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Archives of Applied Science Research, 2010, 2 (6):37-46

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Anxiolytic and explorative behavioral effects of low SAR microwave radiation exposures on *Sprague Dawley* rats

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ABSTRACT

This paper reports the effects of low SAR MW exposures on explorative activities and anxiolytic behaviours Sprague Dawley rats. 30 rats of both sexes, 6-8 weeks old, weighing 90 -130 g were exposed to various values of SAR from MW generator model ER660E from Toshiba UK. Exploratory activity studies were carried out using white-painted wooden board with 4 elevated plus maze (EPM) holes 1 cm diameter and 2 cm depth. Anxiolytic studies were carried out using EPM and Y-maze models. The mean number of dips in the explorative study varied with time after exposure from a minimum of 1.1 in females exposed to 2.39 W.kg^{-1} 6 days post-exposure to 15.4. 1 h post-exposure to SAR of 0.48 W kg^{-1}, the number reduced from 15.6 ±4.88 to 8.5 ±0.58 in males and from 14.8 ± 1.51 to 8.3 ± 0.44 in fema les. In the anxiolytic activity studies, the variation in the percentage time spent at open end of maze models was from a minimum of 3.92 % with SAR of 2.39 W kg^{-1} in males 1 h post exposure, to 75.11 % in females after 15 days. 1 h after exposure, it reduced from 79.13 to 28.45 with females and increased gradually with time to attain the control value after 15 days. These results showed that MW exposures influence the anxiolytic and exploratory behavioural activities in rats.

Keywords: Microwave exposure, Anxiolytic activity, Explorative activity, Elevated plus maze, Y-maze models.

INTRODUCTION

Microwaves (MW) are electromagnetic radiations ranging in frequency from 300 MHz to 300 GHz and they are increasingly present in our environment. They are used in domestic MW ovens, satellite communications, radio and TV transmissions, cellular phone transmission and reception, video display terminals, in occupational, professional and medical applications through thermotherapy, MW ablation and diathermy [1]. These technologies were designed to maximize energy efficiency and for conveniences, not putting into consideration the possible health detrimental effects on the users and the public. Based on recent studies, there is growing evidence about the possible health risks associated with the uses of, and exposure to MW radiations. This is as a result of interactions with biological tissues, absorption of electromagnetic energy is due to either the forces exerted on the molecules and ions in the biosystems or by changing their energy states. These interactions may be described in several different ways depending on the area of interest and the conditions [2]. Performing some vectorial algebra and integrating over the volume in the Poynting theorem yield the power flow in and out of a given volume as

$$\int_s \mathbf{(ExH).ds} = \int_v \mathbf{[J.E} + {}^{\partial}/_{\partial t}\,\mathbf{(½\epsilon_o E)} + {}^{\partial}/_{\partial t}\,\mathbf{(½\mu_o H.H)} + \mathbf{E}\,{}^{\partial}/_{\partial t}\,\mathbf{P} + \mathbf{\mu_o.H}\,{}^{\partial}/_{\partial t}\,\mathbf{\mu]dv} \quad \ldots\ldots\ldots\ldots 1$$

where ds is the element of surface enclosing the volume V, **E** and **H** are the electric and magnetic field strengths respectively, ϵ_o and μ_o are the free space permittivity and permeability respectively. This treatment allows separation of the power dissipated by the flow of free charges (**J.E**-term) from that due to the motion of bound charges ($\mathbf{E}.{}^{\partial}/_{\partial t}\,\mathbf{P}$-term). In biological tissues the dominant conduction mechanism is by ion transport in which at low and moderate E-values, the current density is expressed as:

$$\mathbf{J} = \mathbf{\sigma E} = \mathbf{qn\mu`E} \quad \ldots\ldots\ldots\ldots 2$$

and the power dissipated in the tissue as

$$\mathbf{P_{2J}} = \mathbf{½\sigma E^2} = \mathbf{½\sigma\, J^2} \quad \ldots\ldots\ldots\ldots 3$$

with E = Ecosωt. The Specific Absorption Rate (SAR) in water (the universal soft tissue equivalent medium for electromagnetic radiation interactions) is expressed as [3] (Mcree 1984).

$$\mathbf{SAR} = \mathbf{4118\ C\Delta T/\ dt} \quad \ldots\ldots\ldots\ldots 4$$

where C is the specific capacity of water, ΔT is the temperature change and dt is the time of heating. The SAR is determined from the slope of the curve following eqn. 4 above.

$$\mathbf{SAR\ (mW/g)} = \mathbf{16.67\ C\ dT/dt} \quad \ldots\ldots\ldots\ldots 5$$

with C equal to 3.52 J/g given by [3,4].

