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REPORT №2
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WITH THE AIRES HUMAN GENOME RESEARCH FOUNDATION

Subject: Study of the effects of 5G electromagnetic radiation and the effects of Aires 5G resonators on behavior and genetic and epigenetic processes in the brain: The models were the rat *Rattus norvegicus* and the honey bee *Apis mellifera* L.

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STAGE 2 : **Investigation of the influence of a next generation router and LIFETUNE microprocessors on the feeding behavior of the *Apis mellifera*L. honey bee.**

INTRODUCTION

Today, the telecommunications industry around the world is on the verge of introducing fifth generation (5^{th} Generation, 5G) mobile communications, which should enable the development of the digital economy. 5G connectivity offers advantages such as high data rates, higher data capacity, and improved performance. Specialized developers and equipment manufacturers widely discuss the prospects and problems of 5G. Most attention is directed at the introduction of 5G digital communication technologies, the choice of network infrastructure, financing, and government regulation.

Many countries of Europe and Asia use the 3.4-3.8 GHz band to build 5G networks. In Russia, there are plans to use the 4.4-4.9 GHz and 24.5-29.5 GHz bands.

However, the growth of 5G technologies is raising public concerns regarding their safety. They involve the use of high-frequency millimeter radio waves. A large number of towers must be installed to provide high-quality and reliable service in the 5G network. According to the World Health Organization (WHO), there are no adverse effects on the human body (although some thermal effects are observed). From another point of view, a large number of 5G access points located in close proximity to one another contributes increased exposure levels. At the same time, the power of 5G sources is much less than those of previous generations. The literature does not have many works devoted to studying the effect of EMR on living organisms at 5G frequencies (Simko, Mattsson, 2019). 80% of in vivo studies have shown a response to exposure. 58% of studies have demonstrated in vitro effects. No consistent relationship has been shown between biological effects and power density, duration, or frequency of exposure. The available studies do not provide sufficient information for a meaningful assessment of safety, non-thermal, and thermal effects. What's more, there are no studies of the effect of 5G network frequencies on insects, which are important members of sustainable ecosystems.

Insects are the best experimental subjects for studying the effects of EMR in various bands, since they are highly sensitive to magnetic and electric fields (Kumar et al., 2011). An excellent experimental subject for such studies is the honey bee: with a relatively simple central nervous system, the bee has significant cognitive abilities (Lopatina and Chesnokova, 1992; Menzel, 2012). These qualities make it possible to use bees to study various types of learning, associative and non-associative memory (Bitterman et al., 1983, Menzel, 2012). Previously, it was proven that the effects of high-frequency radiation reduce a queen bee's fertility and also lead to a decrease in the amount of honey and bee bread in the hive (Kumar et al., 2011). The unconditioned food excitability reflex and short-term memory of the honey bee worsens (Lopatina et al., 2019). It is known that a bee can respond to very weak variations in the Earth's constant, local geomagnetic field and use those variations for spatial orientation when foraging. The bee's magnetoreceptors are ferromagnetic crystals (Fe_3O_4) located in the cells of the fatty tissue in the abdomen. The bee's integument acts as a semiconductor. The integument forms the active parts of the bee's magnetoreceptor system. During flight, bees' movements about the honeycomb in the hive generate constant and modulated electric fields, which are detected by the Johnston's organ and other mechanoreceptors located on the antennas and body of the bee. Exposure to EMR can disrupt the receptor mechanisms and thus influence the insect's behavior. There is an obvious need to study the 5G frequency range's influence on the behavior of honey bees, and the large-scale use of EMR in new bands will require the development and study of devices and materials that mitigate their negative impact on living organisms.

The purpose of this stage was the study of the effect of the electromagnetic radiation of a next-generation router operating in the 5G range and the effect of LIFETUNE microprocessors on the feeding behavior of the *Apis mellifera* L.

