



«APPROVE»

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REPORT
UNDER THE AGREEMENT OF 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 CNS and peripheral organs (model organisms : rat (*Rattus norvegicus*) and honey bee (*Apis mellifera* L.)

STAGE FOUR: Study of the effects of the limitation of an external magnetic field, a router electromagnetic radiation and Aires Defender Pro resonators on the behavior in an open field test of rat strains with different levels of nervous system excitability

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During the contractual work (stages 1-3), a Faraday cage (Aires) was used to limit external electromagnetic effects. To determine the exact values of the induction of the magnetic field outside and inside the experimental chambers, measurements were made using Fluxmaster magnetometers (StefanMayerInstruments, Dinslaken, Germany) (1nT-200 μ T, resolution of 1nT) and HB0302.1A (St. Petersburg, Russia) (0.1 μ T-100 μ T, with resolution of 0.1 μ T). The measurements taken in different parts of the chamber and outside it are shown in Table 1 and indicate there are no differences between the magnetic component indices outside and inside the experimental chamber (without a router and with a router included in the network) (ANOVA F-test = 0.36; P=0.704).

Table 1. Measurements of magnetic field induction (μ T) in different parts of the experimental chamber and outside it.

Outside the chamber	Inside the chamber	Inside the chamber with the router turned on
X $\pm$ m		
59.6$\pm$18.1	55.9$\pm$7.9	54.4$\pm$7.8

Considering the results of the measurements, the decision was made to use as an experimental screening chamber covered with several layers of amorphous soft magnetic material, providing a measurable 40-fold decrease in the magnitude of the induction of the Earth's magnetic field inside the chamber (from 48 μ T to 1.2 μ T). In recent years, the biological effects of weak magnetic fields and the Earth's magnetic field weakened by shielding using such equipment have been actively researched. The magnetic field induction inside and outside the chamber were measured by the domestic three-component magnetometer HB0302.1A (0.1-100 μ T). It was important to assess the effect of the animals' exposure under conditions of a limited external magnetic field with clear parameters, primarily on motor activity, emotion, and other components of behavior in an "open field" test. And then we assess the effect of electromagnetic radiation (EMR) of a router and Aires Defender Pro resonators under the given conditions with known LMF values.

The "open field" method was first proposed by Hall (Hall, 1934, 1936). It involved placing a rat in a brightly lit circle, and then observing characteristics of motor activity and emotionality (according to the defecation response, number of boluses). There are presently various modifications of this technique, depending on the tasks of the experiment. A "open field"

(circular) apparatus is designed to study the exploratory and emotional behavior of rodents under conditions of being transferred to a new environment; it allows a comprehensive assessment of natural controlled behavior: assessment of the level of emotion, motor activity (the horizontal is the number of crossed sectors, and the vertical is upright positions reflecting orienting and exploratory activity), and the severity of the anxiety-depressive component (activity in the central, most open and illuminated sector of the field, the number of twists and turns), fear level based on a freezing response, and stereotypical behavior (grooming). Assessing the behavior of rats in an "open field" apparatus is included in the list of tests for conducting preclinical studies of pharmacological substances and the establishing their neuroleptic, antidepressant, and anxiolytic activity.

The nervous system is extremely sensitive to the influence of EMR. The effect of a magnetic field and EMR on the nervous system is characterized by changed behavior, conditioned reflexes, and physiological processes. The body's reaction to these effects largely depends on the initial functional state of the nervous system. Accordingly, it seemed important to use a special rat model with hereditary differences in nervous system excitability, a line with low excitability (high excitability threshold - HT) and a line with high excitability (low excitability threshold - LT).

Purpose and objectives of the fourth stage:

- 1) investigation of the effect of shielding the external magnetic field of a cylindrical chamber and unshielded counterpart;
- 2) investigation of the influence of EMR while a standard Wi-Fi router is operating;
- 3) investigation of the influence of Aires Defender Pro resonators under the action of the EMR of a standard Wi-Fi router
on behavior in an "open field" test of two HT and LT male rat lines

Material and methods

The work was carried out on male rats of the HT and LT lines at the age of 5 months. The rats were bred at the Genetic Laboratory of Higher Nervous Activity. The males were kept in groups of 6 specimens in standard cells on a standard diet.

To create the conditions of a weakened external magnetic field, we used a screening chamber made of non-magnetic material (cardboard) and covered with several layers of amorphous soft magnetic material (AMAG-172), which provided a 40-fold decrease in the magnitude of the induction of the Earth's magnetic field inside the chamber (from 48 μ T to 1.2 μ T). The imitation chamber was made of cardboard, had no shielding, and was covered with

black polyethylene. Both chambers were cylinders with a diameter of 60 cm and a length of 140 cm, closed at one end and open from the other, so that a cage with rats can easily be placed in the chamber.

We used a Wi-Fi router (LinkSys E1200-EE/RU wireless router) with the following technical specifications: wireless carrier frequency: 2.4 GHz, number and type of antennas: 2 internal antennas, gain of the standard antenna(s), dBi: 4 dBi.

The Aires Defender Pro fractal-matrix resonator-converters (special ring diffraction gratings) used in the experiments are a universal Fourier filter. To evaluate the resonators' influence on the damaging effect of the router's EMR, 6 resonators were used, as in the previous experiments on rats. They were placed in the middle of each edge of the cage with the animals (on the outside).

