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Some biological reactions of the organism after exposure to nanosecond repetitive pulsed microwaves

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Abstract. White adipose tissue is an important neuroendocrine organ, it can change the activity of the brain through its biologically active substances, and therefore, it can influence functioning of the body. The effects of nanosecond repetitive pulsed microwaves (RPMs) on the brain and epididymal adipose tissue of mice locally was studied (10 days, 4000 pulses per a day, peak power flux density of 1500W/cm², pulse duration of 100 ns, repetition rates of 6, 13, 16, 22 Hz). The effect of RPM exposure was estimated from the changing of behavioural responses, neuronal activity of brain structures and level of corticosterone in blood serum. The data shows that direct nanosecond RPM irradiation of the brain changes brain activity. The effect is a significant multidirectional behavioural response, changes in the dynamics of general locomotor activity, in the neuronal activation of the hypothalamus and the reticular formation, as well as in the level of corticosterone. Furthermore, direct nanosecond RPM irradiation of the epididymal adipose tissue significantly changed the state of mice. Specifically, the RPMs were able to change the morphometric parameters of the adipose tissue itself, change the content of corticosterone in the blood serum, as well as change the behavioural responses of animals.

1. Introduction

In biological studies at the High current electronics institute of the SB RAS for the last 20 years, it has been shown that nanosecond repetitive pulsed microwave radiation (RPM) causes a variety of bioeffects from the molecular, cellular to the organismic levels of organism [1, 2]. There were no available data on the effect of nanosecond RPMs on brain activity until 2014. The brain has the main role in the regulation of all physiological functions of the body. All this circumstances stimulated experiments in the study of the influence of RPMs on the state and activity of the brain. The activity of the brain is indicated by certain EEG patterns. These patterns are used for assessment the state of brain structures and their changes under the influence of some factors. However, the direct EEG registration to estimate the activity of the brain is difficult. Therefore, behavioural responses of mice in the "open field test" as well as daily locomotor activity were used as an indirect indicator of brain activity. The behavioural activity of animals is a common method to assess the functional state of the central nervous system, including brain reactions to various physical factors. At the same time, adipose tissue reactions after RPM exposure were also studied. Since adipose tissue is an important neuroendocrine organ [3], it capable of altering the activity of the brain and, consequently, changing the functions of the organism through



certain bioactive molecules. Thus, the paper purpose was to experimentally study the effects of local RPM exposure of the brain and epididymal adipose tissue.

2. Materials and methods

The experiments were performed in accordance with the rules of humane treatment of animals [4]. 84 inbred white mice of 25-30 g were used. During the experiment, the region of a head or epididymal adipose tissue of irradiated mice were exposed to RPMs (10 days, one exposure of 4000 pulses per a day, peak power flux density of 1500W/cm², repetition rates of 6, 13, 16, 22 Hz). The source of the RPMs was a laboratory generator based on the magnetron MI-505 (10 GHz, pulse duration of 100 ns). The intensity of the impact was estimated by the method described in [5]. The effects were changes in the indicator value of irradiated animals in comparison with sham-irradiated (false-irradiated) group of mice. There were a control group with naïve mice. The mouse body was covered with radio absorbing material for local irradiation the brain or epididymal adipose tissue. The duration of irradiation varied from 3 to 20 minutes, depending on the pulse repetition frequency. The effects of RPMs were assessed by the behavioural reactions of mice in the "open field" test, the daily dynamics of locomotor activity, the neuronal activity of the brain structures [6] and the level of the hormone corticosterone in the blood serum. The open field activity monitoring test comprehensively assesses locomotor and behavioral activity levels of mice. A horizontal and vertical activity, a hole exploratory behavior, a number of defecations and urinations and a grooming behavior were investigated as the main indicators in the "open field" test. The "open field" test was carried out for experimental and sham-irradiated animals twice: the day before the start of irradiation and the day after the end of the exposure. The estimation of the daily total locomotor activity was carried out round the clock during the experiment. For this purpose, a fixed network video camera (AXIS P1344 connected to a computer) was used, in the field of accessibility of this camera were mice placed 6 animals per a cage. The video was recorded in infrared light at night. Video data obtained was processed using the "Mouse Express" program [7]. Neuronal activity in brain structures after RPM exposure was assessed by immunohistochemical staining of brain sections. This method based on the ability of unlabeled primary antibodies to bind to the desired antigen of the early c-fos protein and then to be detected with labeled secondary antibodies. In present work the conditions of the motor cortex, hypothalamus and reticular formation were studied. The level of neuronal activity was analyzed from brain sections microphotographs obtained with the Axio Imager Z1 fluorescence microscope ("CarlZeiss", Germany). The level of the hormone corticosterone in the blood serum of irradiated and sham-irradiated mice was evaluated with enzyme-linked immunosorbent assay (ELISA). The procedure of ELISA was carried out according to the instructions offered by test system manufacturers (DRG, Germany; IDS, UK). The data obtained were processed with application package Statsoft STATISTICA for Windows 8.0, the arithmetic averages and its error were calculated. The significance of the differences between the indices of irradiated and sham-irradiated mice was estimated using the nonparametric Mann-Whitney U-test.

