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An assessment of the autonomic nervous system in the electrohypersensitive population: a heart rate variability and skin conductance study

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Running title: Evaluation of autonomic nervous system in electrosensitive

ABSTRACT

The aim of the study was twofold: first, to compare the activity of the autonomic nervous system (ANS) between the population self-declared as electrohypersensitive (EHS) and their matched control individuals without intended exposure to electromagnetic fields (EMF). The second objective was to determine whether acute exposure to different radiofrequency signals modifies ANS activity in EHS. For that purpose, two different experiments were undertaken, in which ANS activity was assessed through heart rate variability (HRV) and skin conductance (SC). In the first experiment, a comparison between the EHS group (n=30) and the control group (n=25) showed that the EHS has an increased number of responses to auditory stimuli as measured by skin conductance activity, and that none of the short-term heart rate variability parameters differ between the two matched study groups.

The second experiment, performed in a shielded chamber, involved 10 EHS from the first experiment. The volunteers participated in two different sessions (sham and exposure). The participants were consecutively exposed to four EMF signals (GSM 900, 1800, DECT and Wi-Fi) at environmental level (1 V/m). The experiment was double blinded and counterbalanced. The HRV variables studied did not differ between the two sessions. Concerning electrodermal activity, the data issued from skin conductance and tonic activity did not differ between the sessions, but showed a time variability.

In conclusion, the HRV and SC profiles did not significantly differ between the EHS and control populations under no exposure. Exposure did not have an effect on the ANS parameters we have explored.

New & Noteworthy: This study provided analysis on the skin conductance parameters using a newly developed method (peak/min, extraction of skin conductance responses) which had not been performed previously. Additionally, the skin conductance signal was decomposed, considering tonic and phasic activities to be a distinct compound. Moreover, this is the first time a study has been designed into two steps to understand whether the autonomic nervous system is disturbed in the EHS population.

Keywords: *electrohypersensitivity, symptoms, autonomic balance, idiopathic environmental intolerance, electromagnetic fields, galvanic skin response, electrodermal activity, environmental intolerance*.

INTRODUCTION

Electrohypersensitivity, also known as idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF) (18), is a controversial condition whose pathogenesis and clear etiology is so far unknown. Characterized by a wide range of symptoms (23) and multiple incriminated sources of electromagnetic fields (12), electrohypersensitive (EHS) patients reported that they mainly suffered from symptoms such as tiredness, vertigo, tachycardia, headaches, etc. Some studies have attempted to suggest different mechanisms of triggering symptoms or that individuals are predisposed to become EHS, but nowadays no consensual physiopathological mechanism has been accepted. One of the suspected causes of the appearance of symptoms could be the disturbed activity (or imbalance) of the autonomic nervous system (ANS).

The very few studies that have been conducted on EHS did not find there to be any effect of acute exposure to electromagnetic fields (EMF) on cardiac autonomic control (6, 20, 21, 26, 15, 8). An imbalance of the ANS has been suggested by studies on the neurophysiological characteristics of EHS subjects (16). In terms of visual testing, a significantly higher critical flicker frequency (CFF) and visual evoked potential (VEP) has been found in patients suffering from symptoms related to visual display terminal- and from EHS. Furthermore, an imbalance of the autonomic nervous system and deviation of the circadian rhythm (as assessed by Heart Rate Variability) (16) and the hyper-reactivity of the CNS (25) were reported.

A non-invasive and simple method to analyze sympathovagal balance (at the sinoatrial level) is measuring heart rate variability (HRV). HRV is widely believed to reflect changes in cardiac autonomic regulation. Skin conductance (SC), mostly known as electrodermal activity or sympathetic skin response, results from the activation of the reflex arch to different stimuli; the electrical activity of the eccrine glands is simultaneously analyzed. The latter is thought to reflect sympathetic arousal.

According to the last proceeding of the World Health Organization (WHO) on EHS (18), more studies focusing on the sympathetic nervous system are required to define the role it has to play in the occurrence of EHS or EHS symptoms. Firstly, we aimed

to analyze the characteristics of the ANS in EHS volunteers under baseline conditions (without exposure to EMF), before comparing them to a non EHS (control) group. The HRV and skin conductance (SC) parameters were then analyzed. A second study was performed to identify the possible effects of acute exposure to EMF signals on the HRV and SC of EHS volunteers. To our knowledge, this is the first time a study has been designed into two steps to understand whether the autonomic nervous system is disturbed in the EHS population when compared to the control one, and to characterize the effects of EMF exposure, if there are any, on the autonomic nervous system of EHS individuals.

PATIENTS AND METHODS

Approval

For the whole study, ethical approval was obtained from the appropriate local committee (Comité de Protection des Personnes CPP Nord-Ouest N°: CPP/2014/8; CPP/2015/38) and informed consent was obtained from all participants.

Study Population

In the first part of this study (Experiment 1), a total of 30 EHS (mean age: 47 ± 9) and 25 non EHS (mean age: 46 ± 10) individuals participated in the experimental protocol. EHS patients (aged from 18 to 65), who declared that they suffered from symptom(s) when exposed to various EMF sources such as mobile phones (54%) and ELF-emitting sources (24%), were included in this study. Participants were recruited through self-help groups or from the general population. None of the EHS participants were on medication such as anti-depressants, analgesics or muscle relaxants. They were recruited after they had stopped taking any medication that could affect the autonomic nervous system. The control group of this first experiment was recruited through an advertisement. The control and EHS groups were matched in terms of age, body mass index and gender. The control group was on no medication and presented no evidence of disease. All participants were instructed to abstain from consuming alcohol and coffee for the 24 hours prior to and during the study. The first study protocol, with the results of biological analysis, can be found in (2).

In the second part of our study (Experiment 2), only 10 EHS individuals who had participated in the previous study (Experiment 1) consented to exposure to EMF. They reported being EHS and were aged between 35 and 63 years (mean age: 48 ± 10). The participants reported being sensitive, or attributed their symptoms to any one of the following sources: GSM 900, GSM 1800, DECT and Wi-Fi 2.45 GHz. The study group was composed of eight female and two male participants.

All of the participants gave informed consent and were informed of the purpose, aims and procedure of the experiments.

Experimental conditions

In Experiment 1 (Figure 1), all of the volunteers participated in one experimental day session. No intentional EMF exposure was performed and the experiment took place in a shielded chamber. No EMF source was allowed, and both the EHS and control individuals were advised to switch off all EMF sources and to stop using all possible EMF sources at least two hours before being tested. The participants arrived in the experimental room in the morning for a series of biological tests, the results of which were published in part in (2). All the recordings were taken between 4 pm and 4.30 pm in a noise-free chamber, following a period of acclimatization. Blood pressure (systolic and diastolic) was measured using a commercial device upon the participants' arrival.

Experiment 2 consisted of two sessions (sham and real exposure) (Figures 2 and 3). No external EMF sources were allowed, and the exposure consisted of a series of EMF signals emitted from a generator (Rhode & Schwarz) and a horn antenna (Schwarzbeck BBHA9120b). Consecutive RF signals were applied: GSM 900, GSM 1800, DECT and Wi-Fi signals for five minutes per signal. Each exposure period was followed by 10 minutes' rest. A dosimeter was placed near the participant to record the level of exposure. The participants arrived in the experiment room at 9 am and all recordings began at 10 am. Two blood pressure measurements were taken: the first upon arrival and the second at the end of the experiment. The two sessions (sham and real) were separated by an interval of at least one week.

During the second experiment, the tests were double-blind and the data analysis was performed in blind, without any possible way of recognizing the real or sham exposure.

For both experiments, the temperature and humidity were measured; the average temperature in the laboratory was: 24 ± 1 °C in the morning and 25 ± 0.8 °C in the afternoon. The mean humidity was: 44 ± 8 % during the experiments.

