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ORIGINAL ARTICLES

Effects of Weak Environmental Magnetic Fields on the Spontaneous Bioelectrical Activity of Snail Neurons

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Abstract We examined the effects of 50-Hz magnetic fields in the range of flux densities relevant to our current environmental exposures on action potential (AP), after-hyperpolarization potential (AHP) and neuronal excitability in neurons of land snails, *Helix aspersa*. It was shown that when the neurons were exposed to magnetic field at the various flux densities, marked changes in neuronal excitability, AP firing frequency and AHP amplitude were seen. These effects seemed to be related to the intensity, type (single and continuous or repeated and cumulative) and length of exposure (18 or 20 min). The extremely low-frequency (ELF) magnetic field exposures affect the excitability of F1 neuronal cells in a nonmonotonic manner, disrupting their normal characteristic and synchronized firing patterns by interfering with the cell membrane electrophysiological properties. Our results could explain one of the mechanisms and sites of action of ELF magnetic fields. A possible explanation of the inhibitory effects of magnetic fields could be a decrease in Ca^{2+} influx through inhibition of voltage-gated Ca^{2+} channels. The detailed mechanism of effect, however, needs to be further studied under voltage-clamp conditions.

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Introduction

In spite of the technological progress made by humans, the corresponding increase in the level of electromagnetic fields (EMFs) has also generated new environmental exposures to the radiation (Levallois et al. 1999). EMF exposures are complex and come from multiple sources, which include the home, workplace and high-voltage power lines. The extents of indoor exposures, especially for residences located near power lines, and the potential risks associated with such exposures are still partly unknown (NIEHS 2002b). Interest in the evaluation of health effects due to EMFs has accelerated in the last decades, mostly motivated by the occupational and environmental exposures to humans by such non-ionizing fields. There is great concern regarding the adverse effects on human health of the extremely low-frequency (ELF) sinusoidal 50–60 Hz magnetic fields in the environment introduced via production and transport of electricity (ICNIRP 2001).

The basic restrictions and guidelines for limiting the exposure to electric fields (EFs), magnetic fields (MFs) and EMFs between 0 and 300 GHz are addressed to protect the health of the general population against thermal effects (ICNIRP 2001). An EMF carries very little power and energy and, thus, has no adverse thermal or ionizing effects (NIEHS 2002a). Although increasing experimental evidence shows a wide spectrum of induced biological effects under exposures to static magnetic fields (SMFs) as well as EMFs and ELFs, the nonthermal effects have in fact received little consideration (Azanza and Del Moral 1994).

Initial studies with imposed EMFs in the nervous system centered on modulation of brain ionic mechanisms. The developing vertebrate nervous system exposed to EMFs at specific frequencies (50 or 60 Hz) showed frequency-dependent sensitivities in cerebral 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 pulsed fields at electroencephalographic (EEG) frequencies. An association between occupational exposure to power frequency magnetic fields and Alzheimer disease has been reported (Adey 1988).

According to the literature, effects of pulsed magnetic field (PMFs) on cell function in vitro have implications in altered cellular ion transport. For example, Luben et al. (1982) found that hormone action in bone osteoblasts was altered and, furthermore, clear changes in fibroblast collagen production and cyclic AMP metabolism were also evident after PMF exposure. All or some of these effects might result from altered intracellular calcium levels (Baureus Koch et al. 2003; Farndale et al. 1987), either directly or via other pathways. The most consistent mechanisms to explain the biological effects of magnetic fields, in the of ELF-MFs, are those associated with ELF-MF interaction with the cell plasma membrane that promote changes in calcium flux patterns. It has been described that ELF-MFs induce alterations in the level of intra- and extracellular calcium, as well as in the rates of cellular calcium efflux (Borst and Sakmann 1999). It has experimentally been shown that bioelectric activity in neurons from the brain ganglia of a species of snails, *Helix aspersa*, is modified under exposure to MFs (Azanza 1989; Calvo and Azanza 1999, 2000; Kaviani Moghadam et al. 2008).

However, conflicting results often emerge when different studies are compared. Due to the different biological models, level of structural organization and characterization of EMFs, responses may be seen at different levels, such as at the membrane, cellular or tissue level. The sensitivity of the biological systems to weak MFs has been described in other research, mainly with respect to the dependence of bioeffects on the amplitude or frequency of applied fields.

