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Comparison between low-level 50 Hz and 900 MHz electromagnetic stimulation on single channel ionic currents and on firing frequency in dorsal root ganglion isolated neurons

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Abstract

Alteration of membrane surface charges represents one of the most interesting effects of the electromagnetic exposure on biological structures. Some evidence exists in the case of extremely low frequency whereas the same effect in the radiofrequency range has not been detected. Changes in transmembrane voltages are probably responsible for the mobilization of intracellular calcium described in some previous studies but not confirmed in others. These controversial results may be due to the cell type under examination and/or to the permeability properties of the membranes. According to such a hypothesis, calcium oscillations would be a secondary effect due to the induced change in the membrane voltage and thus dependent on the characteristics of ionic channels present in a particular preparation. Calcium increases could suggest more than one mechanism to explain the biological effects of exposure due to the fact that all the cellular pathways using calcium ions as a second messenger could be, in theory, disturbed by the electromagnetic field exposure. In the present work, we investigate the early phase of the signal transmission in the peripheral nervous system. We present evidence that the firing rate of rat sensory neurons can be modified by 50/60 Hz magnetic field but not by low level 900 MHz fields. The action of the 50/60 Hz magnetic field is biphasic. At first, the number of action potentials increases in time. Following this early phase, the firing rate decreases more rapidly than in control conditions. The explanation can be found at the single-channel level. Dynamic action current recordings in dorsal root ganglion neurons acutely exposed to the electromagnetic field show increased functionality of calcium channels. In parallel, a calcium-activated potassium channel is able to increase its mean open time.

Keywords: Electromagnetic field (EMF); Extremely low frequency (ELF); Radiofrequency (RF); Dorsal root ganglion (DRG); Ca and K single channel; Firing rate

1. Introduction

A broad literature exists on possible biological effects, both in vivo and in vitro, of electromagnetic field (EMF) exposure. In spite of a great deal of results, it is necessary to observe that, even considering the same biological endpoint, it is often difficult to obtain a good repeatability of data among different laboratories, thus generating conflicting results about possible effects. In particular, not only macroscopic analysis on cell survival, using homogeneous primary cultures obtained from humans and animals, have been unable to give a unique response, but also investigations at sub-cellular level failed to explain the sporadic alterations observed after the EMF exposure [1,2]. In fact, if a detectable modification exists, it is not constant and occurs always following activation of complex cellular mechanisms [3]. Some studies on intracellular calcium have been performed using 50 Hz magnetic fields [4–8], even if the few positive results have not been independently replicated [9]. Nevertheless, even in the few cases where the EMF appeared to be involved in cellular

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function modifications, these results did not clarify the mechanism of interaction between EMF and biological structures, hence the scientific debate on this matter is still open.

In a previous study, we have shown that extremely low frequency (ELF) EMFs interfere with the in vitro differentiation of NG108-15 neuroblastoma × glioma cells [10]. The hypothesized mechanism of interaction between EMF and chemical-induced differentiation is based on two antagonist cellular events. ELF EMFs are able to prevent the potential shift due to surface charge alteration linked to the cell commitment to differentiation [11,12]. However, it simultaneously stimulates the increase of intracellular calcium and thus the opening of calcium-dependent potassium channels, re-establishing cell-membrane potential values required for differentiation. In NG108-15 cells exposed to low intensity ELF EMF, the two mechanisms appear to be distinct. The magnetic field counteracts hyperpolarization while intracellular calcium is not sufficiently high.

In the present study, we move from these previous results into testing the hypothesis that the EMF modulates cell firing in dorsal root ganglion (DRG) neurons. The investigation puts side by side exposures to low-level ELF EMF (50 Hz, 125 μT magnetic field) and RF (900 MHz, specific absorption rate (SAR) of 1 W/kg) using the same experimental protocol. The employed values for induced SAR and magnetic field are, in both cases, representative of low-level exposures, being, respectively, below or just above the limits stated by international standards (peak SAR of 2 W/kg at RF; magnetic induction of 100 μT at 50 Hz) [13]. In particular, an ELF magnetic field of 125 μT was already proven to be the most effective in intracellular Ca mobilization [10], whereas a SAR of 1 W/kg has been chosen as representative of a realistic exposure condition. The comparison between two experiments concerns physiological observations at the macroscopic level of cell behaviour (action potential) and molecular protein dynamic events (single ionic channel current). The choice of DRG neurons was not incidental. In these cells, the oscillation frequency of the membrane potential is correlated with the stimulus intensity. Cell firing establishment and modulation respond to a complex equilibrium of membrane ionic currents.

The aim of the present investigation is to highlight possible interactions between EMF and membrane potential oscillations. By dissecting single ionic permeability in channels active during the action potential burst, the intent is to undercover, at the molecular level, the mechanism of action of EMF stimulation at different frequencies.

2. Methods

2.1. Cell cultures

DRG cells of adult rat were prepared by enzymatic digestion, following the procedure of Ferroni and colleagues [12]. After 12 to 24 h in tissue culture medium, and immediately preceding the experiments, we washed the cells in bath solution (in mM): 142 NaCl, 5 KCl, 2 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, adjusted to pH 7.35.

The whole-cell electrode for measuring voltage contained an intracellular-like solution, consisting of 120 KAsp, 20 tetraethylammonium (TEA), 10 NaCl, 2 MgATP, 0.1 BAPTA, 10 HEPES, adjusted to pH 7.35. The cell-attached electrode for measuring patch currents contained (in mM): 20 $BaCl_2$, 70 NaCl, 1 EGTA, 0.003 tetrotodotoxin (TTX), 5 TEA, 10 4-aminopyridine (4-AP), 10 HEPES, adjusted to pH 7.35. All experiments were performed at room temperature.

