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Activation of animal brain neuromediators by interferential transcranial currents

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A method of transcranial electromagnetic stimulation of the mammalian brain is proposed. The method is based on the interference of currents caused by high-frequency orthogonal oscillations of electric fields, which are modulated by low-frequency meander pulses. The effectiveness of the method was confirmed by the results of experiments on stimulating the brain of rats and rabbits.

Keywords: electromagnetic stimulation, interference, alpha-rhythms, theta-rhythms, serotonin, beta-endorphins, neuron.

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Introduction

In the world literature, there is a lot of experimental material on the problem of the influence of electric and magnetic fields on biological processes in a living organism [1–3]. Transcranial magnetic stimulation (TMS) of the mammalian brain occupies a special place in this problem, which is a process of influencing the brain with electric fields formed either by contact [4] or non-contact [5] methods. In the first case, the effect is performed by placing external electrodes (or electrodes implanted under the skin or in the brain structures) on the mammal's scalp, to which alternating electric voltage is applied, resulting in simultaneous electric drift current and bias current flowing between the electrodes, with both currents generally flowing inside the cranium and along the external skin of the head. In the second case, the current influence is carried out by placing the brain in a high-frequency electromagnetic field, which causes induction currents to flow in the outer covers of the head due to the high electrical skin conductivity, determined by an extensive network of microcapillaries. The microcapillaries contain highly conductive blood plasma, cellular elements and erythrocyte aggregates, which through the walls of the vessels interact via transport proteins with the cerebrospinal fluid circulating in the Virchow–Robin space cavities around the microcapillaries [6].

There is no doubt that TMS in most cases has a beneficial effect on the clinical and psychosomatic state of the body, reducing the intensity of the pain syndrome, reducing the severity of the depressive syndrome, panic attacks and some other psycho-emotional disorders. However, the procedure of studying the physical causes of the processes described is very far from being completed, which determines the unflagging interest in this kind of research. Currently, it is generally accepted that the production of serotonin and beta-endorphins by antinociceptive brain structures under the influence of TMS is able to reduce the level of pain syndrome and psycho-emotional disorders, leading to the normalization of neurohumoral imbalance.

The purpose of this work is to describe the TMS algorithm, which is more effective than the existing ones, and to analyze the results of experimental studies of the effect of TMS using the improved algorithm on the activation of serotonergic and betaendorphinergic brain structures in laboratory animals.

The starting point for the new algorithm development was the information [7,8] that the increased content of beta-endorphins in the rabbit brain structures manifested itself as much as possible when using a repetition rate of 78 Hz for TMS stimulating pulses.

The new TMS algorithm proposed in this work provides, in addition to stimulation of serotonergic and beta-endorphinergic brain structures, also the possibility of activation of structures associated with α- and θ-rhythms of the brain, since neurophysiologists have established a correlation between an increase in the amplitude of α- and θ-rhythms of the brain and a decrease in the severity of depression [9] symptoms. This dictates the necessity of using the frequency range from 5 to 14 Hz, which stimulates α- and θ-rhythms of the brain, simultaneously with using the frequency 78 Hz when improving the TMS technique.

1. Experimental procedure

1.1. Technical part of TMS

This section first describes the technical (hardware) part of the technique, followed by the methodological (biophysical) part of the TMS of the animal's brain.

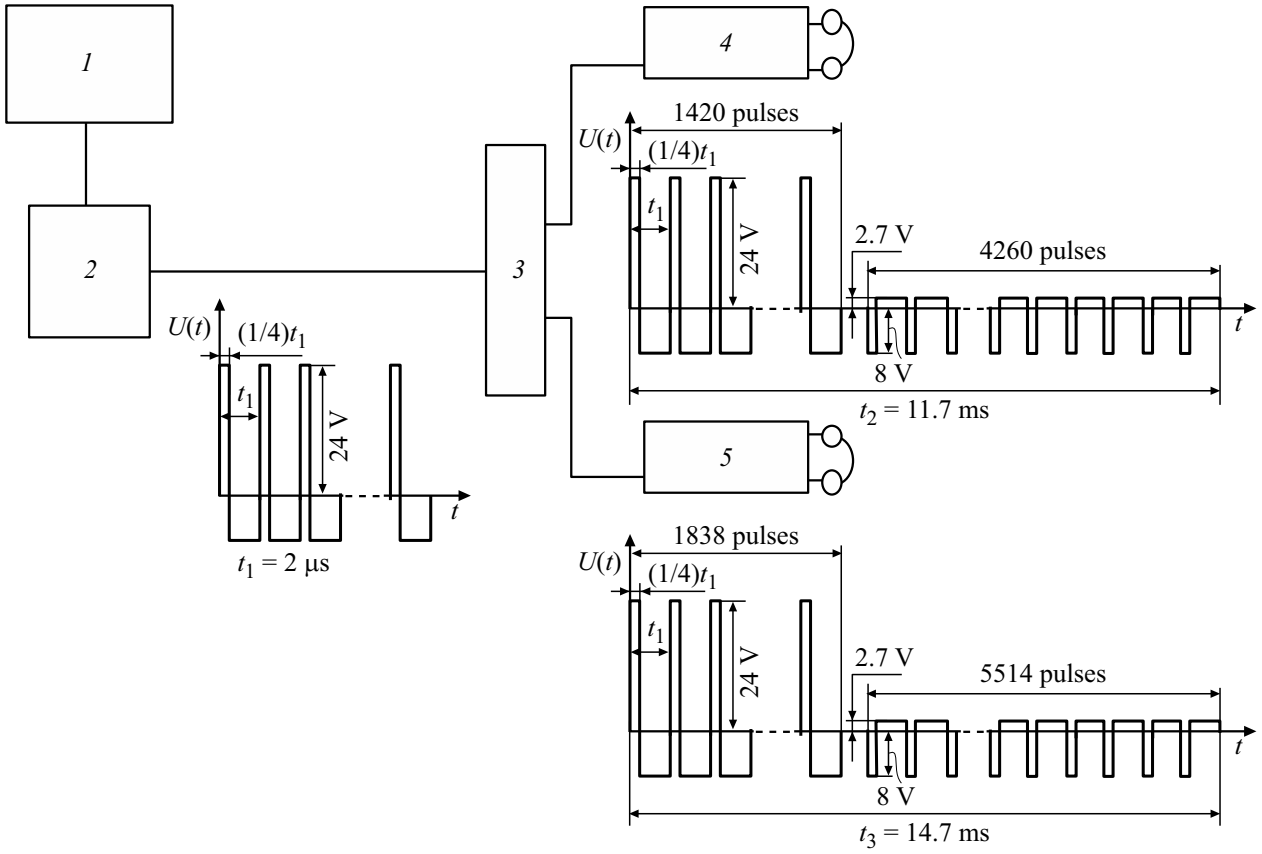

Figure 1. TMS unit block diagram and voltage diagrams. *1* — control computer and control monitor, *2* — alternator 500 kHz, *3* — separator, *4,5* — alternators 88 and 68 Hz with inverters and electrodes.

The new TMS algorithm is reduced to the formation in the animal's brain of two alternating currents mutually perpendicular in the direction of propagation, interfering with each other in the area of current tubes intersection. The proposed TMS algorithm is designed to increase the intensity of serotonin and beta-endorphin production in brain tissues more effectively than the commonly known methods through the process of interference currents in targeted deep brain structures selected for stimulation.

