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«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 inborn eating behavior of honey bees and ability to keep in short and long-term memory an individual obtained experience.

Principal investigators

Chief Research Scientist
Laboratory of Higher Nervous Activity Genetics
Dr.Sci., Prof. N.G.Lopatina

Research Scientist
Laboratory of Higher Nervous Activity Genetics
Ph.D. T.G.Zachepilo

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INTRODUCTION

Technical progress that cut across all spheres of human life support and positively affected development thereof, unfortunately resulted in negative consequences. Influence on non-ionizing electromagnetic radiation (EMR) on human organisms, flora and fauna has increased due the developed global communication systems. As recent studies have shown, the environment electromagnetic field disturbance caused the increase in rate of various abnormalities touching immune, endocrine, and reproductive systems, as well as cognitive activity of humans (Nakhilnitskaya et all, 1983, Stoyan, 1989; Kholodov, 1993; Dunaev, 2007).

Hence, the study of mechanisms of destructive influences, and creation of protection systems thereform, accordingly, throws this issue into sharp relief. As of the moment, devices based on fractal-matrix nanotechnologies of EMR transformation, effectively redistributing EMR and having corrective properties, have been created by Aires Company (http://www.aires.fund/nano.html). The mechanisms of these devices' protective influence on behaviour still need to be studied.

Taking into consideration the difficulties of carrying out these works on humans, a need arises to use model objects, which can be both vertebrate (mammals) and invertebrate animals with rather simple organization of the nervous system, however, highly capable of being trained and long-term keeping individually obtained new information in memory. They can include social insects, and a honey bee, in particular. The mechanisms of training and memorizing with honey bees are being intensively studied at many levels of its nervous system organization, i.e. neuroanatomic, physiological, bioelectric, biochemical, and genetic. Bee's genome is pretty much decoded.

Objectives and tasks of the first stage:

1) study of the effects EMR has on food excitability, ability to develop and maintain acquired eating reflexes for olfactory stimulus in short-term and long-term memory under different operation modes of a standard WiFi router;

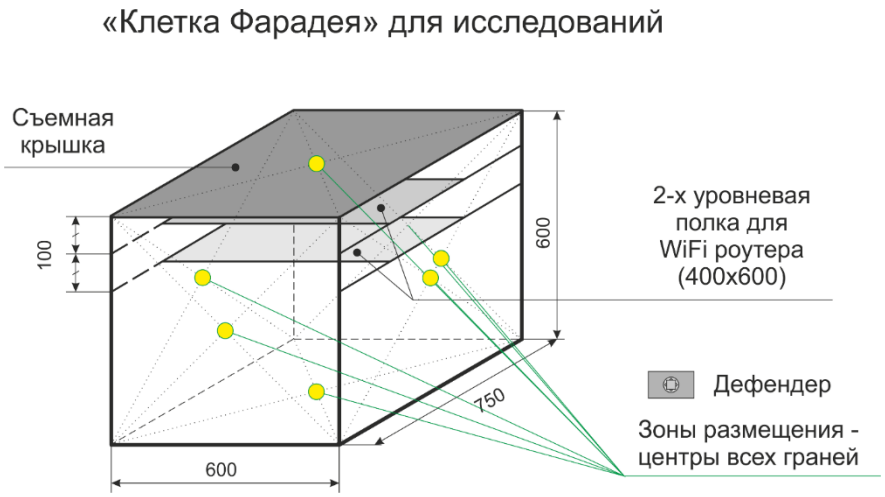
2) evaluation of Aires Defender fractal-matrix resonators' protective effect on the above behaviours.

MATERIALS AND METHODS

The work was performed in honey bees aged 7-30 days. The bees were kept in small beehouses in a mesh cage in a room at t=+20-25ºC. Bees were provided with protein and carbohydrate food and water. Daily cleansing flies were possible.

Use was made of a standard WiFi router (wireless router LinkSys E1200-EE/RU) with the following technical characteristics: wireless communication frequency – 2.4 GHz, number and type of antennas – 2 internal antennas; standard antenna(s) gain power, dBi – 4 dBi.

Aires Defender fractal-matrix resonators (special diffraction ring grids), hereinafter referred to as the protection, being a universal space wave Fourier filter (Zhabrev at all, 2005), were also used in experiments. As a result of interaction with Aires Defender, structural transformation of the electromagnetic field occurs. 6 resonators were used to evaluate the effect they have on the router's EMR noci-influence, which were placed in the center of each Faradey cage's faces (Fig. 1).



