Activity of pyramidal tract neurons in the cat during standing and walking on an inclined plane

A. Karayannidou[1,2], I. N. Beloozerova[1], P. V. Zelenin[2], E. E. Stout[1], M. G. Sirota[1], G. N. Orlovsky[2] and T. G. Deliagina[2]

[1] *Barrow Neurological Institute, Phoenix, AZ 85013, USA*
[2] *Department of Neuroscience, Karolinska Institute, SE-17177 Stockholm, Sweden*

To keep balance when standing or walking on a surface inclined in the roll plane, the cat modifies its body configuration so that the functional length of its right and left limbs becomes different. The aim of the present study was to assess the motor cortex participation in the generation of this left/right asymmetry. We recorded the activity of fore- and hindlimb-related pyramidal tract neurons (PTNs) during standing and walking on a treadmill. A difference in PTN activity at two tilted positions of the treadmill (± 15 deg) was considered a positional response to surface inclination. During standing, 47% of PTNs exhibited a positional response, increasing their activity with either the contra-tilt (20%) or the ipsi-tilt (27%). During walking, PTNs were modulated in the rhythm of stepping, and tilts of the supporting surface evoked positional responses in the form of changes to the magnitude of modulation in 58% of PTNs. The contra-tilt increased activity in 28% of PTNs, and ipsi-tilt increased activity in 30% of PTNs. We suggest that PTNs with positional responses contribute to the modifications of limb configuration that are necessary for adaptation to the inclined surface. By comparing the responses to tilts in individual PTNs during standing and walking, four groups of PTNs were revealed: responding in both tasks (30%); responding only during standing (16%); responding only during walking (30%); responding in none of the tasks (24%). This diversity suggests that common and separate cortical mechanisms are used for postural adaptation to tilts during standing and walking.

(Received 2 February 2009; accepted after revision 1 June 2009; first published online 2 June 2009)
Corresponding author T. G. Deliagina: Department of Neuroscience, Karolinska Institute, SE-17177 Stockholm, Sweden. Email: tatiana.deliagina@ki.se

Abbreviations PTN, pyramidal tract neuron

An efficient control of the body posture is necessary during standing and walking. In either of these motor tasks, the postural control system performs two important functions. First, the system maintains equilibrium by counteracting to all destabilizing influences from the environment. Second, the system maintains a definite body configuration by stabilizing the position of body segments in relation to each other; the system also adapts the animal's posture to the environment (Horak & Macpherson, 1996; Deliagina *et al.* 2006).

We investigated the role of the motor cortex in postural control. In our previous studies, the activity of pyramidal tract neurons (PTNs) was recorded while an unrestrained intact cat stood and kept balance on a platform during sinusoidal tilts (1 Hz) in the frontal plane. It was found that the activity of PTNs strongly correlated with the postural corrections caused by these dynamic postural perturbations (Beloozerova *et al.* 2005). The involvement of the motor cortex in postural corrections was also demonstrated in rabbits (Beloozerova *et al.* 2003*a*). These findings suggest that the motor cortex participates in the maintenance of equilibrium: it sends commands to the spinal cord that contribute to postural corrections (for discussion, see Jacobs & Horak, 2007).

Is the motor cortex also involved in the maintenance of a specific postural body configuration? To answer this question we modified the motor task to constrain the body configuration: in this study, the cat was either standing or walking on an inclined plane. Matsuyama & Drew (2000) showed that the cat easily adapts to walking on an inclined plane by acquiring a specific body configuration during the stance phase of each step: the cat increases the functional length of its limbs on the side tilted down and decreases the functional length on the side tilted upwards. Similar postural adaptations were also observed for standing on a laterally inclined platform, in both cats (Beloozerova *et al.* 2005) and rabbits (Beloozerova *et al.* 2003*b*; Lyalka *et al.* 2005).

DOI: 10.1113/jphysiol.2009.170183

Table 1. Number of pyramidal tract neurons in individual cats with different types of response

	Standing						Walking						S&W		S&W Same tilt				S&W No	
	FL			HL			FL			HL			FL	HL	FL		HL		FL	HL
Cat no.	co	i	no	co	i	no	co	i	no	co	i	no			co	i	co	i		
1	5	6	6	7	4	9	4	5	7	7	5	8	16	20	3	3	3	3	3	5
2	4	7	22	5	7	18	11	9	14	11	11	8	33	30	1	2	3	4	8	7
3	14	23	41	14	20	37	15	27	39	22	21	29	78	71	9	12	6	7	23	15
All	23	36	69	26	31	64	30	41	60	40	37	45	127	121	13	17	12	14	34	27

Abbreviations: FL and HL, neurons from forelimb and hindlimb representations, respectively; co and i, neurons activated with contra- and ipsi-tilt, respectively; no, neurons that were not activated by tilts; S&W, neurons tested during both standing and walking. S&W Same tilt, neurons activated with the same tilt during both tasks. S&W No, neurons that were not activated with tilts in both tasks.

There are data that suggest that a number of supraspinal motor centres participate in postural adaptations performed when standing and walking on an inclined plane. As demonstrated by Matsuyama & Drew (2000), the activity of vestibulospinal and reticulospinal neurons in the cat is modulated in the rhythm of stepping, and the magnitude of this activity depends on the value of the stationary lateral tilt. The involvement of other descending systems (corticospinal, rubrospinal) was not investigated. The aim of the present study was to examine participation of the motor cortex in the maintenance of asymmetrical body configuration during standing and walking on a laterally inclined plane. For this purpose, we recorded the activity of PTNs from the forelimb and hindlimb representations of the motor cortex during these two motor tasks. An important feature of this study was that each neuron was recorded both during standing and during walking, which could help in understanding the function of corticospinal signals.

A brief account of a part of this study was published in abstract form (Karayannidou *et al.* 2008*a*).

Methods

Recordings were obtained from three adult cats. They provided similar contributions to data collection (Table 1). Some of the methods have been described (Beloozerova *et al.* 2005; Prilutsky *et al.* 2005; Karayannidou *et al.* 2008*b*) and will be reported briefly here. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Surgical procedures

Surgery was performed under isoflurane anaesthesia using aseptic procedures. Anaesthesia was induced by an intramuscular injection of ketamine (8 mg kg^{-1}), and a tube was inserted into the trachea. Isoflurane anaesthesia was maintained throughout the surgery by addition of 2–5% isoflurane to the air that the cat inhaled. The isoflurane concentration was titrated according to respiratory rate, heart rate, corneal reflexes, pedal reflexes and muscle tone, which were maintained within parameters recommended by the American Veterinary Medical Association. Bipolar EMG electrodes (flexible Teflon-insulated stainless-steel wires) were implanted bilaterally into the following hindlimb muscles: adductor femoris (hip extensor and adductor), gluteus medius (hip extensor and abductor), vastus lateralis (knee extensor), and gastrocnemius lateralis (ankle extensor). In two cats, EMG electrodes were also implanted into m. triceps (elbow extensor).

The skin and fascia were removed from the dorsal surface of the skull. At 10 points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a wire; the screw heads and the wire were then inserted into a plastic cast to form a circular base. Later, while searching for neurons before behavioural tests, awake cats were rigidly held by this base. The base was also used to fixate connectors, a miniature micro-drive, a pre-amplifier, contacts for stimulating electrodes, and a protective cap.

A portion of the skull and dura above the left motor cortex were removed. The area of the motor cortex was identified by the surface features and photographed. The aperture was then covered by a plastic plate with many small holes filled by wax. The plate was fastened to the surrounding bone. Two 26 gauge hypodermic guide tubes were implanted vertically above the medullary pyramids, at the Horsley-Clarke coordinates (P10, L0.5) and (P10, L1.5), at the depth of H0 for subsequent insertion of stimulating electrodes into the pyramidal tract.

Immediately after surgery (prior to extubation) and then 12 h thereafter an analgesic (buprenorphine, 0.005–0.01 mg kg^{-1}) was administered intramuscularly. After extubation animals were placed in a warm padded cage and respiration and reflexes were monitored until consciousness was regained. Animals were attended bi-hourly during the following 3–4 days. Additional doses of buprenorphine were given as determined by

the attending veterinarian. Antibiotics were used as needed: Baytril (2.5–5.0 mg kg^{-1}) was injected intramuscularly, and bacitracin–neomycin–polymyxin or Neo-Predef with tetracaine was applied topically to wounds. The anti-inflammatory agent dexamethasone was administered subcutaneously in a reducing dose starting at 1–3 mg kg^{-1} as determined by the veterinarian.

Identification of cortical motor area

Experiments were initiated after several days of recovery when the craniotomy was fully healed, and cats resumed their normal preoperative behaviour. The animal was positioned in the restraining device, and its head was restrained using the base. Over several days, a number of sessions of increasing duration were used to accustom the cat to the head restrainer. Methods ensuring the humane treatment of subjects during the immobilization of the head have been described (Karayannidou *et al.* 2008*b*).

A detailed description of the area of recording has been given previously (Beloozerova *et al.* 2005). In brief, the area immediately adjacent to and inside the lateral half of the cruciate sulcus in the cat is considered to be the motor cortex. This is based on a considerable body of data obtained by means of inactivation, stimulation and recording techniques (Nieoullon & Rispal-Padel, 1976; Vicario *et al.* 1983; Armstrong & Drew, 1983, 1985; Beloozerova & Sirota, 1993*a*; Drew, 1993; Martin & Ghez, 1985, 1993), as well as on histological considerations (Myasnikov *et al.* 1994; Ghosh, 1997). In order to delineate the fore- and hindlimb representations of the left motor cortex in each subject, three approaches have been used in different combinations: (1) mapping of somatic receptive fields, (2) observation of neuronal activity during voluntary movements, and (3) intracortical microstimulation. The forelimb and hindlimb representations of the left motor cortex and microelectrode entry points for PTN recording are schematically shown in Fig. 2*G*.

Cell recording and identification

Neuronal activity was recorded extracellularly from the left motor cortex using either platinum–tungsten quartz-insulated microelectrodes (40 μm outer diameter) pulled to a fine tip and mechanically sharpened (Reitboeck, 1983), or commercially available tungsten varnish-insulated electrodes (Frederick Haer & Co). The impedance of the electrodes was 2–4 MΩ. After the electrode reached the depth of the cortex where clear responses of many neurons to limb movements could be observed (presumably layer V), two 200 μm platinum–iridium wires were slowly inserted and lowered into the medullar pyramid through the guide tubes (implanted during surgery). Pulses of graded intensity (0.2 ms duration, up to 0.5 mA) were delivered through this bipolar electrode. The wires were fixed at the position that was most effective in eliciting antidromic responses in neurons of the motor cortex, and served as the pyramidal tract-stimulating electrode. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop *et al.* 1962; Fuller & Schlag, 1976) illustrated in Fig. 2*F*.