To provide interference effects, the new TMS algorithm uses 4 electrodes in pairs: one pair provides a current tube in the basal direction of the forehead–occipital, and another pair of electrodes is superimposed on the area of the mastoid processes, forming the second current tube perpendicular to the first one. All 4 electrodes were implanted under the animal's scalp, and the control experiments established that there was no effect on the experimental results of the stress response arising as a consequence of surgical intervention. The configuration of the electrodes was chosen so that all electrodes lay approximately in the same plane. The use of two pairs of electrodes made it possible to create two intersecting current tubes in the animal's brain with a controlled intersection zone, in which the interference of currents excited in the brain tissues occurred. The length of the high-frequency electromagnetic wave, which plays the role of carrier, is several meters. This was shown by a simple calculation (see below) using literature values of the brain tissue permittivity, from which it follows that current fluctuations in the interference region are summarized, alternately amplifying or weakening each other in time and spatially uniformly covering the entire interference zone. We used bipolar meanders with two different repetition frequencies to modulate the high-frequency carrier voltage — 88 and 68 Hz. The choice of these frequencies was due to the fact that, since in the area of interference, there are oscillations at a frequency equal to the half-sum of the modulating frequencies, as well as low-frequency beats, the interference area is exposed to electromagnetic oscillations with a frequency of half-sum 78 Hz and low-frequency beats 5–14 Hz. The 78 Hz oscillation performed the functions described above during TMS, while the low-frequency beats corresponded to the α- and θ-rhythms of the animal's brain. Electromagnetic exposure with a frequency of 78 Hz increases the concentration of neurotransmitters such as beta-endorphins and serotonin in the brain of animals. Along with this, electromagnetic low-frequency beats also provide an additional increase in the concentration of these neurotransmitters.

Literature data [10] indicate that neutralization of seizure activity, caused, in particular, by previously developed TMS modes, can be achieved by using a weak direct current

flowing along with alternating current in the same electric circuit. Therefore, for effective brain TMS without side effects, the AC voltage must be supplied with a constant electrical bias circuit that ensures that a weak constant component of the electric current flows through the animal's brain between the same electrodes as the pulse [11] component.

Another seizure blocking possibility is the use of a bipolar asymmetrical meander voltage with a 78 Hz repetition rate, modulating the high-frequency carrier voltage. In the present work, we took advantage of this possibility by applying a bipolar asymmetrical pulse voltage of 500 kHz as the carrier. Pulsed electric fields with a frequency of 500 kHz effectively penetrate deep into the brain, as they are weakly shielded by ionic tissue polarization. In addition, bipolar pulses of frequency 500 kHz, as a simple calculation shows, form a constant current component in accordance with the electrical characteristics of brain tissue.

Let's take a closer look at the electrical circuitry of the brain TMS.

Block diagram of the device contains three alternators of alternating voltage in the form of ambipolar meander (Fig. 1), namely a voltage alternator with carrier frequency 500 kHz and two modulating carrier low-frequency alternators with frequencies 88 Hz (feeds basal electrodes) and 68 Hz (feeds mastoid electrodes).

The duration, duty cycle and shape of the pulses are also shown in Fig. 1. Each pair of electrodes consists of two metal plates connected to one of the two channels. The block diagram also contains the control computer and the control monitor. A high-frequency total electric field vector bf E in the brain tissue is shown in Fig. 2, *a*.

A high-frequency bipolar meander with a repetition frequency of 500 kHz has a 1/4 duty cycle and a short pulse amplitude of 24 V, and a longer one — 8 V (Fig. 1). The electromagnetic wave length in a vacuum corresponding to the basic sinusoidal harmonic of the high-frequency carrier is $\lambda = c/\nu = 3 \cdot 10^8/(5 \cdot 10^5) = 600$ m. In the shielding electric field, i.e., in the animal's brain, it is n times less — λ/n, where $n = (\varepsilon)^{1/2} = 50$, and $\varepsilon = 2500$ — the low frequency permittivity of the animal's brain tissue. Taking this into account, the wavelength is equal to 12 m, i.e. much greater than the transverse dimensions of the animal's head. As a consequence, the interference pattern appearing in the animal's brain is a homogeneous spatially limited area in which the oscillations are added and electrical pulsations, i.e. vibrations of electromagnetic fields and currents are performed.

Therefore, further we will talk about oscillatory change of electric field strengths and densities of flowing currents, uniformly overlapping the entire spatially bounded volume of the zone of intersection of orthogonal current tubes. It follows from the above that by changing the location of the electrodes on the scalp, one can control the spatial position of the interference zone, activating different parts of the brain.

It should be noted that the time dependence of the current density amplitude does not coincide with the time dependence of the amplitude of the applied bipolar voltage meander. The fact is that the animal's head has an electrical resistance, which in general is complex. Calculation by the complex method of the simplest equivalent electrical circuit of the animal's brain with superimposed electrodes leads to the identification of significant differences in the shape of current density oscillations and applied voltage. Such an equivalent circuit contains an electrical capacitance, as well as two resistances: charging and discharging, simulating the conductivity of brain tissue. The capacitance is due to a pair of electrodes, between which there is a medium with a sufficiently high permittivity $\varepsilon = 2500$ (dense brain tissue and basal cisterns filled with cerebrospinal fluid). The charging resistance simulates the contact resistance of the scalp and skull bones of the animal, the discharge resistance connected in parallel with the capacitance simulating the ability of the brain tissue to shield the external field represents the electrical resistance of the internal brain tissue. The time constant τ of such a circuit is determined by the expression $\tau = (CR_{\text{charge}}R_{\text{discharge}})/(R_{\text{charge}} + R_{\text{discharge}})$ and is estimated by us at $6\,\mu$s ($C = 100$ pF, $R_{\text{charge}} = 5 \cdot 10^4\,\Omega$, $R_{\text{discharge}} = 10^5\,\Omega$), which is significantly longer than the high frequency voltage period $1/f = (1/5) \cdot 10^{-5}$ s= $2\,\mu$s. This means that the short meander pulses will be reintegrated in percentage terms much more effectively than the time-long pulses of opposite polarity, i.e. during integration in the RC-chain the initial equality of areas of pulses of opposite polarity will change in favor of the time-long part of the bipolar pulse. In other words, there will be a significant shift of the integral midline of the bipolar meander toward its wide pulses, so that, along with the alternating component of the current, a direct current will flow between the electrodes, flowing in one predominant direction.

It should also be taken into account that the addition of electromagnetic field oscillations in the area of intersection of orthogonal current tubes will lead to the appearance in this area of a series of flat Lissajous figures, located in several layers parallel to the plane of the electrodes. The appearance of these figures, which determine the direction of the total current vector and electromagnetic field vector, is determined by the phase difference between the oscillations of the same-name Fourier harmonics (Fig. 2, *b*) of the folding orthogonal currents and electromagnetic fields.

