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Chronic Nonmodulated Microwave Radiations in Mice Produce Anxiety-like and Depression-like Behaviours and Calcium- and NO-related Biochemical Changes in the Brain

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The present study was aimed to investigate behavioural and biochemical effects of chronic exposure of amplitude modulated and non-modulated microwave radiation on laboratory mice. Chronic microwave exposures were executed with 2.45 GHz of either modulated (power density, 0.029 mW/cm^2; specific absorption rate, 0.019 W/Kg with sinusoidal modulation of 400 Hz) or non-modulated continuous sinusoidal wave (power density, 0.033 mW/cm^2; specific absorption rate, 0.023 W/Kg) for 2 hrs daily for 1 month. Mice subjected to non-modulated microwave exposure had significantly increased acetylcholinesterase activity and increased intracellular calcium and nitric oxide levels in the cerebral cortex and hippocampus, and also had increased glucose and corticosterone levels in blood compared to control mice. These non-modulated microwave-exposed mice exhibited anxiety-like and depression-like behaviours. In contrast, mice exposed to modulated microwave for the same period did not show such changes in concomitant biochemical and behavioural analyses. These results suggest that chronic non-modulated microwave, but not modulated microwave, radiation may cause anxiety-like and depression-like behaviours and calcium- and NO-related biochemical changes in the brain.

Key words: modulated microwave, nonmodulated microwave, anxiety, depression

INTRODUCTION

Tremendous advances in recent years have been made in use of mobile telecommunication. These microwave emitting devices, particularly mobile phones and vast base stations presence have raised issues on possible biological and health consequences of long-term exposure of such radiation. These mobile devices are held for hours very close to our body, particularly the head, for conversation and data usage. Microwave is exploited for voice transmission, texting and data transfer. Transmission of signals is achieved via applying different types of modulation to produce modulated signals. Amplitude modulation (AM) is one such type, an earlier means being used in radio. Another is frequency modulation or FM. Presently AM is advanced and employed to provide efficient and speedy data usage through WiMAX and WiFi access. Considering the close proximity of mobile phones

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to our body, non-ionizing microwave radiation induced effects on the brain functions have to be investigated. Human head likely receives higher specific absorption rate (SAR) as compared to the rest of the body [1]. Moreover, brain's bioelectric property is well documented and also accompanied to receive and react to low frequency signals. This neural interaction with external field signals takes place owing to resonance with lower frequencies and making absorption biophysically feasible. Microwave could also elevate head temperature [2]. Previous studies have indicated the effects of modulated microwave on behaviour, hormonal and enzyme alteration [3, 4]. Although reported results are somewhat inconsistent and contradictory, a number of studies reported that following microwave irradiance, alterations occur in brain activity [5], EEG [6, 7], sleep [8, 9], headache, fatigue, dizziness [10] and cognitive functions [11] in humans and in laboratory animals.

Some studies have pointed out the effects of microwave radiation on behaviours in both human subjects and laboratory animals [11, 12]. Many reports have demonstrated alterations in various types of behaviour at different conditions, levels and types of microwave exposures [3, 11-15]. Exposure of microwave radiation induces neuronal changes leading to alteration in behaviour [14], learning and memory [16, 17] and thermoregulation [18, 19]. Microwave radiation also affects neuronal morphology, neural electrical activity, neurochemical, cellular calcium homeostasis, metabolism, genomic responses, neurotransmitter balance and blood–brain barrier permeability [1]. Previous findings stated that microwave radiations are associated with anxiety, depression and stress [20-23]. Despite these reports, however, behavioural aspects associated with amplitude modulation have not been clearly understood.

Imbalances in various biochemical factors in the brain can affect anxiety- and depression-like behaviour. Cholinergic function in the hippocampus and limbic system is involved in anxiety and depression-like behaviour [24, 25]. *In vitro* studies have also claimed alteration in acetylcholinesterase activity and calcium ion dynamics in neurons following microwave exposures [1]. Hippocampus and other forebrain parts like cortex and amygdala regulates emotion-related behaviours, for which calcium-dependent pathways play an essential role [26, 27]. Microwave exposure is also reported to affect calcium oscillation in neurons i.e. influx/efflux which is controlled by ion channels. Calcium ion is associated with the production of nitric oxide (NO) in neurons, which is also involved in the modulation of anxiety and depression-like behaviour [28]. Hippocampus regulates stress via activation of the hypothalamic-pituitary-adrenal (HPA) axis and it is a principle sensing site of glucocorticoids released in circulation from the adrenal gland [29, 30]. Stress responses trigger the release of corticosterone (a glucocorticoid) from cortex of adrenal gland and circulatory corticosterone controls glucose metabolism. Elevated glucose level manifests stress or anxiety and depression like behaviour.

