

Inter-individual and Intra-individual Variation of the Effects of Pulsed RF EMF Exposure on the Human Sleep EEG

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Pulse-modulated radiofrequency electromagnetic fields (RF EMF) can alter brain activity during sleep; increases of electroencephalographic (EEG) power in the sleep spindle (13.75–15.25 Hz) and delta-theta (1.25–9 Hz) frequency range have been reported. These field effects show striking inter-individual differences. However, it is still unknown whether individual subjects react in a similar way when repeatedly exposed. Thus, our study aimed to investigate inter-individual variation and intra-individual stability of field effects. To do so, we exposed 20 young male subjects twice for 30 min prior to sleep to the same amplitude modulated 900 MHz (2 Hz pulse, 20 Hz Gaussian low-pass filter and a ratio of peak-to-average of 4) RF EMF (spatial peak absorption of 2 W/kg averaged over 10 g) 2 weeks apart. The topographical analysis of EEG power during all-night non-rapid eye movement sleep revealed: (1) exposure-related increases in delta-theta frequency range in several fronto-central electrodes; and (2) no differences in spindle frequency range. We did not observe reproducible within-subject RF EMF effects on sleep spindle and delta-theta activity in the sleep EEG and it remains unclear whether a biological trait of how the subjects' brains react to RF EMF exists. *Bioelectromagnetics* 36:169–177, 2015. © 2015 Wiley Periodicals, Inc.

Key words: sleep spindle; slow wave; mobile phones; reproducibility; topography

INTRODUCTION

A number of studies have consistently shown that near head exposure to pulse-modulated radiofrequency electromagnetic fields (RF EMF), such as those emitted by mobile phones, can alter brain physiology during sleep and waking [Kwon and Härmäläinen, 2011]. Even though most published studies vary in terms of exposure conditions (pulse-modulation frequencies, field strength, exposure duration, exposure side, etc.) relatively consistent effects have been observed in sleep electroencephalogram (EEG). Specifically, one of the most reliable effects observed in these studies is the increase of EEG power in the sleep spindle frequency (11–16 Hz) range [Borbély et al., 1999; Huber et al., 2000; Huber et al., 2002; Loughran et al., 2005; Regel et al., 2007; Loughran et al., 2012; Schmid et al., 2012a,]. However, the frequency range affected and the observed time-course of the effects remain variable.

Recent studies also found increased EEG power in the delta (1–4.5 Hz) and theta (5–8 Hz) frequency range

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during sleep [Schmid et al., 2012b; Lustenberger et al., 2013], an effect that might be attributable to the pronounced delta and theta components in the pulse-modulation scheme or the attenuation of higher harmonics [Schmid et al., 2012b]. These studies revealed that (1) the effect of RF EMF exposure can outlast the exposure; and (2) the pulse modulation of the RF EMF is an important factor in generating the observed effects [Huber et al., 2002; Huber et al., 2005; Regel et al., 2007].

An important characteristic of observed field effects are striking inter-individual differences within [Loughran et al., 2005; Loughran et al., 2012; Schmid et al., 2012a;] and between studies in how the subject's brain reacted to field exposure. Mobile phone-induced effects on sleep EEG are therefore difficult to investigate and are presumably statistically undetectable with a small sample size. Why certain subjects show a decrease in power of a specific frequency range after field exposure while a majority showed an increase is unknown. Understanding the origin of these individual differences may be important to investigate the mechanisms underlying effects of EMF on brain activity. Loughran et al. [2012] addressed these inter-individual differences and showed that a group of subjects that demonstrated increased spindle activity in the first study tended to react in a similar way to RF EMF exposure when retested several years later. This result provided a first indication that exposure-related increases in sleep spindle activity might be reproducible in a specific group of subjects. However, whether individual subjects tend to react in a similar way when repeatedly exposed is still unknown.

Our study aimed to investigate inter-individual variation and intra-individual stability of the effects of pulsed RF EMF exposure on human sleep EEG. We, therefore, exposed the same subjects 2 weeks apart to the same pulse-modulated RF EMF and tested the stability of the effect within the subjects. We used the identical exposure system and RF EMF properties used in Schmid et al. [2012b].