Faraday cage used for research.
Detachable cover
2-level shelf for WiFi router (400x600)
Defender
Placement zones –central points of all faces

Fig. 1. Diagram of the Faraday cage used for researches, showing resonator location points.

A mesh test-tube with 18 bees was used to study the router's ERM effect. This test-tube was placed on the Faraday cage floor beneath the router located on the detachable shelf under the top roof to the left of the cage center ot stricktly centered. The router's location to the left of the Faraday cage center was due to more convenient for a researcher conditions to manipulate the test-tubes with bees. At the same time we were on the view that the router will affect bees behaviour independently on its location in the cage.

It should be noted that the extensive works were performed during the preliminary research stage due to searching for efficient conditions for the experiment in no small way convenient for manipulating the mesh test-tubes with bees. First, it was necessary to determine how the behaviour of untreated bees changes subject to a day time and length of isolation from the family (11 tests). Then, how the behaviour of bees not router affected in the Faraday cage

changes subject to a day time, length of stay in the cage, location therein (corners, center, to the left of the center), and bedding quality (foamed rubber bed, paper container) (20 tests). And finally, the behavior of bees exposed to the activated router located either to the left of the cage centre or strictly centered on the detachable shelf under the top roof of the cage. The test-tubes with bees at that were placed on the cage floor immediately beneath the router. In this case, the test-tubes were parallel or perpendicular to the router.

Use of the acquired reflex technique: development of the acquired eating Proboscis Extension Reflex (PER) for olfactory stimulus by a single combination of carnation and food reinforcement (50% sugar solution) and check thereof for ability to keep it in the short-term (1 minute after the training procedure) and long-term (180 minutes after the training procedure) memory through the remote provision of the carnation conditioned stimulus. In order to develop the acquired reflex in cold narcosis immobilized bees, use was made of immobilization of wings with special clamps and placement in a unit enabling testing 18 bees at the same time. The training procedure was as follows. A drop of carnation flavoured sugar solution on a glass rod was brought to the bee antennae (contactless). The bees' voluntary response to the flavour (sensory excitability) was recorded for 5 sec. Then the glass rod with flavoured solution was brought into contact with the antennae, where olfactory and taste receptors of bees are located; at that records were made of the acquired eating reflex (Proboscis Extension – food excitability) or absence thereof. A bee with the extended proboscis then was able to intake sugar solution (food reinforcement of the conditioned signal – carnation) for 2 seconds. Bees demonstrating the voluntary response and non-responders did not participate in further tests.

6 groups of bees were involved: 3 test groups and 3 reference ones. One of the test groups was placed in the Faraday cage with the 24-hour on router located strictly in the cage center. The second group was kept to the left of the center for 2, 4, 4, 6 and 24 hours. The third group was kept in the Faraday cage for 24 hours with the strictly centered router on and the protection. The references groups included that consisting of untreated bees. Two other references groups were kept in the Faraday cage either in the center or to the left of the center for the same test periods as those used for the test groups but without the exposure.

18 family isolated bees were kept in the mesh test-tubes with food that was removed from the test-tube 3 hours before the training procedure to increase the eating stimulus. The test-tubes with bees were placed on the cage bottom as follows: the tested bees – immediately beneath the activated router, the referenced bees – at the same router-free points. The cage was mandatory ventilated between the tests. Bees were trained out of the cage 30 minutes after placement in the unit. The untreated bees' (those directly taken out of the beehouse) ability to learn and reproduce the conditioned response in specific time intervals once the training was

over was evaluated at different day times. The length of keeping the untreated bees in the test-tubes complied with that of the referenced bees kept in the cage but not exposed to the radiation.

