PRELIMINARY RISK ASSESSMENT STUDY- NEUROBIOLOGICAL EFFECTS IN EXPERIMENTAL LONG-TIME EXPOSURE TO LOW GSM RADIATION

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- *Purpose*: Due to continuous rise of mobile phone users, at an increasingly younger age, our preliminary study aimed to assess the possible neurobiological effects of chronic exposure to microwaves, at frequencies and power levels similar to GSM signals. For this purpose, rats were irradiated in their daily habitat. *Materials and Methods*: Twenty male Wistar rats (3 months old) were exposed to GSM 860–890 MHz, 4 hours daily, for 36 weeks. They were compared with sham exposed rats. The medium exposure value of microwave field power density was ≈60 mW/m2 and medium whole body SAR ≈ 0,15 W/kg. Two types of behavioral tests (open field test and elevated pulse maze) and transmission electron microscopy on brain samples were performed after 3 and respectively 9 months of exposure. *Results*: Exposed rats presented decreased locomotor activity and increased emotionality as compared to sham exposed animals. Transmission electron microscopy examination, performed after 3 and 9 months of exposure, showed neurodegenerative alterations in hippocampus and frontal cortex. Severity of alterations seems to be related to duration of exposure. *Conclusion*s: These preliminary results suggest that long-term and low-dose cumulative microwave radiation could cause, in rats, ultrastructural changes in neurons and glia and stress behaviour. Further studies are needed to pursue the interaction of mobile phone radiations with the central nervous system at molecular level.

- *Keywords*: behavioral tests, low power GSM, neurodegenerative damage, increased emotionality, transmission electron microscopy

ABSTRACT

- The biological effects of mobile phone radiation are subject of several decades of scientific research and intense debate; whether or not they are harmful to human body and especially to the nervous system is not yet clarified.
- The aim of our study was to assess the effects of long term exposure to low power GSM signals on behaviour and brain cell ultrastructure in rats. The experimental protocol we propose provides irradiation of the rats in their daily habitat, In our setup, the cage top cover becomes the irradiation antenna.
- In order to assess the effects of the MW exposure, open field test (OFT) and elevated plus maze (EPM) were performed to observe potential behavioral changes. In our study, samples of hippocampus and frontal cortex were analyzed by transmission electron microscopy (TEM) to find possible ultra-structural changes in these brain regions.

INTRODUCTION

MATERIAL AND METHODS

- A total of 30 (20 exposed to MW radiation, 10 controls) male Albino Wistar rats were used in this experiment. At the beginning of the experiment, rats were 3 months old, weighing 230 ± 25g. The animals were kept in standard plastic cages, 5 animals per cage, at a room temperature of 23 ± 0.5^{o}C and at 12/12 hours light/dark cycle, under similar conditions of acoustic noise and ventilation. All animals received standard commercial rodent diet and water ad libitum during the experiment.
- Each animal received an identification number at the beginning of the experiment. They were randomly assigned to the MW exposed groups (two groups of n=10) and to the control groups (two groups n=5). After 3 and respectively 9 months, 10 animals MW exposed and 5 controls were tested in terms of behavior and TEM. Each time, animals were tested, experiments being blind, for locomotor activity one day after the last exposure, and for anxiety–like behavior and locomotor activity the second day after the last exposure.
- On the third day after the last exposure, the animals were anesthetized with a ketamine-xylazine cocktail (60 mg ketamine and 7.5 mg xylazine respectively/ kg b.w.). Under anesthesia, animals were euthanized by decapitation in order to collect cerebral tissue for TEM examination.

MATERIAL AND METHODS

- ***GSM exposure***
- The animals were exposed to MW radiation in the 860 - 890 MHz frequency range, corresponding to the Global System for Mobile Communications GSM-900 uplink (i.e. mobile phone to base) band.The exposure time was of 4 hours per day, from 04:00 to 08:00 daily, for 36 weeks. The MW field power density and whole body SAR (Specific Absorption Rate) values varied between 5 - 120 mW/m^2 and 0.0008 - 0.3 W/kg, respectively. The medium exposure value of microwave field power density was ≈60 mW/m2 and medium whole body SAR ≈ 0,15 W/kg.

- ***Experimental setup***
- The experiment was performed taking into account the *International Commission on Non-Ionizing Radiation Protection* (ICNIRP) recommendations (ICNIRP 1998, 2009a, 2009b) regarding the occupational (SAR < 0.4 W/kg) and general public (SAR < 0.08 W/kg) exposure limits to EMF in the 10 MHz – 10 GHz frequency range. A typical rat cage, i.e. a transparent plastic box with metallic grating cover (Figure 1), was adapted as the MW exposing unit. The metallic cover becomes an omni-directional antenna for the MW radiation. An Agilent N1911A powermeter with an E9321A peak and average power sensor was used for the initial power characterization and the periodical check of the MW power generated in the antenna (Figure 2). The rats were situated in the near field of the transmitting antenna. The MW generator was turned “ON” 4 hours per day, during the rats’ active cycle, by a timer switch. Four identical MW exposure units as described above were used in the experiment, with five rats in each unit (Figure 2).

