The functional state of the neuromotor system during hypogravity in a rat. Support load effects.

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Abstract. The main goal of the research was to estimate the morpho-functional condition of the neuromotor apparatus of the rat soleus muscle under simulated gravitational unloading, as well as under simulated gravitational unloading combined with the action of axial load and support reaction force. Model experiments were performed on male laboratory rats weighing 190-210 g in strict accordance with the accepted bioethical standards. Gravitational unloading was simulated by the antiorthostatic hanging method. Within 7 and 35 days. For the action of the support load, the animals were placed daily on a hard horizontal surface for 90 minutes. The H-reflex and the M-wave of the soleus muscle were recorded; the threshold, maximum amplitude, latency and duration of the evoked potentials were determined. Muscle wet and dry weights were also evaluated. We found that during simulated gravitational unloading occurs an increase in the reflex activity of the m. soleus motoneurons, reduces the reliability of neuromuscular transmission, and initiates atrophic processes. Periodic application of a support load under conditions of short-term (but not long-term) simulated hypogravity prevents alteration in the central and peripheral parts of the neuromuscular apparatus of the rat soleus muscle.

1. Introduction

Research of the neuromuscular apparatus in conditions of weightlessness and its modeling have shown that hypogravity motor syndrome triggers changes in all its structures [1-8] and identified the important role of sensory provision of motions in the pathogenesis of weightlessness-initiated motor disorders [2; 6]. During a space expedition or in conditions of earth based weightlessness analogs, such as immobilization and suspension of the hind limbs in rodents, unloading of the skeletal muscles occurs, which is accompanied by atrophy, especially of the antigravity extensor muscles [4, 9]. At the same time, there is a decrease in muscle mass and strength, a change in myosin composition, a limitation of neuromuscular interactions [2, 10-15]. Maintaining the normal functional state of the lower extremity muscles depends in part on the interaction between support reaction forces and plantar receptors, which activate to transmit these stimuli to the CNS [16]. In experiments with spinal cord injury [17-18], hypodynamia and denervation [19, 20], the need for peripheral afferentation was demonstrated to generate a correct locomotor pattern [21-24]. Under unloading conditions, there is no action of axial loads and the reaction force of the support, which leads to disruption of signals usually transmitted along the nerve pathways between sensory receptors, the central nervous system and effectors. Studies conducted under conditions of real weightlessness in humans [25] and under conditions of terrestrial modeling of hypogravity as in humans [26, 27] and rats [28, 29] demonstrated that increased sensory input from foot support receptors leads to activation of the lower extremity muscles, as well as a significant weakening of the atrophy of the soleus muscle. It is the postural slow
soleus muscle that is the subject of most rodent studies in a changing gravitational environment, as it exhibits rapid, sufficiently pronounced transformations [8, 30-32].

The main goal of the research was to estimate the morpho-functional condition of the neuromotor apparatus of the rat soleus muscle under simulated gravitational unloading, as well as under simulated gravitational unloading combined with the action of axial load and support reaction force. Taking into account the integral role played by the central structures of the motor system, activated by peripheral proprio and cutaneous receptors, we assumed that such motor and support stimulation in time for unloading would prevent or reduced hypogravity effects.

2. Materials and methods
Model experiments were performed on male laboratory rats (n = 30) weighing 190-210 g in strict accordance with the accepted bioethical standards (Declaration of Helsinki, 1975, conclusion of the KFU Commission on Bioethics, Protocol No. 2 of 05/29/2015). Animals were divided into two experimental groups: "GU" - animals under simulated gravitational unloading of the hind limbs (n = 13); “GU + Support” - animals in conditions of simulated gravitational unloading of the hind limbs, combined with the action of axial loading and ground reaction force (n = 12). The rats were placed in special cages designed to simulate gravitational unloading (one rat per cage) in the standard vivar conditions. The experiment started no earlier than one week after placing the rat in the cage. The generally accepted method of antithorostatic hanging of a rat by the tail was used to simulate gravitational unloading [33-35] for 7 and 35 days (GU and GU + Support groups). For the action of the axial load and the reaction force, the animals were daily removed from the antithorostatic position and for 90 min were placed on a solid horizontal surface 15 cm x 15 cm under the control of the experimenter (GU + Support group).