In view of above evidences and the lack of comprehensive understanding of modulated and non-modulated microwave radiation effects, the present study was carried out to examine the effect of amplitude modulated and non-modulated microwave radiation on anxiety and depression-like behaviour and associated calcium- and NO-related biochemical changes in the brain.

MATERIALS AND METHODS

Animals

Adult male Swiss mice were procured from the central animal facility, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Mice were divided into control, and two exposed groups, and housed in polyacrylic cages (29 cm × 21cm × 13 cm) under the condition of controlled room temperature (24±2°C) and humidity (60~65%) with 12:12 hrs of a light-dark cycle. Commercial available rodent food pellets (Kumar Scientific, Varanasi, India) and tap water was provided ad libitum. All experiments were conducted according to the regulations of the Committee for the purpose of control and supervision of experimental animals and the revised Animals (Scientific Procedures) Act of 2002 of the Government of India on Animal Welfare.

Chemicals

Analytical grade chemicals FURA-2AM, acetylthiocholine iodide, 5,5´-Dithiobis 2-nitrobenzoic acid (DTNB), N-(1-naphthyl) ethylenediaminedihydrochloride) and sulphanilamide were purchased from Sigma-Aldrich (U.S.A). Corticosterone-ELISA kit used in this study was purchased from Abcam (U.K).

Microwave exposure

A signal generator or microwave source (frequency range: 250 KHz-20 GHz; Agilent technologies, model No.E8257D) equipped serially with coaxial attenuator, coaxial cables, amplifier (Max. limit 20 dBm; Hewlett Packard, model no. 8349B), isolator and followed by the coaxial cable to waveguide transition (E-plane bend) and pyramidal horn (mouth dimension: 9 cm × 5 cm, throat dimension 7.1 cm × 3.3 cm, and axial length 10 cm) were used for microwave exposure of animals. The frequency 2.45 GHz was selected based on the Industrial Scientific and Medical (ISM) band and 400 Hz in consideration of the modulation typical communication audio bandwidth. Microwave source was tuneable at this frequency by following the manufactures instructions. Output power was measured using a power meter (Hewlett Packard, model no. 8481H) and a make power sensor (Agilent technologies, model no. 836A).

The distance R (25 cm) between pyramidal horn and animal's body was determined according to the far field criterion ($R \geq 2D^2/\lambda$), where D is the maximum aperture dimension of the horn, and λ is the free space wavelength.

Animal cage (size 19.2 cm × 19.2 cm × 15 cm) was designed using pine wood, which had a low dielectric constant and hence provided better impedance matching with free space. The cage was divided into 10 cells configured with 2 rows of five cells. Each cell had many holes in the wall and floor to facilitate air ventilation and discharge of urine. The edges of cell tops and the bed of cells were fitted with thin sheets of carbon-impregnated styrofoam absorber to diminish reflections.

Subject mice were placed individually in different cells during microwave exposure. Each cell contained a space for an animal to be restrained and to receive radiation falling parallel to the electric field. The radiation exposure cage was put in the large wooden anechoic chamber (dimension: 65 cm × 65 cm × 60 cm) fitted with carbon impregnated styrofoam to absorb or avoid interference of external environmental radiations. The transmitted power from the horn was calculated by subtracting the reflected power from the power incident on the antenna (both power levels were measured).

The power density (Pd) was calculated using the following relation: $Pd = Pt.Gt/4\pi R^2$ where Pt is the power transmitted to the exposure cage, Gt is gain of the horn, and R is the distance between the horn aperture to mid plane of mice body. The whole body averaged specific absorption rate (SAR) was calculated using the relation described by Gandhi et al. [31] by keeping the body length of a mouse parallel to the incident electric field. The power density and whole body averaged SAR were 0.029 mW/cm^2 and 0.019 W/Kg for modulated microwave and 0.033 mW/cm^2 and 0.023 W/Kg for non-modulated microwave, respectively. Radiation parameters were carefully measured and assured timely prior to any exposure.

Mice (n=7, for each group) were subjected to continuous non-modulated and modulated microwave exposure of 2.45 GHz (for modulation: modulating signal 400 Hz, modulation index 0.1%) for daily 2 hrs (10:00 A.M.~12:00 A.M., IST) for one month. Sham exposed group was placed in anechoic chamber without any incident microwave radiation.

