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**14. ABSTRACT**

This report results from a contract tasking UNESCO Chair Life Sciences International Educational Center as follows: At present the existence of extremely high-power microwave pulses (EHPP)-specific biological effects remains questionable, in part because of the difficulty in studying such effects in highly organized biological systems. The purpose of the proposed project is to find out whether there are EHPP-specific (non thermal) effects using a simple model less investigated in this aspect, i.e. on the metabolic mechanisms of the neuronal and muscle membrane. Taking into account that Na-K pump activity, Na:Ca exchange, intracellular cAMP, cGMP, cell hydration, and membrane chemosensitivity all play a key role in metabolic regulation of cell function, they would likely reflect any EHPP-specific effect. Therefore their characteristics will be studied before and after EHPP cell exposure. Results will provide data on basic mechanisms of EHPP effects and will also be applicable to evaluation and worldwide harmonization of safety standards.

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## **II. PROJECT TECHNICAL REPORT**

**ISTC A-803P**

### **Final Project Technical Report of ISTC A-803P**

## **Molecular and cellular mechanisms of possible non-thermal biological effect of extremely high-power microwave pulses (EHPP)**

**(From 1 August 2002 to 1 August 2004 for 24 months)**

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**September 2004**

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**Molecular and cellular mechanisms of possible non-thermal biological effect of extremely high-power microwave pulses (EHPP)**

(From 1 August 2002 to 1 August 2004 for 24 months)

**Sinerik Nersesovich Ayrapetyan**  
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The objective of this project is to find out the possible existence of specific (non thermal) effect of EHPP using simple and from this aspect less investigated models, i.e. physicochemical properties of water and water solutions, plant seed hydration and germination, heart muscle contractility and chemo sensitivity, neuronal membrane chemo sensitivity, hydration and Ca adsorption by cell membrane.

Obtained data have shown that after 5-15 min treatment Extremely High Power Pulses (EHPP) have non-thermal effect on water specific electrical conductivity (SEC) and its thermal capacity which remains in water memory after its freezing/thawing in liquid N<sub>2</sub>. A special electronic device with extra sensitive ( $\pm 0,01^{\circ}\text{C}$ ) thermometer and thermostat was constructed, allowing to estimate the non thermal effect of EHPP on water and water solutions.

The non-thermal effect of EHPP on cell bathing aqua medium caused the adequate changes of plant seed hydration and germination potential, snail heart muscle contractility and its acetylcholine (Ach) sensitivity, neuromembrane chemo sensitivity, cell volume and Ca adsorption by neuromembrane.

The obtained data could be considered as strong evidence on the existence of non-thermal biological effect of EHPP, realizing through the change of cell bathing medium.

**Keywords:** (EHPP, non-thermal effect, water, plant, neuron, muscle, heart contractility, chemosensitivity, Ca adsorption)

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### Introduction

The existence of specific (non-thermal) biological effects of extremely high-power microwave pulses (EHPP) still remains the subject of future investigations. The fact that EHPP can certainly produce a thermal effect makes it technically difficult to discriminate its possible specific effect in experiments (Pakhomov *et al.*, 1998). In some elegant and highly technically experiments on isolated heart slices (Pakhomov *et al.*, 2000), no EHPP specific effects were found. One possible difficulty with this study is that the functional activity was recorded in the presence of EHPP thermal effect, which could cover the specific one. As any specific effect of EHPP must be realized by metabolic pathways consisting of the number of enzymatic reactions, the rate of these pathways can be determined by the rate of slowest enzymatic reaction, which can be expressed by Arenus' equation.  $dm/dt = K \exp^{-\frac{E_a}{RT}}$ , where  $dm/dt$  - the rate of enzymatic reaction,  $K$  - the frequency of enzyme molecules' collision with substrates,  $E_a$  - the energy activation,  $R$  - the gas constant (8.314 Voltcoulomb.mol<sup>-1</sup> degree Kelvin<sup>-1</sup>),  $T$  - the absolute temperature. If EHPP has a specific effect, it would be realized by modification of  $E_a$  (modification of enzyme affinity to substrate). Although this equation clearly shows that the  $T$ -dependent reaction rate is much higher than the  $E_a$ -dependent one and there is a big possibility that EHPP thermal effect could cover the non-thermal one. Therefore, in order to record the EHPP specific effect on cells, it is necessary to remove the EHPP-induced thermal effect by recovering the initial temperature and only then study the trace effect of EHPP on simple experimental models, like as water and water solutions, plant seed hydration and germination, snail heart muscle contractility and neuronal activity.

It is well known that the biological effect of any physical (including EMF) and chemical factors before reaching the cell membrane could modify the structure of cell bathing aqua solution. Therefore, it is suggested that the letter can serve as the common target through which the biological effect of EHPP is realized. If there is a non-thermal effect of EHPP, first of all it should become apparent on water structure.

Thus, the working hypothesis for the present project was to consider the cell bathing solution as a target through which the possible non-thermal biological effect of EHPP could be realized. The theoretical basis for such suggestion is that the water molecules are polar and they vibrate when subjected to microwave, causing considerable friction between molecules leading to the possible water structure changes causing the adequate biological effect.

To check this hypothesis the comparative study of the effect of EHPP-induced heating and traditional heating on physico-chemical properties of water and water solutions and their effect on plant seed germination, snail heart muscle and isolated neuron functional activity were studied.