The heart and brain function and are regulated by internal bioelectrical signals. Environmental exposures to artificial MW can interfare with fundamental biological processes in the body. In some cases this may cause discomforts and diseases [5]. Exposure to high intensity MW may cause detrimental effects on the testis, the eye and induce significant changes in the central nervous system (CNS). It can also affect cardiovascular and haematopoietic systems, uteroplacental function, cutaneus perception and development through the thermal and non-thermal actions of MW [6,7]. In animals, behaviour is controlled by the endocrine and the nervous systems and the complexity of the behaviour is related to the complexity of the nervous system. Early experiments seemed to indicate that MW energy at 2.450 GHz was absorbed by water nearly 7,000 times more strongly than at the commonly used short-wave diathermy frequency of 27 MHz [8]. This informed the use of this frequency 2.45 GHz in physical medicine based on its assumed therapeutic value. These studies, however, did not account for such factors as geometry of the body and the depth of penetration of energy into the tissue, which may be more important factors than bulk absorption coefficients alone. This study, conceived out of the concern for the occupationally and professionally exposed to MW and the unsuspecting public, is aimed at studying the effects of 2.45 GHz MW exposures on the possible exploratory and anxiolytic behaviours of mammalian rodents using elevated plus maze, Y-maze and hole- board models. The results hopefully will be useful in predicting the health consequences on man by extrapolation.

MATERIALS AND METHODS

Calibration of Microwave Source

The MW generator model ER660E, Serial No MX704CCR from Toshiba UK Ltd in the Department of Radiation Biology and Radiotherapy, College of Medicine of the University of Lagos was used for irradiation. The detector was the non-interacting thermistor RS 141, which has a resistance of 4.7 kΩ at 25 ^{o}C. The thermistor was calibrated in a 12 cm x 6 cm x 4 cm size water phantom with the aid of a digital readout and a mercury-in-glass thermometer as reference. Details of the calibration procedures have been described elsewhere [9].

Animal and Sample Preparations

30 Sprague Dawley rats of both sexes, 6-8 weeks old weighing 90 -130 g, obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos were used. Their care was in conformity with International, National and Institutional guidelines for care and use of laboratory animals in Biomedical Research according to the Canadian Council of Animal Care [10,11]. The rats were fed with standard rat chou obtained from Livestock feeds, Ikeja, Lagos, Nigeria and they had free access to drinking water. They were initially acclimatized to the laboratory conditions for 5 days and maintained at the standard conditions of 12 h/12 h of dark/light cycle. They were grouped in fives, 5 groups of males and 5 groups of females served as control at various times. All the groups except the control were exposed to whole body MW radiation of various SARs.

Determination of Specific Absorption Rate (SAR)

Measurement of the SAR was done by inserting the thermistor probe into the animal's rectum during exposure following an earlier method of Guy et al. [12] with slight modifications to adapt to local requirements. The irradiation chamber surfaces were lagged with water to minimize the reflective properties which may increase the heating rate [8]. The generator was operated at room conditions of 25 ± 2 ^{o}C and 56 ± 4 % relative humidity. Exposures were total body with the animal at 12 cm from the MW antenna. The SARs were obtained using equations. 4 and 5 with the value of thermal capacity C as 3334 $Jkg^{-1}K^{-1}$ given by previous workers Durney et al. [13], (1980) and Mc Ree and Davies [14] in 1984.

