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Mechanisms of electromagnetic influences and effects on membrane systems in neurons and cardiomyocytes

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Aim

The aim of our studies was to discover responses of the membrane systems in neurons and cardiomyocytes as well as mechanisms of influences and effects produced by the broadband-spectrum stochastic electromagnetic radiation (BBSS EMR) on them according to data on membrane potential (MP) levels and action potential (AP) parameters obtained by us.

Materials and methods

Neurons from the isolated central neural system (CNS) of the snail Helix pomatia were selected to serve as a model for our experiments. We applied an electrophysiological technique implying recording of intracellular potentials of a neuron. The presented research work is of fundamental nature and reveals some intimate mechanisms of actions made by electromagnetic radiation (EMR) on the cytoplasm membrane of a neuron and its channels that is applicable to cardiomyocytes. An absolutely exclusive distinctive feature of our work is that the specific BBSS EMR used in our experiments has unique characteristics and offers broadband stochastic radiated frequencies in the range from 10^n Hz to 10^m Hz at an integral radiation power of only 0,1 μW. Another distinguishing feature of the applied EMR parameters is that the stochastically organized EMR is supplied under such action rhythms, which are close to the natural alpha-, theta- and delta-rhythms of an EEG as well to the natural background base resonance frequency of the Earth, which has been physically measured to be 7,83 Hz, that is evolutionary significant for biological systems.

Results and discussion

We are pioneers in the world science who succeeded in obtaining objective evidence for the effects made by the broadband-spectrum stochastic electromagnetic radiation (BBSS EMR) on intracellularly recorded electrophysiological properties of a CNS neuron. The used BBSS EMR was demonstrated to have the effects on the amplitude, the duration of an action potential and a membrane potential, following the pattern of a regulatory response to weak stimulation, similar to the reaction of training. The effects produced by BBSS EMR on the CNS neurons in the snail Helix pomatia are applicable to cardiomyocytes, since the electrophysiological and ionic mechanisms of firing an action potential and creating the required level of a membrane potential in these cells in the organism are identical; however they differ in their time parameters.

Conclusions

The regulation of the functional state of a neuron in the neuron network with the use of a non-invasive remote method of a control action has been objectively shown herein. According to our philosophy, a neuron is considered as an analogue of the formation of a transmembrane potential by a cardiomyocyte and at the same time as the basic model of the neural cellular auto-regulation of the phase-structured performance of the cardiovascular system. Adhering to this interpretation, a fine tuning instrument to control the functional state of a neuron, CNS, a cardiomyocyte or other cell systems in the organism has been discovered. It offers widest possibilities of a targeted fine-tuning regulation of the control systems in a human organism.

Keywords

Electromagnetic radiation, Cardiomyocyte, Neuron, Action potential, Membrane potential, Ion channels, Endogenous pacemaker activity

Imprint

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Introduction

At present, a multitude of methods and techniques are known which are capable of providing a targeted control over the functional state and individual sub-systems in a human. They can include both contact and contact-free methods to exert an influence on the organism as follows: manual, chemical and verbal methods as well as actions produced by fields of different physical nature. Among the fields, the electromagnetic fields, which belong to the family of the contact-free control methods, are the special case. The range of their actual values stretches from extremely low frequencies (ELF) of $3 \cdot 10^{-3}$ Hz, which are referred to as decamegameter waves with a wave length from 100 Mm in accordance with their accepted international classification, up to and including gamma-rays, having a frequency of $3 \cdot 10^{23}$ Hz with a wave length of $3 \cdot 10^{-15}$ m, which are comparable to the size of an atom and which possess the highest energy within the energy range from 12,4 feV to 5000 keV. A large portion of the said range is used in medicine for the therapy and diagnostics purposes. As a rule, medical instrumentation designed for the therapy and diagnostics practice comprises generators or detectors operating within a narrow band of electromagnetic waves. Electromagnetic radiation (EMR) of different frequencies, even within a narrow band, is perceived or sensed by a human organism in various ways, and for this purpose the organism is "equipped" with the respective EMR sensors, namely, the receptors. The thermoreceptors residing in the skin are sensitive to energy in the infrared region of the EMR spectrum, and the light receptors in the eyes operate as sensors receiving the EMR visible region. The above spectrum regions are found very close to each other.

