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PAVLOV INSTITUTE OF PHYSIOLOGY OF THE RUSSIAN ACADEMY OF SCIENCES

«APPROVE»
Director
of Pavlov Institute of Physiology
of the RAS
corresponding member of RAS
L.P. Filaretova
_____
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REPORT
UNDER THE AGREEMENT ON SCIENTIFIC COOPERATION
WITH AIRES HUMAN GENOME RESEARCH FOUNDATION

Subject: Study of high-frequency electromagnetic radiation impact and Aires resonators influence on behavior, genetic and epigenetic processes in cells of central and peripheral organs (models organisms: rat (*Rattus norvegicus*) and honey bee (*Apis mellifera L.*)

FIRST STAGE: Study of electromagnetic radiation, emitted by the router, and influence of Aires resonators - converters on stability of genetic processes in bone marrow cells of Wistar male rats.

Principal investigator

Senior Research Scientist
Laboratory of Higher Nervous Activity Genetics
_____ Ph.D., Dr.Sci. N.A.Dyuzhikova

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REPORT

ON AGREEMENT FOR SCIENTIFIC COOPERATION BETWEEN FEDERAL STATE BUDGETARY SCIENTIFIC ESTABLISHMENT PAVLOV INSTITUTE OF PHYSIOLOGY OF THE RUSSIAN ACADEMY OF SCIENCE AND AIRES HUMAN GENOME RESEARCH FOUNDATION "research of high frequency electromagnetic radiation effect and Aires resonators influence on behavior, genetic and epigenetic processes in cells of central and peripheral organs (model organisms: rat (Rattus norvegicus) and honey bee (Apis mellifera L.)"

THE FIRST STAGE (April – September 2016): research of router's electromagnetic radiation effect and Aires resonators' influence on stability of genetic apparatus in marrow cells of Wistar male rats

In the context of technological advancement, growth of power generation, development of communications systems there is an increasing negative influence of non-ionizing electromagnetic radiation (EMR) on human organism. Disturbance of environmental electromagnetic background is recorded everywhere, causing steady increase of different diseases, including microwave sickness, electromagnetic hypersensitivity (EHS). The literature on EMR influence on human and animal organism is quite extensive: http://www.bioinitiative.org/report/wp-content/uploads/pdfs/RFR-11_28-research-summary.pdf, http://www.bioinitiative.org/report/wp-content/uploads/pdfs/RFR-free-radical-abstracts.pdf . It has been established that EMR enhancement influences genetic apparatus of cells in different human and animal organs. For the first time mutagenic effect of certain electromagnetic frequencies was demonstrated in 1959 in the paper by Heller, Teixeria-Pinto, published in the journal Nature (Heller, Teixeira-Pinto, 1959). At present, evidence has been collected that electromagnetic fields of different ranges including mobile phones and Wi-Fi can induce a broad spectrum of genetic damage, modify gene expression, affect structural and functional characteristics of cell nuclei (Kryukov, 2000; Deinekina, 2002; Boyko et al., 2010, et al.). For example, it was proven that the effect of microwave radiation (frequency of 7.7 GHz, power of 0,5,10,30 mW/cm2) with an exposure interval from 10 to 60 minutes increases chromosome aberrations in human lymphocytes (Garaj-Vrhovac et al., 1992). With long periods of exposure, signals with a specific absorption rate (SAR) of 5 W/kg also damage chromosomes in blood cells (Tice et al., 2002). However, the mechanisms of those processes are still insufficiently understood.

There is a pressing need to create a system for protection from harmful effect of EMR and study the mechanisms of its influence on genetic processes in the cells of central and peripheral organs of model organisms. At present, Aires has created devices on the basis of the fractal-

matrix EMR conversion nanotechnology that efficiently redistribute EMR and have curative properties
(http://www.aires.spb.ru/nano.html, http://airestech.ru). However, the mechanisms of protective effect of those devices on the cell chromosome apparatus have not been studied.

Purpose and objectives of the first stage:

1) research of standard Wi-Fi router's EMR effect in different modes of operation on destabilization of the genetic apparatus in dividing marrow cells in order to identify the conditions of induction of mitotic disturbances by high-frequency EMR;

2) evaluation of the protective effect of Aires Defender fractal-matrix resonators on the stability of the genetic apparatus in dividing marrow cells of Wistar male rats under router's damaging EMR effect.