The project represents an experimental research which would answer the question of whether the EHPP has specific (non-thermal) effect on physico-chemical properties of water and water solutions and their effect on plant seed germination, snail heart muscle and isolated neuron functional activity. Results will provide data on basic mechanisms of EHPP effects which will be applicable to evaluation and worldwide harmonization of safety standards. The developed new extra-sensitive calorimetric dosimeter could be recommended for calorimetric measurements of EHPP Specific Absorption Rates (SAR) as well as for estimation of non-thermal (specific) effect of EHPP.

### **Method, Experiments, Theory etc.**

For the experiments, involving frequency or power modulation, special modulators were used. Square microwave pulses (9,3 - 9,5 GHz, 1 $\mu$ s width, 50-100 kW) were produced by a Model MH1300 system with an output waveguide (23 x 10 mm). Incident and reflected powers in the waveguide were measured via directional couplers and power meters with power sensors.

The exposure system and dosimeter setup consisted of the following units: power block, MW-generator, buffer, measuring line, wave-measurer, detectors, pointer set up, power measurer, transformers, the object, thermistor head, and the measurer of the crossing power.

In order to measure the temperature in the investigating cell, electronic thermometers with specific parameters necessary for the experiment has been worked out. Besides, the smallest sizes of measuring tip, high precision and sensitivity, the least resistance was also used. In addition, to carry out the investigation in the temperature range of  $-20$  to  $60^{\circ}\text{C}$  and to meet the calibration matters of the worked out thermometers, the special thermostats providing the necessary regulation range of the experimental cell temperature was constructed. For this purpose a miniature EHPP semiconductor *p-n* type diodes 2A517A (Russian production) having a metallized cathode able to created an appropriate thermal contact (cathode diameter- approx. 0.9mm., limited direct current – 0.1A, masse –  $10 \times 10^{-3}$ ) was used. The amplitude of measuring current was  $\leq 0.5 \mu\text{A}$ . The elaborated thermometer gave us a possibility to measure the temperature with high accuracy and sensitivity ( $0.01^{\circ}\text{C}$ ). The sensors had high linearity depending on temperature-dependent rectangular shifting voltage at stable current through *p-n* cross. To measure the temperature in small volumes it was important to provide the operation of these sensors in microregimes (at less than  $10^{-6}\text{W}$  power) in order to exclude the current-induced heating of the sample. To minimize the heating, the sensor was supplied by stabilized current pulses. To increase the sensitivity and interference resistance, a modernized version of the thermometer described by Simonian R. H., (1989, patent No1557458 USSR) was used.

The elaborated thermometer was supposed to measure the temperature in the range of  $-20$  to  $60^{\circ}\text{C}$  with correctness of  $\pm 0.2^{\circ}\text{C}$  at  $0.01^{\circ}\text{C}$  sensitivity. The thermostat was protected from external EMF and controlled by a digital computer. The system had an output signal for measuring the temperature set in chamber by means of external measurer. The most convenient system for such purpose was the equipment described by Simonian R. H. (1989, patient in USSR No3501006). This setup contains a transistor- temperature detector and a heater, an interference resistant signal analyzing system, the possibility to work with small thermo stating volume, and the ability to stabilize the temperature within 1 sec. with accuracy  $0.05^{\circ}\text{C}$ . The thermostat has 2 working regimes: a) the regime of high accuracy follow-up of the temperature and b) the regime of self-thermoregulation. For achieving the high accuracy and noise protection the narrow band filter was used.

In the setup the calorimetric method of EHPP sorption energy measurement was used. It consisted of two identical chambers: chamber N1 contained the medium for EHPP exposure and the identical chamber N2 meant for heating of an object equivalent to the chamber N1. The both chambers were isolated from each other and placed within the thermostat cooled to  $10^{\circ}\text{C}$ .

This system was also used for studying the EHPP effect on thermal capacity of water and water solutions.

To study the electrophysiological properties and chemo sensitivity of the membrane, standard electrophysiological methods, including current and voltage clumps were used (Ayrapetyan 1980). Heart contractions were recorded isotonicly using the special computer technique adapted for this purpose (Azatian *et al.*, 1998).

For studying the isolated cell volume the biological microscope (MB-14, Russian production) connected to PC through the digital video camera (Sony DCR-PC101K) was used.

For determination of intracellular Ca level the FURA 2/AM-induced luminescence method was used. The florescence signals were obtained at 340/380 nm excitation ration. The images were captured with digital video camera (Sony DCR-PC101K) constructed on the biological microscope (MB-15 “Lomo” production, Russia) and recorded in computer.

The Sigma Plot (Version 8.02A) computer program was used for statistical analysis of the experimental data.

The experiments for checking the existence of EHPP-specific effects on the metabotropic membrane mechanisms will be performed on snail neurons and heart muscle before and after EHPP exposure and also in comparison with an adequate heated and sham exposure. The isolated nerve ganglia and the heart muscles to be exposed were transferred into the exposure cell filled with Ringer’s solution. To study the pacemaker activity, Na-K pump activity, Na:Ca exchange, and chemosensitivity of membrane, standard electrophysiological methods, including current and voltage clumps was used (Ayrapetyan 1980). For recording the isolated heart contractility a special setup was used described in Azatian *et al* (1998). Heart contractions were recorded isotonicly by using special computer technique adapted for this purpose (Azatian *et al.*, 1998).

To estimate the cell hydration tissue samples were dried in a thermostatically controlled oven for 24 hours at 105° C. The tissue hydration was measured as (wet weight-dry weight)/ dry weight and expressed as water content g/g in dry weight. Only the data proved by double-blind experiment protocol were considered reliable.

