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ANALYSIS OF BASIC MOTOR BEHAVIORS IN QUADRUPEDS

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On the cover: Original painting of rabbit performing single corrective steps in different directions and mouse locomoting forward with its fore and hindlimbs moving in alternation.
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Analysis of basic motor behaviors in quadrupeds

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"Call it order, call it chaos, it's all in the brain."

— Mission Reality

ABSTRACT

Ability to perform locomotion in different directions and maintain upright body posture is crucial for normal life. At present, mice, which allows employing genetic approaches, are widely used in studying the locomotor system. In these investigations different experimental setups are used to evoke locomotion. *First aim of the present study was to compare kinematics of forward (FW) and backward (BW) locomotion performed in different environmental conditions* (i.e. in a tunnel, on a treadmill and on an air-ball). On all set-ups, average speed, step amplitude and swing duration during BW locomotion were significantly smaller compared to those observed during FW locomotion. The extent of rostro-caudal paw trajectory in relation to the hip projection to the surface (HP) strongly depended on hip height. With high hip height, the trajectory was symmetrical in relation to HP (middle steps). When hip was low, steps were either displaced rostrally (anterior steps) or caudally (posterior steps) in relation to HP. During FW locomotion, predominantly anterior and posterior steps were observed, respectively, on the treadmill and air-ball, while all three stepping forms were observed in the tunnel. We observed only anterior steps during BW locomotion. Intralimb coordination depended on the form of stepping.

Second aim of the present study was to reveal the role of two populations of commissural interneurons ($V0_V$ and $V0_D$ CINs) in control of a number of basic motor behaviours (BW locomotion, scratching, righting, and postural corrections). For this purpose two types of knockout mice (*Vglut2*^{Cre};*Dbx1*^{DTA} mice and *Hoxb8*^{Cre};*Dbx1*^{DTA} mice with only $V0_V$ and all V0 CINs ablated, respectively) as well as wild-type littermates were used. Our results suggest that the functional effect of excitatory $V0_V$ CINs during BW locomotion and scratching is inhibitory, and that execution of scratching involves active inhibition of the contralateral scratching CPG mediated by $V0_V$ CINs. By contrast, $V0_D$ CINs are elements of spinal postural network, generating postural corrections. Finally, both $V0_D$ and $V0_V$ CINs contribute to generation of righting behavior. Thus, our study shows the differential contribution of V0 neuron subpopulations in generation of diverse motor acts.

Single steps in different directions are used for control of balance or body configuration. However, our knowledge about neural mechanisms responsible for their generation is limited. *The third aim of the present study was to characterize postural response to disturbance of basic body configuration caused by forward, backward or outward displacement of the hindlimb.* In intact rabbits, displacement of the hindlimb in any direction caused a postural response consisting of two components. First, a lateral trunk movement towards the supporting (contralateral) hindlimb was performed, and then a corrective step in the direction opposite to the direction of the initial limb displacement was executed. These two components were generated by different mechanisms activated in a strict order by sensory information from the deviated limb signalling distortion of the limb/limb-trunk configuration. We have shown that the integrity of the forebrain was not critical for generation of this postural response. We proposed a hypothesis about operation of mechanisms generating the postural response characterized in the present study.

Key words: backward locomotion, forward locomotion, scratching, righting, postural corrections, corrective steps, commissural neurons, kinematics, rabbit, mouse.

LIST OF SCIENTIFIC PAPERS

This thesis is based on following papers and manuscripts

I. **Vemula M.G**., Deliagina T.G., Zelenin P.V. (2019). Kinematics of forward and backward locomotion performed in different environmental conditions. *J Neurophysiol*. 122:2142-2155.

II. Zelenin P.V*., **Vemula M.G***., Lyalka V.F., Talpalar A.E., Kiehn O., Deliagina T.G. Role of V0 commissural interneurons in control of basic motor behaviors (*Manuscript*).
***Authors with equal contribution**

III. Hsu L.J., Zelenin P.V., Lyalka V.F., **Vemula M.G**., Orlovsky G.N., Deliagina T.G. (2017) Neural mechanisms of single corrective steps evoked in the standing rabbit. *Neuroscience* 347:85-102.

CONTENTS

LIST OF ABBREVIATIONS

FW	Forward locomotion
BW	Backward locomotion
CINs	Commissural interneurons
HP	Hip projection
INs	Interneurons
CNS	Central nervous system
CS	Corticospinal
RS	Reticulospinal
VS	Vestibulospinal
RbS	Rubrospinal
MLR	Mesencephalic locomotor region
CnF	Cuneiform nucleus
PPN	Pedunculopontine
GABA	Gamma-aminobutyric acid
Vglu2	Vesicular glutamate transporter 2
$V0_V$	V0 CINs ventral
$V0_D$	V0 CINs dorsal
Vast	Vastus lateralis
Gast	Gastrocnemius lateralis
Bic	Biceps femoris
Tib	Tibialis anterior
OEA	Oblique externus abdominis
VC	Vertical component
HC	Horizontal component
ES	Electrical stimulation
TM	Treadmill

INTRODUCTION

NEURAL MECHANISMS OF RYTHMIC MOVEMENTS

Locomotion in different directions

''You know the one—its Neil Armstrong's boot print on the surface of the moon. A giant leap, he called it. Arguably just as "giant," though a touch more obscure. It was discovered in a dark slab of rock that hangs on the edge of the North Atlantic in a remote corner of Newfoundland … It's an impression left by another Earthling, an odd-looking ocean dweller that lived roughly 565 million years ago and that was maybe the first creature, certainly the first we know of, to use its own muscles to move from where it was to someplace new'' (Robert Krulwich, National Geographic).

The curiosity to understand the neural mechanisms underlying generation of locomotion dates back to the times of Sherrington in 1883. It was him that first suggested that the nervous system was not, as previously thought, composed of a single interlinked network. The imperative knowledge of his studies comes from experiments on cat. He suggested that locomotion, is a result of a proprioceptor-mediated sequence of movements with reciprocal inhibition of antagonistic musculature, resulting in the generic left-right alternation seen during stepping (Sherrington, 1906). During the next decade Brown G.T demonstrated that sensory inputs during locomotion only play a trivial role, and animals devoid of sensory information do not lose the ability to perform locomotion. It is him that suggested that locomotor-related neuronal circuitry for hindlimbs resides in the lumbosacral spinal cord. The seminal work of Sherrington and Brown laid the groundwork for modern day field of motor control.

Without the ability of an animal or human to displace from one place to another, there would be no life on earth and locomotion has been the driving force of evolution. Therefore, moving around is essential and a large number of species move around using their limbs and the limbs are in turn moved by muscles. How quadrupedals and bipedals move may seem to be a straightforward question, but it emerges from a complex interaction between the nervous system and its passive body dynamics. Although it might not be spontaneous and effortless (biomechanical, energy expenditure reasons), most terrestrial animals are able to walk backwards and sideways if they want to, along with primary form of progression i.e. forward locomotion. The ability to maneuver in different directions helps animals and people to escape out of tight corners and protect them in situations when they feel threatened. I.e. sequential stepping in directions deviated from FW locomotion is performed in context of avoidance behavior.

The crucial components of locomotion include progression, maintaining stability, and adaptation. One must have the strength and control necessary to progress towards a particular location, sufficient dynamic balance to maintain its posture, to overcome the gravitational force or other external forces, and the ability to adjust the locomotor pattern to meet the needs and demands of the environment. Detailed animal studies, confirmed that the fundamental circuitry generating locomotion are localized in the spinal cord; they are initiated and modulated by supraspinal drive through various descending tracts (Grillner, 2011; Garcia-Rill and Skinner, 1987; Lemon, 2008).

Comparison of kinematics and motor pattern during forward and backward locomotion

Structure of the locomotor cycle

During BW locomotion the direction of the limb movement in the locomotor cycle is opposite to that observed during FW locomotion. Thus, during FW locomotion, the limb moves from anterior to posterior position (in relation to the trunk) during stance and from posterior to anterior position during swing, while during BW locomotion - from posterior to anterior position during stance and from anterior to posterior position during swing. In humans, during BW locomotion the limbs travel opposite direction along virtually the same path as during FW locomotion (Thorstensson, 1986). However, in terrestrial quadrupeds the path is different due to difference in body configuration with which FW and BW stepping is performed (Buford et al., 1990; Herbin et al., 2004). It was reported that in humans, the average speed of progression during BW locomotion is similar (Thorstensson, 1986) or lower than that during FW locomotion (Katsavelis et al., 2010). In terrestrial quadrupeds (cats and rats) the speed of progression is lower during BW locomotion compared to that during FW locomotion (Buford et al., 1990). In human adults, infants, as well as in cats the swing/stance proportions in the locomotor cycle are the same during FW and BW locomotion (Lamb et al., 2000; Nilsson et al., 1985; Buford et al., 1990), but at the same locomotor speed absolute swing and stance duration are shorter during BW locomotion than those during FW locomotion (Vilensky et al., 1987; Buford et al., 1990). In both humans and cats, regardless of direction of locomotion, with an increase in locomotor speed, the duration of swing phase remains relatively constant, whereas the duration of stance phase and stride length decreases (Leblond et al., 2003; Frigon et al., 2015; Nilsson et al., 1985).

Interlimb coordination

Human’s exhibit left-right alternation during FW and BW locomotion. In humans two main gaits, walk and run are observed. Walk is characterised by periods of double-support and executed during low speed locomotion, while during run only one of the feet is in contact with the support. This interlimb coordination is characteristic for high speed locomotion. Run could be further subdivided into jog (run at low steady speed) and sprint (run at maximal speed characterised by periods without support when two legs do not touch the ground) (Van deursen et al., 1998; Grasso et al., 1998; Ackermann et al., 2012). It was found that, interlimb coordination observed during FW locomotion is largely preserved during BW locomotion in primary school-aged children and adults (Pieter Meyns et al., 2013). The gait of bipedal runners and walkers is simple, while quadrupedal animals have more complex and varied gaits (Fig. 1). During FW locomotion, cats, rats and mice have used three different patterns of interlimb coordination: walk at slow speeds (three legs are in contact with surface at all times), trot at moderate speeds (two diagonal legs move synchronously and movements of opposite diagonal limbs alternate) and gallop at fastest speeds (limbs within each girdle move almost synchronously and movements of forelimbs and hind limbs alternate). Mice also exhibits bound at fastest speeds (all feet are off the ground together in each stride, limbs within each girdle move synchronously and movements of forelimbs and hind limbs alternate) (Bellardita et al., 2015). The difference between gallop and bound is that the contralateral limbs of both pairs contact the ground at different times. At medium speeds of locomotion, some animals (e.g. horses) also use pace (two legs on the same side move synchronously and movements of the left and right limbs alternate) (Fig. 1) (Engberg and Lundberg, 1969; Halbertsma, 1983). During walking the following sequence of paw landings are generally observed: right hindlimb, right forelimb, left hindlimb and left forelimb. This pattern is known as diagonal walk. Along with this, a reverse pattern of that seen during diagonal walk is also observed in all studied quadrupeds. Such interlimb coordination is called lateral walk

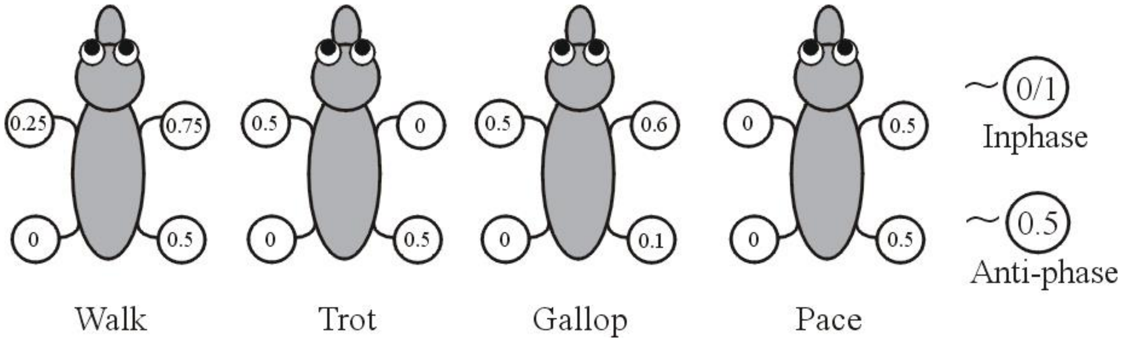

Figure 1. Four different gaits: the numbers in the feet of the animal show when a particular foot hits the ground, as a fraction of a complete cycle. Left hindlimb is the reference limb and the locomotor cycle is defined as beginning of stance to the next stance.

(Grillner et al., 1981; Buford et al., 1990; Lemieux et al., 2016; Eilam et al., 1992). Gaits are also generally classified as symmetrical and asymmetrical based on limb movement. In a symmetrical gait, the left and right limbs of a pair alternate, while in an asymmetrical gait, the limbs move together. Diagonal walk, lateral walk, trot and pace are considered as symmetrical gaits, while gallop and bound is considered as asymmetrical gaits (Thota et al., 2005; Kloos et al., 2005; Leblond et al., 2003; Eilam et al., 1992).

It was found, in humans (adults and infants) and cats that during FW locomotion there is a strong relationship between speed of locomotion and consistency of inter limb coordination, i.e. steps were less consistent at the slowest speeds compared with faster speeds of locomotion (Krasovsky et al., 2014; Frigon et al., 2015; Buford et al., 1990).

Among terrestrial quadrupeds, interlimb coordination during BW locomotion was analysed in cats and mole rats. It was found that during BW locomotion their gait corresponds to lateral walk (Buford et al., 1990; Eilam and Shefer, 1992).

It was suggested that in bipeds, a sequence of movements of limbs during BW locomotion is a simple reversal of that observed during FW locomotion. This meant that inter-limb coupling remained invariant in relation to the direction of locomotion (Eilam and Shefer., 1992; Miller et al., 1975).

Intralimb coordination

Intra-limb coordination during FW and BW locomotion was studied in humans and cats. It was found that in humans, the waveforms of vertical displacement of the thigh, shank and foot segments during BW locomotor cycle are almost the mirror image of those observed during FW cycle (Grasso et al., 1998). In both cats and humans, the hindlimb position and joint angles at the rostral extreme position are similar during FW and BW locomotion, but the limb is more extended at caudal extreme position during FW locomotion (Vilensky et al., 1987; Thorstensson, 1986; Buford et al., 1990). Thus, in both bipeds and quadrupeds the amplitude of the rostro-caudal excursion of the limb during FW locomotion is larger than that during BW locomotion.

In both humans and cats, movement at the hip joint is monophasic in each phase of BW and FW locomotor cycle, but the direction of the movement is opposite (i.e. flexion during FW while extension during BW swing and extension during FW while flexion during BW stance) (Miller et al., 1975; Grasso et al., 1998; Buford et al., 1990). By contrast, movement at the ankle and knee joint is biphasic in each phase during FW locomotion, but mostly monophasic during BW locomotion (Thorstensson, 1986; Vilensky et al., 1987; Miller et al., 1975; Grasso et al., 1998; Buford et al., 1990).