Explorative activity study

The rats were exposed to MW radiation of SAR 0 (as control), 0.95, 1.43, 1.91 and 2.39 Wkg^{-1} for the study of exploratory and anxiolytic behaviours. Exploratory behavioural activity studies were carried out using a white-painted wooden board (40 cm x 40 cm) with 4 equidistant elevated plus maze holes 1 cm diameter and 2 cm depth. The rat was placed at one corner of the board and allowed to move about and dip its head into the holes, which is a demonstration of exploratory behavior [15]. The number of dips every 7.5 mins was observed [16] for 21 days post-exposure.

Anxiolytic activity study

Anxiolytic study was carried out using elevated plus maze (EPM) and Y-maze (YM) models, 120 cm x 12 cm x 12 cm each arm, placed at 60 cm above the floor. The rat was placed at the centre of the Y–shaped wooden runway. The EPM of the same dimensions as YM was made of wood consisting of two open and two closed arms according to Yemitan and Adeyemi [15]. The rat was placed in the central position facing an open arm and the cumulative time spent in each open and closed arm for 5 mins was recorded. Measurements were carried at 1 h and 1 to 21 days after exposure to MW radiation and analyzed using the ANOVA and the results recorded as mean and standard error of mean (SEM).

RESULTS AND DISCUSSION

Figures 1 and 2 present the measured number of dips as a function of time over 3 weeks for male and female rats respectively, after exposures to different SAR of MW. The variation in the measured value was from a minimum of 1.1 in females exposed to 2.39 W kg^{-1}, 6 days post exposure to 15.4, while in the control groups throughout the study period, it ranged between 14.5 ± 0.44 and 16.2 ± 0.48 for both males and females. 1 h after exposure to 0.48 W.kg^{-1}, the number of dips reduced from a mean value of 15.6 ± 4.88 to 8.5 ± 0.58 in males and then decreased till the third day, after which it increased again gradually to attain the control value about 3 weeks later. 1 h after exposure to 0.48 W kg^{-1}, the number of dips reduced from a mean value of 14.8 ± 1.51 to 8.3 ± 0.44 in females, and it decreased till the fourth day after which it increased again gradually to attain the control value after about 3 weeks. Similar trend was observed in all other exposed groups up to other SAR values. The variation in the number of dips with SAR showed that the number of dips is both time- and SAR-dependent.

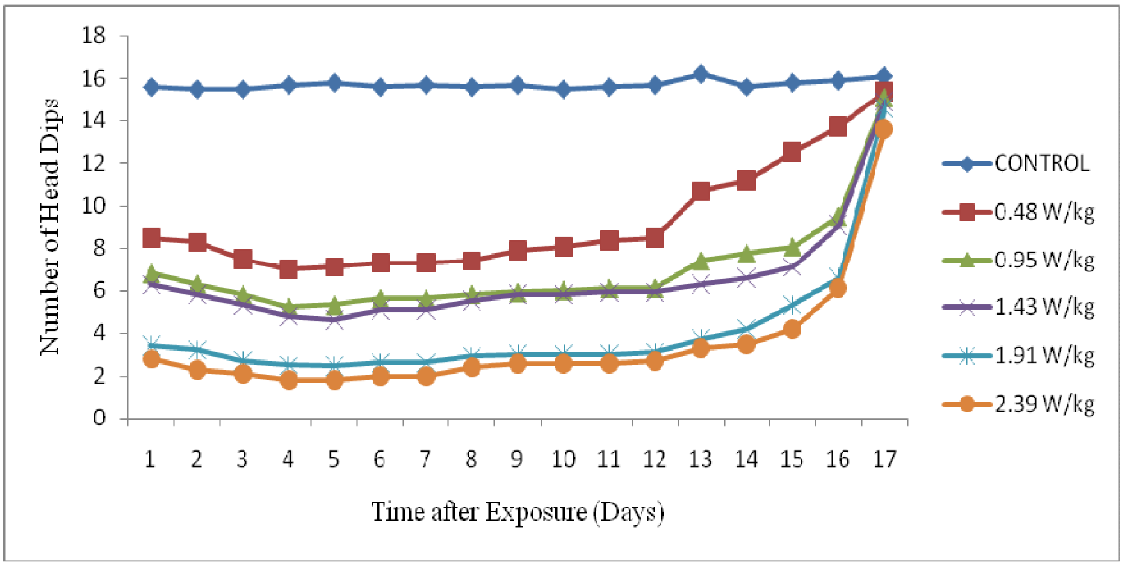

Fig. 1: Microwave Effects on the Exploratory Behaviour in Male Rats

Figures 3 and 4 show the variation in the percentage of duration of cumulative time spent in the open arms of the EPM with the different applied SAR values for males and females respectively. The overall variation was from a minimum of 3.92 % with 2.39 W kg^{-1} in males 1 h post exposure, to 75.11 % with same SAR in females after a period of 15 days.