Despite these detailed analyses which provide a wealth of relevant data on the effects of EMFs on nerve cells, a clear picture of the mechanisms and sites of action of ELF-MFs in the range of environmental flux densities is far from accomplished and the ionic or metabolic processes underlying the observed effects have not been fully explained.

We investigated the effects of 50-Hz MFs in the range flux densities which are relevant with respect to our current environmental exposures, on action potential (AP), after-hyperpolarization potential (AHP) and neuronal excitability in neurons of land snails, *H. aspersa*. F1 neurons of the right parietal ganglion of land snails were exposed to various applied MFs, and the effects on their bioelectric parameters were monitored using conventional intracellular recording in the current-clamp mode. Changes were compared with the characteristic spontaneous activities of neurons under normal conditions. These investigations attempt to mimic real-life situations and may give some insight into the effects of ELF-MFs on living biological systems at the molecular level.

Materials and Methods

Experiments were performed on single F1 neuron units, dissected from subesophageal ganglia of land snails, *H. aspersa*. Neurons were visually identified under the stereomicroscope (SZH-10; Olympus, Tokyo, Japan) by their position, size, color and appearance within the left visceral ganglion (Kerkut et al. 1975) (Fig. 1a). The ganglion mass was isolated by cutting away all peripheral nerves and tissues and then pinned in a Sylgard-grounded recording chamber with a volume capacity of 1 ml (Dow Corning, Midland, MI). To denude neurons, the overlying layers of connective tissue were gently pulled away using fine forceps, without any pretreatment with proteolytic enzymes. Each neuron was used only once, and all individual experiments were independent of each other. Following microelectrode insertion, if for any reason microelectrode came out, we did not attempt to continue recording from the same cell. Recordings were made in snail Ringer (NaCl, 80 mM; $CaCl_2$, 10 mM; $MgCl_2$, 5 mM; KCl, 4 mM; glucose, 10 mM; HEPES, 5 mM; pH adjusted to 7.4 with TRISMA-base; all chemicals were from Merck, Rahway, NJ). All experiments were performed at room temperature (21–24°C). These procedures were in accordance with the guidelines of the Institutional Animal Ethics Committee at Shahid Beheshti University of Medical Sciences.

The brain ganglion preparation was placed between a pair of Helmholtz coils, and homogenous sinusoidal magnetic fields were applied. The coils, 20 cm diameter and separated by 10 cm, consist of 325 turns of 0.5-mm-diameter copper wire. They were connected to a 50-Hz power signal generator (XR2206 Monolithic Function Generator; EXAR, Fremont, CA) and a home-made power signal amplifier. The MF was measured using a Gauss Meter (TES-1394; TES Electrical Electronic CORP., Ontario, Canada), and normal background flux density at 50 Hz was below 0.1 μT.

A series of electrophysiological recordings were obtained at 50 Hz with various applied flux densities of magnetic field (B field; 2.83, 6.02, 14.91, 45.87, 109.34 and 207.20 μT). All flux densities are given as root mean squares, and these intensities correspond to monitored working fields with homogeneity found to be better than

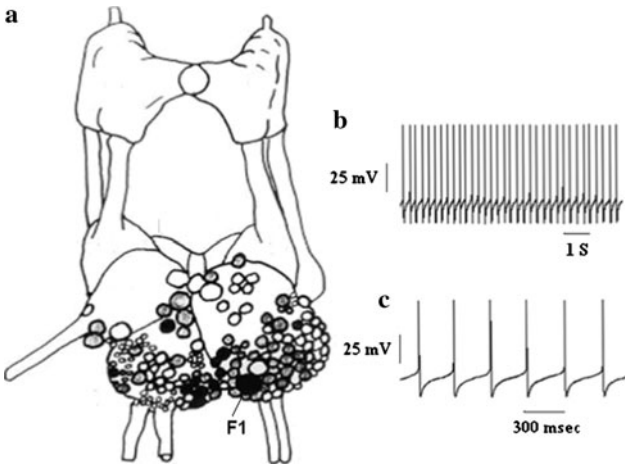

Fig. 1 **a** Schematic representation of the subesophageal ring and different cerebral nervous ganglia in posterodorsal view of *H. aspersa*. The F1 neuron is on the *left* (Kerkut et al. 1975). **b** Typical electrophysiological recording showing spontaneous activity of the F1 neuron under control conditions. *Vertical axis* is the amplitude (millivolts) and time is shown on the *horizontal axis*. **c** Train of action potentials in **b** at higher time resolution