All encountered neurons were tested for antidromic activation using the same current pulses and the criterion before, during and after standing and walking tasks. In addition, the waveform analysis was employed to discriminate and identify the spikes of a single neuron using the Power-1401/Spike-2 system waveform-matching algorithm. Only the neurons with a stable response latency and spike shape that consistently satisfied the collision test were used for analysis.

Postural tests

In both motor tasks, the animal was positioned on the belt of the treadmill, which either did not move (standing task, Fig. 1*C*) or moved at a speed of 0.5 m s^{-1} (walking task, Fig. 1*A* and *B*). The treadmill was periodically tilted between the two stationary positions, 15 deg to the left and 15 deg to the right (Fig. 1*B* and *C*). During standing, a transition from one stationary position to the other lasted for 0.5–0.7 s, and each position was maintained for 2–3 s (as in Fig. 3*A*); during walking, these values were 3–4 s and 8–20 s, respectively (as in Fig. 4*C*).

Cats were trained to maintain equilibrium on the tilted treadmill both during standing and during walking. They were rewarded by a paste-like food continuously ejected from a feeder (Karayannidou *et al.* 2009). The feeder (plastic tube of 18 mm outer diameter and 6 mm inner diameter) was positioned in front of the cat at a height of 21–23 cm (Fig. 1*A*). All cats were easily engaged in these postural tasks and maintained equilibrium during tilts and in the tilted positions of the treadmill. They tended to compensate for tilts by producing lateral displacements of their body in relation to the supporting surface (postural corrections), which allowed them to hold their mouth against the feeder and keep licking food despite the tilts (Fig. 1*B* and *C*).

The main factor affecting the animal's posture during tilts is the vertical displacement of the support surface under the standing limbs. This vertical displacement is proportional to the distance of the foot from the axis of treadmill tilting. The latter value is determined by the width of support, i.e. by the distance between the left and right feet (when standing) or between their projections on the left–right axis (when walking). An observation in the present study, common for all three cats, was that the width of support for the forelimbs was smaller than that for the

hindlimbs both during walking and during standing. Also, the width of support during walking was smaller than that during standing (Fig. 1*B* and *C*). Any systematic analysis of this issue was not performed, however.

Four mechanical sensors monitored the anterior–posterior (AP) position of the limbs during walking (Karayannidou *et al.* 2008*a*); two of them are shown in Fig. 1*A*. Limb movements were also monitored using the Visualeyez system (3-D real-time motion capture and analysis system, Phoenix Technologies Inc., Canada). It detected the positions of light-emitting photodiodes in 3-D space and made calculations of various kinematical parameters. The photodiodes were attached to the skin projections of the main limb joints and to the foot, either on the right hindlimb (Figs 3 and 4) or on the right forelimb (Fig. 5). The diodes attached to the foot allowed measuring of the vertical displacement of the support surface under the standing limb.

In addition, a photodiode was attached to the spine near the midline, either in the pelvic or in the shoulder region. The period of frame sampling was 28.8 ms. In selected trials, the cat's movements were also videotaped (30 frames s^{-1}).

Signals from the microelectrode pre-amplifier, as well as from the treadmill position and body position sensors were amplified (CyberAmp 380, Axon Instruments), digitized with a sampling frequency of 30 kHz (microelectrode), and 400 Hz (sensors), displayed on-screen and recorded to a computer disk drive with data acquisition software (Power-1401/Spike-2).

Data collection and processing

Most PTNs were recorded both in the standing task (as in Fig. 2*A*) and in the locomotor task (as in Fig. 2*D*). From 5 to 10 tilt cycles were performed to collect data for the standing task, and from 3 to 6 tilt cycles (which included 30–60 step cycles) were performed to collected data for the locomotor task.

To evaluate the response of a PTN to tilts in the standing task, the tilt cycle was divided into six bins (Fig. 2*C*) reflecting different phases of movement (bin 1, tilting from right to left; bins 2 and 3, maintained left position; bin 4, tilting from left to right; bins 5 and 6, maintained right position). For each PTN, a histogram of spike activity during each tilt cycle (Fig. 2*B*) was generated. The activity was then averaged over all consecutive cycles of the test (Fig. 2*C*). A difference between the activities in the left tilted positions (bins 2 and 3) and in the right tilted position (bins 5 and 6) was considered as the static (positional) response. The activity during a change of the tilt angle (bins 1 and 4) was considered as a dynamic response.

During the locomotor task, the PTN activity was modulated in the rhythm of stepping (Fig. 2*D*). Circular statistics were performed to ascertain the significance of the coupling between a neuron firing and the locomotor movements (Batschelet 1981). The latency to each spike was measured relative to the step cycles (the onset of the swing phase was set as 0.0). The spikes were represented in vectorial form and the average vector

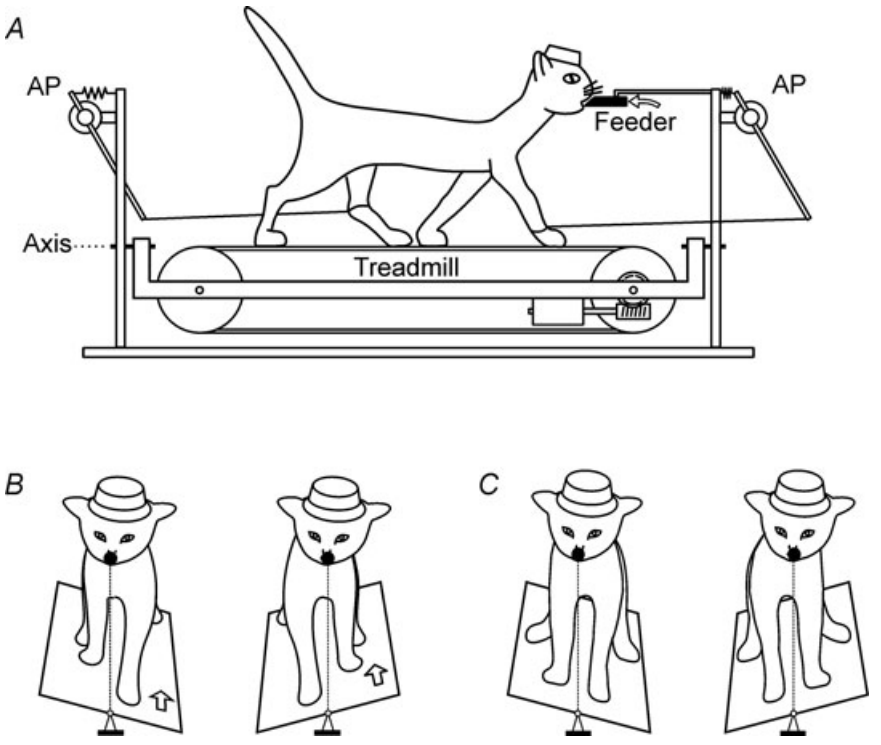

Figure 1. Postural tests used during standing and walking
A and *B*, experimental design for postural tests during locomotion. The cat was walking on the moving treadmill belt (*A*), which could be inclined to the right or to the left about the longitudinal axis (*B*). The anterior–posterior movements of limbs during stepping were recorded by mechanical sensors (AP). *C*, postural tests during standing. The same set-up as in *A* was used, but the treadmill belt was not moving. The cat was standing on the treadmill, which could be inclined to the right or to the left. During both tasks, the cat was continuously licking food from the feeder (feeder position is indicated by black bar in *A*, and by black circles in *B* and *C*).

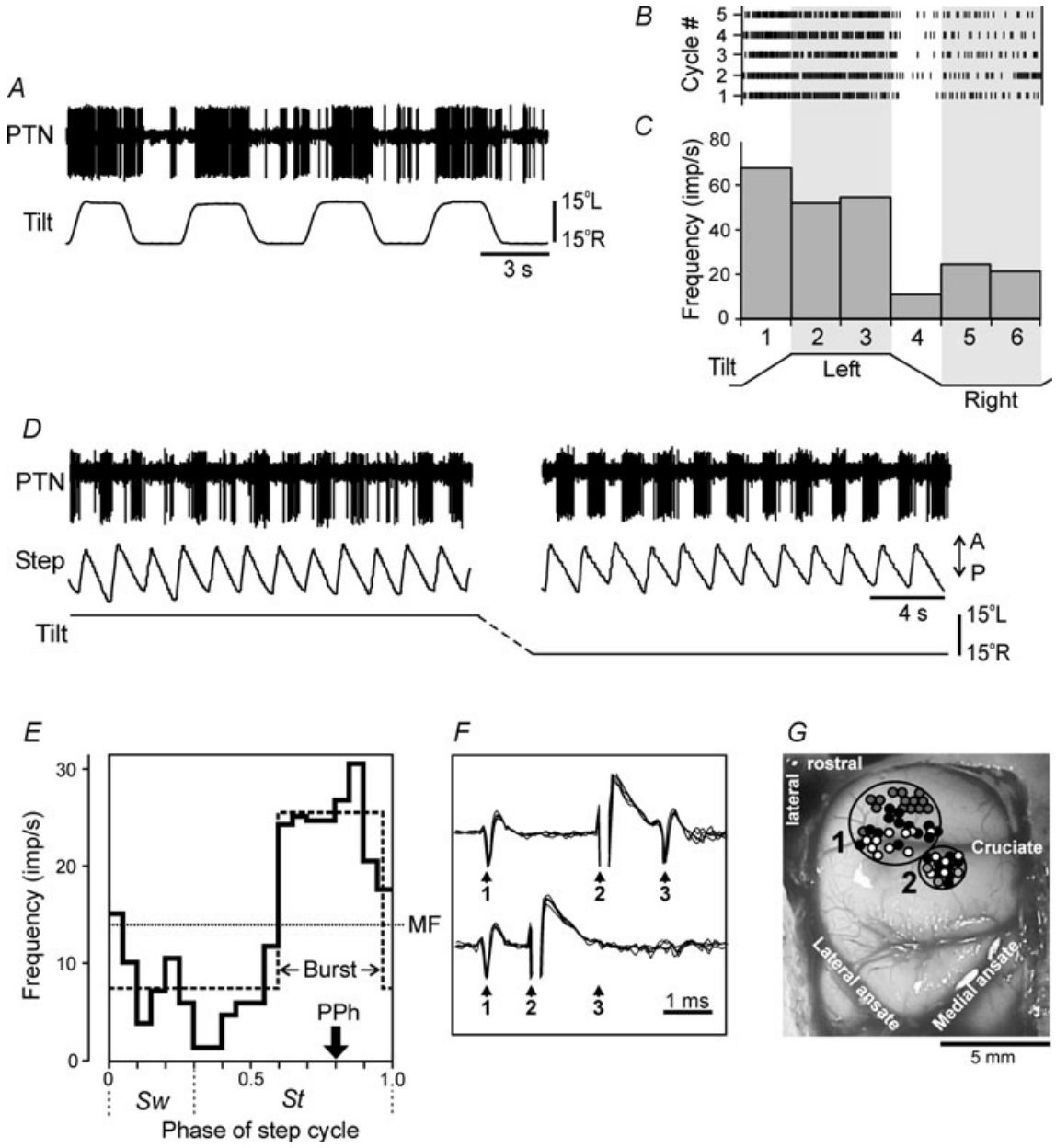

Figure 2. Characterization of pyramidal tract neuron (PTN) responses to tilts during standing and walking