MATERIALS AND METHODS

Participants

A total of 20 healthy male subjects (age: 23.3 ± 0.5 years; mean $\pm$ standard error of mean [SEM]) gave written informed consent to participate in the experiment and completed the study. Only male subjects were included because in female subjects spindle activity is significantly modulated by the menstrual cycle [Driver et al., 1996]. Thus, by measuring female subjects at weekly intervals, sleep spindle

activity would vary significantly due to the menstrual cycle, lowering the probability to detect an exposure-related effect. Participants underwent a telephone and questionnaire screening to exclude personal or family history of psychopathology, chronic diseases, sleep disorders, and current use of psychoactive agents or other medications. Subjects were normal sleepers, which was verified during a screening night (to exclude subjects suffering from sleep apnea, myoclonus, or low sleep efficiency ($<80\%$)). Subjects were right handed, non-smokers, free of drugs and medication, and were moderate mobile phone users (phoning <2 h per week). Subjects had to adhere to regular bedtimes (8 h time in bed, according to scheduled bedtime in the lab) and abstain from caffeine, naps, and alcohol 3 days before study nights. Compliance with the instructions was controlled by breath alcohol test, wrist-worn actometers, and sleep logs. During the day of the study night, physical exercise and use of mobile phones were prohibited. The procedures were approved by the cantonal ethic commission in Zurich, Switzerland, and the study was performed according to the Declaration of Helsinki.

Procedure

Each participant spent four study nights (one per week on the same weekday) in the sleep laboratory of the Institute of Pharmacology and Toxicology, University of Zurich. In two of those nights, subjects were exposed for 30 min to the same RF EMF (field; FD1 and FD2) prior to their scheduled bedtime. The two other nights served as sham condition (sham; SH1 and SH2). In a randomized, double-blind crossover design, subjects had one of the two condition orders: SH1-FD1-SH2-FD2 or FD1-SH1-FD2-SH2. After real or sham exposure, a high density EEG cap was applied to the subjects resulting in a 30 min time window between end of exposure and start of sleep recording (lights out). We recorded two subjects simultaneously. Therefore, exposure times were shifted because only one exposure unit was available. Thus, bedtimes were either 10:50 p.m. to 06:50 a.m. or 11:40 p.m. to 07:40 a.m. Prior to exposure, subjects performed a 30 min declarative learning task and conducted an implicit motor sequence learning task during exposure. Subjects were retested on these tasks in the morning (results not shown). After exposure, subjects were asked whether they perceived a field or not and completed questionnaires concerning sleep quality and mood in the morning.

Exposure Conditions

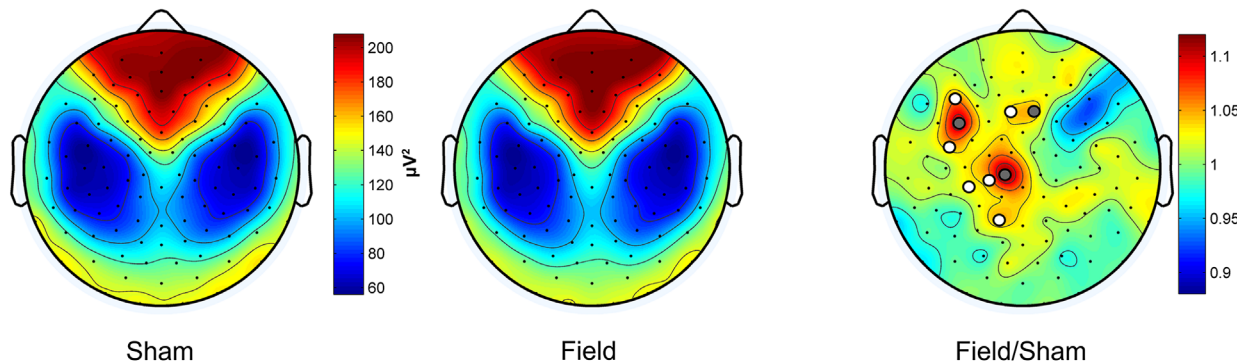
The 2 Hz pulse-modulated RF signal of Schmid et al. [2012b] was applied in this study. The signal