To evaluate the neurophysiological specifications of the neuromotor apparatus m. soleus was subjected to electromyographic testing (EMG). EMG in the GU + Support group started no earlier than 3 hours after the final landing procedure. Before testing, the animals were anesthetized (intraperitoneal injection with a mixture of zoletil 0.5 mg / kg and xylavette 0.05 ml / kg). Animals of groups GU and GU + Support were anesthetized in an antithorostatic position, excluding the interaction of the hind limbs with the support. The research equipment used and the EMG protocol have been described previously [36]. Briefly: H-reflex and M-wave of m.soleus caused by sciatic nerve irritation were recorded; determined the threshold of occurrence, maximum amplitude, latency and duration of the induced potentials; the ratio of maximum amplitudes H-reflex and M-wave (H/M ratio) was calculated. Also, the M-wave decrement was determined during stimulation of the sciatic nerve with supramaximal stimuli with a frequency of 3 Hz and 50 Hz. With low-frequency stimulation (3 Hz), determined the changes in the amplitude of the 5th potential in comparison with the amplitude of the 1st potential, which was taken as 100%; with high-frequency stimulation (50 Hz), the amplitudes of the 1st and 200th potentials were compared.

To evaluate the atrophic changes in muscle fibers, a raw and dry weight analysis of m.soleus was performed. To do this, after euthanasia of the animal (decapitation with guillotine), m.soleus was prepared, isolated within tendons, immediately weighed, then kept for 24 hours in a thermostat at a temperature of 90 ° C and weighed again.

The time of the experimental exposure remained the same throughout the experiment. The animal's belonging to a particular experimental group remained unknown until the analysis of the research results began. The control data were the values found in the group of intact animals (n = 5). All data obtained in the experiments are presented as mean ± se as a percentage of the control values, which were taken as 100%. Student's t-test was used for statistical data analysis. Significance level: * p <0.05.
3. Results

3.1. Examination of the H-reflex

After 7 days of exposure to experimental conditions, the threshold of the H-reflex of m.soleus in the GU and GU + Support groups did not change significantly (p > 0.05). When assessing the amplitude of the H-reflex, it was found that in the GU group the value of the reflex potential increased significantly and reached 225 ± 45% (p < 0.05) in relation to control, however, in the GU + Support group, no significant changes were recorded (p > 0.05). Evaluation of the ratio of the maximum amplitudes of reflex and motor responses revealed a significant increase in the indicator in the GU group, where the H / M ratio increased to 202 ± 40% (p < 0.05), in the GU + Support H / M ratio it approached the control values (p > 0.05). The latency and duration of the H-reflex in experimental groups did not change significantly (p > 0.05). The data are presented in Figure 1.

![Figure 1](image-url)

Figure 1. Analysis of the H-reflex parameters of the rat soleus muscle during hypogravity and hypogravity combined with periodic support load.

After 35 days of exposure to experimental conditions, the threshold of the H-reflex of m.soleus in the GU group decreased to 81 ± 8% (p < 0.05), and in the GU + Support group - to 82 ± 9% (p < 0.05). Amplitude of the H-reflex of m. soleus rise in experimental groups, so in the GU group the amplitude reached 130 ± 15% (p < 0.05), in the GU + Support group - 118 ± 6% (p < 0.05). The H / M ratio in the GU group was 141 ± 8% (p < 0.05), in the GU + Support group - 119 ± 10% (p < 0.05). Latency and duration of the soleus muscle H-reflex corresponded to the control data (p > 0.05) (Figure 1).
3.2. Examination of the M-wave

After 7 days of exposure to experimental conditions, no significant changes was found in the parameters of the rat soleus muscle M-wave. The results are shown in Figure 2. During the decrement test, it was recorded that there were no significant changes with low-frequency stimulation of the sciatic nerve (p > 0.05). The data are presented in Figure 3A. High-frequency stimulation led to a significant depression of the motor potential in the GU group, the decrement reached 39 ± 6% (p < 0.05); no changes in the M-wave amplitude were found in the GU + Support group (p > 0.05). The data are presented in Figure 3B.

![Figure 2](image)

**Figure 2.** Analysis of the M-wave parameters of the rat soleus muscle under hypogravity and hypogravity combined with periodic support load.

Notes: designations as in fig. 1.

