Motor reorganization during simulation of gravitational unloading

Artur Fedianin
Kazan Federal University
Kazan, Russia
artishock23@gmail.com

Tatiana Zaytceva
Kazan Federal University
Kazan, Russia
tatana.nikolaevna@ya.ru

Maxim Baltin
Kazan Federal University
Kazan, Russia
baban.bog@mail.ru

Sofya Bikeeva
Kazan Federal University
Kazan, Russia
sofya.bikeeva01@mail.ru

Victoria Smirnova
Kazan Federal University
Kazan, Russia
yaikovavictoriya@mail.ru

Oscar Sachenkov
Kazan Federal University
Kazan, Russia
4works@bk.ru

Tatiana Baltina
Kazan Federal University
Kazan, Russia
tvbaltina@gmail.com

Anton Eremeev
Kazan Federal University
Kazan, Russia
2anton.eremeev@mail.ru

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***Abstract*— The structures of the central nervous system and, in particular, the neuronal circuits of the spinal cord, by integrating afferent and efferent signals, play a key role in the organization of motor skills. The functional state of the motor systems of the rat calf muscle after simulated gravitational unloading of the hind limbs was assessed by the methods of registration of MEP during magnetic stimulation of the spinal cord and video analysis of movements. It is shown that limitations in the intensity of peripheral afferentation, including the support one, initiate the reorganization and change in the functional state of the spinal neuronal networks that regulate and adapt motor activity in accordance with external conditions.**

Keywords—antiorthostatic hanging, movement analysis, motor neuronal networks

I. Introduction

The key role in the control of morphofunctional characteristics of motor systems belongs to the neuronal networks of the spinal cord [1,2]. Despite the encouraging results of studies aimed at increasing the efficiency of the compensatory reorganization of neuromotor systems when the gravitational environment changes, the preventive methods used do not provide a significant improvement in the motor qualities of astronauts. In recent years, transcranial magnetic stimulation has been used in experiments to study descending influences on the excitability of the motor neuron pool of the lumbar enlargement in humans. Most studies using this method are devoted to studying the mechanisms of movement control [3,4], studying the excitability of cortical motor neurons and their effect on spinal structures [5], assessing the electrical and mechanical activity of tired muscles [6], and analyzing electromyographic characteristics during walking [7], cortico-spinal mechanisms of adaptation during sensorimotor training [8]. For a more complete analysis of motor function, an adequate system for determining changes in coordination and the motor ability itself is also needed. For this purpose, “motion capture” (Mo-cap) systems are mainly used. This technique can significantly improve gait study protocols, specifically select the treatment of injuries, and allow creating individual conditions for the recovery of patients with disorders of the musculoskeletal system and a wide range of CNS disorders [9].

In the present study, during magnetic stimulation of spinal cord and video analysis of movement was assessed the functional condition of motor system of rat calf muscle after modelling gravitational unloading of the hind limbs.

II. Matherials and Methods

The study was carried out on non-linear laboratory sexually mature male rats weighing 180–220 g (n=15). The experimental protocol was approved by the Local Ethics Committee of the Kazan Federal University Protocol No. 30 dated 06.28.2021. Animals were withdrawn from the experiment in accordance with the principles of the Basel Declaration and ARRIVE instructions [10].

To simulate gravitational unloading in rats (n=9), the generally accepted method of antiorthostatic hanging by the tail for 7 days was used [11,12].

After completion of exposure to experimental conditions, the functional state of the following neuromotor systems was assessed: m. soleus, m. gastrocnemius, m. tibialis anterior - the corresponding spinal motor centers. The motor evoked potential (MEP) of the studied muscles was recorded during magnetic stimulation of the cervical and lumbar enlargement of the spinal cord (C5-Th1 and L1-L4). The center of the coil was placed 3-5 mm from the dorsal surface of the animal's body along the midline in the projection of the corresponding thickening of the spinal cord. The threshold of occurrence, maximum amplitude, latency, and duration of potentials were determined. To immobilize the animal, intramuscular analgesia was used. The experiments used a research setup (Neurosoft, Russia) including a «Neuro-MS/D» magnetic stimulator, a «Neuro-MVP»-4 4-channel electroneuromyograph, and the «Neuro-MVP.NET» software.

