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Synaptic neurone activity under applied 50 Hz alternating magnetic fields

Ana C. Calvo, María J. Azanza *

Instituto de Bioelectromagnetismo, Alonso de Santa Cruz, Facultad de Medicina, Universidad de Zaragoza, 50008 Zaragoza, Spain

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Abstract

The effect of 50 Hz alternating magnetic fields of 10–150 Gauss (1–15 mT) intensity on neurone synaptic activity for glutamate and acetylcholine has been studied. The applied 50 Hz alternating magnetic field does not modify the synaptic activity induced by glutamate or acetylcholine on neurones. It has been observed that both caffeine and glutamate induce similar effects, either stimulation or inhibition, on different neurone types. It is shown that applied 50 Hz alternating magnetic fields mimic the synaptic effect of glutamate. A mimic effect has also been observed between the induced effect by applying 50 Hz alternating magnetic field on neurones and the one induced by caffeine and glutamate on the same neurone. The application of Ringer solutions with different concentrations of Ca^{2+}/K^{+} ions suggest that Ca^{2+} ions are involved in the elicited responses to either caffeine, glutamate or 50 Hz magnetic fields. Our conclusion is that the observed mimic induced effects for 50 Hz alternating magnetic fields, caffeine and glutamate on neurones corroborate that Ca^{2+} ions are the cytosolic effectors of the applied 50 Hz alternating magnetic fields interaction with neurone plasma membrane.

Keywords: Neurons activity under ELF; Biological electromagnetic field effects; Synaptic effects under 50 Hz magnetic fields; Glutamate activity under ELF exposure; Acetylcholine activity under ELF exposure; Caffeine effects under ELF exposure; Ca^{2+}, neurons and ELF

1. Introduction

The effect of an alternating magnetic field (MF), in the range of extremely low frequencies (ELF-MF), on the synaptic activity of single unit neurones is reported.

So far experiments have been carried out by applying ELF-MF on mollusc neurones [16], muscle cells [21] and axons [42]. But the outcoming results seem rather contradictory, quite controversial and the processes underlying the observed effects, not fully explained. It has been described that ELF-MF induces alterations in the levels of intra- and extracellular calcium, as well as in the rates of cellular calcium efflux [12]. A continuous exposure for 10 min to electromagnetic fields of 0–60 Hz, 0.2–1 Gauss intensity in human brain, resulted in a detectable persistent change in brain electrical activity following termination of the field. It was reported that, on average, the 10 Hz component of the electroencephalogram was partially blocked by the applied 1 Gauss, 10 Hz [10].

Little work has been made in relation with synaptic events. ^{3}H-noradrenaline release was potentiated by low intensity pulsed magnetic fields (1.6–8.5 G) with a magnitude comparable with certain cholinergic stimuli applied to isolated PC12 cells in culture. The proposed hypothesis suggested that cooperative changes in Ca^{2+} membrane binding induced by the applied field could promote the calcium entrance and vesicular release increased [18]. The effects of an acute (45 min) exposure to a 60 Hz, ≥ 0.75 mT intensity, magnetic field on sodium-dependent, high-affinity choline uptake in the brain of the rat has been investigated. Decreases in uptake were observed in the frontal cortex and hippocampus. These effects of the magnetic field were blocked by pretreating the animals with naltrexone, but not by the peripheral opioid antagonist, naloxone me-

* Corresponding author. Tel.: +34-976-37-39-84; fax: +34-976-76-17-54.

E-mail address: mjazanza@posta.unizar.es (M.J. Azanza)

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thiodide. These data indicate that the magnetic-field-induced effect were mediated by opioids [29].

The most consistent mechanisms in explaining the biological effects of magnetic fields, in the range of ELF-MF, are those considering that ELF-MF interaction with cell plasma membrane promote changes in calcium flux patterns. Different exposed cells show such behaviour [6]. Calcium ions are known to be second messengers in signalling systems, conveying signals from the cell surface to the interior of the cell, and could provide a mechanism to explain a way of amplifying the electromagnetic signal impact on plasma membrane. Liboff [31] has suggested that ELF fields may indirectly affect the signalling pathway by either reinforcing or altering the frequency of the intrinsic cytosolic Ca^{2+} oscillations. The low-frequency oscillatory behaviour referred to as Ca^{2+} oscillations is not limited to excitable cells, but is actually a somewhat common property of most, if not all living cells [2,6,11,23].

We have described that under exposure to static magnetic fields (SMF) neurones are either inhibited or excited by processes seemingly Ca^{2+}-dependent. Neuronal '*magnetosensitivity*' was expressed in terms of ion kinetic changes across the neurone plasma membrane promoted by calcium ions [5,6,17]. It is very well known that synaptic neurotransmitter liberation is also a calcium dependent process. We have considered the interest in investigating the possible effect of applying 50 Hz MF in the neurone response to two different neurotransmitters: acetylcholine and glutamate. In selecting these molecules we have taken into account not only the Ca^{2+}-dependent liberation of the neurotrasmitters but also the postsynaptic induced effect. Acetylcholine can mediate excitatory and inhibitory transmission effects on the postsynaptic membrane of *Helix aspersa* neurones. The excitatory effect is mainly mediated by Na^+ ions while the inhibitory effect is mediated by Cl^- ions [30]. Glutamate can also excite, inhibit or produce biphasic responses on certain gastropod neurones [25,38]. For glutamate three kinds of receptors have been described: Q and K receptors, mainly permeable to Na^+ ions, the most specific agonists being quisqualate and kainate, respectively. The third one is the NMDA-receptor, the most specific agonist is *N*-methyl-D-aspartate and it is permeable to both monovalent cations and to Ca^{2+} ions [22]. In the experiments described in the present paper, we show the effect of 50 Hz-MF of 10–150 Gauss intensity on the synaptic activity mediated by acetylcholine and glutamate. The mimic effect observed for the applied 50 Hz-MF, glutamate and caffeine suggest that the neurones behaviour under alternating magnetic fields can have many common aspects with respect to their behaviour under applied SMF.

