



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.))

STAGE THREE: Study of electromagnetic radiation, emitted by the router, and influence of resonators - converters on behavior and neurodegenerative processes in the brain of male Wistar line rats.

Principal investigator

Head of Laboratory of Genetics of Higher Nervous Activity

N.A. Dyuzhikova

Saint Petersburg, 2017

REPORT

UNDER THE AGREEMENT ON SCIENTIFIC COOPERATION BETWEEN FSBIS PAVLOV INSTITUTE OF PHYSIOLOGY OF RAS AND AIRES HUMAN GENOME RESEARCH FOUNDATION "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 of *Rattus norvegicus* rat and *Apis mellifera* L. honey bee)".

STAGE THREE (May 2017 - October 2017): Study of electromagnetic radiation, emitted by the router, and influence of resonators - converters on training and neurodegenerative processes in the brain of male Wistar line rats.

In today's world natural sources of electromagnetic radiation (EMR) are extensively replenished by anthropogenic fields and radiation. Exposure to radiation, emitted by mobile devices, significantly affects humans and animals (Paul et al., 2015; Kwon et al., 2011; Daniels et al., 2009).

The important indicator of the adverse negative effect of the environment, in particular, EMR, is impairment of cognitive functions, ability to learn and remember in humans and animals (Pavlova et al., 2013). A number of works shows that EMR causes changes in cognitive functions and intellectual disability in children (Guxens et al., 2016; Hawkes et al., 2014), depression of cognitive abilities in elderly people (Ng et al., 2012), impacts sleep quality (Fritzer et al., 2007; Hutter et al., 2006). The change of cognitive function related to spatial orientation in men was revealed upon exposure of infants to ionizing radiation (Hall et al., 2004). At the same time, some human and rodents studies revealed no effects of exposure to radiation, emitted by mobile phones, on the cognitive functions (Besset et al., 2005; Haarala et al., 2005). Therefore, information on EMR impact on human's and animals' cognitive performance is scarce and contradictory, requiring further research.

There is evidence of the negative effect EMR of mobile phones produces on the function of the central nervous system, which is caused by damage of neurons in the brain. It is shown that EMR generated by mobile devices causes damage of neurons in the cerebral cortex, hippocampus and basal ganglion (Salford et al., 2003).

At the present time there are devices, efficiently redistributing EMR and having protective properties, which are created based on the effects of conversion of incident EMR

range by self-affine relief (Serov et al., 2006a, 2006b; Kopyltsov et al., 2007; Zhabrev et al., 2005). However, the mechanisms of protective action of such devices on the learning ability and memory, as well as neurodegenerative processes in the brain have not been studied.

The goal and objectives of stage three:

1) study of EMR impact and resonators - converters influence on conditioning and maintenance of passive avoidance conditioned reflex in male Wistar line rats under conditions of the standard Wi-Fi router mode of operation (4 days, 6 h a day).

2) study of EMR impact and resonators - converters influence on neurodegenerative processes in the hippocampus and neocortex in male Wistar line rats under conditions of the standard Wi-Fi router mode of operation (4 days, 6 h a day).

Material and Methods

The work was performed in male Wistar line rats, weighing 250 - 300 g. The rats were taken from the collection of FSBIS Pavlov Institute of Physiology of RAS (No Г3 0134-2016-0002). After the animals were received, they were held in the laboratory animal facility for no less than two weeks to ensure adaptation thereof. The males were kept in groups of 6 in standard cages on standard diet.

The study was performed, using the W-Fi router (LinkSys E1200-EE/RU wireless router) with the following technical specifications: wireless communication frequency: 2.4 GHz, number and type of antennas: 2 internal antennas, standard antenna(s) gain factor, dBi: 4 dBi. To study EMR effects, the cage where the animals were initially kept, was placed into the Faraday cage, whereas the router was placed in the center, under the upper cover of the cage, on the removable shell. The experimental groups were exposed to the router impact during 4 days, 6 hours a day (8 a.m. - 2 p.m.) with no resonators and with resonators - converters. The controls were groups of rats, which were placed into the Faraday cage at the same time, however, were not exposed to the router, and animals, kept in the laboratory facility. Aires Defender fractal-matrix resonators-converters (special annular diffraction grids), used in the experiments are universal Fourier spatial and wave filter (Zhabrev et al., 2005). The interaction of electromagnetic field with resonators results in structural conversion of the field.

