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Radiofrequency electromagnetic radiation exposure effects on amygdala morphology, place preference behavior and brain caspase-3 activity in rats

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ABSTRACT

The purpose of the study was to evaluate the changes in amygdala morphology and emotional behaviors, upon exposure to chronic RF-EMR in adolescent rats. Four weeks old male albino Wistar rats were exposed to 900 MHz (power density:146.60 μW/cm2) from a mobile phone in silent-mode for 28 days. Amygdala morphology was studied using cresyl violet, TUNEL and Golgi-Cox staining. Place preference behavior was studied using light/dark chamber test and following this brain caspase-3 activity was determined. Number of healthy neurons was decreased in the basolateral amygdala and cortical amygdala but not in the central amygdala after RF-EMR exposure. It also induced apoptosis in the amygdala. RF-EMR exposure altered dendritic arborization pattern in basolateral amygdala but not in the central amygdala. Altered place preference and hyperactivity-like behavior was evident after RF-EMR exposure, but brain caspase-3 activity did not change. RF-EMR exposure perturbed normal cellular architecture of amygdala and this was associated with altered place preference.

1. Introduction

As a result of technological revolution, cell phones, which emit and receive radiofrequency electromagnetic radiation (RF-EMR) have become ubiquitous with an estimated 6.9 billion subscriptions globally (WHO, 2011). Majority of the children and adolescents carry at least a phone and uses it extensively. Recent reports suggest that, children as young as 13 years have been treated for addiction to mobile phones and this depicts the extent of impact of this technology amongst children (Lee et al., 2017). RF-EMR effects on various biological system is one of the major health concerns over the globe. As a consequence, researchers have extensively studied the effects of various frequencies of RF-EMR (such as 50 MHz, 1800 MHz, 2.5 GHz etc.) including the most widely used 900 MHz band that may cause changes on nerve cells in different brain regions.

RF-EMR is a form of energy and there are considerable reports suggesting that, RF-EMR specific types may affect biological tissues, particularly the brain (Barthélémy et al., 2016; Wang and Guo, 2016; Zhang et al., 2017; Kim et al., 2017). RF-EMR exposure for 50 min could increase the brain glucose metabolism and this increase was in the parts of brain nearest to the antenna. However, the brain metabolism on the whole was almost same between on and off modes (Volkow et al., 2011). According to another report, three months of RF-EMR exposure induced increased MDA levels and reduction in antioxidant parameters in the rat hippocampus and cerebellum. Additionally, neuronal degeneration was found in the hippocampus and cerebellum. These changes were associated with significant over expression of cyclooxygenase-2 apoptotic gene and fragmentation of DNA (Hussein et al., 2016). RF-EMR exposure for 2 h/day for a month induced impaired spatial memory performance and lead to morphological changes in the hippocampal CA1 region such as degeneration of mitochondria, decreased synapses, and shorter postsynaptic densities in the rats (Li et al., 2012). As per another report, RF-EMR emitted from mobile phone affects the auditory potential in human subjects (Singh, 2015). In another study, it was found that, RF-EMR exposure for a month induced altered spatial learning and memory (Narayanan et al., 2009) and affected surviving cell count in the hippocampal CA3 region along with changes in dendritic arborization pattern in exposed group (Narayanan et al.,

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2015). In a report, it has been documented that, one month of exposure to RF-EMR had induced significant behavioral changes in rats as evident from elevated plus maze (EPM) test (Kumar et al., 2009). Additionally, RF-EMR exposure for a month lead to changes in oxidant and antioxidant status in hippocampus, amygdala, prefrontal cortex and cerebellum (Narayanan et al., 2014) and induced altered passive avoidance behavior and morphological changes in the hippocampus (Narayanan et al., 2010). As a result of substantial number of publications demonstrating the impact of RF-EMR on biological systems, researchers have opinioned that, there is an immediate need to evaluate the health implications of RF radiation (in the range 30 kHz–300 GHz) particularly on neurological disease, physiological addiction, cognition, sleep, and behavioral problems in addition to its role in cancer (Hardell, 2018).

Most of these reports have focused on RF-EMR induced changes in the cortex and hippocampus. Reports on the effect of RF-EMR on amygdala morphology, particularly nerve cell damage and surviving cells have not been reported so far. The amygdala is a collection of nuclear groups and is located deep in the temporal lobe (Johnston, 1923). The amygdaloid complex in rat consists of thirteen regions. As per 'Price's' nomenclature, amygdala nuclei are categorized in to three major groups such as, the basolateral group, the cortical group and the centromedial group (Price et al., 1987). The different nuclei can be very well distinguished on the basis of cytoarchitecture and are referred as amygdaloid complex (Price et al., 1987; Amaral et al., 1992). This complex regulates memory, attention, emotions etc. (Davis, 1992; Kapp, 1992). However, the most studied and the best understood function of amygdala is its contribution to the detection of emotional events and the production of appropriate responses (emotional processing).