2.2. Electrophysiology

We used standard current-clamp and single-channel recording techniques. The bath solution (pH 7.32) contained (in mM): 133 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 5 Glucose. The electrode solution (pH 7.32) for action potential detection contained (in mM): 10 NaCl, 120 KAsp, 2 $MgCl_2$, 4 $CaCl_2$, 10 HEPES, 10 EGTA, Mg-ATP 3. To record single Ca current, we filled the electrode with (in mM): 72 Choline-Cl, 3 KCl, 1 $MgCl_2$, 20 $BaCl_2$, 10 HEPES, 10 TEA-Cl, 10 4AP, 24 Glucose. For single K-channel recording, we used in the pipette the bath solution (see above). We used an Axon 200B patch-clamp amplifier (Axon Instruments, Novato, CA, USA) to record membrane voltage, action potentials and single-channel currents. Experimental traces were digitized (0.2 ms sampling rate), filtered at 1000 Hz, and analyzed using PClamp 8 programs (Axon Instruments, Novato, CA, USA).

Data are presented as mean ± S.E.M. Values obtained from different experiments were tested for statistical differences using independent two population *t* test (Origin software; Origin Lab, Northampton, MA).

2.3. Hz EMF exposure

50 Hz EMF was continuously provided to the cell under observation on the inverted microscope head stage. In order to perform the electrophysiological measurements in the presence of EMF, the experimental chamber was encircled with a Helmholtz device using coils made out of isolated copper wire each field intensity being calibrated. The field was measured at the beginning and at the end of each experiment. Throughout all experiments we used an EMF at 50 Hz with a magnetic induction of 125 μT_{rms}, since, in the previous paper [10], this was the most effective field intensity to induce mobilization of intracellular Ca after 2-min stimulations. For control experiments the current flowing in the coil was zeroed by switching off the waves generator. The sequence on/off of EMF was randomly decided during each experiment.

The maximum value of the induced electric field was negligible, being about 0.48 mV/m. All experiments were performed at room temperature. However, the Petri dish temperature was monitored throughout a few complete experiments to make sure there was no unwanted bath solution temperature changes. The resulting working range was between 24.6 and 26.2 °C.

2.4. 900 MHz EMF exposure

The exposure system used [14] is based on a coplanar waveguide (CPW), an open propagating structure with a dielectric substrate on which three metallic strips are deposited [15]. Choosing glass as the dielectric substrate, it was possible to design a system suitable to replace the microscope head stage. This permits the usual operating mode in patch-clamp recordings, and, at the same time, guarantees the concentration and focusing of EMF lines close to the exposure zone. Moreover, in a CPW, the direction of the E field is almost parallel to the Petri surface and, as a consequence, orthogonal to the glass microelectrode. In this way it is possible to minimize the interference of the field with the electrode (i.e. with the current being measured).

The frequency operating range of the system is between 800 and 2000 MHz and its efficiency, experimentally evaluated, is around 20 W/kg/W at 905 MHz. Further technical details can be found in the literature [14].

During the experimental recordings, exposure conditions have been guaranteed by a microwave radiation set-up [15], which is able to implement different signal patterns, like continuous wave (CW) and GSM standard [16], through an automated procedure. In these investigations we use a 900 MHz CW at a SAR value of 1 W/kg. All experiments were performed at room temperature. However, Petri dish temperature during 900 MHz continuous

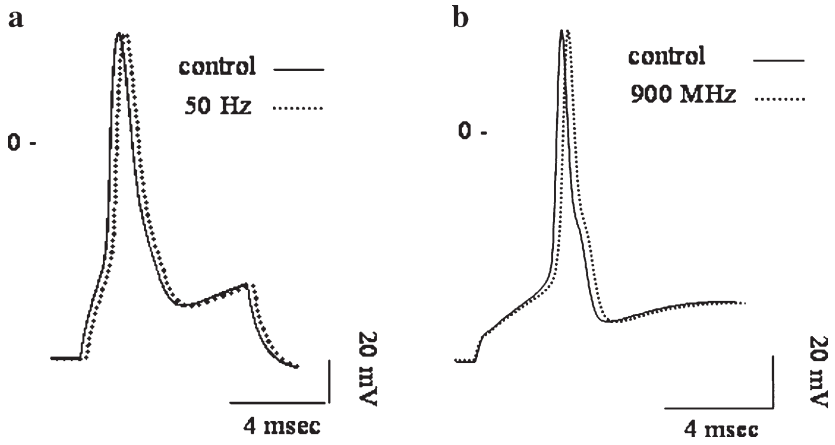

Fig. 1. Single action potentials under EMF exposure. (a) Average of 20 stimulated action potentials from DRG neurons under control conditions (continuous line) and during exposure to 50 Hz EMF (dotted line). (b) Same experiment as in panel a with exposure to 900 MHz EMF.

stimulation was monitored in 4 separate cases. Culture media did not change the temperature after 30-min exposure. The average temperature during this time was 24.2±0.6 °C without any correlation between control and exposed culture cells.

3. Results

The purpose of this study was a qualitative comparison, using the same preparation, patch-clamp unit, and experimental protocols, between the effects of two different EMF stimulations on the electrophysiological properties of isolated adult rat DRG.