MATERIALS AND METHODS.

The research was performed on Wistar male rats weighing 250-300 g received from the animal quarters of the Federal State-Funded Establishment of Science Pavlov Institute of Physiology of the Russian Academy of Sciences. Rats of an outbread Wistar population are a generally recognized model used in medical and biological research. Upon arrival, the animals were maintained in a laboratory animal quarters for at least two weeks for adjustment. The males were kept in groups of 6 in standard cages on a standard food ration.

The research employed a standard Wi-Fi router (LinkSys E1200-EE/RU wireless router) with the following characteristics: wireless frequency: 2.4 GHz, number and type of antennas: 2 internal antennas, standard antenna gain factor, dBi: 4 dBi.

To study router's EMR effect, the «home» cage with animals was placed in a Faraday cage (Fig. 1), a router was placed under the top lid of the cage in the center of a removable shelf. The experimental groups were exposed to the router's effect in the following modes:

1) once for 2 hours (8:00-10:00);
2) 4 days, 6 hours a day (8:00-14:00);
3) 3 weeks, 6 hours a day (8:00-14:00).

The reference was groups of rats placed in a Faraday cage at the same time, but with no router, as well as intact animals.

The experiments also employed Aires Defender fractal-matrix resonators (special circular diffraction grating), which are a universal space-wave Fourier filter (Zhabrev et al., 2005). Due to interaction of the electromagnetic field with the Aires Defender, its structural transformation occurs. To evaluate resonators' influence on the router's damaging EMR effect, 6 resonators were

used. They were placed in the center of each side of the Faraday cage (Fig. 1). One of the experimental groups was exposed to router's effect in the Faraday cage as per the schedule, 6 hours a day for 4 days.

Making human marrow cell preparations. 24 hours after exposure, human marrow cells were fixated in freshly made fixative (1 part of glacial acetic acid : 3 parts of ethanol) for at least an hour. The material was kept at a temperature of +4□C until specimens were prepared following the routine method (Makarov, Safronov, 1978; Dayev et al., 2009).

Analysis of marrow preparations. Squash marrow preparations were analyzed using a Micromed-3 microscope with x640–1600 . Chromosome aberrations at the anaphase–telophase stage were factored in (the standard ana-telophase method) with consideration of additional recommendations (Dayev et al., 2009). At least 200 cells from each animal were analyzed. The study considered the number of normal and aberrant anaphase-telophases with the following types of disturbances: single reorganizations (a fragment, a bridge, a lagging chromosome), multiple reorganizations (two or more disturbances of any type per cell) (Fig. 2).

Statistical processing. The data was checked for homogeneity using the nonparametric method of multi-field χ2. Based on the check, individual data was united within groups. To present the findings in tables, frequencies of detected disturbances in mitosis were calculated and expressed as per cent with and error percentage. Among cells with chromosome reorganization, shares of cells with different types of disturbances were determined, which characterized the spectrum of chromosome aberrations (Dayev et al., 2009). Significance of differences between the versions was determined and the spectra of mitotic disturbances were compared by means of the χ2 method (Glotov et al., 1982), as well as a ranking test and ANOVA using Statgraphics Centurion XV11 Statistica 6.0 software.