High-frequency voltage modulation was carried out in our applied electric circuit by bipolar low frequency voltage meanders (88 and 68 Hz) with a duty cycle of 4. As a result of the modulation, mutually orthogonal currents of alternating polarity flowed in the animal's brain along with unidirectional mutually orthogonal currents. The latter in the area of crossing the tubes of currents excited mainly low-frequency beats of oscillations. The shape of Lissajous figures and their changes in time are very difficult to analyze due to multi-factoriality of the processes going on: presence of different modulation frequencies, complex law of changes in time of phase shifts between different Fourier-harmonics generated by the whole set of voltage sources, complex

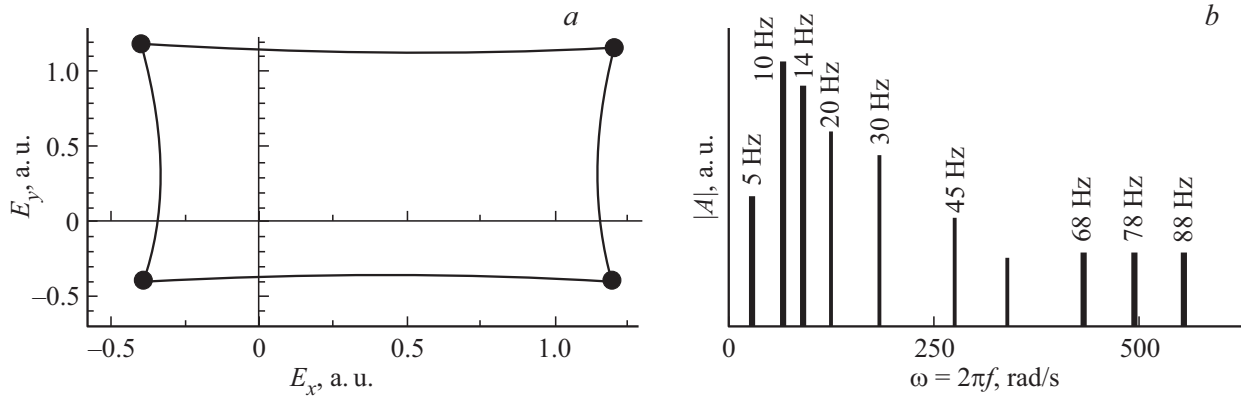

Figure 2. *a* — the change in the hodograph of the high-frequency electric field intensity vector bfE for one period $t_1 = 2\mu$s. The hodograph contains all directions **E**, but the main directions **E** are marked with points in the corners of the hodograph, since the voltage to the electrodes is applied as rectangular meanders. The hodograph is shifted in the positive direction along the coordinate axes due to the difference in the values of positive and negative amplitudes of the high-frequency meander. *b* — modulus of amplitudes of Fourier-harmonics of low-frequency signal obtained as a result of vector addition of electric fields for two meanders with frequencies 88 and 68 Hz.

branching in the animal's brain of currents into separate „flows" etc. Nevertheless, the analysis shows that the main frequencies of low-frequency oscillations are the half-sum of frequencies 68 and 88 Hz, modulating the high-frequency carrier, namely 78 Hz and low-frequency beats of the range 5–14 Hz, the aggregate of which is a set of optimal frequencies for effective brain TMS.

Let us note the positive technical aspects of the implementation of the interference algorithm of animal brain TMS proposed in this paper:

1) The use of two pairs of electrodes, ensuring the presence of a summation process in a limited volume area, reduces the magnitude of the total current flowing through the animal's brain. The reason for this is the pumping of energy into the working zone from the areas of the brain not captured by the interference zone, according to the principles of coherent interference summation.

2) Selecting the position of electrodes on the skin surface allows changing the spatial location of the limited in volume area of intersection of mutually perpendicular current tubes inside the animal's head, which expands the research possibilities.

3) Use of two pairs of electrodes with voltages of different frequencies (88 and 68 Hz) to modulate the amplitude of the high-frequency voltage allows in one procedure of influence to generate effective for TMS oscillation frequency 78 Hz and periodic current pulsations in the low frequency range 5–14 Hz.

4) The use of an asymmetrical high-frequency bipolar meander allows, along with alternating currents, the possibility of unidirectional currents flowing in the brain tissues, contributing to the suppression of foci of convulsive activity of the animal's central nervous system.

5) Rapidly alternating beats in the form of multidirectional Lissajous figures engage different micro areas of the brain within the interference area, which reduces the „habituation" of brain tissue to the TMS. In early variants of TMS implementation, habituation forced to continuously increase the operating voltage on the electrodes in order to counteract the decrease of TMS efficiency as a result of the „compensatory" habituation mechanism [11].

6) Changing the direction in space of current and field vectors (Lissajous figures) allows to prevent electric ionic polarization of the basal cistern cerebrospinal fluid. The polarization process forced, in order to avoid the „habituation effect", to change the polarity of the direct current every 15–20 min TMS. The use of current interference also makes it possible to reduce such a negative effect as uncontrolled spontaneous growth of bipolar pulse amplitude during the first 5–10 min of the TMS with its drop during the next 5–10 min.

1.2. Biophysical part of the animal brain TMS technique

The most convenient way to confirm the effectiveness of the proposed new stimulation algorithm is to experimentally test the effects of the interference variant of TMS on the brains of laboratory animals such as rats and rabbits. Two variants of the technique are discussed in this section: the development of the conditioned reflex (CR) of electric shock avoidance in rats and the suppression of the pain syndrome (reduction of pain sensitivity) in rabbits.

1.2.1. Dividing the rats into groups. Technology for producing CR Four groups of sexually mature rats (Wistar males weighing about 130 g) were used in the experiments, with 11 animals in each group. Animals were assigned to experimental groups by randomization procedure.

The first group was the control one; for it, the development of stable CR of electric shock avoidance on intact rats was evaluated.

The second group was also a control one, but differed from the first in that the animals in it had two pairs of electrodes implanted under the scalp: fronto-occipital and behind-the-ear microcurrent channels. Electrode implantation was performed under inhalation anesthesia (4.5% fluorotane, oxygen 1 l/min, duration 5 min). In this group, the experiments were performed one day after electrode implantation in order to level out the pain syndrome of the implantation process.

In animals of the third group, TMS was performed for 30 min immediately before the CR generation procedure.

Finally, animals of the fourth group were injected with kytril 30 min before TMS, and then TMS was performed for 30 min. Injection of kytril was dictated by the fact that this drug is a serotonin blocker. The results of experiments with animals of the third and fourth groups unambiguously showed that brain TMS interferes with the formation of CR. Kytril blocks the sensitivity of serotonergic receptors, which manifests itself in a more rapid formation of CR even during brain TMS. Let us justify this statement in more detail.

The procedure for developing CR in rats is as follows. The animal is placed in a test enclosure. A series of voltage pulses is applied to the animal's legs simultaneously with the bell (a series of 70 pulses with an amplitude of 100 mV and duration of 8 ms, sound frequency of 50 Hz with a volume level of 50−60 dB). The animal jumps onto the electrically safe shelf of the cage almost instantly. But when a single call is made after that, without an electric shock, there is no jumping in. Then, the combined effect of the electric shock and the bell is repeated N times. After that, only the bell is rung and the animal jumps up on the shelf, but not every time. The percentage of $\alpha = (\Delta n/n_0) \cdot 100\%$ of the number of Δn check jumps only by sound without electric shock to the total number of no sound jumps is calculated. It turned out that when $N = 3$, on average $\alpha = 30\%$. After that, the number N was increased to the value $N = N_{st}$ until $\alpha = 90\%$ was reached, which was proposed to be considered the completion of the formation of a stable CR of pain avoidance.