The kinematic characteristics of the locomotor motor activity of the pelvic limbs of rats were assessed by the method of video analysis of movements. Three-dimensional data were obtained using six Vicon MX cameras (UK) placed on special mounts in a semicircle. An Active Wand calibration marker (UK) was used to calibrate and synchronize the cameras. A Rekam DVC-340 video camera (Canada) was used to obtain a standard video image. Passive reflective markers were placed in the projections of the joints of the pelvic limbs of the rat. During video capture, the rats moved freely on a flat horizontal surface (1.5 x 1.5 m). Spline interpolation was used to resample the Vicon data up to 30 Hz before analysis. The phases of the gait cycle were

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determined with time marks of gait events - foot separation and resumption of contact with the surface, angles in the joints. Kinematic analysis was performed for the complete walking cycle of each study rat. When processing the obtained data, the Vicon Nexus 2.9 software (UK) was used to manually complete the 3D motion model and remove artifacts from the recording. The data obtained by Vicon Nexus 2.9 were converted into text format using the ASCII module (Schneider Electric, France), and then processed using the MATLAB software (MathWorks, USA).

The data obtained from the study of intact animals (n=6) were used as controls. When assessing the parameters of evoked motor potentials, the experimental data were expressed as a percentage of the values recorded in the control group and taken as 100%. Statistical analysis was performed using ANOVA analysis of variance and Student's t-test. Significance level: *p<0.05.

III. Results

A. Parameters of evoked motor potentials of rat calf muscles during magnetic stimulation of the spinal cord

When analyzing the parameters of motor potentials caused by magnetic stimulation of the cervical thickening of the spinal cord, it was found that the amplitude of MEP m. soleus was 75±10% (p<0.05), amplitude of MEP m. gastrocnemius decreased to 66±9% (p<0.05); MEP latency m. soleus decreased to 79±11% (p<0.05), MEP latency m. gastrocnemius was 69±11% (p<0.05). Other analyzed parameters of m. soleus and m. gastrocnemius, as well as MEP parameters m. tibialis anterior did not differ significantly from control values (Fig. 1).

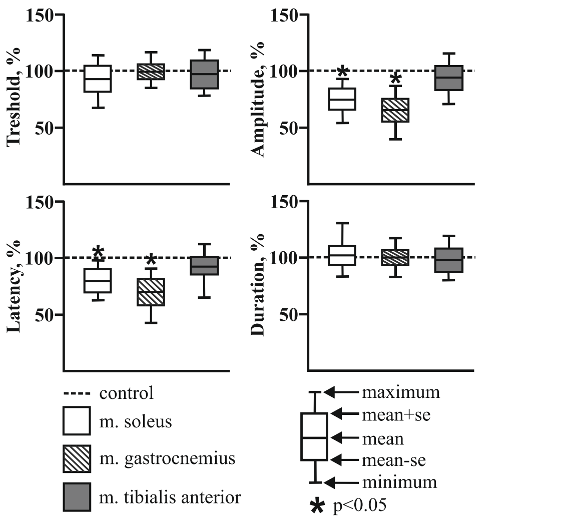

Fig. 1. Parameters of evoked motor potentials of the calf muscles of the rat leg, during stimulation of the cervical thickening of the spinal cord. The y-axis indicates the parameters of evoked motor potentials, expressed as a percentage relative to the control values taken as 100%.

When analyzing the parameters MEP of the lumbar enlargement of the spinal cord, the threshold of MEP of m. soleus was 73±8% (p<0.05), the threshold of MEP of m. gastrocnemius - 65±8% (p<0.05); the amplitude of MEP of m. soleus decreased to 75±10% (p<0.05), the amplitude of MEP of m. gastrocnemius was 48±10% (p<0.05); the latency of MEP of m. soleus reached to 69±11% (p<0.05) the latency of MEP of m. gastrocnemius - 64±8% (p<0.05); the duration of MEP of m. soleus increased to 117±6% (p<0.05); the duration of MEP of m. gastrocnemius was 127±6% (p<0.05). Significant changes in MEP parameters of m. tibialis anterior was not found (Fig.2).

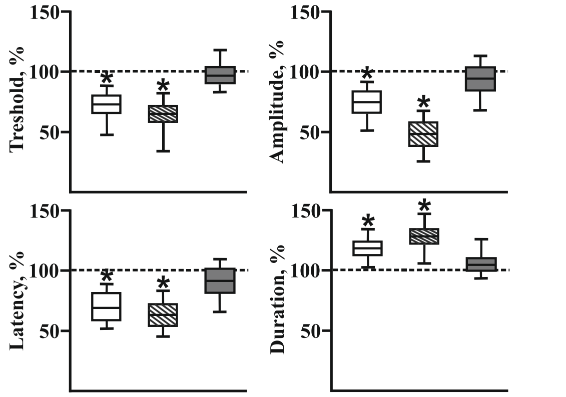

Fig. 2. Parameters of evoked motor potentials of the calf muscles of the rat leg, during stimulation of the lumbar enlargement of the spinal cord. Designations as in fig. 1.