had a carrier frequency of 900 MHz with a basic modulation frequency of 2 Hz and a peak-to-average ratio of 4. Higher harmonics were reduced by applying a 20 Hz Gaussian low-pass filter to a rectangular pulse sequence (see Fig. 1 of Schmid et al. [2012b] for details of the signal characteristics). In contrast to other studies, the EEG electrodes were mounted after the RF EMF exposure, as the utilized high density EEG net would significantly increase the uncertainty of induced fields due to coupling with incident fields. A detailed description of the exposure system and its dosimetry are provided in Schmid et al. [2012b] and Murbach et al. [2012], and the evaluation of the potential artifacts in Murbach et al. [2014]. In brief, a lateral patch antenna (SPA860/65/9/0/V, Huber & Suhner, Herisau, Switzerland; 110 mm distance to the head) in a plastic box-casing was used to obtain left hemispheric 900 MHz exposure of 2 W kg^{-1} peak spatial SAR averaged over 10 g (psSAR10g) in the whole head. An identical box-casing was installed on the right side to prevent knowledge about the RF EMF origin. For the targeted 2 W kg^{-1} psSAR10g, the

forward power to the antenna was controlled ($4.0 \text{ W} \pm 6\%$ standard deviation [SD]). The corresponding SAR efficiency was numerically estimated with anatomical human head models from the Virtual Population [Christ et al., 2010], using the simulation platform SEMCAD X (SPEAG, Zurich, Switzerland). Validation measurements were performed with dosimetric SAR probes in the specific anthropomorphic mannequin (SAM, [IEEE Std 1528, 2003]) with the near-field scanner DASY5 NEO (SPEAG). The uncertainty for the psSAR10g assessment stayed below 8% (SD, $k=1$); largest contribution from the dielectric tissue property and segmentation uncertainties. This corresponds well to the obtained 10.4% deviation between simulation and measurement of the SAM head. The variability of the psSAR10g remains below 15% (SD, $k=1$), mainly due to different head models (three different models from the Virtual Family were included in the variability analysis).

With the utilized planar patch antenna, the lateral cortex is exposed to a psSAR1g of up to $2.12 \text{ W kg}^{-1} \pm 12\%$ (SD, $k=1$). The corresponding 0.62 W

A Delta-theta



B Spindle

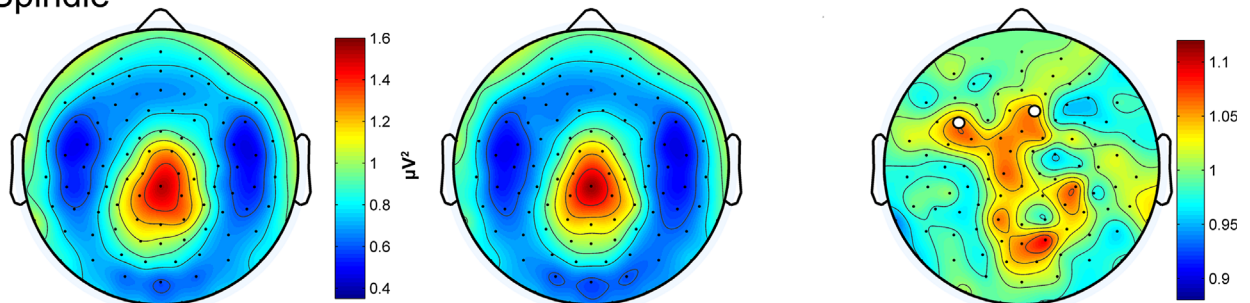


Fig. 1. Topographical distribution ($n=19$) of delta-theta (A, 1.25-9 Hz) and spindle activity (B, 13.75-15.25 Hz) of the averaged sham and field condition, and the ratio between field and sham condition. Topography is based on NREM sleep stages 2 and 3 of the entire night. Values are color coded (maxima brown, minima blue) and plotted on the planar projection of the hemispheric scalp model. Electrodes (black dots) that showed significant exposure effects ($P < 0.05$) are marked with grey dots and electrodes that tended to be higher in power after exposure are highlighted in white ($0.05 \leq P < 0.1$). These electrodes, however, did not remain significant (or trend level) after supra-threshold cluster analysis.

$\text{kg}^{-1} \pm 35\%$ (SD, $k=1$) of the thalamus indicates limited RF penetration depth at 900 MHz. For a robust double blind protocol, the physical exposure system was fully computer controlled.