In both humans and cats, the primary contributor for horizontal displacement of the body during BW locomotion is knee extension, while during FW locomotion - hip extension (Grasso et al., 1998; Buford et al., 1990; Thorstensson, 1986). It was found that during swing, hip joint displacement during BW locomotion was nearly similar to knee joint displacement during FW locomotion, and more strikingly, the knee joint angles during BW locomotion resembled the hip joint angles during FW locomotion. In this sense, the hip and knee joints exchanged their functional roles with the change in walking direction (Buford et al., 1990; Thorstensson, 1986).

In humans, the range of joint motion during BW locomotion is considerably smaller in all joints compared with that during FW locomotion (Perry, 2000). By contrast in cats, range of joint motion during FW and BW locomotion is similar (Buford et al., 1990). It was suggested that the aberrant inclination and flexion of the lumbar spine used by the cats during BW locomotion accounts, in large part, for the differing kinematic results between humans and cats.

EMG patterns

In a number of studies in humans and cats, muscle activity patterns observed during FW and BW locomotion were compared (Grasso et al., 1998; Buford and Smith, 1990; Nilsson et al., 1985). It was demonstrated that during FW and BW locomotion, in general flexors are active during swing and extensors during stance (Nilsson et al., 1985; Buford and Smith, 1990; Grasso et al., 1998). However, the EMG pattern during BW locomotion differs from that observed during FW locomotion. It was found that, though the EMG pattern differs, the pattern of muscular synergies are common (Grasso et al., 1998). This means that the time sequence of activation of different muscles is common and muscles that behave as antagonists in one direction could also behave as antagonists in the opposite direction, but the phase of activation could be exchanged. For example, activation of knee extensors in one phase during FW locomotion could be replaced by the activation of knee flexors in the opposite phase during BW locomotion and *vice versa* (Grasso et al., 1998; Buford and Smith, 1990). It was suggested that some common networks could contribute to generation of FW and BW locomotion. The magnitude of EMG activity generated during BW locomotion is higher than that during FW locomotion. It was therefore suggested that the level of energy expenditure during BW locomotion is greater than during FW locomotion (Buford and Smith, 1990). During both FW and BW locomotion with increase in speed, exponential increment of muscle activity is reported (Nilsson et al., 1985; Buford and Smith, 1990).

Comparison of locomotor movements performed in different environments

For investigating the locomotor system, different set-ups (treadmill, air-ball, and immovable surface) are used interchangeably. There are considerable differences between these set-ups e.g. treadmill determines locomotor speed of the animal, air-ball provides unstable support surface and on both treadmill and air-ball the animal locomotes in a static surrounding. This difference in the exteroceptive and interoceptive information during air-ball and treadmill locomotion as compared with those during over ground locomotion (Dunbar, 2004) could result in differences in some aspects of locomotor movements. Earlier studies devoted to comparison of treadmill and over ground FW locomotion in humans and mice did not find any clear differences (Matsas et al., 2000; Schieb, 1986; Taylor et al., 1996). During tunnel locomotion in rats, there was a tendency of increased hip, knee and ankle flexion in stance phase. Treadmill locomotion in rats was associated with increased hip extension at initial contact (Pereira et al., 2006). At the same time the overall hip height patterns appeared very similar and only subtle discrepancies were evident around mid-stance and push-off. Treadmill locomotion was associated with longer step cycle and stance durations compared to tunnel locomotion. Studies on mouse (Herbin et al., 2004) suggested that, training and familiarizing them would give insignificant differences in locomotion patterns produced on over ground and treadmill. Otherwise it’s well reported that the mechanics of locomotion achieved on treadmill are significantly different from tunnel locomotion, and that kinematic measurements taken in these conditions are not equivalent for humans, cats, rats or mice (Charteris and Taves, 1978; Herbin et al., 2007; Pereira et al., 2006; Wetzel 1975).

While during past several decades the cat was the main animal model to study locomotor system, today it gave way to the mouse that allows the use of new advanced and highly promising genetic, optogenetic and chemogenetic methods. However, kinematics of FW and BW locomotion performed by mice on different set-ups has never been compared. *One of the aims of the present study was to compare kinematics of FW and BW locomotion performed by mice in different environmental conditions (that is on treadmill, air-ball and in the tunnel).*

Neuronal mechanisms controlling forward locomotion

The neural mechanisms controlling FW locomotion were intensively studied over the past few decades. FW locomotion is thought to be controlled by a tripartite neural system, which includes a *central pattern generator (CPG)* that is a network, which primarily provides the basic motor pattern in the absence of movement related afferent signals from the limb (Grillner and Zanggner, 1979). This network can be modulated by input from *supraspinal centers* and *motion-related feedback* (sensory input from the limbs), thus adapting its activity to different behavioral tasks and environmental conditions (Sherrington, 1906; Grillner and Zanggner, 1979; Pearson, 1987; Perret, 1980; Orlovsky et al., 1999).

Spinal locomotor networks

In all quadrupedal animals, spinal locomotor networks generating stepping movements by fore and hindlimbs are located in the cervical and lumbosacral enlargements, respectively. Midline transection of the spinal cord abolishes left-right coordination, but rhythmic locomotor activity with appropriate coordination between ipsilateral flexor and extensor motor neurons still remained, suggesting that locomotor movements of each limb are generated by own ipsilateral network (Kjaerulff and Kiehn, 1996; Cowley and Schmidt, 1997; Cangiano and Grillner, 2005; Moult et al., 2013; Jankowska, 2001).

It was demonstrated that during FW locomotion, activity of majority of spinal interneurons located in different parts of the grey matter of lumbosacral and cervical enlargements correlates with locomotor movements suggesting that they contribute to generation of locomotor movements, and thus, represent crucial elements of the locomotor network (Zelenin et al., 2016; Orlovsky et al., 1999). However, a majority of these interneurons are unidentified interneurons and their functional role in control of specific aspects of locomotion is unknown. Earlier studies demonstrated that a few groups of electrophysiologically identified spinal neurons [1a inhibitory interneurons, Renshaw cells, commissural interneurons CINs) and alpha and gamma-motoneurons] contribute to generation of locomotor movements. All these neurons are elements of the locomotor CPG since they are modulated in locomotor rhythm during fictive locomotion. Thus, Ia inhibitory interneurons are rhythmically bursting in phase with the muscle supplying them with 1a afferent input (Feldman and Orlovsky, 1975; Jordan, 1983; McCrea et al., 1980). By inhibiting the antagonistic motoneurons, the 1a interneurons contribute to generation of the basic locomotor pattern, i.e. alternating activity of flexor and extensors. Gamma-motoneurons are modulated in phase with alpha-motoneurons innervating the same muscle, thus contributing to phase-dependent modulation of the stretch reflex gain during locomotion (Murphy et al., 1984). Finally, it was suggested that locomotion-related modulation of commissural interneurons is important for interlimb coordination during locomotion (Edgley and Jankowska, 1987).

During last decade, there has been a rapid progress in genetic identification of neurons. Application of genetic approaches allowed knockout of specific identified neurons, as well as their temporal activation and inactivation in both *in-vitro* and *in-vivo* conditions leading to understanding of specific functional role of these neurons in control of locomotor movements. At present, role of a few genetically identified interneurons in control of FW locomotion have been studied.

Within CINs, a number of different groups were genetically identified. V0 CINs represent a major class of CINs in the ventral spinal cord (Lanuza et al., 2004; Pierani et al., 2001). The V0 population consists of two sub-populations, the excitatory (glutamatergic) V0 ventral or $V0_V$ and the inhibitory (GABAergic and glycinergic) V0 dorsal or $V0_D$ population (Griener et al., 2015; Lanuza et al., 2004; Moran-Rivard et al., 2001; Pierani et al., 2001; Talpalar et al., 2013). The functional role of V0 CINs in control of left-right coordination during locomotor-like activity *in-vitro* (Lanuza et al., 2004) and forward locomotion *in-vivo* (Talpalar et al., 2013) has been investigated. Selective ablation of $V0_D$ and $V0_V$ sub-populations showed that the inhibitory $V0_D$ neurons are responsible for left-right hindlimb alternation at low locomotor speed corresponding to walk while the excitatory $V0_V$ neurons are responsible for execution of hindlimb alternation at higher locomotor frequencies corresponding to trot (Fig. 2, Talpalar et al. 2013). *One of the aims of the present study was to reveal the role of V0 CINs in generation of BW locomotion.*

A different population of genetically identified CINs include glutamatergic V3 CINs (Briscoe et al., 1999; Goulding et al., 2002). *In-vivo* studies have shown that when V3 CINs are inhibited, the locomotor rhythm becomes less steady, indicating that this neuron class is involved in producing a robust locomotor pattern as well as harmonizing the activity between left and the right sides of the spinal cord (Zhang et al., 2008). It has been hypothesized that V3 CINs may contribute to synchronous left and right limb movements at highest locomotor speeds (during gallop and bound, Fig. 2) (Quinlan and Kiehn, 2007; Talpalar et al., 2013; Bellardita and Kiehn 2015; Crone et al., 2008).

Within interneurons with ipsilateral projections, V1 and V2 classes were genetically identified. V1 class are inhibitory interneurons which include Renshaw cells and 1a inhibitory interneurons (Sapir, 2004; Alvarez et al., 2005; Benito-Gonzalez and Alvarez, 2012). Selective ablation of all V1 class of neurons leads to slower cycle duration and a lengthening in the locomotor burst duration (Gosgnach et al., 2006; Nishimaru, 2006). V2 class consists of two populations, the excitatory V2a neurons and inhibitory V2b neurons. It was demonstrated that V2a neurons are involved in control of left-right alternation (Crone et al., 2008; Crone et al., 2009), while V2b neurons are involved in ipsilateral flexor – extensor alternation (Del Barrio et al., 2013).

There are a number of models explaining the generation of locomotor movements. The earlier hypothetical models suggest that locomotor rhythm can be generated by three or more neuronal elements forming a closed ring (Kling and Székely, 1968). However, at present, the generally accepted point of view (based on numerous studies of locomotor networks in animal models of different complexity) is that a rhythm-generating part of the locomotor network consists of just two populations (half-centers) of neurons with mutual inhibitory connections (Graham Brown, 1911; Lundberg, 1981; Ijspeert, 2008). One of the locomotor network models (McCrea, D. A. & Rybak, 2008; Rybak et al., 2015) includes a rhythm generator of the half-center type, and an output stage responsible for formation of the locomotor EMG pattern. Another one (Grillner, 1985, 2006) suggests that the locomotor network contains a set of rhythm-generating units, each controlling muscles of a particular joint, and specific interactions between these units result in generation of a particular locomotor pattern.

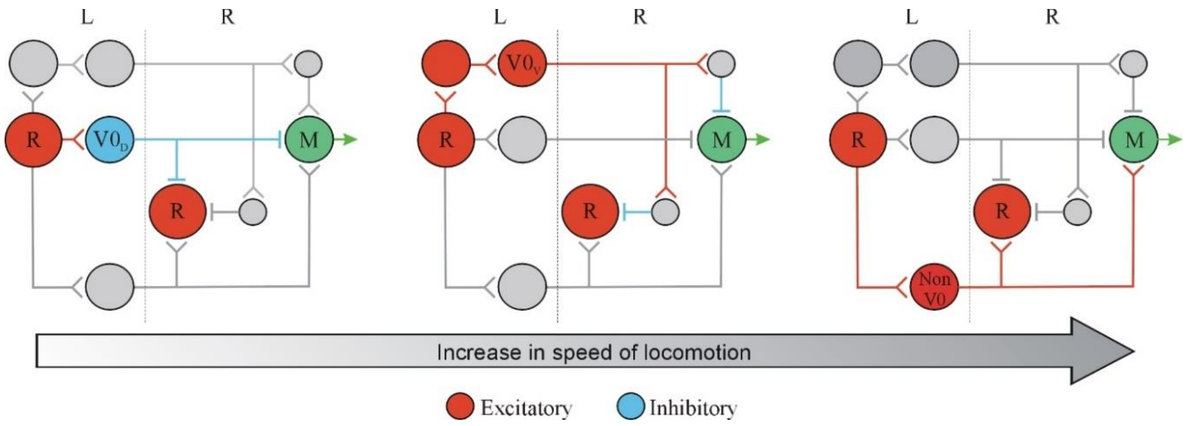

Figure 2. Proposed recruitment of V0$_D$, V0$_V$ and non-V0 CINs in response to increased locomotor frequency. M – Motor neuron, R - Rhythm generator. Open triangles - excitatory synapse, blunt line – inhibitory synapse. The grey dashed line separates the left (L) and the right (R) sides of the cord. The grey circles represent the neurons that do not play a decisive role during a particular locomotor speed.

Supraspinal control of locomotion

In all studied vertebrates, FW locomotion can be initiated and speed of progression is set by mesencephalic locomotor region (MLR) (Shik et al., 1966), which serves as a command centre for FW locomotion (Musienko et al., 2012). Activation of MLR leads to formation of reticulospinal (RS) commands, which specifically activate spinal locomotor networks generating FW locomotion (Orlovsky, 1970). It was shown recently that activation of glutamatergic neurons of cuneiform (CnF) and pedunculopontine (PPN) nucleus in mice evokes slow locomotion with left-right limb alternation, whereas activation of CnF – produces high-speed locomotion with synchronous left-right limb movements (Caggiano et al., 2018). Recently Capelli and colleagues (2017) have shown that the glutamatergic neurons within the lateral paragigantocellular nucleus in the caudal brainstem are essential for supporting higher speeds of locomotion. One can expect that they are activated by CnF glutamatergic neurons. Besides populations of glutamatergic RS neurons contributing to activation of FW locomotion, a population of glutamatergic V2a RS neurons located in the rostral medulla that terminates FW locomotion was found (Bouvier et al., 2015). It was demonstrated that these neurons inhibit interneurons of the rhythm-generating network and thus, one can expect that they stop locomotion in other directions as well. Command centres for locomotion in other directions (e.g. backward and sideward) are unknown.

Cregg and colleagues, 2019 reported the role of Chx10-lineage RS neurons in control of steering during FW locomotion. They have shown that a selective unilateral activation and inactivation of Chx10 neurons causes ipsilateral and contralateral turns, respectively.

It was demonstrated that during locomotion the activity of neurons of main descending tracts originating from the brainstem (VS, RS and RbS) is modulated in locomotor rhythm (Arshavsky et al., 1978, 1986; Wetzel and Stuart, 1976; Grillner, 1981; Garcia-Rill, 1986; Armstrong, 1986). It was reported that neurons of the VS tract has a peak of its activity in stance phase, while neurons of RS and RbS tracts – in the swing phase of locomotor cycle. Electrical stimulation of individual tracts during locomotion demonstrated that in general, VS tract increases activity of extensors during stance, while RS and RbS tracts – preferentially activate flexors during swing. It was shown that modulation of RS, VS and RbS tracts is caused by spino-cerebellar loop (Orlovsky, 1972d, e).