2. Materials and methods

We have studied 40 neurones. The experiments were performed on single neuron units from the suboesophageal ganglia of the snail *Helix aspersa*. The dissected brain ganglia was mounted in a 12-ml capacity bath and immersed in Ringer solution (NaCl 80 mM; KCl 4 mM; $CaCl_2$ 7 mM; $MgCl_2$ 5 mM; Tris–HCl buffer 5 mM; pH 8), enriched with 10 mM pyruvic acid. The neurones were viewed using an Olympus zoom binocular microscope. Different Ringer solutions have been applied, in different sets of experiments. Ringer K^+ free (NaCl 80 mM; $CaCl_2$ 7 mM; $MgCl_2$ 5 mM; sucrose 4 mM;Tris–HCl buffer 5 mM; pH 8); Ringer with five times the normal K^+ concentration (NaCl 64 mM; KCl 20 mM; $CaCl_2$ 7 mM; $MgCl_2$ 5 mM; Tris–HCl buffer 5 mM; pH 8); Ringer Ca^{2+} free (NaCl 80 mM; KCl 4 mM; $MgCl_2$ 5 mM; sucrose 7 mM; Tris–HCl buffer 5 mM; pH 8); and Ringer with five times the normal Ca^{2+} concentration (NaCl 52 mM; KCl 4 mM; $CaCl_2$ 35 mM; $MgCl_2$ 5 mM; Tris–HCl buffer 5 mM; pH 8). The brain methacrylate bath was placed between a pair of Helmholtz coils and 50 Hz alternating MF of peak intensity ranging between 10–150 G (1–15 mT) were applied at time intervals of 1 min. The different applied field intensities were attained in 15 s for each field value. All the experiments have been performed at room temperature (r.t.). Temperature was monitored during the experiments and no variation in the bath solution was observed under alternating MF exposure within ± 1°C. Intracellular recordings were made using glass microelectrodes, tip diameters of < 0.5 μm and tip resistance of 2–20 MΩ, filled with 1M potassium acetate, pH 6.8. The peak magnetic field intensity was previously calibrated in the centre of the coils, and monitored during the experiment with a Hall probe. The brain ganglia were placed in such a way that the applied magnetic field was set perpendicular to the geomagnetic field. All the above set-up and recording equipment was kept into a Faraday cage, to filter for the environmental electromagnetic noise. The electrophysiological recordings were made in real time by testing natural neuronal activity, i.e. bursting and non-bursting (silent) neurones, before (control), during and after magnetic field exposure [9]. The potentials were amplified using a negative capacity cathode follower (Medistar-A-35) and displayed on an oscilloscope (HAMEG, HM 205). Permanent recordings were made on a pen recorder (MD2 Palmer BioScience). The following compounds were used in this study: caffeine (Merck), acetylcholine (Sigma) and glutamate (Sigma).

3. Results

We have observed that the neurones physiological activity is strongly modified under applied 50 Hz alternating MF. Firing frequency changes are observed under our experimental conditions in the 63% of the studied neurones. The 34% of the neurones exposed to an applied 50 Hz-alternating MF are inhibited showing a decrease of the firing frequency (Fig. 1). The 18% of the neurones show spike frequency increases (Fig. 2). The 11% of the neurones show a bimodal response of 'a' type: stimulation followed by a decrease of the spikes frequency [14] (Fig. 3). The 5% of the neurones show a bimodal response of 'b' type: spike frequency decreases followed by stimulation of the bioelectric activity [14] (Fig. 4). Very frequently oscillations of the average recorded frequency rate are observed (Figs. 2 and 4). All those neurones excited by ELF applied alternating MF showed in the end of the recordings a decrease of the firing frequency.

In order to study the possible effect of the applied 50 Hz alternating MF on the synaptic interneurones activity we have performed a series of experiments by applying glutamate and acetylcholine as putative neurotransmitters. We have also applied caffeine for comparison. On Fig. 5 we show a control experiment. In the graphic is shown that in this kind of neurone neither caffeine nor glutamate induce important changes in the bioelectric activity while acetylcholine stimulates the neurone.

