

## RESEARCH ARTICLE

# The role of load-dependent sensory input in the control of balance during gait in rats

Alexander Popov<sup>1,2,\*</sup>, Vsevolod Lyakhovetskii<sup>2,\*</sup>, Elena Bazhenova<sup>1</sup>, Oleg Gorskii<sup>1,2</sup>, Daria Kalinina<sup>1</sup>, Natalia Merkulyeva<sup>1,2</sup> and Pavel Musienko<sup>1,2,3,†</sup>

## ABSTRACT

Locomotor activity requires fine balance control that strongly depends on the afferent input from the load receptors. Following hindlimb unloading (HU), the kinematic and EMG activity of the hindlimbs is known to change significantly. However, the effects of HU on the integrative control mechanisms of posture and locomotion are not clear. The goal of the present study was to evaluate the center of mass (CoM) dynamic stabilization and associated adaptive changes in the trunk and hindlimb muscle activity during locomotion after 7 days of HU. The EMG signals from the muscles of the low lumbar trunk [m. longissimus dorsi (VERT)] and the hind limb [m. tibialis anterior (TA), m. semitendinosus (ST), m. soleus (SOL)] were recorded together with the hindquarter kinematics during locomotion on a treadmill in six rats before and after HU. The CoM lateral shift in the step cycle significantly increased after HU and coincided with the enhanced activity of the VERT. The mean EMG of the TA and the ST flexor activity increased significantly with reduction of their burst duration. These data demonstrate the disturbances of body balance after HU that can influence the basic parameters of locomotor activity. The load-dependent mechanisms resulted in compensatory adjustments of flexor activity toward a faster gait strategy, such as a trot or gallop, which presumably have supraspinal origin. The neuronal underpinnings of these integrative posture and locomotion mechanisms and their possible reorganization after HU are discussed.

**KEY WORDS:** Hindlimb unloading, Locomotion, Posture, Rat, Sensory feedback, Load-dependent afferent input, Evolution of the sensorimotor system

## INTRODUCTION

Locomotor activity requires fine balance control that strongly depends on the afferent input from the load receptors (Deliagina et al., 2000; Duysens et al., 2000). The regulation of body posture is mainly determined by the resistance to the Earth's gravity, and the particular perturbations of antigravitational control lead to certain disturbances in locomotion itself. Normal control of locomotion is


based on spinal locomotor networks called 'central pattern generators' (CPGs) located within the left and right sides of the cervical (for forelimbs) and lumbar (for hindlimbs) regions of the spinal cord, and these are modulated by sensory and supraspinal inputs (Grillner, 1985). CPG control expands to the muscles of the limbs and beyond to the epaxial muscles of the trunk (Delvolvé et al., 1997; Wada et al., 2006; Grillner and Jessell, 2009), which participate in both locomotor and postural functions in various classes of animals. The significance of epaxial muscles is extremely high in all chordates, and in tetrapods in particular. Starting from the primary forms of non-mammalian tetrapods, the epaxial muscles changed their main function from the formation of a lateral trunk tilt in amphibians (Frolich and Biewener, 1992) to maintenance of equilibrium during terrestrial locomotion in lizards (Ritter, 1996), but they still have only one burst activity per cycle. The transition to permanent ground locomotion in tetrapods is provided by the bilateral double burst activity of the epaxial pelvic girdle muscles at push-off and paw contact (Carlson et al., 1979; Ritter et al., 2001; Schilling and Carrier, 2010). Thus, the epaxial muscles in terrestrial mammals have become important elements in postural control. In all likelihood, this change in the main function of the epaxial muscles was associated with an increasing residence time of new species under conditions of ground reaction force, thereby increasing the influence of load-dependent input on the operation of locomotor networks.

The importance of load-dependent sensory input to the control of locomotion in ground mammals has been repeatedly confirmed (Duysens and Pearson, 1980; Pearson et al., 1992). The non-weight-bearing condition in the hindlimb unloading (HU) model (Morey-Holton and Globus, 2002) is well known to change locomotor control (Canu and Falempin, 1996; Canu et al., 2005; Tajino et al., 2015; Popov et al., 2019). In particular, hyperextension of the ankle (Canu et al., 2005; Canu and Garnier, 2009) and knee joints (Tajino et al., 2015), increased cycle duration and m. soleus (SOL) burst duration (Canu and Falempin, 1996), and decreased m. tibialis anterior (TA) burst duration (Canu and Falempin, 1997) have been described after HU in treadmill locomotion. The locomotion after HU is also characterized by postural instability in the form of poor lateral stability and abduction of the hindlimbs (Canu and Falempin, 1998), as well as changes in the activity of neck (rhomboides capitis) and trunk (internal oblique) muscles in rats performing air-righting tasks (Kawano et al., 2004); these responses could also be evidence of posture mechanism disturbance. However, the kinematic characteristics of postural stability in locomotion after HU have not yet been evaluated. Furthermore, the specific changes in the activity of epaxial muscles that occur after HU during locomotion are not yet known. Assessment of the role of load-dependent sensory input in the reorganization of the spinal neural network responsible for locomotor and postural control could assist in explaining the changes described above following HU.

<sup>1</sup>Institute of Translational Biomedicine, Saint-Petersburg State University, 7-9 Universitetskaya emb., 199034 Saint-Petersburg, Russia. <sup>2</sup>Pavlov Institute of Physiology RAS, 6 Makarov emb., 199034 Saint-Petersburg, Russia. <sup>3</sup>Sirius National Technical University, Neuroscience Program, 1 Olympic pr., 354340 Sochi, Russia.

\*These authors contributed equally to this work

†Author for correspondence (p.musienko@spbu.ru)

 A.P., 0000-0003-4336-5942; V.L., 0000-0001-5948-0991; E.B., 0000-0003-3690-9905; O.G., 0000-0001-7247-8263; D.K., 0000-0002-7587-9924; N.M., 0000-0003-1276-1918; P.M., 0000-0002-7324-9021

One aspect that is not yet clear is whether the epaxial muscles of mammals produce a direct lateral tilt of the pelvis during walking or whether they only stabilize the pelvis. Carlson et al. (1979), in experiments on cats, excluded the participation of the caudal epaxial muscles in the formation of lateral trunk tilt. However, Schilling and Carrier (2010), who explored the locomotion of dogs, suggested that the formation of a lateral tilt by the epaxial muscles is theoretically possible. The assessment of epaxial muscle activity after HU, in our opinion, will not only provide a better understanding of the mechanisms of postural instability, as identified earlier, but may also provide greater clarity in understanding the role of the epaxial muscles in producing the lateral tilt of the pelvis.

Another unknown factor is the locomotor activity of the hip muscles after HU. The knee joint is known to exist in hyperextension in the second part of the stance phase, as well as in the swing phase (Tajino et al., 2015; Popov et al., 2019). Hyperextension of the knee joint in the stance phase is likely to be caused by the increased activity of the unique main extensor of the knee joint, the m. quadriceps femoris. However, no data have been published regarding the activity of the knee flexors after HU. We believe this information is important for evaluation of the knee flexor activity, beginning with the activity of the m. semitendinosus (ST), which reproduces pure flexor bursts (Engberg and Lundberg, 1969; English and Weeks, 1987; Smith et al., 1993). This assessment will help in understanding the role of the ST flexor activity in the previously detected kinematic changes in the swing phase. It will also determine whether the two-joint muscle ST activity shows the same changes depending on the phase of the locomotor cycle after HU. Assessment of the activity of the two hindlimb flexors, ST and TA, after HU, together with that of the postural SOL and epaxial muscles, could also provide more insight into the integration of posture and locomotion mechanisms.

The goal of the present study was to evaluate the center of mass (CoM) dynamic stabilization and associated adaptive changes of the trunk and hindlimb muscle activity during locomotion after 7 days of HU. Analysis of epaxial muscle activity and its relationship to postural control and the locomotor changes after HU was expected to further expand our knowledge of integrative sensorimotor control and the evolutionary development of postural and locomotor systems.

## MATERIALS AND METHODS

The study was performed on  $n=6$  adult male Wistar rats (200–350 g body mass). The number of animals used for the EMG analysis were chosen based on power calculations (G\*Power 3.1.9.7 program; Faul et al., 2007). All experimental procedures were approved by the Ethics Commission of the Pavlov Institute of Physiology. Experiments were performed in full accordance with the requirements of the Council Directive 2010/63EU of the European Parliament on the protection of animals used for experimental and other scientific purposes. The rats were housed in individual cages with free access to food and water. All surgical procedures were conducted under aseptic conditions under anesthesia.

### Implantation of EMG electrodes

Rats were implanted with stainless steel wire electrodes (AS632, Cooner Wire, Chatsworth, CA, USA) under intra-abdominal anesthesia [a mixture of 7 mg kg<sup>-1</sup> Zoletil100 (tiletamin+zolazepam, Virbac, France) and xylazine 2% (Interchemie werken 'De Adelaar' BV, The Netherlands), with additional injections during surgical procedures, when necessary] (Musienko et al., 2011). A skin incision was made along the sagittal suture of the skull. Three screws were anchored firmly to the skull,

and a nine-pin Amphenol head-plug was cemented (with dental cement) to the skull and screws. Common ground wires (with 1 cm of the Teflon removed distally) were inserted subcutaneously in the mid-back region. Animals were implanted with bipolar electrodes in the TA, ST and SOL of the left hindlimb and in the m. longissimus dorsi of the lower lumbar spine (VERT) at the L4–L6 level on the left side ( $n=6$  rats). In two rats, the VERT was additionally implanted in the right side. The mid-belly of the muscles was exposed, and two electrodes (with a 1 mm portion of the Teflon insulation removed) were inserted into each muscle with a needle.

The EMG electrodes were fixed together with an Ethylon 4 suture at the entrance and exit from the muscle. The proper placement of the electrodes was verified during surgery by stimulating through the head connector and post-mortem via dissection. Analgesic and antibiotic treatments were provided for 3–5 days after surgery.

### Hindlimb unloading

HU was carried out according to the method of Morey-Holton and Globus (2002). A rotating mechanism was attached with adhesive tape to the tail of the animal, and the rats were suspended from the ceiling of the cage. This mechanism allowed the animals to contact the forelimbs with the floor of the cage and to move in all directions. The hindlimbs were free from external influences and had no possibility of contact with the surface of the cage. The body angle was approximately 30 deg.

