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Comparison of the Behavior of Rats after Prolonged Immobilization with Structural Changes in the Motor Cortex and Hippocampus

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Behavioral and neuron-glial ratios in the motor neocortex and hippocampus after stress induced by discontinuous (7–8 h/day for three weeks) immobilization were compared in Wistar rats (n = 23). Immobilization led to suppression of motor and orientational activity in the open field test and increases in the numbers and durations of episodes of freezing. Morphometric measurements demonstrated significant increases in the density of neurons with hypoxic changes in hippocampal field CA3 and a three-fold increase in this value in the motor zone of the cortex in both hemispheres of experimental animals as compared with controls. No clear glial cell reaction was seen in the motor cortex. Increases in the density of glial elements and the number of multinucleolar neurons in field CA3 provide evidence of compensatory processes occurring in the brain. Hypoxic changes to neurons were functional in nature.

KEY WORDS: open field test, behavior, rats, immobilization, motor cortex, hippocampus, neurons, glia.

Decreases in physical activity associated with modern lifestyles are accompanied by increases in mental loading, which lead to imbalance of various functions and the appearance of negative body states in humans. The severity of the changes in the functional state of the body depend partly on the duration of the decrease in motor activity [5, 18, 19]. Chronic restriction of mobility in humans is known to lead to hypokinetic illness [5, 19].

Studies of the effects of restriction of movement activity in model experiments on animals are relevant from the point of view of explaining the pathogenesis of the resulting functional disorders and in terms of the possibility of compensating them and providing prophylaxis. Immobilization of animals is regarded in the literature as an emotional stress whose sequelae differ from those of physical stress [13, 26, 28, 30].

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We have reported studies of the sequelae of restriction of motor activity (discontinuous immobilization) of rats for one week [13]. This immobilization induced activation of the animals' behavior in the open field test. Morphometric measurements demonstrated significant increases in the density of neurons with hypoxic changes and the absence of any significant changes in the density of glial cells in the motor zone of the cortex of the right hemisphere of the experimental animals as compared with values in control animals. These two observations probably indicate a redistribution of functional activity in the brain, with a predominant functional increase in the role of the left hemisphere [13]. It was suggested that the proportion of neurons with hypoxic changes in the rat motor cortex increases with longer-lasting immobilization. This would provide evidence for a major role of hypoxia in the pathogenesis of functional disorders accompanying hypokinesia and may provide a target for treatment. It is also known from published data that execution of various motor programs by the body is associated with significant activation of structural elements in the motor fields of the neocortex and hippocampal field CA3 [7, 8, 16, 21].

TABLE 1. Measures of Motor Activity in Rats of the Experimental and Control Groups in the First (1) and Second (2) Tests in the Open Field. Numbers before Parentheses Are Medians; Numbers in Parentheses Are Minima and Maxima (at the 0.25–0.75 levels)

Behavioral measure	Test No.	Control group	Experimental group
Latent period, sec	1	1.6 (1–3)	1 (1–3)
	2	3 (1–3)*	2.5 (1–4)
Number of squares	1	112 (78–138)	121.5 (54–170)
	2	110 (52–131)**	71 (30–112)*
Number of rearings	1	14 (7–20)	9.5 (5–28)
	2	2 (0–12)	6 (0–8)***

Note. Significant differences in the second test (2) as compared with the first test (1) within each group of rats are identified (Wilcoxon test): *$p < 0.03$; **$p < 0.0025$; ***$p < 0.005$.

The aim of the present work was to study the behavior of rats in an open field test and to compare nerve and glial cells in the motor cortex of the brain and hippocampal field CA3 after prolonged discontinuous immobilization (21 days) of the animals.

The parameters of the immobilization (duration of daily sessions and duration of the whole series) were selected on the basis of results from our previous studies and published data [17, 24].

