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Does exposure to extremely low frequency magnetic fields produce functional changes in human brain?

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Abstract Behavioral and neurophysiological changes have been reported after exposure to extremely low frequency magnetic fields (ELF-MF) both in animals and in humans. The physiological bases of these effects are still poorly understood. In vitro studies analyzed the effect of ELF-MF applied in pulsed mode (PEMFs) on neuronal cultures showing an increase in excitatory neurotransmission. Using transcranial brain stimulation, we studied noninvasively the effect of PEMFs on several measures of cortical excitability in 22 healthy volunteers, in 14 of the subjects we also evaluated the effects of sham field exposure. After 45 min of PEMF exposure, intracortical facilitation produced by paired pulse brain stimulation was significantly enhanced with an increase of about 20%, while other parameters of cortical excitability remained unchanged. Sham field exposure produced no effects. The increase in paired-pulse facilitation, a physiological parameter related to cortical glutamatergic activity, suggests that PEMFs exposure may produce an enhancement in cortical excitatory neurotransmission. This study suggests that PEMFs may produce functional changes in human brain.

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Introduction

The presence of electromagnetic fields (EF) in our environment is a fact but biological effects of EF are still poorly understood. During recent years, there has been increasing scientific evidence for biological effects of EF and public concern on potential health risks of EF (Hardell and Sage 2008). Large public and occupational populations are exposed to two types of EF: the extremely low frequency magnetic fields (ELF-MF), generally produced by electrical and electronic appliances and power lines, and radio frequency magnetic fields (RF-MF) produced by wireless devices such as cell phones and cordless phones, cellular antennas and towers, and broadcast transmission towers.

When applied in vivo ELF-MF seem to affect several brain functions:

Deterioration in memory and learning processes after acute ELF-MF exposure have been described both in animals (Jadidi et al. 2007; Lai et al. 1998; Sienkiewicz et al. 1998) and in humans (Podd et al. 2002). Different findings have been reported after chronic ELF-MF exposure with positive effect in social recognition memory (Vazquez-Garcia et al. 2004) and spatial learning (Liu et al. 2008) suggesting a crucial role for the length of exposure.

Extremely low frequency magnetic fields related effects were described also on the motor system. Rat's locomotor activity, initially enhanced by ELF-MF, was attenuated when exposure became chronic (Janac et al. 2005). Preliminary studies in humans also suggest some effects on motor control: Thomas et al. (2001) showed that MF

improves standing balance. The sensory system seems also targeted: many authors have documented the influence of ELF-MF on pain threshold, showing hyperalgesia occurrence for different frequencies, in animals (Choleris et al. 2002; Kavaliers et al. 1990) and in humans (Ghione et al. 2005). The opposite effect with induction of hypoalgesia has also been reported using pulsed ELF-MF both in animals (Thomas et al. 1997) and humans (Shupak et al. 2004).

The ELF-MF influence on brain activity is also supported by some neurophysiological studies that show changes in electroencephalographic activity after ELF-MF exposure with an increase in alpha rhythm (Bell et al. 1992, 1994; Cook et al. 2004; Lyskov et al. 1993). However, the significance of these data and their correlation with clinical and behavioral reports remain unclear. In vitro studies showed that ELF-MF modify gene-expression in neuron-like cells (Campbell-Beachler et al. 1998; Pirozzoli et al. 2003), promote neurite outgrowth (Blackman et al. 1993; McFarlane et al. 2000), reduce apoptosis (Oda and Koike 2004) and facilitate neuronal differentiation of neural stem/progenitor cells by upregulating Calcium-channel expression and function (Piacentini et al. 2008). Recently, Manikonda et al. (2007) demonstrated modifications in NMDA receptor function mediated by alteration of Ca^{2+} signaling in rat hippocampus exposed to 50 Hz magnetic field.

Most of research studies conducted with ELF-MF address the concern for electromagnetic environmental pollution, the waveform of the signal is sinusoidal and the peak values of the magnetic field are in the range of microTesla. Pulsed electromagnetic fields (PEMFs) are generated by pulsed signals, the peak value of the magnetic field is in the range of milliTesla (mT) and the rate of change of the magnetic field ($d\Phi/dt$) is fast (milliseconds).

