Weak Environmental Magnetic Fields Inhibit Spontaneous Bioelectrical Activity in Snail Neurons

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Abstract : The interest in the evaluation of health effects due to EMF has accelerated in the last decades, mostly motivated by the occupational and environmental exposures to humans by such non-ionizing fields. The increasing exposure to electromagnetic fields has been suspected to contribute to the rising incidence of cancer in industrialized countries Despite detailed analysis which provide a wealth of accurate data on the effects of electromagnetic fields on nerve cells, a clear pictures of the mechanism and sites of action of ELF magnetic field in the range of environmental intensities is far from being accomplished and the ionic or metabolic processes underlying the observed effects have not been fully explained. The aim of this study was to evaluate the effects of 50 Hz magnetic fields in the range of environmental intensities on spike and neuronal excitability in snail neurons. We measured the bioelectric parameters of F1 neuron with the conventional intracellular recording in current clamp mode.

Key -Words : Ion Channels, Neuronal Excitability, Extremely Low Frequency Magnetic Fields, *Helix aspersa,* Electrophysiology, Current Clamp

1 Introduction

In Spite of the technological progress made by man the corresponding increase in the amount of electromagnetic fields (EMF) has also generated new environmental exposures to the radiation [1]. EMF exposures are complex and come from multiple sources which include the home, workplace and also high voltage power lines. The extents of indoor exposures, especially for residences located near a power lines and the potential risks associated with such exposures are still partly unknown [1,2].

The interest in the evaluation of health effects due to EMF has accelerated in the last decades, mostly motivated by the occupational and environmental exposures to humans by such non-ionizing fields [3].

EMF carries very little power and energy and thus has no thermal or ionizing effects [4]. Although increasing experimental evidence show a wide spectrum of induced biological effects under exposures to static magnetic fields (SMF), EMF or ELF magnetic fields, the non-thermal effects have in fact received little consideration [5].

Initial studies with imposed EM fields in the nervous system centered modulation of brain ionic mechanisms. The developing vertebrate nervous system expose to EM fields at specific frequencies

(50 or 60 Hz) showed frequency-depended sensitivities in cerebel calcium binding. More recent epidemiological studies have reported developmental defects in motor skills, memory and attention in children who have been exposed throughout life to high intensity radar fields pulsed at electroencephalograph (EEG) frequencies. An association between occupational exposure to power frequency magnetic fields and Alzheimer' disease has been reported [6]. The increasing exposure to electromagnetic fields has been suspected to contribute to the rising incidence of breast cancer in industrialized countries. Electromagnetic fields reduce the efficacy of treatment of breast cancer [7].

The most consistent mechanism in explaining the biological effects of magnetic fields, in the range of ELF-MF, are those associated with ELF-MF interaction with cell plasma membrane that promote changes in calcium flux patterns [8]. Several experiments have been performed using time-varying magnetic fields to study possible effects on the electrophysiological activity of different cells, tissue, organs and behavior. The targets seem to be those ions controlling the motile activity, mainly Ca^{2+} fluxes, and the corresponding metabolic signal transduction chain [9].

Despite this detailed analysis which provide a wealth of accurate data on the effects of electromagnetic fields on nerve cells, a clear pictures of the mechanism and sites of action of ELF magnetic field in the range of environmental intensities is far from being accomplished and the ionic or metabolic processes underlying the observed effects have not been fully explained.

The aim of this study was to evaluate the effects of 50 Hz magnetic fields in the range of environmental intensities on spike and neuronal excitability in snail neurons. We measured the bioelectric parameters of F1 neuron with the conventional intracellular recording in current clamp mode.

2 Material and Methods

These experiments were performed on signal-neuron units, F1 neuron, in subesophageal ganglia of *Helix aspersa*. The F1 neuron was recognized by its size, position and electrophysiology properties [10]. The brain ganglia were dissected by cutting all peripheral nerves and pinned by the nerve and edges of the connective tissue into a small Sylgard-grounded recording chamber with a total volume of 1 ml (Dow Corning Midland, MI, USA) where it was submerged in normal snail Ringer,(84 mM NaCl, 10 mM $CaCl_2$, 5 mM M **A** 4 mM KCl:, 5mM HEPES and pH a(d to 7.4 with TRISMA –base (all from Merck). F1 neuron is positioned in the lower part of left parietal ganglion (Fig 1A) and it was visually identified under the stereomicroscope (Olympus SZH-10, Japan). To denude neurons, the connective tissue was gently torn off by fine tweezers without any proteolytic enzyme pretreatment.