But a large portion of the EMR range cannot objectively be perceived by a human, despite the fact that the unperceivable EMR regions sometimes may produce essential effects on the organism, up to fatal outcomes. Effects of this sort are exerted by some EMR regions like gamma radiation, microwave and UHF electromagnetic radiation, when power flux densities reach extremely high values. In contrast thereto, the absence of EMR, as it is the case in experiments modeling the disappearance of the Earth's natural magnetic field, is another matter. The peculiarities of the influence exerted by magnetic field on the human organism show fundamental differences from any other action provided by chemical exposure, heat, radiological impact or electricity. For example, musculature and circulation circuit in the human body are capable of partly shunting hazardous currents, or, to take one more example: ionizing radiation can be absorbed to some extent by the body surface, while magnetic field can freely penetrate the entire living organism. The Earth's magnetic field patterns act in the ultra-low frequency range, and therefore they meet the basic physiological rhythms in a human organism: the heart and brain rhythmic patterns as well as the respiration rhythm. As opposed to other physical actions and factors, a human individual is not able to sense magnetic fields, but however the human organism always responds to a magnetic field exposure by initiating primarily certain functional changes in the performance of the nervous and cardiovascular system as well as the brain activity. And it is Dr. Kyoichi Nakagawa's opinion that magnetic field deficiency syndrome occurs due to a decrease in the Earth's magnetic field so that it is responsible for appearance of many abnormalities like sleep disorders, appetite abnormalities, decreased immunity performance, increased susceptibility to diseases, including joint- and skin diseases, urogenital disorders, nervousness and generalized weakness. The concept offered by K. Nakagawa was given the name "magnetic field deficiency syndrome". Actually, a magnetic field deficiency can be created by artificial means: an exemplary illustration is a space flight; another example of the said conditions is a submarine, where magnetic field shielding effects are used. In individuals, who have experienced a long-term magnetic field deficiency, observed are considerable deviations of the functional parameters from their normal levels, metabolic deceleration, a decrease in the total number of leukocytes; prenosological and premorbid states are found in them, too. Every human organism has its own integrative electromagnetic field surrounding the body, which interacts with external electromagnetic fields in the environment. The modern knowledge and expertise in the area of electromagnetic fields and radiation allow stating that one of the most advanced fundamental line in research and development of medical instrumentation, theoretical & practical biophysics and bioinformatics is an investigation of the life performance and the associated information processes in a human organism, so that, following this way, research on endogenous electromagnetic fields (EMF) of biological entities and their interplays should be considered as a highest priority task [1].

Recent scientific explorations have reinforced the conceptual idea that information exchange among separate units within the nervous system, i.e. among the neurons, in an organism is provided not only by chemical and electrical signals, but also via electromagnetic fields generated by the neurons, their dendrites and axons, through which the excitation current flows.

When designing most advanced medical equipment for therapy & diagnostics, it is of great importance to properly assess the patient's own integrative electromagnetic field along with generators and conductors creating EMF, responses and properties of the systems responsible for the regulation in the organism: principally it should be applied to the constituents of the nervous system, the neurons and their networks as well as some other cell structures, for instance, cardiomyocytes, which reside in the heart; and such assessment should be completed by an estimation of their interactions with the physiotherapeutic EMR sources applied.

As to the two above cell populations, i.e. the neurons and the cardiomyocytes, which represent one of the most important regulatory operation systems in the organism, they share a common property: it is their pacemaking activity. Pacemaker (origin: pace as a rate of activity, tempo + maker) is a natural heart beat controller governing the proper heart rhythm. It might be a single cell or a group of the cells, which are capable of generating rhythmic excitation pulses propagating through the neighboring cells. It should be noticed that the pacemaking activity of the neurons is found occasionally, whereas pacemaking of the cardiomyocytes is their permanent mode of operation designed to provide the normal performance of the organism throughout its entire biological life span. Variations in frequencies and shapes of potentials in the pacemaking activity are interconnected with the respective changes in the functional state of the organism in general. Abnormalities in the cardiomyocyte pacemaking rhythmicity result in pathological outcomes in the organism. Migration of the supraventricular pacemaker, as an example, takes place, when the natural cardiac pacemaker locus shifts in a gradual manner between the sinoatrial node and the atrioventricular node. The shifting of the pacemaker can be detected on an ECG by tracing the same ECG lead record with assessing changes in the P-wave configurations, amplitudes and polarity as well as alterations in the P-P and P-Q time intervals.

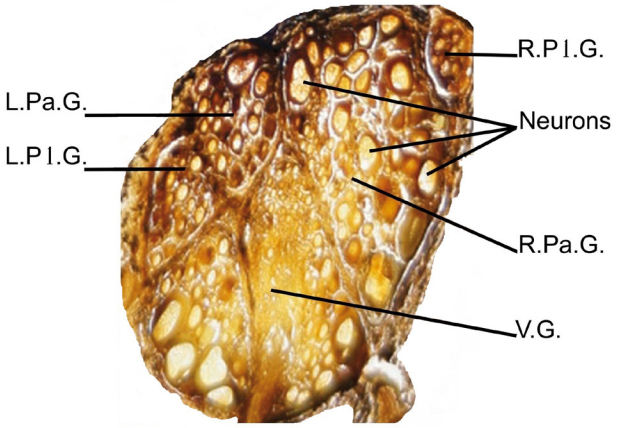


Fig. 1. The subesophageal ganglion complex of CNS in the snail Helix pomatia. LPlG, RPlG, LPaG, RPaG and VG are the ganglia of the subesophageal ganglion complex in CNS. The surface neurons of the dorsal side of the ganglion complex are clearly seen herein. Prepared histological specimen. Golgi's silver staining technique improved by Ramón y Cajal. Photo made by V.I. Orlov.

Based on the above, actually, development of a contact-free physiotherapy instrumentation designed for generation of superweak EMR in a wide band of frequencies, which are objectively not sensed by a human individual, but which are adequate to the endogenous resonance frequencies produced by the cells, organs and systems in the human organism and which are capable of normalizing their performance, appear to have considerable promise [2].

Materials and methods

Bioelectrical activity of the neurons in mollusks and warm-blooded animals is mainly provided by the activity of sodium, calcium and potassium ion channels, which are built and operate according to the same general principles [3–8]. It enable us to use the neurons of mollusks as eligible models [9–18], in parallel with the neurons of warm-blooded animals, for experimental research on the performance of the ion channels and the mechanisms of influences on them made by various physical factors, chemical substances and pharmaceutical drugs either of already known nature or being designed.

