Chronic Exposure of Low Power Radio Frequency Changes the EEG Signals of Rats

Low power radio frequency alters EEG

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Abstract

Central nervous system of mammals is known to be highly vulnerable to the environmental stimulus, including radio-waves. The aim of this work is to evaluate the chronic effect (one hour daily for 21 days) of low power radio frequency of 1 KHz square wave modulated 2.45 GHz on the electroencephalogram (EEG) in animal model. Experiments were carried out on male rats (n=10), weighing 100(20) gm and age of 9-10 weeks divided in two groups: (i) control (n=4) and (ii) experimental (n=6). After 21 day of experiments, three hours of single channel bipolar EEG signals were recorded from parietal cortex of rats' brain on 22nd day continuously, under anesthetized condition (Urethane anesthesia: 1.6 gm/kg of body weight, *i.p.*). The power spectra of the EEG signals were calculated for analysis of changes in EEG frequency spectrum. The core body temperature was also recorded and evaluated for the thermal nature of the exposure. Student t-test was applied to the EEG spectral results as well as on temperature data to analyze the significance of changes. The analyses suggest that this experimental setup of exposure produces non-thermal effects, however, significantly increased ($P<0.05$ or better) the power of EEG signals. The EEG spectral changes were found most prominent on the higher frequency side. On the basis of results of this study, it can be suggested that the chronic exposure of low power radio frequencies have sufficient energy to change the brain function that may lead to the development of different psychopahological disorders.

Keywords

Chronic Exposure; Electroencephalogram; Low Power Radio Frequency; Rats

Introduction

With increase in technologies related with the high frequency radio-waves, the exposure of living subjects to this is evident. In past, many reports have been published in support of its hazardous effect, however, with contradictions (Lin, 2003; Hossmann & Hermann, 2003; D'Andrea et al., 2003). It is now established that the nervous system of the mammals is highly vulnerable to the exposure to external as well as internal environmental stimuli (Sinha, 2004). The primary effect of high frequency radio-waves on nervous system has been evaluated and it is confirmed that even low power radio-waves have sufficient energy to open the blood-brain barrier of the exposed subjects (Chou et al., 1992). Along with the confirmed extravagation of blood-brain barrier, alterations in neurotransmitter levels have also been reported that might have correlations with changed neuronal firing of the brain (Hinrikus et al., 2005).

The review of literature also revealed that the altered firing of neuron ultimately alters the electroencephalogram (EEG) in effect to radio frequency exposure (Lin, 2003; D'Andrea et al., 2003; Huber et al., 2002; Hinrikus et al., 2008; Wu et al., 2009), however, highly dependent on the exposure power, time, frequency, modulation frequency and the vigilance state of the subject (Fritzer et al., 2007; Sinha et al., 2008). The uses of 2.45 GHz radio frequency in physiotherapy, domestic as well as in industrial cooking are very common. Now days, this band of radio frequency is also widely used for the wireless communications (Mahatthanajatuphat et al., 2009). In all of these applications, humans are chronically exposed to the varied power of radio-waves, which may have hazardous effects on the exposed subjects. It is now more or less established that even the low power non-thermal radio frequency exposure is sufficient to produce functional changes at cellular level (Lee et al., 2005) that may be reflected with the alterations in physiological parameter (Crouzier et al., 2007). However, very few literatures are available demonstrating the effects of low power and non-thermal exposure of 2.45 GHz radio frequencies on the brain signals.

Therefore, an experiment has been designed with the chronic, low power, non-thermal exposure of 2.45 GHz radio frequency to analyze the EEG spectral alterations on young rats. The EEG signals are highly non-linear and its spectral variations are dependent on many psychophysiological effectors including the vigilance state of the subject. Thus, avoiding the frequent alterations in vigilance states, the experiment has been designed to evaluate the changes in EEG signals of the rat under the irreversible anesthetic condition with exposure to low energy radio frequency.