Numerous exposure systems have been developed and used to explore biological effects and even potential therapeutic applications. These devices can generate PEMFs characterized by the repetitive variation of the field amplitude in the time, with rapidly increasing and falling waveform. It has been shown that pulsed EF may improve bone healing (Bassett et al. 1982; Dimitriou and Babis 2007; Nelson et al. 2003) and reduce osteoartritic pain (Fini et al. 2005; Massari et al. 2007): these methods are currently used in the orthopedic practice.

While a large number of studies investigated both in vitro and in vivo the effects of high frequencies (MHz) and strong power (1–2 W) PEMFs on brain function using a mobile phone-like model (Ferreri et al. 2006; Valentini et al. 2007), only few in vitro works have analyzed the influence of low frequency–low intensity PEMFs on neuronal activity. Wieraszko studied the effects of low frequency–low intensity PEMFs in hippocampus focusing on glutamatergic synapsis between Schaffer collateral-commissural fibers and CA1 pyramidal cells (Wieraszko 2004). Exposure to PEMFs of 15 mT, produced frequency-dependent amplification of evoked potentials mediated by an increase of cAMP in neurons (Hogan and Wieraszko 2004) and glutamate concentration in synaptic cleft (Wieraszko et al. 2005). Also different neurotransmitter systems seem to be influenced: using rat and human brain slice preparations, Massot et al. (2000) demonstrated that weak magnetic fields (0.1–1 mT) induce reversible changes in 5HT-1b receptor; Varani et al. (2002, 2003, 2008) evidenced that PEMF exposure increases the number of $A2_A$ adenosine receptors available for the ligand with adenosine analogs, thus demonstrating an adenosine $A2_A$ receptor agonist like activity; Tebano et al. (2005) showed that the adenosine $A2_A$ receptor agonist (CGS 21680) and mGlurR synergistically reduced the slope of excitatory postsynaptic field potential that may result in facilitation of intracortical communication. The effects of PEMFs on neurotransmission seem to be related to changes in function and distribution of membrane proteins of exposed cells (Chiabrera et al. 2000).

The aim of this paper was to evaluate whether PEMF exposure may produce changes in the excitability of human cerebral cortex. To this end we used non invasive techniques of transcranial brain stimulation. The recently introduced transcranial magnetic stimulation (TMS) of the brain has provided the opportunity to study mechanisms of cortical physiology (Chen et al. 2008). Several TMS protocols provide functional information about excitatory and inhibitory neurotransmission in circuits of the human cerebral cortex (Chen et al. 2008). Considering the high variability of the results of previous studies that, at least in part, is due to the variable physic characteristics of the EF, we used a highly standardized exposure system in order to obtain comparable stimulation in all subjects. We evaluated the effects of PEMFs on different TMS measures, because in vitro studies have shown that the exposure to these fields for less than 1 h produces consistent effects on neurotransmission. In order to obtain information on different neurotransmitter systems, we evaluated the following TMS parameters: (1) threshold of motor evoked potentials (MEPs), which reflect the excitability of motor cortex involving both axonal and synaptic mechanisms; (2) short interval intracortical inhibition (SICI) to paired pulse TMS which is believed to be mediated by GABA-A receptors (Chen et al. 2008); (3) intracortical facilitation (ICF) that represents a complex phenomenon reflecting the activity of some still poor defined cortical circuits independent from those involved in SICI, whose function is, at least in part, related to glutamatergic activity (Chen et al. 2008); (4) short interval afferent inhibition (SAI) which is believed to be regulated by muscarinic cholinergic and GABAergic cerebral circuits (Chen et al. 2008).

Materials and methods

Subjects

Twenty-two (9 male and 13 female) healthy volunteers [mean age 27.6 ± 9 (SD) years] participated in the experiments. All gave their informed consent. The study was performed according to the Declaration of Helsinki and was approved by the Ethics Committee of the Medical Faculties of the Catholic University of Rome.