DRG neurons are able to produce either single or repetitive action potentials (AP) upon sudden membrane depolarization. Fig. 1depicts induced APs under ELF and RF EMF exposure. We analyzed a total of 6 experiments composed of several action potentials, induced every 10 s, alternating control conditions and exposures to ELF or RF EMFs. In Table 1, we compare the values of several biophysical parameters (resting membrane potential (MP), threshold potential (dV/dt), and dV/dt_{max}), averaged over 20 APs in exposed and unexposed conditions for the two frequencies. As evident from Table 1, the exposures do not significantly modify the examined parameters. If there were no apparent influence of EMF exposure on the single AP, results are different for stimulated firing. In Fig. 2, we show the effect of both ELF and RF exposures during membrane potential sustained depolarization. In control conditions (top of Fig. 2a), 500 ms of current injection produced an action potential burst of 30 Hz frequency. Subsequent depolarizations were applied every 10 s. When the ELF EMF was present, we first observed an increase in the firing rate frequency (40 Hz) (Fig. 2a, second from the top). However, the following action potential bursts showed a marked decrease of the frequency, failure in repetitive firing (Fig. 2a, third from the top) and, most of the time, a complete silencing of the action potential burst (Fig. 2a, fourth from the top). The removal of the field was sufficient to re-establish the initial firing frequency (Fig. 2a, control trace at the bottom). The same experiment was repeated using 900 MHz field exposure. Even after 15 consecutive 500 ms depolarizations, the firing burst was unaltered and did not present any apparent modification in firing frequency (Fig. 2b).

To investigate whether one or more ionic conductance played a role during the modification of the firing rate caused by the exposure, we monitored the behaviour of single-channel events under the influence of the EMF. The first ion pathway we considered was the L-type calcium channel. In previous works, we extensively characterized this channel permeability in DRG neurons, both during the spontaneous action potential [17,18] and in steady state conditions [19]. In such investigations, we first fixed the resting potential of the cell to 0 mV using an external solution where Na was replaced by K. Under this condition, we clamped the patch membrane potential to −60 mV and stepped the voltage to −20, −10, 0 and +10 mV (data not shown). We calculated a single channel conductance of 22.4±1.5 pS, in agreement with the typical value for L-type calcium channel under these experimental conditions [19]. Fig. 3adepicts, on the left, three examples of 2 s single-channel recordings at 0 mV membrane potential, obtained under control conditions. The current traces in the centre are relative to 50 Hz exposure. It is quite visible that ELF stimulation increases the open probability of the channel. After EMF exposure (right column), the re-establishment of initial behaviour of the channel is observable. Panel (b) shows results of the same experiment repeated exposing the cell to 900 MHz EMF. In Fig. 4, we present an example of amplitude histograms, open and closed times distributions, for a single calcium channel before, during and after EMF exposure. In panel (a), the plots are relative to control conditions before exposure (left

Table 1
Single action potential biophysical parameters

	Control 1	50 Hz	Control 1	900 MHz
MP (mV)	62.0±4.1	62.0±4.1	60.0±5.0	62.0±3.4
dV/dt (mV/ms)	41.0±3.6	40.0±4.5	47.0±6.2	45.0±7.0
dV/dt_{max} (mV/ms)	60.0±3.7	62.0±4.5	58.0±4.0	61.0±3.8

The table reports resting membrane potential (MP), threshold potential (dV/dt), and dV/dt_{max}. Reported values are relative to control (control 1) and exposure conditions (50 Hz or 900 MHz).

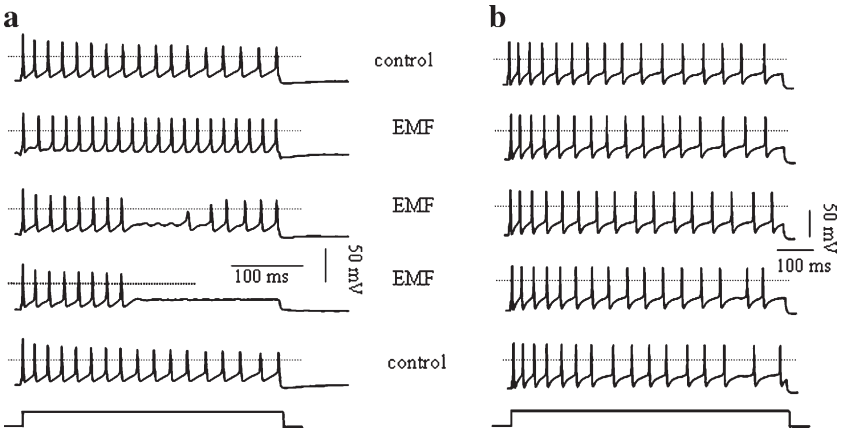

Fig. 2. EMF effect on DRG-stimulated firing properties. (a) Following a 500 ms current pulse injection (bottom), the single neuron is able to produce an action potential train. Current injection was delivered every 10 s. Once the firing rate was stabilized, we applied 50 Hz EMF. The second trace from the top, recorded after 10 s of EMF exposure, shows a firing rate increase. However, in the following recordings (3rd and 4th traces from the top), the firing rate decreased, presented some failure and finally stopped. The bottom voltage trace represents the recovery in the absence of EMF after 2 min. (b) Firing rate did not experience any changes when 900 MHz EMF was applied to a stimulated firing neuron (2nd, 3rd, and 4th voltage traces). Firing rate remained unaltered before, during, and after EMF exposure.