Our studies were carried out on the large identified (100–200 μm) neurons of the parietal ganglia (LPaG and RPaG) (Fig. 1) and the adjacent non-identified neurons in the subesophageal ganglion complex of the isolated CNS in the mollusk Helix pomatia. The neurons in the ganglia of this mollusk kind have a pigmented edge in the region of the axon hollock and are clear-

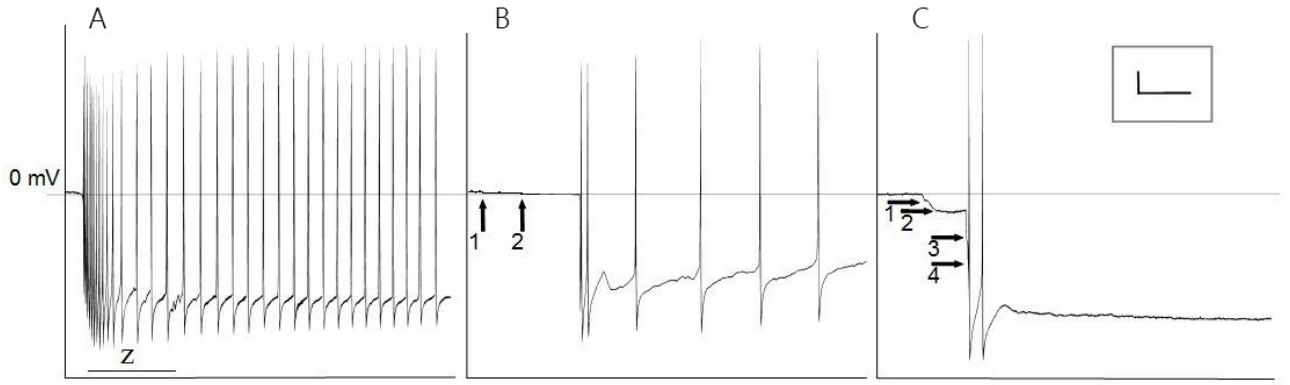
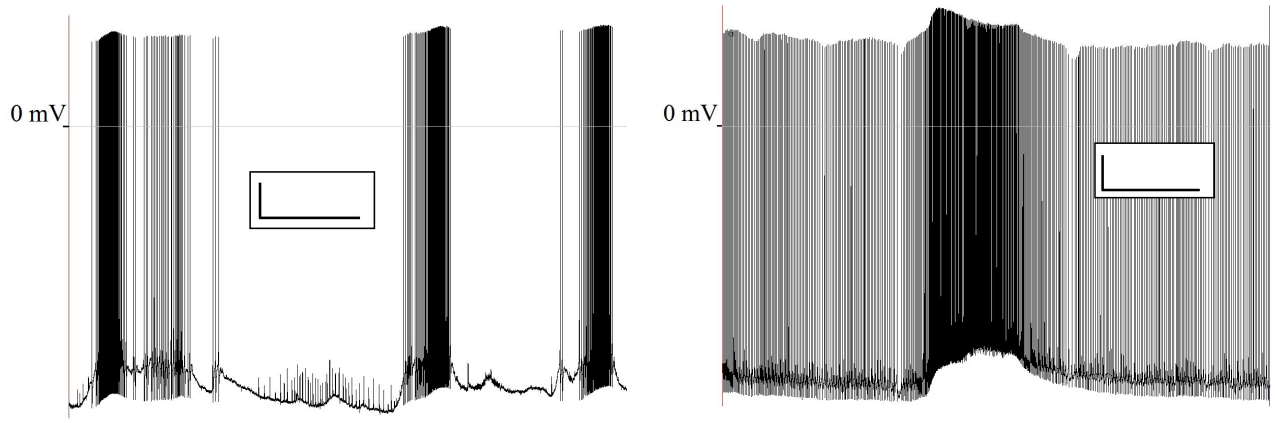


Fig. 2. Legend: A. Disintegration of the neuron membrane due to insertion of the conventional ME: strong response to the ME invasion into the neuron as frequent multiple action potentials (AP) and prolonged endogenous pacemaking generation of them. Z is the time of the ME invasion into the neuron and "the capture" of the electrode by the membrane. B. Disintegration of the neuron membrane with the use of a pulse electromagnetic micromanipulator system designed by Ivlev S.A. at al. [24]. C. Disintegration of the neuron membrane due to insertion of a superfine microelectrode applied by us in our experimental studies. The picture exhibits two APs only, which are produced due to the SME invasion into the neuron; subsequently the resting functional state of the silent neuron is found, and the stable level of its membrane resting potential (MRP) is noted. Arrows in the picture identify individual steps of advance of the microelectrode tip being inserted into the membrane. Calibration for A, B and C: 10 mV/1 s

ly visible under a binocular magnifying glass. Prior to the studies, we had succeeded in developing our own original technique of preparation of the required Helix pomatia test samples [19]. For the studies described herein we used only a snail organism section comprising the CNS. From the mollusk body we isolated the circumaesophageal ganglion complex and fixed the latter onto a silicon slide with brackets inside a recording non-magnetic chamber designed by us for the experimental purposes. The recording chamber had a volume of 0,5 cm^3 and contained physiological saline composed as follows (in mmol/L): NaCl – 50; KCl – 2; $CaCl_2$ – 4; $MgCl_2$ – 1,5; Tris-OH – 10; pH – 7,5. From the dorsal side of the subesophageal ganglion complex we removed the thick neural sheath made of connective tissue and the arachnoid sheath in the projection of the neurons under studies. The experimental equipment system designed and manufactured by us, the scope of which incorporated the recording chamber and its auxiliaries, has made it possible to carry out chronic experiments on freshly prepared single neurons taken from the CNS of the snail Helix pomatia with continuous long-term recording of electrophysiological potentials of neurons up to three 24-hour days.

