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Effects of radiofrequency electromagnetic radiations (RF-EMR) on sector CA3 of hippocampus in albino rats- A light and electron-microscopic study.

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Abstract

The interaction of mobile phone radio-frequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. Because of growing use of mobile phone, there is increasing concern of such interaction with many human organs in general and brain in particular. In this study, the effect of RF-EMR on sector CA3 of hippocampus was investigated. Thirty five adult albino rats were divided into one control and 4 experimental groups namely E25, E50, E75 and E100which received 25, 50, 75 and 100 missed calls per day respectively for a period of 4 weeks. At the end of experiment the animals were euthanized and perfusion fixed in karnovsky's fixative. Light microscopy of 10 µm thick paraffin and semithin (0.5 µm thick) resin sections from CA3 region revealed few congestion and signs of hemorrhage with enlarged perivascular spaces; apparent shrinkage neurons and deformation of their nuclei. Transmission electron microscopy confirmed presence of shrunken cells with condensed and increased electron density of both cytoplasm and nucleoplasm. The mitochondria were swollen, vacuolized and had reduced number of distorted cristae. The synapses had fewer synaptic vesicles in their presynaptic terminals, synaptic clefts widened and postsynaptic densities were reduced in thickness. These findings indicated that excessive mobile phone radiation leads to demonstrable light and electron-microscopic morphological alterations in sector CA3 of hippocampus which may in long term, lead to both cognitive and behavioral changes.

Keywords: Hippocampus, Sector CA3, RF-EMR, TEM, Mitochondria, Synapse

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Introduction

Because of enormous increase in mobile phone usage throughout the world the effect of its radiation on human health is the subject of recent interest and study. It is estimated that as of November, 2011, there were more than 5.981 billion subscriptions worldwide (International Telecommunication Union) and a large proportion of users consist of children and teenagers. The close proximity of a mobile telephone antenna to the user's head leads to the deposition of a relatively large amount of radiofrequency energy in the head region. After repeated exposure (3 hr/day for 30 days) to the radiation, myelin degeneration and glial cell proliferation has been reported in the brains of exposed guinea pigs [1].

Animal models focusing on histopathological and functional alterations have indicated that EMR causes progressive defects in hippocampal-dependent learning and memory [2-3]. In rodents, performance on hippocampal-dependent spatial learning and memory tasks is compromised after whole-brain radiation. Specifically, radiation of young adult rodents leads to impaired performance on the Morris water maze (MWM) [3-4], Barnes maze [5], passive avoidance [4&6] and T-maze tasks [7]. With the dramatic increase in mobile phone use, establishing the long-term cognitive consequences of brain radiation exposure is important. Previous studies have shown that performance on the MWM task is dependent on hippocampal integrity [8].

Lesion studies have indicated that partial or complete hippocampal ablations lead to impaired performance in spatial learning and memory [8-9]. The underlying mechanisms for such radiation-related cognitive deficits have not been defined clearly, but they likely involve changes in multiple neural processes, such as synapse formation, pruning, and synaptic plasticity [10-11]. Synaptic changes may be reflected either by a loss of synapses in the dentate gyrus [10&12]or by changes in synaptic function, such as the impaired synaptic transmission and long-term potentiation observed in the hippocampus of old rats [11&13]. Synaptic function changes are also reflected in alterations in the morphology of synaptic configurations, permitting an indirect assessment of synaptic function in which synaptic profiles can be quantified [14].

Material and Methods

The present study was carried out on thirty five adult albino rats of either sex weighing 180-200 gm. The rats were housed in plastic cages of size 36 cm × 23 cm × 21 cm (three/four rats in each cage) inside a temperature- and humidity-controlled environment & provided with standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. All the experiments were carried out with prior approval from the institutional animal ethics committee. Animals were divided into five groups: one control and four experimental. Based on the number of missed calls/day they were grouped as E100, E75, E50, & E25 receiving 100, 75, 50, & 25missed call/day. The rats were exposed to RF-EMR by giving complete missed calls of 45 seconds duration each, one after the other, everyday for 4 weeks, keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in silent mode (no ring tone & no vibration) in the cage.