### Treadmill stepping

Recordings began 5 days after surgery on a motor-driven treadmill with locomotion at walking speeds (20 cm s<sup>-1</sup>) prior to HU and after 7 days of HU. After 7 days of HU, the recording was initiated 5 min after the rat was freed from the suspension mechanism and performed for 5–10 min. The rats were trained to walk on a treadmill and were selected according to their ability to walk at regular speed.

### Swimming

Swimming was recorded in  $n=2$  animals for qualitative assessment of muscle activity before and after HU. The swimming task method was as previously described (Gruner and Altman, 1980; Roy et al., 1991) using a 1.0×0.3×0.4 m water tank with a water temperature of 37°C. Each animal was trained to swim in the correct direction before the experiment. Recording uninterrupted locomotion in water included an average of 4–5 cycles. Between recordings, the animals rested for about 1 min. The earliest records were used for analysis to exclude the influence of physical fatigue on muscle activity. The swim cycle was divided into protractor (hip flexion) and retractor (hip extension) phases of the left hindlimb. Swimming speed was determined based on video analysis, as the ratio of the distance traveled by the marker point on the head plug to the time required to pass this distance. Segments of a known length were initially marked on the walls of the pool.

### Kinematic analysis

Video recording was performed with video cameras (Basler daA1280-54uc with global shutter, triggered by an external synchronization signal) located on the left and right side and rear of the treadmill for kinematic analysis of selected stepping sequences from  $n=6$  rats. Five identifying marks were drawn with a marker on shaved skin on the iliac crest, the trochanter major, the knee, the lateral malleolus and the fifth metatarsophalangeal joint for the lateral view and the center of the sacrum for the rear view. The video recording was analyzed frame by frame (100 frames s<sup>-1</sup>; 21 pixels cm<sup>-2</sup>). The step cycle was divided into the swing and the

stance phases. Two subphases were identified in the swing phase: flexion (F), when a flexion occurs in three (hip, knee, ankle) joints of the hindlimb, and extension 1 (E1), when an extension in the knee and ankle joints occurs (Philippson, 1905). The onset of the swing phase was marked as push off (po), and the onset of the stance phase was marked as paw contact (pc). We measured the maximum pelvic shift in each step cycle as the distance from one extreme point (shift to the left) to the other extreme point (shift to the right) with the help of custom-written software. The extreme points were in the middle of swing and at paw contact.

### Analysis of EMG activity

The EMG activity was recorded from the left VERT ( $n=6$  rats), left ST ( $n=5$  rats), left SOL ( $n=6$  rats), left TA ( $n=5$  rats), and right VERT ( $n=2$  rats) muscles. The EMG signal was differentially amplified (A M Systems USA, model 1700, bandwidth 10 Hz to 5 kHz) and digitized at 20 kHz with a National Instruments A/D board. The burst onset and offset times of  $10\pm 2$  steps of each rat were determined manually.

Custom scripts written in MATLAB were used to measure the mean peak amplitude, the duration and the mean EMG for a rectified burst signal. The mean EMG of burst activity (TA, VERT, ST and SOL) was calculated by dividing the integrated area of a burst by its duration. The mean EMG of non-burst activity (ST) was calculated by dividing the integrated area by the duration of three intervals: (1) from ST burst offset to the middle of the swing phase, (2) from the middle of the swing phase to the paw contact moment, and (3) from the paw contact moment to the ST burst onset. All parameters were averaged and normalized per individual rat.

### Statistics

A hierarchical linear model with a constant slope and random intercept (Aarts et al., 2014) was used to compare all investigated

values. The individual distributions of these were normal in almost all cases, as determined by the Lilliefors test. All data are reported as means $\pm$ s.e.m. The criterion level for the determination of statistical difference was set at  $P<0.05$ .

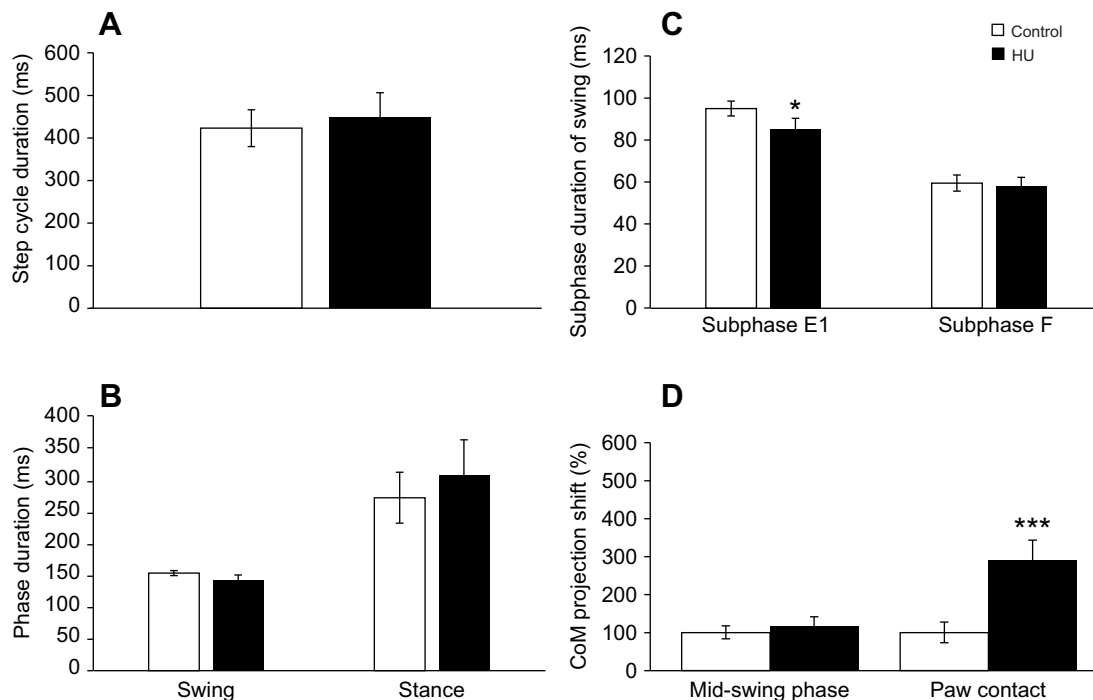
## RESULTS

### General observations

All animals after the 7 day HU were able to step, with maintenance of equilibrium, on a treadmill belt, as well as to support their posture while standing, but there was a noticeable swaying and their hindlimbs were more extended during locomotion. The HU rats had shorter episodes of uninterrupted locomotion (3–4 consecutive steps) than were observed in the control animals. In addition, a change was noted in the sequence of movements of the ipsilateral forelimbs and hindlimbs after HU in the form of a reduced double support state period. The stance phase for this type of walking was provided by the contralateral forelimbs and hindlimbs, and it sometimes lasted up to 50 ms. Similar to previous observations (Canu and Falempin, 1996), some animals had a galloping stride after HU while they were trying to return quickly to the center of the treadmill belt.

### Step cycle kinematics

The time parameters of the step cycle are presented in Fig. 1. The step cycle duration after HU was not changed (Fig. 1A) ( $429\pm 44$  ms versus  $454\pm 60$  ms,  $P=0.72$ ). Stance phase duration tended to increase (Fig. 1B) ( $274\pm 40$  ms versus  $310\pm 56$  ms,  $P=0.07$ ). Swing phase duration was not changed after HU ( $154\pm 8$  ms versus  $143\pm 8$  ms,  $P=0.20$ ), but one component, subphase E1, was significantly decreased (Fig. 1C) ( $95\pm 4$  ms versus  $85\pm 5$  ms,  $P=0.02$ ), while the duration of subphase F remained unchanged ( $60\pm 4$  ms versus  $58\pm 4$  ms,  $P=0.97$ ).



**Fig. 1. Effect of hindlimb unloading (HU) on step cycle kinematics.** (A) Step cycle duration, (B) phase duration of the step cycle, (C) subphase duration of the swing phase (E1, early extension; F, flexion) and (D) center of mass (CoM) projection shift (means $\pm$ s.e.m.,  $n=6$ ). Note that the histogram in D was normalized on a per rat basis to the initial shift values. \* $P<0.05$ , \*\*\* $P<0.001$ .

### Dynamic balance control during treadmill locomotion

The treadmill locomotion of each rat, both before and after HU, was characterized by a lateral shift of the pelvis (CoM projection in Fig. 2) toward the contralateral side of the swinging hindlimb. Before HU, the maximum value of the lateral shift occurred while the ipsilateral limb was in the middle of the swing phase (Fig. 2). By the time of paw contact of the ipsilateral limb, the pelvis had almost completely returned to the central line. In contrast, after HU, at the paw contact of the ipsilateral hindlimb, the lateral shift of the CoM was maintained and significantly increased (to  $191 \pm 47\%$ ,  $P < 0.001$ ). The lateral shift value in the middle of the swing phase after HU did not change significantly (to  $18 \pm 21\%$ ,  $P = 0.23$ ) (Fig. 1D). Thus, the kinematic changes in pelvic movement were observed only in the late period of the swing phase (E1), which simultaneously had a significantly reduced duration (see above).

### Back muscle activity

Before HU, the paravertebral muscles had a double-burst pattern of activity in the step cycle. The first period of activity (VERT push-off) began during late stance and ended after the paw-off moment of the ipsilateral limb. The second period of activity (VERT paw contact) began during late swing before touching the ipsilateral limb of the treadmill surface and ended at the beginning of the stance phase (Fig. 2). Although all animals revealed both bursts distinctly in a step cycle, in some cases these bursts were less clear because of the presence of simultaneous tonic activity. As described earlier in experiments on cats (Carlson et al., 1979; English, 1980), back muscles also had two bursts of activity in the step cycle, occurring during paw-off and paw contact of the ipsilateral limb. The activity of the left back muscle was completely co-active with that of the right side (Fig. 3).

After HU, the double-burst activity of the paravertebral muscles was maintained with unchanged burst duration. Only the VERT push-off had a significant increase in maximum amplitude ( $48 \pm 14\%$ ,  $P = 0.01$ ) and mean EMG activity ( $26 \pm 14\%$ ,  $P = 0.04$ ) (Fig. 4A). Thus, the same significant changes in the amplitude of paravertebral muscle activity on the contralateral side occurred when the ipsilateral limb was in the late swing phase and close to the moment of paw contact. Consequently, the increased pelvic shift to the contralateral side at the moment of paw contact in HU animals was well matched to the increased activity of the contralateral VERT push-off.