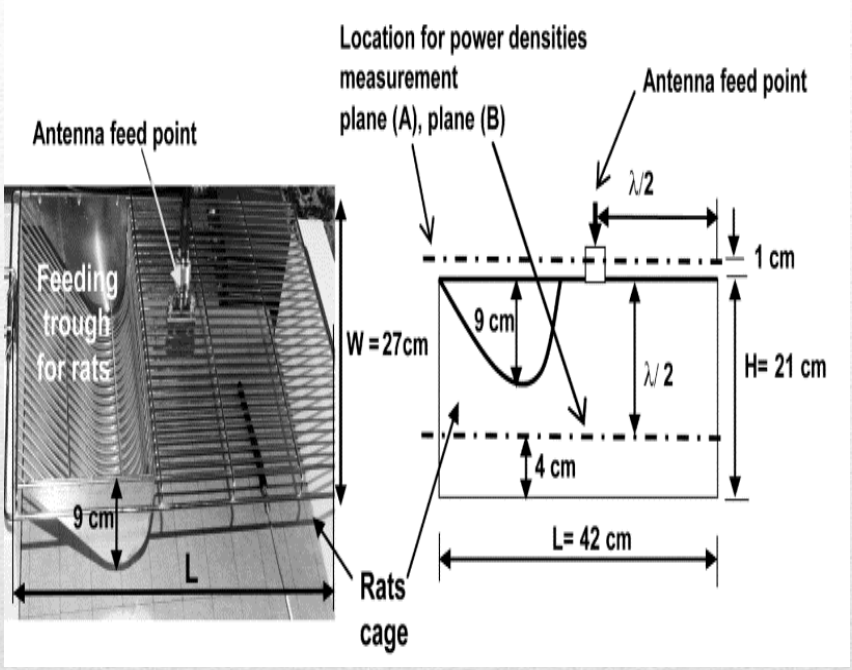


Fig. 1. Antenna configuration and locations for MW power density measurements in the rats' cage

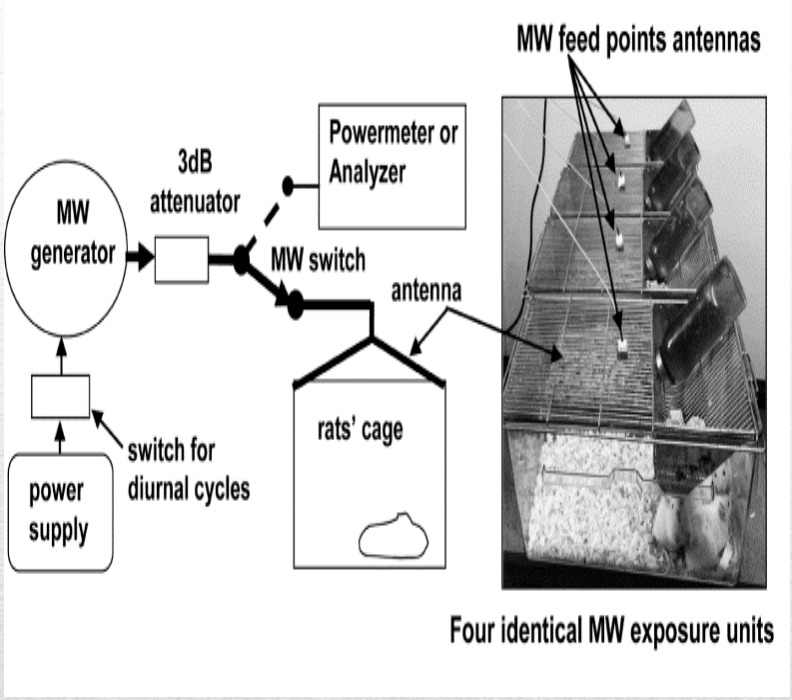
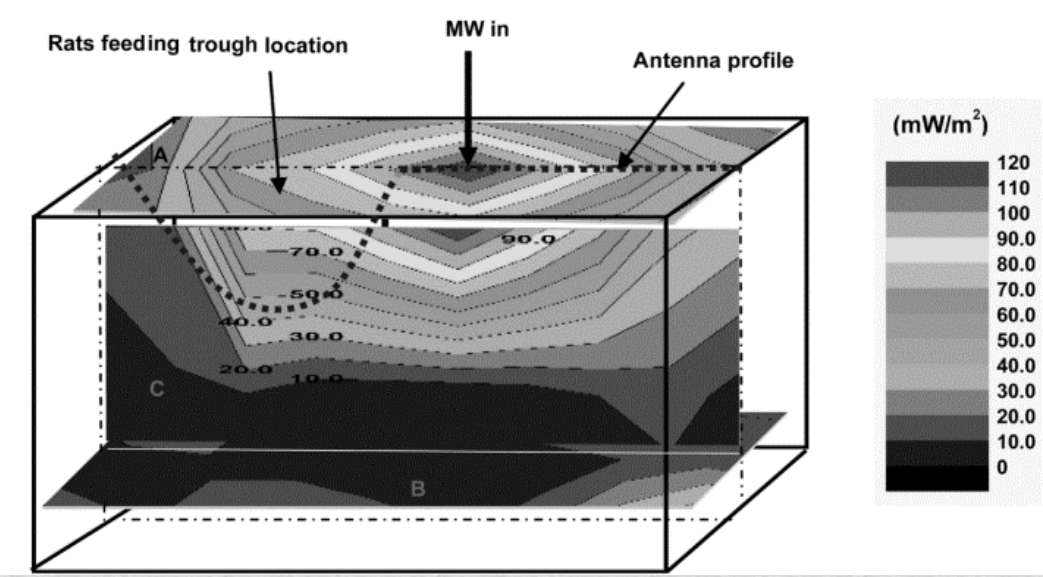


Fig. 2.The experimental setup for the MW irradiation of rats.