After 35 days of exposure to experimental conditions, a decrease in the M-wave of m.soleus threshold of the rat in the GU group was recorded, the M-wave threshold was 75 ± 13% (p < 0.05); in the GU + Support group, the M-wave threshold did not differ from the control (p > 0.05). The M-wave amplitude decreased in both experimental groups.

Thus, in the GU group, the M-wave amplitude was 73 ± 11% (p < 0.05), in the GU + Support group - 78 ± 10% (p < 0.05) (Fig. 2). The decrement test showed no changes in the M-wave amplitude during low-frequency stimulation (p > 0.05) (Fig. 3A). With high-frequency stimulation, the decrement of the M-wave amplitude significantly differed from the control values: in the GU group, the decrement reached 50 ± 11% (p < 0.05), in the GU + Support group - 51 ± 11% (p < 0.05) (Fig. 3B).
3.3. Examination of changes in muscle weight
After 7 days of exposure to experimental conditions, wet weight m. soleus in the GU group decreased and amounted to 67 ± 8% (p < 0.05), statistical differences in the wet weight of m. soleus between the GU + Support group and the control group was not noted (p > 0.05). Dry weight of m. soleus decreased in both experimental groups and in the GU group it was 58 ± 12% (p < 0.05), in the GU + Support group - 78 ± 11% (p < 0.05) compared with the control data. The results are shown in Figure 4.

After 35 days of exposure to experimental conditions, a decrease in the wet and dry weight of m. soleus continued in both experimental groups. Thus, the wet weight in the GU group was 37 ± 11% (p < 0.05), compared with the control, in the GU + Support group - 59 ± 11% (p < 0.05); dry weight in the GU group was 35 ± 12% (p < 0.05), in the GU + Support group - 55 ± 11% (p < 0.05) (Fig. 4).

4. Discussion
Our previous study showed that stimulation of the rat's foot eliminates muscles atrophy of the hind limbs after 7 and 14 days of antioorthostatic suspension [37]. The obtained positive result allowed us to form a hypothesis that with a longer exposure to simulated microgravity conditions, the daily action of the support reaction force and axial load, partially or completely will lead to the prevention of alteration in the central and peripheral parts of the neuromuscular apparatus of the rats m. soleus.

The examination of the soleus muscle H-reflex indicate a change in the functional state of the corresponding motor center under simulated hypogravity: after 7 days in the GU group, and after 35 days in both experimental groups (GU and GU + Support). Numerous research works have shown that
the characteristics of the H-reflex are due to the reflex excitability of motoneurons of the spinal motor center [38-40], and the H / M ratio demonstrates the number of alpha motoneurons activated by Ia afferent stimulation in the entire motor neuron pool of the muscle [41-43]. Limitation of presynaptic inhibition of Ia, increased excitability of α-motoneurons are probably reasons for a decrease in the threshold and an increase in the amplitude of the H-reflex found in the EMG study [36, 44-46]. The increase in H / M ratio recorded in experiments indicates that afferent stimulation of Ia activates most of the entire motoneuronal pool [47]. Similar results were obtained in other studies. De-Doncker L. et al., [48] showed that 14 days of unloading of the hind limbs caused a decrease in the threshold of the neurographic analogue of the H-reflex caused by afferent excitation of the rat soleus muscle. In the experiments of A.V. Kirenskaya et al. [49], using a dry immersion model, it was found out that after unloading, the electrical activity of fast high-threshold motor units increased. In studies carried out after long-term space flights, most of the crew members showed signs of a sharp relief in the system of spinal reflex mechanisms [50]. In the works of Y.P. Gerasimenko et al., [51], S. Harkema et al., [52]; Lavrov et al., [53], Gill et al., [54] and others, note the important role of activation of spinal neural networks in the probability / efficiency of recovery of voluntary movements after spinal cord injury. The formation of a new motor skill can determine the activity of various reflex pathways [55]. Motor activity after exposure in conditions of real or simulated weightlessness should be due to the action of compensatory mechanisms that intensify the functional capabilities of the locomotor apparatus [56]. It is assumed that it is the central nervous mechanisms that play the main role in the gravitationally dependent motor transformations [57, 58].

