ISTC Project No. G-1187

Research of different frequency Electromagnetic Field effects on the neuron

Annual Project Technical Report

on the work performed from 02.01. 2006 **to** 02. 01. 2007

Legal entity of Public Law Institute of Cybernetics

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Title of the Project:	Research of different frequency Electromagnetic Field effects on the neuron
Contracting Institute:	Legal entity of Public Law Institute of Cybernetics
Participating Institutes:	Legal entity of Public Law Institute of Cybernetics
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1. Brief description of the work plan: objective, expected results, technical approach

The Project is fully studying of influence of electromagnetic fields (EMF) utilized in GSM cell phone on the single neuron.

The following are the objectives of the Project:

- To investigate influence of EMF irradiated directly from the cell phone on the single neuron habituation ability;
- To investigate influence 900 MHz RF EMF punctuated by Low frequencies on the single neuron habituation ability;
- To study influence of Extremely Low Frequencies Electromagnetic fields (ELF) utilized in cell phones on the single neuron habituation ability.

Expected Results

Project represents applied research**.** Expected results are:

- Project may lead to new knowledge of influence of EMF on neuron activities.
- To expand studies about interaction of Cell Phone irradiation with neuron operations processes. To determine sensitivity of different plastic systems to Cell Phone irradiation;
- To get new knowledge about influence of ELFs utilized in cell phones on the functional operations of the single neuron.
- To get new knowledge about influence of ELFs utilized in cell phones on single neuron plasticity.

Technical approach

Used scientific-technical approaches are defined by the contents of a concrete task and are brought to the following:

Task 1

Stage A-1

- TEM Cell is been designed on the basis of computer modeling. For modelling TEM cell and EM field interaction with living tissue the proprietary computer program package based on the Finite-Difference Time-Domain (FDTD) method for Electromagnetic (EM) and Thermal analysis is developed.
- The powerful 3D geometry editing tool is incorporated into the application.
- Generated grid geometry visualization and editing method is developed.
- The Core EM Solver is used for EM analysis based on the explicit Yee algorithm, for the FDTD solution of Maxwell equations.
- Electromagnetic properties of TEM Cell (hardware) are determined using the HP 8341B Synthesized Sweeper and Automatic network Analyser HP 8757A.

Stage A-2.

- The electrical circuit is designed and created for compatibility of the cell phone antenna circuit with the TEM cell.
- The test card was used to compel Cell phone to irradiate constant power.

Stage A-3.

The specific RF generator (900 MHZ) was designed and created for modeling of cell phone irradiation. Low frequency modulation is performed separately with: 8,34Hz, 2Hz and 217Hz extremely low frequencies (ELF) s.

Stage B. **Stage B-1.**

- Microelectrode technique was used for investigation of rules of habituation to stimulation at sham case.

Right Parietal ganglion (RPG) and Left parietal ganglion (LPG) were used in experiments. Each of the ganglions contains several giant neurons. Neuron #3 of the LPG and neuron #3 of RPG were selected for investigations. The criteria of selection were; size of the neurons-average 150-200 MKM, good pigmentation and invariant electrophysiological properties. Ganglions together with afferent nerves were separated from the body. The connective tissue was carefully removed with fine micro scissors. After this ganglion were placed in the 0, 5% Pronaze solution for 40 min at room temperature. After the proteolytic treatment ganglion were washed several times with pure Ringer solution. . This solution consists of: NaCl - 80 mM, KCl - 4 mM, $CaCl_2$ - 35 mM, $MgCl_2$ 6 H_2O 5 mM, Tris 7 mM, pH=7,5. Left Pallial nerve was used for synaptic stimulation. This nerve has well identified two offsets innerving the muscles. This offsets were soaked into suction electrodes, which in turn were connected to the outputs of the pulse generator. This output was completely insulated from the ground. This measure conditioned invariance of nerve stimulation. For exposure to the EMF ganglion were placed in the special chamber (capacity) made from foam plastic and filled with Ringer solution. Dimensions of the chamber were 8mmx8mmx10mm. Creation of such kind of chamber was conditioned by following circumstances: Electrical properties (dielectric permeability ε and conductance τ) are approximately one and the same for air and for the foam plastic. By these reason electrical parameters of the chamber walls were neglected during SAR modeling.

- The borosilicate tubing (PYREX©,) with filament of Sutter Instrument Co. were used for preparing of microelectrodes ME. In the neuron were immersed two microelectrodes: one for registration, another for applying of the intracellular stimulus (current impulses).
- ME were prepared on the puller ME-4. ME were filled with 2, 5 M KCl solution. Each ME was mounted in special holder containing chlorinated silver electrode. This measure was necessary to reduce contact potential between electrode and solution. Usually contact potential was approximately 1-2 MV and was compensated.
- The PM-20 piezo-micromanipulators were mounted on the basis manipulator and were used for microelectrode impaling into neuron.
- Pico ampere source K 261 was used for applying intracellular currents to the neuron. This device provides constant current flow for very large range of resistances. We decided to apply to the neuron current impulses (trains) with constant amplitude as stimulus. For this purpose the scheme was designed and created. This scheme provides opportunity to convert constant current on the output of the K 261 into impulses with desired parameters.

- Output of the amplifier was connected to oscilloscope - D 1010 HP- for visual observation and to the pen recorder H338/8 for a permanent recording. (It has to be appointed that at this stage of project execution the data acquisition system was not purchased yet.)
- The TEM cell was used for exposure of the ganglion and neurons to RF EMF. Source of irradiation was commercially available cell phone ("Philips twist"). Output of the cell phone via coaxial cable was connected to the TEM cell input. Output power of the phone was not controlled. The output of the TEM cell was loaded with 50 Ohm resistance.
- Commercially available cell phone ("Motorola timeport") was used as irradiate source. Output power was controlled using test card.
- SAR determination and temperature increment was numerically calculated using FTDT method.
- Power emitted by cell phone and time of exposure were altered.
- Data acquisition system "Powerlab ML 866" was used for acquisition of experimental data.
- "Cart 5" software with "Peak parameters extension" was used for data recording and analyzing.

Stage B-2.

- The TEM cell was used for exposure of the ganglion with neurons to RF EMF. Source of irradiation was RF (900 MHZ) generator. Modulation frequency was 2Hz or 8,34 Hz. Output of RF generator via coaxial cable was connected to the input of the TEM cell. The output of the TEM cell was loaded with 50 Ohm resistance.
- SAR distribution and temperature increment were numerically calculated using FTDT method.

- Power emitted by RF generator and time of exposure were altered.
- Habituation dynamics of identified neurons at sham and actually irradiated cases were compared.

2. Technical progress during the first year (for 2nd annual reports)

The year being reviewed is the first for the Project.

3. Technical progress during the year of reference

In the course of I – IV Quarters of the Project were completed the basic part of Task 1 and Task 2 and commencement of the activities envisaged in Task 3.

Task 1

Task description and main milestones	**Participating Institutions**
Task #1- **Preparation of the Experiment Base** • The TEM cell design and creation. Determination of the electrical parameters and features of the TEM Cell. • Modification of the Cell phone for irradiation constant output power. • Creation of 900 MHz RF Generator with low frequency punctuation.	1-Institute of Cybernetics

Description of deliverables	
1	**Scientific-technical reference "TEM CELL modeling for experimental study of electromagnetic field influence on the mollusk neurons."**
2	**Scientific-technical reference "Report of cell phone and 900 MHz generator modifications"**
3	**Scientific-technical reference "Protocol of TEM CELL examination"**

Task 2

Task description and main milestones	Participating Institutions
Task 2, **Investigation effects of neuron exposure to cell phone irradiation:** • Investigation of rules of neuron habituation to intracellular stimulations. • Investigation of influence of cell phone irradiation on the single neuron habituation to intracellular stimulation by current impulses. • Investigation of influence of cell phone irradiation on the single neuron habituation to synaptic stimulus.	1- Institute of Cybernetics

	Description of deliverables
1	**Protocols of Experiments**
2	**Manuscript of the scientific article "**EFFECTS OF CELL PHONE IRRADIATION ON MOLLUSK SINGLE NEURONS" And "NUMERICAL INVESTIGATION OF SAR AND TEMPERATURE RISE IN MOLLUSK NEURON EXPOSED TO ELECTROMAGNETIC RADIATION AT 900MHZ."

Task 3

Task description and main milestones	Participating Institutions
Task #3, **Investigation effects of neuron exposure to punctuated 900 MHz generator irradiation:** • Investigation of influence of 900 MHz RF punctuated by 2 Hz on the single neuron habituation to intracellular current impulses. • Investigation of influence of 900 MHz RF punctuated by 8, 34 Hz on the single neuron habituation to intracellular current impulses.	1- Institute of Cybernetics.

	Description of deliverables
1	**Protocols of Experiments**

Equipment and Materials

The equipment necessary for the Project activities for the 1st year are fully purchased and installed in the corresponding laboratory premises.
The basic part of the required materials is purchased.

Repairing of Experimental Premises

The door from the steel sheets is installed on the room #604 for purpose of saving of purchased equipments.

Stage A – 1.
Design and creation of TEM Cell for neuronal ganglion radiation exposure and dosimetry.

TEM Cell design and creation
TEM Cell for neuronal ganglion radiation exposure and dozimetry was designed and modeled on PC.
While thermal effects are well established, non-thermal effects at present are still subject of investigation. These are mostly complex experimental studies which require very accurate estimation of exposure level and temperature control. In this regard, an accurate dosimetry is one of the key elements.
For dosimetry computer modeling is one of most adopted method. To accomplish this goal user friendly program package based on Finite Difference time Domain Method [1-3] is developed [4-8].

TEM CELL MODELING FOR EXPERIMENTAL STUDY OF ELECTROMAGNETIC FIELD INFLUENCE ON MOLLUSK NEURONS.

Developed Software package

For modeling TEM cell and EM field interaction with living tissue the proprietary computer program package based on the Finite-Difference Time-Domain (FDTD) method for Electromagnetic (EM) and Thermal analysis is developed. This software involves RF radiation and RF power absorption in complex environments including the models of living organisms. Formulation accounts for frequency dispersion and temperature dependence of tissue properties are accomplished, to enable narrow and broadband dosimetry.

In the developed program package problem geometry can be imported from the variety of conventional formats such as STL, NASTRAN or generated manually in the program. The powerful 3D geometry editing tool is incorporated into the application. Generated grid geometry visualization and editing is also available. It allows constructing the complex objects from the simple shapes, thin wires, plates, etc. One can rotate, translate, scale, and deform the objects separately or joined in the group. All performed transformations are stored for possible later modifications. Operation with the object can be performed using mouse and direct keyboard input, as well as using the command console or script file.

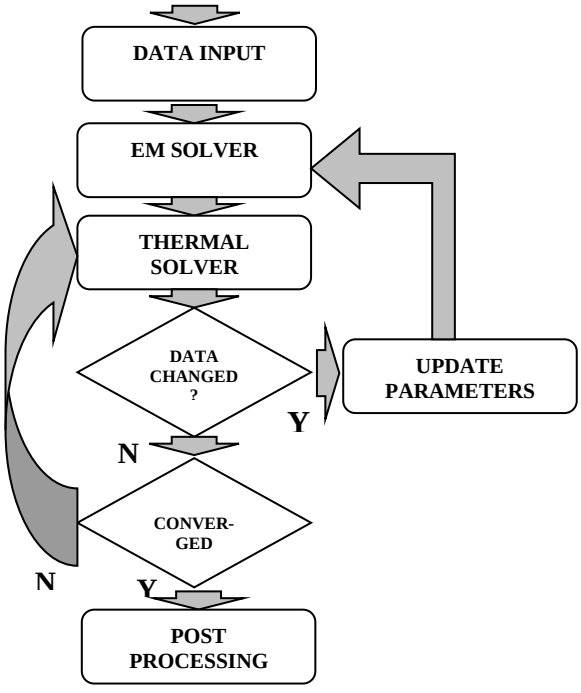


Fig. 1

The Core EM Solver is advanced tool for EM analysis based on the explicit Yee algorithm, for the FDTD solution of Maxwell equations. Developed EM solver allows to use all necessary radiation sources, all geometry structures, allows to define material properties in such way to represent their frequency and temperature effects. Solver can use several boundary conditions, and has advanced features as such adaptive grid. It performs all necessary EM calculations and generates the requested data. Also it has possibility to choose MAS for

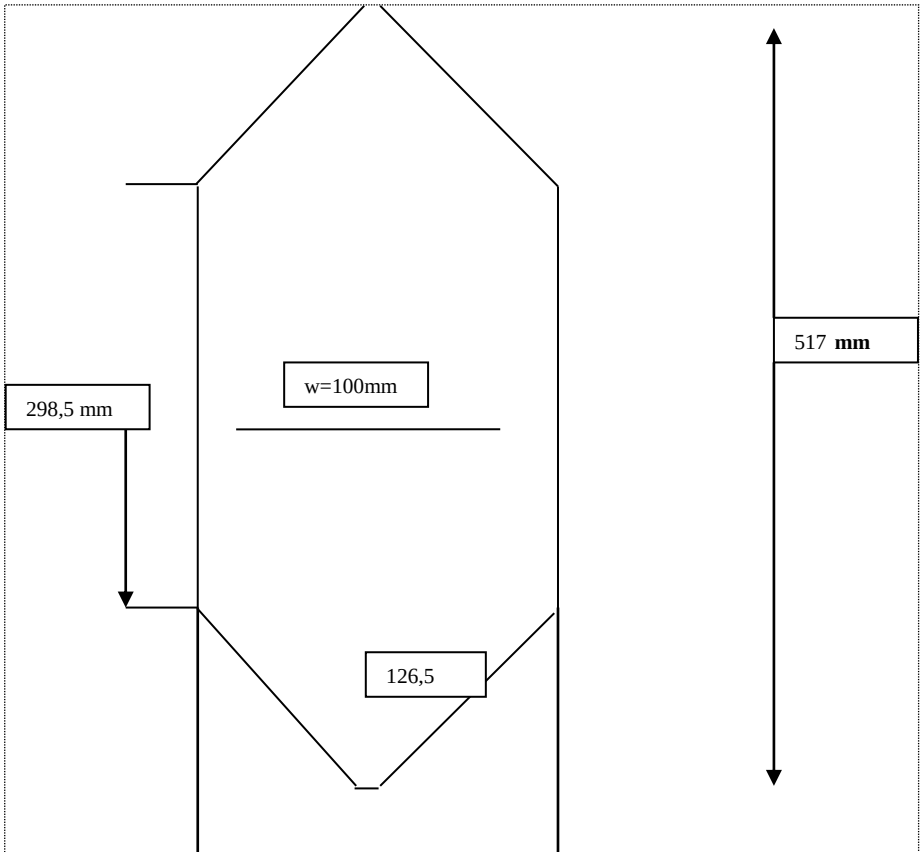
appropriate geometry (partial homogeneous problems) as more effective (less resource demanding and much fast). Once the fields are solved for, all point and integral quantities are derived. In particular, the SAR distribution is determined and the volume averaging operations required to access compliance related quantities are performed.