Researchers have used various tools to measure the emotional process in rats. The emotional processes are evaluated in rats by putting them into a situation of conflict. Rodents prefer darker areas over lighter areas but when presented to a novel environment they have a tendency to explore (Crawley and Goodwin, 1980). Although there are several tools used to measure the emotionality, one of the best studied and validated tools is light dark chamber (LDB) test. The advantage of LDB test is that it is being fast and easier to use. Moreover, prior training of animals is not required in this test.

Evidences suggest that, caspase-3 is vital for processes particularly associated with cell death or dismantling of certain cells and also the formation of apoptotic bodies (Hyman and Yuan, 2012). In addition to this, caspase- 3 is also involved in cellular remodeling processes and these functions are independent of apoptosis. They include, dendritic pruning, metaplasticity and long term depression. One of the recent reports suggests that, in relation to the whole organism, caspase-3 deficiency in mice disturbs specific aspects of cognition and behavior, particularly attention and inhibitory control that results in a behavioral phenotype similar to attention deficit/hyper activity disorder (ADHD) (Lo et al., 2016).

Although the effect of RF-EMR on various brain regions has been studied, evidences on its effect on amygdala morphology and related behaviors is scanty. Hence, in the current study we investigated the effects of RF-EMR on morphology of amygdala nuclei, activity of caspase-3, and its possible impact on place preference behavior in rats.

2. Materials and methods

2.1. Animals and maintenance

Four week old male albino Wistar rats (50–60 g) were acquired from Central Animal Research Facility of our university. The rats were taken from six different litters and were kept in plastic cages which measured 41cm × 28cm × 14 cm. Each of the cage housed three rats. Water and food was given to them *ad libitum* and a 12:12 h L:D environment was maintained in an air-conditioned room. Institutional animal ethics committee approval pertaining to the current research was obtained.

2.2. Experimental groups and study design

Rats were grouped into; A: Control, B: Sham exposed and C: RF-EMR exposed. The *animals of group A* were housed in the home cage throughout the entire experimental period (28 days). *Animals of group B* were exposed to a cell phone in switch-off mode (1 h/day). *Animals of group C* were exposed to 900 MHz RF-EMR from a mobile phone (1 h/day, in silent mode, without any ring tone) for 28 days. The study was carried on in two separate stages. In stage-I; the morphology of amygdala was studied. 36 rats (n = 12/group) were used in this stage and they were divided into various groups as stated earlier. On 29th experimental day, 6 animals from each group were killed, brain was removed and processed for cresyl violet staining, Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, and the other animals were used for modified Golgi-Cox staining. In stage-II; place preference behavior was studied. Thirty six rats (n = 12/group) were used in this stage, and they were divided into different groups as cited above. To study their place preference, on 29th experimental day, all rats were tested on light-dark chamber and then euthanized. The brain was shelled out to study caspase-3 activity in the amygdala, hippocampus, and prefrontal cortex.

2.3. RF-EMR exposure and power measurements

The mode of RF-EMR exposure and the dose were used in the current study was in accordance with previous publications (Narayanan et al., 2009, 2013). Animals (3/cage) of the RF-EMR group were subjected to 900 MHz radiation through a cell phone with a maximum power level output 2W/kg (SAR; 1.15 W/kg). The cell phone was placed in a small wire-mesh cage with a wooden bottom, in the center of the home cage. This was particularly done to prevent rats being in contact with the phone. Rats were exposed to radiation for 7 days per week (1 h/day) for 28 days. To expose rats with 900 MHz radiation, the phone which was placed in the cage was activated repeatedly by giving unattended calls (50 calls/h). This was performed with the help of an auto dialer unit (indigenously made to befit our needs) which can dial four phone at a time. All these phones were acquired from the same manufacturers with the same SAR specifications. Measurement of power density was performed in the cage by SPECTRAN HF-2025E with MCS real-time spectrum analyzer software, Aaronia AG, (Germany). Peak power density was noted to be 146.60 μW/cm^2 at 3 cm distance from the cell phone when the phone was ringing (Narayanan et al., 2013).

2.4. Cresyl violet (CV) staining and quantification of surviving neurons

CV stain was made as per the previous published reports (Narayanan et al., 2015). The sections were embedded with paraffin and then stained with cresyl violet as reported in the earlier research (Narayanan et al., 2015; Bancroft and Stevens, 1990). The slides were coded by an independent person before quantitative analysis. The method reported earlier (Narayanan et al., 2015) was followed to count viable cells in different amygdala nuclei (basolateral amygdala, central amygdala, and cortical nuceli). Briefly, viable neuron count was done using a light microscope (Magnus MLX, Microscope) which was equipped with an ocular micrometer and a stage micrometer (Erma, Tokyo, Japan). Hundred divisions in the micrometer measured 250 μm on the stage. Ten brain sections of each animal were counted for the number of viable neurons in 250 μm^2 area. Decoding of the slides was done only after the statistical comparison of the groups was completed.

2.5. Golgi-Cox staining and dendritic quantification

After the experimental period animals were euthanized. The brain