PEMF exposure

We used a coil custom made by IGEA with the following characteristics: rectangular shape (22 × 20 cm) with rounded corners, 1,400 turns of copper wire, 0.2 mm ∅, 540 ohm resistance and weight 280 gr. The coil that is flexible and thus adaptable to the head assuming an approximately round shape, was positioned tangential to the inion and to a point 3 cm above nasion and was kept in place by a Velcro strap (Velcro, Manchester, New Hampshire) (Fig. 1). The coil was positioned to orient the positive pole of the magnetic field toward the top of skull. The pulse generator (B-01; IGEA, Carpi, Italy) supplied the coil with a single-pulsed signal at 75 ± 2 Hz, with a pulse duration of 1.3 ms. The peak intensity of the magnetic field was 1.8 ± 0.2 mT, the amplitude of the induced electric field was 3 ± 1 mV. The magnetic field was measured using a Hall effect transverse gaussmeter probe (HTD61-0608-05-T, F.W. Bell line, Sypris Solutions Inc., Louisville, KY) and a gaussmeter (DG-500, Laboratorio Elettrofisico, Milan, Italy). Magnetic field distribution was modeled using COMSOL Multiphysics (COMSOL, Palo Alto, USA) (Fig. 2), the induced electric voltage was measured using a standard pick-up coil probe (50 turns, 0.5 cm internal diameter of the coil probe, 0.2 mm diameter copper wire), and the temporal pattern of the electromagnetic signal was evaluated using a digital oscilloscope (LT322, WaveRunner Series, LeCroy Inc., Chestnut Ridge, NY) (Fig. 3).

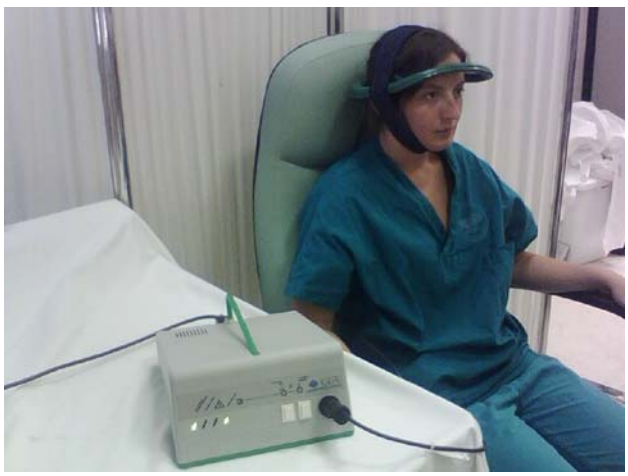

Fig. 1 The coil positioned on the head and the pulse generator used to expose the subjects to pulsed electromagnetic fields

In 14 of the subjects we also evaluated the effects of sham field exposure. For sham field exposure the coil was applied in the same position but the pulse generator was not turned on. Subjects were blinded for stimulation conditions. PEMF exposure does not give any sensation; for this reason it is impossible for the subject to distinguish between real from sham exposure.

Evaluation of cortical excitability

Magnetic stimulation was performed with a high-power Magstim 200 (Magstim Co., Whitland, Dyfed, UK). A figure-of-eight coil, with external loop diameter of 9 cm, was held over the right motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous muscle (FDI). The induced current flowed in a posteroanterior direction. MEPs were recorded via two 9-mm-diameter Ag–AgCl surface electrodes with the active electrode over the motor point of the left FDI and the reference on the metacarpophalangeal joint of the index finger. The EMG was amplified and filtered (bandwidth 3 Hz–3 kHz) by D360 amplifiers (Digitimer, Welwyn Garden City, Herts, UK). Data were collected on a computer with a sampling rate of 10 kHz per channel and stored for later analysis using a CED 1401 A/D converter (Cambridge Electronic Design, Cambridge, UK).

