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Effect of 2.45 GHz Pulsed Microwave Radiation on Hippocampus Mediated Behaviour: in a Specific Relation to the Expression of Rac1, Cdc42 and pCREB

Manoj Kumar¹, Surya Pal Singh², Chandra Mohini Chaturvedi³

M. Sc., Department of Zoology, Banaras Hindu University, Varanasi, India¹.

Ph.D., Department of Electronics Engineering, Indian Institute of Technology (Banaras Hindu University),
Varanasi, India²

Emeritus Professor, Department of Zoology, Banaras Hindu University, Varanasi, India³

ABSTRACT: Present study was aimed to investigate the effect of pulsed microwave exposure on anxiety & depression like behaviour and spatial memory function of laboratory mouse. Mice were exposed to 2.45 GHz frequency with 16 and 217 Hz pulse modulation at the rate of 1 msec, pulse width 500 μ sec & 50 % duty cycle; specific absorption rate (SAR) of 0.017 W/Kg for 30 days (2 hrs daily, 10.00 to 12.00 IST). Mice behaviour was monitored in different behavioural paradigms and hippocampal tissue was processed for expression analysis of Rac1, Cdc 42 and pCREB^(S-133). Our results denote that 16 and 217 Hz did not induce anxiety and depression like behaviour analysed in OFT and FST respectively. Spatial memory function was remained unaffected following pulsed 16 and 217 MW exposures (except decline in time spent in target quadrant of 217 Hz). Expression levels of the above proteins complied with the behavioural findings and remain unaltered following 16 and 217 MW exposure. Findings of the present study suggested that 2.45 GHz with the 16 and 217 Hz modulation is unable to trigger any adverse effect on behavioural function of laboratory mouse.

KEYWORDS: microwave, spatial memory, Hz, anxiety and depression like behaviour,

I. INTRODUCTION

Many investigations have led to address the possible ill biological effects of microwave (MW) on central nervous system and brain functions [1]. Hippocampus is a brain's crucial structure involved in the behavioural functions i.e. learning, memory, stress and emotions [2-4]. Plastic nature of hippocampus confers its vulnerability towards structural and functional changes involved in behavioural regulation following MW irradiation [5]. Furthermore, bioelectric nature of brain also renders its susceptibility to MW irradiation and induces changes upon absorption of MW either emitted from mobile phones [6, 7]. Recent studies were also focused to unravel MW exposure related adverse effects on hippocampal physiology [8].

Several evidences have suggested that mobile phones exposure induces effects on brain's neuronal and encephalographic activity as well as on sleep [9-12]. Exposure of pulse MW radiation triggers alteration of monoamine level of different brain areas and documented the deleterious effect of mobile phone radiation on hippocampal dependent cognitive functions in the experimental models [13, 14]. In contrast, there are contradictory findings claiming no adverse change in behavioural function following pulse MW radiation [15]. In line of further evidences, few other studies mentioned that exposure of mobile phone radiation did not alter spatial and non-spatial memory functions [16]. In spite of determining role of hippocampus in learning and memory function, it also plays a role in

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Vol. 6, Issue 10, October 2017

anxiety and depression like behaviour [3]. Some of the recent findings are in agreement with claiming no effect on behavioural functions of pulsed MW exposure. Similarly, Cosquer et al [17] showed that 2.45 GHz pulse MW radiation unable to produce any anxiety responses of rats in plus maze test.

Many researchers have developed an interest to investigate the effect of pulsed MW radiation on brain functions including the role of hippocampus in memory, anxiety and depression behaviour [8]. Rac1 (Ras-related C3 botulinum toxin substrate 1) and Cdc 42 (Cell division control protein 42 homolog) acts as critical regulators of cytoskeleton dynamics in hippocampus, responsible for the regulation of synaptic plasticity underlying the memory formation [18-20]. Similarly, cAMP response element binding (CREB) protein is a nuclear transcription factor involved in cognitive function, associated with depression and neuronal survival [21-23]. The rationale for selecting the 16 and 217 Hz lies in the fact that 16 Hz may interact with brain waves (EEG) and 217 Hz is emitted from mobile phone during operation mode. In view of the above reports, present study was hypothesized to determine the effect of pulsed MW on some proteins [Rac1, Cdc 42 and pCREB^(s-133)] in hippocampus and behavioural functions.