Polysomnography

A high density EEG net (Electrical Geodesics Sensor Net for long-term monitoring, 128 electrodes, Electrical Geodesics, Eugene, OR) was adjusted to the Vertex (Cz) and Nasion, and the cap electrodes were filled with gel electrolyte. These nets combined the superior temporal resolution of EEG recordings with high spatial resolution [Lustenberger and Huber, 2012]. Therefore, this method allows a topographical analysis of sleep EEG and can be used to assess regional differences. EEG, electrooculogram, and submental electromyogram were continuously recorded during the 8 h nighttime sleep period. The signals were digitized at 500 Hz (band-pass filter 0.01–200 Hz), referenced to Cz. Offline, signals were band-pass filtered (0.5–40 Hz).

The sleep stages were scored for 20 s epochs according to standard criteria [Iber et al., 2007]. Artifacts were identified on a 20 s basis by visual inspection and if power exceeded a threshold based on a sliding mean of power in 0.75–4.5 and 20–30 Hz bands [Huber et al., 2000].

Spectral power of consecutive 20 s epochs was computed using a Fast Fourier routine (Hanning window, average of five 4 s epochs, frequency resolution 0.25 Hz), and frequencies between 0.75 and 20 Hz were analyzed. Mean power density spectra were calculated for the whole-night non-rapid eye movement (NREM) sleep and averaged for both field (FDmn) and sham nights (SHmn), respectively. Spindle and delta-theta activity of the whole-night NREM sleep were calculated as the spectral EEG power between 13.75–15.25 Hz and 1.25–9 Hz, respectively, according to Schmid et al. [2012b]. These activities were investigated for all electrodes of the high-density EEG net to reveal topographical distribution. EEG data were re-referenced to average reference after exclusion of EEG channels of insufficient quality (on average, seven channels per subject were excluded). Thereafter, power values of bad channels were interpolated using a spherical interpolation provided by “EEGLAB” toolbox [Delorme and Makeig, 2004].

Statistics

All EEG spectral power values were first log transformed to approximate normal distribution prior to testing. A 2-tailed paired Student's *t*-test was used to compare polysomnographic variables derived from visual scoring. The following sleep variables were

analyzed: sleep latency (interval from lights off to the first occurrence of stage 2), amount of REM, stage 1, stage 2, and stage 3 NREM sleep, sleep efficiency (total sleep time percentage of time in bed), and amount of wakefulness after sleep onset. Based on the findings of previous studies, one-tailed Student's *t*-tests were applied to investigate whether field condition (average FD1 and FD2; FDmn) leads to higher mean power density in the frequency range of 0.75–20 Hz, including spindle activity and delta-theta activity, compared to the sham (average SH1 and SH2; SHmn) condition. To assess topographical differences in spindle activity and delta-theta activity, we applied statistical nonparametric mapping (SnPM) using a supra-threshold cluster analysis to control for multiple comparisons [Nichols and Holmes, 2002; Huber et al., 2004; Ferrarelli et al., 2007; Ringli et al., 2013]. Briefly, neighboring electrodes above a specific *t*-value (1.33 for a one sided Student's *t*-test to reach a $P < 0.1$) were considered a cluster. Using permutation analysis, we generated new datasets (50,000) by randomly relabelling the condition label from original data, performed Student's *t*-tests, and counted the maximal size of resulting clusters with neighboring electrodes reaching a *t*-value of 1.33 from these new datasets. The given cluster sizes were sorted and the 95th percentile cluster size was determined. In case our cluster of the original data has been above this determined threshold it was considered significant [Nichols and Holmes, 2002; Huber et al., 2004; Ringli et al., 2013]. To investigate whether the field effect follows a specific time-course, additional analyses were conducted for power in the two frequency bands for three consecutive intervals (thirds of NREM sleep, i.e., all NREM sleep epochs from sleep onset until final awakening). Therefore, a one-way repeated ANOVA of the relative power value changes (FDmn/SHmn) with the factor interval (NREM third 1 to 3) was performed. To test whether RF EMF effects are reproducible within subjects, we calculated Pearson's correlations between RF EMF related EEG changes of the first and second exposure condition.

One subject was excluded from statistical analysis due to poor sleep quality in sham night 1 (only one sleep cycle) and bad signal quality in many electrodes of the high density net during field condition 1 leading to a final sample size of 19 subjects.