Heart rate variability measurement

The electrocardiogram (ECG) signal was recorded according to the instructions of the European Society of Cardiology (14). Leads, attached to the left and right wrists and ankles, were connected to a Biopac system and a laptop with a rate of 1000 samples/sec. Kubios HRV® signal analysis software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) was used to automatically detect QRS complexes in ECG signals. A visual inspection was first performed to avoid any misdetection and to ensure that the records did not contain any ectopic beats or artifacts.

Non-stationary trends were removed from the HRV signals by using a detrending procedure based on smoothness prior to regularization. Next, time and frequency

domain parameters were extracted. The participants were seated in a comfortable position in a normal, resting state. Due to the circadian rhythm of HRV, the recordings were performed at the same time of day for each participant for Experiment 1 and Experiment 2.

Skin conductance and electrodermal responses measurement

Two Ag/AgCl electrodes were attached to the phalanges of the non-dominant hand and linked to a Biopac MP 36 system to record SC and the resulting skin conductance responses (SCR). Auditory stimuli were generated to elicit specific SCRs, which could then be analyzed and characterized. For Experiment 1, SC was recorded without auditory stimulation for Run 1 and Run 7, and with stimulation from Runs 2 to 6 (10 * auditory burst for each run) (Figure 1). For Experiment 2, SC was continuously recorded before exposure, during all exposure runs (both real and sham for GSM 900, GSM1800, Wi-Fi and DECT) and after exposure with repetitive auditory stimuli (5 * auditory burst for each run) (Figure 2 and Figure 3). The software used was Ledalab, which allowing continuous decomposition analysis (CDA), trough to peak (TTP) or the general linear method. After visual inspection and artifact correction, analysis was performed on the recordings. The software can separate SC signals into phasic and tonic activities. The CDA method has been shown to be beneficial for analyzing data with apparent superposition of SCR signals (3).

Variables

The RR intervals were acquired from the ECG recordings seven times and five times respectively for Experiment 1 and Experiment 2 (Figure 1 and Figure 2). The time domain parameters analyzed were: the average RR interval (RR), heart rate (HR), the standard deviation of the instantaneous heart rate values (STD HR), the standard deviation of the RR intervals (SDNN), the square root of the mean squared differences between the successive RR intervals (RMSSD), the number of successive RR interval pairs that differ by more than 50 ms (NN50) and NN50 divided by the total number of RR intervals (pNN50). Power spectral analysis was then conducted using the non-parametric Fast Fourier Transform (FFT) method. The frequency-domain metrics (ms^2) were: low frequency band (LF: 0.04 – 0.15 Hz), high frequency band (HF: 0.15 - 0.4 Hz) powers. Furthermore, the LF in normalized units (nu), the HF nu and the LF/HF ratio were extracted. The latter represents the frequency at which the power spectral density is highest within the HF band.

Ledalab was used to extract SC, and hence tonic and phasic activities, after applying the CDA method. The software was employed to extract the following parameters: the total number of SCRs above the threshold, the response latency of the first significant SCR within response window (wrw), the sum of amplitudes of significant SCRs wrw, the average phasic driver wrw (SCR amplitude), area (i.e., time integral) of the phasic driver wrw (ISCR) and mean tonic activity wrw (tonic amplitude). The amplitude threshold of the SCR was defined as 0.02 µS for both non-specific and

event-related SCRs. The responses to auditory stimuli were summed for Experiment 1.

For non-specific SCRs (i.e., without auditory stimuli), the total number of SCRs and hence the average peak number over a one-minute time frame (mean peak/min) were retrieved. For event-related SCRs (i.e., with auditory stimuli), a time window relative to events between one and four seconds' duration was set up.

For blood pressure, arterial systolic (PAS) and diastolic (PAD) pressure were directly obtained from the device, and breathing frequency from respiratory belt monitoring.

Statistics

Normally-distributed HRV data were analyzed using a two-way repeated measures ANOVA (RM-ANOVA), followed by Bonferroni adjusted post-hoc analysis. There were two factors: Stage (seven levels: Rest 1, Stim 1, Stim 2, Stim 3, Stim 4, Stim 5 and Rest 2) and Group (two levels: EHS and control). In Experiment 1, some non-normally distributed variables were log (ln) transformed. These parameters include: RR, RMSSD, SDNN, LF, HF and LF/HF ratio.

For Experiment 2, the two factors for the RM-ANOVA were Session (two levels: real and sham) and Stage (five levels: Pre-expo, GSM 900, GSM 1800, DECT and Wi-Fi).

Data with non-normal distribution were analyzed using non-parametric tests for Stage or Group differences (Experiment 1) and paired rank tests were performed for inter sessions (i.e., Wilcoxon tests with multiple comparisons adjustment) or intra-session differences (i.e., Friedman tests followed by Bonferroni adjusted post-hoc analysis if significant) (Experiment 2).

For RED analysis under Experiment 1, a proportion of the significant responses between groups was compared by using the chi-squared test. SC, tonic and phasic components under auditory stimulation and the characteristics of SCRs were compared using the Mann-Whitney test between the groups. Times comparison was performed using the Friedman test.

The Wilcoxon t-test was applied to compare respiration rates and blood pressure readings acquired before/after and between sessions.

The statistical analyses were performed and plotted using SPSS IBM Statistics version 20, Graph Pad Prism version 5.0 and Excel software.

RESULTS

Experiment 1

Respiratory rate

Respiratory frequency was not available during the first Experiment.

Blood pressure

The blood pressure readings recorded during the first Experiment did not show any significant difference between EHS and non EHS groups. Neither systolic (SBP: 115 ± 12 mmHg, SBP: 108 ± 13 mmHg, p= 0.27) nor diastolic pressure (DBP: 73 ± 10 mmHg, DBP: 71 ± 8 mmHg, p= 0.64) differ between groups.

HRV parameters

Time-domain parameters

Among all of the analyzed parameters, no significant differences between the groups were found. Indeed, RR, RMSSD and SDNN did not seem to be different in the EHS group when compared to the control one (Figure 4).

Frequency-domain parameters

LF, HF and the LF/HF ratio were not significantly different between the groups. It should be noted that HF did not vary over the course of the Experiment, while LF and the LF/HF ratio were modified in both groups (Figure 5). There was a trend of an increase in LF/HF ratio due to LF increase. Also, the comparison of LFnu or HFnu between the EHS and control groups did not show any significant difference ($p > 0.05$) (Figure 6).

Skin conductance (SC) and SCR parameters (SCRs or RED)

Statistical analysis did not show any significant differences ($p > 0.05$) between the EHS and control groups for SC (3.8 ± 0.22 vs 2.93 ± 0.18 µS), tonic (3.33 ± 0.21 vs 2.86 ± 0.17 µS) and phasic activity levels (0.05 ± 0.01 vs 0.07 ± 0.01 µS). The data were expressed as a mean over a three-minute recording period ± SD.

Over all recording periods, including auditory bursts (Run 2 to Run 6), there was a higher percentage of responses to the auditory bursts in the EHS group compared to the control group (36% of responses in the EHS group versus 29% in the control).

Due to the fact that the participants rapidly acclimatized to the bursts, the SCR analysis presently shown was only based on the first auditory burst analysis, but not on 10 bursts (significant differences between bursts). Due to the presence of non-specific responses during runs, an analysis on Run 2 (the first run with bursts) was considered as appropriate to compare the EHS group with the non-EHS group. The results have shown no difference between the groups.

Other methods of analysis, including the TTP method and general methods, did not show there to be any difference between the groups during Run 2 and after the first stimulation.