II. MATERIALS AND METHODS

1. Animals

Swiss male mice (weighing 30-35 gms) were used in the experiments and procured from Central Animal facility, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Mice were divided into 3 groups i.e. control (sham), 16 and 217 Hz (n=10) and kept under standard housing condition with 12:12 hrs light and dark (LD) cycle. Mice were fed on chow pellet and water *ad libitum*. Mice were exposed to 2.45 GHz with 16 and 217 Hz pulse modulation (pulse rate 1 millise., pulse width 500 μ sec. 50 % duty cycle), power density 0.029 mW/cm² for 30 days (2 hrs daily 10.00 to 12.00 IST). All experimental procedures were carried out according to the recommendations of committee for the purpose of control and supervision of experiments on animals (CPCSEA, Govt. of India) and approved institutional ethical committee guidelines.

2. MW exposure set up

MW generator (250 KHz-20 GHz, Agilent technologies, model No.E8257D) was serially fixed with coaxial attenuator, coaxial cables, amplifier (model no. 8349B, Hewlett Packard Co., USA), isolator, coaxial adapter and H-plane waveguide (s-band). MW pass through pyramidal horn antenna (mouth dimension: 9 cm X 5 cm, throat dimension 7.1 cm X 3.3 cm and axial length 10 cm, S-band) in E-bend position to the animal cage. Mice were placed in pine wood cage beneath 25 cm to the pyramidal horn. Incident power falling on wooden cage was measured in power meter (Model no. 8481H, Hewlett Packard Co., USA) using a power sensor (Model no. 836A, Agilent Technologies). The distance of animal from the horn aperture was selected according to far field criterion ($R \geq 2D^2/\lambda$). This criterion defines angular field distribution is independent of distance from antenna and radiation intensity is far field parameter. The pine wood cages or animal exposure cages (size 19.2 cm X 19.2 cm X 15 cm) with low dielectric constant were structured with 2 parallel rows of 10 cells/tiny compartments. Animal's body orientation was kept parallel to E-plane of applied microwave. Cell's space was adequate to restrain animals during exposure and provided with proper air ventilation (holes in the wall of cell and beneath to discharge of urine). Cells bed and mouth was furnished with thin sheets of carbon impregnated Styrofoam absorber to reduce reflections of MW. The animal exposure cage was enclosed by a wooden anechoic chamber (65cm X 65 cm X 60 cm) fabricated with Styrofoam to absorb environmental radiations. Incident power was calculated by subtracting the reflected power of antenna from the measured units of power meter output. Power density was calculated with the relation: $= Pt.Gt/4\pi R^2$ Where Pt: power transmitted to the exposure cage, Gt: gain of the horn, R is the distance between horn aperture to mid plane of mice body [24, 25]. Prior to any exposure, power density and whole body SAR were measured. Measured incident power density was 0.029 mW/cm² with whole body averaged SAR 0.017 W/Kg.

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Vol. 6, Issue 10, October 2017

III. BEHAVIOURAL ASSESSMENT

3.1 Open field test (OFT)

OFT was performed as described by previous studies to assess anxiety like behaviour [25, 26]. The apparatus consisted of base area (48X48 cm) divided into identical marked sectors and enclosing walls of 18 cm height. The squares further divided into peripheral and centre sectors. Mice were placed at the centre and allowed to explore the arena for 5 min. Entries, time spent either in central and grooming were scored to evaluate the anxiogenic effect of pulsed MW irradiation.

3.2 Forced swim test (FST)

Mice were subjected to FST for evaluation of depression like behaviour as per the method described from previous studies [25, 27]. Mice were allowed to swim in a glass beaker (15 cm in diameter and 20 cm in height) filled with tap water. Immobility time was observed to measure of depression like behaviour in mice.

3.3 Morris water maze (MWM)

Morris water maze was used to evaluate the spatial memory function [28], comprised of the round water tank usually plastic (diameter 1.3 m, height 60 cm). To test the spatial memory, water tank was visually marked in the four quadrants and filled with tap water to certain heights with slightly submerged platform in target quadrant. Mice are allowed to swim in water pool and find the submerged platform. Mice spent time to locate the platform called as latency time and 1 min cut off time set for the each trial for mice. Significant reduction in the latency time over consecutive trials indicates learning and spatial memory. Probe trials also provide the measures for mice made entries and time spent in the target quadrant (quadrant without platform).

4 Brain tissue collection

Mice were sacrificed after behavioural assessment by decapitation. Brains were dissected out quickly and placed immediately on ice. Hippocampus was isolated from the whole brain as mentioned in our previous study [25].