Experimental setup

Microwave field power density characterization

- The MW power density (P_D) distribution measured in the cage is presented in Figure 3.
- Fig. 3. MW field power density distributions in the cage:(A) close to the antenna (1 cm above the antenna);(B) at 4 cm from the bottom; (C) in the vertical central plane



Behavioral Testing
OFT and EPM methods

- Two different tests, largely accepted for studying animal behavior, were used in this study, in order to assess the locomotor activity and anxiety–like behavior in rodents: Open Field Test (OFT) and Elevated Plus Maze (EPM). Testing was conducted each time between 09:00 and 14:00.
- A visual tracking system (Smart Basic Software version 3.0 Panlab Harvard Apparatus) automatically recorded the animals behavior over a 5 minute period, while the rats freely explored the specific mazes (Ugo Basil Animal Mazes for Video-Tracking). After the test, the system automatically generated a folder containing the results.

Statistical Analysis

- All statistical analyses were conducted using ANOVA GraphPad Prism software, version 6.0 (GraphPad, San Diego, California, USA). The results were expressed as the mean ± standard deviation (SEM). Two way analysis of variance (repeated measures of ANOVA) was used, followed by Bonferonni`s post hoc test, to determine the statistical significant among two groups. A p value below 0.05 was considered to be statistically significant.

- After euthanizing the animals, 2 small samples, 1.5 mm^3 each, of frontal cortex and respectively hippocampus were collected from each rat and further processed for TEM (Hayat, 2000; Watt, 2003). Samples were fixed with 2.7% glutharaldehyde (Electron Microscopy Sciences, Hatfield, USA) in 0.1 M phosphate buffer pH 7.4 for 2 hours, washed four times with the same buffer, then postfixed 2 hours with 1.5% OsO4 (Sigma-Aldrich, St. Louis, USA) in 0.15 M phosphate buffer, and finally washed twice with the later buffer for 2 hours. After being washed twice with the latter buffer, the samples were dehydrated with an acetone (Nordic Invest SRL, Bucharest, Romania) series, and infiltrated with Epon 812 (Fluka AG, Buchs, Switzerland). One sample of frontal cortex and one of hippocampus were analysed for each animal, while the others were preserved as backup. Ultrathin sections were cut in at least two different regions of each sample, with glass knives using a LKB Ultrotome III Bromma 8800 ultramicrotome (LKB Produckter AB, Stockholm-Bromma, Sweden), and collected on 300 mesh Cu grids. The sections were contrasted with saturated solution of uranyl acetate (Merck, Darmstadt, Germany) in ethanol 50% (18 min), and 2.8% lead citrate (Fluka AG, Buchs, Switzerland) (5 min). Examination was performed with a JEOL JEM 1010 microscope (Jeol Ltd., Tokyo, Japan) operating at 80 kV, and the images were photographed with a Mega VIEW III system (Olympus, Soft Imaging System, Münster, Germany). All TEM analyses were performed blind to the test situation.

Tissue sampling and Transmission electron microscopy (TEM)

RESULTS

Behavioral tests

- The effects of MW exposure on the rats' general locomotor activity, tested in both OFT and EPM, are illustrated in Figures 6 and 7.
- In OFT, our results showed that 3 months exposure to MW significantly decreased the locomotor activity of rats (total travelled distance and travelled distance in periphery, $p < 0.01$; total number of entries, $p = 0.001$; number of entries in periphery, $p < 0.001$).
- After 9 months of exposure, the animals travelled significantly less and made fewer entries in OFT (total travelled distance and total number of entries, $p < 0.05$; travelled distance in periphery, $p = 0.05$; number of entries in periphery, $p = 0.01$).
- In EPM, neither 3 nor 9 months of exposure to MW statistically influenced the total travelled distance and distance in closed arms ($p > 0.05$), even though both parameters tended to decrease in the exposed groups. On the other hand, the exposure of rodents to MW, significantly diminished the total number of entries (3 and 9 months), as well as the entries in the closed arms (9 months) ($p < 0.05$).