Tasks:

Study the influence of the following devices on bees' sensory and food excitability, and the ability to maintain a conditioned food reflex in short-term and long-term memory:

1. Faraday cages covered with metal mesh and aluminum foil;

2. Xiaomi Pro AC2600 router (with 2.4 and 5 GHz modes) placed in a Faraday cage with a different covering (see item 1), in two operating modes, when only connected to the network and when also connected to the Internet for a 3-hour and 24-hour period;

3. LIFETUNE microprocessors in combination with operating the router in different operating modes (see item 2).

Material and methods

The study was carried out on the *Apis mellifera* L. honey bee, on workers 7-30 days old in the summer-autumn (July-October) of 2020. The bees were kept in observation hives in a room, in a mesh chamber at t = 20-25°C. The bees were provided with protein, carbohydrates, and water. The bees could make cleaning flights every day. Lighting conditions were maintained automatically: 8:00 AM - 8:00 PM (day), 8:00 PM - 8:00 AM (night).

The tests were carried out in an adjacent room, where the temperature was also maintained at 20-25°C, with round the clock lighting.

Two versions of the Faraday cage were used as the experimental chamber: a wooden frame wrapped in: a) a metal mesh ("Faraday Cage 1"), b) a metal mesh and aluminum foil ("Faraday Cage 2") (Fig. 1).

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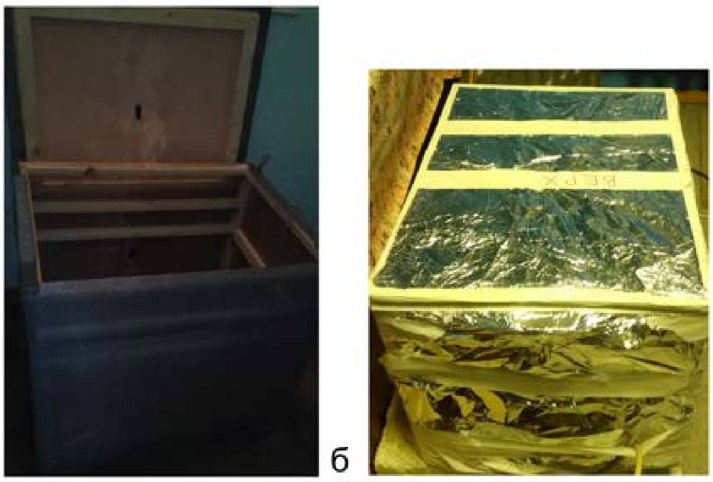

б

Fig. 1. A Faraday cage covered in (a) a metal mesh, (b) a metal mesh and aluminum foil.

We analyzed the radiation of a Xiaomi Pro AC2600 router (the "router") in two operating modes: a) when connected to the network, b) when connected to the network and connected to the Internet.

Fig. 2. A Xiaomi Pro AC2600 router was used in the experiments.

We studied the protective properties of LIFETUNE microprocessors (one device is 10x10mm) (the "microprocessors") (Fig. 3a) while the router is operating. LIFETUNE microprocessors form the backbone of a product line specially produced by American Aires Inc. to protect people from the negative influence of radiation from 5G networks. At all stages of the experiment, 8 microprocessors were placed on adhesive tape across a mesh test tube with bees, equidistant from the ends of the test tube (Fig. 3b).

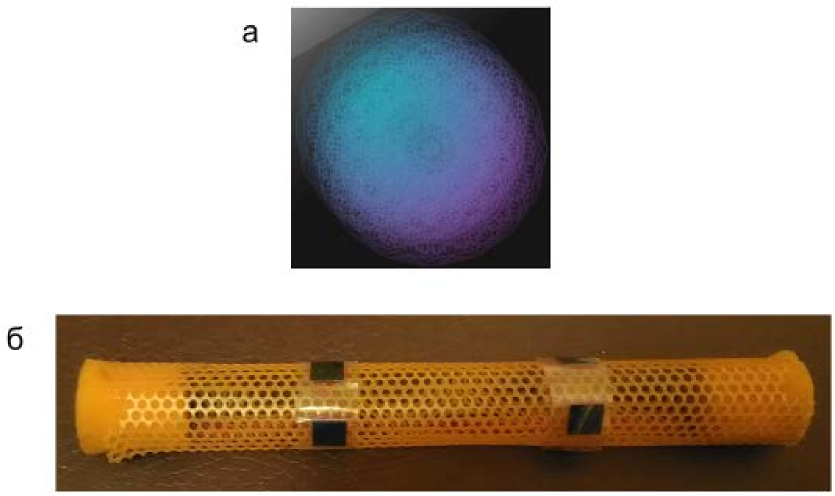


Fig. 3. A LIFETUNE microprocessor (a) and an experimental mesh test tube for bees with microprocessors located on it (b).