METHODS

Studies were performed using 23 male Wistar rats weighing 220–250 g. The control group consisted of 17 rats: 11 animals were used to study behavior in the open field and six intact rats served as controls for morphological studies. The experimental group consisted of six animals. These were subjected to daily immobilization for 7–8 h/day in specially made perforated Plexiglas tubes (length 170 mm, diameter 75 mm). The tubes had two moveable delimiters, the front one prevented the rat from putting its head into the tube and the back one restricted the length of the tube to the length of the rat's body. The animal's head and tail remained free. This prevented the animal from overheating and compressing the blood vessels running to and from the head. During immobilization of the experimental rats, the control animals were kept in the same room in their home cages. Testing in the open field and assessment of the rats' reactions to the experimenter's approaching hand were performed before immobilization and one day after the end of immobilization. Experiments were performed in accordance with the principles of humane treatment laid down in European Community Directive 86/609EC.

The open field consisted of an arena 1 m in diameter, divided into 32 squares. The field was illuminated at about 30 Lx during the experiment. The rat was placed at the center of the field. The observation time was 5 min. The whole experiment was recorded using a video camera and recordings were analyzed. The number of square crossings was counted (horizontal activity), along with the total number of rearings (with and without support on the wall; vertical activity), and the numbers and durations of episodes of grooming and freezing. Animals of the experimental group (six rats) and rats of the control group (11 animals) were tested in the open field on two occasions: before the start of immobilization of rats in the experimental group and one day after the end of immobilization.

One day after the second test in the open field, the animals were decapitated and brains were extracted and fixed in 4% paraformaldehyde solution and, after 14 days, were embedded in paraffin-celloidin using standard methods. The brains of a control animal and an experimental animal were placed in the same block, which ensured identical conditions (temperature, chemical, vibration) for embedding and preparation of sections. The motor zone of the neocortex was studied (coordinates from a stereotaxic brain atlas [28]): from 1.7 to 0.4 mm from the bregma, 1 mm from the midline, and 2 mm from the surface; field CA3 of the dorsal hippocampus was also studied.

A microtome was used to prepare frontal sections of the brain of thickness 8 μm and every tenth section in the motor cortex and hippocampus was collected, placed on a slide, and stained using the Nissl method. It should be emphasized that brain sections from control and experimental animals were mounted on a single slide, so all stages of staining the sections were performed simultaneously. Neurons with pathological changes were regarded as classical hypoxic neurons, i.e., hyperchromic neurons with nuclei and well stained apical dendrites withcharacteristic corkscrew tortuosity [23, 27, 29]. A total of 140 frontal sections were prepared from the study areas of the brain (motor cortex and hippocampal field CA3) and examined. An ocular grid with squares of side 130 μm (ocular ×20, objective ×40) was used to count the total number of neurons on each section, along with the numbers of nerve cells with hypox-

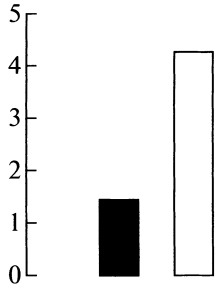

Fig. 1. Numbers of "pathological" neurons in layer V of the motor cortex per unit area by hemispheres (fields of 130 × 130 μm). Mean values for the control (dark column) and experimental (white column) groups. Significant differences are identified in the text.

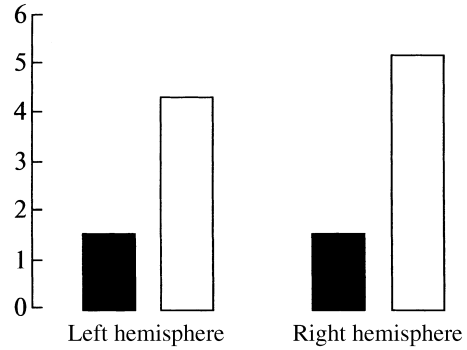

Fig. 2. Numbers of "pathological" neurons in layer V of the motor cortex of the left and right hemispheres (fields 130 × 130 μm). White columns show experimental animals; dark columns show controls. The vertical axis shows the number of neurons. Significant differences are identified in the text.

ic changes, glial satellite cells, and so-called free glial cells (these are glial cells separated from neuron bodies by distances greater than the diameter of the glial cell nucleus) [27]. Counts of cellular elements on sections were performed separately in the right and left hemispheres of the rats' brains (10 fields per section).

