

Radiofrequency signal affects alpha band in resting electroencephalogram

Rania Ghosn, Lydia Yahia-Cherif, Laurent Hugueville, Antoine Ducorps, Jean-Didier Lemarechal, György Thuroczy, René de Seze, Brahim Selmaoui

► **To cite this version:**

Rania Ghosn, Lydia Yahia-Cherif, Laurent Hugueville, Antoine Ducorps, Jean-Didier Lemarechal, et al.. Radiofrequency signal affects alpha band in resting electroencephalogram. *Journal of Neurophysiology*, American Physiological Society, 2015, 113 (7), pp.2753-2759. 10.1152/jn.00765.2014 . ineris-01852915

HAL Id: ineris-01852915

<https://hal-ineris.archives-ouvertes.fr/ineris-01852915>

Submitted on 27 Sep 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Radiofrequency signal affects alpha band in resting electroencephalogram

Rania Ghosn^{1,2}, Lydia Yahia-Cherif^{3,4,5,6}, Laurent Hugueville^{3,4,5,6}, Antoine Ducorps^{3,4,5,6},
Jean-Didier Lemaréchal^{3,4,5,6}, György Thuróczy⁷, René de Seze^{1,2}, Brahim Selmaoui^{1,2}.

¹ Institut National de l'Environnement Industriel et des Risques (INERIS), Department of Experimental
Toxicology, Verneuil-en-Halatte, France

² Université de Picardie Jules Verne (UPJV), Peritox Laboratoire de Périnatalité & Risques Toxiques UMR-I-01
Unité mixte INERIS, Amiens France

³ UPMC, Univ Paris 06, Centre MEG-EEG, Centre de Recherche de l'Institut du Cerveau et de la Moelle
Epinrière (CRICM) et Centre de Neuroimagerie de Recherche (CENIR), UMR_S 975, 75013 Paris, France.

⁴ CNRS, Centre MEG-EEG, CRICM et CENIR, UMR 7225, 75013 Paris, France.

⁵ Inserm, Centre MEG-EEG, CRICM et CENIR, U 975, 75013 Paris, France.

⁶ ENS, Centre MEG-EEG, CRICM et CENIR, UMR_S 975, 75013 Paris, France.

⁷ National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

Corresponding author:

Dr. Brahim SELMAOUI.

Department of experimental toxicology,
INERIS, Parc ALATA BP.2, 60550
Verneuil-en-Halatte, France

Email : brahim.selmaoui@ineris.fr

Tel. : +33 3 44 61 82 68

Fax : +33 3 44 55 67 67

Abstract

Objective: The aim of the present work was to investigate the effects of the radiofrequency (RF) electromagnetic fields (EMFs) on human resting EEG with a control of some parameters that are known to affect alpha band such as electrode impedance, salivary cortisol and caffeine. **Methods:** Eyes open and eyes-closed resting EEG data were recorded in 26 healthy young subjects under two conditions: sham exposure and real exposure in double-blind, counterbalanced, crossover design. Spectral power of EEG rhythms was calculated for the alpha band (8-12Hz). Saliva samples were collected before and after the study. Salivary cortisol and caffeine were assessed respectively by Enzyme linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC). The electrode impedance was recorded at the beginning of each run. **Results:** Compared with sham session, the exposure session showed a statistically significant ($p < 0.0001$) decrease of the alpha band spectral power during closed eyes condition. This effect persisted in the post-exposure session ($p < 0.0001$). No significant changes were detected in electrode impedance, salivary cortisol and caffeine in the sham session when compared to the exposure one. **Conclusions:** These results suggest that GSM-EMFs of a mobile phone affect alpha band within spectral power of resting human EEG.

Keywords: electroencephalogram, radiofrequency, mobile phone, alpha band

Running Title: GSM signal affects alpha band

1. Introduction

Emerging technologies in mobile telecommunications such as radio frequency fields (RF) and microwave radiation are widely used in our modern society. Prominent examples are the wireless internet network and mobile phone communications, which are particularly widespread. The extensive use of mobile phones (MP) increases the exposure of human beings to radiofrequency electromagnetic fields. During a phone call, given the close proximity of the MP to the user's head, a part of the electromagnetic field (EMF) can be absorbed by the head and the brain (Schönborn et al. 1998). This exposure to EMF has raised questions about possible effects of the EMF of mobile phones on brain activity.

Some earlier studies have investigated the effects of EMFs on resting cerebral activity with somewhat mixed results, but more recently there has been consistent data indicating the existence of exposure effects on the alpha bands of the resting EEG.

Indeed, data reported by some authors showed an increase in EEG power in the alpha frequency band (Reiser et al. 1995; Croft et al. 2002; Huber et al. 2002; Kramarenko and Tan 2003; Cook et al. 2004; Curcio et al. 2005; Regel et al. 2007; Croft et al. 2008; Hinrikus et al. 2008; Croft et al. 2010), whereas other studies reported a decrease in EEG power or coherence in the alpha band (Maby et al. 2006; Vecchio et al. 2007; Vecchio et al. 2010; Vecchio et al. 2012; Perentos et al. 2013). Finally, other studies failed to show an effect on EEG power in the alpha bands (Röschke and Mann, 1997; Hietanen et al. 2000; D'Costa 2003; Perentos et al. 2007).

As the literature cited demonstrates, the most consistent effect observed is a change in alpha band power. However, these changes sometimes correspond to an increase in alpha power and sometimes to a decrease. The reason why alpha band power reacts differently to RF exposure remains unclear. The main problem lies in the use of different methods, different experimental

protocols and/or different intensities or frequencies (van Rongen et al. 2009), thus making the comparison of data more difficult. As also reported by Loughran et al. (2012), individual variability is also one of the important factors that may explain the discrepancies between the results.

Moreover, several other parameters, could impact the EEG results as confounding factors. Among these parameters are electrode impedance changes. The battery and electronics of the phone causes it to heat up, which in turn causes heating of the skin and underlying tissue (Straume et al. 2005; Anderson and Rowley 2007; Ghosn et al. 2012). As exposure to heat causes the dilation of blood vessels, this phenomenon may result in a change in the skin impedance (Luck 2005), which in turn could explain some observed changes in the recorded EEG power.