6 resonators were used to assess the resonators influence on harmful effects of EMR, generated by the router. They were placed in the center of each face of the Faraday cage (Fig. 1).

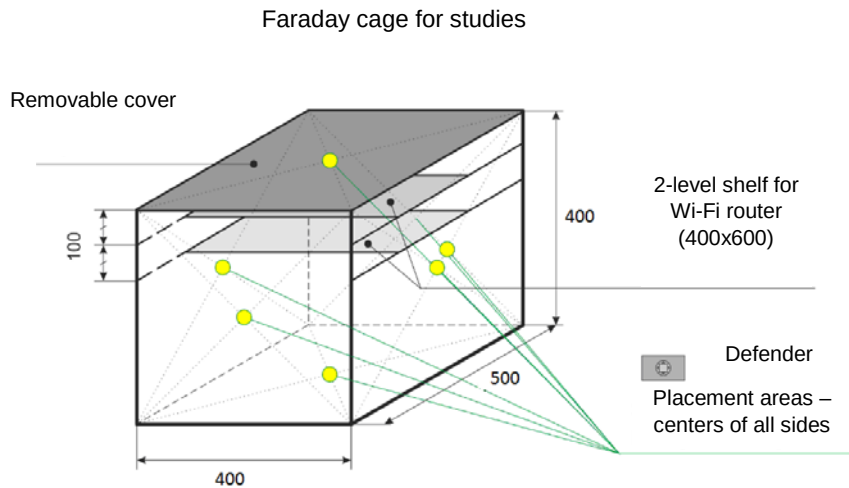


Fig. 1. The diagram of the Faraday cage (shield) used for a study with a layout of the resonators placement.

Method of passive avoidance conditioning in rats. Passive avoidance was conditioned by single negative pain reinforcement 24 hours after the end of 4-day exposure of the animals in the Faraday cage with Wi-Fi router (experimental group 1), Wi-Fi router and resonators-converters (experimental group 2), after exposure in the Faraday cage, without exposure (control 2) and in animals, which were kept in the laboratory facility and were not exposed to EMR (control 1). The study was performed using the unit, consisting of two chambers: the well-lit - light one, and the dark one, where there was no lighting, the chambers were connected by the passage. As is known, generally, rats spend most of their lives in the dark chamber, which is attributed to their instinctive urge to stay in a dark and confined space - a den (the burrow reflex). The method is based on conditioning of passive avoidance of the dark chamber in rats as a response to unconditional electrodermal pain stimulus.

The rat was set out in the center of the light chamber with its tail to the passage to the dark chamber. The animal was given 2 minutes to examine the chambers; during the provided time it found the passage to the dark chamber and entered it. In the dark chamber the animal received 1 minute-long 1mA electrodermal pain stimulus.

The following behavior characteristics were recorded during passive avoidance conditioning: latent period of entering and time of the animal's stay in the dark chamber and the percentage of the animals who entered and did not enter the dark chamber. Passive avoidance reflex maintenance was checked 24 hours and 7 days after conditioning. The animals were also placed into the light chamber of the unit and their behavior was monitored during 2 minutes.

Method of paraffin embedding of the brain and brain slices preparation.

24 hours after the procedure of 4-day (6 hours a day) rats exposure in the Faraday cage with no router, with router, with router and resonators, the rats were killed, the brain was extracted and dissected. The material was subject to fixation (4% solution of paraformaldehyde in the fridge during 3 days), 1-hour washing in the phosphate buffer and passing through a series of alcohols with increasing concentration, methyl benzoate (within 1 day), 1:1 methyl benzoate and paraffin mixture (within an hour at 56°C), paraffin (twice per 1 hour at the same temperature), afterwards, it was embedded into paraffin.