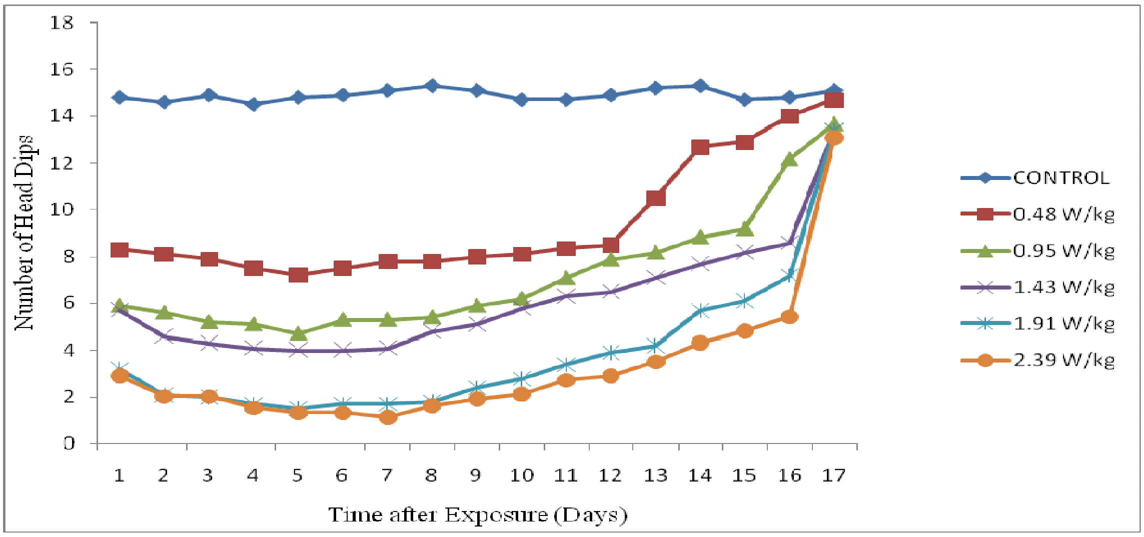

Fig. 2: Microwave Effects on the Exploratory Behaviour in Female Rats

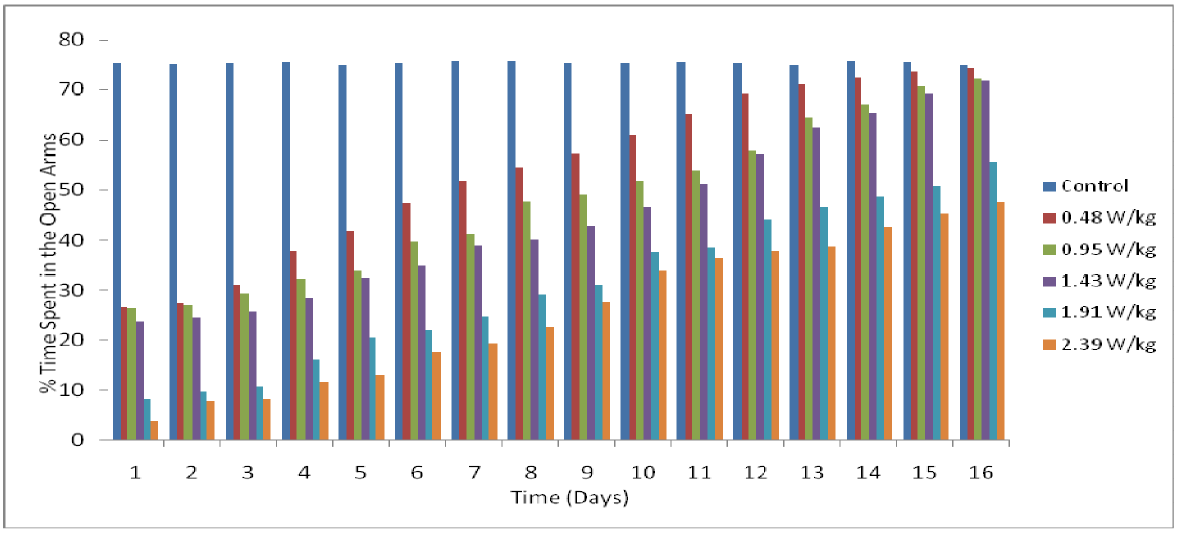

Fig 3: Behaviour of the Male Rats in the Elevated Plus Maze

The control values ranged from 74.93 % to 75.77 %. 1 hr after exposure to 0.48 W.kg^{-1} the percentage of time spent reduced from a mean of 75.30 % to 26.61 % with males, and it increased gradually with time to attain the control value after about 15 days. 1 h after exposure to the same SAR, the percentage of time spent also reduced from a mean value of 79.13 % to 28.45 % with females, and it increased gradually with time to attain the control value after 15 days. Similar trend was observed in all other exposed groups up to SAR value of 2.39 Wkg^{-1}. The minimum percentage time spent found with males was 3.92 % with SAR of 2.39 W kg^{-1} 1 h after exposure, while the maximum was 75.11 % with SAR 0.48 W kg^{-1} at 15 days post-exposure. By implication, longest cumulative time spent in the closed arms of the EPM was obtained in the group exposed to 2.39 W.kg^{-1} 1 h after exposure, while the shortest time is found in the group with SAR of 0.48 W.kg^{-1} 15 days after exposure.