The experimental protocol was as follows: We isolated bees from the colony and placed them in 120 cm^3 mesh tubes with food. 9-10 individuals in each test tube. A test tube with bees was placed on the bottom in the center of the Faraday cage, directly under the antenna of the router, which was placed on a removable shelf under the top cover of the cage.

We evaluated the influence of 3-hour and 24-hour (the main part of the experiments) router exposure on the behavior of bees. The cage was ventilated between tests. The bottom of the cage was thoroughly cleaned. The tests were carried out in three consecutive stages.

Stage I

a. After 3 and 24 hours in Faraday Cage 1;

b. After 3 and 24 hours in Faraday Cage 1 with a powered-on router;

c. After 24 hours in Faraday Cage 1 with a powered-on router and microprocessors.

Stage II

a. After 24 hours in Faraday Cage 2;

b. After 24 hours in Faraday Cage 2 with a powered-on router;

c. After 24 hours in Faraday Cage 2 with a powered-on router and microprocessors.

Stage III

a. After 24 hours in Faraday Cage 2 with a powered-on router connected to the Internet;

b. After 24 hours in Faraday Cage 2 with a powered-on router connected to the Internet and microprocessors.

We recorded the bees' feeding behavior. The bees' behavior was assessed according to the following parameters: sensory (olfactory) and food excitability, as well as the bees' ability to retain the developed conditioned reflex in short-term and long-term memory. We used the conditioned reflex method: We developed a conditioned food reflex involving stretching of the proboscis toward an olfactory stimulus (Proboscis Extention Reflex, PER) (Bitterman et al., 1983) (Fig. 10, Appendix) by combining the smell of cloves with food reinforcement (50% sugar solution) once and checked its retention in short-term (1 minute after the training procedure) and long-term (3 hours after the training procedure) memory by presenting the conditioned stimulus — the smell of cloves — at a distance. The time intervals for the different phases of honey bee memory formation are shown in Fig. 11 (Appendix). Food was removed from the tubes 3 hours before the training procedure. The experiment was repeated from 2 to 5 times.

To develop a conditioned reflex, we immobilized the bees using a 3-minute cold anesthesia, immobilized their wings using special clamps, and placed them in an apparatus that allowed the simultaneous testing of 18 individuals. The training procedure was as follows. A drop of a clove-flavored sugar solution was brought to the bees' antennae (without touching them) on a glass spatula. For a period of 5 seconds, we recorded the bees' spontaneous response of stretching their proboscis toward the smell (sensory olfactory excitability). Then a drop of a flavored sucrose solution was brought into contact with the antennae, where the bees' olfactory and taste receptors are located, while registering the presence/absence of an unconditioned food reflex involving proboscis stretching (food excitability). Next, by sticking out its proboscis, a bee could suck in a sugar solution for 2 seconds (the smell of clove is the conditioned signal's food reinforcement). Bees showing a spontaneous reaction and, on the contrary, those not responding with an unconditioned food reflex, did not participate in further experiments.

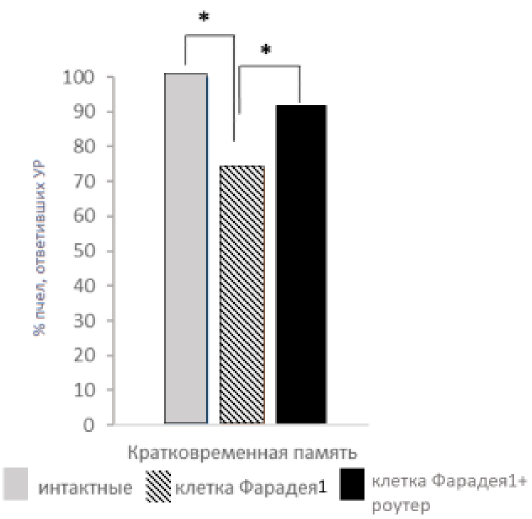
Untreated individuals isolated from the colony in the same room in containers of the same size and for the same time period as the experimental ones served as the control. A second control consisted of bees placed in a Faraday cage not exposed to any influences.