column), 50 Hz stimulation (central column) and control after stimulation (right column). In Fig. 4b, the central column is relative to 900 MHz EMF stimulation. Among 14 experiments at 50 Hz, effects were detected only in 5 of them that were subsequently analyzed. In 22 experiments at 900 MHz, we never detected any alteration in channel property. All the data reported in Table 2 are average±S.E.M. (see Methods) of 5 experiments at both 50 Hz and 900 MHz in which we recorded single calcium channel openings at −10 mV test potential. All the parameters considered in the table did not show any significant changes concerning RF stimulation. No changes occurred also in the single channel conductance and in the mean open time during ELF EMF exposure. However, both the open probability and the mean close time resulted statistically different ($n=5$; $P<0.05$) from unexposed channel openings. To confirm our hypothesis of a possible membrane potential shift caused by 50 Hz EMF via mobilization of plasma membrane surface charges [10], we examined the P_o as a function of the voltage. In Fig. 5, we show the calculated P_o versus membrane potential (from −20 mV up to +10 mV) under control conditions (squares) and 50 Hz exposure (circles). Although changes in P_o are statistically different only for 0 and −10 mV ($n=5$; $P<0.05$), there is an evident shift towards negative voltage values for the EMF related curve.

As a further investigation, we analyzed the delayed rectifier (DR) potassium channel activity. The single channel current is visible during the spontaneous action potential using the experimental procedure previously described [17]. The average single-channel conductance is 35.50±0.03 pS ($n=4$) according

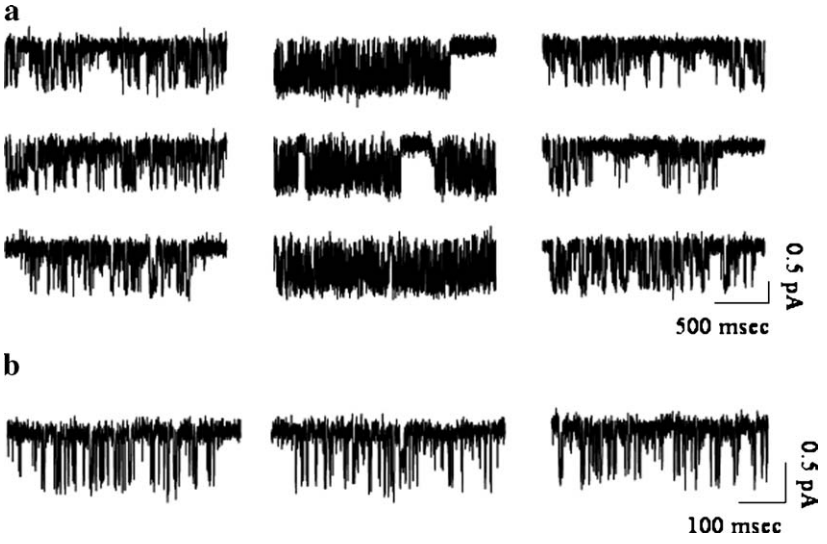

Fig. 3. Effect of EMF exposure on single calcium channel. (a) Three examples of ionic single Ca channel current traces recorded under control conditions before (left), during (centre), and after (right) 50 Hz EMF exposure at 0 mV test potential. In panel b, the same experiment with exposure to 900 MHz EMF (trace in the centre).