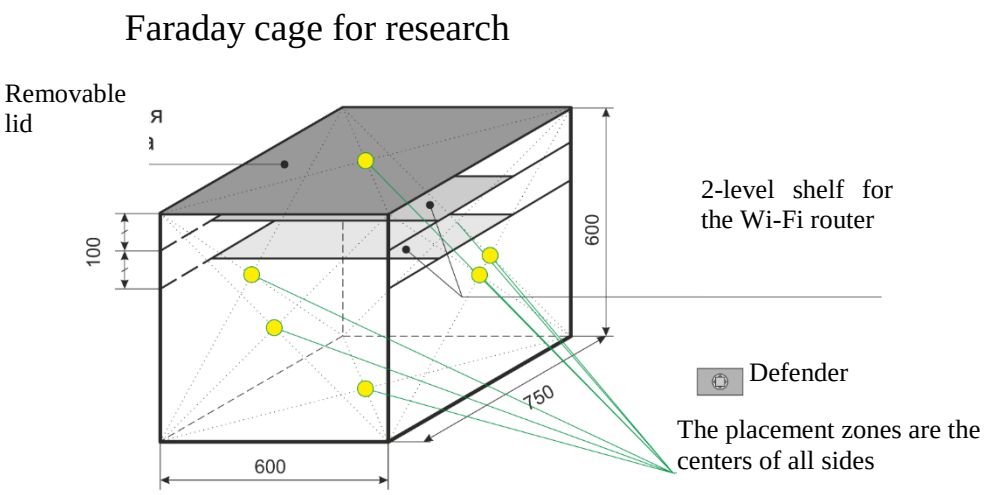

Fig. 1. The diagram of the Faraday cage used in the research showing the location of resonators.