Although integrity of motor cortex is not necessary for generation of FW locomotion in regular environment, activity of CS neurons is modulated in locomotor rhythm. It was demonstrated that modulation of the majority of CS neurons from forelimb area is caused by spinal locomotor mechanisms generating locomotor movements of the forelimbs. By contrast, both fore- and hindlimbs locomotor mechanisms contribute to modulation of CS neurons from hindlimb area. It was suggested that such grouping of inputs allows the motor cortex to contribute to the left-right limb coordination within the girdles during locomotion, and that it also allows hindlimb neurons to participate in coordination of the movements of the hindlimbs with those of the forelimbs (Zelenin et al., 2011). It was found that integrity of motor cortex is critically important for adaptation of locomotor movements to irregular environments for precise foot placement. It was demonstrated that CS tract transmits commands for the corresponding modification of locomotor movements, which are formed on the basis of visual information (Beloozerova et al., 2005; Drew et al., 2004).

It has been demonstrated that majority of CS neurons contribute to control of both FW and BW locomotion but their modulation patterns during FW and BW locomotion are different (Zelenin et al., 2011). Most neurons are modulated by locomotor mechanisms of their projection girdle when this girdle was leading and by locomotor mechanisms of both girdles when this girdle was trailing. Such reconfiguration of the sources of modulation suggests flexibility of the functional roles of individual cortical neurons during different forms of locomotion.

Locomotor networks generating stepping in different directions

Functional organization of neural mechanisms generating stepping in different directions in relation to the trunk was studied in experiments with direct unspecific activation of spinal networks in the decerebrate cat by means of epidural electrical stimulation of the spinal cord (Musienko et al., 2012). It was shown that epidural stimulation of definite sites caused stepping and the direction was determined by the direction of the treadmill belt movement, and on stationary surface or in the air, in-place stepping was performed. This suggests that the locomotor system generating steps in any direction consists of two principal mechanisms: one - generating vertical component of the step (VC, for limb elevation and lowering), and the other generating horizontal component (HC, for limb transfer between extreme positions). Mechanism generating HC consists of the networks that generate the horizontal component of steps in different directions. It receives sensory input signaling direction of the limb movement during stance; reaching an extreme position in the end of stance triggers the limb lifting and transfer in the opposite direction. It was suggested that VC-mechanism contains rhythm-generating network while HC-mechanism – pattern formation networks. Mapping the efficacy of epidural electrical stimulation of different areas of the lumbosacral enlargement to elicit FW and BW locomotion combined with c-*fos* immunostaining (Merkulyeva et al., 2018), led to suggestion that circuits generating the VC of steps and the HC for FW locomotion are distributed throughout the entire lumbosacral enlargement, while the circuit generating the HC for BW locomotion is located in a confined zone (from caudal L5 to L7; Fig. 3).

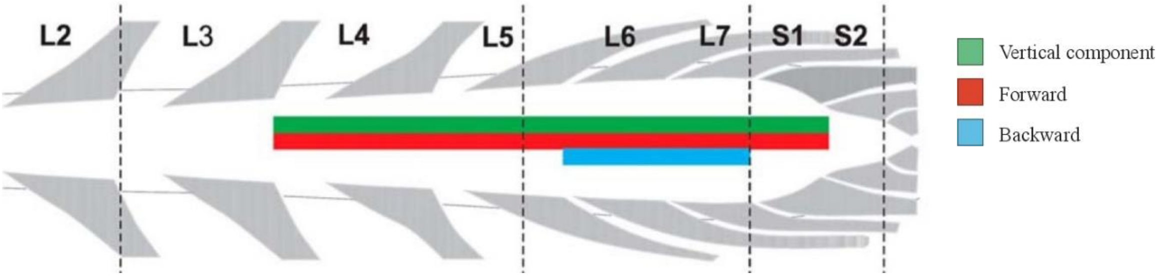

Figure 3. Rostro-caudal distribution of a network generating vertical component of the step (green line), a network generating horizontal component for FW steps (red line) and a network generating horizontal component for BW steps (blue line) in the lumbosacral enlargement.

Recently, Zelenin and colleagues (2016) have recorded activity of individual spinal neurons during both FW and BW locomotion performed by decerebrate cat. According to their activity, the neurons were divided into three groups. Group 1 neurons had the same phase of modulation during both FW and BW locomotion, suggesting that they belong to the networks generating VC of the step. Group 2 neurons were modulated only during FW locomotion or only during BW locomotion, suggesting that they belong to networks generating HC of FW and BW step, respectively. Group 3 neurons changed the phase of their modulation in locomotor cycle depending on the direction of locomotion, suggesting that they control muscles of the hip joint. Since, direction of the movements in swing and stance phase are opposite during FW and BW locomotion, it was suggested that Group 3 neurons also belong to networks generating HC of FW and BW steps.

Neural mechanisms controlling scratching movements

Scratching is a spinal rhythmical reflex, which was studied in considerable detail (Berkinblit et al. 1978a; Deliagina et al. 1983; Sherrington, 1906, 1910; Deliagina et al. 1975; Stein 1983). In intact mammals, scratching behavior consists of two components (Sherrington, 1906, 1910; Deliagina et al., 1975; Kuhta and Smith 1990). The first (postural) component is a protraction of the ipsilateral to stimulation side hindlimb towards the stimulated skin area accompanied by body and neck bending towards this hindlimb. The second (rhythmical) component is fast alternating flexion and extension of ankle, knee and hip joints that results in repetitive scratching of the irritated area of the skin. Scratching is activated by signals from skin mechanoreceptors. In cat, receptive field of scratching covers the pinnas and neck area, but in dogs it also occupies the side of the trunk (Sherrington, 1906, 1910; Kuhta and Smith 1990).

In cats, different neural mechanisms activated by commands transmitted by different pathways, generate the postural component and the rhythmical component of scratching (Deliagina, 1977). Rhythmical component is generated by spinal CPG located in lumbo-sacral enlargement unilaterally (Berkinblit et al., 1978a; Deliagina et al., 1983). The pathway, which activates scratching CPG, represents chains of propriospinal neurons with long descending axons. It crosses the midline in the rostral cervical segments and descends along the contralateral spinal cord until middle thoracic segments where it again crosses midline and descend in the ipsilateral side in the caudal thoracic and lumbar region. By contrast, the pathway activating postural component of scratching descends ipsilaterally (Deliagina, 1983).

For purposeful scratching (which removes irritant from the skin) sensory movement-dependent feedback and normal operation of the spino-cerebellar loop is required (Deliagina et al., 1975; Arshavsky et al., 1983). It was also shown that sensory feedback from the limb signaling about execution of the postural component (the hindlimb protraction) gates the command addressed to scratching CPG (Deliagina et al., 1975).

In all studied mammals, unilateral stimulation of the receptive field evokes scratching movements performed by the ipsilateral hindlimb (Sherrington, 1906, 1910; Deliagina et al., 1975; Stein, 1983), while bilateral stimulation causes alternation in scratching episodes performed by the left and right limb, suggesting strong reciprocal inhibition between the left and right scratching CPGs presumably mediated by commissural interneurons. *One of the aims of the present study was to reveal the role of V0 commissural interneurons in control of scratching behaviour.*

CONTROL OF POSTURE

Principles and strategies of postural control

The activity of postural control system allows animals and humans to sustain basic standing posture (upright in humans and dorsal-side-up orientation in quadrupeds). An active and adequate control of the basic body posture is equally important for standing and during locomotion (Orlovsky et al., 1999; Macpherson et al., 1997a; Macpherson, 1999), as well as for providing support during voluntary limb, trunk and head movements (Massion,1998). Maintenance of the basic body posture is involuntary and rooted mainly in inborn neural mechanisms (Massion, 1998; Vinay et al., 2005).

The feed-back and feed-forward modes are two principal modes of operation of the postural system. The executed mode depends on the nature of postural perturbations (Horak and Macpherson, 1996; Macpherson et al., 1997a). *The feed-forward mode of postural control* is a compensation for the destabilizing consequences of voluntary movements or for the expected perturbations of posture caused by external factors. This mode is used for generation of anticipatory postural adjustments. *The feed-back mode of postural control* is a compensation for deviations from the desired posture caused by unexpected external factors. This mode is used for generation of compensatory postural adjustments. Deviation from the desirable body orientation is detected by different sensory systems (somatosensory, vestibular and visual) and their signals cause generation of a corrective movement (compensatory postural adjustment). The importance of a particular sensory system depends on the postural task and its circumstances (Beloozerova et al., 2003; Deliagina et al., 2000b). Two main groups of strategies are used for generation of compensatory postural adjustments: fixed-support strategies and change-in-support strategies (Horak et al., 2009; Maki and McIlroy, 1996). *Fixed-support* strategies are used for generation of postural corrections, which does not lead to a change of the support area. By contrast, *change-in-support strategies* are used for generation of postural corrections which lead to a change of the of the support area (e.g. a corrective step or arm movement aimed to reach the support for regaining equilibrium). *In the present study only compensatory postural adjustments were investigated.*

Postural corrections related to fixed-support strategy

Postural corrections related to fixed-support strategy are generated in response to different unexpected postural perturbations, caused by lateral tilt of the support surface, translation of a supporting surface, and lateral push of the trunk (Macpherson and Fung, 1999; Karayannidou et al., 2008, 2009; Musienko et al., 2008; Deliagina et al., 2012; Honeycutt et al., 2009; Honeycutt and Nichols, 2010; Deliagina et al., 2006; Beloozerova et al., 2003; Musienko et al., 2010). Out of these, neural mechanisms underlying postural corrections caused by lateral tilts were the most studied.

A lateral tilt of the support surface in a standing subject causes a lateral body sway, thus moving the dorso-ventral trunk axis away from the vertical. This evokes a compensatory postural correction which includes extension of limbs on the tilt side and flexion of the limbs on the contralateral side, thereby the trunk axis shifts towards the vertical (Fig. 5J; Deliagina et al., 2006). The somatosensory inputs from the limbs are crucial for evoking postural reaction to the tilts (Deliagina et al., 2000, 2006; Inglis et al., 1995; Stapley et al., 2009; Karayannidou et al., 2009) except for very fast tilts (Macpherson et al., 2007).

Postural corrections in response to lateral tilts of the support surface are generated by the postural system responsible for stabilization of the dorsal-side-up trunk orientation in the transverse plane. It was shown that this system consists of two relatively independent sub-systems stabilizing anterior and posterior parts of the trunk (Fig. 4; Beloozerova et al., 2003; Deliagina et al., 2006). Each sub-system contains a mechanism (limb controller) generating corrective movement of the limb in response to tilt-related somatosensory input from the same limb. However, the amplitude of this corrective movement is smaller than in control. It was suggested that tilt-related sensory signals from contralateral limb of the girdle are required for generation of full amplitude limb corrections (Deliagina et al., 2006). These sensory signals could be transmitted by spinal commissural interneurons. *One of the aims of the present study was to reveal the role of V0 commissural interneurons in generation of postural correction caused by lateral tilts.*

The basic networks underlying operation of both sub-systems reside in the brainstem, cerebellum and spinal cord. However, in decerebrate animals the efficacy of postural corrections generated by these networks is lower than in control conditions, suggesting that forebrain contributes to their activation (Musienko et al., 2008). Tilt-related somatosensory information directly affects spinal neurons and transmits to the brain forming a supraspinal postural command. It was demonstrated that spinal cord contains the postural network, which is capable in generating EMG pattern of postural corrections (Musienko et al., 2010). It was shown that though the modulation of a majority of neurons forming this network is determined by tilt-related sensory signals from ipsilateral limb, a substantial part of these neurons also receive tilt-related sensory inputs from contralateral limb, suggesting that spinal commissural interneurons are important elements of spinal postural network (Zelenin et al., 2016). The efficacy of EMG pattern of postural corrections generated by spinal postural network to move the trunk is close to zero, since the activity of the muscles is very weak (Musienko et al., 2010). It was demonstrated that both phasic supraspinal postural commands and tonic supraspinal drive are critically important for generation of effective postural corrections (Zelenin et al., 2013; Musienko et al., 2010). Activity of RbS and CS neurons during postural corrections caused by lateral tilts of the support surface have been analysed (Zelenin et al., 2010; Beloozerova et al., 2005; Karayannidou et al., 2008; Tamarova et al., 2007). It was demonstrated that these neurons belong to neuronal network, which generates corrective movements of the limb on the basis of tilt-related somatosensory information from the same limb (Karayannodou et al., 2008).

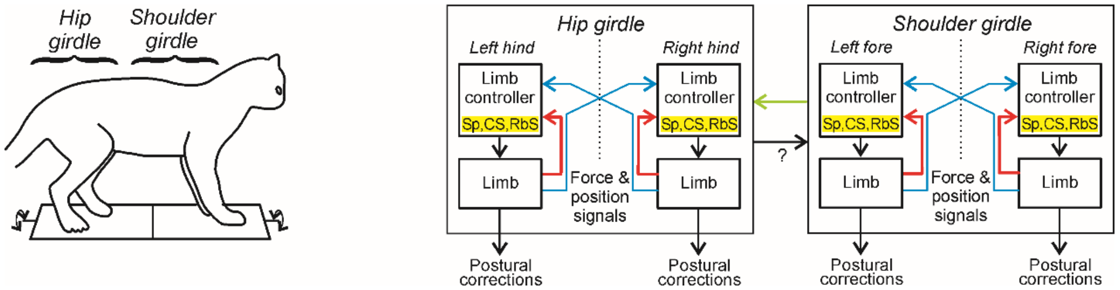

Figure 4. Functional organization of the postural system stabilizing the trunk orientation in the transverse plane. The dorsal side-up orientation of the anterior and posterior parts of the body (shoulder and hip girdles) is maintained by two relatively independent subsystems. Each subsystem contains two limb controllers generating a part of corrective limb movement in response to sensory signals from the same limb (red arrows). Neurons of spinal postural network (Sp), as well as rubrospinal (RbS) and corticospinal (CS) neurons are elements of this neural mechanism. Another part of corrective limb movement is generated in response to sensory signals from the contralateral limb (blue arrows). Coordination between two subsystems is achieved mainly due to influences of the anterior sub-system on the posterior one (green arrow).