The time of the central motor conduction averaged 74±3% (p<0.05) (when registering the MEP of m. soleus - 73±8% (p<0.05), while registering the MEP of m. gastrocnemius 69±8% (p<0.05), when registering the MEP of m. tibialis anterior 79±9% (p<0.05).

B. Kinematic characteristics of locomotor motor activity of rat hind limbs during movement on a horizontal surface

As a result of the analysis of locomotor motor activity, changes in the angular ranges in the joints of the hind limbs were found. On average, the angle in the knee joint after the simulated gravitational unloading decreased by almost two times, while jumps in the values of the angles appeared, which is associated with a sharp / “twitchy” nature of the movement. The position of the limbs during the movement of animals on a horizontal surface is shown in Fig. 3.

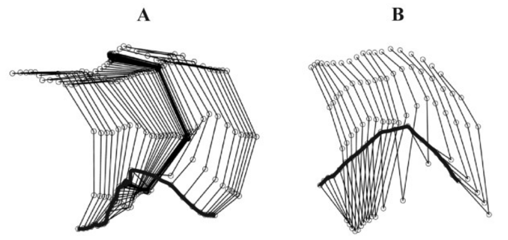

Fig. 3. The position of the limbs during the movement of animals on a horizontal surface: A - normal, B - after simulated gravitational unloading.

In control animals, the range of angles in the hip joint was from 131°±2° to 142°±3°, for the knee joint - from 102°±4° to 127°±4°. After simulation of gravitational unloading, the range of angles in the hip joint was from 154°±2° to 168°±7° (p<0.05), for the knee joint – from 66°±16° to 168°±7° (p<0.05). Differences for upper and lower joint angle thresholds were evaluated. All values were found to be significantly different. For the hip joint, after simulating gravitational unloading, the range of angles increased by 18% (p<0.05), and for the knee joint, the lower threshold decreased by 35% (p<0.05), and the upper threshold increased by 31% (p<0.05). The results are shown in Fig. 4. Movements in the hip joint remained approximately the same volume, but the angle of maximum extension increased. In the knee joint, the nature of movements also changed - the range of motion increased and an extensor setting was formed in the joint.

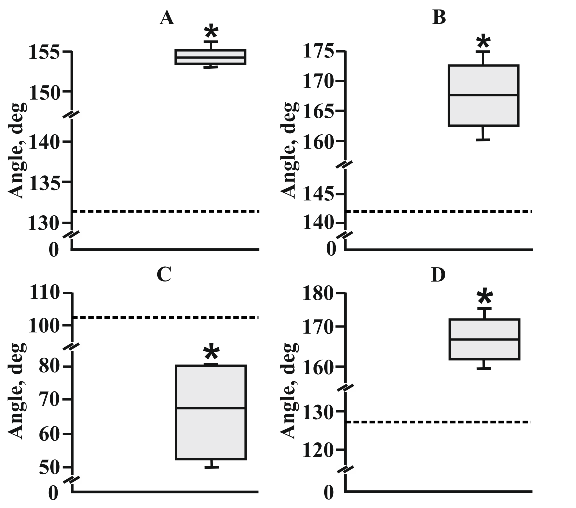

Fig. 4. Angles in the joints of the hind limbs of a rat during movement on a horizontal surface. The y-axis indicates the angles in the joints of the hind limbs in degrees: A - lower threshold, B - upper threshold for the angle in the hip joint; C - lower threshold, D - upper threshold for the angle at the knee joint. Boxing - data obtained after gravitational unloading. Other designations as in fig. 1.

DISCUSSION

Magnetic stimulation is able to penetrate deeply and directly activate the interneuronal networks of the spinal cord [13,14]. During electromagnetic stimulation of the human lumbar spinal cord, rhythmic stepping movements of the lower limbs arose almost immediately after the first stimulus was applied, which can be considered as a result of direct activation of the spinal locomotor networks [15]. Changes in the parameters of rat leg muscle potentials induced by magnetic stimulation of the spinal cord, found in our study during unloading of the hind limbs, probably characterize the adaptive reorganization of motor activity at the level of spinal neuronal networks. It is the motor spinal neuronal structures that play a central role in the organization of motor activity, integrating efferent and afferent signaling and adapting movements to environmental conditions [16]. Significant changes of MEP in the m. soleus (SM) and m. gastrocnemius (GM) recorded in our experiments and the absence of significant changes in the m. tibialis anterior (TA) are consistent with the data described in the literature. It has been shown that the neuromotor systems of the antigravitational (postural) extensor muscles are primarily and to a greater extent subject to functional unloading [17,18].

