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Exploring the Effect of Chronic Exposure to Radio-Frequency Electromagnetic Radiation on Neurotransmitters and Acetylcholinesterase activity in rat Brain

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Abstract

Today there is an upsurge in the public and media concern about the potential health risk of radiofrequency field exposure. Interaction of radio frequency electromagnetic radiation with the brain is a precarious apprehension in our society. The current study was aimed to evaluate the possible implications on neurotransmitter and AChE activity levels on exposure to radio frequency-electromagnetic radiation (RF-EMR) in male rats at 900 MHz and 1800 MHz frequencies for 2 h per day for 3 months. Wistar rats were categorised into three groups (n=6) viz., sham exposure, exposure to 1800 MHz and exposure to 900 MHz. At the end of the study alterations in neurotransmitters level and histopathology of brain were analysed. The brain level of dopamine, nor-adrenaline, GABA and AChE enzyme activity was found to be significantly lowered. Histopathological findings of brain showed a marked congestion of the cerebral blood vessels and few of the pyramidal cells and neuroglial cells show degenerative changes with mild inflammatory infiltration. Thus, our results suggest that exposure to RF-EMR certainly causesinhibition of AChE activity, alteration in brain transmitter levels and changes in the architecture of brain parenchymal tissue.

Keywords: RF-EMR, Dopamine, Nor-Adrenaline, GABA, Acetylcholinesterase Enzyme

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1. Introduction

In everyday life, we are constantly surrounded by electromagnetic fields (EMF). There are two means of sources: natural sources of EMF, such as the earth's magnetic field and man-made sources, such as power cables, domestic appliances, radio stations, mobile phones, wireless networks and radars. Among them mobile phones use modulated radio frequency electromagnetic fields (RF-EMF) to transfer information. Mobile phones have been a part of everyday life and been an unprecedented growth in the global communication industry that has resulted in a dramatic increase in the number of wireless devices. Mobile services are one of the fastest growing mobile telephonic industries in the world. According to the Telecom Regulatory Authority of India, the composition of telephone subscribers using wireless form of communication in urban area is 63.27% and rural area is 33.20%[1].As on May 2014, the number of mobile phone users in India was 910 million (second highest in the world) and that of mobile towers was more than 6 lac. This has led to the mushrooming of supporting infrastructure in the form of cell towers, which provide the link to and from the mobile phone[2].

Mobile phones emit RF-EMF using specified frequency bands during the transmission phase.While speaking during a phone call the rate at which radio frequency (RF) energy is actually absorbed in a body is called the "Specific Absorption Rate (SAR)".It is usually expressed in Watts per kilogram (W/kg) or milliwatts per

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gram (mW/g). SAR is set at 1.6 W/kg averaged over 1 g of body tissue in the US and Canada and 2 W/kg averaged over 10 g of body tissue in countries that adopted the ICNIRP guidelines.

There is an increasing concern about the interactions of electromagnetic radiation with the human organs, particularly with the brain. The brain and nervous system have long been considered as sensitive targets for the effects of RF fields. Calcium ions play an essential role in brain function, because a small amount of calcium must enter the cytosol of the neuron before it can release its neurotransmitters. Electromagnetically-induced membrane leakage would increase the calcium levels in the neurons so that they quickly release their neurotransmitters. This improves the reaction time towards simple stimuli, but it can also trigger the spontaneous release of neurotransmitters to transmit spurious signals, which makes the brain hyperactive and less able to concentrate. [2,3].

Studies by Finnie et al. have detected no consistent evidence of any field-induced changes in gene and protein expression following low-level exposures. There was no evidence that short- or long-term exposure to 900 MHz field produced microglial activation in the cortex or hippocampus of mice[4]. There was no evidence of apoptosis and/or increase in degenerationof (dark) neurons in the brain of juvenile rats after acute, low-level exposure to pulsed 900 MHz fields[5]. Previously, it had reported that an equivalent exposure had caused widespread damage to neurons[6]. Tarantino et al.had investigated the delayed effects of long-term, continuous, whole-body exposure of rabbits to 650 MHz, typical of some broadcasting signals. No effects were observed on body weight and health of the animals, but progressive changes were observed in brain morphology with a gradual increase in apoptosis[7].

