Brain sub-Region-Specific Effect of Mobile Phone Radiofrequency Radiation

Kumari Vandana Singh[1]*, J.P. Nirala[1], Paulraj R[1]

School of Environmental Sciences, Jawaharlal Nehru University,
New Delhi 110067, India

Abstract

With more than 6.8 billion mobile phone users world-wide, health effects due to continuous exposure to radiofrequency radiation emitted from mobile phones (MP-RFR) has generated serious concern. Brain due to its proximity to mobile phones while talking and its specific physiology, is particularly vulnerable. There are no of reports available regarding MP-RFR induced effect on brain ranging from headache, fatigue to more severe effects like cognitive impairment and brain tumor. Most of these effects are reported to be mediated via oxidative stress mechanism. In most of these studies effect of MP-RFR was assessed on whole brain. However different regions of brain greatly vary in their molecular and cellular composition and controls different cognitive functions. In this regards objective of this study was to assess impact of MP-RFR on redox environment and functionality of brain in a region-specific manner. For experimental procedure 6-week-old male Wistar rats were divided into two independent groups sham and exposed, animals of exposed group were subjected to MP-RFR of 1915MHz at power density and SAR value of 4 mW/cm^2 and 0.36 W/Kg respectively for 16weeks (2hrs/day, 5days/week). Animals of sham group was kept in similar condition except MP-RFR exposure. Towards the end of exposure period behavioural testing was performed and the animals were sacrificed to dissect out hippocampus, cortex, striatum and cerebellum region of brain for estimation of oxidative stress markers. We observed a significant increase in reactive oxygen species, lipid peroxides and protein carbonyl level in both hippocampus and cortex region of brain. There was no change in the any of the oxidative stress parameters in striatum and cerebellum region of brain. Decrease in hippocampus dependent fear memory was also not significant. Outcomes of this study indicate that the hippocampus and cortex region of brain is more sensitive to MP-RFR induced changes and these two regions can further be investigated for actual effect occurring at molecular level and their functional implications.

1 Introduction

Over the past few decades there is an exponential growth in the number of mobile phone subscription worldwide. Having the number of mobile phone users reaching 6.8 billion in 2019 (www.statistica.com), exposure to radiofrequency (RF) radiations emitted from these devices has become an unavoidable concern. As the mobile phones (MP) are kept in close proximity to brain while talking, RF radiations emitted can be absorbed at a depth which can affect neuronal activity [1]. Reports regarding effect of RF radiations on central nervous system (CNS) ranges from common fatigue and headache to more severe effects including behavioural abnormality, cognitive impairment, and brain tumor [2]. Since the intensity of RF radiation emitted from mobile phones are much below the limiting guidelines, possibility of thermal mechanism for afore said effects on CNS has been ruled out [3]. However, certain oscillating frequencies of MP-RF signal which are within the range of certain oscillating frequencies in CNS, may interfere and thus impact the neuronal signalling process [4]. Further oxidative stress is the most hypothesized mechanism of action for harmful effects of MP-RFR [5]. There are number of studies which have reported MP-RFR induced increase in concentration of free radical and several oxidative stress markers in CNS and their consequential impact on cognitive functions [6]. Most of these studies have estimated an overall impact of MP-RF radiation on whole brain. However, different subregions of brain differ in their molecular and cellular composition and play different role in controlling various behavioural and memory functions [7]. So, in this study we have examined the effect of MP-RFR exposure at a dose duration and exposure condition mimicking the real human uses on redox environment of Wistar rat brain in a region-specific manner and whether these changes culminate into altered behavioural or memory functions controlled by that specific region of brain.