1.2.2. Pain avoidance time technique. Division of rabbits into groups Since it is known [15] that brain TMS is capable of activating not only serotonergic but also beta-endorphinergic brain structures, this paper also describes the results of experiments to test the improved interference algorithm of TMS on laboratory rabbits.

Before the experiments, the animals were suspended in a special hammock with slits for the limbs, the stay in which for 3−4 h did not result in immobilization analgesia. Radiant heat of the light beam was used for thermal irritation. In this method, a spot of intense IR light from a halogen lamp was projected onto the nose of an immobilized rabbit at time t_0, which caused painful irritation. After a time interval $\Delta t = t_R - t_0$ (Δt — avoidance time), the rabbit reacted to the painful stimulus by jerking its head away. The time point t_R was fixed as the moment of the rabbit's reaction, and the position of this point on the timeline was dependent on various influences on its body (in particular, on the frequency of TMS)

Four groups of rabbits were used in the experiments, with 12 animals in each group. Animals were assigned to experimental groups by randomization procedure, i.e., randomly. A total of 48 sexually mature male New Zealand rabbits were used in the experiments.

The first control group was not exposed to any stressors. The measurement of measured Δt — the reaction time of avoidance of painful stimulus — was executed in it.

The second control group differed from the first one in that the animals in it had two pairs of electrodes implanted under the scalp: fronto-occipital (sagittal) and behind-the-ear (mastoidal) channels of microcurrent influence. Electrode implantation was performed under inhalation anesthesia (4.5% fluorotane, oxygen 1 l/min, duration of operation 5 min). In the second control group, experiments to measure the rabbit's reaction time to thermal pain were performed one day after the implantation of the electrodes. This was done in order to even out the pain caused by the implantation process. Statistical analysis showed no statistically significant difference in the time between the moment when the heat source was turned on and the pain avoidance reaction in the group with implanted electrodes compared to the group without electrodes.

The third group of animals was used to measure the degree of increase in pain threshold (decrease in pain sensitivity) against the background of TMS. This group was divided into two subgroups, in one of which experiments were performed on a standard three-electrode TMSA instrument, and in the other — on a modified four-electrode interference instrument.

Animals in the fourth group received naloxone — a beta-endorphin receptor sensitivity blocker 30 min before the start of brain TMS [13]. It should be noted that since analgesia is one of the most important results of direct stimulation of the antinociceptive system [14], in the present work analgesia was used as an indicator of beta-endorphinergic systems activation during and after brain TMS.

2. Experimental results

2.1. Rat brain TMS

Fig. 3, *a* shows a graph of the dependence of α on the number of repetitions N of the operation „electric shock + sound supply". This graph is used to determine the value of N_{st} when $\alpha = 90\%$ is reached.

Fig. 3, *b* shows Nst values for animals of the first and second groups, i.e. without electrodes *1* and with implanted electrodes *2*. The figure shows that one day after anesthesia of electrodes under the animal's scalp, there are no statistically significant differences N_{st} for the control group of animals from N_{st} for animals with implanted electrodes (the course of the N_{st} dependence for both groups is almost the same). Possible inflammation of the

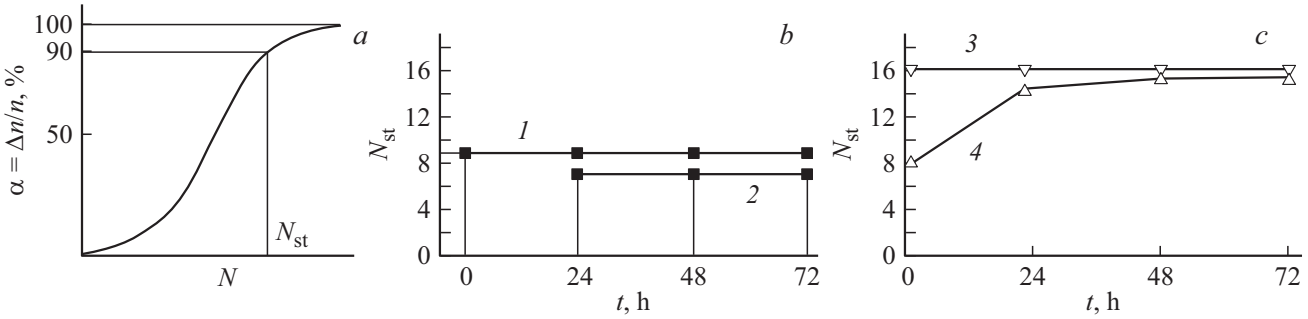

Figure 3. *a* — the dependence of the averaged percentage α of the number of verified realizations of the developed CR of pain avoidance on N — the number of repetitions of simultaneous exposure to the current pulse and the sound signal in the experiments with rats.*b* the number of repetitions N_{st} of simultaneous current pulse and audio signal for two groups of rats: without electrodes — *1* and with implanted electrodes — *2* necessary to develop a stable CR. *c* — comparison of repetition numbers N_{st} to produce sustained CR after short-term TMS (30 min) for two groups of rats: without kytril — *3* and with injected kytril — *4*.

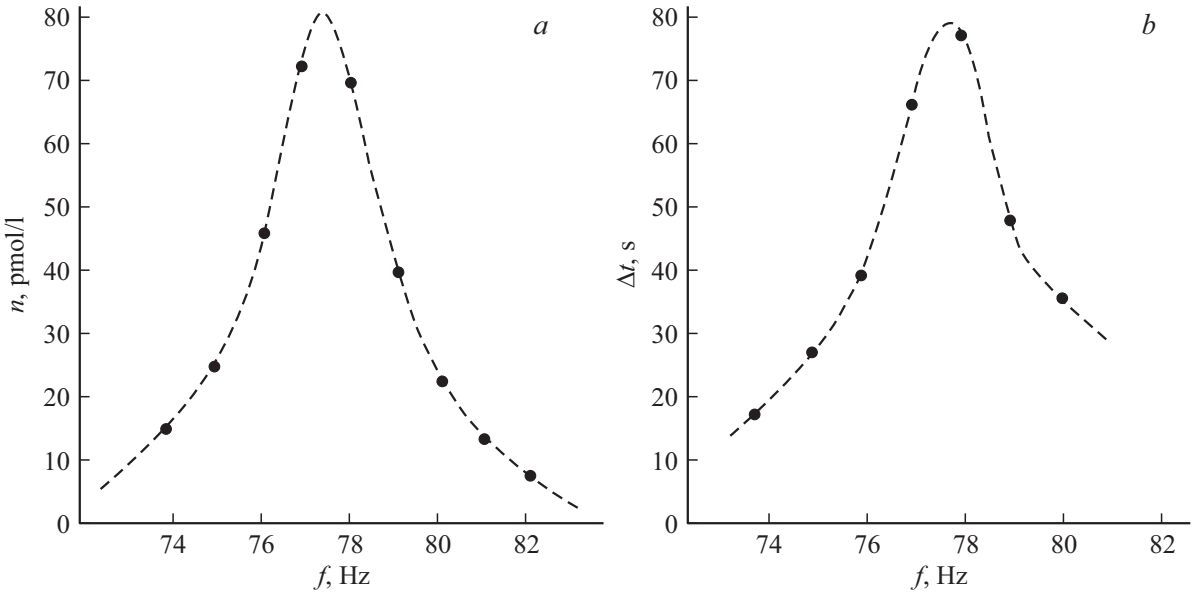

Figure 4. *a* — dependence of the concentration of n beta-endorphins (pmol/l) in the cerebrospinal fluid of rabbits on the frequency of the stimulating current. This frequency dependence has a resonance character, and the resonance is reached at frequencies 77–78 Hz [17].*b* — dependence of the time interval $\Delta t = t_R - t_0$ between the moment t_0 of the onset of painful thermal stimulus and the moment t_R of the impulsive motor reaction of avoidance of painful stimulus by the animal on the frequency of repetition of TMS pulses. The curve peaks at frequencies 77–78 Hz.

surgical areas was also not detected. These preliminary results suggest that it is possible to perform brain TMS in animals with implanted electrodes.