## **Results**

### ***1. The effect EHPP on physico-chemical properties of water and water solutions***

10 min. exposure to EHPP led to the increase of temperature of DW and PS from 20 °C until 40 and 60 °C, correspondingly. After the exposure the temperature of DW and PS returned back to its initial value after 85 min. This temperature changes were accompanied by the according changes of SEC of DW and PS (Fig.1B). Following after the exposure the time dependant decrease of the both parameters was not adequate and their rates were different. It was suggested that such differences could be connected to the phenomena of temperature anomaly of water structure (Drost- Hansen, 1957). The comparative study of EHPP-exposure and adequate heating on temperature-dependence of DW SEC changes have shown a significant differences between them. The heating –induced elevation of SEC in case of EHPP was ~20% less than in traditional heating one.

It is well known that water structure (SEC) could keep memory on its previous thermodynamic state (Klassen, 1982). The different trace effect of EHPP exposure and adequate heating on SEC of DW also was recorded.

In the next series of experiments we have tried to find out an additional evidence for existence of specific (non thermal) effect of EHPP on water: the malting point of water after its freezing in liquid N<sub>2</sub>. As the melting point of liquids raises with the increasing polarity of their molecules and especially with the formation of a hydrogen bond, in present experiments the melting (freezing) point of distilled

water (DW) frozen in liquid N<sub>2</sub> after 15 minutes exposure to EHPP was determined. The freezing (melting) point of DW was 0.35 °C. The freezing (melting) point of DW preliminary heated in water bath was 0.48 °C, while in preliminary EHPP-treated water this index was 0.40 °C.

DW had initial conductivity at room temperature (20 °C) in the range of 1 – 10 µs/cm. The letter depended on “age” of DW i.e. time passed after water distillation (Stepanian et al. 1999).

The temperature-dependence of SEC of DW heated in water bath and exposed to EHPP was studied. 3 min. of exposure to EHPP led to the temperature increase in 5 °C (from 18 °C to 23, 35 °C). The SEC of DW exposed to EHPP was increased by 70 ± 4%, while the SEC of DW heated in water bath was changed only by 55 ± 5%.

After the exposure to EHPP the temperature of DW returned back to the room temperature faster, that in case of heated DW. In case of EHPP exposure the water plays the role of heater of vessel body, while in case of water bath heating it was vice versa. From this we can conclude that in physiological experiments it is practically impossible to compensate the thermal effect of EHPP. However, the kinetic of SEC of DW heated in the mentioned ways has opposite character, i.e. the SEC of heated DW returned to its initial level faster, than the SEC of EHPP-exposed DW.

It is well known that the temperature has thermal anomaly properties, which is expressed more pronounced in 4 °C when the water density is sharply changed. It is obvious to predict that these changes could be accompanied by the according changes of water SEC. If the water underwent to any structural changes (hydrogen bounds) the thermal capacity will change, too.

The melting process of non-treated PS started earlier than in case of heated PS. In EHPP-treated PS this process also started earlier than in case of heated one. These differences also demonstrate the different level of temperature anomaly of SEC.

Thus, on the basis of the obtained data we can strongly suggest on the existence of non-thermal effect of EHPP on water and water solutions.

## ***2. The effect EHPP on plant seeds hydration and germination***

The water uptake (hydration) by barley seed during the period of germination (after 72 hours of incubation at room temperature) was significantly increased in DW preliminary exposed to EHPP during 5 min., while the adequate heated DW had slightly depressing effect comparing to the control.

The study of seed dry weight, which can be the marker for germination potential of seed incubated in the above mentioned conditions showed that in EHPP-treated DW seed dry weight was significantly lower, while in the heated DW it was increased, i.e. the germination of barley seed was depressed in EHPP-treated DW.

Thus, the obtained data strongly suggest on the existence of non-thermal biological effect of EHPP on seed metabolic dependent water uptake and its germination potential.

## ***3. The effect EHPP on heart muscle contractility***

Two types of experiments were performed:

1. The heart contractility was continuously recorded upon the direct exposure by EHPP
2. Only the intra-cordial perfuse PS was treated by EHPP.

After 30 sec. of direct expose of heart, the depressing effect on heart contractility with 1 min. latent period was observed. After twice prolonging the exposure time (1 min.) the latent period of its depressing effect on heart contractility was not significantly changed (1 min.), although, this effect was

much longer than the previous one. Such exposure time independency of latent period of EHPP-induced depression of heart contractility could be the subject for special investigation. If this inhibitory effect of EHPP is due to the increase of temperature of intra-cordially perfused solution, which was less than  $0.3^{\circ}\text{C}$  when exposure time was 0.5 min, the most probable candidate for the metabolic mechanism through which the temperature could cause the inhibition of heart pacemaker activity is the electrogenic Na pump (Carpenter 1969, Ayrapetyan 1969, Levengood and Kusano 1972). EHPP-induced inhibitory effect on heart contractility was present in K-free solution also, when pump was in inactive state (Skou, 1957). Therefore, the contribution of temperature-induced activation of pump could be excluding in EHPP-induced inhibition of heart contractility.

The comparative study of EHPP-induced depressing effect on heart contractility at the beginning and at the end of the experiment showed the absence of EHPP-induced depressing effect on heart contractility, even after 3 min. of direct exposure of EHPP on heart. The absence of EHPP-induced depressing effect on heart contractility after 10 hours staying in vitro state could serve as additional evidence on the metabolic nature of this effect.

In the next series of experiments the effect of EHPP on Ach-induced inhibition of heart muscles contractility was studied. To exclude Na-pump induced modulation on Ach sensitivity of muscle (Ayrapetyan & Arvanov 1979), the experiments were carried out in K-free solutions. The exposure time of heart was chosen less than 20 sec. which had no significant effect on heart contractility. In this case the temperature of intracordial perfused PS was increased in no more than  $0.5^{\circ}\text{C}$ . Before and after 20 sec. of exposure to EHPP,  $5\mu\text{l } 10^{-3}\text{ M}$  Ach was injected into intracordial perfusion PS. The interval between the end of exposure and beginning of Ach application was 2 min.