To study the influence of the limitation of the external magnetic field, the influence of the EMR of the router and resonator-converters, the "home" cage with animals was placed in the shielding chamber either without additional influences (LMF group) or with a router located on a tray in the center of the cage's lid (LMF+Router groups), and the router and resonators (LMF+Router+Resonators groups). The experimental groups were exposed for 12 hours (10:00 PM to 10:00 AM). The control groups were groups of rats placed in the imitation chamber at the same time, without the router (WLMF groups), with a router (WLMF+Router groups), with a router and resonators (WLMF+Router+Resonators groups). The animals' behavior in the "open field" test was assessed one hour after the end of the animals' exposure in the chambers.

The animals kept in the vivarium and not exposed to the effects served as the naive control ("Control 1").

The "open field" apparatus used was a circle with a diameter of 160 cm, bounded by a border that was 35 cm high. The floor of the circle was divided into 20 cm squares. Above the center of the floor at a height of 60 cm a 500 W lamp was suspended with a mirror reflector, providing illumination at the floor level from 2000 lx in the center to 1500 lx at the edges. The apparatus was placed in a darkened room. During the test, a rat was placed in the central square of the circle and its behavior was monitored for 5 minutes. After each animal was tested, the floor was wiped with 35-40% alcohol solution and then a dry napkin. The following behavior parameters were recorded:

Latency of the first movement (s)

Horizontal motor activity (number of intersected squares)

Vertical motor activity (raising up on the hind legs, number of upright positions)

Emotionality (number of boluses)

Grooming (number of acts)

Freezing (number of acts)

Turns to the left (number of acts)

Turns to the right (number of acts)

Twists (number of acts)

Statistical processing

To present the results in tables, we calculated mean values with the error of the mean and median. The medians are shown in all the figures. The accuracy of the differences between the variants was determined using the Mann-Whitney test, as well as ANOVA using Statgraphics Centurion XV11 and Statistica 6.0.

Results and discussion

Analyzing the behavior of the naive animals in the "open field" test made it possible to identify interlinear differences in the level of vertical motor activity (Fig. 3) and acts of freezing (Fig. 6). The number of upright positions is higher, and the number of acts of freezing is lower for highly excitable rats of the LT line compared with the low-excitable HT line. This is associated with genetically determined features of the rats' excitability and reflects the linear characteristics of elements of innate behavior. It should be noted that the rats of these lines behave differently depending on the level of excitability of their nervous systems, and their behavior has different strategies. This is confirmed by the data we have obtained.

It was found that exposing the animals in the chambers for 12 hours, regardless of additional experimental conditions, affects the behavior of the studied rat lines in different ways and leads to a change in the various elements of behavior in one and the other line compared to the naive control: for the HT line — fewer acts of freezing (Fig. 6) and a larger number of turns (Fig. 7); for the LT line — shorter latency of the first movement (Fig. 1), less emotionality (Fig. 4), and more acts of grooming (Fig. 5). This data indicates that the animals' very presence in the closed space of the cylinders affects their subsequent reactions in the "open field" apparatus and the characteristics of these reactions depend on the animals' genotype.

The experimental results made it possible to establish that the attenuation factor from the screening of the external magnetic field (LMF) affects only highly excitable rats of the LT line. The LMF's influence was an increase in the number of acts of freezing (Fig. 6), which indicate a possible increase in the fear response in the new environment, as well as more left and right turns (Figs. 7 and 8), which indicates an increase in the animals' anxiety (chaotic movements) (compared to the corresponding WLMF groups and naive controls).

The radiation of the router under conditions of an external magnetic field weakened by the shielding, and without it, also affects only certain elements of behavior in the "open field" test, which differ in highly excitable and low-excitable rats. For the HT line under the influence of the router, an increase in the number of right turns was observed (Fig. 8), whereas for the LT line, there was an increase in the number of acts of freezing (Fig. 6) and twisting (Fig. 9) in comparison with the LMF and WLFM groups, respectively, and the naive control.

However, the router's influence in the conditions of LMF leads to a selective decrease in the emotionality in rats of the HT line (Fig. 4) and in the horizontal motor activity in rats of the LT line (Fig. 2). These are the most significant elements of normal behavior associated with different levels of hereditary nervous system excitability in the animals.

The action of the Aires resonators was tested on only one HT line. Under the conditions of the shielding chamber, the resonators resulted in a decrease in the number of right turns (Fig. 8) in comparison with the action of the router. That is, the resonators influenced precisely the trait that changed (increased) under the influence of the router.

Under the conditions of the imitation chamber, the resonators amid the influence of the router caused a sharp increase in horizontal motor (exploratory) activity (Fig. 2), acts of grooming (Fig. 5), and reduced freezing (Fig. 6). However, the number of twists under the influence of the router and resonators increased regardless of the type of chamber (Fig. 9).

Thus, our results allow us to infer that the resonators have a selective positive effect, provided that the external magnetic field is limited to only one component of the behavior, i.e. the number of right turns, but not left turns. This may be due to lateralization of the to receive and respond to EMR. However, it is still difficult to establish specific patterns.

Without a weakening of the external magnetic field, the resonators influenced other behavioral components and increased motor activity and grooming, while decreasing fear in the animals, which can generally be considered the animals' adaptive responses to the new conditions. Regardless of the operating conditions of the external magnetic field, the resonators caused an increase in acts of twisting.