We have observed that neurones inhibited by caffeine are also inhibited by glutamate. The inhibitory effect is also induced by applied 50 Hz alternating magnetic field, on the same neurone, as shown on Fig. 6. On the other hand those neurones stimulated by applied 50 Hz alternating magnetic field are also stimulated by caf-

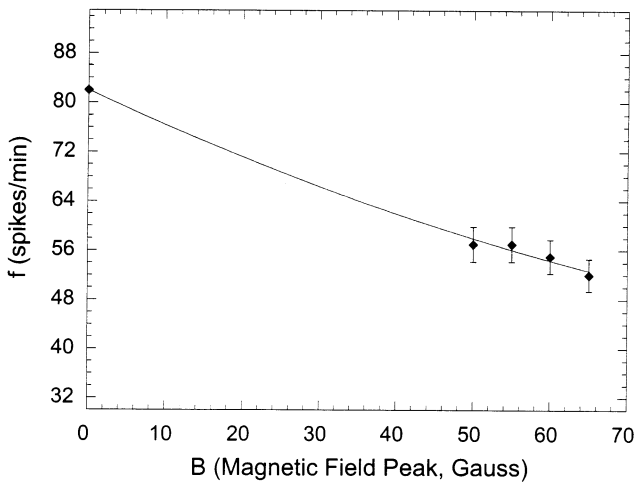


Fig. 1. Variation of the average neurone spike frequency, *f*, with the applied MF peak intensity, B: inhibitory effect. Bursting neurone from the visceral ganglion identified as V10 (adapted from Kerkut et al. [26]) ($E_m \approx -22$ mV). The symbols are (◆) for the average frequency changes recorded. The bars mean the estimated error. The continuous line is an eye guide. Notice that the average frequency decreases under 50 Hz applied alternating MF, the activity decrease is maintained for increasing applied MF.

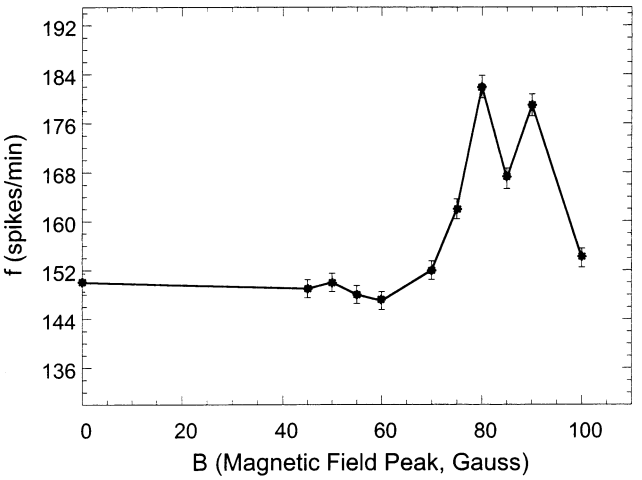


Fig. 2. Variation of the average neurone spike frequency, *f*, with the applied MF peak intensity, B: excitatory effect. Bursting neurone from the visceral ganglion identified as V21 ($E_m \approx -90$ mV). The symbols are (*) for the average frequency changes recorded. The bars mean the estimated error. The continuous line is an eye guide. Notice that the average frequency progressively increases with increasing applied alternating MF. The highest average frequency value is recorded for 80 G. The average frequency values oscillates up to the highest applied 50 Hz MF of 100 Gauss. The average frequency values are always maintained over the spontaneous ones.

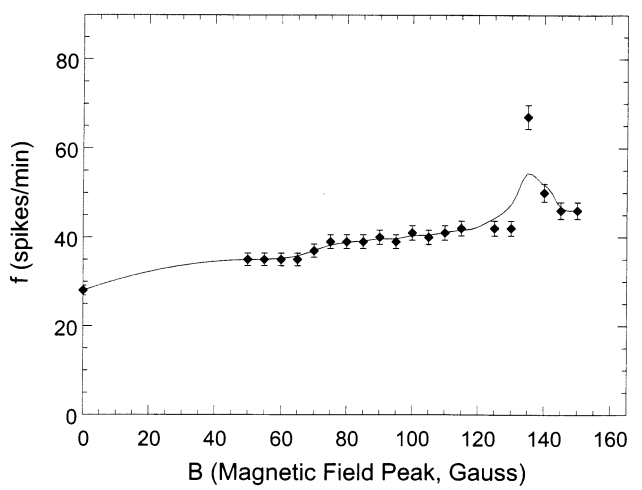


Fig. 3. Variation of the average neurone spike frequency, *f*, with the applied MF peak intensity, B: bimodal 'a'-type [14]. Bursting neurone from the visceral ganglion identified as V20 (adapted from Kerkut et al. [26]) ($E_m \approx -40$ mV). The symbols are (◆) for the average frequency changes recorded. The bars mean the estimated error. The continuous line is an eye guide. It is observed a continuous increase of the frequency with the increased applied field intensity. The maximum average frequency is attained for 135 G. Spike frequency decreases for 150 Gauss, maximum applied MF, but the frequency values are always maintained over the spontaneous activity recorded for 0 Gauss.

