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IMPACT OF ELECTROMAGNETIC UHF RADIATION ON GENOME DESTABILIZATION IN BONE MARROW CELLS OF RAT STRAINS WITH CONTRAST NERVOUS SYSTEM EXCITABILITY

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❀ Chromosomal machinery of highly excited animals with low threshold of the nervous system excitability (LT strain) is more susceptible to the damaging effect of high frequency EMR compared against the animals with high threshold of the nervous system excitability (HT strain). High nervous system excitability determines greater decrease in chromosome aberrations level in the presence of additional reflecting elements – Aires Defender Pro resonators under UHF-waves of standard Wi-Fi router. It is shown that the genotype of animals and the functional state of their nervous system affect susceptibility to the UHF EMR and the action of resonators.

❀ **Keywords:** electromagnetic UHF radiation; resonators; chromosome aberrations; bone marrow; nervous system excitability; rat strains.

ВЛИЯНИЕ ЭЛЕКТРОМАГНИТНОГО ИЗЛУЧЕНИЯ УВЧ-ДИАПАЗОНА НА ДЕСТАБИЛИЗАЦИЮ ГЕНОМА КЛЕТОК КОСТНОГО МОЗГА КРЫС ЛИНИЙ С КОНТРАСТНОЙ ВОЗБУДИМОСТЬЮ НЕРВНОЙ СИСТЕМЫ

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❀ Хромосомный аппарат высоковозбудимых крыс линии НП (с низким порогом возбудимости) более подвержен повреждающему действию высокочастотных электромагнитных излучений по сравнению с линией ВП (с высоким порогом возбудимости). Высокая возбудимость нервной системы определяет большую выраженность снижения митотических нарушений в присутствии отражающих элементов — резонаторов Aires Defender Pro в условиях действия излучения ультравысокочастотного диапазона стандартного Wi-Fi-роутера. Показано, что генотип животных и функциональное состояние их нервной системы влияют на восприимчивость к электромагнитным излучениям ультравысокочастотного диапазона и действию резонаторов.

❀ **Ключевые слова:** электромагнитное излучение ультравысокочастотного диапазона (УВЧ-диапазона); резонаторы Aires Defender Pro; хромосомные аберрации; костный мозг; возбудимость нервной системы; крысы.

INTRODUCTION

The impact of technogenic electromagnetic fields on the Earth's biosphere is constantly increasing. This phenomenon is associated with increase in the number of artificial sources of electromagnetic radiation (EMR). EMR parameters differ from those of the Earth's geomagnetic field; the nature of their effect on biological objects differs as well. Man-made electromagnetic fields are characterized by their increased power and higher radiation frequencies. Their effect on humans is impacted by a reduction in penetration depth and an increase in the energy factor of the effect. Insufficient data about the effects of such EMR on animals and humans means there is an urgent need for the study of potential mechanisms of magnetobiological effects (MBE), assessment of their after-effects, and the development of potential methods and tools for electromagnetic shielding.

Wireless communication systems such as Wi-Fi routers are one of the major sources of technogenic electromagnetic fields. Different shielding strategies against such types of irradiation are being developed, in particular, by power reduction and change in the spatial properties of EMR sources. Recently, a new direction has appeared in terms of device manufacturing—resonator-reflectors [1], which, together with the main sources of irradiation, produce different magnetobiological responses in living systems.

Because of the wide-spread use of home appliances, cellular phones, and wireless routers, EMR effects on the genetic apparatus of cells of different organs in humans and animals have increased. It has been demonstrated that electromagnetic fields of different frequencies, including the range of cellular phones and Wi-Fi, can induce a wide range of genetic damage, modify gene expression, and affect the structural and functional properties of the cell nucleus [2–7]. However, the mechanisms behind EMR effects on the genetic material as well as the whole organism are still difficult to understand [5, 9, 10]. Individual peculiarities of organism responses to the electromagnetic fields' effects are almost unknown, as is the role of the functional condition of its nervous system in determining the sensitivity/resistance to their effects.