0.1 μT in the region of brain ganglia within the coils. Recordings were made in real time by monitoring spontaneous neuronal activity, before exposure (control) and after exposure to the MFs. A first exposure at the chosen magnetic field was applied for 18 min, after which the bioelectrical activities were recorded for 2 min. Then, a second exposure of 2 min was applied and the neuron activity recorded for a further 2 min (Fig. 2 depicts the procedure). For studying any reversible effects of the applied field, the bioelectric parameters were measured 12 min after the last exposure (data not shown).

The Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) was used to record membrane potentials and to apply current under current-clamp conditions. Intracellular recordings were made using glass microelectrodes (Clark Instrument, Reading, UK), which were prepared using a horizontal microelectrode puller (Stoelting Instrument, Wood Dale, IL). Electrodes were filled with 3 M KCl, and only 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 setup and recording equipment were kept in a Faraday cage to eliminate any electrical noise. The collected data were filtered at 30 kHz; voltage records were sampled at 20 kHz and digitized online using a 16 bit A/D converter (ADInstrument, Sydney, Australia) and stored for further analysis.

Quantitative parameters of spikes were measured using chart5 software (ADInstrument). The firing frequency (hertz) was determined from a 1-min sample of spontaneous activity. The amplitude of AP (millivolts) was measured from the resting potential to the maximum peak height of the pulse, and the AHP (millivolts) was the value from the resting membrane potential to the peak negativity of AP. Data are presented as mean ± SEM, and statistical differences, as determined by a two-way ANOVA followed by a Bonferonni post hoc, were considered statistically significant at $P < 0.05$.

Results

All F1 neurons showed spontaneous intrinsic activity in the form of trains of regular APs.

In order to get the average patterns of bioelectrical activity, neural excitability and associated parameters were first recorded under normal conditions for 10 min. In control conditions, all of the recorded neurons ($n = 42$) exhibited regularly spaced spontaneous activity with a mean resting membrane potential (RMP) of -39.28 ± 0.97 mV, amplitude of 73.82 ± 1.21 mV and mean frequency of 2.98 ± 0.20 Hz. APs were followed by an AHP with mean amplitude of -15.73 ± 0.45 mV (Fig. 1b, c). When the neurons were exposed to MF at various flux densities, marked changes in neuronal excitability, AP firing frequency and AHP amplitude were seen. These effects seemed to be related to the flux density, type (single and continuous or repeated and cumulative) and length of exposure (18 or 20 min).

Effect of MF on Firing Frequency of AP

Neurons were inhibited by the applied MF, and this inhibition of bioelectrical activity was recorded as a decrease

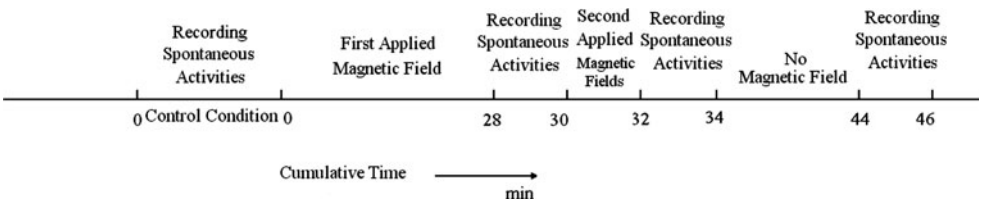

Fig. 2 Method flowchart. Electrophysiological recordings of spontaneous neuronal activity were made in real time, before (control) and after exposure to MFs. Activity under control conditions was recorded for 10 min. First exposure was applied for 18 min, after which bioelectrical activity was recorded for 2 min. Thereafter, a second exposure of 2 min was followed by recording for 2 min. For studying any reversible effects of the applied field, the bioelectrical parameters were measured 10 min after the last exposure