Behavioural assessments

Rotarod test

All mice were habituated to the experimental environment for 30 min prior to behavioural tests. Motor coordination behaviours of control and exposed groups were examined using a rotarod apparatus (Orchid Scientific, India). After training for three consecutive days at 2 revolutions/min, animals were placed on the rotating rod at the speed of 15 rpm for 5 min and separately at the accelerating speeds of 5 - 40 revolutions/min for 5 min and the latency to falling was measured as described previously [32, 33]. This motor activity was examined prior to behavioural tests.

Elevated plus maze test

Elevated plus maze (EPM) consisted of four equal arms (30 cm × 8 cm) made of wood with thin plastic coating. Two arms in the EPM were closed with walls (height, 12 cm) and two arms are open (without walls). Open and close arms were fixed at right angle and elevated up to 60 cm from the ground. For the evaluation of the anxiety, mice were placed gently in the central point of the EPM apparatus and allowed to explore arms for 5 min. Time spent in each arm; number of entries into arms were measured manually. The percentage of entries and time spent in the open arms was considered as a measure for the anxiety-like behaviour as described previously [34].

Open field test

The open field test (OFT) was used to assess anxiety-like behaviour as described previously [34, 35]. Dimension of the OFT apparatus was 48 cm x 48 cm with height of walls by 18 cm. It was demarcated with peripheral and central zones marked with an equal size of squares on the floor of the apparatus. Mice were initially placed in the central square of the central zone and allowed to explore the open field for 5 min. Entries in central and peripheral zones, grooming, and stretch attend postures (SAP for exploratory behaviour) were measured for each animal.

Forced swim test

Individual mice were placed softly in glass beaker (15 cm in diameter and 20 cm in height) filled to 1/3 volume (or 15 cm in depth) with water (~25 °C) with a care not to dip head and nose of an animal in water. Time spent in immobility was scored for 5 min and was considered as depression-like behaviour [36].

Brain tissue preparation

Mice were sacrificed with cervical dislocation at the end of behavioural tests. Whole brain was isolated and kept in petri dish in phosphate buffer saline (PBS) on ice. Immediately, hippocampus was dissected out according to the method described by Glowinski and Iversen [37].

Measurements of acetylcholinesterase activity

Fresh homogenate was made in 0.03 M phosphate buffer saline (1:10 w/v) using a plastic-glass homogenizer (Universal/REMI Motors Ltd.) at a speed of 6,000 rpm at 4°C. Homogenate was

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mixed with an equal volume of 1% Triton X-100 (1% v/v in 0.03 M PBS, pH 7). Samples were processed to ultracentrifuge at 100,000 g at 4°C for 1 hr using a Hitachi Micro-ultracentrifuge (CS150NX, Japan) with a fixed-angle rotor. Supernatants were collected and kept at 4°C for estimation of acetylcholinesterase activity as described previously [38, 39]. Supernatants were diluted in 1:10 ratio in 0.03 M Phosphate Buffer Saline, and 50 µl of this sample was added with 2.9 ml of 0.1 mM Phosphate Buffer Saline (pH 8). After 15 min incubation time at 37°C, 40 µl of acetylthiocholine iodide (154.38 mM) and 10 µl of di-thiobis-nitrobenzoic acid (DTNB, 10 mM) were added and O.D. measured. Kinetic profile of the enzyme activity was measured at an interval of 15 sec for 150 sec each at 412 nm wavelength using a spectrophotometer (UV-VIS spectrophotometer, ECIL, India). The unit of acetylcholinesterase activity was defined as a number of micromoles of acetylthiocholine iodide hydrolysed per min per mg of protein and expressed as micromoles/min/mg of protein.

Protein quantification was done following the method described by Lowry et al. [40]. Bovine serum albumin (1 mg/ml) was selected as a standard to determine the quantity of protein in the range of 0.01 mg~0.1 mg/ml.

Measurements of nitrate-nitrite levels

As an indicator of NO production in the brain, nitrate and nitrite levels were measured using a Griess reagent (0.1% N-(1-naphthyl) ethylenediaminedihydrochloride, 1% sulfanilamide, 2.5% phosphoric acid) and concentration was measured as described in the method by Sastry et al [41]. One volume of Griess reagent preparation and one volume of brain tissue supernatant were mixed and incubated at 37°C for 30 min in a hybridization chamber (Biometra OV3, Germany). Absorbance of samples at 542 nm was noted in MicroScan spectrophotometer (MS5608A, Electronics Corporation of India Limited, India). The values were expressed as nmoles/mg of protein.

