



Mobile Phone Emissions Modulate Brain Excitability in Patients with Focal Epilepsy

Mario Tombini^{a,*}, Giovanni Pellegrino^a, Patrizio Pasqualetti^b, Giovanni Assenza^a, Antonella Benvenga^a, Emma Fabrizio^a, Paolo Maria Rossini^{c,d}

^aNeurology, Campus Biomedico University, Rome, Italy

^bAFAr, Department of Neuroscience, Hosp, Fatebenefratelli, Isola Tiberina, Rome, Italy

^cNeurology, Catholic University of Rome, Italy

^dCasa di Cura S. Raffaele, and IRCCS S. Raffaele-Pisana, Rome, Italy

ARTICLE INFO

Article history:

Received 10 March 2012

Received in revised form

16 June 2012

Accepted 14 July 2012

Available online 11 August 2012

Keywords:

Brain excitability

Electromagnetic fields

Epilepsy

Mobile phone

TMS

ABSTRACT

Background: Electromagnetic fields (EMFs) emitted by mobile phones had been shown to increase cortical excitability in healthy subjects following 45 min of continuous exposure on the ipsilateral hemisphere.

Objective: Using Transcranial Magnetic Stimulation (TMS), the current study assessed the effects of acute exposure to mobile phone EMFs on the cortical excitability in patients with focal epilepsy.

Methods: Ten patients with cryptogenic focal epilepsy originating outside the primary motor area (M1) were studied. Paired-pulse TMS were applied to the M1 of both the hemisphere ipsilateral (IH) and contralateral (CH) to the epileptic focus before and immediately after real/sham exposure to the GSM-EMFs (45 min). The TMS study was carried out in all subjects in three different experimental sessions (IH and CH exposure, sham), 1 week apart, according to a crossover, double-blind and counter-balanced paradigm.

Results: The present study clearly demonstrated that an acute and relatively prolonged exposure to GSM-EMFs modulates cortical excitability in patients affected by focal epilepsy; however, in contrast to healthy subjects, these effects were evident only after EMFs exposure over the hemisphere contralateral to the epileptic focus (CH). They were characterized by a significant cortical excitability increase in the exposed hemisphere paired with slight excitability decrease in the other one (IH). Both sham and real EMFs exposure of the IH did not affect brain excitability.

Conclusion: Present results suggest a significant interaction between the brain excitability changes induced by EMFs and the epileptic focus, which eliminated the excitability enhancing effects of EMFs evident only in the CH.

© 2013 Elsevier Inc. All rights reserved.

Introduction

The growing use of mobile phones (MPs) has driven neuroscientists to focus their attention on the potential impact of this technology on human health [1] and brain functions [2]. An interaction between electromagnetic fields (EMFs) due to

radiofrequency and cerebral activity could be hypothesized, given the fair amount of radiofrequency emitted by mobile phones absorbed by the skull and reaching the brain. Nevertheless, in recent years only a few studies have investigated the effects of MP exposure on brain metabolism or cortical excitability [3–5].

These data usually refer to the signal generated by the Global System for Mobile Communication (GSM), which operates at frequencies around 900 MHz. Huber et al. [6,7] investigated the potential effect of a GSM signal on cerebral blood flow through a PET study, showing for the first time, how the regional cerebral blood flow (rCBFs) increased significantly on the dorsolateral prefrontal cortex on the side of EMFs exposure. More recently Volkow [8] demonstrated via PET that in healthy subjects the 50-min mobile phone exposure is associated with an increase of

There are not potential or real conflicts of interest.

Mario Tombini: No financial interest/arrangement, Giovanni Pellegrino: No financial interest/arrangement, Patrizio Pasqualetti: No financial interest/arrangement, Giovanni Assenza: No financial interest/arrangement, Antonella Benvenga: No financial interest/arrangement, Emma Fabrizio: No financial interest/arrangement, Paolo Maria Rossini: No financial interest/arrangement.

* Corresponding author. Tel.: +39 06 225411286; fax: +39 06 225411028.

E-mail address: m.tombini@unicampus.it (M. Tombini).

brain glucose metabolism in the region closest to the antenna. This is consistent with a previous transcranial magnetic stimulation (TMS) study from our group [3], which evidenced significant effects of GSM 900 EMF exposure on brain excitability in healthy subjects. In particular, it showed how intracortical excitability is significantly increased, short-interval intracortical inhibition (SICI) is reduced, and intracortical facilitation (ICF) is enhanced in an acutely exposed (45 min) human cerebral hemisphere if compared with the non-exposed contralateral hemisphere or with sham.

Given the previous evidence of the effects of EMFs generated by GSM (GSM-EMFs) on cortical excitability and brain metabolism, it becomes of public health interest to investigate whether such EMFs-mediated cortical excitability changes behave in those patients suffering from a pathophysiological increase of cortical excitability, as in epilepsy disorders.

The aim of our study was to investigate for the first time the effects of GSM 900 EMFs exposure on brain excitability in patients affected by cryptogenic focal epilepsy as evaluated through a paired-pulse TMS paradigm.