3. Results

The analysis of the obtained results allowed to establish that the direct RPM influence on the brain and epididymal adipose tissue of laboratory mice changes the functional state of the its organism. Effects were presented in a multidirectional change of behavioural responses (open field test) and daily dynamics of general locomotor activity, as well as increase in neuronal activity in the hypothalamus and in the reticular formation and change of corticosterone in the blood serum.

Several behavioral responses of animals in the "open field" changed after the irradiation of the brain and epididymal adipose tissue of mice. In particular, the grooming act frequency increased by 62% (figure 1A) and the number of defecations / urinates increased by 50% of (figure 1B) after exposure to RPMs with a repetition rate of 6 Hz. Analysis of active search behavior and investigative reflex of animals showed that the hole exploratory behavior decreased by 68% and 10% respectively after RPM exposure with repetition rates of 6 and 22 Hz. Horizontal component of the locomotor activity decreased by 61% after RPM exposure with 22 Hz.

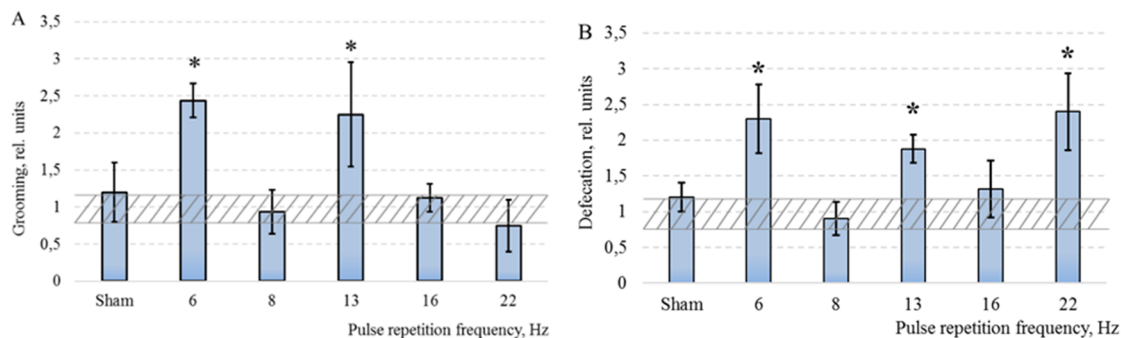


Figure 1. The number of grooming behavior (A) and acts of defecations / urinations (B) after RPM irradiation of the brain by an open field test. Note: the indicators are normalized with respect to the control group (indicators of control group is taken as 1). Shaded space is 95% confidence interval of the averages of the indicator in the sham-group. * – the differences are statistically significant with respect to corresponding indicators of sham-animals ($p \leq 0,05$).