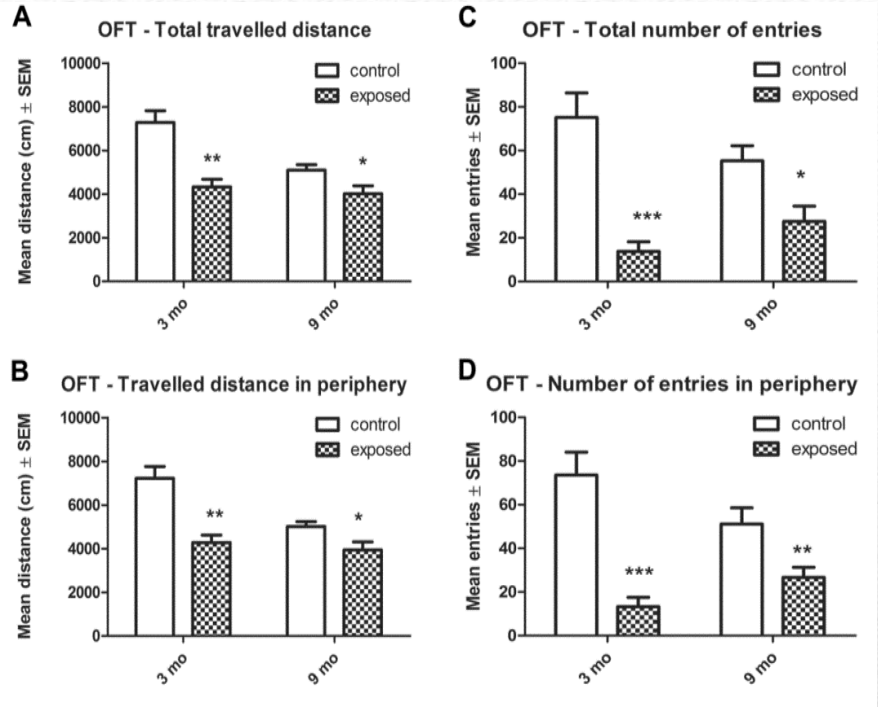


Fig. 4. The effects of GSM radiation on total (A)and peripheral (B) travelled distance and total (C) and peripheral (D) number of entries in open field test (OFT).Each exposed group consisted of 10 rats and each control group consisted of 5 rats.Results are expressed as mean ± SEM.

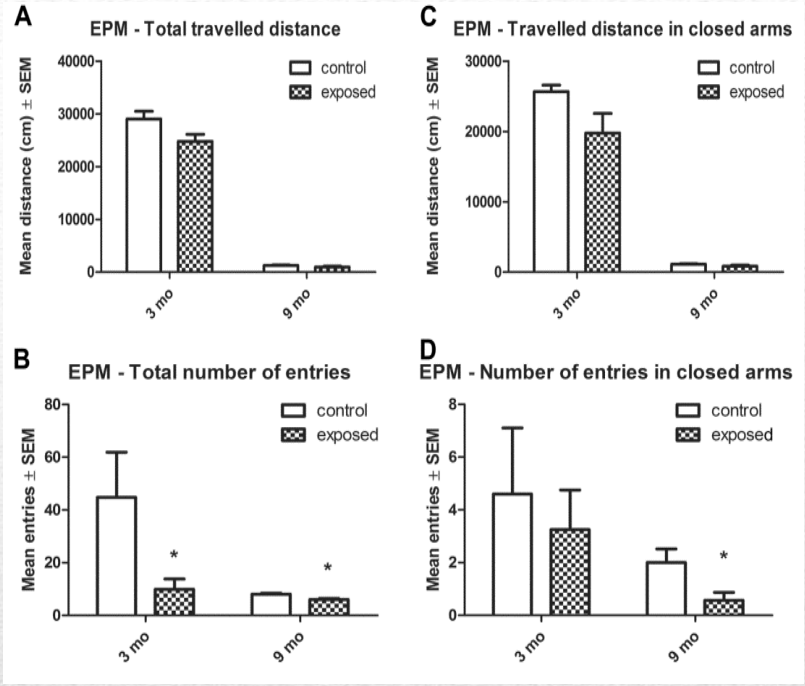


Fig. 5. The effects of GSM radiation on the total (A) and peripheral (C) travelled distance and the total (B) and peripheral (D) number of entries in elevated plus maze (EPM). Each group consisted of 10 rats and each control group consisted of 5 rats. Results are expressed as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001 as compared with control.

