NEURAL MECHANISMS OF SINGLE CORRECTIVE STEPS EVOKED IN THE STANDING RABBIT

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Abstract—Single steps in different directions are often used for postural corrections. However, our knowledge about the neural mechanisms underlying their generation is scarce. This study was aimed to characterize the corrective steps generated in response to disturbances of the basic body configuration caused by forward, backward or outward displacement of the hindlimb, as well as to reveal location in the CNS of the corrective step generating mechanisms. Video recording of the motor response to translation of the supporting surface under the hindlimb along with contact forces and activity of back and limb muscles was performed in freely standing intact and in fixed postmammillary rabbits. In intact rabbits, displacement of the hindlimb in any direction caused a lateral trunk movement toward the contralateral hindlimb, and then a corrective step in the direction opposite to the initial displacement. The time difference between onsets of these two events varied considerably. The EMG pattern in the supporting hindlimb was similar for all directions of corrective steps. It caused the increase in the limb stiffness. EMG pattern in the stepping limb differed in steps with different directions. In postmammillary rabbits the corrective stepping movements, as well as EMG patterns in both stepping and standing hindlimbs were similar to those observed in intact rabbits. This study demonstrates that the corrective trunk and limb movements are generated by separate mechanisms activated by sensory signals from the deviated limb. The neuronal networks generating postural corrective steps reside in the brainstem, cerebellum, and spinal cord. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: corrective steps, muscle synergy, postural control, rabbit, sensory feedback.

INTRODUCTION

When standing, bipeds and quadrupeds maintain a specific basic body posture (the body orientation in space and the body configuration) due to activity of the postural control system. This system is driven by sensory feedback signals and generates corrective motor responses when the body posture deviates from the desired one (for review, see Horak and Macpherson, 1996; Massion, 1998; Deliagina et al., 2012, 2014). Both bipeds and quadrupeds use different strategies to stabilize the body orientation when standing. These strategies depend on the type and strength of postural perturbations, as well as on other factors. They could be divided into two groups: fixed-support strategies and change-in-support strategies (Horak, 2009; Maki and McIlroy, 1997). Fixed-support strategies include postural corrections caused by redistribution of the activity of the muscles which does not lead to a change of the support area. Change-in-support strategies include postural corrections resulting in a change of the support area, such as performing a corrective step or movement of the arm aimed to reach the support. It was found that the same types of postural perturbations (e.g. caused by tilt or translation of the support surface, by lateral push applied to the trunk) can evoke execution of the fixed-support strategy or the change-in-support strategy (the corrective step) (Beloozerova et al., 2003; Karayannidou et al., 2009). It was also shown that when the change-in-support strategy including the corrective step was generated in response to translation of the support surface in human, the functional muscle synergies characteristic for the fixed-support strategy were observed in the supporting limb (Chvatal et al., 2011; Burleigh et al., 1994).

It was suggested that change-in-support strategy is used when the fixed-support strategy is insufficient, and there is a risk of falling down (Horak and Nashner, 1986). However, later it was shown that in human, a corrective step could be initiated well before the moment when the center of mass appeared near the stability limits of the base of support (Maki and Whitelaw, 1993; Maki and McIlroy, 1997).

Fixed-support strategies executed in response to different types of postural perturbations were studied in considerable detail (Macpherson and Fung, 1999; Musienko et al., 2008, 2010; Honeycutt et al., 2009; Honeycutt and Nichols, 2010; Beloozerova et al., 2003; Deliagina et al., 2006, 2012; Karayannidou et al., 2008, 2009). By contrast, our knowledge about the operation of the postural systems generating change-in-support strategies, in particular those which include a corrective step, is extremely limited. It was shown that in humans, translation of the support surface in any particular direction evoked, first, the body weight shift toward one of the legs and then a corrective step performed by the unloaded limb in the direction opposite to the direction

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of the surface translation (Maki et al., 1996). It was also demonstrated that manipulation of sensory signals from receptors of the foot sole affected the probability of initiation of the corrective step (Perry et al., 2000).

Recently we found that distortion of the basic body configuration caused by displacement of the limb in the standing rabbit, evoked postural response restoring the initial body configuration (Hsu et al., 2014). This postural response included a single corrective step performed by the displaced limb in the direction opposite to the direction of the initial displacement while the other limbs remained standing. The goal of the present study was to analyze the neural mechanisms underlying the generation of postural responses to distortion of the basic standing body configuration caused by displacement of a single limb in different directions. For this purpose, *first*, these postural responses were characterized in details in intact standing rabbit. We found, that the limb displacement evoked a lateral movement of the trunk toward the contralateral (supporting) limb, and then a corrective step. During the corrective step the direction of the trunk movement was reversed and to the end of the step the body configuration returned to the initial one. *Second*, to determine the location in the CNS of the basic networks underlying generation of the postural response under the study, the motor response to displacement of the limb in relation to the trunk was examined in decerebrate premammillary and postmammillary rabbits. It was found that integrity of higher levels of CNS was not necessary for generation of the postural reaction to distortion of the basic body configuration.

A brief account of a part of this study has been published in abstract form (Hsu et al., 2014).

EXPERIMENTAL PROCEDURES

Experiments were carried out on 16 adult New Zealand rabbits (weight 2.5–3.5 kg). All experiments were conducted with approval of the local ethics committee (Norra Djurförsöksetiska Nämden) in Stockholm.

Surgical procedures

All animals were subjected to a surgery performed under Hypnorm-midazolam anesthesia, using aseptic procedures. Bipolar EMG electrodes (0.2-mm flexible stainless steel Teflon-insulated wires) were implanted bilaterally into four selected muscles of the trunk and/or hindlimbs. The recorded muscles, as well as the number of animals in which individual muscles were recorded, are listed in Table 1. The wires were led subcutaneously toward the head and then through a small incision in the skin on the dorsal aspect of the neck. The wound was sutured so that the wires were fastened to the skin. A small connector was soldered to each wire at a distance of 2–3 cm from the skin.

In 1–2 days, when the animal had recovered completely from the surgery, it was subjected to postural tests (see below). After few days of testing, seven animals were taken to acute experiment. The animal was injected with propofol (average dose 10 mg kg-1 i.v.) for induction of anesthesia, which was

Table 1. Recorded muscles and their function

Muscle	Abbreviation	Function	N
Sartorius	Sart	Hip flexor	3
Adductor longus	*Add*	Hip adductor	2
Gracilis	Grac	Hip extensor and adductor	3
Gluteus medius	Glut	Hip extensor and abductor	3
Abductor cruris caudalis	*Abd*	Caudal abductor of shin	3
Rectus femoris	(RF)	Hip flexor and knee extensor	2
Vastus medialis	*Vast*	Knee extensor	4
Bicep femoris	Bic	Hip extensor and knee flexor	3
Semimembranosus	Sm	Hip extensor and knee flexor	3
Semitendinosus	(St)	Hip extensor and knee flexor	4
Tibialis anterior	Tib	Ankle flexor	10
Extensor digitorum longus	EDL	Ankle flexor and extensor of digits	2
Gastrocnemius	(Gast)	Ankle extensor	4
Oblique externus abdominis	OEA	Ipsilateral bending and contralateral twisting of the spine	2
Erector spinae	ES	Ipsilateral bending and contralateral twisting of the spine	3
Multifidus	MF	Ipsilateral bending and contralateral twisting of the spine	3

In Abbreviation column, parenthesis indicate that the muscle was not active in the standing limb, italic indicates that the phase of the muscle activity in the stepping limb varied in different animals, underline indicates that the phase of the muscle activity in the standing limb varied in different animals. *N*, the number of animals in which a particular muscle was recorded.

continued on isoflurane (1.5–2.5%) delivered in O_2. The trachea was cannulated. For all subsequent procedures, six animals were positioned in a metal frame, and its head and vertebral column were rigidly fixed (Fig. 1G). Then they were decerebrated at the precollicular-postmammillary level (Musienko et al., 2008). One animal was decerebrated at the precollicular-premammillary level (Musienko et al., 2008) without the body fixation. After decerebration, the anesthesia was discontinued. During the experiment, the rectal temperature and mean blood pressure of the animal were continuously monitored and were kept at 37–38 °C and at greater than 90 mmHg, respectively. Recordings in decerebrated animals were started no less than 1 h after cessation of anesthesia. The experiments were terminated by a lethal dose of anesthetic (pentobarbital sodium).

Experimental design

Experiments on intact rabbits and on premammillary rabbit. No special training of the animal was performed prior to postural testing. During the test, the animal was freely standing on four horizontal platforms (Fig. 1A–C). A movable thin plastic plate (23 × 10 × 0.3 cm) with
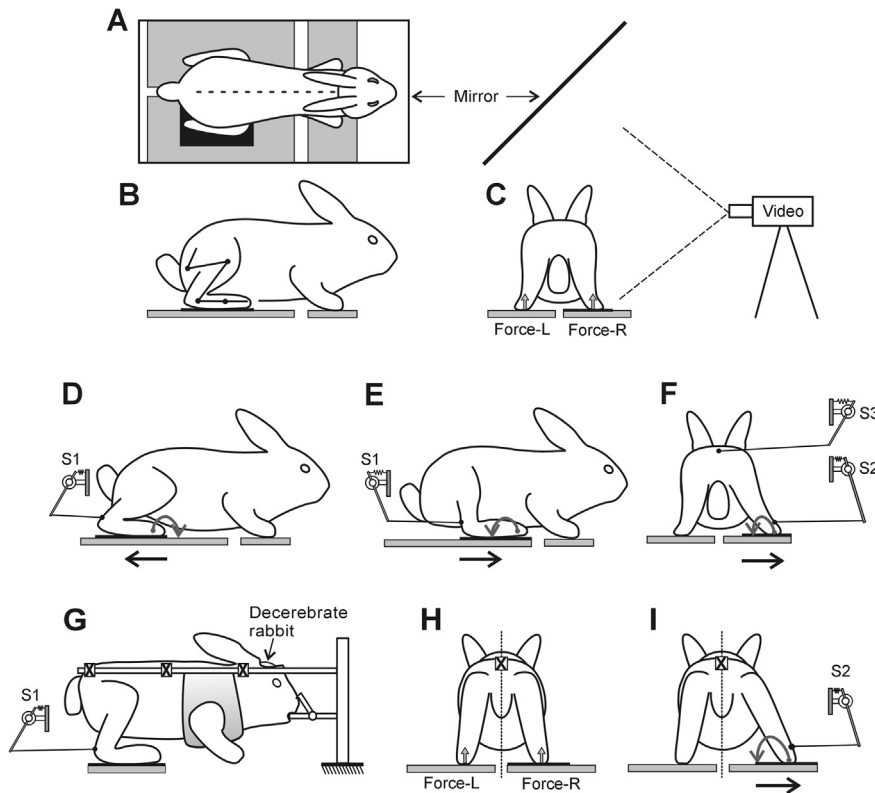
Fig. 1. Experimental designs. (A–F) Experimental design to study postural responses to displacement of the hindlimb in intact rabbits and in premammillary rabbit. The rabbit was freely standing on horizontal platforms, with a movable plastic plate (indicated by black) under one of the hindlimbs. The plate could be translated in the horizontal plane (indicated by black arrows in D–F) backward (D), forward (E) or outward (F) to evoke a corrective step in the opposite direction (indicated by gray arrows in D–F). Video recording (50 frames/s) from the top (by means of a mirror) along with either the rear view or the side view (A, C) was performed. The contact force under each hindlimb was measured by the force sensor (Force-L and Force-R in C). The anterior-posterior position of the stepping limb was recorded in tests with forward (D) and backward (E) corrective steps by means of a mechanical sensor S1. The medio-lateral position of the stepping limb was recorded in tests with inward step by means of a mechanical sensor S2 (F). The lateral displacement of the caudal part of the trunk was recorded by means of a mechanical sensor S3 (F). (G–I) Experimental design for acute experiments on postmammillary rabbits. The head and vertebral column of the decerebrate rabbit were firmly fixed (points of fixation are indicated by X). The hindlimbs were positioned on horizontal platforms, with a movable plastic plate (indicated by black) under one of the hindlimbs. The contact force under each hindlimb and the movement of the stepping limb were recorded by the force sensors (Force-L and Force-R in H) and mechanical sensors S1 (shown in G) and S2 (shown in I), respectively.

rough surface to increase friction, was placed under one of the hindlimbs (black plate in Fig 1). The plate was translated manually in the horizontal plane either backward (Fig. 1D) or forward (Fig. 1E) or outward (Fig. 1F), causing limb displacement in the corresponding direction, which resulted in distortion of the basic body configuration. This evoked a corrective step in the opposite direction (indicated by gray arrow in Fig. 1D–F), while the other limbs remained standing.