Experiment 2

Respiratory rate

The statistical analysis for comparing between sessions (sham versus real) showed that the respiratory rates at each stage (i.e., pre-exposure, GSM900, GSM1800, DECT, Wi-Fi or post-exposure) were not significantly different. The global mean values (±SD) calculated for all participants and for each session (sham or real) were: 13.05 ± 2.34 and 12.74 ± 2.18 bpm respectively.

Blood pressure

Under each exposure, the blood pressure readings taken before and after exposure were not significantly different between the real and sham sessions for SBP ($p > 0.05$).

HRV parameters

Time-domain parameters

None of the time-domain parameters have shown any difference between sham and real sessions after the Bonferroni correction was performed for multiple comparisons. The corrected alpha values were obtained by taking 0.05/number of comparisons (data not shown).

Frequency-domain parameters

None of the frequency-domain parameters has shown any difference between sham and real sessions, with or without the Bonferroni correction being performed. The LF/HF ratio was not significantly different between the two sessions (Figure 7).

Skin conductance (SC) and SCR parameters (SCRs)

Exposure to EMF

The non-specific SCR parameters (the number of non-specific SCRs, the average number of non-specific SCRs in a one-minute time frame) were not significantly different between sessions and between stages of exposure. SC, as well as tonic activity, was found to be time-variant after exposure for both the real and sham sessions as shown in Figure 8. There is a trend towards an increase for both parameters in both sessions.

Exposure to auditory stimulation

All activities (tonic and phasic) analyzed over a 120 second period decreased during exposure to auditory sounds for both the sham and real exposures ($p < 0.0001$), but not for conductance during the sham exposure. There is no significant difference between runs under stimulation between SC ($p = 0.838$) or tonic activity ($p = 0.800$) in both sessions.

Analysis was performed on the responses to the first signal and first stimulation to burst, which showed a higher response to stimulation. Only five repetitions of auditory stimulation were used. There are no significant differences between the sham and

real exposures in terms of latency, the number of SCRs and the amplitude of responses ($p > 0.05$) (results not shown).

DISCUSSION

There are few reports investigating the regulatory functioning of the cardiovascular and electrodermal systems in EHS patients. These studies suggested possible arousal and hyper-reactivity in EHS subjects (24, 16). Most of those studies were not replicated and despite an interesting hypothesis, an imbalance of the ANS or hyper-reactivity in EHS subjects was not confirmed. The majority of this research focused on the effects of exposure to several electromagnetic sources. The results of these studies indicate that the link between exposure, symptoms and alterations to the activity of the autonomic nervous system remains to be proven.

According to the baseline records, the HRV results do not indicate any differences in autonomic cardiac control between the EHS and control (non-EHS) groups. However, in response to successive auditory stimulation, the LF/HF ratio seems to gradually increase over time in both populations. For both groups, a stage effect is found for LF nu and HF nu. A decrease in HF nu reflects a parasympathetic withdrawal, and an increase in sympathetic activity (LF nu) can explain this variation. Similar findings have been reported by a study on the LF/HF ratio during sleep-deprivation (19) under EMF exposure. The LF/HF ratio may also increase or decrease in response to stressful situations (1). However, in this experiment, heart rate monitoring did not show the presence of stress. This alteration was found for both groups, and means that the responsiveness of the ANS is similar for the two study groups. Indeed, this increase in the LF/HF ratio can be attributable to the presence of sound stimuli, but psychological stress can increase the LF/HF ratio (11). In a study on magnetic fields, in which participants were asked not to move during a 64-minute experiment, an increase in the LF/HF ratio (13) over the stages of the experiment was found.

The results from the first experiment indicated that the LF/HF ratio was unaffected in EHS subjects, which does not corroborate previous studies (16,17). In their first study, they found differences in the heart rate, electrodermal activity (latency and amplitude), diastolic pressure, critical fusion frequency and LF/HF ratio of EHS volunteers in comparison to healthy subjects. These tests were significant both during baseline tests and after stress induced by mathematical tasks. In their second study, they used functional tests (22), including deep breathing, standing in a position for one minute, followed by visual acuity and contrast sensitivity. They found that the mean heart rate was different during baseline tests, whereas the LF/HF ratio and the response to standing test were decreased for the EHS group. The EHS group also had a faster onset and higher amplitude electrodermal response to auditory tones. Moreover, in a study evaluating the effects of GSM signals, the LF/HF ratio was elevated during memory tests and the critical fusion flicker threshold

measurement, but not in relaxed conditions (27). This discrepancy with our results can be partially explained by the different tests and methodology used by the studies (i.e., the absence/presence of functional or cognitive tests), but also by the difference in the characteristics of the population. The nature of the challenge to the autonomic nervous system leads to different neural responses. However, in our study, we did not include mental tasks which can induce non-autonomic task-related influences. Likewise, in the first study reported by Lyskov, participants reported to suffering from skin-related symptoms, whereas in our study EHS subjects generally reported symptoms related to mobile phone and/or extremely low frequency fields. In Wilén's study (27), only mobile phone-related subjects were involved in the study. None of the RR and HR parameters differ between our two populations. This contradicts the previous studies, which reported a higher HR associated with a shorter RR in EHS patients. In this study, the healthy subjects were matched for age, gender and body mass index, in order to obtain comparability in the groups.

According to our findings, a globally high number of skin responses to auditory stimuli are found for electrohypersensitive subjects, although the parameters of skin conductance responses (amplitude and latency) were not modified. We presently provided a further analysis of the skin conductance parameters using a newly developed method (peak/min, extraction of skin conductance responses) which had not been performed previously. Previous studies on the effects of electromagnetic fields mainly focused on skin conductance levels (7), skin resistance (19), the latency or onset of electrodermal activity (16) or the number of spontaneous peaks (17). Our analysis provided the same parameters, but additionally the skin conductance signal was decomposed, considering tonic and phasic activities to be a distinct compound.

An elevated number of spontaneous peaks during rest conditions or while performing mathematical tasks for a 10-minute duration was reported by Lyskov and colleagues (17). Combined with their previous findings on the amplitude and latency of electrodermal activity, they suggest that electrohypersensitive subjects are predisposed to physiological sensitivity to various physiological and psychosocial stressors. A higher number of responses from EHS patients can be attributable to the higher attention of electrohypersensitive individuals when compared to a control group (non-electrohypersensitive individuals). Several hypotheses, such as the individual difference hypothesis or the so-called 'electrodermal lability" hypothesis, could explain this high number of responses. In 1958, Lacey and Lacey first used the term "electrodermal lability" for subjects with high levels of non-specific responses to frequency. In contrast, individuals with few electrodermal responses were called "electrodermal stabiles". The related higher number of EDR has been associated with the acclimatization process. Multiple individual studies have explored this difference, and found that electrodermal lability is associated with neuroticism.

This "electrodermal lability" in the electrohypersensitive group could explain the absence of the effects of EMF during Experiment 2. The response rates varied significantly, as each subject represented his own control. The absence of any effects

of EMF exposure can be attributable to this characteristic. Indeed, it is also hypothesized that exposure did not have any effect on SCRs. We can also say that variability in electrodermal activity was the same during the two Experiments, and no hyper-reactivity is found in response to EMF exposure.

Our experimental studies have some limitations. Our ECG recordings in Experiment 1 were of a short duration (duration < five minutes). This makes it difficult to compare between both experiments. However, in the second study, a five-minute ECG recording was performed. Based on the spectral analysis of short-term HRV fluctuations, a sequential time of two to five minutes is useful, as reported by the literature. The recordings performed during the first experiment were also influenced by auditory stimulation, resulting in a higher LF/HF ratio, whereas in Experiment 2, there is a trend towards a decrease in the LF/HF ratio in the absence of such stimulation. These findings support the effective sensitivity of HRV to external stimulation and stress. Indeed, this could be a confounding factor for the determination of the effects of EMF, and has to be taken into account for future studies.