In addition, changes in the alpha band power are related to changes in parameters such as cortisol or caffeine which, to our knowledge, have never been concretely measured in relation to EMF exposure. Changes in cortisol and ECG could result from stress linked to the experimental environment and protocol, and therefore these parameters need to be controlled.

The aim of the present study was to examine the potential impact of GSM (global system for mobile) RF (radiofrequency) exposure to the alpha band of the resting EEG under controlled parameters and to thus bring additional information to fill certain gaps in our current knowledge of the effects of GSM RF exposure. This study was conducted on awake volunteers in two different conditions: open and closed eyes. In addition, some parameters that are known to affect alpha band such as electrode impedance, cortisol levels and caffeine concentrations were also investigated to ensure that if any effect was observed, that it was not attributable to one of the aforementioned parameters. Hence, electrode impedance was

checked after each block of EEG recordings, and caffeine and cortisol were concurrently evaluated in the saliva.

2. Methods

2.1 Participants

Twenty-six healthy volunteers participated in the experiment (13 females and 13 males, mean age = 23.5 ± 3.1 years). All women reported having regular menstrual cycles (25–32 days) during the year preceding the study, no vasomotor complaints (i.e. hot flashes, night sweats). These women were studied in the laboratory during the follicular phase of their menstrual cycle to avoid any interference with EEG rhythms and hemispheric activity. All participants provided informed written consent and were compensated for their participation. All procedures were approved by the local ethics committee (ID N° = RCB: 2011, A01455-36). The volunteers were selected following a routine clinical examination. The mean body mass index of the subjects was 22.3 ± 1.8 . Systolic and diastolic blood pressures were 113.3 ± 9.2 and 74 ± 7.7 mmHg (mean \pm SD) respectively. Inclusion criteria included regular sleep habits, no medication, no chronic disease or disability, no recent acute illness, no smoking, and no neurological or psychiatric illness. All participants were right-handed and had normal or corrected-to-normal vision. Those selected were instructed to abstain from consuming alcohol and coffee for 24 h before and during each experimental session. They were instructed to abstain from using a mobile phone on the day of the experiment. Participants declared that they did not use the mobile phone at all on the day of the experiment. Moreover, we are quite sure that they did not use their phones 2 to 3 hours before the start of the experiment since they were admitted into the facility of the hospital to fill some documents related to the experiment 2 to 3 hours prior to the exposure.

2.2 Experimental design

Participants attended two EEG recording sessions in a crossover, randomized, double-blind and counterbalanced design experiment. During each session, the subject was exposed to 26 min 15 s of sham or real GSM RF exposure (Fig. 1). In the case of sham exposure, the mobile phone was switched “on” but without RF radiation, while for real exposure, the mobile phone was switched “on” with RF radiation. For the same subject, the two sessions were at a one-week interval. Both the subjects and experimenters were unaware of the exposure condition. The experiment was conducted in a dimly lit, electrically shielded room. Subjects were seated in a comfortable chair, and a screen was placed one meter in front of the volunteer to keep their eyes in a well-defined direction. In addition to the EEG recordings, electrocardiograms (ECG) and galvanic skin responses (GSR) (also called electrodermal response (EDR)) were simultaneously recorded (EDR data will not be reported in the present paper). During the recordings, volunteers were asked to fix their eyes on a center point on the screen represented by a white square in the center of a black background. Each recording session was composed of 7 experimental blocks distributed across the 3 experimental conditions: pre-exposure, exposure and post-exposure. Each block consisted of three recordings: EDR assay (2 min 45 s), resting EEG with open eyes (3 min) and resting EEG with closed eyes (3 min) (Fig 1). Vocal instructions were previously recorded by the experimenter. Loudspeakers placed on either side of the screen in front of the volunteer connected to a computer in the acquisition room allowed instructions to be sent to the volunteers. Auditory instructions to inform the volunteers when recording starts, when to open or to close their eyes, and the fixation point were given with Omnistim (stimulus presentation software developed at the MEG-EEG Center). TTL pulses were used to synchronize stimulus presentation and the EEG / BIOPAC systems. Instructions were at the beginning of the recording block, the open eyes and the closed eyes periods, and at the end of the block.

Timeline of the two experimental sessions is presented in Fig. 1.

The pre-exposure period consisted of two blocks of recordings (run 1 and run 2) with no mobile phone (baseline). Three blocks (run 3, run 4 and run 5) were recorded during the exposure period in which the actual mobile phone (genuine) was positioned and activated for the exposure session, and the sham phone was used in the sham session. The mobile phone was then removed, and two blocks were recorded in the post-exposure period (runs 6 and 7).

2.3 Exposure System and dosimetry

Subjects were exposed to RF EMF by a commercial dual band GSM mobile phone (Nokia 6650). The mobile phone was positioned against the left ear. To set the standard exposure parameters, the phone was connected to a personal computer to control the required frequency and RF power by service software (Phoenix, Nokia Corp., Finland). The sham or genuine exposure was carried out using a “load” or a “dummy load”, respectively. For this purpose an external power load was connected to the external antenna connector of the phone. A 50-ohm resistive load and an open-circuit dummy load were developed for sham/exposed conditions with the same shape and structure to allow for the double-blind protocol of the study. This implied that, when the telephone was on, the internal circuitry was regularly active, but no radiofrequency power was delivered in space by the antenna. The participants received GSM modulated exposure with the full power of the mobile phone (2 W peak, 250 mW average, pulse modulated with 1/8 duty cycle) at 900 MHz for 26 min. The maximum specific absorption rates (SARs) were averaged on 10 g tissue, 1 g tissue, and the peak value was measured at 0.49 W/kg, 0.70 W/kg and 0.93 W/kg, respectively. The SAR of the “sham” phone was below the detection level of the system (0.001 W/kg) at any position of the phantom, and no electric field was detected on the surface of the “sham” phone (for more details see: Ghosn et al. 2012).

2.4 EEG recording and data acquisition

Electroencephalography data were recorded using BrainCap (EASYCAP Products GmbH, Herrsching, Germany) with 29 passive electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, PO3, PO4, O1 and O2) placed according to the international extended 10/10 system. The reference electrode was the AFz and the ground electrodes were placed on the right shoulder of each participant. Repeated EEG blocks were recorded with respect to the AFz reference at a sampling rate of 1000 Hz. The signal was amplified and band-pass filtered online between 0.016 Hz and 250 Hz. We used three bipolar derivations to monitor eye movements: one electrode was placed below the right eye for vertical eye movements, and two electrodes were placed at the outer canthi of the eyes for horizontal movements. Data acquisition was performed using BRAINAMP MR plus Amplifiers (Brain Products GmbH, Munich).