2 Methodology

Male Wistar rats aged 6-7 weeks were obtained from Central Laboratory Animal Resources, Jawaharlal Nehru University-New Delhi and kept in the animal room in a standard housing condition (12-12hr light dark cycle, 25°C room temperature) with food and water *ad libitum*. Prior approval for all the experimental procedures performed on animals was taken from institutional animal ethical committee (IAEC) of Jawaharlal Nehru University, New Delhi, India (Application code # 23/2016). After one week of acclimatization period animals were randomly divided in to two independent groups (n=4/group) Sham and Exposed. Animals of exposed group were subjected to RF radiation for 16 weeks (2hrs/day; 5days a week). Exposure of 1915MHz frequency at a power density of 4mW/cm^{2}was given in a specially designed exposure chamber through a horn antenna connected to signal generator and amplifier. Frequency and power were periodically measured during the course of exposure by power meter (NBM-520, Narda, Germany) and spectrum analyser (Field fox, N9912A, Agilent technology, USA) to see any fluctuation. Whole body average specific absorption rate (SAR) for each animal was calculated using empirical formula of average SAR [8]. Whole body average SAR comes out to be 0.36 W/Kg. Animals from sham group are kept in exactly similar condition as exposed except any radiation exposure. Six days before the end of exposure period animals were subjected to 5-day contextual fear conditioning protocol while exposure was continued as usual. One day after the behavioural testing protocol and just after the end of exposure animals were sacrificed by CO_2 asphyxiation to avoid any behavioural procedure related effect. Within 10 minutes of sacrificing the animal, brain was harvested and various sub-regions viz. hippocampus cerebral cortex, striatum and cerebellum was dissected out, flash frozen in liquid nitrogen and stored at -80°C for further biochemical analysis. Various oxidative stress markers viz reactive oxygen species (ROS), ferric reducing ability of plasma (FRAP) a measure of total antioxidant capacity, protein carbonyl a derivative of oxidized protein and malonaldehyde (MDA) a measure of total lipid peroxides was estimated in each region of the brain using standard protocols [9,10,11,12]. Contextual fear conditioning test was performed according to earlier described protocol [13] with few changes. Percentage freezing was considered as measure of contextual memory. For all the analysis p-value were calculated using unpaired students t-test and values less than 0.05 considered significant. All the experimental data was analysed using GraphPad Prizm software (GraphPad Prism 8.3.1, USA)

3 Results

In this study we observed that exposure to MP-RF signal at 1915MHz frequency and SAR of 0.36 W/Kg for 16weeks has generated oxidative stress in hippocampus and cortex region of brain. All the oxidative stress markers viz ROS ($p=0.002$) lipid peroxides ($p=0.003$) and protein carbonyl ($p=0.004$) were significantly upregulated (Figure 1A, B &C) while total antioxidant level was significantly down regulated ($p=0.006$) in hippocampus of exposed group as compared to sham (Figure 1D). In cerebral cortex region of brain ROS ($p=0.009$), lipid peroxide ($p=0.02$) and protein carbonyl ($p=0.01$) level was significantly increased in exposed group as compared to sham (Figure 1A, B &C) while there is no change in antioxidant level ($p>0.05$) between the groups (Figure 1D). Striatum and cerebellum region of brain have shown no sign of oxidative stress as changes in the oxidative stress markers are not significant ($p=>0.05$) between the groups (Figure 1A, B, C & D). The effect of MP-RF radiation was particularly pronounced in the hippocampus region of brain as observed by the increased level of all the oxidative stress marker measured. So, we further investigated the animals for any impact of RF signal on hippocampal functionality by subjecting them to hippocampus dependent memory test viz contextual fear memory. We observed a 33% decrease in percentage freezing (a measure of fear memory retrieval) in exposed group as compared to sham on testing day however it is not significant. Decrease in percentage freezing at base line and training day was also not significant in the exposure group as compared to control (Figure 2).

4 Discussion

In the present study we found that exposure to the cell phone RF signal is primarily affecting the redox environment of hippocampus and cortex region, making them more oxidizing, however effect on other two regions striatum and cerebellum was not significant. Hippocampus region of brain is particularly sensitive to any of the environmental stressors including MP-RFR as it controls major stress related behavioural, memory functions, site of adult neurogenesis, and also rich in glucocorticoid and cytokine receptors [14, 15]. Further, there are several reports available which have shown that impact of stress condition on hippocampus specific functions like neurogenesis, cognition etc. are mediated via altered oxidative environment of the hippocampus and vice-versa [16, 17]). These facts corroborate our findings where effect of MP-RFR measured in terms of oxidative stress markers were particularly pronounced in the hippocampus region of brain. Moreover, it is reported that RF radiations emitted from mobile phone antennae can penetrate 4-6cm deep in brain [18] and cortex region of brain is closest to the source of RF radiation (mobile

phones) while talking thus it absorbs maximum radiation. This might be the region for increased oxidative stress in this region of brain upon MP-RFR exposure. In this study we also observed a 33% decrease in fear memory however decrease was not significant enough to be called as impairment of hippocampal memory. This might be due to the small sample size. Further, hippocampal function depends on several other factors as well like level of cytokine, stress hormone etc. which is not measured in this study and these factors have a mutually opposing impact on hippocampal functionality. So, in conclusion this study provides an interesting insight about which region of brain is particularly vulnerable to harmful impact of RF radiations emitted from cell phones and further studies looking in to molecular targets of MP-RFR in hippocampus and cortex region of brain can be done to get precise information about actual interaction at molecular level.