in AP firing rate. A maximum reduction in firing frequency of 60.08 ± 0.40%, from the control value of 2.98 ± 0.2 to 1.19 ± 0.12 Hz, was recorded when 14.91 μT was applied for 18 min (Fig. 3a). The minimum significant reduction of 10.07 ± 0.25%, from the control value of 2.98 ± 0.2 to 2.68 ± 0.15 Hz, occurred after an 18-min MF exposure of 109.3 μT. An effect on the AP amplitude was also recorded. For instance, at 14.91 μT for 18 min, a decrease from the control value of 73.82 ± 1.21 to 61.39 ± 1.36 mV was seen. The reduction in the firing rate abolished the regularity of the AP, leading to an irregular, progressive and substantial increase in interspike intervals (ISIs) (Fig. 3b). The magnitude of the effects seems to be related to exposure density in a nonmonotonic and "parameter-dependent window effect" manner, and the effects were also dependent on the type of exposure. Repeated exposures at the lower densities, below 14.91, seem to give a less marked (although still significant) effect compared to the single 18-min exposure. At higher intensities, multiple exposures had similar effects on the AP firing frequency as single continuous magnetic exposure.

The mean and standard deviation of ISIs were calculated, and the coefficient of variation (CV) was derived from the ratio of SD and mean ISI. We found that the clear and regularly spaced AP peaks for the firing F1 cells under control conditions were associated with a low CV (0.08) where the neurons under MF exposure had a higher CV (0.12), a 42.85% increase in the case of 18-min exposure at 207.2 μT. Further analysis confirmed this observation.

Effects of MFs on AHP

Following exposures to MFs of various intensities, the mean amplitude of AHP was seen to shift toward more hyperpolarized (negative) voltages compared to control conditions (Fig. 4a). The effects, significant when compared to the control values, show a nonmonotonic and parameter-dependent type with critical effective flux densities (at 50 Hz) lying in the range 14.91–45.87 μT. A U-shaped response with the maximal effect occurred at a flux density of 14.91 μT for 18 min, inducing a 25.56 ± 0.24% (from −15.73 ± 0.45 to −19.75 ± 0.56 mV) increase in the peak amplitude of the AHP compared to control conditions. At 45.87 μT for 18 min, a 24.09 ± 0.26% (from −15.73 ± 0.44 to −19.52 ± 0.56 mV) increase in the peak amplitude of the AHP compared to control conditions was seen (Fig. 4a, b). The effects for the flux density outside this window seem to be dependent on the type (single or repeated) of exposure as shown by the divergence of the 18- and 20-min plots (Fig. 4a). It can be seen that the mean minimum nonsignificant increase in hyperpolarization of the neurons was after 18 min at 6.02 μT intensity, 2.67 ± 0.58% (from −15.73 ± 0.45 to −16.15 ± 0.71), and comparable to that at the other end of the window, at 109.34 μT, where a 2.92 ± 0.58% increase (from −15.73 ± 0.45 to −16.19 ± 0.19) was recorded. Table 1 shows a summary of the results.

Discussion and Conclusions

Accumulating evidence demonstrates that ELF-MFs are capable of modifying neuronal functions in living organisms. There is substantial evidence indicating that very weak alternating MFs, in the nanotesla range, are also capable of inducing marked biological effects (Belova and Lednev 2001; Blackman et al. 2001; Juutilainen et al. 1987; Novikov et al. 2009). Here, we examined the effects of low-density ELF-MF exposures on neuronal excitability of F1 nerve cells of the land snail *H. aspersa*. The results obtained in this study indicate that the ELF-MF exposures affect the excitability of F1 neuronal cells in a nonmonotonic manner, disrupting their normal electrophysiological characteristic and synchronized firing patterns by interfering with the cell membrane electrical properties.

Any factors that interfere with membrane voltage can alter the gating kinetics of voltage-dependent ion channels and, thus, may have profound effects on physiological function.

ELF-MFs are thought to produce, at most, microvolt changes in neuronal membrane potential, changes too small to significantly influence neuronal signaling. However, in the central nervous system, a number of mechanisms exist which may amplify the signals and translate such small changes into effects that induce a cascade of significant physiological alterations (Mathie et al. 2003).

Although in many studies data do not allow a conclusion about the precise molecular mechanism for the effects of MFs, one possibility is that MFs influence the intermolecular interactions important for signal transduction, such as protein–protein or protein–lipid membrane interactions (Lindstrom et al. 1995).

The present findings describe the cellular effects of low-frequency MFs on neuronal excitability, its AP characteristics. The AP firing frequency was found to be decreased in the presence of applied ELF-MF, suggesting that MFs inhibit neuronal activity. This decreased neuronal activity is in line with the observed increase in AHP amplitude. A stronger and longer AHP renders the nerve unresponsive to subsequent impulses and inhibits it from firing APs.