The variations in the percentage cumulative time spent in the open or closed arm of the EPM show both time- and SAR-dependence. These observations demonstrated that MW exposures may have caused fear and anxiety at open and elevated areas. These results correlate with those reported by Yamaguichi et al. [17]. Also, MW exposures altered the exploratory behaviour in male and female rats compared with control. There was no significant difference between both sexes in the measured values, indicating that the effects of exposures are not sex-dependent. For a given SAR value, the number of head dips increased with time to approach the value for the control at the end of the third week. The same trend was observed changes in both male and female in the exploratory behaviour in terms of SAR and time variation over the 3-week period. Some other reported consequences of MW exposures include changes in locomotive behaviour due to SAR as low as 1.2 $W.kg^{-1}$ [18]. Reductions in conditioned behaviour due to SAR of 2.5 $W.kg^{-1}$ and such behaviour ceased at SAR of 10 $W.kg^{-1}$ [19]. Behavioural alterations have been reported to be reversible with time [20]. Bornhausen and Scheingrahen [21] did not record any significant change in operant behaviour in rats prenatally exposed to a 900-MHz. Sienkiewicz et al. [22] reported no significant effect on performance in an 8-arm radial maze in mice exposed to a 900-MHz MW pulsed at 217 Hz with a whole body SAR of 0.05 Wkg^{-1}.