Materials and Methods

Subjects

Experiments were carried out on 12 healthy male rats (9-10 weeks of age and 100(20) gm of weight) for two groups: (i) control group (n=4) and (ii) Exposure group (n=8). All rats (n=12) were individually housed in polypropylene cages (30 cm × 20 cm × 15 cm) with drinking water and commercial laboratory food pellet *ad libitum*. The room temperature was artificially illuminated with a 12L:12D (light cycle during 7:00 A.M. to 19:00 P.M. Indian Standard Time (IST)) cycle at 24±1°C. The EEG data were recorded from four control and six exposed subjects for further analysis. Two exposed rats were died during the surgery. All procedures in this study have been conducted after ethical approval from institutional ethical committee and in compliance with 'committee for purpose of control and supervision of experiments on animals (CPCSEA), India as well as with internal institutional policies and guidelines.An easy way to comply with the journal paper formatting requirements is to use this document as a template and simply type your text into it.

Exposure Setup

The radio frequency work bench for 1 KHz square wave modulated 2.45 GHz (Source: SSS 940S, SICO, India) has been used for the exposure of low power chronic exposure of 1 hour duration (3:00 to 4:00 PM) daily for 21 days. The control group of rats were also subjected to the exposure setup, however, without switching-on the microwave source and similar procedure was followed as with the exposed group of subjects. The animal holder (35 cm × 34 cm × 26 cm) was made with the Perspex glass to hold 4 animals at a time and the partition has been given by pine wood to keep the animals in separate chamber as suggested in Sinha et al. (2008). Holes were made on each side of the four chambers of the animal holder to provide proper ventilation to the rats during the exposure. The base of the animal holder was made of Styrofoam and the exposure is given from the bottom of the animal holder. For the radio frequency exposure, the animal holder with four rats was directly placed on the top of horn antenna heading towards the roof (Fig. 1). The base of the designed animal holder was having the same dimension as of the antenna, so that it can be properly fixed on the antenna top. The power density of the radiation was 7.37×10^{-4} mW/cm^2 and the specific absorption rate (SAR) was calculated as 1.16 mW/Kg using the standard method (Kubacki and Sobiech, 2006) using USB based power meter (EmVib Technologies, India).

FIG. 1 MICROWAVE EXPOSURE SETUP USED FOR THIS STUDY

Preparation and Data Collection

Surgical Procedure and EEG Recording: Single channel EEG has been recorded from control as well as from radio frequency exposed subjects from the rat brain under irreversible Urethane anesthesia (1.6 gm/kg of body weight, *i.p.*) obtained from Sigma Chemicals Co., USA (Sinha & Aggarwal, 2007). Three stainless steel screw electrodes of 1 mm diameter were placed aseptically on the rat's skull under the stereotaxic guidance (Inco, India). Two bipolar, bilateral screw electrodes were connected 2 mm posterior and 4 mm lateral to bregma. One ground electrode was fixed on the anterior most region of the skull. Electrodes were fixed with the help of dental acrylic. The surgical procedure for data recording was performed on 22nd day of the experiment. One hour stabilization time was given to each of the subjects after surgical procedure before the electrophysiological recording. The EEG signals were recorded continuously for three hours from 9.00 AM to 12.00 noon from each rat on separate day (Sinha & Aggarwal, 2007) with the help of 2-channel bioamplifier mobile unit (MP 45) and its compatible 'Biopac Student lab. 3.7.5 Lessons & Pro, and processed with the help of 'Acknowledge 4.0' software (obtained from Biopac Systems Inc., USA).

Temperature Recording: The core body temperature has also been monitored five minutes before subjecting and within five minutes after removal of rats from the animal holder during 21 days of experiment. It was recorded using thermistor probe connected to 6-channel telethermometer (Scan-96, Micron, India) for both the exposure as well as the control groups. The probe marked at 4 cm was inserted into the rectum of the animal and kept static for 1 minute to record the body temperature.