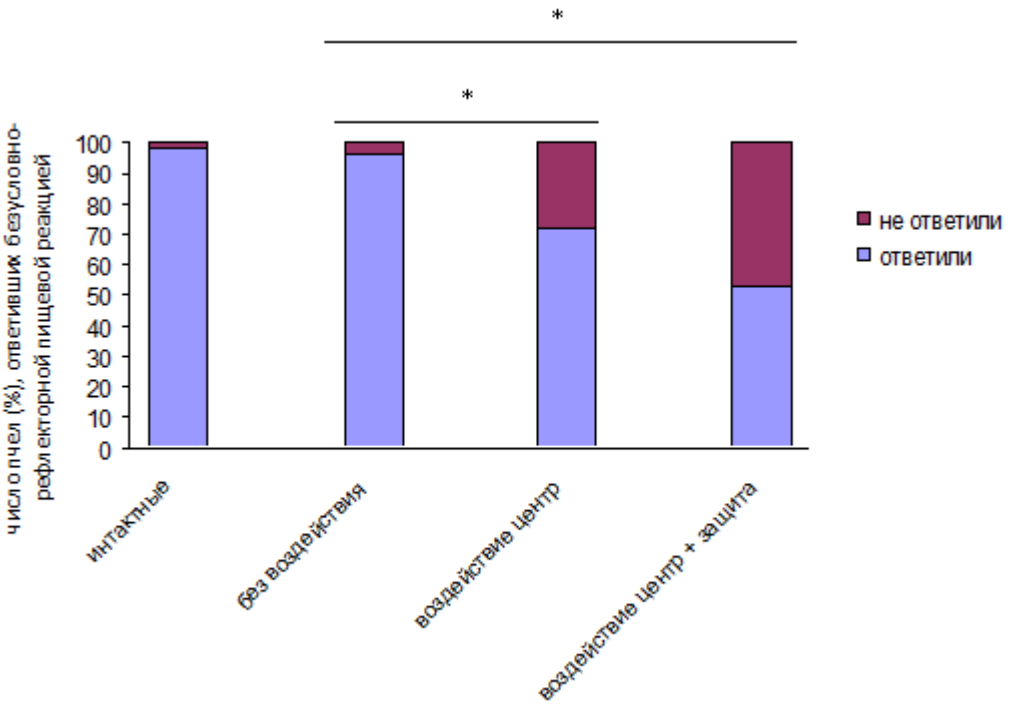
The measured data (test and reference groups) was compared using the non-parametric methods: Mann–Whitney–Wilcoxon test for independent samplings, $\chi 2$ criterion, and Fisher's exact test. Based on the check the individual data was combined within a group. Results were presented as graphs showing true differences (stars).

FINDINGS

The measured data (primary) of all tests is given in Table 1; replications and limits of researched signs variation is given in brackets. The comparison of the test and reference data on the researched indicators, i.e. a number of bees (%) responded with the unconditional eating reflex and conditional reflex in different test series is given in the diagrams (Fig. 2-4). Only that data demonstrating the true differences between the studied signs out and after exposure to EMR shall be given in the diagrams.

Analysis of the obtained data first of all enabled to come to the conclusion that all the researched groups of bees demonstrated no clear sensory excitability (voluntary activity)- test conditions dependence.

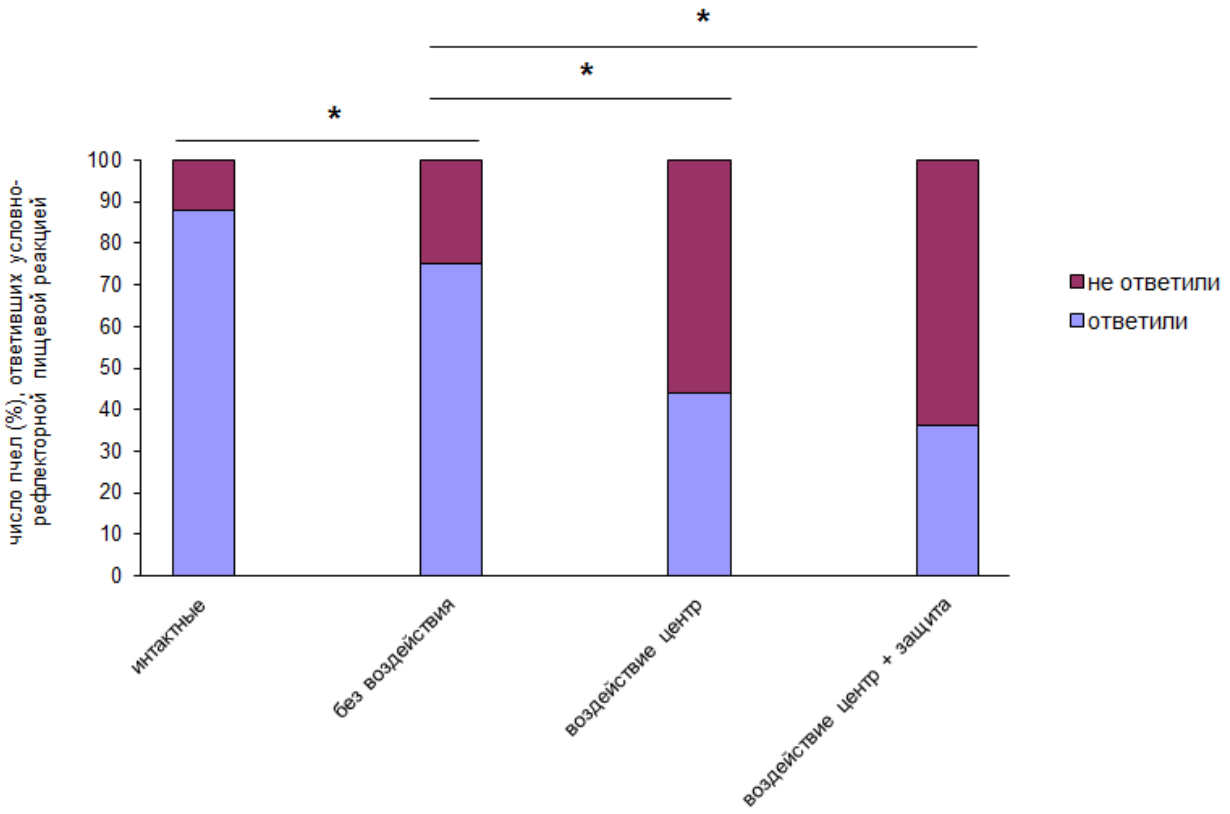
Results obtained on the untreated bees show high stability of both unconditional eating and conditional reflexes independent on a day time or family isolation length (at least, within 24 hours). At that, the unconditional reflexes varied from 94 to 100% (98% in average), while the conditioned reflexes varied from 82% to 93% (88% in average) (Fig. 2).