RESULTS

All included subjects ($n=19$ out of 20, see methods) showed good sleep quality with high sleep efficiency (SHmn: $95.3 \pm 0.65\%$ SEM; FDmn: $95.3 \pm 0.62\%$ SEM). There was no exposure effect on

sleep variables derived from visual scoring (all $P > 0.19$). Specifically, no significant field effect was found for sleep variables that showed so far inconsistent field effects in literature (Table 1) [for a review table, see Danker-Hopfe et al., 2011].

In a first step, we looked at the topographic distribution of EEG power in frequency ranges that were affected in the study by Schmid et al. [2012b] that used the same exposure system and RF EMF properties. The topographical analysis of delta-theta activity revealed a typical frontal maximum for both conditions. Some electrodes in a fronto-central region showed increased delta-theta activity after exposure compared to the sham condition (Fig. 1a). However, when controlling for multiple comparisons (see Methods) we did not find a significant cluster of electrodes.

As expected, spindle activity was most pronounced over central electrodes in both conditions. Spindle activity of two electrodes in a fronto-central region tended to be higher under field conditions compared to sham. However, these increases also did not remain significant after performing a supra-threshold cluster analysis (Fig. 1b).

Next, we defined a specific fronto-central cluster including those electrodes that under field exposure showed a significant or trend level increase (not corrected) in either the delta-theta or spindle range (electrodes marked with a grey/white dot in Fig. 1). We then compared the field and sham condition using again a one-tailed paired Student's *t*-tests to see whether field exposure led to increased power density in specific frequency bands of this cluster.

The delta-theta activity was significantly higher in the field compared to the sham condition in this specific cluster of electrodes (SHmn: $13.93 \pm 1.07 \mu\text{V}^2\text{Hz}^{-1}$ SEM; FDmn: $14.64 \pm 1.23 \mu\text{V}^2\text{Hz}^{-1}$ SEM; $P < 0.01$). Spindle activity did not differ between the two conditions (SHmn: $0.47 \pm 0.05 \mu\text{V}^2\text{Hz}^{-1}$ SEM; FDmn: $0.47 \pm 0.04 \mu\text{V}^2\text{Hz}^{-1}$ SEM; $P > 0.1$). Figure 2 supports this finding and shows that only frequency

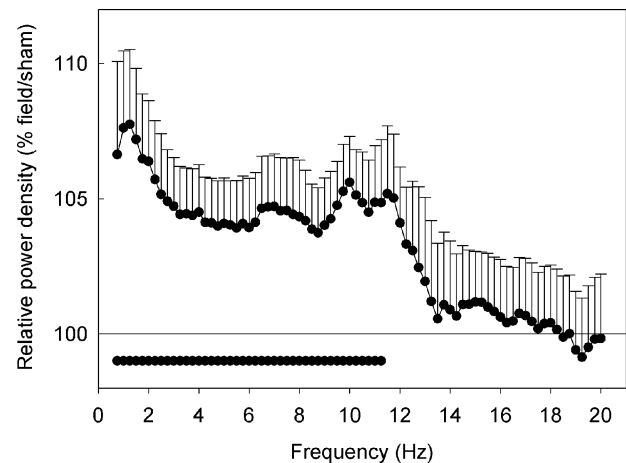


Fig. 2. Mean relative EEG ($n = 19$) power density spectrum of non-rapid eye movement (NREM) sleep of the entire night of a specific fronto-central cluster. Power density ($\mu\text{V}^2/\text{Hz}$) of the averaged field conditions (night 1 and 2, FDmn) is expressed as percentage of averaged sham conditions (night 1 and 2, SHmn). Filled black dots in the lower panel illustrate significant frequency bins ($P < 0.05$) after one-tailed paired Student's *t*-tests were performed comparing SHmn and FDmn conditions. Vertical lines illustrate SEM.

bins from 0.75 Hz to 11.5 Hz were significantly increased after exposure.

We further investigated whether the field effect follows a specific time-course. One-way repeated ANOVA with factor interval (thirds of NREM sleep) showed no significant time-effect for the FDmn/SHmn ratio values, for both delta-theta ($F = 2.51$, $P = 0.1$) and spindle activity ($F = 1.09$, $P = 0.35$).