The effects of treatment between groups were compared using the non-parametric Mann–Whitney test and effects for each group before and after treatment were compared using the Wilcoxon test for dependent groups.

RESULTS

In the initial tests, animals of both groups moved calmly along the walls and made "excursions" by one body length toward the center of the field. Of the 11 rats of the control group, eight reached the center (two of them twice); of the six rats of the experimental group, four reached the center. In the second test, five rats of the control group reached the center, while none of the experimental group did. This difference in the behavior of the animals of the two groups was significant ($p = 0.027$, differential assessment with one-tailed test). In the second test, the style of moving across the "floor" was no different in control rats from that seen in the first test. Rats of the experimental group pressed themselves against the wall on moving in the open field after immobilization. Quantitative measures of motor activity and latent periods are shown in Table 1. After three weeks of discontinuous immobilization, rats of the experimental group showed significant reductions in horizontal and vertical activity as compared with the pre-immobilization test. The control group showed an increase in the time to departure from the center of the field (latent period, LP) and a decrease in the number of rearings.

The largest changes following immobilization occurred in relation to open field behavioral measures such as grooming and freezing reactions. In all rats in the first test and control rats in the second test, grooming included washing the snout and short episodes of scratching with the hindpaw. Rats of the experimental group showed a predominance of prolonged grooming procedures in the second (post-immobilization) test, usually with abnormal sequencing: after licking the abdomen, the rat could return to washing or start grooming immediately, with licking of the genitals. Comparison of measures of grooming in rats of the control group in the first and second tests revealed no significant differences in the number of episodes (the median was three in both cases) or their mean duration (the median was 9 sec in the first test with a range of 1–22 sec, while the median in the second test was 12 sec, with an interval of 1–45 sec, $p = 0.55$).

The number and duration of grooming acts in rats of the experimental group in the first (pre-immobilization) test were not significantly different from values in rats of the control group. In the second test, rats of the experimental group (after immobilization) showed no change in the average number of grooming episodes from that in the first test (median 1.5, range 1–2 sec, $p = 0.13$). However, there was an increase in the average duration of individual episodes of grooming: the median of 8 sec increased to 120 sec, with a range of 100–240 sec ($p = 0.028$). In terms of the average duration of grooming episodes, the second test in the experimental group was significantly different from the value in the control group ($p = 0.009$).

There were no significant differences in the total duration of grooming between the first and second tests in rats of the control group (medians of 25 and 20 sec, respectively). In the first test, the total duration of grooming by rats of the experimental group was no different from that in rats of the control group; the median was 22.5 sec. In the second test, rats of the experimental group showed a significant increase in the total duration of grooming both in comparison with the first test in this group (median 218 sec, range

TABLE 2. Median Differences in Behavioral Measures in the Second and First Tests in the Open Field in Rat Groups. Ranges Are Shown in Parentheses

Behavioral measure	Control group	Experimental group	Significant differences*
Latent period, sec	1.36 (0, +3)	1.3 (0, +3)	$p = 0.9$
Number of squares	–7 (–16, +8)	–44 (–93, 0)	$p = 0.02$
Number of rearings	–6 (–13, +4)	–9 (–22, 0)	$p = 0.02$
Grooming, sec	5 (–30, +20)	149 (27, +229)	$p = 0.002$
Freezing, sec	10 (–40, +20)	94 (65, +190)	$p = 0.002$

Note. *Significance levels (p) are given for differences in measures in the experimental group compared with the control group (Mann–Whitney test).

TABLE 3. Mean Densities of Neurons with Hypoxic Changes, Neurons with Increased Numbers of Nucleoli, and Free and Satellite Glial Cells per Unit Area 130 × 130 μm in Hippocampal Field CA3

Structural unit	Control group	Experimental group	Significant differences*
Neurons with hypoxic changes	3.36 (2.48–4.8)	6.13 (4.52–8.0)	$p = 0.004$
Multinucleolar neurons	3.32 (3.0–3.7)	5.07 (4.06–6.01)	$p = 0.003$
Total glia	11.25 (10.5–12.6)	13.92 (13.2–14.7)	$p = 0.002$
Satellite glial cells	7.24 (6.88–7.92)	9.49 (8.64–10.6)	$p = 0.002$

Note. *Significance levels (p) are given for differences in measures in the experimental group compared with the control group (Mann–Whitney test). Minima and maxima (at the 0.25–0.75 levels) are given in parentheses.

