Parametric Modeling of Nerve Cell under the Sinusoidal Environmental 50 Hz Extremely Low Frequency Magnetic Field

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Abstract: The development of technology has naturally given rise to an increase in environmental low frequency electromagnetic fields and consequently has attracted scholars attention. Most of the studies have focused on transmission lines and power system distribution with 50 Hz. This research is an attempt to show the effect of 50 Hz magnetic fields on bioelectric parameters and indicate the possible influence of this change in F1 cells of *Helixaspersa*. In this research neural cell F1 snails from the garden to the effects of magnetic fields on the peripheral nervous system were used. The control group was selected because of the consideration of time passing, electrode entrance and membrane torn. The sham group has been selected as because of consideration of the probable effect of moderating of environmental factors. Experiment to identify the effect of the magnetic field was considered. To apply sinusoidal magnetic fields, a pair of Helmholtz coils, to get a homogenous magnetic field. Electrophysiological recording from cells under current clamp conducted to show the effects of magnetic fields on ion channels of the cell model Hodgkin-Huxley used. Two-way ANOVA was used statistical tests with the significance level of $p<0.05$. To estimate the parameters PSO algorithm was used. No statistically significant difference was found between control and sham groups in different time intervals. Once the 109.34 microtesla was applied significant differences were observed 12 minutes after the application. The highest amount of change happened 16 minutes after the application of more fields. No significant changes were observed in different time intervals, whereas significant differences were seen in frequency of action potential during different time intervals. The amplitude of AHP show no significant changes. The results indicated that low frequency magnetic fields with 50 Hz frequency will directly lead to change in bioelectric activities neurons through a change in amount and rate of open and close ionic channels. Conductivity of reduced sodium and potassium channels and potassim dependent calcium channel (AHP) increases.

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Keywords: Nerve Cells, Current Clamp, Hodgkin-Huxley Model, Ionic Channels, Environmental Electromagnetic Fields.

1. Introduction

Technological development has led to increasing environmental low frequency magnetic fields, so it has attracted the scientific communities attention to biologic effects of these fields. Key discussion is on the transmission and electricity power distribution, but there are many resources in the work and life environment that create electrical and magnetic fields adjacent to their environments and the radiation amounts of these fields increase by increasing the energy use level [1, 2, 3].

There are low frequency magnetic fields with the range of 3-3000 Hz and by regarding the low energy, they can't end to ionization thermal effects when they encounter with biologic systems. So, other effective mechanism have been gone through in the encountering these fields with active systems that findings of these mechanisms and consideration of the effects of these fields on the human beings active systems demand scientific researches [3, 4, 5]. Animals experiments, clinic consideration and computer simulation can be accounted as these ways and can be as the clue for finding the answer to this question [6].The scientific evidence has proved the neurological system sensibility to magnetic fields. The neurological system responses to these fields followed different patterns are the results of ion channels and their numbers in the membrane of neurological cell [7, 8]. Researches show that low frequency electromagnetic fields effects have been as the results of the effect of these fields on the permeability of cell membrane. So, the study of events when the neurological messages transition happen in the field ensues has paved the way for understanding the biologic effects of these fields [9].

There are different ionic channels in membrane cell that their activeness and inactiveness by opening and closing, on cell parameters including membrane respite potential, performance potential positive and negative domain peak, changes in the membrane permeability will play significant role. So, the process like voltage change will be stimulus for the trend of ionic channel by effecting and opening and closing of them [10].

In considering of effective mechanism in the appearing of biologic effects of low frequency magnetic fields it is pointed that cell membrane and ionic channel has significant role in the instigation and simulative cell activities including neurons and they can be the main contract place of low-frequency fields with active systems. So, the performance changes of these channels may be effective on the appearance of some side effects like changes in the activity level of neurons bases[11].

By regarding the traits and appropriate parameters in addition to regard the source and the effective way, derived from the effectiveness to magnetic field performance and effect on the whole, we can be assured (Homogenous parameters) and such model that is able to show the resulted changes in certain and purposeful parameters can help in understanding the way of effectiveness of this field on the biologic systems [12]. Since the source and cause of animals and biologics' behavior are relevant to the membrane ensue which have been started from membrane ionic channel performance, isomorph parameters in performance potential at the period of non-performance and maximum performance of field are derived by offered parameters in HH (Hodgkin-Huxley) cell model and PSO algorithm, then the field effectiveness on the model of parameters in question are identified, APH are compared by speed and delayed Sodium and Potassium conductive models.