We evaluated threshold, SICI, ICF and SAI. The execution of the complete battery of tests required about 25 min. The order of execution of the different tests was counterbalanced within the subjects.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 50 μV in 50% of ten trials) at rest. Active motor threshold (AMT) was defined as the minimum stimulus intensity that produced a liminal MEP (about 200 μV in 50% of ten trials) during isometric contraction of the tested muscle. A constant level of voluntary contraction was maintained with reference to an oscilloscope display of the EMG signal in front of the subject. Auditory feedback of the EMG activity was also provided. Trials contaminated by EMG activity were discarded. RMT and AMT are given in percentage of maximum stimulator output (% MSO). SICI and ICF were studied using the technique of Kujirai et al. (1993) and Ziemann et al. (1996). Two magnetic stimuli were given through the same stimulating coil over the right motor cortex and the effect of the first (conditioning) stimulus on the second (test) stimulus was investigated. The conditioning stimulus was set at an

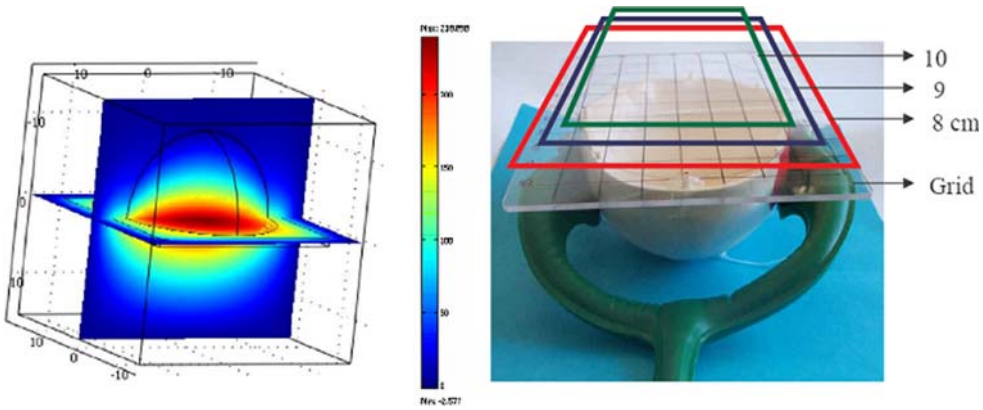

Fig. 2 *Left* magnetic field peak value distribution modeled with COMSOL software. *Right* set-up used to actually measure, with the pick-up coil, the values of the induced electric field at different distances, centimeters, from the coil plane; the grid, 1 × 1 cm, was used to map the electric field in the plane

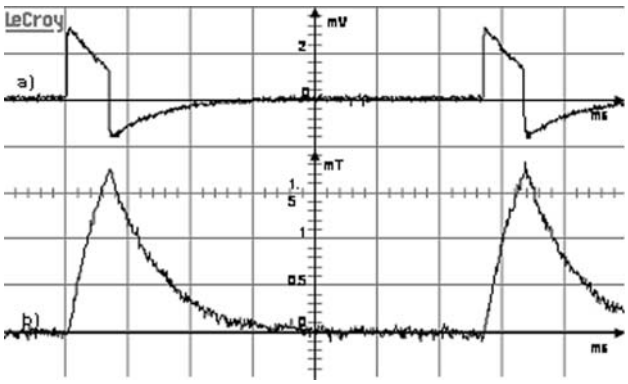

Fig. 3 **a** Waveform of the electrical field induced in the pick-up coil probe described in the text, **b** waveform of the induced magnetic field

intensity of 5% of MSO below AMT. The intensity of the test stimulus was adjusted to elicit an unconditioned test MEP in the relaxed left FDI of approximately 1 mV in peak-to-peak amplitude. ISIs of 2 and 3 ms (for SICI) and 10 and 25 ms (for ICF) were investigated. Five stimuli were delivered at each ISI in pseudo-randomized order. Subjects were provided with audio-visual feedback of the EMG at high gain (50 μV/D) to assist in maintaining complete relaxation. The amplitude of the conditioned MEP was expressed as a percentage of the amplitude of the unconditioned test MEP. The SICI and ICF data were averaged across the two inhibitory and the two facilitatory ISIs to obtain one grand mean single value for SICI and for ICF.

After PEMF and sham field exposure the intensity of the test stimulus was adjusted whenever necessary to ensure that the test MEP matched the amplitude to the baseline test MEP before stimulation.