RESULTS

Behavioral tests

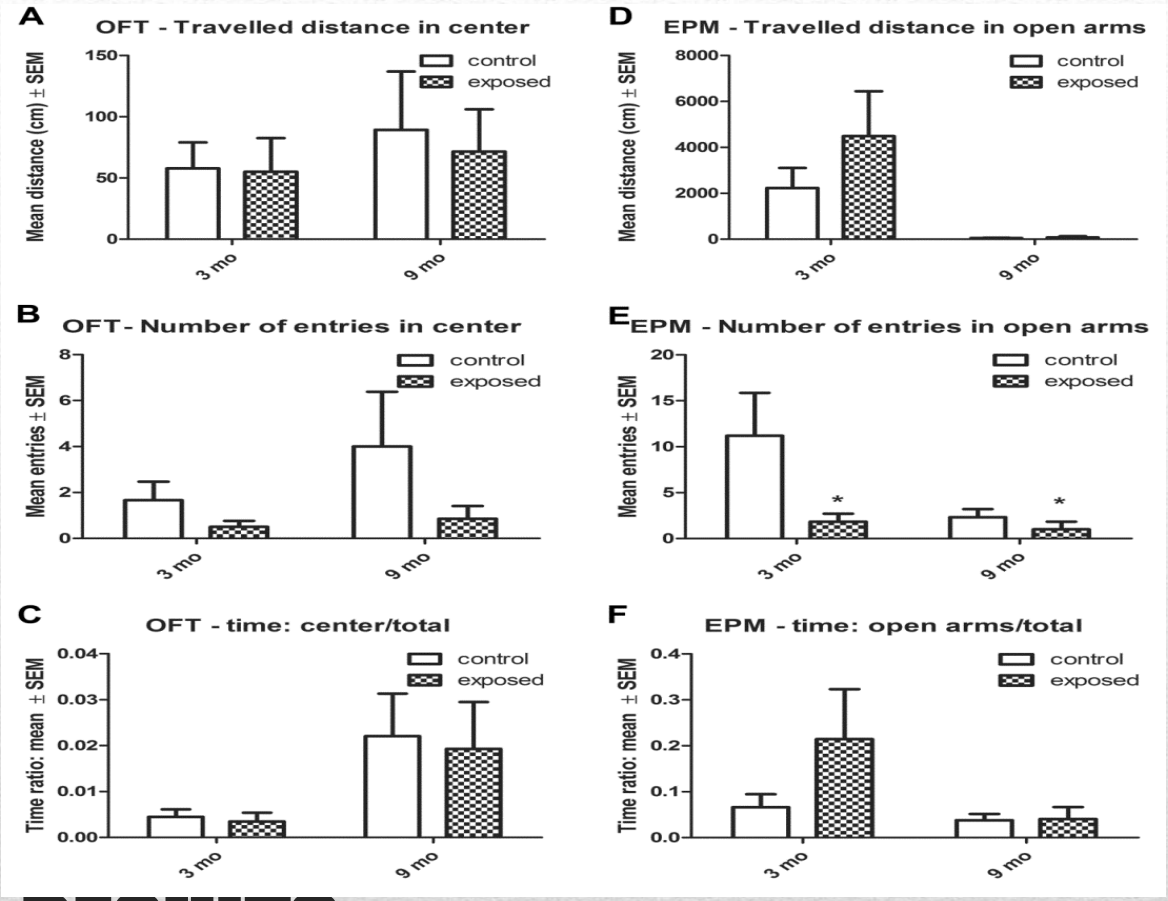


Fig. 6. The effects of GSM radiation on emotionality in open field test (OFT) (A, B, C) and in elevated plus maze (EPM) (D, E, F). Each group consisted of 10 rats and each control group consisted of 5 rats.Results are expressed as mean ± SEM; *p < 0.05, **p < 0.01, *** p< 0.001 as compared with control.

RESULTS

Behavioral tests

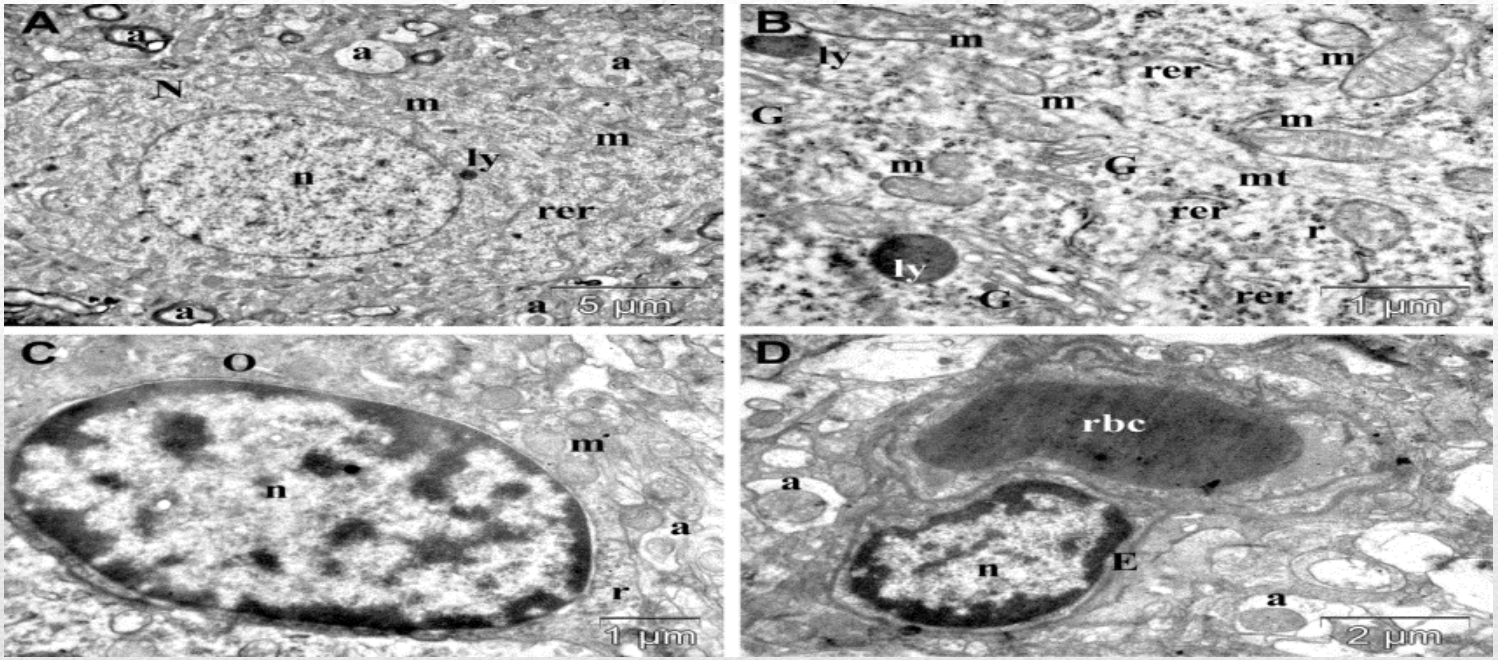


Fig. 7. TEM images showing the typical (normal) architecture of the frontal cortex in control group (n=5). General A) and detailed (B) views of a neuron, oligodendrocyte (C) and blood capillary (D). N, neuron; n, nuclei; a, axons; m, mitochondria; rer, rough endoplasmic reticulum; ly, lysosomes; mt, microtubules; G, Golgi apparatus; O, oligodendrocyte; r, ribosomes; E, endothelial cell; rbc, red blood cell

RESULTS

Tissue sampling and Transmission electron microscopy -TEM

TEM -Frontal cortex: 3 months exposure

After 3 months of daily exposure to the MW field, important ultrastructural degenerative changes were identified in the frontal cortex samples of all tested animals. The neurons showed various degrees of alterations. The most affected ones had highly indented nuclei, but still contained prominent nucleoli (Fig. 10A).