Patients and methods

Patients

Ten patients affected by focal epilepsy (5 M/5 F; age range 19–67 years, mean age 39.9 ± 18.1 years) were studied. The study protocol was approved by local Ethics Committee and a written informed consent was obtained from all patients. On the basis of neuroimaging data (CT scan and/or MRI), clinical and EEG features, all patients were diagnosed as having cryptogenic partial epilepsy. Epilepsy onset was between 5 and 57 years (mean age 24.6 ± 18 years). Eight patients were on single drug therapy, 2 patients were newly diagnosed as having epilepsy and were not taking antiepileptic drugs during the study recordings. The inclusion criteria considered patients affected by focal epilepsy, whose epileptogenic zone was localized outside the primary motor area (M1). The drug dosages were not changed throughout the duration of the trial and the level of drugs – when possible – were monitored during all duration of the study and resulted stable within the therapeutic range. The exclusion criteria established by international safety standards for TMS were followed [9,10]. In addition, we excluded: i) patients who had recently suffered from a status epilepticus (less than 1 week); ii) patients who had experimented seizures in the 24 h preceding recording session; iii) drug-resistant patients; iv) patients with more than 2 antiepileptic drugs (AEDs) and/or other drugs affecting the nervous system to avoid the excessive interference in the cortical excitability; v) patients with organic or psychiatric disorders other than epilepsy.

All patients were right-handed, as assessed by the Edinburgh Handedness Inventory [11] with an average score of 94.9 (range

84.4–100.0). The clinical features of patients were summarized in Table 1.

Experimental protocol

Subjects were instructed to abstain from caffeine/alcohol and to maintain their regular sleep–wake schedule on the 3 days before each experimental session. No mobile phone use was allowed on the day of the experiments. Considering the influence of ovarian hormones on human cortical excitability [12], all experimental sessions with female patients – except two of them who were in menopause (pts 6 and 9, Table 1) – were always performed during the early follicular phase (low estradiol, low progesterone; Days 5–10, being Day 1 the first menstrual day).

To enhance ecological validity, we used a standard and commercially available mobile phone. It was set by a test card to transmit a typical GSM signal, working with a frequency of 902.40 MHz, modulated at 217 Hz, with a maximum power of 2 W (equivalent to an average power of 0.25 W). The mobile phone was always mounted to the side of the subject's head using a modified helmet maintaining a constant distance of 15 mm between phone and ear to avoid any heating or buzzing effects produced by the device [4]. In this experimental condition, a maximum value of specific absorption rate (SAR) equal to 0.5 W/kg was measured [3].

An identical phone, but with no battery, was positioned on the other side of the head to balance the phone's weight and make the subject blind on the eventual side of stimulation. Both phones were in the normal position for use over the ears in such a way that the aerial near the head in the parieto-temporal area was about 40 mm from the scalp surface.

All subjects underwent three sessions, 1 week apart, according to a crossover, double-blind and counter-balanced paradigm (Fig. 1): i) the signal was turned “on” and MP was placed on the side of the hemisphere ipsilateral (IH) to the epileptic focus (EMF_IH); ii) the signal was turned “on” but the MP was placed over the contralateral hemisphere (CH; EMF_CH); iii) SHAM exposure, where the signal was turned “off”. The duration of exposure was 45 min in all cases. To avoid any possible uncontrolled effect due to the presence/absence of the mobile, the subjects wore the helmet holding both phones in all sessions.

Motor-evoked potentials (MEPs) were recorded during motor cortex TMS via a paired-pulse paradigm [13] (see later) before and immediately after exposure to the EMFs. MEPs were recorded after separate TMS of both hemispheres.

TMS paradigm

The paired-pulse TMS procedure was performed twice during each session according to standardized methods [13] with the subject wearing earplugs masking the stimulus noise and lying

Table 1
Clinical features of patients.

Pt	G/Age	Epilepsy onset (y)	Brain MRI	EEG (irritative zone)	Seizure type	Seizure frequency (last 4 months)	AEDs
1	M/27	5	Normal	FT right	SG	0	CBZ
2	F/36	21	Normal	T left	CPS, SG	0	CBZ
3	M/64	55	Normal	FT right	SG	0	VPA
4	M/19	18	Normal	T left	SG	2	CBZ
5	M/20	6	Normal	TO right	CPS	0	OXC
6	F/67	57	Normal	P right	SPS; CPS	0	LTG
7	M/43	12	Normal	P right	CPS, SG	0	PB
8	F/28	27	Normal	PT right	SG	1	–
9	F/61	20	Normal	T right	CPS	1	–
10	F/34	25	Normal	T right	SPS, CPS	4	TPM

F = frontal region; T = temporal region; P = parietal region; O = occipital region; AEDs = antiepileptic drugs; TPM = topiramate; CBZ = carbamazepine; LTG = lamotrigine; OXC = oxcarbazepine; PB = phenobarbital; VPA = valproate; SPS = simple partial seizures; CPS = complex partial seizures; SG = secondarily generalized seizures.

