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Rats exposed to 2.45 GHz of non-ionizing radiation exhibit behavioral changes with increased brain expression of apoptotic caspase 3

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ABSTRACT

In recent years there has been a tremendous increase in use of Wi-Fi devices along with mobile phones, globally. Wi-Fi devices make use of 2.4 GHz frequency. The present study evaluated the impact of 2.45 GHz radiation exposure for 4 h/day for 45 days on behavioral and oxidative stress parameters in female Sprague Dawley rats. Behavioral tests of anxiety, learning and memory were started from day 38. Oxidative stress parameters were estimated in brain homogenates after sacrificing the rats on day 45. In morris water maze, elevated plus maze and light dark box test, the 2.45 GHz radiation exposed rats elicited memory decline and anxiety behavior. Exposure decreased activities of super oxide dismutase, catalase and reduced glutathione levels whereas increased levels of brain lipid peroxidation was encountered in the radiation exposed rats, showing compromised anti-oxidant defense. Expression of caspase 3 gene in brain samples were quantified which unraveled notable increase in the apoptotic marker caspase 3 in 2.45 GHz radiation exposed group as compared to sham exposed group. No significant changes were observed in histopathological examinations and brain levels of TNF-α. Analysis of dendritic arborization of neurons showcased reduction in number of dendritic branching and intersections which corresponds to alteration in dendritic structure of neurons, affecting neuronal signaling. The study clearly indicates that exposure of rats to microwave radiation of 2.45 GHz leads to detrimental changes in brain leading to lowering of learning and memory and expression of anxiety behavior in rats along with fall in brain antioxidant enzyme systems.

1. Introduction

Mobile phones have been a part of everyday life for most people in the world since the late 1990s. There has been an unprecedented growth in the global communication industry in recent years which has resulted in a dramatic increase in the number of wireless devices. It has been suggested that the widespread use of cellular telephones and wireless devices like Wi-Fi may result in increased health risks resulting from brain exposure to electromagnetic radiation (EMR) [1]. Mobile phones and Wi-Fi make use of Non-Ionizing electromagnetic radiation (NI-EMR). Non-ionizing radiation is the term given to radiation in the part of the electromagnetic spectrum where there is insufficient energy to cause ionization. It includes electric and magnetic fields, radio waves, microwaves, infrared, ultraviolet, and visible radiation. Microwaves with the frequency range of 300 MHz–300 GHz are used in the telecommunication system.

There are several health hazards associated with microwave radiations. One amongst them is the thermal effects of the radiations that lead to localized increase in tissue temperature, which may cause disruption of cell function. The extent of heating depends on the specific absorption rate (SAR), and power density of the electromagnetic waves that are emitted from the radiation emitting devices. Non-thermal effect mainly results from the stress generated in the cells and tissues due to the interaction between the radiation and cells in the body. The non-thermal effects include changes in the activity of Ca^{2+} dependent K^{+} channels, alterations of membrane structure and function, permeability modifications of liposomes and isolated cells Non-thermal effects of microwave radiation accumulate over time and their harmful effects are more evident after 8–10 years of exposure and as in the initial years

Abbreviation: TNF-α, Tumor necrosis factor-α.

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of exposure body's defense mechanisms and the stress proteins, specifically the heat shock proteins come into play. These situations has prompted many investigations to probe interaction between EMR and neuronal cells, even at intensities not able to produce thermal effects. Studies on the potential health and biological effects of mobile phone radiation have been extensively conducted over the years. Epidemiological as well as *in vivo* and *in vitro* approaches have been applied to examine the potential health and biological effects of mobile phone radiation [2–4]. Furthermore, human studies have been carried out to examine the effects of these radiations on sleep, cognitive functions and behavioral aspects [5,6]. The brain is the organ which gets affected by the use of cell phones as the device is held often in proximity to the head. Also brain is the most sensitive target organ for microwave radiation where mitochondrial injury occurs early and more severely in comparison to other organs. Thus, among other organs the effect of microwave radiation on brain is the most researched area [3,7].

Considerable amount of research has been focused on the behavioral impairments particularly, learning and memory deficits in rats, following exposure to EMR [7]. For this, various behavioral paradigms are utilised. This includes Morris water maze test for spatial memory evaluation, object recognition test to study recognition memory, Elevated plus maze test and light dark test to evaluate anxiety. All these paradigms have been used in the current study. A number of studies have investigated the effects of NI-EMR on spatial memory and place learning tasks in rodents mainly using radial arm mazes or water mazes [8–11]. Despite numerous studies, there is no definitive conclusion till date that high frequency EMR can affect the memory performance. Also there have been no reliable reports on the effect of these radiations on anxiety behavior in rats. The present study was thus an attempt to study the effects of NI-EMR on learning and memory along with the effect on anxiety behavior in rats.