The output of the EM calculation in terms of SAR distribution due to RF exposure serves as an input data for the thermal solver. The flow chart of the simulation based on proprietary computer program package is shown in Fig. 1.
Temperature rise calculation is based on discretization of Bioheat equation (Pennes model - [1]) with convective boundary conditions. The initial temperature distribution is evaluated. Then temperature rise due to the SAR is obtained based on the calculated equilibrium-state temperature distribution the. If thermo effects lead to the significant change in dielectric properties of materials during the simulation, the process flow can update the parameters and perform the EM calculations with the new properties, until the steady state temperature is achieved.

The process of the calculations is assumed to be script-driven so the implemented in it decision making logic based on EM or thermo data post processing can change the process flow towards the thermo simulation or just the input data correcting and EM problem re-calculation. This makes program more extendable. Pos-processing block allows representing all calculation results in convenient way.

TEM Cell Modeling

Large scale rectangular transmission lines are widely used for maintaining Transverse Electromagnetic field. TEM cell is characterized by air dielectric and thin centered conductor called septum shielded with another rectangular conductor. Goal is to have uniform Transverse EM field in studied test space of the transmission line. Properties of such rectangular transmission lines are discovered and studied by Crawford [2], Claudie [3] and others. To create TEM cell working with cutoff frequency at 900 MHz we have followed Claudie [4]. In notations of [11] parameters of designed TEM cell are given on Fig 2. and Fig. 3



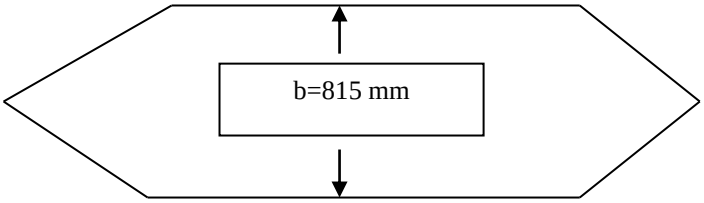


Fig.3

thickness of septum t=1mm. Changing of w we found optimal size of septum for 900MHz. For validation of TEM cell FTDT simulation was applied. Excitation between septum and walls of TEM cell is applied as 1V voltage to R=50 Ohm resistance. On other end of septum we have another 50 Ohm resistance to maintain impedance matching. On fig 4-5 Modeled TEM cell is presented.

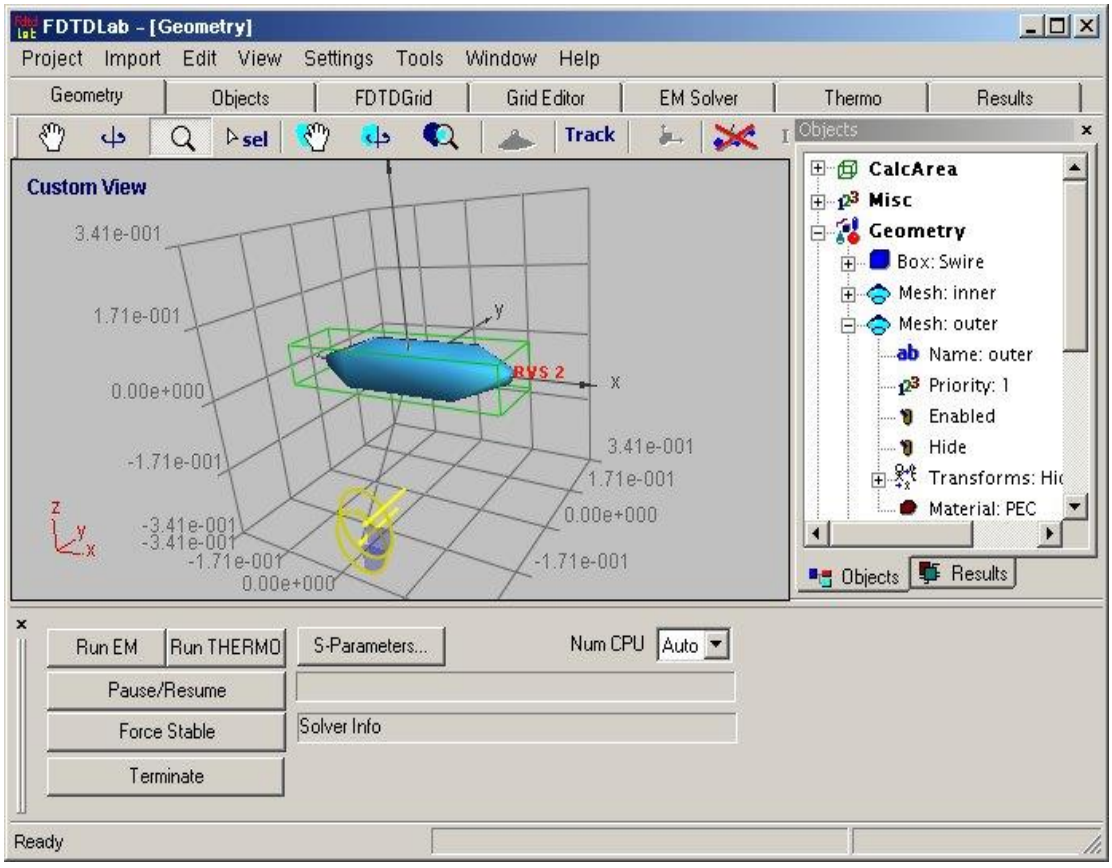


Fig.4 . TEM Cell view in modeler

Ez component of simulated field is presented on Fig. 6. Impedance, Power and S11 parameters are calculated for each R element. As results show SWR for such TEM cell is 1.30 and transmitted power 98,4%. As simulations show (Fig. 7) we have uniform field (acceptable for experiments) in middle of TEM Cell 4cm x 4cm x 2cm area. This completely satisfies our goal for mollusk experiments and modeling.

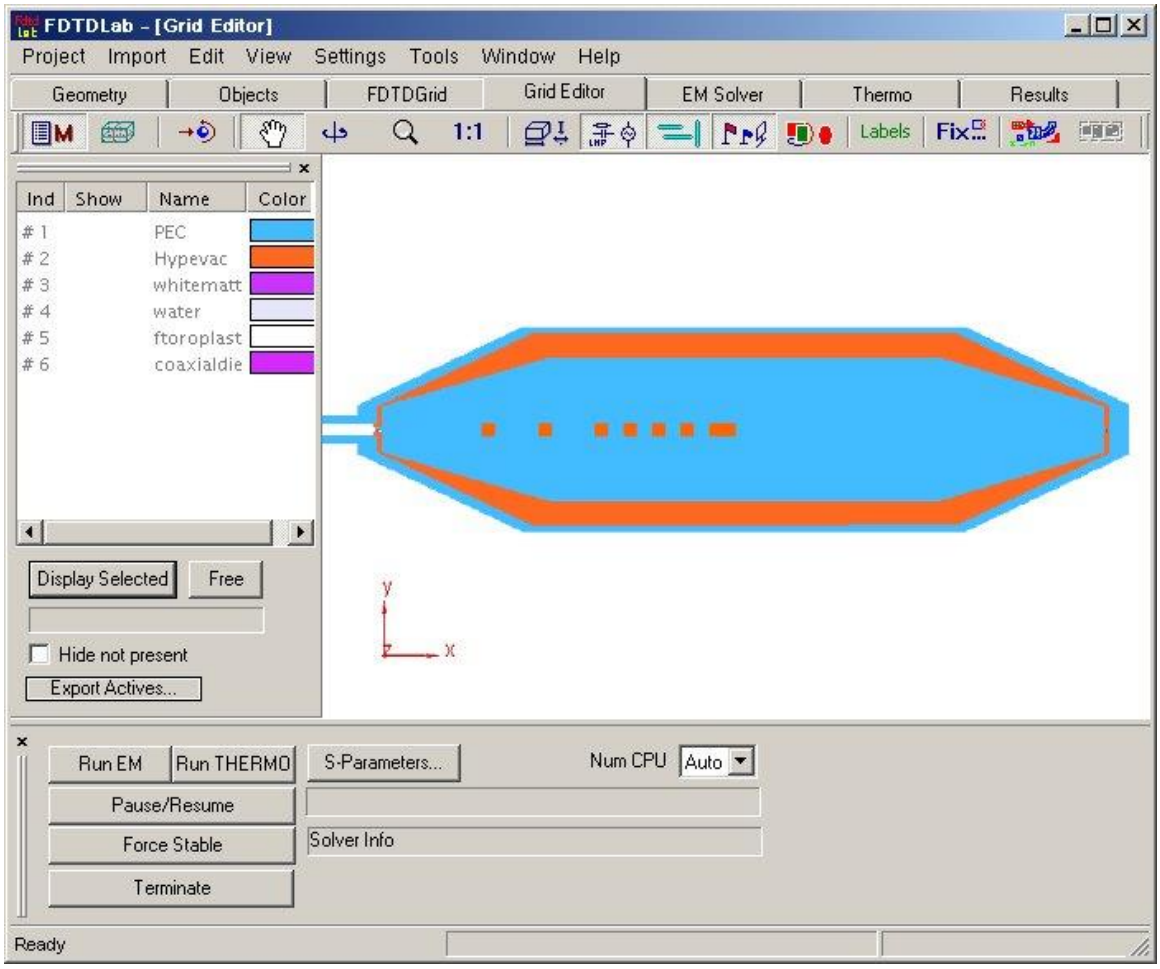
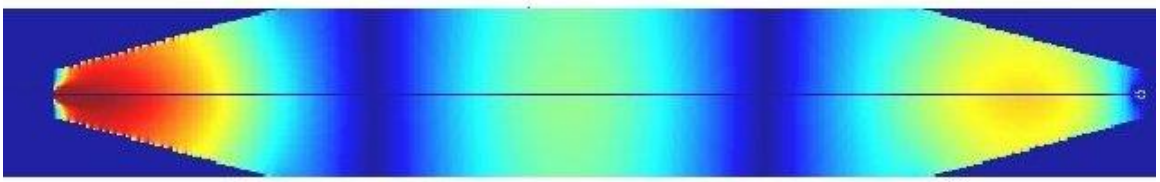
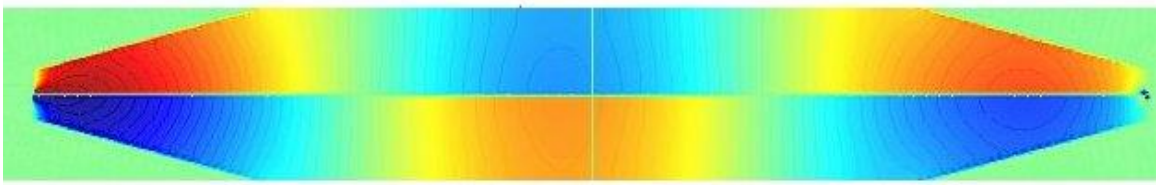


Fig.5 Generated FDTD grid (Top view).

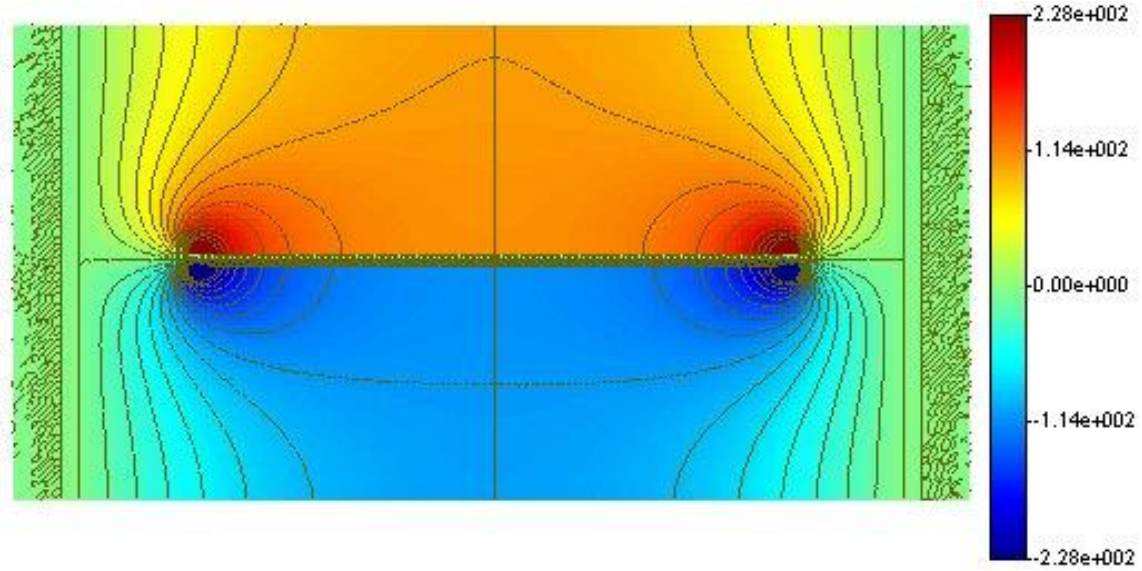


a)

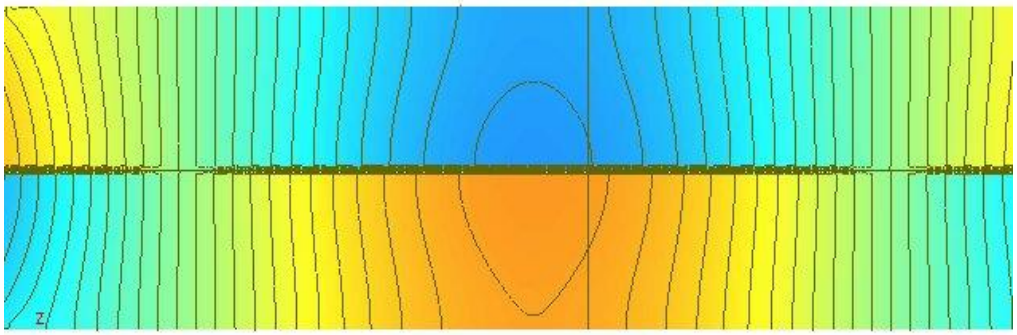


b)

Fig.6 . Ez Field propagation in TEM cell in different color palettes a) and b)



a)



b)

Fig.6 . Ez Field propagation in the middle of TEM cell in Y
a) and Z cross sections b)

References.