Data Processing and Analysis

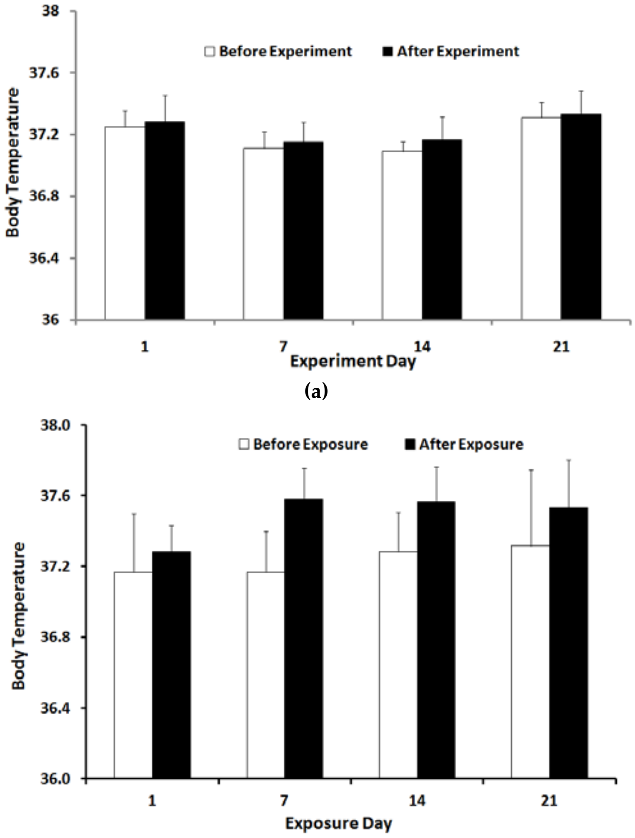
The EEG data were band-pass filtered (1-40Hz) and the power spectrum density (PSD) was calculated for the 6 segments each of 30 minutes duration (1st is for first 30 minutes and 6th is for last 30 minutes) for the 3 hours recording. In each segment, 10 epochs were randomly selected to calculate the PSD and the average PSD of each segment were studied. The EEG frequency spectrum was divided in four conventional frequency sub-bands; delta (0.5-3.99 Hz), theta (4.0-7.99 Hz), alpha (8.0-12.99 Hz) and beta (13.0-30.0 Hz). From each epoch, maximum power of each of the four EEG frequency sub-bands was taken for the statistical evaluation. The student's t-test was applied to analyze the statistical significance in EEG signals. Similarly, the student's t-test was also applied to the temperature data taken to analyze the temperature variations in the rats due to radio frequency exposure of 2.45 GHz.

Results

The analyses of results have been presented in two sections for the changes, if any, in body temperature and EEG frequency spectrum.

Analysis of Changes in Body Temperature

The core body temperature data of radio frequency exposure and control groups of rats were analyzed on 1st, 7th, 14th and 21st day of experimental exposure (Fig. 2a&b). The statistical analysis of the data suggested insignificant changes in core body temperature on all four days of analysis with the used exposure power of 1 KHz square wave modulated 2.45 GHz radio frequency exposed for one hour duration. Therefore, the exposure effect of the used experimental radio frequency power is found to be non-thermal. However, minor rise in body temperature were observed in both exposed and control groups of rats after the experimental procedure.



(a)

(b)

FIG. 2 COMPARISON OF CHANGES IN BODY TEMPERATURE (°C) OF RATS (N=8) DUE TO ONE HOUR OF EXPOSURE OF 1 KHZ SQUARE WAVE MODULATED 2.45 GHZ RADIO FREQUENCY. BODY TEMPERATURE DATA WERE RECORDED AND ANALYZED FOR 1ST, 7TH, 14TH AND 21ST DAY OF EXPERIMENT AND PRESENTED AS MEAN±S.E FOR (a) CONTROL AND (b) EXPOSURE GROUPS OF RATS

Analysis of Changes in EEG Frequency Spectrum

From the analysis of results (Table 1), it can be revealed that 2.45 GHz radio frequency, modulated with 1 KHz square wave changes the EEG frequency spectrum significantly. Overall, the power of EEG spectrum was found to be increased in each of the frequency sub-bands due to the exposure. However, the increase in power was highly significant and dominant in higher frequency bands, *i.e.*, beta ($P<0.05$). Increase in power of EEG frequency was also evident on second hour of EEG recording in delta ($P<0.1$) and