### TA and SOL muscle activity

In both conditions, the purely flexor TA muscle was always strongly activated for a short period at the end of stance phase, followed by some activity in the early flexion phase, and then another short burst late in the first extension phase. This phenomenon was previously mentioned in intact cats (Engberg and Lundberg, 1969) and in rats before and after HU (Canu and Falempin, 1997). In the present study, this pattern of TA activity was maintained after HU, but some features of TA activity were altered by HU: (1) burst duration was decreased ( $15 \pm 2\%$ ,  $P < 0.001$ ) (similar to Canu and Falempin, 1997); and (2) both maximum amplitude and mean EMG activity were significantly increased after HU ( $66 \pm 13.7\%$ ,  $P < 0.001$ , and  $43 \pm 85\%$ ,  $P < 0.001$ , respectively) (Fig. 4C). SOL burst duration had a tendency to increase ( $25 \pm 11\%$ ,  $P = 0.07$ ) (Fig. 4B).

### ST activity

The ST is a two-joint muscle that had a distinct burst of activity at the end of stance phase (STpo, push-off) in our experiment. This burst has also been described in previous locomotion studies in rats,

dogs and cats (Engberg and Lundberg, 1969; Nicolopoulos-Stourmaras and Iles, 1984; English and Weeks, 1987; Smith et al., 1993; Deban et al., 2011). After HU, this pattern of ST activity was unchanged (Fig. 3). After HU, the maximum amplitude and mean EMG activity of the STpo burst were strongly and significantly increased ( $321 \pm 240\%$ ,  $P < 0.001$ , and  $215 \pm 144\%$ ,  $P < 0.001$ , respectively) (Fig. 4D). The STpo burst duration was slightly but significantly decreased ( $13 \pm 2\%$ ,  $P = 0.003$ ) similar to the TA burst duration.

All HU animals also exhibited a highly variable non-burst activity of the ST that extended from the beginning of the early extension phase E1 to the onset of STpo flexion burst in late stance phase (Fig. 5).

We analyzed the duration, maximum amplitude and mean EMG of this non-burst activity by dividing it into sections corresponding to all subphases of the step cycle, with the exception of the STpo burst duration (Fig. 3). In the E1 subphase and throughout the stance phase, the ST non-burst maximum amplitude and mean EMG activity were significantly increased (Fig. 4E), notably at the moment of paw contact. In general, a marked inter-individual variation was noted in non-flexor burst ST activity, but it displayed an increase in all animals after HU.

### Timing of flexor and extensor activity during the cycle period

Before HU, during treadmill locomotion in the late stance phase, the moment of maximum extension of the hindlimb joints preceded the initiation of swing phase and was followed immediately by almost simultaneous joint flexion and lifting of the limb. The STpo activity onset always occurred prior to the TA activity onset. The push-off moment always started after the TA activity onset. The SOL burst activity was always strictly reciprocal with the STpo burst and the TA burst activity (Fig. 3). The gait diagrams and time relationships between flexor and extensor activity within the step cycle are presented in Fig. 6. A significant decrease occurred in the delay between TA onset and the initiation of the swing phase. After HU, the initiation of knee flexion usually outpaced ankle flexion. A specific feature of the locomotion after HU was the significantly extended period of simultaneous unsupported state of the ipsilateral forelimbs and hindlimbs in the late E1 subphase of the ipsilateral hindlimb (Fig. 6C).

### Swimming performance

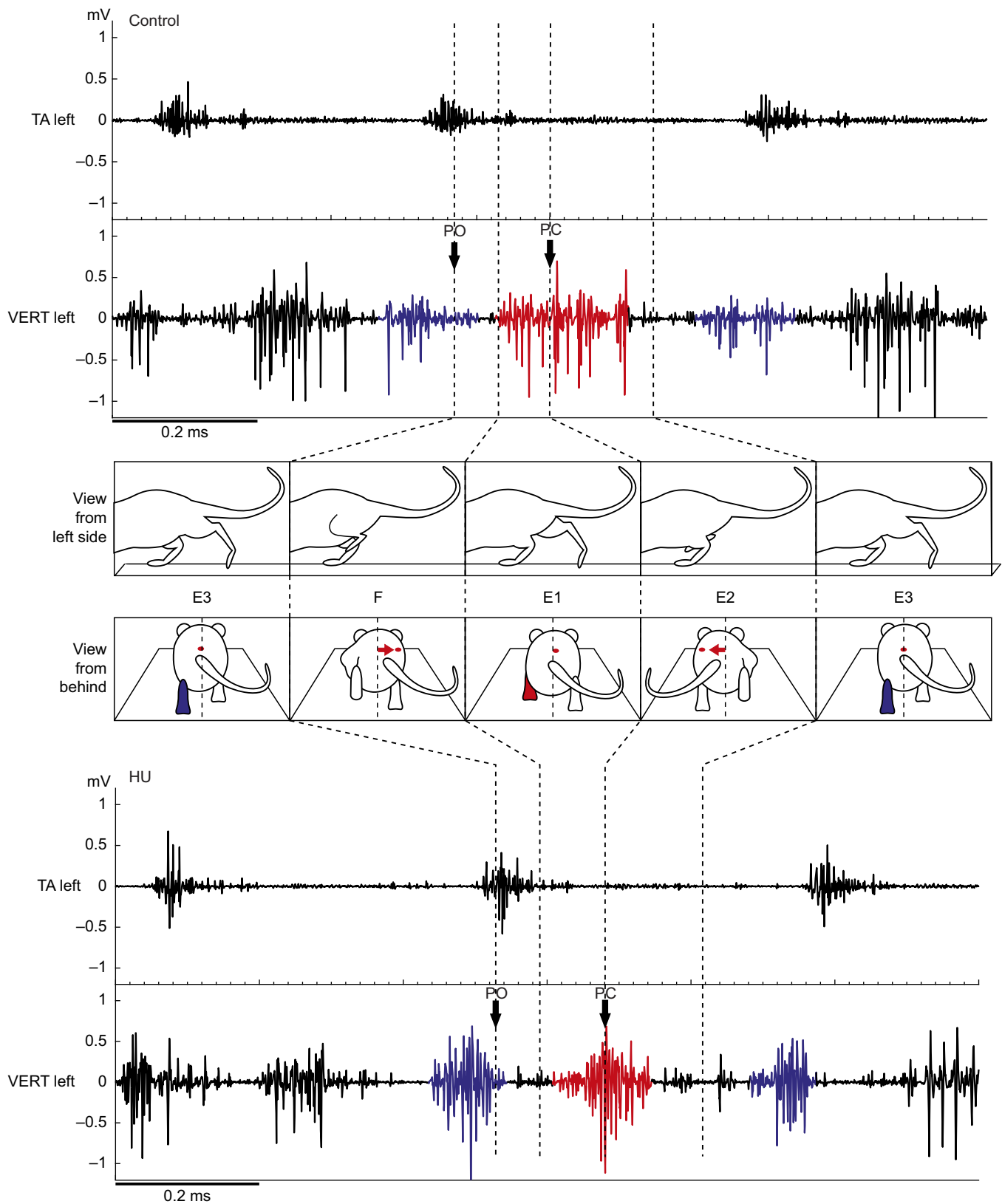
The load-dependent mechanisms influencing locomotor ability in gravitationally unloaded conditions were tested in supplementary swimming experiments ( $n = 2$  rats) (Gruner and Altman, 1980; Roy et al., 1991). Before HU, the activity of the paravertebral muscles during swimming was strictly co-active on both sides. The bursts of the EMG activity were clearly identified and had the same pattern seen during treadmill locomotion: at the beginning of hindlimb protraction and at the beginning of hindlimb retraction, the movements matched well the VERT push-off and VERT paw contact obtained previously (Fig. 7). The average speed before and after HU was  $38.0 \pm 3.0 \text{ cm s}^{-1}$  and  $23.3 \pm 1.4 \text{ cm s}^{-1}$ , respectively. More alterations were revealed for the paravertebral muscles during swimming compared with treadmill stepping. A clear tendency was noted for unifying the two bursts of activity into one consisting of two bursts from different cycles (Fig. 7). This occurred more often in the second half of the swimming distance, probably when the rats had tired.