Oxidative stress is a biochemical condition characterised by a relatively high levels of toxic reactive species mainly reactive oxygen species (ROS) and the natural anti-oxidant defence mechanism in body is unable to remove this excess free radicals [12]. Excessive ROS is detrimental to brain energy metabolism. Electromagnetic radiations are known to increase the activity of free radicals in the cells. EMR could initiate a state of oxidative stress in the brain [13]. This may accelerate neuronal degeneration and can cause molecular damage to other cell organelles [14]. The present study attempted to profile the activities of different anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and determine the extent of lipid peroxidation and protein carbonyl levels in the brains of rats exposed to NI-EMR of 2.45 GHz for 4 h/day for 45 days.

Apoptosis plays a crucial role in homeostasis during the development of embryo. It also plays a fundamental role in organogenesis and during the entire life time of multi cellular organisms. Several *in vitro* studies have been carried out in this context and have reported that exposure to non-ionizing electromagnetic radiation can activate the caspase dependent apoptosis pathway. [15]. TNF-α is a pro-inflammatory cytokine and it is released from the cells during inflammation. It is involved in extrinsic apoptosis pathway and ultimately stimulates the effector caspase which is caspase 3 [16]. The present study has therefore focused on the involvement of the apoptotic markers in the brain when exposed to radiofrequency radiation.

The present study was undertaken to investigate and explore the effects of NI-EMR especially the radiation frequency used in Wi-Fi devices, on the brain of rats focussing on some of the parameters of oxidative stress and apoptosis. The 45 day study was designed to shed light on the impact of these radiations on memory performance and behavioral changes.

2. Materials and methods

2.1. Animals

Female Sprague Dawley rats of body weight 180–220 g were used in the study. They were obtained from Glenmark laboratories Ltd., Mumbai, India. The rats were acclimatised to the laboratory environment for a period of one week before handling them. All the animals were housed in the humidity (40–50%) and temperature (25–27 °C) controlled room, in polycarbonate cages. The animals were kept on 12–12-h light-dark cycle and were provided with water and standard rat chow diet. For the animal experimentation protocols, prior approval was obtained from 'Institutional Animal Ethics Committee' (IAEC, CPCSEA-BCP/2015-01/05 dated 6 June 2015), and all studies were performed in accordance with 'Committee for the Purpose of Control and Supervision on Experiments on Animals' (CPCSEA) guidelines.

2.2. Experimental design and exposure system for the rats

Animals for the study were randomized into following two groups:

Group1: Sham exposed group; Rats unexposed to NI-EMR. A total of 6 rats (n = 6) were placed in the exposure cage during the exposure hours but with the radiation source kept off. Group 2: Rats exposed to NI-EMR of 2.45 GHz (n = 6). The animals of group 2 were exposed to the 2.45 GHz radiation for 4 h/day for 45 days between 10 am to 6 pm, at a power density of 7.88 W/m^2.

During the exposure hours, rats in the group were placed in the individual square Plexiglas cage of dimension 15 × 15 × 15 cm. The cage was perforated with sufficient holes of 1 cm diameter for proper ventilation. A monopole antenna was placed at the centre and the six individual cages were placed surrounding it. The rats were positioned in such a way that every animal received equal intensity of radiation (Fig. 1.). The power absorbed by the rat is given by the formula:-Power density x Surface area of the rat x 0.5. Surface area of the rat was calculated to be 0.012 m^2. Using 0.5 as the reflection coefficient in biological media at 2.45 GHz, the radiated power absorbed by the body of rat was calculated to be 0.04728 W.

The radiation exposure system was fabricated in the Antenna lab of Electrical Engineering Department of IIT Bombay, Powai. A Printed Circuit Board (PCB) consisting of Voltage Controlled Oscillator (VCO) and amplification stages produced 24 dBm (about 0.252 W) of microwave energy at 2.45 GHz of frequency. Output was measured using spectrum analyser. A printed monopole antenna with a gain of 1 dB to radiate at 2.45 GHz was designed using IE3D simulation tool. The generator was thereafter terminated with this antenna to radiate at designated frequency.

2.3. Behavioral studies

Four different behavioral paradigms were used in the study namely, Elevated Plus Maze test (EPM), Light Dark Box test (LDT), Object Recognition Task (ORT) and Morris Water Maze test (MWM). Rats from each group were subjected to all the four behavioral paradigms.

2.3.1. EPM

The EPM consisted of two open arms (40 × 40 cm) and two closed arm of similar dimension with a wall of 40 cm height. The maze was elevated 50 cm above the ground and was placed in a quiet room. Rats were placed individually in the centre of the maze facing the open arm. The following parameters were calculated:

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