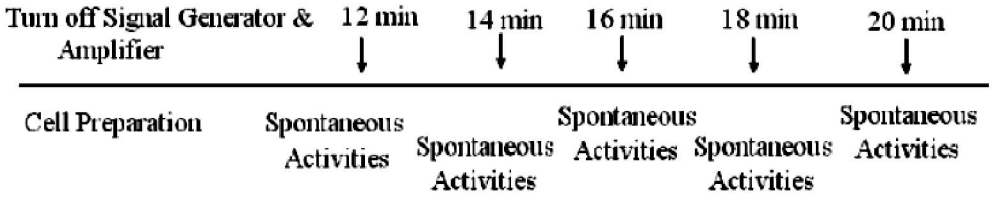
2. Materials and Methods

We appealed to Dr. Kaviani Mogadams inner cell data which was collected in Shahid Beheshti University of Medical Sciences and Tarbiateh Modaress University. These data are relevant to neuron F1 in Helix aspersa. Helix aspersa, which has fairly simple neurological system, paves the way for massive understanding of complicated neurological process[11]. After the provision and cell preparation steps, one microelectrode was filled with 3 ML KCL solution inside and another microelectrode was laid outside of the cell and all experiments was carried out in FARADY Cage [13]. This has been done by one electrode Current Clamp technique, the performance potential was recorded by spontaneous and instigated forms. spontaneous activity recording was measured right away after depolarizing current infusion and hyperpolarizing by square wave and intensity of -5 to +5 nAby microelectrode and by the use of D/Aconverter and Axoclamp device. The cell disturbing response has been registered by amplifier device and A/D.

2.1. Experimental groups

Participants in this study were three groups control, sham and experimental. The control group was selected because of the consideration of time passing, electrode entrance, membrane torn and probable ionic leakage by electrode entrance and the effect of useful magnetic field on cell bioelectric activities.

In this group the recordings of electric activity of cell was in standard ringer without electromagnetic performance. (Figure 1)



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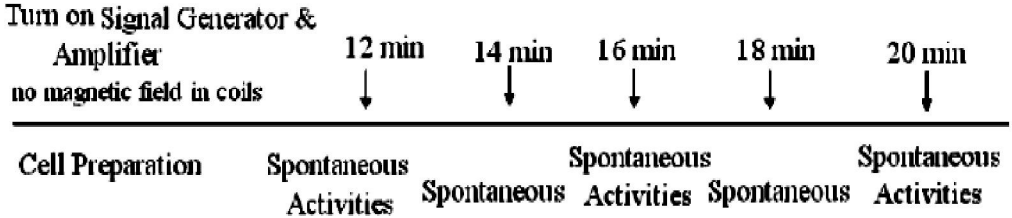


Figure 2. Time pattern in cell inside register steps within intuition group

2.2. Hodgkin-Huxley model in nerve cell

Neural neurons have been studied extensively. For modeling cell in neural net, the kind and channel number, cell trait in relation to instigation and cell innate performance are important factors in the model identification. This model includes minimum collection of ionic current which is depending on voltage of gained data.

Cell inner registration in neuron F1 in Helix aspersa at standard ringer could be used as to show input sodium and calcium current, outside potassium and potassium dependent on calcium for adjusting frequency shooting and subsequent potential domain in hyperpolarizing (AHP). In addition, leakage current resulted from potassium current in cell has been taken into account. The Input Calcium current would be appeared if we control sodium current, unless they would be affected by perfect sodium current. So, all of the following current in cell will be taken into account.