Secondly, we did not measure respiration rates in Experiment 1, which this would have provided important information on skin conductance and heart rate variability, since respiration could affect these parameters. In the context of our study, the results from our SCR analysis could be affected by the absence of this data and may result in an overestimation of skin conductance responses. Also, the mechanism for the generation of SCRs is complex and needs further analysis. This analysis should take into account respiratory effects and the results have to be confirmed. It is known that breathing frequency influences the spectral powers of HRV (10, 4). As a matter of fact, changes in breathing frequency (i.e., a lower or higher frequency) induces a shift in sympathetic activity to adapt to the new frequency. It is known and clearly established that a single deep breath can evoke an electrodermal response (9) and is presently used as a control response. Here, inspiratory-related SCRs have been visually inspected during Experiment 2. More generally, in order to develop a clear psychophysiological inference on signals, Cacioppo and Tassinary have suggested that the method of analysis should account for other physiological signals or events of interest (5). Future studies, including those on skin conductance responses and heart rate variability as a measure of the ANS, should take into account the interaction between different signals.

Finally, the major limitation of our study is the small number of participants in Experiment 2, as well as the absence of an additional control group, which rendered the statistical power of the analyses performed relatively low. The volunteers represented their own control. According to the perceived and potential invasive character of exposure to electromagnetic fields for people reporting symptoms, many participants declined to be exposed. Respecting the inclusion criteria (e.g., the absence of chronic illness) due to the biological analysis performed in parallel also contributed to the restricted number of participants.

CONCLUSION

We still reiterate that the generation of SCR is a rather complex phenomenon involving cognitive and respiratory processes. Meanwhile, according to our protocol, electrosensitivity is not associated with an imbalance in the regulation of the autonomic nervous system. Exposure to electromagnetic fields did not have any effect on those parameters. Due to the relative low number of participants, our study results have to be confirmed with a higher number of participants and a comparative group, which will be very interesting to support findings on the cardiovascular modulation of autonomic nervous system.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions

B.S. conceived and designed research

S.A. and L.H. performed experiments

S.A. and JG performed statistics and prepared figures

B.S., S.A., J.G., E.S.B and F.T. interpreted results

B.S. And S.A. drafted manuscript

B.S., S.A., F.T. edited and revised manuscript

B.S., E.S.B and F.T. approved final version of manuscript.

REFERENCES

1. **Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ.** Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213:220–222, 1981.
2. **Andrianome S, Hugueville L, de Seze R, Hanot-Roy M, Blazy K, Gamez C, Selmaoui B.** Disturbed sleep in individuals with Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF): Melatonin assessment as a biological marker. Bioelectromagnetics. 37 (3) : 175–182, 2016.
3. **Benedek M, Kaernbach C.** A continuous measure of phasic electrodermal activity. *J Neurosci Methods* 190:80–91, 2010.
4. **Brown TE, Beightol LA, Koh J, Eckberg DL.** Important influence of respiration on human R-R interval power spectra is largely ignored. *J Appl Physiol* Bethesda Md 1985 75:2310–2317, 1993.
5. **Cacioppo JT, Tassinary LG.** Inferring psychological significance from physiological signals. *Am Psychol* 45:16–28, 1990.
6. **Eltiti S, Wallace D, Ridgewell A, Zougkou K, Russo R, Sepulveda F, Fox E.** Short-term exposure to mobile phone base station signals does not affect cognitive functioning or physiological measures in individuals who report sensitivity to electromagnetic fields and controls. *Bioelectromagnetics* 30:556–563, 2009.
7. **Eltiti S, Wallace D, Ridgewell A, Zougkou K, Russo R, Sepulveda F, Mirshekar-Syahkal D, Rasor P, Deeble R, Fox E.** Does short-term exposure to mobile phone base station signals increase symptoms in individuals who report sensitivity to electromagnetic fields? A double-blind randomized provocation study. *Environ Health Perspect.* 115:1603–1608, 2007.
8. **Furubayashi T, Ushiyama A, Terao Y, Mizuno Y, Shirasawa K, Pongpaibool P, Simba AY, Wake K, Nishikawa M, Miyawaki K, Yasuda A, Uchiyama M, Yamashita HK, Masuda H, Hirota S, Takahashi M, Okano T, Inomata-Terada S, Sokejima S, Maruyama E, Watanabe S, Taki M, Ohkubo C, Ugawa Y.** Effects of short-term W-CDMA mobile phone base station exposure on women with or without mobile phone related symptoms. *Bioelectromagnetics* 30:100–113, 2009.
9. **Hay JE, Taylor PK, Nukada H.** Auditory and inspiratory gasp-evoked sympathetic skin response: age effects. *J Neurol Sci* 148:19–23, 1997.
10. **Hirsch JA, Bishop B.** Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am. J. Physiol* 241:H620-629, 1981.
11. **Hjortskov N, Rissén D, Blangsted AK, Fallentin N, Lundberg U, Søgaard K.** The effect of mental stress on heart rate variability and blood pressure during computer work. *Eur. J Appl Physiol* 92:84–89, 2004.

12. **Kato Y, Johansson O.** Reported functional impairments of electrohypersensitive Japanese: A questionnaire survey. *Pathophysiol. Off. J. Int. Soc. Pathophysiol. ISP* 19:95–100, 2012.
13. **Kim SK, Choi JL, Kwon MK, Choi JY, Kim DW.** Effects of 60 Hz magnetic fields on teenagers and adults. *Environ. Health* 12:42, 2013.
14. **Kligfield P, Gettes LS, Bailey JJ, Childers R, Deal BJ, Hancock EW, van Herpen G, Kors JA, Macfarlane P, Mirvis DM, Pahlm O, Rautaharju P, Wagner GS.** Recommendations for the Standardization and Interpretation of the Electrocardiogram: Part I: The Electrocardiogram and Its Technology A Scientific Statement From the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society Endorsed by the International Society for Computerized Electrocardiology. *J Am Coll Cardiol* 49:1109–1127, 2007.
15. **Kwon MK, Choi JY, Kim SK, Yoo TK, Kim DW.** Effects of radiation emitted by WCDMA mobile phones on electromagnetic hypersensitive subjects. *Environ Health* 11:69, 2012.
16. **Lyskov E, Sandström M, Hansson Mild K.** Neurophysiological study of patients with perceived "electrical hypersensitivity." *Int J Psychophysiol Off J Int Organ Psychophysiol* 42:233–241, 2001a
17. **Lyskov E, Sandström M, Mild KH.** Provocation study of persons with perceived electrical hypersensitivity and controls using magnetic field exposure and recording of electrophysiological characteristics. *Bioelectromagnetics* 22:457–462, 2001b.
18. **Mild KH, Repacholi M, van Deventer E, Ravazzani P.** Electromagnetic hypersensitivity, in: proceedings of the International Workshop on EMF Hypersensitivity, Prague, Czech Republic, October 25–27, 2004, World Health Organization, 2006.
19. **Nam KC, Kwon MK, Kim DW.** Effects of posture and acute sleep deprivation on heart rate variability. *Yonsei Med J* 52:569–573, 2011.
20. **Nam KC, Lee JH, Noh HW, Cha EJ, Kim NH, Kim DW.** Hypersensitivity to RF fields emitted from CDMA cellular phones: a provocation study. *Bioelectromagnetics* 30:641–650, 2009.
21. **Oftedal G, Nyvang A, Moen BE.** Long-term effects on symptoms by reducing electric fields from visual display units. *Scand. J Work Environ Health* 25:415–421, 1999.
22. **Ravits JM.** AAEM minimonograph #48: autonomic nervous system testing. Muscle Nerve 20:919–937, 1997.