Estimation of intracellular calcium

Cytosolic fractions from tissue homogenate were prepared using the procedure described previously with slight modifications [42]. In brief, tissues were homogenized in ice cold isolation buffer (IB, 0.15 M KCl, 20 mM potassium phosphate, pH 7.6) in 1:10 (w/v) ratio with a motorized glass homogenizer (Universal REMI motors) at the speed of 8,000 rpm. Homogenate was transferred into centrifuge tubes for centrifugation (2,000 g, 3 min, 4 °C) in a cooling centrifuge (Remi Electronic Ltd, C-30 plus). Supernatant was aspirated in separate tube. Pellet was re-suspended in another tube, briefly homogenized and centrifuged (2,000 g, 3 min, 4°C). The combined supernatant was retrieved from previous steps and subjected for centrifugation (20,000 g, 10 min, 4°C).

The resultant supernatant was obtained as cytosolic fraction to measure intracellular calcium by the ratio-metric dye Fura2-AM. The cytosolic fractions were incubated with the dye (5 µM) in HEPES buffer (145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM $MgCl_2$, 5 mM glucose, 5 mM HEPES, and 0.1 mM EGTA, pH 7.4) for 30 min at 37°C in a hybridization chamber (Biometra OV3) protected from light. Intracellular calcium levels in brain samples were analysed by using the method of Grynkiewicz et al. [43]: $[Ca^{2+}]i$ (nM) = Kd× [(R – Rmin)/Rmax – R)] × Sfb where Kd (for Ca^{2+} binding to fura-2 at 37°C) = 225 nM, R = 340/380 ratio, Rmax = 340/380 ratio under Ca^{2+}-saturating conditions, Rmin = 340/380 ratio under Ca^{2+}-free conditions, and Sfb = ratio of baseline fluorescence (380 nm) under Ca^{2+}-free and -bound conditions. Calcium level in samples were assayed in cuvette at 37°C in fluorometer (Perkin Elmer, LS45) with dual excitation wavelengths; 340 and 380 nm, alternately with slit widths 10 nm and an emission wavelength of 510 nm (slit width 10 nm).

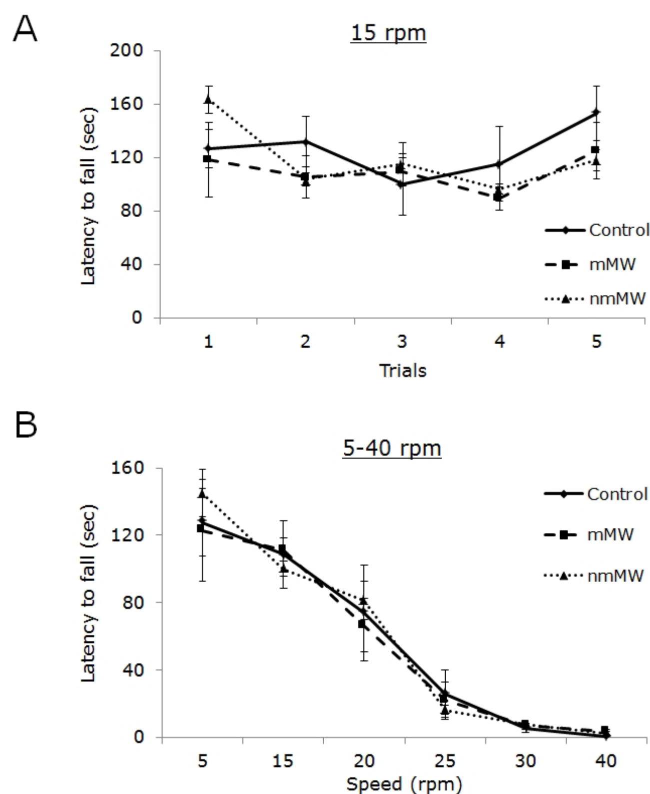

Fig. 1. Chronic microwave treatment did not change in motor behaviours in the rotarod test. (A, B) Motor coordination behaviours on the rotarod rotating at the speed of 15 rpm (A) and at the accelerating speeds of 5-40 rpm (B), among mice subjected to chronic non-modulated microwave (nmMW) or modulated microwave (mMW) radiation and their control. The latency to falling during the 5-min period was measured. Data values are expressed as mean±SEM.

Measurements of corticosterone and glucose levels in blood

Whole blood was collected after decapitation at the end of microwave exposure. Blood samples in tubes were allowed to stand at room temperature for few minutes and centrifuged at 4,000 rpm for 15 min at 4°C. After centrifugation, plasma was collected for measurement of corticosterone using ELISA based kit (AB108821) as described previously [44].