To characterize kinematics of postural corrections, video recording (50 frames/s) from the top (by means of a mirror) along with either the side view (Fig. 1A, C) or the rear view was performed. The fur on the limbs and on the back of the animals was shaved, and black paper stickers were attached to the skin projections of the main hindlimb joints and along the midline of the

spine (Fig. 1A, B). The video camera was positioned at a distance of ∼2 m from the rabbit.

Mechanical sensors were used to record movements of the limb performing corrective steps, as well as the trunk movements. The mechanical sensor consisted of a variable resistor whose axis was rotated by means of a long lever; the latter was connected to the limb or trunk by means of a long string. In the tests with forward and backward corrective steps, the anterior–posterior hindlimb movements were recorded by means of a mechanical sensor S1 (Fig. 1D, E). In the test with inward corrective step, the medial–lateral limb movements, as well as the medial–lateral trunk movements were recorded by means of sensors S2 and S3, respectively (Fig. 1F). To record movements of the limb, the string of the mechanical sensor was attached to the skin at the level of the ankle joint, and to record movements of the trunk – at the midline of L5 vertebra. The vertical forces under hindlimbs were recorded by means of two force plates (Force-R, Force-L in Fig. 1C). The signals from mechanical sensors, force plates, and EMG electrodes were synchronized with the video recording.

To clarify if sensory feedback from the limb contributes to generation of the corrective step, in three intact animals at the end of the outward limb displacement, we applied a lateral force (up to 0.15 kg) to the distal part of the limb by means of the elastic string of 10 cm in length (Fig. 6F). The applied force did not evoke displacement of the limb in relation to the plate (Fig. 6G) but produced a lateral, gradually increasing force affecting the limb during the corrective step (Fig. 6H).

Acute experiments on postmammillary rabbits. To examine if sensory signals about the limb displacement alone can evoke a corrective step, acute experiments were performed in six postmammillary rabbits. The head and vertebral column of the decerebrate rabbit were rigidly fixed (Fig. 1G). The forequarters were suspended in a hammock, whereas the hindlimbs were positioned on two horizontal plates (Fig. 1G, H). The hindlimb configuration and the distance between the feet (Fig. 1G, H) were similar to that observed in intact freely standing rabbits (Fig. 1B, C; Beloozerova et al., 2003). The methods to evoke forward, backward and inward

corrective steps in the decerebrate animal were the same as in intact one (see above). Mechanical sensors were used to record movements of the limb performing corrective steps (S1 and S2 in Fig. 1G, I). The contact forces under the limbs were measured by means of force sensors (Force-L and Force-R in Fig. 1H). Video recording (50 frames/s) of the forward and backward corrective steps was performed from the side. Inward corrective steps were video recorded from the rear. The signals from mechanical sensors, force plates, and EMG electrodes were synchronized with the video recording.

Recording and data analysis

The signals from the EMG electrodes, the mechanical sensors, and the force plates were amplified, digitized with a sampling frequency of 5 kHz (EMGs) and 1 kHz (sensors), and recorded on a computer disk using data-acquisition and analysis software (Power1401/Spike2, Cambridge Electronic Design, Cambridge, UK). EMG signals were rectified and smoothed (time constant, 10 ms).

The video recordings were analyzed frame-by-frame. In the intact and decerebrate animals, video data were used to calculate the parameters of the limb and/or the trunk movement during the postural tests.

All quantitative data in this study are presented as mean ± SD. Student's t-test was used to characterize the statistical significance when comparing different means; the significance level was set at $P = 0.05$. To test correlations between parameters, the linear regression method was used (Figs. 2E–J, 7C–H) and the coefficient of determination R^2 was indicated. The significance level P was presented if the correlation was statistically significant.

RESULTS

Characteristics of motor response to distortion of the basic body configuration

All tested animals ($N = 16$) were easily engaged in the postural tasks and exhibited stereotypic sequence of postural responses to disturbance of the basic body configuration caused by translation of one hindlimb in a specific direction (forward, backward or outward). An example of the motor response to outward translation of the hindlimb is shown in Fig. 2A. At the beginning of recording (before time point 1), the animal maintained the basic body configuration. Positions of the right hindlimb and trunk in the transverse plane were monitored by the sensor S2 and S3, respectively, along with contact forces produced by left and right limbs, and EMGs of right and left tibialis. A gradual displacement of the right hindlimb to the right (started at time point 1) led to gradual rightward displacement of the trunk (started at time point 2) and resulted in disturbance of the basic standing body configuration. These passive movements of the limb and trunk were accompanied by a gradual decrease and increase in loading of the right and left limb, respectively. Displacement of the right limb, first, initiated leftward corrective movement of the trunk

(started at time point 3) and then about 0.2 s later (at time point 4) a corrective leftward step of the right hindlimb. During the corrective step, the right tibialis was activated. These corrective trunk and limb movements resulted in a rapid drop to zero of the force under the right limb and in an increase in the force under the left limb. One can see also that the corrective step brought the right limb close to the initial position (at time point 6), while the corrective trunk movement resulted in an overshoot the initial trunk position (at time point 5), which was gradually compensated later. Thus, the body configuration close to the initial one was attained.

In all tested animals, translation of the hindlimb in any of three directions (forward, backward or outward) evoked, first, a corrective movement of the trunk, and then, with some delay, a corrective step. As one can see in Table 2, the value of the time difference between onsets of the corrective step and the onset of the trunk corrective movement was always positive [$(Min − Max)$]. This value varied considerably (about 10 fold) between tests in individual animals, suggesting that the corrective trunk movement and the corrective step were controlled independently. In the following sections, corrective steps and corrective trunk movements are characterized in detail.

Kinematic characteristics of corrective steps. Fig. 2B–D shows representative stick diagrams of the left hindlimb lateral view obtained during tests with inward (B), forward (C) and backward (D) corrective steps. One can see that outward, backward and forward displacement of the limb caused a change in the limb configuration (compare 1 and 2 in B, C and D, respectively) and evoked a corrective step in opposite direction which led to restoration of the initial limb configuration (compare 1 and 8, 1 and 9, 1 and 11 in B, C, and D, respectively).

To characterize kinematics of corrective steps, we calculated the following parameters indicated in Fig. 2A: (i) the amplitude of the limb displacement in a particular direction (Limb displ) required for initiation of the postural response, (ii) the corrective step length (Step length), and (iii) the corrective step duration (Step duration).

In Fig. 2E–G, the amplitude of the limb displacement is plotted against the step length for the trials with inward (E), forward (F) and backward (G) corrective steps. We found a positive correlation between these parameters for steps in all three directions suggesting that the limb tended to return to its initial position. However, the majority of data points in Fig. 2E, F, are located above the diagonal (shown by interrupted gray line), indicating that in most trials at the end of the inward steps, the limb landed medially, and at the end of the forward step – rostrally to the initial position. In most trials at the end of the backward step, the limb landed rostrally to the initial position (the majority of data points are located below the diagonal in Fig. 2G). Thus, forward and inward corrective steps caused overcompensation, while backward corrective steps – undercompensation of the disturbed limb position. We found that the length of inward, forward as well as
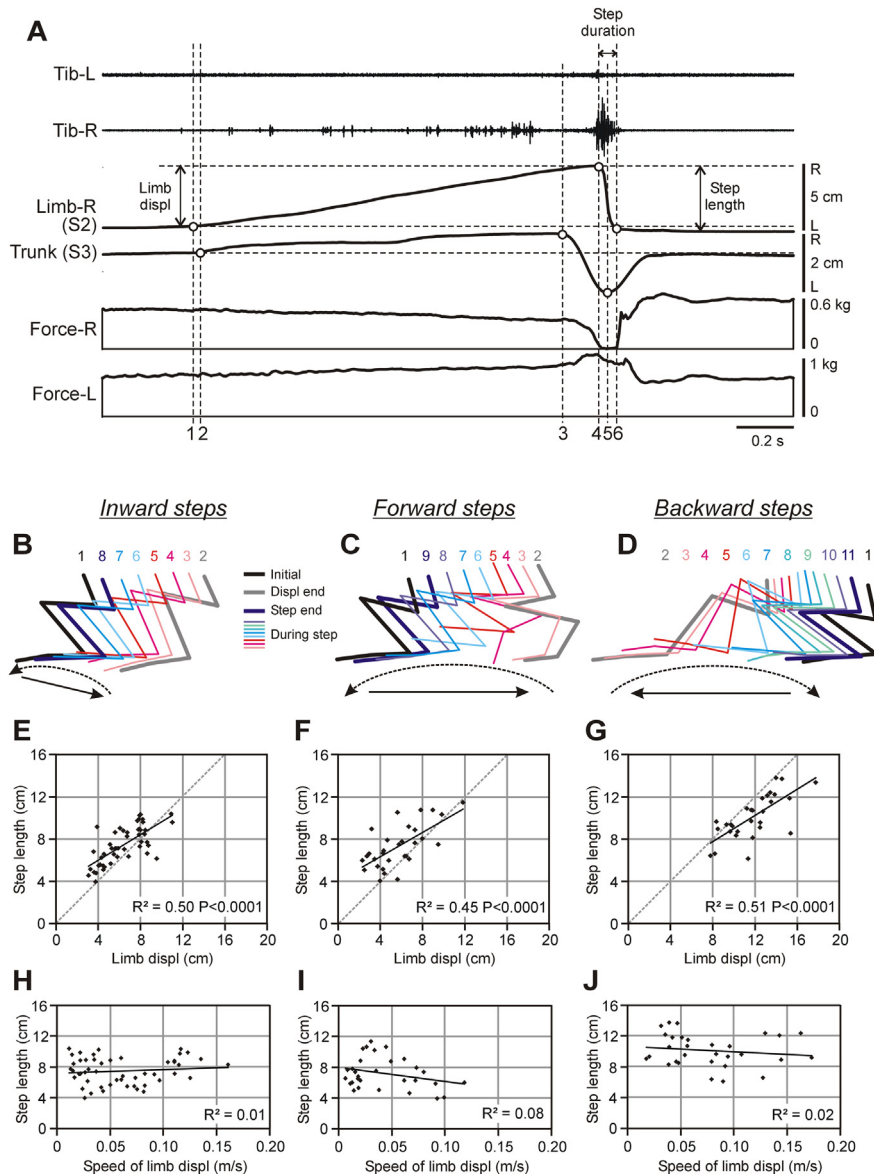
Fig. 2. Characterization of the motor response to the hindlimb displacement in the intact rabbit. (A) A representative example of the motor response to the right hindlimb outward displacement. White circles and interrupted vertical lines (1–6) indicate important time points of the limb and trunk movement (see text for explanations). (B–J) Kinematics of corrective steps. (B–D) Configuration of the left hindlimb (lateral view) at different moments of the inward step (B, 1–8), forward step (C, 1–9) and backward step (D, 1–11). A time interval between sequential configurations during inward (2–8), forward (2–9) and backward (2–11) steps is 20 ms. The amplitude of the outward, backward and forward limb displacement from the initial position causing inward (B), forward (C) and backward (D) corrective steps was 7 cm, 7 cm and 14 cm, respectively. Solid and interrupted arrows indicate the direction of the limb displacement and the direction of the step, respectively. Initial, initial limb configuration; Displ end, configuration at the end of the limb displacement; Step end, configuration at the end of the step; During step, configurations during the step. (E–G) Positive correlation between the amplitude of the limb displacement (Limb displ) and the step length in tests with inward (E), forward (F) and backward (G) corrective steps. (H–J) No correlation between the speed of the limb displacement and the step length in tests with inward (H), forward (I) and backward (J) corrective steps. In E–J, $N = 5$. In E, H, $n = 50$; in F, G, I, J, $n = 30$.

required for initiation of the inward and forward corrective steps, as well as the mean values of corresponding step lengths were similar. By contrast, mean values of both these parameters of backward corrective steps were significantly larger. We found that on average, duration of forward steps was slightly (but significantly) longer than that of inward steps, and duration of backward steps was slightly (but significantly) longer than those of inward and forward steps.