SAI was studied using the technique that we have described previously (Tokimura et al. 2000). Conditioning electrical pulses (constant current square wave pulses; duration, 200 μs) were applied through a bipolar electrode to the left median nerve at the wrist (cathode proximal).

The intensity of the conditioning stimulus was set to evoke a just visible twitch of the thenar muscles. The intensity of the TMS test pulse over the right motor cortex was adjusted to evoke an unconditioned MEP in the relaxed FDI of approximately 1 mV in peak-to-peak amplitude. The conditioning stimulus to the median nerve preceded the TMS test pulse by interstimulus intervals (ISIs) that were related to the individual latency of the N20 component of the median nerve somatosensory evoked potential. To record somatosensory-evoked potentials, the active electrode was attached 3 cm posterior to C4 (according to the 10–20 International EEG system) and the reference was 3 cm posterior to C3, respectively. Five hundred responses were averaged to identify the latency of the N20 peak. ISIs corresponding to the N20 latency plus 2, 3, 4, 5, 6, 7 and 8 ms were investigated (Tokimura et al. 2000) with five repeats per ISI in a pseudo-randomized order. Subjects were given audio-visual feedback of the EMG signal at high gain (50 μV/D) to assist in maintaining complete relaxation of the left FDI. The mean amplitudes of the conditioned MEPs at the various ISIs were expressed as a percentage of the mean amplitude of the unconditioned test MEP. These data were averaged across all ISIs to obtain a grand mean single value of SAI. After stimulation the intensity of the test stimulus was adjusted whenever necessary to ensure that the test MEP matched the amplitude to the baseline test MEP before stimulation.

Experimental design and data analysis

The tested electrophysiological parameters (AMT, RMT, SAI, SICI, ICF) were evaluated before (baseline) and after 45 min of exposure to PEMFs or to sham field. At the beginning of the experiments the subjects were asked whether they agreed to undergo two sessions of the study (one with real and one with sham exposure) at a distance of at least 1 week. Fourteen subjects accepted to participate in both studies and underwent both real and sham exposure while the remaining eight subjects underwent only real

exposure. Thus, we had a larger number of subjects undergoing real exposure than sham. Although this strategy decreases the power of the study (because less information from the sham group is provided), it reflects a conventional strategy both in randomized phase II trials (which are often quite small) (Peto 1978) and in electrophysiological studies (Fregni et al. 2006) because it can offer additional information regarding the active treatment. The subjects received PEMF exposure and sham field exposure in a randomized order.

Statistical analysis was performed separately for each electrophysiological parameter by means of the Mann-Whitney tests. Conditional on a significant difference between PEMFs and sham field exposure groups, measures before and after exposure were compared by means of Wilcoxon matched-pair tests. All data are expressed as mean ± 1 standard error of the mean (SEM). The level of significance was set at 0.05.

Results

Mean values are reported in Fig. 4, 5, and 6.

No side effects were observed in all the subjects after stimulation.

No statistically significant difference between PEMF and sham exposure was found for RMT, AMT, SAI, and SICI ($P > 0.05$, Mann–Whitney test, N1 = 44, N2 = 28).

There was a significant difference between PEMF and sham exposure for ICF ($P < 0.05$, Mann–Whitney test, N1 = 44, N2 = 28). Post hoc analysis using Wilcoxon matched-pair tests showed that ICF was significantly increased after PEMF exposure; it increased by about 20% from the baseline value (pre-stimulation 119.5 ± 4.8% of the test response evoked by single pulse TMS; post-stimulation 140.2 ± 7.8% of the test response evoked by single pulse TMS; $P = 0.005$), while ICF was not modified after sham exposure (pre-stimulation 117.2 ± 8.2% of the test response evoked by single pulse TMS; post-stimulation 117 ± 10.7% of the test response evoked by single pulse TMS; $P > 0.05$).