1.

2. H. H. Pennes, “Analysis of Tissue and Arterial Blood Tempera ture in the Resting Human Forearm,”J. of Applied Physiology, Vol. 1, pp. 93-102, 1948.
3. M.L. Crawford, “Generation of standard EM fields using TEM transmission cells.” IEEE Trans. Electromagn. Compat. Vol EMC-16,pp. 189-195,Nov 1974
4. M. Claudie, “The characteristic impedance of rectangular transmission lines with thin center conductor and air dielectric.” IEEE Trans. Microwave Theory Tech., Vol. MTT-26, N4 pp 238-242., Apr 1978

Creation of the TEM Cell

TEM CELL hardware was created on the basis of developed software and computer modeling. Dimensions of the septum were determined experimentally also. Very important electromagnetic characteristic of the TEM Cell is ratio of standing waves (SWR). SWRs for the wide range of electromagnetic waves were measured. For this purpose were used The HP 8341B Synthesized Sweeper and Automatic network Analyzer HP 8757A

Result of measurements is given in the table 1. Using formula (1) ratio P_{in}/P_{out} was calculated for all the range of electromagnetic waves:

$$P_{in}/P_{out} = 1 - [(SWR-1)/ (SWR +1)]^2 = 4\ SWR / (SWR +1)^2 \qquad (1)$$

Where SWR is standing waves ratio,
P_{in} is input power in to the TEM Cell
P_{out} is output power in the TEM Cell.
Results of measurements are given in table #1

Table #1

Frequency MHz	KSW	Pin/Pout
750	1,413	0,97
795	1,393	0,97
830	1,372	0,98
870	1,353	0,97
905	1,300	0,98
915	1,315	0,98
935	1,448	0,96
985	1,480	0,96
990	1,481	0,96
1000	1,519	0,95
1050	1,743	0,92
1025	1,678	0,93
1850	1,316	0,98
1295	1,501	0,95
1300	1,274	0,98
1460	1,741	0,93
1580	1,381	0,97
1630	1,911	0,90
1680	2,215	0,85
1950	2,20	0,85
2200	2,355	0,83
2370	1,80	0,98
2380	1,225	0,91

The table clarifies that maximal conductivity of electromagnetic waves is at frequency range of 830-915 MHZ.
Thus, the works of Stage A-1 are fully completed.

Stage A-2. Modification of Cell phone for constant power irradiation.

The commercially available cell phone "Phyllips twist" was used as irradiation source at the first stage of experiments. A compatible circuit was designed for connection of cell phone antenna circuit to the TEM Cell input. The linkage coaxial cable was ended with N type socket for connecting to the TEM Cell.
Regulation of compatible circuits was performed for achievement of certain compromise: On the one hand it is necessary good compatibility for achievement of maximal power irradiation. On the other hand it is necessary to receive signals from the base station. Possible minimal signal has to be set out to the receiver. This provides maximal power emission on the output of device.
At the cecond stage of experiments for irradiation was used cell phone "Motorola timepotr". The cable was connected by the analogues way, however, the emmitted power was controlled by test card using controlling commandds.

For neuron intracellular stimulation with current impulses it was necessary to modulate the output of Picoampere source K 282. For this purpose: The relaxation generator was designed and created. The buffer and waiting generators determines the frequency of repeat. Time of each generator is regulated. Cut is performed by magnetically operated sealed switch (MOSS) relay. A connection to output circuits is performed with screened cables.

Thus, the works of Stage A-2 are fully completed.

Stage A-3. Creation of 900 MHz generator for modeling cell phone irradiation
Tasks of the stage B-2 conceives irradiation of the neuron with 900 MHz EMF separately modulated with low frequencies: 2Hz, 8,34 Hz or 217 Hz. For this purpose was created 900 MHz generator, modeling the cell phone irradiation. All ELF are present simultaneously in real cell phone. Designed generator provides modulation of RF 900 MHZ separately with different ELF. Device is completely autonomic and has regulated parameters. Outputs of the generator are connected to the counted decoder. Shallowness is selected by the means of switchers. Meanings of shallowness 1/8 and ¼ are correspondly selected. VCO900 is regulated generator, which controlls output PF0814 cascade. Comperaison of so called "falled sygnal" and controlling levels is performed by the means of logarithmic detector PF0814 and directed splitter from the output. This conditiones stabilization of output sygnal. Feeding of performed with 220->8V pulse stabilizator. Additionnal 7805 5V feeds common part. VCO is feeded with 3,3V stabilizator.

Stage B-1.

Comparison of sham and actually irradiated neuron habituation abilities.

Investigation of habituation rules to stimulation.
First part of the Task 2 conceives investigation of rules of neuron habituation to intracellular stimulations (sham irradiation).

It was shown that neurons reaction depends on the stimulus parameters. Stimulus (outward current impulse) amplitude and duration determines the level of neuron reaction. Neuron's simplest reaction on the stimulus (ST) is that, neuron generates one action potential (AP) on one intracellular current impulse. Such reactions could be achieved by gradually increasing of stimulus amplitude from the very low meaning to threshold one. With repeat of ST apply neuron declines some stimulus and habituation is beginning (i.e. neuron does not react some

stimulus with AP). This event might be regarded as partial habituation. Continuing of apply of intracellular stimulus causes gaining of the process i.e. more stimulus are declined by neuron. At last appears situation, when neuron declines all applied intracellular stimulus. This might be regarded as complete habituation, i.e. neuron does not respond the ST with APs at all. Rest causes partial recovery of reactions (i.e. de-habituation). However, continuing of stimulation causes relief the new habituation. If such series of ST are applied for several times, it could be reached situation when neuron declined all stimulus, i.e. rest does not cause dehabituation. One sample recording of habituation dynamics of sham irradiated neuron is shown in the fig. 7.

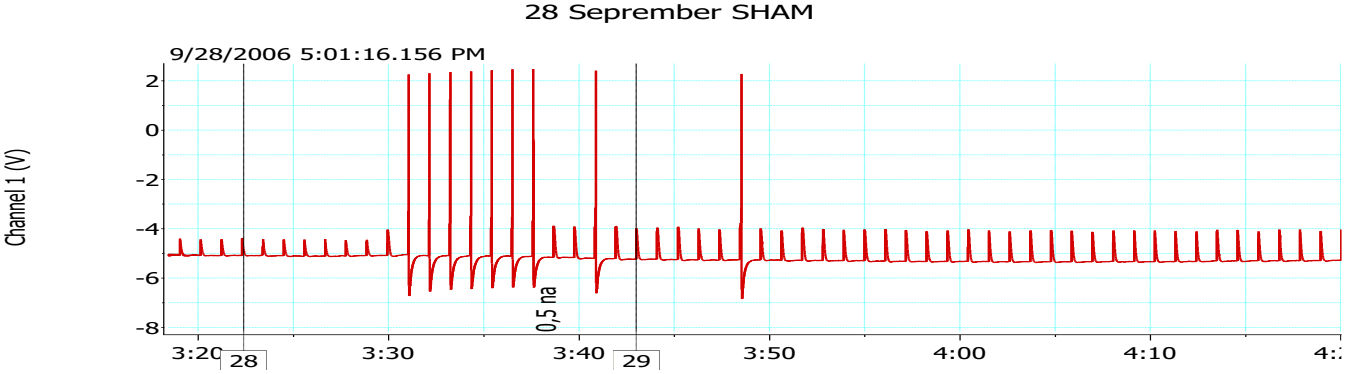


Fig. 7. ST with the amplitude of 0,1 NA was below the threshold. Stepping increase of ST amplitude reveals that threshold meaning is 0,5 NA. Complete habituation is arises in 47 seconds. On the abscises axes is plotted the time in seconds, on the ordinate voltage. One division on Y axes corresponds to 10 MV.
The moments of ST apply is not appointed on the recordings, since they correspond to artifacts on recordings.

Increase of latency period is proved o by the chart of dependency of latency and threshold from the AP number, given in the fig.8.

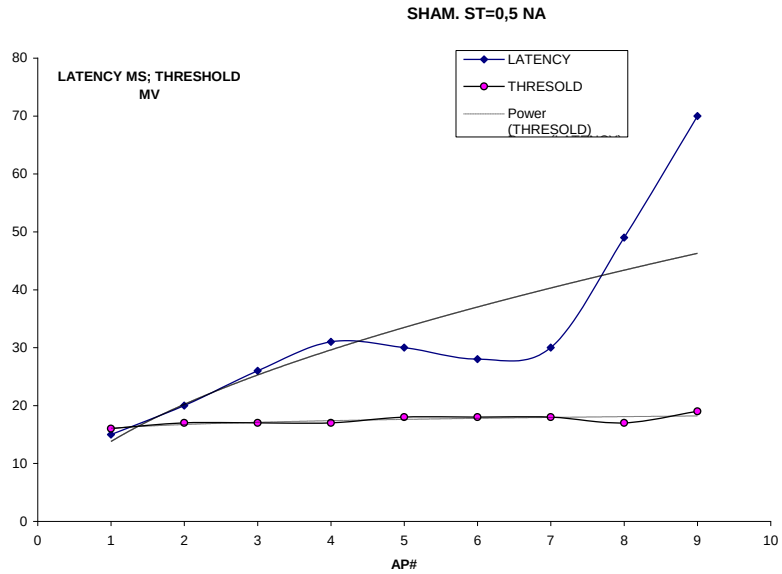


Fig. 8. Latency and threshold dependence on the AP #.
Lines without dots are trends for latency and threshold, calculated by PC.

Exploring of peak parameters of the action potentials revealed that these parameters are changed (increased) slightly. For example the width of the APs at all 20 and 50 % levels increases, however this gain is tenth parts of msec. The same could be said about rising and falling times of the action potentials.

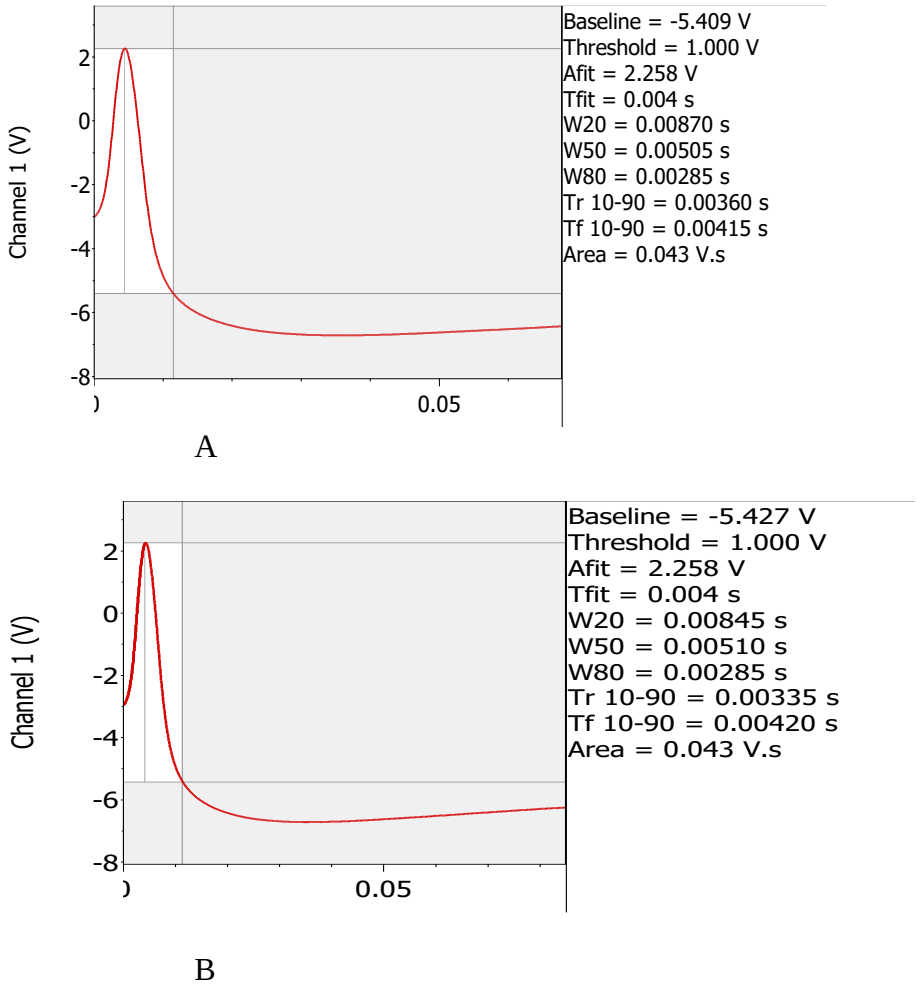


Fig. 9. A-peak parameters of the 1st AP. B- peak parameters of the last AP in reactions.

Rest usually facilitates recovery of reactions on stimulus, however new habituation arises much sooner.

Effects of exposure of the neuron to RF EMF of cell phone in the TEM Cell.

Intracellular stimulation

Research of single neuron plasticity to intracellular stimulus. Exposure source - cell phone irradiation.

Second phase of the stage B-1 conceives investigations of influence cell phone irradiation on the single neuron habituation to intracellular stimulations with outward current impulses. Researched ganglions were placed in the center of the TEM Cell for RF EMF exposure. SAR depends on the power emitted by cell phone. Firstly was investigated influence of cell phone irradiation effect on the neuron plasticity when cell phone is switch on into GSM net. Cell phone was shielded with screen. This conditioned maximum power emission. Correspondingly SAR was biggest in such circumstances.

On the next phase the test card was used. The test card provided constant power emission regime for cell phone. Effects of these two series of experiments were similar. Namely: At high

(possible maximal) SARs neurons reaction on intracellular stimulations are prolonged for several dozen minutes.

Effect of maximal power emission. Maximum power carrying by electromagnetic waves in the TEM cell, is equal to 1,94 watts. Exposure time was 60 min. Ganglion is modeled as cylinder with diameter of 2,5 mm and high-2mm. Calculation of SAR using FTDT method gave that SAR=0,63 W/KG. Temperature increment calculation gave that ΔT= 0,1 º C. Results of modeling are shown in the fig.10.