Postural corrections related to change-in-support strategy

While postural corrections related to fixed-support strategies were studied rather intensively (Beloozerova et al., 2003; Macpherson and Fung, 1999; Musienko et al., 2008; Honeycutt and Nichols, 2010; Honeycutt et al., 2009; Deliagina et al., 2006; Musienko et al., 2010; Karayannidou et al., 2008, 2009; Deliagina et al., 2012), our knowledge about neural mechanisms generating postural corrections related to change-in-support strategies is scarce. It was reported that similar postural perturbations (e.g. caused by tilting or translation of the support surface on which the subject is standing or lateral push of the trunk) can evoke either fixed-support strategy or change-in-support strategy (Beloozerova et al., 2003; Karayannidou et al., 2009). It was suggested that change-in-support strategy is triggered only when the fixed-support strategy is insufficient for regaining balance (i.e. projection of the centre of mass appears close to the border of the support area delimited by limbs), and there is a risk in falling down (Horak and Nashner, 1986). However, later studies in human demonstrated that a corrective step could be generated well before the time point when the centre of mass reached the border of the support area (Maki and McIlroy, 1996; Maki and Whitelaw, 1993).

It was reported (Maki et al., 1996) that in humans, horizontal translation of the support surface in a particular direction, caused, first, a shift of the body weight towards one of the legs and then, second, a corrective step executed by the another (unloaded) leg in the direction opposite to the direction of the surface translation. In the supporting limb, the functional muscle synergies distinctive for the fixed-support strategy postural corrections in response to corresponding translation were executed (Chvatal et al., 2011; Burleigh et al., 1994). Also, importance of sensory information from receptors of the foot sole for the probability of initiation of the corrective step has been demonstrated (Perry et al., 2000).

Hsu and colleagues (2014) from our lab found that disturbance of the basic body configuration produced by horizontal translation of one of the hindlimbs in standing rabbit elicited a postural response leading to restoration of the initial body configuration. This postural response contained a single corrective step generated by the displaced hindlimb in the direction opposite to the direction of initial displacement whereas the other limbs continued to stand. *One of the aims of the present study was to analyse the neural mechanisms underlying generation of the postural response to disturbance of the basic standing body configuration by displacement of a single hindlimb in different directions.*

Righting behaviour

When positioned on their side or in upside-down position, majority of quadrupeds exhibits a set of righting reflexes and rapidly assume the basic standing posture (Magnus, 1925; Musienko et al., 2010; 2008). Similarly, when the cat is released in upside-down position in the air, due to a set of righting reflexes it lands on four limbs assuming standing posture (Magnus, 1914). Depending on environmental conditions, sensory signals of different modalities contribute to generation of righting behavior. In falling cat, vestibular signals evoke righting. In cats, kittens and rabbits, during righting from the side position the sequence of righting is as follows - righting of the head orientation followed by trunk (Magnus, 1914; Musienko et al., 2010), vestibular signals largely contribute to the head righting while somatosensory signals – to the trunk righting (Magnus, 1914; Sylvestre and Cullen et al., 2006; Nguyen et al., 2005; Jusufi et al., 2011; Sechzer et al., 1984; Cremieux et al., 1984; Ponomarev et al., 2002 and Delval et al., 2012).

Movements causing righting of the body orientation in space are very fast, depend on environmental conditions, vary in different species, as well as in sequential trials which creates problems for their kinematic analysis. Analytical models of righting behavior suggest importance of trunk movements (its lateral bending combined with twisting of the anterior part of the body in relation to the posterior one) for rotation of the body towards the dorsal-side-up orientation (e.g., Kane and

Scher 1969; Edwards 1986; Marsden and Ostrowski 1998). In rats and cats, twisting of the trunk during righting performed, respectively, on the surface and in the air was reported (Schönfelder 1984; Laouris et al. 1990a). One can expect that the trunk lateral bending and twisting observed during righting require reciprocal coordination in activity of homonymous axial muscles bending or twisting the trunk in opposite directions. This reciprocal coordination could be mediated by commissural interneurons. *One of the aims of the present study was to reveal the role of V0 commissural interneurons in generation of righting behavior.* Contribution of limb movements to rotation of the animal toward dorsal side-up orientation during righting is unknown (Arabyan and Tsai, 1998).

It was demonstrated that when decerebrated at the precollicular-premammillary level, cats and rabbits exhibit righting behavior (Kellar and Hare, 1934; Musienko et al., 2010), suggesting that the basic networks generating righting behaviour reside in the brainstem, cerebellum and spinal cord. However, the duration of righting behaviour generated by these networks is longer than in control, suggesting that forebrain contributes to their activation.

AIMS

I. To compare kinematics of forward and backward locomotion performed by mice in different environmental conditions (*Paper 1*).

II. To reveal the role of V0 commissural interneurons in control of a number of basic motor behaviours (backward locomotion, scratching, righting and postural corrections) (*Paper 2*).

III. To analyse the neural mechanisms of postural responses to distortion of the basic standing body configuration in rabbit (*Paper 3*).

METHODS

Experiments were conducted on two animal models – the mouse and the rabbit. Analysis of kinematics of FW and BW locomotion in different environmental conditions as well as experiments devoted to studying the role of V0 CINs in control of basic motor behaviours were performed on mice. Neural mechanisms underlying postural response to disturbance of the basic body configuration were studied on rabbits.

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

In this section, the main methodological approaches are briefly discussed. Detailed experimental procedures are presented in the corresponding papers.

Experiments on mice

Kinematics of FW and BW locomotion performed on different set-ups (tunnel, treadmill, air-ball) was studied in wild-type (WT) mice. The study devoted to reveal the role of V0 CINs in control of basic motor behaviors (BW locomotion, scratching reflex, righting behavior and postural corrections) was performed on knockout mice [$Hoxb8^{Cre}$;$Dbx1^{DTA}$ mice with all V0 CINs ablated in the spinal cord caudally to C4 segment ($V0^-$ knockouts), and $Vglut2^{Cre}$;$Dbx1^{DTA}$ mice with $V0_V$ CINs ablated in the entire CNS ($V0\text{-}Glu^-$ knockouts; Talpalar et al., 2013)] and their littermates. All experiments were conducted on C57 mouse strain.

Surgical procedures: Surgery was performed under general anesthesia (ketamine in combination with medetomidin, i.p.) under aseptic conditions. In some animals, chronic implantation of EMG-electrodes was performed. Bipolar EMG electrodes were implanted bilaterally into two-four selected muscles of the hindlimbs in varying combinations. A small head-plate was implanted in some WT animals. It was used for fixation of the head when the animal performed locomotion on the air-ball. The head fixation was necessary to prevent the animal from falling off the air-ball. We performed the experiments 1-2 days post-surgery.

Experimental designs: Experimental designs are shown in Fig. 5. Both mutant mice and WT mice performed the following basic motor behaviours: FW/BW locomotion, scratching, righting and postural corrections.

FW and BW locomotion was performed in the tunnel (Fig 5A,B), on the treadmill (Fig. 5C) and on an air-ball (Fig. 5D). *In the tunnel set-up*, the mouse was placed in the entrance box, after opening the door to the entrance of the tunnel, the animal performed sequential FW stepping along the length of the tunnel. When the mouse reached the end of the tunnel, since it could not turn around in the narrow tunnel, the mouse performed BW locomotion. In the majority of attempts the animal spontaneously exhibited at least 3-4 sequential episodes of FW and BW locomotion per recording. FW and BW locomotion *on the treadmill* was evoked, respectively, by backward and forward direction of the treadmill belt movement in relation to the animal. The speed of the belt movement was adjusted to the animal's performance. To evoke locomotion *on the air-ball*, the mouse was placed on the fixed ball and the implanted head-plate was used for the head fixation. Then, the air-ball was released and the mouse performed spontaneous FW locomotion. BW locomotion was elicited by slight tactile stimulation of the snout or the animal spontaneously performed BW locomotion. To evoke walking straight FW or straight BW on the air-ball and on the treadmill, the mouse was placed in a corridor with transparent Plexiglas walls (Fig. 5C,D).

To evoke *scratching* (Fig. 5I), a gentle mechanical stimulation of the pinna was given to a freely standing mouse.

To evoke *righting reflex* (Fig. 5H) the experimenter placed the mouse on its back on a horizontal surface. When released, the animal immediately assumed normal (with back-up) body orientation characteristic for standing.

To evoke *postural corrections*, the mouse was placed on the tilting platform with the sagittal plane of the animal aligned to the axis of the platform rotation (Fig. 5J). The platform with the animal standing on it was tilted periodically in frontal (transverse) plane of the animal (roll tilt α, Fig. 5J,K) with an amplitude of ±20°. A trapezoid tilt trajectory with the transitions between extreme positions lasting for ~0.5–1 s and each position maintained for ~1-1.5 s was engaged (Fig. 5J). A mechanical sensor was used for monitoring the tilt angle of the platform.

<u>Recording and data analysis</u>*:* During tunnel (Fig. 5A.B) treadmill (Fig. 5C) locomotion, scratching (Fig. 5I) and righting (Fig. 5H), we simultaneously video recorded side and bottom view of the animal, while during postural corrections (Fig. 5J), back and bottom view was recorded. During locomotion on an air-ball, only side view was recorded. Markers were attached to the skin projections of the main hindlimb joints, rostral and caudal point of the pelvis and on the midline of the spine. Video recording was synchronized with EMG and position sensors from the tilt platform. The signals from the EMG electrodes were amplified, digitized with a sampling frequency of 5 kHz and recorded on a computer disk. They were then rectified and smoothed (time constant, 10ms). The video recordings were analyzed frame by frame. Video data were used to calculate the kinematics parameters of studied behaviours.

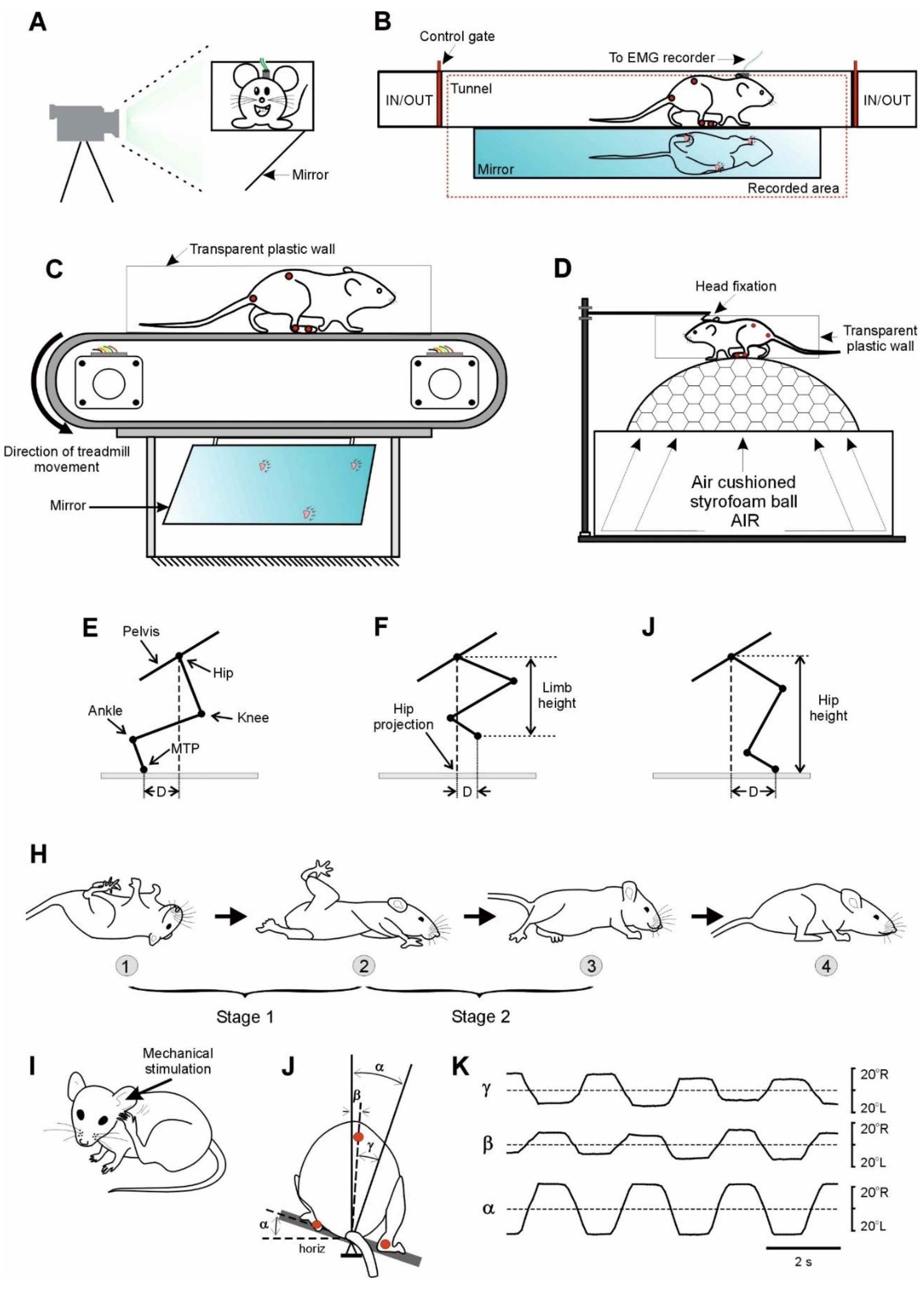

Figure 5. Experimental designs. **A-D.** A frontal (**A**) and lateral view (**B**) of the mouse walking in a narrow tunnel. **C,D**. Lateral view of the mouse walking on a treadmill (**C**), and the mouse with fixed head walking on an air-ball (**D**). . **E-G.** The right hindlimb configuration in the beginning of swing (**E**), in the middle of swing (**F**) and at the end of swing (**G**). **H.** Sequential positions of the mouse (*1-4*) during righting behavior. Two stages of the righting (*Stage 1* and *Stage 2*) are indicated. (**I**)**.** The body posture during scratching. **(J).** The rear view of the mouse executing postural correction caused by the lateral tilt of the supporting platform. α, the angle of the platform tilt; β, the angle of the dorso-ventral trunk axis tilt in relation to vertical; γ, the angle of the dorso-ventral trunk axis tilt in relation to the platform. The dorso-ventral trunk axis was determined as a line connecting the marker positioned on the spine at the level of the rostral pelvis (red circle) and the axis of rotation. **(K).** Changes of β and γ angles caused by trapezoid tilts of the platform (α). To estimate efficacy of postural corrections, we calculated the coefficient of postural stabilization, K_{STAB}= 1 – β/α. With perfect stabilization, K_{STAB} = 1; with no stabilization, K_{STAB} = 0.

Experiments on Rabbits

Surgical procedures: All animals were subjected to chronic implantation of EMG-electrodes. The surgery was performed under Hypnorm-midazolam anaesthesia, under aseptic conditions. Bipolar EMG electrodes were chronically implanted bilaterally into four selected muscles of the trunk and/or hindlimbs in varying combinations. 1–2 days post-surgery when the animal completely recovered, it was subjected to various postural tests (see below). After a few days of testing, few animals were subjected to acute experiments. For induction of anaesthesia, the rabbit was injected with propofol, then anaesthesia continued on isoflurane. The trachea was cannulated and decerebration was performed either at precollicular-postmammillary level or at precollicular-premammillary level. After decerebration, the anaesthesia was discontinued. Recordings in decerebrate rabbits were started not earlier than in 1 h after cessation of anaesthesia.