The physiological saline supplying system and the system of discharge of waste fluid were designed for creating and maintaining of a stream of the saline flowing through the recording chamber at a constant flow rate. The material used for the chamber was capable of withstanding steam-and-pressure sterilization, and the chamber design was intended for multiple uses. To record electrophysiological characteristics, utilized were special superfine microelectrodes (SME) filled with 2,5 moles of KCl, with a tip diameter under 0,05 μm, having a resistance up to 5 GΩ. The bio-potentials were recorded and measured by the unipolar method with the use of an indifferent electrode being removed from the neuron to a distance $r_{ie} >> ø_n$, where: r_{ie} is the distance between the indifferent electrode and the neuron, and $ø_n$ is a diameter of the neuron soma. This determines the position of the zero line on a recording.

In our experimental studies, biological potentials were recorded with a 12 digit analog-to-digital converter (ADC) at a time quantization of 40 milliseconds. A special preamplifier developed by us for input currents to 3 fA, an input resistance of 100 GΩ and a bandwidth from 0 to 10 kHz, provided the proper operation with our SME and the adequacy in recording of amplitude-frequency responses of the intracellular potentials measured. Following that way, we investigated the dynamics of changes in a resting potential (RP) and pulse activity (PA) as well as parameters of an action potential (AP). The original software developed by the Neurocybernetics Research Institution at the Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don, Russia, allowed us obtaining the proper visualization of the intracellular potentials captured from a single neuron, their recording and measuring accompanied by production



A) B)

Fig. 3. Legend: A. Natural background activity of the LPaG2 neuron after the 43 hour-residence of the superfine ME in the neuron. B. Natural background activity of the LPaGx neuron upon expiration of 52 hours from the beginning of intracellular potential recording. Calibration 10 mV/60 s

of slides. The BBSS EMR generator employed in the experiments had the following parameters: the frequency range from 10^n Hz to 10^m GHz; an integral radiation power of 0,1 μW; the regimes of rhythmic modulation of the radiated frequency spectrum were as given below: regime 1: m_1 Hz and m_2 Hz; and regime 2: m_3 Hz and m_4 Hz, respectively.

Results and discussion

The chronic experiments offered stabilization of the functional state of the neurons. Our research work was carried out based on "the Rule of Initial State" formulated by S.M. Leites, the former Soviet pathophysiologist and endocrinologist. Delivery of stimuli to the neurons was provided upon expiration of 4–5 hours after completion of the sample preparation. According to our long-term observations, a stable background state in neurons is reached upon expiration of 3–4 hours from the time of sample preparation. Sometimes the stabilization process takes a shorter time because it depends on the initial functional state of the biological object and specificity of the technique applied to prepare the CNS samples as well as procedures of insertion of a microelectrode into the neuron. When a tip of the conventional microelectrode (ME) is contacting the neuron surface, and when the ME is being moved towards the center of the neuron, the neuron membrane is being bent and stretched. At that time, when, upon application of a pressure produced by the ME tip, the membrane surface is being disintegrated, an opening is appearing in the membrane letting the ME tip be inserted into the neuron, so that drastic vibration of the neuron membrane takes place, and, as a consequence, mechanical activation of the ion channels in the neuron membrane occurs, and pacemaking action potentials are fired (see Figure 2 herein).

It is explained by the fact that, when a microelectrode is penetrating the membrane, between them a gap, a large pore opening, is appearing, through which an ion exchange between the intercelluar cytoplasm fluid and the external solution washing the neuron takes place. By this means, the mechanism of a long-term operation of the mechanically sensitive ion channels is initiated. The pore opening appeared in such a manner around the microelectrode is quickly shrinking, and finally the contacting surfaces are being sealed by the membrane and securely kept thereby. In Figure 2, fragment A, this process is designated by line Z. In some cases, under fluctuations in the functional state (FS) of the neuron during many hours' intracellular recording, or upon exposure to some chemical substances, the membrane may release the captured conventional-type microelectrode, so that the latter may even leave the neuron body. In our test studies, we used our original SME with a high electrical resistance. Researchers, who deal with recording of intracellular activity, know that this sort of technique is always associated with overcoming of a number of drastic engineering challenges. In case, when the diameter of a channel conductor is reduced to 0,05 μm, we face a sharp increase in the resistance of the microelectrode. As a consequence, sensitivity of the high-ohmic input of the amplifier to external electromagnetic noise, and first and foremost, to network interference, is raised. The input members (an object under studies, the microelectrode and the