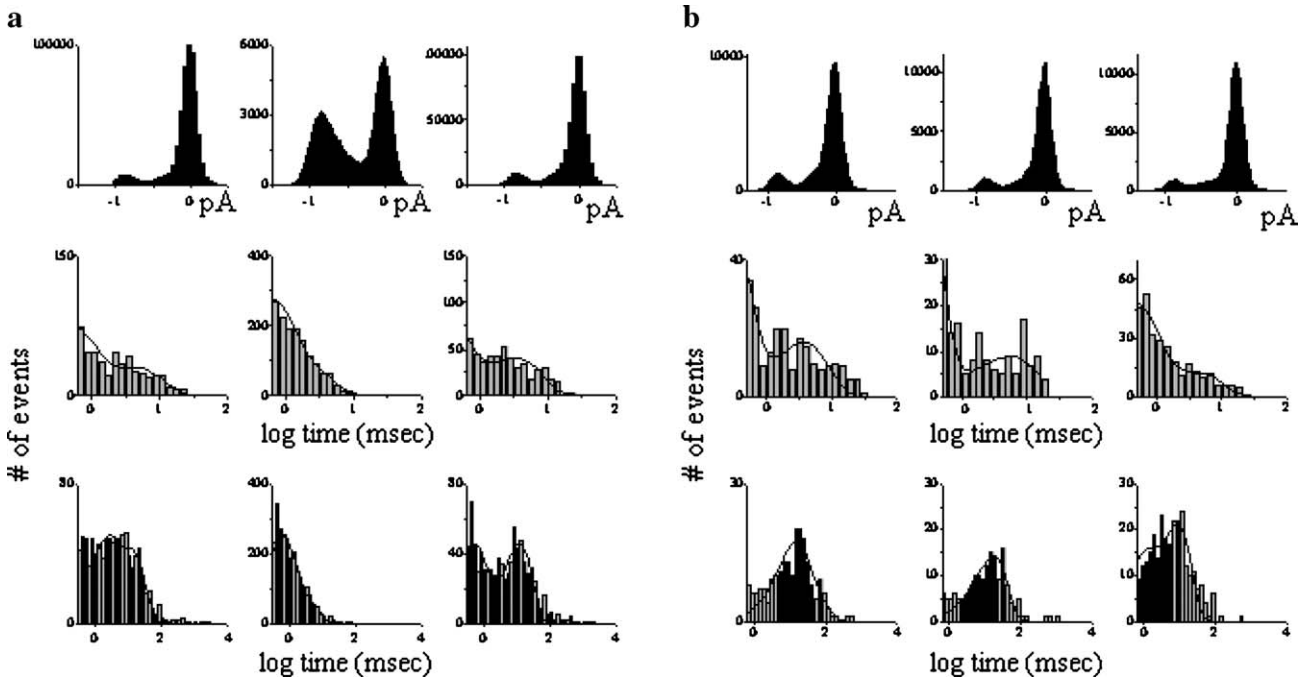

Fig. 4. Analysis of calcium single channel current. In panel a we show amplitude histograms (top), open-time (middle), and close-time (bottom) plots calculated for single channel current traces under control conditions before, and after the exposure (left and right columns), and during the exposure to 50 Hz EMF (central column). Panel b shows the same type of graphs. The central column concerns data obtained during exposure to 900 MHz EMF.

to previous measurements [20]. The values of channel current, open probability and open and close times in control conditions and under 50 Hz and 900 MHz EMF exposures are reported in Table 3. Changes in calculated parameters are not statistically significant ($n=4$), therefore, it appears clear that the DR ionic channel is not involved in the decrease of firing frequency observed when the cells were exposed either to an ELF or to an RF EMF.

Taking into account that 50 Hz EMF exposure induced changes in dynamic behaviour of the high threshold calcium channel and an increase in intracellular calcium concentration [10], it is reasonable to think that ELF EMF exposure could indirectly interfere with calcium-activated potassium channels (K_{Ca}). In DRG neurons the channel conductance value is 149±8.4 pS. In Fig. 6, patch-clamp on-cell recordings with single K_{Ca} channels exposed to EMF are shown. Fig. 6a depicts on the left three examples of 2 s single-channel recordings at −10 mV membrane potential obtained under control conditions before EMF exposure. The current traces in the centre are relative to 50 Hz exposure, where it is quite visible that ELF stimulation increases the open probability of the channel. After EMF exposure (right), the initial behaviour of the channel is re-established. Panel (b) depicts results of the same experiment repeated exposing cells to RF EMF. In Fig. 7, we present an example of amplitude histograms and distributions of open and closed times for a single K_{Ca} channel, before, during, and after EMF exposure. In panel (a) the plots are relative to control before exposure (left column), 50 Hz stimulation (central column), and control after stimulation (right column). In panel (b) the central column is relative to 900 MHz field stimulation. The values of analyzed parameters, reported as mean±S.E.M. ($n=3$; $P<0.05$), are summarized in Table 4, showing an evident increase in the open probability for the exposure to 50 Hz EMF. This is related to changes in channel dynamic behaviour evidenced by longer open times and correspondent

Table 2
Single L-type calcium channel biophysical parameters

		Control 1	50 Hz	Control 2	Control 1	900 MHz	Control 2
pA		0.838±0.09	0.852±0.07	0.825±0.1	0.844±0.08	0.885±0.06	0.829±0.12
P_o		0.119±0.016	0.191±0.02	0.115±0.04	0.12±0.02	0.13±0.04	0.11±0.01
τ_o	1	0.29±0.05	0.62±0.03	0.23±0.05	0.17±0.04	0.15±0.02	0.23±0.04
	2	3.61±0.08	3.15±0.09	3.20±0.03	3.60±0.11	5.57±0.09	4.21±0.13
τ_o	1	0.88±0.1	0.51±0.1	0.65±0.1	0.32±0.1	0.41±0.1	0.60±0.1
	2	10.3±0.26	1.99±0.08	11.78±0.17	12.68±0.22	14.76±0.32	9.30±0.21

The table reports channel amplitude (pA), open probability (P_o), open (τ_o) and close (τ_c) channel constants at −10 mV membrane test potential. The values are shown before (control 1) during (50 Hz or 900 MHz) and after (control 2) EMF exposure.

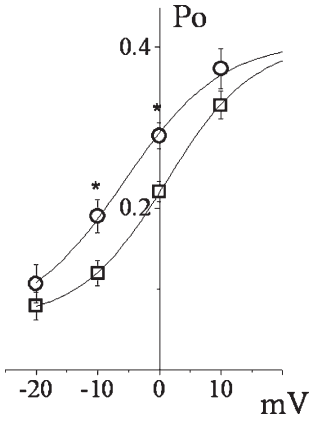

Fig. 5. Comparison between the open probability plots of a single L-type calcium channel in control condition (squares) under exposure to 50 Hz (circles) EMF.

shorter closed times. These results are in agreement with the hypothesis that the effect of the ELF EMF on K_{Ca} channel is indirect and mediated by intracellular calcium concentration. The increase in the open probability of a single K_{Ca} channel during the ELF exposure was detected in 3 of 12 experiments.