Experiments on intact and premammillary rabbits. To evoke postural response to disturbance of basic body configuration, the animal was placed on four horizontal platforms (Fig. 6A-C). A movable plastic plate was positioned under one of the hindlimbs (black plate in Fig. 6). During experiments the plate was moved horizontally either backward (Fig. 6D) forward (Fig. 6E) or outward (Fig. 6F), evoking limb displacement that followed the direction of the surface translation, which resulted in disturbance of the basic standing configuration. This disturbance elicited a corrective step performed by the displaced limb in the direction opposite to the direction of the displacement (indicated by grey arrow in Fig. 6D-F).

During postural response the animal was video recorded from above along with either side (Fig. 6A,C) or from back. Markers were placed along the spine midline and on the main hindlimb joints (Fig. 6A,B). During forward and backward corrective steps, the anterior–posterior hindlimb movements were recorded by mechanical sensor S1 (Fig. 6, D and E). During inward corrective steps, the medio–lateral hindlimb and trunk movements were recorded by means of sensors S2 and S3, respectively (Fig. 6F). Two force plates (Force-R, Force-L in Fig. 6C) were used to record the vertical force generated under the hindlimbs. The signals from force plates, mechanical sensors and EMG electrodes were synchronized with the video recording.

To clarify if sensory feedback from the limb alone is sufficient to generate a corrective step, in 3 intact animals at the end of the outward limb displacement, we applied lateral force to the distal part of the limb using an elastic string (Fig. 6G). The applied force produced a gradually increasing lateral force affecting the limb during the corrective step (Fig. 6H-J).

Acute experiments on postmammillary rabbits. To clarify whether sensory information about hindlimb displacement alone can evoke postural corrections, the head and vertebral column of a decerebrate rabbit were rigidly fixed to a frame (Fig. 6K), forequarters were suspended in a hammock, and hindlimbs were placed on two horizontal plates with a configuration similar to that observed in freely standing intact rabbits (Fig. 6L,M). The method to evoke forward, backward and inward corrective steps in the postmammillary decerebrate animal were the same as that used for intact animals. The video recording from side allowed us to capture forward and backward corrective steps, while inward corrective steps were video recorded from the rear. The signals from position sensors, EMG electrodes and force plates were synchronized with the video recording.

__Recording and data analysis__: The signals from the position sensors, EMG electrodes and force plates were amplified, digitized with a sampling frequency of 5 kHz (EMGs) and 1 kHz (sensors), and recorded on a computer disk. EMG signals were rectified and smoothed (time constant, 10ms). The video recordings were analysed frame-by-frame. In intact and decerebrate animals, video data were used to calculate kinematics of limb and/or the trunk corrective movement during various postural tasks.

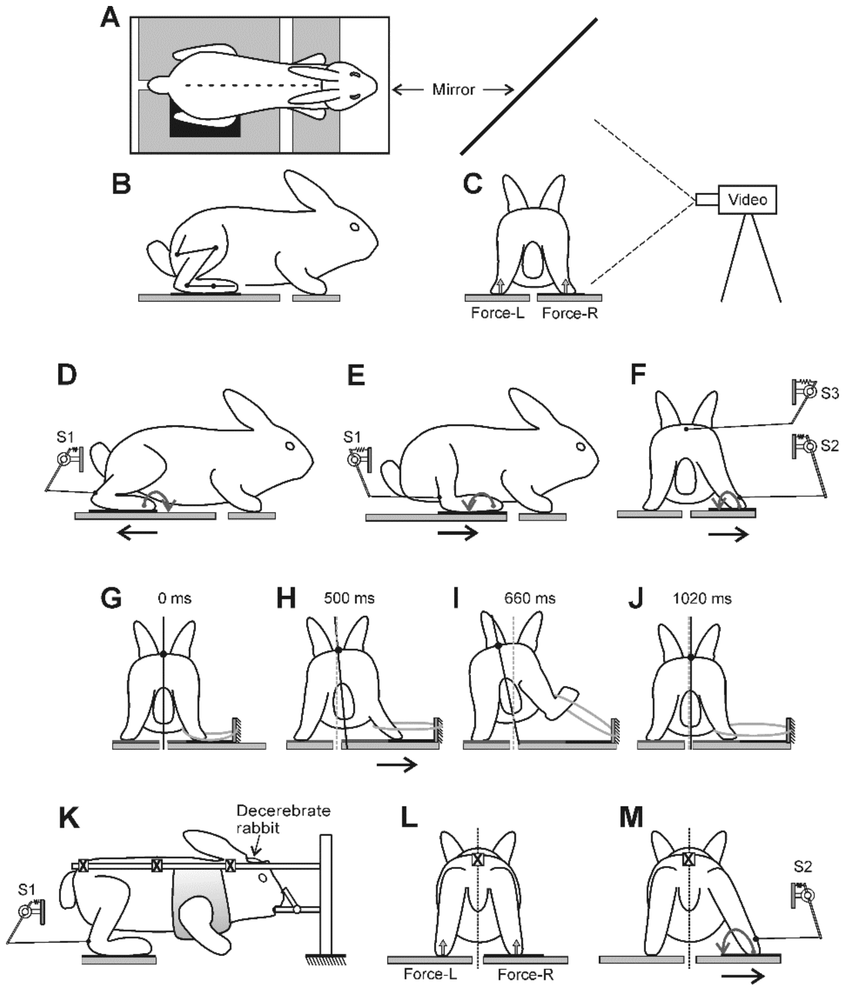

Figure 6. Designs for experiments devoted to analysis of postural response to distortion of the basic body configuration (see text for explanations).

RESULTS AND DISCUSSION

Kinematics and motor patterns during FW and BW locomotion performed by mice in different environmental conditions

The aim of the present study was to compare kinematics of FW and BW locomotion performed in different environmental conditions - in a tunnel, on a treadmill and on an air-ball. The motor behavior was video recorded and hindlimb kinematics were analyzed.

Basic parameters of FW and BW locomotion performed on different experimental setups

We found that on each of the three setups, mice performed both FW and BW stepping with a wide range of speeds, step lengths and cycle durations which substantially overlapped. Similar results were reported earlier while comparing kinematics of FW locomotion in an open field and on a treadmill in horses (Barrey et al., 1993), humans (Riley et al., 2007), rats (Pereira et al., 2006), cats (Miller et al., 1975) and mice (Herbin et al., 2007).

On all three experimental setups, both mean values of the step amplitude (i.e. the distance between two points corresponding to foot lift-off and landing) and speed of BW locomotion were significantly (approximately twice) smaller than those observed during FW locomotion. Similar results were reported for other animals and for humans (Ashley-Ross and Lauder, 1997; Buford et al., 1990; Thorstensson, 1986; Van Deursen et al., 1998)

The mean values of cycle, stance and swing durations during locomotion performed in a specific direction on different experimental setups were similar. However, on a given setup, the mean values of swing duration during BW locomotion was significantly (almost twice) smaller as compared with that observed during FW locomotion. One can expect that reduced step length and a specific body configuration resulting in very rostral hindlimb stepping position (that leads to reduction of the base of support), reduce lateral stability during BW locomotion. A shorter swing duration benefits the animal to improve its balance, since three limbs support the body during a large proportion of the locomotor cycle. We found that in mice, as in all studied humans and animals (Hruska et al., 1979; Thorstensson, 1986; Halbertsma, 1983; Buford et al., 1990; Grasso et al., 1998; Clarke and Still, 1999; Vilensky and Cook, 2000), an increase in cycle duration during FW or BW locomotion was caused by an increase in duration of the stance phase, while duration of the swing phase remained unchanged.

Thus, different environments provided by three experimental conditions did not affect the basic parameters of locomotor movements. However, basic characteristics of locomotor movements performed in opposite directions (FW and BW) differ substantially.

Sequences of stepping during FW and BW locomotion

Interlimb coordination was analysed only for FW and BW locomotor episodes obtained in the tunnel. During FW locomotion, we observed only one type of interlimb coordination that is walk with lateral sequence of limb movements (Fig. 7A; Gambaryan, 1975; Hildebrand, 1980). This result is consistent with the suggestions of Bellardita and Kiehn (2015) for similar range of locomotor speeds (less than 60 cm/s). It was suggested that walk with lateral sequence, employed by majority of mammals, provides superior postural stability (Gray, 1944) compared to gait corresponding to walk with diagonal sequence used by a few mammals (Hildebrand, 1980).

During BW locomotion we observed three different interlimb coordination's – walk with lateral sequence (Fig. 7B), walk with diagonal sequence (Fig. 7C) and trot (Fig. 7D). The same mouse was able to perform BW locomotion with different interlimb coordination's and sometimes even switch from one pattern to another within the same locomotor episode. We did not find any correlation between the velocity of BW locomotion and the observed interlimb coordination.

We found that during different sequences of FW and BW locomotion the mean SD values of the phase shift between movements of the reference hindlimb and contralateral hindlimb were similar, suggesting that the strength of coupling between limb movements of the pelvis girdle was similar during both FW and BW locomotion regardless of the chosen stepping sequence. By contrast, the mean SD value of the phase shift between locomotor movements of the reference hindlimb and ipsilateral forelimb during FW locomotion was significantly smaller compared to that during BW locomotion, suggesting that the strength of coupling between girdles was stronger during FW than during BW locomotion. One can suggest that during BW locomotion in mice, postural stability is provided by short swing duration, therefore an exact stepping sequence is not essential, and as a result the inter-girdle coordination is erratic. During BW locomotion a rather irregular interlimb coordination that does not compare to any definitive gait pattern has been described in salamanders (Ashley-Ross and Lauder, 1997). By contrast, it has been reported that cats and mole rats have a stable interlimb coordination during BW locomotion (walk with diagonal sequence) (Buford et al., 1990; Eilam and Shefer, 1992).

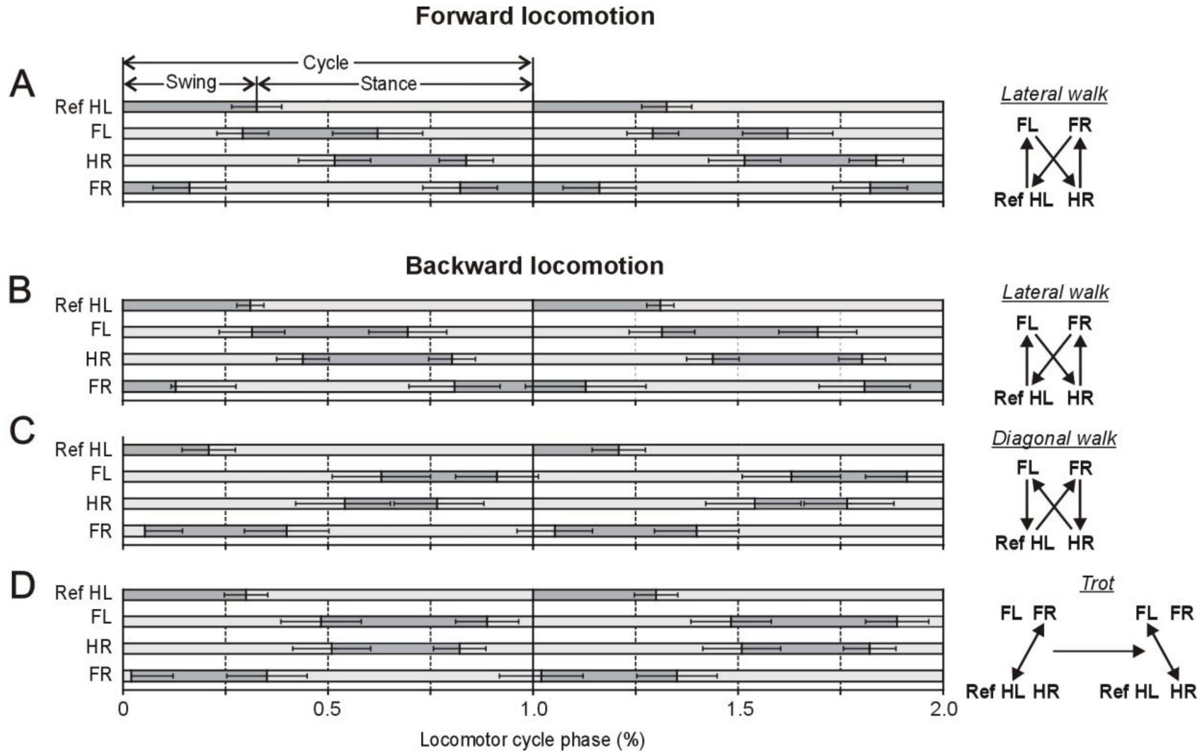

Figure 7. Different types of interlimb coordination's revealed during FW (**A**) and BW locomotion (**B**-**D**) in the tunnel. **A**. Scheme of FW walk with "lateral sequence" **B**-**D**. Schemes of interlimb coordination's ("lateral sequence" (**B**), "diagonal sequence" (**C**), and "trot" (**D**)) observed during BW locomotion. The phase (mean ± SD) of the swing beginning and end of the left forelimb (FL), right hindlimb (HR) and right forelimb (FR) normalised to the cycle of the reference left hindlimb (Ref HR) are shown. Swing and stance are indicated by dark and light grey bars, respectively. In **A**-**D**, right panels represent schemes of the pattern of limb movements.

Body configurations during FW and BW locomotion on different setups

Mice can perform locomotion with various body configurations, i.e., with different pelvis height (that could be characterised by hip height), as well as with different location of the paw trajectory in relation to the trunk. We found that during a particular FW or BW locomotor episode the hip height is maintained constant (Fig. 8A), and there is a relationship between hip height and the position of the paw trajectory in relation to the hip. When the hip height was high, the extreme rostral and extreme caudal points of the paw trajectory were symmetrical in relation to the hip ("middle steps", Fig. 8B), when the hip was low, either rostral (Fig. 8C) or caudal (Fig. 8D) displacement of the paw trajectory in relation to the hip was observed ("anterior" and "posterior" steps, respectively).

We found that mice exhibits different forms of FW stepping in different environmental conditions. Thus, during locomotion on an air-ball, middle or posterior steps were observed. Since posterior stepping leads to an increase in the base of support, one can expect that by using this form of stepping the mouse increases its postural stability on unstable surface provided by the floating air-ball. By contrast on treadmill mice used middle or anterior steps. Moving treadmill belt represents rather insecure environment from a behavioural point of view, and possibly, anterior stepping allows the mouse to quickly change direction of locomotion from FW to BW and escape out of threat that may suddenly appear in front of it. Finally, in the tunnel (a rather natural environment for the mouse that lives in tight burrows) all forms of stepping were observed. In mole rats and cats, posterior and anterior stepping were observed, respectively, during upslope and downslope FW locomotion (Smith et al., 1998; Carlson-Kuhta et al., 1998; Eilam et al., 1995). It was also reported that cats use posterior stepping during crouched FW walking (Trank et al., 1996).