2.5 EEG Interference with RF-EMF

To test possible interference between radio frequencies emitted by the mobile phone and EEG signals recorded during exposure, a polystyrene phantom head was constructed to simulate a complete EEG chain. Time-frequency analysis was performed on the three recordings blocks (without a phone, with the 'sham' phone, and the 'real' phone) to detect any interference signals. Results showed no disturbance in the recording in the absence or presence of the two phones for the frequencies between 1 and 20 Hz. The two 'sham' and 'real' phones used in our experiment seem not to have disturbed the EEG recordings assessed during exposure.

2.6 Measurement of electrode impedance

Electrode impedance was checked to be below 5 k Ω and recorded along the experiment at the beginning of each run.

2.7 Heart rate data acquisition

Heart rate was recorded by BIOPAC MP150 (GSR100C and ECG100C modules) at a sampling rate of 1000 Hz by using two electrodes. One was placed at the base of the neck (above the right clavicle) and the other on the left forearm.

2.8 Enzyme Linked Immuno Sorbent Assay (ELISA) for salivary cortisol

Saliva was collected using a Salivette device (Sarstedt, Inc.) then centrifuged and immediately frozen. Each volunteer provided two saliva samples, the first before starting the experiment (T₀) and the second after the recordings were complete (T_f). The cortisol was quantified in two samples collected at T₀ and T_f using commercialized sandwich ELISA kits (Human cortisol) according to the manufacturer's instructions.

Samples were centrifuged (1000 g/20 min/4°C) and the supernatant was collected. Raw data were presented for sham and exposed groups.

2.9 Salivary caffeine concentration using high performance liquid chromatography (HPLC)

Salivary caffeine concentration was assessed in T₀ samples. A rapid high performance liquid chromatography (HPLC) method was used for the salivary caffeine analysis. The HPLC system consisted of a Spectra SYSTEM Pump and a Spectra SYSTEM UV detector (Ultimate 3000 Photodiode Array detector, USA). An Envirodur C18 (3 µm) column (250 x4.6 mm, Macherey Nagel) was used for the separation. The mobile phase was made of 85% of a 0.012 M KH₂PO₄ and 15% acetonitrile. The flow rate was set at 1 ml/min, and the injection volume was set at 20 µl. The detection wave length was set at 280 nm. The caffeine solution concentrations used for the standard curves were 1, 1.5, 2, 5, 8, 15, 25, 50 and 100 µg/ml.

Standard curves were constructed by plotting concentration versus area under the curve. Caffeine retention time was 5.2 min.

2.10 EEG data analysis

Resting EEG data were analyzed for the periods “open eyes” and “closed eyes,” which lasted three minutes each for each run. In total, 7 runs were performed: the first two runs (runs 1 and 2) consisted of the pre-exposure period, the three following runs (runs 3, 4 and 5) constituted the exposure period, and the last two runs (runs 6 and 7) represented the post-exposure period. Markers were placed in the data at 4-s intervals and then we performed the time-frequency wavelet transform on individual EEG epochs comprising data from -2.5 to 2.5 ms around each marker. We used a family of complex Morlet wavelets, with an m parameter of 10 and a Blackman window of 100 ms, resulting in an estimate of signal power at each time sample and at each frequency between 1 and 20 Hz, with a frequency step of 1 Hz. The time-frequency transformed data were then averaged across epochs for each experimental eye condition, each run and for each subject, separately for the baseline trials and the exposure and post-exposure trials to obtain spectral power, which were then subsequently averaged in the Alpha (8-12 Hz) bands. The alpha band was divided into two sub-bands: the upper (10-12 Hz) and the lower (8-10 Hz) and were then analyzed. The log-transformation of the data was used to approach a normal distribution. Finally the data were averaged over the 3 conditions of interest: pre-exposure (baseline), during exposure, and post-exposure period, for each subject and for the grand mean of the 26 volunteers.

2.11 Statistical Analysis

A four-way repeated measures ANOVA was run to determine the effect of exposure (sham/exposed), frequency bands (delta-theta-alpha), period (before/during/after) and Eyes conditions (Closed/Open) across subjects. Then, we restricted the analysis to alpha band (8-

12Hz) in closed eyes condition as follows: for each period (before, during and after), we performed a paired t-test for each electrode across subjects in the two conditions (real exposure/sham exposure). Then we averaged frequency power values for each portion of the alpha band (8-12Hz, 8-10Hz and 10Hz-12Hz) on each electrode across subjects and performed a paired t-test in the two conditions (sham/real exposure). The family wise error rate (FWER) was controlled via permutations tests as showed in (Groppe et al. 2011) which is at most as conservative as Bonferroni.

Heart rate, impedance and cortisol data analyses were performed using two-way ANOVA repeated measures. Statistical significance was set for $p < 0.05$.

3. Results

3.1 EEG Interference with RF-EMF

No disturbance was seen in any recording in the absence or presence of the phones (actual or genuine) for the analyzed frequencies between 1 and 20 Hz. The two 'sham' and 'real' phones used in our experiment did not disturb EEG recordings assessed during exposure (data not shown).

3.2 Alpha spectral power

There were significant differences between frequency bands and eyes conditions over all the electrodes. Period levels (before, during, after) were statistically significantly different on all electrodes except in the frontal region (Fp1, Fp2, F7, F3, Fz, F4, FC1, FC2).

In closed eyes condition, a significant difference between sham and real exposure was found in alpha band power (8-12Hz) for all electrodes during the exposure (except FP2, FC5 and

P8) and post-exposure period (except Cz, CP2, P7). Indeed, a paired permutation t-test analysis detected a significant and important decrease in alpha band power (8-12 Hz) ($p < 0.0001$) during the exposure and post-exposure period ($p < 0.001$) (Table 1).