The modulatory effect of ELF EMF on calcium channels, and consequently on calcium-activated potassium permeability, and the absence of effects of RF EMF can be also tested employing the action-current clamp mode [21] to prevent cytoplasmic manipulation due to the whole-cell electrode. In this configuration, it is possible to highlight the influence of 50 Hz field on the spontaneous firing frequency. Fig. 8a shows 50 s continuous on-cell recording from spontaneous oscillating DRG neurons, where the capacitive transient, corresponding to a cell potential overshoot, gives a precise measurement of the firing activity. After 2 s of action currents recordings, we applied a 50 Hz EMF for 15 s (grey horizontal line) and a drastic reduction of the spontaneous excitability rate was observed after 7–8 s from field application, whereas recovery occurred 10 s after the field removal. We then reactivated the EMF exposure for 2 s. In this case, the cell was able to fire for 10 s at the same rate, showing that the effect of the field is related to the exposure duration. In Fig. 8b 20 s of current trace under control conditions (the first 18 s are not reported in the figure) were followed by 10 s under 900 MHz exposure. In this case, the firing frequency remained constant at 3.00±0.27 Hz, during the whole recording. A more quantitative analysis of the firing frequency under the influence of EMF is only possible using current-clamp experiments, where we can control parameters, such as the membrane potential level, on which the neuronal cell firing rate depends. In Fig. 9, we show stimulated action potential trains under control condition and under the influence of EMF. In Fig. 9a, three examples of stimulated current-clamp recordings in control conditions are compared with three action potential trains during the ELF exposure. A statistically significant increase of action potentials number is shown at the beginning of the train (100 ms) when the 50 Hz field is present ($n=4$; $P<0.05$). After 1 s, the tendency to reduce action potential rate is augmented by persistent field exposure even if this phenomenon is not statistically significant. Such a behaviour is quantitatively summarized in the histograms at the bottom, representing the number of action potentials in the first 100 mss (left) and after 1 second (right). The same experiment (Fig. 9b), performed using RF EMF, left unaltered the number of action potentials for both short and long time exposures.

Table 3
Single DR potassium channel biophysical parameters

		Control 1	50 Hz	900 MHz
pA		1.37±0.08	1.37±0.08	1.37±0.08
P_o		0.58±0.08	0.54±0.13	0.48±0.11
τ_o	1	7.12±0.16	6.5±0.4	7.54±0.3
	2	49.08±0.17	47.03±0.13	52.04±0.35
τ_o	1	5.6±0.12	5.8±0.12	5.9±0.28
	2	60.5±0.13	62.9±0.25	57.81±0.21

The table reports channel current amplitude (I), open probability (P_o), open (τ_o) and close (τ_c) times. Reported values are relative to control (control 1) and exposure conditions (50 Hz or 900 MHz).

4. Discussion

The main objective of the present study was to observe, in parallel investigations, the effects of ELF and RF EMF on the same preparation and under the same experimental conditions. Although the two EM exposures are not directly comparable, due to the dielectric properties of biological cultures, we had the opportunity to evaluate the impact, at the molecular level, that low-intensity EMFs at different frequencies could have on non-stationary biological events.

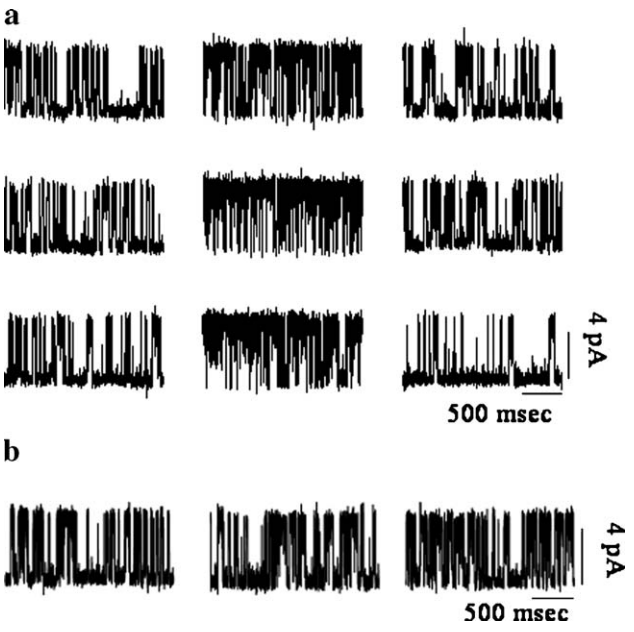

Fig. 6. Effect of EMF exposure on single calcium-activated potassium channel: (a) three examples of ionic single potassium channel current traces recorded in control conditions before (left), during (centre), and after (right) 50 Hz EMF exposure at −10 mV test potential. In panel b, the same experiment with exposure to 900 MHz EMF (trace in the centre).