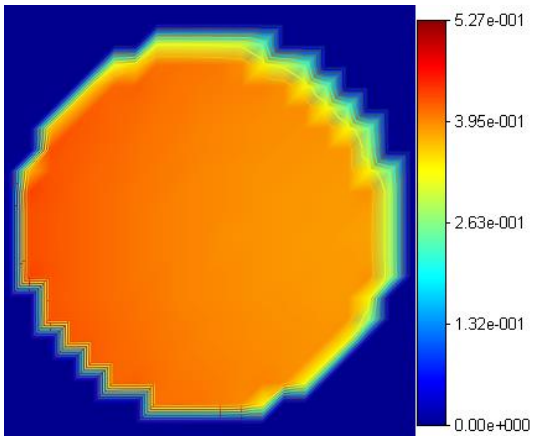


Fig.10. a) XY plane view of SAR distribution

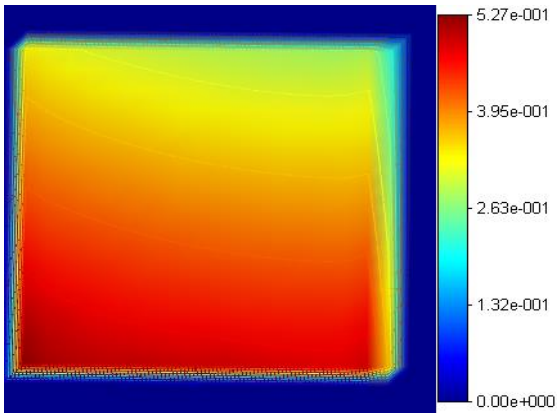


Fig.10.b) XZ plane view of SAR distribution

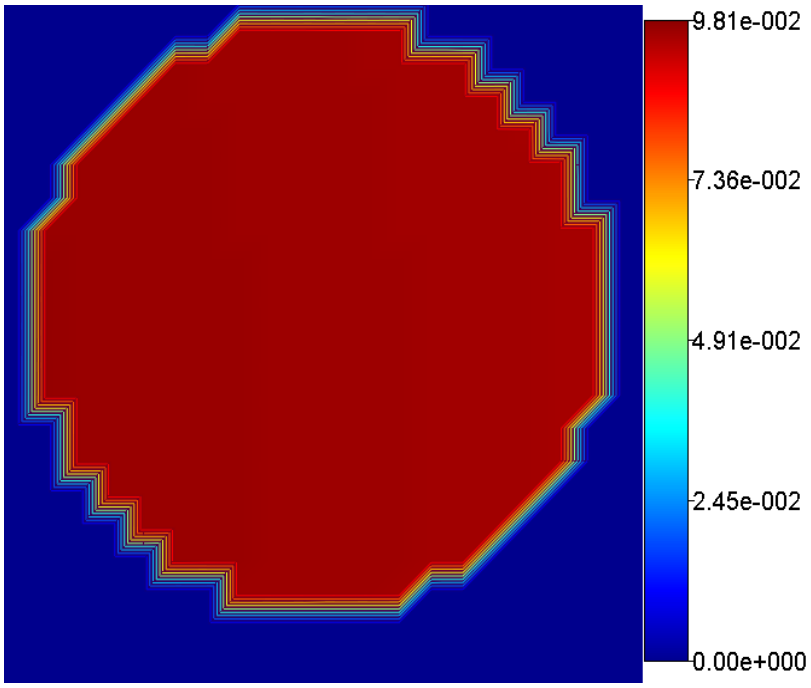


Fig10.c. Temperature rise in mollusk ganglion. XY plane view

Cell phone irradiation effect was habituation ability loss by neuron. Neuron continues to react on the ST more than for 20 min. Latency suffers irregular variation, however, trend of the graphic does not show tendency of increase. The threshold deviation is extremely small and the threshold might be regarded as constant.

Fig 11. A – illustrates sample recordings of the neuron reactions on the intracellular stimulus at the 1st min of stimulation. Intracellular current impulse amplitude consists 0,1 NA, duration of each impulse was 4 msec. B - illustrates neuron reactions on ST at 9th min of stimulation; Reactions at 20th min of stimulation are shown in the fig. 11 C. Absence of habituation is obvious.

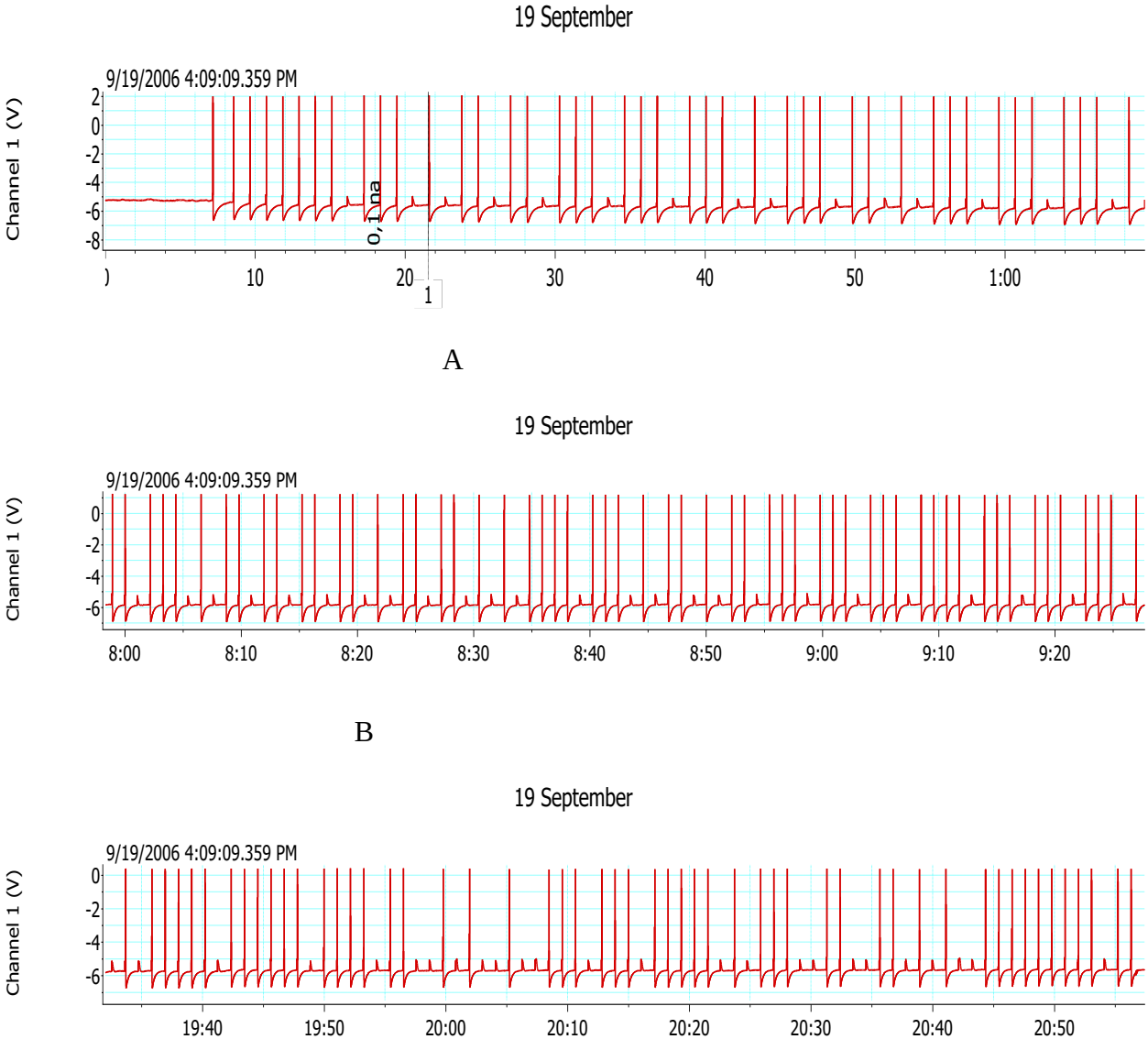


Fig. 11.
Neuron was exposed to cell phone irradiation in the TEM Cell. Exposure time 60 min. Neurons reactions on stimulations with 0,1 NA intracellular stimulus.
A - reactions at 1st min of stimulation. B-reactions at 9th min of stimulation. C-reactions at 20 th min of stimulation. Absence of habituation.

On the abscises axes is plotted the time in seconds, on the ordinate voltage. One division on Y axes corresponds to 10 MV.

Dependence of latency and the threshold on the AP number is shown in the fig. 12. Latency suffers irregular variation, however, trend of the graphic might be regarded as parallel to the abscises axis i.e. does not show increase trend. A variation of the threshold is neglect small.

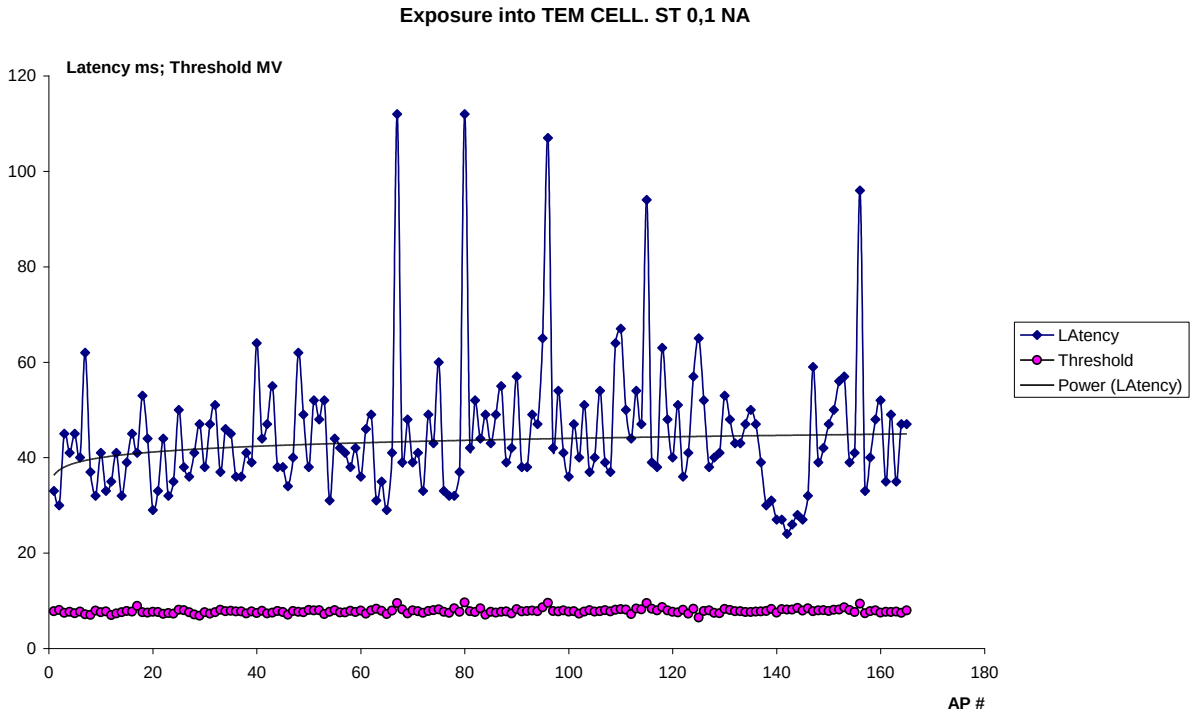
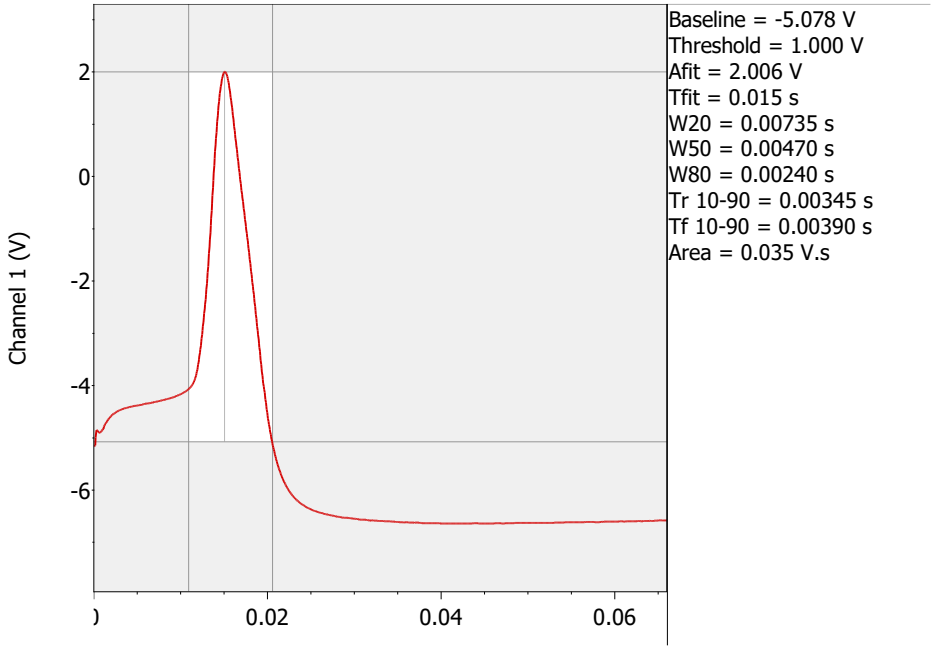
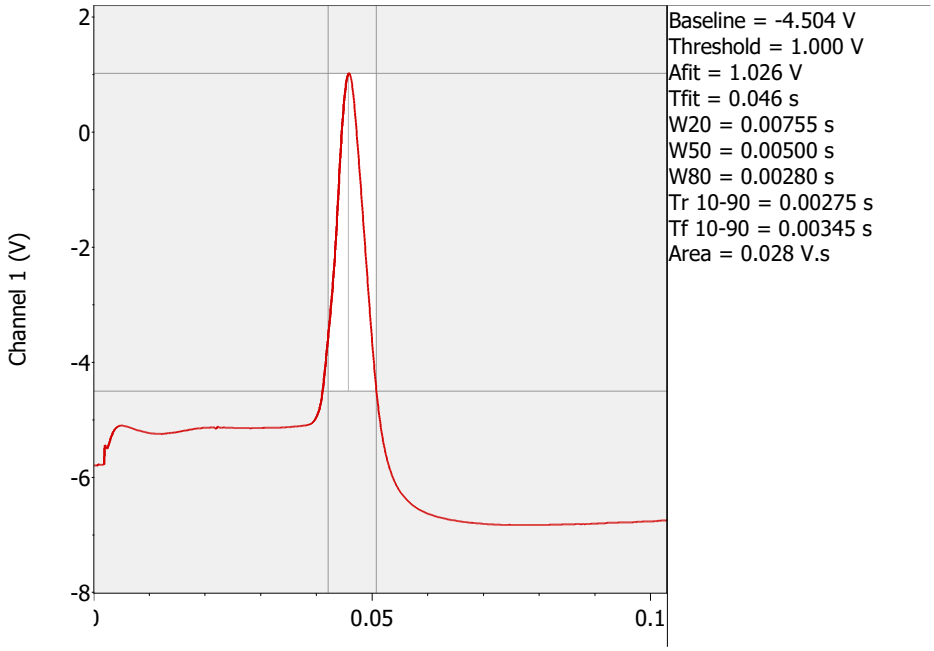


Fig. 12.
Dependence of latency and threshold on the AP number. (SAR 0,67 w/kg. Exposure time 60 min.).