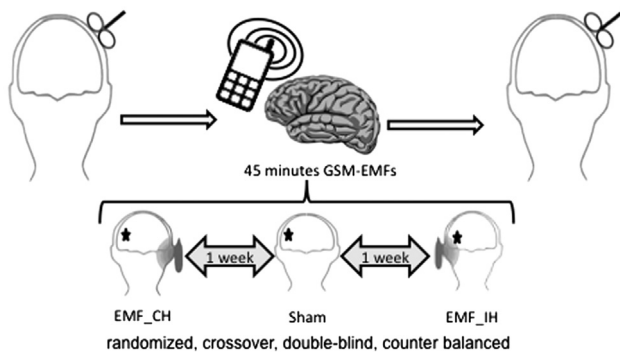


Figure 1. Experimental protocol. Motor-evoked potentials (MEPs) were recorded after separate TMS of both hemispheres via a paired-pulse paradigm before and immediately after 45 min exposure to the electromagnetic fields (EMFs) generated by a mobile phone (MP). According to a randomized, crossover, double-blind, counter-balanced paradigm, each patient underwent three sessions, 1 week apart: 1) MP turned “on” and placed over the side of the hemisphere ipsilateral (IH) to the epileptic focus (EMF_IH); 2) MP turned “on” and placed over the contralateral hemisphere (CH; EMF_CH); 3) SHAM exposure, the signal was turned “off”.

supine on a bed. Two magnetic stimulators were connected to a Bistim device (Magstim Company, Dyfed, United Kingdom) and to an eight-shaped coil with an inner diameter of 70 mm for each wing, which was used for stimulation over the hand representation area, precisely on the designated hotspot [10]. Muscle twitches from either hand triggered by TMS were recorded from the first dorsal interosseus muscle. Signal recording was conducted using GALILEO equipment (EBNeuro) via 1–2,000 Hz filter setting and a post-stimulus analysis time of 50 ms with a 5 kHz sampling rate.

The resting motor threshold (RMT) was identified according to international guidelines as the lowest intensity able to elicit reproducible MEPs (at least 50 μ V in amplitude) in about 50% of 10–20 consecutive stimuli [10]. The stimulus intensity for the first conditioning pulse was set at 80% of the resting motor threshold. The second test pulse was given suprathreshold with an intensity of 120% of the resting motor threshold. Interstimulus intervals (ISIs) of 1, 3 ms were selected to test short-interval intracortical inhibition (SICI) [14]. Meanwhile, ISIs of 7, 9, 11, 13 ms were used to test intracortical facilitation (ICF). Amplitudes of the “test” MEPs were measured between the two major and stable peaks of opposite polarity and compared with those of a series of “baseline,” unconditioned MEPs. Up to a maximum of eight trials with paired TMS were recorded for each ISI during complete muscle relaxation, and the amplitude of the conditioned MEPs was expressed as the ratio of the control MEP elicited by the test stimulus alone. As motor thresholds and MEP amplitudes might change over time, at the beginning of each session (SHAM, EMF_IH, EMF_CH), we identified RMT and measured ‘baseline’ unconditioned MEPs.

Statistical analysis

After checking the frequency distributions, MEP values needed log-transformation to better approximate gaussianity and reduce outliers.

The main statistical analysis aimed to assess the effects on MEP of four possible sources of MEP variation: *Hemisphere* (IH, CH), *Condition* (SHAM, EMF_IH, EMF_CH), *ISI* [1,3,7,9,11,13], *Time* (pre-, post-stimulation). Since several MEP values were available for each patient in each cell of the design (*Hemisphere*, *Condition*, *ISI*, *Time*), we chose to use all data points and applied a General Estimating Equation model, with patient as “Subject” (or cluster)

variable, MEP values as dependent variable and *Hemisphere*, *Condition*, *ISI* and *Time* as predictors. Since MEP values were registered consecutively, we chose the autoregressive (lag = 1) as the working correlation within subjects. The alpha-inflation due to multiple comparisons was faced according to Bonferroni-Sidak’s procedure.

Moreover, as 8 out of 10 patients revealed an epileptic focus in their non-dominant hemisphere (NDH), on the basis of previous studies, showing anatomical and electrophysiological differences between dominant (DH) and non-dominant hemispheres [15–17], a specific analysis addressed this comparison in order to rule out the possibility that hemispheric dominance could explain our findings, so being a confounding factor. In particular, in order to take into account the effect of the presence of the epileptic focus in the DH or NDH, the “epileptic focus side” was added as covariate in the above model.