Activity of muscles in standing (supporting) and stepping hindlimb. In 11 animals, EMG activity of hindlimb muscles during tests with inward, forward and backward corrective steps was recorded. Fig. 3A, B shows an example of EMG activity of muscles in the standing (Limb-L) and stepping (Limb-R) hindlimb recorded in the test with inward step. We characterized the activity of individual muscles in relation to different phases of the corrective step. To determine these phases, the recording of the vertical force produced by the stepping limb (Force-R in Fig. 3B) was used. The time points defining different phases of the corrective step are indicated in Fig. 3B. The *phase of unloading* the limb starts at the moment of the rapid drop of the force value (time point 1) and ends at the moment when this value reaches zero (time point 2) reflecting lift of the limb above the surface, *the swing (transfer) phase* starts at the time point 2 and ends at the moment of foot contact manifested by fast increase in the force (time point 3), and finally *the phase of re-loading* is between the time point 3 and the moment of termination of the rapid increase in the force value (time point 4). As one can see in Fig. 3B, individual muscles recorded in the stepping (right) limb had specific patterns of activity, i.e. Bic was inactivated during last two-thirds of the unloading phase as well as during swing and re-loading phases, Glut generated a burst of activity in the middle of the unloading phase, and Vast had maximal activity in the second half of the swing phase, as well as during and after re-loading phase. By contrast, in general the phases of activity of muscles recorded in the standing (left) limb

backward steps practically did not depend on the speed of the limb displacement (Fig. 2H, I and J, respectively).

Table 2 shows mean values of the main kinematical parameters for different types of corrective steps. One can see that the mean values of the limb displacement

Table 2. Kinematical characteristics of inward, forward and backward corrective steps in intact rabbits

Type of corrective step	Limb displacement (cm)	Step length (cm)	Step duration (s)	$T_{step} - T_{trunk}$ (s)(Min − Max)	N	n
Inward	6.47 ± 2.02	7.45 ± 1.72	0.11 ± 0.02	0.18 ± 0.09 (0.04–0.42)	5	50
Forward	5.73 ± 2.38	7.27 ± 2.04	0.13 ± 0.04[**]	0.16 ± 0.12 (0.02–0.56)	5	30
Backward	11.95 ± 2.39[***]	10.17 ± 2.08[***]	0.16 ± 0.03[***]	0.20 ± 0.14 (0.06–0.54)	5	30

The values represent mean ± SD of different parameters of inward, forward and backward corrective steps. $T_{step} - T_{trunk}$, the time difference between onsets of the corrective step and trunk corrective movement. (Min − Max), the range of the time differences observed in different trials. N and n are number of tested animals and number of analyzed steps. Significance of difference among group means was revealed with a one-way ANOVA with $P = 0.05$; then the means were compared pairwise using t-test with Bonferroni's correction for multiple comparisons. Indication of significance level.
[**] $P < 0.01$.
[***] $P < 0.001$.

were similar. They were co-activated before and during unloading, as well as during swing phase.

We found that the phase of activity of individual muscle in stepping, as well as in standing limb could vary considerably in different animals. Of the total list of the recorded muscles (Table 1) only those, which had relatively similar phases of activity in the majority of trials in at least two animals were analyzed. Phases of activity of these muscles in the stepping and standing limb (during inward, forward and backward corrective steps) are shown in Fig. 3C, D, respectively. In the stepping limb (Fig. 3C), some muscles contributed to the same phases of corrective steps performed in different directions. For example, Glut (hip abductor), Sart (hip flexor), RF (hip flexor and knee extensor), St (hip extensor and knee flexor) as well as Tib (ankle flexor) were active during the phase of unloading (delimited by interrupted lines 1 and 2) thus contributing to lift of the limb above the surface during inward, forward and backward steps. In addition, Sart and Tib together with EDL (ankle flexor) were continuously active during swing phase (delimited by lines 2 and 3) keeping the limb above the ground.

Some muscles had clear pattern of activity only during steps in a specific direction. For example, Grac (hip adductor) was consistently activated (in the swing phase) only during inward steps thus moving the limb toward the initial position. Since in the swing phase of forward and backward steps movements at the hip and knee joints are performed in opposite directions (Buford et al., 1990), RF (the hip flexor and knee extensor) was active in the swing phase of the forward step (thus moving the limb forward), but was inactivated in the swing phase of the backward step. By contrast, Bic (the knee flexor and hip extensor) had clear pattern of activity during backward, but not during forward corrective steps. It was activated in the phase of limb re-loading (delimited by lines 3 and 4) of the backward step (thus contributing to landing the limb). Some muscles of the stepping limb (e.g. Grac, RF, St, Sm, Bic, EDL, Gast) were active before the phase of unloading (between lines 0 and 1). Usually they were gradually activated at the end of the limb displacement (like Bic-R in Fig. 3A) most likely in response to their passive stretching.

We found that in the standing limb, muscles had similar phases of activity during steps in different directions (Fig. 3D). The majority of them were activated at the end of the limb displacement and continued to be active during the phase of unloading and during the

swing phase of the corrective step. These muscles included flexor (Sart), extensor (Vast), adductor (Grac) and abductor (Glut), as well as bifunctional muscles (Bic, EDL). Co-activation of these muscles increased the stiffness of the standing limb supporting the body during a corrective step.

Muscles of the stepping and standing limb which had different phases of activity in different animals, as well as muscles of the standing limb which were not active during corrective steps in any of three directions, are marked in Abbreviation column of Table 1 by italic, underline and parentheses, respectively.

Corrective trunk movements. Fig. 4A–L shows the top view of four characteristic positions of the rabbit's hindquarters during the response to inward (A-D), backward (E–H) and forward (I–L) displacement of the left hindlimb. The body outlines immediately before the limb displacement are shown in Fig. 4A, E, I (Initial). The limb displacement caused distortion of the initial trunk configuration, i.e. a passive lateral bending of the caudal part of the trunk with its maximal value (immediately before the trunk corrective movement) depicted in Fig. 4B, F and J, (Distortion). One can see that in tests with outward and forward limb displacement, the caudal part of the spine midline (indicated by solid black line) was deviated from its initial position (indicated by gray interrupted line) toward the displaced (left) limb (Fig. 4B, F, respectively). By contrast, in the test with backward limb displacement, it was deviated toward the supporting (right) limb (Fig. 4J). Then, due to the activation of the mechanisms of postural corrections, in tests with outward and forward limb displacement, the caudal part of the trunk started to move toward the initial position, but overshot this position. The positions corresponding to the peak overshoot in these two tests are shown in Fig. 4C, G (Corr peak), respectively. As one can see, in the caudal part of the trunk the distance from the middle spine to the right edge of the trunk was much smaller than that to the left one, indicating that the corrective trunk movement includes both bending (toward the stepping left limb) and twisting (toward the supporting right limb) components. The twisting component of the trunk corrective movement caused by outward displacement of the limb is also clearly seen on the rear body view in Fig. 6C. In contrast, in the test with backward limb displacement, rapid straightening and rightward twisting of the caudal spine were observed. They led to further
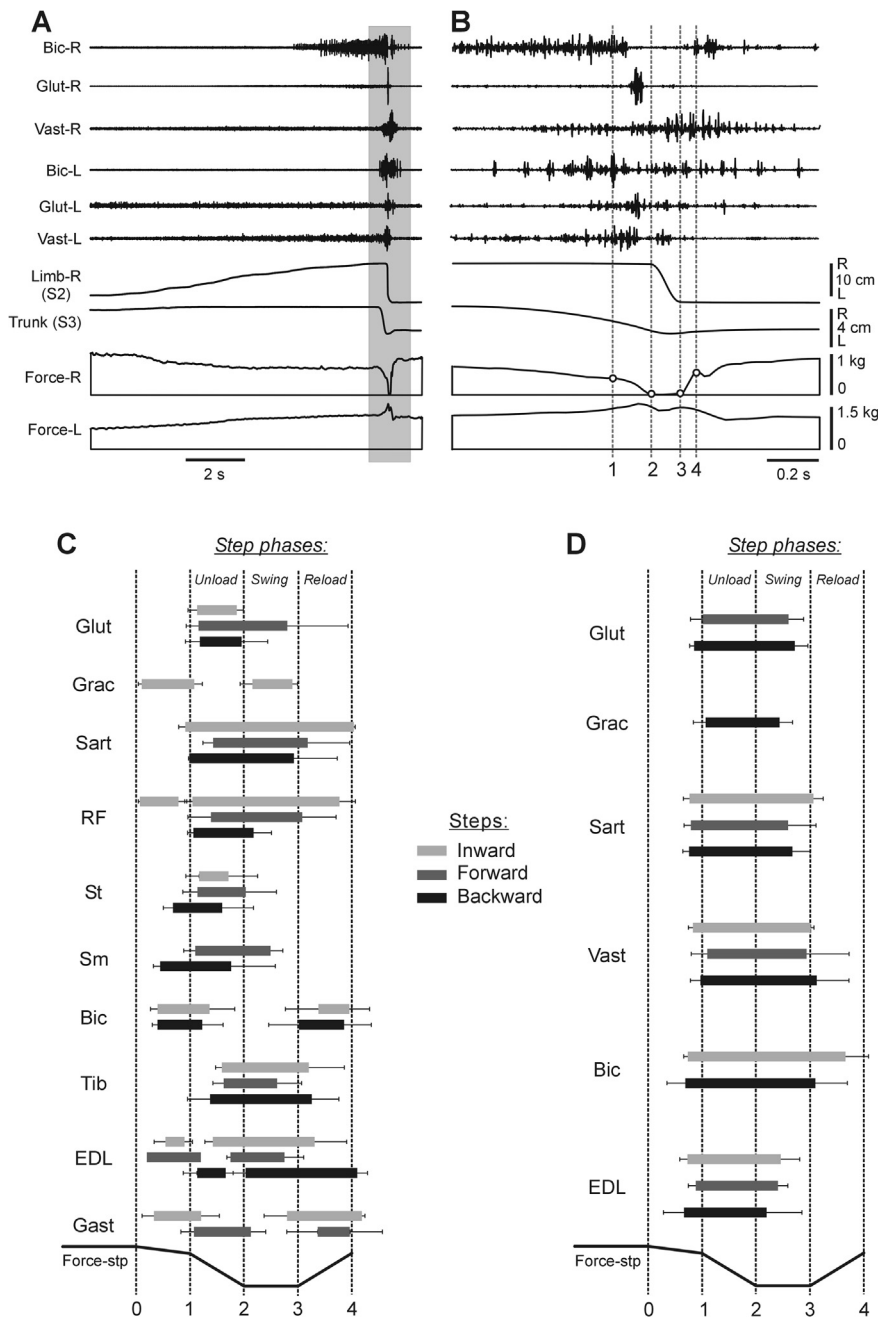
Fig. 3. Activity of hindlimb muscles during corrective steps performed in different directions. (A, B) A representative example of EMG responses to outward displacement of the right hindlimb in selected muscles of the right (performing inward corrective step) and left (supporting) hindlimb. A part of the recording indicated by thick gray line in A is shown with higher time resolution in B. Interrupted lines 1–4 in B indicate the time points delimiting different phases of the corrective step determined using the signal from the force sensor under the stepping limb (Force-R). 1–2, phase of unloading the limb; 2–3, swing (transfer) phase; 3–4, phase of loading the limb. (C, D) Activity of individual muscles recorded in the stepping (C) and standing (supporting, D) hindlimb during inward, forward and backward corrective steps. Each thick line shows averaged over 4–8 steps activity phase of an individual muscle in relation to the phases of the corrective step (delimited by interrupted lines 1–4). The period of slight unloading of the limb during its displacement is delimited by interrupted lines 0–1. Force-stp, schematically shown signal from the force sensor under the stepping limb.