Fig. 3, *c* shows the N_{st} values for the third and fourth groups of animals with implanted electrodes. The animals of the third group underwent TMS for 30 min one day after the implantation of electrodes. This detects a blockage in the process of generating CR. That is, with TMS, the value of N_{st} rose from $N_{st} = 8$ without TMS (Fig. 3, *b 2*) to $N_{st} = 16$ (Fig. 3, *c 3*). The effect of CR suppression lasted for 24 hours.

The animals of the fourth group were injected with kytril once a day after the implantation of electrodes, and 30 min after kytril was injected, TMS was also performed for 30 min. Then we repeatedly determined N_{st} during the first day, one day later, two days later, and finally three days after the kytril injection. The graph *4* in Fig. 3, *c* shows that immediately after the injection of kytril TMS is not able to increase N_{st} from 8 to 16, as it happened in the group without kytril, i.e. kytril blocks the result of TMS of the animal's brain, which consists in the inhibition of the CR production. The experiment also showed a gradual disappearance of the blocking effect of kytril. After one day, the effect of kytril practically disappeared, and TMS statistically significantly increased N_{st} to its numerical value $N_{st} = 16$, as in the group of animals without kytril.

Thus, the above results indicate that the brain TMS interferes with the formation of CR. Kytril partially blocks

the sensitivity of serotonergic receptors, which manifests itself in a more rapid formation of CR even against the background of brain TMS.

Based on the above results, it can be considered proven that the process of blocking the ability to produce CR is due to the effect of increased doses of serotonin produced under the influence of TMS on the serotonergic receptors of the brain, since the introduction of kytril deactivates the overexcitation of serotonergic receptors [15,16]. Experiments have also shown that the serotonin produced in the TPP process only affects the process of CR production, making it difficult, but does not prevent the preservation of CR over time. Specifically: one day after the administration of kytril, the effect of serotonergic receptor blockade disappeared, and the TMS again statistically significantly increased the number of combinations of the conditioned stimulus and unconditioned reinforcement necessary for the formation of a stable reflex reaction.

2.2. Rabbit brain TMS

Changes in beta-endorphin concentration depending on TMS operating frequency were recorded by direct measurements. Fig. 4, *a* shows that the maximum concentration is observed when using a frequency of 78 Hz in the standard TESA [17] device. This result was confirmed by us using a new device with an interference effect algorithm, using the frequencies 68 and 88 Hz to obtain interference. The results of experiments on the reduction of pain sensitivity in rabbits under TMS is the identification of the dependence of the time $\Delta t(f) = t_R - t_0$ between the moment t_0 of pain stimulus onset and the moment t_R of the motor response to pain stimulus avoidance by the rabbit on the operating frequency of modulating voltage, which has a resonance curve with a maximum at 78 Hz (Fig. 4, *b*).

Experiments on the second and third groups of rabbits showed that the most pronounced analgesic effect occurred when using a narrow range of frequency characteristics (68 ± 1) and (88 ± 1) Hz (the value of the half-sum of frequencies is 78 Hz). Deviation in any direction from the declared parameters by more than 2 Hz statistically significantly reduced the analgesic effect.

The data shown in Fig. 5, *a* correspond to the results obtained with the rabbit brain TMS by two devices with the interference algorithm: the device operating at frequencies 88 and 68 Hz, and the device operating at frequencies 44 and 34 Hz. These results reflect the change over time in the relative value of $\delta(t)/\Delta t_{st}$ (%), where $\delta(t) =$ $Deltat - \Delta t_{st}$ — the difference between the time Δt of the motor response to pain stimulus avoidance by the rabbit under TMS and the time Δt_{st} of the response under normal conditions without TMS. You can see that in the first minute after the start of the TMS, when there is still virtually no stimulation, $\delta(t)/\Delta t_{st} = 0$. After 30 min of brain TMS at 88 and 68Hz, the value of $\delta(t)/\Delta t_{st}$ increases to 65% compared to the normal reaction time Δt_{st} without TMS (curve *1* in Fig. 5, *a*/). At that moment, the TMS is turned off and the change of $\delta(t)/\Delta t_{st}$ in time is monitored. The curve *1* shows that the efficiency of brain TMS gradually decreases: after 5 h $\delta(t)/\Delta t_{st}$ decreases from 65 to 30%. The curve *2* describes similar results of the TMS for the device operating at frequencies 44 and 34 Hz with the only difference being that the efficiency of this device is about 2 times lower.

In the fourth group of rabbits (Fig. 5, *b, c*), the effect of naloxone — the brain beta-endorphin receptor blocker — was studied. Experiments in the fourth group of rabbits were performed based on the assumption that if after naloxone administration TMS would not lead to an increase in avoidance time, i.e. would not increase the pain threshold, then the mechanism of the analgesic effect of TMS is based on the activation under the influence of TMS of endorphinergic brain structures. This assumption is based on the fact that, according to the literature [16], naloxone is an antagonist of beta-endorphin receptor sensitivity in the brain, and therefore it can block the sensitivity of these receptors and the development of the analgesic effect that occurs with TMS due to increased beta-endorphin generation in brain structures.

Curve *1* in Fig. 5, *b*, similar to curve *1* in Fig. 5, *a*, demonstrates the efficiency of brain TMS (the $\delta(t)/\Delta t_{st}$ value is several dozen percent) and is shown here for comparison with curve *2*. Curve *2* in Fig. 5, *b* corresponds to the case of injecting naloxone into the animal's peritoneum when the TMS is simultaneously activated at 88 and 68 Hz. In the first 10 min there is an increase in motor response latency to a value of $\delta(t)/\Delta t_{st} = 7\%$ from the standard, and then it begins to decline, which is due to the activation of the blocking action of naloxone. By the time of TMS turning off $\delta(t)/\Delta t_{st}$ falls, passing through a minimum of 2%. Thereafter, a slow increase in motor reaction time, reaching 45% of the standard after several hours, is recorded, which is associated with the gradual disappearance of the blocking effect of naloxone.