Sample size justification

We gathered a minimum of 5 (up to 8) MEP values for each *Time/Condition/ISI* (mean number: 7.1). Since the levels of the factors were two for *Time*, three for *Condition* and six for *ISI*, we gathered about 180 (up to 288) measurements in each subject. The main expected findings were both interactions “*Condition*Time*” and “*Condition*Time*ISI*”. With 5 measurements for each cell, the power was 99% of detecting an expected large effect size ($f = 0.40$) of *Time * Condition* interaction, and 94% of detecting a large effect size ($f = 0.40$) of *Time * Condition * ISI* (at bilateral alpha levels set at 0.05). Considering that the power was about 80% even for detecting a medium effect size ($f = 0.25$) of *Time * Condition* interaction, we concluded that the available data for each subject allowed to reach a good power, i.e. an adequate probability of detecting at least medium effect sizes. Such within-subject considerations allow performing a conjunction analysis [18], that indicates that 10 subjects are enough to detect an 80% (or above) typicality.

Results

TMS parameters before EMF exposure

Resting motor threshold (RMT)

RMT did not show any interhemispheric difference (CH mean = 56.5%; IH mean = 56.7%; $P = 0.883$) and any difference among type of stimulation successively administered (pre-sham mean = 57.9%; 95% CI: 52.1%, 63.6%; pre-EMF IH mean = 56.8%; 95% CI: 51.7%; 61.8%; pre-EMF CH mean = 55.2%; 95% CI: 48.2%; 62.1%).

MEP amplitude (single-pulse transcranial magnetic stimulation)

MEP parameters did not show any dependence on *Hemisphere* and type of stimulation, successively delivered (373.2 μ V; 95% CI: 230.2, 604.9 in the CH and 390.2; 95% CI: 306.4, 496.8 in the IH; $P = 0.833$). Again, MEP values before EMF stimulation were similar with respect to type of stimulation successively delivered ($P = 0.732$).

MEP ratio (paired-pulse transcranial magnetic stimulation)

Considering MEP ratio as dependent variable and *ISI*, *Hemisphere* and *Condition* as predictors in a GEE model with patients as “subject” variable, the only significant factor was *ISI* (Wald chi-square = 237.7, $df = 6$, $P < .001$), confirming the well-known inhibition–facilitation curve. In particular, we observed inhibition at ISI 1 (67%, 95% CI: 61%, 73%) and at ISI 3 (76%, 95% CI: 71%, 82%), lack of inhibition/facilitation between ISI 7 and ISI 11 (corresponding 95% CI consistently included the 100% reference line) and facilitation at ISI 13 (106%, 95% CI: 102%–110%). We also found

a significant $ISI * Hemisphere$ interaction (Wald chi-square = 4E13, $df = 10, P < .001$), suggesting that inhibition/facilitation inter-hemispheric differences could vary across ISI. Post-hoc analysis indicated that only at ISI 1 the inhibition in the ipsilateral hemisphere (75%; 95% CI: 66%, 83%) was lower (more excitable) than in the contralateral one (60%; 95% CI: 51%, 69%); however, the statistical significance of this difference did not survive after Bonferroni-Sidak's correction (even considering only the 6 interhemispheric comparisons).

Finally, taking into account the effect of the presence of the epileptic focus in the DH or NDH, the “epileptic focus side” was added as covariate in the above model. Its main effect was not significant ($P = 0.188$) and did not change the significance of the other model effects.

TMS parameters changes after EMF exposure

Resting motor threshold (RMT)

Three-way ANOVA (*Time, Condition, Hemisphere*) showed that there were no significant changes of RMT depending on different experimental conditions (consistently, $P > .05$). In particular, the threshold means changed from 54.8 to 55.5 in the CH after EMF exposure of the same hemisphere and from 57.2 to 58.3 in the IH after EMF_IH (in both cases, $P > 0.70$).

MEP amplitude (single-pulse transcranial magnetic stimulation)

Considering the effect of MP exposure on the test-MEP amplitudes in different experimental conditions, we found a significant triple interaction $Hemisphere * Condition * Time$ ($P < .001$). The post-hoc analysis showed only in EMF_CH a significant reduction of MEP amplitude after EMF exposure in the same hemisphere (CH: from 437 μV to 224 μV , $P = .001$).

MEP ratio (paired-pulse transcranial magnetic stimulation)

To test the excitability changes across time (before and after EMF exposure), we considered all double/single-pulse ratios in the two hemispheres across different ISIs. The GEE model with patient as “Subject variable” and *Time, Condition, Hemisphere* and *ISI* as predictors revealed $Time * Condition * Hemisphere$ interaction ($P < .001$).

Taking into account the post-hoc analysis, we observed: i) no significant excitability changes related to SHAM and EMF_IH conditions (consistently, $P > 0.05$; Fig. 2); ii) a significant excitability modulation related to EMF_CH condition consisting of an increase of global excitability in the CH (exposed hemisphere, Fig. 2). More precisely, the ratio increased from 0.93 to 1.06 ($P < .001$). Since this increase could be due to the single-pulse MEP amplitude changes after the same exposure, we added this measure as covariate in the GEE model and found that the ratios slightly changed but confirmed the same pattern (from 0.90 to 1.02, $P < .001$). In other terms, the increased excitability was not influenced by the observed decrease of single-pulse MEP amplitude.