Condition of the motor centers probably determines the characteristics of the peripheral structures of the neuro-motor system under their control. The neurogenic nature of alterations in striated muscles during gravitational unloading is evidenced by data on morphological and functional transformations in motor neurons innervating skeletal muscles [59]. A decrease in the tonic activity of extensor motoneurons causes atony and atrophic changes in the muscle [57, 58]. In our study, the state of the peripheral structures of the locomotor apparatus was determined by the parameters of the M-wave and the analysis of the soleus muscle weight. The M-wave is a direct response of muscle fibers to stimulation of efferents and may reflect the state of the sarcolemma [60]. It was found that during unloading, the excitability of muscle fibers decreases [61]. In connection with these data, the decrease in the M-wave threshold recorded in our study (group GU, 35 days of unloading), in our opinion, is caused by an increase in the excitability of efferents due to the transformation of the state of the corresponding motoneurons. The recorded decrease in the amplitude of the M-wave after prolonged unloading indicates a decrease in the number of the soleus muscle motor units caused by the development of atrophic processes. The development of atrophy is also indicated by a decrease in the wet and dry weight of m. soleus. Similar changes recorded during muscle inactivation have been described by many researchers [30, 62-65]. It is known that in microgravity conditions, the loss of muscle wet weight is caused by the loss of fluid [58]; it has also been found that gravitational unloading significantly suppresses protein synthesis [31]. The values of the M-wave decrement of the soleus muscle that we found, indicate a violation of the reliability of neuromuscular transmission: so with high-frequency stimulation, the M-wave decrement significantly exceeded the values corresponding to the norm (group GU, 7 and 35 days of unloading; group GU + Support, 35 days of unloading). A significant decrement of the M-wave during stimulation of 50 Hz and the absence of changes during stimulation of 3 Hz indicate disorders localized in the zone of the presynaptic membrane. The results obtained are consistent with the literature data: it was shown that imitation of hypogravity leads to a reduction or complete absence of synaptic vesicles in the terminals of neuromuscular synapses [66], instability in the functioning of neuromuscular synapses [64, 65], a decrease in the total area of the end plate [67].

A number of mechanisms of hypogravitational changes in motor systems are discussed in the literature. De-Doncker L., et al., [48] associate an increase in the reflex excitability of the spinal motoneurons with a reduction in the soma and a decrease in the level of presynaptic inhibition. Also, limitation of afferent signals from the limbs during gravitational unloading [68, 69] can cause the
soleus muscle motoneurons excitability up [70]. It was found that support sensitivity is the basic afferent apparatus of the "tonic" motor system [6, 58]. Signaling from cutaneous receptors initiates central reflex processes [71] that modulate postural activity [72]. Mechanical irritation of the foot restrict or completely prevent changes in skeletal muscles caused by the limitation of the action of gravity [26, 28, 29, 73, 74]. The important role of peripheral afferentation, including support afferentation, is confirmed by our data. Animals in which gravitational unloading was combined with the daily action of the support reaction force and axial load (group GU + Support) did not show alterations in the H-reflex and M-wave m. soleus under short-term exposure to experimental conditions. In addition, after 7 days of hypogravity modeling in the GU + Support group, no significant decrease in wet weight of m. soleus. However, daily support loads did not prevent the effects of prolonged hypogravity. In the GU and GU + Support groups, after 35 days of suspension, similar significant changes were recorded. But when the support reaction force and axial load are applied under simulated weightlessness, changes in the state of the neuromotor apparatus m. soleus were less pronounced.

5. Conclusion
Thus, the results of this study indicate an important role of proprio and support reception in the control of the functional state of the motor systems. The use of the axial and support load under the conditions of short-term simulated gravitational unloading (7 days) counteracts the change in the state of the central and peripheral links of the neuro-motor apparatus of the rat's postural soleus muscle (the results of electromyographic testing and wet weight correspond to the data of the intact group of animals). However, there is no prevention of a reduction the muscle protein under these conditions (the dry weight of the soleus muscle is reduced). Daily support load does not exclude the effects of prolonged imitation of weightlessness, but it limits the intensity of hypogravitational transformations. Further research is required aimed at a more detailed identification of the mechanisms of motor function control when the gravitational environment changes. Also, important to select the effective therapeutic protocols (identify the duration, intensity and type of preventive actions) that can prevent changes in the state of motor systems during long periods of gravitational unloading.

6. References
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