deviation of the spine from the initial position to the right (Fig. 4K). Then, in all three tests, the caudal part of the trunk shifted to the left until it reached a position close to the initial one (Fig. 4D, H, L, Final).

Fig. 4M–O shows mean ± SD values of different characteristics of the corrective trunk movements (indicated by black arrows in Fig. 4B–D) in tests with inward, backward and forward corrective steps. Despite the fact that values of these characteristics varied considerably, the same tendency was revealed in all tested animals: (i) the limb displacement caused deviation of the spine from its initial position (Distortion) toward the displaced limb in tests with inward and backward steps (Fig. 4M, N, respectively) and toward the supporting limb in test with forward steps (Fig. 4O). (ii) In all three types of tests, the first trunk corrective movement was performed toward the supporting limb and terminated when the caudal spine was deviated from its initial position toward the supporting limb (Corr peak in Fig. 4M–O). (iii) In all three types of tests, in the majority of cases at final position the caudal spine was slightly shifted from the initial position toward the supporting limb (Final in Fig. 4M–O).

Out of three recorded muscles affecting the spine (erector spinae, multifidus and oblique externus abdominis, Table 1) only in oblique externus abdominis (OEA) the limb displacement evoked a robust pattern of EMG response. To clarify its role in generation of trunk movements, in two animals we recorded the EMG activity of the left and right OEA during passive trunk bending in the horizontal plane and during passive rotation in the transverse plane (twisting). For this purpose, in the rabbit standing with its forelimbs on the horizontal surface, the hindquarters were suspended and manually periodically bent (the right panel in Fig. 5A) or rotated (the right panel in Fig. 5B). We found that the OEA responded to passive contralateral bending (Fig. 5A) and to ipsilateral rotation (Fig. 5B), indicating that unilateral OEA activation would cause simultaneously ipsilateral bending and contralateral rotation of the caudal trunk.

A representative example of the response of the left and right OEA to outward displacement of the left limb is shown in Fig. 5C. One can see that left OEA (OEA-L)

generated a large amplitude burst of activity which started before the onset of the trunk rightward corrective movement (indicated by arrow on the Trunk trace) and terminated at its end thus causing the leftward bending and rightward rotation of the caudal trunk resulting in displacement of the body toward the supporting (right) limb (as in Figs. 4C, 6C). During rightward body displacement the inward (rightward) corrective step by the left limb was initiated, and the left Tib (Tib-L) was activated (Fig. 5C). The right OEA (OEA-R) was activated with some delay after onset of the rightward corrective trunk movement and generated the burst of the activity of a smaller amplitude. It terminated the rightward trunk corrective movement.

Fig. 5D–F shows activity of OEA during tests with ipsilateral (dark gray trace) and contralateral (light gray trace) limb outward (D), forward (E) and backward (F) displacement averaged over eight trials. With each of three directions of the limb displacement, the pattern of the ipsilateral and contralateral OEA activity was similar. Ipsilateral OEA was activated before the onset of the trunk corrective movement (indicated by black arrow in D–F) and had a high level of activity until the end of the corrective step. By contract, contralateral OEA had lower level of activity, which started with some delay after onset of the trunk corrective movement. One can also see that activation of OEA-ipsi preceded Tib-ipsi burst during steps in each of three directions. Similar EMG patterns were observed in all tested animals ($N = 2$).

Role of the sensory feedback from the stepping limb. To analyze the role of sensory feedback from the stepping limb in generation of postural response to hindlimb displacement, in the tests with inward steps, we applied a lateral force to the stepping limb (by means of an elastic string attached to the ankle joint, Fig. 6F). This elastic string did not affect the limb during its displacement (Fig. 6G) but produced a lateral, gradually increasing force affecting the limb during the corrective step (Fig. 6H). The lateral force evoked changes in response to limb displacement that were similar in all tested rabbits ($N = 3$). Characteristic positions of the rabbit during the response to outward displacement of the right hindlimb in the trial without (Control) and in the trial with an elastic string are shown in Fig. 6A–E and F–J, respectively. The body outline (the rear view) immediately before the limb displacement is shown in Fig. 6A, F. Outward displacement of the right limb with similar amplitude caused similar distortion of the body configuration (at the moment immediately before the step initiation) in both trials (Fig. 6B, G). Fig. 6C, H, shows the body outline at the moment of the highest heel lift during the swing phase of the corrective step in the control trial and in the trial with the elastic string, respectively. The value of the twisting component of the trunk corrective movement (rotation toward the supporting limb), as well as the value of the heel lift exhibited by the right limb were larger in the trial with elastic string as compared to those in control. The higher heel lift was partly caused by increased (as compared with control) functional length of the supporting (left) limb (due to its extension),
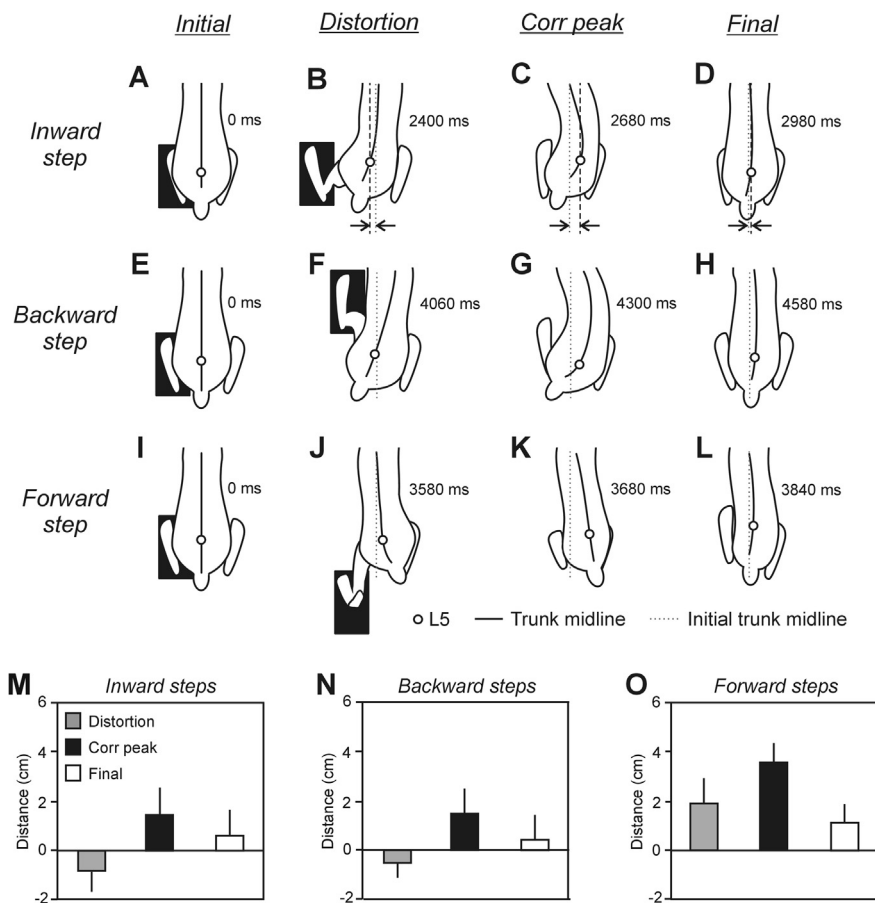
Fig. 4. Characterization of the trunk corrective movements. (A–L) The top view of four characteristic positions of the rabbit's hindquarters during the response to outward (A–D, Inward step), forward (E–H, Backward step) and backward (I–L, Forward step) displacement of the left hindlimb. The body outlines immediately before the limb displacement (Initial), at maximal value of the passive lateral bending of the caudal part of the trunk caused by the limb displacement (Distortion), at the peak of the trunk corrective movement (Corr peak) and at the end of the corrective step (Final) are shown. (M–O) Mean values (± SD) characterizing the deviation of the caudal trunk from its initial position at different moments (Distortion, Corr peak, Final) of postural tests with inward (M), backward (N) and forward (O) steps. Positive and negative values correspond to the displacement of the trunk from its initial position toward the supporting and stepping limb, respectively. The deviation of the caudal trunk from its initial position (indicated by black arrows in B–D) was determined as the displacement of the L5 vertebra (shown by a circle in A–L) from the initial position of the spine (indicated by dotted gray line in B–D, F–H and J–L). For M, N and O, $N = 5$; $n = 50$, $n = 30$, and $n = 30$, respectively.
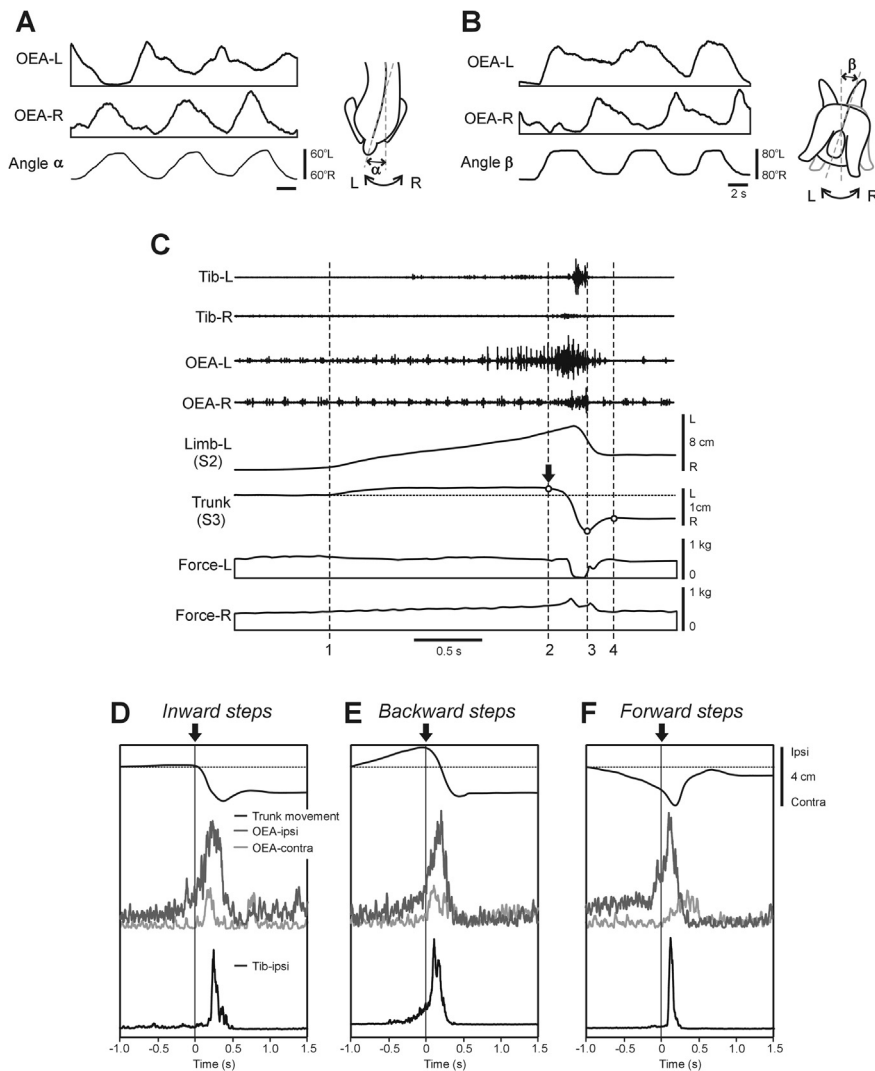
Fig. 5. Contribution of the muscle oblique externus abdominis (OEA) to trunk corrective movements. (A, B) A representative example of responses of the left and right OEA (OEA-L and OEA-R, respectively) to periodic passive lateral bending (A) and twisting (B) of the trunk. Angle α is the bend angle, angle β is the twist angle. (C) Activity of the left and right OEA and Tib during the postural test with inward step performed by the left hindlimb. Time points 1, 2, 3 and 4 (indicated by interrupted lines) show the beginning of the passive trunk movement caused by the outward displacement of the left hindlimb, the beginning of the trunk corrective movement, the peak of the corrective trunk movement and the moment when the trunk orientation returned close to the initial position, respectively. (D–F) Averaged EMG activity in OEA and Tib muscles during trunk corrective movements in postural tests with inward (D), backward (E) and forward (F) corrective steps. The EMG amplitude is expressed in arbitrary units. Upper black line shows averaged lateral deviation of the trunk from its initial position (indicated by horizontal dotted line). Ipsi and Contra, ipsilateral and contralateral to the stepping limb, respectively. In C–F, thick black arrow indicates the beginning of the trunk corrective movement. In D, E and F, average over four trials.