The changes detected in the AP characteristics of the F1 neurons caused by MFs may be the result of changes in some membrane proteins such as ion channels and/or ionic pumps.

Bawin and Adey (1976) suggested that a stimulus capable of modifying the efflux of an ion essential in

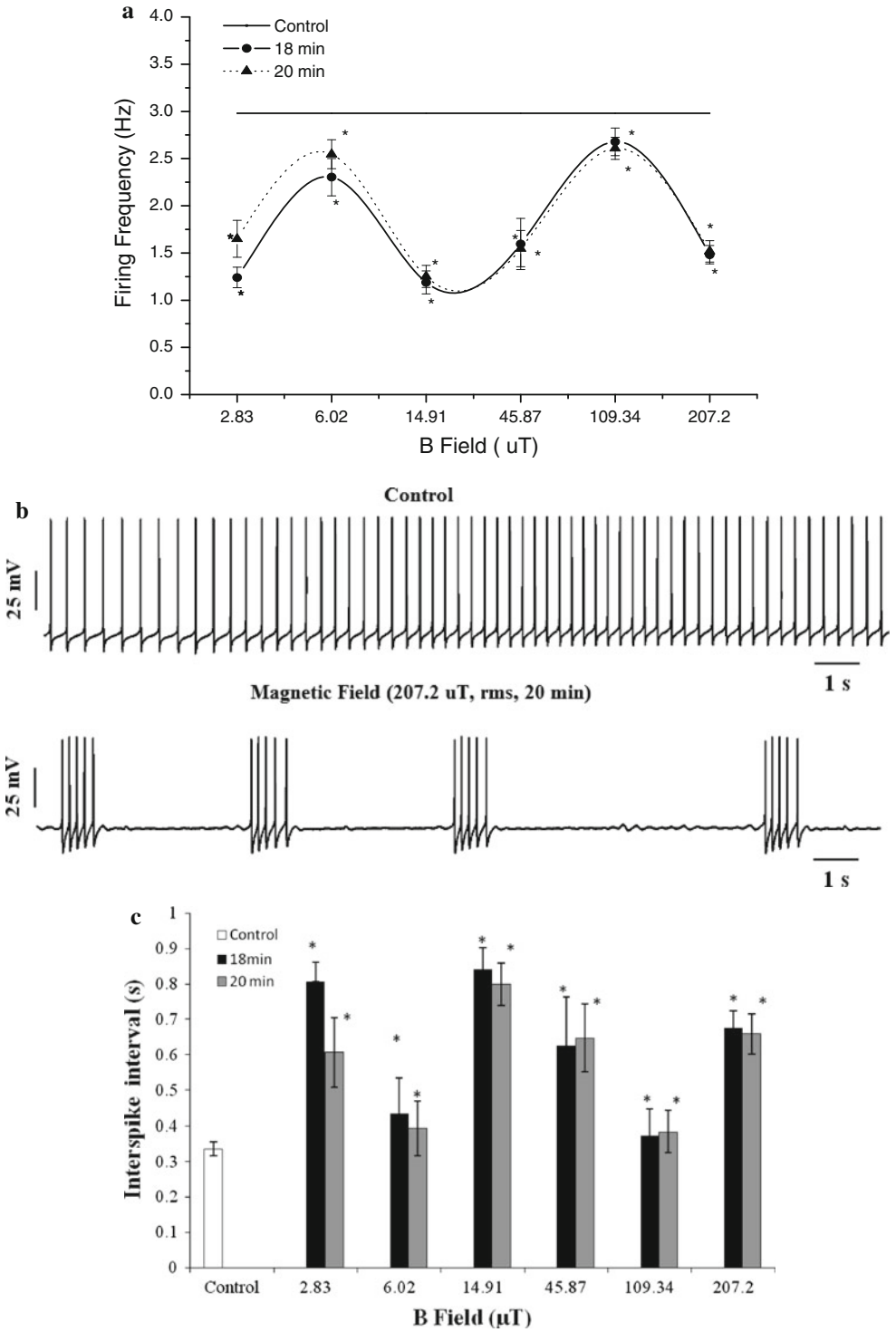

Fig. 3 **a** Dependence of firing frequency of spontaneous AP on the intensity of MFs, B Field (μT, 50 Hz, sinusoidal). Inhibitory responses from F1 neurons were recorded after 18- and 20-min exposures. Changes in firing frequency were in a nonmonotonic fashion as the applied MF intensity increased. Control line shows an average of a train of APs recorded under control conditions (2.98 ± 0.2 Hz). Experiments lasted 18 and 20 min. **b** Pattern of spontaneous firing under control conditions and in the presence of applied MF (207.2 μT, rms, 20 min). The MF clearly decreased the firing frequency and disrupted the regular pattern of neuronal spontaneous activity. **c** Histogram generated from interspike intervals (s) of neuronal activity after 18 and 20 min under control and various exposure (B field, μT) conditions. The long interval was apparent after MF exposure. Values are means ± SEM. *Significance at $P < 0.05$