Thus, it is important to study the mechanisms of EMR effects on genetic processes in the cells of the central and peripheral organs of models with respect to the genetically determined properties of the nervous processes, particularly nervous system excitability, as well as creation of shielding mechanisms against the harmful effects of EMR. At present, Aires Human Genome Research Foundation has developed devices—resonators Aires Defender Pro (hereinafter called resonators) for reflecting EMR [1]. Total EMR effects of wireless communication systems and resonators on animals can be weaker and could potentially be completely neutralized by changing the parameters of the electromagnetic field. However, the mechanisms of resonator protection and how they affect the chromosome apparatus of the cells with regard to the peculiarities of the genetically determined level of excitability of the nervous system of animals have not been studied yet.

The goals of this study are as follows:

1) Perform a comparative study of EMR effects of standard Wi-Fi routers of 2.4 GHz, operating 4 days, 6 h per day, on the state of the genetic apparatus of dividing cells of the bone marrow of male rats of two strains with low and high threshold of the nervous system excitability (LT and HT accordingly) and Wistar rats;
2) Assess EMR effects of standard Wi-Fi routers in the presence of a resonator and those of the resonator itself on the state of genetic apparatus of dividing cells of the bone marrow of the LT and HT strain rats and Wistar rats.

MATERIALS AND METHODS

The objects of examination were rats of two strains, selected by the value of their nervous system excitability threshold—HT (high threshold, low excitability) and LT (low threshold, high excitability) [11, 12], and the Wistar rats of the original strain (body weight: 300–350 g) from the biocollection of the FSBIS Pavlov Institute of Physiology RAS (No. 013420180003). Rats of HT and LT strains were grown in standard conditions of the animal facility of the Laboratory of Genetics of Higher Nervous Activity of the Pavlov Institute of Physiology RAS and were given free access to water and food. Wistar rats were obtained from the central animal facility of FSBIS Pavlov Institute of Physiology RAS. After receipt, the animals were held in the conditions of the laboratory animal facility minimum for 2 weeks for adaptation . Males were kept in groups, six species each, in standard cages with a standard dietary intake. About 8–10 animals were used per option.

The international principles of the Declaration of Helsinki were adhered to when working with the animals.

A standard Wi-Fi router (wireless router LinkSys E1200EE/RU) was used with wireless communication frequency of 2.4 GHz, two internal antennas with amplification factor 4 dBi, output power of 16.5 dBm, and average distance to animals of 40 cm.

To study router EMR, the cage with animals was put inside a Faraday shield for protection against external low frequency electric fields. The Wi-Fi router was placed under the top cover on a removable shelf in the center [39]. It was previously demonstrated that the router operation mode of 4 days, 6 h per day, had the highest damaging effect on the chromosome apparatus of the bone marrow of Wistar rats [39]. Therefore, experimental groups of animals were subjected to the ultra-high frequency (UHF) radiation of the Wi-Fi router daily for a period of 6 h (8:00–14:00) for 4 days (Group "R"). The groups of rats placed in the Faraday shield and treated in the same way but without a Wi-Fi router (C2) as well as the intact animals that were not subjected to any effects (C1) served as the control animals.

To assess the effect of the resonator [15], one experimental group of each strain under examination was subjected to a similar (4 days, 6 h per day) treatment with a Wi-Fi router in the Faraday shield with resonators (option "R + Rzt"). Six resonators placed in the center of each side of the Faraday shield [39] were used.

One more group of animals of the Wistar strain was placed in the Faraday shield with resonators but without the router (option "Rzt").

Preparation of the bone marrow cells

Twenty-four hours after completion of effect, the bone marrow samples of animals were fixed in the freshly prepared Clarke's fluid (1 part glacial acetic acid: 3 parts ethanol) and stored at +4 °C. Samples of the bone marrow were stained with 2 % aceto–orcein solution and squashed preparations were prepared according to the standard technique [16, 17].