Ach sensitivity after 20 sec. of heart muscle exposure to EHPP was significantly decreased. The effect of Ach sensitivity was estimated by the duration of Ach-induced inhibition of heart contractility. After exposure to EHPP Ach sensitivity was depressed unreversibly.

#### **b) The effect of EHPP pretreated PS on heart contractility and Ach-sensitivity**

The inhibition of heart contractility was recorded also in the case when only the intra-cordial perfused PS was pretreated by EHPP. The latter effect could not be connect with EHPP thermal effect, because the inhibitory effect of EHPP treated PS was recorded after the returning the temperature of perfused PS back to its initial one. It is interesting to note that the continuous perfusion (5 min) by EHPP-treated PS led to full inhibition of heart contractility and after removing the treated solution the heart contractility was recovered and in the first 1 min its amplitude was higher than before it.

The study of the effect of EHPP pretreated PS on Ach-induced inhibitory effect on heart contractility have shown that Ach had irreversible poisoning effect on heart contractility after application of EHPP pretreated PS.

#### ***4. The effect of EHPP treated PS on neuronal activity***

The effect of EHPP-treated PS on resting membrane potential (RP), Ach-sensitivity of membrane, neuronal hydration, Ca adsorption by neurons were studied.

The replacement of non- treated PS by EHPP-pretreated PS did not cause a statistically significant changes of RP value of 50 studied neurons in normal PS at room temperature, while in K-free medium the slowly developing (1-1,5 mV/min) hyper-polarization effect (3-5 mV) of EHPP-pretreated PS on RP in 39 neurons was observed.

It is well known that three types of neurons according their sensitivity to Ach can be distinguished in CNS of snails: a) insensitive, b) depolarizing (D) and c) hyperpolarizing (H). (Sakharov et al, 1969). It has been found also, that *Helix* neurons are distinguishable by their sensitivity to inhibitors of

electrogenic Na-pump, ouabain and  $K^+$  free solution and by the ionic dependence of their Ach responses (Ayrapetyan, 1980; Arvanov et al., 1984). The Ach responses of A-type neurons are almost completely blocked by ouabain and K-free solution and are due to an increase of membrane permeability for  $Cl^-$  and  $Na^+$  ions, while Ach responses of B-type neurons are insensitive to ouabain and are due to an increase of permeability for  $Na^+$  and  $K^+$  ions.

The present experiments were carried on neurons having D-responses. The applied Ach concentration was  $10^{-5}$  M. The neurons having resting membrane potential from -40 to -55mV and Ach-induced D-responses with constant amplitude (with 5 min. application intervals) were chosen for experiments. After recording the control Ach-induced current in normal PS the cells were perfused by PS 10 min preliminary treated by EHPP. It was studied 15 B-type and 5 A-type neurons. EHPP-treated PS had no effect on Ach-induced current in A-type neurons (data are not presented), while in B-type neurons it was partly (10-50%) depressed.

Thus, the obtained data allow us concluding on the non thermal effect of EHPP on Ach-sensitivity of membrane, which was realized through the cell bathing solution. However, the nature of the metabolic pathways through which the non thermal effect of EHPP is realized is staying unclear.

### ***5. The effect of EHPP-treated PS on the volume of snail neuron***

The comparative study of EHPP-treated and water bath heat-treated physiological solution (PS) on volume of isolated single neurons was performed.

Isolated neurons were incubated in special experimental glass micro-chamber containing PS. This chamber was placed under the microscope (MB-14, Russian production) with digital video camera (Sony DCR-PC101K). This chamber can be continuously perfused by non-treated (sham exposed), preliminary heat-treated and EHPP-treated PS.

The effect of heat and EHPP-treated PS on cell volume was studied on 35 neurons. Preliminary heat-treated PS had no significant effect on cell volume, while EHPP-treated PS has the following results: cell volume of 15 neurons was increased, in 6 neurons it was decreased, in 5 neurons it had biphasic (shrinkage-swelling) effect and 9 neurons has no significant volume changes. The cell volume changes were accompanied by changes of axon diameter in opposite manner, i.e. cell soma increase was accompanied by axon diameter decrease.

Preliminary EHPP-treated PS-induced cell volume changes and its insensitivity to adequate heat-treated PS can be considered as an evidence on the existence of EHPP specific (non-thermal) effect on cell volume, which is realized through EHPP-induced specific structural changes of perfuse PS. Non-adequate effect of EHPP on cell volume of different neurons could be explained either by initial functional state of neurons or different sensitivity of metabolic cascade of individual neurons to EHPP-induced PS structural changes. From literature data on electrophysiological experiments it is well known that mollusk neurons have non-adequate (membrane hyper-polarizing and depolarizing) responses to different physical and chemical factors. Therefore, these non-adequate responses of cell volume are not unexpected data. However, for final conclusion on the mechanism responsible for EHPP specific effect on cell volume it is necessary to carry out more detailed investigation.

### ***6. The effect of EHPP-treated Physiological Solution on intracellular Ca ion concentration of snail neuron.***

The isolated snail neurons were incubated for 60 minutes in FURA 2/AM-containing physiological solution medium at 27-30°C. Then cells were 3 times washed by normal physiological solution and placed in the Petri's dish where they were perfused. The florescence signals were obtained at 340/380 nm excitation ratio. The images were captured with digital video camera (Sony DCR-PC101K)

constructed on the biological microscope (MB-15 “Lomo” production, Russia) and recorded in computer.