## DISCUSSION

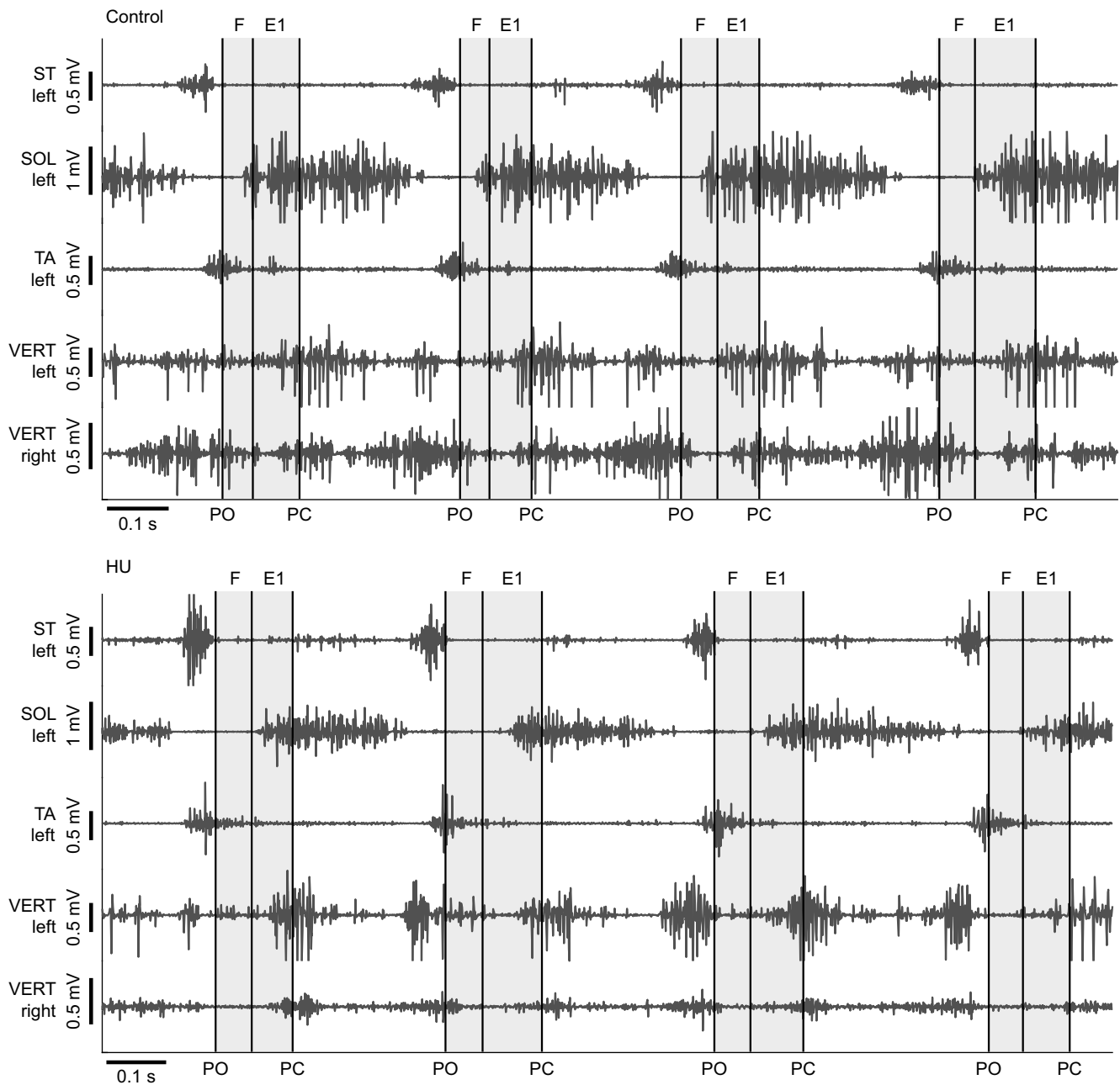
### Epaxial muscle activity after hindlimb unloading

The kinematic analysis showed that during treadmill locomotion, through subphase F of the ipsilateral limb before HU, the CoM





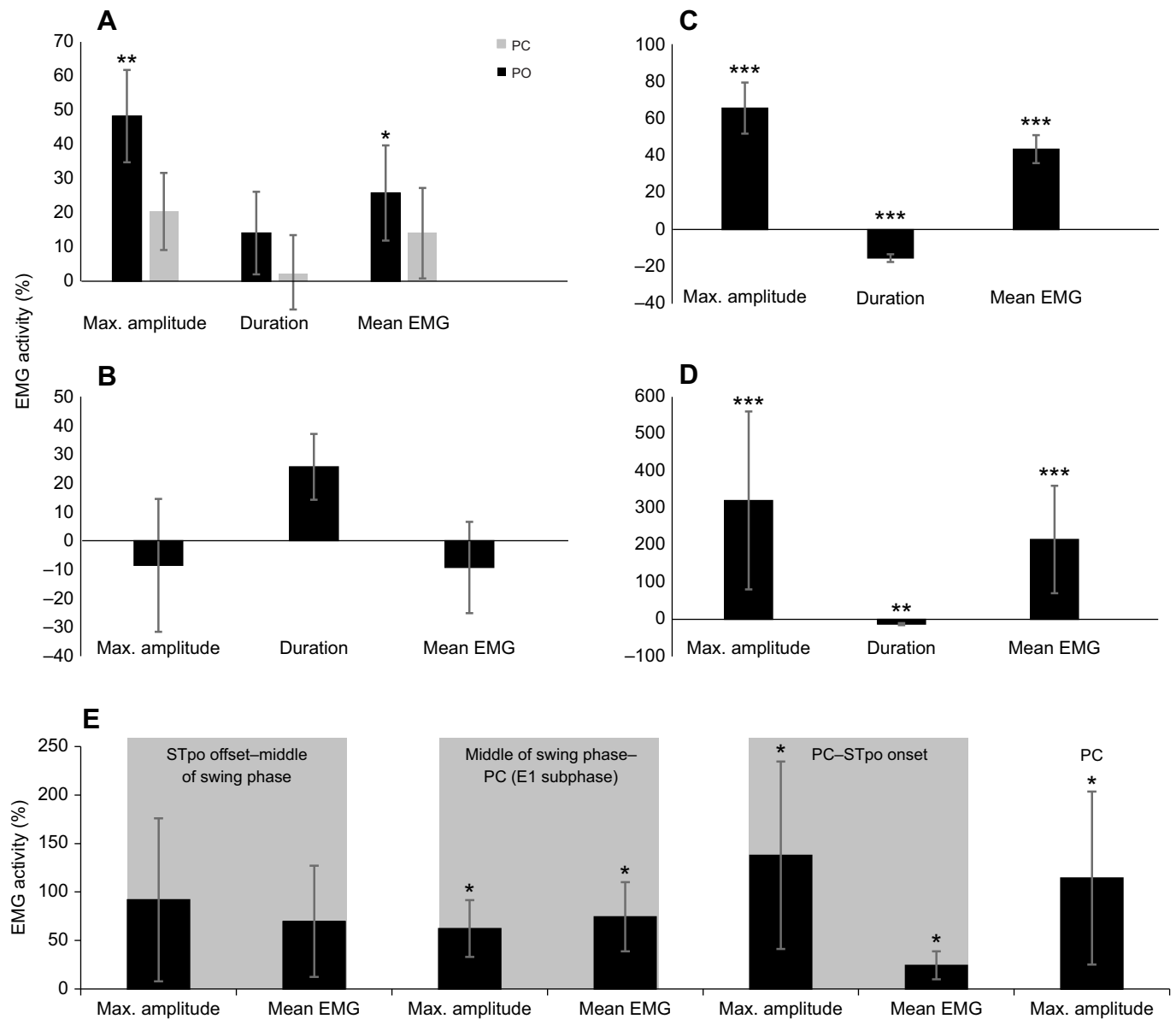
**Fig. 2. Effect of HU on back muscle activity and balance control.** M. longissimus dorsi (VERT) activity in the control condition (top) and after 7 days of HU (bottom) in one rat, synchronized with the CoM projection shift (red dot) scheme when walking. The corresponding animal views from the left side and from the rear are shown in the center (F, flexion; E1–3, extension). VERT push-off (PO) activity is in blue, and VERT paw-contact (PC) activity is in red. After HU, the VERT push-off burst activity clearly increased. After HU, the CoM projection shift also increased (red arrow) at the moment of paw contact. M. tibialis anterior (TA) activity is shown for identification of the step cycle phases.



**Fig. 3. Effect of HU on extrinsic hindlimb and trunk muscle activity.** A representative example of the activity patterns of extrinsic hindlimb muscles (ST, m. semitendinosus; SOL, m. soleus; TA) and a trunk muscle (VERT) in the control condition (top) and after 7 days of HU (bottom). The gray area indicates the swing phase. PO, push-off; PC, paw contact. After HU, the double-burst pattern of VERT activity was unchanged and strictly co-active for the two VERT bursts on the left and right sides.

shifted toward the supporting contralateral limb (Fig. 2). From the onset of the E1 subphase of the ipsilateral limb, the CoM started to shift to the central axis of the movement trajectory, and by the time of paw contact, it was located near the central line of the trajectory. After HU, a delay in returning CoM to the center line and a significant increase in the CoM shift amplitude toward the contralateral side were observed at paw contact of the ipsilateral hindlimb. Hence, when the ipsilateral hindlimb was in the late E3 phase and the contralateral hindlimb had just touched the surface, CoM was increasingly shifted to the direction of this ipsilateral supported hindlimb. Thus, the rats after HU demonstrated lateral instability when walking, as described previously (Canu and

Falempin, 1996, 1998). This increased shift of the CoM toward the supporting contralateral limb at paw contact of the ipsilateral limb presumably was not due to limb bending and weakness of the muscles of the supporting limb, which was observed after HU (Winiarski et al., 1987). Firstly, the supporting limb at that moment was at the second part of the stance phase and, accordingly, in the active period of the limb elongation and joint extension that was clearly shown even 14 days after HU (Tajino et al., 2015). Secondly, at the end of the stance phase, the knee joint (Tajino et al., 2015; Popov et al., 2019) and ankle joint (Canu and Garnier, 2009; Canu et al., 2005) were in hyperextension after HU. Hindlimb abduction was also observed (Canu and Falempin, 1996), probably to

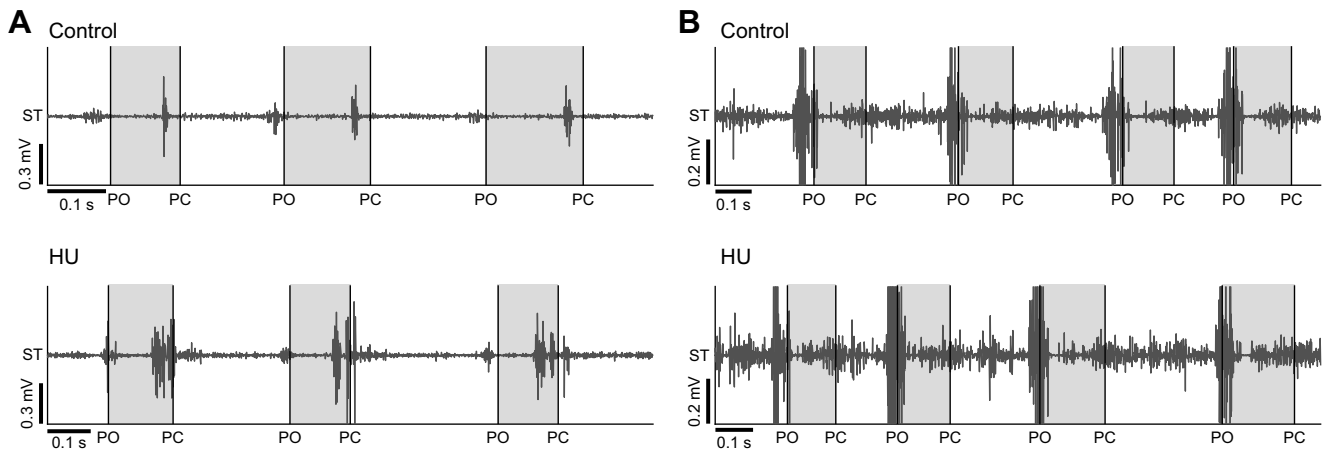


**Fig. 4. Adaptive adjustments of hindlimb and trunk muscle EMG activity after 7 days of HU.** (A–D) Changes in the EMG bursts of activity in (A) VERT at paw contact (PC) and push-off (PO) ( $n=6$ ), (B) SOL ( $n=6$ ), (C) TA ( $n=5$ ) and (D) ST at push-off (STpo) ( $n=5$ ). (E) Maximal amplitude and mean EMG of non-burst ST activity for three intervals (gray), and at the moment of paw contact ( $n=5$ ). All data (means $\pm$ s.e.m.) were normalized on a per rat basis to initial values. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .

compensate for the instability in walking. Therefore, the changed position of the CoM projection after HU should primarily be a result of changes in trunk muscle activity, which, as previously reported (Kawano et al., 2004), modify their work in the air-righting condition after 9 weeks of HU. Accordingly, we observed an increase in the amplitude and mean EMG activity of the VERT push-off in HU rats (Fig. 4A), raising the question of whether the CoM shift, the change in VERT push-off activity and the hypothetically related lateral flexion of the pelvis are due to the HU.