which elevated the body (with flexed stepping right limb) up, as well as by larger leftward trunk rotation. In both trials, at the end of the corrective step, the right limb position was close to the initial one with small overshoot and small undershoot in control and in the trial with elastic string, respectively (compare Fig. 6A and D, F and I, respectively). Also in both trials, at the end of the corrective step, the trunk position was close to the initial one with small undershoot (Fig. 6D, I) which was then compensated (Fig. 6E, J).

Fig. 6K shows that with similar amplitude of the limb displacement (Limb displ), the average values of the maximal heel lift during the step (Max-H), the trunk corrective movement peak (Corr peak), the increase in the functional length of the supporting limb (ΔMax-L) and the step duration (Step durat) were significantly larger in trials with elastic string than those in control. By contrast, the mean value of the step length in trials with elastic string was significantly smaller than that in control. One can conclude that sensory feedback, signals about external force applied to the limb, affected all components of postural response to limb displacement to counteract the resistance caused by this force and to restore the initial limb position.

Fig. 6L, M shows a representative example of activity of individual muscles recorded in the stepping and standing limbs during control trial (L) and during the trial with an elastic string (M). In both trials, the inward step was evoked by outward displacement of the right limb with the amplitude of 5 cm. The onset of the corrective step (phase of unloading the limb) is manifested by a rapid drop of the force value under the right limb (Force-R), and the onset of the swing phase is indicated by interrupted line. One can see that EMG bursts generated by all recorded muscles during the step, were substantially increased in the trial with the elastic string (M) as compared to control (L).

To evaluate these changes, we performed corrective step-linked averaging of individual EMGs in control and in trials with the elastic string (gray and black traces, respectively, in Fig. 6N, O). Only trials with the same amplitude of the limb displacement causing the corrective step were used. In the stepping limb (Fig. 6N), EMG burst of Grac (hip extensor and adductor) and EMG burst of Vast (knee extensor) were almost tenfold larger, and EMG burst of Glut (hip extensor and abductor) was substantially larger in tests with the elastic string as compared to control. One can suggest that due to the strong activation of the adductor muscle (Grac) in the swing phase of the step, the limb overcame the resistance of the elastic string and moved toward the initial position. An increase in the activity in the knee
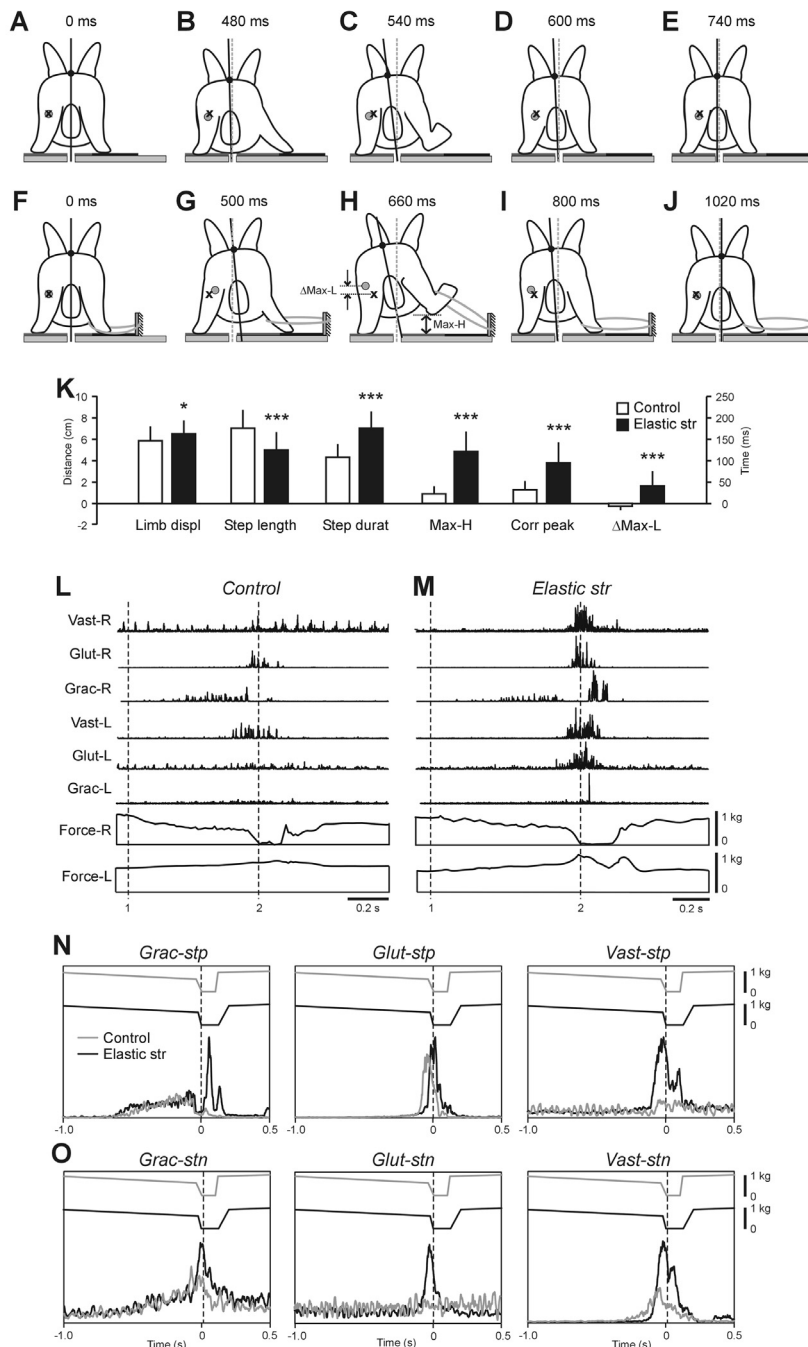
Fig. 6. The role of sensory feedback from the stepping limb in generation of postural response to hindlimb displacement. (A–J) Five sequential positions of the rabbit in response to outward displacement of the right hind limb in the trial without (A–E) and in the trial with an elastic string which produced a lateral force affecting the limb during the corrective step (D–J). The gray circle and the black cross indicate the current and initial position of the left hip joint, respectively. Solid black and interrupted gray lines show the current and the initial position of the spine, respectively. (K) Comparison of the mean (±SD) values of different kinematic parameters of the postural responses to outward displacement of the hindlimb in trials without (Control, $N = 2$, $n = 26$) and in the trials with an elastic string (Elastic str, $N = 2$, $n = 41$). Indication of significance level: $^*P < 0.05$, $^{***}P < 0.001$. (L, M) A representative example of EMG responses to outward displacement of the right hindlimb in selected individual muscles of the right (performing inward corrective step) and left (supporting) hindlimb in the trial without (Control) and in the trial with an elastic string (Elastic str). Interrupted lines 1 and 2 indicate the onset of the limb displacement and the onset of the swing phase of the corrective step, respectively. (N, O) Averaged EMG response to outward displacement of the hindlimb in three selected muscles of the stepping (stp) and standing (stn) limb in trials without (Control, $n = 5$) and in the trials with an elastic string (Elastic str, $n = 5$). The EMG amplitude is expressed in arbitrary units. Upper traces in panels, the averaged force responses produced by the stepping limb. The interrupted black line indicates the onset of the swing phase of the corrective step.

(Vast) and hip (Glut) extensors during the phase of unloading and in the beginning of the swing phase, most likely contributed to an increase in stiffness of the stepping limb, which is necessary to resist the destabilizing effect of the lateral force generated by the elastic string.

In the standing limb (Fig. 6O), EMG burst of Grac, as well as EMG burst of Vast were substantially larger in tests with the elastic string as compared to control. Glut, which had no specific pattern of activity in control trials, generated a large burst of activity during the corrective step in trials with an elastic string. Co-activation of extensor muscles in the standing limb during the corrective step caused an extension of this limb observed in trials with an elastic string (Fig. 6H), and co-activation of adductor and abductor muscles resulted in an increase in the hip joint stiffness. Thus, one can suggest that the changes in activity of muscles in the standing limb resulted in extra support for the trunk, which was required at condition, when a resisting force was applied to the stepping limb.