A–C, standing task. *A*, an example of responses to tilts in a forelimb-related PTN. *B* and *C*, the raster and histogram of the PTN activity in different phases (1–6) of the tilt cycle. *D* and *E*, locomotor task. *D*, an example of the step- and tilt-related modulation of activity of a PTN. Step: anterior(A)–posterior(P) movements of the stepping right hindlimb. *E*, histogram (continuous line) of the step-related modulation of the PTN activity, and its two-level rectangular approximation (interrupted line), with the burst period (upper level) and inter-burst period (lower level). MF, mean frequency; PPh, preferred phase. *F*, collision test determines if a PTN response is antidromic. Top trace, PTN spontaneously discharges (arrow 1), and pyramidal tract is stimulated ∼2 ms later (arrow 2). PTN responds with a latency of ∼1 ms (arrow 3). Bottom trace, PTN spontaneously discharges (arrow 1) and pyramidal tract is stimulated ∼1 ms later (arrow 2). PTN does not respond (arrow 3) because in 1 ms its spontaneous spike was still *en route* to pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred. *G*, areas of recording within representations of the fore- and hindlimb in the left motor cortex (1 and 2, respectively). Microelectrode entry points into the cortex are combined from cats 1, 2 and 3 and shown by white, grey and black circles, respectively.

r was calculated (the direction of r gives the preferred phase of an individual neuron). The neurons with significance values $P < 0.05$ were considered modulated. The P values for the significance of r were taken from Zar (1974). To characterize this modulation, the step cycle was divided into 20 equal bins, and the onset of the swing phase was taken as the cycle onset. For this purpose, the signal from the sensor monitoring the limb anterior–posterior position (Fig. 2*D*) was used. The extreme posterior position of the limb was considered as a swing onset. A phase histogram of PTN spike activity was generated. The activity was then averaged over all consecutive cycles of the test (Fig. 2*E*). Since most of PTNs during locomotion have one peak of activity in the cycle (Armstrong & Drew, 1984), to evaluate the step-related modulation of PTN activity, we used the best two-level rectangular fit for the spike distribution within the step cycle; the upper level was defined as a 'burst', and the lower level as an 'inter-burst period' (Fig. 2*E*). For the burst and inter-burst periods, average frequencies were calculated. We also evaluated the mean frequency of a PTN in the cycle. All values were determined separately for the two tilted positions (left and right) as well as for the non-tilted position. The difference between the activities (frequency in the burst) in the two stationary tilted positions (left and right) was considered as the positional response.

All quantitative data are presented as the mean ± S.E.M. Statistical comparisons were made using Student's t test, with the significance level $P = 0.05$.

To confirm that two groups of data were statistically different, we fitted two regression lines to the data, and tested if they were significantly different from each other. The following statistical model was used: $y_i = \beta_0 + \beta_1 x_i + \beta_2 z_i + \beta_3 x_i z_i + e_i$, where y is a response variable, x is an explanatory variable, z is an indicator variable ($z = 1$, if a data point is from one group; $z = 0$, if a data point is from another group), $\{\beta_k\}$ are regression coefficients, e is a random error. ANOVA was used to test the significance of the indicator variable z and the interaction term xz, simultaneously. The null hypothesis (the hypothesis of coincidence) was H_0: $\beta_2 = \beta_3 = 0$.

Histological procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of the motor cortex from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 10% formalin solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. The position of stimulation electrodes in the medullar pyramids was verified by observation of electrode track gliosis. Positions of recording tracks in the motor cortex were estimated in relation to the reference lesions.

Results

Results obtained from individual cats were similar (Table 1) and are considered together.

Postural adaptations to inclined support surface during standing and walking

Adaptive changes of limb configuration and EMG pattern during standing and walking on an inclined plane were partly described previously (Matsuyama & Drew, 2000; Beloozerova *et al.* 2003*a*,*b*, 2005). In the present study, we characterized the motor and EMG pattern for both motor tasks, and in general confirmed the previous results.

Hindlimbs. During standing, lateral tilts of the supporting surface caused asymmetry in the functional length of the two hindlimbs. Stick diagrams in Fig. 3*A* show a configuration of the right hindlimb for the two positions of the support under the limb (up and down). One can see that the vertical displacement of the support was compensated for by a change in the functional length of the limb, so that the hip and spine vertical displacements (less than 0.5 cm) were much smaller than the support displacement (about 5 cm).

The tilt-induced changes in functional length were produced by modulation of extensor activity: the extension of the limb with ipsilateral downward tilt was caused by an increase of extensor activity, whereas its flexion with upward tilt was associated with a decrease of extensor activity. This is illustrated in Fig. 3*B*, where adductor femoris and gluteus medius (hip extensors), as well as vastus lateralis (knee extensor) increased their activity with ipsilateral downward tilt and decreased their activity with ipsilateral upward tilt. The tilt-related modulation of these extensors, as well as m. gastrocnemius (ankle extensor) was apparent after the tilt-triggered averaging of their EMGs, as illustrated in Fig. 3*C* for one of the cats.

During walking, lateral tilts of the supporting surface also caused asymmetrical changes in the limb configuration, but these changes were incorporated into a step cycle. Figure 4*A* shows the stick diagrams of the right hindlimb, which were obtained in 22 sequential time points during the stance phase, when the limb was supporting the body. This was done for two positions of the support area under the limb (up and down). One can see that vertical displacement of the support was compensated by a change in the length of the limb, so that the hip and spine vertical displacements in any point during the

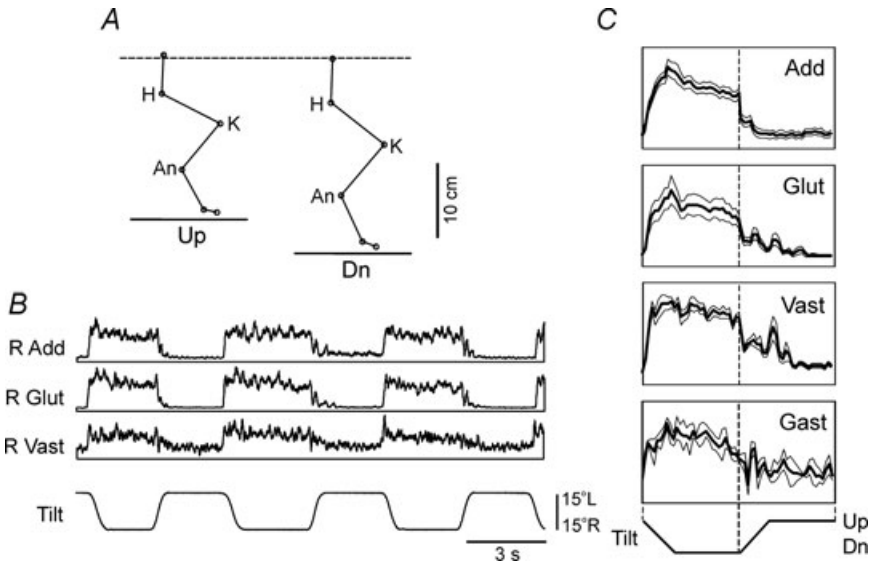

Figure 3. Adaptive changes in the right hindlimb configuration and EMG activity during standing on an inclined plane
A, configuration of the right hindlimb during standing (stick diagram) for two positions of the support under the limb. An interrupted horizontal line is a reference for evaluating the vertical displacement of the spine. *B*, example of EMG responses to tilts. *C*, tilt-triggered averaging of EMG responses (averaging over 20 responses in one cat; expressed as mean ± S.E.M.; arbitrary units). Designations and abbreviations: H, hip; K, knee; An, ankle; Dn, down; Add, adductor femoris; Glut, gluteus medius; Vast, vastus lateralis; Gast, gastrocnemius lateralis.

stance phase of the step were less than 0.5 cm, that is, much smaller than the support displacement (about 4 cm). Limb trajectories during the swing phase were also different at the up and down support positions (Fig. 4*B*).

The changes of limb configuration during the stance phase were associated with specific modifications of the extensor EMG activity: the peak EMG value increased with ipsilateral downward tilt and decreased with ipsilateral upward tilt (Fig. 4*C*). These tilt-related changes of the step-related modulation in four hindlimb extensors are apparent after the step-triggered averaging of their EMGs, which was done separately for the two support positions (up and down). As shown in Fig. 4*D*, an increase of extensor activity in the down-position of the support occurred mainly at the beginning of the EMG burst, i.e. in the second half of the swing phase and (in Add and Glut) also in the beginning of the stance phase.