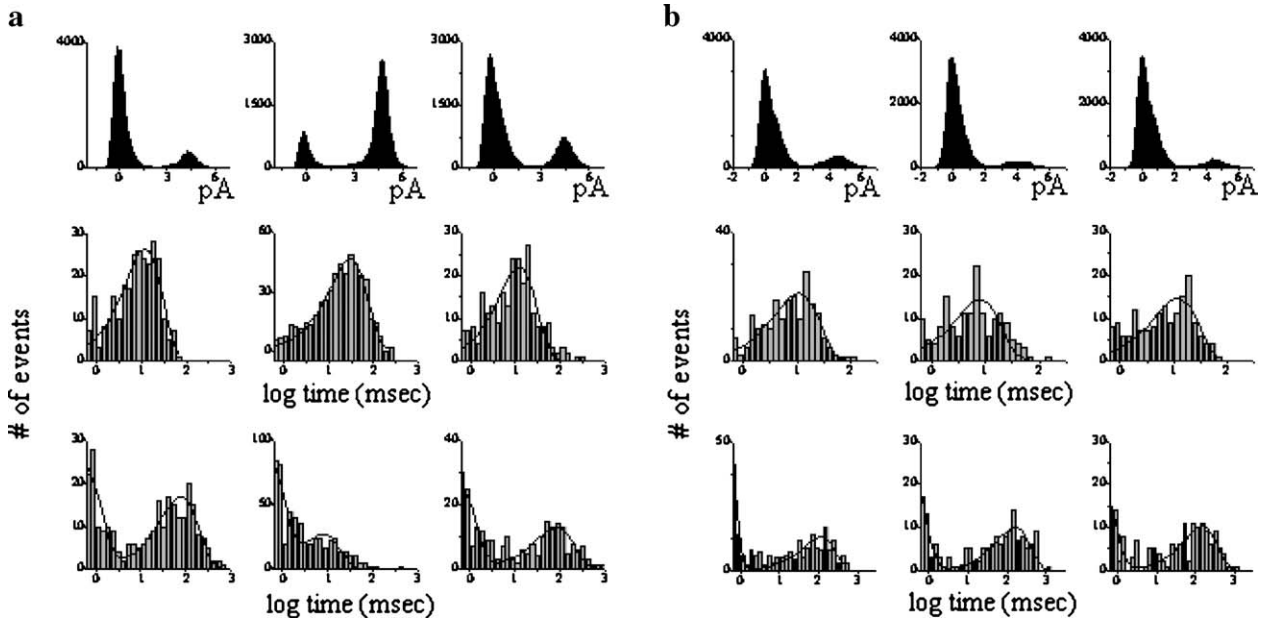

Fig. 7. Analysis of calcium-activated potassium single channel current. In panel a, we show amplitude histograms (top), open-time (middle), and close-time (bottom) plots calculated for single channel current traces under control conditions before, and after the exposure (left and right columns), and during the exposure to 50 Hz EMF (central column). Panel b shows the same type of graphs. The central column concerns data obtained during exposure to 900 MHz EMF.

The action potential represents the dynamic basic unit of neuronal firing and results from the combination of the activities of two main ionic currents: the voltage- and time-dependent fast TTX-sensitive sodium and the delayed rectifier potassium current.

According to our results, none of the two currents is influenced by ELF or RF EMFs (Fig. 1), since all the values of considered parameters (MP, dV/dt, dV/dt_{max}) never showed a significant difference between control and EMF exposed cells. Regarding the more complex mechanism of cell firing, our results indicate that low-intensity ELF EMFs are able to modulate firing frequency (Fig. 2) in two opposite ways. At first, the exposure induces an increase of the action potential frequency. This early phase is followed by a decrease of DRG cells firing frequency that, in some cases, reaches a complete silencing. Control cells, as well as RF exposed neurons, did not show any significant modification in firing frequency, but membrane potential oscillations lasted for several minutes (Figs. 2, 8, 9). The behaviour of action potential repetitive firing under the influence of the ELF EMF could be explained by the potentiation of Ca and alteration of the ionic permeability of the K_{Ca} channel. Our previous studies suggested that the ELF EMF behaves like the calcium channel agonist Bay K 8644 [22], modifying the voltage difference felt by the protein in the membrane. Therefore, the effect of the field on calcium current is probably mediated by the action on the membrane surface charges [10].

Our experiments at single channel level took in consideration ionic channels active during the repetitive action potential [21,23]. More specifically, we focused our attention on high-threshold calcium channel [18], being responsible for the modulation of interspike interval during action potential burst [17]. ELF EMF is able to modify the kinetics of the single calcium channel, increasing the mean open time during the exposure. On the basis of results obtained by Tonini and colleagues [10], who showed that there was a shift in current activation curve in whole-cell high-threshold calcium current recordings under 50 Hz EMF exposure, the observed effect on single channel current can be related to a shift in open probability behaviour as a function of membrane potential (Fig.

Table 4
Single calcium-activated potassium channel biophysical parameters

		Control 1	50 Hz	Control 2	Control 1	900 MHz	Control 2
pA		4.72±0.7	4.85±0.8	4.63±0.6	4.83±0.8	4.68±0.4	4.79±0.12
P_o		0.17±0.03	0.79±0.18	0.24±0.06	0.15±0.03	0.09±0.05	0.09±0.06
τ_o		12.13±0.07	30.71±0.03	12.15±0.09	10.8±0.08	8.06±0.14	11.88±0.12
τ_c	1	0.54±0.14	0.4±0.15	0.51±0.16	0.16±0.14	0.32±0.17	0.35±0.22
	2	78±0.94	7.84±0.19	81.88±0.67	110.63±0.14	156.2±0.11	143.61±0.42

The table reports channel current amplitude (I), open probability (P_o), open (τ_o) and close (τ_c) times. Reported values are relative to control conditions before (control 1) and after (control 2) the EMF exposure, and to exposure conditions (50 Hz or 900 MHz).