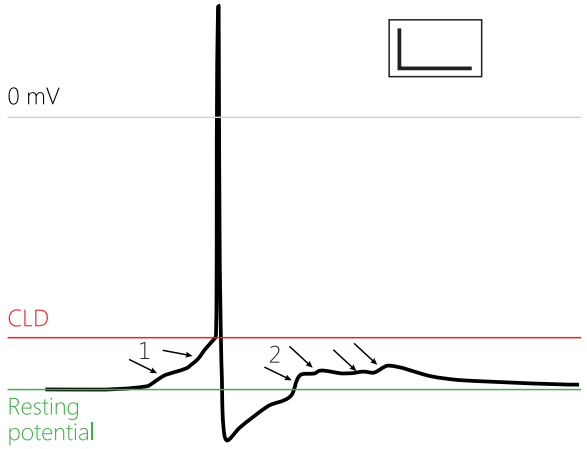


Fig. 4. The shape and the amplitude of the pacemaker potentials (arrows 1) and AP of neuron LPaG1 caused by them as well as the following excitatory postsynaptic potentials (EPSP) (arrows 2) in 35 minutes upon the 5-minute BBSS EMR exposure have shown no deviations from the norm. Calibration 10 mV/100 ms. CLD – critical level of depolarization.

conductors connecting an object with the amplifier input) become extremely sensitive both to static charges and their changes. The input in the circuitry responds even to some slight fluctuations in humidity of the ambient air in the testing room, and it might be illustrated by the case, for example, when other two or three colleagues enter the testing room with one operating researcher already present therein: their overall breathing changes the actual ambient air humidity. In this connection, the question arises of whether it is generally reasonable to apply the SME technique because of a plethora of complicated tasks to be solved. We say definitely: yes: the application of the technique is quite justified. Our superfine microelectrode (SME) enters freely, without any hindrance or friction, the neuron cytoplasm, as if SME were abundantly lubricated (see Figure 2, fragment B). The process of inserting SME into the neuron does not produce any violent reaction by the neuron: in this case the inserted microelectrode is "not detected" or "not seen" by the neuron. There is another noticeable matter: the very small diameter of the tip makes possible to exclude electrolyte infiltrations from the SME body into the internal fluid of the neuron, so that the natural functional state of the neuron is retained unchanged for several days. To illustrate this fact, we present Figure 3 herein, which gives fragment A to exhibit a recording of the potentials of the LPaG2 neuron upon the 43-hour residence of the SME in the neuron soma. We can trace the natural intracellular activity pattern: excitatory postsynaptic potentials (EPSP), endogenous slow-wave potentials and endogenous pacemaker action potentials.

The background state of the LPaGx neuron in the prepared isolated CNS specimen, upon expiration of more than 2 days (52 hours) after preparation, as illustrated in Figure 3, fragment B, that represents the norm, is characterized by a specific language of communication of the neuron with the other neuron units within the network: it comprises slow and fast endogenous waves of de- and hyperpolarization of the cytoplasm membrane of the neuron, the stability of the AP parameters, the constant duration and some minor amplitude fluctuations, a wide range of frequencies in the generation of APs initiated by mixed endogenous pacemaker activity and EPSP, and some MP variations within the physiological limits. In the first set of the experiments we considered whether pathology in the shape of an action potential is available or not as well as the dynamics of the synaptic potentials arrived at the neuron under studies in a distant post-pulse period, at an interval within 60 minutes upon the EMR exposure. An action on the neurons by BBSS EMR was produced in the standard first regime of the generator operation. The time of delivery of the stimulus was 5 minutes. The mode of the rhythmic modulation of the radiated flux covered m_1 Hz and m_2 Hz. The spacing between the EMR source and the neuron was 50 mm. The axis of the EMR source was perpendicular to the surface of the ganglia and the neuron. Utilized was an integral radiation power of 0,1 μW. We traced the post-firing activity of the neuron on the BBSS EMR action within an interval of 1 hour, and there were no significant variations in the bio-potentials of the examined neuron detected. In Figure 4 shown is a recording segment to illustrate the intracellular activity of the Helix pomatia CNS LPaG1 neuron in 35 minutes after the BBSS EMR exposure. The state of the membrane potential, the endogenous pacemaker potentials, the amplitude, the shapes and durations of AP as well as the excitatory postsynaptic potentials (EPSP), arriving at the neuron, enable us to conclude that the functional state of the neuron has not experienced any pathological alterations. The functional state of the neuron has been found to be in correspondence with the respective physiological norm, as it has been the case before the BBSS EMR exposure.

In the second set of the experiments we estimated the effects made by electromagnetic radiation produced by our generator in another regime of operation, which