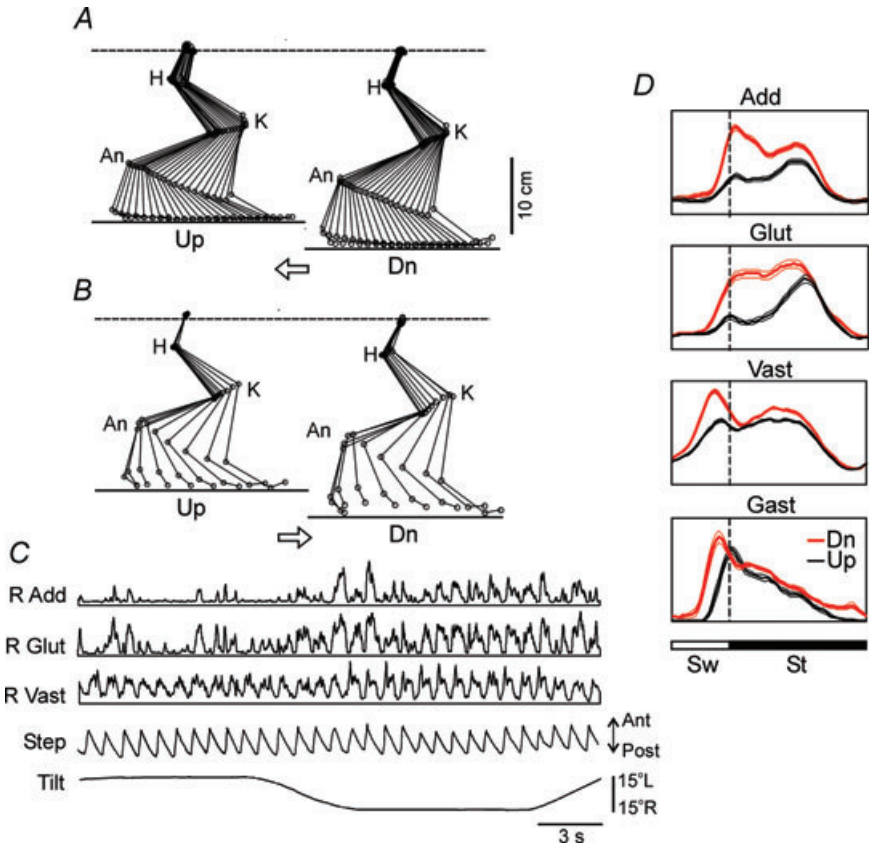

Figure 4. Adaptive changes in the right hindlimb configuration and EMG activity during walking on an inclined plane
A and *B*, configuration of the right hindlimb during stance phase of the step (*A*) and during swing phase (*B*), for two positions of the support under the limb (up and down). Superposition of 22 (in *A*) and 9 (in *B*) stick diagrams recorded (by Visualeyez system) sequentially during the stance and swing phase, respectively, with a time interval of 28.8 ms. *C*, example of EMG responses to tilts (Step, the AP movement of the stepping right hindlimb). Note the step-related modulation of EMGs, and the tilt-related modulation of the EMG level. *D*, step-triggered averaging of EMGs in 4 hindlimb muscles (mean ± S.E.M.) at two positions of the supporting area under the limb: up (black traces) and down (red traces). Averaging was over 60 cycles. Stance and swing phases are shown by the filled (black) and the open bar, respectively. Designations and abbreviations as in Fig. 3.

Forelimbs. Adaptive changes in the forelimb configuration were similar to those observed in the hindlimb. Figure 5*A* shows the right forelimb configuration during standing, for two positions of the limb support area (up and down). As in the hindlimbs, the vertical displacement of the support was compensated by a change in the functional length of the forelimb, so that the shoulder and spine vertical displacement (less than 0.5 cm) was much smaller than the support displacement (about 3 cm). Extension of the limb with ipsilateral downward tilt was associated with an increase of the activity of m. triceps (elbow extensor), whereas its flexion with upward tilt was associated with a decrease of this activity (Fig. 5*B* and *C*).

During walking, vertical displacements of the support were also compensated for by changes in the functional length of the limb, which were observed throughout the stance phase of the step (Fig. 5*D*). An increase of the length

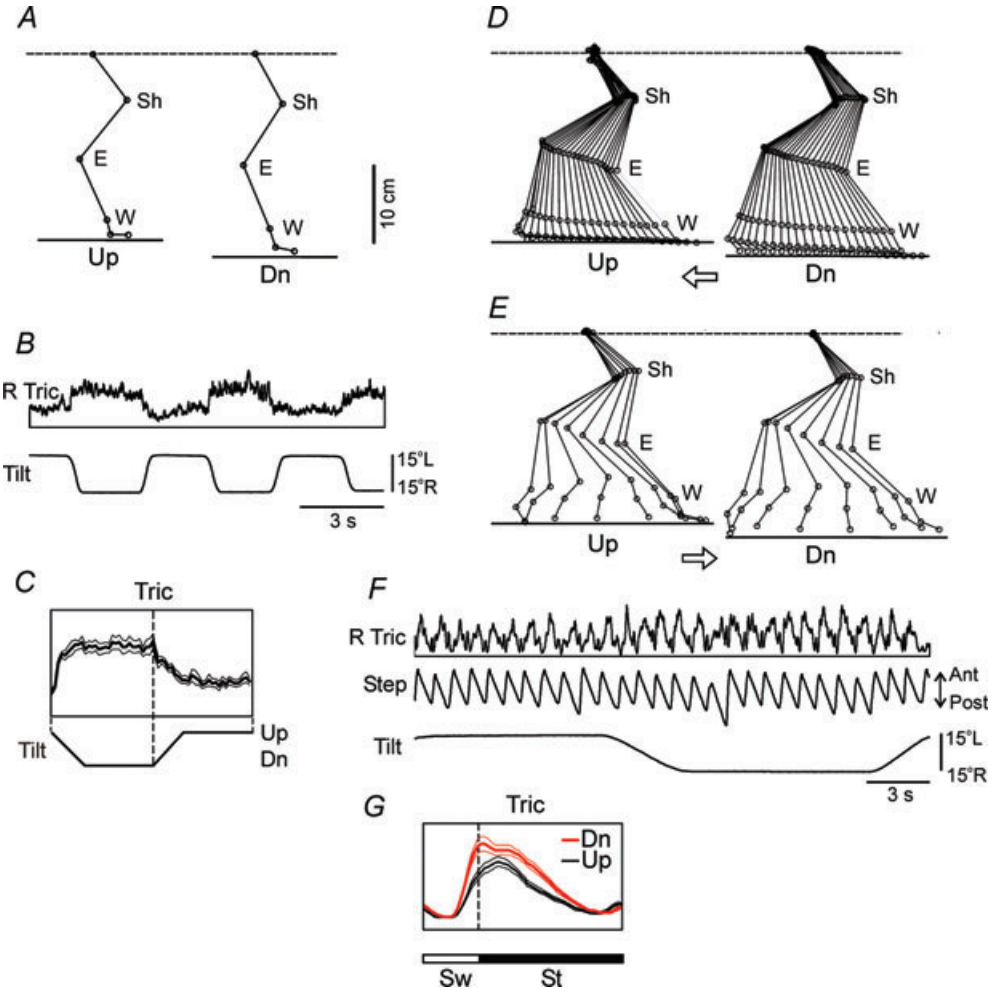

Figure 5. Adaptive changes in the right forelimb configuration and EMG activity during standing and walking on an inclined plane

A–C, responses to tilts during standing. *A*, configuration of the right forelimb during standing (stick diagram), for two positions of the support under the limb (up and down). *B*, example of EMG responses to tilts in the right triceps (R Tric). *C*, tilt-triggered averaging of triceps EMG responses (mean ± S.E.M., averaging over 20 responses in cat 1). *D–G*, responses to tilts during walking. *D* and *E*, configuration of the right forelimb during stance phase of the step (*D*) and during swing phase (*E*), for two positions of the support under the limb (up and down). Superposition of 22 (in *D*) and 8 (in *E*) stick diagrams recorded sequentially during the stance and swing phases, respectively, with a time interval of 28.8 ms. *F*, example of EMG responses to tilts in R Tric (Step, the AP movement of the stepping right forelimb). *G*, step-triggered averaging of triceps EMGs (mean ± S.E.M.) at two positions of the support: up (black traces) and down (red traces). Averaging over 40 cycles in cat 1. Designation of joints: Sh, shoulder; E, elbow; W, wrist. Note that inter-limb distance (in the frontal plane) for the forelimbs was smaller than that for the hindlimbs (see Fig. 1), which resulted in smaller vertical excursions of the support area under the forelimbs (in *A*, *C* and *D*) than under the hindlimbs (Figs 3 and 4).

was accompanied by a larger burst in m. triceps (Fig. 5*F* and *G*). As in the hindlimb (Fig. 4*B*), the limb trajectory in the swing phase was also affected by tilt (Fig. 5*E*).

Activity of PTNs during standing

Altogether, 249 PTNs were recorded in three cats in the task of standing on an inclined plane, including 128 forelimb PTNs and 121 hindlimb PTNs (Table 1). We will consider them separately.

Forelimb PTNs. Among the forelimb PTNs ($n = 128$), a large group of neurons (59 cells, or 46%) exhibited a static (positional) response to tilt, i.e. their frequencies during ipsi-side down tilt and during contra-side down tilt were significantly different ($P < 0.05$, paired t test), as in a PTN shown in Fig. 2*A*. (In this and subsequent sections, terms ipsi- and contra- are used in relation to the side of PTN recording.) The tilt-related activity of PTNs of this group is characterized in Fig. 6*A*. In this scatter diagram, the values of each point on the y- and x-axes show the frequency of individual PTNs during ipsilateral and contralateral downward tilt, respectively. The majority of data points occurred within the zone of 0–30 impulses (imp) s^{-1}.

All 59 statically responding forelimb PTNs were classified into two sub-groups, depending on the direction of tilt at which the neurons were more active. In the diagram (Fig. 6*A*), these sub-groups are positioned below and above the line $y = x$, respectively. Twenty-three neurons (18% of the total number) were activated with contra-tilt, and 36 neurons (28%) were activated with ipsi-tilt (Fig. 7*A*). The response characteristics (i.e. the activity in different parts of the tilt cycle, mean ± S.E.M. for these two sub-groups are given in Fig. 7*B* and *C*. In each subgroup, there was an almost threefold difference between the activities during ipsi-tilt and contra-tilt.

Another large group of forelimb PTNs (69 cells, or 54%), exhibited no static response to tilt, i.e. there was no significant difference between their frequencies during ipsi-side down tilt and during contra-side down tilt. The majority of these PTNs (39 cells, or 57%) responded dynamically, however.