Mausset-Bonnefont et al. had investigated the effects of acute exposure of the head of rats to high power (but not hyperthermic) 900 MHz field on the levels and binding properties of N-methyl-D aspartate (NMDA), GABA-A receptors and dopamine transporters in the cortex, striatum and hippocampus. Significant changes were reported, particularly for the decreased levels of GABA-A receptors in the hippocampus, increased dopamine transporters in the striatum and decreased NMDA receptors in the cortex[8].

In our study, we investigated the effect of long-term exposure of RF-EMR (900 MHz and 1800MHz) on behavioural and cognitive function and neurotransmitter level in male Wistar rats.

2. Materials and methods

2.1 Experimental animals

Inbred healthy male Albino Wistar rats (aged 6–8 weeks old) were used in this experiment. The rats were housed in polypropylene cages inside a temperature- and humidity-controlled environment with free access to food and water ad libitum, with a 12 h light and 12 h dark cycle. All the experiments were carried out with prior approval from the institutional animal ethics committee. Care was taken to handle the rats in a humane manner, and all precautions were taken to use the minimum number of animals required to generate significant data.

2.2 Experimental design

Animals were categorised into three groups: group I (n = 6), normal control; group II (n = 6) exposed to RF-EMR 900 MHz and group III (n=6), exposed to RF-EMR 1800 MHz. The radio frequency electromagnetic radiation generator (manufactured by AND Solutions, India) was used to emit 900MHz and 1800MHz frequencies, SAR set at 1.6 W/kgand power density was 0.9 watt/m^2(which was fixed statically by manufacturer as per IEEE guidelines) via monopole antenna that directed the signal to the cages.The antenna generated uniform RF radiations as a genuine GSM mobile phone. The length of the monopole was set so that the antenna is resonant at the operating frequency. The antenna had an omni-directional pattern in the azimuth plane through which the rats were to be uniformly distributed. Male Wistar rats were placed individually in a hexagonal acrylic plastic cage (40 cm in diameter) which was divided into six compartments and exposed to RF-EMR daily 2 h for 3 months. The cage was designed in such a way that the animals were not restrained and ventilated by drilling holes. The animals were left for 3 daysfor acclimatisation. Control animals underwent the same transportation, habituation and handling procedure without switching on the signal generator i.e. no RF-EMR exposure. The antenna was placed 30 cm above the ground. At the end of the study rats were sacrificed by

cervical dislocation to estimate neurotransmitter levels, acetylcholinesterase enzyme activity in brain and to study the histopathology of the brain.

2.3 Estimation of dopamine and nor-adrenaline in rat brain homogenate

Estimation of dopamine and nor-adrenaline was followed according to Schlumpf et al. Dopamine and nor-adrenaline levels were measured at 330 and 395 nm as excitation and 375 and 485 nm as emission wavelength, respectively, using spectrofluorimeter.

On the day of experiment, rats were sacrificed by cervical dislocation, whole brain was dissected out and the sub-cortical region (including the striatum) was separated. Briefly, 0.5g of tissue was weighed and homogenized in 25 ml of HCl–butanol for 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (5 ml) was removed and added to centrifuge tube containing 12.5 ml of heptane and 8 ml 0.1 M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the aforementioned conditions to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (1ml) was then extracted for dopamine and nor-adrenaline assay. The extraction procedure was carried out at 0°C[9].

2.4 Estimation of GABA content in brain homogenate

The brain gamma amino butyric acid (GABA) content was estimated as specified by Lowe et al. Rats were sacrificed by decapitation, the brains were rapidly removed, blotted, weighed and transferred in ice cold 5 mL trichloroacetic acid (10% w/v), homogenized and centrifuged at 10,000 g for 10 min at 0°C. A sample (0.1 mL) of tissue extract with 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.9), kept in a water bath at 60°C for 30 min then cooled and treated with 5 mL of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min, the fluorescence reading was measured at 377 and 451 nm as excitation and emission wavelength, respectively.

GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with 0.14 M ninhydrin in an alkaline medium, in the presence of glutamate. The GABA content in brain was expressed in µg per mg of wet brain tissue.[10]

2.5 Estimation of acetylcholinesterase in rat brain homogenate

Acetylcholinesterase (AChE) enzyme activity was estimated by Elman method. The rats were decapitated by cervical dislocation, brains are removed quickly and placed in ice-cold saline. Thalamus, hypothalamus and hippocampus are quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed (250 mg) and homogenized in 0.1M Phosphate buffer (pH 8). Further 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm using a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance was recorded for 10 minutes at an interval of 2 min. Change in the absorbance per minute is thus determined. The enzyme activity was calculated using the following formula.[11]

Calculation:

$$\text{Acetylcholinestease activity (µ moles/minute/mg tissue)} = \frac{\text{A/min X Vt X 1000}}{\text{abs. coeff X b X Vs X mg tissue / ml}}$$

A/min = Absorbance/min
Vt= Total volume (3.1 ml)
Abs. coeff. = absorption coefficient (1.361 X10^4 $M^{-1}cm^{-1}$)
B= pathlength (1 cm)
Vs= sample volume (0.4 ml)

2.6 Histopathology

The brain was carefully dissected and fixed in 10% buffered formalin (with pH 7.4) for 24 h. It was then dehydrated in ethanol, defatted in xylene and embedded in paraffin. Care was taken to ensure that the brains of all rats were oriented in the same direction during embedding to minimize the differences in the angles at which the brains were sectioned. A single investigator processed all brains to maintain consistency in histological analysis and stained with haematoxylin and eosin (H&E) according to the standard procedure and observed under the magnification 400X. The brain parenchyma from cerebral region was studied and identified for pyramidal cells, neuroglial cells and blood vessels.

2.7 Statistical analysis

All data were expressed as mean ± SEM and analysed with one way analysis of variance (ANOVA) between the groups and followed by Tukeys Multiple Comparison Test to assess differences between the groups using graph pad prism (Ver. 5). Probability values $p<0.05$,$p < 0.01$ and $p < 0.001$ were considered significant.

3. Results

3.1 Effect on brain neurotransmitters level.

Significant changes were observed in brain neurotransmitters level. Rats exposed to RF-EMR at 1800 MHz and 900 MHz frequencies had brain dopamine levels of 4.743 ± 3.64 ng/mg and 38.49 ± 2.50ng/mg, respectively, of which 1800 MHz was found to be significantly lower than sham-exposed rats (47.69 ± 5.61 ng/mg)(Fig. 1).

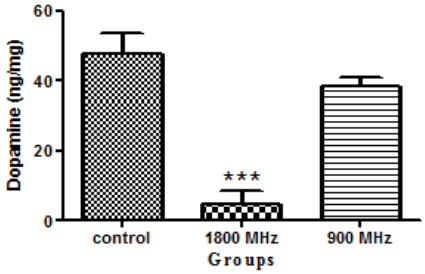


Figure 1. Effect of RF-EMR on brain dopamine levels in rats. *** denotes significant difference $p < 0.001$.

Moreover, rats exposed to RF-EMR 1800 MHz and 900 MHz frequency had brain noradrenaline levels of 6.660 ± 1.083 ng/mg and 3.855 ± 0.521 ng/mg, respectively,which was found to be significantly lower as than sham-exposed rats (12.04 ± 0.625 ng/mg)(Fig. 2).

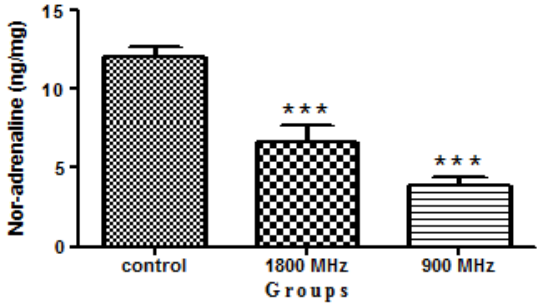


Figure 2. Effect of RF-EMR on brain nor-adrenaline levels in male rats. *** denotes significant difference $p < 0.001$.

Furthermore, rats exposed to RF-EMR at 1800 MHz and 900 MHz frequency had brain GABA levels of 1.103 ± 0.06518 µg/mg and 0.9860 ± 0.09917 µg/mg, respectively, which was significantly lower than sham-exposed rats (1.690 ± 0.07864 µg/mg) (Fig. 3).

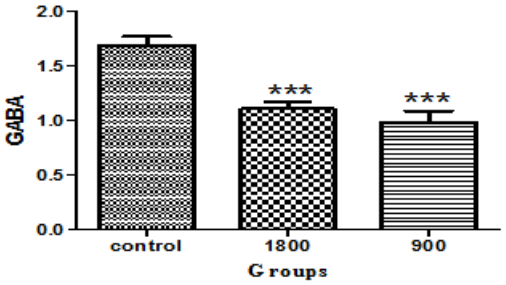


Figure 3. Effect of RF-EMR on brain GABA levels in rats. *** denotes significant difference $p < 0.001$.