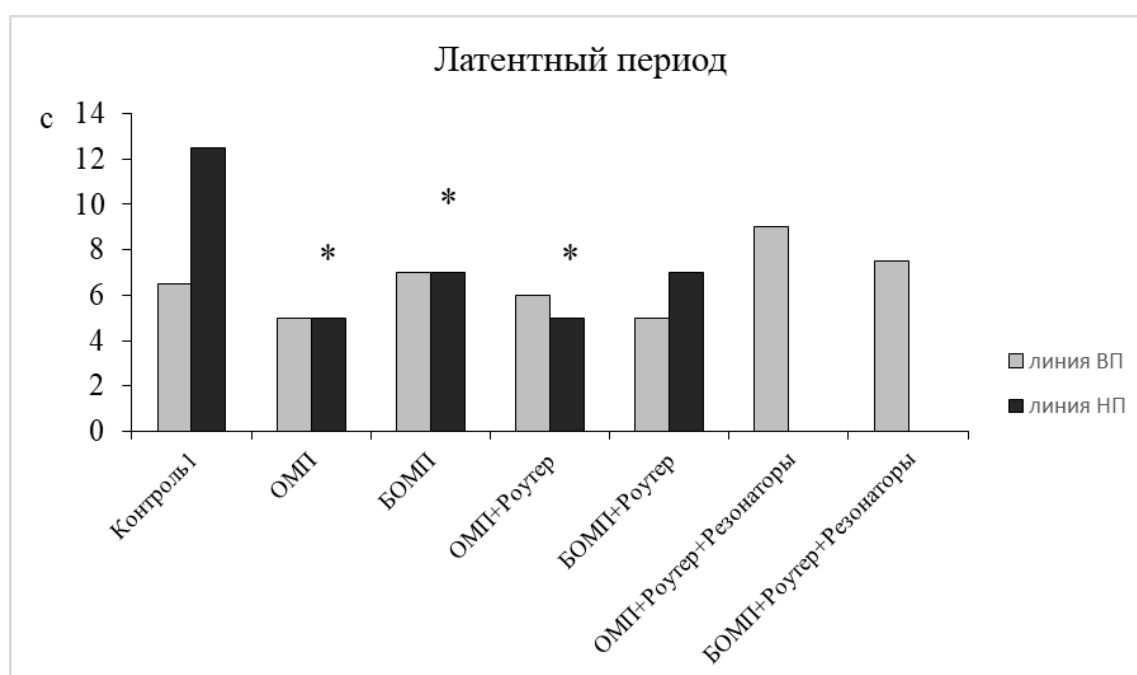
Thus, given the combination of two EMR factors (the router and the resonators), elements of the animals' general motor and exploratory activity were strengthened and the fear response, which can also be observed after stressful influences, was suppressed.

The increased grooming may have two biological meanings. On the one hand, grooming is classified as a comfort behavior, which is confirmed by the well-known increase in the number of acts of grooming as an animal becomes accustomed to the environment, accompanied by a decrease in indicators of stressful behavior. On the other hand, in rodents grooming, a behavioral marker of stress, also becomes more active under the influence of stress. Next, it seems

important to use correlation analysis to determine the relationship between the behavioral elements under investigation.

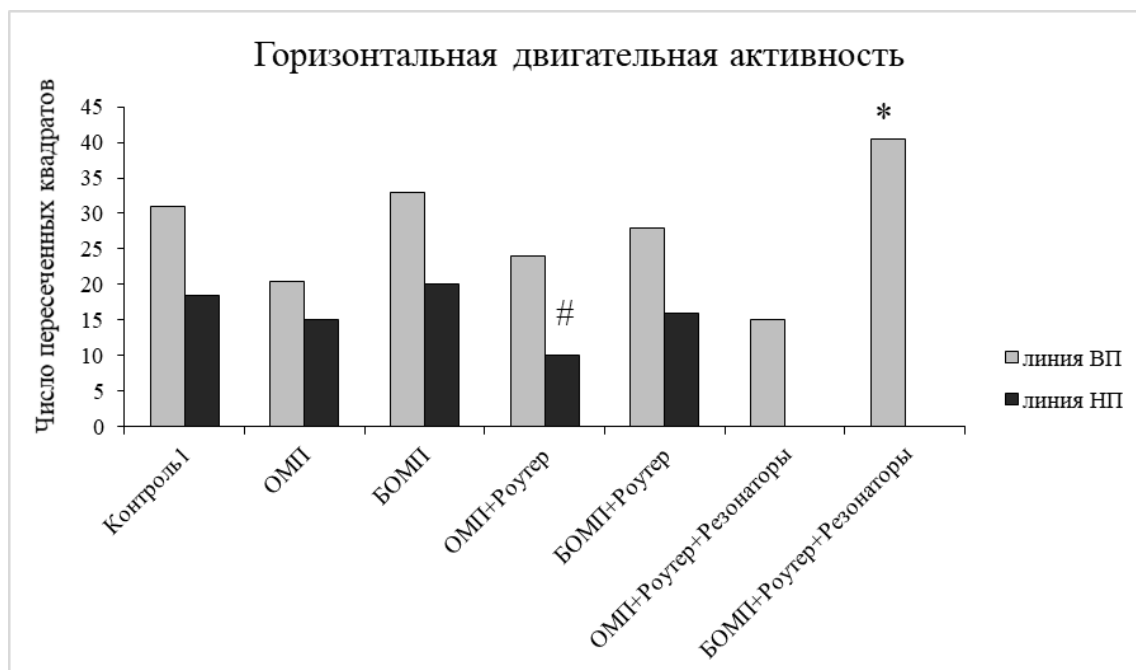
In general, our experiments indicate that all of the studied factors influence different components of rat behavior in the "open field" test. The nature of the reaction to exposure in cylinders, to the weakening of the external magnetic field, and to the router's EMR depends on the animals' hereditary nervous system excitability and affects various components of behavior.