In contrast to FW locomotion, we found that during BW locomotion mice exhibited strong ventral flexion of the spine and adopted anterior stepping on all three setups. Such body configuration was also reported in cats when they walked BW (Buford et al., 1990).

It has been shown that displacement of epidural electrical stimulation in the rostro-caudal direction along the midline of the lumbosacral enlargement in decerebrate cat performing FW locomotion, evoked, respectively, rostro-caudal displacement of the paw trajectory in relation to the hip (Merkulyeva et al., 2018). It was suggested that this rostro-caudal shift of hindlimb stepping was caused by predominate increase in excitability of motoneuronal pools of hip flexors (located in the rostral segments of the lumbosacral enlargement) and hip extensors (located in the caudal segments of the lumbosacral enlargement), respectively (Vanderhorst and Holstege, 1997). One can suggest that in intact animals, specific supraspinal commands set the ratio of the excitability level of hip extensor/flexor motoneuronal pools that leads to a definite form of FW stepping. By contrast, epidural stimulation of the spinal cord evoked only anterior BW steps in decerebrate cat (Musienko et al., 2012; Merkulyeva et al., 2018), suggesting that most likely, anterior stepping during BW locomotion is hardwired in corresponding spinal networks and not determined by supraspinal commands.

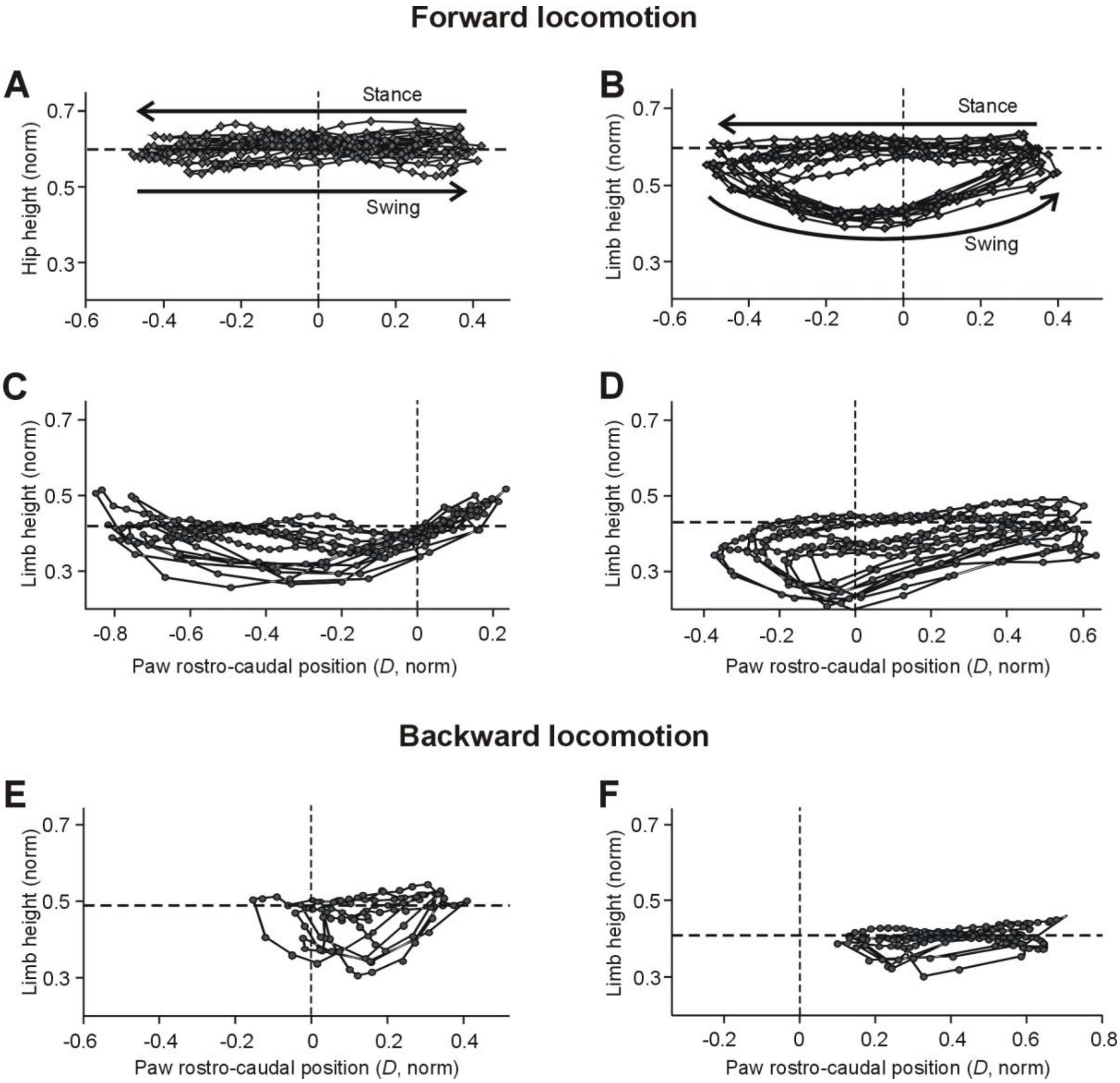

Figure 8. Examples of FW (**A-D**) and BW (**E-F**) locomotor episodes performed with different body configurations. Hip height (**A**) and limb height (**B-F**) at different paw rostro-caudal positions. Arrows indicate direction of the limb movement during stance and swing. The time interval between sequential points in **A-F** is 10ms. Hip height, limb height and paw horizontal position were normalized to the maximal limb length. On abscissa, the position of the hip projection to the surface is taken as zero (marked by vertical interrupted line). The negative and positive D-values indicate caudal and rostral paw positions in relation to the hip projection, respectively. Horizontal interrupted line indicates the mean value of the hip height during a locomotor episode.

Intralimb coordination during FW and BW locomotion performed with different body configurations

In mice during FW locomotion, intralimb coordination robustly depended on the form of stepping (anterior, middle or posterior). While joint angle profiles of the hip were qualitatively similar during different forms of stepping (flexion during swing and extension during stance, Fig.9A), joint angle profiles of the knee and ankle (Fig. 9, B and C, respectively) were substantially different. Thus, during stance, almost pure extension of ankle and knee joints was observed during posterior stepping (dotted line in B,C), relatively pure flexion – during anterior stepping (solid line in B,C) and weak flexion-extension– during middle stepping (dashed line in B,C). Joint angle profiles of the knee and ankle joints similar to those observed in mice during FW anterior and posterior stepping, were also reported in cats that performed anterior and posterior stepping during upslope/crouched, downslope FW walking, respectively (Smith et al., 1999; Carlson-Kuhta et al., 1998; Trank et al., 1996). We found that in mice the intralimb coordination during BW walking was similar to that previously reported in cats (Buford et al., 1990): the hip joint profile was reversed (compare A and D in Fig. 9), while knee and ankle joints exhibited flexion during swing and extension during stance (Fig. 9E,F).

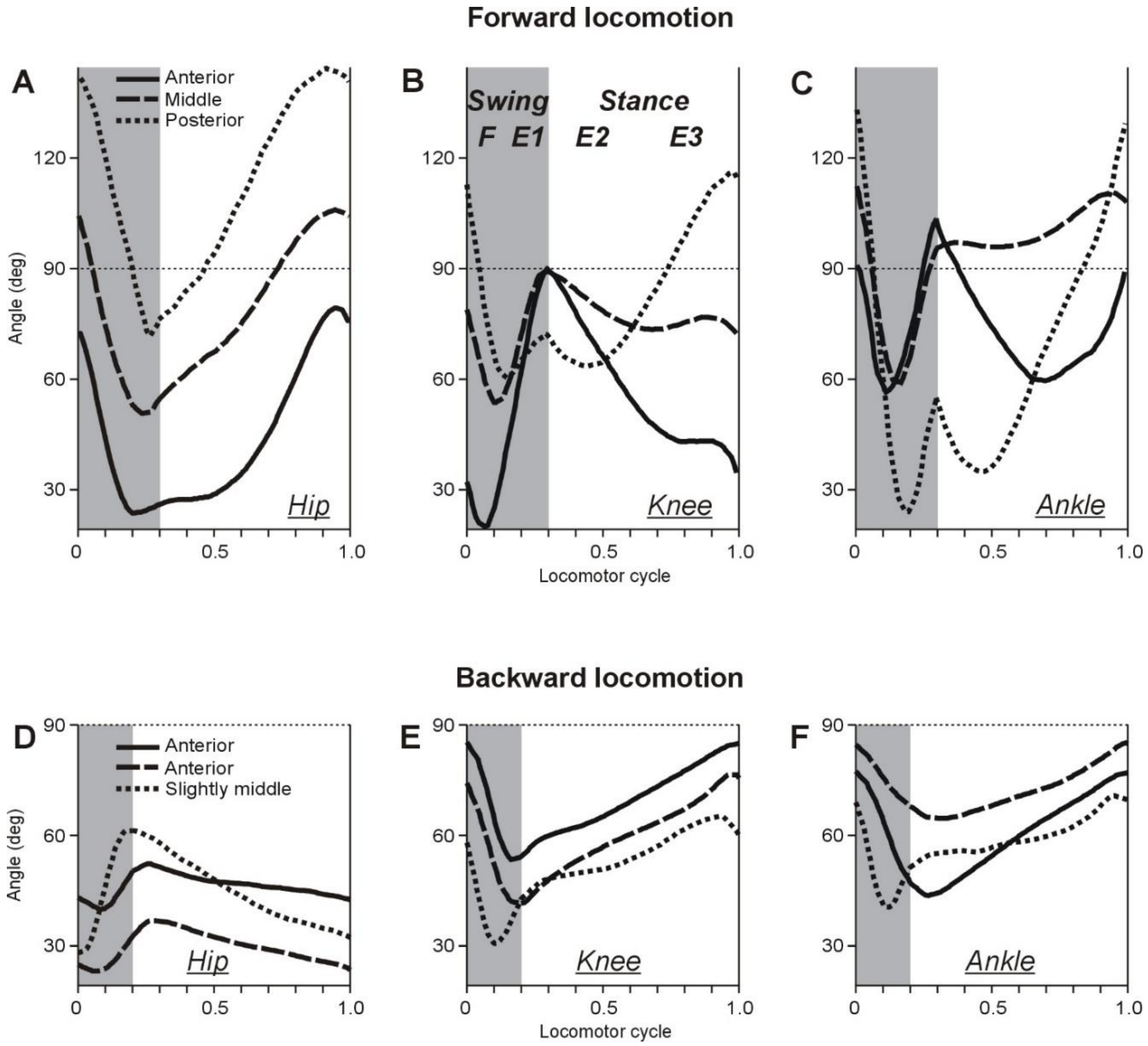

Figure 9. Joint angle profiles in mice during FW and BW locomotion performed with different body configurations. Averaged profiles of joint angles change at the hip (**A and D**), knee (**B and E**) and ankle (**C and F**) joints. The line style indicates the type of stepping.

Because mice and cats perform FW and BW locomotion with different trunk configuration, different joints contribute to generation of FW and BW propulsive force. During FW locomotion in mice and cats the lumbar spine is maintained at horizontal position, and therefore, the thigh segment has vertical orientation at mid-stance. Thus, extension of the hip joint during stance is effective for providing thrust to generate FW propulsion. By contrast, during BW locomotion, the adaptive posture of flexed lumbar spine results in a more horizontal thigh position, whereas the shank is more vertical, and thus knee extension provides BW propulsion. By contrast, humans and dogs maintain the same trunk configuration during both FW and BW locomotion, their BW joint angle profiles represent reversed FW joint angle profiles and the hip joint has a major contribution to generation of both FW and BW propulsive force (Thorstensson, 1986; Grasso et al., 1998; Vilensky and Cook, 2000).

Comparison of EMG patterns during FW and BW locomotion

Figure 10 shows the average activity of four muscles during FW and BW locomotion in tunnel as a function of the step phase. For average, the locomotor episodes were used in which we did not find any substantial differences in amplitude, phasing and the burst shape of the recorded muscles although the animals performed locomotion with slightly different hip heights. In general, regardless of direction of locomotion, tibialis anterior (Tib, ankle flexor) was active mainly during swing, while gastrocnemius lateralis (Gast, ankle extensor) and vastus lateralis (Vast, knee extensor) - during stance, although during BW locomotion, burst onset in each of three muscles occurred slightly earlier. Thus, during both FW and BW locomotion, Tib contribute to flexion of the ankle joint observed in swing, while Gast and Vast – to extension of the ankle and knee joints, respectively, observed in stance during both FW and BW locomotion.

In contrast to aforementioned muscles, the activity of biarticular posterior biceps femoris (Bic, hip extensor and knee flexor) strongly depended on direction of locomotion. During BW locomotion, the EMG burst in Bic was almost entirely confined to the swing phase, while during FW locomotion, its amplitude was the highest in the second half of stance. Most likely this muscle contribute to hip extension observed during swing phase of BW locomotion and to knee flexion observed during second half of stance phase of FW locomotion.

Overall the amplitude of EMG bursts was higher during BW locomotion and this might be due to shorter cycle duration that demanded higher accelerations and higher forces. Also, during BW locomotion, interburst activity in all four muscles was noticeably higher than during FW locomotion, suggesting that during BW locomotion the joint stiffness is higher throughout the cycle as compared to that observed during FW locomotion.

Our results obtained in mice are similar to those obtained earlier in cats (Buford and Smith, 1990; Musienko et al., 2012).

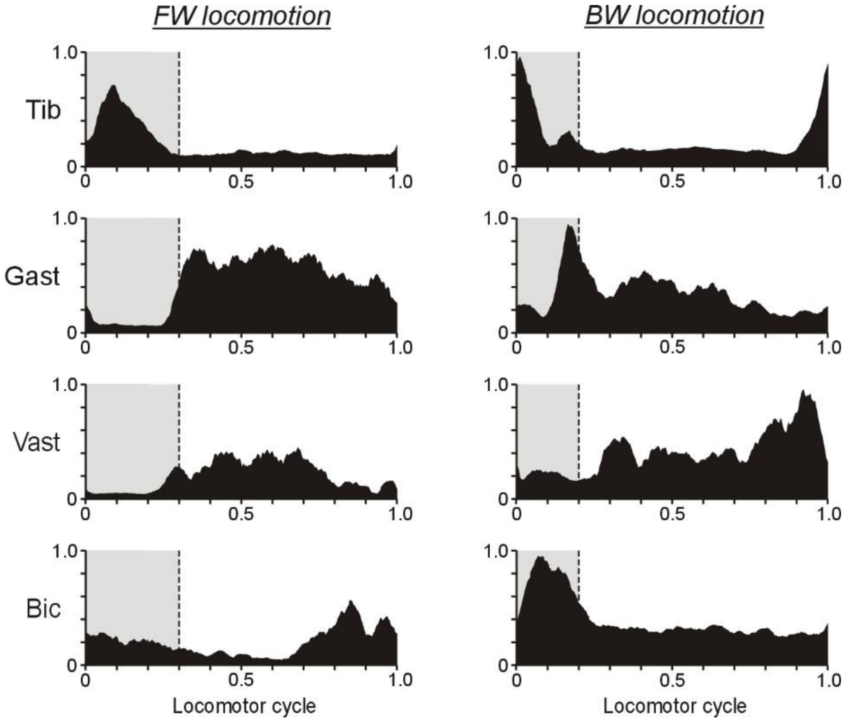

Figure 10. Comparison of average EMG patterns of hindlimb muscles recorded during of FW and BW locomotion performed in the tunnel. The step cycle was normalized to 1.0; the beginning of the swing phase was taken as the cycle onset. Swing phases are highlighted. Abscissa: phase of step cycle; ordinate: EMG (arbitrary units).