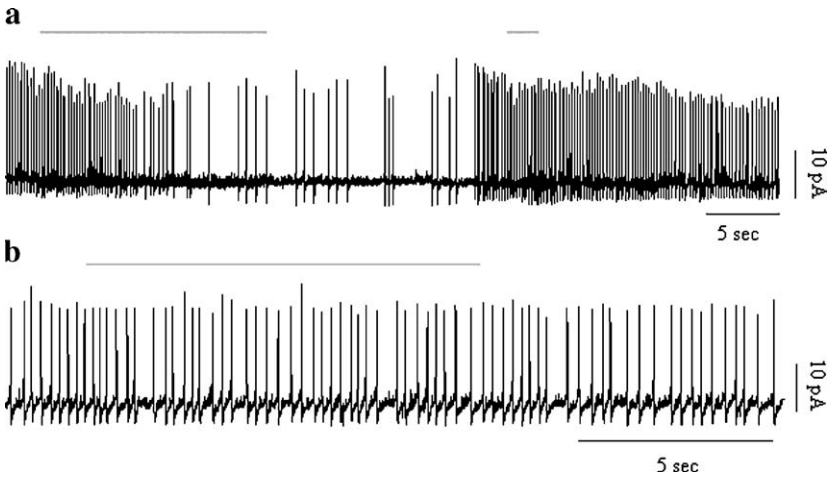

Fig. 8. Repetitive action current in the presence of EMF. Cell-attached experiment recording of DRG neuron spontaneous activity. The horizontal bar indicates when the 50-Hz EMF (a) and 900-MHz EMF (b) were applied.

5). In the present experiments we believe we are facing the same cellular mechanism. ELF EMF, through the interaction with surface charges, is able to change channel perception of the voltage difference across the membrane of about 5/10 mV more depolarized. It is known that high threshold calcium channel open probability is upregulated by membrane depolarization [19]. Thus, the charges moved through calcium channels during field exposure increase, modifying, not only the depolarizing slope between action potentials, but also the concentration of calcium ions in the microdomains just below the plasma membrane. There are two consequences due to the increase of calcium entry: (a) a transitory increase of firing rate due to a modification in the dV/dt preceding the upstroke (Fig. 9), and (b) the activation of calcium-dependent potassium channels (Fig. 6), resulting in a delayed reduction of firing frequency (Fig. 9).

All the experiments on single ionic channels were done also under EMF exposure to 900 MHz but, at least in our experimental conditions and for the parameters adopted in the present investigation, RF EMF does not show any interaction with non-stationary biological events.

Conflicting results obtained for the two different exposure conditions can be traced back to the specific response of cell membrane to various frequencies. In particular, phenomena underlying surface charges distribution under the action of an EMF, such as counterions polarization and changes in the

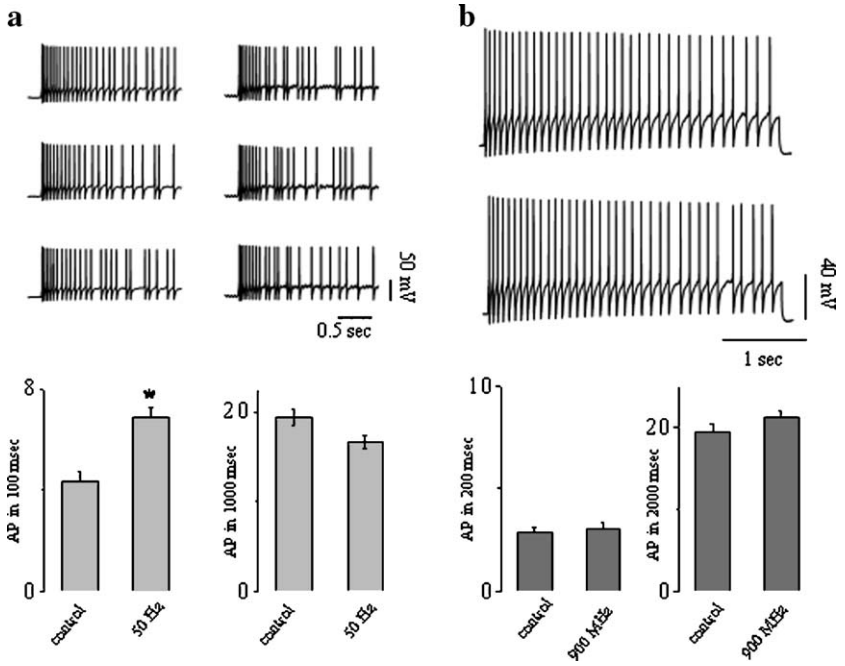

Fig. 9. (a) DRG firing burst elicited by current injection, before (top left), and during the exposure to 50 Hz EMF (top right). At the bottom, the histograms show the comparison between action potential number in the first 100 (left) and 1000 ms (right) for control and ELF EMF exposed recordings $n=9$). (b) DRG firing burst elicited by current injection, before (top left), and during the exposure to 50 Hz EMF (top right) the bottom, the histograms show the comparison between action potential number in the first 200 (left) and 2000 ms (right) for control and ELF EMF exposed recordings ($n=7$).

orientation of hydrophilic phospholipids heads, have already relaxed at 900 MHz [24]. This is because charged particles inertia prevents them from following rapid oscillations of the EMF.

In conclusion, a RF EMF, inducing a SAR of 1 W/kg in the biological specimen, does not interact, either directly or indirectly, with ionic membrane permeability. On the contrary, ELF EMF modulates the currents flowing through at least two ionic channels: high threshold calcium channel and one of the calcium-activated potassium channels. We believe this modulation is indirect and mediated by changes occurring at surface charge level. Although it is always possible a direct action of EMF on membrane proteins, we have never observed modifications in single-channel behaviours to justify such a hypothesis.

Physiologically, DRG cell firing modulation by ELF EMF could suggest a modification in the proprioceptive ability to transduce external stimuli. Since we do not have, at the moment, any precise indication with this respect, we are designing experiments using nerve-skin preparation to test such a suggestive hypothesis.

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