We also found that the above triple interaction evaluated the effects of EMF_CH condition on the intracortical inhibition/facilitation curve introducing the factor *ISI*. We found a significant $Hemisphere * Time * ISI$ interaction that revealed a different effect of EMF exposure across ISIs in the two hemispheres (Fig. 3). This interaction is related to an enhancement of ICF in the CH (hemisphere GSM-EMFs exposed): we found significant increase at $ISI = 9, 11, 13$ (consistently $P < .003$), still there even after taking into account the changes observed on single-pulse MEP amplitude, added as covariate in the GEE model ($ISI 9 P = 0.076$, $ISI 11 P < .001$, $ISI 13 P < .001$). In addition, a slight increase of SICI was found in the IH (opposite to the EMF exposure) at $ISI 3$ ($P = .038$), but the significance did not survive after correction for multiple comparison ($P > .20$, as shown in Fig. 3).

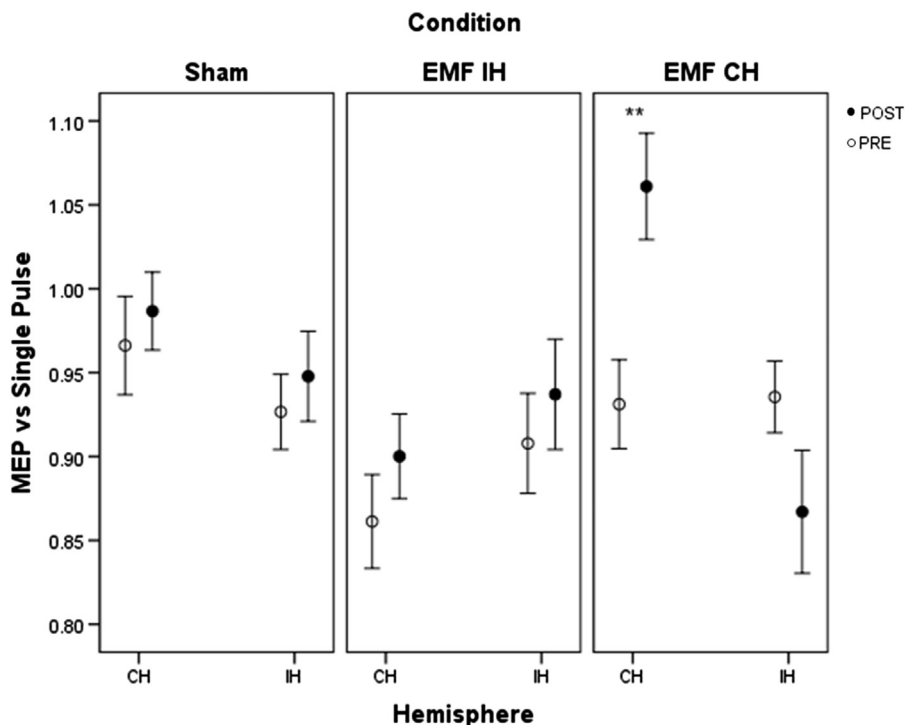


Figure 2. Excitability changes in different conditions. Time course of the double/single-pulse ratio before (empty circle, PRE) and after (filled circle, POST) EMFs exposure in the two hemispheres (ipsilateral –IH– and contralateral –CH– to the epileptic focus) in each condition (from the left: SHAM exposure, exposure of the IH –EMF_IH–, exposure of CH –EMF_CH–, after averaging across different interstimulus intervals (ISIs). Error bars correspond to 95% confidence intervals after pooling subjects together. MEP = motor-evoked potential. ** $P < .001$.