23. **Röösli M, Moser M, Baldinini Y, Meier M, Braun-Fahrländer C.** Symptoms of ill health ascribed to electromagnetic field exposure--a questionnaire survey. *Int J Hyg Environ Health* 207:141–150, 2004.
24. **Sandström M, Lyskov E, Berglund A, Medvedev S, Mild KH.** Neurophysiological effects of flickering light in patients with perceived electrical hypersensitivity. *J Occup Environ Med Am Coll Occup Environ Med* 39:15–22, 1997.
25. **Sandström M, Lyskov E, Hörnsten R, Hansson Mild K, Wiklund U, Rask P, Klucharev V, Stenberg B, Bjerle P.** Holter ECG monitoring in patients with perceived electrical hypersensitivity. *Int J Psychophysiol Off J Int Organ Psychophysiol* 49:227–235, 2003.
26. **Wallace D, Eltiti S, Ridgewell A, Garner K, Russo R, Sepulveda F, Walker S, Quinlan T, Dudley S, Maung S, Deeble R, Fox E.** Do TETRA (Airwave) Base Station Signals Have a Short-Term Impact on Health and Well-Being? A Randomized Double-Blind Provocation Study. *Environ Health Perspect* 118:735–741, 2010.
27. **Wilén J, Johansson A, Kalezic N, Lyskov E, Sandström M.** Psychophysiological tests and provocation of subjects with mobile phone related symptoms. *Bioelectromagnetics* 27:204–214, 2006.

552 Figures

553

554 Figure 1. Experimental procedures for measuring physiological changes during Experiment
555 1. SC: skin conductance, no auditory stimulation; HRV: heart rate variability; RED:
556 electrodermal response to auditory stimulation.

557

558 Figure 2. Experimental procedures for measuring physiological changes during Experiment 2
559 (sham or real sessions). SC: skin conductance, no auditory stimulation; HRV: heart rate
560 variability; RESP: measurement of breathing frequency.

561

562 Figure 3. Procedures during the post-exposure period (Experiment 2). SC: skin conductance,
563 no auditory stimulation; RED: electrodermal response to auditory stimulation; HRV: heart rate
564 variability; RESP: measurement of breathing frequency.

565

566 Figure 4. Time-domain parameters of heart rate variability during Experiment 1. Data are
567 presented as mean ± SEM, RM-ANOVA: No significant effect on groups for RR, SDNN and
568 RMSSD.

569

570 Figure 5. Frequency-domain parameters of heart rate variability during Experiment 1. Data
571 are presented as mean ± SEM, RM-ANOVA: No significant effect on groups for LF, HF and
572 LF/HF ratio.

573

574 Figure 6. Variations of LF nu and HF nu during Experiment 1. Data are presented as Mean ±
575 SEM. No significant effect on groups.

576

577 Figure 7. Inter-session comparisons of LF/HF ratio (a) and HR (b) in EHS participants
578 exposed to different radiofrequency signals during Experiment 2. Data are presented as
579 mean ± SEM.

580

581 Figure 8. Inter-session comparisons of skin conductance (a) and tonic activity (b) in EHS
582 participants exposed to different radiofrequency signals during Experiment 2. Data are
583 presented as mean ± SEM.

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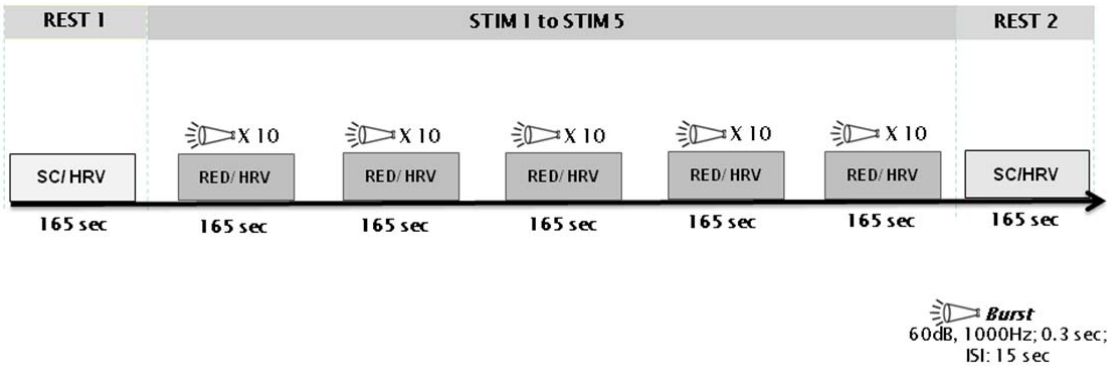

REST 1
STIM 1 to STIM 5
REST 2
X 10
X 10
X 10
X 10
X 10
SC/HRV
RED/HRV
RED/HRV
RED/HRV
RED/HRV
RED/HRV
SC/HRV
165 sec
165 sec
165 sec
165 sec
165 sec
165 sec
165 sec
Burst
60dB, 1000Hz; 0.3 sec;
ISI: 15 sec

Fig.1

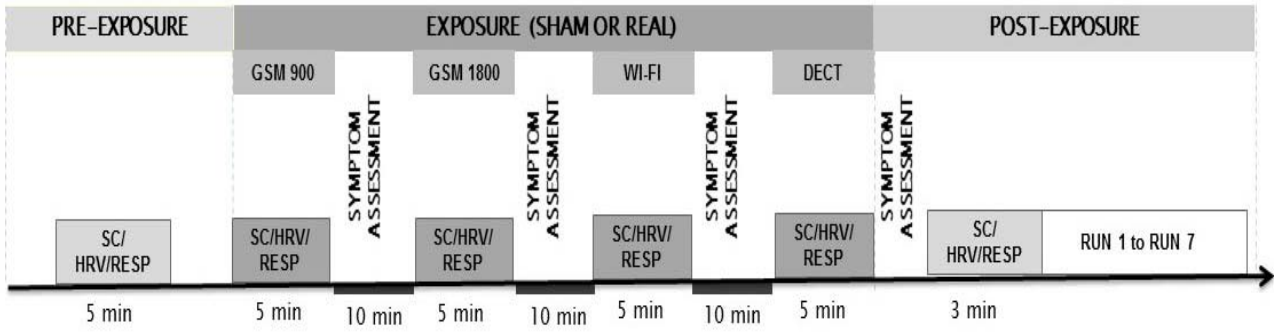

PRE-EXPOSURE
EXPOSURE (SHAM OR REAL)
POST-EXPOSURE
GSM 900
GSM 1800
WI-FI
DECT
SYMPTOM ASSESSMENT
SYMPTOM ASSESSMENT
SYMPTOM ASSESSMENT
SYMPTOM ASSESSMENT
SC/ HRV/RESP
SC/HRV/ RESP
SC/HRV/ RESP
SC/HRV/ RESP
SC/HRV/ RESP
SC/ HRV/RESP
RUN 1 to RUN 7
5 min
5 min
10 min
5 min
10 min
5 min
10 min
5 min
3 min