The 30% decrease in horizontal activity in mice was observed after a ten-day irradiation of epididymal adipose tissue with nanosecond RPMs with frequencies of 6, 13 and 22 Hz. That corresponded to a similar decrease after brain irradiation. However, changes in the number of hole exploratory activity and acts of defecation/urination were different from the same behavioral components in experiments with the influence on the brain. Namely, there was an increase in the orienting and research behavior (hole exploratory activity), as well as a decrease in the number of acts of defecation/urination after RPM irradiation with a frequency of 22 Hz of adipose tissue. The number of acts of defecation/urination also decreased after RPMs of 6 Hz. Consequently, anxiety and a depressed state were formed in the animal organism after nanosecond RPM exposure of the brain. But there was a lack of anxiety after nanosecond RPM exposure of the adipose tissue, that, apparently, indicated the development of adaptive responses.

The significant dependence on the time of day was shown in analysis of the general locomotor activity dynamics of mice after brain irradiation. Irradiated and sham-irradiated animals had the same night dynamics. The daily dynamic of the locomotor activity of sham-irradiated had an oscillation in the circadian septal rhythm range (6-7 days) but the dynamic of irradiated animals had an oscillation in the infradian rhythm (3-4 days). Similar change in the oscillations period and an increase in the locomotor activity was observed after exposure to RPMs with repetition frequencies of 6, 13 and 16 Hz, (figure 2). In particular, the amplitude of the oscillations increased by 35-45% after irradiation with a repetition rate of 6 Hz. Apparently, nanosecond microwave pulses can influence the brain structures responsible for the formation of infradian rhythms [8].

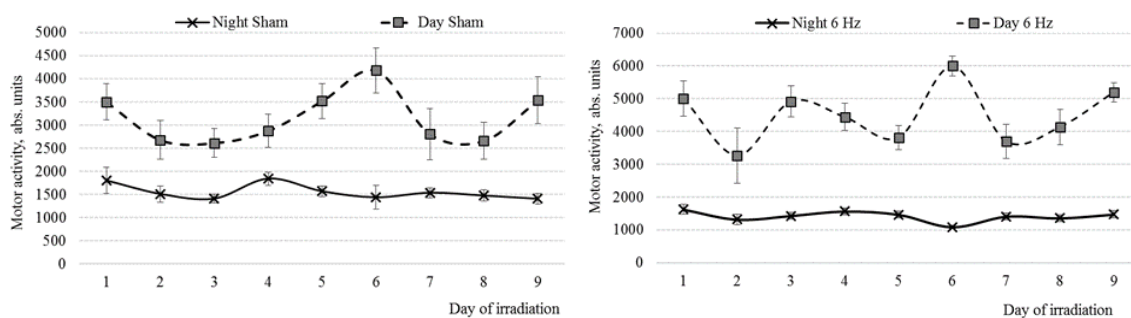


Figure 2. Daily dynamics of the general motor activity of sham-irradiated (left) and irradiated (right) mice after RPMs of 6 Hz.

There were no statistically significant differences in the dynamics of general motor activity both at night and daytime after the local RPM exposure of adipose tissue. As noted above, the development of an anxiety state was observed after the RPMs irradiation of the brain and adipose tissue of mice. Therefore, it was possible to assume the stress development in the body. An indicator of stress is the high level of the hormone corticosterone in the blood. In fact, a statistically significant increase of the hormone level (table 1) after local RPM of 6 and 13 Hz exposure of the brain and adipose tissue of animals was showed. The data obtained confirmed the assumption that the RPM exposure promotes the development of stress in the animals.

Table 1. The level of corticosterone in the blood serum of mice after RPM irradiation of the brain or epididymal adipose tissue.

Exposure mode	After brain irradiation	After epididymal adipose tissue irradiation
	Concentration, ng/ml	
Sham	21.06±4.7	36.57±9.0
6 Hz	69.31±16.2* ↑	69.27±16.2* ↑
13 Hz	66.96±12.8* ↑	55.95±10.33* ↑
22 Hz	4.32±0.3* ↓	22.03±2.7* ↓

The averages of the indicators ± average error are presented; * – the differences are statistically significant with respect to the indices in the sham-group ($p \leq 0.05$).