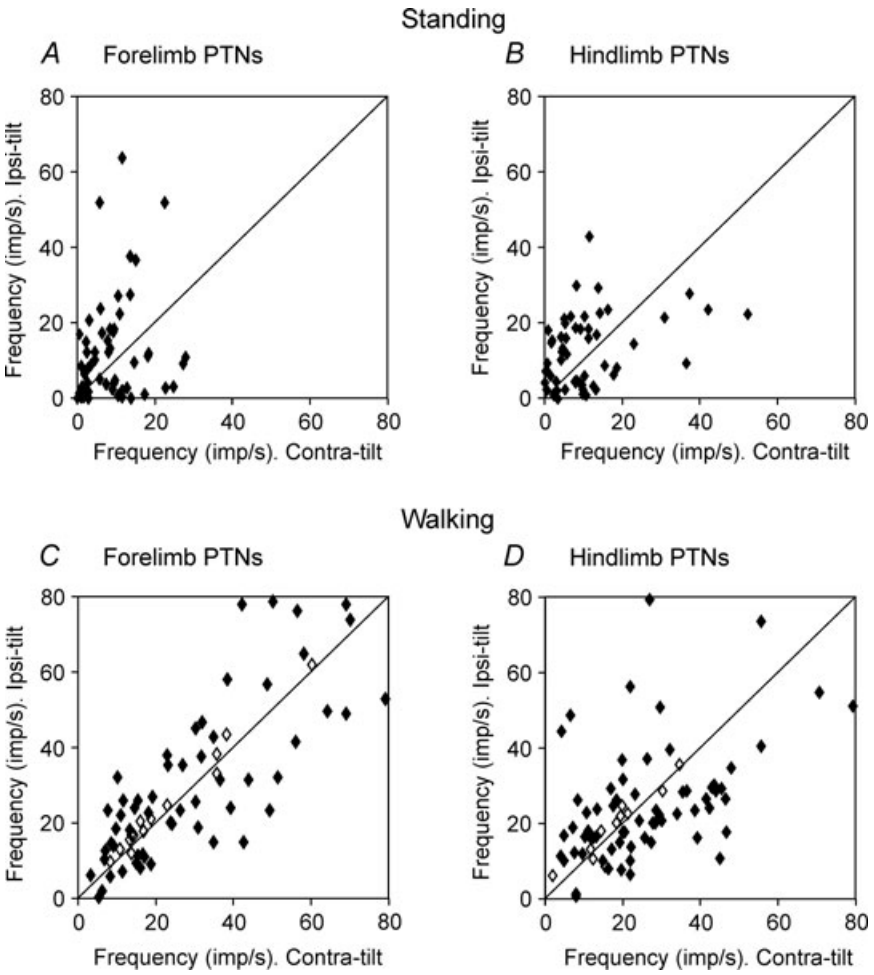

Figure 6. Comparison of PTN activity during ipsilateral and contralateral tilt
The x and y values of each point show the frequency of individual PTNs during contralateral and ipsilateral tilt, respectively. *A* and *B*, mean frequency during standing, for the forelimb PTNs (*A*) and hindlimb PTNs (*B*). *C* and *D*, burst frequency during walking, for the forelimb PTNs (*C*) and hindlimb PTNs (*D*). In *C* and *D*, filled diamonds represent the PTNs in which the difference between their mean burst frequencies was statistically significant; open diamonds, those in which the difference between their mean cycle frequencies was statistically significant. To confirm that the group of data below the x–y diagonal is statistically different from the group above the diagonal, we fitted two regression lines to the data and tested if they were significantly different from each other. The hypothesis that the two lines are the same was rejected in all these cases. In *A*, $F(2,55) = 48.5$, $P < 0.001$; in *B*, $F(2,53) = 40.5$, $P < 0.001$; in *C*, $F(2,66) = 37.0$, $P < 0.001$; in *D*, $F(2,53) = 30.0$, $P < 0.001$.

Hindlimb PTNs. Similar results were obtained for the hindlimb PTNs. Among these PTNs ($n = 121$), a large group (57 cells, or 47%) exhibited a static response to tilt. The frequencies of individual neurons of this group during ipsilateral and contralateral tilt are shown in Fig. 6*B*. The majority of data points occurred within the zone of 0–30 imp s^{-1}.

We classified all 57 statically responding hindlimb PTNs into two sub-groups, depending on the direction of downward tilt at which the neurons were activated. In the diagram (Fig. 6*B*), these sub-groups are positioned below and above the line $y = x$, respectively. There were 26 neurons (21% of total number) activated with contra-tilt, and 31 neurons (26%) activated with ipsi-tilt (Fig. 7*D*). The response characteristics for these two sub-groups are given in Fig. 7*E* and *F*. In each sub-group, there was a more than twofold difference between the activities during ipsi-tilt and contra-tilt.

Another large group of hindlimb PTNs (64 cells, or 53%) exhibited no static response to tilt. Some of these PTNs (26 cells, or 41%) responded dynamically, however.

Thus, about half of PTNs from the fore- and hindlimb representations of the motor cortex had a static component in their response to tilt, suggesting their participation in the maintenance of asymmetrical limb configuration during standing on the inclined surface.

Activity of PTNs during walking

Step-related modulation. Altogether, 253 PTNs were recorded during walking, including 131 forelimb PTNs and 122 hindlimb PTNs (Table 1). It is known that the

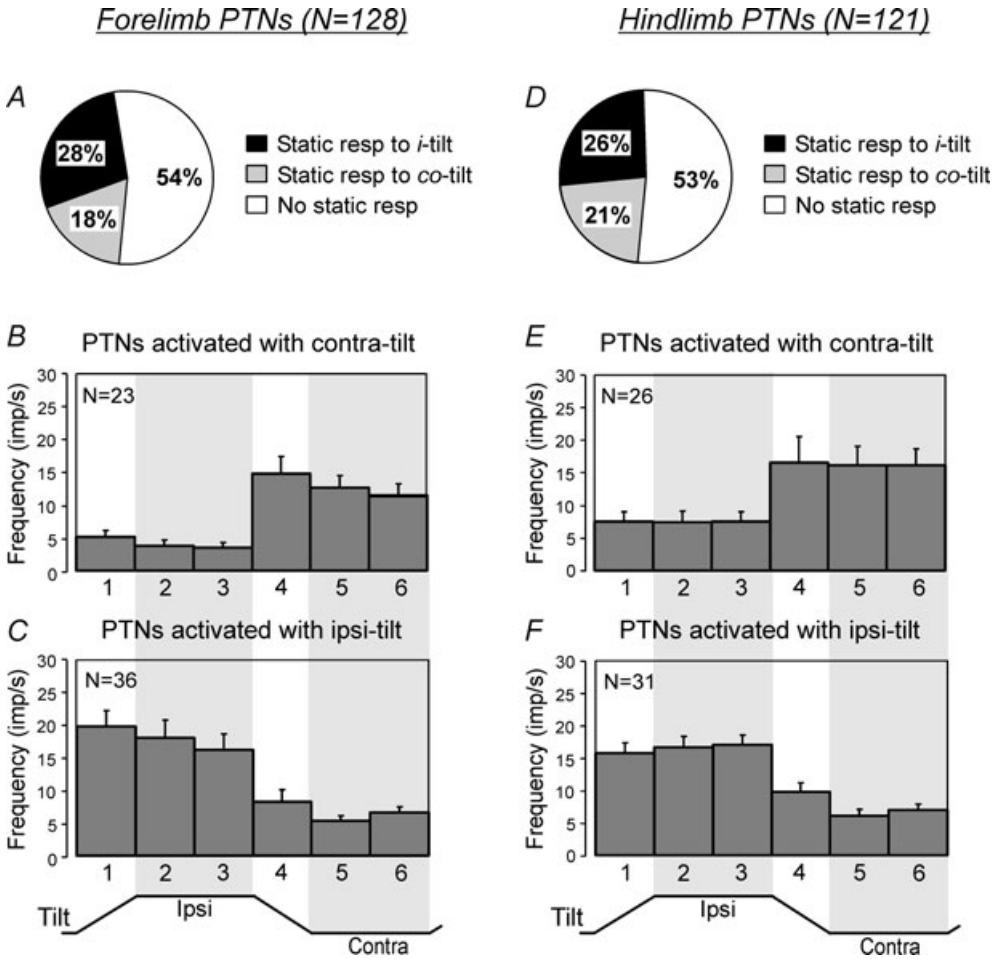

Figure 7. Population characteristics of forelimb and hindlimb PTNs when standing on an inclined plane
A–C, forelimb PTNs. *A*, expression of static responses to tilts in the population of forelimb PTNs. *B* and *C*, response characteristics for the sub-population activated statically with contra-tilt (*B*), or with ipsi-tilt (*C*). *D–F*, hindlimb PTNs. *D*, expression of static responses to tilts in the population of hindlimb PTNs. *E* and *F*, response characteristics for the sub-population activated statically with contra-tilt (*E*), or with ipsi-tilt (*F*). The histograms show the mean frequency (± S.E.M.) in different phases of the tilt cycle. Periods of stationary tilts and static responses are shaded.

activity of PTNs during locomotion is modulated in the rhythm of stepping (see e.g. Drew, 1993; Beloozerova & Sirota, 1993*a*,*b*). This rhythmic modulation was also characteristic for our sample of neurons (as in the neuron in Fig. 2*D*). During locomotion on the horizontal surface, modulation was observed in 130 (99%) of forelimb PTNs and in 121 (99%) of hindlimb PTNs. Figure 8*A* shows the burst and inter-burst frequency (mean ± S.E.M.) for the populations of forelimb and hindlimb PTNs. For each of the populations, the preferred phases of individual PTNs were very diverse and distributed over the whole step cycle (Fig. 8*B* and *C*). These results are in good agreement with the previous studies (Drew, 1988, 1993; Beloozerova & Sirota, 1988, 1993*a*,*b*).

Effect of tilt. Among the forelimb PTNs ($n = 131$), a large group of neurons (71 cells, or 54%) exhibited a positional response to tilt. The tilt-related activity of PTNs of this group is characterized in Fig. 6*C*. In this diagram, the values of each point on the *y*- and *x*-axes show the mean burst frequency of individual PTNs during ipsilateral and contralateral downward tilt, respectively. Most of these neurons (shown by filled diamonds) had statistically significant differences between their mean burst frequencies during ipsi-side down tilt and during contra-side down tilt, as in a PTN shown in Fig. 2*D*. In a small proportion of neurons ($n = 13$, shown by open diamonds), the difference between their mean burst frequencies was not significant. However, we classified these PTNs as responding ones since they had statistically significant differences in the mean frequency over the locomotor cycle. In Fig. 6*C* they are positioned close to the $y = x$ line. On average, the activity of forelimb PTNs during walking (frequency in the bursts) was considerably higher than their activity during standing (compare Fig. 6*A* and *C*).