Analysis of the bone marrow preparations

Preparations of the bone marrow cells were analyzed using microscope Micromed-3 (400–1000× magnification) with camera MS-300 (additional 1.6× magnification). Chromosome aberrations were studied at the stage of anaphase–telophase (standard ana-telophase method) with regard to additional recommendations [17]. Minimum of 200 cells were analyzed from every animal. Normal and aberrant anaphases–telophases with the following types of disturbances were analyzed: single rearrangements (fragment, bridge, laggard) and multiple rearrangements (two or more disturbances of any type per cell).

Statistical methods

Percentage frequencies of detected mitosis disturbances were calculated. Data were verified for homogeneity and normality of distribution of the examined indicators using chi-square test; the data were considered statistically dissimilar at the level of significance $p < 0.05$ [18]. Significance of differences among options was determined by means of ANOVA tests (one-way analysis of variance) and Mann–Whitney test using software Statgraphics Centurion XV11 and Statistica 6.0.

RESULTS

Verification of the obtained primary data detected the intra-group homogeneity at the absence of normality of distribution. This allowed combining results in every option; however, non-parametric methods were used for further comparative analysis.

Experiment results for the assessment of the effect of EMR of a Wi-Fi router and the effect of resonators on the stability of the genetic apparatus in the bone marrow cells of Wistar rats demonstrated that Wi-Fi router exposure for 4 days, 6 h per day, led to destabilization of genetic apparatus of the dividing cells of the bone marrow. Total frequency of mitotic disturbances was found to increase by 3.9 and 3.6 times in the experimental group compared with the control groups C2 and C1, respectively (Fig. 1)

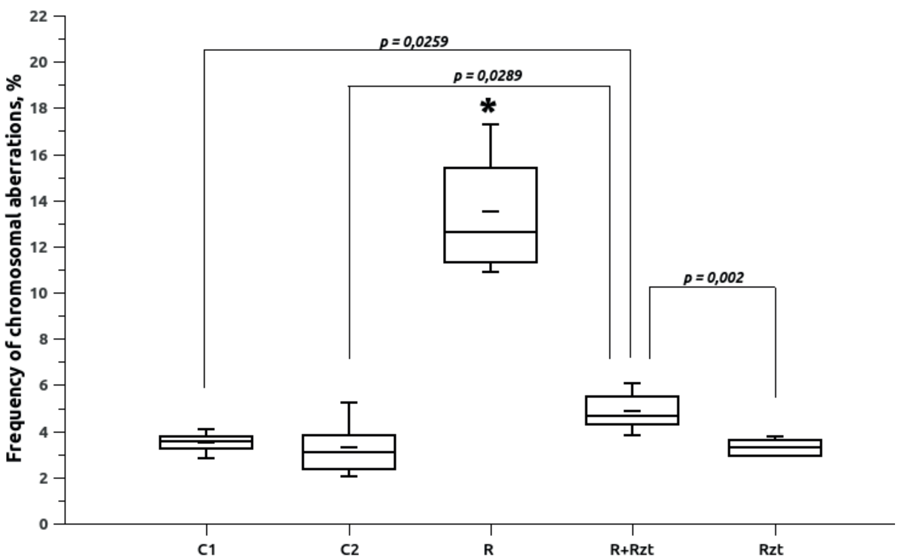

Fig. 1. The frequency of chromosomal aberrations in the bone marrow cells of male Wistar rats after the action of electromagnetic radiation of the router and resonators. The values of medians, means (short line), quartile boundaries, minimum and maximum values in the analyzed groups are given. C1 — intact rats, C2 — rats placed in a Faraday cage, R, Rzt and R + Rzt — animals subjected to the appropriate effects of the router, resonators or their joint action. * difference from all other options (Mann–Whitney test, p <0.0005)