Motor response to distortion of the body configuration in decerebrate rabbits

To find out if sensory signals from the deviated limb alone can evoke a corrective step, acute experiments were performed in six postmammillary rabbits with rigidly fixed head and spine (see Methods, Fig. 1G). We found that deviation of the hindlimb in any of three tested directions (outward, forward and backward) could evoke a single step in the opposite direction in the decerebrate rabbit. An example of the forward step caused by the backward displacement of the hindlimb in individual rabbit before and after decerebration is shown in Fig. 7A, B, respectively. One can see that the sequence of events caused by deviation of the limb from its normal standing position is similar in both intact and decerebrate rabbit. In both cases a gradual displacement of the left hindlimb backward (started at time point 1) was accompanied by a gradual decrease and increase in the contact
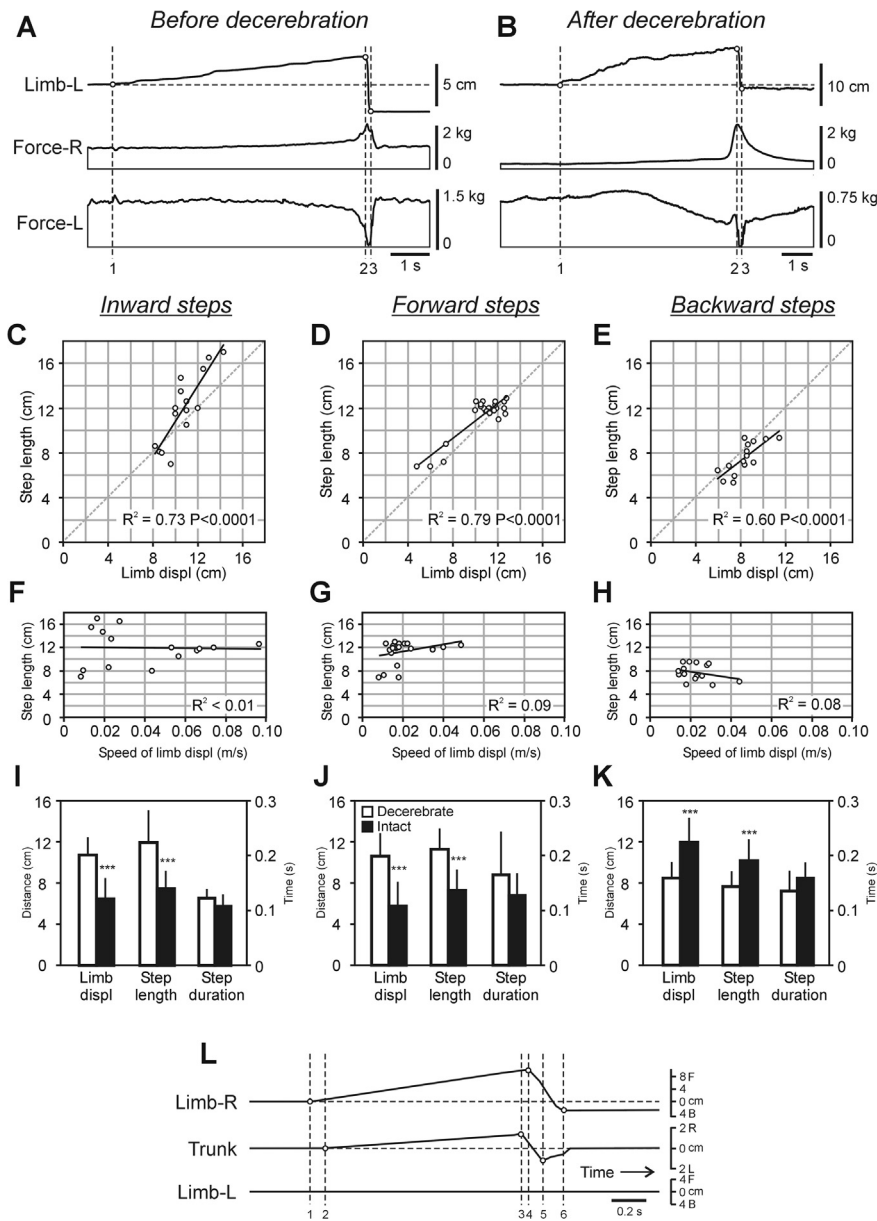
Fig. 7. Characterization of the motor response to the hindlimb displacement in decerebrate rabbits. (A, B) An example of the forward corrective step evoked in individual animal before (A) and after decerebration at the precollicular-postmammillary level (B). The corrective step was evoked in the freely standing animal before decerebration (A) and in the fixed animal after decerebration (B). (C–H) Kinematic characteristics of inward (C, F), forward (D, G) and backward (E, H) corrective steps evoked in fixed decerebrate rabbits. (C–E) Positive correlation between the amplitude of the limb displacement (Limb displ) and the step length. (F–H) No correlation between the speed of the limb displacement and the step length. For C, F, $N = 3$, $n = 15$; for D, G, $N = 3$, $n = 24$; for E, H, $N = 2$, $n = 15$. (I–K) Comparison of different kinematic characteristics of inward (I), forward (J) and backward (K) corrective steps evoked in freely standing intact rabbits ($N = 5, 5, 5$ and $n = 50, 30, 30$, respectively) and in fixed decerebrate rabbits ($N = 3, 3, 2$ and $n = 15, 24, 15$, respectively). *** indicated that $P < 0.001$. (L) An example of motor response to forward displacement of the right hindlimb in the freely standing rabbit decerebrated at the precollicular-premammillary level. Based on video data, deviation of limbs in anterior-posterior direction and deviation of the caudal trunk in the lateral direction from their initial position are shown. In A, B, L, time points indicated by interrupted vertical lines show important moments in the movement of the right limb and in the movement of the trunk (marked by white circles).

force produced by the left and right limb, respectively. Displacement of the left limb initiated a corrective forward step of that limb (at time point 2) resulting in a rapid drop to zero of the force produced by the left limb

and in an increase in the force produced by the right limb. In both cases the corrective step brought the left limb close to its initial position (at time point 3).

Sometimes, deviation of the limb in a particular direction did not evoke a step in some trials or in some rabbits, most likely due to the low level of excitability of the corresponding networks. Out of six postmammillary rabbits, in five rabbits deviation of the limb in two out of three directions evoked a step in the opposite direction. These directions were different for different rabbits. In one rabbit, a step was generated to deviation of the limb only in one direction. In cases when limb deviation did not evoke a step, to increase the level of excitability of corresponding networks, we applied a pinch to the tail with a strength, which did not evoke any movement when applied alone. Combination of the limb deviation with the tail pinch evoked a step in the opposite direction in all trials in all tested postmammillary rabbits. However, for comparison of different characteristics of corrective steps performed by decerebrate and by intact rabbits we used only those trials in which deviation of the limb alone evoked a step.

In Fig. 7C–E, the amplitude of the limb displacement required for the initiation of the step in decerebrate rabbits is plotted against the step length for the trials with inward (C), forward (D) and backward (E) corrective steps. Like in intact rabbits (Fig. 2E–G), a positive correlation between these parameters for steps in all three directions (suggesting that the limb tended to return to its initial position) was found. As in intact rabbits (Fig. 2E–G), in most trials with inward and forward steps overcompensation was observed (the majority of data points in Fig. 7C, D, are located above the diagonal), and in most trials with backward steps undercompensation was revealed (the majority of data points in Fig. 7E are located below the diagonal). We found that as in intact rabbits, the length of inward, forward and backward steps in decerebrate rabbits practically did not depend on the speed of the limb displacement (compare Fig. 2H, I and J, with Fig. 7F, G and H, respectively).
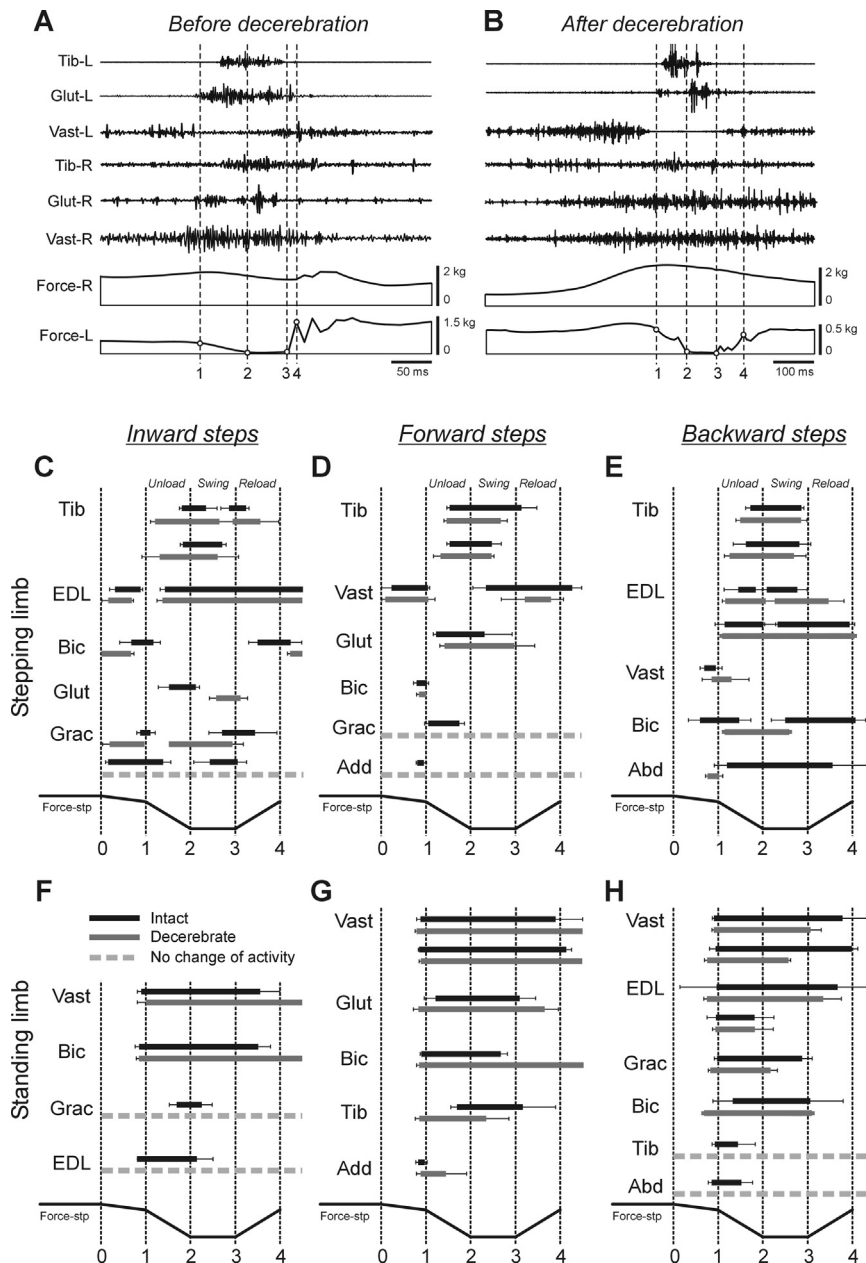
Fig. 8. Comparison of EMG patterns of the hindlimb muscles during corrective steps evoked before and after decerebration. (A, B) An example of activity of selected hindlimb muscles during forward corrective step (performed by the left limb) evoked in the same animal before (A) and after (B) decerebration. The amplitude of the limb displacement initiating the step was 4 cm in A and 10 cm in B. (C–H) Comparison of EMG patterns of the same muscles during corrective steps performed by individual rabbits before and after decerebration. Activity of individual muscles in the stepping (C–E) and standing (F–H) limbs averaged over three trials are shown in relation to the phases of the inward (C, F), forward (D, G) and backward (E, H) corrective step. Phases of the corrective step reflected in the contact force signal (Force-stp) schematically shown at the bottom in C–H. Vertical interrupted lines (1–4) delimit phases of the corrective step: 1–2, phase of unloading the limb; 2–3, swing (transfer) phase; 3–4, phase of loading the limb. 0–1, slight unloading of the limb during its displacement. In A-H, corrective steps were evoked in freely standing animals before decerebration and in fixed animals after decerebration.