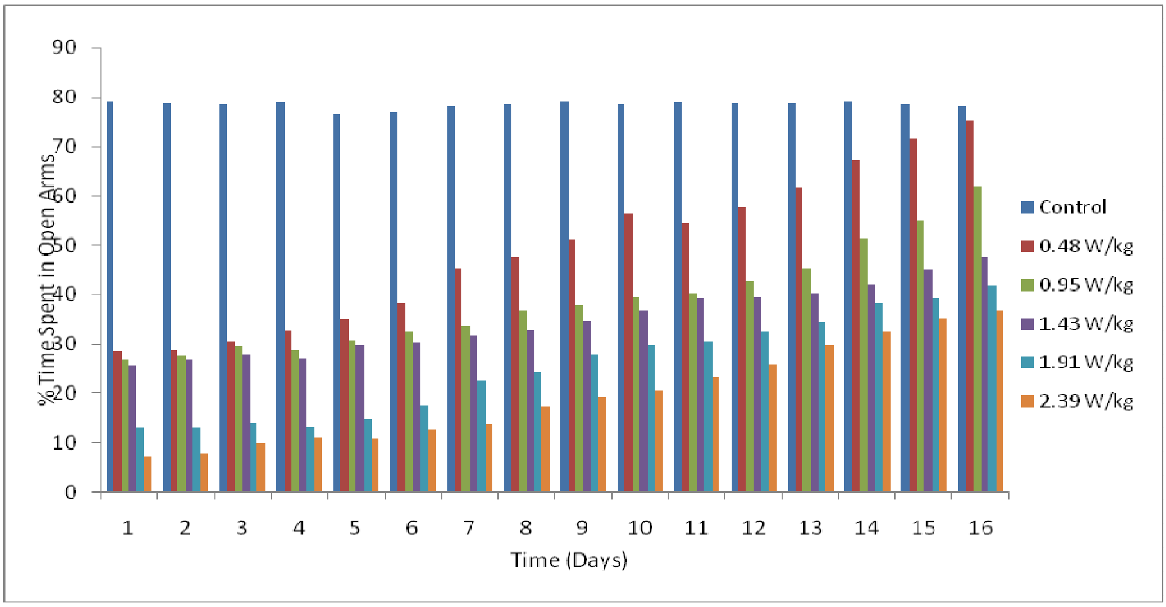

Fig 4: Behaviour of Female Rats in the Elevated Plus Maze

Dubreuil et al. [23,24] found no significant change in radial maze performance and open-field behaviour in rats with heads exposed only for 45 min to a 217-Hz modulated 900 MHz MW at SARs of 1 and 3.5 Wkg^{-1}. Yamaguichi et al. [17] reported a change in T-maze performance in the rats only after exposure to a high whole body SAR of 25 $W.kg^{-1}$. Lovely and Guy [25] reported that rats that were exposed to continuous-wave 918-MHz for 10 min at power densities greater than 25 $mW.cm^{-2}$ and then allowed to drink saccharin solution, showed a significant reduction in saccharin consumption when tested 24 h later. No significant effect was found in rats exposed to 5 or 20 $mW.cm^{-2}$. Rudnev et al. [26] studied the behaviour of rats exposed to 2375-MHz RF at 0.5 $mW.cm^{-2}$, 7 h/day for 1 month. They reported decreases in food intake, balancing time in a treadmill and inclined rod, and motor activity in an open-field after 20 days of exposure. Interestingly, the open-field activity was found to be increased even 3 months post-exposure. In a long-term exposure study [27], rats were exposed to pulse 2.450 GHz MW from 8 weeks to 25 months old. The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 Wkg^{-1}. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12 week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session and a rise in the blood corticosterone level was also observed.