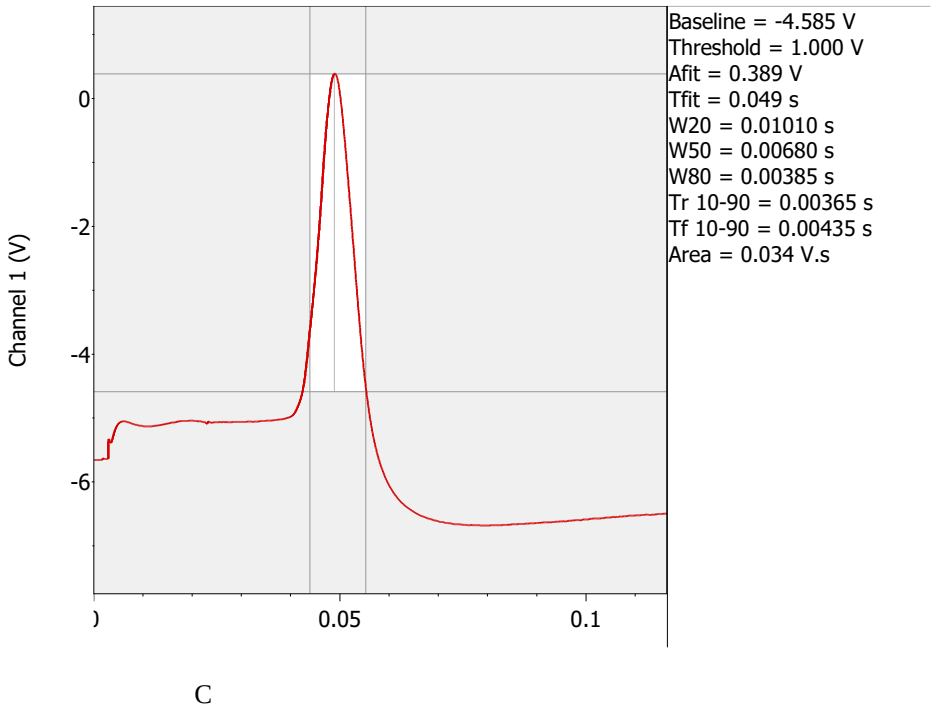
Comparison of peak parameters of the APs at the beginning middle and end of stimulation show that changes of the parameters are not significant: Width of AP suffers some increase, Increases also rising and falling times of the APs however absolute meaning of this changes are small.


Channel 1 (V)
2
0
-2
-4
-6
0.02
0.04
0.06
Baseline = -5.078 V
Threshold = 1.000 V
Afit = 2.006 V
Tfit = 0.015 s
W20 = 0.00735 s
W50 = 0.00470 s
W80 = 0.00240 s
Tr 10-90 = 0.00345 s
Tf 10-90 = 0.00390 s
Area = 0.035 V.s

A


Channel 1 (V)
2
0
-2
-4
-6
-8
0.05
0.1
Baseline = -4.504 V
Threshold = 1.000 V
Afit = 1.026 V
Tfit = 0.046 s
W20 = 0.00755 s
W50 = 0.00500 s
W80 = 0.00280 s
Tr 10-90 = 0.00275 s
Tf 10-90 = 0.00345 s
Area = 0.028 V.s

B



C

Fig. 13 A - peak parameters of the 1st AP; B- peak parameters of the AP at 9th min of stimulation. C- Peak parameters of the AP at 20th min of stimulation.

In the table 1 is given ratios of peak parameters for AP's of different stimulation moments.

Table 1.

	W20	W50	W80	Tr	Tf
AP_9/AP_1	1,1761	1,111	1,26	1,021	1,059
AP_{20}/AP_9	1,20	1,32	0,79	1,1505	1,20
AP_{20}/AP_1	1,4150	1,4747	1	1,2142	1,4150

Remark: APn is action potential at n-th min of stimulation

We explored effects of SAR change. It was established that when SAR was lowered neuron reacted to the stimulus for minutes, however at least habituation took place. It worth mentioning, that in any case of EMF exposure time necessary for arising of habituation was much greater, than in the sham case.

Comparison of sham and actually irradiated neurons habituation ability to the synaptic stimulations:

Sham irradiation case

For stimulation was selected Left Pallial nerve LPN (out-coming from Left Parietal ganglion). Selection was conditioned by following circumstances: This nerve has two very well identified offsets, which innerves muscles. This two offsets were soaked into pear of glass suction

electrodes, which in turn were connected to stimulator of voltage impulses. Apply stimulus on this offsets give opportunity to make invariant stimulation operation from animal to animal. Frequency of stimulus (ST) voltage impulses was1,26 Hz, duration of each square voltage impulse was 30 msec. The outputs of generator are insulated from the ground. Stimulation conditions were invariant.

Sample recordings of neuron habituation dynamics to the nerve stimulation at sham irradiation case is sown in the fig. 14. Amplitude of stimulant impulses was 0,8 V. It is clear, that neuron began to decline ST completely at 54th second of stimulation-fig. 14A. 2nd series of ST was applied after 21 min of rest. Simulation was not reacted with APs. Habituation was complete. This event is shown in the fig.14 B. Increase ST to 2V caused firing only one AP, i.e. habituation is saved completely- fig. 14 C.

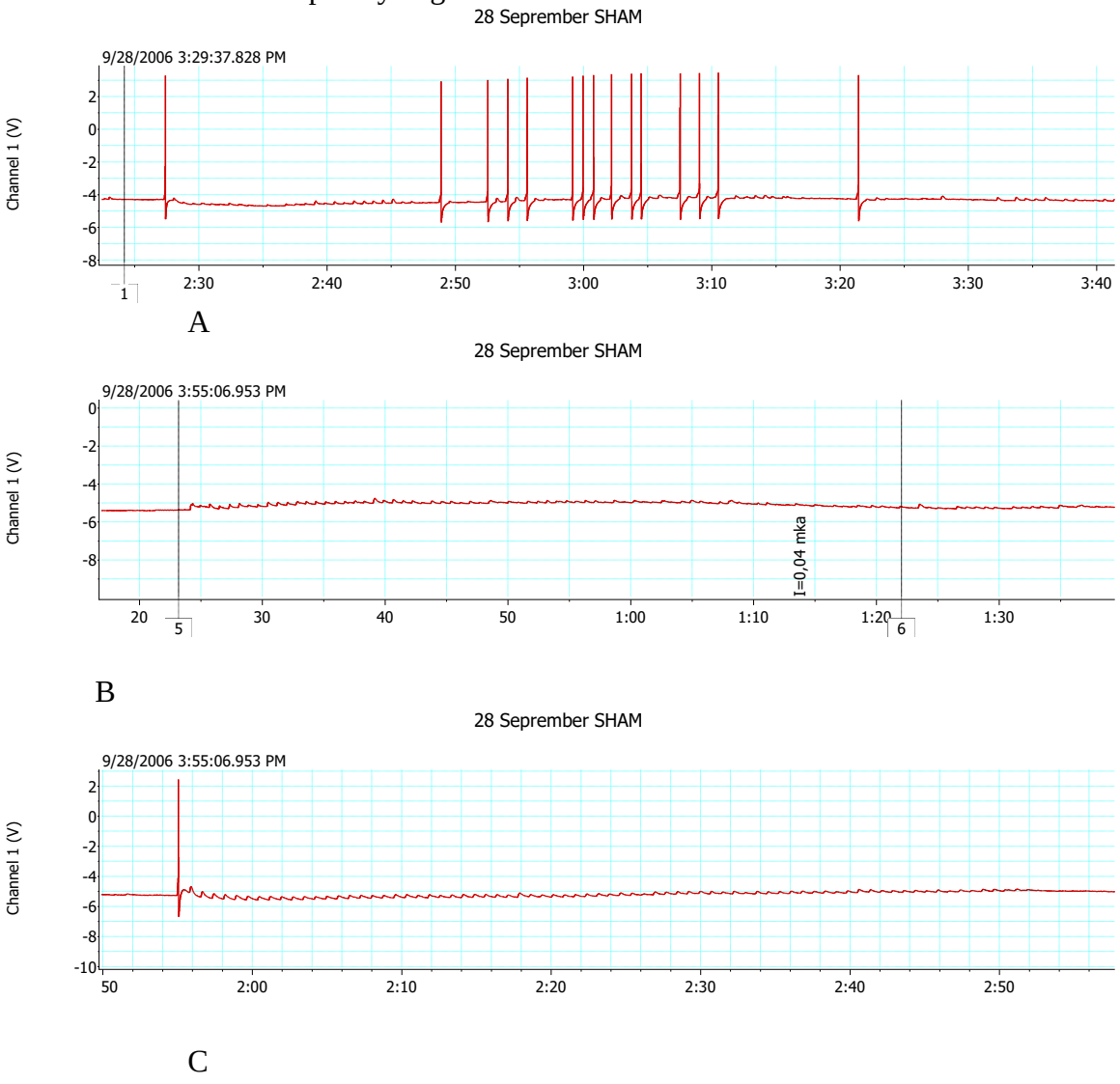


Fig. 14. A-habituation to ST 0,8 V impulse train, applied on LPN. B- Repeat of the ST series after 22 min of rest did not cause neuron AP reactions. Habituation is saved. C- Increase ST up to 2V caused generation only one AP; i.e. habituation is saved.
On the abscises axes is plotted the time in seconds, on the ordinate voltage. One division on Y axes corresponds to 10 MV.

Peak parameters explore revealed that changes are not significant:

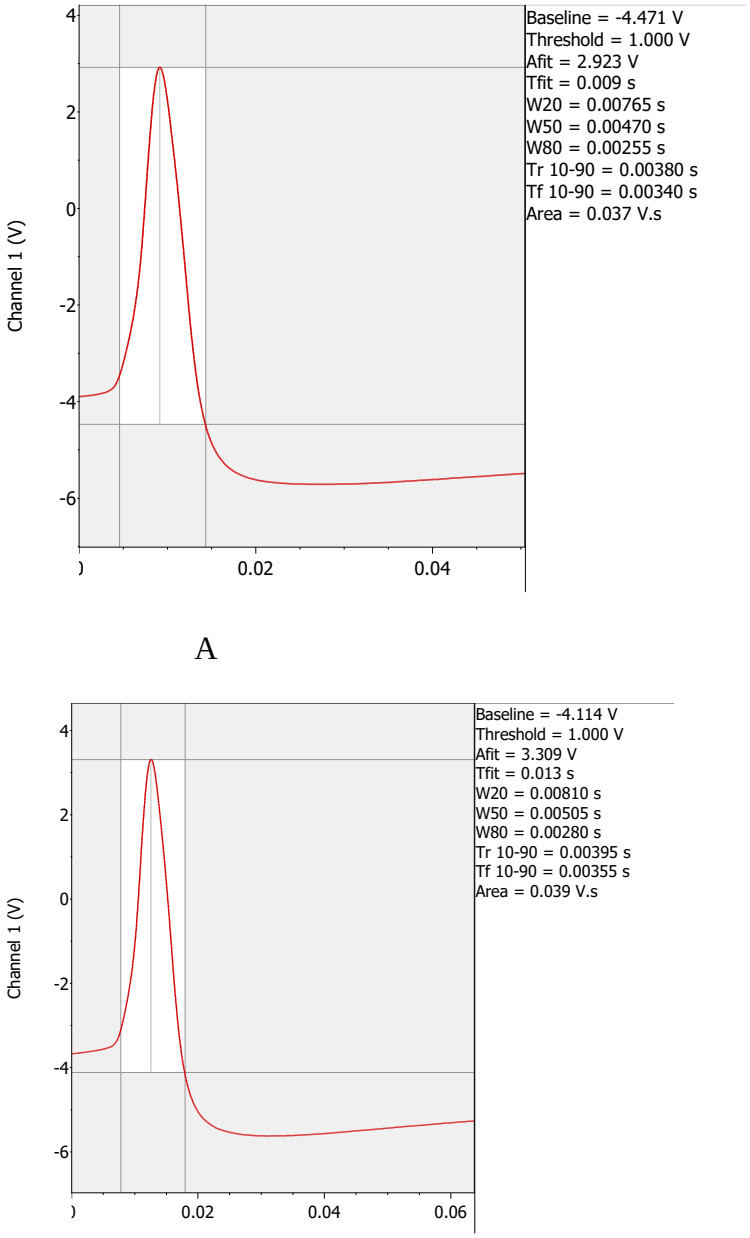


Fig. 15. A- peak parameters of the 1st AP in reactions. B- Peak parameters of the last AP in reactions.

Effects of exposure to EMF in the TEM Cell

Exposure to the EMF of the cell phone in the TEM Cell causes prolongation neuron reactions for many minutes, in opposite to sham case when during 1 minute habituation arises. An example recording of such reactions is shown in the fig. 16. It worth mentioning, that the view of reactions remains unchanged. In both cases two phases with AP firing are divided by silent on background of EPSPs.