TABLE 1 EVALUATION OF RELATIVE CHANGES IN POWER OF EEG SIGNALS IN FOUR FREQUENCY SUB-BANDS DUE TO ONE HOUR OF EXPOSURE OF 1 KHZ SQUARE WAVE MODULATED 2.45 GHZ RADIO FREQUENCY. DATA ARE PRESENTED AS MEAN(S.E.). DATA WERE STATISTICALLY COMPARED FOR SIGNIFICANCE OF CHANGES WITH *(P<0.1) AND **(P<0.05). THE T-VALUE FOR ANALYZING THE SIGNIFICANCE OF CHANGES IS PRESENTED IN THE TABLE

Time (Min)	Delta (0.5-3.99) Hz		Theta (4.0-7.99) Hz		Alpha (8.0-12.99) Hz		Beta (13.0-30.0) Hz	
	Control Group	RF Exposed Group	Control Group	RF Exposed Group	Control Group	RF Exposed Group	Control Group	RF Exposed Group
0-30	2.621(0.895)	4.115(0.691)	1.597(0.240)	2.314(0.421)	0.905(0.197)	1.351(0.271)	0.933(0.235)	1.530(0.305)
30-60	2.919(1.001)	4.194(0.726)	1.563(0.315)	2.094(0.348)	0.767(0.097)	0.994(0.113)	0.566(0.047)	1.007(0.129)**
60-90	1.758(1.085)	4.963(1.112)*	0.952(0.499)	2.484(0.570)*	0.487(0.229)	1.371(0.263)**	0.424(0.174)	1.282(0.218)**
90-120	2.301(1.242)	5.412(1.426)	0.752(0.251)	2.810(0.650)**	0.651(0.264)	1.406(0.192)**	0.595(0.206)	1.520(0.232)**
120-150	3.745(2.182)	5.439(1.668)	0.920(0.385)	2.735(0.619)*	0.731(0.343)	1.124(0.167)	0.684(0.268)	1.150(0.158)
150-180	2.606(1.730)	4.011(1.362)	0.739(0.264)	2.385(0.715)*	0.719(0.330)	1.295(0.287)	0.527(0.203)	1.280(0.193)**

alpha ($P<0.05$) sub-bands. However, it was very interesting to note that theta frequency band ($P<0.05$ for second hour and $P<0.1$ for third hour, respectively) shown greater increase than that of delta and alpha sub-bands as the significant increase was recorded on second and third hour of recording. Further, it was also analyzed that increase in EEG power on different sub-bands were significantly apparent on the second hour; however, it was generally insignificant on the first hour of recording.

Discussion

It is evident from the observations that even though no statistical difference was analyzed due to radio frequency exposure, slight increase in the body temperature of the subjects were apparent in both exposure and control groups. The experiments were performed in daytime, during which, the nocturnal animals like rats were observed to remain in sleep conditions during most of the experiment period. The core body temperature of rats was analyzed before and after subjecting them to the animal holder of the experimental setup. Slight increase in body temperature in this experiment may have observed due to thermoregulatory responses during the sleep as suggested earlier (Thomas and, Kumar, 2000; Sinha & Ray, 2006). The results demonstrate that this experimental exposure pattern produces significant effects on the power of EEG frequency spectrum of the rats, while the maximum variations were observed in the higher frequency band, *i.e.*, the beta frequency band. In contrary to the present work, past research on chronic exposure to hot environment reveals that the power of higher frequency components (higher β components: 18-30 Hz) are sensitive to ambient heat and reported to decrease significantly in all three sleep-wake states following exposure to chronic heat stress (Sinha & Ray, 2004). The analysis suggests that the chronic exposure to radio frequency produced varied results in comparison to the exposure to chronic heat stress. Simultaneously, insignificant changes in body temperature of the rats have been analyzed from day 1 to 21st day of exposure, which suggests that the used exposure pattern produces non-thermal effects. Thus, it can reveal that the changes in the power of EEG frequency spectrum observed in this experiment have occurred due to the non-thermal interactions of radio frequency with the brain, not due to its heating effects.