After 3 min. incubation in EHPP solution the Ca accumulation near the cell membrane and in cytoplasm can be clearly observed. However, in the following period of incubation, the fluorescent excitation was dimmed down, probably because of the long period of light exposure and heating. Although, after 7 min incubation the cell volume was significantly increased.

It is well known that Ach application leads to the increase of intracellular Ca ion concentration. Therefore, it was interesting to study the effect of EHPP treated PS on Ach-induced elevation of intracellular Ca concentration.

The experiments performed on 10 neurons have shown that Ach-induced increasing of the intracellular Ca was more pronounced in EHPP-treated solution than in the case of non-treated PS (sham exposed).

As previously it was shown that cyclic nucleotide-dependent Na:Ca exchange played a crucial role in regulation of Ca homeostasis in snail neuron (Azatyan et al., 1998), in order to find out the role of cyclic nucleotides in the realization of EHPP-treated PS-induced intracellular Ca elevation, in the next series of experiments the effect of Guanylyl Cyclase inhibitors (LY 83583 and Methylene Blue) and Adenylate Cyclase inhibitors (SQ 22536) were studied.

In the investigated 10 neurons we were unable to demonstrate any significant effect of Guanylyl Cyclase and Adenylate Cyclase inhibitors on EHPP-induced elevation of florescent intensity.

However, these results cannot be considered as the final because the question if the absence of the effect of inhibitors is due to the low sensitivity of our methods or the effect of EHPP-treated PS is insensitive to these inhibitors is still unanswered.

## Conclusion

The fact that EHPP can certainly produce a thermal effect makes it technically difficult to discriminate its possible specific effect in experiments (Pakhomov *et al.*, 1998). In some elegant and highly technically experiments on isolated heart slices (Pakhomov *et al.*, 2000), no EHPP specific effects were found. One possible difficulty with this study is that the functional activity was recorded in the presence of EHPP thermal effect, which could cover the specific one. As any specific effect of EHPP must be realized by metabolic pathways consisting of the number of enzymatic reactions, the rate of these pathways can be determined by the rate of slowest enzymatic reaction, which can be expressed by Arenus' equation.  $dm/dt = K \exp^{- (E_a/RT)}$ , where  $dm/dt$  - the rate of enzymatic reaction,  $K$  - the frequency of enzyme molecules' collision with substrates,  $E_a$  - the energy activation,  $R$  - the gas constant (8.314 Voltcoulomb.mol<sup>-1</sup> degree Kelvin<sup>-1</sup>),  $T$  - the absolute temperature. If EHPP has a specific effect, it would be realized by modification of  $E_a$  (modification of enzyme affinity to substrate). Although this equation clearly shows that the  $T$ -dependent reaction rate is much higher than the  $E_a$ -dependent one and there is a big possibility that EHPP thermal effect could cover the non-thermal one. Thus, the existence of EHPP specific biological effect could be determined by the comparative study of trace effect of EHPP-exposed and traditional heated experimental models. If there is a specific (metabolic) effect of EHPP, this effect could be realized first of all through the structural changes of cell bathing aqua solution, which serves as a general medium for cell metabolic cascades (Klassen 1982, Bistolfi, 1991; Ayrapetyan *et al.*, 1994a). Because of water molecules are polar and they vibrate when subjected to microwave energy, causing considerable friction between molecules it is suggested that EHPP-induced water structure changes must be different from traditional heated one and such differences could underlay in the ground of generation of non-thermal biological effect of EHPP.

The obtained data in the present project on the comparative study of the effect of preliminary heat- and EHPP-induced treatment of water and water solution on its physical properties (specific electrical

conductivity, thermal capacity, thermal anomaly properties), and the effect of such water (or PS) on plant seed germination potential, muscle and nerve cells functional activities clearly demonstrated the existence of no thermal effect of EHPP on water physico-chemical properties, which had modulating effect on metabolic dependent water uptake by seed, plant germination potential, heart muscle contractility and its Ach-sensitivity, cell volume of neurons, its Ach-sensitivity and Ca adsorption by neuro-membrane.

If the specific effect of EHPP on water structure could be explained by its molecule vibration induced by EHPP, the metabolic pathway and membrane sensor able to transfer the EHPP-induced water structure to cell metabolic cascades stain as a subject for future investigation, which has great fundamental and practical interest. The existence of non thermal effect of EHPP allow as to predict that by shortening the pulses the non-thermal (specific) effect would prevail on the thermal one, like as in nanosecond impulse duration, in which the obtained data on non thermal biological effect would be more pronounced. Therefore, the study of the biological effect of nanosecond pulses will be the subject of our future investigation.

Thus, on the basis of the obtained data it is suggested that EHPP-induced water structural changes can serve as one of the main pathway through which the specific (non-thermal) biological effect of EHPP is realized.

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### **List of published papers and reports with abstracts**

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### **List of presentations at conferences and meetings with abstracts**

- Ayrapetyan S.N. Cell Bathing solution as target for specific biological effect of Microwaves. The Bioelectromagnetics Society (BEMS) 26th Annual Meeting. Washington D.C. USA p 20 (P-C-84).