Discussion

This is the first in vivo demonstration that pulsed, extremely low frequency, EF influence cortical excitability in humans. The effect seems to be specific: there is a pronounced increase in intracortical facilitation produced by paired pulse stimulation (ICF) while cortical excitability to single pulse stimulation (RMT and AMT) and measures of intracortical inhibitory activity (SICI and SAI) are not modified. Sham field exposure had no effects. ICF, is a

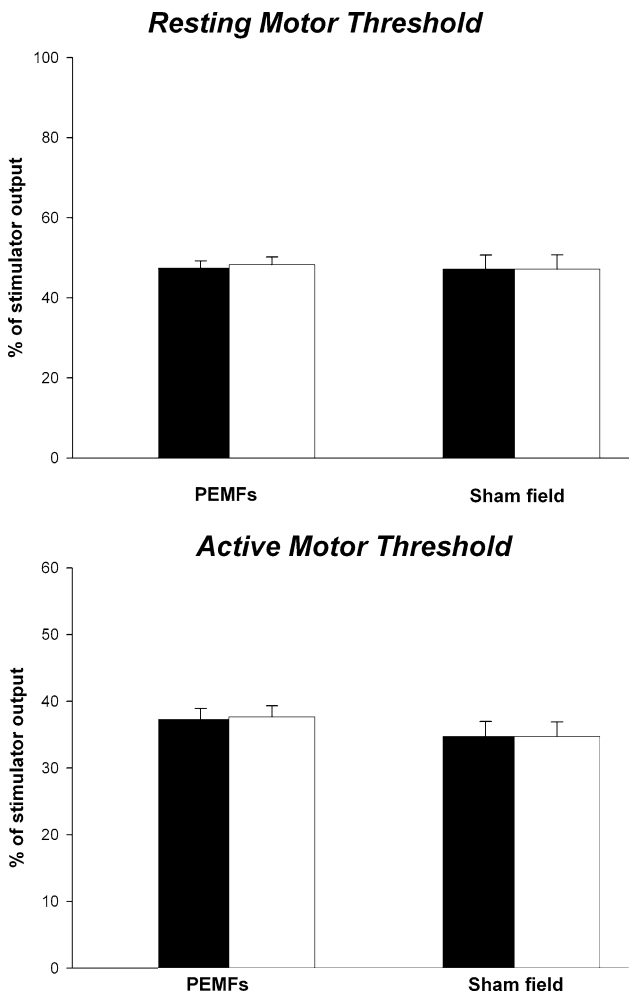

Fig. 4 Histograms showing mean resting motor threshold (RMT) and mean active motor threshold (AMT) in baseline conditions and after 45 min of exposure to pulsed electromagnetic fields (PEMFs) in 22 subjects and after 45 min of exposure to sham field in 14 of the subjects. *Error bars* are standard errors. There is no statistical significant difference between PEMF and sham exposure data ($P > 0.05$, Mann–Whitney test, N1 = 44, N2 = 28)

cortical phenomenon produced by paired pulse cortical stimulation (Kujirai et al. 1993; Ziemann et al. 1996). The exact mechanism of ICF is still unclear (Chen et al. 2008; Di Lazzaro et al. 2006): pharmacological studies suggest that ICF reflects excitatory neurotransmission largely mediated by the NMDA receptor (Paulus et al. 2008). Together with an enhanced excitatory glutamatergic neurotransmission, a reduced cortical inhibition (GABA-A receptor mediated) has also been suggested to explain ICF. In this study, the increase of ICF was associated with no change in SICI, a putative marker of GABA-A related inhibitory activity (Paulus et al. 2008). Thus, the results of the present study, taken together with previous evidence of a similar dissociated behavior of SICI and ICF under several pharmacological manipulations (Strafella and Paus 2001; Ziemann et al. 1996), support the idea that PEMF