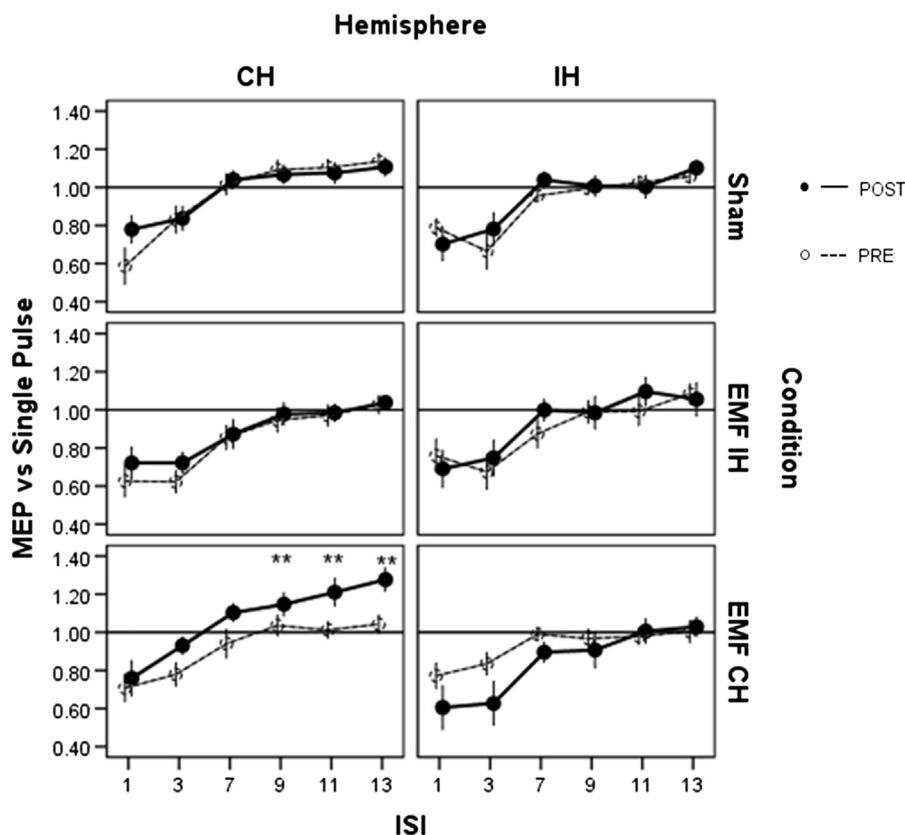


Figure 3. Inhibition–facilitation curves. Inhibition–facilitation curves for each combination of the experimental design. Rows represent *condition*; from bottom: exposure of the hemisphere contralateral to the epileptic focus (CH; EMF_CH), exposure of the hemisphere ipsilateral (IH) to the epileptic focus (EMF_IH), SHAM exposure. Columns represent *hemisphere* (CH, IH) and *time* (dashed line indicates the session before EMFs exposure – PRE; continuous and bold line indicates the session after EMFs exposure – POST). Error bars correspond to 95% confidence intervals after pooling subjects together. MEP = motor-evoked potential; ISI = interstimulus interval. *** $P < .003$.

Finally, when the “epileptic focus side” was added as covariate in the above model, its main effect was not significant ($P = 0.077$) and did not change the significance of the other model effects, in particular the *Time * Condition * Hemisphere* interaction (the P -value remained < 0.001).

Discussion

The present study clearly demonstrated that an acute and relatively prolonged exposure (45 min) to GSM-EMFs modulates cortical excitability in patients affected by focal epilepsy; however, in contrast to the healthy subjects, these effects were evident only after EMFs exposure over the hemisphere contralateral to the epileptic focus (CH). They were characterized by a significant cortical excitability increase in the exposed hemisphere paired with slight excitability decrease in the other one (IH).

Baseline brain excitability asymmetries in epileptic patients

Epilepsies are a complex group of conditions characterized by a cortical excitability dysfunction with a variety of background mechanisms. The epileptogenic properties of neuronal tissues can be summarized in two main characteristics: hyperexcitability and hypersynchrony of firing discharge [19]. Focal epilepsies arise from a localized brain area – the epileptogenic zone or focus – where neurons share these features. Several studies have demonstrated that the excitation/inhibition imbalance not only affects the seizure focus, but may also involve more distant areas, including

motor cortex [20]. In this regard, TMS has been already used in epileptic patients to evaluate excitatory and inhibitory processes underlying different epileptic disorders, but with results somewhat conflicting [21,22].

Our TMS study in a sample of patients affected by cryptogenic focal epilepsy did not show significant interhemispheric asymmetries of RMT. On the other hand, taking into account the intracortical excitability curve before EMFs exposure, a slight reduction of SICI at 1 ms in the ipsilateral hemisphere was observed. Intracortical excitability curves at short ISIs (1–5 ms) seem to reflect the activity of GABAergic interneurons, probably GABA_A receptor mediated, located within the motor cortex [23]. Nevertheless, the significance of SICI at very short ISIs is still debated. Even if previous studies [24] suggested that SICI at ISI of 1 ms could be attributed to the axonal refractoriness of neural elements in the cortex activated by the conditioning pulse, synaptic inhibition may also be involved [25], possibly revealing a defective GABA inhibition in focal epilepsy [26,27].

Brain excitability changes after GSM-EMFs exposure

Real GSM-EMFs exposure induced different behaviours in the two hemispheres. Surprisingly, in IH exposure there were no significant effects like in sham condition. On the other hand, the real CH exposure caused a significant increase of brain excitability in the same hemisphere exposed with a similar pattern previously reported in healthy subjects [3]. Focusing on the effect of GSM-EMFs across ISIs, we observed that the excitability enhancement in the CH was explained by an increase of ICF, mediated by

glutamatergic N-methyl-D-aspartate (NMDA) receptors [28]. As already suggested [3], these effects could be due to changes of the cellular oxidative status, the evidence being that both acute [29] and chronic GSM 900 EMF exposure [30] could modify it. In this regard, oxidative stress has been demonstrated to interact with NMDA receptors and ion transportation proteins [31,32], it impairs redox-sensitive glutamate uptake in membrane transport systems of neurons and astrocytes [33] and selectively increases NMDA transmission, activating normally silent NMDA receptors and Ca^{++} influx [34].