During walking, the trunk shifts mediolaterally in a frontal plane, with maximal amplitude at low speeds (Carlson et al., 1979). Back muscles are hypothesized to act synergistically with abdominal muscles, in step lengthening, pushing the pelvic girdle forward in the direction of movement of the hindlimb (English, 1980; Schilling and Carrier, 2010). In cats, lateral motion of the body is produced by lift-off of the forelimb and landing of the hindlimb during walking

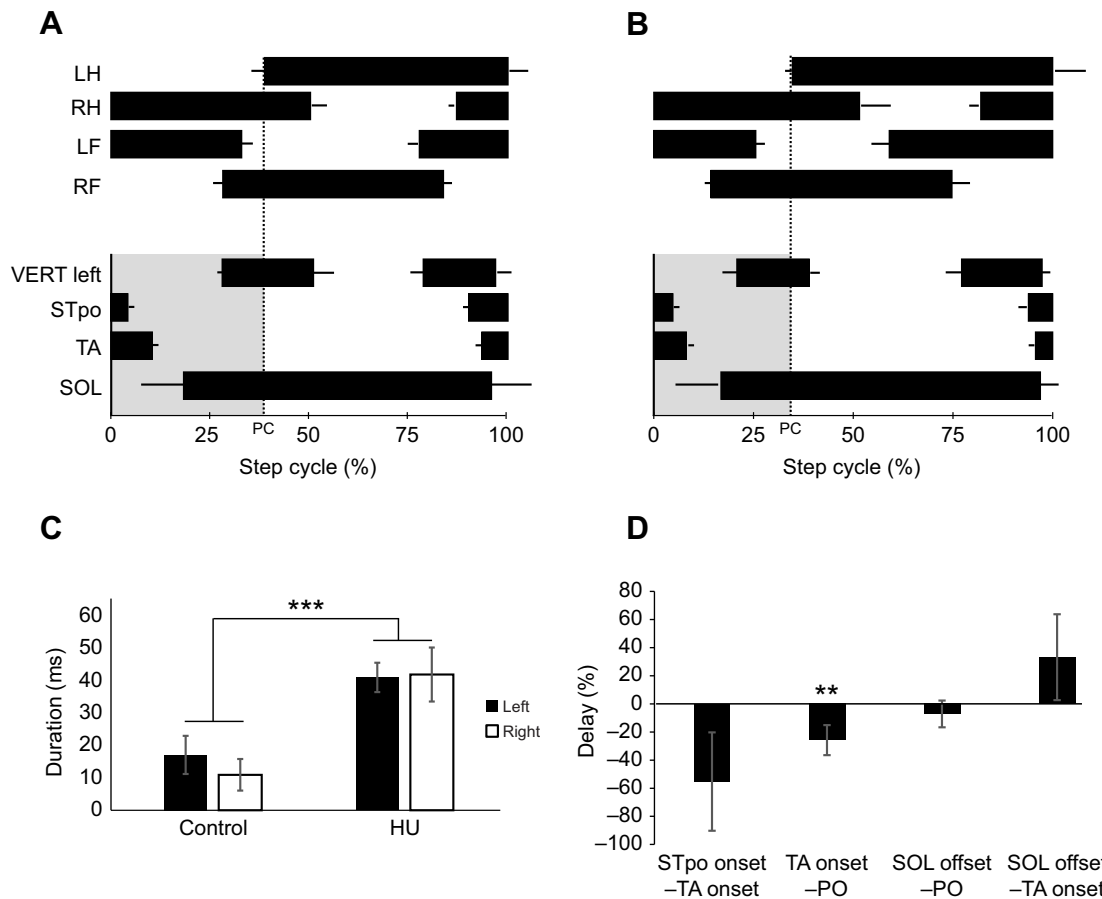
(Wada et al., 2006). Studies on cats (Carlson et al., 1979; English, 1980) and dogs (Ritter et al., 2001) have shown that activity of the caudal trunk muscles occurs before the start of hindlimb swing phase and therefore performs a stabilizing role. Our results for the VERT push-off activity are consistent with these studies (Carlson et al., 1979; English, 1980; Ritter et al., 2001): the major part of the VERT activity is observed before the onset of the hindlimb swing phase. Meanwhile, this activity remained till ipsilateral STpo activity (to a greater extent) and TA activity (to a lesser extent) began, both before and after HU; that is, till the onset of swing phase. This theoretically can initiate the formation of an ipsilateral pelvic lateral flexion at the push-off moment. In addition, according to Schilling and Carrier (2010), the trunk muscle activity in dogs, when walking and trotting, may still contribute to the formation of ipsilateral bending. Our results also point to another function of VERT activity: at the moment of paw contact of the ipsilateral



**Fig. 5. Effect of HU on ST activity.** A representative example of ST activity in two rats: (A) one which increased non-burst ST activity presumably in late swing; and (B) one which increased non-burst ST activity presumably in stance. The amplitude of the STpo burst is not entirely displayed in B because it was significantly greater than the non-burst activity. The gray area indicates the swing phase. PO, push-off; PC, paw contact.

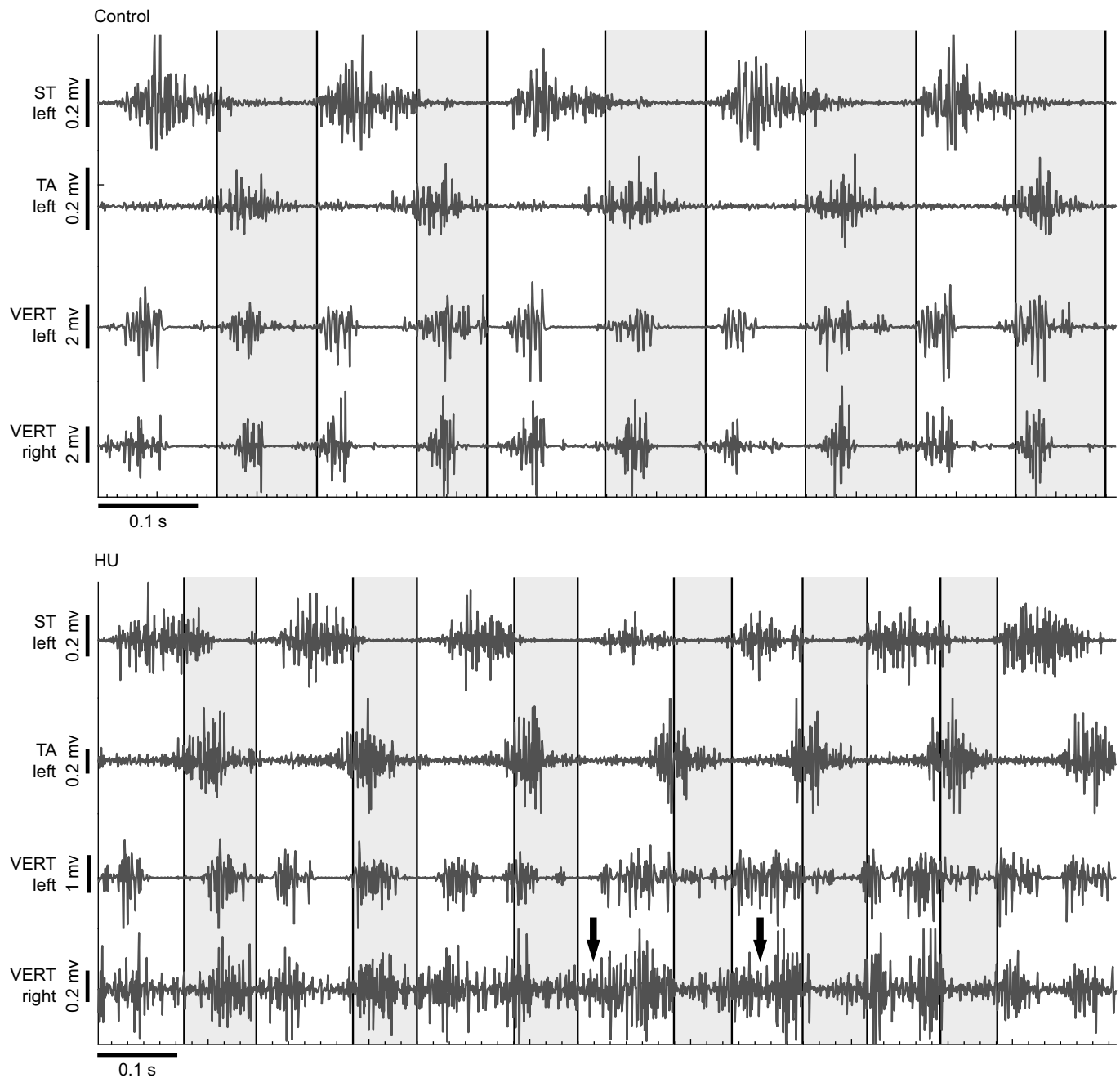
hindlimb, the CoM projection after the HU was shifted toward the supporting contralateral limb, which was in the second part of the stance phase. Wada et al. (2006) suggested that one possible function of the epaxial muscles is to counteract the formation of inertial lateral displacement of the trunk caused by limb movements.