At present the existence of specific (non-thermal) biological effect of microwaves still remains discussable and it serves as subject for intensive investigations. The fact that extremely high power pulses (EHPP) can certainly produce thermal effect makes it technically difficult to discriminate its possible specific effect in experiments. As the most probable pathways through which the possible specific effect of EHPP could be realized is the modulation of cell metabolic activity (enzymatic cascades), according to the Arrhenius' equation, predicting that temperature-dependence of chemical reaction rate must be much higher than the energy activation (E<sub>a</sub>)-dependence one, which is main target for non-thermal effect of EHPP. Therefore, there is high possibility that in the presence of EHPP thermal effect to record its specific effect on cells is experimentally difficult. Thus, the comparative study of the trace effect of EHPP and adequate heating effect on living systems will make possible to separate non-thermal and thermal biological effects of EHPP. Although, water is the main medium, where the measure part of biochemical reactions of different metabolic cascades of cell and organism take place, our knowledge on the biological effect of microwave-induced water structure changes is rather weak. Because of water molecules are polar and they vibrate when subjected to microwave energy, causing considerable friction between molecules it is suggesting that EHPP-induced water structure changes must be different from traditional heated one and such differences could underlay in the ground of generation of non-thermal effect of EHPP.

At our laboratory comparative study of the effect of preliminary heat- and EHPP-induced treatment of water and

water solution on its physical properties (specific electrical conductivity, thermal capacity, thermal anomaly properties), and the effect of treated water on plant seeds germination potential, yeast growth, cell volume, neuromembrane chemo-sensitivity and snail isolated heart contractility was performed.

Water and water solution were preliminary exposed to EHPP source (MIG 9.3 generator Russian production, Peak SAR = 220 kW/g., 1  $\mu$ sec = 9.3 GHz). In all experiments the equivalent heating of control sample was performed in water bath.

The both EHPP and heat-treatment-induced increase of SEC of water and water solution was not recovered fully after returning back its temperature to the initial level. This trace effect for EHPP was higher in about 15–20 % than in heat-treated one. These differences depend on concentration of CO<sub>2</sub> in water and water solution. The rates of spontaneous temperature recovery after heating was significantly different than it was in case of EHPP-induced treatment. Preliminary EHPP- and heat-treated water and water solution have different effects on plant seeds germination potential, cell volume of *Helix* neurons, chemo sensitivity of neuronal membrane, contractility of snail isolated heart, however, the significant differences between the effect of heat and EHPP-treated nutrient medium on yeast growth did not observed, although, the preliminary treatment of nutrient medium in both cases has significant depressing effect on yeast growth comparing to the non-treated one.

On the basis of obtained data it is suggested that EHPP-induced water structural changes in result of its molecule vibration can serve as one of the main pathway through which the specific (non-thermal) biological effect of EHPP is realized.

- Hunanyan A. Sh., Ayrapetyan S.N. The effect of extremely high power pulses (EHPP) –treated physiological solution on Ach-induced current in snail neuronal membrane. The Bioelectromagnetics Society (BEMS) 26th Annual Meeting. Washington D.C. USA. p 17 (P-B-59).

**INTRODACTION:** At present the existence of specific (non-thermal) biological effects of RF-EMF still remains discussable. However, by resent study at our laboratory it was shown that at room temperature specific electrical conductivity (SEC) of distilled water (DW) exposed to EHPP was higher comparing to the control one [1]. On the basis of these data it was suggested that EHPP- induced structural changes of physiological solution (PS) could modulate neuromembrane chemosensitivity. To check this hypothesis the effect of preliminary EHPP-treated PS on Ach-induced current in snail neuronal membrane was studied.

**OBJECTIVE:** The comparative study of the effect of preliminary EHPP-treated and heat-treated PS at room temperature on Ach-induced current in snail neuronal membrane in voltage clamp experiment was performed.

**METHODS:** PS was frozen at –1<sup>0</sup>C during 1 hour after which was defrost at room temperature (20-21<sup>0</sup>C). After temperature stabilization (after 1 hour) PS was divided into two samples (Vol: 5ml): control and experiment. Experimental sample was exposed to EHPP source (MIG 9.3 generator, Russian production) during 15 minutes (Peak SAR = 220 kW/g., 1  $\mu$ sec = 9.3 GHz). EHPP exposure leads to temperature increase to 50,5<sup>0</sup>C. The equivalent heating of control sample was performed in water bath.

The experiments were carried out on giant neurons from the right paratal ganglion of *Helix pomatia*. To record the Ach-induced current through the neuronal membrane, the usual Voltage-Clamp method combining with Digidata acquisition system (Axon Instruments) connected to personal computer was used. PS contains (in mM): NaCl-80, KCl-4, CaCl<sub>2</sub>-7, MgCl<sub>2</sub>-13, Tris-HCl (pH 7.8)-10, glucose-10.

**RESULTS:** Previously it was shown, that Ach-responses of *Helix* neurons can be distinguished according their pharmacological properties and ionic nature: A-type neuron responses are depressed by Na-K pump inhibition and they are generated by the increase of membrane permeability for Cl and Na ions. While B-type responses are due to increase of K and Na ions membrane permeability and they are pump-insensitive. PS preliminary treated by EHPP in A-type neurons has non-reversible depressing effect on amplitude of Ach-induced current. The typical record of EHPP-treated PS-induced depressing effect on Ach response at room temperature is presented on Figure 1a. The B-type neurons in normal PS have Ach-induced response with constant amplitude (interval between Ach applications-5 min). The RP1neuronnes having bursting peacemaker activity have B-type Ach responses, which are depressed in K-free solution (Fig. 1b). As it can be seen on Fig. 1b, there are

spontaneous miniature responses which were also depressed in K-free solution. EHPP-treated PS has depressing effect on Ach-induced current, while the amplitude of miniature potential was not depressed (Fig. 1c).