Interestingly, in the same EMF_CH condition we found a slight increase of SICI at 3 ms in the non-exposed hemisphere (IH), possibly expressing a decrease in cortical excitability. We hypothesize that this finding could be due to interhemispheric transcallosal modulation of inhibitory mechanisms. This inter-hemispheric inhibition circuits probably relies on transcallosal excitatory connections activating inhibitory interneurons in the contralateral motor cortex [35–37]. Thus, the EMFs-induced hyperexcitability in the CH could inhibit the opposite hemisphere (IH) through activation of transcallosal fibres.

Finally, there are no obvious explanations for the lack of significant effects after IH exposure. Significant asymmetries and differences in the anatomy and excitability properties of motor cortex have been extensively demonstrated also in healthy subjects. In particular, through TMS it has been shown that muscles in the dominant hand have larger representations than those in the non-dominant hand [15], and the DH has lower motor threshold [16] and less SICI than NDH [17]. In our patients' sample, contrary to what described in healthy subjects, in the pre-exposure condition we detected a slight decrease of SICI in the IH (i.e. NDH in most of our patients). Moreover, after EMF exposure we observed no significant effect in IH, and notable changes of ICF in the CH (i.e. DH in our patients). Nevertheless, as in the majority of our patients the epileptic focus is localized in the NDH, we cannot definitely rule out that the different behaviour observed in the IH and CH after EMF exposure could be affected by the hemispheric dominance rather than the epileptogenic zone. Thus, even if our analysis specifically addressing this question does not support this hypothesis, and our findings do not perfectly fit with previous data in healthy subjects, this issue should be considered a limitation of our study. Alternatively, an attractive hypothesis could be that the excitation/inhibition imbalance involving the IH might disable the excitability changes induced by EMF exposure. This finding could be interpreted in the context of the emerging concepts of 'homeostatic plasticity'. This "homeostatic plasticity" enables selective modification of synaptic activity while maintaining total synaptic strength within a physiological range: high levels of activity favour synaptic depression, whereas low levels of activity favour facilitation [38,39]. These mechanisms have been evidenced also in human motor cortex [40], suggesting the existence of an M1 homeostatic mechanism that stabilizes corticospinal excitability. On this basis, we might speculate that cortical excitability derangement of the IH may disable the excitability enhancing effect of GSM-EMFs on the same exposed hemisphere. Finally, we cannot exclude that the effects of anti-epileptic drugs could be more evident in the epileptogenic zone and in the IH, thus making the modulation of the GSM-EMFs ineffective.

Conclusions

Our data demonstrate that an acute exposure to GSM-EMFs can significantly modulate cortical excitability in patients affected by focal epilepsy. As already observed in healthy subjects, the real exposure over the CH induced a significant increase of brain

excitability in the exposed hemisphere, probably mediated by NMDA receptors. On the contrary, real EMFs exposure of the IH did not produce significant effects. Thus, present findings suggest a significant interaction between the brain excitability changes induced by GSM-EMFs and the epileptic focus, which disables the excitability enhancing effects of the EMFs.

Future studies will be required to confirm the present results on a larger group of patients, and to understand the clinical impact of GSM-EMFs.

Acknowledgments

The authors are grateful to Dr. Giuseppe Curcio who gave us the access to the modified helmet for the delivery of GSM-EMFs.