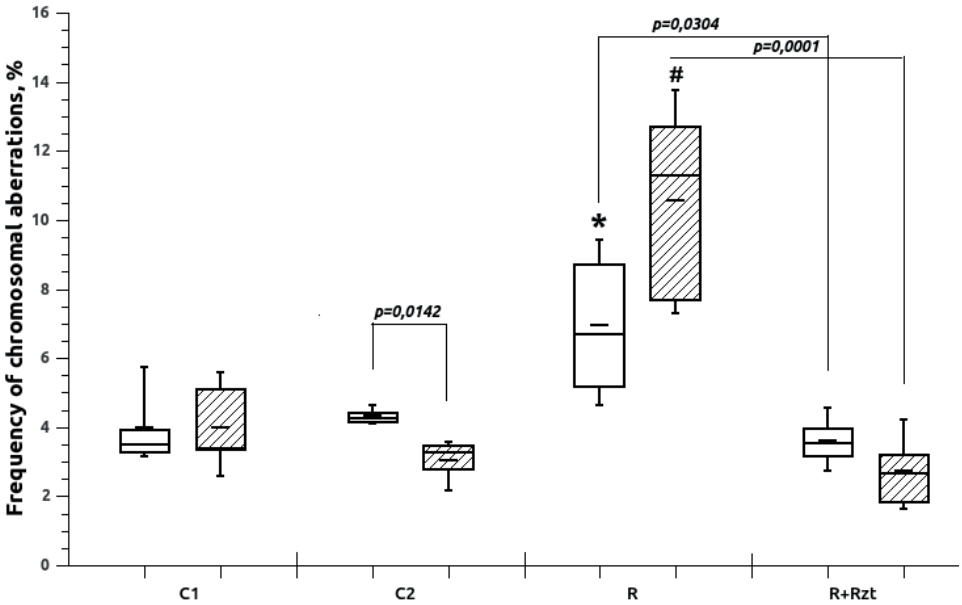

Fig. 2. The frequency of chromosomal aberrations in the bone marrow cells of male rats of strains with high and low thresholds of excitability (highlighted in gray) after the action of electromagnetic radiation from the router and resonators. The designations are the same as for fig. 1. * difference from the C1 and C2 variants of the EP line are reliable (Mann – Whitney test, p <0.0005), # difference from the C1 and C2 variants of the NP line are reliable (Mann–Whitney criterion, p <0.0005)

($p < 0.0005$). The presence of resonators resulted in a reduction in the frequency of chromosome disturbances by 2.8 times in comparison with the EMR effect of the Wi-Fi router (see Fig. 1). However, the level of chromosome aberrations was 1.3 and 1.4 times higher in the experimental groups than in the control groups C2 ($p = 0.029$) and C1 ($p = 0.026$), respectively.

Exposure of animals in the Faraday shield to only resonators did not affect the frequency of chromosome disturbances compared with both control groups, which indicates the absence of destabilizing effects of resonators on the genetic material.

EMR in the UHF range (in the mode of Wi-Fi router operation within 4 days, 6 h per day) resulted in destabilization of genetic apparatus of the dividing cells of the bone marrow of male rats of both the examined strains, HT and LT. The total frequency of mitotic disturbances in animals from the low-excitable HT strain increased 1.6 and 1.9 times in comparison with C2 and K1, respectively (Fig. 2). The frequency of chromosome aberrations in rats of the high-excitable LT strain in the same conditions increased 3.2 times in comparison with those of group C2 (Faraday shield, 4 days, 6 h per day), and 2.5 times in comparison with those of group C1 (see Fig. 2), which had a high degree of significance in all used statistical criteria. Though statistically significant interstrain differences in the frequency of chromosome disturbances after EMR effects of the Wi-Fi router treatment were not detected in the used samples, the degree of destabilization of the genome of the bone marrow cells of LT rats nevertheless was two times higher than that of HT rats (see Fig. 2), which indicates higher sensitivity of the LT rats to EMR effects in the range under examination ($t = 3.38$, $p < 0.01$).

Bone marrow cell sensitivity to router effects in Wistar rats was higher (level of chromosome aberrations was increased 3.9 times) than that of HT strain rats (increase of 1.6 times, ANOVA: $F = 19.04$ $p = 0.0018$), but was comparable to that of LT strain animals, which demonstrated an increase in the sensitivity by 3.3 times.

It should be noted that higher levels of chromosome damage in the HT strain rats were detected in comparison with the alternative LT strain during exposure in the Faraday shield; however, differences with appropriate groups of intact controls were not detected.