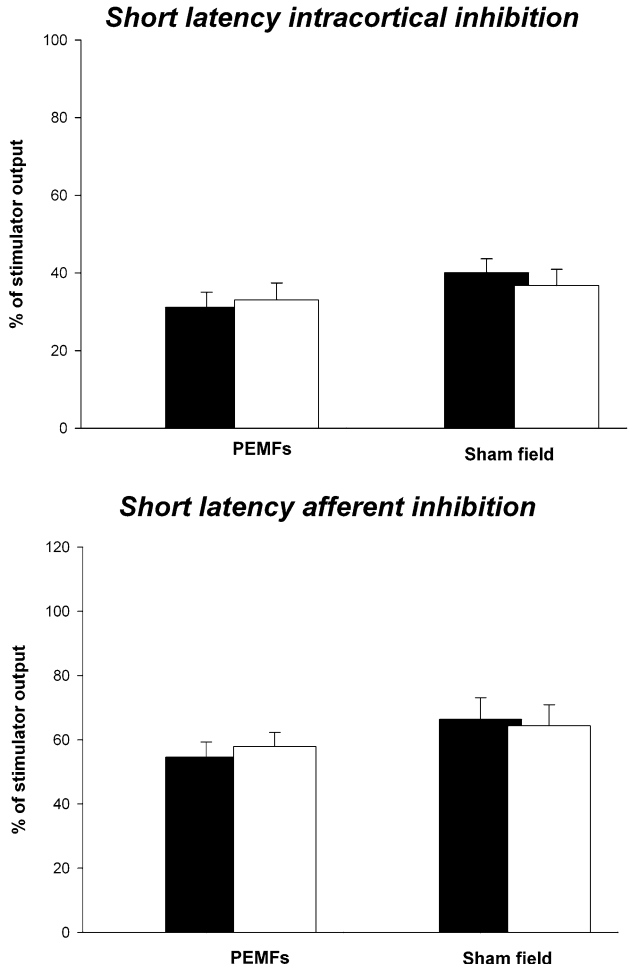

Fig. 5 Histograms showing mean short latency intracortical inhibition (SICI) and mean short latency afferent inhibition (SAI) in baseline conditions and after 45 minutes of exposure to PEMFs in 22 subjects and after 45 min of exposure to sham field in 14 of the subjects. *Error bars* are standard errors. There is no statistical significant difference between PEMF and sham exposure data ($P > 0.05$, Mann–Whitney test, N1 = 44, N2 = 28)

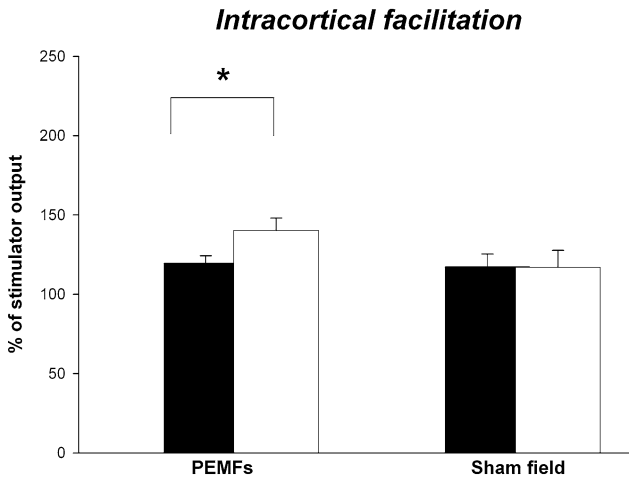

Fig. 6 Histograms showing mean intracortical facilitation (ICF) in baseline conditions and after 45 min of exposure to PEMFs in 22 subjects and after 45 min of exposure to sham field in 14 of the subjects. *Error bars* are standard errors. Statistical analysis showed a significant difference between PEMF and sham exposure ($P < 0.05$, Mann–Whitney test, N1 = 44, N2 = 28). Post hoc analysis using Wilcoxon matched-pair tests shows that ICF is significantly increased after PEMFs exposure ($^*P = 0.005$), while ICF is not modified after sham field exposure ($P > 0.05$)

exposure may produce a selective enhancement of glutamatergic neurotransmission in human brain. ICF was increased by about 20% after PEMF exposure; interestingly a comparable change in ICF, that was statistically significant, was observed after pharmacological manipulation of glutamatergic transmission (Ziemann et al. 1998). These authors evaluated the effects of dextromethorphan that is a NMDA receptor antagonist capable of reducing the activity of the excitatory glutamatergic circuits in the normal human brain. As expected, though the amount of change was comparable to the change observed in present study, they found a reduction of ICF instead of an increase.