1. Sodium current
2. Potassium current
3. Potassium dependent on calcium current
4. Leakage current

$$I_L = g_L(V - V_L)$$

Finally, cell model will be accepted as the following formula

$$C_m \frac{dV}{dt} = g_{Na} m^3 h (V \updownarrow V_{Na}) + g_K n^4 (V \updownarrow V_K) + g_A a^3 b (V \updownarrow V_A) + g_L (V \updownarrow V_L)$$

$$I_{Na} = g_{Na(MAX)} m^3 h (V - V_{Na})$$

$$I_K = g_{K(MAX)} n^4 (V - V_K)$$

$$I_A = g_{a(MAX)} a^3 b (V - V_A)$$

2.3. Data Analysis

Referring to the relevant articles of the samples of each group, the electrophysiological and modeling results of 4 to 6 samples have been obtained. On the spontaneous activity of each neuron, resting potential (RMP) was measured and their mean values were calculated. The calculation of distance between Spikes (ISI) was carried out in the form of the distance between two peaks of action potential and action potential duration at 50% spike amplitudes. Furthermore, the action potential amplitude and Hyperpolarization following resting membrane potential level are also calculated (Figure 3).

Data analysis was performed using the software Chart 6. Quantitative values are expressed as SEM $\pm$ Mean. Test mean comparisons using two-way ANOVA were done by the MinTab 16 software.

P<0.05 was considered as the significant level. Matlab software environment for the implementation of the PSO algorithm is used to estimate parameters.

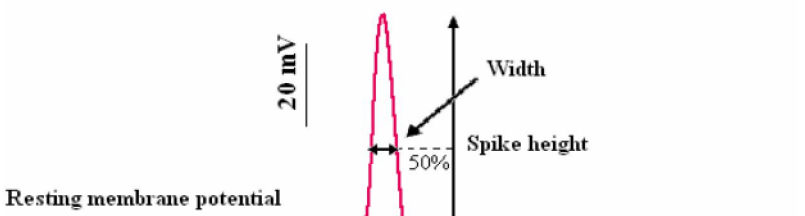


Figure 3.The ways of measuring the characteristics of action potential

3. Results

3.1. Electrophysiological properties of nerve cells in the control group

In order to investigate the intrinsic properties of nerve cell membrane and the used solution (ringer) and also the effect of time duration, the spontaneous activities' data of control group for providing the results of electrophysiologic and modeling have been used. To provide the electrophysiologic results for this group, 6 samples of F1 nerve cells were recorded, 30 action potential characteristics of each period have been investigated and the results are presented as S.E.M $\pm$ Mean (Table 1).

Table 1. Electrophysiological properties of F1nerve cells in control group

Parameter / Time (min)	0	12	14	16	18	20
Resting membrane potential (mV)	-41.9 ± 0.53	-42.2 ± 0.4	-42.1 ± 0.29	-41.21 ± 0.31	-40.97 ± 0.25	-40.87 ± 0.24
Firing frequency of action potentials (Hz)	2.29 ± 0.08	2.29 ± 0.07	2.28 ± 0.06	2.27 ± 0.07	2.12 ± 0.07	2.29 ± 0.05
Positivepeak amplitude of action potential (mV)	41.39 ± 3.93	41.37 ± 3.34	41.72 ± 3.00	41.73 ± 2.98	41.72 ± 3.12	41.77 ± 3.79
Negative peak amplitude of actionpotential (mV)	-50.86 ± 0.39	-50.72 ± 0.41	-50.53 ± 0.55	-50.57 ± 0.58	-50.57 ± 0.59	50.55 ± 0.57
Duration of action potential (ms)	5.71 ± 0.13	5.72 ± 0.13	5.71 ± 0.14	5.74 ± 0.14	5.77 ± 0.16	5.75 ± 0.15

In this group, of the characteristics of 30 selected action potentials of each time interval, the parameters of the resting membrane potential, firing frequency of action potential, action potential peak amplitude, negative peak amplitude of action potential and the action potential duration using the two-way ANOVA in accordance with the time interval and the primary record were conducted, and as a result no significant difference was observed. Therefore, in bioelectric activity of F1 nerve cells there is no significant difference over the time ($P<0.05$, $n=30$).