Number (%) of bee responders with unconditional eating reflex
Untreated
Non-exposed
Exposed: center
Exposed: center+protection
Non-responders
Responders

Fig. 2. Router's EMR effect on unconditional food excitability.* - true differences, $p \leq 0{,}05$, Mann-Whitney criterion, $\chi 2$ criterion, and Fisher's exact test.

Processing of data on the bees kept in the cage but not exposed to the router's effect has shown no dependence of the bees' unconditioned food responses on the test conditions. A per cent of bee responders varied from 56 to 88% (94% in average) (Fig.2). However, a per cent of bees responded with the proboscis extension to the conditioned stimulus was insignificant for the group of bees in the test-tube placed on the cage bottom (13%), though significantly lower than that of the untreated bees (Fig.3).

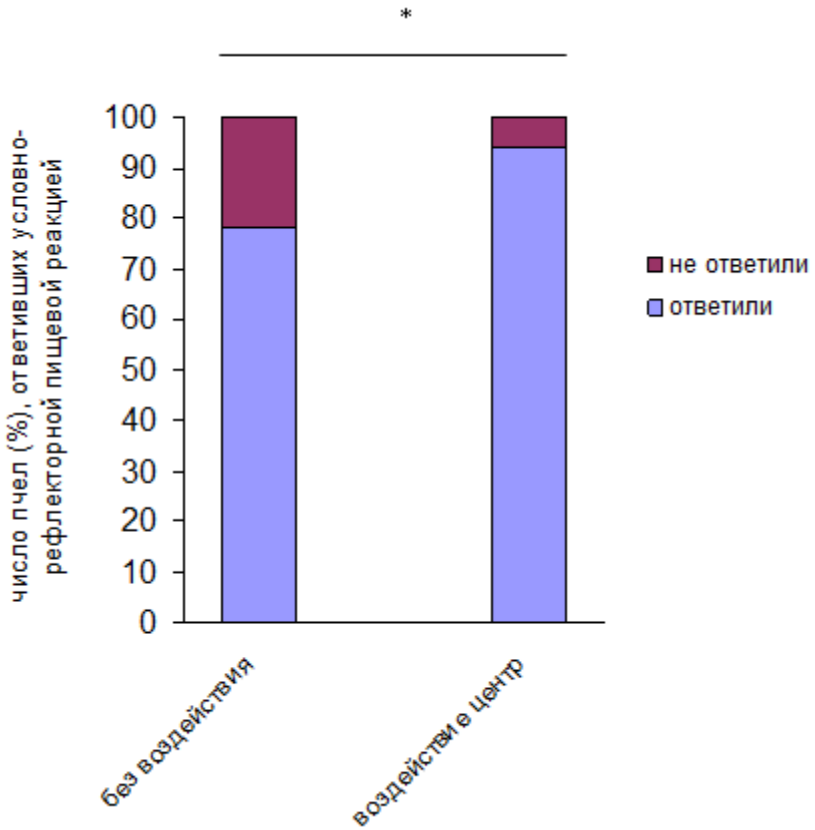


Number (%) of bee responders with conditioned food reflex
Untreated
Non-exposed
Exposed: center
Exposed: center+protection
Non-responders
Responders

Fig. 3. Router's EMR effect on the ability of bees to keep the developed food reflex to the olfactory stimulus.* - true differences, $p \leq 0{,}05$, Mann-Whitney criterion, $\chi 2$ criterion, and Fisher's exact test.