The presence of resonators with a working Wi-Fi router reduced the destabilizing effect of the latter on the HT strain rats from 6.7 % to 3.6 %, and the effects on LT strain were reduced from 9.8 % to 2.7 %. The frequency of chromosome aberrations in both HT and LT strains did not differ from that in the respective controls C2 and C1 (see Fig. 2). It should be noted that despite the absence of statistically significant interstrain differences in the frequency of chromosome damages with simultaneous treatments with a Wi-Fi router and resonators, reduction of frequency of mitosis disturbances was more apparent in the LT strain males (3.6 times) than in the HT strain (1.9 times) ($t = 5.86$; $p < 0.001$).

DISCUSSION

In the structure of this experiment, it is reasonable to consider the running Wi-Fi router as the unit radiator (despite the fact that the spectrum of its irradiation was not reduced to single frequency) to differentiate the nature of its effect on biological objects from other sources of radiation, including the ones similar to the source under examination. Introduction of an additional factor in the form of resonators acting as reflectors allows the study of the formation of the modified structure of EMR [19]. The effect of EMR different in structure will have different MBE on biological objects. Possible mechanisms of the effect of high frequency irradiation on biological objects have been described and discussed in detail in other studies [20–23].

The depth of EMR penetration in biological media is specified by its magnetic component and does not exceed ¼th of the wavelength. A wavelength of 12.5 cm corresponds to a frequency of 2.4 GHz. For this wavelength, the depth of penetration in any biological media does not exceed 3.125 cm. Additional factors in penetration reduction depend on the type of biological medium or tissues (bone, muscles, fats, etc.) through which radiations penetrate.

The maximum output power of irradiation did not exceed 16.5 dBm = 44.67 mW = 0.04467 J/s for the specific type of router used in this study (LinkSys E1200EE). Biological objects (rats) were placed at 40 cm from the source of irradiation (router), which allowed reducing the power of the affecting radiation in inverse proportion to square of the distance. The value of heat barrier for one act of molecules i.e. chemical transformation (local heat response) at 25 °C (298 K) amounts to $kT = 4.11 \cdot 10^{-21}$ J. It is clear that the level of radiation of this specific type of router at this distance from the animals does not exceed the kT value. The issue of the value of kT level in magnetobiology has been comprehensively discussed in the fundamental work of V.N. Bingi and A.V. Savin [24].

Thus, as the intensity of used EMR does not cause any temperature changes in the biological object, its response to such effects can be specified by the biological structures located at the depth of radiation penetration in which MBEs are possible. Different elements of the neural circuitry or neurons can be considered as major candidates of such biological structures, which is stipulated by their structural arrangement.

When several different sources of radiation (in frequency and power) affect complicated biological systems, each of them can affect different structural fragments, and different elements of one structural fragment can cause their own MBE. Overlapping of specific MBEs can result in multidirectional changes of the registered effects caused by one source.

Possibly, this particular case of such overlap is the reduction of the effect of chromosome destabilization in the rat bone marrow cells due to the radiation of the Wi-Fi router acting together with the resonators.

The results of assessment of the level of chromosome aberrations in the bone marrow cells of Wistar male rats confirmed that the EMR from the Wi-Fi router working 4 days, 6 h per day, has cytogenetic activity and induces chromosome aberrations [39]. Our experiments demonstrated that resonators did not affect the level of chromosome damage in the bone marrow cells of Wistar rats in comparison with the control groups.

Results of assessment of the level of chromosome aberrations in the bone marrow cells of rats of LT and HT strains with contrasting excitability of the nervous system demonstrated that EMR from the Wi-Fi router running 4 days, 6 h per day, induces mitotic disturbances in animals of both strains. Statistically significant interline differences in the frequency of chromosome damages were not detected; however, the level of destabilization of the genome of the bone marrow cells of HT strain rats is higher in comparison with the LT strain, indicating dependence of their sensitivity to the effect of EMR in the examined range on the functional condition of the nervous system. Sensitivity of the bone marrow cells of Wistar rats was higher in comparison with the LT strain but it was comparable to the HT strain. Thus, the nature of EMR's damaging effect on the chromosome apparatus of the bone marrow cells depends on the genotype and is associated with the level of excitability of the animals' nervous system.