5 Western blotting

Hippocampal tissues were lysed in RIPA buffer (1% (v/v) with 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulfate (SDS) in phosphate-buffered solution (PBS) containing protease inhibitor aprotinin and sodium orthovanadate) to extract protein. Protein concentrations were determined by BCA proteins assay kit. In brief, protein samples were resolved by 12% (w/v) SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidenedifluoride (PVDF) membranes and were detected using primary antibody (Rac1, Cdc 42 and pCREB (serine 133) rabbit monoclonal 1:800) followed by incubation with IgG- HRP conjugated secondary antibodies (Rac1, Cdc42, pCREB: Anti-Rabbit IgG, 1:3000). Membranes with ECL (chemoluminescent kit) treated were processed for signals detection in Chemilimager/ documentation system. Changes in the intensity of Rac1, Cdc42 and pCREB proteins were normalized using the intensity obtained of the internal control β actin bands.

6 Statistical analysis

One way ANOVA (followed by Tukey's post hoc test) was used to analyse significance among the groups. Values were expressed as Mean \pm S.E.M. * $p < 0.05$ considered as significant.

IV. RESULTS

Effect of pulsed MW on anxiety and depression like behaviour

OFT results denote that only 16 Hz exposed mice showed increased centre entries compared to control, while no difference was seen in time spent in centre of 16 Hz exposed mice. Whereas, 217 Hz pulsed exposed mice did not exhibit change in centre entries and time spent as compared to control mice. Similarly in FST test, immobility time was also found statistically insignificant compared to control. Grooming activity of pulse MW exposed mice was also not found significant compared to control (Table 1).

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Vol. 6, Issue 10, October 2017

Groups	Centre entries (OFT)	Time in centre (OFT)	Grooming	FST (Immobility time)
Control	2.7±0.44	7.86±0.98	3.5±0.76	34.71±4.33
16 Hz	4.5±0.51*	9.133±1.38	4.5±1.23	24.29±4.29
217 Hz	3.5±0.41	10.2±1.15	3.8±1.01	29.86±4.13

Table: 1 Effect of pulsed MW (16 and 217 Hz) irradiation on anxiety & depression like behaviour and grooming. Data values were expressed as Mean±S.E.M. *p<0.05 considered significant.

Effect of pulsed MW exposure on spatial memory function

There was significant reduction observed in the latency time of all groups on subsequent trials or sessions compared to S1 (Fig. 1A). Probe test results showed that only 217 Hz exposed group has shown significant reduction in time spent in target quadrant as compared to control group. Whereas, 16 Hz exposed mice showed no significant difference compared to control (Fig. 1B). Number of entries in the target quadrant was also found insignificant among groups (Fig. 1C).