References

- [1] Repacholi MH. Health risks from the use of mobile phones. *Toxicol Lett* 2001; 120:323–31.
- [2] Marino AA, Carrubba S. The effects of mobile-phone electromagnetic fields on brain electrical activity: a critical analysis of the literature. *Electromagn Biol Med* 2009;28:250–74.
- [3] Ferreri F, Curcio G, Pasqualetti P, De Gennaro L, Fini R, Rossini PM. Mobile phone emissions and human brain excitability. *Ann Neurol* 2006;60:188–96.
- [4] Curcio G, Ferrara M, Moroni F, D'Inzeo G, Bertini M, De Gennaro L. Is the brain really influenced by a phone call? An EEG study of resting wakefulness. *Neurosci Res* 2005;53:265–70.
- [5] Valentini E, Curcio G, Moroni F, Ferrara M, De Gennaro L, Bertini M. Neurophysiological effects of mobile phone electromagnetic fields on humans: a comprehensive review. *Bioelectromagnetics* 2007;28:415–32.
- [6] Huber R, Treyer V, Borbély AA, Schuderer J, Gottselig JM, Landolt H-P, et al. Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking EEG. *J Sleep Res* 2002;11:289–95.
- [7] Huber R, Treyer V, Schuderer J, Berthold T, Buck A, Kuster N, et al. Exposure to pulsed modulated radio frequency electromagnetic fields affects regional cerebral blood flow. *Eur J Neurosci* 2005;21:1000–6.
- [8] Volkow ND, Tomasi D, Wang GJ, Vaska P, Fowler JS, Telang F, et al. Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. *JAMA* 2011;305:808–13.
- [9] Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* 2009;120:2008–39.
- [10] Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. *Electroencephalogr Clin Neurophysiol* 1994;91:79–92.
- [11] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971;9:97–113.
- [12] Smith MJ, Adams LF, Schmidt PJ, Rubinow DR, Wassermann EM. Effects of ovarian hormones on human cortical excitability. *Ann Neurol* 2002;51(5): 599–603.
- [13] Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol* 1993;471:501–19.
- [14] Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. *J Physiol* 2001;530:307–17.
- [15] Wassermann EM, McShane LM, Hallett M, Cohen LG. Noninvasive mapping of muscle representations in human motor cortex. *Electroenceph Clin Neurophysiol* 1992;85:1–8.
- [16] Triggs WJ, Calvanio R, Macdonell RA, Cros D, Chiappa KH. Physiological motor asymmetry in human handedness: evidence from transcranial magnetic stimulation. *Brain Res* 1994;636:270–6.
- [17] Ridding MC, Flavel SC. Induction of plasticity in the dominant and non-dominant motor cortices of humans. *Exp Brain Res* 2006;171:551–7.
- [18] Friston KJ, Holmes AP, Worsley KJ. How many subjects constitute a study? *NeuroImage* 1999;10:1–5.
- [19] McCormick DA, Contreras D. On the cellular and network basis of epileptic seizures. *Annu Rev Physiol* 2001;63:815–46.
- [20] Spencer SS. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia* 2002;43:219–27.
- [21] Cicinelli P, Mattia D, Spanedda F, Traversa R, Marciani MG, Pasqualetti P, et al. Transcranial magnetic stimulation reveals an interhemispheric asymmetry of cortical inhibition in focal epilepsy. *Neuroreport* 2000;11:701–7.
- [22] Tassinari CA, Cincotta M, Zaccara G, Michelucci R. Transcranial magnetic stimulation and epilepsy. *Clin Neurophysiol* 2003;114:777–98.
- [23] Boroojerdi B. Pharmacologic influences on TMS effects. *J Clin Neurophysiol* 2002;19:255–71.
- [24] Fisher RJ, Nakamura Y, Bestmann S, Rothwell JC, Bostock H. Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. *Exp Brain Res* 2002;143:240–8.

- [25] Roshan L, Paradiso GO, Chen R. Two phases of short-interval intracortical inhibition. *Exp Brain Res* 2003;151:330–7.
- [26] Varrasi C, Civardi C, Boccagni C, Cecchin M, Vicentini R, Monaco F, et al. Cortical excitability in drug naive patients with partial epilepsy: a cross sectional study. *Neurology* 2004;63:2051–5.
- [27] Badawy RA, Curatolo JM, Newton M, Berkovic SF, Macdonell RA. Changes in cortical excitability differentiate generalized and focal epilepsy. *Ann Neurol* 2007;61:324–31.
- [28] Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *J Neurosci* 1998;18:7000–7.
- [29] Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH, Ali FM. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. *J Pharm Biomed Anal* 2001;26:605–8.
- [30] Irmak MK, Fadillioglu E, Güleç M, Erdoğan H, Yağmurca M, Akyol O. Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. *Cell Biochem Funct* 2002;20:279–83.
- [31] Avshalumov MV, Rice ME. NMDA receptor activation mediates hydrogen peroxide-induced pathophysiology in rat hippocampal slices. *J Neurophysiol* 2002;87:2896–903.
- [32] Himi T, Ikeda M, Yasuhara T, Murota SI. Oxidative neuronal death caused by glutamate uptake inhibition in cultured hippocampal neurons. *J Neurosci Res* 2003;71:679–88.
- [33] Volterra A, Trotti D, Floridi S, Racagni G. Reactive oxygen species inhibit high-affinity glutamate uptake: molecular mechanism and neuropathological implications. *Ann N Y Acad Sci* 1994;738:153–62.
- [34] Klann E, Thiels E. Modulation of protein kinases and protein phosphatases by reactive oxygen species: implications for hippocampal synaptic plasticity. *Prog Neuropsychopharmacol Biol Psychiatry* 1999;23:359–76.
- [35] Ferbert A, Priori A, Rothwell JC, Erdoğan H, Yağmurca M, Akyol O. Interhemispheric inhibition of the human motor cortex. *J Physiol* 1992;453:525–46.
- [36] Meyer BU, Roricht S, Graf von Einsiedel H, Kruggel F, Weindl A. Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum. *Brain* 1995;118:429–40.
- [37] Lee H, Gunraj C, Chen R. The effects of inhibitory and facilitatory intracortical circuits on interhemispheric inhibition in the human motor cortex. *J Physiol* 2007;580:1021–32.
- [38] Huang YY, Colino A, Selig DK, Malenka RC. The influence of prior synaptic activity on the induction of long-term potentiation. *Science* 1992;255:730–3.
- [39] Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci* 2004;5:97–107.
- [40] Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency Repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *J Neurosci* 2004;24:3379–85.