Fig. 5, *c* shows a comparison of the degree of blocking effect of naloxone in three groups of animals: without TMS — curve *1*, exposed to interference TMS at 88 Hz and 68 Hz — curve *2* and three-electrode TMS without interference at 78 Hz — curve *3*. Curves *2* and *3* in Fig. 5, c are similar to curve *2* in Fig. 5, it b, and they show that the non-interference TMS demonstrates significantly less resistance to the blocking effect of naloxone compared to the interference TMS, even though the average current for it is double that of the interference TMS. The course of curve *1* (in the absence of TMS) shows that after naloxone administration, the delay in the motor response to pain decreases relative to the standard, so that the difference $\delta(t) = \Delta t - \Delta t_{st}$ becomes negative because $\Delta t < \Delta t_{st}$ due to naloxone. This indicates the initial production in the body of a healthy animal of some normal concentration of beta-endorphins, determined by standard homeostasis. After 2 h the lag Δt reaches the minimum, and the value $\delta(t)/\Delta t_{st}$ reaches the minimum negative value — 12%. As the naloxone concentration decreases, the lag time Δt not only returns (after 3 h) to the standard value $\Delta t_{st}(\delta(t) = 0)$ but begins to exceed it, and after 5 h the value $\delta(t)/\Delta t_{st} = 25\%$.

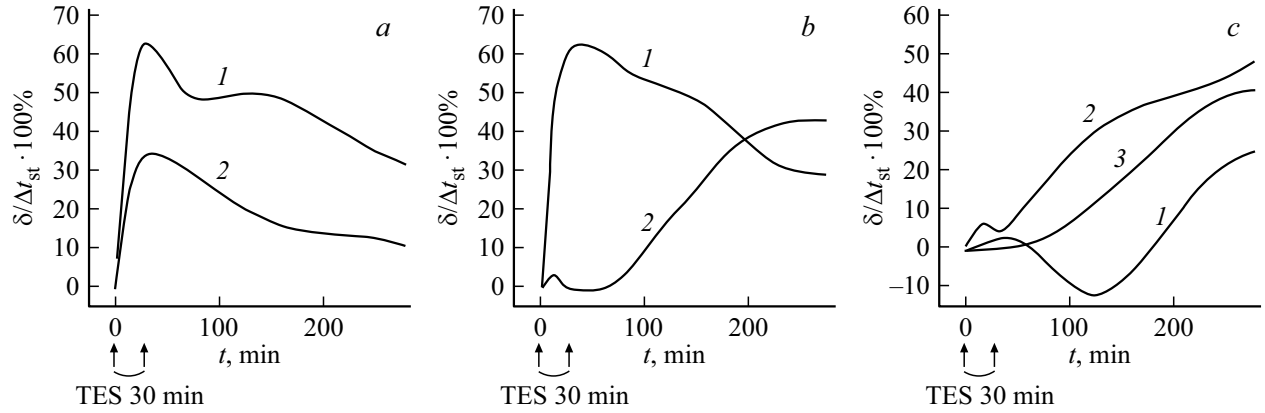

Figure 5. Time dependence for rabbits of the value $\delta(t)/\Delta t_{st}$, where $\delta(t) = \Delta t - \Delta t_{st}$ — the difference between the time Δt of motor avoidance reaction of the rabbit pain stimulus under TMS and the time Δt_{st} of avoidance reaction under normal conditions without TMS. *a* — comparison of the $\delta(t)/\Delta t_{st}$ dependences for four-electrode interference devices with frequencies of 68 and 88 Hz (curve it1) and 34 and 44 Hz (curve *2*). *b* — time dependence of $\delta(t)/\Delta t_{st}$ after TMS for rabbits without naloxone (curve *1*) and with naloxone injected (curve *2*). This result was obtained with a modified interference device equipped with an automatic voltage control system to maintain a constant average current. The system compensates for the increase of electrical resistance of the brain at the initial stage of TMS exposure and its decrease at the subsequent stage. It can be seen that TMS increases the reaction time (pain threshold), which gradually decreases with time after TMS. On the contrary, after the administration of naloxone the reaction time is short (pain threshold is low), despite the presence of TMS. After cessation of TMS, the reaction time gradually increases as the effect of naloxone decreases. *c* – -time dependence of $\delta(t)/\Delta t_{st}$ after TMS for rabbits with naloxone injected (curve *1*) and without naloxone (curve *2*) for TMS modified interference device and curve *3* for TESA device.

Thus, the experiment confirmed that the use of naloxone leads to a statistically significant blocking of the development of the analgesic effect, which is restored after naloxone cessation, i.e., the assumption of the most effective activation of the endorphinergic brain structures when using the interference algorithm of TMS and thus providing the analgesic effect was confirmed.

3. Discussion

The results obtained in the present work can be related to the modern ideas about the mechanisms of CR formation and suppression of the unconditioned reflex.

3.1. CR formation

CR formation in rats involves the creation of a stable connection in the brain between the endings of two nerve circuits: perception of sound and tactile influences (bell and electric shock). In the interaction of these circuits, signal transmission within the brain from one circuit to another can be carried out both through external perception sensors and through internal influence arising from direct point contact of dendrites of one circuit with neurons of the other cycle. The signal transmission between both circuits is performed by neurotransmitter vesicles releasing neurotransmitters (active substances released by the vesicle that activate the formation of the electrical response of dendrite cell receptors) into the synaptic gap (a micrometer gap between the neurotransmitter vesicle — end of axon — and dendrite), i.e., signal transmission from the extrasynaptic receptors of the tactile circuit neurons to the dendrites of the sound circuit neurons.

During the development of the CR, a new temporal connection is formed between the cortical parts of the auditory analyzer in the brain and the central link of the unconditioned reflex [18], i.e. a reflex arc (reflex ring [19]) is formed, which consists of the following elements: sound receptors (sensors), the afferent link (the nerve pathway from the sound receptor to the central nervous system), the central link (the nerve pathway linking the auditory center of the brain to the nerve center of the brain responsible for storing information about unconditioned reflexes), the efferent link (the nerve pathway linking the structure responsible for having unconditioned reflexes to the executive organ), the effector — the executive organ (muscles). The unconditioned reflex, unlike CR, is an evolutionary stable connection between the receptors (sensors) of external action and the effector. When CR is formed, the synaptic apparatus structures are activated, resulting in the emergence of a new channel of information transmission through the temporal reflex arc (ring).

Normally, a standard proportional sensor has an S-shaped characteristic, i.e. it has a sensitivity threshold, a linear section of the transfer characteristic and a sensitivity saturation area. Therefore, we believe that the serotonin generated by TMS puts the synaptic receptors of sound (acting similarly to the sensor) connected to the afferent and further to the central link into a saturation mode, making their functioning difficult. The TMS does not completely suppress, as the experiment shows (curves *3, 4* in Fig. 3, *c*), the production of CR, but only complicates it, leaving almost intra-slit neurotransmitter mechanism of the

central link-$efferentlink$−effector line almost untouched. Of course, some interchange of neurotransmitters between intra-slit and extra-slit receptors does occur [19].