The average of 75% of bees was able to reproduce the conditioned reflex 1 minute after the training procedure. This number varied from 40% to 82%. The effect did not depend on the test-tubes location in the cage, however significantly worsened when the test-tubes with bees were placed in the container. Due to this fact, further tests were carried out on bees in the test-tube placed in the cage bottom.

Activation of the router located to the left of the cage center did not cause any changes in the behaviour of bees compared to those kept in the cage but not exposed to the router's EMR. On the other hand, 24 hour on router located in the cage center affected the behaviour of bees in the test-tube placed in the cage bottom: inhibition of food excitation and the bees' ability to keep the conditioned response in the short-term memory (Fig.3), as well as stimulation of the bees' ability to keep the conditioned response in the long-term memory compared to those placed in the cage but not exposed to EMR (Fig.4).



Number (%) of bee responders with conditioned food reflex
Non-exposed
Exposed: center
Non-responders
Responders

Fig. 4. Router's EMR effect on the ability of bees to keep the developed conditioned food reflex to the olfactory stimulus in the long-term memory.* - true differences, $p \leq 0,05$, $\chi 2$ criterion, and Fisher's exact test

The "exposure+protection" test series seemingly confirmed the inhibitive effect of the router's EMR on the short-term memory. However, the bees responded to the protection activation also, as the variety of the responses, unconditional food reflexes (from 17% to 94%, 53% in average), and conditioned reflexes (from 0% to 87%, 36% in average) increased. It seems, that in order to reveal and improve the protection's positive role, the tests in selecting the relevant conditions should be continued. One of the conditions, as we believe, may be placement of bees in a mesh cage larger than a test-tube, where they could move freely.

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What mechanisms are at the origin of the revealed changes in the ability to keep the experience obtained when exposed to the router's EMR and router's EMR + protection in the short-term and long-term memory is still early to predict. Even superficial acquaintance with EMR effect related literature permits to accept the researchers' opinion on that fact that the EMR biological influence effects are various, insufficiently studied, and unpredictable, as evidenced by a short list of EMR exposed processes in the organism: biochemical reaction course, enzymic activity in the brain and other organs, genetic information transfer processes (transcription and translation processes), which may result in the partial proteins (ferments) synthesis. The pattern of neuron generated pulse flows, functional activity of receptors and different ion channels may change under the influence of EMR. Any of the above disturbances may affect the memory processes. With a huge background in studying glutamate receptors in honey bees (Lopatina, Zachepilo, Ryzhova at all, 1993-2014), separation of the receptor component response to EMR is offered, namely, a study of L-glutamate receptors functional state and related thereto epigenetic mechanisms of gens activity regulation (DNA methylation, post-translation modifications of histones) in the brain ganglion neurons using the behaviour (conditioned), immunocytochemical, and other molecular biological methods in bees exposed to the router's EMR and router's EMR + protection. CNS degenerative factors of bees are also offered for studying.

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Таблица1. Первичные данные по безусловно- и условно-рефлекторной деятельности пчел в эксперименте.

Регистрируемый показатель	Интактные пчелы	Пчелы в клетке Фарадея							
		Без воздействия		Воздействие					Воздействие + защита
		периферия	центр	периферия				центр	
			24 часа	2 часа	4 часа	6 часов	24 часа	24 часа	24 часа
Число пчел (%), ответивших безусловно-рефлекторной пищевой реакцией	98 (11) (94-100)	98 (18) (56-100)	94 (2)	97 (4) (94-100)	94 (1)	100 (1)	90 (3) (72-94)	72 (4) (50-88)	53 (8) (17-94)
Число пчел (%), ответивших условно-рефлекторной пищевой реакцией через 1 минуту после процедуры обучения	88 (11) (82-93)	75 (18) (40-82)	76 (2)	80 (3) (76-88)	93 (1)	59 (1)	82 (3) (70-100)	44 (4) (30-60)	36 (8) (0-87)
Число пчел (%), ответивших условно-рефлекторной пищевой реакцией через 180 минут после процедуры обучения	-	-	78 (2)	-	-	-	-	94 (2)	-

Примечание: в скобках указано число повторностей и границы изменчивости изучаемых признаков.