We classified all 71 responding forelimb PTNs into two sub-groups, depending on the direction of downward tilt in which the neurons increased their burst frequency. Thirty neurons (23% of total number) were activated with contra-tilt, and 41 neurons (31%) were activated with ipsi-tilt (Fig. 9*A*). In the diagram (Fig. 6*C*), these sub-groups are positioned below and above the line $y = x$, respectively. Their response characteristics (i.e. the burst and interburst frequency at different tilts, mean ± S.E.M.) are given in Fig. 9*B* and *C*. In each sub-group, there was about a 30% difference between the burst frequencies during ipsi-tilt and during contra-tilt.

Another large group of forelimb PTNs (60 cells, or 46%) exhibited no positional response to tilt, i.e. there was no significant difference ($P > 0.05$) between their burst frequency during ipsi-side down tilt and during contra-side down tilt.

Similar results were obtained for the hindlimb PTNs. Among these PTNs ($n = 122$), a large group (77 cells, or 63%) exhibited a positional response to tilt. Most of these neurons (shown by filled diamonds in Fig. 6*D*) had statistically significant differences between their mean burst frequencies during ipsilateral and contralateral tilt, whereas a small proportion ($n = 10$) was classified as responding to tilts on the basis of statistically significant differences in their mean cycle frequencies. On average, the activity of hindlimb PTNs during walking (burst frequency) was considerably higher than their activity during standing (compare Fig. 6*B* and *D*). Another large

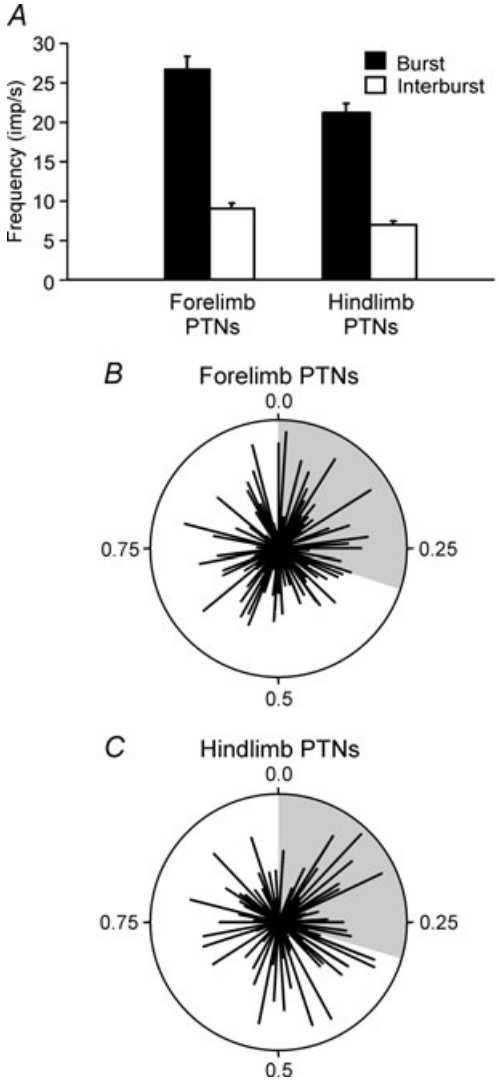

Figure 8. Population characteristics of PTNs when walking on the horizontal plane
A, burst and interburst frequency for the populations of forelimb and hindlimb PTNs during walking (mean ± S.E.M.). *B* and *C*, circular plots of firing of forelimb (*B*) and hindlimb (*C*) PTNs. Averaged vector of firing and its phase is indicated for individual neurons, the radius of the circle is set equal to 1. Swing phase of the corresponding fore- or hindlimb (contralateral to a neuron) is indicated with a grey sector.

group of hindlimb PTNs (45 cells, or 37%) exhibited no positional response to tilt.

Among 77 responding hindlimb PTNs, 40 neurons (33% of total number) were activated with contra-tilt, and 37 neurons (30%) were activated with ipsi-tilt (Fig. 9*D*). In the diagram (Fig. 6*D*), these sub-groups are positioned below and above the line $y = x$, respectively. The response characteristics (i.e. the burst and inter-burst frequency at different tilts, mean ± S.E.M.) for these two sub-groups are given in Fig. 9*E* and *F*.

We also characterized the effect of tilt on the phase of modulation of PTNs in the step cycle. For this purpose, we compared preferred phases of PTN activity during left tilt with those during right tilt. Figure 10 shows the histograms of algebraic difference between the preferred phases of individual PTNs at the left and right tilted positions, for two sub-groups of forelimb PTNs (*A* and *B*) and for two sub-groups of hindlimb PTNs (*C* and *D*). In each sub-group, the shift of the preferred phase in the majority of neurons was less than 10% of the step cycle, suggesting that the effect of tilt on the phase of modulation was weak, in contrast to the effect on the magnitude of PTN activity.

Thus, about half of PTNs from the fore- and hindlimb representations of the motor cortex had a positional response to tilts, i.e. the magnitude of their activity depended on an inclination of the supporting surface. These PTNs may contribute to the adaptation of stepping movements when walking on an inclined plane.

Relationships between step-related and tilt-related modulation of PTNs during walking

In the previous section, we divided all the forelimb PTNs responding to tilt during walking into two sub-groups, which were activated with contra-tilt and with ipsi-tilt, respectively. Similar classification was done for the hindlimb PTNs. The preferred tilt direction presumably characterizes the role of a PTN in the control of postural body configuration. On the other hand, all PTNs were modulated in the rhythm of stepping, and

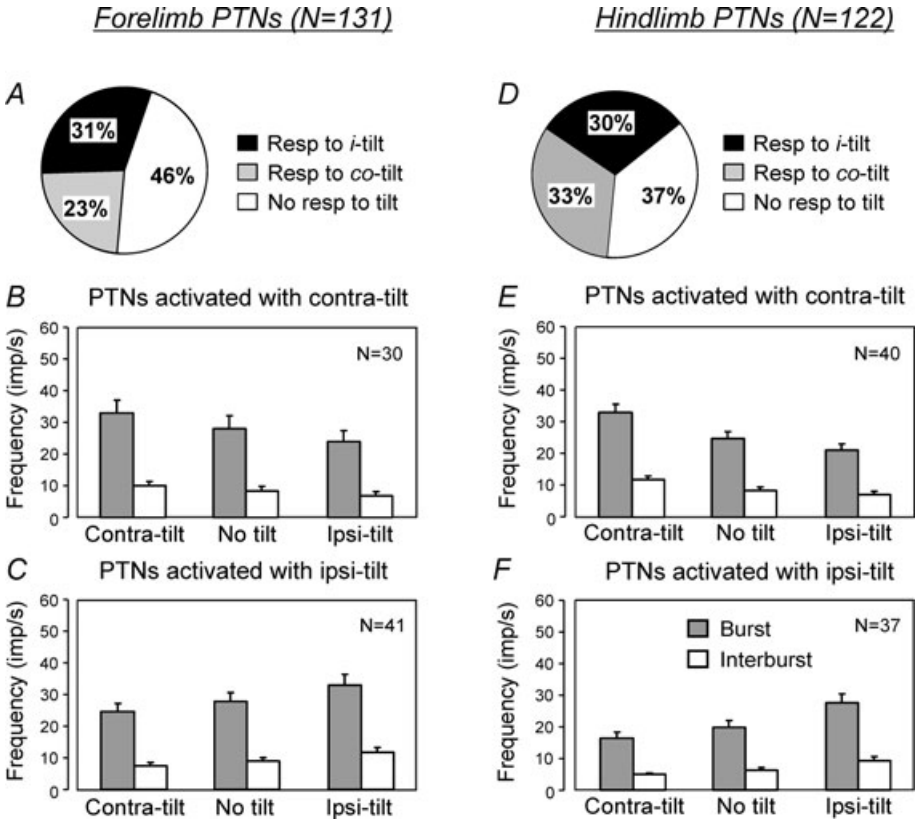

Figure 9. Population characteristics of forelimb and hindlimb PTNs when walking on an inclined plane
A–C, forelimb PTNs. *A*, percentage of forelimb PTNs with different patterns of response to tilts. *B* and *C*, response characteristics for the sub-populations activated with contra-tilt (*B*), or with ipsi-tilt (*C*). *D–F*, hindlimb PTNs. *D*, percentage of hindlimb PTNs with different patterns of response to tilts. *E* and *F*, response characteristics for the sub-populations activated with contra-tilt (*E*), or with ipsi-tilt (*F*). The histograms show the burst and interburst frequency (mean ± S.E.M.) for different orientations of the treadmill: contra-tilt, no tilt, ipsi-tilt.

individual PTNs had a specific modulation pattern, with their peak activity in either the swing or stance part of the step. The preferred phase presumably characterizes the role of a PTN in the control of stepping. Do these two characteristics correlate? To answer this question, we calculated separately the number of PTNs with their preferred phase in the swing and stance parts of the step cycle. In the graph (Fig. 11), the number of neurons in each sub-group, i.e. activated by ipsi-tilt or contra-tilt (these numbers are indicated under the bars) was taken as 100%, and the proportion of swing-related and stance-related PTNs is shown by black and grey parts of the bar, respectively. This was done separately for the forelimb and hindlimb PTNs. One can see that the proportions of swing-related and stance-related PTNs were similar in the subgroup activated by contra-tilt (50% and 50%) in both fore- and hindlimb populations. Thus, the step-related characteristics of PTNs of this subgroup did not correlate with their tilt-related characteristics. By contrast, the proportion of stance-related PTNs in the sub-group activated by ipsi-tilt was larger than the proportion of swing-related PTNs (71% *vs.* 29%, and 58% *vs.* 42% for the fore- and hindlimb populations, respectively) (Fig. 11).