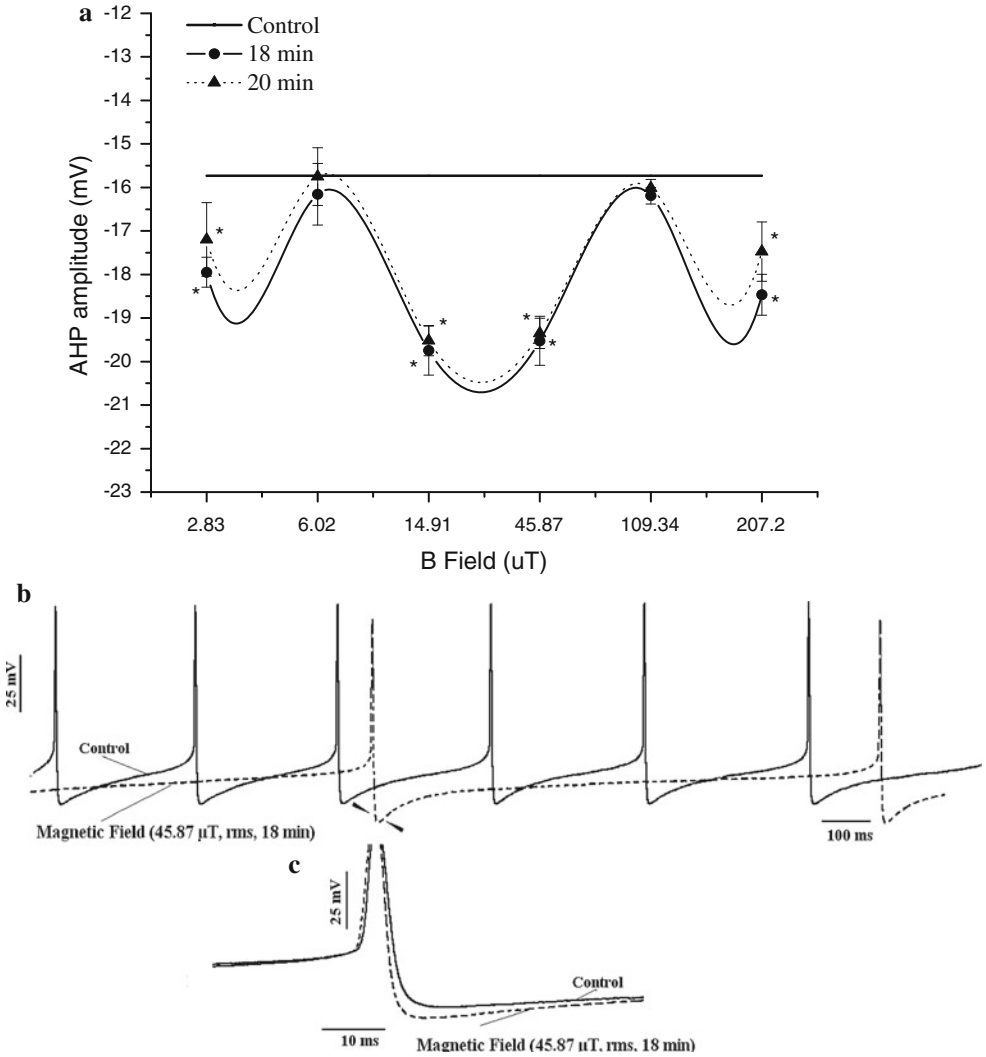

Fig. 4 **a** Dependence of AHP amplitude (millivolts) of spontaneous AP on the intensity of MFs (50 Hz, sinusoidal) applied for 18 and 20 min. Control line shows an average of -15.73 ± 0.45 mV. AHP amplitude decreased significantly on MF exposure in a nonmonotonic fashion and an amplitude window effect was evident. **b** Spontaneous activity showing AP of F1 neuron under control conditions (*solid line*) and after MF exposure (*broken line*; 45.87 µT, 18 min). **c** Superimposed AHPs under control condition (*solid line*) and after MF exposure (*broken line*; 45.87 µT, 18 min). The MF increased the AHP amplitude following the single AP spike. Values are means ± SEM. *Significance at $P < 0.05$

Table 1 Values of firing frequency and AHP amplitude of spontaneous AP in control conditions and after MF exposure

B field (µT)	Firing frequency (Hz)		AHP amplitude (mV)	
	18 min	20 min	18 min	20 min
2.83	1.24 ± 0.11	0.61 ± 0.19	−17.95 ± 0.34	−17.20 ± 0.85
6.02	2.30 ± 0.20	1.55 ± 0.15	−16.15 ± 0.71	−15.75 ± 0.66
14.91	1.19 ± 0.12	1.25 ± 0.12	−19.75 ± 0.56	−19.52 ± 0.35
45.87	1.60 ± 0.27	1.55 ± 0.19	−19.52 ± 0.56	−19.35 ± 0.35
109.34	2.68 ± 0.15	2.61 ± 0.12	−16.19 ± 0.19	−16.01 ± 0.20
207.2	1.48 ± 0.17	1.52 ± 0.11	−18.46 ± 0.47	−17.47 ± 0.68

Firing frequency in control condition 2.98 ± 0.20 Hz; AHP amplitude in control condition −15.73 ± 0.44 mV

excitation and regulatory functions by 15% would be associated with dramatic changes in perception and behavior. Along the same lines, Nikolic et al. (2008) believed that the increase in AP amplitudes of F1 cells after exposure to SMFs could be a result of either increased activity of channels involved in the depolarizing phase of AP or a decrease in channels responsible for after-hyperpolarization. However, considering that the nervous system is a complex system of small units, it is possible that some other factors propagated these changes in membrane resistance which caused the increases in AP amplitude. One alternative candidate mechanism investigated involves a Ca^{2+}-dependent nonspecific cation current, a