As a serotonin inhibitor, kytril adsorbs to the membrane surface of the free external receptors of the auditory neural circuit [20,21], shifting their potential to the positive side enough to block the interaction of serotonin with these receptors. In this case, the process of interaction of neurotransmitters with receptors is not complicated, because their action potential is even higher. Due to the exchange between the external and intralesional receptors, the action of kytril, which blocks the oversaturation of the receptors with serotonin, spreads over the entire reflex arc (ring) within 30 min. The effect of the serotonin produced during the TMS, which inhibits the production of CR, ceases, which is observed in the experiment. On the other hand, kytril, as a blocker or inhibitor (as opposed to a catalyst), is continuously consumed [21] and eliminated from the body, and after 24 hours it ceases to block the serotonin-inhibiting effect of CR production, which is observed in the experiment as a renewed inhibition of CR production in TMS (Fig. 3, *c*).

3.2. Mechanism of suppression of the unconditioned reflex

The mechanism for suppressing the unconditioned reflex in rabbits is as follows. In the rabbit's body there is already a stable connection between the tactile receptors and the effector. The motor response of avoiding a painful stimulus is characterized by the natural time it takes for the signal to travel that way. Animal brain TMS activates the generation of not only serotonin, but also beta-endorphins. Therefore, rabbit brain TMS, just like serotonin, supersaturates endorphinergic receptors of neurons, inhibiting their work. In experience, this leads to an increase in the rabbit's motor response time to the painful stimulus (Fig. 6,*a*, curve *1*). Injection of naloxone 30 min before the start of TMS affects the external endorphinergic receptors, shifting their potential so much as to block the action of beta-endorphins. After 30 min due to the exchange between the extrasynaptic and synaptic receptors, the effect of naloxone spreads to all neurons of the neural circuit, which ensures the triggering of the unconditioned reflex of avoiding pain stimuli. Naloxone, like kytril, is an inhibitor and is consumed during the chemical reaction of interaction with receptors. Therefore, after a few hours, its influence ceases, which is manifested in the experiment in the form of a renewed increase in the motor reaction time to pain stimuli (decrease in pain sensitivity) (Fig. 5, *a*, curve *2*).

It should be noted that administration of naloxone without subsequent TMS in the rabbit's brain leads to a decrease in the motor reaction time of pain stimulus avoidance (an increase in pain sensitivity). The fact is that the animal's body normally produces some amount of beta-endorphin, providing natural avoidance time. Administration of the inhibitor — naloxone — blocks the slight inhibitory effect of beta-endorphin of normal concentration, resulting in shorter motor reaction times. The experiment of injecting naloxone (curve *3* in Fig. 5, *c*) also confirms that nerve impulse transmission along the neuronal circuit is not impaired by beta-endorphin inhibitor, that is, naloxone does not block the action of neurotransmitters necessary for nerve impulse transmission.

4. Technical part of the proposed method

— Brain TMS gives pronounced results when using pulsed electromagnetic exposure as opposed to using sinusoidal exposure. The point is that an external electromagnetic impulse, when applied to a biological system, triggers a cascade of electrochemical reactions, each of which is characterized by its own characteristic time. Thus, the action potential of a neuron consists of several phases and lasts several ms, and the first phase, the beginning of which is determined by the triggering threshold, has a duration of less than 1 ms [22] (Fig. 6).

Therefore, pulsed electrical influence, on the one hand, triggers the formation of the action potential of the neuron, and, on the other hand, due to the high steepness of the leading edge, creates the possibility of sequential flow for all phases of action potential activation without any interference. Immediately after the completion of the whole reaction cascade, the external electrical influence changes polarity also with a high steepness of the posterior front of the pulse, which increases the intensity and duration of trace hyperpolarization (remembered electrical response of the neuron) [22], giving the neuron an opportunity to extend the hyperpolarization period until the arrival of the next TMS pulse. Therefore, it is clear that a pulse with a duration shorter than 0.1 ms does not cause any effects [23]. The pulse duration in our experiments was 12 ms, which is longer than the activation time of the neuron action potential. At the same time, the sinusoidal voltage,

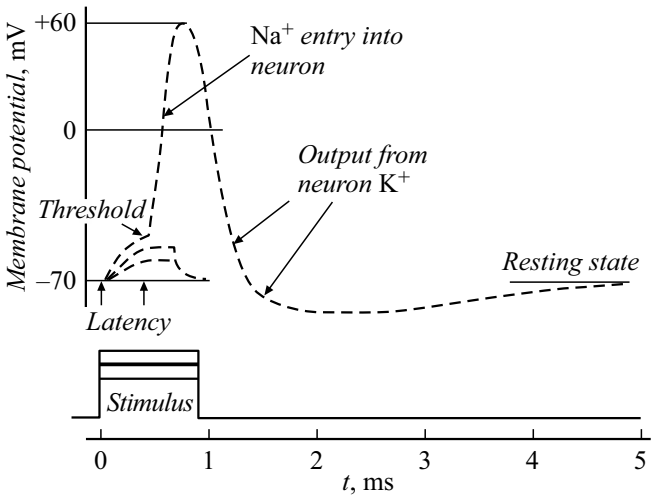

Figure 6. Neuron action potential [22].

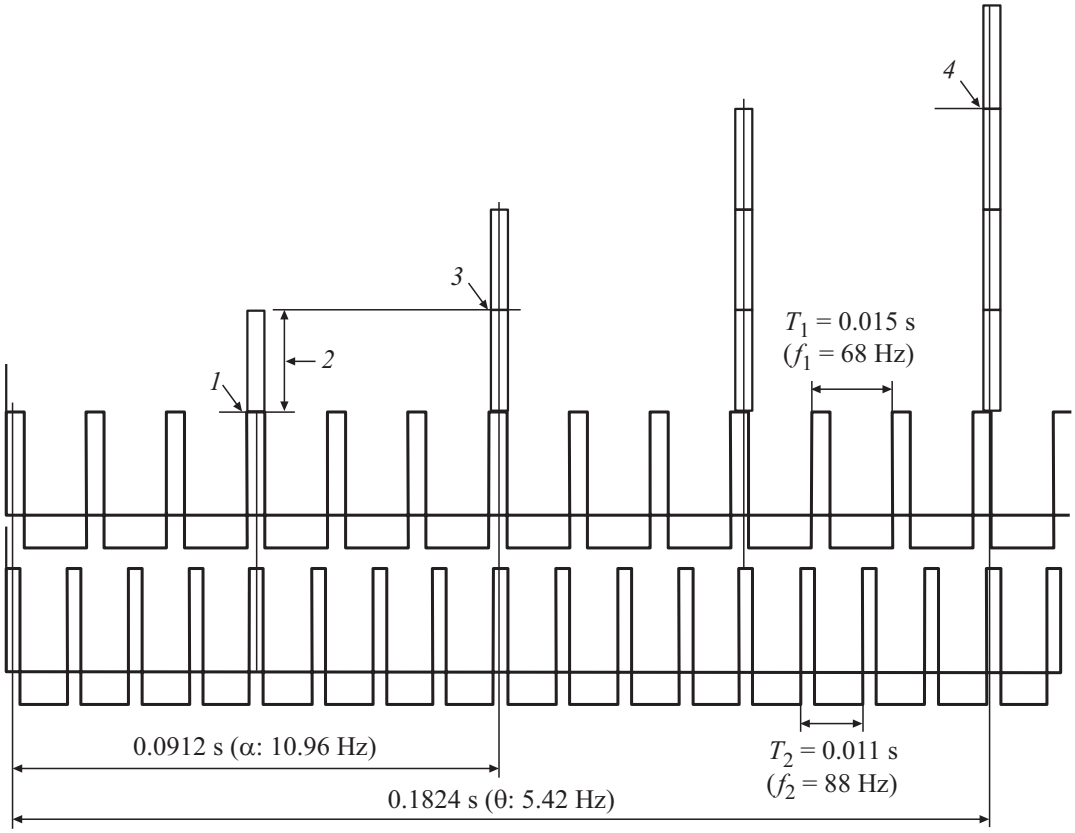

Figure 7. Addition of pulses 68 and 88 Hz

due to the gradual increase of the voltage value, does not provide triggering of electrochemical reactions with a sharp threshold activation character [24]