3.2. Electrophysiological properties of F1 nerve cells in the sham group

In order to remove noise from the intracellular records, the experiments were carried out in the Faraday cage. The entire environmental field within the cage was small and only two signal generator and audio amplifier are available in the cage. Therefore, to investigate the effect of environmental noise on bioelectric activity of nerve cells, the intuition has been taken into consideration. In this group the signal generator and audio amplifier are on, but their relationship with field-generating coils is cut off. The trends and characteristics of this group are like control group and the characteristics of 30 action potentials have been investigated. The results are presented as S.E.M $\pm$ Mean (Table 2).

Table 2 - The electrophysiological properties of F1 nerve cells in the sham group

Parameter / Time (min)	0	12	14	16	18	20
Resting membrane potential (mV)	-41.99 ± 0.51	-42.07 ± 0.52	-42.12 ± 0.52	-41.06 ± 0.53	-42.97 ± 0.54	-42.2 ± 0.49
Firing frequency of actionpotentials (Hz)	2.44 ± 0.08	2.41 ± 0.07	2.42 ± 0.08	2.40 ± 0.07	2.41 ± 0.08	2.43 ± 0.09
Positive-peak amplitude of action potential (mV)	38.19 ± 3.64	27.26 ± 3.34	39.17 ± 2.98	37.21 ± 3.11	38.14 ± 3.23	39.57 ± 3.91
Negative peak amplitude of action potential (mV)	-52.61 ± 0.67	-53.02 ± 0.78	-53.01 ± 0.61	-52.96 ± 0.59	-52.75 ± 0.58	52.87 ± 0.59
Duration of action potential (ms)	5.22 ± 0.12	5.19 ± 0.09	5.17 ± 0.14	5.23 ± 0.19	5.77 ± 0.16	5.19 ± 0.14

Similar statistical analysis, which had been done in control group, was conducted in the sham group and similar to the results obtained from control group, the procedure of gradual differences in action potential characteristics were observed, but no significant difference was observed in any of the intervals ($P<0.05$, $n=30$). So, bioelectric activity of the F1 nerve cells is not affected by electromagnetic fields and noise caused by turning on the signal generator equipment and audio amplifier.

Therefore, if any significant statistical difference in the parameters derived from action potential due to the employment of external magnetic field is seen, this would be due to the application of magnetic field.

3.3. Employment of magnetic field109.34 micro tesla with sinusoidal frequency of 50 Hz.

3.3.1. Resting potential of membrane

The intensity of the applied magnetic field leads to the nerve cell membranes resting potential depolarization. By applying the magnetic field for 12 minutes, based on the statistical tests, significant differences were observed and the greatest difference was seen 16 minutes after the application of the field ($P<0.05$, $n=30$); in a way that the average resting membrane potential of such cells in controlled conditions was at -44.71 ± 0.9 mV, and 16 minutes after applying the field it reached to -34.19 ± 0.82mV. With the increase of duration in applying the magnetic field, the amount of the changes of resting potential would decrease.

3.3.2. Amplitude of sodium action potential

Applying magnetic field with this intensity leads to the decrease of action potential amplitude. After applying the field for 12 minutes, in comparison with control group, no significant difference was seen in the potential amplitude and by increasing the duration to 16 minutes the difference became significant ($P<0.05$, $n=30$) in a way that the average sodium action potential in controlled conditions was at 36.63 ± 0.88 mV; after 16 minutes of applying the magnetic field the average reached to 25.98 ±1.34 mV and after 20 minutes to the 25.11 ± 1.36 mV.

3.3.3. Duration of action potential

Applying magnetic field with this intensity has no significant effect on the duration of action potential. In control group, the duration of action potential was 4.79 ± 0.12 ms and at different time intervals no significant difference was seen($P<0.05$, $n=30$).