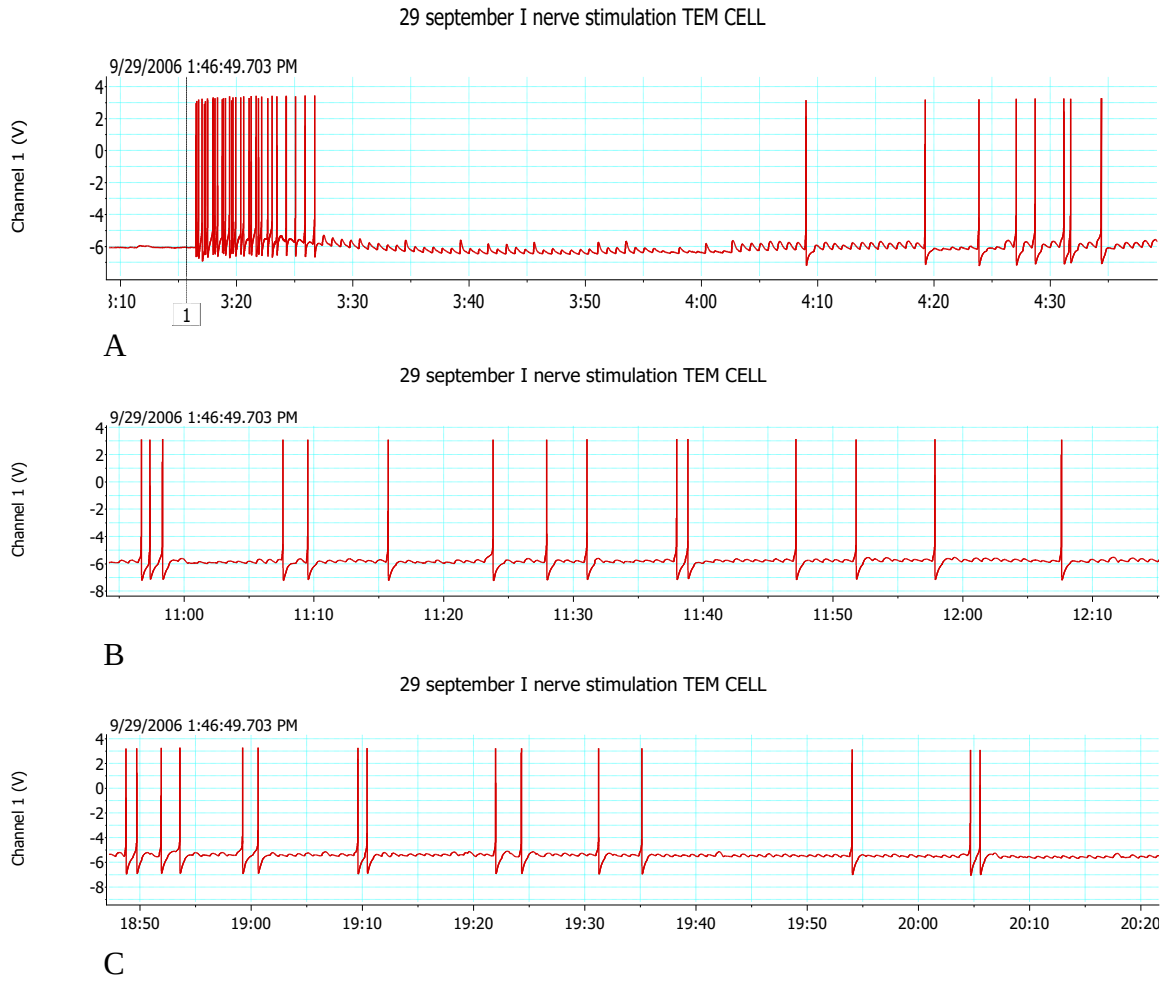
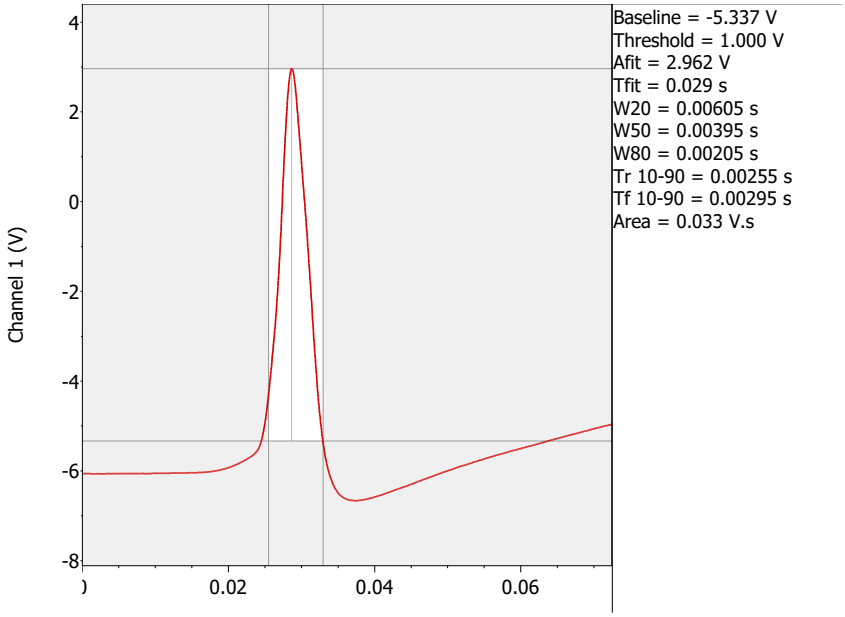
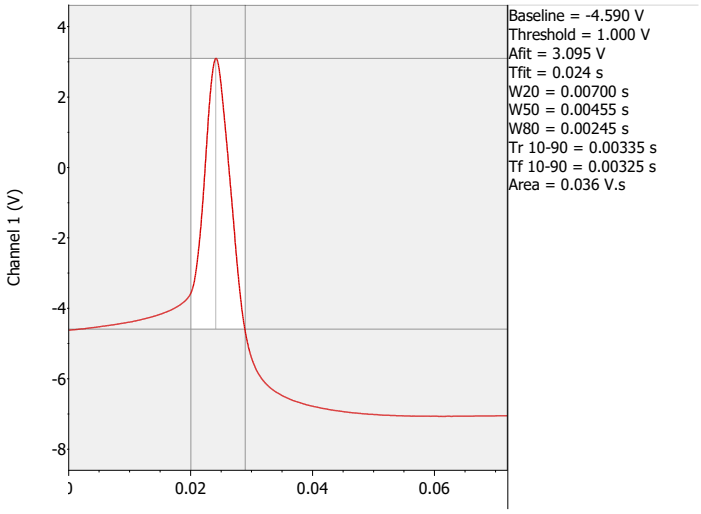


Fig. 16. Effect of Exposure in the TEM CEL. SAR=0,63 w/kg. Temperature increment ΔT=0,1ºC.
A-reactions at the 1st min of stimulation.
B- reactions at 8th min of stimulation.
C – reactions at 15th min of stimulation.
On the abscises axes is plotted the time in seconds, on the ordinate voltage. One division on Y axes corresponds to 10 MV.

Peak parameter analyze revealed that changes are in all cases similar to each other and not significant.



A



B

Fig. 17 A- peak parameters of the 1st AP in reactions.
B- peak parameters of the AP at 15th min of stimulation.

In the case of smaller SAR (SAR 0,33 w/kg ΔT= 0,05ºC, exposure time 62 min) habituation appeared. However, repetitions of the stimulus (after some rest) cause increase of reactions and consequently harden of habituation. This is opposite to sham habituation order, according which repeat of stimulation does cause de-habituation and neuron does not react on the same stimulus with APs. As in previous cases stimulus represented train of voltage impulses applied on LPN.

Stage B-2

Effects of exposure of the neuron to 900 MHz EMF modulated by 2,0 HZ and 8,34 HZ in the TEM Cell.

This stage of project conceived use as irradiate source 900 MGZ generator. Modulation of the generator had to be performed with low frequencies separately. In this series experiments modulated frequencies were 2,0 Hz or 8,34 Hz.
Below is illustrated effect of 900 MHz EMF modulated with 2,0 HZ low frequency. Exposure time was 60 min. Numerical determination of SAR and ΔT gave following: SAR=0,65w/kg, ΔT= 0, 11ºC
Examined neuron is #3 of RPG. At the beginning neuron expressed background activity for short period -2 min. 54 s after which it turned on into silence -fig. 18 A. Stimulation was launched from 0,1 NA, but is occur under threshold. Increase amplitude of intracellular stimulus impulses up to 0,3 AN caused firing of few APs. By this reason amplitude was risen to 0,5 NA. Reactions appeared – fig 18 B. However, in 2 min 20 seconds neuron began to decline stimulus and complete habituation was arisen fig. 18 C.

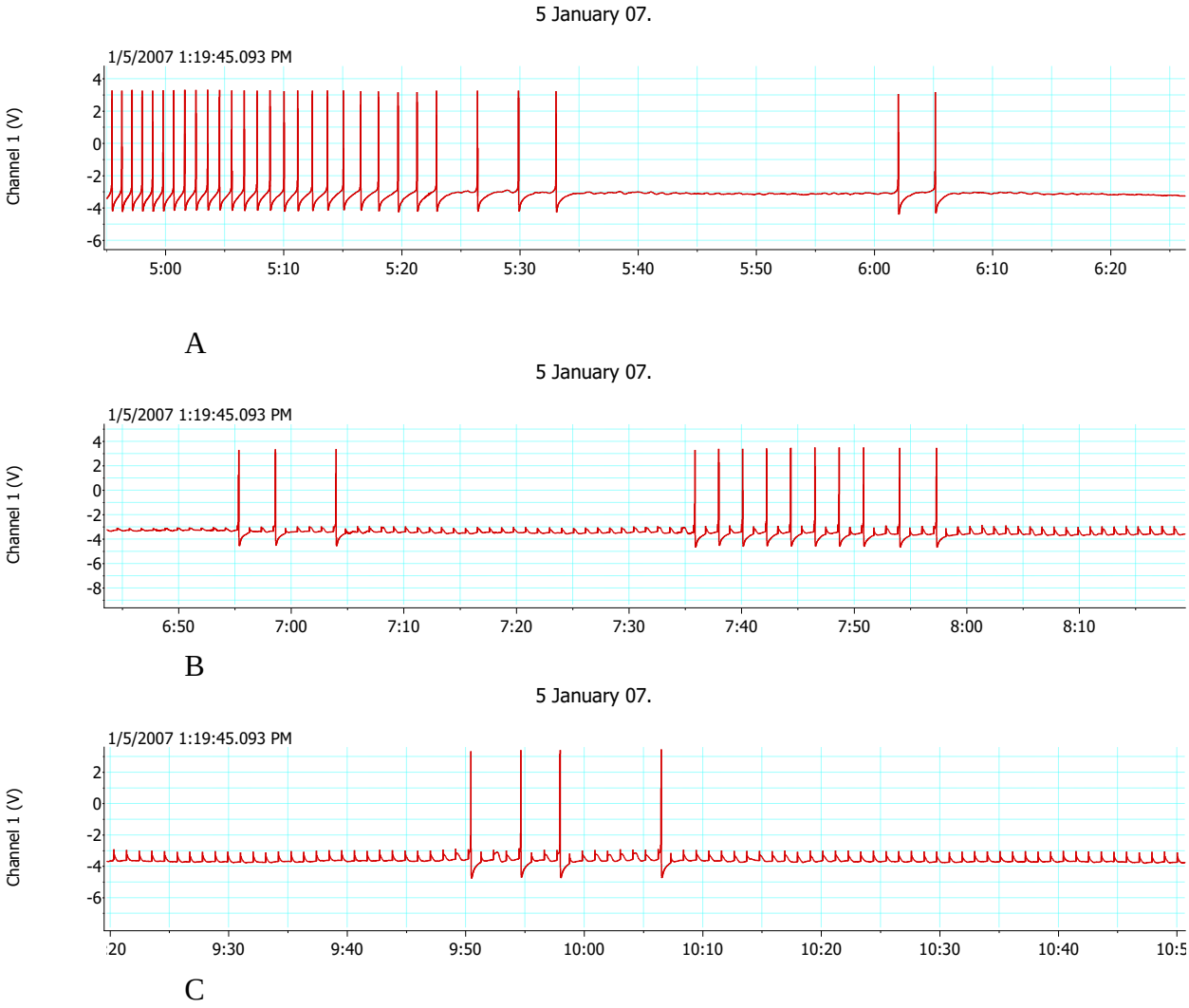


Fig. 18.

A- background activity of the neuron after immersing MEs into neuron.
B- Firs 3 APs are reactions on the ST with amplitude 0,3 NA, following 10 APs are reactions on the ST with 0,5 NA amplitude
C- Beginning of compete habituation on the same stimulus. This is continuance of recordings B.
Axis X corresponds to time in seconds, Axis Y corresponds to voltage – one division equals to 10 MV.

Latency dependence is smoother graphic with comparison to latency dependences for same neurons, which were irradiated with real cell phone. Only at the end of this graphic is observed small deviation - fig. 19: Trend of this graphic represents increased function.

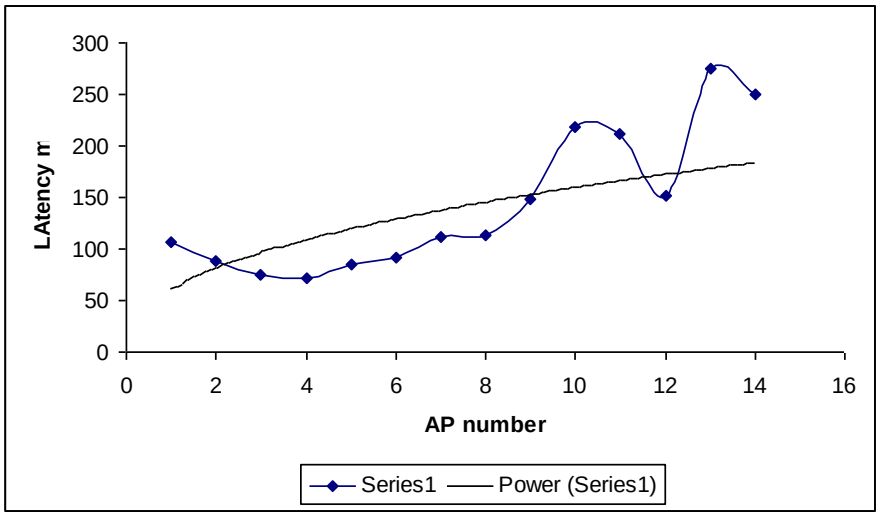


Fig. 19.
Latency dependence on AP number for neuron reactions shown in the fig. 34. Series 1 corresponds to latency; Power is trend calculated by PC.

Dependence of the threshold on AP number is shown in the fig. 20. No deviations are noticeable as in latency case and the trend is parallel line to OX axis i.e. average meaning of the threshold remains constant.