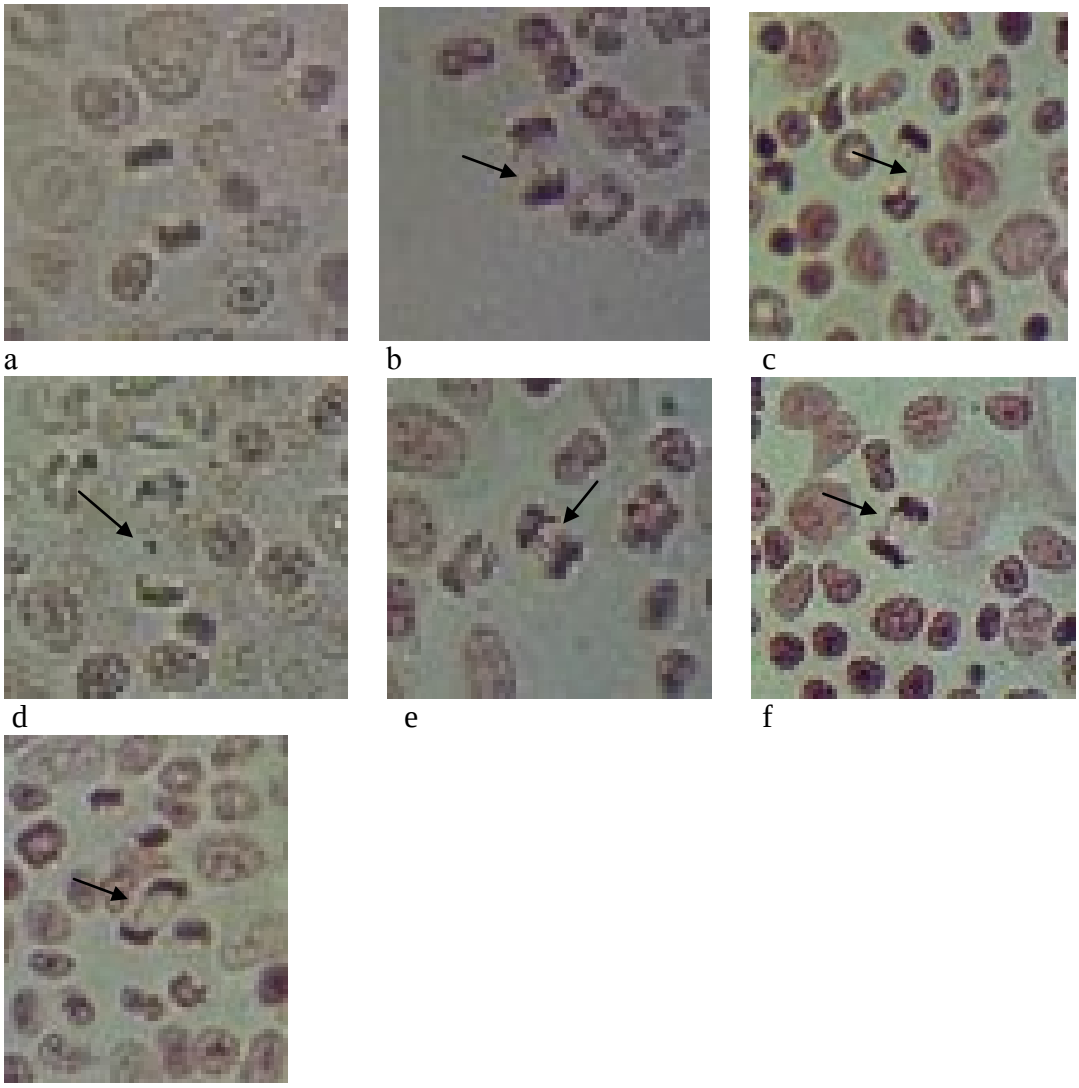

Fig. 2. Dividing marrow cells in rats at the ana-telophase stage.

There are examples of analyzed reorganizations (labeled with arrows): a- normal ana-telophase, b- lagging chromosome, c- bridge, d- fragment, d,e-multiple reorganizations with the bridge and lagging chromosomes, f- double bridge.

RESULTS

It is demonstrated that high frequency electromagnetic radiation of a router in the exposure mode of 4 days, 6 hours a day cause d the highest destabilization of genetic apparatus of the dividing marrow cells in Wistar male rats: the overall frequency of mitotic disturbances rises by 4.5 times as compared to Reference2 (Faraday cage, 4 days x 6 hours) and by 3.9 times as compared to Reference1 (Table 1), which has a high degree of significance in all statistical criteria applied. One-time 2-hour long exposure to router's effect raises the number of chromosome aberrations by 1.9 times as compared to the intact Reference 1 (Table 1), significance of differences is confirmed by the multiple range test (Multiple Range Test ,

Diff.=6,03, +/-Limits=3,33) and ANOVA (F= 19,65 , p< 0,004), but not $\chi 2$. At the same time, as compared to the group of animals exposed to router's effect for 4 days, 6 hours a day, the frequency of disturbances reduced by 2.1 times (Table 1). After the animals were exposed for 3 weeks, 6 hours a day in the conditions of router's electromagnetic radiation, the frequency of mitotic disturbances rises by 1.8 times as compared to Reference 3 (Faraday cage, 3 weeks, 6 hours a day) (Multiple Range Test , Diff.=5.02, +/-Limits=3.63; ANOVA , F= 3,13 , p< 0,01) and by 1.5 times as compared to the intact Reference 1 (Multiple Range Test, Diff.=4,1, +/-Limits= 3,9; ANOVA , (F= 2,49 , p< 0,04), but it decreases by 2.6 times as compared to the group of animals exposed to the router's effect for 4 days, 6 hours a day (Table 1).

The effect of Aires Defender fractal-matrix resonators was evaluated using the pattern of 4 days, 6 hours a day, which caused the most damage to the marrow cell chromosome apparatus. Using resonators in combination with router's effect reduces fourfold the frequency of damage to mitosis in dividing marrow cells as compared to router's effect without protection, and that level is comparable to Reference 2 (Faraday cage, 4 days, 6 hours a day) and intact Reference 1 (Table 1).

Comparison of the spectra of spontaneous mitotic disturbances and those induced by router's EMRs revealed that router's effect of 4 days, 6 hours a day changes the spectrum of chromosome disturbances due to increase in the share of lagging chromosomes and decrease in the share of bridges among all types of disturbances, as compared to Reference 2 and Reference 1 (Table 2). Here it is important to note that the Reference 1 and Reference 2 groups differ by the share of lagging chromosomes and multiple reorganizations among all types of disturbances (Table 2). Using resonators changes the spectrum of disturbances owing to all types of aberrations in question as compared to router's effect alone (Table 2).

Table 1. The frequency of mitotic disturbances in marrow cells of Wistar male rats after exposure to router's electromagnetic radiation in different modes.