Ca^{2+}-dependent K^+ current and a transient K^+ current on the Br neuron of snails. Whether the net effect of the MF exposure would be an increase in the K^+ current or a decrease in some inward current carrying Ca^{2+}, one of the expected consequences of such a change would indeed be a change in membrane potential.

Based on observations in current-clamp mode, we found that the applied MF led to an increase in the amplitude of AHPs. In snail neurons, spike repolarization and AHP are determined by a set of potassium channels which underlie fast and delayed outward K^+ currents (Bal et al. 2000; Sakakibara et al. 2005; Solntseva 1995; Thompson 1977). There are two classes of Ca^{2+}-activated K^+ channels: large-conductance (BK channels) and small-conductance (SK) (Crest and Gola 1993; Hermann and Erxleben 1987). These channels are activated during the AP, causing a transient hyperpolarization of the cell membrane. This produces the AHP, which in turn inhibits further AP firing. The cell is incapable of further stimulation until the membrane potential is restored to its resting potential and AP firing may be resumed. Thus, AHP that follows APs is a key determinant of cellular excitability and an important intrinsic negative feedback mechanism in excitability and control of regular firing pattern in neurons. Alternations in the AHP amplitude have been shown previously to influence neuronal excitability in many different neurons. An increase in AHP amplitude results in decreases in neuronal excitability (Kawai and Watanabe 1986; Madison and Nicoll 1986). While voltage-dependent Na^+ and K^+ channels are essential for the generation of APs in most neurons, the functional role of Ca^{2+} channels and Ca^{2+}-dependent K^+ is more controversial. It is suggested that Ca^{2+}-dependent K^+ channels are mainly involved in the shape, frequency and pattern of APs (Bennett et al. 2000; Crest and Gola 1993; Faber and Sah 2002; Goldberg and Wilson 2005).