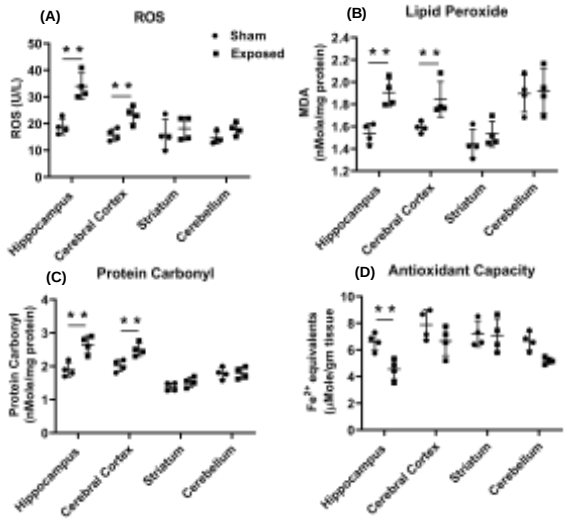

Figure 1. Exposure to radiofrequency radiation of 1915MHz frequency at a SAR value of 0.36W/Kg for 16 weeks (2hrs/day, 5days/week) caused oxidative stress in hippocampus and cortex region of brain however redox environment of striatum and cerebellum region remain unaltered. **(A)** Reactive oxygen species (ROS) level expressed in Units (U) where one unit is equal to 1mg/L H_2O_2. **(B)** Lipid peroxidation measured in terms of malonaldehyde and expressed as nmole MDA/mg protein. **(C)** Level of protein carbonyl, a measure of total oxidized protein expressed as nmol/mg protein and **(D)** Total antioxidant capacity expressed in terms of µmol of FeSO4 equivalents/g tissue in different brain subregions. Results were expressed as mean ±Standard deviation (SD) and compared with Sham by two tailed unpaired t-test (n=4). p-values represents as *; $P < 0.05$; **; $P < 0.01$.

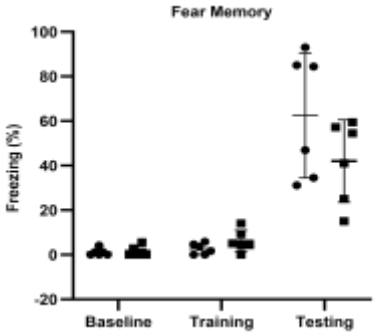

Figure 2. Exposure to radiofrequency radiations of 1915MHz frequency at a SAR value of 0.36W/Kg for 16 weeks (2hrs/day, 5days/week) does not have any impairing effect on hippocampal dependent fear memory. Results were calculated as percent time the rat expressed freezing behaviour during the 5 min observation period for context. Decrease in freezing (%) was taken as measure of impaired fear memory. Values were expressed as mean ±SD and compared with control by two tailed unpaired t-test (n=6).

5 Acknowledgement

The authors are thankful to Council of Scientific and Industrial Research (CSIR, Award no. 09/263(1005)/2013-EMR-I), New Delhi for the fellowship and Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi for assistance in experiments.

6 References

[1] F. Schönborn, M. Burkhardt, and N. Kuster, "Differences in energy absorption between heads of adults and children in the near field of sources", *Health Phys.*, **74**, 2, February 1998, pp. 160-168, doi: 10.1097/00004032-199802000-00002.

[2] M. H. Repacholi, "Health risks from the use of mobile phones", *Toxicol. Lett.*, ***120***, 1-3, March 2001, pp. 323-331, https://doi.org/10.1016/S0378-4274(01)00285-5.

[3] P. Wainwright, "Thermal effects of radiation from cellular telephones", *Phys. Med. Biol.*, **45**, 8, February 2000, pp. 2363, https://doi.org/10.1088/0031-9155/45/8/321.