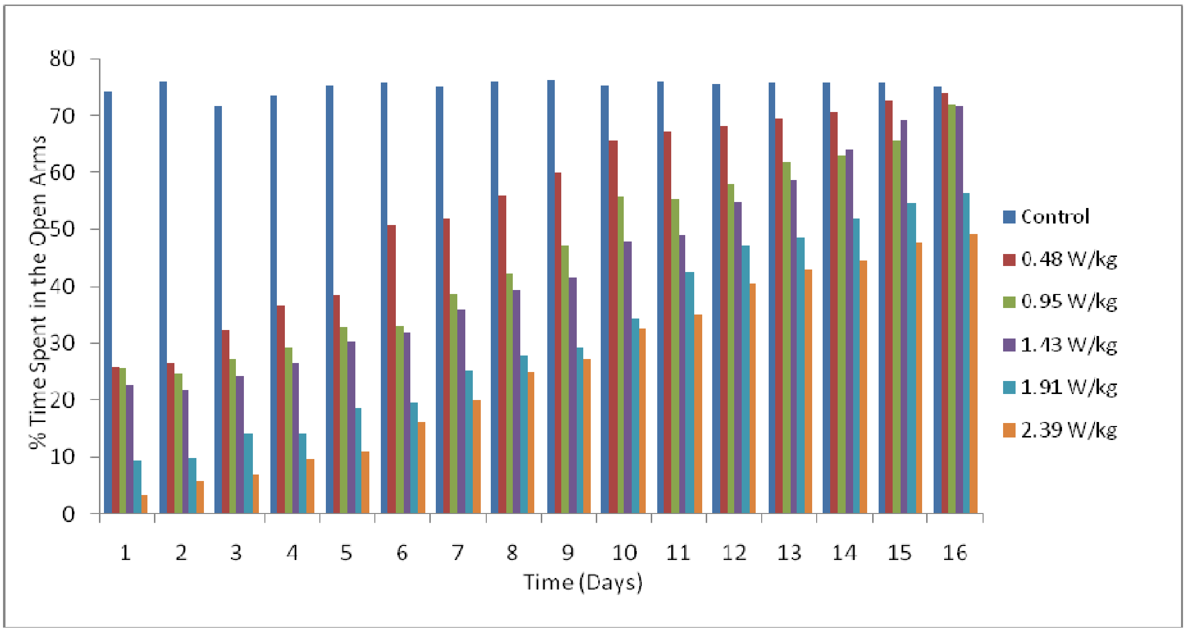

Fig. 5: Behaviour of the Male Rats in the Y-Maze

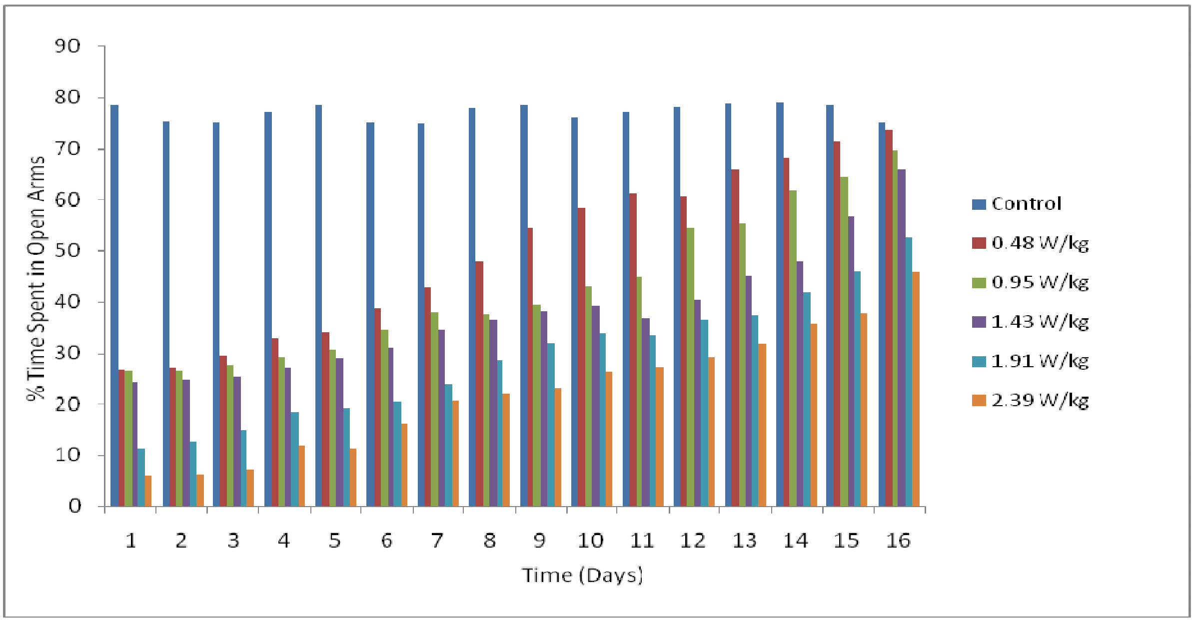

Fig. 6: Behaviour of the Female Rats in the Y-Maze

Figures 5 and 6 show the variation in the percentage of duration of cumulative time spent in the open arms of the YM for the different applied MW SARs with males and females respectively. The overall variation was from a minimum of 3.49 % with SAR of 2.39 W kg^{-1} found among the males 1 h post-exposure, to 73.94 % with same SAR also in males after 15 days. The control values ranged from 74.22 % to 78.88 %. 1 h after exposure to SAR of 0.48 W.kg^{-1}, the percentage of time spent reduced from a mean of 74.22 to 25.85 in males, and it increased gradually with time to attain the 73.94 after 15 days. 1 h after exposure to the same SAR, the percentage of time spent reduced from a mean value of 78.55 to 26.75 with females, and it increased gradually with time to attain 73.60 after 15 days. Similar trend was observed in all the other exposed groups up to SAR value of 2.39 Wkg^{-1}. The shortest percentage time spent found with males for the YM study was 3.49 % with SAR of 2.39 Wkg^{-1} 1 h after exposure, while the longest was 73.94 %, also among males with SAR 0.48 Wkg^{-1} at 15 days post-exposure. By implication, longest cumulative time spent in the closed arms of the YM was obtained in the group exposed to 2.39 Wkg^{-1} 1 h after exposure, while the shortest time was found in the group with SAR of 0.48 Wkg^{-1} 15 days after exposure. The variations in the percentage cumulative time spent in the open or closed arm of the YM show both time- and SAR-dependence.

Absorption of MW energy may cause an increase in tissue temperature following equation 4 above. The initial rate of temperature increase is proportional to the SAR, according to Schwan and Foster [28]. Separato et al. [29] showed that biological effects, such as the heat killing of cells, depend on the temperature profile in time. A well-established athermal mechanism of interaction at frequencies below a few tens of MHz is through electrical stimulation of excitable membranes of nerve and muscle cells [30,31]. RF fields can induce current sufficient to stimulate excitable tissue for frequencies below 1 MHz. This study hereby hypothesizes that the various interaction mechanisms of MW radiation observed in this and previous studies producing behavioural changes may be due to any or some of the following:

1. Effects of MW interactions on nerve cells that may increase or decrease the amount of neurotransmitters released at the synaptic cleft which may also increase or decrease the rate of generation of action potentials, increase the conduction implies greater excitability which may be revealed in form of fear, ectasy or increase in secretion from gland etc.