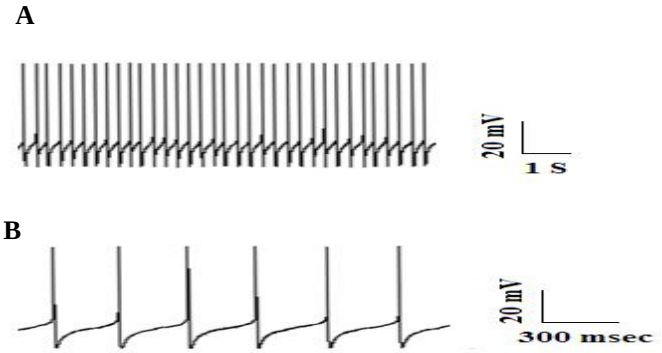

Fig 1. (A) Spontaneous activity of F1 neuron under control condition (B) Train of action potentials in B at higher time resolution

To apply sinusoidal magnetic fields, a pair of Helmholtz coils, to get a homogenous MF in the coil centre where the brain ganglia are placed. The coils have a diameter of 20 cm, were separated 10 cm. They consisted of 325 turns of 0.5 mm diameter copper wire. The coils were energized from 50 Hz power signal generator (XR 2206, Monolitic Function Generator, EXAR Corporation) and home-made power signal amplifier. Then 50 Hz MF of flux intensity ranging between 2.83 to 207.2 μT (rms) were applied for 12 min with 2 min intervals. Variation in the chamber bath solution under ELF magnetic field exposure was within ±1º C. The time duration of the bath solution was observed 20 min. For studying the reversible effects we measured the bioelectric parameters 10 min after last exposure (data not shown). Field intensity (B), was measured by a probe (TES 1394, Electronic, Electronic Electrical Corp). Stray ambient ac field is below 0.1 μT.

Conventional intracellular recording in current clamp method were performed using the Axoclamp 2B amplifier (Axon instrument, Foster City, CA, USA). Intracellular recording were

made by using glass microelectrode (Clark Instrument, UK) were prepared using a horizontal microelectrode puller (Stoleting, USA), filled with 3M KCl and those with a resistance of 5-7 MΩ were used for recording. The reference electrode in all experiments was a silver-silver chloride wire within an agar bridge (4% agar in snail Ringer). The above set-up and recording equipment were kept in Faraday cage. The electrophysiological recordings were made in real time by testing spontaneous neuronal activity, before (control), and after exposure to magnetic fields. Data were filtered at 30 kHz, voltage record were sampled at 20 kHz and digitized online using a 16 bit A/D converter (AD Instrument Pty Ltd., Sydney, Australia) and stored for further analysis using Chart 5 (AD Instrument Pty Ltd, Sydney, Australia).

Further analysis was carried out by measuring the parameters of action potential: the firing frequency of spike (Hz), the duration (ms) of action potential, resting membrane potential (mV) and also the amplitude of after hyperpolarization potential (AHP) (mV).

The duration of spikes were measured from the resting potential to the peak and at half maximal amplitude. The amplitude of AHPs was measured from the resting membrane potential to the peak of AHP. Numerical results are given as mean±SEM with n being the number of cells on which the measurement was made. Statistical significance was calculated by a two-way ANOVA followed by a Boneferonie post hoc to determine the effects of 50 Hz magnetic fields on the action potential properties. Differences with $P<0.05$ were considered statistically significant.