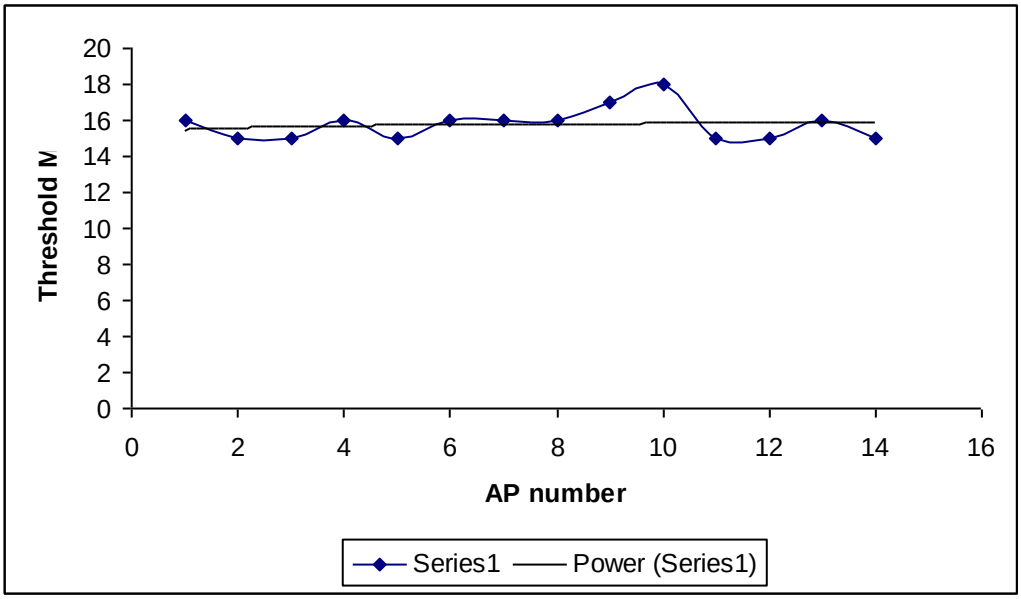
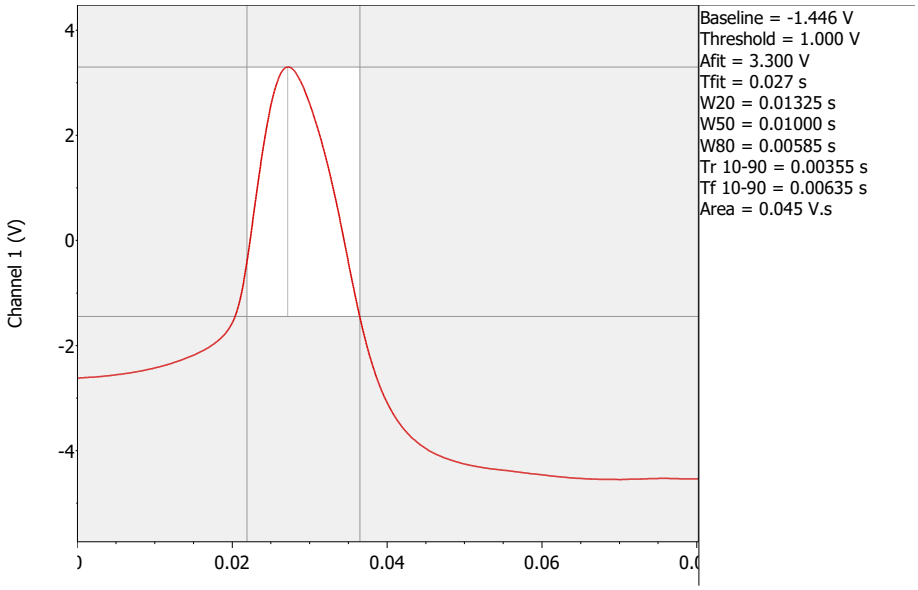


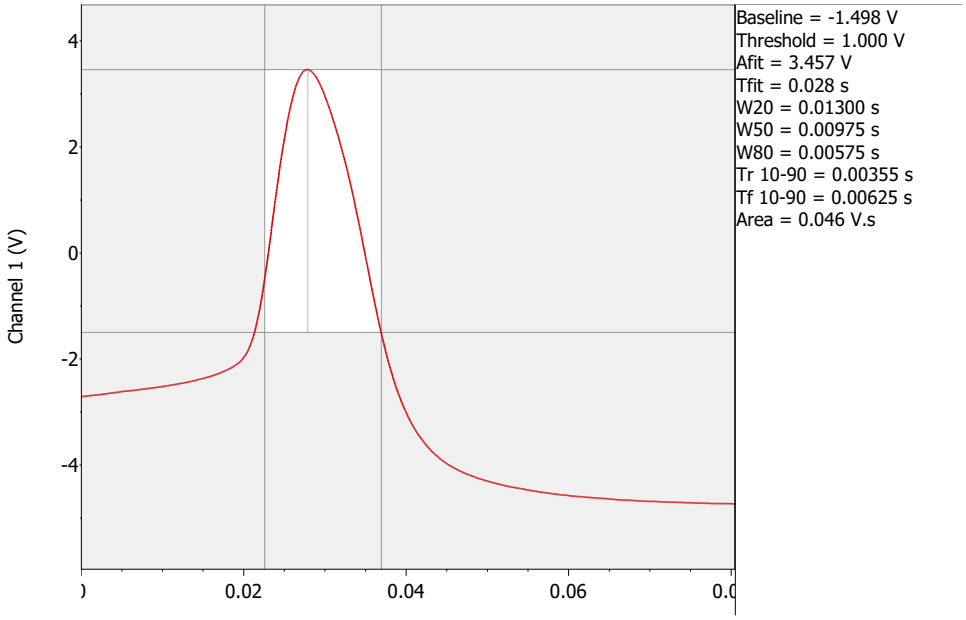
Fig. 20.
Threshold dependence on the AP number. Series 1 corresponds to threshold, Power represents trend calculated by PC.

Peak parameters were also explored: Width at 20% level is changed only on 0,25 msec, at 50% level also on 0,25 msec, and on 80 level only on 0,1 msec. This changes might be regarded in the region of experiment fallibility and it might be stated that APs this parameters are not changed under exposure to EMF. Rising times are equal to each other. According falling times

which differ from each other only on 0,1 msec could be said the same. Peak parameters of 1st and last APs in reactions are shown in the fig.21.



A



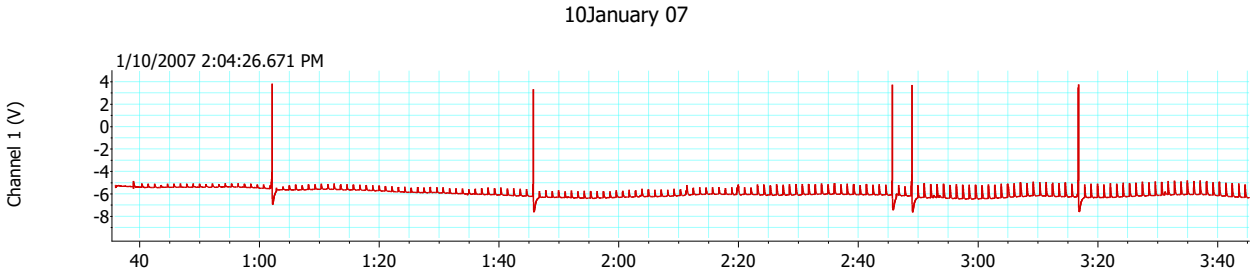
B

Fig. 21. Peak parameters of APs.
A- 1st AP in reactions
B- Last AP in reactions

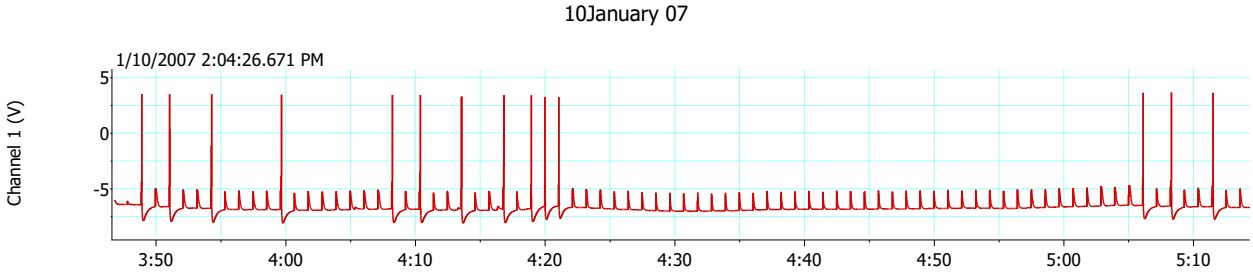
This result suggests the idea that, EMF effects the passive processes and has no effect on the active processes of the neuron, particularly on processes of AP firing.

Effects of modulation of 900 MGZ with 8, 34 Hz .
Calculation of SAR gives meaning of 0,63w/kg. Temperature rising was equal to 0, 1 ºC. Exposure time was 60 min. Neuron first reaction appeared on ST with amplitude 0,2 NA, however reaction was only 1 AP, and neuron declines all other stimulant impulses. The same happened with ST with amplitudes 0,3NA-0,7 NA – fig. 22 A. Even 0,7 NA occur very weak-3 APs firing. Reactions with only 3 APs could not be regarded as such reactions which can be transferred into habituation in other words this reaction is not informative - fig. 22 B. Habituation can be raised on such stimulus, which is informative, i.e., reaction has to be quite strong and changes might be developed on the background of changes of this reaction. By the reason ST was risen to 1,0 NA. Interesting is that in this case reactions were continued for 25 min 6 second, after which complete habituation was arisen. Dynamics of reactions are shown in the fig. 22B-G: It is interesting that at the beginning of stimulation with 1,0 NA, neuron declines large amount of STs-fig 22B. Reactions were enhanced beginning from 3rd min, and neuron fires APs on most intracellular impulses and declines small amount fig 22C.

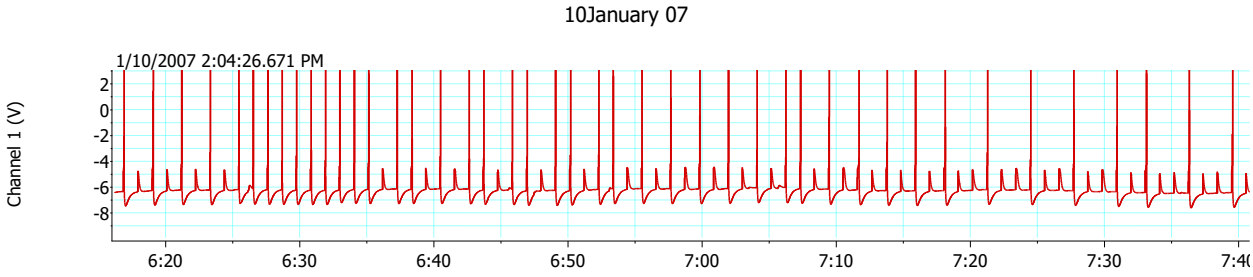
Complete view of the reactions is illustrated in the fig. 22G.



A



B



C.

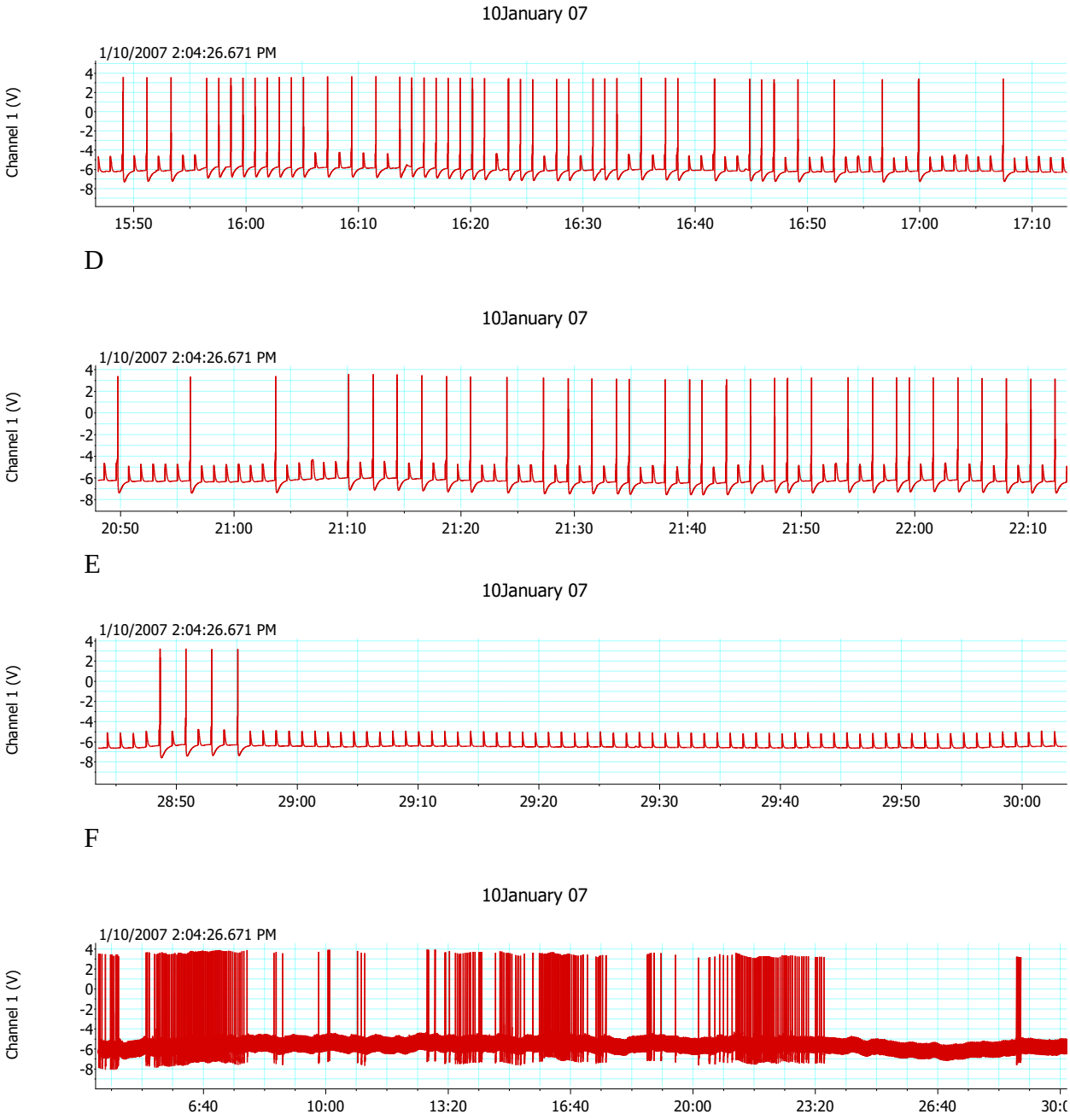


Fig. 22

A- Reactions on the 0,3 NA-1st AP; 0,5 NA- 2nd AP and 0,7 NA 3rd-5th APs.
B- Beginning of stimulation with 1,0 NA amplitude intracellular impulses.
C- Enhancing of reactions at 3rd min of stimulation
D- Reactions at 12th min. of stimulation.
E- Reactions at 17th min of stimulation
F- Beginning of habituation at 27th min of stimulation
G- Complete view of reactions

Caliber is the same as in above figures.