With stimulation of the cervical thickening and, as a result, activation of the supra- and propriospinal pathways, a decrease in the maximum amplitude and latency of the MEP of SM and GM was recorded. During stimulation of the lumbar thickening and, as a result, direct activation of the neuronal networks that control the muscles of the lower limbs, a decrease in the threshold, maximum amplitude, latency, and an increase in the duration of the SM and GM were recorded. Normally, in rodents, in contrast to humans, descending efferent influences are carried out mainly through interneurons and synaptic connections mediated by them [19]. Under conditions of limited motor activity, with a decrease in corticospinal descending influences, regressive changes are observed [20], which can cause activation of direct monosynaptic connections with motor neurons, bypassing interneurons and reducing synaptic delay. An increase in the speed of the efferent signal, a decrease in the number of motor units activated by stimulation of the spinal cord can cause a decrease in the latency and amplitude of MEP. A decrease in the MEP threshold, in our opinion, indicates an increase in the excitability of motor neurons in SM and GM. An increase in the activity of motor centers with limited peripheral afferentation has been repeatedly shown in previous studies [21,22]. Also, under these conditions, a transformation in the ratio of slow and fast motor units has been shown [23], changes in the pattern of their recruitment [24]. The asynchronous activation of individual motor neurons, which innervate morphologically and functionally different muscle fibers, caused by such a reorganization, probably causes a recorded increase in the duration of MEP. It is clear that the parameters of muscle potentials can also be affected by processes in the peripheral structures of the motor apparatus: the conduction of excitation along efferents, neuromuscular synaptic transmission, excitability and the rate of involvement of muscle fibers. However, it was shown that after 7 days of simulated microgravity, the analysis of the parameters of the M-response of SM and GM caused by stimulation of the peripheral efferents of the sciatic nerve did not reveal changes in the maximum amplitude, latency and duration, a decrease in the threshold was recorded only for the M-response of GM [25, 26]. Limitation of locomotor activity, changes in the intensity of afferent signals from proprioreceptors of extensor and flexor muscles, and limitation of signaling from support receptors probably primarily determine the state of spinal cord neural networks. Sensory afferent feedback plays a crucial role in the regulation of muscle activity, controlling short-term and long-term reorganization of motor neuronal circuits [27].

The registered changes in the functional state of motor neuronal networks probably determine the locomotor pattern. The revealed extensor positioning of the hind limbs during locomotion after gravitational unloading is consistent with the literature data [28]. With a decrease in the activity of primary afferents from the corresponding extensor calf muscles, which are in a state of contraction, hyperexcitability of extensor motor neurons and a discrepancy in the length of extensors and flexors occur [29], which, in turn, leads to hyperextension due to the forced position of the rat's foot during suspension [30]. Restriction of the activity of motor neurons and Ia interneurons should affect the switching of activity from flexion to extensor motor neurons [31]. In recent studies, a hypothesis has been formulated that the transformation of the system of proprioceptive connections under conditions of a modified configuration of the position of the body and joints of the limbs during hanging, as well as the limitation of afferent signaling from the support receptors of the feet, can cause changes in the kinematic characteristics of movements. In addition, the sensitivity of plantar skin receptors involved in maintaining postural activity [32] may be increased after gravitational unloading. Sensory deprivation of the rat hind limb reduces the threshold intensity of skin receptor stimulation and increases the sensitivity of cortical somatosensory neurons [33]. A sharp activation of peripheral afferent influences, including those reciprocal from antagonist muscles, when interacting with the support after unloading, an increase in descending supraspinal signaling, probably also determines the characteristics of the locomotor pattern.

Thus, under conditions of muscle unloading and, as a result, changes in the intensity of peripheral afferentation, including the support one, there is a reorganization and change in the functional state of the spinal neuronal networks that regulate and adapt motor activity in accordance with external conditions. A detailed understanding of the principles of adaptive reorganization of spinal interneuronal circuits requires further research, the results of which will contribute to the development of innovative targeted strategies for the rehabilitation of the musculoskeletal system in cases of various disorders and space expeditions.

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