0.5 hour of perfusion by normal PS did not recover the initial level of Ach-induced current (before the application of EHPP-treated PS) (Fig. 1d). However the miniature responses were not significantly affected by EHPP-treated PS. These different sensitivities of Ach responses and spontaneous synaptic responses to EHPP-treated PS seems extremely interesting and can be the subject for special investigation. Preliminary heat-treated PS has no significant effect on Ach-induced current and amplitude of miniature potential.

**DISCUSSION:** The obtained data on existence of depressing effect of preliminary EHPP-treated PS on amplitude of Ach-induced current in A-type neurons and the absence of such effect in heat-treated PS allow us to suggest that EHPP-treated PS has specific (non-thermal) effect on Ach-induced current in snail neuronal membrane. The membrane mechanism of damaging effect of EHPP-treated PS is a subject for current investigation.

- Amyan A. M., Ayrapetyan S.N. The effect of extremely high power pulses (EHPP) - treated water on barley seeds' DRY weight kinetics during their awakening. The Bioelectromagnetics Society (BEMS) 26th Annual Meeting. Washington D.C. USA p 17 (P-A-58).

**INTRODUCTION:** It was shown that dry weight (d.w.) and wet weight (w.w.) kinetics of barley seeds during their awakening can be modulated by preliminary treatment of aqua bathing solution by low frequency electromagnetic fields (LF EMF) [1]. Seeds d.w. and w.w. dynamics gives information on the osmotic properties of seeds, dissolvent of water soluble components of seeds and water binding in them, as well as metabolic-dependent cell hydration, root formation and germination. At the same time by recent study at our laboratory it was shown that at room temperature specific electrical conductivity (SEC) of distilled water (DW) exposed to EHPP was higher comparing to the control one [2]. These data allow us to suggest that EHPP-induced water structure changes could serve the mechanism, through which the possible specific (non-thermal) biological effect of EHPP is realized.

**OBJECTIVE:** To estimate the physiological meaning of EHPP-induced water structure changes on seeds germination potential, the comparative study of preliminary EHPP-treated and heat-treated distilled water (DW) on seeds d.w. and w.w. kinetics during their awakening process (72 hours incubation) was performed.

**METHODS:** The seeds of spring barley (sort- Nutans 115, Gramineae family, class Monocotyledoneae, forms fibrous root system, cleistogamous) were used. The distilled water was obtained using the device- DE-4-2M (Russian production, State Standard 64-1-721-91). Fresh distilled water (DW) was frozen at  $-1^{\circ}\text{C}$  during 1 hour after which was defrost at room temperature ( $18^{\circ}\text{C}$ ). The water was poured out into three identical glass test tubes for control, heat-treated and EHPP-treated DW. DW was treated by EHPP (MIG 9.3 generator Russian production) during 15 minutes (Peak SAR =  $180\text{ kW/g.}$ ,  $1\ \mu\text{sec} = 9.3\ \text{GHz}$ ). The equivalent heating of the second sample was performed in water bath, after which 5ml of DW was added to each Petri's dishes (vol.~45-50 ml.), containing 20 seeds and this moment was considered as a starting time of seeds incubation. Before and after DW treatment its SEC was measure by special conductometer (Production of Institute of Radiophysics and Electronics of Armenian NAS). To exclude the side effect of light and to create the natural conditions for seeds, the experiments were performed in dark conditions (at  $18^{\circ}\text{C}$ ). The determination of w.w. and d.w. was carried out separately for each seed. Seeds d.w. was determined by 24 hours seeds heating at thermostat (at  $104^{\circ}\text{C}$ ).

**RESULTS:** At  $18^{\circ}\text{C}$  SEC of DW before and after 15 min. heat- and EHPP-treatment were  $145 \times 10^{-3}\ \text{sim.}$ ,  $73 \times 10^{-3}$  and  $97 \times 10^{-3}$ , correspondingly. DW exposure to EHPP leads to temperature increase to  $35^{\circ}\text{C}$ . The results of studying the time-dependence of changes of d.w. in process of seed swelling in non-treated (control), heat- and EHPP-treated DW are presented on Fig 1. As it can be seen from these data, in case of heat- and EHPP-treated DW the rate of d.w. decrease was slower than in control one. However, this process in EHPP-treated DW was much slower than in heat-treated medium. It is interesting to note that these differences between d.w. kinetics were more pronounced after 2 hours of seeds incubation. As it can be seen on presented figure, during 48-72 hours incubation, when the process of seeds germination was activated, the decrease of d.w. of seeds incubated in heat-treated DW was more significant comparing to the control one, meanwhile it must be noted that in EHPP-treated DW this decrease was more pronounced than in case of heat-treated DW.

**DISCUSSION:** The obtained data on EHPP-treated DW depressing effect in period of 2-48 hours incubation and accelerating effect in period of 48-72 hours incubation on time-dependant decrease of seeds d.w. comparing

to heat-treated and non-treated DW could serve as strong evidence on the existence of specific effect of EHPP on seeds awaking which is realized through EHPP-induced structural changes of bathing water.

- Baghdasaryan N. S., Ayrapetyan S.N..The effect of extremely high power pulses- treated wort on growth and development of yeasts. The Bioelectromagnetics Society (BEMS) 26th Annual Meeting. Washington D.C. USA. p 20 (P-C-81).

**INTRODACTION:** The existence of possible non-thermal biological effect of MW still remains discussable. Recent studies at our laboratory were shown that EHPP-induced heat treatment of water and water solution caused different effect on their physico-chemical properties than similar heat-treatment one [1]. Such differences were demonstrated by studding their biological effect on plant seeds germination potential and neuronal cell volume [2,3]. However, EHPP-induced non-thermal effect on other biological objects could be the subject for future investigations.