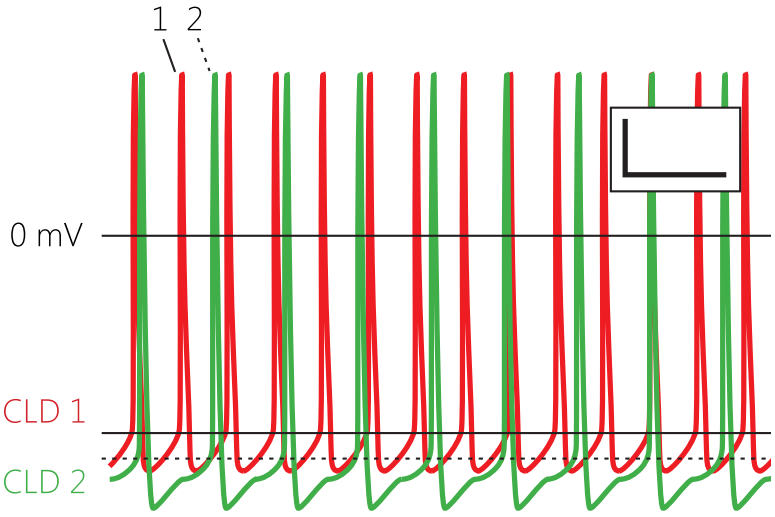


Fig. 5. Legend: Red curve (arrow 1): the activity pattern of the neuron RPaGx in its background state before EMR exposure. Parameters of AP before BBSS EMR delivery: T_{ap} – 8 ms, level of MP – minus 67±2 mV. Green curve (arrow 2): the activity pattern of the same neuron after BBSS EMR exposure. Parameters of AP after BBSS EMR exposure: T_{ap} – 6 ms, level of MP – minus 75±3 mV. F_2 = 9 Hz. Tap is a pulse duration of an action potential. F_1 and F_2 are frequencies of AP. CLD1 and CLD2 – critical level of depolarization of the neuron membrane before and after the EMR exposure, respectively. Calibration 10 mV/250 ms.

differed from the first one in the modulation frequency of the radiated electromagnetic flux, utilizing the following frequencies: m_3 Hz and m_4 Hz. Under the conditions, the neuron operating in the pacemaking mode was exposed to the above specific EMR [20]. The dynamics of the background-active non-identified neuron RPaG in the snail Helix pomatia CNS is presented in Figure 5, curve 1, to portray the initial background functional state of the neuron in question.

Upon completion of the electromagnetic exposure, the firing rate decreased from 14 to 9 pulses as indicated in Figure 5, curve 2, illustrating the neuron FS after the EMR exposure, and the level of the membrane potential was getting more negative: from minus 67±2 mV to minus 75±3 mV that indicated that there was hyperpolarization of the neuron membrane available. Upon the BBSS EMR action on the neuron, there was a change of the critical level of the membrane depolarization denoted by Threshold 2 referred to Threshold 1 of the initial state. Figure 5 herein depicts some fragments of the recordings of the neuron activity before the EMR exposure and after it. The event of the BBSS EMR delivery starting is intentionally ignored herein due to large artifacts interfering with a signal to be recorded.

The third set of the experiments was designed to deliver the BBSS EMR stimulation against the background of the pathological activity pattern of the non-identified neuron LPaGx. It was decided to test the influence made by BBSS EMR on the pathological activity of the neuron, which usually appeared upon an action of some chemical substances resulting in over-excitation of a cell. An example of production of a pathological activity pattern occurred in our experiment is given in Figure 6, fragment 1 herein, where a degradation of the neuron performance upon action of a chemical factor is demonstrated. We can find therein a steady-state pathological condition represented by fluctuating-amplitude spikes reaching values from 8 mV to 19 mV.

Some measures taken by us to eliminate the artifacts induced by BBSS EMR allowed us minimizing the signal distortions and improving in the quality of recordings of the potentials during the EMR exposure sessions. That has made possible to trace the bio-potentials immediately during the course of the EMR action. Figure 6, fragment 2, exhibits the intracellular potentials of a neuron recorded during the BBSS EMR exposure session. The given set of the experiments was dedicated to the application of the BBSS EMR stimulating signal at a signal modulation frequency of m_1 Hz. An exposure time from switching-on of the BBSS EMR generator was limited to 30 seconds. The other parameters remained unchanged. Upon completion of the electromagnetic exposure, the amplitude of some large spikes reached 21 mV; the value of the membrane potential increased and became more electronegative, and the spiking pattern was found to be more rhythmic that was a marker of a relative stabilization and improvement in the functional state of the neuron under study.

Conclusion

Our experimental studies aimed at discovery of influences and effects produced by BBSS EMR under the above specific parameters on the intracellular potentials of the central nervous system neurons within the neuronal network in the circumaesophageal ganglion complex in the snail Helix pomatia, and, following our philosophy, on the intracellular potentials of the cardiomyocytes, have demonstrated the presence

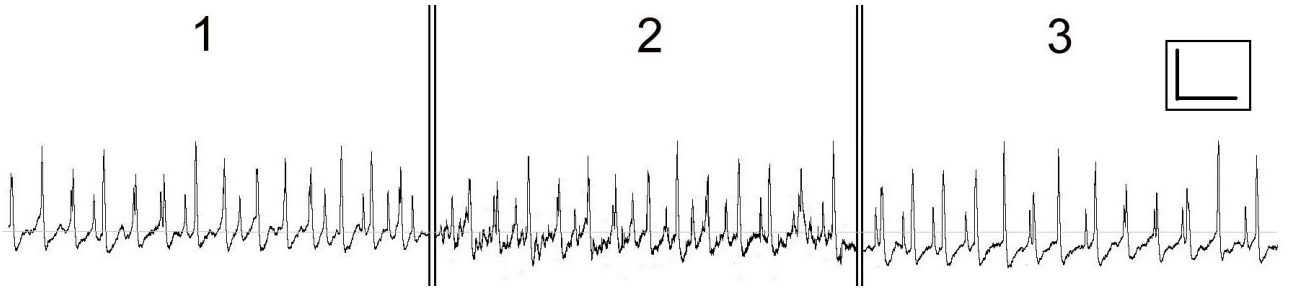


Fig. 6. Legend: 1. Non-identified neuron LPaGx in a steady pathological functional state prior to BBSS EMR exposure. 2. BBSS EMR exposure session for 30 seconds. We can trace the dynamics in variations in miniature potentials in the pathology state, produced before the EMR exposure. 3. Intracellular activity of the neuron upon completion of the BBSS EMR exposure. Please, refer to our comments in the paper text. Calibration 10 mV/ 1 s.

of responses given by the neurons and changes of their functional state. The detected responses have been found to be reversible, and they should be classified as reactions of training according to the Kvakina-Garkavi-Ukolova scale for assessing the activity level in biological systems. This is the evidence that the neurons and the entire nervous system recognize and sense the specific BBSS EMR as a soft regulatory and hyperpolarizing action ranked by us at this stage of the studies as producing a calming effect and allowing the neuron not consume, but store up its internal energy.