We compared the experimental data (control and experimental groups) using nonparametric methods: Mann-Whitney-Wilcoxon test for independent samples and a $\chi 2$ test. Based on the check, individual data were combined into groups. The data are presented in the graphs (statistically significant differences are indicated when $p \leq 0.05$). All the results obtained are included in Table 1 (Appendix), where significantly different values (when $p \leq 0.05$) are indicated by vertical lines.

RESULTS AND DISCUSSION

The experimental results are presented in Figures 4-9 and in Table 1 (Appendix).

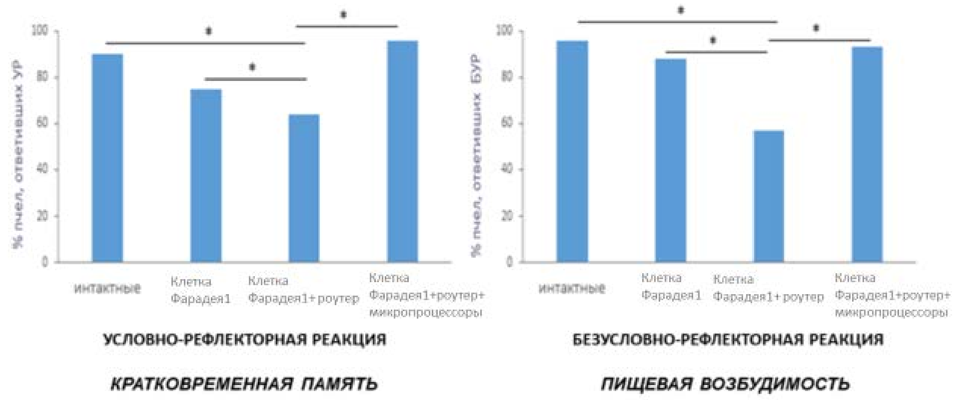
An analysis of the obtained data indicates that the confinement of bees in Faraday Cage 1 for both 3 hours and 24 hours (**Stage I**) led to a decrease in the bees' ability to retain the conditioned response in short-term memory, without affecting the level of sensory and food excitability (Fig. 4). The bees' responded in different ways to radiation from a router connected to the network, depending on the duration of exposure in the Faraday cage. A 3-hour exposure to the router normalized short-term memory to the level of untreated bees, having a stimulating effect compared to how Faraday Cage 1 affected this trait (Fig. 4).



Russian	English
% пчел, ответивших УР	% of bees that responded with a conditioned reflex
Кратковременная память	Short-term memory
Интактные	Untreated
Клетка Фарадея1	Faraday Cage 1
Клетка Фарадея1 + роутер	Faraday Cage 1 + router

Fig. 4. Influence of a router's EMR on short-term memory (conditioned reflex) of honey bees after 3-hour exposure in Faraday Cage 1.

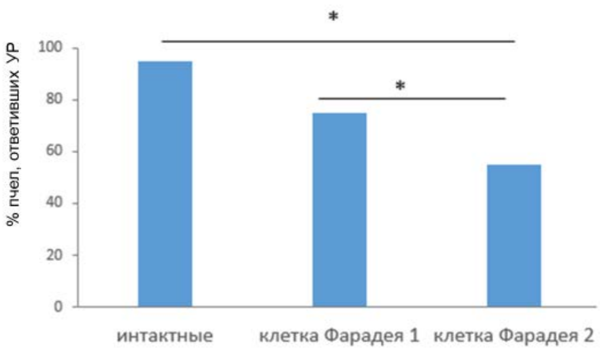
24-hour exposure to the router caused a decrease in short-term memory compared with Faraday Cage 1's effect and with untreated individuals, and also suppressed food activity (Fig. 5).