Fig. 2

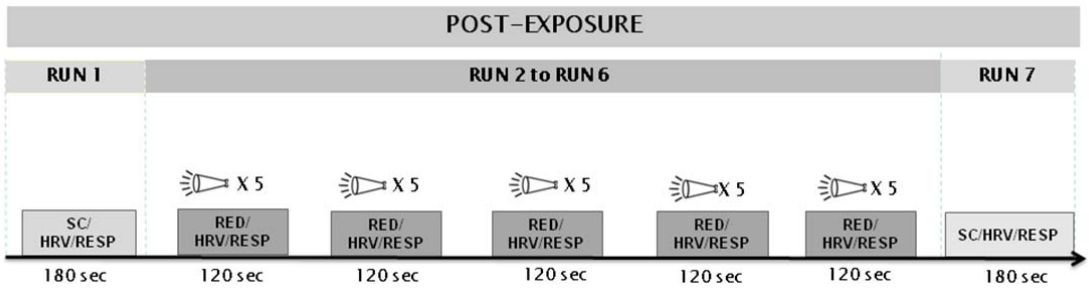

POST-EXPOSURE
RUN 1
RUN 2 to RUN 6
RUN 7
X 5
X 5
X 5
X 5
X 5
SC/
HRV/RESP
RED/
HRV/RESP
RED/
HRV/RESP
RED/
HRV/RESP
RED/
HRV/RESP
RED/
HRV/RESP
SC/HRV/RESP
180 sec
120 sec
120 sec
120 sec
120 sec
120 sec
180 sec

Fig.3

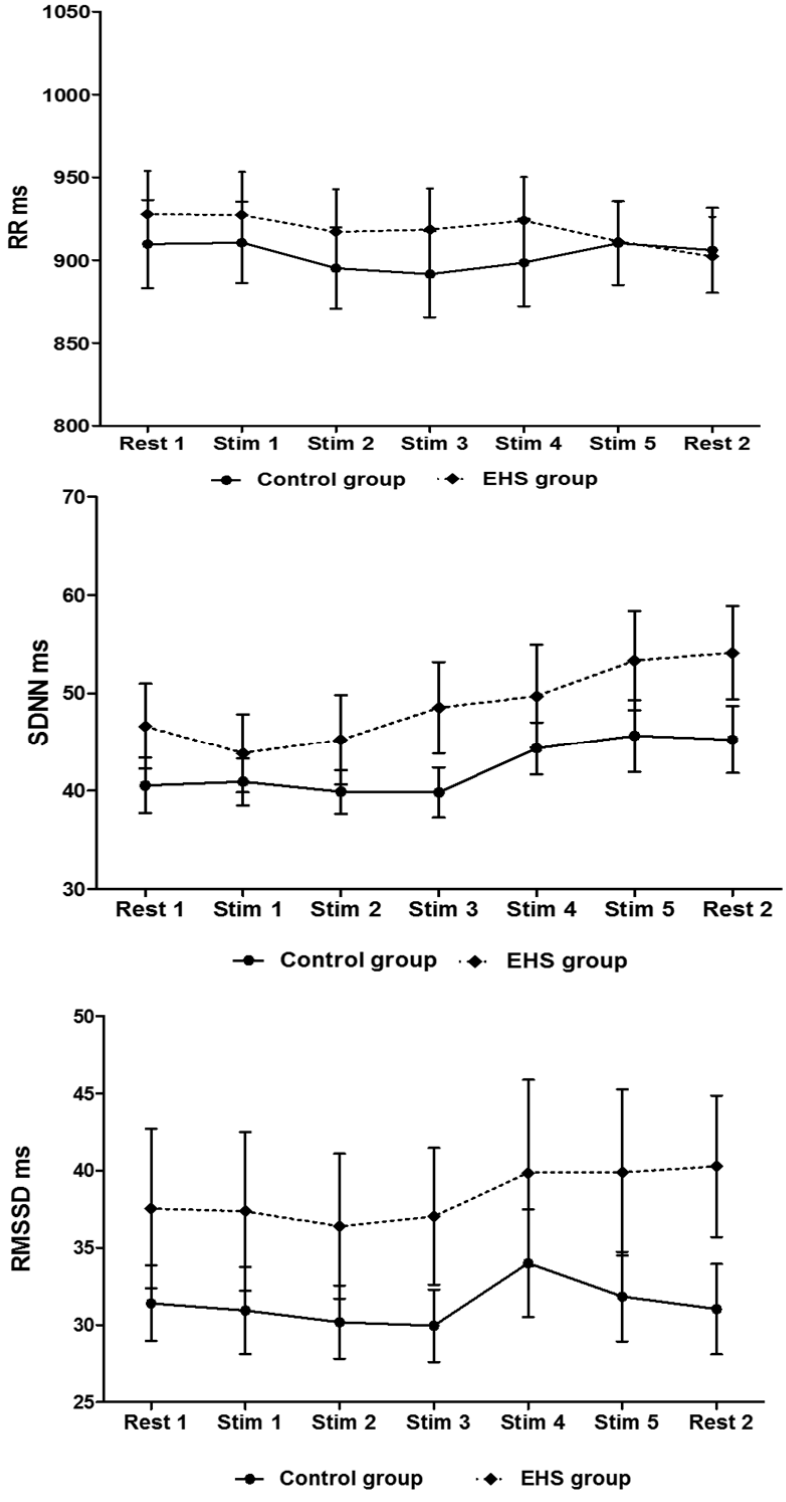
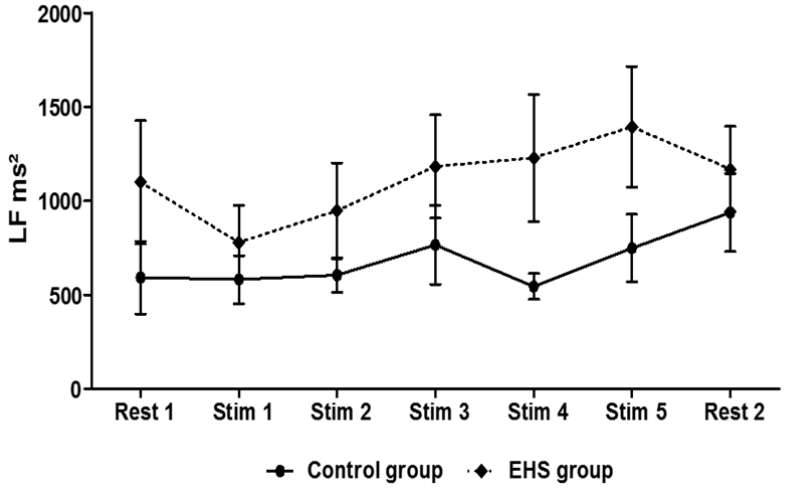
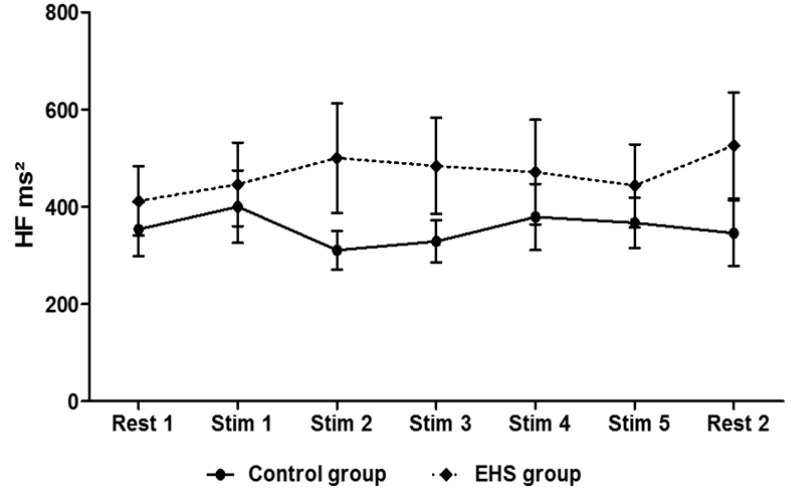

1050
1000
950
900
850
800
RR ms
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group
70
60
50
40
30
SDNN ms
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group
50
45
40
35
30
25
RMSSD ms
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group

Fig.4


2000
1500
1000
500
0
LF ms²
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group


800
600
400
200
0
HF ms²
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group