After experimental period of 4 weeks exposure the animals were sacrificed by giving overdose of diethyl ether vapor, and brain was dissected and divided into two equal halves by median incision.

Five coronal sections of 2-3 mm thickness were made in the cerebrum to include whole of the hippocampus, which all were embedded in paraffin to cut in 8-μm sections using rotary microtome which were stained with hematoxylin and eosin according to standard procedure. For *transmission electron-microscopy* about 1x2 mm-thick tissue blocks were cut from Karnovsky's fixed brain tissue to include the CA3 area of hippocampus and processed for resin embedding.

Semi-thin sections were cut with diamond knives using LKB ultramicrotome for light microscopy and thin sections (70 nm) lifted on 200 mesh copper grid for TEM after staining with Uranyl acetate and Lead citrate and examined with JEOL JEM -2100 Transmission Electron Microscope in university sophisticated instrument facility (USIF), AMU, Aligarh.

Observations

Light microscopy

In comparison to the control animals, the RF-EMR-exposed rats showed marked morphological changes were detected in the CA3 region of the hippocampus. The RF-EMR-exposed rats showed apparent changes in the form of darker staining and shrinkage of neurons (Figure 1B). The vessels appeared congested and showed signs of hemorrhage with an enlarged perivascular space (Figure 1B). Most of the above-mentioned changes were noticed in all experimental groups with varying intensity depicting a close dose-dependent relationship. Thus, group E100 was affected most while group E25 the least. The light microscopic features in *semithin* sections of experimental groups were in the form of relatively loose packing of neuropil, congested capillaries, swelling and irregularity in the shape of neuronal nuclei and heterogeneity of nuclear chromatin as well as less prominent nucleolus as compared to control (Figure 1B).

Here also, the severity of changes reflected a dose-dependent change. Thus features were seen more prominently in group E100 and very subtle in group E25. *Ultrastructure* - normal neurons in the hippocampus exhibited a large eurchromatic nucleus with a distinct nuclear envelope and nucleoli, and numerous rough endoplasmic reticulum and mitochondria in the cytoplasm with regular cristae (Figure 2A). In the hippocampus of RF-EMR - exposed rats, many neurons appeared shrunken with increased electron density of both cytoplasm and nucleus (Figure 2B). The mitochondria were swollen with fewer cristae having less regular arrangement (Figure 2D).

Compared with the synapses of hippocampal neurons in control rats, RF-ER exposed animals exhibited a decreased number of synaptic vesicles in the presynaptic terminals. The postsynaptic density was less prominent and synaptic clefts were widened or blurred (Figure 2C). These ultra-structural changes were increased with increasing dose of RF-EMR exposure, revealing a dose-dependent effect on the subcellular structures.