2. Effects on the normal synthetic and metabolic activities of cells. Production of reactive oxygen species and hence athermal effects having consequences on the nucleus; damage to organelles, DNA and chromosomes which can lead to genetic effects and inadequate production of neurotransmitters by the Golgi apparatus.

3. Delition of receptors for the neurotransmitters on the post synaptic membrane. This event reduces rate of generation of impulses (action potential).

4. If the glial cells serving as a myelin sheath, as seen in oligodendocytes-CNS and Schwann cells-PNS get affected, it may produce a degenerative effect on those lipid coatings or even lead to production of free radicals.

5. If the ependima cells and menange are affected, there may be problem cerebrospinal fluid production or excretion or inadequate carrying out of produce meningitis.

6. If some tissues in the hippocampus are damages by the MW exposures, similar conditions seen in Alzheimer's disease may show up.

7. The heating effect of the radiation can raise or readjust the biological thermostat in the hypothalamus, thus giving the brain a higher than normal temperature. Local warming of the interior hypothalamus triggers physiological and behavioural heat loss mechanism. The animal tries to loose more heat and cool its temperature beyond the normal body temperature because the body thermostat has been readjusted. This results in hypothermia. Persistent hypothermia reduces brain metabolism.

CONCLUSION

Exposures to MW radiation produce bioeffects which influence the anxiolytic and exploratory behavioural characteristics in rats. These observation are consequences of MW radiation interactions with the nervous systems. The results from this study pose a challenge and show the dire need for critical study and understanding of the various interaction mechanisms responsible for the observed effects. The rapidly expanding scope of MW devices, technology and applications are a welcome development for their socio-economic values and convenience. The results from this study however calls for caution in these diverse applications while strict adherence to the recommended safe SAR limits by the various relevant national and international organizations [32,33,34] on radiation protection against non-ionizing radiations.

Acknowledgements
This study was supported by the Third World Organization for Women in Science (TWOWS FR: 3240154345) in collaboration with the Institute of Modern Physics, Chinese Academy of Science, Lanzhou, China.

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