The action of the Aires Defender Pro resonators changed the behavior of low-excitability rats of the HT line and caused their activity to increase when placed in a new environment. Specific behavioral components of the animals' reaction to the action of Aires resonators were also identified, indicating their selective positive influence on specific elements of behavior.



Russian	English
Латентный период	Latency
с	s
Контроль 1	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

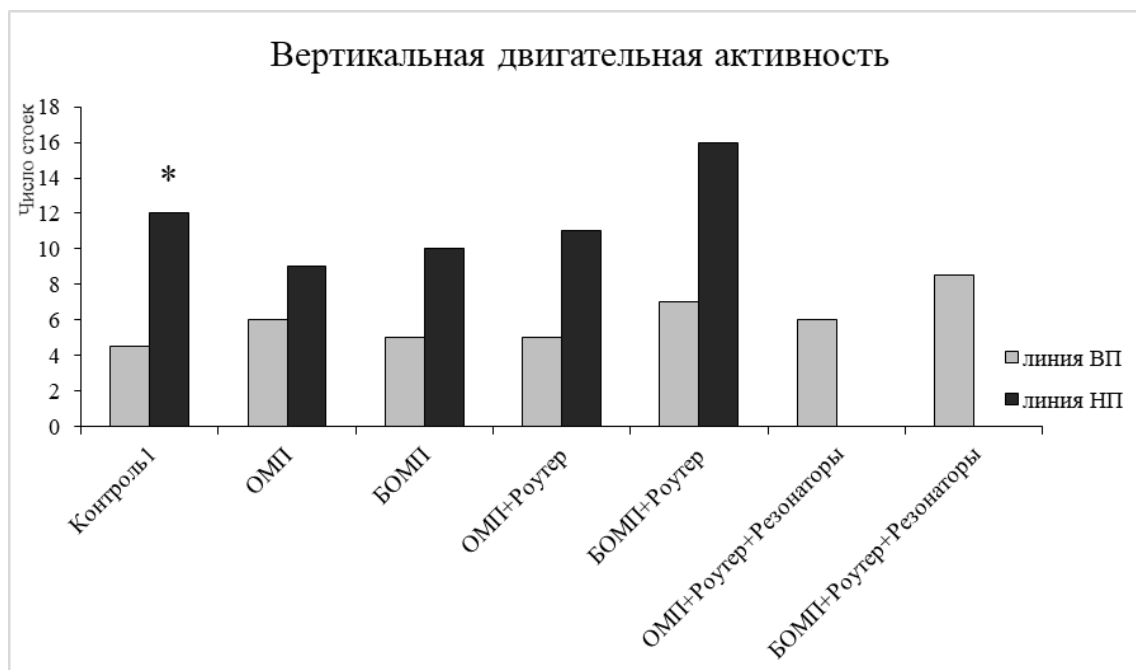
Fig. 1 Latency of reactions in the "open field" test of male rats of the HT and LT lines
Key: *- The differences with Control 1 (naive) of the LT line are significant ($P < 0.05$).



Russian	English
Горизонтальная двигательная активность	Horizontal motor activity
Число пересеченных квадратов	Number of intersected squares
Контроль 1	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 2. Horizontal motor activity (number of intersected squares) in the "open field" test of male rats of the HT and LT lines.

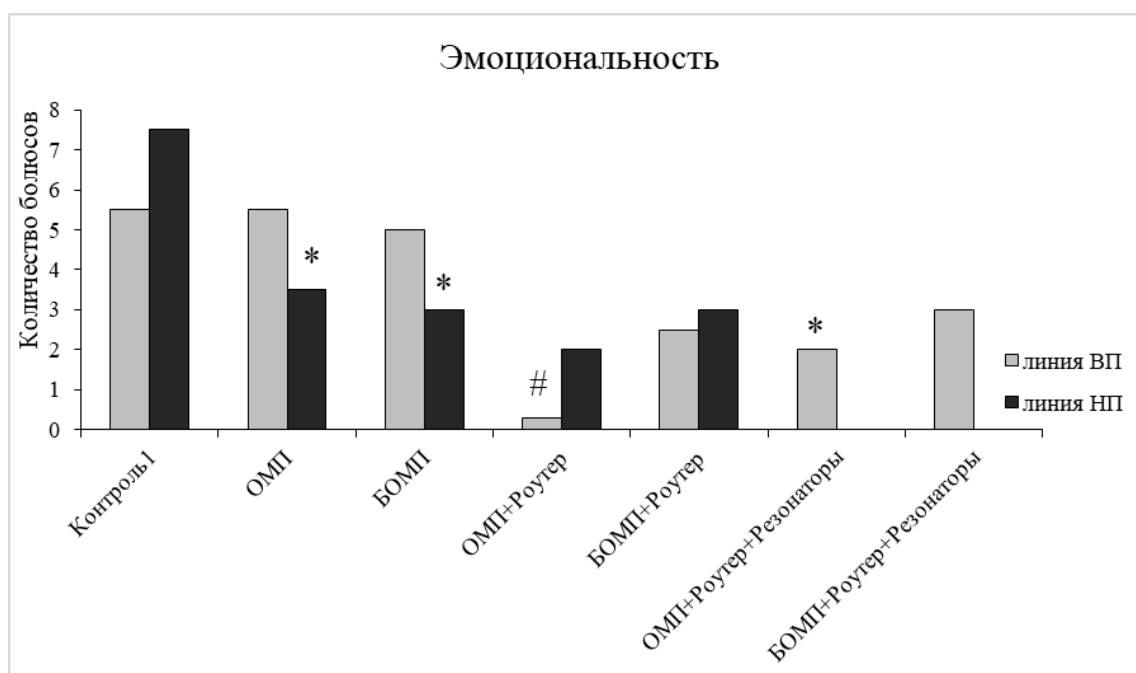
Key: *- The differences with the "LMF+Router+Resonators" group of the HT line are significant ($P < 0.05$); #- The differences with the remaining groups of the LT line are significant ($P < 0.05$)



Russian	English
Вертикальная двигательная активность	Vertical motor activity
Число стоек	Number of upright positions
Контроль 1	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 3. Vertical motor activity (number of upright positions) in the "open field" test of male rats of the HT and LT lines.