96–240 sec, $p = 0.003$) and with the second test in rats of the control group.

The characteristics of freezing when rats were placed in the open field were assessed using several measures: the average number of cases of freezing, the average duration of individual episodes of freezing, and the total duration of freezing averaged for the group. In the first test, freezing was seen in eight of the 11 rats of the control group, seven showing freezing in the second test. In the experimental group three of six froze in the first test, while all showed freezing in the second test. This change in the experimental group was significant ($p = 0.037$, differential assessment with one-tailed test).

The number of cases of freezing seen per animal showed no significant change in the control group in the second test as compared with the first: group medians were 1.0 (range 0–2) and 2.0 (range 0–3), $p = 0.68$. In the experimental group, the median number of freezing episodes in the first test was 0.67 (range 0–2), and this was not significantly different from the value in the control group ($p = 0.36$). Testing of rats of the experimental group after immobilization revealed an increase in cases of freezing, with a median of 2.67 (range 2–4). This result was significantly different from the first test in rats of this group ($p = 0.028$) and the second test in rats of the control group ($p = 0.043$).

The mean duration of individual freezing episodes in rats of the control group in the first test increased slightly (median 25 sec, range 0–130 sec) as compared with 16 sec (range 0–78 sec), though this increase was not significant. In rats of the experimental group, the median in the first test was not different from that in rats of the control group (20 sec, range 0–40 sec), $p = 0.8$. In the second test, the average duration of freezing episodes in experimental rats increased significantly (median 41 sec, range 20–139 sec) and was significantly different from the value in rats of this group in the first test and the value in rats of the control group in the second test ($p = 0.041$).

There were no significant differences in the total duration of freezing between the first and second tests in rats of the control group (medians of 35 and 42 sec, respectively). In the first test, the total duration of cases of freezing in rats of the experimental group was not different from the value in rats of the control group: the median was 33.5 sec. In the second test, rats of the experimental group showed a significant increase in the total duration of freezing in comparison with both the first test of rats of this group (median 117.5 sec, range 92–235 sec, $p = 0.002$) and the second test of rats of the control group (median 46.9 sec, range 0–110 sec).

Individual changes in behavioral measures following immobilization in the experimental group were significantly more extensive than those in rats of the control group. The differences between the two tests (with the correspond-

ing sign) were taken for each animal and medians for the group were then determined. For convenience, the results in Table 2 compare the medians of these groups.

Morphometric measurements showed that the density of neurons with pathological changes, using the mean for both hemispheres, was significantly greater in the experimental animals (Fig. 1) than in controls ($p < 0.0005$). The density of anomalous neurons in the motor zone of the cortex by hemisphere are shown for control and experimental rats in Fig. 2. A significant increase in the density of hypoxic neurons in the motor zones of the cortex of both the right ($p < 0.008$) and left ($p < 0.002$) hemispheres were seen in experimental rats as compared with values in controls. It also follows from Fig. 2 that the densities of anomalous neurons in the motor zones of the cortex showed no difference between the right and left hemispheres either in the control rats ($p = 0.8$) or in rats subjected to immobilization ($p = 0.17$). Glial cell reactions were not seen in the motor cortex.

The densities of neurons with hypoxic changes and neurons with increased numbers of nucleoli were measured in hippocampal field CA3, as were the densities of free and satellite glial cells.

As shown in Table 3, hippocampal field CA3 in rats subjected to immobilization showed, along with an increase in the density of hypoxic neurons, significant increases in the numbers of multinucleolar neurons and glial elements.

DISCUSSION

The literature contains extensive data on changes in the behavioral reactions and biochemical processes in brain structures in various stressful conditions [6, 11], though there are few reports of studies of structural rearrangements of brain nervous tissue and comparison with changes in behavior [13–15, 21].