Blood glucose (mg/dl) level was measured at the end of microwave exposure using a digital glucometer (Bayer's Contour) and tail vein puncturing method, which required only a minute drop of blood for glucose estimation as described previously [45].

Statistical analysis

Graph Pad Prism 7 software (Graph Pad Software, Inc., USA) was used for statistical analysis. The values were expressed as mean±S.E.M. One-way ANOVA and Tukey's post hoc test were used for the analysis of significance among controls, modulated and non-modulated microwave groups. $p<0.05$ was considered as a significant value.

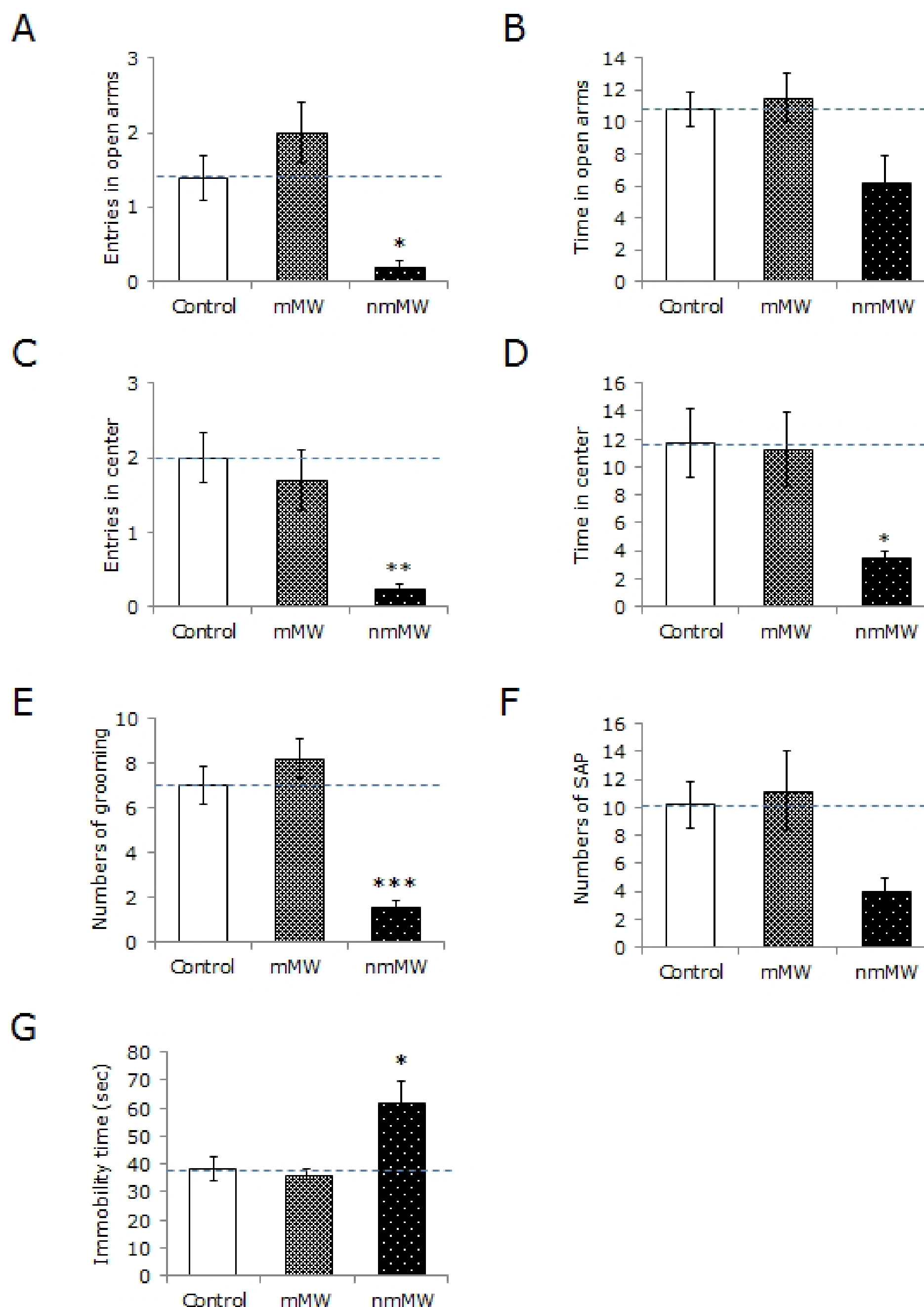

Fig. 2. Chronic microwave treatment produced anxiety-like and depression-like behaviour. (A, B) Entries (A) and time spent (B) in the open arms of the EPM, among mice subjected to chronic non-modulated microwave (nmMW) or modulated microwave (mMW) radiation and their control. (C, D) Entries (C) and time spent (D) in the center zone of the open field, among mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. (E, F) The number of grooming (E) and stretch attend posture (SAP) (F) in the open field, among mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. (G) Immobility time in the forced swim test, among mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. Data values are expressed as mean±SEM. *, ** and ***, significant difference from control at $p<0.05$ or $p<0.01$ or $p<0.001$, respectively.