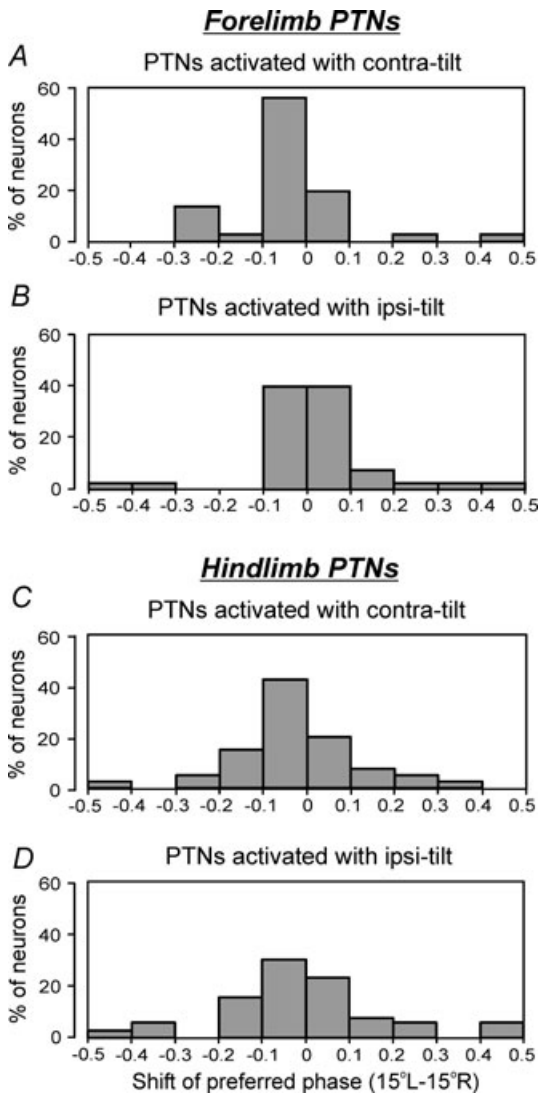

Figure 10. Effect of tilts on the preferred phase of PTNs during walking
The histograms show the algebraic difference between the preferred phases of individual PTNs at the left and right tilted positions, for the forelimb PTNs (*A* and *B*) and hindlimb PTNs (*C* and *D*), separately for the PTNs activated by contra-tilt (*A* and *C*) and ipsi-tilt (*B* and *D*).

Relationships between PTN responses to tilt during standing and during walking

In the motor task of standing on the inclined plane, three main types of static (positional) PTN responses to tilts were observed: activation with contra-tilt, activation with ipsi-tilt, and no response to tilts (Fig. 7*A* and *D*). Similarly, in the task of walking, there were observed three types of positional responses: activation with contra-tilt, activation with ipsi-tilt, and no response to tilts (Fig. 9*A* and *D*). Do individual PTNs have the same or a different type of response in the two motor tasks? Figure 12 shows the proportion of PTNs with different combinations of responses, observed in the population of PTNs recorded in both tasks (fore- and hindlimb PTNs were taken together, $n = 248$). This proportion was basically similar in individual cats (see Table 1). About a quarter of PTNs (24%) did not respond either during standing or during walking, whereas 16% responded only during standing, and 30% only during walking. About one-third of PTNs (30%) responded in both tasks, including the PTNs

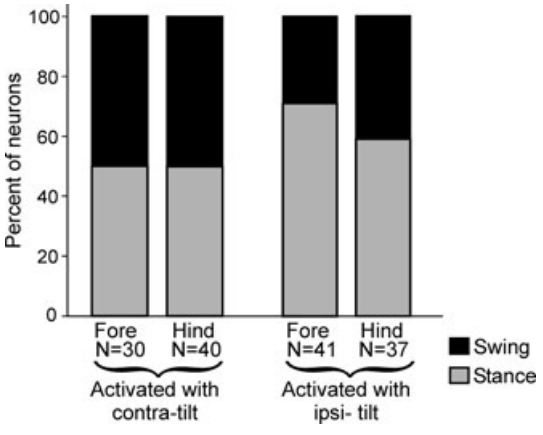

Figure 11. Relationships between the step-related and tilt-related modulation of PTN activity
The forelimb and hindlimb groups of PTNs each consisted of two sub-groups, activated with contra-tilt and with ipsi-tilt, respectively. The number of neurons in each sub-group (indicated under the bars) was taken as 100%. Each sub-group included the PTNs with the preferred phase in the swing part of step (black bars) and in the stance part (grey bars).

responding to opposite tilts in the two tasks (7%) and PTNs responding to the same tilt (23%). In the latter group, about half of PTNs (10% of the whole population) were activated by contra-tilt, and another half (13%) by ipsi-tilt. It is possible that the group of PTNs with similar responses to tilts during standing and during walking participates in both tasks by performing similar postural adaptations.

Discussion

Freely behaving animals are capable of adapting to different features of the terrain on which they are standing or walking. One of these is inclination of the supporting surface. As shown by Matsuyama & Drew (2000), walking cats easily adapt their posture to the support inclination in any plane, and modify the activity in both limbs of the same girdle either symmetrically (for pitch tilts) or asymmetrically (for roll tilts). How are these adaptations controlled? The existing data suggest that supraspinal mechanisms play an important role in the motor pattern modifications that are required for postural adaptations. *First*, the animals with extensive spinal lesions have almost no ability to maintain the normal posture of their hindquarters or to modify this posture during locomotion (Grillner, 1973; Brustein & Rossignol, 1998; Rossignol *et al.* 2000) or during standing (Pratt *et al.* 1994; Lyalka *et al.* 2005). *Second*, the activity of reticulo- and vestibulospinal neurons during locomotion correlates with the value of stationary tilt of the walkway (Matsuyama & Drew, 2000), suggesting that these descending systems send commands to the spinal cord that affect the postural configuration and cause adaptive modifications.

It is known that the motor cortex participates in some aspects of the control of body posture (for review, see Jacobs & Horak, 2007). As shown by Beloozerova *et al.* (2005), the activity of PTNs in the standing cat strongly correlates with postural corrections caused by dynamic postural perturbations (sinusoidal lateral tilts), suggesting that the motor cortex participates in the feedback control of equilibrium and generates commands contributing to postural corrections with any deviation from the stabilized body orientation. Does the motor cortex also participate in the control of postural adaptations? To answer this question, in the present study we characterized the activity of PTNs during two motor tasks, i.e. during standing and walking on the surface inclined in the roll plane. The main result of this study was that, in both motor tasks, many PTNs had a positional response to tilt, that is, their activity depended on the value of stationary tilt. In both motor tasks, a 30 deg change in the stationary tilt caused a $\sim$10 imp s^{-1} change of the frequency in the population of responding PTNs (Figs 7 and 9). These results suggest that the motor cortex, along with other descending systems, e.g. reticulo- and vestibulospinal ones (Matsuyama & Drew, 2000) sends commands to the spinal cord and medulla that determine a postural body configuration and its adaptive modifications. Thus, the motor cortex participates in the performance of both principal postural functions formulated by Horak & Macpherson (1996): the maintenance of a definite body configuration and the maintenance of equilibrium.

Both standing and walking on an inclined plane require specific modifications of the body configuration, with asymmetry in the functional length of the opposite limbs. As demonstrated previously (Matsuyama & Drew, 2000; Beloozerova *et al.* 2005; Lyalka *et al.* 2005) and confirmed in the present study (Figs 3–5), these changes in the limb configuration are caused by specific modifications of the EMG pattern. During standing, the extensor activity

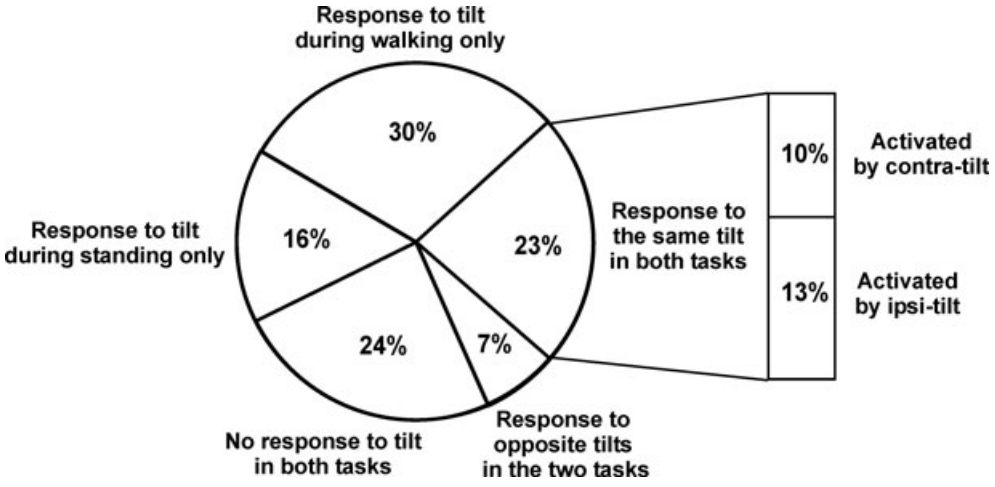

Figure 12. Proportions of PTNs exhibiting different combinations of responses to tilt in the tasks of standing and walking

increased when the ipsi-side was tilted down and the limb was extended, and decreased when the ipsi-side was tilted up and the limb was flexed. During walking, these changes were incorporated into a step cycle, so that the extensor bursts in the stance part of the step increased when the ipsi-side was tilted down, and decreased when it was tilted up. These changes did not perturb the basic pattern of stepping, with rhythmical alternation of its swing and stance phases (Orlovsky *et al.* 1999).

It is likely that in both motor tasks, adaptations to the inclined support plane are caused by similar influences on the motor output, i.e. by activation of extensor motoneurons on the side tilted down, and by reduction of their activity on the opposite side. In the locomotor task, these influences are rhythmically modulated (gated) so that they are effective only in the end of the swing phase and beginning of the stance phase of the step. Can the corticospinal commands, which are transmitted by PTNs, contribute to these changes in the extensor activity?

To assess a possible role of corticospinal commands for postural adaptations during standing and walking, we compared the PTN activity with the adaptive changes in the motor pattern. In the task of standing, about half of PTNs exhibited a positional (static) response to tilt: they increased their activity either with contra-tilt or with ipsi-tilt (Fig. 7*A* and *D*). We suggest that this population of PTNs, by sending tilt-related commands to the spinal cord, contributes to modifications of the motor pattern necessary for standing on an inclined plane. If so, the main attribute of the corrective motor response, that is, activation of extensors on the side tilted down, requires an excitatory action upon the extensor motoneurons from the sub-group of PTNs activated with contra-tilt (taking into account crossed PTN projections). The sub-group activated with ipsi-tilt should have an inhibitory action on extensors, or no action.

During locomotion, the PTNs were modulated in the rhythm of stepping, with the preferred phases of activity distributed over the step cycle. Tilts of the supporting surface caused changes in the magnitude of this step-related modulation. About half of PTNs exhibited a positional response to tilt, i.e. they increased their burst of activity either with contra-tilt or with ipsi-tilt (Fig. 9*A* and *D*). The tilts only slightly affected the phase of the step-related modulation in these PTNs. We suggest that this population of PTNs, by sending step-related and tilt-related commands to the spinal cord, can affect the output stage (motoneurons) of the spinal locomotor mechanism and, by increasing or decreasing the extensor bursts, can contribute to the modifications of stepping movements necessary for walking on the inclined plane. To increase the extensor bursts on the side tilted down, the sub-groups of PTNs, activated with contra-tilt and with ipsi-tilt, should have an excitatory and inhibitory action on the extensor motoneurons, respectively. It should be noted, however, that only some of the PTNs, with their burst located in the stance phase of the step cycle, could participate in the control of extensors. Such neurons compose from 50% to 71% of different sub-groups of PTNs (Fig. 11). Another part of PTNs, which were active in the swing phase of the step cycle, could be responsible for the modifications of the swing motor pattern observed during walking on an inclined plane (Figs 4*B* and 5*D*).