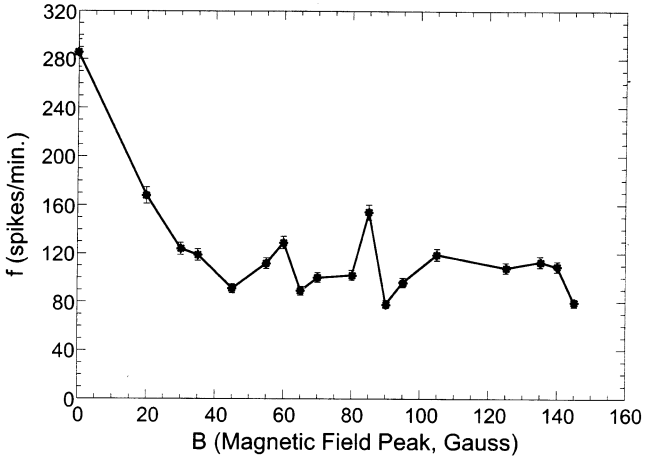


Fig. 4. Variation of the average neurone spike frequency, *f*, with the applied MF peak intensity, B: bimodal 'b'-type [14]. Bursting neurone from the visceral ganglion identified as V19 (adapted from Kerkut et al. [26]) ($E_m \approx -65$ mV). The symbols are (*) for the average frequency changes recorded. The bars mean the estimated error. The continuous line is an eye guide. The firing frequency drops strongly under 20 G applied MF. The frequency values oscillate reaching the minimum value, average frequency 79 spikes min^{-1}, for maximum applied MF (145 Gauss).

feine and glutamate as shown on Fig. 7 on a silent neurone. From recording (e) it seems that glutamate induces a biphasic response on this neurone: excitation followed by inhibition. After washing with normal Ringer solution (recording (f)) the continuous neuronal

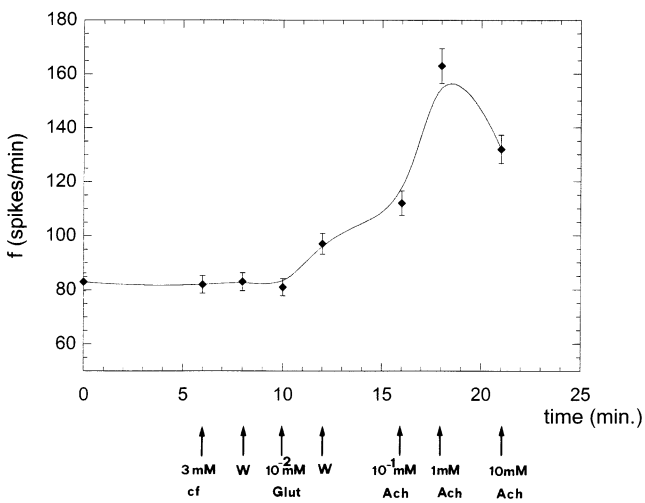


Fig. 5. Control experiment. Bursting neurone from the visceral ganglion identified as V12 (adapted from Kerkut et al. [26]) ($E_m \approx -60$ mV). The symbols are (◆) for the average frequency changes recorded. The bars mean the estimated error. The continuous line is an eye guide. In this neurone neither 3 mM caffeine (↑ cf) nor 10^{-2} mM glutamate (↑ Glut) induce important changes in the bioelectric activity while acetylcholine (↑ Ach) stimulates the neurone activity. Three different concentrations of acetylcholine were applied: 10^{-1} mM, 1 mM and 10 mM. It is observed that the applied increasing acetylcholine concentration solutions desensitize the acetylcholine receptors reversing in turn the excitatory effect.

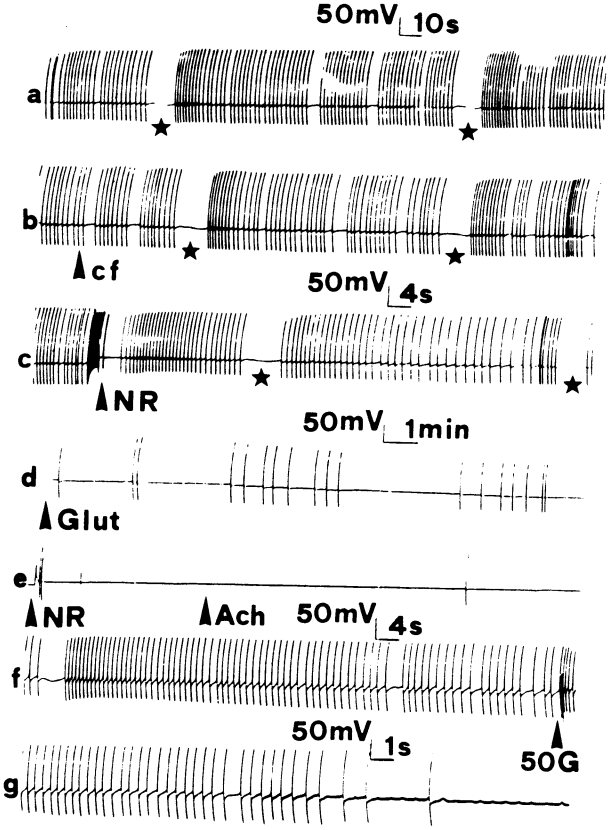


Fig. 6. Applied 50 Hz alternating MF mimics the inhibitory effect of caffeine and glutamate on the neurone. Bursting neurone from the visceral ganglion identified as V17 (adapted from Kerkut et al. [26]) ($E_m \approx -35$ mV), time scale is expressed in seconds (s), the applied MF intensity is attained in 15 s. This neurone shows interspikes ILD activity (✱). Recording (a), spontaneous cell activity, 32 spikes min^{-1} average frequency. Recording (b) 3 mM caffeine (▲ cf) induces the decrease of the firing to 27 spikes min^{-1} average frequency. Recording (c), after washing with normal Ringer solution (▲ NR) the spikes average frequency rises to 46 spikes min^{-1}. Recording (d), glutamate 10^{-2} mM (▲ Glut) induces the decrease of the average frequency to 3 spikes min^{-1}. Recording (e) acetylcholine 1 mM (▲ Ach) induces no response. Recording (f) 50 G applied MF (▲) induce a decrease of the spikes firing frequency being the neurone activity completely inhibited (recording (g)).

bioelectric activity is recovered. On recording (g) it is observed that once the applied MF is switched-off the spike frequency increases. Glutamate biphasic effect seems to be mimic by applied 30 G alternating MF.