**OBJECTIVE:** The objectives of the present work was to study the effect of adequate heat and EHPP-induced wort treatment on growth and development of yeasts.

**METHODS:** During the experiments three samples of wort were used: control (non-treated), heat-treated and EHPP-treated. Experimental sample (tube vol: 5ml) was exposed to EHPP (MIG 9.3 generator Russian production), (Peak SAR = 180 kW/g., 1  $\mu$ sec = 9.3 GHz) during 10 min in sterile conditions. The equivalent heating of the second sample was performed in water bath. The temperature of the experimental samples was returned back to the initial room temperature (18  $^{\circ}$ C) in water bath during 30min. Than in each samples 300 $\mu$ l leaven yeast- containing medium (*Saccharomyces cerevisiae*, strain L-41), grown one day before the experiment, was add. Immediately after sowing to check the equal dilution of yeasts the optical transmission factor ( $\tau$ ) of samples was measured by spectrometer (SF-48 Russian production)  $\lambda=540$  nm. All three groups (each contains 10 samples) were incubated in thermostat at 30  $^{\circ}$ C during 24 hours. After which their optical transmission factor was measure again.

**RESULTS:** Heat and EHPP-treatment of yeast-free nutrient medium did not produce any significant changes of its optical transmission factors. Therefore the differences between optical transmission factors in yeast-containing medium in control and experiments are due to the differences in intensities of yeasts growth. Data presented on Figure 1 clearly show that yeast growth in heat-treated mediums was depressed comparing to the control (non-treated), while there is no differences between EHPP-induced heat-treated and water bath-heat treated mediums.

**DISCUSSION:** The obtained data show the absence of specific (non-thermal) effect of EHPP on yeast growth, while its existence was demonstrated on water and water solutions [1], plant seeds germination [2] and neuronal volumes [3]. The absence of specific effect of EHPP on yeast growth can be explained either by insensitivity of investigated yeasts to specific effect of EHPP, or by absence of this effect on nutrient medium depending on its own physico-chemical properties. The nature of the disagreement of mentioned experimental data will be the subject for future investigations.

- Khachatryan M. G., Ayrapetyan S.N. The specific effect of extremely high power pulses (EHPP)- treated physiological solution on cell volume of snail neuron. The Bioelectromagnetics Society (BEMS) 26th Annual Meeting. Washington D.C. USA. p 19 (P-C-78).

**INTRODACTION:** The existence of possible non-thermal biological effect of MW still remains discussable. Recent studies at our laboratory were shown that EHPP-induced heat treatment of water and water solution caused specific effect on their physico-chemical properties [1]. Earlier it was shown that neuronal volume is controlled by cell metabolic activity and it is extra sensitive to weak environmental factors having chemical and physical nature. Therefore, it was suggested that cell volume could be convenient experimental model for studying the biological effect of factor-induced changes of physico-chemical properties of cell bathing medium.

**OBJECTIVE:** The objectives of the present work is the comparative study of EHPP-treated and water bath heat-treated physiological solution (PS) on volume of isolated single neurons.

**METHODS:** The neurons were isolated from *Helix pomatia* nerve ganglia by special micro-scissors and needles under the binocular microscope. Isolated neurons were incubated in special experimental glass micro-

chamber containing PS. This chamber was placed under the microscope (MB-14, Russian production) with digital video camera (Sony). This chamber can be continuously perused by non-treated (control), preliminary heat-treated and EHPP-treated PS. PS was treated by EHPP (MIG 9.3 generator Russian production) during 15 minutes (Peak SAR = 180 kW/g., 1  $\mu$ sec = 9.3 GHz) in Petri's dishes (vol.~45-50 ml.). After EHPP exposure PS temperature rose from 18  $^{\circ}$ C to 26  $^{\circ}$ C. The equivalent heating of the second sample was performed in water bath. After PS treatment the temperature of both of the samples was returned back to 18  $^{\circ}$ C and only after it they were used for perfusion of neuronal bath medium.

**RESULTS:** The effect of heat and EHPP-treated PS on cell volume was studied on 35 neurons. Preliminary heat-treated PS has no significant effect on cell volume, while EHPP-treated PS has the following results: cell volume of 15 neurons was increased, in 6 neurons it was decreased, in 5 neurons it had biphasic (shrinkage-swelling) effect and 9 neurons has no significant volume changes. On figure 1 is presented a picture of the same neuron in normal PS (A), after 2 min. (B) and 5 min. (C) incubation in preliminary EHPP-treated PS. As it can be seen from these data cell volume changes were accompanied by changes of axon diameter in opposite manner, i.e. cell soma increase was accompanied by axon diameter decrease.

**DISCUSSION:** Preliminary EHPP-treated PS-induced cell volume changes and its insensitivity to adequate heat-treated PS can be considered as evidence on the existence of EHPP specific (non-thermal) effect on cell volume, which is realized through EHPP-induced specific structural changes of perused PS. Non-adequate effect of EHPP effect on cell volume can be explained either by initial functional state of neurons or different sensitivity of metabolic cascade of individual neurons to EHPP-induced PS structural changes. From literature data on electrophysiological experiments it is well known that mollusk neurons have non-adequate (membrane hyper-polarizing and depolarizing) responses to different physical and chemical factors. Therefore, these non-adequate responses of cell volume are not unexpected data. However, for final conclusion on the existence of EHPP specific effect on cell volume it is necessary to carry out more detailed investigation of cell volume in combination with other methods giving information on its metabolic activity.

**Hasmik L. Manukyan**  
**The Signatory of the Project Agreement**



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