Russian	English
% пчел, ответивших УР	% of bees that responded with a conditioned reflex
Кратковременная память	Short-term memory
Условно-рефлекторная реакция	Conditioned reflex reaction
Интактные	Untreated
Клетка Фарадея1	Faraday Cage 1
Клетка Фарадея1 + роутер	Faraday Cage 1 + router
Клетка Фарадея1 + роутер + микропроцессоры	Faraday Cage 1 + router + microprocessors
% пчел, ответивших БУР	% of bees that responded with an unconditioned reflex reaction
Кратковременная память	Short-term memory
Безусловно-рефлекторная реакция	Unconditional reflex reaction
Интактные	Untreated
Клетка Фарадея1	Faraday Cage 1
Клетка Фарадея1 + роутер	Faraday Cage 1 + router
Клетка Фарадея1 + роутер + микропроцессоры	Faraday Cage 1 + router + microprocessors

Fig. 5. Effect of 24-hour exposure to a router in Faraday Cage 1, in isolation and in combination with microprocessors, on bees' short-term memory (conditioned reflex reaction) and food excitability (unconditional reflex reaction).

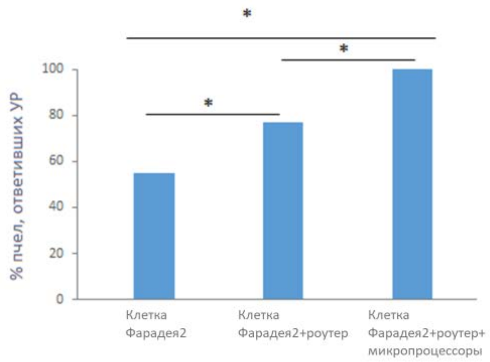
Faraday Cage 2 with an aluminum foil covering (**Stage II**) dramatically affected the bees' cognitive activity, in particular almost double the level seen in untreated bees and 1.4 times compared with bees kept in Faraday Cage 1 for a day (**Stage I**). It reduced the number of individuals that responded with a conditioned reaction involving stretching of the proboscis toward a smell 1 minute after the training procedure (Fig. 6).



Russian	English
% пчел, ответивших УР	% of bees that responded with a conditioned reflex
Интактные	Untreated
Клетка Фарадея 1	Faraday Cage 1
Клетка Фарадея 2	Faraday Cage 2

Fig. 6. Effect of 24-hour exposure under the conditions of Faraday Cage 1 and Faraday Cage 2 on bees' short-term memory (conditioned reflex reaction)

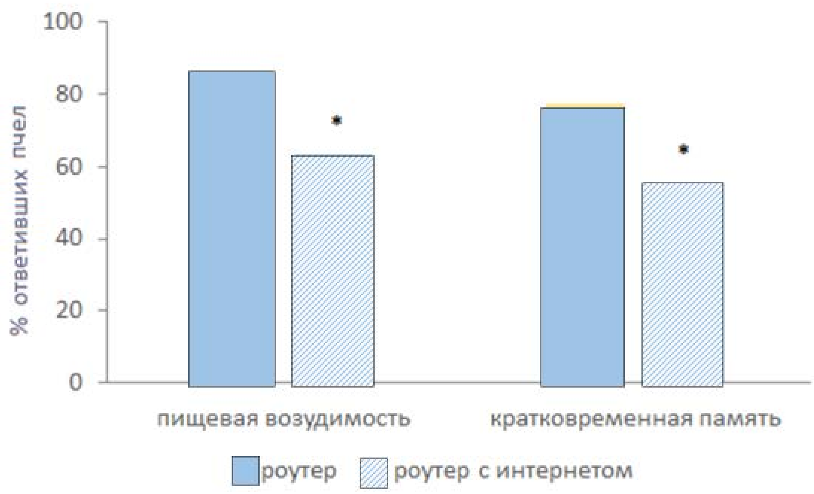
Paradoxically, a 24-hour exposure to a router under the conditions of Faraday Cage 2 stimulated short-term memory, increasing by a factor of 1.4 the number of bees that retained the conditioned reaction in their memory compared to the memory-inhibiting effect under the conditions in Faraday Cage 2 (Fig. 7).