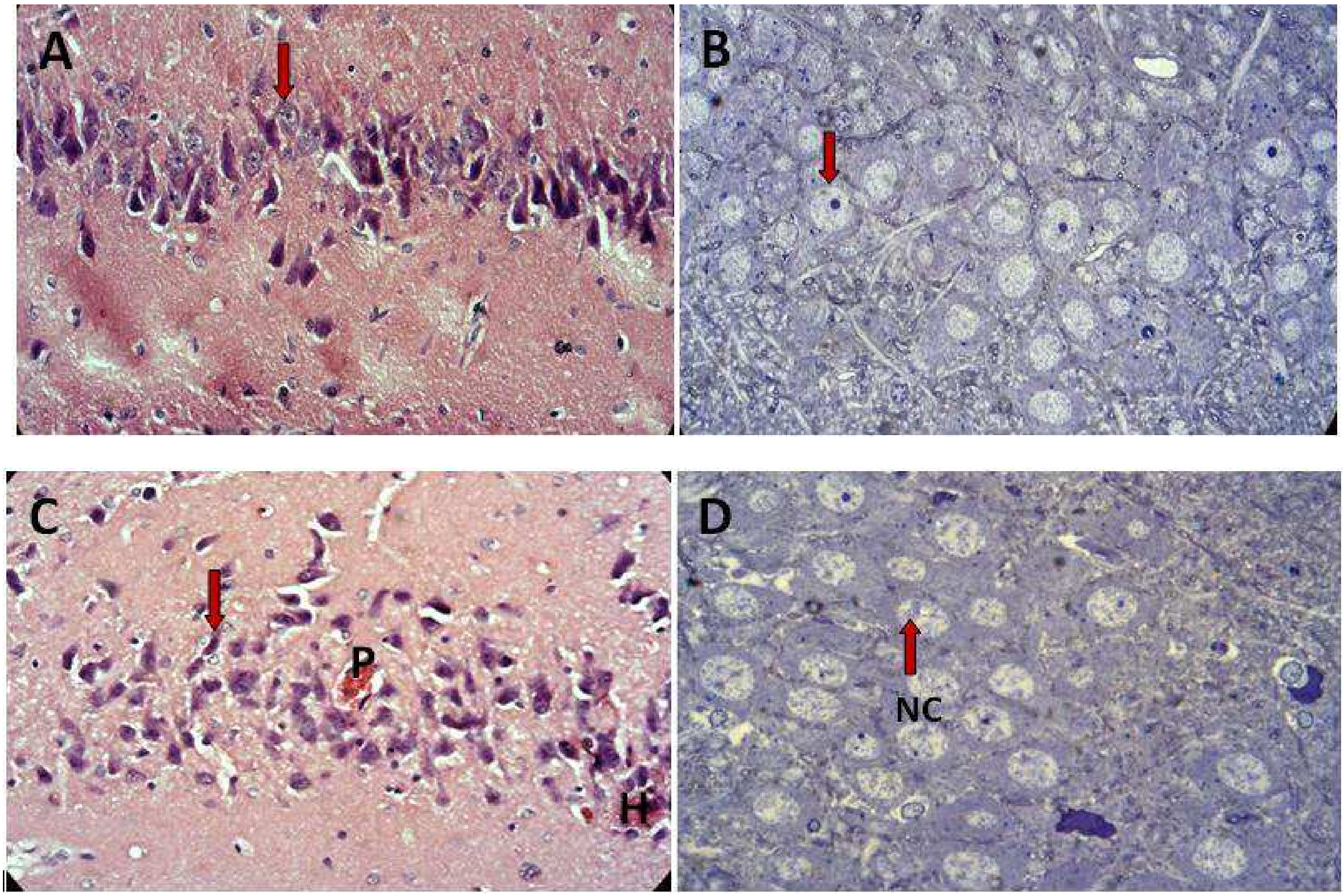

***Figure 1.** Sample photomicrograph of CA3 region of hippocampus, showing normal neurons (arrow) in control group (A) and RF-EMR affected neurons in E100 group (C). The hippocampal neurons are relatively darkly stained and loosely arranged. There is also a significant shrinkage of neurons (arrow) which are irregularly arranged with evidence of karyopyknosis. The vessels appear congested and show signs of hemorrhage (H) with an enlarged perivascular space (P). (A & C - paraffin sections, H & E stain, X400). The hippocampal neurons in semi-thin sections from control group (B) exhibit a regular arrangement, with distinct outline, and eurchromatic nucleus with prominent nucleolus while in E100 group (D) neurons exhibited nuclear swelling, clumping of nuclear material (NC) & nucleolus appear less prominent. (B & D - araldite sections, Toluidine stain, x1000*