Versions of exposure	Number of analyzed cells (those with disturbances among them)	Overall frequency of disturbances in mitosis	Significance of differences $\chi 2$ ($\nu = 1$, $p < 0{,}01$)
Reference1 (intact)	1378 (92)	6.7 ± 0.7	
Reference2 (Faraday cage, 4 d x 6 hrs)	1986 (114)	5.7 ± 0.5	75.8
Router (4 d x 6 hrs)	1360 (354)	26.0 ± 1.2	18.9
Router+resonator (4 d x 6 hrs)	1961 (127)	6.5 ± 0.6	16.8
Reference3 (Faraday cage, 3 weeks x 6 hrs;	1714 (96)	5.6 ± 0.5	8.9
Router (3 weeks x 6 hrs)	1789 (180)	10.1 ± 0.7*#	6.5
Router (2 hours)	1175 (149)	12.7 ± 0.9*	

Legend: vertical lines mean significantly different values ($\chi 2$ criterion), # – differences from Reference3 are significant (Multiple Range Test, ANOVA ($p<0{,}01$), *- differences from Reference1 are significant (Multiple Range Test, ANOVA ($p<0{,}01$) .

Table 2 The spectrum of mitotic disturbances detected in the marrow cells of Wistar male rats after exposure to router's electromagnetic radiation in different modes (%).

Versions of exposure	Number of cells with mitotic disturbances	Fragments	Bridges	Lagging chromosomes	Multiple reconstructions	Significance of differences χ^2 (ν= 3, p< 0,01)
Reference1 (intact)	92	5	37	46	12	10.3
Reference2 (Faraday cage, 4 d x 6 hrs)	114	8	32.5	32.5	27	9.6
Router (4 d x 6 hrs)	352	9	18	59	14	16.5
Router+ resonators (4 d x 6 hrs)	127	5	33	30	32	22.1
Reference3 (Faraday cage, 3 weeks x 6 hrs;	96	9	26	40	25	
Router (3 weeks x 6 hrs)	180	8	28	44	20	
Router (2 hours)	149	7	29	33	31	

Legend: vertical lines mean significantly different values.

DISCUSSION

The findings of the evaluation of chromosome aberrations in marrow cells of Wistar male rats revealed that router's electromagnetic radiation using different exposure modes (2 hours, 4 days x 6 hours a day, 3 weeks x 6 hours a day) has cytogenetic activity, can induce mitotic disturbances. They can affect the functioning of immune system links related to the marrow's operation.

The most significant changes in the chromosome apparatus were detected after exposure of the animals in a Faraday cage with a plugged in router for 4 days, 6 hours a day – from 8 am till 2 pm. That is the very mode to use for pronounced induction of mitotic disturbances in

Wistar rats by means of high-frequency EMR for the purpose of subsequent research of the efficiency of protective devices, as well as mechanisms underlying their protective properties.

Differences between the spectra of spontaneous EMR caused by exposure of animals in the Faraday cage, as well induced EMR of a router in combination with the effect of matrix resonators of chromosome disturbances can indicate a specific response of the animals' organisms to the applied effects.

Change in stability of the genetic apparatus of dividing marrow cells under the influence of a router's EMR can be viewed as the result of cell oxidation stress, whose mutagenic activity is based on the genotoxic effect of internal causes of humoral nature and free-radical products of peroxidation (Achudume et al., 2010). Those mechanisms can cause immunosuppression, inhibition of immunopoesis and haematopoesis, which will affect the state of the organism. However, information that long-term, 3-week-long exposure of animals to EMR conditions decreases the level of mitotic disturbances relative to a 4-day-long session indicates that there may be adaptive mechanisms causing elimination of cells with disturbances and/or activation of reparative processes.

It should be noted that we were the first to demonstrate that Aires Defender fractal-matrix resonators have a protecting effect on the genetic apparatus of dividing marrow cells in Wistar male rats under router's EMR effect. Its interaction with an Aires Defender causes structural transformation of electromagnetic field (Zhabrev et al., 2005). In a counting number of directions, electromagnetic field strengths concentrate, and in the other directions the electromagnetic field reduces significantly, which in general weakens the sum-total electromagnetic effect exceeding the organism's sensibility threshold to electromagnetic radiation. Thus, resonators' protective properties are evidently based on restructuring (conversion) of incident EMR, which reduces its damaging effect on dividing cells.

Significantly, among rats from the group using Aires Defender resonators there were several subjects whose chromosome aberration level reduced even in comparison with the intact reference. It is a very interesting fact that calls for further research and special experiments to evaluate the effect of resonators proper on stability of the genetic apparatus in marrow cells of rats.