Acetylcholinesterase activity also showed a significant difference in the brain. Rats exposed to RF-EMR at 1800 MHz and 900 MHz frequencies showed 1.735 ± 0.01867 µ moles/min/mg tissue and 1.687 ± 0.2013 µ moles/min/mg tissue, respectively, which was significantly lower than sham-exposed rats (2.398 ± 0.2284 µ moles/min/mg tissue). (Fig. 4).

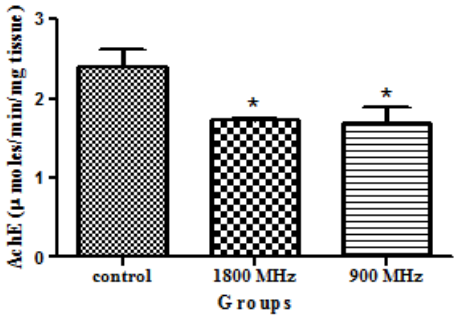


Figure 4. Effect of RF-EMR on brain acetylcholinesterase in rats. * denotes significant difference $p < 0.05$.

3.2 Histopathological study

There were observable histological changes in the rats exposed to radiation when compared to the sham-exposed rats. The photomicrograph of brain parenchyma of rats exposed to 1800 MHz and 900 MHz showed (Figs. 5 and 6) a marked congestion of the cerebral blood vessels and few of the pyramidal cells and neuroglial cells showed degenerative changes (10A and 11A, long arrow) with mild inflammatory infiltration (5B and 6B, arrow). Few of the blood vessels appeared congested (5A and 6A, short arrow).

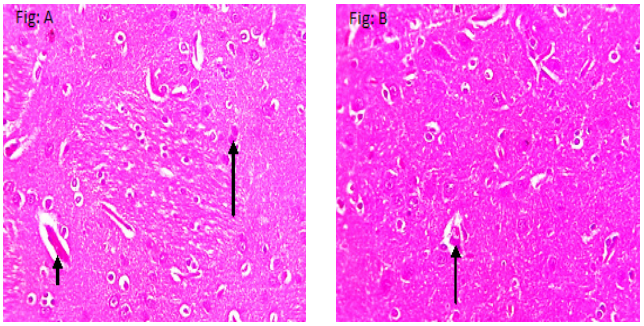


Figure 5. Section of H & E stained brain exposed to 1800 MHz rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells show degenerative changes [A, long arrow], mild inflammatory infiltration [B, arrow], congested blood vessels [A, short arrow].

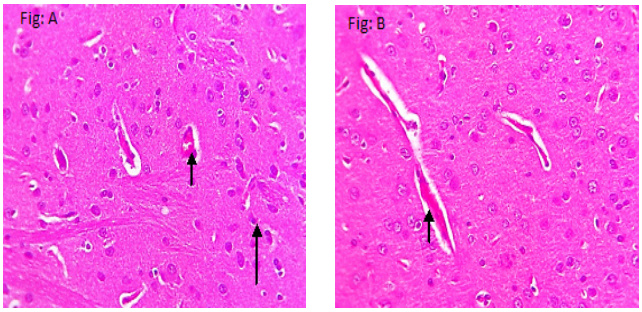


Figure 6. Section of H & E stained brain exposed to 900 MHz rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells show degenerative changes [A, long arrow], mild inflammatory infiltration [B, arrow], congested blood vessels [A, short arrow].

The photomicrograph of a rat brain of the control(Fig. 7) showed intact architecture of brain parenchyma. Most of the pyramidal cells and neuroglial cells appeared intact (Fig.7A, arrow). The blood vessels appear unremarkable (Fig.7B, arrow).

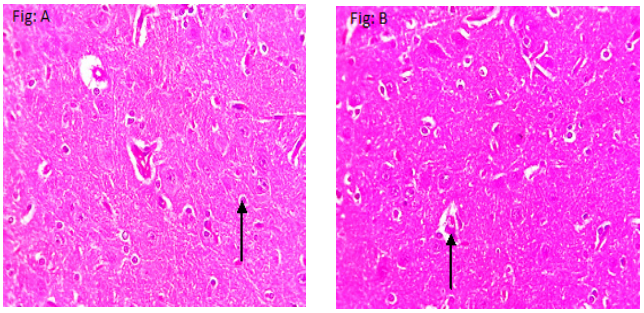


Figure 7. Section of H & E stained brain of normal rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells appear intact [A, arrow]. Blood vessels appear unremarkable [B, arrow].