Experimental data support an origin from glutamatergic synaptic activity enhancement for the ICF increase. In vitro studies by Wieraszko (2004), using hippocampal model of glutamatergic synapsis between Schaffer collateral–commissural fibers and CA1 pyramidal cells, showed that 30 min of exposure to pulsed fields of 15 mT, produced frequency-dependent amplification of evoked potential and that 0.16 Hz was the most effective frequency. The increase in neuronal excitability was associated with increase in concentration of cAMP (Hogan and Wieraszko 2004) and modulation of glutamate turnover: enhancement in release and reduction in reuptake (Wieraszko et al. 2005). Interestingly, Varani et al. (2002, 2003, 2008), using PEMFs with identical field characteristics of the present study, found an increase in density and functionality of adenosine receptors ($A2_A$ and A3) expressed by non neuronal cells (neutrophils, chondrocytes, fibroblast-like synoviocytes); remarkably, the effect seems to be specific (A1, $A2_B$, opioid and adrenergic receptors are not modified), evocable by brief stimulation (also evident after 30 min exposure), determining metabolic consequences (increase in cAMP and reduction in superoxide anion concentration, cellular proliferation). Adenosine is a neuromodulator of CNS that acts by four types of G-protein coupled receptor (A1, $A2_A$, $A2_B$, A3) (Fredholm et al. 2005). In particular, A1 and $A2_A$ modulate glutamatergic transmission with opposite effects: A1 receptor activation reduces glutamate release and hiperpolarizes neurons, while $A2_A$ activation potentiates neurotransmission (Cunha 2005). In hippocampal synapsis (Sch. Coll—CA1), it has been demonstrated that activated $A2_A$ receptor, interacting with mGLU5 receptor, induces transduction-pathways (both cAMP/PKA and Ca/PKC related) that determine

NMDA receptors mediated effects (Tebano et al. 2005). The presence of adenosine receptors and their influence on glutamatergic transmission (Rebola et al. 2005) have been evidenced also in cortical cultured neurons. The above studies suggest that low frequencies–low intensity pulsed magnetic fields specifically interact with $A2_A$ receptor located in glutamatergic synapses activating signalling-pathways. This enhancement in excitatory neurotransmission might also explain the increase in ICF observed in the present study. However, this hypothesis needs to be confirmed by further studies because different mechanisms cannot be excluded: for example, there is evidence that ELF-MF can modulate neuronal functional state influencing nitric oxide system in the brain (Bawin et al. 1996; Kavaliers et al. 1998).

Adenosine receptors, in particular $A2_A$, expressed in neurons, glia and inflammatory cells are thought to be implicated in many neurological disorders and they might have a role in neuroprotection (Chen et al. 2007). The neuroprotective role of PEMF has also been reported in an experimental model of ischemia (Grant et al. 1994). These preliminary experimental data about the possible neuroprotective role of PEMF, together with the evidence of an effect of these fields in intact human brain provided by present study, encourage further investigations about the possible application of PEMFs as a neuromodulation tool. PEMF exposure devices could be added to the armamentarium of the neuromodulatory techniques under investigation as potential therapeutic tools for neurological disorders like repetitive TMS of the brain (Ridding and Rothwell 2007) and direct current brain stimulation (Fregni and Pascual-Leone 2007).

Because ICF, as other TMS related parameters, reflects both axonal and synaptic excitability (Paulus et al. 2008), an increase in ICF might also be explained by a change in axonal excitability. It has been suggested that paired-pulse ICF results from the recruitment of cortical axons in addition to those recruited by single pulse TMS (Di Lazzaro et al. 2006); thus, it can be hypothesized that PEMF exposure lowers the threshold of these axons making ICF more pronounced.

In conclusion, our research demonstrates that magnetic fields, even low-energy, can modulate neuronal activity of the human cerebral cortex. The functional significance of the excitability changes is not clear, but considering the number of people exposed everyday to EF, further studies are urgently needed to clarify the correlates of observed changes for the possible impact on public health. More studies addressing the evaluation of the potential therapeutic application of EFs are also warranted.

Conflict of interest statement Ruggero Cadossi is President and Director of IGEA S.p.A. that produces and distributes the PEMF exposure device. Stefania Setti is an employee of IGEA S.p.A. that produces and distributes the PEMF exposure device

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