Role of V0 commissural interneurons in generation of different motors behaviors

The aim of the present study was to reveal the functional role of two subpopulations of V0 commissural interneurons ($V0_V$ and $V0_D$ CINs) in control of a number of basic motor behaviors observed in the majority of higher vertebrates (i.e. BW locomotion, scratching, righting and postural corrections). For this purpose two types of knockout mice were used: *Hoxb8*^{Cre};*Dbx1*^{DTA} mice ($V0^-$ knockouts) with V0 CINs ablated in the spinal cord only (caudally to C4 segment), and *Vglut2*^{Cre};*Dbx1*^{DTA} mice ($V0\text{-}Glu^-$ knockouts) with $V0_V$ CINs ablated in the entire CNS (Talpalar et al., 2013). Kinematics of movements performed during a particular behavior by two types of mutant mice was compared with that performed by their wild-type littermates.

The majority of V0-KO mice had postural asymmetry when standing on a horizontal surface in contrast to V0v-KO mice and wild-type littermates (control mice) which had symmetrical body posture. In twelve out of fifteen V0-KO mice the dorso-ventral axis of the hindquarters was tilted to the left while it was titled to the right in one animal. In addition, asymmetrical animals had bending of the tail to the opposite side of the hindquarter tilt. We did not find any significant difference between results obtained in V0-KO mice with and without hindquarters tilt and they were therefore pooled. Similarly, if a difference between control data obtained in wild-type littermates of $V0_V$-KO and V0-KO mice was insignificant, they were pooled for analysis.

Role of $V0_V$ and $V0_D$ CINs in control of FW and BW locomotion

In the present study, mice performed FW locomotion with low frequency (<4Hz) and BW locomotion with higher frequency (6-10 Hz). We found that in $V0^-$ knockouts, majority of parameters of FW and BW locomotor movements (step amplitude, frequency, and stance duration) differed significantly from those observed in control mice, while in V0-Glu^- knockouts they were similar to those in control mice. These results suggest that $V0_D$ CINs may contribute to setting of these characteristics of locomotor movements.

In our study (Fig. 11), control mice exhibited alternation of fore and hindlimbs during both FW and BW locomotion. In $V0^-$ knockouts, synchronous hindlimbs movement was observed, while V0-Glu^- knockouts exhibited rather irregular hindlimb alternation (with double and missed steps, as well as hops in more than 30% of reference hindlimb cycles). Thus, our results confirm the dominating role of inhibitory $V0_D$ CINs in control of hindlimbs alternation at low (< 4 Hz) frequencies of FW walking (Bellardita and Kiehn, 2015; Talpalar et al., 2013), but suggest that $V0_V$ CINs may also contribute to execution of hindlimb alternation at low frequency, but do not play a decisive role.

Previous study (Bellardita and Kiehn, 2015) suggested that after ablation of all V0 CINs causes in-phase forelimb coordination at all FW locomotor speeds. However, we found that $V0^-$ knockouts did not exhibit forelimb hopping during FW locomotion, but their alternation was substantially distorted (double and missed steps, hops in about 40% of reference hindlimb cycles). Thus, most likely non-V0 CINs play a crucial role in alternation of forelimbs at low FW locomotion frequencies.

Both V0-Glu^- and $V0^-$ knockouts exhibited hindlimbs hopping, respectively, in 70 % and 90% of cycles during BW locomotion, suggesting that $V0_V$ CINs determine hindlimbs alternation, while $V0_D$ CINs may also contribute though do not play decisive role. Finally, in $V0^-$ knockouts forelimbs hopped while in V0-Glu^- knockouts - alternated during BW locomotion, suggesting that $V0_D$ CINs determine alternation of forelimbs.

Thus, the role of two subpopulations of V0 CINs in control of fore and hindlimbs coordination during BW locomotion performed with frequency 5-10 Hz is similar to that reported earlier during FW locomotion performed with the same frequency range (Bellardita and Kiehn, 2015): $V0_V$ CINs determine alteration of the hindlimbs and inhibitory $V0_D$ CINs – alternation of the forelimbs. It is well documented that at the same frequency of locomotor movements in quadrupeds the speed of BW locomotion is significantly lower than that of FW locomotion (Buford et al., 1990; Vilensky et al., 1987). The observation that the same subpopulation of V0 CINs determines hindlimb coordination during both FW and BW locomotion performed at the same frequency but with different speed show that interlimb coordination depends on locomotor frequency and not on speed. We suggest that the supraspinal command that set a definite frequency of locomotor movements leads recruitment of a specific subpopulation of CINs, which is responsible for left-right limb coordination at the set frequency. Most likely, this command is addressed to the part of the spinal locomotor network involved in generation of both FW and BW locomotion. It was demonstrated in cats that this network generates the vertical component of the step and contains the rhythm generating mechanism (Deliagina et al., 2019; Musienko et al., 2012; Zelenin et al., 2016).

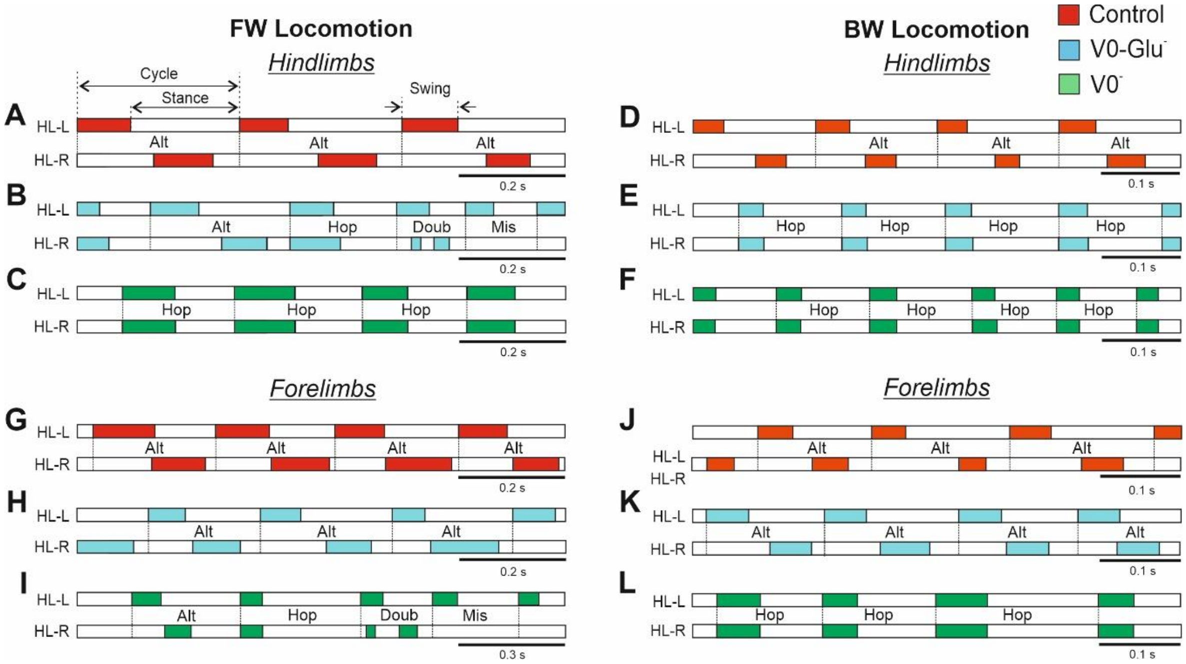

Figure 11. Interlimb coordination during FW (**A-C, G-I**) and BW (**D-F, J-L**) locomotion in hindlimbs (**A-F**) and forelimbs (**G-L**). Note: Hindlimb and forelimb alternation in control animals, hopping and mixed gait in knockouts. (Alt: alternation, Hop: hopping, Mis: missed, Doub: double steps).

Role of V0$_V$ and V0$_D$ CINs in generation of scratching

We found that as in all studied mammals (Stein, 1983), in control mice, unilateral stimulation evoked scratching performed by the ipsilateral hindlimb. By contrast, both V0 and V0-Glu knockouts in response to unilateral stimulation performed scratching by both hindlimbs. These results suggest that during scratching in control mice, excitatory V0$_V$ CINs (which are only absent in V0-Glu knockouts) actively inhibit (through contralateral inhibitory interneurons, Quinlan et al., 2007; Nishimaru et al., 2006) network generating scratching movements of the contralateral limb. Existence of a strong reciprocal inhibition between the left and right scratching networks was suggested earlier, since bilateral stimulation causes alternation in scratching episodes performed by the left and right hindlimb (Stein et al., 1995). We now provide definite evidence that this is the case.

In knockouts the bilateral scratching movements were synchronous and the average frequency of scratching movements in V0$^-$ knockouts was similar to that in control mice, while in V0-Glu$^-$ knochouts it was slightly lower. However, in both knockouts the amplitude of scratching movements in all planes in the hindlimb ipsilateral to the side of stimulation was significantly larger but in the hindlimb contralateral to the side of stimulation – significantly smaller than in control mice. This suggests that the ipsilateral scratch network is activated first which in turn activates and synchronizes the contralateral scratch network through excitatory (presumably V3) populations of CINs.

We found that in V0-Glu$^-$ knockouts, the amplitude of the ipsilateral and contralateral limb protraction, which preceded the rhythmical movements were similar to that observed in control mice, while the corresponding amplitudes of protraction in V0$^-$ knockouts were significantly smaller than those in V0-Glu$^-$ knockouts. These results suggest that V0$_D$ CINs (which are absent only in the V0$^-$ knockouts) contribute to generation of the postural component of scratching (hindlimb protraction). It was also shown that sensory feedback from the limb signaling execution of hindlimb protraction gates the command addressed to spinal network generating the rhythmical component of scratching, so the rhythm is generated only if the hindlimb is protracted (Deliagina et al., 1975). In V0$^-$ knockouts, despite dramatic reduction of the protraction of both limbs, the rhythmic component was generated. This suggests that V0$_D$ CINs may be involved in gating of the descending command activating the rhythm-generating network.

Role of V0$_V$ and V0$_D$ CINs in generation of righting behavior

In both control and knockout mice, righting of upside-down position was performed in two Stages. During Stage 1 and Stage 2, respectively, righting of the forequarters and hindquarters was observed. We found that the latency of Stage 1 in V0-Glu$^-$ knockouts was twofold longer than in control mice, and in V0$^-$ knockouts (in trials with the first successful attempt) it was twofold longer than in V0$_V$$^-$ KO mice, suggesting that both V0$_V$ and V0$_D$ CINs contribute to processing of sensory information causing initiation of Stage 1. V0-Glu$^-$ knockouts performed Stage 1 in first attempt and duration of Stage 1 was similar to that in control, suggesting that V0$_V$ CINs do not contribute to generation of Stage 1. By contrast, in majority of V0$^-$ knockouts the animal performed 1 to 8 unsuccessful attempts before successfully performing Stage 1. The duration in successful attempts was significantly longer than in control suggesting that V0$_D$ CINs are essential elements of the network generating Stage 1. We found a dramatic increase in duration of Stage 2 in both V0$^-$ and V0-Glu$^-$ knockouts as compared to control with the longest duration in the V0$^-$ knockouts, suggesting that both V0$_V$ and V0$_D$ CINs contribute to generation of Stage 2.

Most likely body movements observed during Stage 1 (ventral flexion of the trunk accompanied by twisting and lateral bending of the forequarters in relation to the hindquarters) and Stages 2 (hindquarter twisting in relation to the forequarters) require reciprocal coordination in activity of homonymous axial muscles. One can suggest that V0$_D$ CINs substantially contribute to reciprocal coordination in activity of axial muscles causing the body configurations leading to leftward and rightward body rolling during Stage 1, while both $V0_V$ and $V0_D$ CINs contribute to reciprocal coordination in activity of muscles causing leftward and rightward rotation of the hindquarters in relation to the forequarters during Stage 2. Ablation of V0 CINs decreases asymmetry in activity of muscles causing movements in opposite directions leading to an increase in duration of movements performed during Stage 1 and Stage 2.

The majority of V0-KO mice had postural asymmetry and developed scoliosis. Scoliosis usually develops in animals spinalized in the thoracic region (Ouellet and Odent, 2013) because these animals for a long time maintain an asymmetrical body posture with the hindquarters laying on the side because of inability to perform Stage 2 of righting. It is possible that in the absence of all V0 neurons newborns can accomplish - although with difficulty - Stage 1 of righting, but cannot perform Stage 2. As a result, they maintain an asymmetrical body posture leading to development of scoliosis.

Role of V0 CINs in generation of postural corrections

Previous studies (Beloozerova et al., 2003; Deliagina et al., 2006) demonstrated that the postural system stabilizing the dorsal side-up orientation of the trunk consists of two relatively independent sub-systems responsible for stabilization of the anterior and posterior parts of the trunk, and driven by somatosensory inputs from the fore and hindlimbs, respectively. To compare efficacy of postural corrections stabilizing the dorsal side-up orientation of the posterior part of the trunk in knockouts and their littermates, we calculated the coefficient of their hindquarters stabilization (see Materials and Methods for details). We found that the coefficient of stabilization in $V0\text{-}Glu^-$ knockouts was similar to that in their wild-type littermates, suggesting that $V0_V$ CINs do not contribute to generation of postural corrections. By contrast, in $V0^-$ knockouts, it was significantly lower than that observed in their wild-type littermates, suggesting that $V0_D$ CINs are essential elements of the postural networks responsible for generation of corrective movements.

We found that as in cats, rabbits and rats (Beloozerova et al., 2003; Deliagina et al., 2000, 2006), in control mice corrective movements of the hindlimbs evoked by platform tilts are caused by reciprocal coordination of extensor activity in left and right hindlimbs with activation of extensors on the tilted side and inactivation of extensors in the opposite limb. One can suggest that $V0_D$ CINs mediate sensory input from the limb loaded by the tilt that contribute to inactivation of extensors in the opposite limb. Thus, ablation of $V0_D$ CINs cause disinhibition of extensor motor neurons leading to a decrease in the amplitude of the limb corrective movement and resulting in a decrease in efficacy of postural corrections.