TEM- Frontal cortex: 9 months exposure

The effects of 9 months exposure to the MW radiation were even more severe in all the animals of this group.

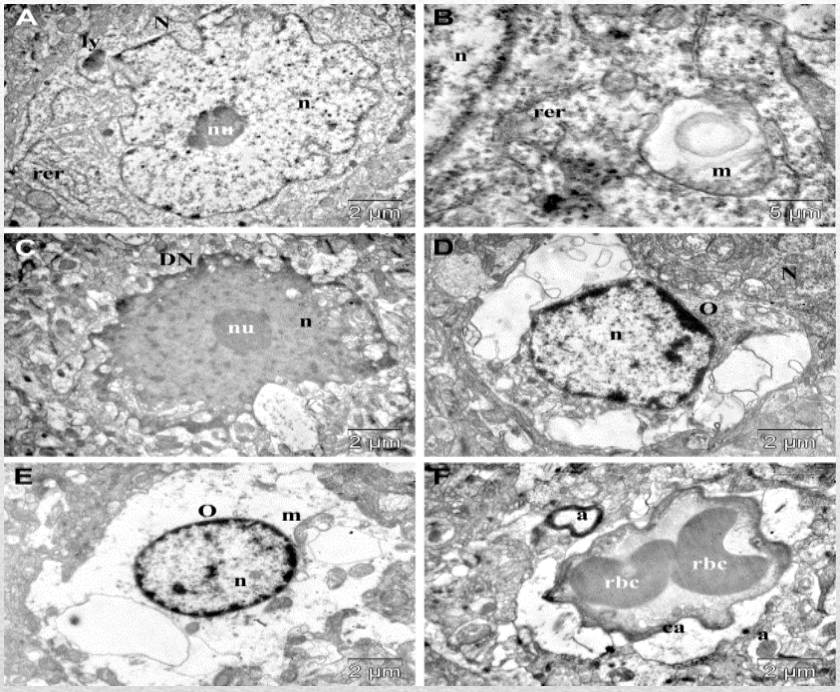


Fig. 8. TEM images of the frontal cortex in animals exposed for 3 months to GSM radiation (n=10). Degenerated neuron with indented nucleus (A). Swollen mitochondrion with electron lucent matrix and abnormal, circular cristae (B). Dark neuron with irregular nucleus cytoplasmatic vacuolations (C). Highly altered (D) and necrosed (E) oligodendrocytes surrounded by extracellular vacuolations. Capillary with normal endothelium surrounded by perivascular oedema due mainly to enlargement of the end feet of astrocyte (F). N, neurons; n, nuclei; nu, nucleoli; a, axons; m, mitochondria; rer, rough endoplasmic reticulum; ly, lysosomes; DN, dark neuron; O, oligodendrocytes; ca, capillary; rbc, red blood cells.

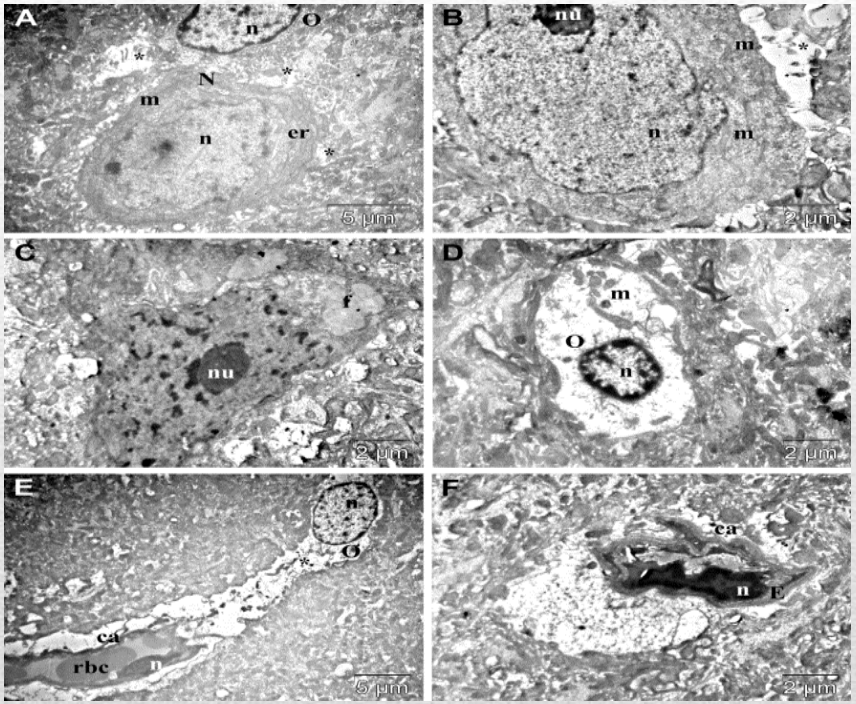


Fig. 9. TEM images of the frontal cortex in animals exposed for 9 months to GSM radiation(n=10). Rarefaction areas (*) of the frontal cortex tissue and altered neurons and oligodendrocytes (A,B). Dark neuron with indented nucleus and increased heterochromatin, and with cytoplasmic deposits of fibrillar inclusions (C). Necrosed oligodendrocytes (D,E), continued with perivascular oedema (E). Blood capillary with normal aspect, but surrounded by large electron transparent areas (E,F). N, neurons; n, nuclei; nu, nucleoli; m, mitochondria; er, endoplasmic reticulum; O, oligodendrocytes; ca, capillaries; E, endothelial cell; rbc, red blood cells.