— The brain TMS is „ resonant“ i.e. it is effective at the modulation frequency (78±2) Hz. The explanation of this fact can be summed up as follows. Since the neuron has the ability to memorize the electrical stimulus (which is exactly established [25]), the arrival of the next impulse must take place before the neuron has completely forgotten its state, i.e. the excitation period must be shorter than the time of „forgetting“. This dictates that a strictly defined pulse repetition rate must be used. In our case this corresponds to a frequency of 78 Hz. Besides, as our Fourier analysis shows, only the sequence of pulses of maximum steepness of fronts and frequency 78 Hz provides a greater amplitude of Fourier harmonics in the region of low frequencies (5−10 Hz), coinciding with the frequencies of α- and θ-rhythms of the brain.

— The brain TMS in the new four-electrode version of the device has twice the efficiency of its interference algorithm compared to the three-electrode version of the TESA [17] device. The reason is that, according to [25], brain TMS at frequencies 5−10 Hz, coinciding with the frequencies of α- and θ-rhythms of the brain, also leads to the effective generation of serotonin and beta-endorphin, doubling the efficiency of TMS, launching an additional cascade of electrochemical reactions due to the subliminal state [26] memorization effect.

— Brain TMS at 78 Hz can activate a neuron action potential at lower frequencies. This is due to the fact that the neuron has a trigger threshold, and if the amplitude of the excitatory pulse is less than this threshold, the launch of electrochemical reactions in the neural circuit does not happen, but the fact of the pulse arrival below the threshold is remembered by the neuron (positions *1, 2* in Fig. 7). When subsequent pulses arrive, their mutual addition triggers the formation of the neuron action potential, but already under the action of low-frequency pulses. This frequency is determined by the coincidence in time of the moments of arrival of pulses of frequencies 88 and 68 Hz. For example, if every seventh pulse of frequency 68 Hz coincides with every ninth pulse of frequency 88 Hz (position *3* in Fig. 7) and triggers a trigger, then the time interval between coinciding pulses corresponds to the frequency 10 Hz, i.e. α-rhythm of the brain. If other coincidences give a total amplitude below the trigger threshold, then the neuron functions as a memory frequency divider. If the 13th and 17th pulses coincide (position *4* in Fig. 7), it corresponds to the θ-rhythm of the brain.

— The ambipolar form of TMS pulses suppresses the convulsive activity of muscles. The occurrence of seizure activity is explained as follows. The cell membrane of the neuron comes in contact with a large number of Na^+, K^+ and Cl^- ions present in the cerebrospinal fluid. Under the influence of external electric influence in the form of low-frequency alternating current of symmetric form (used in

the early modifications of TMS equipment), which is of resonance nature, the diffusion of Na^+ ions through sodium channels inside the neuron increases, resulting in a decrease of the negative internal charge, to which the neuron reacts by avalanche depolarization and an act of electrical impulse release into the neuronal network. After that the equilibrium potential is restored (repolarization) [16]. If an excessive amount of sodium ions enters the cell during symmetric pulse electric stimulation and the threshold level of negative internal charge compensation is reached with its transition to the zone of positive values, a „salvo" depolarization of the neuron — the neuron impulse response to the critical value of the potential difference between the membrane inner and outer surfaces [22] occurs. There will be a conversion of normally functioning neurons into pacemakers — generating uncontrolled nerve impulses [26]. Neurons will spontaneously begin to generate impulses, sending a series of „unplanned" nerve impulses to the neural network. Reaching the nerve pathways to the control centers of the — animal's muscle activity, these impulses will provoke convulsive pathological activity.

Seizure activity is suppressed by a sequence of asymmetrical ambipolar pulses, which causes, as mentioned above, a constant component of current to flow between the electrodes. The fact is that when direct current, along with alternating current, is passed through the brain, there is a directional movement of sodium and chlorine ions, which are part of the cerebral basal cisterns' liquor. The direct current organizes two counterflows of ions of opposite signs, and creates a preferential entrainment of the entire liquid mass in favor of Cl^- ions due to the larger midsection of the hydrate shells of Cl^- ions and the quadratic dependence of the stokes force on the flow rate. The cross section of the hydrate shells of negative Cl^- ions is much larger than [27] the cross section of positive Na^+ ions, since they are formed by hydrogen bonds of large negative Cl^- ions with water molecules. There is a unidirectional fluid flow due to a sharp difference in the midsection of the ionic conglomerates. At the same time, the fluid flow takes Na^+ ions out of the mouths of the sodium channels, according to Bernoulli's equation, in a process similar to that of a waterjet pump. This effect makes it difficult to saturate the internal cavities of neurons with positive Na^+ charges without bringing the membrane potential to a threshold value (+60 mV), blocking spontaneous depolarization and thus preventing the generation of convulsive activity impulses.

— Finally, the use of the interference TMS algorithm suppresses the „effect of habituation". This is due to the fact that the complex shape of the hodograph of the vectors of total currents and electromagnetic fields (Fig. 2, *a*) leads to a continuous change of groups of activated neurons, preventing the effect of neurons getting used to the TMS process.

Conclusion

We have found that the activation of serotonin and endorphin production in brain structures is caused not only by the activation of serotoninergic and endorphinergic brain structures by TMS at 78 Hz, but is also connected with the resonance amplification of α- and θ-waves of the brain, which can cause a neuromodulatory effect. This turns out to be possible as a result of the occurrence of Fourier harmonics with frequencies of 10 and 5 Hz when using the interference pulse algorithm of TMS. The increase in amplitude of α-waves caused by resonance activation causes an increase in the concentration of beta-endorphins, resulting in a decrease in the stressful effects on the body, particularly those associated with pain.

The maximum degree of CR suppression in rats and the maximum analgesic effect, which was assessed in rabbits by the reaction of thermal avoidance (pain threshold) were noted when using TMS frequency 78 Hz. In our proposed transcranial electromagnetic stimulator, such frequency was formed as a result of using high frequency currents modulated by meanders with frequencies 88 and 68 Hz. We emphasize that the modified brain stimulator requires half the current (0.1 mA versus 0.2 mA, Fig. 5, *c*) to achieve results equivalent to those of the previous design.

Crucially, both the suppression of the ability of rats to produce CR and the increase in pain threshold in rabbits are caused by interference beats occurring in the proposed TMS device in the zone of intersection of mutually perpendicular current tubes. The absence in animals of the seizures previously experienced with TMS is due to the flow of direct current through the animal' brain resulting from the partial rectification of the alternating high frequency component (500 kHz) due to the valve properties of the „electrode−brain−electrode" system.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for animal care and management were observed.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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