Neural mechanisms underlying single corrective steps in different directions

The aim of the present study was to analyse the neural mechanisms underlying generation of postural responses to disturbance of the basic standing body configuration evoked by outward, forward and backward displacement of a single hindlimb. The postural responses were analysed in freely standing intact, decerebrate premammillary standing rabbit, as well as in fixed decerebrate postmammillary rabbits.

Two components of motor response to distortion of the basic body configuration

We found that distortion of the basic body configuration in intact rabbits evoked by displacement of one of the hindlimbs evoked a postural response that consisted of two components which were executed in a strict sequence. First, a trunk displacement towards the supporting limb (that caused supporting limb loading and simultaneous unloading the contralateral displaced hindlimb) was observed. Then, the displaced and unloaded hindlimb performed a corrective step in the direction opposite to the direction of its displacement. During the corrective step the direction of the trunk movement was reversed (towards the stepping hindlimb) and the trunk returned to the initial position at the end of the step, while the corrective step returned the displaced hindlimb to the initial position. Thus the initial basic body configuration was regained. Similar postural reaction was described in humans. Horizontal translation of the support surface caused, first, the body weight shift towards the supporting leg and then a corrective step was performed by the unloaded leg in the direction opposite to the direction of the surface displacement (Maki et al., 1996).

The time difference between onsets of two components of the postural response (the corrective trunk movement and corrective step) varied considerably. In addition, we found that premammillary rabbit can perform the corrective step without execution of the corrective trunk movement. Altogether, these results suggest that two separate mechanisms generate the corrective trunk movement and the corrective step, as schematically shown in Fig.11. Since in intact rabbit they are activated in a particular order (first, the trunk mechanism and then the step mechanism), one can suggest that sensory signals about supporting hindlimb loading (indicated by grey arrow in Fig. 12) gate the command activating the step mechanism. Thus, the corrective step is executed only when the trunk displacement creates a strong asymmetry in loading of the supporting and displaced hindlimbs. Fluctuations in the excitability level in the trunk and step mechanisms could explain a large time variability between onsets of the execution of the corrective trunk movement and a corrective step.

Sensory control of the postural response to the limb displacement

Postural response to limb displacement is caused by sensory information signalling disturbance of basic body configuration. We found a positive correlation between the amplitude (but not velocity) of the limb displacement and the step length that indicates that sensory information signalling the limb-trunk/limb configuration (most likely transmitted by group II afferents) activates the mechanisms generating corrective steps in different directions.

Earlier it was shown (Rademaker, 1931) that inward (medial) or outward (lateral) displacement of the hindlimb in relation to the trunk evokes a lateral or medial step, respectively. It was suggested that these responses are evoked by sensory signals from stretch receptors of abductor and adductor muscles (Rademaker, 1931, Rademaker and Hoogerwerf, 1930). We found that in postmammillary rabbits with fixed spine, displacement of the hindlimb not only evoked a corrective step, but also activated extensors in the contralateral hindlimb, suggesting that both a corrective step and an increase in the stiffness of the contralateral (supporting) hindlimb were elicited by sensory signals from the deviated hindlimb. However, in intact rabbits, one cannot exclude contribution of load-compensating reflexes generated in response to limb loading produced by the corrective trunk movements.

We found that displacement of limb in any direction distorts not only the limb/limb-trunk but also trunk configuration. Displacement of limb caused passive bending of the trunk in different directions [towards the supporting limb during forward and outward limb deviation and towards the stepping limb during backward limb deviation] but the corrective trunk movements always remained the same - bending towards the deviated limb and twisting towards the supporting limb). These results suggest that the mechanism generating the trunk movement is triggered by sensory information signalling disturbance in the limb/limb-trunk configuration (as shown schematically in Fig.11) but not about the disturbance in the trunk configuration.

To evaluate the role of sensory feedback from the displaced limb during the corrective step, we applied a gradually increasing lateral force by means of an elastic string attached to the ankle joint. We demonstrated that the sensory feedback from the limb performing a corrective step can alter all aspects of the postural response (the amplitude of the stepping limb lift, the amplitude of the trunk corrective movement, the functional length of the supporting limb, duration and amplitude of the corrective step) to secure landing of the displaced limb close to its initial position. It was reported that during FW locomotion the sensory feedback from the limb during swing phase signaling the current extent of hip adduction or abduction changes the swing trajectory to ensure that the foot position in relation to the trunk at the beginning of stance (the moment of the limb loading) is always the same (Musienko et al., 2014).

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

Steps in different directions in relation to the trunk, as well as a lateral movement of the trunk can be generated not only in context of postural corrections executed by standing subject. Majority of quadrupeds and bipeds are capable to perform sequential stepping in different directions (forward, backward, sideward) generated in context of locomotion (Stein et al., 1986b; Buford and Smith, 1990; Buford et al., 1990; Rossignol, 1996; Deliagina et al., 1997; Zelenin et al., 2011; Musienko et al., 2012). Inward and outward single steps are also used for regaining balance during FW walking (Karayannidou et al., 2009; Musienko et al., 2014; Hof et al., 2010; Hof and Duysens, 2013). It was reported that the trunk exhibits lateral oscillations in the rhythm of stepping during undisturbed stationary FW locomotion (Karayannidou et al., 2009; Misiaszek, 2006). One of the important questions is whether the same or different neuronal mechanisms generate trunk movements and limb stepping in context of locomotion (i.e. during sequential stepping in a specific direction in relation to the trunk) and in context of postural reactions to disturbance of posture.

At present there are some indirect evidences supporting the hypothesis that the neural mechanisms are the same. Thus, during FW locomotion the trunk movement towards the contralateral limb starts before initiation of swing in the ipsilateral limb and the direction of the trunk movement reverses during swing performed by ipsilateral limb (Karayannidou et al., 2009; Misiaszek, 2006), like during corrective steps. Furthermore, the trunk muscle (oblique externus abdominis, OEA) that evokes ipsilateral bending and contralateral twisting the trunk during postural response, is active rhythmically during FW locomotion. The period of its activity in the cycle of the ipsilateral limb (from the second half of the stance to the middle of swing; Deban et al., 2012) is similar to that observed during postural response. Also, back muscles (erector spinae and multifidus) which do not contribute to trunk corrective movements during postural response, do not exhibit step-related modulation during locomotion (Musienko et al., 2014) and most likely they are involved in control of back stiffness (Carlson et al., 1979). Finally, the activity phases of the hip and ankle flexors, as well as of the posterior biceps (hip extensor and knee flexor) which we observed in the limb performing forward and backward corrective steps are similar to those reported earlier during forward and backward locomotion in the intact cat (Buford and Smith, 1990).

Recently it was found that during locomotion in postmammillary cat was initiated by epidural stimulation of the spinal cord, the direction of stepping (in relation to the trunk) was determined by the sensory feedback from the limb signalling direction of movement (in relation to the trunk) performed by the limb during stance. These signals evoked the step in opposite direction. In the absence of these sensory signals, in place stepping was performed. It was suggested, that locomotor system contains two mechanisms generating the vertical (limb elevation and lowering), and horizontal (limb transfer from one extreme position to the other) components (Step mechanism in Fig. 12). The mechanism generating the horizontal component of the step is activated by the sensory feedback signalling direction of the displacement of the limb in relation to the trunk during stance. When during stance the limb reaches an extreme position these sensory signals evokes the limb transfer in opposite direction (Musienko et al., 2012). One can suggest that sensory information signalling about direction of the limb deviation in relation to the trunk in standing animal activate both mechanisms that results in generation of both components of the postural response – a corrective trunk movement and a single corrective step (Fig. 12).

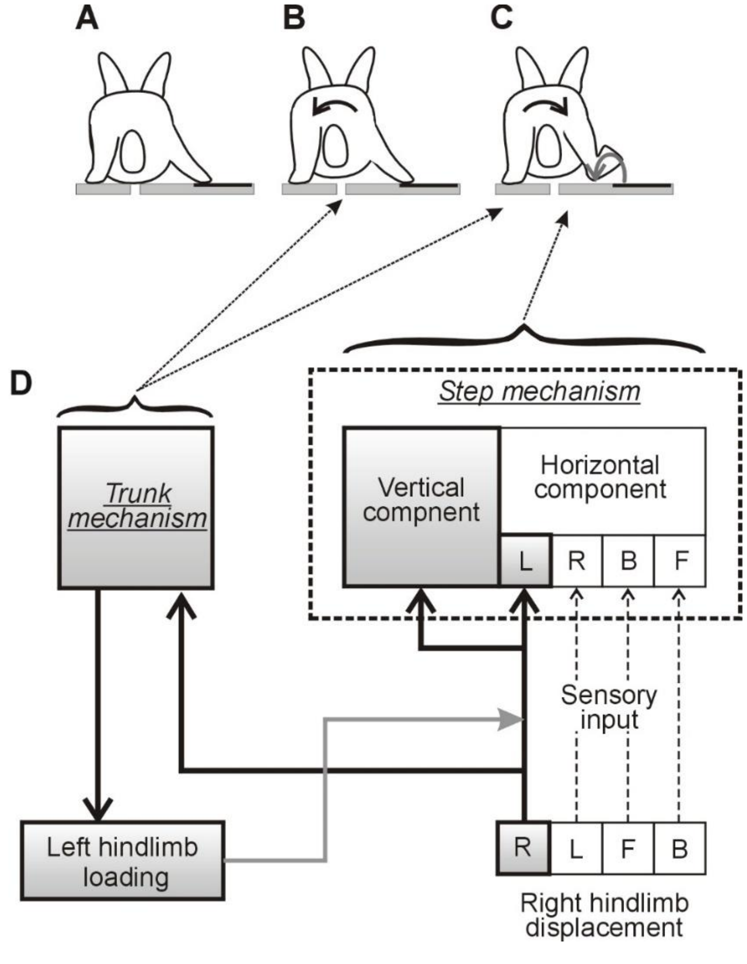

Figure 12. Hypothesis about mechanisms generating postural response to disturbance of the basic body configuration. Sensory signals caused by rightward displacement of the right hindlimb (**A**) addressed to both Trunk and Step mechanisms (**D**) cause, first, activation of the Trunk mechanism since symmetrical loading of the limbs prevent activation of the step mechanism. Activation of the trunk mechanism causes the trunk displacement to toward the left hindlimb (indicated by arrow in **B**) that results in its loading. Sensory signals about left limb loading (grey arrow in **D**) allows sensory signals from deviated right limb to activate the step mechanism (open the gate), which generates a leftward corrective step of the right limb (**C**). During the corrective step the direction of the trunk movement reverses (indicated by arrow in **C**).

CONCLUSIONS AND FUTURE PERSPECTIVES

Locomotion and control of posture are vital motor function for any living being. Most terrestrial quadrupeds are capable in performing not only FW, but also BW and sideward locomotion. Single corrective steps are used in context of postural corrections to restore distorted balance during standing and locomotion. Despite the fact that FW locomotion was intensively studied during past few decades, our understanding of organization and operation of the networks generating locomotor movement in mammals are still very far from complete. In contrast to FW locomotion, our knowledge about neural mechanisms generating stepping in other directions (backward, sideward) are extremely limited. In the present study neural mechanisms contributing to control of stepping in different direction, control of balance and body configuration were investigated.

Due to development of genetic approaches for analysis of neuronal networks, nowadays mice are widely used for investigation of neuronal mechanisms of locomotion. In the present study, kinematics of FW and BW locomotor movements performed by mice on a number of different setups, which are generally used for analysis of locomotor system (i.e. on the treadmill, air-ball and in the tunnel), has been characterized for the first time. We found that during BW locomotion on all setups, the average step amplitude, speed and swing duration were significantly reduced compared with those observed during FW locomotion. We demonstrated that the hip height maintained during a locomotor episode determines the rostro-caudal position of the paw trajectory in relation to the hip. When the hip height was low, the trajectory was displaced either caudally (posterior steps) or rostrally (anterior steps) in relation to the hip, while when the hip height was high, the trajectory was symmetrical in relation to the hip projection to the surface (middle steps). During FW locomotion, we observed all three forms of stepping in the tunnel and mostly posterior and anterior stepping on the air-ball and treadmill, respectively. By contrast, during BW locomotion, only anterior stepping was observed on all setups. Intralimb coordination strongly depended on the form of steps. Hindlimb joints were coordinated to maintain the hip at constant height during stance and during swing to obtain smallest functional limb length when the limb passed under the hip. The obtained data represent a benchmark for future studies based on optogenetic and chemogenetic manipulations with activity of specific populations of spinal, supraspinal and brain neurons aimed to reveal their roles in control of particular aspects of locomotor movements analyzed in the present study.

Comparison of characteristics of movements performed by control, V0-Glu$^-$ and V0$^-$ knockout mice during a number of basic motor behaviors (FW and BW locomotion, scratching, righting, postural corrections) allowed us to suggest the functional role of $V0_V$ and $V0_D$ CINs in control of these movements. We found that $V0_V$ CINs determine alternating movements of hindlimbs during BW locomotion, play a crucial role in inhibition of contralateral scratching CPG, as well as contribute to initiation of righting and in generation of Stage 2 of righting. In all these tasks, the functional effect of excitatory $V0_V$ CINs is inhibitory, which is mediated by inhibitory interneurons of contralateral side. We have shown that inhibitory $V0_D$ CINs determine alternating movements of forelimbs during BW locomotion, contribute to initiation and generation of both Stages of righting as well as in generation of postural corrections. Thus, our study shows the differential contribution of two subpopulations of V0 CINs to different motor behaviors as well as in control of different aspects of the same behavior. However, it should be noted that ablation of V0 CINs can lead to some adaptive plastic changes in developing CNS. Suggestions in the present study are based on an assumption that these plastic changes did not affect the principle organization of the networks underlying studied behaviors. To test the validity of this assumption, future studies with temporal activation and inactivation of $V0_V$ and $V0_D$ subpopulations of CINs are necessary.

In the rabbits, we characterized the postural response to distortion of the basic body configuration caused by forward, backward or sideward displacement of the hindlimb. We have shown that the response included the corrective trunk movement and a corrective step that resulted in restoration of the basic standing body configuration. We found that these two components of the postural response were generated by different mechanisms, which were activated, however, in a strict order by sensory input from the deviated limb signalling disturbance of the limb-trunk/limb configuration. We demonstrated that main networks generating the postural reaction to disturbance of the basic body configuration are located in the brainstem, cerebellum and spinal cord. We hypothesized that the same networks generate a single corrective step (in context of postural reactions) and sequential stepping (in context of locomotion) in a particular direction. To test this hypothesis, activity of the same individual neurons during both single corrective steps and sequential stepping in a particular direction should be recorded in future studies.

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