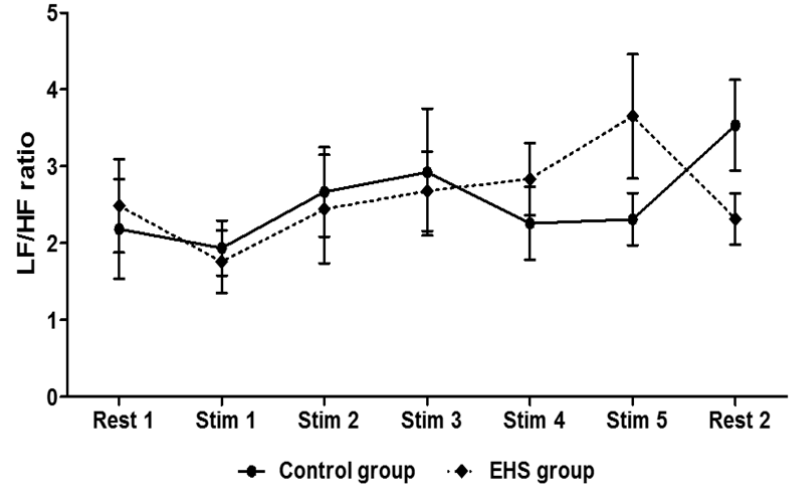
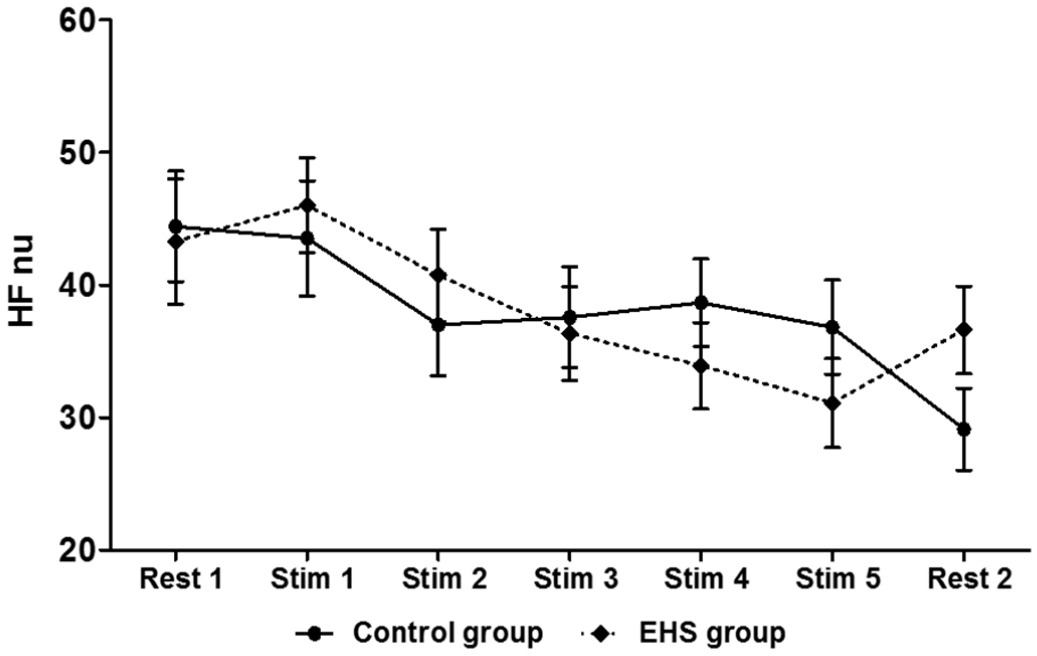

5
4
3
2
1
0
LF/HF ratio
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group

Fig.5


60
50
40
30
20
HF nu
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group

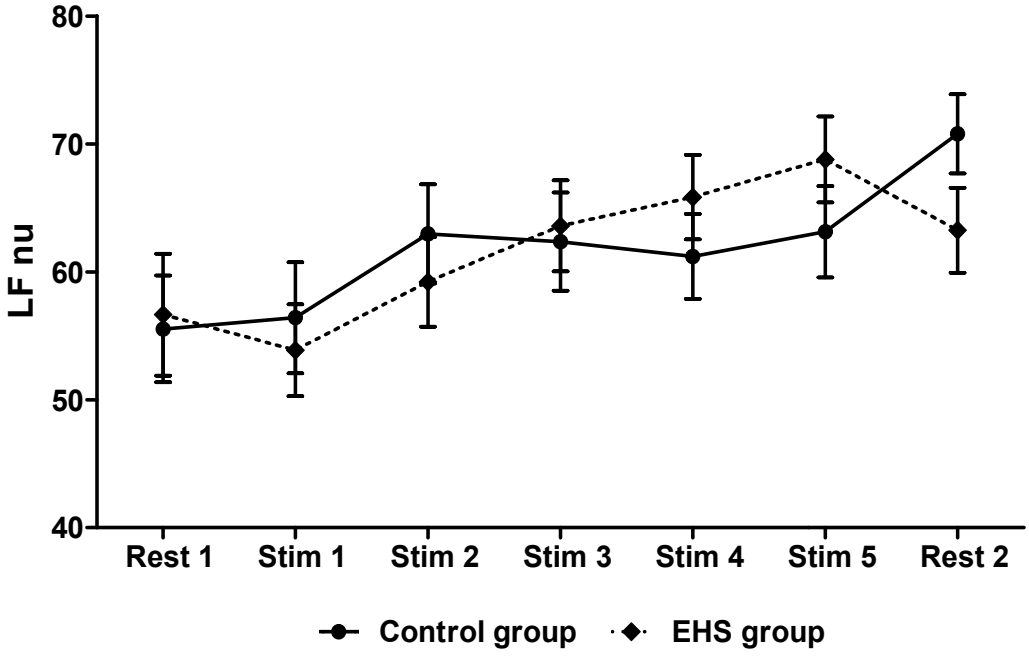
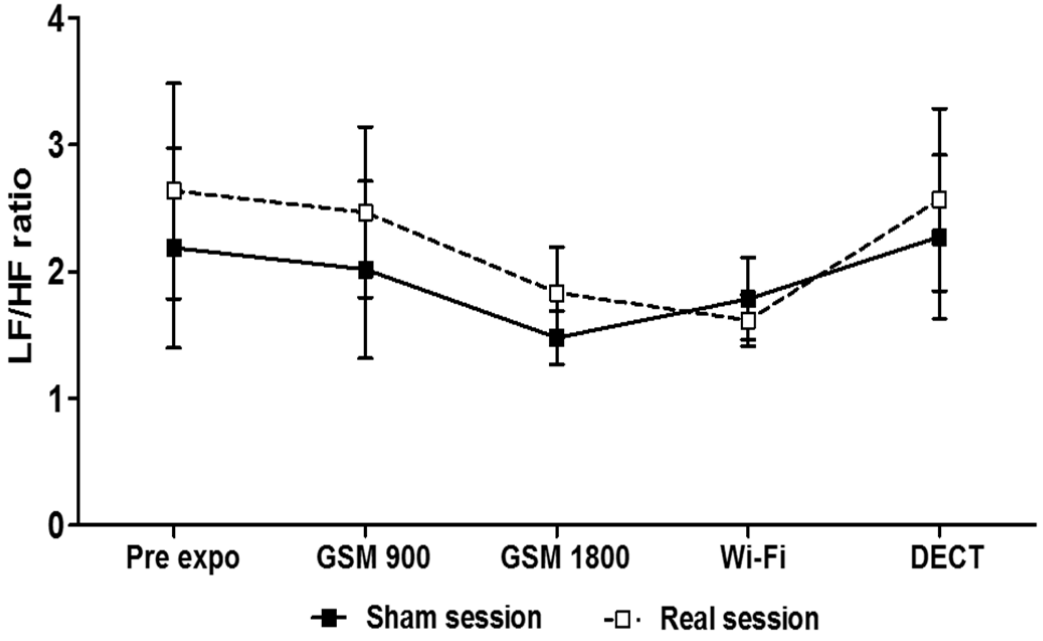