Fig. 7I–K compares mean ± SD values of the main kinematical parameters for different types of corrective steps performed by freely standing intact rabbits and by fixed postmammillary rabbits. One can see that the mean values of the limb displacement required for initiation of the inward and forward corrective steps, as

well as the mean values of corresponding step lengths were significantly larger in decerebrate rabbits (I and J, respectively). By contrast, mean values of these parameters in tests with backward corrective steps were significantly smaller in decerebrate rabbits (K). These differences could be caused by some difference in the initial limb-trunk configuration of the fixed and freely standing rabbits (compare Fig. 1B, G, respectively). Slight differences in the mean values of duration of the corrective steps observed in intact and decerebrate rabbits were insignificant (Fig. 7I–K).

Fig. 8A, B, shows an example of EMG activity of muscles in the standing (right) and stepping (left) hindlimb recorded in the test with forward step performed before and after postmammillary decerebration of the rabbit, respectively. One can see that the activity of individual muscles before and after decerebration was similar. Thus, in the left (stepping) limb, Tib was activated in the middle of the unloading phase of the step (between time points 1 and 2) and was active until the end of the swing phase (time point 3), Glut was activated at onset of the unloading phase (time point 1) and was active until the end of the loading phase (time point 4), and Vast was inactivated at the onset of the unloading phase (time point 1) and activated again at the end of the swing phase – beginning of the loading phase (around time point 3) in both tests. Muscles recorded in the standing (right) limb were co-activated before and during the unloading phase, as well as during the swing phase of the forward corrective steps before (A) and after decerebration (B). Slight increase in the amplitude of EMG response in the stepping limb after decerebration (as compared to that before decerebration) could be explained by a larger displacement of the limb required for initiation of the corrective step (10 cm vs 4 cm, respectively), and as a result to generation of the corrective step of a larger length due to stronger activation of muscles.

As one can see in Fig. 8C–H, the phases of activity of the majority of individual muscles recorded in stepping (C–E) and standing (F–H) limb during inward (C, F),

forward (D, G) and backward (E, H) steps performed by individual animals before (black bars) and after (gray bars) decerebration were rather similar. These results suggest that the neuronal networks generating EMG pattern for both stepping and supporting limb reside in the brainstem, cerebellum, and spinal cord; they can be activated by afferent signals from the deviated limb.

To examine if the integrity of the higher levels of the CNS is not critical also for generation of the corrective trunk movements, the postural reactions to displacement of the hindlimb were studied in the freely standing rabbit decerebrated at the precollicular-premammillary level. A characteristic feature of premammillary rabbits is that they exhibit spontaneous periodical changes in the level of their excitability, which are reflected in alterations of their motor activity including postural and locomotor ones (Musienko et al., 2008; Orlovsky, 1969). We subjected the premammillary rabbit to postural tests during the periods when it had the "intermediate" level of excitability: it maintained a basic standing body configuration similar to that of intact rabbits but did not exhibit the spontaneous bouts of loco-motion. An example of motor response to the forward displacement of the right hindlimb in the freely standing premammillary rabbit is shown in Fig. 7L. Based on video data, deviation of limbs in anterior–posterior direction and deviation of the caudal trunk in the lateral direction from their initial position are shown. One can see that a gradual displacement of the right hindlimb forward (started at time point 1) led to a gradual rightward displacement of the trunk (started at time point 2) and resulted in disturbance of the basic standing body configuration. As in intact rabbits, displacement of the limb, first, initiated leftward corrective movement of the trunk (started at time point 3) and then about 80 ms later (at time point 4) a corrective backward step of the right hindlimb. One can see also that the corrective step brought the right limb close to the initial position with some overshoot (at time point 6). As in the intact rabbit, the corrective trunk movement resulted in an overshoot the initial trunk position (at time point 5), and then during the corrective step, the direction of the trunk movement was reversed and the trunk reached the initial position around the end of the corrective step. Thus, the body configuration close to the initial one was attained.

Because of continuous fluctuation of the excitability level in the premammillary rabbit, we were able to evoke only five backward and six forward corrective steps in response to forward and backward limb displacement,

respectively. The trunk corrective movements were observed in all tests with the backward corrective steps and in one test with the forward corrective step, suggesting that most likely the level of excitability in the networks generating the trunk movement and the corrective step could fluctuate independently.

In cases when the limb deviation alone did not evoke a step, combination of the outward, forward or backward limb displacement with the tail pinch evoked a step in opposite direction in the majority of trials (Corr steps in Table 3) and sometimes jumps (Jumps in Table 3). In the majority of the trials in which the corrective step was generated, the corrective trunk movements were observed (Trunk in Table 3). Like in intact rabbits, the onset of the trunk corrective movement in the premammillary rabbit always preceded the onset of the corrective step and the time difference between these two events substantially fluctuated between trials [see (Min−Max) in Table 3].

DISCUSSION

In the present study we investigated the effects of postural perturbations – outward, forward and backward displacement of the hindlimb – in the standing rabbit. Such perturbations happen in natural habitat, e.g. when interacting with moving animals or standing on a slippery surface.

Distortion of the basic body configuration caused by displacement of the hindlimb resulted in a robust postural response and return to the initial body configuration (Figs. 2A, 6A–L, 6A–E). The response included the corrective movement of the trunk and the corrective step. These two components of the postural response were initiated in a consistent sequence: first, a lateral displacement of the trunk toward the supporting limb started (that resulted in loading the supporting limb and unloading the limb, which would perform the step) and then, the corrective step in the direction opposite to the direction of the limb displacement was initiated. During the corrective step the direction of the trunk movement was reversed and the trunk reached the initial position at the end of the step. The corrective step returned the limb close to the initial position. Thus the basic symmetrical standing body configuration was restored. We found that the time difference between onsets of these two components (the trunk movement and the corrective step) of the postural response varied considerably (Table 2). We also observed that in

Table 3. Responses to the hindlimb displacement in the premammillary rabbit

Direction of limb displ	Limb disp − corr step (n)	Limb disp + tail pinch			
		Corr steps (n)	Trunk (n)	$T_{step} - T_{trunk}$ (s) (Min − Max)	Jumps (n)
Outward	0	8	8	0.22 ± 0.10 (0.12–0.44)	6
Backward	6	10	7	0.23 ± 0.11 (0.08–0.32)	3
Forward	5	17	14	0.25 ± 0.11 (0.12–0.48)	0

Limb disp − corr step, trials in which the limb displacement caused the corrective step. Trunk, trials in which corrective movements of the trunk were observed. Limb disp + tail pinch, trials in which the limb displacement was combined with the tail pinch. $T_{step} - T_{trunk}$, the time difference (mean ± SD) between onsets of the corrective step and trunk corrective movement. (Min − Max), the range of the time differences observed in different trials. n, number of trials with corrective steps, trunk corrective movements or jumps.

premammillary rabbit the corrective step could be generated without the corrective trunk movement. These findings suggest that two components of the postural response (the corrective trunk movement and the corrective step) are generated by different mechanisms. In intact subject they are activated in a strict sequence. One can suggest that sensory signals about hindlimbs loading gate the command activating the mechanism generating a corrective step, and only when a strong asymmetry in loading of the left and right hindlimbs (caused by displacement of the trunk toward the supporting limb) is created the corrective step is generated by the unloaded limb. The large time variability between the onset of the trunk movement and the onset of the corrective step [*(Min−Max)* in Table 2) could be explain by independent fluctuations of the level of excitability in the trunk and in the step mechanisms.

Postural reactions which include a corrective step could be aimed not only at restoration of the basic body configuration but also at restoration of the equilibrium. In terrestrial quadrupeds, such reactions could be caused by the lateral push applied to the trunk (Karayannidou et al., 2009), by the tilt (Beloozerova et al., 2003) or translation of the support surface. However, these postural reactions have not been analyzed in details. In human, the postural reaction to translation of the support surface is similar to that described in the present study. It was shown that translation evoked, first, the body weight shift toward the supporting limb and then a corrective step performed by the unloaded limb in the direction opposite to the direction of the surface translation (Maki et al., 1996).

Sensory control of the postural response to the limb displacement

We demonstrated that displacement of the limb distorts not only the limb/limb-trunk but also trunk configuration (Fig. 4B, F, J). The fact that displacement of the limb in different directions caused passive bending of the trunk in different directions [toward the supporting limb during the limb deviation forward (Fig. 4F) and toward the stepping limb during the limb deviation backward (Fig. 4J)] but the corrective trunk movements were always the same (bending toward the stepping and twisting toward the supporting limb, Fig. 4C, G, K), one can suggest that most likely, the mechanism generating the trunk movement is activated by proprioceptive information signaling about distortion in the limb/limb-trunk configuration but not about the distortion in the trunk configuration, as shown schematically in Fig. 9B, D.

It was shown in earlier studies (Rademaker, 1931) that a lateral or medial step can be evoked by medial or lateral displacement of the limb in relation to the trunk, respectively (hopping reactions). It was suggested that these reactions are triggered by proprioceptive input from the afferents of abductor and adductor muscles (Rademaker, 1931; Rademaker and Hoogerwerf, 1930). Our experiments on decerebrate rabbits with fixed spine have shown that sensory input from the deviated limb not only triggered the corrective step, but also evoked activation of extensors in the contralateral limb (Fig. 8B, F–H). Thus, an increase in the stiffness of the
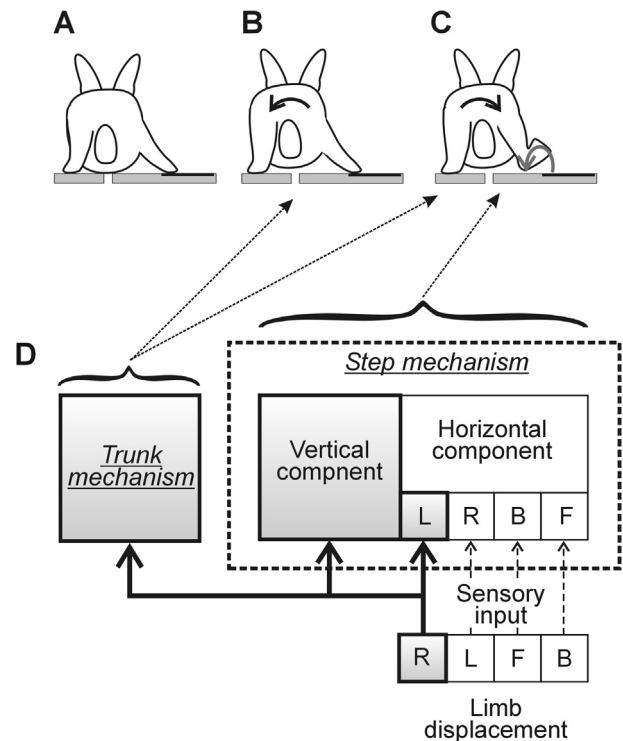
Fig. 9. Mechanisms generating postural response to distortion of the basic body configuration during standing. (A–C) Outward displacement of the right hindlimb (A) initiates the leftward corrective movement of the trunk (black arrow in B) and then the inward corrective step (gray arrow in C). During the corrective step the direction of the trunk movement reverses (black arrow in C). (D) Elicitation of the postural response to distortion of the body configuration caused by the limb displacement. The postural response is generated by two mechanisms: Trunk mechanism and Step mechanism which are activated by sensory input signaling the limb displacement in relation to the trunk (distortion of the limb/limb-trunk configuration). The Step mechanism includes two principal types of circuits, one generating a vertical component of step (limb elevation and lowering) and the others generating a horizontal component of step in different directions (limb transfer from the deviated limb position to the initial one). For simplicity, the circuits generating steps in four directions (F, forward; B, backward; R, rightward; L, leftward) are shown. These circuits receive the sensory input signaling the limb displacement in relation to the trunk in a specific direction. When this sensory signal reaches the threshold value it triggers a limb transfer in the opposite direction. The circuit generating the vertical component of a step is activated by the sensory input signaling a limb displacement in any direction. Thus, a displacement of the limb (e.g., to the right) will activate, first, the trunk mechanism causing a displacement of the trunk toward the contralateral limb (black arrow in B) and with some delay the circuit generating the vertical component of a step and the circuit L, which will evoke the leftward corrective step (gray arrow in C). The reverse in the direction of the trunk movement during the transfer phase of the corrective step (black arrow in C) can be caused by the sensory feedback from the stepping limb signaling returning the limb to the initial position or by the signal from the step generating mechanism. Gray, activation of a given mechanism.

supporting limb was caused mainly by the sensory input from the deviated limb though in intact rabbits, load-compensating reflexes in response to limb loading caused by the corrective trunk movement can also contribute.