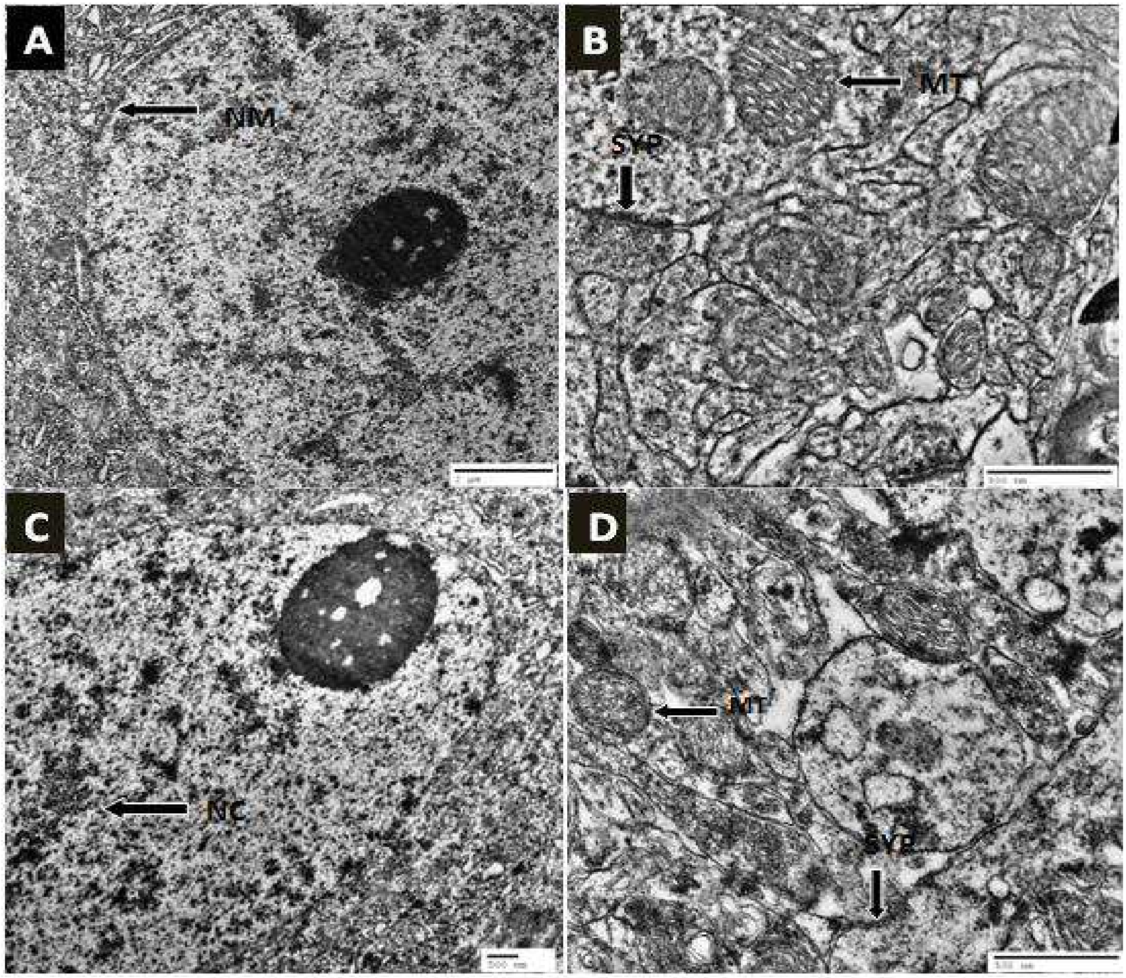

Figure 2: *Sample electron-micrograph of CA3 region of hippocampus, showing nerve cell body [A] and part of soma with neuropil [B] respectively in control group. Neuron exhibited a large round nucleus with a distinct nuclear envelope (NM), nucleolus and mitochondria (MT). There is an axosomatic excitatory synapse with (SY) with distinct pre-and post-synaptic profiles and synaptic cleft. In E100 group [C)] a shrunken neuronal cell with ovoid nucleus with condensed nucleoplasm (NC) and nucleolus can be seen. The mitochondria (MT) appear swollen and vacuolized (arrow), and the cristae appear disordered and fewer in number [D]. The synapses (SYP) exhibited fewer presynaptic vesicles, hazy appearance of organelles and poorly defined postsynaptic membrane densities. Scale bar A=2µm; B,C, D= 500 nm*

Discussion

Rapid advances in the communication involving EMF-technologies have greatly increased the human populations' exposure to EMFs and it possible adverse effects on nervous system. Since hippocampus is concerned with memory, emotion as well as navigation, the present study was planned to evaluate the effects of EMF on the CA3 region of hippocampus.