Changes in measures of behavior in the open field in rats of the experimental group after immobilization can be characterized in two ways. In particular, there was an increase in the passivity of the animals, which was apparent as a significant decrease in horizontal movement activity as compared with the unchanging activity of rats in the control group (Table 1). The decrease in motor activity in rats of the experimental group is best interpreted in association with other behavioral characteristics. The increase in the duration of grooming episodes (scratching and licking) without any change in the number of episodes in rats after immobilization would appear to be linked with the direct effects of manipulations – massaging of the muscles after prolonged hypokinesia and changes in pain sensitivity. The directionality of changes in pain sensitivity after immobilization depends on the duration of immobilization. Discontinuous immobilization for 5–6 days increases the pain thresholds for a variety of traumatic factors. Prolonged restriction of mobility decreases pain thresholds and increases the duration of pain responses [25], which may be associated with decreased enkephalin secretion [20]. The emotional state of the animals must also be considered. The leading role of fear in the behavior of the experimental animals in the second test is indicated by the significant increase in the proportion of rats in the experimental group displaying freezing reactions, as compared with the level in controls. This reaction is regarded as a marker of the emotional fear reaction [22]. In control rats, freezing accounted for no more than 15% of the time spent in the open field, while in the experimental group this proportion approached 40%.

In addition, altered behavioral features were noted, which were not included in the Results section, as they are not quantitative. Each "session" of immobilization was accompanied by clear signs of defensive and aggressive behavior: the rats squeaked, rotated their heads and tails, and tried to bite items close to their snouts. Signs of aggressive-defensive behavior increased not only as the animals were placed in the tube, but also when they tried to escape from the tube. Changes in the rats' behavior probably reflected the development of the anxiety stage of the stress response [25].

Immobilization of rats is known to be accompanied by changes in the activity of the stress-mediating (sympathoadrenal and hypothalamic-hypophyseal) and stress-limiting (endogenous opioid peptides) systems [4, 16, 20]. Rearrangements in the transmitter and hormonal status of the animals are accompanied by significant changes in the vascular system [2, 5, 9, 12, 19]. As a result, dystonic vascular disorders lead to oxygen deficiency in the tissues, from depletion of the vascular system in the limbs [18, 19] to hypoxic changes in nerve cells [1, 3, 10, 13]. This was the starting point for comparing the post-immobilization state of nerve tissue with changes in behavior in immobilization of different duration.

The sequelae of one-week immobilization [13] consisted of increases in those measures of rat behavior in the open field providing evidence of its activation, which was in good agreement with the morphological pattern seen in the motor cortex: greater preservation of structural units in the motor cortex of the left hemisphere and increases in the density of neurons with hypoxic-type changes in the motor cortex of the right hemisphere. This distribution of densities is evidence of an increase in the functional role of the left hemisphere in regulating the behavior of the experimental animals and is reflected in activation of the rats' behavior in the open field. Increases in the experimental period are known to be accompanied by increases in the effectiveness of hypokinesia [7]. Three-week immobilization altered the proportion of neurons with decreased functional activity and their distribution across zones of the motor cortex in both hemispheres. In particular, the ratio of the densities of hypoxic neurons changed in the experiment as compared with the controls: after immobilization for

one week, the increase in density in the cortex as compared with controls was no more than l.5-fold, while three-week immobilization led to a three-fold increase. Prolonged immobilization eliminated the difference in the density of hypoxic neurons between the left and right hemispheres which was clearly apparent after one-week immobilization. An increase in the density of neurons with hypoxic changes was seen in hippocampal field CA3 in the brains of rats subjected to immobilization.

Functional changes developing on restriction of mobility are well known from published data to occur not only in the motor system, which is directly involved in the perception of movement afferentation, but also in nonspecific formations at different levels of the CNS. These changes affect both the structural and ultramicroscopic levels [7, 8]. Quantitative and qualitative changes in the brain resulting from movement restriction provide evidence of decreases in the functional activity of the brain, which make a contribution to changes in the animals' behavior which are particularly apparent on testing the rats in the open field. It is important to note that pathological rearrangements in the brain in hypokinesia are accompanied by compensatory processes. These are supported by increases in the densities of total glial cells and satellite glial cells in hippocampal field CA3. The increases in free glial cells and neurons with increased numbers of nucleoli in the hippocampus provide evidence of active repair processes in the brain [14], with no clear "pathological" glial reaction in the motor cortex of the experimental rats.