The protractors of the swinging hindlimb and the retractors of the supporting hindlimb cause a horizontal moment on the pelvis that must be stabilized by unilateral epaxial muscle activity ipsilateral to the supporting hindlimb (Schilling and Carrier, 2009). Therefore, when the ipsilateral swinging hindlimb is at the moment of paw



**Fig. 6. Timing of muscle activity.** (A,B) Summary bar diagram of EMG activity in hindlimb muscles relative to the gait diagram of the step cycle, which was determined by video analysis before (A) and after HU (B) ( $n=6$ ). LH, left hindlimb; RH, right hindlimb; LF, left forelimb; RF, right forelimb. The gray area indicates the swing phase of the left hindlimb. (C) The histogram shows the duration of the unsupported state of the ipsilateral forelimbs and hindlimbs during the step cycle before and after HU ( $n=6$ ). (D) The histogram shows the effect of HU on the delay between the onset and offset of the burst activity of the hindlimb muscles (SOL  $n=6$ , TA  $n=5$ , ST  $n=5$ ). In this histogram, all parameters were normalized on a per rat basis to the initial values (means $\pm$ s.e.m.). \*\* $P<0.01$ , \*\*\* $P<0.001$ .





**Fig. 7. Effect of HU on swimming performance.** A representative example (one rat) of the activity patterns of extrinsic hindlimb muscles (ST, TA) and a trunk muscle (VERT) in the control condition (top) and after 7 days of HU (bottom) during swimming. The gray area indicates the protraction phase. After HU, VERT activity was still strictly co-active on the left and right sides but the double-burst pattern was somewhat changed. The black arrows indicate the one-burst pattern of VERT activity after HU. One rat (this example) had an unexplained decrease in overall activity on the right side. This change was not observed in the second rat.

contact, an increase in the contralateral VERT push-off activity can explain the increased shift of the CoM toward the contralateral hindlimb after HU.

The increasing locomotion speed requires an increase of sagittal stability (Schilling and Carrier, 2010; Schilling et al., 2009; Ritter et al., 2001). The co-activation is particularly characteristic of bilateral trunk muscle activity in the presacral region (Schilling and Carrier, 2010). The HU differentially influenced the activity of the VERT at push-off and paw contact, with an increase in VERT at push-off only (Figs 4A and 2). This typical asymmetric activity ratio was previously described in trotting versus walking dogs (Schilling

and Carrier, 2010; Ritter et al., 2001). A greater activity of the caudal back muscles is assumed to contribute to the stabilization of the pelvis in the horizontal (Schilling and Carrier, 2010) and sagittal (Ritter et al., 2001) planes and is required for the trotting gait because of the increased ipsilateral protractor and contralateral retractor activity of the lower extremities.

The alteration in the ST extensor (retractor) activity observed after HU is similar to the pattern of its activity in a trotting gait (see below). Increased extensor activity of the ST in the late swing phase, with a combined decrease in the E1 subphase duration after HU, seems to require increased activity of the VERT at push-off and

therefore stabilization of the CoM projection in the sagittal plane to the push-off of the ipsilateral limb. Increased VERT push-off activity after HU was almost synchronous with the onset of the increased ST extensor activity of the contralateral hindlimb in the E1 subphase. From this point of view, after HU, the change in activity of epaxial muscles, as well as the increased activity of the ST, resembles the trot-like or symmetrical running gait pattern.

After HU, all animals experienced an extended period of a simultaneous unsupported state of the ipsilateral forelimbs and hindlimbs in the late E1 subphase of the ipsilateral hindlimb. In that position, in the late E1 subphase, there is a risk of falling not only for the pelvis but also for the entire body toward the ipsilateral side, as mentioned by Schilling and Carrier (2010) for all quadrupedal animals. With the condition of reduced limb muscle strength after HU (Winiarski et al., 1987), this locomotor pattern seems unsuccessful for maintaining balance. Muscle recruitment was distinctly asymmetrical at T13 and L3 in walking dogs, pointing to a net lateral bending/torsional moment at these vertebral levels but a greater bilateral symmetry during trotting than during walking (Schilling and Carrier, 2010). This suggests a relatively greater extensor moment in the sagittal plane at all vertebral levels during trotting, but particularly at T13, which is nearest the center of the body mass. Conversely, in combination with the activation of epaxial muscles at the T13 region, which is possibly symmetrical during a walk–trot transition, this can create additional muscular effort on the contralateral side to counteract a trunk collapse in the direction of the simultaneously unsupported hindlimbs and forelimbs. This could also explain the shift in the CoM after HU and counteraction of the trunk collapse in the direction of the simultaneously unsupported hindlimbs and forelimbs. Thus, an increased time of the unsupported condition of the ipsilateral forelimbs and hindlimbs, the increased VERT push-off activity, and, possibly, symmetrical activation of the nearest T13 epaxial muscles could be a centrally programmed walk–trot (walk–symmetrical running) transition process. The walk gait is consistent with a traveling wave, while synchronized activity of epaxial muscles during trotting is consistent with a standing wave of the trunk bending (O'Reilly et al., 2000; Bennett et al., 2001; Schilling et al., 2009; Schilling and Carrier, 2010). As a hypothesis, if two patterns of activity of the epaxial muscles from different types of gait (i.e. walk and trot) exist at the same time after HU, the summation of the mechanical traveling and standing waves of the trunk movement could lead to an increase in the inertial lateral shift of the CoM, which is not found before HU.

### Swimming performance

We analyzed locomotor activity in the swimming of two rats before and after HU. The main feature that distinguishes locomotor performance in swimming after HU was the frequent synchronous protractor movement of the hindlimbs, similar to the gallop or bound gait. When swimming after HU, the pattern of epaxial muscle activity was much more difficult to determine, and the activity with these gallop (half-bound)-like movements was more like a single fused burst in a cycle, reciprocal to the TA activity. Note that the double-burst pattern of epaxial muscle activity was maintained during alternating hindlimb movements. Accordingly, the pattern of the single burst activity of the back muscles is defined in normal and decerebrated locomoting cats when switching to a gallop (English, 1980; Zomlefer et al., 1984). Similarly, in fictive cat locomotion, the activity of the back muscle motoneurons demonstrated only a single burst of activity (Koehler et al., 1984). Notably, Zomlefer et al. (1984) showed a typical pattern of a double-burst activity on a low-

spinalized cat only with appropriate foot contact. Therefore, after HU, the motor system seems adjusted toward a faster gait strategy. These changes are better manifested during swimming, where the support afferentation is reduced and the balance control is minimized in comparison with treadmill locomotion.

### Hindlimb muscle activity and time parameters of the step cycle after unloading

The results showed a tendency to increase the duration of the step cycle and the stance phase after HU. In previous work, Canu and Falempin (1996) also found that a speed of 20 cm s<sup>-1</sup> did not cause a significant increase in the duration of the step cycle. The pattern of movement of all four limbs after HU was changed. As noted above, all animals had a significantly extended period of simultaneous unsupported state of the ipsilateral forelimbs and hindlimbs in the late E1 subphase of the ipsilateral hindlimb. This phenomenon has also been described in rats after 14 days of HU (Canu and Garnier, 2009). Górska et al. (1999) showed a similarly increased duration of the simultaneous unsupported state of the ipsilateral limbs with an increase in walking speed up to 40 cm s<sup>-1</sup> (Górska et al., 1999, Fig. 4). However, in our work, a similarly increased duration of the simultaneous unsupported state after HU resulted in the same walking speed (20 cm s<sup>-1</sup>) in the rats as before HU. The duration of the stance phase after HU also demonstrated a tendency to increase, with an increase in the duty factor (Hildebrand, 1965), which cannot be a sign of an accelerated gait. These facts show a duality of changes that are the opposite of each other. On the one hand, the type of gait during stance phase shows signs of slowing down, while on the other hand, the type of gait during swing phase shows acceleration.

### Timing parameters and SOL activity

Previous studies indicate strong correlation between cycle duration, support duration (Halbertsma, 1983) and SOL duration (Canu and Falempin, 1996; Nicolopolus-Stourmaras and Iles, 1984). Canu and Falempin (1997) reported a significant increase in the SOL burst duration for some locomotor speeds after HU. In our work, the SOL burst duration had a tendency to increase.

The offset of SOL activity may be associated with a disturbance of the trunk muscle activity through load-dependent sensory input. The main role for regulating the duration of the stance phase belongs to the Golgi tendon organ triceps surae via the Ib affected fibers (Duyssens and Pearson, 1980; Pearson et al., 1992). The activity of these fibers depends on the strength of muscle contraction, particularly in the work to maintain body weight (ground reaction force). After HU, in the second part of the stance phase, the CoM is excessively shifted toward the supporting ipsilateral limb. Thus, the ground reaction force applied to this one limb is increased and could prolong the excitatory force feedback from tendon organs of the triceps surae, thereby delaying the limb lift off. As a result, the duration of the stance phase and the duration of SOL activity increase, and the hindlimb joints are in hyperextension at push-off, as described earlier (Canu and Falempin, 1996; Canu et al., 2005; Tajino et al., 2015; Popov et al., 2019). Thus, we assume that changes in the work of the epaxial muscles can directly affect the duration of the stance phase and, accordingly, the duration of SOL activity. In addition, changes in the activity of the Golgi tendon organ (Treffort et al., 2005) may enhance the mechanism described above.

### TA and ST activity

The TA and ST changed their activity after HU. The duration of the STpo burst was slightly but significantly reduced, while the

maximum amplitude and mean EMG were dramatically and significantly increased after HU. Absolutely similar changes were observed in the TA after HU, but with a lower degree of severity. We assume that the similar changes in the two in-phase flexors, the STpo and TA, after HU may have a single mechanism. Changes in mean EMG muscle activity occur when the task or conditions of locomotion are altered (e.g. during the walk–trot–gallop transition or with inclined surface locomotion) (Engberg and Lundberg, 1969; Hutchison et al., 1989; Smith et al., 1993; Carlson-Kuhta et al., 1998; Schilling and Carrier, 2009; Deban et al., 2012). Stepping cats (fig. 4 of Engberg and Lundberg, 1969) do not increase their STpo activity when changing their gait from walking to a trot. However, when locomotion occurs with an upwards-sloping surface, both cats (Carlson-Kuhta et al., 1998) and dogs (Schilling and Carrier, 2009) increase the STpo activity significantly. From this point of view, the increased STpo activity in rats after HU is similar to walking on an upwards-sloping surface. The TA activity in cats (fig. 6 of Pierotti et al., 1989) and rats (fig. 6 of Roy et al., 1991) tends to increase, but to a lesser extent, when the speed is increased. The mean EMG TA also shows no dependence on the speed of movement after HU (Canu and Falempin, 1997). By contrast, when the upwards slope is increased, the mean EMG TA increases proportionally (Carlson-Kuhta et al., 1998), which is highly likely to be similar to changes after HU. When walking on an inclined surface, the TA burst duration and the step cycle duration ratio also decrease, which also agrees with our results after HU.