Furthermore, the alpha band (8-12 Hz) was divided into two sub-bands—the upper (10-12 Hz) and lower (8-10 Hz) alpha bands— which were analyzed separately. Results showed that in the 8-10 Hz frequency band, alpha spectral power significantly decreased during the exposure and post-exposure period ($p < 0.001$ and $p < 0.0001$ respectively). Likewise, data within the upper alpha band (10-12 Hz) showed a decrease in the spectral power during and also after exposure ($p = 0.0001$ and $p < 0.0001$ respectively) (Table 1)

3.3 Electrode Impedance

Figure 2 represents electrode impedance recorded at the beginning of each run. No significant differences have been detected when comparing sham and real exposure between runs. Repeated two-way measures ANOVA and Bonferroni post tests were applied. p and F values are given in Table 2. Impedance was not affected by the factor session (sham/real exposure) recorded one week apart in all runs. Moreover, no significant differences were found in all electrode impedances when comparing the seven runs separately in the sham sessions and in the exposure sessions.

3.4 Heart rate

There were no significant variations in heart rate, (Fig. 3) whether it be between the two sessions (sham and real exposure), eye condition (open eyes / closed eyes) within and between sessions (two-way ANOVA: exposure ($F = 0.1$, $p = 0.75$) and eye condition ($F = 0.58$, $p = 0.71$)).

3.5 Salivary cortisol

Figure 4 shows the salivary cortisol concentration in sham and exposed sessions separately for participants recorded in the morning or in the afternoon. ANOVA analyses showed no significant differences in cortisol concentrations when comparing sham to exposed sessions, no differences between volunteers, and no significant interaction between exposure x subjects in the morning respectively in T0 and Tf ($F = 2.72, p = 0.12$; $F = 0.08, p = 2.27$; interaction $F = 0.42, p = 0.87$). In the afternoon no significant difference was observed between T0 and Tf when comparing sham to exposed sessions ($F = 0.67, p = 0.78$), but a significant difference was noted between subjects ($F = 2.08, p = 0.04$) and no exposure x subjects interaction ($F = 0.55, p = 0.89$).

3.6 Salivary caffeine

Results showed that caffeine concentrations in all samples were negligible and below the detection limit of 2 µg/ml.

4. Discussion

The present study evaluated the effect of GSM (global system for mobile) signals of a mobile phone on the electrical activity of the human brain especially in the alpha band of the resting EEG in young adults. In this study, healthy adults underwent two sessions of EEG recordings one week apart as a wash out period. Results showed that alpha spectral power decreased during exposure period to GSM signals. These results concur with previous findings on the effects of GSM signals on alpha power of resting EEG in humans (Croft et al. 2002; Kramarenko and Tan 2003; Curcio et al. 2005). When analyzing lower (8-10 Hz) and upper (10-12 Hz) alpha bands separately, results showed a similar significant decrease. This effect persisted in the post-exposure period (Table 1), suggesting that the effect is sustained with

lasting physiological changes and not solely during immediate interaction between exposure and the target tissue. This is in line with the results obtained in other studies that have exposed participants prior to the EEG recording (Reiser et al. 1995; Huber et al. 2002; Curcio et al. 2005), and where an effect of RF-EMF has been observed on brain activity. The persisted effect of RF-EMF on brain activity was also observed on the EEG during sleep in where some authors have reported a modification following the active period of exposure (Loughran et al. 2005; Regel et al. 2007; Loughran et al. 2012).

As we know, interpreting alpha wave activity from the amplitude/power measurement is dependent on several factors, mainly the experimental conditions under which the amplitude is measured such as open or closed eyes (Bazanov and Vernon 2013). Indeed, it was reported that an increase in the amplitude seen with closed eyes indicates less activation, whereas when eyes are open, there is a decrease in amplitude, indicating an increase in activation (Barry et al. 2007). It was assumed that neuronal activity generating the alpha rhythm is associated with areas of cortex that are not processing information at rest. This is the usual explanation of why the rhythm may disappear when the eyes are open while processing the visual information. Similarly, when a subject concentrates on a particular modality, the EEG activity in the alpha band specifically decreases in the corresponding brain region. Also, reduction in the power of alpha rhythms has been related to the speed of information processing, the subject's global attention, and cognitive performance (Neubauer and Freudenthaler 1995; Klimesch 1997; Klimesch et al. 1998; Klimesch 1999; Vogt et al. 1998; Krause et al. 2000a, 2000b; Klimesch et al. 2003).

The possible reasons for why an effect was found only for eyes closed but not for eyes open may reside in the fact that amplitudes of alpha waves diminish when subjects open their eyes and thereby the effect of radiofrequency could not be significantly detected. While, in the

opposite, alpha rhythm is prominent when subject is awake and relaxed with eyes closed facilitating thereby the observation of any effect.

According to these data, it seems that the effects observed in our study mimic, to some extent, the global reductions in alpha-band power observed in eyes-opened versus eyes-closed conditions. One would suggest that the power decrease in alpha band frequency resulting from the GSM signal exposure could be beneficial for memory process, global attention and cognitive performance. The potential clinical significance of this effect, in this area, could be assessed in further studies.

The mechanisms behind these exposure-induced changes still remain unclear. However, based on earlier reported data, it has been shown that intracortical excitability of the motor cortex was modified by acute exposure to GSM 900 (Ferreri et al. 2006). Intracortical inhibitory/facilitatory (ICI/ICF) curves were investigated, and results showed that ICI is reduced and ICF is enhanced after exposure to GSM signal (Ferreri et al. 2006). It has been suggested that ICI is mediated by GABA-A receptors (Hanajima et al. 1998), ICF is mediated by glutamatergic N-methyl-D-aspartate (NMDA) (Ziemann et al. 1998), and an imbalance between ICI and ICF may lead to changes in the intracortical excitability (Sanger et al. 2001). It has also been suggested that oxidative stress may play a role in this phenomenon since it reduces the release of GABA and the activity of GABA-A receptors at pre-synaptic and post-synaptic sites (Sah et al. 1999; 2002), which correlates with the observed decrease in EEG amplitude.

The data reported in the present study were obtained while controlling certain parameters considered as confounding factors. Indeed, alpha rhythm is known to be sensitive to several factors, including caffeine and cortisol. To our knowledge, previous studies on RF effect on EEG did not concretely and concurrently measure such factors that may modify alpha power. Therefore, our study was designed to include and assess salivary cortisol and caffeine.