Frontal brain slices (7 μ m) were made at the level from -2.80 mm to -4.0 mm from the bregma subject to the stereotaxic atlas of the rat's brain (Paxinos, Watson, 2007) to analyze neurodegenerative processes in hippocampus and neocortex. The slices were placed on water-wet gelatin-coated object glasses, flattened out and dried. Ready slices were stained.

Method of slides staining with FluoroJade stain (Millipore)

All manipulations were carried out according to the manufacturer's recommendations (Millipore).

After paraffin removal by holding in xylol (2 times, 5 minutes each time), absolute alcohol (5 minutes) and 70° alcohol (5 minutes), the slides were held in 0.06 water solution of potassium permanganate (20 minutes) and were washed with distilled water one more time (1 minute). Thereupon, the slices were stained with FluoroJade B fluorescent stain within 30 minutes with subsequent washing in distilled water (three times, one minute each time) and 20-minute drying in the temperature-controlled cabinet ($t=50^{\circ}\text{C}$). The basic (0.01%) solution was made at the rate of 1 mg of FluoroJade B preparation per 10 ml of distilled water. The work (0.001%) solution was made of the basic solution in 0.1% acetic acid at the rate of 1:9. Dried glasses were treated with xylol and put into DPX (Fluka) medium.

The ready slices were analyzed with Altami fluorescent microscope.

Statistical analysis

Findings of behavioral experiments were checked for homogeneity by nonparametric multiple-field chi square method. Individual data were combined within groups based on check results. To present the results in the tables, average values with error in mean and error rate were calculated and expressed as a percentage. Significance of differences among variants was determined by chi square method (Glotov et al., 1982) and by the rank test and analysis of variance (ANOVA), using Statgraphics Centurion XV11 and Statistica 6.0 software.

Results and Discussion

Analysis of the findings of the study on conditioning and maintenance of passive avoidance conditioned reflex after exposure to the router and exposure to the router in combination with resonators-converters has shown the following. The experimental exposure generally had no influence on rats' behavior upon passive avoidance conditioning (day 1, conditioning). In each of two experimental and control groups 100% of the animals entered the dark chamber, and no statistically significant differences were revealed with regard to the total time of staying in the dark chamber between the groups (Table 1). It should be noted that in the group of experimental rats, who had previously been exposed to the router in the presence of resonators-converters in the Faraday cage, the latent period of entering the dark chamber was 2.5 times less than in control group 1 (rank test, MRT = 21.8, Diff(+/-Lim=21.1). No differences were revealed between other groups.

In control group 1 conditioning persisted 24 hours and 7 days after training in 83% of the animals (the rats did not enter the dark chamber). Only 17% of the rats entered the dark chamber (Table 1). In control group 2, which was placed in the Faraday cage and was not subject to additional exposure, conditioning persisted in 36% of the animals, 64% did not remember the previous experience and entered the dark chamber.

Exposure to the router caused significant suppression of passive avoidance conditioning. 24 hours after training 77% of the animals entered the dark chamber. It is important to note that seven days after the training, the effect intensified: 10% of experimental group 1 entered the dark chamber, which differed from the control 1 values under study (Table 1).

The resonators prevented negative impact of the router and the Faraday cage, which resulted in maintenance of passive avoidance conditioned reflex, comparable with control 1, 24 hours after training. However, seven days later the conditioned reflex was suppressed in 50% of the animals.

Therefore, exposure of the animals in the Faraday cage without any additional influence and in combination with the router's impact resulted in failure to preserve passive avoidance conditioned reflex in male Wistar line rats. Resonators-converters prevented negative impact on the memory of the animals, exposed in the Faraday cage, 24 hours after conditioning. Seven days later the protective effect subsided.

Table 1 Main behavior characteristics upon conditioning and maintenance of passive avoidance conditioned reflex after exposure to router and resonators-converters in male Wistar line rats.