4. Discussion

The brain and nervous system have long been considered sensitive targets for the effects of low RF fields. Depending on the amount of movement of the animal, the energy absorption pattern in its body could become either more complex and unpredictable or more uniform. The pattern of energy absorption inside an irradiated body is non-uniform, and biological responses are dependent on distribution of energy and the body part that is affected. RF-EMR emitted from mobile phone could influence anxiety like behaviour in rats [12].Critical analysis of the current study revealed that chronic exposure to RF-EMR from mobile phone could affect the well-being of rats.

A number of clinical and experimental studies have shown the role of hippocampal formation and related structures in the medial temporal lobe in learning and memory [13][14]. The radiation from cell phones, even at one-hundredth of the permitted SAR value, can open the blood brain barrier in rats so that the protein molecules as large as albumin could enter their brains [15]. Later experiments by Salford showed that this was associated with the death of neurons [16]. An immediate effect may not be expected because the brain has spare capacity. However, prolonged or repeated exposure to cell phone or similar radiation would be expected to cause a progressive loss of functional neurons and result in early dementia and Alzheimer's disease in humans.

In the present study, significant change was observed in brain neurotransmitter levels. Level of dopamine was significantly decreased in rats exposed to 1800 MHz.The chemical is involved in controlling emotions, pleasure, movement and incoming information. Low dopamine levels in the motor areas of the brain may result in abnormal nerve-firing patterns within the brain that causes impaired movement leading to consequences,such as like Parkinson's disease, restless leg syndrome, anxiety, attention deficits, cognitive impairment, confusion, depression, blunted effect, fatigue, sleepiness and memory impairment. Significant decrease in nor-adrenaline levels in rats was observed and may linked to lower arousals, lower alertness and depression. It helps promote vigilant concentration and is different from dopamine in the fact that dopamine has a greater influence on cognition. In a stressful condition, norepinephrine is secreted by the sympathetic nervous system that leads to an increased amount of heart contractions. Decrease in the locomotor

activity of rats shows the animal was prone to stress and depression condition, which can be correlated with the decrease level of noradrenaline[17].

Brain GABA levels were also found to be significantly lowered in rats exposed to 1800 MHz and 900 MHz. GABA is primarily known for its ability to keep in a relaxed state; it actually plays a crucial role in regulating many aspects of mood, attention, cognition, and sleep. It control nervous signals in the retina and the central nervous system;therefore, insufficient GABA usually leads to insomnia, depression, anxiety, mood disorder, excessive stress, hypertension, motion sickness, low level of digestive enzymes, epileptic seizures, panic disorders and low growth hormone levels. Acetylcholinesterase (AChE) activity is important to maintain normal brain physiological function and learning-memory ability.Dutta et al.hadshowed the modifications in the activity of the acetylcholinesterase; there is, therefore, well deterioration of the cell functions [18]. Kunjilwar and Behari found modifications of the AChE by long-term application of these modulated RF-EMR[19].Significant inhibition of the activities of AChE was detected in brain tissue of the rats as compared to controls

Histopathology of brain showed structural changes in the brain cells of the rats exposed to mobile phone radiation as compared with sham-exposed rats. These changes in the histology is in consonance with the report given by other researchers, such asLaila K. Hanafy et al., where they observed histological changes in the different visceral organs after exposing 15 rats to mobile phone radiation daily for 4 weeks (1h/day)[20]. Hence, the possible mechanism could be due to increased vulnerability to free radicals generation. Boris et al., have also shown that the first changes occurred at the neuronal structure, followed by glial and endothelial cell damages[21].

Thus, alteration in neurotransmitter levels could affect the cognitive function and behaviour of the exposed rats, which could be detrimental to overall health. But the study is not asserted by our research and it has to be endorsed by doing further research and more findings.

5. Conclusion

Analyses showed various changes in the neurotransmitter levels in rats exposed to RF-EMR of 1800 MHz and 900 MHz. The most significant observation was the alteration of brain neurotransmitter levels of dopamine, noradrenaline and GABA and brain acetylcholinesterase activity levels with marked congestion of cerebral blood vessels and mild inflammatory infiltration in pyramidal and neuroglial cells of brain parenchyma.

6. Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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