Finally, we evaluated whether subjects reacted in a similar way when exposed twice to the same pulse-modulated RF EMF. Figure 3 shows field-induced individual changes in EEG power of the 1.25–9 Hz and the 13.75–15.25 Hz frequency bands in a fronto-central cluster (see above). As in previous studies, we observed large individual differences in exposure induced power changes. However, subjects did not react with a similar change in the first and second exposure condition (absence of a significant correlation between the relative EEG power values $\text{FD1}/\text{SH1} \sim \text{FD2}/\text{SH2}$; 1.25–9.0 Hz: $r = 0.01$, $P > 0.9$; 13.75–15.25 Hz: $r = 0.02$, $P > 0.9$). We further performed a group comparison as done by Loughran et al. [2012]. Thus, we divided our subjects into “increasers” and “decreasers” based on their RF EMF related changes in the sleep EEG of the first exposure condition. “Increases” were defined as those participants showing an increase in spectral power in delta-theta or spindle range, respectively, and “decreasers” vice-versa. However, when performing an unpaired Student's *t*-test for the second exposure condition there was not more of an increase in spindle ($P = 0.3$) or

TABLE 1. Polysomnographic Variables Derived from Visual Scoring of the Sleep Period

	SHmn	FDmn	<i>P</i> -values
Sleep latency [min]	13.3 (1.3)	14.7 (2.1)	0.4
REM sleep latency [min]	92.8 (4.6)	101.4 (5.2)	0.2
REM sleep [min]	111.9 (4.0)	114.0 (4.2)	0.7
Wake after sleep onset [min]	13.4 (2.9)	11.7 (2.1)	0.5

Mean values $\pm$ SEM ($n = 19$) are reported. Sleep latency: latency to the first occurrence of stage 2 sleep. REM sleep latency: interval from sleep onset to first occurrence of REM sleep. REM: Rapid eye movement sleep. SHmn and FDmn: average values of the 2 sham and field conditions, respectively.

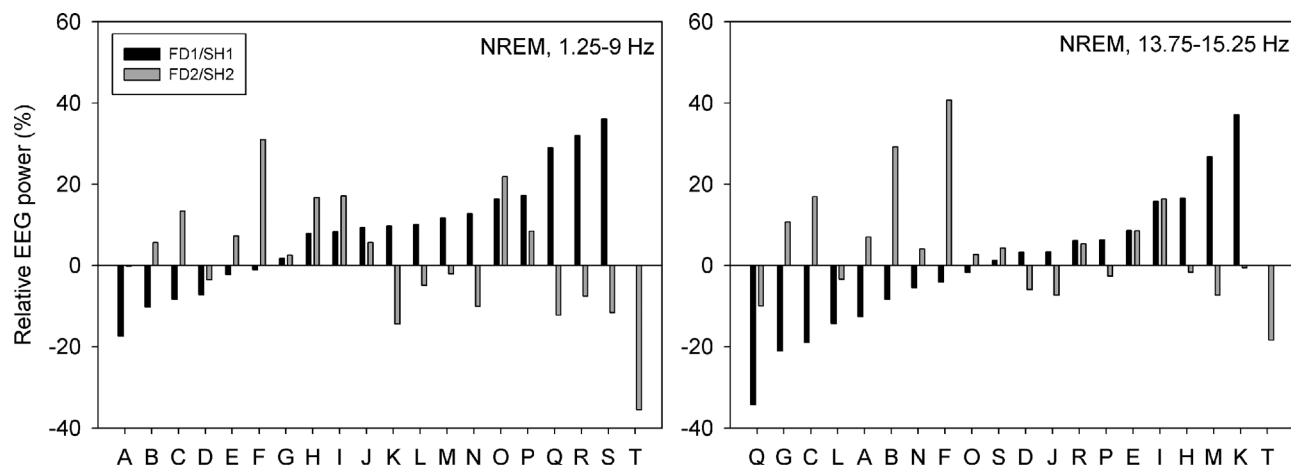


Fig. 3. Inter-individual variability and intra-individual stability of the field effect. Individual percentage change in whole-night spectral power relative to sham (sham = 0%) during non-rapid eye movement sleep (NREM sleep: 1.25-9 Hz, 13.75-15.25 Hz) for the first field to sham night (FD1/SH1, black bars) and the second field to sham night (FD2/SH2, grey bars). Subjects were sorted according to the increase in the respective frequency band in the FD1/SH1 condition ($n = 20$, A-T, for subject T FD1/SH1 values are missing, q.v. methods). Averages across channels of a specific fronto-central cluster were analyzed.

delta-theta ($P = 0.9$) activity in the “increasers” than in the “decreasers.”