A complex experiment is shown on Fig. 8(A and B) which confirm, on the one hand the stability of the experimental model chosen and on the other hand the great specificity of the neurone responses to applied MF. The experiment was made on the same neurone but recording results shown on Fig. 8b were taken 4 h (240 min) after the recording results shown on Fig. 8a. The neurone is the one identified as V19 characterized by a biphasic, or bimodal response to the applied 50 Hz-MF: inhibition followed by stimulation as shown on Fig. 4. On Fig. 8a it is observed that caffeine and

glutamate induce an small frequency decrease while increasing concentrations of acetylcholine stimulate the neurone. Acetylcholine receptors are desensitized decreasing in turn the firing frequency. After washing with normal Ringer the average frequency is restored to values near to those recorded at the beginning of the experiment. After 2 h (240 min), Fig. 8b, the firing frequency is maintained in the same value. But now caffeine and glutamate stimulates the neurone (bimodal induced effect). The neurone behaviour for caffeine and glutamate is quite parallel in both experiments. The response to acetylcholine is quite similar to the stimulatory one observed on Fig. 8a being the firing frequency smaller. Under 35 Gauss (Fig. 8b) the spike frequency increases giving similar response with respect to the one observed for caffeine and glutamate. We have studied the effects induced on the neurone activity by changing the Ringer ionic concentrations under 35 G continuous exposure. It is observed that in Ringer calcium free the firing frequency decreases. The spike frequency also decreases in Ringer potassium free but it increases in Ringer K^+ 'rich'. After washing with normal Ringer the firing activity is restored. It is interesting to notice that under 35 G the neuronal response to acetylcholine is not modified.

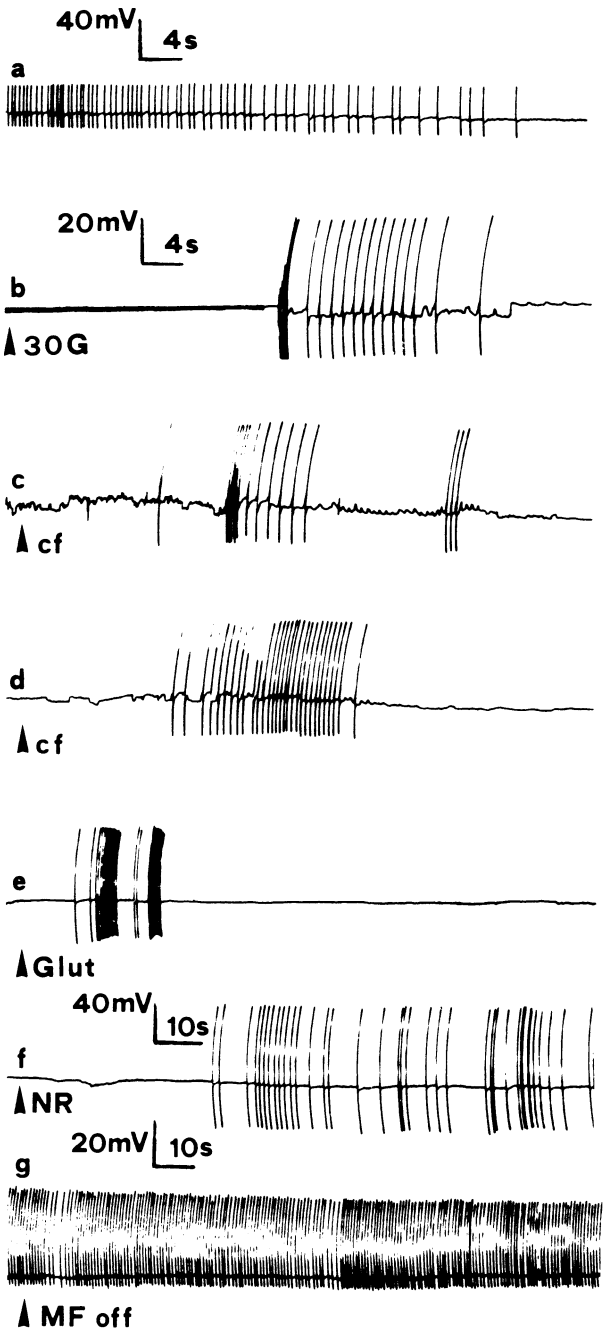


Fig. 7.