BIBLIOGRAPHY

Boyko O.V., Lantushenko A.O., Lukyanchuk G.A., Salamatin V.V., Shkorbatov Yu.G. Effect of microwave radiation on WIMAX mobile communication frequencies on the state of chromatin in human buccal cells.// Academic Proceedings of Taurida National V.I. Vernadsky University. Series «Biology, Chemistry». V.23(62), No. 4. P.56-65. (Бойко О.В., Лантушенко А.О., Лукьянчук Г.А.,Саламатин В.В.,Шкорбатов Ю.Г. Влияние микроволнового излучения на

частотах мобильной связи и сети WIMAX на состояние хроматина клеток буккального эпителия человека.//Учебные записки Таврического национального университета им. В.И.Вернадского. Серия «Биология, химия». Т.23(62), №4. С.56-65.)

Glotov N.V., Zhivotovsky L.A., Hovanov N.V., Hromov-Borisov N.N. Biometrics. Leningrad, 1982. (Глотов Н.В., Животовский Л.А., Хованов Н.В., Хромов-Борисов Н.Н. Биометрия. Л. 1982.)

Dayev Ye.V., Vyborova A.M., Kazarova V.E., Dukelskaya A.V. Influence of pheromone-like pyrazine-containing compounds on stability of the genetic apparatus in marrow cells of male Mus musculus L. // Journal of Evolutional Biochemistry and Physiology. 2009. V.45, No. 5. P.486-491. (Даев Е.В., Выборова А.М., Казарова В.Э., Дукельская А.В. Влияние феромоноподобных пиразинсодержащих соединений на стабильность генетического аппарата в клетках костного мозга самцов домовой мыши Mus musculus L. // Журнал эволюционной биохимии и физиологии. 2009. Т.45, №5. С.486-491.)

Deynekina T.A. Influence of electromagnetic fields on cytophysiological parameters of human and animal cells. Author's Abstract, Candidate of Biological Sciences. 2002. 42 p. (Дейнекина Т.А. Влияние электромагнитных полей на цитофизиологические параметры клеток животных и человека. Автореф. дисс… канд.биол.наук. 2002. 42 С.)

Zhabrev V.A., Lukyanov G.N., Margolin V.I., Potehin M.S., Tupik V.A., Serov I.N., Soshnikov I.P. Study of fractal structures C built by method of ionic magnetron sputtering. //Structures of Composite Materials. 2005. No. 4. (Жабрев В.А., Лукьянов Г.Н., Марголин В.И., Потехин М.С., Тупик В.А., Серов И.Н., Сошников И.П. Исследование фрактальных структур Си, полученных методом ионного магнетронного распыления. //Конструкции из композиционных материалов. 2005. №4.)

Kryukov V.I. Genetic effects of electromagnetic fields.// Messenger of New Medical Technology. 2000. V.V11, No. 2. P.8-13. (Крюков В.И. Генетические эффекты электромагнитных полей.// Вестник новых медицинских технологий. 2000. Т.V11, №2. С.8-13.)

Makarov V.B., Safronov V.V. Cytogenetic methods of chromosome analysis. Moscow, 1978. (Макаров В.Б., Сафронов В.В. Цитогенетические методы анализа хромосом. М. 1978.)

Achudume A., Onibere B., Aina F., Tchokossa P. Induction of oxidative stress in male rats subchronically exposed to electromagnetic fields at non-thermal intensities.// J. Electromagnetic Analysis and Applications. 2010. V.2. No. 8. P.482-487.

Garaj-Vrhovac V., Fucic A., Horvat D. The correlation between the frequence of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwaves// Mutation Research. 1992. V.281. P.181-186.

Heller J., Teixeria-Pinto A. A new physical method of creating chromosomal aberration. // Nature. 1959. V.183. P.905.

Tice R.R., Hook G.G., Donner M. Genotoxicity of radiofrequency signals.1. Investigation of DNA damage and micronuclei induction in cultered human blood cells // Bioelectromagnetics. 2002. V.23. P.113-126.