DISCUSSION

The present study aimed to explore topographical distribution of 2 Hz pulse-modulated RF EMF induced effects on the sleep EEG to extend the findings of an earlier study that used the same RF EMF properties [Schmid et al., 2012b]. Moreover, we aimed to investigate the inter-individual variation and intra-individual stability of these effects.

Topographical analysis revealed several significant or trend-level changes in electrodes of fronto-central regions that showed increased power in delta-theta frequency range after exposure. In the spindle frequency range, only at two electrodes, we observed a trend of an EMF exposure-related increase. However, none of these electrodes remained significant when a supra-threshold cluster analysis was applied. Our study might have been underpowered to statistically detect such subtle topographical differences. We, thus, defined a specific fronto-central cluster that contained all significant, trend-level electrodes of the delta-theta, and spindle frequency range. Interestingly, earlier studies mainly found effects in left central electrodes that were also included in our cluster. Delta-theta activity but not spindle activity was significantly higher in field condition compared to sham for this specific fronto-central cluster. Thus, the previously observed increase in EEG power between 1.25 Hz and 9 Hz in the study by Schmid et al. [2012b] was similar/reproducible in our

study, though slightly lower in magnitude (5.3% vs. ~9%). It remains elusive whether such small changes in delta-theta activity are biologically relevant and therefore behavioral consequences of small delta-theta changes should be investigated in future studies. Interestingly, the extremely low frequency magnetic field (ELF MF) exposure (2 Hz pulsed with the same characteristics as the RF envelope) used in Schmid et al. [2012b] also led to an increase in this low-frequency range whereas spindle activity was not affected. Thus, a pulse-modulation (e.g. 2 Hz) seems to be essential for an increase in delta-theta activity. Another possible explanation might be that the applied attenuation of higher harmonics restricting modulation components to the low frequency range led to an effect on delta-theta activity during sleep as discussed in Schmid et al. [2012b]. Of course, these are still speculative explanations and alternative interpretations might apply.

Spindle activity was not significantly higher in the fronto-central cluster after field exposure as would have been expected from earlier studies [Borbély et al., 1999; Huber et al., 2000; Huber et al., 2002; Loughran et al., 2005; Regel et al., 2007; Loughran et al., 2012; Schmid et al., 2012a;]. Using an established spindle detection algorithm [Ferrarelli et al., 2007], additional analysis revealed that spindle density, amplitude, duration, and power were not affected by exposure (data not shown). Even though the exposure-related increase in sleep spindle activity is one of the most robust effects reported, a number of studies failed to find exposure-related increases in sleep spindle activity likely explainable by methodological issues [Mann and Rösche,

1996; Wagner et al., 1998; Wagner et al., 2000; Hinrichs et al., 2005; Fritzer et al., 2007; Lowden et al., 2011]. For instance, due to pronounced inter-individual differences in how subjects react to the field, effects on sleep EEG are presumably undetectable with small sample sizes [Loughran et al., 2012]. To determine the minimal number of subjects needed to detect a probable significant increase in EEG spindle activity in this fronto-central cluster with our specific study design, we performed a power analysis for a paired one-sided Student's *t*-test [Morris and DeShon, 2002]. Calculation of the sample size necessary to achieve a statistical power of 80% revealed that a minimum number of 121 subjects would have been needed to detect a significant effect ($P < 0.05$). Such a large number of subjects is not feasible to investigate with our extensive study design. Our results may imply that the effect of applied pulse-modulated RF EMF might be too small compared to the influence of other factors that may lead to high intra-individual spindle variability and might thus have masked EMF effects on spindle activity. This interpretation is supported by the fact that sleep spindles are known to be sensitive to many different factors such as learning-related changes, circadian influences, and sleep pressure [De Gennaro and Ferrara, 2003]. Thus, high intra-individual variability in spindle activity might be explainable by methodological differences, for instance in exposure conditions or general study procedure as discussed below.