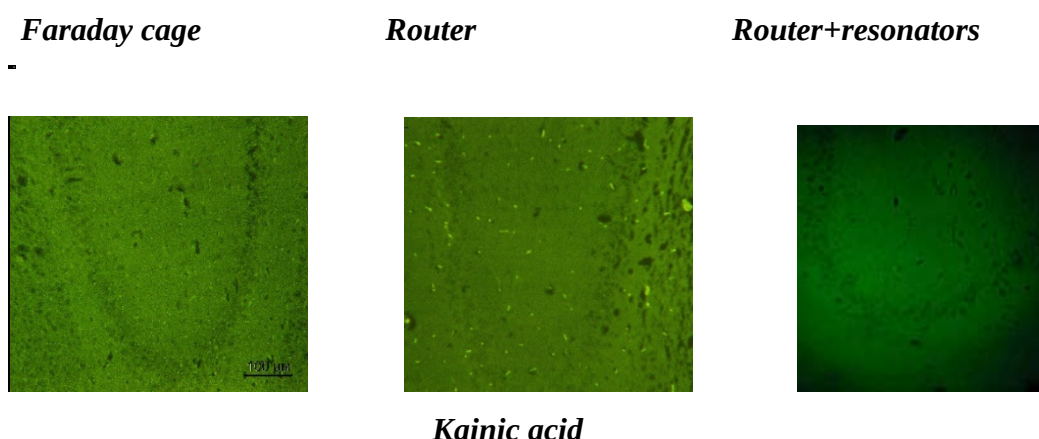
Groups	Training			24 hours			the 7th day		
	LP, sec	DC, sec	%	LP, sec	DC, sec	%	LP, sec	DC, sec	%
Control 1	37 ±7 (29)	134±11 (143)	100/ 0	–	–	17/ 83	–	–	17/ 83
Control 2 (Faraday cage)	23,7 ±7,8	134±13 (149)	100/ 0	52±22 (42)	101±3 0	64/3	15±9 (14)	121±34 (156)	77/

	(16)				(136)	6#			33*
Experimental group 1 (router)	24±8 (22)	135±12 (148)	100/ 0	54±27 (29)	111±3 3 (138)	77/3 3#	30±9 (15,5)	117±24 (139)	100/ 0*
Experimental group 2(router + resonators)	15±3* (12,5)	136±12 (130)	100/ 0	—	—	17/ 83	7±4 (5)	—	50/ 50

Legend: DC - time spent in the dark chamber, X+m(M); LP - latent period of entering the dark chamber, X+m(M);% - percentage of animals, who entered the dark chamber/percentage of animals, who did not enter the dark chamber; * - differences with control 1 are valid ($p<0.05$); # - difference from control groups 1 and experimental group 2 is valid ($p,0.05$)

It should be emphasized that the findings show that limitation of the natural electromagnetic field in the Faraday cage influences cognitive abilities of the male Wistar line rats per se therefore it was not possible to single out negative impact of EMR, emitted by the router, to the studied behavior characteristics of rats under the given experiment conditions. Nevertheless, protective effect of resonators-converters against the background of memory suppressing Faraday cage's impact, was detected.

Images of hippocampus and neocortex of rats, exposed in the Faraday cage without additional influences, with router, without router and in combination with resonators, are given in Figures 2 and 3 correspondingly. No neurodegenerative processes were detected in the hippocampus of all three groups of the animals. However, there are cases of high negative staining (see Fig. 2, router). As a comparison, Fig. 2 shows an example of degenerating hippocampus cells (fields CA2, CA3) after cell death induced by excitotoxin - kainic acid.



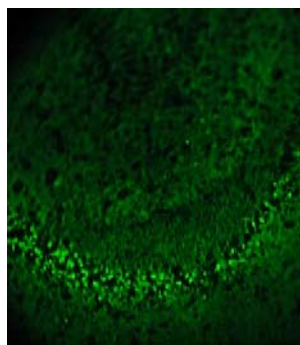


Fig. 2 Slides of rats' hippocampus, stained with FluoroJadeB neurodegeneration marker: "Faraday cage" - group of rats, placed into the Faraday cage; "Router" - group of rats, exposed to the router in the Faraday cage; "Router+resonators" - group of rats, exposed to the router and resonators in the Faraday cage; "Kainic acid" - group of rats in which neurodegenerative processes are induced by excitotoxin - kainic acid.