We found that the amplitude, but not the velocity of the limb displacement, was positively correlated with step length (Figs. 2E–J, 7C–H). This result suggests that

afferents signaling the limb/limb-trunk configuration (most likely group II afferents) were responsible for triggering the mechanisms underlying generation of single corrective steps in different directions.

In experiments with the lateral force applied to the stepping limb during swing, we found that the sensory feedback from the stepping limb can modify all components of the postural response to ensure landing of the limb close to its initial position (Fig. 6). It was shown that during forward locomotion the sensory feedback from the limb during swing also plays an important role. It is signaling the current value of hip abduction or adduction and modifies the swing trajectory to secure that the final foot position in relation to the trunk at the moment of the limb loading is always the same (Musienko et al., 2014).

Distribution of basic neuronal networks generating the postural response to the limb displacement

It has been known for a long time that quadrupeds (e.g., cats and rabbits) decerebrated at the premammillary (or more rostral) levels are capable of standing with close to normal basic body configuration without losing the balance. By contrast, postmammillary animals cannot stand (Bard and Macht, 1958; Magnus, 1924).

We have found that premammillary rabbit can compensate for perturbation of the basic body configuration caused by the hindlimb displacement by generating the postural response similar to that observed in intact animals. The relative number of trials in which the limb displacement alone caused the full postural response including the trunk and limb movements was relatively small (Table 3). However, when the limb displacement was combined with exteroceptive stimulation, i.e., pinching the tail, the full postural response was initiated in the majority of trials (Table 3).

These findings taken together suggest that, after decerebration at the premammillary level, the neural mechanisms compensating for the distortion of the basic standing body configuration remained mostly undamaged, though the level of their activity was considerably reduced. It seems likely that excitatory drive which they receive from brainstem structures is insufficient to fully activate them. It was demonstrated that premammillary rabbits contain also the basic postural mechanisms maintaining the lateral stability and generating fixed-support postural responses to lateral tilts of the supporting surface and to the lateral push applied to the trunk (Musienko et al., 2008).

Despite the fact that postmammillary rabbit cannot stand (Bard and Macht, 1958; Magnus, 1924), we demonstrated that if its spine was fixed, deviation of the limb in relation to the trunk could evoke a corrective step in opposite direction, and thus the limb returned to its initial position. Kinematic characteristics of the corrective steps generated by intact and postmammillary rabbits were remarkably similar. As in intact rabbits, in postmammillary rabbits the amplitude but not the speed of the limb displacement positively correlated with the step length (compare Fig. 2E–J and Fig. 7C–H, respectively). Similar to intact rabbits, the corrective forward and inward steps generated by postmammillary rabbits caused overcompensation and the backward steps undercompensation. The duration of the corrective steps in the corresponding directions, was also similar in intact and postmammillary rabbits (Fig. 7I–K).

We have found that displacement of the limb in postmammillary rabbit evoked activation of the muscles not only in the stepping limb but also in the contralateral limb, and the pattern of their activity was similar to that observed during corresponding corrective steps evoked before decerebration (Fig. 8C–H). Thus, postmammillary rabbit contains neuronal networks generating EMG patterns of stepping and supporting limbs in response to sensory information from the limb signaling about distortion of the limb-trunk configuration. Previously it was demonstrated that animals decerebrated at postmammillary level contain also neuronal networks generating EMG patterns of fixed-support postural responses to sensory information signaling about the lateral tilt and about the translation of the supporting surface (Musienko et al., 2010; Honeycutt et al., 2009; Honeycutt and Nichols, 2010).

Thus, our experiments on decerebrate rabbits demonstrated that the basic networks generating postural response to distortion of the body configuration during standing are located in the spinal cord, brainstem and cerebellum. Despite the fact that sensory information signaling deviation of the limb in relation to the trunk directly affects the spinal networks, most likely supraspinal signals formed by spinocerebellar loop contribute to generation of the postural response. It was shown that dorsal spinocerebellar tract supplies cerebellum with information about the limb end point position in relation to the trunk (Bosco and Poppele, 1997; Casabona et al., 2004) and output of the cerebellum affects activity of the descending pathways (Orlovsky et al., 1999). To reveal contribution of the spinocerebellar loop to generation of postural response to distortion of the basic body configuration is a topic for future studies.

Mechanisms generating trunk and limb movements during change-in-support postural response and during locomotion

Steps in different directions, as well as a lateral movement of the trunk loading the supporting limb during the step can be initiated not only by postural perturbations during standing. Most bipeds and quadrupeds, in addition to the main form of locomotion, forward stepping, are also capable of backward and sideward stepping (Stein et al., 1986; Buford and Smith, 1990; Buford et al., 1990; Rossignol, 1996; Deliagina et al., 1997; Zelenin et al., 2011; Musienko et al., 2012). Steps with a lateral component are also used for correcting perturbations of balance during forward walking (Karayannidou et al., 2009; Musienko et al., 2014; Hof et al., 2010; Hof and Duysens, 2013). During undisturbed stationary locomotion the trunk exhibits lateral oscillations in the rhythm of stepping (Karayannidou et al., 2009; Misiaszek, 2006). One of the principle questions is if the same or different

neural mechanisms generate trunk and limb movements in context of locomotion performed in a specific direction (in relation to the trunk) and in context of postural responses to distortion of the basic body posture in the standing animal.

We found that in the standing rabbit, the hindlimb displacement in any direction caused a rapid lateral movement of the caudal trunk toward the contralateral limb (Fig. 4), which started 0.02–0.56 s before onset of the corrective step (Table 2). It consisted of bending of the trunk toward the displaced limb and twisting toward the contralateral limb. This trunk movement resulted in unloading of the displaced limb and loading of the contralateral one. During the corrective step the direction of the trunk movement was reversed and it reached its initial position at the end of the corrective step. Similarly, it was shown that during forward locomotion the trunk movement toward the contralateral limb started before the swing initiation (causing the unloading of the limb about to step and loading the supporting limb which performed stance) and its direction was reversed in the second part of the swing (Karayannidou et al., 2009; Misiaszek, 2006). Moreover, we demonstrated that trunk movement during postural response was caused mainly by activation of the abdominal muscle (oblique externus abdominis, OEA) which caused ipsilateral bending and contralateral twisting the trunk. It was shown that OEA was also rhythmically active during forward walking and the phase of its activity in the cycle of the ipsilateral limb was similar to that observed during postural response (from the second half of the stance to the middle of swing) (Deban et al., 2012). In addition, it was demonstrated that OEA exhibited the rhythmical activity with the same phase during fictive locomotion, suggesting that it is activated by locomotor central pattern generator (Koehler et al., 1984). Finally, we found that other back muscles (erector spinae and multifidus) did not contribute to trunk corrective movements caused by the limb displacement in the standing rabbit. These muscles also did not exhibit consistent step-related modulation during locomotion (Musienko et al., 2014), and it was suggested that their role during locomotion is to control the stiffness of the back (Carlson et al., 1979). Thus, one can suggest that the same mechanism generates the trunk movements during change-in-support postural corrections and during locomotion. This mechanism is activated by sensory input from the limb signaling displacement of the limb (standing or performing the stance) in relation to the trunk (Fig. 9B) during both postural tasks and during locomotion. The reverse of the direction of the trunk displacement observed during the step could be caused by the sensory feedback from the stepping limb or by the signal from the step generating mechanism.

Phases of the corrective step (the limb unloading, transfer and loading) represent a part of the locomotor cycle. Despite a large variability in activity of muscles during corrective steps which can be explained by some differences in the basic body configuration of individual animals, as well as by difference in amplitudes of the corrective steps, the phase of activity of some muscles was similar to that observed during locomotion performed in the corresponding direction. For example, we found that during corrective steps in different directions, the hip and ankle flexors in the stepping limb were activated during unloading and during transfer phases of the step (to clear the limb off the ground) with specific activation period during step in a particular direction. The phases of the activity of these two groups of muscles during forward and backward walking in the intact cat (Buford and Smith, 1990) were similar to those observed during corrective steps performed in corresponding directions. Also, Bic (hip extensor and knee flexor) was activated during the transfer phase of the backward (but not the forward) corrective step, and thus contributed to the hip extension and knee flexion which retracted the limb back to its initial position. Similar phase of its activity was observed during backward locomotion in the intact cat (Buford and Smith, 1990).

The basic locomotor pattern is generated by a spinal network (the central pattern generator) which in the intact animal is activated by supraspinal commands (Orlovsky et al., 1999). Recently we demonstrated that when locomotor mechanisms in postmammillary cat were activated by stimulation of the spinal cord, the direction of generated stepping was determined by the sensory information from the limb signaling deviation of the limb in relation to the trunk during the stance. These signals caused generation of the step in opposite direction. In the absence of this information stepping in place was observed. It was suggested, that locomotor system contains the mechanisms generating the vertical component of step (limb elevation and lowering), and the other generating a horizontal component (limb transfer from one extreme point to the other). The latter is activated by the sensory input signaling displacement of the limb in relation to the trunk during stance; reaching an extreme position triggers a limb transfer in opposite direction (Musienko et al., 2012). One can suggest that sensory information signaling about deviation of the limb in relation to the trunk in standing animal activate both mechanisms leading to generation of a single corrective step (Step mechanism in Fig. 9D).

In conclusion, in the present study, for the first time, the postural response to distortion of the basic body configuration caused by displacement of the hindlimb has been characterized. It was shown that the response consisted of the corrective trunk movement and the corrective step which led to restoration of the basic standing body configuration. It was found that these two components of the postural response were generated by separate mechanisms which were activated, however, in a strict sequence by sensory input from the deviated limb signaling distortion of the limb/limb-trunk configuration. It was demonstrated that integrity of the highest levels of CNS was not necessary for generation of the postural reaction to distortion of the basic body configuration. It was shown that sensory input from the deviated limb activated networks generating EMG patterns of both the stepping and supporting limb. These networks reside in the brainstem, cerebellum and spinal cord. Finally, the hypothesis about the mechanisms underlying generation of the postural

response characterized in the present study has been proposed (Fig. 9). To test this hypothesis is the goal of future studies.

CONFLICT OF INTEREST

There is no conflict of interest.

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