It is known that bridge and fragment types of chromosome damages fixed in the anaphase and telophase of mitosis result from faulty repair of damaged DNA at the stage of interphase and/or from erroneous replication [26]. EMR possibly induces DNA damage at the initial stages of its effect. Consequences can be observed at the chromosome level 24 h after beginning of the exposure with regard to the average duration of the cell cycles of rats and mice [27].

Despite a significant amount of work demonstrating the availability or absence of biological effects of UHF EMR, the specific mechanisms of their impact on biological systems are still unknown. HF EMR likely affects weak electromagnetic links used to support conformation of biomolecules and supramolecular structures [5]. EMR effects on the cell in the high frequency range can be associated with the change in activity of the inner cell signal systems and the effect on the secondary messengers, DNA, and enzymes [9, 10]. Participation of the neuroendocrinal system in response to EMR is believed to mediate its effect on the operation of the individual organs as well as the entire organism [5]. An increase in the level of stress hormone and pathological changes in the suprarenal glands of Wistar rats were detected because of the long-term (4–8 weeks) effect of the radio frequencies of cellular communication [25]. It is well known that corticosterone, adrenaline, and noradrenaline are the most important neuroendocrine factors of DNA damage induction during stress [29, 30].

Because there is evidence of cell oxidative stress development under the effect of high frequency EMR [28], it is possible that the stability of the genetic apparatus of dividing cells of the bone marrow changes under the effect of the EMR router in our experiments. It is well known that the mutagenic activity of oxidative stress is based on genotoxic activity of endogenous factors of a humoral nature and free-radical products of peroxidation [28].

It can be assumed that detected chromosomal disturbances are capable of affecting the functional links of the immune system associated with operation of the bone marrow, leading to immune suppression and inhibition of immunopoesis and hematopoesis [31]; however, these issues require further study.

Examination of the individual features of sensitivity/resistance to EMR effects and protection tools against EMR on the genetic apparatus of cells is of great interest. This aspect is directly associated with the development of methods of prevention and correction of disturbances caused by EMR and is based on a personalized approach. Genotype effects on the degree of chromosome damage under the effect of mutagens, genotoxicants, and stress factors of different natures are well known [32–34]. Information is limited regarding genotype specificity of EMR effects [35, 36]. This work presented the different sensitivities of rat strains (Wistar, LT, HT), which was evaluated by the degree of increase in the chromosome aberration frequency after exposure to the EMR of a wireless router. The chromosome apparatus of highly excitable animals of the LT strain was more susceptible to EMR damage in comparison with the HT strain. Reduction of mitotic damage relative to the higher level of chromosome aberrations induced by EMR in the used range was better for the LT strain rats with resonators. Previously, we have demonstrated that rats of the highly excitable LT strain are more sensitive in comparison with the low-excitable HT strain to the effect of cyclophosphane mutagen and to the effect of psychoemotional stress, which is also stated by an increase in the quantity of cytogenetic damage to the bone marrow cells [37, 38]. Likely, a high tonus of the nervous system which was genetically induced, correlated with higher metabolic activity of the organs and tissues, with more active functioning of the thyroid gland and hypothalamus [11], and determines higher sensitivity of the chromosome apparatus to the number of damaging effects such as UHF EMR. For example, enhancement of the mutagenic effect of microwaves in the case of the level of thyroid hormones is known [40]. It is also possible for selection to have an effect on the increase of mutability of highly excitable rats [37].

In summary, the level of effect of UHF EMR can depend on the following:

a) The nature of MBE in structural elements of biological objects formed under the effect of EMR of several sources—router and resonators;

b) The functional condition of the nervous system determined by the difference of genotypes of animals of the used strains.

In general, the peculiarities of the effects of radiation sources with different properties on the various structural elements of biological objects along with the use of the animal strains of certain genotypes, such as those with contrast properties of the nervous processes, can help understand the mechanism behind EMR effects and the methods of reducing their damaging effects and the study of the methods of reducing their damaging effect.

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