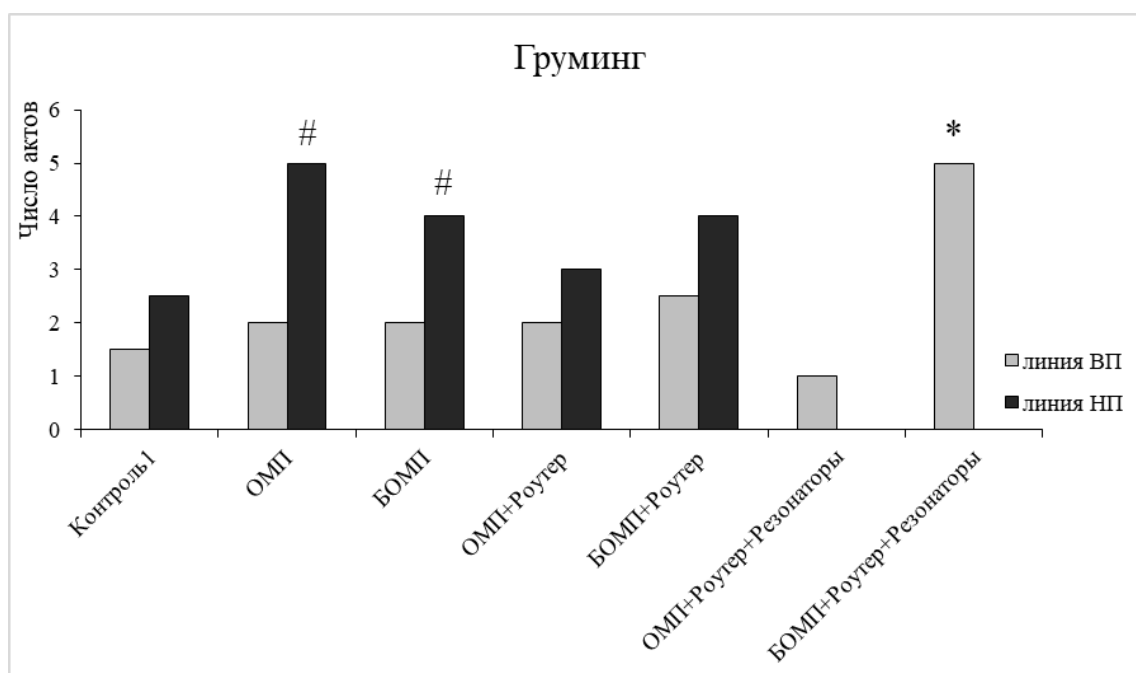
Key: *- The differences with the (naive) Control 1 group of the HT line are significant ($P < 0.05$).



Russian	English
Эмоциональность	Emotionality
Количество болюсов	Number of boluses
Контроль1	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

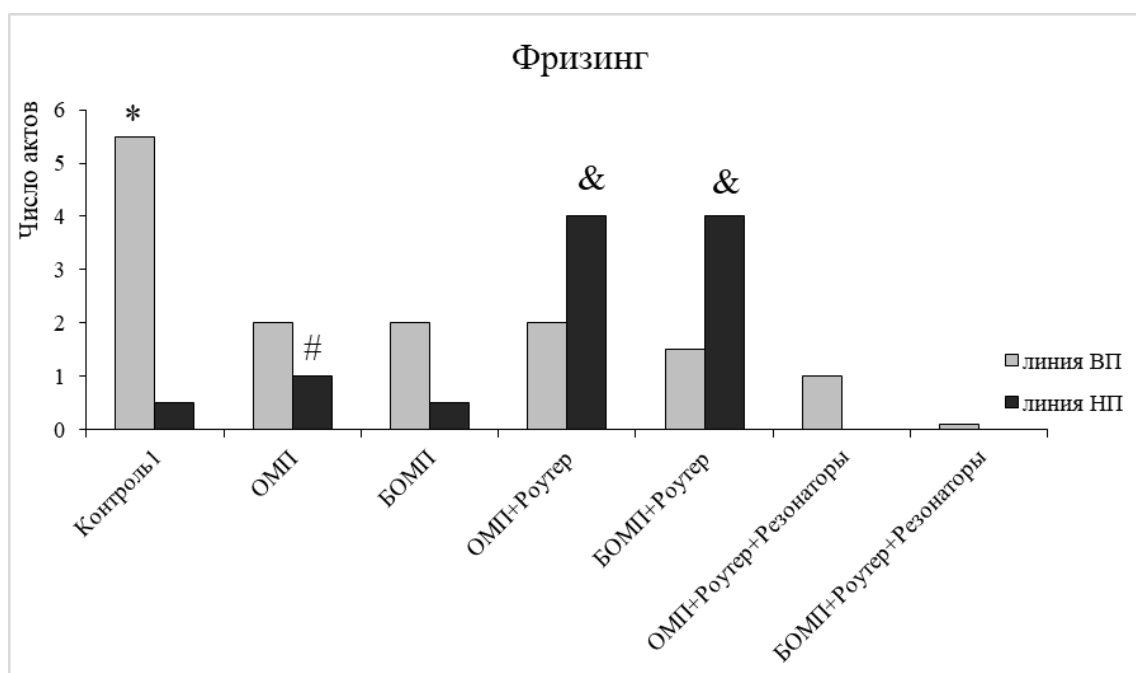
Fig. 4. Emotionality (number of boluses) in the "open field" test of male rats of the HT and LT lines.