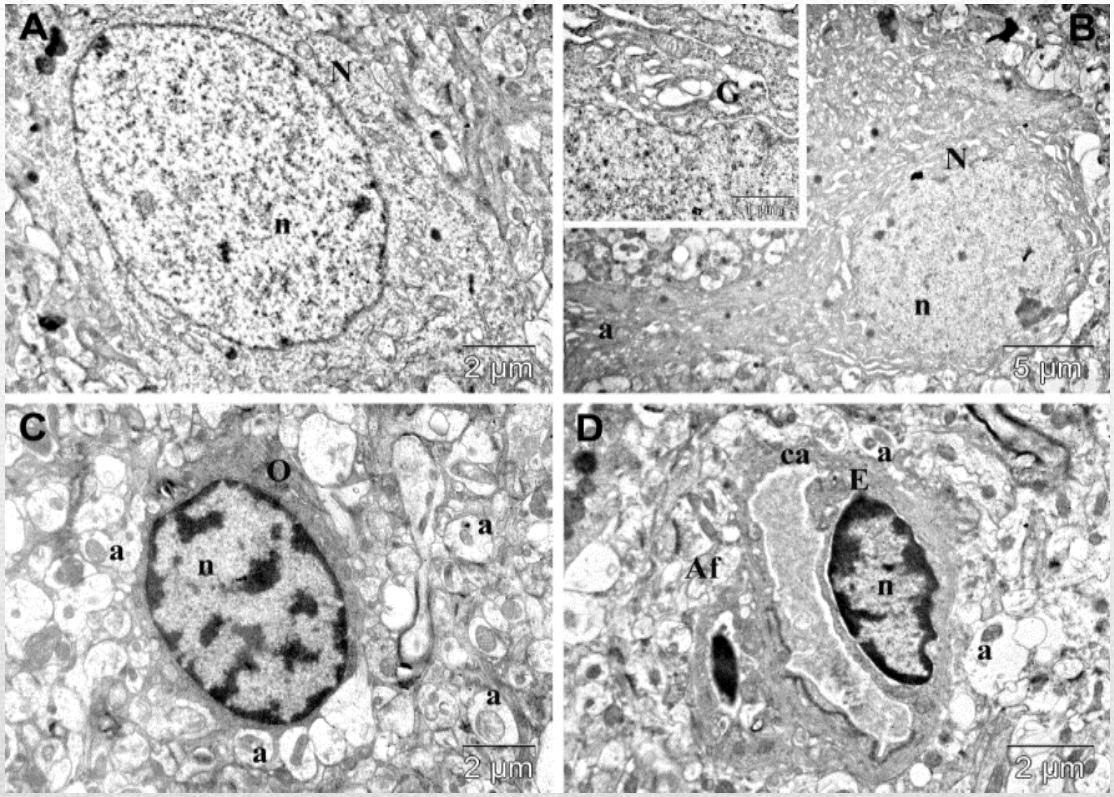


Fig. 10. TEM images showing the typical architecture of hippocampus in control group(n=5). General views of neurons (A,B), oligodendrocyte (C) and blood capillary (D). N, neurons; n, nuclei; a, axons; m, mitochondria; G, Golgi apparatus; O, oligodendrocyte; ca, capillary; E, endothelial cell; Af, astrocyte feet.

TEM

Hippocampus: Control group

TEM- Hippocampus: 3 months exposure

The density of the nervous tissue was extensively reduced on hippocampus sections as consequence of astrocytes necrosis and extracellular vacuolation, in all rats in this group