3 Results

To determine the effects of magnetic fields on spike and neural excitability, magnetic fields (50 Hz, sinusoidal) were applied in the range of 2.83 to 207.2 μT (rms). The initial activity was recorded for several minutes, in order to get the average bioelectrical parameters as a control for comparison with induced changes under applied magnetic fields. In control condition, all of the examined neurons (n= 39) exhibited regular activity with a frequency of 2.98±0.20 Hz. Single action

potentials were followed by AHP with a mean amplitude of -13.20± 0.37 mV and a mean duration of 4.90 ± 0.24 ms. Neuronal activities recorded under control conditions and after exposure to magnetic field to analyze the effects of magnetic ac field on spontaneous bioelectrical activity and action potentials configuration of F1 cells. At all intensities, the neuronal excitability and some of action potential parameters were significantly were by changed. The neurons were inhibited. The inhibition of bioelectric activity was recorded as a decrease in the spike frequency and an increase in interspike interval of action potential.

3.1 Effects of magnetic fields on firing frequency

Each neuron shows intrinsic properties which determine its firing frequency this defined as the neurons characteristic spontaneous firing, which changes among the different *Helix aspersa* neurons. The pattern activity of F1 neuron consists of spontaneously train of action potentials (Fig 1B).

Application of ac magnetic field (50 Hz) decreased the firing frequency from 2.98±0.20 Hz to 1.33±0.05 Hz in 2.83 μT; to 1.57±0.4 Hz in 6.02 μT; to 1.57±0.12 Hz in 14.91μT; to 1.12±0.13 Hz in 45.87μT; to 2.35±0.12 Hz in 109.34 μT; and to 1.41±0.18 Hz in 207.2 μT after 18 min ($P<0.05$) (Fig 2A). Magnetic field was seen to have a suppressive effect on the electrical activity of F1 neurons.

The experiments were repeated after a 20 min. In spite of that the field exposure changed the spontaneous firing activity of F1 cells, the percentage reduction were not the same at the remain intensities of magnetic fields (Fig 2A). Thus the neurons responses indicate an “amplitude window” effect. The minimum reduction was in the presence of an applied field of (109.34 μT, rms) and after 18 min (by 10.07 ±0.25 %) (Fig 2A).