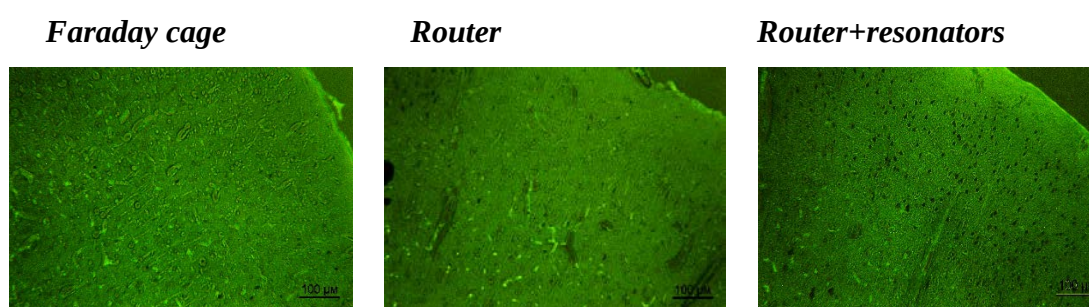


Fig. 3 Slides of rats' neocortex, stained with FluoroJadeB neurodegeneration marker: "Faraday cage" - group of rats, placed into the Faraday cage; "Router" - group of rats, exposed to the router in the Faraday cage; "Router+resonators" - group of rats, exposed to the router and resonators in the Faraday cage

FIJ-positive cells in the neocortex are detected mostly in the lower cortex layer in all three studied groups. Qualitative visual analysis showed that there are more degenerating cells in the neocortex of "Faraday cage" and "Router" groups and that they are more prominent in comparison with "Router+resonators" group. The findings of the pilot experiment suggest resonators' protective influence, weakening neurodegenerative processes in the neocortex of male Wistar line rats, which requires confirmation by further experiments, involving methods of accurate quantitative assessment of dying cells.

BIBLIOGRAPHY

1. Zhabrev V.A., Lukyanov G.N., Margolin V.I., Potekhin M.S., Tupik V.A., Serov I.N., Soshnikov I.P., Study of Cu fractal structures, produced by ion magnetron deposition method// Composite materials structures 2005, No 4
2. Kopyltsov A.V., Serov I.N., Lukyanov G.N. Mathematic simulation of electromagnetic radiation interaction with self-affine silicon surface. INZHEKON Bulletin, Technical Sciences series, 2007, P. 199-205.
3. Pavlova L.N., Zhavoronov L.P., Dubovik B.V. Impact of low-intensity broadband pulse-modulated electromagnetic field on cognitive functions of rats' brain.//Radiation and risk. 2013. V. 22, No 2, P. 91-100.