3.3.4. Firing frequency of function potential

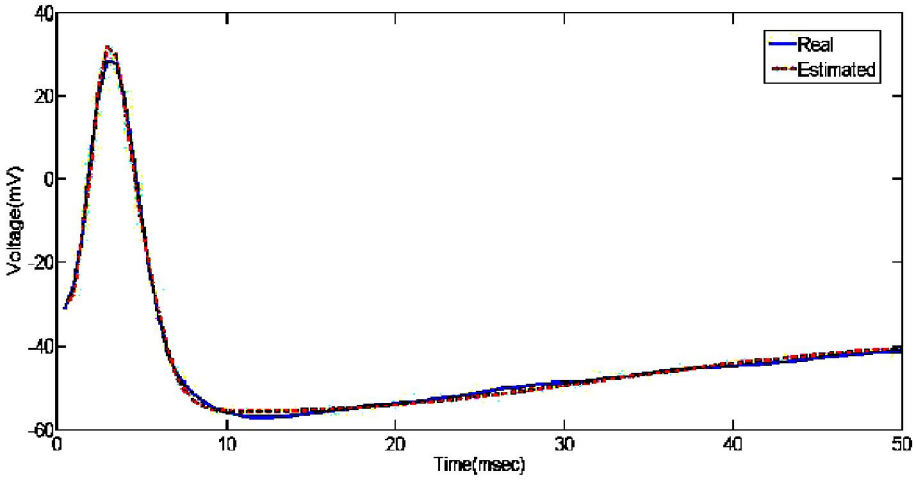
Comparing to control group, applying the magnetic field with this intensity has no significant effect at different time intervals.

3.3.5. The subsequent hyperpolarization amplitude of action potential

Applying the intensive magnetic field leads to the increase of the subsequent hyperpolarization amplitude of action potential. The statistical analysis in this intensity didn't show any significant difference at different time intervals ($P<0.05$, $n=30$). It happened in a way that the subsequent hyperpolarization amplitude of action potential of cell in controlled conditions was -53.07± 3.11 mV, and after 16 minutes of applying external magnetic field it reached to -53.79 ± 2.99 mV.

As a result,16minutes after applying the field is chosen for the field 109.34 microtesla with 50 Hz sinusoidal frequency as a critical point.

For cell modeling, of the time intervals of control conditions as many as the registered samples (n=6) and of the time intervals of the greatest effect of the field, like the control conditions, the function potential of the cell was chosen. In the considered model, all the constant numbers were considered as parametric(15 parameters for sodium conductivity, 9 parameters for fast potassium conductivity, 17 parameters for AHP current conductivity, 2 parameters for leakage current and 1 parameter for capacitor capacity). By conducting the PSO algorithm, 44 parameters were calculated (figures 4, 5) and of the obtained values, sodium conductivity rate, quick and delayed potassium conductivity rates were computed(Table 4).



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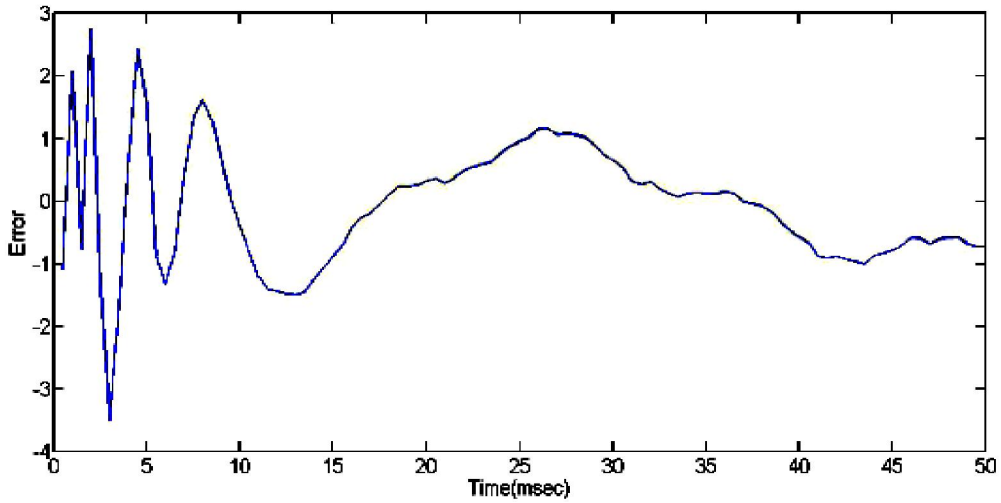

Figure 5. The instant error rate of the actual and estimated action potential in the conditions of applying 109.34microtesla field after 16 minute applying of the field

Table4.The average parameters of Hodgkin-Houxly F1 nerve cell in control conditions and 16 minutes after applying sinusoid field 109.34 micro tesla 50 Hz.