Rats are known to be able to acquire operant defensive reflexes after discontinuous three-week immobilization [17]. Repair processes in the brain on movement restriction probably dominate over pathological (hypoxic) processes, so the functional capacities of the cortex and hippocampus are preserved.

CONCLUSIONS

1. Three weeks of immobilization led to suppression of motor-orientational activity in rats in the open field and the development of an anxious-defensive state.

2. Morphometric changes showed a significant increase in the density of hypoxic neurons in hippocampal field CA3 and a three-fold increase in this parameter in the motor zone of the cortex of both hemispheres in experimental animals as compared with control animals, contributing to changes in the rats' behavior.

3. Increases in the densities of glial elements and multinucleolar neurons in field CA3 in experimental animals provides evidence for the development of compensatory processes in the brain. In experimental animals, the motor cortex showed no clear glial cell reaction.

4. Hypoxic changes to neurons were functional in nature.

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REFERENCES

1. M. G. Airapetyants, "Mechanisms of the pathogenesis of neuroses," *Zh. Vyssh. Nerv. Deyat.*, **55**, No. 5, 734–746 (2005).
2. M. G. Airapetyants, V. V. Aleksandrin, E. V. Kurochkina, I. P. Levshina, and P. N. Aleksandrov, "Microcirculatory lesions in the rat brain in neurosis," *Byull. Eksperim. Biol. Med.*, **118**, No. 11, 521–522 (1994).
3. M. M. Aleksandrovskaya and A. V. Koltsova, "Structural and functional rearrangements of neurons and glial cells in the sensorimotor cortex in experimental neurosis," *Zh. Vyssh. Nerv. Deyat.*, **30**, No. 9, 529–532 (1980).
4. M. A. Gilinskii, S. V. Goryakin, T. V. Latysheva, G. M. Petrakova, and N. V. Prokopieva, "Mechanisms of formation of adaptive traces in graded stressing," *Byul. Sib. Otdel Ros. Akad. Med. Nauk.*, **112**, 141–147 (2004).
5. B. A. Dushkov, *Motor Activity in Humans in a Hermetic Chamber and Space Flight* [in Russian], Meditsina, Moscow (1969).
6. N. V. Gulyaeva and I. P. Levshina, "Relationship between individual-typological behavioral characteristics and the state of the lipid components of cerebral membranes in stress," in: *The Individual Brain: Structural Bases of Individual Behavioral Characteristics* [in Russian], Nauka, Moscow (1993), pp. 82–91.
7. M. G. Zhvaniya, "Ultrastructural rearrangements in a number of end-brain formations in the rat in conditions of decreased motor activity not inducing stress. Morphology," *Arkh. Anat. Gistol. Embriol.*, **109**, No. 3, 10–13 (1996).
8. M. G. Zhvaniya and M. G. Bliadze, "Effects of hypokinesia on the ultrastructure of the emotiogenic formations of the cerebrum," *Arkh. Anat. Gistol. Embriol.*, **98**, No. 1, 27–34 (1990).
9. D. Krants, M. Poppai, A. Vollenberger, and K. Gekht, "Metabolic processes in the heart and blood vessel walls in stress," *Zh. Vyssh. Nerv. Deyat.*, **27**, No. 2, 355–356 (1977).
10. V. N. Larina and I. P. Levshina, "Structural-functional changes in the central nervous system in white rats on exposure to asthenia-inducing white noise," in: *The Systems Properties of Tissue Organizations* [in Russian], Meditsina, Moscow (1977), pp. 145–147.
11. I. P. Levshina and N. V. Gulyaeva, "Changes in energy metabolism in a number of brain areas and autonomic reactions in white rats during neuroticization," *Zh. Vyssh. Nerv. Deyat.*, **34**, No. 3, 554–559 (1984).
12. I. P. Levshina and N. V. Gulyaeva "Changes in local blood flow rate and cytochrome contents in various areas of the brain in white rats during neuroticization," *Zh. Vyssh. Nerv. Deyat.*, **34**, No. 5, 967–971 (1984).
13. I. P. Levshina, V. N. Mats, N. V. Pasikova, and N. N. Shuikin, "Comparison of the behavior of rats after immobilization with structural changes in the motor cortex," *Zh. Vyssh. Nerv. Deyat.*, **58**, No. 4, 498–505 (2008).
14. V. N. Mats, *Neuron-Glial Interactions in the Rat Neocortex during Learning* [in Russian], Nauka, Moscow (1994).
15. V. N. Mats, O. L. Segal, and R. I. Kruglikov, "Changes in the dry weight of the nuclei of pyramidal nuclei in the motor cortex during acquisition of a local motor-feeding conditioned reflex," *Izv. Akad. Nauk. SSSR Ser. Biol.*, No. 2, 282–289 (1979).
16. F. Z. Meerson, "The stress-limiting systems of the body and their role in preventing ischemic cardiac lesions," *Byul. Vses. Kardiol. Nauch. Tsentra Akad. Med. Nauk. SSSR*, **1**, 34–43 (1985).
17. M. Poppai, K. Gekht, and L. Morits, "Integrative activity of the brain and blood pressure in rats during hypokinetic stress," *Zh. Vyssh. Nerv. Deyat.*, **27**, No. 2, 348–349 (1977).