Key: *- The differences with the (naive) Control 1 group of the HT line are significant ($P < 0.05$);
 #- The differences with the remaining groups of the HT line are significant ($P < 0.05$)



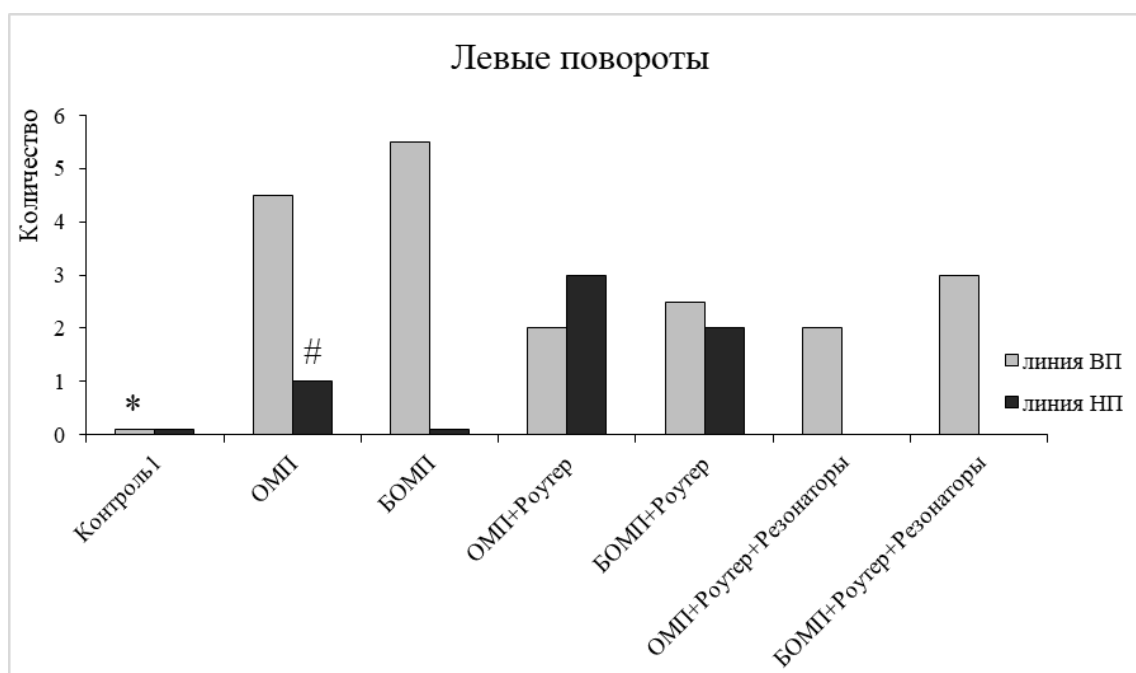
Russian	English
Груминг	Grooming
Число актов	Number of acts
Контроль 1	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 5. Number of acts of grooming in the "open field" test of male rats of the HT and LT lines.
Key: *- The differences with the "LMF+Router+Resonators" group of the HT line are significant ($P < 0.05$); #- The differences with the (naive) Control 1 group of the LT line are significant ($P < 0.05$)



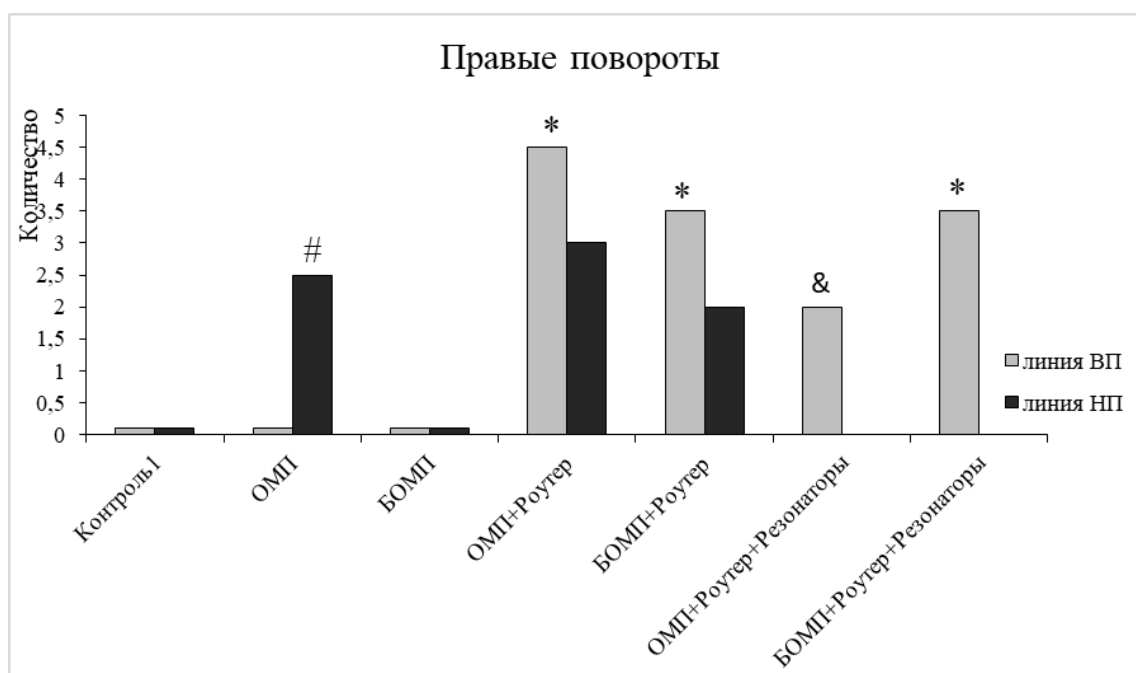
Russian	English
Фризинг	Freezing
Число актов	Number of acts
Контроль I	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 6. Number of acts of freezing in the "open field" test of male rats of the HT and LT lines.
Key: *- The differences with Control 1 of the NT line and with all other groups of the HT line are significant ($P < 0.05$); #- The differences with the WLMF group of the LT line are significant ($P < 0.05$); &- The differences with the corresponding LMF and WLMF groups of the LT line are significant ($P < 0.05$)



