ipsilateral limb and a lower level during its extension (Fig. 3*C*). In the majority of F1-neurons, the modulation was deep (Fig. 3*E*), which well corresponded to the deep modulation of the motor output, i.e., extensor EMGs and contact forces (Fig. 2). As in extensor motoneurons, the activity in F-neurons increased with cathodal GVS and decreased with anodal GVS (Fig. 6, *B* and *D*). Such a close similarity allows us to suggest that F1-neurons are excitatory interneurons contributing to activation of extensor motoneurons during PLRs, as well as mediating the effects of

GVS on extensor motoneurons. As shown in Fig. 7, 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. A part of F1-neurons (located in the dorsal horn) could receive vestibulospinal influences through other interneurons. The F1-neurons are included in the conceptual model of the trunk stabilization system as the last-order premotoneuronal interneurons (Fig. 8*A*). However, we cannot exclude a possibility that close similarity between activities of F1-neurons and extensor motoneurons (at least in some cases) was due to a common synaptic input.

Since F1-neurons were found in all three zones of the gray matter, one can suggest that they perform different functions, from the processing of afferent signals to the direct control of extensor motoneurons. Additional information about afferent inputs and axonal projections of these neurons is needed to identify individual neurons and to reveal their specific functions.

In other F-neurons affected by GVS (a small F2-subgroup), the effects of anodal and cathodal GVS were opposite to those in extensor motoneurons and much weaker than in F1-neurons (Fig. 6*B*). Participation of F2-neurons in formation of motor output during PLRs seems less likely than participation of F1-neurons. These neurons can perform many other functions, such as formation of crossed and ascending influences.

Neurons of the E-group were active in the anti-phase to extensor motoneurons. Such neurons (if they are inhibitory) could contribute to the formation of PLR motor output by disinhibition of extensor motoneurons during limb flexion. To mediate the effects of GVS, these neurons should also have the responses to GVS, opposite to those in extensor motoneurons. Such responses were observed in E2-neurons (Fig. 6*C*). These neurons are incorporated into the conceptual model of the trunk stabilization system (Fig. 8*A*). Neurons of this subgroup are located mainly in the ventral horn (Fig. 7); they could comprise interneurons mediating reciprocal inhibition (Hultborn et al. 1976).

In other E-neurons affected by GVS (E1-subgroup), the effects of anodal and cathodal GVS were opposite to those in E2-neurons and much weaker (Fig. 6*C*). Participation of these neurons in the formation of motor output during PLRs seems less likely than participation of E2-neurons.

The proportion of neurons without GVS influences (*subgroups F3* and *E3*) was almost two times larger in *segment L4* than in *segment L5* (Fig. 7, *A–D*). This finding suggests stronger vestibulospinal influences on L5 than on L4. In the cat, more intense vestibulospinal projections to the central segments of the lumbosacral enlargement have been reported (Petras 1967).

To conclude, this study has demonstrated that the GVS-induced changes in the tonic vestibulospinal drive considerably affect the gain of PLRs in decerebrate rabbits. The GVS causes asymmetry in the right/left PLRs, which can be responsible for the GVS-induced, sustained lateral body sway observed in normal subjects. Several groups of spinal interneurons have been characterized, whose activity was differently correlated with the motor output during PLRs and with the effects of GVS, suggesting their different roles in the control of postural reactions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.V.Z., G.N.O., and T.G.D. conception and design of research; L.-J.H., P.V.Z., and T.G.D. performed experiments; L.-J.H. and T.G.D. analyzed data; L.-J.H., P.V.Z., G.N.O., and T.G.D. interpreted results of experiments; L.-J.H. and G.N.O. prepared figures; L.-J.H. and G.N.O. drafted manuscript; L.-J.H., P.V.Z., G.N.O., and T.G.D. edited and revised manuscript; L.-J.H., P.V.Z., G.N.O., and T.G.D. approved final version of manuscript.

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