Parameter	conditions	Before applying the field (control conditions)	After applying the field (critical points)
Positive peak amplitude $G_{Na} = G_{Na(\max)} m^3 h$ (mS)		0.3129	0.2898
Positive peak amplitude V_{Na} (mV)		49.7042	52.6921
Negative peak amplitude $G_K = G_{K(\max)} n^4$ (mS)		0.0591	0.0628
V_{Na} (mV)		-58.8146	-61.7035
$G_{a(AHP)} = G_{a(\max)} a^3 b$ (mS)		0.00491	0.00493
$V_{a(AHP)}$ (mV)		-70.8933	-73.7822
G_L (mS)		0.0012	0.0019
V_L (mV)		-20.8436	-25.7325
$C_m (\mu F / cm^2)$		1.004	1.010
MSE		0.45034	0.49200

4. Discussion and Conclusion

Accumulated evidences demonstrate that extremely low frequency magnetic fields (ELF-MFs) are capable of modifying neuronal function.

At the cellular level, one of the possible methods for demonstrating the biological effects of magnetic fields on cell membranes is to investigate it on the membrane of that cell, mainly because of the existence of the ionic channels inside the cell membrane [14].

Neuronal ion channels are gated pores whose opening and closing actions are usually regulated by factors such as a voltage or/and ligands. They are often selectively permeable to ions such as sodium, potassium and calcium. Anything that interferes with the membrane voltage can alter channel gating and comparatively small changes in the gating properties of a channel can have profound effects. Extremely low frequency electrical or magnetic fields are thought to produce, at most, microvolt changes in neuronal membrane potential. At first sight, such changes in membrane potential seem too small in orders of magnitude to significantly influence neuronal signaling [15].

However, in the central nervous system, a number of mechanisms exist that amplify signals. This may allow such small changes in membrane potential to induce significant physiological effects[15].

Although in many studies, data do not allow a conclusion about the precise molecular mechanism for the effect of MF, but one possibility is that the MF influences intermolecular interactions that are important for signal transduction, either in protein-protein or protein-lipid membrane interaction forms [16].

The present findings described the cellular effects of low frequency magnetic fields on the neuronal excitability and action potential characteristics. It decreased firing frequency of action potentials, thereby causing neuronal inhibition. The inhibitory induced by magnetic fields could be mediated through inhibition of Ca^{2+} channels or voltage and/or calcium dependent K^+ channels. In explaining the elicited bioelectric activity changes under static magnetic field exposure two mechanism were proposed, namely the Ca^{2+}-dependent and the Ca^{2+}-independent metabolic processes[17].

We assume that the changes detected in the membrane resting potential of the F1 caused by magnetic field are the result of the change in membrane proteins (ion channels and ionic pumps) which are altered by magnetic field. Ljiljana.*et al* believe that the increase in the spike amplitude of theF1 could be a result of increased activity of channels involved in the depolarizating phase of AP, or a decrease in channels responsible for spike hyperpolarization. However, it is possible that other changes in membrane resistance could cause to an increase in the amplitude of the action potential [17,18].

The decrease in the frequency of action potentials indicates that the magnetic field has an inhibitory influence on the F1 neuron activity, and

similar finding were reported on cultured mammal neurons. The same effect on action potential frequency was also found in a study on spontaneous active snail neurons from *Helix lucorum*[17,18].

Inactivation of Na^+ channels leads to a progressive decline in the Na^+ conductance available for generating of action potentials which in turn can lead to a delay in the onset of successive spikes and progressive decline in the frequency.

In conclusion, on the basis of the present data in combination with the previous work on snail neurons [16], it can be suggested that magnetic field induce inhibitory effects through inhibition of K_{ca}channels. The use of different flux intensity as in this study seems to be an essential as a contributing factor in the decrease of frequency in snail neurons [19].

5. Acknowledgements

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References

1- Adey WR. Biological effects of electromagnetic fields. Cell Biochem. 1993 Sep; 51(4):410-6.

2- Levallois P, Gauvin D, Gingrad S. Comparison between personal exposure to 60 Hz magnetic fields and stationary home measurements for people living near and away from a 735kV power line. Bioelectromagnetics. 1999 Oct; 20:331-337.