Below we would like to outline some considerations relating to the mechanisms of neuronal responses to the above EMR exposure. According to the findings in the reference literature [21], this role is assigned to the pacemaker potential-dependent membrane channels of hyperpolarization, which also can be found in different structures in the brain, including the cortex region, according to current knowledge. Specifically, based on the data in the reference literature, the appearance of rhythmic burst discharges of the neurons in the thalamus is treated to be an endogenous intrinsic property, arising due to joint activation of the potential-dependent K^+ channels of hyperpolarization and the low-threshold Ca^{2+} and Na^+ currents initiating the burst discharge. So, mentioned should be some research papers which outline activation of the h-channels (hyperpolarization-activated cyclic nucleotide-gated ion channels) that results in local generation of intrinsic pacemaker currents, and then oscillations of this sort can propagate both into the other cortical and subcortical structural regions in the brain. This activation of the pacemaking h-channels, as we may think, and as it is evidenced by the reference publications, is initiated as a genetically determined adaptive response of the neurons to an excessive hyperpolarization of the neuronal membrane due to a reduction in tonus of the reticular formation in the brain in order to provide a restoration by them of their initial level of the resting potential with elevating their excitability level towards the spindle termination. This assumption is supported by researches which have established that the dendritic h-channels play a critically important role in the regulation of excitability of the cortical cells. At the same time, there is another way of looking at this mechanism as discussed below. Electrical potential is an intrinsic property of all excitable tissues (the nervous, muscle fiber and gland tissues). An essential feature of the excitability is its close interplay with the specific sensitivity of the cell membranes and their property to respond to stimuli by certain specific changes in the ionic permeability and the membrane potential. In this case, the excitability should not be confused with the excitation, since the excitation is a response by a biological cell to a stimulus, when the living system experiences a conversion from the state of its physiological resting into the specific activity state peculiar to a given cell or tissue. If a cell is placed in an electrical field, it becomes polarized, i.e in one region of the membrane the sign of the resting potential is identical to that of the field strength, while the opposite membrane region has the same potential, but with the opposite sign. What this means is, that, on the one hand, the membrane potential in one half of the cell has increased (the membrane has been hyperpolarized), while, on the other hand, the potential in the other half of the cell in question has decreased (the membrane has been depolarized). An excitation appears as soon as depolarization of the membranes reaches or exceeds a threshold level under an action of a current applied from the outside. This process is also referred to as stimulation. Following this way of

the interpretation, we suggest that one of the methods of an artificial change in the level of excitability for different cells, individual structures in the brain and the general performance as well as formation of a predominance in the model experiment is polarization of some brain tissue regions produced by an electromagnetic field applied from the outside.

In this connection, the following points can be highlighted:

a) EMR under the specific parameters, as indicated above, being a stimulus can be exactly graduated in strength, time and pattern effects produced on a living tissue;

b) EMR under the above specific parameters and the current induced by the latter are found to be close to the natural mechanisms responsible for generation and propagation of excitation in living tissues;

c) EMR under the above specific parameters is remote and contact-free in its effects.