Russian	English
% пчел, ответивших УР	% of bees that responded with a conditioned reflex
Клетка Фарадея2	Faraday Cage 2
Клетка Фарадея2 + роутер	Faraday Cage 2 + router
Клетка Фарадея2 + роутер + микропроцессоры	Faraday Cage 2 + router + microprocessors

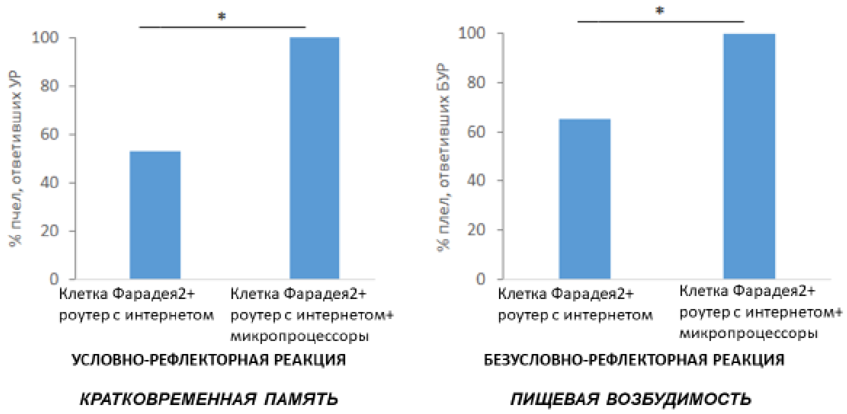
Fig. 7. Effect of 24-hour exposure with a router in Faraday Cage 2, in isolation and in combination with microprocessors, on bees' short-term memory (conditioned reflex reaction).

Connecting the router to the Internet (**Stage III**) significantly suppressed both bees' feeding and cognitive activity in comparison with the effect of a router without an Internet connection, and had a effect comparable to the effect of Faraday Cage 2 (Fig. 8).



Russian	English
% ответивших пчел	% of bees that responded
Пищевая возбудимость	Food excitability
Кратковременная память	Short-term memory
Роутер	Router
Роутер с интернетом	Router with Internet

Fig. 8. Effect of 24-hour exposure to a router without an Internet connection and with an Internet connection in Faraday Cage 2 on bees' food excitability and short-term memory.



Russian	English
% пчел, ответивших УР	% of bees that responded with a conditioned reflex
Клетка Фарадея2 + роутер с интернетом	Faraday Cage 2 + router with Internet
Клетка Фарадея2 + роутер с интернетом + микропроцессоры	Faraday Cage 2 + router with Internet + microprocessors
Условно-рефлекторная реакция	Conditioned reflex reaction
Кратковременная память	Short-term memory
% пчел, ответивших БУР	% of bees that responded with an unconditioned reflex reaction

Клетка Фарадея2 + роутер с интернетом	Faraday Cage 2 + router with Internet
Клетка Фарадея2 + роутер с интернетом + микропроцессоры	Faraday Cage 2 + router with Internet + microprocessors
Безусловно-рефлекторная реакция	Unconditional reflex reaction
Пищевая возбудимость	Food excitability

Fig. 9. Effect of 24-hour exposure to a router connected to the Internet, in isolation and in combination with microprocessors, in Faraday Cage 2 on bees' short-term memory (conditioned reflex reaction) and food excitability (unconditional reflex reaction).

It should be emphasized that at all stages of the study and under the conditions of all the applied influences, when the microprocessors were attached directly to the mesh tube with the bees, the microprocessors normalized their behavioral activity (Fig. 5, 7, 9). At the same time, under the influence of microprocessors, we observed an increase in the olfactory sensitivity of the bees (Table 1, Appendix).

It should be noted that at all stages of the work, the number of bees responding with the conditioned reaction was counted not only after 1 minute, but also 3 hours after the training procedure. The obtained data (Table 1, Appendix) indicate that the applied influences have a similar effect on the processes underlying the formation of both short-term and long-term memory. Since the experiment to assess long-term memory was only repeated once, statistical differences are not presented.