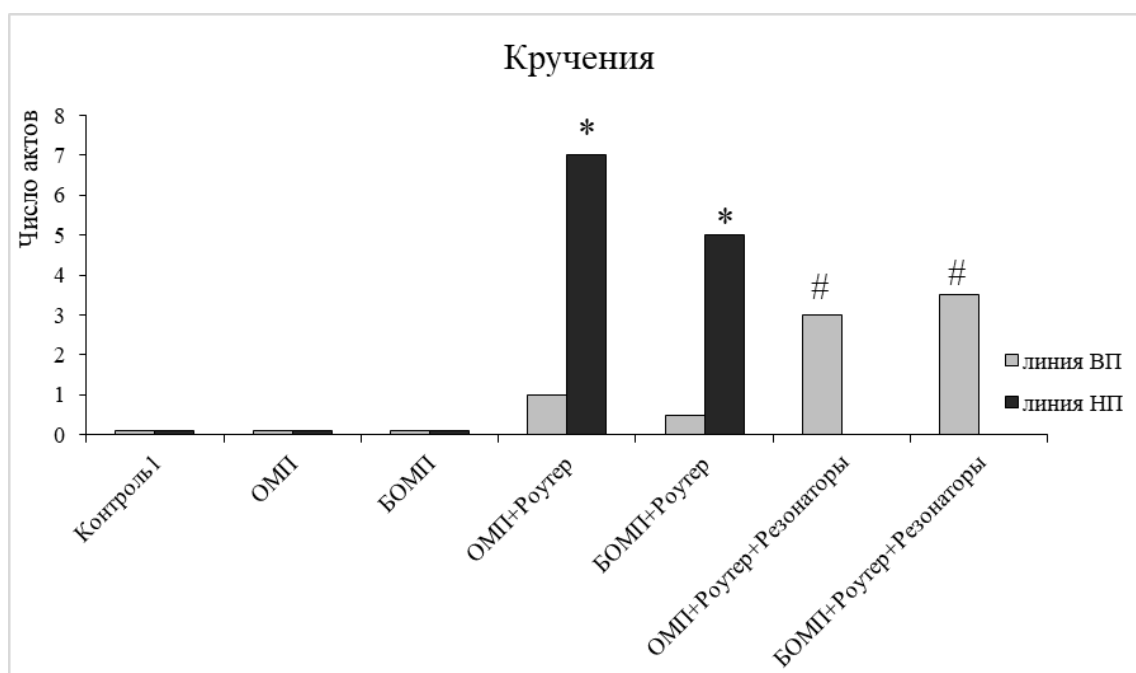
Russian	English
Левые повороты	Left turns
Количество	Number
Контроль I	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 7. Number of left turns in the "open field" test of male rats of the HT and LT lines.
Key: *- The differences with all the remaining groups of the HT line are significant ($P < 0.05$); #-
The differences with the WLMF group of the LT line are significant ($P < 0.05$).



Russian	English
Правые повороты	Right turns
Количество	Number
Контроль I	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 8. Number of right turns in the "open field" test of male rats of the HT and LT lines.
Key: *- The differences with the Control 1, LMF, and WLMF groups of the HT line are significant ($P < 0.05$); #- The differences with the WLMF group of the LT line are significant ($P < 0.05$), &- The differences with the LMF+Router group are significant ($P < 0.05$).



Russian	English
Кручения	Twists
Число актов	Number of acts
Контроль I	Control 1
ОМП	LMF
БОМП	WLMF
ОМП+Роутер	LMF+Router
БОМП+Роутер	WLMF+Router
ОМП+Роутер+Резонаторы	LMF+Router+Resonators
БОМП+Роутер+Резонаторы	WLMF+Router+Resonators
линия ВП	HT line
линия НП	LT line

Fig. 9. Number of twists in the "open field" test of male rats of the HT and LT lines.

Key: *- The differences with the Control 1, LMF, and WLMF groups of the LT line are significant ($P < 0.05$); #- The differences with the remaining groups of the HT line are significant ($P < 0.05$).

Appendix

Table 1. Initial results of measurements of the magnetic field (μT) in different parts of the experimental chamber and outside it.