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RESULTS

Chronic microwave exposure did not alter motor behaviour in the rotarod test

Mice were subjected to microwave exposure with 2.45 GHz of either amplitude modulated (power density, 0.029 mW/cm^2; specific absorption rate, 0.019 W/Kg with sinusoidal modulating frequency 400 Hz) or amplitude non-modulated continuous sinusoidal wave (power density, 0.033 mW/cm^2; specific absorption rate, 0.023 W/Kg) for 2 hrs daily for 1 month. At the end of microwave exposure, motor coordination activity was evaluated in the rotarod test by measuring the latency to falling at the fixed rotating speed (15 rpm) and at an acceleration mode (at 5~40 rpm). Analyses of behavioural performance showed no significant difference in motor activity on the rotarod among mice exposed with modulated and non-modulated microwave compared to the control (Fig. 1A and B).

Chronic microwave exposure induced anxiety-like behaviour

We examined whether chronic microwave exposure affect anxiety-related behaviours using the EPM and OFT on one day before the ending of respective microwave exposures. In the EPM test, the entries and time spent in open arms of apparatus was used to assess the level of anxiety in mice. Mice exposed to chronic non-modulated microwave showed reduced entries in the open arms ($p<0.05$) and insignificant reduced time spent in the open arm compared to the control. In contrast, mice exposed to chronic modulated microwave did not exhibit such reduction in entries and time spent in the open arms compared to the control ($p>0.05$) (Fig. 2A and B).

In the open field test, mice exposed to chronic non-modulated microwave showed significant reduction in entries and time spent in the centre zone of the open field compared to control ($p<0.05$) (Fig. 2C and D). Grooming behaviour was suppressed in non-modulated microwave group compared to control ($p<0.05$) (Fig. 2E). Stretch attend postures (SAP), a measure for exploratory behaviour, was slightly, but insignificantly, reduced in non-modulated microwave exposed group compared to control ($p>0.05$). In contrast, mice exposed with modulated microwave did not show such changes (Fig. 2F). Collectively, these results suggest that chronic exposure of non-modulated, but not modulated, microwave induces anxiety-like behaviours.

Chronic microwave exposure induced depression-like behaviour

The forced swim test (FST) was performed to evaluate depression-like behaviour [46]. Mice exposed to chronic non-modulated microwave showed increased immobility in the FST compared to control ($p<0.05$), whereas mice exposed with modulated microwave did not exhibit any significant effect on depression like behaviour (Fig. 2G). These results indicate that chronic exposure of non-modulated, but not modulated, microwave induces depression-like behaviour.

Chronic microwave exposure induced alteration in acetylcholinesterase activity, NO, and intracellular calcium levels in the brain

Acetylcholinesterase activity (μmol/min/mg protein) in the cerebral cortex and hippocampus was estimated. Mice exposed