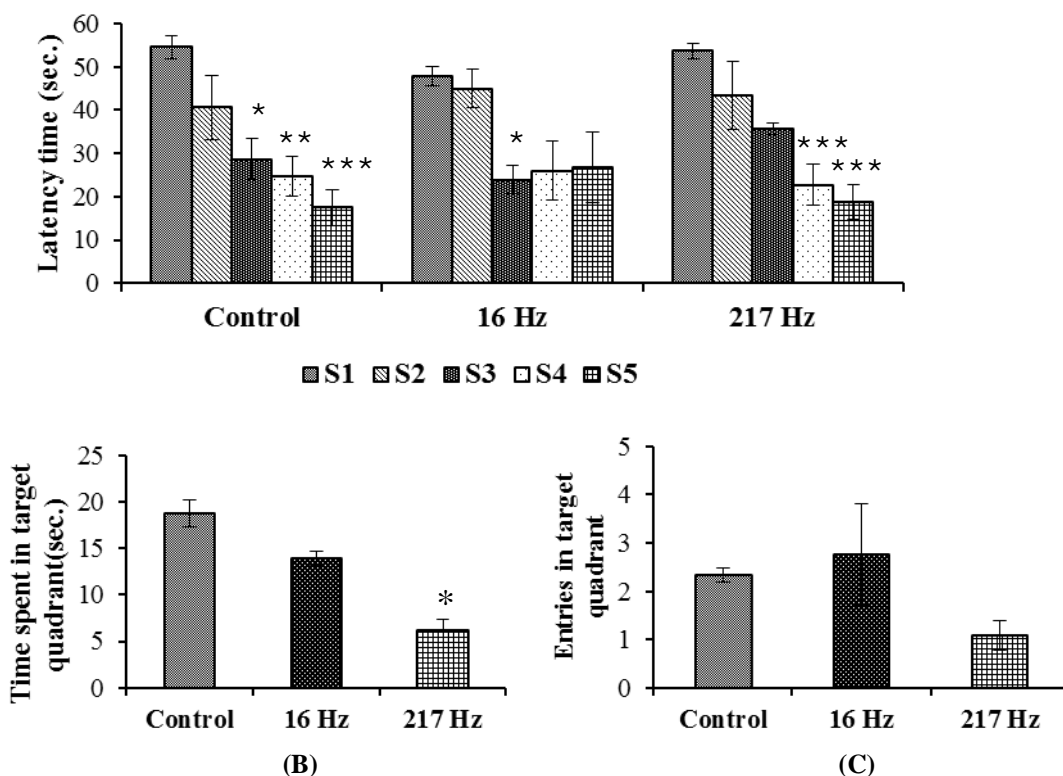


Fig. 1:- Effect of pulsed MW on spatial memory of mice. Significant reduction in latency time among groups showed intact spatial memory (A) (sessions compared to S1 within each group). In Probe test, only 217 Hz showed reduced time spent in target quadrant (B).Whereas, entries in target quadrant of 16 and 217 Hz were insignificant difference from control (C). (S represents sessions, S1, S2, S3, S4 and S5). Data values were expressed as Mean±S.E.M. *p<0.05, **p<0.01, ***p<0.001 considered significant.

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Vol. 6, Issue 10, October 2017

Effect of pulsed MW on the expression of Cdc 42, Rac1 and pCREB proteins in hippocampus

We observe no significant difference in the expression of Cdc 42, Rac1 and pCREB between exposed groups compared to control (Fig.2 A-C).