18. V. V. Portugalov, E. I. Ilyina-Kakueva, and V. I. Starostin, "Structural and cytochemical changes in skeletal muscle during restriction of mobility," *Arkh. Anat. Gistol. Embriol.*, **11**, No. 61, 82–90 (1971).
19. M. G. Prives, "Some achievements and outlook for cosmic anatomy of the vascular system," *Arkh. Anat. Gistol. Embriol.*, **11**, No. 61, 5–16 (1971).
20. M. G. Pshennikova, "The role of opioid peptides in the reactions of the body to stress," *Patol. Fiziol. Eksperim. Terap.*, **2**, 85–90 (1987).
21. O. L. Segal and V. N. Mats, "Studies of protein content in hippocampal neurons during acquisition of local motor-feeding conditioned reflexes," *Biol. Nauki*, No. 7, 82–87 (1978).
22. P. V. Simonov, *The Emotional Brain. Physiology. Anatomy. The Psychology of Emotions. Brain. Emotions. Needs. Behavior. Selected Works* [in Russian], Nauka, Moscow (2004), Vol. 1.
23. P. E. Snesarev, *Theoretical Grounds for the Pathological Anatomy of Mental Diseases* [in Russian], Medgiz, Moscow (1950).
24. L. Khettei, M. Poppai, and K. Gekht, "Actions of chronic stress on energy metabolism in cortical synaptosomes in rats," *Zh. Vyssh. Nerv. Deyat.*, **27**, No. 2, 352–354 (1977).
25. E. N. Chuyan and T. V. Zayachnikova, "Modification of pain sensitivity in rats in hypokinetic stress," *Neirofiziologiya*, **39**, 174–183 (2007).
26. T. A. P. Femke, G. Wolterink, and J. M. van Ree, "Physical and emotional stress have differential effects on preference for saccharine and open field behaviour in rats," *Behav. Brain Res.*, **139**, No. 1–2, 131–138 (2002).
27. M. B. Graever, W. F. Blakemore, and G. W. Kreutzberg, "Cellar pathology of the central nervous system," in: *Greenfield's Neuropathology*, D. I. Graham and P. L. Lantos (eds.), Edward Arnold, London (2002), Chapter 3, 7th Edition, pp. 123–191.
28. E. Y. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, San Diego (1997).
29. M. Polak, "Morphological and functional characteristics of the central and peripheral neuroglia (light microscopic observations)," *Progr. Brain Res.*, **15**, No. 1, 12–33 (1965).
30. E. Sahin and S. Gümülü, "Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models," *Behav. Brain Res.*, **155**, No. 2, 241–248 (2004).