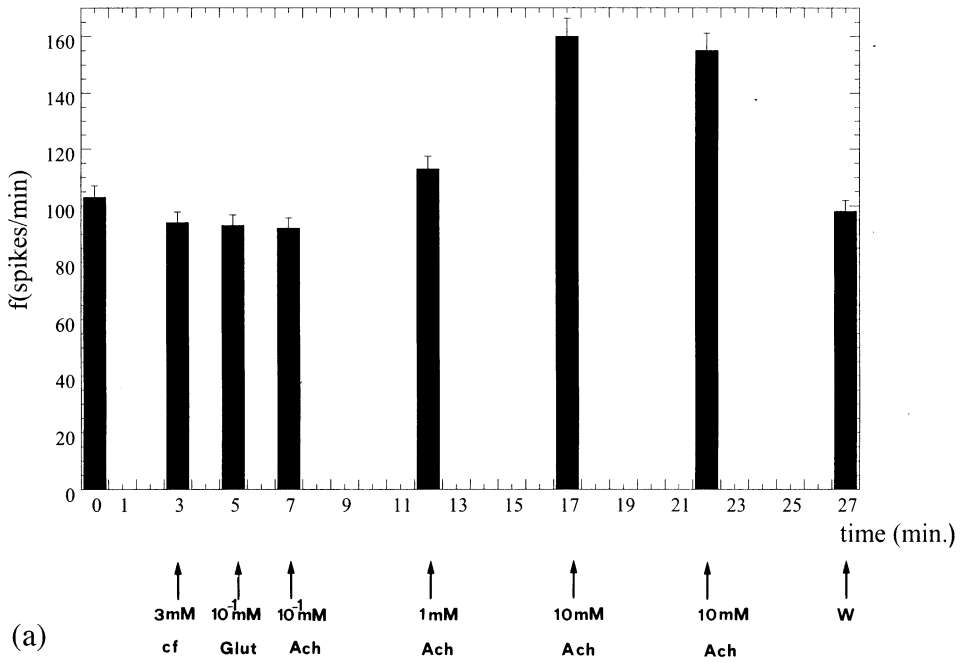
4. Discussion

Comparing the above results with our previous results by applying static magnetic fields (SMF) on neurons [4], we observe a similar behaviour under exposure to 50 Hz alternating magnetic fields.

Some characteristics should be emphasized. The threshold magnetic field inducing a change in the bioelectric activity can be, for some neurones, of the same order of magnitude, 20 G (2 mT) for 50 Hz applied alternating fields (Fig. 4), 50 G (5 mT) for applied SMF [7].

Under SMF, 70% of the studied neurones showed some spike frequency modification, i.e. 50% of the neurones were inhibited while 20% were excited [2,7]. The rest, 30%, showed important amplitude spikes modifications [8]. Under 50 Hz alternating magnetic fields 63% of the neurones showed firing frequency modifications being the 34% inhibited, the 18% stimulated and the rest 16% showing bimodal responses, as

Fig. 7. Caffeine and glutamate mimic the excitatory effect of 30 G 50 Hz-alternating MF. Silent neurone from the visceral ganglion identified as V32 ($E_m \approx -40$ mV), time scale is expressed in seconds (s), the applied MF intensity is attained in 15 s. Recording (a), spontaneous neurone activity of 71 spikes min^{-1} average frequency, recorded 1 min after introducing the microelectrode. After 1 min, the neurone becomes silent. Recording (b) the application of 30 G-50 Hz alternating MF intensity (▲), induces a transitory stimulation of the neuronal activity. The restored activity to 20 spikes min^{-1} average frequency is dramatic. The neurone becomes silent after 22 s. The 30 G-MF is maintained during recordings (c),(d),(e) and (f). Recording (c) 3 mM caffeine (▲ cf) induces again the stimulation of the neuronal activity to 16 spikes min^{-1} average frequency. Recording (d) 3 mM caffeine (▲ cf) stimulates the neurone to 30 spikes min^{-1} average frequency. Recording (e) glutamate 10^{-2} mM (▲ Glut), induces an excitatory response similar to the one observed with caffeine, average frequency 27 spikes min^{-1}. Recording (f) after washing with normal Ringer (▲ NR) the neuronal firing activity is recovered, average frequency of 22 spikes min^{-1}. Recording (g), after switching off the applied MF the frequency increases to 77 spikes min^{-1}, a value similar to the spontaneous activity shown on recording (a).