ST activity changes typical of a high velocity gait pattern were observed in the E1 subphase and at paw contact after HU. The ST maximum amplitude and the mean EMG increased significantly in the E1 subphase and at paw contact. Walking rats, as non-cursorial mammals (in contrast to cursorial cats), show ST extensor activity just after paw contact in the stance phase but without clearly defined burst boundaries (Nicolopoulos-Stournaras and Iles, 1984), and when walking at low speed, this activity is absent (Gruner and Altman, 1980). Before HU, we observed this activity, but at a very low level. A pronounced increase in ST activity in the E1 subphase when gait changes from walking to trot has been described in cats (Engberg and Lundberg, 1969; Hutchison et al., 1989; Smith et al., 1993) and dogs (Schilling et al., 2009; Deban et al., 2011). Retractor (extensor) ST activity when trotting in subphase E1 requires a deceleration of the more rapid forward swing of the limb in trot (Engberg and Lundberg, 1969). When cats walk on an inclined surface, ST activity also increases in the stance phase (Carlson-Kuhta et al., 1998), which may partially correspond to our results in rats after HU. However, after HU, the ST activity was observed exactly before paw contact, and in some cases it resembled a burst-like activity, which more likely indicates a change in activity specifically when changing the gait.

In addition, the decrease in the swing phase duration during the walk–trot transition and the dependence of ST activity in the E1 subphase on the velocity of walk and trot (Wisleder et al., 1990) are consistent with our results. Thus, the assumption can be made that, strictly during the swing phase, when the support afferentation stops, the nervous system begins to reproduce a pattern similar to a running walk or trotting, and just after paw contact, this pattern is interrupted by the appearance of load-dependent input.

Notably, when their gait changes from walking to a trot, non-cursorial rats show a similar increase in the knee joint angle at paw contact (Gillis and Biewener, 2001) to hyperextension of the knee joint angle at paw contact after HU (Tajino et al., 2015; Popov et al., 2019). Taken together, the changes in the activity of the epaxial trunk and hindlimb muscles after HU show that, at swing phase and

without load-dependent input, the locomotor pattern becomes very similar to that of an accelerated gait, which may reflect changes in supraspinal control after the absence of a load-dependent input. The changes observed in flexor activity in the stance phase, when the support afferentation is activated, are very similar to those seen when walking on an inclined surface and are thus directly related to the load-dependent input and its intensity and duration. When walking on an upward-sloping surface, the ground reaction force applied to the limb in the late stance phase increases, which could prolong the lb afferentation and increase the stance phase duration. Hence, an increased stance phase and the activity of the load afferents can produce the effect of an upward-sloping surface, thereby changing the flexor activity prior to swing phase.

## Conclusions

In summary, we have shown a relationship between the mechanisms of posture and locomotion. We cannot exclude vestibulospinal influences or the occurrence of altered feedback from the axial muscles of the thoracic region, which is in hyperextension as a result of the change in the spine configuration during HU. For example, a head tilt in decerebrated cats is known to alter the pattern of the hindlimb muscle activity for a short time during locomotion (Gottschall and Nichols, 2007). However, similarities are apparent between the morphological and biochemical changes in muscles (Winiarski et al., 1987), as well as in the changes in EMG activity (Alford et al., 1987) after a modified body configuration (HU), after conditions such as changes in gravity during parabolic flight or space flight (Kawano et al., 2002), or, conversely, when gravity increases in a centrifuge (Tajino et al., 2015). All these changes allow us to assume that the lack of load-dependent input during HU presumably leads to a reorganization of the posture–locomotor systems.

The patterns of muscle activity obtained during locomotion after HU in the stance and swing phase change differently, which may indicate independent nervous control of one muscle by different sources of activity, as previously suggested (Wetzel, 1981). One of these, which may have a supraspinal origin, formed a locomotor pattern of accelerated movement during the 7 day absence of load-dependent input. Its influence was manifested in the muscle activity in the E1 subphase and at paw contact. Another one immediately reacted to the arising load-dependent input after paw contact and thus controlled locomotor activity during stance phase. Locomotor changes that lack a requirement for maintaining body balance when swimming indicated that this control depends on the degree of load-dependent input. Thus, we assume that the nervous system may possess a certain pattern of muscle activity, with simultaneous inhibition of its manifestation by load-dependent input. From this point of view, the standing position could also block the manifestation of various muscle activity patterns.

The loading influenced the locomotor pattern during stance phase after paw contact, and this influence was related to the ground reaction force and to the changes in trunk muscle activity, which was part of the new locomotion strategy after HU. However, the pattern of accelerated gait suggested by the nervous system in the abnormal conditions of muscle atrophy and the reduced strength and speed of muscle contraction of the hindlimbs after HU cannot manifest in the stance phase. Thus, from this point of view, the treadmill locomotion after HU represents a constant attempt by the rats to switch to a faster gait, with a failure to perform this task by the musculoskeletal system, possibly due to altered receptor activity.

Finally, we suggest that the mechanisms of a postural control during movements may be based on various locomotor strategies



(walk, trot, gallop, etc.) which were formed by evolution during the transition from water to land in the earliest tetrapods. Under the conditions of the new Earth gravity environment and the requirements to support a body above the surface, the possibility of the occurrence of individual adaptive mutations into the already formed programming mechanisms of locomotion seems more logical than the occurrence of a new complex system of postural control.

#### Acknowledgements

We thank Olga Ptitsyna for the design and layout of figures.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: A.P., P.M.; Methodology: A.P., V.L., E.B., O.G., D.K., P.M.; Software: V.L., O.G.; Validation: A.P., V.L.; Formal analysis: A.P., V.L.; Investigation: A.P., E.B., O.G., D.K., N.M., P.M.; Resources: P.M.; Data curation: V.L.; Writing - original draft: A.P., P.M.; Writing - review & editing: V.L., N.M., P.M.; Supervision: V.L., P.M.; Project administration: P.M.; Funding acquisition: P.M.

#### Funding

This work was performed within project ID: 73025408 of St Petersburg State University, St Petersburg, Russia (for N.M., E.B. and O.G.), supported by the Russian Foundation for Basic Research grant no. 17-29-01034\_ofi\_m (for electrophysiological testing) and grant no. 20-015-00568 (for data analysis).