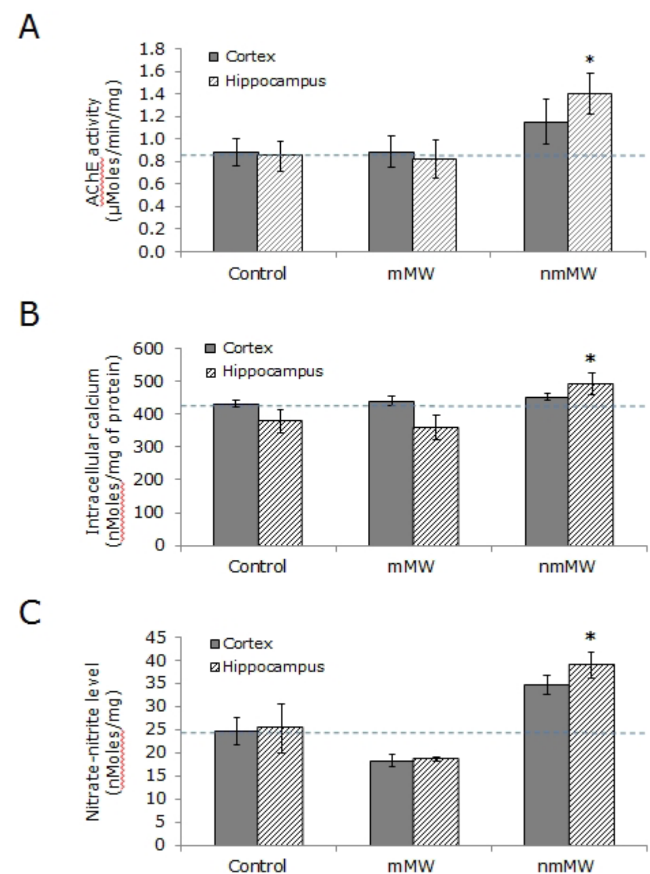

Fig. 3. Chronic microwave treatment produced biochemical changes in AChE activity, NO, and intracellular calcium levels in the brain. (A) Acetylcholinesterase (AChE) activity in the cerebral cortex and hippocampus, among mice subjected to chronic non-modulated microwave (nmMW) or modulated microwave (mMW) radiation and their control. (B) Cytosolic calcium levels in the cerebral cortex and hippocampus, among mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. (C) Nitrate-nitrite levels in the cerebral cortex and hippocampus, among mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. Data values are expressed as mean±SEM. *, significant difference from control at $p<0.05$.

to chronic non-modulated microwave showed increased acetylcholinesterase activity in the hippocampus compared to the control ($p<0.05$), although its effect on the cerebral cortex was insignificant. Whereas, mice exposed to chronic modulated microwave did not show any significant alteration in acetylcholinesterase activity (Fig. 3A).

Mice exposed to chronic non-modulated microwave showed increased intracellular calcium level in the hippocampus compared to the control ($p<0.05$), whereas its effect on the cerebral cortex was not significant. In contrast, mice exposed to chronic modulated microwave tended to show insignificant change in calcium level in the cortex and hippocampus compared to the control (Fig. 3B).

Mice exposed to chronic non-modulated microwave showed increased nitrate-nitrite level in the hippocampus compared to the control ($p<0.05$), whereas mice exposed to chronic modulated microwave exhibit no such significant effect (Fig. 3C).

Chronic microwave exposure induced changes in corticosterone and glucose levels in blood

Mice exposed to chronic non-modulated microwave showed increased glucose and corticosterone levels in blood compared to the control ($p<0.05$), whereas mice exposed to chronic modulated microwave did not exhibit any alteration (Fig. 4A and B).

DISCUSSION

In the present study, we demonstrate that chronic microwave exposure induces anxiety-like and depression-like behaviours and increased acetylcholinesterase activity, NO, and intracellular calcium levels in the brain. These behavioural and biochemical alterations were induced by chronic exposure of non-modulated microwave radiation, but not of modulated microwave. Concerning the modulated microwave radiation effects on behaviours, our result is in part consistent with previous reports [3, 21, 47, 48]. Exposure of pulsed 2.45 GHz microwave radiation, with a whole body averaged SAR of 0.6 W/kg, in rats did not produce any change in anxiety [47]. Exposure of pulsed 2.45 GHz microwave radiation with 100 mW power intensity in mice was ineffective [48]. However, there are contradictory reports on the effects of 2.45 GHz modulated microwave. The 2.45 GHz modulated microwave exposure in rats had affected behaviour [3]. Although what makes this discrepancy is not clearly understood, considering that the calculated averaged whole body SAR in the present study was 0.019 W/Kg and power density was 0.029 mW/cm^2 at the modulated frequency of 400 Hz, it may be attributed to relatively lower SAR than those (0.05~0.6 W/Kg) used in previous reports. Regardless of this insignificant effect of modulated microwave radiation, chronic exposure of non-modulated microwave radiation induced behavioural and biochemical changes, suggesting that non-modulated microwave radiation is harmful to brain function.