The study was planned carefully so that brain is not unduly disturbed by other factors eg. sound or vibration, during the course of study. Therefore, in this study whatever morphological changes have been observed they can be considered to be primarily due to EMF Keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in silent mode (no ring tone & no vibration).

Light microscopic findings of the present study show that pyramidal cell size of hippocampal sector CA3 of RF-EMR exposed rats decreased in comparison to control groups. Individual cells could be seen with condensed cytoplasm and nucleus. These changes in size could be due to effects of EMF on the genetic material. Such effects have been reported in various studies after exposure to RFR [15-16]. Recently, several studies have reported cytogenetic changes in brain cells by RFR, and these results could have important indicator on the health effects of RFR. Singh [17] reported significant decreases in poly-ADP-ribosylation, a process involved in chromatin functions, in the brain of rats after sixty days of exposure to 2450-MHz RFR. Sarkar [18] reported changes in DNA sequences in mouse brain cells after exposure to RFR 2 hr/day for 120, 150, and 200 days. Lai and Singh [19] reported an increase in single strand DNA breaks in brain cells of rats after 2 hours of exposure to 2450-MHz RFR. Genetic damages to glial cells which retain mitotic potential can result in carcinogenesis. However, since neurons do not undergo mitosis, a more likely consequence of neuronal genetic damage is changes in functions and cell death, which could either lead to or accelerate the development of neurodegenerative diseases. In this study, the vessels appear congested and showed signs of hemorrhage with an enlarged perivascular space. These finding are in partial agreement with other studies like those of Finnie [20] that showed RFR increases blood-brain barrier (BBB) permeability. At the molecular level EMF produces biological stress and free radical, which can make the susceptible animal population prone to increase permeability of BBB and its consequences like congenital malformation, tissue and cell damage or death [21]. Moreover, free radical**s** can cause oxidative stress at the cellular level interfering with protein synthesis. These elements also play an important role in acute inflammation, endothelial destruction, resulting in tissue edema. It has been postulated that EMF-exposure produces high levels of oxidative stress as a result of its effect on the immune response [22] and long-term exposure to EMF may be linked to even higher levels of oxidative stress [23].

Electron microscopy in the present study also revealed shrinkage of neuron with condensed cytoplasm and nucleus. The mitochondria which were swollen and vacuolized, and the cristae were disordered and fewer in number. These findings were in accordance with the study of sanders [24], who studied the components of the mitochondrial electron transport system that generates high energy molecules for cellular functions. The compounds nicotinamide adenosine dinucleotide (NAD), adenosine triphosphate (ATP), and creatine phosphate (CP) were measured in the cerebral cortex of rats exposed to RFR. In one study, Sanders [24] exposed the head of rats to 591-MHz continuous-wave RFR and observed a decrease in concentrations of ATP and CP and an increase in NADH in the cerebral cortex. These changes were found at both power densities of exposure. They concluded that the radiation decreased the activity of the mitochondrial electron transport system. The synapses of hippocampal neurons in exposed animals exhibited a decrease in the quantity of synaptic vesicles and the synaptic clefts were widened or blurred. Synapses are the places where information is transferred between neurons, and nervous system function is based on their activity. Synaptic loss and synaptic structural changes indicate radiation induced injury & may be associated with reduced synaptic function which may be demonstrated by concomitantly impaired cognitive abilities [25], which is an important function of hippocampus. Thus, the morphological changes in the synaptic structure may indicate radiation related synaptic dysfunction which is evident long before neuronal loss due to synaptic dysfunction may take place [26-27].

Conclusion

The findings of the present study show that RF-EMR from mobile phones are undoubtedly capable of producing demonstrable morphological changes in sector CA3 at both cellular and synaptic levels. These changes though subtle are likely to produce behavioral and cognitive changes which require to be evaluated in future study to ascertain the extent of damage caused by such RF-ER- exposures.

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