This experiment is simulated for the leakage power of some important domestic and medical radio frequency equipment. The effects of chronic exposure for one hour daily, continuously for 21 days of low power radio-waves of 1 KHz square wave modulated 2.45 GHz on EEG is evaluated. It is already established that 21 days of chronic exposure produces acclimatization of the animals to the experimental environmental conditions and numerous physiological parameter changes toward setting a new 'set point' (Day, 2000; Sharma et al., 1998). It has been established that chronic exposure to non-thermal radio frequency irradiations can affect vital organs; however, its effects depend on radiation intensity, frequency of exposure, modulation frequency and the exposure duration (D'Andrea et al., 2003). Similar protocol for radio frequency exposure has also been followed earlier to evaluate the effects of microwave on sleep-EEG of rats (Sinha et al., 2008). Further, to evade the acute effects of the exposure pattern, the recording was performed on 22nd day of exposure. In these experiments, the metallic electrode implantation during the exposure period is not recommended as the radio-waves interact with the metallic electrodes and produce local changes in the cortex of brain. Thus, the intermediate recording of EEG (*i.e.*, recording of EEG on different days of exposure) is not done in this study. Further, to overcome the spectral variations due to shift in vigilance states in non-linear EEG signals, in this study, data were recorded from the anesthetized subjects.

The effects of low power radio frequency of 2.45 GHz on pathophysiology of the brain have already been reported in past (D'Andrea et al., 2003; Sinha et al., 2008). However, no report has come to the authors' knowledge with 2.45 GHz radio frequency that experiments with the EEG signals on the anesthetized subjects. Central nervous system of mammals is known to be highly sensitive to the external and internal stimuli, which controls all the psychophysiological and behavioral activities (Sinha, 2006). The ill effects of high frequency radio-waves on the behavior and brain electrophysiology of mammals have already been reported (Sienkiewicz et al., 2005; Hinrikus et al., 2010; 2011; Schmid et al., 2012). Nevertheless, the interaction mechanism of radio frequencies with the nervous system is still in controversy. Some of the well-known mechanisms, through which the radio frequencies may alter the neuronal electrophysiology and its firing patterns, are based on the changes in extravasations in blood-brain barrier as well as the alterations in synaptic properties of neurons with quantitative changes in different neurotransmitter levels (Neubauer et al., 1990; Chou et al., 1992; D'Andrea et al., 2003; Salford et al., 2003; Cosquer et al., 2005; Sinha et al., 2008). Considering these hypothesis, it can be suggested that the chronic exposure of radio-waves in the present experiment changes the extravasations of blood-brain barrier that further altered the synchronization/desynchronization of neuronal firing in sub-cortical region of the rat brains and hence, resulted in the changes in power of EEG frequency spectrum. Although, the exact biological mechanism and correlations of the alterations in EEG power spectrum with other biological variables in this study has not been identified in this work, it is assumed that various neuochemicals and other factors are participating in enhancing the frequency spectrum of EEG signals. Furthermore, intermittent monitoring of body temperature of the subjects was not done during the EEG recording that may also provide insight regarding the correlation, if any, between the variations in body temperature with the alterations in brain signals. However, it is evident that the Urethane anesthesia produces subject wise hypothermic effects that lowers the body temperature from 0.5 to 1°C (Sinha & Aggarwal, 2007). These hypothermic effects have been observed in both exposure and control groups of subjects.

Conclusion

With the gained importance of radio-wave applications in industry, communication, military, medical and domestic uses; it is utmost necessary to evaluate its hazards on living systems. Nevertheless, with this experimental finding, the hazardous effects of low power 2.45 GHz radio frequency on EEG signals can definitely been suggested that may also be correlated with occurrence of many psychopathophysiological disorders to the subjects exposed chronically to the microwave and other radio frequencies.

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