We demonstrate that chronic exposure of non-modulated microwave radiation, but not modulated microwave, have also induced physiological and biochemical changes. Despite that chronic exposure of modulated microwave radiation did not affect glucose and corticosterone level in blood, non-modulated microwave

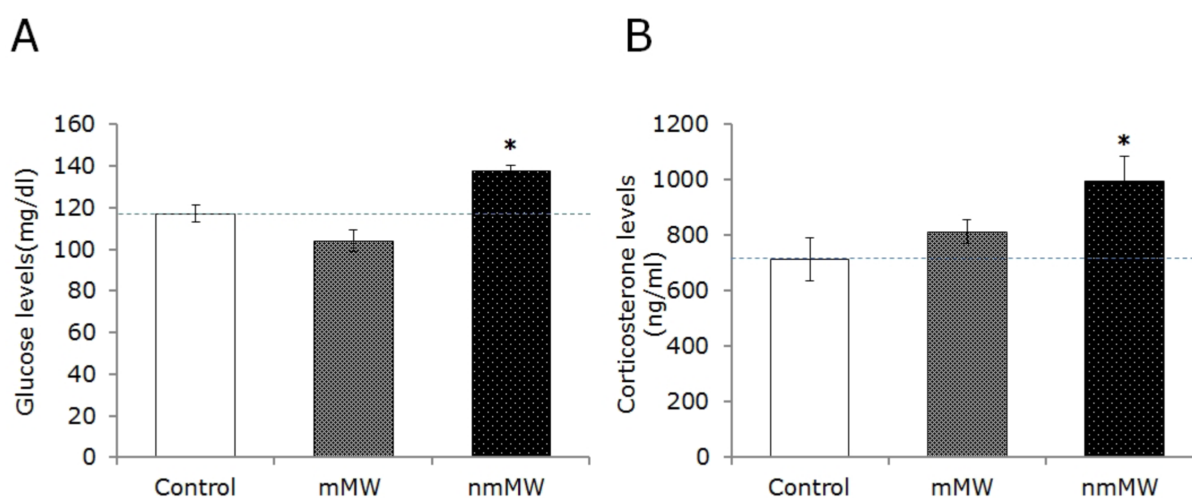

Fig. 4. Chronic microwave treatment produced changes in glucose and corticosterone levels in blood. (A) Blood glucose levels of mice subjected to chronic non-modulated microwave (nmMW) or modulated microwave (mMW) radiation and their control. (B) Plasma corticosterone levels of mice subjected to chronic non-modulated microwave or modulated microwave radiation and their control. Data values are expressed as mean±SEM. *, significant difference from control at $p<0.05$.

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radiation significantly increased glucose and corticosterone levels in blood. Blood glucose and plasma corticosterone levels are closely related to stress responses [23, 49].

Chronic non-modulated microwave exposure has increased acetylcholinesterase activity in the hippocampus (Fig. 3A). Although, physiological significance of this change is unknown at present, a role of the cholinergic system in the modulation of anxiety-like and depression-like behaviours has also been suggested [50] in association with cerebral cortex and hippocampus [25, 30]. Blockade of cholinergic receptors exerts antidepressant-like effects [50]. In regards to this, it may be worthy of noting that increase of intracellular calcium increases the expression of acetylcholinesterase activity in brain cells [51]. Microwave radiation can act on voltage gated calcium ion channels, and thereby enhance the release of excessive calcium ions [52]. Although it was *in vitro* assay, describes mobile phone radiation modulating the activity and expression of acetylcholinesterase [53]. These evidences suggest that exposure of microwave radiation can bring alteration in the intracellular calcium, which could trigger various calcium dependent pathways, including changing acetylcholinesterase activity.

Nitric oxide (NO) is a short lived gaseous molecule, produced by nitric oxide synthase (NOS) and is readily converted to nitrite in tissues. Its signalling in the brain cells, mediates a mechanism involved in depressive disorders and anxiety [54, 55]. Inhibition of NOS enzyme confers an anxiolytic effect [56]. The nitrate/nitrite levels were markedly increased in the brain following chronic exposure of non-modulated microwave radiation, but not modulated microwave (Fig. 3C). Activation of NOS can be triggered by intracellular free calcium levels. Hence, it is possible that NO increase is related to increased intracellular calcium levels in brain.

Our results demonstrate that chronic exposure of non-modulated microwave radiation resulted biochemical and behavioural impairments, whereas chronic exposure of modulated microwave radiation did not produce such changes. Although the precise mechanism remains unknown, the ineffectiveness of modulated microwave exposure on behaviours and biochemical changes could be attributed to the duration of exposure and the type of modulation used. Varying results concerning modulated microwave effects could be due to differences in selected tasks, exposure systems, modulation parameters, frequency discrepancies, experimental models and/or exposure duration. On the other hand, non-modulated microwave exposure produced changes in behaviours, and cholinergic and NO signalling in the brain. Further studies are necessary to evaluate whether chronic exposure of modulated microwave radiation given for a longer duration (>1 month) or in a stronger intensity could affect anxiety and depression-like behaviours.

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