By the end of the working period, in untreated individuals in one of the experimental families (hive 2), both food excitability and short-term memory decreased 1.4-1.3 times in comparison with untreated bees from hive 1. The bees of hive 2 and hive 1 did not differ from one another in the nature of their responses to the applied influence. However, we observed the following in bees in hive 2: 1) high sensitivity in terms of food excitability to the applied influences, 2) the microprocessors' normalizing effect was not manifested in all the experimental series (only 3 out of 5) and 3) no increase in olfactory sensitivity under the influence of microprocessors. These data indicate the role of the functional state of the bees' nervous system in the specifics of the reaction to the surrounding electromagnetic field.

CONCLUSIONS

1. The natural electromagnetic background is important for maintaining the optimal tone in the bees' nervous system, which is necessary for cognitive activity, and in case of pathological conditions in the insect, also for innate food activity. Isolating the bees from the influence of natural electromagnetic fields had a detrimental effect on memory formation processes.
2. The router used in the work had a depressing effect on the bees' cognitive activity. When the router was connected to the Internet, the influence suppressed both food and cognitive activity. The router's operating mode had a stimulating effect on the studied bee behavior parameters while they were depressed in a Faraday cage covered with aluminum foil.
3. The next generation of LIFETUNE microprocessors, when placed on the walls of the experimental test tubes, had a universal protective effect on the bees. Under the conditions of all the experimental influences, bee behavior returned to the normal untreated level across all parameters. Moreover, the LIFETUNE microprocessors increased the bees' olfactory sensitivity.

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APPENDIX

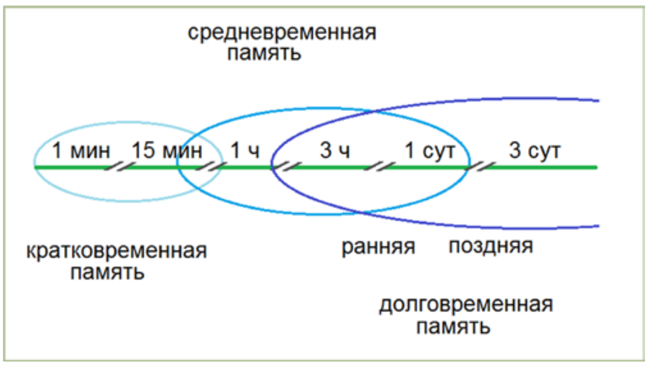
Table 1

Comparison of the feeding behavior of a honey bee under normal conditions and under conditions of the action of various electromagnetic fields

Effect 24 hours	Response, %			
	Sensory excitability	Food excitability	Short-term memory	Long-term memory
Stage I				
Untreated	0 (4)	96 (4)	95 (4)	85 (2)
FC1	7 (3)	88 (3)	75 (3)	73 (1)
FC1+R	0 (5)	57 (5)	64 (5)	78 (1)
FC1+R+MP	11	93	96	100
Stage II				
FC2	17 (4)	90 (4)	55 (3)	60 (1)
FC2+R	11 (5)	81 (5)	77 (5)	78 (1)
FC2+R+MP	18 (2)	94 (2)	100 (2)	100 (1)
Stage III				
FC2+RwI	12 (4)	65 (5)	53 (4)	44 (1)
FC2+RwI+MP	22 (1)	100 (1)	100 (1)	89 (1)

Key: FC1 - Faraday Cage 1, FC2 - Faraday Cage 2, R - router connected to the network, RwI - router connected to the Internet, MP - microprocessors. The table shows the percentage of bees that responded by stretching their proboscis under the specified experimental conditions. The number of repetitions is given in brackets. Vertical lines show statistically significant differences (p < 0.05).

Fig. 10. Demonstration of a conditioned proboscis extension response in a honey bee (Girling et al., 2013).



Russian	English
Средневременная память	Medium-term memory

1 мин	1 min
15 мин	15 min
1 ч	1 h
3 ч	3 h
1 сут	1 day
3 сут	3 days
Кратковременная память	Short-term memory
Ранняя	Early
Поздняя	Late
Долговременная память	Long-term memory

Fig. 11. Phases of olfactory associative memory in the honey bee (Menzel, 2012).