As alpha rhythm has long been known to be sensitive to overall attentional states (i.e., intensity aspects such as arousal) (Adrian and Matthews 1934) and is also involved in the biasing of selective attention (Foxe et al. 1998; Kelly et al. 2006), we instructed subjects to refrain from any caffeinated drinks (coffee, tea, caffeinated soft drinks, etc.) 24 hours before the study. It has been reported that caffeine increases alertness and speeds reaction time, dominant factors in relation to alpha power (Fredholm et al. 1999; Smith 2002). In addition, previous studies reported a drop in absolute alpha power during rest with eyes open when caffeine was ingested at high doses (Siepmann and Kirch 2002; Deslandes et al. 2005). In our study, caffeine assessed in the saliva did not show detectable values (above the device's quantification limit = 2µg/ml), suggesting that caffeinated drinks did not bias the observed results.

Moreover, salivary cortisol was assessed because it has been shown that concentrations of cortisol within the blood or saliva can vary spontaneously with EEG power across a range of 6.5–14.0 Hz, which includes the alpha rhythm (Sannita et al. 1999). Our results showed no significant variations in salivary cortisol between sham and real exposure.

In regards to electrical impedance, no differences were detected in all runs when comparing sham to real exposure sessions.

Thus, the reported effects could not be related to differences in electrode impedance throughout the experiment, caffeine consumption before the experiment, or cortisol differences between groups.

Conclusions

Exposure to GSM-EMFs of a mobile phone can influence human dominant alpha rhythms in a resting state. Our results showed a power decrease of alpha band during and after exposure to

GSM-EMFs compared with sham exposure in an eyes-closed condition. These findings were not correlated with impedance electrodes, cortisol or caffeine, factors that can influence alpha power. However, extended post-exposure duration should be tested since the observed effect persisted until the end of the post-exposure period. Furthermore, it is also important to stress the potential clinical significance of this effect.

Acknowledgements

This study has been fully funded by INERIS and the program 190 “post Grenelle” for the “pôle applicatif en Toxicologie et Ecotoxicologie” (French Ministry of Ecology and Sustainable Development). Sponsors have not been involved in the collection, analysis and interpretation of data and in the writing of the manuscript. The authors wish to thank the staff of the MEG-EEG center CENIR at Paris Salpêtrière Hospital (where the study was conducted) for their technical support. We also wish to thank Dr. Alexandre Vallée for clinical examination and Jean-Pierre Gomez for the fabrication of the phantom head at the INERIS workshop.

Conflict of Interest Statement

None of the authors have conflicts of interest.

References

1. **Adrian ED, Matthews BHC.** The Berger Rhythm: Potential changes in the occipital lobes in man. *Brain* 57:355-385, 1934.
2. **Anderson V, Rowley J.** Measurements of skin surface temperature during mobile phone use. *Bioelectromagnetics* 28:159-162, 2007.
3. **Barry RJ, Clarke AR, Johnstone SJ, Brown CR.** EEG differences between eyes-closed and eyes-open resting conditions. *Clin Neurophysiol* 118:2765-2773, 2007.
4. **Bazanov OM, Vernon D.** Interpreting EEG alpha activity. *Neurosci Biobehav Rev* 2013 pii: S0149-7634(13)00127-9. doi: 10.1016/j.neubiorev.2013.05.007.
5. **Cook CM, Thomas AW, Prato FS.** Resting EEG is affected by exposure to a pulsed ELF magnetic field. *Bioelectromagnetics* 25:196-203, 2004.
6. **Croft RJ, Chandler JS, Burgess AP, Barry RJ, Williams JD, Clarke AR.** Acute mobile phone operation affects neural function in humans. *Clin Neurophysiol* 113:1623-1632, 2002.
7. **Croft RJ, Hamblin DL, Spong J, Wood AW, McKenzie RJ, Stough C.** The effect of mobile phone electromagnetic fields on the alpha rhythm of human electroencephalogram. *Bioelectromagnetics* 29:1-10, 2008.
8. **Croft RJ, Leung S, McKenzie RJ, Loughran SP, Iskra S, Hamblin DL, Cooper NR.** Effects of 2G and 3G Mobile Phones on Human Alpha Rhythms: Resting EEG in Adolescents, Young Adults, and the Elderly. *Bioelectromagnetics* 31:434-444, 2010.
9. **Curcio G, Ferrara M, Moroni F, D'Inzeo G, Bertini M, De Gennaro L.** Is the brain influenced by a phone call? An EEG study of resting wakefulness. *Neurosci Res* 53:265-270, 2005.
10. **D'Costa H, Trueman G, Tang L, Abdel-rahman U, Abdel-rahman W, Ong K, Cosic I.** Human brain wave activity during exposure to radiofrequency field emissions from

mobile phones. *Australas Phys Eng Sci Med* 26:162-167, 2003.

11. **Deslandes AC, Veiga H, Cagy M, Piedade R, Pompeu F, Ribeiro P.** Effects of caffeine on the electrophysiological, cognitive and motor responses of the central nervous system. *Braz J Med Biol Res* 38:1077-1086, 2005.
12. **Ferreri F, Curcio G, Pasqualetti P, De Gennaro L, Fini R, Rossini PM.** Mobile phone emissions and human brain excitability. *Ann Neurol* 60(2):188-96, 2006.
13. **Foxe JJ, Simpson GV, Ahlfors SP.** Parieto-occipital approximately 10 Hz activity reflects anticipatory state of visual attention mechanisms. *Neuroreport* 9:3929-3933, 1998.
14. **Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE.** Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 51:83-133, 1999.
15. **Ghosn R, Thuróczy G, Loos N, Brenet-Dufour V, Liabeuf S, de Seze R, Selmaoui B.** Effects of GSM 900 MHz on middle cerebral artery blood flow assessed by transcranial Doppler sonography. *Radiat Res* 178(6):543-50, 2012.
16. **Groppe, D.M., Urbach, T.P., & Kutas, M.** Mass univariate analysis of event-related brain potentials/fields I: A critical tutorial review. *Psychophysiology* 48(12): 1711-1725, 2011.
17. **Hanajima R¹, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I.** Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. *J Physiol* 509 (Pt 2):607-18, 1998.
18. **Hietanen M, Kovala T, Hämäläinen A M.** Human brain activity during exposure to radiofrequency fields emitted by cellular phones. *Scand J Work Environ Health* 26:87-92, 2000.
19. **Hinrikus H, Bachmann M, Lass J, Tomson R, Tuulik V.** Effect of 7,14 and 21 Hz modulated 450 MHz microwave radiation on human electroencephalographic rhythms.