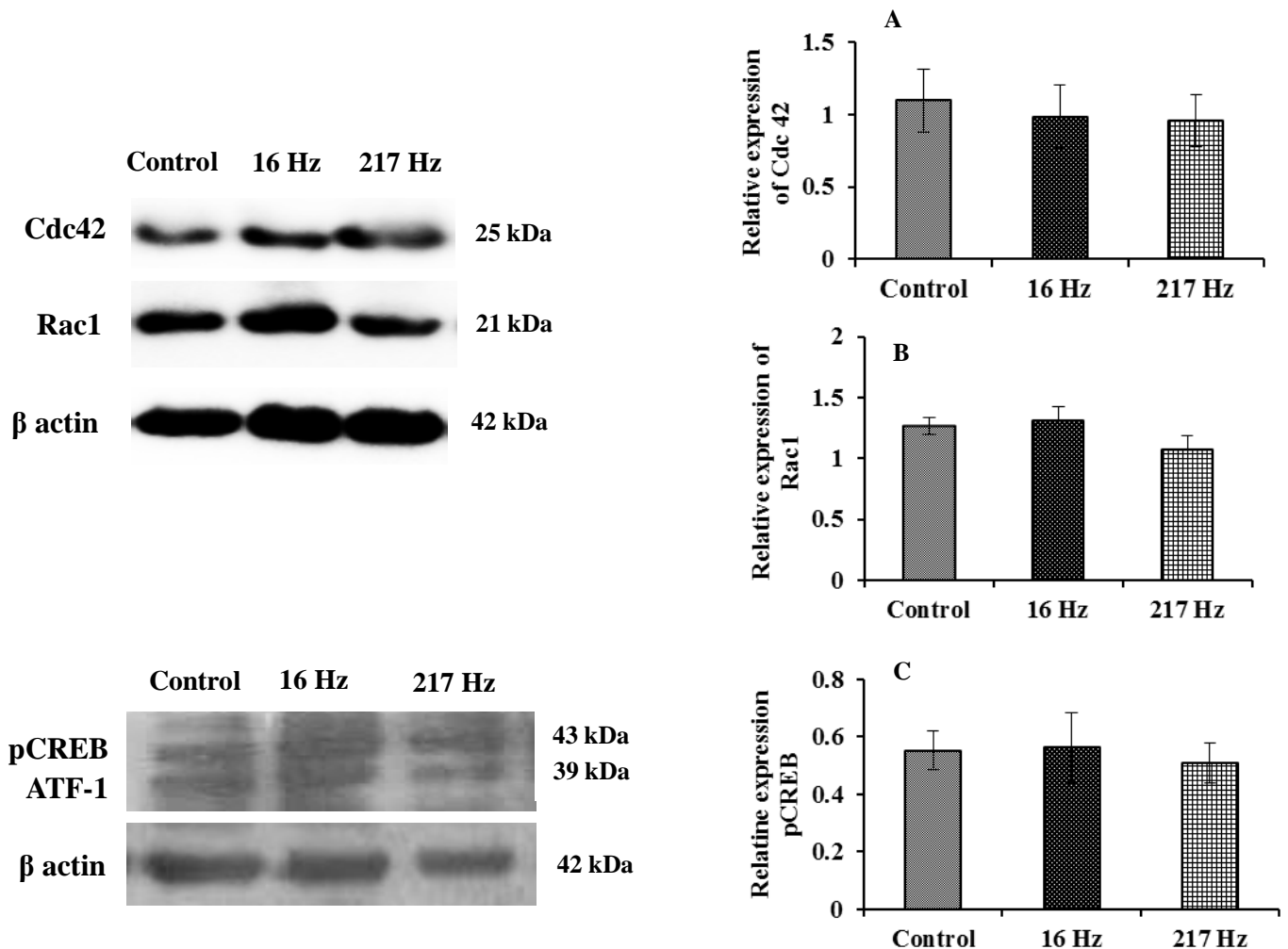


Fig.2: Effect of pulsed MW irradiation on Cdc-42 (A), Rac1 (B) and pCREB^(s-133) (C) expression in hippocampus. The expression of these molecules was found unchanged in hippocampus following pulsed MW exposure. Values expressed as Mean±S.E.M. (pCREB primary antibody (Catalog. No. 87G3) also detects pCREB related protein, ATF-1)

V. DISCUSSION

Present study demonstrated the effects of pulsed MW (16 and 217 Hz) exposure on anxiety & depression like behaviour and spatial memory with any relation to the expression of Rac1, Cdc42 and pCREB molecules. Our results indicated that 16 and 217 Hz exposure did not affect adversely studied behaviour and expression level of investigated proteins remained unchanged in hippocampus following pulsed MW exposure.

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Vol. 6, Issue 10, October 2017

OFT results denoted that 217 pulsed MW did not produce any significant change in time in centre and entries. Whereas, only 16 Hz group showed increased centre entries but did not affect time spent in centre suggesting no anxiogenic effect of 16 Hz. Grooming is an index to stress level and was observed insignificant among exposed groups versus control. Similarly, FST data showed no effect of either types of MW on immobility time, indicating no adverse effect on depression like behaviour. MWM results indicated that both the types of MW radiation showed decline in latency time over consecutive sessions and probe data confirmed no adverse effect on spatial memory. However, decrease of time spent in target quadrant was noted only in 217 Hz. Though, there are scarce findings and limited knowledge pertaining to pulsed MW induced effect on behavioural functions in rodents as well as health effects in human [29-31]. In addition to this, some studies noted the effect of pulsed MW or mobile phone radiation resulting in alteration of neuronal activity and cognitive function in humans [32-34]. Contrary to this, some studies also negate the findings of pulsed MW or GSM signals effects on spatial memory on non-spatial tasks suggesting that these exposure types did not affect such behaviour [16].

At molecular level, we have detected no significant change in the expression level of Rac1, Cdc 42 and pCREB. Western blot analysis showed that cytoskeleton remodelling proteins Rac1 and Cdc 42 was found unchanged following pulsed MW exposure. It can be suggested that neuronal actin organization remain unchanged complying with no disruption in the studied behavioural functions (anxiety & depression like behaviour and spatial memory). Rac1 and Cdc 42, GTPase molecule participates in the regulation of actin skeleton and also modulates synaptic plasticity [18]. Further, expression of both proteins was reported abundant in cortex, hippocampus and thalamic region of brain, suggesting their critical role in neuronal functions and behaviour. The knockout study for Rac 1 protein has lend deeper understanding in the regulation of cortical and hippocampal neurons development and hence deletion laid the basis for impaired memory functions by compromising plasticity in hippocampus (i.e. reduced spine size and density) [35].

Our results suggested that pulse MW exposure did not induce any change in expression level of active CREB (i.e. phosphorylated at serine 133 residues). Though, mobile phone exposure related effects on the pCREB signalling pathway is not well understood. Earlier studies have stemmed evidences regarding the mobile phone exposure effects on genomic response or gene expression [36, 37]. Some recent reports have stated that mobile phone exposure delimit the neurodegeneration via CREB activation (phosphorylation). An investigation of Inoue et al [38] also showed beneficial effect of mobile phone exposure enhanced neurite outgrowth and neurosurvival of cultured cells, mentioning the exposure induced activation of p38 MAPK (mitogen activated protein kinases) pathway via CREB activation that facilitates neurite outgrowth.

VI. CONCLUSION

Above results summarize that behavioural functions along with associated proteins of neuronal plasticity in hippocampus were unaltered following pulsed MW irradiation. Overall, our findings suggest that pulsed MW radiation of 2.45 GHz at SAR 0.017 W/Kg did not induce any anxiety & depression like behaviour, spatial memory and the unaltered level of cytoskeleton/nuclear proteins in hippocampus have strengthened behavioural findings. Therefore, it might be possible that in our designed experimental specifications of pulsed MW exposure, there is no such adverse effect of pulsed MW on hippocampus dependent behavioural functions.

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International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 10, October 2017

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Vol. 6, Issue 10, October 2017

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