



ORIGINAL ARTICLE

Non-thermal continuous and modulated electromagnetic radiation fields effects on sleep EEG of rats[☆]

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Abstract In the present study, the alteration in the sleep EEG in rats due to chronic exposure to low-level non-thermal electromagnetic radiation was investigated. Two types of radiation fields were used; 900 MHz *unmodulated* wave and 900 MHz *modulated* at 8 and 16 Hz waves. Animals has exposed to radiation fields for 1 month (1 h/day). EEG power spectral analyses of exposed and control animals during slow wave sleep (SWS) and rapid eye movement sleep (REM sleep) revealed that the REM sleep is more susceptible to modulated radiofrequency radiation fields (RFR) than the SWS. The latency of REM sleep increased due to radiation exposure indicating a change in the ultradian rhythm of normal sleep cycles. The cumulative and irreversible effect of radiation exposure was proposed and the interaction of the extremely low frequency radiation with the similar EEG frequencies was suggested.

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Introduction

The widespread of radiofrequency radiation (RFR) sources in domestic use has increased over the last decades, especially in the communication field, and public concern has been raised to

quantify the health hazard problems that may occur due to the exposure to such type of non-ionizing radiation.

Tissue heating is the most widely accepted mechanism of microwave radiation with biological systems. These effects can result from elevations of tissue temperature induced by radiofrequency (RF) energy deposited or absorbed in biological systems through local, partial-body or whole-body exposures. However, a large bulk of literature have evidenced that several biological effects of RF can be formed without tissue heating which are known as non-thermal biological effects of radiation [1].

EEG considered to be a sensitive tool to assess quantify and classify sleep stages as well as study their changes due to radiation interaction with the brain. In human and most animals, EEG appears as low-amplitude fast waves during awake state, high-amplitude slow waves during SWS and low amplitude fast waves during REM sleep.

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It has also been repeatedly reported that exposure to low-level microwaves produces alterations in the resting or sleep EEG signal and brain physiology [2–4]. It has been demonstrated that exposure to pulse-modulated microwaves alters not only the EEG but also regional cerebral blood flow [5,6]. Furthermore, it has been reported that modulation is crucial for radiofrequency electromagnetic field-induced alterations in brain physiology [6].

Sleep function is hypothesized to be the reprocessing and consolidation of memory traces [7,8]. There is also some recent evidence suggesting that sleep may help to protect declarative memories from subsequent associative interferences [9].

Sleep is one of the biological phenomena that can be affected by RF radiation exposure. Mann and Roschke [10] reported reduction in latency to sleep onset and the percentage of REM sleep due to exposure to GSM-like signals. Loughran et al. [11] reported a decrease in REM sleep latency after 30 min of 894.6 MHz radiation exposure.

In the present study, several aims have been addressed. First, the non-thermal effect of electromagnetic radiation was studied by the application of low-level radiation (0.025 mW/cm^2). Second, the differences in the effect of the continuous and the modulated wave's electromagnetic radiation were checked out by application of these two types of radiation. The modulation frequencies were selected to be within the physiological range of the brain's EEG signals to assess the interaction of these similar frequencies. Finally, the chronic exposure of radiation rather than the acute exposure was used to investigate the cumulative nature of radiation effects on the biological system.

Material and methods

Experimental animals

The experimental animals used in the present study were adult male Wistar albino rats, weighing 175–250 g. The animals were obtained from the animal house of the National Research Center, Egypt. They were maintained on stock diet and kept under fixed conditions of housing and handling. They were under controlled light-dark cycle (on at 7 a.m. and off at 7 p.m.) and temperature conditions ($25 \pm 2^\circ\text{C}$). All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center, Egypt which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Experimental design

A total of 40 rats were divided into four groups. Three groups were irradiated with electromagnetic radiation either 900 MHz continuous wave or frequency-modulated (8 and 16 Hz) wave on a daily basis, (1 h per day) for 1 month. The fourth group served as a control group with the same experimental conditions except radiation exposure.

The exposure setup

The radiofrequency (RF) generator (Aeroflex company, Model: 2025, UK) connected to a power amplifier (Stealth

Microwave, Model: SM 0520-36, SSB Technologies, Inc., NJ, USA) was used to generate the electromagnetic radiation. The amplifier, in turn, was connected to a circular monopole antenna designed so that the reflection coefficient at its input should not more than -12 dBm and fed by a coaxial line through a Bayonet Neill-Concelman (BNC) connector. The spatial distribution of the electromagnetic radiation power density was measured with a field meter (Narda, EMR200, frequency from 0 to 4 GHz, Germany).

The specific absorption rate (SAR) distribution in the rat head was determined by using the finite different time domain (FDTD) method, with the aid of the XFDTD Bio-pro software (version: 6.3.8.4, NY, USA). Geometric/electric model was constructed for the animal's head from the stereotaxic atlas of Paxinos and Watson [12]. An ellipsoid model with the internal anatomic layers was used. The standard dielectric properties [13] were assigned to each layer. The animal head model was subjected to RFR with the same power density as that measured by the field meter through the experimental exposure process. The FDTD algorithm was then applied to calculate the electric field distribution everywhere inside the head model. The SAR was calculated at the desired points as $\sigma E^2 / 2\rho$, where E is the electric field peak value at the point (V/m), σ is the conductivity of the tissue at this point (S/m) and ρ is the density of the tissue (Kg/m^3). The calculated spatial peak SAR averaged over 1 g was found to be 0.245 W/kg .

As shown in Fig. 2, rats were housed in a circular plastic tray (50 cm diameter) which is divided into equal sectors to ensure that all rats were equally exposed to radiation. The antenna emitting the electromagnetic radiation was fixed in the center of the tray. To avoid stress, an aperture (1.5 cm in diameter) was made in the upper lid of each sector tip toward the antenna for animal breathing and this design make the animals freely direct their heads toward the radiation antenna.

EEG recording and analysis

Under Na-pentobarbital anesthesia (40 g/kg of animal), animals were positioned in the stereotaxic device (David Kopf instruments, Tujunga, California, USA) and implanted with three epidural stainless steel electrodes, of 1 mm diameter. Electrodes were implanted over the frontal cortex at 3.9 mm anterior to the Bregma and 2 mm lateral (right) to the midline, the other electrode was implanted at 6.4 mm posterior to the Bregma and 4 mm lateral (right) to the midline, whereas, the third electrode (reference electrode) was implanted over the cerebellum 1 mm posterior to Lambda, on the extension of the midline [12]. The three electrodes were connected to a multipin connector base, and the entire assembly was fixed to the skull and isolated with dental cement (zinc polycarboxylate non-irritating dental cement, purchased from Spofa-Dental-Praha, Czech Republic).

During EEG recordings, rats were housed in a sound attenuated, aerated and electrically shielded cage ($25 \times 25 \times 30 \text{ cm}$). They were left 30 min prior to recording for acclimatization to the laboratory environment. EEG recordings were performed at fixed time of the day under the following conditions; 50 Hz notch filter and sampling rate of 200 sample/s.

REM sleep was characterized by low-voltage (desynchronized) EEG activity and continuous high theta power (4–8 Hz) [14,15]. SWS was characterized by high-voltage (syn-

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