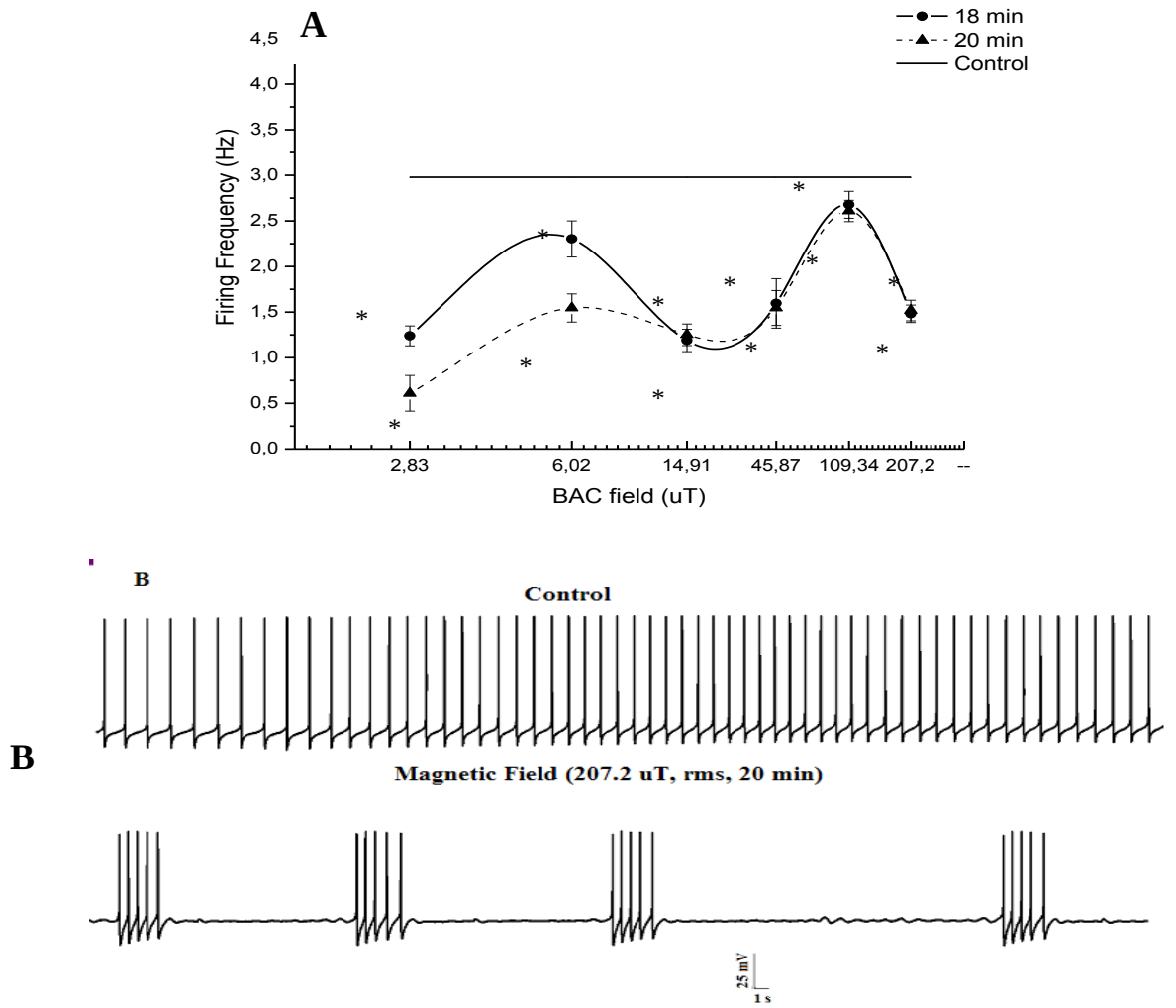

Fig 3. (A) The figure shows records before exposure to magnetic fields (control),18 and 20 min exposure to different flux density (rms) (2.83, 6.02, 14.91, 45.87, 109.34 and 207.2 µT) of magnetic field. Values are mean±SEM * denote P<0.05 . (B) Two traces are superimposed, indicating that the duration of action potential increased 18 and 20 min after exposure to magnetic field with 207.2 µT flux density compare to control
.

4 Discussion

Accumulating evidence demonstrates that extremely low frequency magnetic fields (ELF-MFs) are capable of modifying neuronal function. Here we examine the effect of ELF-MF exposure on neuronal excitability in F1 nerve cells of *Helix aspersa*. In this study, not only did the magnetic field affect action potential properties of nerve cells post exposure, but it did so in an oscillating manner.

Neuronal ion channels are gated pores whose opening and closing is usually regulated by factors such as 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

orders of magnitude too small to significantly influence neuronal signaling. However, in the central nervous system, a number of mechanisms exist which amplify signals. This may allow such small changes in membrane potential to induce significant physiological effects [11].

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 influence intermolecular interactions important for signal transduction, either protein-protien or protein-lipid membrane interactions [12].

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 SMF exposure two mechanism were propose, namely the Ca^{2+}-dependent and the Ca^{2+}-independent metabolic processes [13].

calcium entry might be enhanced to trigger downstream signal transduction events that overcome in inhibitory effects of treatment of cancer. Calcium is a potentially interesting research indicator to elevate effects of magnetic field.

We have observed that when flux intensity of magnetic fields increases, the bioelectrical parameter such as firing frequency of spike strongly changes as so called “amplitude window”. A similar effect was early observed by Bawin *et al* on chicken brain in vitro at a central frequency of 16 Hz [14]. These findings are in good agreement with previous studies that showed Jurkat cells responded to an applied MF by intracellular Ca^{2+} oscillations over a wide low frequency range (5-100 Hz). The response had a threshold with no effect observed at 0.04 mT, maximum effect at 0.15 mT and no further increase at 0.3 mT. The applied sinusoidal MF induced oscillatory changes of $[Ca^{2+}]_I$ in the leukemic cell line Jurkat in a manner similar to that seen with stimulation by antibodies [12].

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) are altered by magnetic field. Ljiljana.*et al* believe that the increase in the spike amplitude of the F1 could be a result of increased activity of channels involved in the depolarizating phase of AP, or decrease in channels responsible for after spike hyperpolarization. However, it is possible that other changes in membrane resistance caused the increase in the amplitude of the action potential [15].

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* [15].

Inactivation of Na^{+} channels leads to a progressive decline in the Na^{+} conductance available for generation 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 [12], 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.

Together, these findings provide support to the theory that environmentallevel

magnetic fields can act to modify the action of a drug or hormone on regulation of cell proliferation.

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