Outside the chamber	Inside the chamber	Inside the chamber with the router turned on
52.2	59.6	59.6
55	59.6	62.4
52.3	61.8	49.6
52.7	47.1	48.3
85.6	51.2	52.2

Table 2. Results of the assessment of the behavior of rats of the HT and LT lines in an "open field" test after exposure under conditions of a limited external magnetic field, and electromagnetic radiation from a router and resonator-converters.

Groups/behaviors	LP, s	HMA, s	VMA, s	Number of boluses	Grooming, s	Freezing, s	Left turn,	Right turn	Twisting	
HT line										
X $\pm$ m (M)										
Naive control	7.2 $\pm$ 5.6 (6.5)	29.3 $\pm$ 16.3 (31)	4.8 $\pm$ 3.2 (4.5)	5.2 $\pm$ 1.8 (5.5)	1.7 $\pm$ 1.4 (1.5)	5.2 $\pm$ 2.6 (5.5)	-	-	0.8 $\pm$ 1.7 (0)	
LMF	5.8 $\pm$ 3.5 (5)	21.5 $\pm$ 4.4 (20.5)	6.3 $\pm$ 2.2 (6)	4.0 $\pm$ 3.3 (5.5)	2.7 $\pm$ 2.1 (2)	2.2 $\pm$ 1.7 (2)	3.5 $\pm$ 3.0 (4.5)	-	-	
WLMF	6.8 $\pm$ 1.8 (7)	34.2 $\pm$ 21.9 (33)	5.3 $\pm$ 4.4 (5)	4.8 $\pm$ 1.8 (5)	2.7 $\pm$ 2.2, 2.3 (2)	2.2 $\pm$ 1.6 (2)	5.5 $\pm$ 3.0 (5.5)	-	-	
LMF+Router	7.0 $\pm$ 4.0 (6)	31.2 $\pm$ 22.1 (24)	6.2 $\pm$ 4.9 (5)	0.9 $\pm$ 1.4 (0)	2.3 $\pm$ 2.1 (2)	1.3 $\pm$ 1.8 (2)	2 $\pm$ 2.4 (2)	4.3 $\pm$ 1.2 (4.5) #	2 $\pm$ 2.3 (1)	
WLMF+Router	6.2 $\pm$ 2.8 (5)	33.3 $\pm$ 17.5 (28)	7.7 $\pm$ 5.4 (7)	2.8 $\pm$ 2.4 (2.5)	3.7 $\pm$ 3.7 (2.5)	1.7 $\pm$ 0.9 (1.5)	2.7 $\pm$ 0.9 (2.5) #	3.7 $\pm$ 1.7 (3.5) #	0.8 $\pm$ 1.2 (0.5)	
LMF+Router+Resonators	8.1 $\pm$ 2.9 (9)	20.0 $\pm$ 10.2 (15)	6.4 $\pm$ 4.0 (6)	1.7 $\pm$ 1.3 (2) #	1.8 $\pm$ 1.7 (1)	1.7 $\pm$ 1.5 (1)	2.0 $\pm$ 0.5 (2)	2.0 $\pm$ 0.5 (2) #	3.0 $\pm$ 1.1 (3)?	
WLMF+Router+Resonators	7.2 $\pm$ 2.0 (7.5)	49.3 $\pm$ 29.1 (40.5)	8.5 $\pm$ 2.5 (8.5)	2.7 $\pm$ 2.1 (3)	4.7 $\pm$ 1.6 (5)	0.5 $\pm$ 1.3 (0) #	3.0 $\pm$ 0.0 (3)	3.5 $\pm$ 0.6 (3.5)	2.8 $\pm$ 2.0 (3.5)?	

Line LT $X \pm m$ (N)									
Naive control	10.7±5.6 (12.5)	17.2±6.6 (18.5)	11±3.9 (12)	5.7±3.9 (7.5)	2.3±1.8 (2.5)	0.7±0.09 (0.5)	-	-	0.5±0.8 (0)
LMF	5.3±3.6 (5)	20.2±13.5 (15)	9±3.1 (9)	3±2.3 (3.5)	4.5±2.1 (5)	1.7±1.1 (1)	1±1.5 (1)	2.5±1.1 (2.5)	-
WLMF	6.0±3.9 (7)	21.0±10.8 (20)	10.7±4.5 (10)	2.3±2.1 (3)	5.2±3.7 (4)	0.5±0.1 (0.5)	-	-	-
LMF+Router	6.6±5.4 (5)	9.0±1.7 (10)	9.8±4.4 (11)	3±4.3 (2)	2.4±2.4 (3)	4.2±1.3 (4) #	2.2±1.6 (3)	1.8±1.6 (1)	6.8±3.7 (7) #
WLMF+Router	9.2±9.1 (7)	15.2±3.7 (16)	12.6±8.3 (12)	3.8±2.8 (3)	3.6±2.8 (4)	3.8±3.2 (3) #	1.6±1.4 (2)	1.8±0.6 (2)	5.2±1.0 (5) #
LMF+Router+Resonators									
WLMF+Router+Resonators									

Key: The results are presented as an average with the error of the mean ($X \pm m$) and median (M)

Key

Values with statistical differences are highlighted in color



- differences with the alternative line in the naive control groups



- differences in experimental groups with the corresponding naive control groups



- differences with all other groups (naive control, LMF, WLMF, WLMF+Router) = combined effect of LMF and router



- differences with the WLMF group = intrinsic influence of LMF



- Differences with the alternative LMF+Router+Resonators group



- influence of the resonators



- influence of the router