#### References

- Aarts, E., Verhage, M., Veenliet, J. V., Dolan, C. V. and Van Der Sluis, S.** (2014). A solution to dependency: using multilevel analysis to accommodate nested data. *Nat. Neurosci.* **17**, 491-496. doi:10.1038/nn.3648
- Alford, E. K., Roy, R. R., Hodgson, J. A. and Edgerton, V. R.** (1987). Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. *Exp. Neurol.* **96**, 635-649. doi:10.1016/0014-4886(87)90225-1
- Bennett, W. O., Simons, R. S. and Brainerd, E. L.** (2001). Twisting and bending: the functional role of salamander lateral hypaxial musculature during locomotion. *J. Exp. Biol.* **204**, 1979-1989. doi:10.1242/jeb.204.11.1979
- Canu, M. H. and Falempin, M.** (1996). Effect of hindlimb unloading on locomotor strategy during treadmill locomotion in the rat. *Eur. J. Appl. Physiol. Occup. Physiol.* **74**, 297-304. doi:10.1007/BF02226924
- Canu, M. H. and Falempin, M.** (1997). Effect of hindlimb unloading on two hindlimb muscles during treadmill locomotion in rats. *Eur. J. Appl. Physiol. Occup. Physiol.* **75**, 283-288. doi:10.1007/s004210050162
- Canu, M.-H. and Falempin, M.** (1998). Effect of hindlimb unloading on interlimb coordination during treadmill locomotion in the rat. *Eur. J. Appl. Physiol. Occup. Physiol.* **78**, 509-515. doi:10.1007/s004210050453
- Canu, M.-H. and Garnier, C.** (2009). A 3D analysis of fore- and hindlimb motion during overground and ladder walking: comparison of control and unloaded rats. *Exp. Neurol.* **218**, 98-108. doi:10.1016/j.expneurol.2009.04.009
- Canu, M.-H., Garnier, C., Lepoutre, F.-X. and Falempin, M.** (2005). A 3D analysis of hindlimb motion during treadmill locomotion in rats after a 14-day episode of simulated microgravity. *Behav. Brain Res.* **157**, 309-321. doi:10.1016/j.bbr.2004.07.009
- Carlson, H., Halbertsma, J. and Zomlefer, M.** (1979). Control of the trunk during walking in the cat. *Acta Physiol. Scand.* **105**, 251-253. doi:10.1111/j.1748-1716.1979.tb06338.x
- Carlson-Kuhta, P., Trank, T. V. and Smith, J. L.** (1998). Forms of forward quadrupedal locomotion. II. A comparison of posture, hindlimb kinematics, and motor patterns for upslope and level walking. *J. Neurophysiol.* **79**, 1687-1701. doi:10.1152/jn.1998.79.4.1687
- Deban, S. M., Schilling, N. and Carrier, D. R.** (2012). Activity of extrinsic limb muscles in dogs at walk, trot and gallop. *J. Exp. Biol.* **215**, 287-300. doi:10.1242/jeb.063230
- Deligiagina, T. G., Beloozerova, I. N., Popova, L. B., Sirota, M. G., Swadlow, H. A., Grant, G. and Orlovsky, G. N.** (2000). Role of different sensory inputs for maintenance of body posture in sitting rat and rabbit. *Motor Control.* **4**, 439-452. doi:10.1123/mcj.4.4.439
- Delvolvé, I., Bem, T. and Cabelguen, J.-M.** (1997). Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt, *Pleurodeles walli*. *J. Neurophysiol.* **78**, 638-650. doi:10.1152/jn.1997.78.2.638
- Duysens, J. and Pearson, K. G.** (1980). Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* **187**, 321-332. doi:10.1016/0006-8993(80)90206-1
- Duysens, J., Clarac, F. and Cruse, H.** (2000). Load-regulating mechanisms in gait and posture: comparative aspects. *Physiol. Rev.* **80**, 83-133. doi:10.1152/physrev.2000.80.1.83
- Engberg, I. and Lundberg, A.** (1969). An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta Physiol. Scand.* **75**, 614-630. doi:10.1111/j.1748-1716.1969.tb04415.x
- English, A. W.** (1980). The functions of the lumbar spine during stepping in the cat. *J. Morphol.* **165**, 55-66. doi:10.1002/jmor.1051650106
- English, A. W. M. and Weeks, O. I.** (1987). An anatomical and functional analysis of cat biceps femoris and semitendinosus muscles. *J. Morphol.* **191**, 161-175. doi:10.1002/jmor.1051910207
- Faul, F., Erdfelder, E., Lang, A.-G. and Buchner, A.** (2007). G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**, 175-191. doi:10.3758/BF03193146
- Frolich, L. M. and Biewener, A. A.** (1992). Kinematic and electromyographic analysis of the functional role of the body axis during Terrestrial and Aquatic Locomotion in the Salamander *Ambystoma Tigrinum*. *J. Exp. Biol.* **162**, 107-130. doi:10.1242/jeb.162.1.107
- Gillis, G. B. and Biewener, A. A.** (2001). Hindlimb muscle function in relation to speed and gait: *in vivo* patterns of strain and activation in a hip and knee extensor of the rat (*Rattus norvegicus*). *J. Exp. Biol.* **204**, 2717-2731. doi:10.1242/jeb.204.15.2717
- Górska, T., Zmysłowski, W. and Majczyński, H.** (1999). Overground locomotion in intact rats: interlimb coordination, support patterns and support phases duration. *Acta Neurobiol. Exp. (Wars)* **59**, 131-144.
- Gottschall, J. S. and Nichols, T. R.** (2007). Head pitch affects muscle activity in the decerebrate cat hindlimb during walking. *Exp. Brain Res.* **182**, 131-135. doi:10.1007/s00221-007-1084-z
- Grillner, S.** (1985). Neurobiological bases of rhythmic motor acts in vertebrates. *Science* **228**, 143-149. doi:10.1126/science.3975635
- Grillner, S. and Jessell, T. M.** (2009). Measured motion: searching for simplicity in spinal locomotor networks. *Curr. Opin. Neurobiol.* **19**, 572-586. doi:10.1016/j.conb.2009.10.011
- Gruner, J. A. and Altman, J.** (1980). Swimming in the rat: analysis of locomotor performance in comparison to stepping. *Exp. Brain Res.* **40**, 374-382. doi:10.1007/BF00236146
- Halbertsma, J. M.** (1983). The stride cycle of the cat: the modelling of locomotion by computerized analysis of automatic recordings. *Acta Physiol. Scand. Suppl.* **521**, 1-75.
- Hildebrand, M.** (1965). Symmetrical gaits of horses. *Science* **150**, 701-708. doi:10.1126/science.150.3697.701
- Hutchison, D. L., Roy, R. R., Bodine-Fowler, S., Hodgson, J. A. and Edgerton, V. R.** (1989). Electromyographic (EMG) amplitude patterns in the proximal and distal compartments of the cat semitendinosus during various motor tasks. *Brain Res.* **479**, 56-64. doi:10.1016/0006-8993(89)91335-8
- Kawano, F., Nomura, T., Ishihara, A., Nonaka, I. and Ohira, Y.** (2002). Afferent input-associated reduction of muscle activity in microgravity environment. *Neuroscience* **114**, 1133-1138. doi:10.1016/S0306-4522(02)00304-4
- Kawano, F., Ishihara, A., Stevens, J. L., Wang, X. D., Ohshima, S., Horisaka, M., Maeda, Y., Nonaka, I. and Ohira, Y.** (2004). Tension- and afferent input-associated responses of neuromuscular system of rats to hindlimb unloading and/or tenotomy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R76-R86. doi:10.1152/ajpregu.00694.2003
- Koehler, W. J., Schomburg, E. D. and Steffens, H.** (1984). Phasic modulation of trunk muscle efferents during fictive spinal locomotion in cats. *J. Physiol.* **353**, 187-197. doi:10.1113/jphysiol.1984.sp015331
- Morey-Holton, E. R. and Globus, R. K.** (2002). Hindlimb unloading rodent model: technical aspects. *J. Appl. Physiol.* **92**, 1367-1377. doi:10.1152/jappphysiol.00969.2001
- Musienko, P., van den Brand, R., Märzendorfer, O., Roy, R. R., Gerasimenko, Y., Edgerton, V. R. and Courtine, G.** (2011). Controlling specific locomotor behaviors through multidimensional monoaminergic modulation of spinal circuitries. *J. Neurosci.* **31**, 9264-9278. doi:10.1523/JNEUROSCI.5796-10.2011
- Nicolopoulos-Stournaras, S. and Iles, S. F.** (1984). Hindlimb muscle activity during locomotion in the rat (*Rattus norvegicus*) (Rodentia:Muridae). *J. Zool.* **203**, 427-440. doi:10.1111/j.1469-7998.1984.tb02342.x
- O'Reilly, J. C., Summers, A. P. and Ritter, D. A.** (2000). Evolution of the functional role of trunk muscles during locomotion in adult amphibians. *Am. Zool.* **40**, 123-135. doi:10.1093/icb/40.1.123
- Pearson, K. G., Ramirez, J. M. and Jiang, W.** (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp. Brain Res.* **90**, 557-566. doi:10.1007/BF00230939
- Philippson, M.** (1905). *L'Autonomie et la Centralisation dans le Systéme Nerveux des Animaux*. Trav Lab Physio Inst Solvay (Bruxelles), **7**, 1-208.
- Pierotti, D. J., Roy, R. R., Gregor, R. J. and Edgerton, V. R.** (1989). Electromyographic activity of cat hindlimb flexors and extensors during locomotion at varying speeds and inclines. *Brain Res.* **481**, 57-66. doi:10.1016/0006-8993(89)90485-X

- Popov, A. A., Lyakhovetskii, V. A., Merkulyeva, N. S., Gorski, O. V., Bazhenova, E. J. and Musienko, P. E.** (2019). Kinematics of rat locomotor movements during 7-day hindlimb suspension. *Russian J. Physiol.* **105**, 447-455.
- Ritter, D.** (1996). Axial muscle function during lizard locomotion. *J. Exp. Biol.* **199**, 2499-2510. doi:10.1242/jeb.199.11.2499
- Ritter, D. A., Nassar, P. N., Fife, M. and Carrier, D. R.** (2001). Epaxial muscle function in trotting dogs. *J. Exp. Biol.* **204**, 3053-3064. doi:10.1242/jeb.204.17.3053
- Roy, R. R., Hutchison, D. L., Pierotti, D. J., Hodgson, J. A. and Edgerton, V. R.** (1991). EMG patterns of rat ankle extensors and flexors during treadmill locomotion and swimming. *J. Appl. Physiol.* **70**, 2522-2529. doi:10.1152/jappl.1991.70.6.2522
- Schilling, N. and Carrier, D. R.** (2009). Function of the epaxial muscles during trotting. *J. Exp. Biol.* **212**, 1053-1063. doi:10.1242/jeb.020248
- Schilling, N. and Carrier, D. R.** (2010). Function of the epaxial muscles in walking, trotting and galloping dogs: implications for the evolution of epaxial muscle function in tetrapods. *J. Exp. Biol.* **213**:1490-1502. doi:10.1242/jeb.039487
- Schilling, N., Fischbein, T., Yang, E. P. and Carrier, D. R.** (2009). Function of the extrinsic hindlimb muscles in trotting dogs. *J. Exp. Biol.* **212**, 1036-1052. doi:10.1242/jeb.020255
- Smith, J. L., Chung, S. H. and Zernicke, R. F.** (1993). Gait-related motor patterns and hindlimb kinetics for the cat trot and gallop. *Exp. Brain Res.* **94**, 308-322. doi:10.1007/BF00230301
- Tajino, J., Ito, A., Nagai, M., Zhang, X., Yamaguchi, S., Iijima, H., Aoyama, T. and Kuroki, H.** (2015). Intermittent application of hypergravity by centrifugation attenuates disruption of rat gait induced by 2 weeks of simulated microgravity. *Behav. Brain Res.* **287**, 276-284. doi:10.1016/j.bbr.2015.03.030
- Treffort, N., Picquet, F., Petit, J. and Falempin, M.** (2005). The structure and response properties of golgi tendon organs in control and hypodynamia-hypokinesia rats. *Exp. Neurol.* **195**, 313-321. doi:10.1016/j.expneurol.2005.05.010
- Wada, N., Akatani, J., Miyajima, N., Shimojo, K. and Kanda, K.** (2006). The role of vertebral column muscles in level versus upslope treadmill walking-an electromyographic and kinematic study. *Brain Res.* **1090**, 99-109. doi:10.1016/j.brainres.2006.01.132
- Wetzel, M. C.** (1981). Independently controlled EMG responses in treadmill locomotion by cats. *Am. J. Phys. Med.* **60**, 292-310.
- Winiarski, A. M., Roy, R. R., Alford, E. K., Chiang, P. C. and Edgerton, V. R.** (1987). Mechanical properties of rat skeletal muscle after hind limb suspension. *Exp. Neurol.* **96**, 650-660. doi:10.1016/0014-4886(87)90226-3
- Wisleder, D., Zernicke, R. F. and Smith, J. L.** (1990). Speed-related changes in hindlimb intersegmental dynamics during the swing phase of cat locomotion. *Exp. Brain Res.* **79**, 651-660. doi:10.1007/BF00229333
- Zomlefer, M. R., Provencher, J., Blanchette, G. and Rossignol, S.** (1984). Electromyographic study of lumbar back muscles during locomotion in acute high decerebrate and in low spinal cats. *Brain Res.* **290**, 249-260. doi:10.1016/0006-8993(84)90942-9