[4] G. J. Hyland, "Physics and biology of mobile telephony" *The Lancet*, **356, 9**244, November 2000, pp. 1833-1836, https://doi.org/10.1016/S0140-6736(00)03243-8.

[5] I. Yakymenko, O. Tsybulin, E. Sidorik, D. Henshel, O. Kyrylenko, S. Kyrylenko, "Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation" *Electromagn. Biol. Med.*, **35**, 2, April 2016, pp. 186-202. https://doi.org/10.3109/15368378.2015.1043557.

[6] Y. B. Chen, J. Li, J. Y. Liu, L. H. Zeng, Y. Wan, Y. R. Li & G. Z. Guo, "Effect of Electromagnetic Pulses (EMP) on associative learning in mice and a

preliminary study of mechanism" *Int. J. Radiat. Biol.*, **87**, 12, September 2011, pp. 1147-1154, https://doi.org/10.3109/09553002.2011.584937.
[7] K. Sharma, S. Schmitt, C. G. Bergner, S. Tyanova, N. Kannaiyan, N. Manrique-Hoyos,…& M. J. Rossner, " Cell type–and brain region–resolved mouse brain proteome", *Nat. Neurosci.*, **18**, 12, November 2015, pp. 1819, https://doi.org/10.1038/nn.4160.
[8] C. H. Durney, M. F. Iskander, H. Massoudi, C. C. Johnson, "An empirical formula for broad-band SAR calculations of prolate spheroidal models of humans and animals", *IEEE T. Microw. Theory*, **27**, 8, August 1979, pp. 758-763, doi: 10.1109/TMTT.1979.1129720.
[9] I. Hayashi, Y. Morishita, K. Imai, M. Nakamura, K. Nakachi, & T. Hayashi, "High-throughput spectrophotometric assay of reactive oxygen species in serum" *Mutat. Res.*, **631**, 1, July 2007, pp. 55-61, https://doi.org/10.1016/j.mrgentox.2007.04.006.
[10] H. Ohkawa, N. Ohishi & K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". *Anal. Biochem*., **95**, 2, 1979, pp. 351-358, doi: 0 1979.
[11] A. Dubey, M. J. Forster, H. Lal, & R. S. Sohal, "Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse", *Arch. Biochem. Biophys.*, **333**, 1, September 1996, pp. 189-197, https://doi.org/10.1006/abbi.1996.0380.
[12] I. F. Benzie & J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay", *Anal. Biochem*., **239**, 1, July 1996, pp. 70-76, https://doi.org/10.1006/abio.1996.0292.
[13] A. C. Pawlyk, S. K. Jha, F. X. Brennan, A. R. Morrison, & R. J. Ross, "A rodent model of sleep disturbances in posttraumatic stress disorder: the role of context after fear conditioning", *Biol. Psychiatry*, **57**, 3, February 2005, pp. 268-277, https://doi.org/10.1016/j.biopsych.2004.11.008.
[14] P. Andersen, R. Morris, D. Amaral, T. Bliss, J. O'Keefe, *The hippocampus book*. Oxford university press: 2006.
[15] N. Prochnow, T. Gebing, K. Ladage, D. Krause-Finkeldey, A. El Ouardi, A. Bitz, J. Streckert, V. Hansen, R. Dermietzel, "Electromagnetic field effect or simply stress? Effects of UMTS exposure on hippocampal longterm plasticity in the context of procedure related hormone release" *PLoS One*, **6**, May 2011, e19437, doi: 10.1371/journal.pone.0019437.
[16] T. F. Yuan, S. Gu, C. Shan, S. Marchado & O. Arias-Carrión, "Oxidative stress and adult neurogenesis", *Stem Cell Rev. Rep.*, **11**, 5, October 2015, pp. 706-709, https://doi.org/10.1007/s12015-015-9603-y.
[17] V. A. Gault, R. Lennox & P. R. Flatt, "Sitagliptin, a dipeptidyl peptidase-4 inhibitor, improves recognition memory, oxidative stress and hippocampal neurogenesis and upregulates key genes involved in cognitive decline", *Diabetes Obes. Metab.*, **17**, 4, January 2015, pp. 403-413, https://doi.org/10.1111/dom.12432
[18] E. Odaci, O. Bas & S. Kaplan, "Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study", *Brain Res.*, **1238**, October 2008, pp. 224-229, https://doi.org/10.1016/j.brainres.2008.08.013