The effects produced by BBSS EMR on the Helix pomatia CNS neurons are also applicable to the performance of cardiomyocytes. Similar to AP of a neuron, in case of cardiomyocytes, the EMR influence will be manifested in an increase in growth rate of the rapid depolarization phase. This is encouraged by opening of a greater number of the influx sodium and calcium ion channels, and, as a consequence, an increase in the amplitude of the action potential and a rise in the initial phase repolarization rate with a steeper fall that is provided by opening of a greater number of the fast potential-dependent potassium ion channels and a faster efflux of the K^+ from the cell. The ionic balance between the Ca^{2+} entering the cell and the K^+ exiting the latter keeps the potential of the membrane at the level corresponding to the plateau portion of the action potential of the cardiomyocyte. The effect made by BBSS EMR is manifested in an increase in steepness of the phase of the rapid end repolarization of the membrane due to activation of most fast potassium channels, determining the outward potassium current, as well as due to an increase in the inward calcium and chlorine currents that results in a shift of the phase of the recovery of the membrane resting potential towards more negative values (hyperpolarization). In consequence of the above, the slow repolarization phase, the plateau portion, should become somewhat shorter, and a pulse-to-pulse interval between the two successive action potentials of the cardiomyocyte should be longer that has been evidenced by our model studies. Hyperpolarization of the cells under the BBSS EMR exposure can be explained by an enhancing of contribution of the electrogenic component of the sodium-potassium pump to the MP value and either by a decrease in the passive permeability to sodium and calcium ions, or an increase in the permeability to potassium ions. Changes in the total ion currents, the AP and PA parameters of the neurons, exposed to BBSS EMR, are essentially attributed to the respective variations in the MP value and a marginal direct influence on the potential-controlled ion channels. Besides, a minor depolarization of the neurons upon the BBSS EMR exposure and the stabilization of the frequency of electrical activity may indicate that there is a slight activating effect of the ionic channels available. A quite interesting effect produced by the above specific BBSS EMR is initiation of delayed-rectifier currents. The basis for effects of this sort might be activation of the processes of phosphorylation – dephosphorylation of the channel proteins and the stabilization of the membranes that facilitates faster conformational mutual transitions of the ion channels between the states (closed – open – inactivated). The cause of the prolonged changes detected in the neurons might be an EMR effect exerted on the expression of the ion channels and embedding of them in the plasma membrane. The membranotropic activity of BBSS EMR traced in our experimental study sets was reported to be considerably less as compared to that revealed by us in the mollusk neurons for anesthetics and anti-arrhythmia drugs, applied immediately onto the neuron membrane [22, 23]. Many anti-hypoxants produce their pharmacological effects at least at three levels: at the neuronal, vascular and metabolic ones. They may promise a wide spectrum of pharmacological activity offering effects of anti-hypoxia, stress protection, nootropy, anticonvulsant and anxiolytic actions, inhibition of the free-radicals processes of lipid oxidation, a rise in the resistance of the organism to exposures of various damaging factors as well as to oxygen-dependent pathological conditions (shock, hypoxia and ischemia, brain circulation disorders, alcohol and anti-psychotic intoxication, including neuroleptical intoxication). By this means we can conclude that BBSS EMR under the above specific parameters, similar to anti-hypoxants, by changing MPs and ion currents of the cardiomyocytes and neurons modulates their functional state. BBSS EMR has both "calming" effects and activating influences on the performance (electrical activity) of the neurons that is similar to the effects produced by anti-hypoxia medication.

Based on the evidence obtained in our experiments, we may conclude the following:

1. We have conducted pioneering research on influences and effects made by some rhythmic patterns, similar to those found in an electroencelogram, or the natural resonance frequencies of the Earth of the rhythmic broad-band stochastic electromagnetic field patterns, on intracellular potentials of a neuron within the isolated central nervous system. Treating the neuron from the isolated CNS in the snail Helix pomatia as an exemplary case, we have demonstrated the responses by the neurons to the BBSS EMR exposure and the BBSS EMR action mechanisms, based on the data on the membrane potential levels and the action potential parameters measured.

2. The rhythmic BBSS EMR initiates reversible hyperpolarization of the cytoplasm membrane of the neuron with maintaining it at the level of the residual hyperpolarization under the action of the field. The recovery of the membrane resting potential is accompanied by an enhanced operation of the Na^+/K^+-ATPasa to remove the sodium ions influxed in the first phase of depolarization of AP. Besides, an accelerated restoration of the concentration of the calcium ions owing to operation of the $3Na^+/1Ca^{2+}$ antiporter and the Ca^{2+} ATPasa takes place.

3. The influence of the rhythmic-pattern BBSS EMR raises the rate of growth of the rapid depolarization phase of the action potential. This is promoted by opening of a greater number of the influx sodium and calcium ion channels, and, as a consequence, an increase in the AP amplitude and a rise in the repolarization rate with a steeper fall provided by opening of a greater number of the potassium ion channels and a faster efflux of the K+ from the cell.

4. The effect produced by BBSS EMR is manifested in a greater steepness of the rapid end phase of polarization because of faster closing of the sodium channels and activation of the fast potassium channels, governing the outward potassium current, as well as in an increase in the inward calcium and chlorine ion currents, which lead to the shift of the membrane resting potential recovery phase towards more negative values (residual hyperpolarization) and maintenance of the membrane potential at this level as in the course of the specific EMR action.

5. The regulation of the functional state of a neuron in the neuron network with the use of the non-invasive remote method of control has been objectively shown herein. The neuron is treated by us as an analogue for the formation of the transmembrane potential of the cardiomyocyte and at the same time as the basic model of the neuronal cellular auto-regulation of the phase-structured performance of the cardiovascular system. In doing so, a fine-tuning instrument to control the neuron, CNS and other cell systems in the organism has been discovered. EMR of this sort, being a stimulus, can be exactly graduated in strength, time and effects produced on a living tissue. Besides, the employed BBSS EMR and the current induced by the latter have been found to be close to the natural mechanisms of generation and propagation of excitation in living tissues.

6. A favorable effect made by the specific BBSS EMR therapy produced on the functional state of a neuron has been demonstrated. It finds its reflection in the "calming" dynamics traced in the neuron membrane potential changes and the hyperpolarizing influence. An approximation of the results obtained at the cellular level makes possible to assume that there is a possibility available to control the performance of the autonomous highly specialized nervous system of the heart, the integrated system, CNS and development of physiological reactions at the level of the human organism.

The results of our research offer widest possibilities of a targeted fine tuning and regulation of the control systems in a human organism.

We have studied one of the aspects of the mechanism of the targeted cardiac regulation that has been analyzed on the basis of the cardiac cycle phase analysis in the context of cardiometry, but it might involve new challenges in science related to further investigations of much finer subcellular mechanisms responsible for the regulation in the high-specialized cell systems in the organism.

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Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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