4. Serov I.N., Kopyltsov A.V., Lukyanov G.N., Interaction of semiconductor plate with self-affine relief of the surface with electromagnetic radiation//Nanotekhnika (engineering magazine) 2006a, No 4(8), Page 44-49.
5. Serov I.N., Kopyltsov A.V., Lukyanov G.N. Interaction of electromagnetic radiation with self-affine relief surfaces.//White Book "Nanoparticles, nanostructures and nanocomposite materials studies in the Russian Federation", M: 2006b, P.165-166.
6. Barth A., Ponocny I., Gnambs T., Winker R. No effects of short-term exposure to mobile phone electromagnetic fields on human cognitive performance: a meta-analysis.//Bioelectromagnetics. 2012. V.33(2).P.159-165.
7. Besset A., Espa F., Dauvilliers Y., Billiard M, de Seze R. No effect on cognitive function from daily mobile phone use.//Bioelectromagnetics. 2005.V.26(2).P.102-108.
8. Daniels W.M., Pitout I.L., Afullo T.J., Mabandla M.V. The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat.//Metab Brain Dis. 2009. V.24(4). P. 629-641.
9. Deshmukh P.S., Nasare N., Megha K., Banerjee B.D., Ahmed R.S., Singh D., Abegaonkar M.P., Tripathi A.K., Mediratta P.K. Cognitive impairment and neurogenotoxic effects in rats exposed to low-intensity microwave radiation.//Int. J. Toxicol. 2015. V.34(3).P.284-290.
10. Fritzer G., Göder R., Friege L., Wachter J., Hansen V., Hinze-Selch D., Aldenhoff J.B. Effects of short- and long-term pulsed radiofrequency electromagnetic fields on night sleep and cognitive functions in healthy subjects.//Bioelectromagnetics. 2007.V.28(4).P.316-325.
11. Guxens M., Vermeulen R., van Eijdsden M., Beekhuizen J., Vrijkotte T.G., van Strien R.T., Kromhout H., Huss A. Outdoor and indoor sources of residential radiofrequency electromagnetic fields, personal cellphone and cordless phone use, and cognitive function in 5-6 years old children.//Environ. Res.2016. V.150. P.364-374.
12. Haarala C., Bergman M., Laine M., Revonsuo A., Koivisto M., Hämäläinen H. Electromagnetic field emitted by 902 MHz mobile phones shows no effects on children's cognitive function.//Bioelectromagnetics. 2005;Suppl 7:S144-50.
13. Hall P., Adami H.-O., Trichopoulos D., Pedersen N.L., Lagiou P., Ekblom A., Ingvar M., Lundell M., Granath F. Effect of low doses of ionising radiation in infancy on cognitive function in adulthood: Swedish population based cohort study.//BMJ. 2004.V. 328(7430).P. 19.
14. Hawkes N. Study will look at effect of mobile phones on children's cognitive development.//BMJ. 2014. V.348:g3407.
15. Hutter H.P., Moshhammer H., Wallner P., Kundi M. Subjective symptoms, sleeping problems, and cognitive performance in subjects living near mobile phone base stations.//Occup Environ Med. 2006.V.63(5).P.307-313.
16. Kwon M.S., Hämäläinen H. Effects of mobile phone electromagnetic fields: critical evaluation of behavioral and neurophysiological studies.//Bioelectromagnetics. 2011.V.32(4).P.253-272.
17. Ng T.P., Lim M.L., Niti M., Collinson S. Long-term digital mobile phone use and cognitive decline in the elderly.//Bioelectromagnetics. 2012.V.33(2).P.176-185.
18. Paul B., Saha I., Kumar S., Samim Ferdows S.K., Ghose G. Mobile phones: time to rethink and limit usage.//Indian J. Public Health. 2015. V.59(1). P. 37-41.
19. Paxinos G., Watson C. The rat brain in stereotaxic coordinates – 6th edition. - Ac.Press, 2007.
20. Salford L.G., Brun A.E., Eberhardt J.L., Malmgren L., Persson B.R. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones.//Environ Health Perspect.2003.V.111(7).P.881-883.

PUBLICATIONS BASED ON FINDINGS OF THE STUDIES:

Puchkova V.A., Dyuzhikova N.A., Serov I.N. Impact of high-frequency electromagnetic radiation on stability of genetic apparatus in the bone marrow cells of Wistar line rats.//In papers of the 23rd Conference of Pavlov Physiological Society. Voronezh. September 17-22, 2017. Radiation physiology. P. 825 (poster report and abstract in the Conference Papers)

<http://www.vrngmu.ru/upload/iblock/19c/19c3af15a1650df8c7a276f73fb80c44.pdf>

Dyuzhikova N.A., Kopyltsov A.V., Korshunov K.A., Lukyanov G.N., Puchkova V.A., Serov I.N.

Impact of high-frequency electromagnetic radiation and influence of resonators-converters on frequency of chromosome aberration in the bone marrow cells of male Wistar line rats//Electromagnetic waves and electronic systems. 2018. No 1 (the article is accepted for publication).