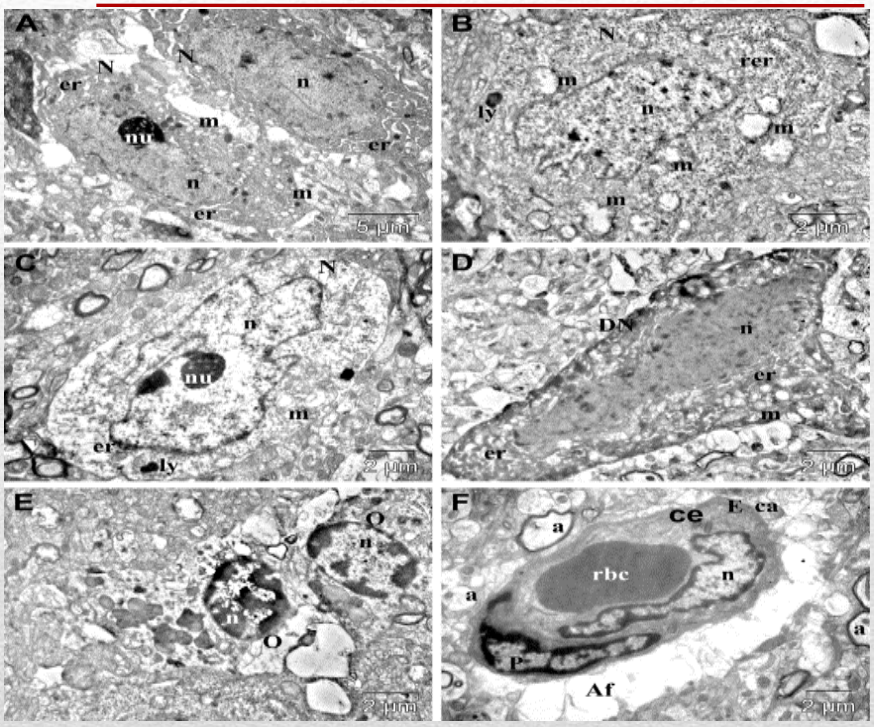


Fig. 11. TEM images of hippocampus in animals exposed for 3 months to GSM radiation(n=10). Neurons with major shape alterations containing swollen mitochondria with highly altered cristae (A-C). Dark neurons with cytoplasm vacuolation (D). Normal and necrosed oligodendrocytes (E). Capillary with normal aspect, surrounded by dilated end feet of astrocytes (F). N, neurons; n, nuclei; nu, nucleoli; a, axons; m, mitochondria; er, endoplasmic reticulum; rer, rough endoplasmic reticulum; ly, lysosome; DN, dark neuron; O, oligodendrocytes; ca, capillary; E, endothelial cell; ce, centrioles; rbc, red blood cells; Af, astrocyte feet.

TEM- Hippocampus: 9 months exposure

Both neurons and olygodendrocytes suffered ultrastructural degenerations in this group.

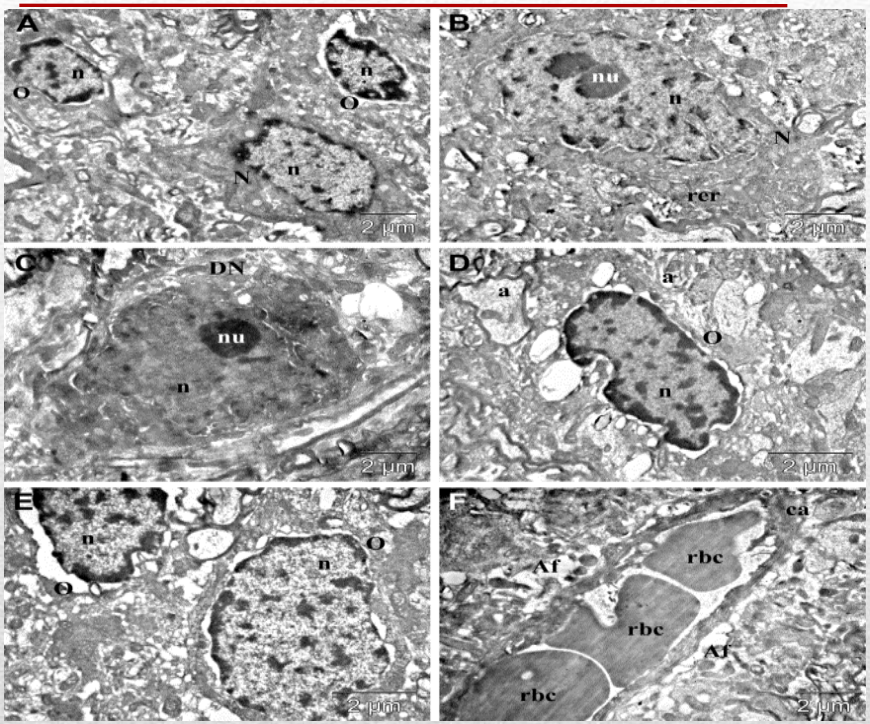


Fig. 12. TEM images of hippocampus in animals exposed for 9 months to GSM radiation(n=10). Highly degenerated neurons (A,B) and olygodendrocytes (A,D,E). Dark neuron with indented nucleus and with a few organelles (C). Normal blood capillary surrounded by dilated end feet of astrocytes (F). N, neurons; n, nuclei; nu, nucleoli; a, axons; m, rer, rough endoplasmic reticulum; DN, dark neuron; O, oligodendrocytes; ca, capillary; rbc, red blood cells; Af, astrocyte feet.

CONCLUSIONS

- The cognitive function in relationship with GSM MW exposure, while examined in numerous experimental studies, is highly controversial. Although there is increasing evidence regarding biological effects induced by weak radiofrequency radiations (microwaves), there is a lack of indisputable available data and this matter is not clarified so far.
- Our study highlights that long term exposure to MW radiation in the 860 - 890 MHz frequency range, corresponding to the GSM-900 uplink band, may produce neurobiological effects. The results of the present study suggest that long-term and low-level cumulative MW radiation could determine significant ultrastructural damage at cerebral substructures level that could trigger behavioral alterations. Our findings indicate that cells injuries could be directly related with the exposure time, being more severe as the duration of exposure increased.
- This issue should deserve a special consideration, because neuronal damage may not have immediate demonstrable consequences, but in time it may lead to late degenerative nervous diseases. We aim to replicate our findings in future advanced research, in order to elucidate the fundamental mechanisms underlying the interaction of MW radiation and the central nervous system at cellular and molecular level. Based on our results, further behavioral studies are also needed to investigate the effect of MW on the animals' cognitive performance.