Int J Radiat Biol 84:69-79, 2008.

20. **Huber R, Treyer V, Borbély AA, Schuderer J, Gottselig JM, Landolt HP, Werth E, Berthold T, Kuster N, Buck A, Achermann P.** Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking EEG. *J Sleep Res* 11:289-295, 2002.
21. **Kelly SP, Lalor EC, Reilly RB, Foxe JJ.** Increases in alpha oscillatory power reflect an active retinotopic mechanism for distracter suppression during sustained visuospatial attention. *J Neurophysiol* 95:3844-3851, 2006.
22. **Klimesch W, Russegger H, Doppelmayr M, Pachinger T.** A method for the calculation of induced band power: implications for the significance of brain oscillations. *Electroencephalogr Clin Neurophysiol* 108:123-130, 1998.
23. **Klimesch W, Sauseng P, Gerloff C.** Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. *Eur J Neurosci* 17:1129-1133, 2003.
24. **Klimesch W.** EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Brain Res Rev* 29:169-195, 1999.
25. **Klimesch W.** EEG-alpha rhythms and memory processes *Int. J Psychophysiol* 26:319-340, 1997.
26. **Kramarenko AV, Tan U.** Effects of high-frequency electromagnetic fields on human EEG: A brain mapping study. *Int J Neurosci* 113:1007-1019, 2003.
27. **Krause CM, Sillanmäki L, Koivisto M, Häggqvist A, Saarela C, Revonsuo A, Laine M, Hämäläinen H.** Effects of electromagnetic field emitted by cellular phones on the EEG during a memory task. *Neuroreport* 11:761-764, 2000a.
28. **Krause CM, Sillanmäki L, Koivisto M, Häggqvist A, Saarela C, Revonsuo A, Laine M, Hämäläinen H.** Effects of electromagnetic fields emitted by cellular phones on the

- electroencephalogram during a visual working memory task. *Int J Radiat Biol* 76:1659-1667, 2000b.
29. **Lorenzano C, Gilio F, Inghilleri M, Conte A, Fofi L, Manfredi M, Berardelli A.** Spread of electrical activity at cortical level after repetitive magnetic stimulation in normal subjects. *Exp Brain Res* 147:186-192, 2002.
30. **Loughran SP, McKenzie RJ, Jackson ML, Howard ME, Croft RJ.** Individual differences in the effects of mobile phone exposure on human sleep: rethinking the problem. *Bioelectromagnetics* 33(1):86-93, 2012.
31. **Loughran SP, Wood AW, Barton JM, Croft RJ, Thompson B, Stough C.** The effect of electromagnetic fields emitted by mobile phones on human sleep. *Neuroreport* 16(17):1973-6, 2005.
32. **Luck SJ.** An Introduction to the Event-Related Potential Technique. Chapter 4 – Averaging, artifact rejection and artifact correction. Cambridge, MA: MIT Press. p: 166, 2005.
33. **Maby E, Le Bouquin Jeannes R, Faucon G.** Short-term effects of GSM mobiles phones on spectral components of the human electroencephalogram. *Conf Proc IEEE Eng Med Biol Soc* 1:3751-3754, 2006.
34. **Neubauer AC, Freudenthaler HH.** Ultradian rhythms in cognitive performance: no evidence for a 15-h rhythm. *Biol Psychol* 40:281-298, 1995.
35. **Perentos N, Croft RJ, McKenzie RJ, Cvetkovic D, Cosic I.** Comparison of the effects of continuous and pulsed mobile phone like RF exposure on the human EEG. *Australas Phys Eng Sci Med* 30:274–280, 2007.
36. **Perentos N, Croft RJ, McKenzie RJ, Cosic I.** The alpha band of the resting electroencephalogram under pulsed and continuous radio frequency exposures. *IEEE Trans Biomed Eng* 60:1702-1710, 2013.

37. **Regel SJ, Gottselig JM, Schuderer J, Tinguely G, Rétey JV, Kuster N, Landolt HP, Achermann P.** Pulsed radio frequency radiation affects cognitive performance and the waking electroencephalogram. *Neuroreport* 18:803-807, 2007.
38. **Reiser H, Dimpfel W, and Schober F.** The influence of electromagnetic fields on human brain activity. *Eur J Med Res* 1:27-32, 1995.
39. **Röschke J, Mann K.** No short-term effects of digital mobile radio telephone on the awake human electroencephalogram. *Bioelectromagnetics* 18:172-176, 1997.
40. **Sah R, Galeffi F, Ahrens R, Jordan G, Schwartz-Bloom RD.** Modulation of the GABA(A)-gated chloride channel by reactive oxygen species. *J Neurochem* 80(3):383-91, 2002.
41. **Sah R, Schwartz-Bloom RD.** Optical imaging reveals elevated intracellular chloride in hippocampal pyramidal neurons after oxidative stress. *J Neurosci* 19(21):9209-17, 1999.
42. **Sanger TD, Garg RR, Chen R.** Interactions between two different inhibitory systems in the human motor cortex. *J Physiol* 530(Pt 2):307-17, 2001.
43. **Sannita WG, Loizzo A, Garbarino S, Gesino D, Massimilla S, Ogliastro C.** Adrenocorticotropin-related modulation of the human EEG and individual variability. *Neurosci Lett* 262:147-150, 1999.
44. **Schönborn F, Burkhardt M, Kuster N.** Differences in energy absorption between heads of adults and children in the near field of sources. *Health Phys* 74:160-168, 1998.
45. **Siepmann M, Kirch W.** Effects of caffeine on topographic quantitative EEG. *Neuropsychobiology* 45:161-166, 2002.
46. **Smith A.** Effects of caffeine on human behavior. *Food Chem Toxicol* 40:1243-1255, 2002.
47. **Straume A, Oftedal G, and Johnsson A.** Skin Temperature Increase Caused by a Mobile Phone: A Methodological Infrared Camera Study. *Bioelectromagnetics* 26:510-519, 2005.