3- Ahmed N, Asaad A, Aboul E, Radwan N. Effects of exposure to electromagnetic radiation from the mobile phone on acetylcholinesterase activity in the hippocampus and striatum of young and adult male rats. Proceeding of the 3rd International Workshop of Biological Effects of Electromagnetic Fields, Kos, Greece. 2004 Oct. 4-8; 924-930.

4- Ainsbury EA, Conein E, Henshaw DL. An Investigation into the vector ellipticity of extremely low frequency magnetic fields from appliances in UK homes. Phys Med Biol. 2005 Sep; 50(13): 3197-3209.

5- Valentini E, Curcio G, Moroni F, Ferrara M, De Gennaro L, Bertini M. Neurophysiological effects of mobile phone electromagnetic fields on humans: a comprehensive review. Bioelectromagnetics 2007 May; 28(6): 415-9

6- Kaviani-Moghaddam M. The 50Hz extermeley low ferquency electromagnetic fieds effect on bioelectrical activites of helix aspersa nerve cells [Dissertation], Tarbiat Modares University. 2002. (Full Text in Persain)

7- Shu-Min H, Yu-Wen L, Fung-Chang S, Chung-Yi L, Ming-Fong C, Pei-Chun C. Occupational exposure of dentists to extermeley-low-frequency magnetic field. Joccup Health. 2011 Oct; 53(1):130-136.

8- Weaver CM, Susan L, Wearne SL. The role of action potential shape and parameter constraints in optimization of compartment models. Neurocomputing. 2006 Nov; 69: 1053-1057.

9- Haughes G, Kerkut G. Electrical activity in a slug ganglion in relation to the concentration of lock solution. J Gen Physiol. 1955 May; 44(2): 282-294.

10- Calvo AC, Azanza MJ. Synaptic neuron activity under applied 50 Hz alternating magnetic fields. Electro and Magneto. 1999 Jun; 124 : 99-107.

11- Calvo AC, Azanza MJ. Electrophysiological responses of snail brain neurons under applied 50 Hz alterating magnetic fields. Eleotro and Magneto. 1999 Jun;8(3);305-315.

12- Balcavage WX, Alvager T, Swez J, Goff CW, Fox MT, Abdullyava S, et al. A mechanism for action of extremely low frequency electromagnetic fields on biological systems. Biochem Biophys Res Commun. 1996 Oct;222(2):374-8.

13- Chung SH, Andersen OS, Krishnamurthy V. Biological Membrane Ion` Channels Dynamics Structure and Applications. 1 th ed. NewYork: Elsevier: 2007: 420-560.

14- Ebrahimian H, Firoozabadi M, Janahmadi M. Effect of 50Hz magnetic Field on Non-Uniforn (Fractal) Parameters of Nerve Cell. The 1st Conference on Electromagnetic Field Effect on Live Tissues, Amirkabir University, Tehran, Iran, 2008 May: 27-29. (Full Text in Persain)

15- A. Mathie, L. E. Kennard and E. L. Veale. Neuronal Ion Channels and Their Sensitivity to Extremely Low Frequency Weak Electric Field Effects. Radiation Protection Dosimetry Vol. 106, No 4, pp. 311–315 (2003).

16- E. Lindström, Lindström P, Berglund A, Lundgren and Mild H.K. Intracellular calcium oscillations in a T-cell line after exposure to extremely low frequency magnetic fields with variable frequencies and flux densities. *Bioelectromagnetic*.. 16 (1995) 41-47.

17-Azanza MJ, Calvo AC. Snail neuron bioelectric activity induced under static or sinusoidal magnetic fields reproduces mammal neuron responses under transcanial magnetic stimulation. Electro-magnetbiol. 2000; 19: 303-319.

18- Azanza MJ, Moral AD. Frequency resonance effect of neurons under low frequency weak magnetic field. J Magn Mater. 2007; 310(2): 2865-2867

19- N, Ljiljana. K, Gordada and N, Miodrag. Effects of static magnetic fields on bioelectric properties of the Br and N1 neurons of snail Helix pomatia, Comparative Biochemistry and Physiology part A, 151(2008)657-663.