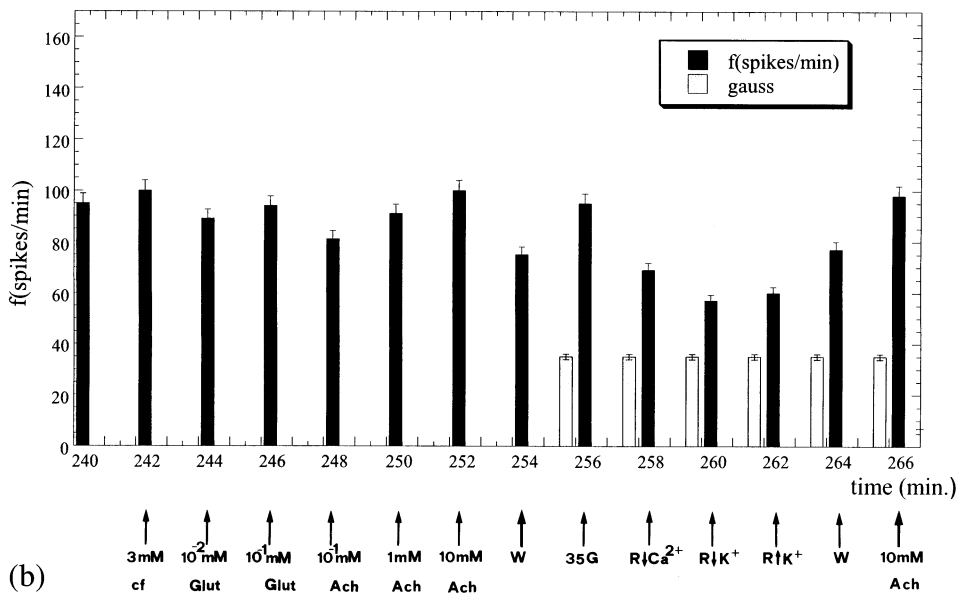


Fig. 8. (A) Bursting neurone from the visceral ganglion identified as V19 (adapted from Kerkut et al. [26]) ($E_m \approx -65$ mV). Caffeine 3 mM (↑cf) induces a small decrease of the average frequency, from the spontaneous average frequency of 103 spikes min^{-1} to an average frequency of 94 spikes min^{-1}. Glutamate 10^{-1} mM (↑Glut) induces an small decrease to an average frequency of 93 spikes min^{-1}. Acetylcholine (↑Ach) is applied at concentrations of 10^{-1} mM (average frequency of 92 spikes min^{-1}), 1 mM (↑ average frequency of 113 spikes min^{-1}), and 10 mM (↑ average frequency of 165 spikes min^{-1}). The neuron is desensitized (average frequency decrease to 155 spikes min^{-1}). After washing (↑W) with normal Ringer solution, the average frequency recovers to near the initial, spontaneous average frequency (98 spikes min^{-1}). (b) It is the same experiment shown on Fig. 8a but the recordings were taken 4 h after the recording shown on Fig. 8a (240 min value on graphic). The spontaneous average frequency after 4 h is 98 spikes min^{-1}, the same value recorded at the end of the experiment shown on Fig. 8a. Caffeine 3 mM increases the frequency to 100 spikes min^{-1} (on recording Fig. 8a, 3 mM caffeine (↑cf) induces an average frequency of 94 spikes min^{-1}). Glutamate 10^{-2} mM (↑Glut) induce an slight decrease to an average frequency of 89 spikes min^{-1}. Glutamate 10^{-1} mM (↑Glut) induce an increase to an average frequency of 94 spikes min^{-1}. The neurone behaviour for caffeine and glutamate is quite similar. The responses to acetylcholine (↑Ach) are maintained in both experiments with similar characteristics, increase of the activity (81 spikes min^{-1} for 0.1 mM ↑; 91 spikes min^{-1} for 1 mM ↑; 100 spikes min^{-1} for 10 mM ↑) being the neurone desensitized for repetitive increased concentrations of acetylcholine. After washing (↑W) the average frequency decreases to 75 spikes min^{-1}. Under 35 Gauss (↑) the average frequency increases to 95 spikes min^{-1} (similar behaviour with respect to the one observed for caffeine and glutamate). Under 35 G continuous exposure the induced effects by changing the Ringer ionic concentrations are studied. In Ringer calcium free (R↓Ca^{2+}) the average frequency decreases to 69 spikes min^{-1}. In Ringer K^+ free (R↓K^+) the average frequency decreases to 57 spikes min^{-1}. In 'rich' K^+ Ringer solution (R↑K^+) the average frequency increases to 60 spikes min^{-1}. Washing with normal Ringer solution (↑W) the frequency rises to 77 spikes min^{-1}. The response to acetylcholine is not modified by applied 35 G. Ach 10 mM (↑Ach) induces an average frequency of 98 spikes min^{-1} (the same value observed before applying alternating MF).

shown on the results. We have described that in ≅27% of the neurones studied a firing rhythm is generated with 50 Hz-MF, for ≅7 mT, which resembles *synchronus* oscillations activity. The possibility that ELF-MF could generate neuronal networks synchrony firing does exits as an explanatory physical model shows [9].

We also show, as we did for the case of applied SMF [6], that the responses elicited under alternating-MF are specific of the kind of neurone under scrutiny.

The diverse above described neuronal responses must be a consequence of the *metabolic* properties of the neurone itself, as we also discussed for the results under applied SMF [2]. Being the metabolic properties of one neurone in turn the result of the neurone structural characteristics expressed in terms of plasma membrane structure, i.e. kind and density of ionic channels and pumps; calcium ions as second messengers and calcium cytosolic homeostasis. In explaining the elicited bioelectric activity changes under applied alternating-MF, Ca^{2+} ions could be the cytosolic effectors of the alternating-MF interaction with its target, i.e. the neurone plasma membrane. This would happen in the same way as it was demonstrated for SMF induced effects [5,6,17].

With the experiments shown on Fig. 8b it is possible to induce the ionic basis underlying the bioelectrical modifications observed under applied 50 Hz-alternating MF. As calcium concentration in the bathing solution decreases the firing frequency induced by applied 35 G decreases. It means that the stimulatory action of applied alternating MF is a calcium-dependent process. As potassium ion concentration decreases in the superfusate the spikes frequency decreases. In this experimental condition, the electrochemical gradient for K^+ ions favours the sorting of these ions out from the neuron hyperpolarizing the membrane, the firing frequency in turn drecreasing. As extracellular K^+ concentration ($[K^+]_o$) increases the firing frequency also increases. It is known that as $[K^+]_o$ increases voltage regulated Ca^{2+} channels are opened [13] and Ca^{2+} ions move inside the cell down their electrochemical gradient. If the firing frequency is a calcium-dependent process the expected result is the increasing of the spike frequency as shown on Fig. 8b. The observed results fit with the shown frequency decrease as extracellular calcium concentration decreases. In this way, with these experiments, the involvement of calcium ions in the responses elicited by 50 Hz-MF find a new support. It has already been suggested that calcium is necessary in human HL-60 cell response to ELF electromagnetic fields (ELF-EMF). Under exposure to ELF-EMF and under conditions of low extracellular calcium the activation of transcript levels for c-fos and c-myc was tested. The EMF signals could be amplified by Ca^{2+} flux modification which in turn could led to the observed gene activation [24].