48. **Trunk A, Stefanics G, Zentai N, Kovács-Bálint Z, Thuróczy G, Hernádi I.** No Effect of a Single 3G UMTS Mobile Phone Exposure on Spontaneous EEG Activity, ERP Correlates, and Automatic Deviance Detection. *Bioelectromagnetics* 34:31-42, 2013.
49. **Van Rongen E, Croft R, Juutilainen J, Lagroye I, Miyakoshi J, Saunders R, de Seze R, Tenforde T, Verschaeve L, Veyret B, Xu Z.** Effects of radiofrequency electromagnetic fields on the human nervous system. *J Toxicol Environ Health B Crit Rev* 12(8):572-597, 2009.
50. **Vecchio F, Babiloni C, Ferreri F, Curcio G, Fini R, Del Percio C, Rossini PM.** Mobile phone emission modulates interhemispheric functional coupling of EEG alpha rhythms. *Eur J Neurosci* 25:1908-1913, 2007.
51. **Vecchio F, Babiloni C, Ferreri F, Buffo P, Cibelli G, Curcio G, van Dijkman S, Melgari JM, Giambattistelli F, Rossini PM.** Mobile phone emission modulates inter-hemispheric functional coupling of EEG alpha rhythms in elderly compared to young subjects. *Clin Neurophysiol* 121:163-171, 2010.
52. **Vecchio F, Buffo P, Sergio S, Iacoviello D, Rossini PM, Babiloni C.** Mobile phone emission modulates event-related desynchronization of alpha rhythms and cognitive–motor performance in healthy humans. *Clin Neurophysiol* 123:121-128, 2012.
53. **Vogt F, Klimesch W, Doppelmayr M.** High-frequency components in the alpha band and memory performance. *J Clin Neurophysiol* 15:167-172, 1998.
54. **Ziemann U, Hallett M, Cohen LG.** Mechanisms of deafferentation-induced plasticity in human motor cortex. *J Neurosci* 18(17):7000-7007, 1998.

Figure legends

Fig. 1: The experimental protocol included three periods: pre-exposure, exposure and post-exposure. Each volunteer participated in two recording sessions (sham and active exposure) in a crossover randomized double-blind design. Electrodermal response (EDR), open eyes (OE) and closed eyes (CE) during resting EEG recordings.

Fig. 2: Changes in the electrical impedance of EEG electrodes during sham (white circles) and exposed (black squares) sessions. The impedances were maintained below 5 kOhms. No significant differences were detected comparing sham and real exposure in all runs.

Fig. 3: Heart rate during open eyes (OE) and closed eyes (CE) periods in sham and exposed sessions. Results are expressed as mean \pm SEM.

Fig. 4: Salivary cortisol concentration (ng/mL) before starting the study protocol (T0) and after the end of the protocol (Tf) in sham and exposed sessions for the volunteers who attended the experiment in the morning or in the afternoon.

Table 1: Statistical analyses of alpha band spectral power. For each period (before, during and after), we performed a paired t-test for each electrode across subjects. Then the frequency power values were averaged across subjects and a paired t-test was performed on the averaged electrodes values in the two conditions (sham/exposure). Comparisons were made between sham vs. real exposure (i.e. $t > 0$ corresponds to a decrease and $t < 0$ corresponds to a power increase in the truly exposed condition).

Table 2: Statistical findings. Electrode impedance with two factors: session (sham and exposed), electrodes (29 electrodes) and interaction between the two factors.