However, there was a statistically significant decrease in the level of corticosterone after RPM exposure at a frequency of 22 Hz. The natural pool of this hormone can be depleted as a result of the development of severe stress. All the above point to the complicated nature of the RPM influence on the animal organism and, in particular, on the activity of the brain and adipose tissue and the metabolic processes under its control.

C-fos protein expression (neuronal activity) in neurons of the hypothalamus and the reticular formation was analyzed to identify possible brain reaction zones after local exposure to RPMs.

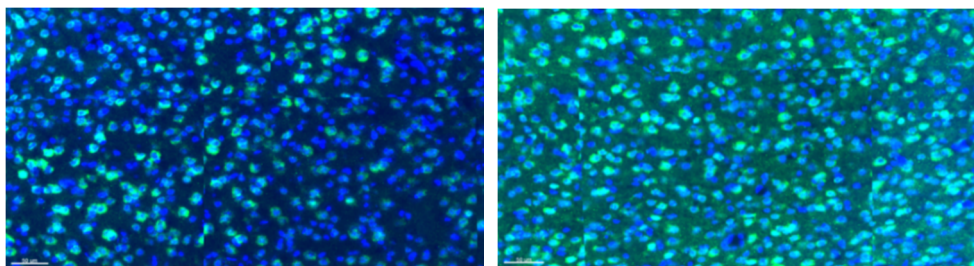


Figure 3. Microphotographs of hypothalamic nuclei sections of mice brain. Cells with c-fos protein expression (green) of sham-irradiated (left) and RPM irradiated animals (right) (pulse repetition frequency of 16 Hz).

An increase in the number of activated c-fos cells in the ventromedial and dorsal nuclei of the hypothalamus after PMM exposure (of 16 Hz) of the brain was found (figure 3). There were no significant changes in the level of activated neurons in the hypothalamus after irradiation with pulse repetition rates of 6 and 13 Hz. The number of activated neurons of the reticular formation was significantly increased after brain irradiation with a pulse repetition rate of 13 Hz.

4. Conclusions

The research revealed the effect of nanosecond RPM on the brain and adipose tissue. The decrease in the active-search component against the increase in the passive-defensive component were the main behavioural effects observed in the "open field test" after the impact of the RPMs on the brain. The most physiological effective frequencies of exposure were 6, 13 and 22 Hz. Such reactions indicated the

depressive state development and increased emotional reactivity in animals after a ten-times irradiation. The increase in the number of acts of grooming (which is a nonspecific indicator of the level of emotionality and is a mechanism for removing the excessive activity of the central nervous system) indicates a strong emotional stress in irradiated mice – a fear or an anxiety. The obtained stressor activation of grooming in experimental animals is a compensatory reaction related to adaptive necessity to reduce the level of excitation caused by irradiation. An increase in corticosterone after RPM with repetition rates of 6 and 13 Hz, and a decrease after 22 Hz is just confirmed the possibility of stress development in response to RPM exposure. It cannot be excluded that specific metabolic situation is created in the body of animals after RPM irradiation of the brain and fatty tissue, that situation is the result of a change in the interaction of hormones with neuronal receptors in the hypothalamus nuclei [9]. The effect can be mediated through the reduction of ligand-receptor reactions (by change of receptor affinity to hormones that provide feedback between the leptin, the Y-peptide of the hunger center and the ghrelin hormone of digestive tract) or reducing the number of membrane receptors on the hypothalamus neurons. The obtained data revealed involvement of the hypothalamus and the hypothalamo-pituitary-adrenal axis into effect of brain and adipose tissue irradiation. That data ensures the production of corticosterone and, accordingly, the formation of stress. Furthermore, the brain irradiation seemed to activate the hippocampus and, accordingly, enhance its reciprocal relationship with the hypothalamus and the reticular formation. As a result, there is a change in activity of brain structures responsible for the infradian rhythm formation, which than changes in daily motor activity. Thus, nanosecond RPMs can have an adverse effect on the body, with the development of stress, or oxidative stress [10], as well as the formation of a chronophysiological effect in the form of desynchronosis. Such circumstances should be considered in the operating of sources of nanosecond repetitive pulsed microwave radiation to eliminate undesirable effects on the staff.

Acknowledgments

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