Latency dependence on the AP number occur irregular oscillations with very weak increase trend – fig 23.

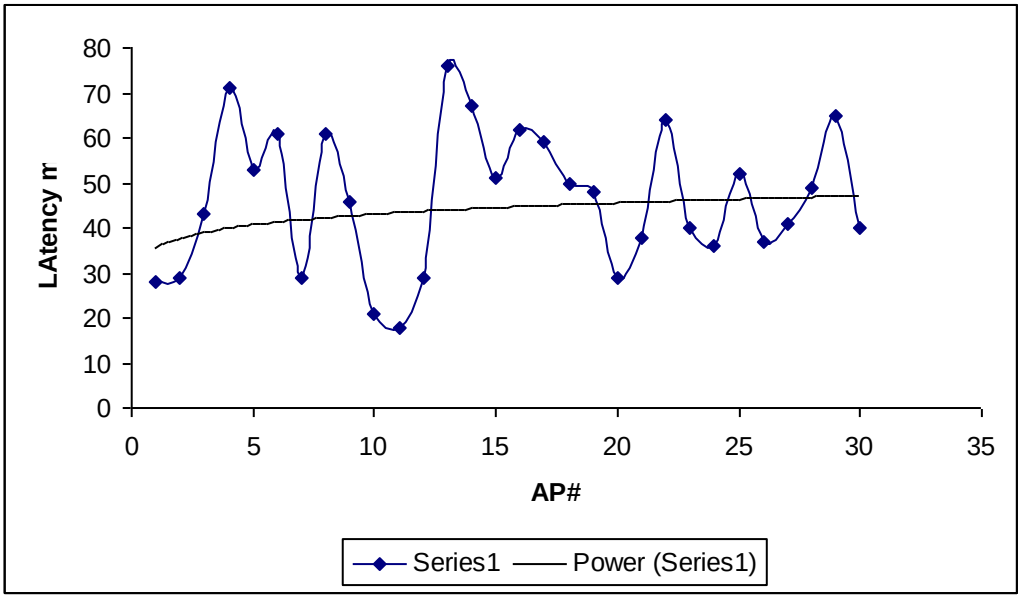


Fig. 23
Latency dependence on AP number. Series 1 corresponds to latency; "Power" is trend calculated by PC.

Threshold changes are not significant and the trend is constant function i.e. it is parallel to X axis- fig. 24;

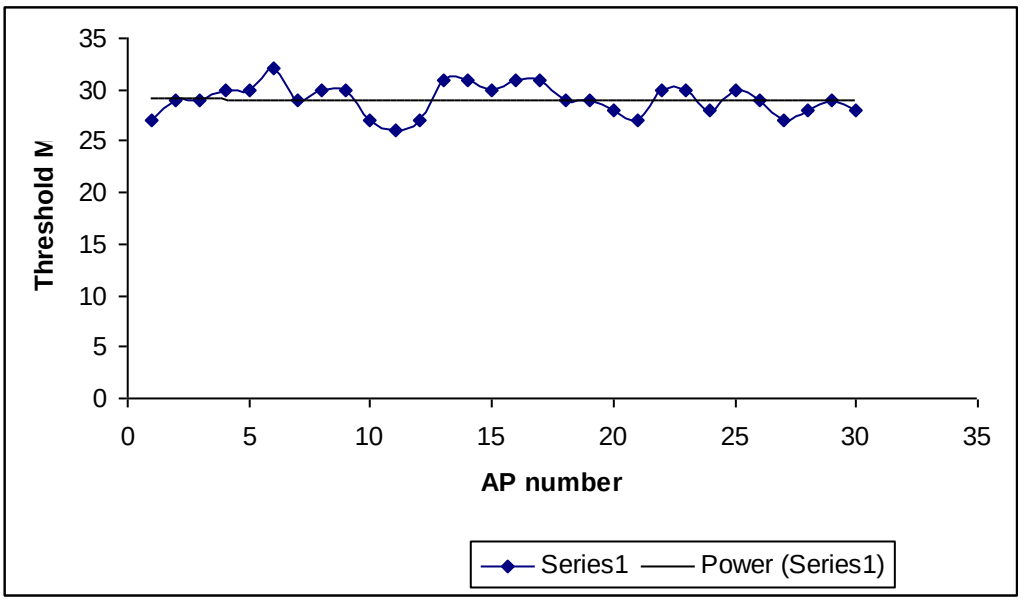
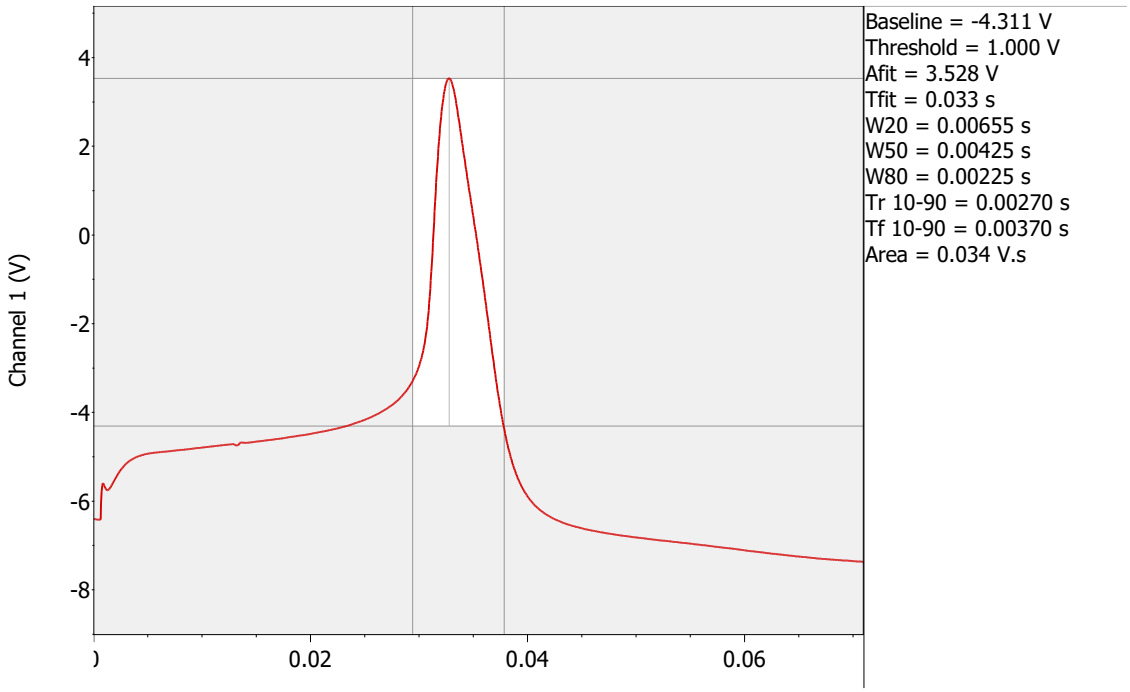
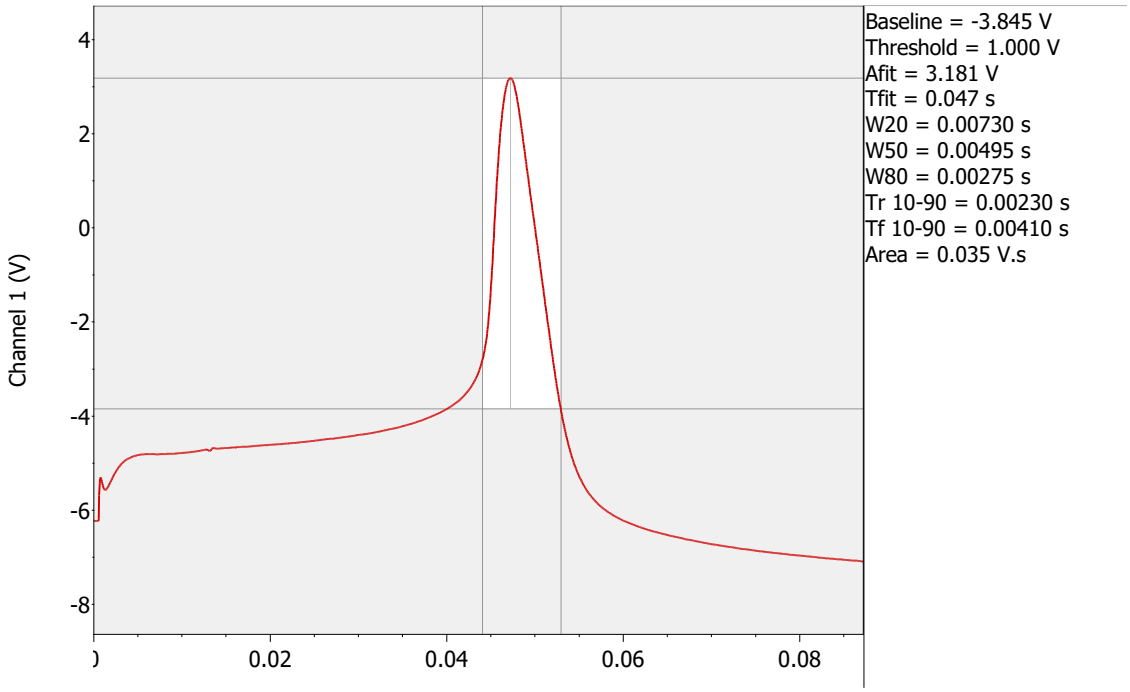


Fig. 24.
Threshold dependence on the AP number. Series 1 corresponds to threshold, Power represents trend calculated by PC.

Peak parameters of APs at different moments of stimulation are shown in the fig. 25:
These figures indicate that parameters such are; width at 20, 50 and 80 % levels are practically unchanged. The same could be said about rising and falling times of APs and areas under the plots. Only latency periods are different, however this parts of graphic are not under analyze of peak parameter extension.



A



B

Fig. 25
A peak parameters of the 1st AP in reactions
B- peak parameters of last AP in reactions.

Thus the effect of 900 MHZ EMF modulated with one low frequency has following features:

Latency dependence is smooth graphic. This differs from the same dependence for cell phone case where latency exerts nonregular variations. However the trend of the dependence graphic is similar –i.e. increased functions.
Average meaning of the threshold remains constant. This concerns as 2,0 as 8,34 Hz.
In both case of modulation (2,0 Hz or 8,34 HZ) peak parameters of APs are c constant.

This result suggests idea that EMF has no effect on the active processes of the neuron, particularly on processes of AP firing.

EMF modulated with 8,34 Hz exerts larger effect on the neuron then EMF modulated with 2,0Hz. This is expressed first of all in difference of reactions duration and appearance of habituation.
Modulation with 8,34 HZ causes similar to Cell phone effect - what is reflected in prolongation reactions for several dozen minutes. However there is difference also- in the case of expose to 900 MHZ modulated with 8,34 habituation appears at last, what could not be said for the case of irradiation with cell phone.
Behavior of the latency looks like each other in both cases: for exposure to cell phone and for 900 MHZ generator modulated with 8,34 HZ, whereas threshold dependence on AP number are different. Particularly in the case of 900 MHZ generator irradiation average meaning of the threshold remains strongly unchanged, while at cell phone case this function is slightly increased function. Entirely different are EMFs relationships with APs. In cell phone irradiation case peak parameters are slightly changed, while at 900 MHz generator irradiated case (modulated with 8,34 Hz) APs peak parameters do not change during whole period of stimulation. This circumstance urges to suggestion that RF EMF affects passive characteristics of the membrane and does not involve active characteristic.

4. Current technical status

The works of the first year of the Project correspond to the Work Plan and are carried out on schedule.

5. Cooperation with foreign collaborators

The following are the international collaborators of the Project:

1. Professor Jukka Juutilainen, University of Cuopio, Department of Environmental Sciences- officially.
2. Dr. Henry C. Lai , Research Professor University of Washington, Seattle, WA
3. Dr. Carl F. Blackman, Chief, Cellular Toxicology Branch Environmental Carcinogenesis Division, United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory.

The first year of the Project was dedicated to: preparation of experimental base, experimental investigations of the rules of the sham irradiation and investigations of influence of cell phone and 900 MHC generator irradiation on the single neuron habituation abilities. Cooperation with the international collaborators such as exchange of scientific materials (information, software and data,), business trips to the organizations of the international collaborators will be actively realized starting from Quarter VI. During the reported period, the current information was delivered to international collaborators through e-mails. To International collaborators were send:

Scientific-Technical Referencec:

1) Experiment result of neuron habituation to the intracellular and synaptic stimulations Sham Case:

2) TEM Cell Modeling for Experimental Study of Electromagnetic Field Influence on Mollusk Neurons.

3) Comparison of sham and actually irradiated neuron habituation abilities.

Technical reference:

Report of cell phone modification and creation of 900 MHz generator punctuated with ELF separately

The manuscript of the following scientific articles are sent for this time

1) "**Influence of Cell Phone Irradiation on the Single Neuron Responses to Stimulation**". and

2) "**Numerical Investigation of SAR and Temperature Rise in Mollusk Neuron Exposed to Electromagnetic Radiation at 900 MHZ**."

During the first year of the Project, weekly topical discussions, seminars were organized. The issues related to the scientific problems were discussing.

6. Problems encountered and suggestions to remedy

During the first year there were no problems able to affect execution of planned works of the Project. It has to be appointed not receiving of materials- Suction electrode and Calibrated Suction/Pressure Source; however this does not be reflected negatively on the project execution.

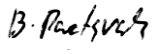
7. Perspectives of future developments of the research/technology developed

Attachment 1: Abstracts of papers and reports published during the year of reference- **Appendix**

8. Conclusions:

- TEM Cell for EMF exposure is designed and created.
- Output circuit of the Cell phone is modified and output power is controlled.
- 900 MHZ generator with separate ELF modulation is designed and created.
- Rules of habituation for sham irradiated neurons are investigated.
- Influence of cell phone irradiation on the single neuron habitation to intracellular stimulation is investigated. It is shown that cell phone EMF suppress neuron habituation ability.
- Influence of cell phone irradiation on the single neuron habitation to synaptic stimulation is investigated. It is shown that cell phone EMF suppress neuron habituation ability.
- Influence of 900 MHZ EMF punctuated with 2,0 HZ on the single neuron habitation to intracellular stimulation is investigated.
- Influence of 900 MHZ EMF punctuated with 8,34 HZ on the single neuron habitation to intracellular stimulation is investigated. Both modulation causes prolongation of the reactions, however effect of 8,34 Hz is greater than effect of 2HZ modulation

Manager of the project

G-1187  Besarion Partsvania