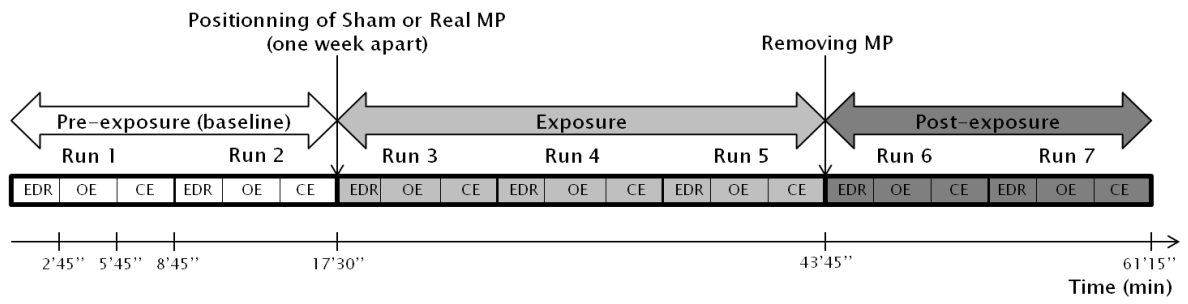


Fig.1

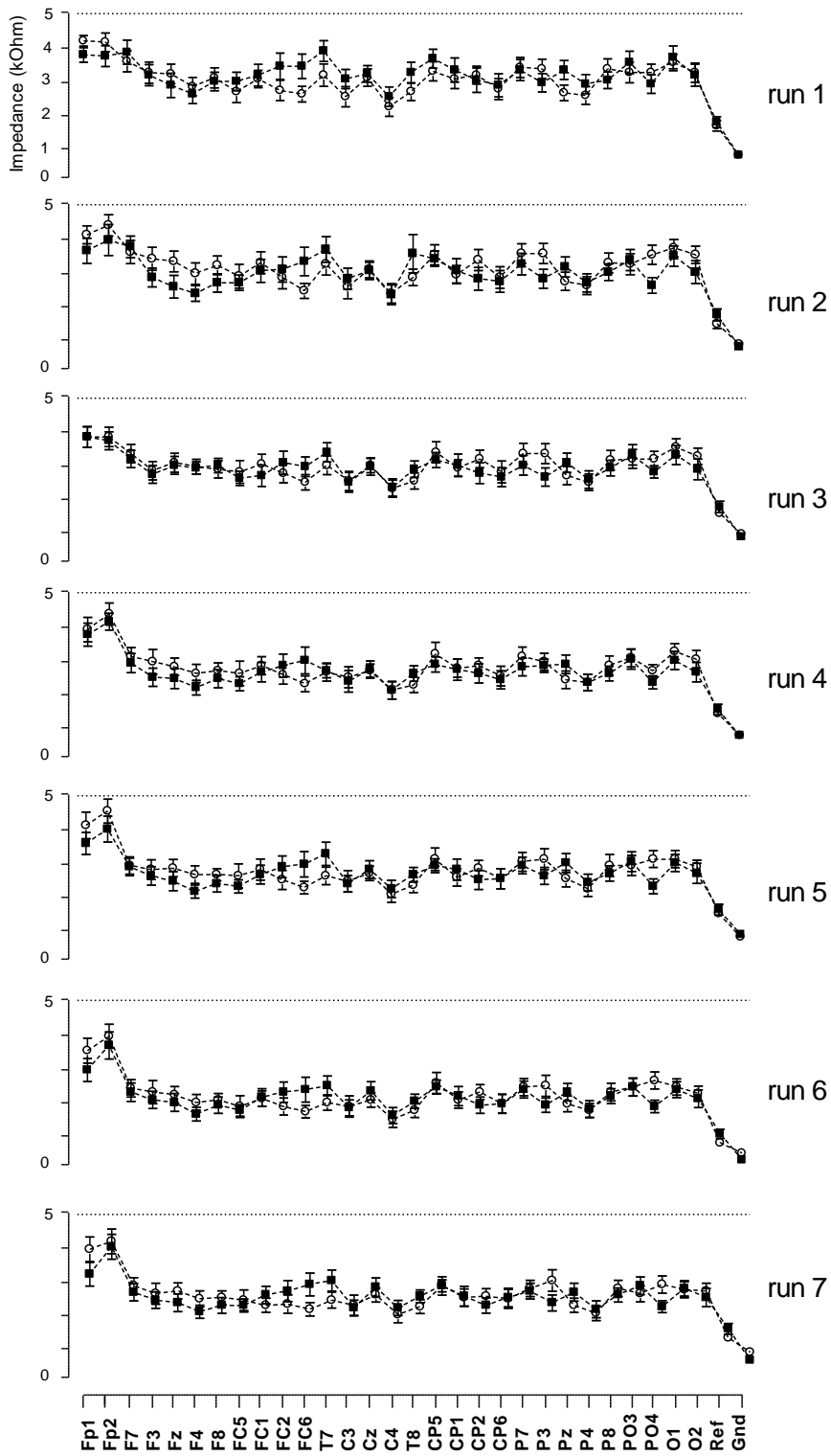


Fig.2

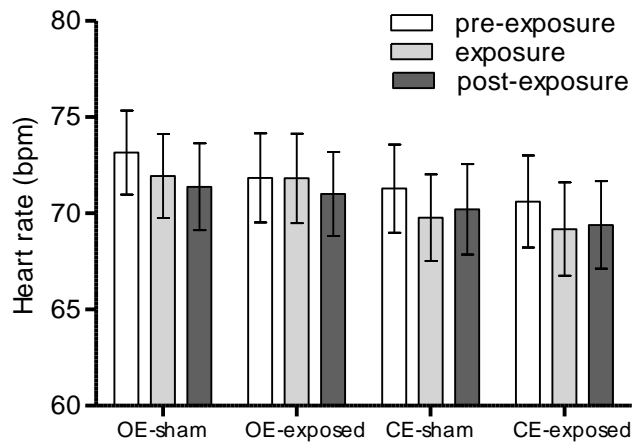


Fig.3

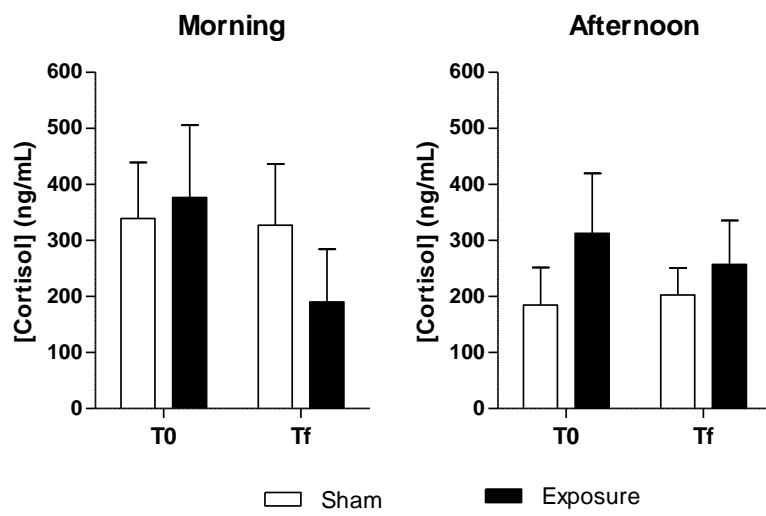


Fig.4

Exposure to GSM 900 signal	Alpha band		
	8-12	8-10	10-12
Closed Eyes			
Before	$p = 0,1116 \rightarrow$	$p = 0,9412 \rightarrow$	$p = 0,210 \rightarrow$
	t = 1,6606	t = -0,0758	t = 1,2868
During	$p < 0,0001 \downarrow\downarrow$	$p < 0,001 \downarrow\downarrow$	$P < 0,0001 \downarrow\downarrow$
	t = 4,8816	t = 5,1514	t = 4,1656
After	$p < 0,001 \downarrow\downarrow$	$p < 0,0001 \downarrow\downarrow$	$p < 0,0001 \downarrow\downarrow$
	t = 5,2655	t = 5,3638	t = 4,4889

Table 1

	Source of Variation	p-value	F
Run 1	Interaction	0,7391	0,8313
	session	0,0696	3,296
	elecrodcs	< 0.0001	18,89
Run 2	Interaction	0,5268	0,9639
	session	0,0734	3,208
	elecrodcs	< 0.0001	18,47
Run 3	Interaction	0,9862	0,5348
	session	0,2490	1,330
	elecrodcs	< 0.0001	19,66
Run 4	Interaction	0,9944	0,4832
	session	0,0912	2,857
	elecrodcs	< 0.0001	20,09
Run 5	Interaction	0,6613	0,8816
	session	0,1883	1,732
	elecrodcs	< 0.0001	18,43
Run 6	Interaction	0,8481	0,7492
	session	0,3986	0,7129
	elecrodcs	< 0.0001	20,22
Run 7	Interaction	0,5234	0,9659
	session	0,4799	0,4992
	elecrodcs	< 0.0001	18,74

Table 2