By comparing the positional responses to tilts in individual PTNs recorded in the two motor tasks (standing and walking), four groups of PTNs have been revealed (Fig. 12): responding to tilts in both tasks (30%); responding only during standing (16%); responding only during walking (30%); responding in none of the tasks (24%). This striking diversity suggests that both common and separate cortical mechanisms are used in the two motor tasks (standing and walking) for the control of postural adaptations to inclinations of the supporting surface. Among the PTNs responding in both tasks (30%), the majority of neurons (23%) were activated by the same tilt in both tasks, either 'contra' (10%) or 'ipsi' (13%). One can suggest that this group of PTNs performs similar functions during both standing and walking, that is, they affect the value of extensor tone (in standing) in the same way that they affect the value of periodical extensor bursts (in walking). The diversity of relationships between the activity of cortical neurons on the one hand and the variables characterizing the motor pattern on the other is a common finding in many studies of the motor cortex (see e.g. Drew, 1993; Kakei *et al.* 2003), including those devoted to postural control (Beloozerova *et al.* 2005; Karayannidou *et al.* 2008*b*).

What is the origin of the positional responses to tilts observed in PTNs during standing and walking? It seems unlikely that these responses were caused by the vestibular and visual inputs signalling changes in the head position. Indeed, during both motor tasks in these experiments, the cat was continuously licking food from the feeder (see Methods). To do this, the cat stabilized its mouth position against the feeder with high precision, despite the tilts of the supporting surface (Beloozerova *et al.* 2005). Under these conditions, it is not likely that the tilts are reflected in the position of the head and labyrinths, and could be monitored by vestibular and visual systems. However, it can be expected that load and stretch receptors in the limbs are strongly affected by differential loading and flexion/extension movements of the limbs through tilt-related changes in the spatial orientation of the animal. These limb receptors are most likely responsible for the dynamic reactions to different perturbation of posture during standing (Inglis & Macpherson, 1995; Deliagina *et al.* 2000; Stapley *et al.* 2002), as well as the dynamic PTN responses to tilts in standing subjects (Karayannidou *et al.* 2008*b*). Whether this afferent input is also responsible for

the positional reactions to tilts during standing and during walking remains an open question.

References

Armstrong DM & Drew T (1983). Topographical localization in the motor cortex of the cat for somatic afferent responses and evoked movements. *J Physiol* **350**, 33–54.

Armstrong DM & Drew T (1984). Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. *J Physiol* **346**, 471–495.

Armstrong DM & Drew T (1985). Forelimb electromyographic responses to motor cortex stimulation during locomotion in the cat. *J Physiol* **367**, 327–351.

Batschelet E (1981). *Circular Statistics in Biology*. Academic Press, New York.

Beloozerova I & Sirota M (1988). Role of motor cortex in control of locomotion. In *Stance and Motion. Facts and Concepts*, ed. Gurfinkel VS, Ioffe ME, Massion J & Roll JP, pp. 163–176. Plenum Press, New York, London.

Beloozerova IN & Sirota MG (1993*a*). The role of the motor cortex in the control of accuracy of locomotor movements in the cat. *J Physiol* **461**, 1–25.

Beloozerova IN & Sirota MG (1993*b*). The role of the motor cortex in the control of vigour of locomotor movements in the cat. *J Physiol* **461**, 27–46.

Beloozerova IN, Sirota MG, Orlovsky GN & Deliagina TG (2005). Activity of pyramidal tract neurons in the cat during postural corrections. *J Neurophysiol* **93**, 1831–1844.

Beloozerova IN, Sirota MG, Swadlow HA, Orlovsky GN, Popova LB & Deliagina TG (2003*a*). Activity of different classes of neurons of the motor cortex during postural corrections. *J Neurosci* **23**, 7844–7853.

Beloozerova IN, Zelenin PV, Popova LB, Orlovsky GN, Grillner S & Deliagina TG (2003*b*). Postural control in the rabbit maintaining balance on the tilting platform. *J Neurophysiol* **90**, 3783–3793.

Bishop PO, Burke W & Davis R (1962). The identification of single units in central visual pathways. *J Physiol* **162**, 409–431.

Brustein E & Rossignol S (1998). Recovery of locomotion after ventral and ventrolateral spinal lesions in the cat. I. Deficits and adaptive mechanisms. *J Neurophysiol* **80**, 1245–1267.

Deliagina T, Beloozerova IN, Popova LB, Sirota MG, Swadlow HA, Grant G & Orlovsky GN (2000). Role of different sensory inputs for maintenance of body posture in sitting rat and rabbit. *Motor Control* **4**, 439–452.

Deliagina TG, Orlovsky GN, Zelenin PV & Beloozerova IN (2006). Neural bases of postural control. *Physiology* **21**, 216–225.

Drew T (1988). Motor cortical cell discharge during voluntary gait modification. *Brain Res* **457**, 181–187.

Drew T (1993). Motor cortical activity during voluntary gait modifications in the cat. I. Cells related to the forelimbs. *J Neurophysiol* **70**, 179–199.

Fuller JH & Schlag JD (1976). Determination of antidromic excitation by the collision test: problems of interpretation. *Brain Res* **112**, 283–298.

Ghosh S (1997). Identification of motor areas of the cat cerebral cortex based on studies of cortical stimulation and corticospinal connections. *J Comp Neurol* **380**, 191–214.

Grillner S (1973). Locomotion in the spinal cat. In *Control of Posture and Locomotion*, ed. Stein RB, Pearson KG, Smith RS & Redford JB, pp. 515–535. Plenum Press, New York.

Horak F & Macpherson J (1996). Postural orientation and equilibrium. In *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*, ed. Rowell LB & Shepherd JT, pp. 255–292. Oxford UP, New York.

Inglis JT & Macpherson JM (1995). Bilateral labyrinthectomy in the cat: effects on the postural response to translation. *J Neurophysiol* **73**, 1181–1191.

Jacobs JV & Horak FB (2007). Cortical control of postural responses. *J Neural Transm* **114**, 1339–1348.

Kakei S, Hoffman DS & Strick PL (2003). Sensorimotor transformations in cortical motor areas. *Neurosci Res* **46**, 1–10.

Karayannidou A, Beloozerova IN, Zelenin PV, Stout EE, Sirota MG, Orlovsky GN & Deliagina TG (2008*a*). Participation of pyramidal tract neurons from the motor cortex in control of standing and walking on inclined surface. *Soc Abstr Neurosci* **34**, 860.3.

Karayannidou A, Deliagina T, Tamarova Z, Sirota M, Zelenin P, Orlovsky G & Beloozerova I (2008*b*). Influences of sensory input from the limbs on feline corticospinal neurons during postural responses. *J Physiol* **586**, 247–263.

Karayannidou A, Zelenin PV, Orlovsky GN, Sirota MG, Beloozerova IN & Deliagina TG (2009). Maintenance of lateral stability during standing and walking in the cat. *J Neurophysiol* **101**, 8–19.

Lyalka V, Zelenin P, Karayannidou A, Orlovsky G, Grillner S & Deliagina T (2005). Impairment and recovery of postural control after spinal cord lesions. *J Neurophysiol* **94**, 3677–3690.

Martin JH & Ghez C (1985). Task-related coding of stimulus and response in cat motor cortex. *Exp Brain Res* **57**, 427–442.

Martin JH & Ghez C (1993). Differential impairments in reaching and grasping produced by local inactivation within the forelimb representation of the motor cortex in the cat. *Exp Brain Res* **94**, 429–443.

Matsuyama K & Drew T (2000). Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane. *J Neurophysiol* **84**, 2257–2276.

Myasnikov AA, Dykes RW & Avendano C (1994). Cytoarchitecture and responsiveness of the medial ansate region of the cat primary somatosensory cortex. *J Comp Neurol* **349**, 401–427.

Nieoullon A & Rispal-Padel L (1976). Somatotopic localization in cat motor cortex. *Brain Res* **105**, 405–422.

Orlovsky GN, Deliagina TG & Grillner S (1999). *Neuronal Control of Locomotion: From Mollusc to Man*. Oxford UP, Oxford.

Pratt CA, Fung J & Macpherson JM (1994). Stance control in the chronic spinal cat. *J Neurophysiol* **71**, 1981–1985.

Prilutsky BI, Sirota MG, Gregor RJ & Beloozerova IN (2005). Quantification of motor cortex activity and full-body biomechanics during unconstrained locomotion. *J Neurophysiol* **94**, 2959–2969.

Reitboeck HJ (1983). Fibre microelectrodes for electrophysiological recordings. *J Neurosci Methods* **8**, 249–262.

Rossignol S, Belanger M, Chau C, Giroux N, Brustein E, Bouyer L, Grenier C, Drew T, Barbeau H & Reader T (2000). The spinal cat. In *Neurobiology of Spinal Cord Injury*, ed. Kalb E, pp. 57–87. Humana, Totowa, NJ, USA.

Stapley PJ, Ting LH, Hulliger M & Macpherson JM (2002). Automatic postural responses are delayed by pyridoxine-induced somatosensory loss. *J Neurosci* **22**, 5803–5807.

Vicario D, Martin J & Ghez C (1983). Specialized subregions in the cat motor cortex: A single unit analysis in the behaving animal. *Exp Brain Res* **51**, 351–367.

Zar JH (1974). *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ, USA.

Author contributions

I.N.B., M.G.S., G.N.O. and T.G.D. contributed to the conception and design of the experiments. All authors contributed to the analysis and interpretation of data, writing and revision of the manuscript. The experiments were performed at Barrow Neurological Institute, AZ, USA.

Acknowledgements

We thank Peter Wettenstein for exceptional engineering assistance. This study was supported by grants from the National Institutes of Health (R01 NS-049884), from the Swedish Research Council (M, 11554), from Gösta Fraenckels Foundation to T. G. Deliagina; by grants from the National Institutes of Health (R01 NS-058659) and from Barrow Neurological Foundation to I. N. Beloozerova; and from the Swedish Research Council (M, 21076) to P. V. Zelenin.