Vatanparast and Janahmadi (2009) revealed that inhibition of the small-conductance Ca^{2+}-dependent K^+ channels using apamin, a compound that specifically blocks these channels, eliminates one of the major components of AHP in snail neurons, thus increasing the frequency of Ca^{2+} spikes. In many neurons, Ca^{2+} influx through voltage-dependent Ca^{2+} channels and consequent activation of Ca^{2+}-dependent K^+ channels is a major determinant of AHP amplitude.

The decrease in the frequency of APs indicates that the MF has an inhibitory influence on F1 neuron activity, and similar findings were reported in cultured mammalian neurons (McLean et al. 1995).

It has been described that ELF-MFs induce alterations in the levels of intra- and extracellular Ca^{2+}, as well as in the rates of cellular calcium efflux, which caused many research groups to attend to this field and focus on the influence of weak MFs on Ca^{2+} metabolism in living cells.

MFs have been shown to influence the release of neurotransmitters, which may be due to changes in neuronal calcium and in the stability of calcium binding to neuronal membranes (Bawin and Adey 1976; Bawin et al. 1978; Blackman et al. 1985a, 1985b).

Therefore, a possible explanation of the inhibitory effects of MFs could be a decrease in the Ca^{2+} influx through inhibition of voltage-gated Ca^{2+} channels, but this needs to be further investigated using voltage-clamp techniques.

Another issue which was confirmed in the present study is the dependence of flux density of MFs on bioelectrical parameters such as firing frequency and amplitude of AP and AHP, which strongly change in a so-called amplitude window effect. The bioelectrical parameters of AP as a function of flux density of the MF were studied at 50 Hz. We found that at low intensity repeated, cumulative, longer exposure had a stronger effect than single exposures and that this difference was not evident at relatively higher densities. These findings are in good agreement with previous studies which showed that Jurkat cells, an immortalized line of T-lymphocyte blood cells, responded to an applied MF of a wide range of low frequencies (5–100 Hz) by oscillations in their intracellular Ca^{2+} levels. The response had a threshold, with no effect observed at 0.04 mT, maximal effect at 0.15 mT and no further increase at 0.3 mT (Lindstrom et al. 1995). A similar effect was earlier observed by Bawin and Adey (1976) in cerebral tissue from chick forebrain in vitro at a central frequency of 16 Hz.

The magnitude of the effects in the window enclosed by the critical effective flux densities appears to remain approximately constant on repeated cumulative exposures. This implies that repeated exposures are neutralized and have a lesser effect, maybe due to recovery, adaptation and compensation mechanisms which may come into play during long-term exposures. It could also be that the maximal effect (threshold) is reached and any further exposure is irrelevant in this sense.

An additional feature of the flux density response is the narrow range of effective flux densities. If this relationship holds for lower and higher densities as well, then future experiments should investigate the narrower regions of effective intensities thoroughly.

It seems that under certain exposure conditions a biological system can exchange energy with the environment and may achieve a new stationery state, remaining stably there for a certain period of time. Alternatively, some other MFs (lower or stronger) would transit the system to a state different from the stationery one which appears to be unstable; therefore, the effects are lower and last for a shorter time. This window should be considered an opportunity for a given biological system to react to an exogenous MF. In particular, the

cyclotron resonance model affirmed that there exists a special combination of applied AC and DC for particular ions, such as calcium, potassium and magnesium. Later, other resonance models were proposed by Lednev, Blanchard and Blackman. All of these models are based upon consideration of the importance of the ionic charge/mass ratio in establishing the appropriate resonance frequency of the AC signal. Under resonance conditions, the system can exchange the energy with the environment and, therefore, the effects are smaller and disappear quickly at these nonpermitted states (Baureus Koch et al. 2003; Markov 2004).

The findings of the present study suggest that a nonlinear phenomenon may help to transfer information between neurons, thus transmitting and propagating signals over a distance.

This implies that the MFs not only affect the target neuron (direct effect) but, via synaptic propagation, may also have indirect effects on the neighboring neurons and then modulate the ganglia as a whole.

In conclusion, on the basis of the present data in combination with previous work on snail neurons (Kaviani Moghadam et al. 2008), it can be suggested that MFs induce inhibitory effects on biological membranes, most possibly through inhibition of Ca^{2+}-dependent K^+ channels. The use of different flux densities of MFs as in this study seems to be an essential contributing factor in decreasing the firing frequency of APs in F1 neurons of the land snail.

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