80
70
60
50
40
LF nu
Rest 1
Stim 1
Stim 2
Stim 3
Stim 4
Stim 5
Rest 2
Control group
EHS group

Fig. 6


LF/HF ratio
4
3
2
1
0
Pre expo
GSM 900
GSM 1800
Wi-Fi
DECT
Sham session
Real session

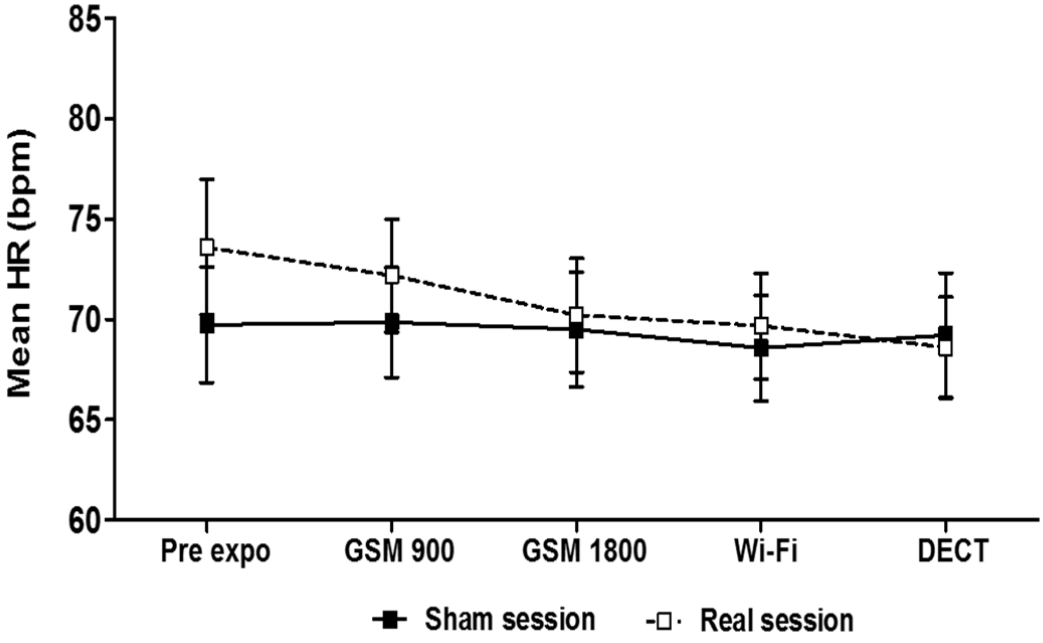
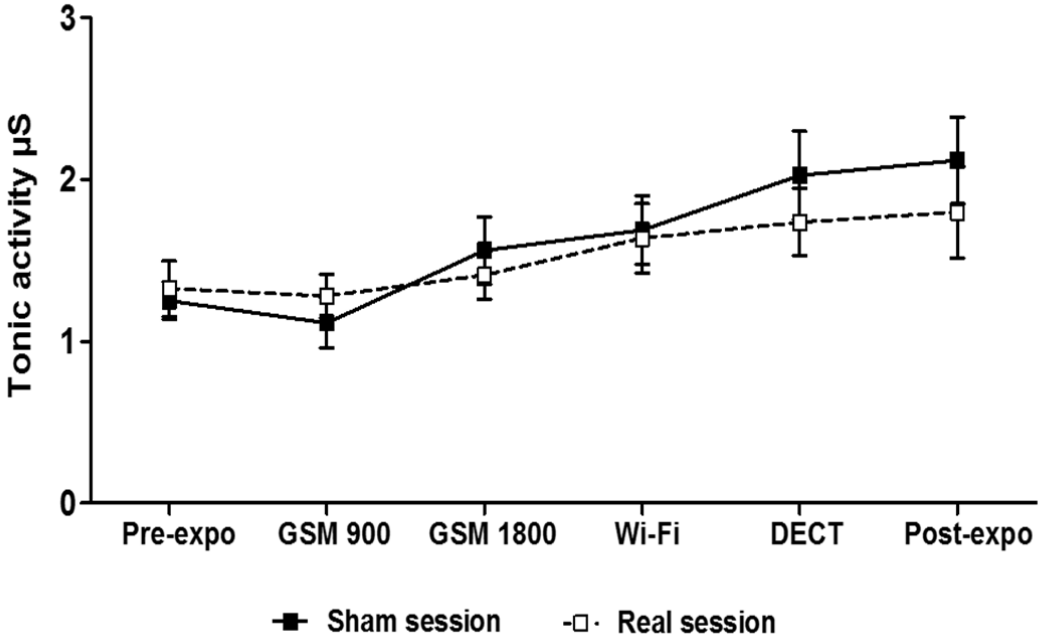

Mean HR (bpm)
85
80
75
70
65
60
Pre expo
GSM 900
GSM 1800
Wi-Fi
DECT
Sham session
Real session

Fig.7


Tonic activity µS
3
2
1
0
Pre-expo
GSM 900
GSM 1800
Wi-Fi
DECT
Post-expo
Sham session
Real session

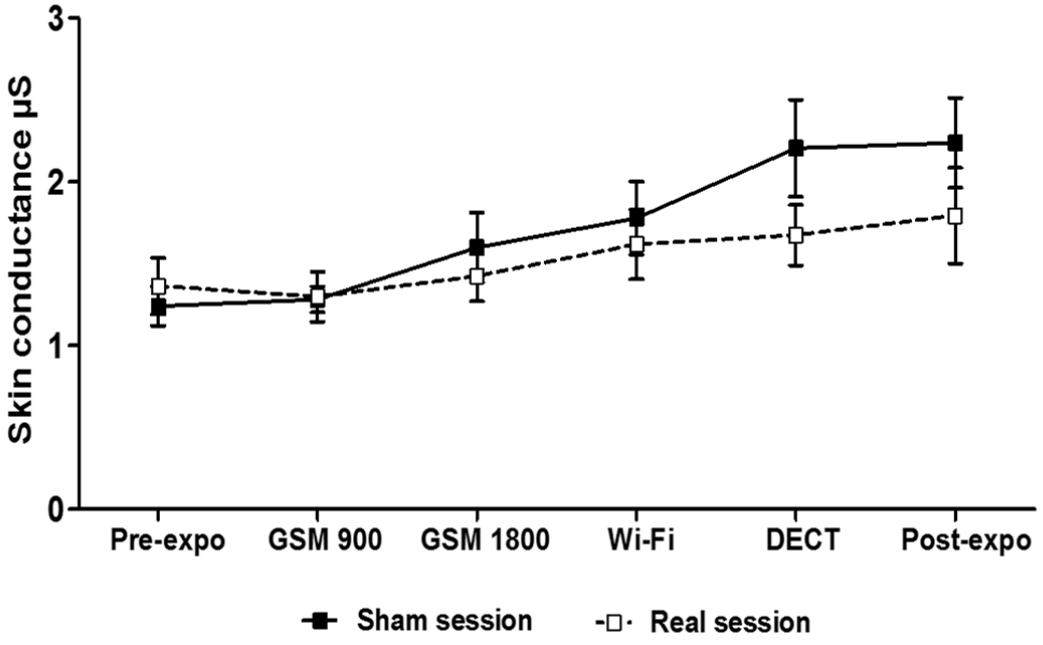

Skin conductance µS
3
2
1
0
Pre-expo
GSM 900
GSM 1800
Wi-Fi
DECT
Post-expo
Sham session
Real session

Fig.8