Our study used the same RF EMF properties as Schmid et al. [2012b]. Nevertheless, we were not able to reproduce the findings on sleep spindle activity. A possible explanation could be that our experimental procedure slightly differed from the one of Schmid et al. [2012b]. First, subjects performed two different learning tasks, one before and one during exposure (sham or real EMF). The word-pair declarative learning task that was performed by the subject before exposure is known to change spindle density in the subsequent sleep period compared to a baseline night [Rasch and Born, 2013]. Thus, learning induced changes in spindle activity, which have been reported in earlier studies using the same task [Gais et al., 2002; Schmidt et al., 2006], might have interfered with exposure-related changes in sleep spindle activity. Of note, we used four parallel, equally difficult versions of this task for all four measurements and therefore would expect a similar effect of the learning task on spindle activity. If, indeed, a 30 min word-pair learning session can mask the effect of RF EMF exposure on sleep spindle activity, it would mean that EMF effects are rather small and easily masked.

Second, we had a 30 min time window between end of exposure and lights out whereas in Schmid

et al. [2012b] and several other studies the interval was only 10 min [Huber et al., 2002; Regel et al., 2007; Schmid et al., 2012a] or 20 min [Loughran et al., 2005]. Longer time intervals between the end of exposure and sleep might reduce the EMF induced effects. Lowden et al. [2011] had a time window of 1 h and also did not find a significant effect on sleep spindle activity. Nevertheless, we would not expect that postponing sleep after exposure for additional 10–20 min would weaken the RF EMF effect to such an extent that 100 subjects more (power analysis) would be needed to reach significance. This argument is also supported when considering the time-course of RF EMF effect of our specific RF EMF exposure [Schmid et al., 2012b]. They reported that the EMF effects of spindle and delta-theta activity were most pronounced (mean difference to sham condition) in the fourth and third NREM sleep episode, respectively, i.e., approximately 3.8 and 5.5 h after the end of RF EMF exposure. This finding indicates that our specific RF EMF exposure should provide long-lasting effects. However, if time awake from exposure end until sleep onset is the limiting factor to see an effect in spindle activity one should also take the sleep latency into account. Comparing sleep latencies between our study and Loughran et al. [2012] our subjects needed on average 10 min less to fall asleep. Thus, time until sleep onset after the end of exposure was similar in both studies (on average 30 min).

Third, several earlier studies applied electrodes to the subject's head prior to exposure to minimize the time window between exposure and bed time. Our high-density EEG net acted as a Faraday's cage and we had to apply the net post-exposure. It is still debated whether possible effects might be partly explained by locally induced currents in skin or cortex by electrodes during exposure, although highly unlikely [Murbach et al., 2014]. However, Loughran et al. [2005] applied electrodes after exposure and found a significant exposure-related increase in spindle activity.

Fourth, Loughran et al. [2012] found the effect to be largest in females. Thus, our null finding in sleep spindle activity might be explainable by the exclusion of female subjects in our study. Hence, further studies should investigate gender specific aspects of RF EMF effects on sleep spindle activity.

Finally, our participants had no adaptation night prior to experimental nights. Thus, the null finding in spindle activity might be related to increased variance of a "first night" effect. The first night effect, however, mainly affects REM sleep, but not sleep spindle activity [Curcio et al., 2004]. In addition, subjects could adapt to the EEG net and the environ-

ment during a screening night, and their sleep behavior was controlled 3 days before the experimental night verifying that subjects slept for 8 h and had good sleep quality. Furthermore, we applied a counterbalanced design and the first field exposure was either in the second or third night of measurement (including the screening night). Therefore, it is rather unlikely that a “first night” effect would be responsible for the null result in sleep spindle activity. Nevertheless, if this limitation still holds true, the reported spindle effect might be weak and easily masked by conditional changes (e.g. learning tasks).

Previous research indicated that exposure-related effects on sleep EEG are reproducible in a specific group of participants [Loughran et al., 2012]. We took advantage of this finding for our specific study design and further addressed whether individual subjects (in addition to a group of subjects) react in a similar way by performing correlational analysis. Thus, even though there was no overall field effect in our study in the spindle frequency range, subjects might react similarly to the field when exposed again after 2 weeks. Our data clearly showed that subjects did not react in a similar way in the first and second exposure condition since no correlation between the effects in the spindle frequency range of these two exposures was observed as revealed by the correlation analysis and the group analysis. In addition, even though delta-theta activity was significantly higher after RF EMF exposure in a fronto-central cluster, we also found no correlation or group difference between the two exposure conditions in this frequency range. Our study did not provide any evidence that some subjects were more susceptible/sensitive to our specific RF EMF. Thus, it remains unclear whether a biological trait exists that predicts how subjects’ brains would react to EMF exposure.

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