It is to notice the shown mimic effect between caffeine and alternating applied MF. Such a mimic effect was also described under SMF [1]. We show here that the mimic effect is extended to alternating MF, caffeine and also glutamate. The depolarization associated with caffeine is caused by an increase on permeability to both Ca^{2+} and Na^+ ions [28]. There has already been described in *Helix pomatia* [40] a pacemaker current in bursting neurones due to the intracellular Ca^{2+} elevation, in turn due to the Ca^{2+} entry during action potential membrane depolarization. It activates a non-specific depolarizing membrane current that initiates the burst (CAN currents, [37]). Therefore we consider that the cytosolic increased free Ca^{2+} ions are by themselves able to depolarize the cell beyond the firing level. For neurones giving responses defined as bimodal, the ionic basis for the observed effects would be explained in the same way. The effect of caffeine on neurones has also been shown to be biphasic [36].

We have observed on the experiment shown in Fig. 6 that in this kind of neurone, spontaneous activity shows interspikes ILD activity (inhibition of long duration) what could express a high membrane density of Ca^{2+}-dependent-K^+-channels. It is well known the calcium-dependent potassium channels activation in nervous tissues with concomitant activity inhibition [33]. In cell-attached patches performed in voltage-clamped *Helix* neurones, Gola and Crest [20] have shown that in the cell body of *Helix* neurones, large conductance active Ca^{2+}-dependent-K^+-channels are colocalized in clusters together with Ca^{2+}-voltage-operated channels. This structural organization would explain the Ca^{2+}-dependent fast responses observed under the experimental conditions here shown.

Several authors have shown in different cell types increases in the cytosolic Ca^{2+} oscillations under applied alternating MF [15,19,27,32,41]. Different experimental data confirm that calcium plays a role in the response of cells to electromagnetic fields. One of them being related to cell differentiation of chromaffin cells toward neuronal-like cells subjected to the application of either nerve growth factor (NGF) or ELF-MF. When the L-Ca^{2+} channel blocker nifedipine was applied simultaneously with ELF-MF, this differentiation did not take place, but it did when an N-Ca^{2+} channel blocker was used. In contrast, none of the Ca^{2+} channel blockers prevented differentiation in the presence of NGF. In addition, Bay K-8644, an L-Ca^{2+} channel agonist, increased both the percentage of differentiated cells and neurite length in the presence of ELF MF. This effect was much weaker in the presence of NGF [34].

We consider that the shown mimic effect between glutamate and applied 50 Hz alternating magnetic field is quite important. We have described that neurones are magnetosensitive under applied SMF in the intensity range of the ones applied for clinical diagnosis MRI [3]. Taking into account that glutamate is the most important excitatory neurotransmitter on vertebrate brain the implications of the experimentally effects here described should be taken into account and needs further research, the pharmacological characterization of the glutamate receptors is in progress to go further in this study.

Accordingly to our results, being the processes induced under applied 50Hz alternating MF calcium-dependent it would be expected some changes on synaptic events studied as described by other authors. Increased ^{3}H-noradrenaline released under alternating MF [18] and neuromuscular junction potentiation under SMF [39] have been observed. It seems that electromagnetic fields stimulation alters catecholamine metabolism, as shown for PC12 cells, where dopamine levels were significantly reduced within 10–15 min under 60 Hz 8 μT sinusoidal field [35]. Nonetheless we consider that our experimental conditions are different. We are working with the whole ganglia and taking intracellular recordings from the neuronal soma. The neurone under study is receiving stimulatory and inhibitory neurotrasnmitters from neurones also exposed to the applied MF. We are in this way measuring a summary effect of the postsynaptic events. The subtle effects described at the synapses by other authors could be masked under our experimental conditions.

In summary, we consider that the applied 50-Hz-alternating magnetic field is not able to transfer time varying energy to the neurone. The induced effects on neurones are then metabolic, the increased transmembrane ionic conductances are as well under neurone metabolic control. In this way inhibition, excitation and bimodal responses are the result of the intrinsic structural and metabolic characteristics of the different studied neurone subpopulations.

An explanatory physical model was developed to explain the induced neuronal responses under 50 Hz *alternating* MF [9]. This model follows the trends of the one so far proposed, in order to explain the response of neurones to applied *static* magnetic fields (SMF) [6,17]. The proposed model assumes that a cooperative magnetic dipole orientational process takes place, because of the plasma membrane phospholipid and protein strong anisotropic diamagnetism, an effect so-called superdiamagnetism. Such a superdiamagnetic molecular clusters rotation is capable, through a particular electrostatic interaction between membrane Ca^{2+} ions at both membrane sides, of liberating under a coulomb explosion, the membrane trapped Ca^{2+} ions.

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