Electromagnetic field induces cellular adaptation in the rat spinal cord ependyma

Lamiaa L.M. Ebraheim[1] and Mohamed M.M. Metwally[2]

[1]Department of Histology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
[2]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt.
Email address: lamiaavet@yahoo.com

Abstract: This study was designed to investigate the cellular changes induced by EMF (electromagnetic field) in ependymal cells of the central canal of the rat spinal cord. Twenty adult male and female albino Wistar rats were randomly divided into two equal groups. Animals in the control group were kept in EMF free space and animals in the second group was exposed to 900 megahertz (MHz) EMF for two hours/day for 60 consecutive days. The rats were anesthetized and transcardial perfusion was applied. Spinal cords specimens were taken out, processed for light, transmission and scanning electron microscopy. Numerous cellular alterations represented by; decrease in cell height, staining affinity, microvilli number and number of cilia in ciliary tufts with abnormal clumping of the cilia, besides cellular detachment, glial aggregations and dilatation of the lumens of central canals were observed in the examined specimens taken from rats exposed to EMF in comparison with the control group.
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Key words: electromagnetic field, spinal cord, rat.

1. Introduction

The biological effects of electric and magnetic fields are a controversial issue (**Polk and Postow 1995, Knave 2001 and Koyu et al., 2005)**. In 1996, WHO has established the international EMF project to assess the scientific evidence of possible health effects of EMF in the frequency range from 0 to 300 GHz. Many studies have previously reported structural and/or functional changes as cell response to exposure to EMFs (**Rothman et al., 1996; Somosy, 2000; Bortkiewicz, 2001; Bartsch et al., 2002; Leszczynski et al., 2002; Cox, 2003; Demsia et al., 2004)**. The mechanisms by which EMFs induce histological alterations still need more clarification, but based upon currently available literature, EMFs exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than the International Commission on Non-Ionizing Radiation Protection (ICNIRP) recommended values (**Demsia et al., 2004; and Hardell and sage 2008)**. A number of studies have suggested that the nervous system is one of the most affected systems by EMFs (**Tattersall et al., 2001; Huber et al., 2003; WHO 2003; Hardell and Sage 2008; Odaci et al., 2008).** The effects of EMF on ependymal cells of the central canal of rat spinal cord are not established yet, and the currently available literatures describe the histological effects of EMF on ependymal cells of the spinal cord are scarce. Ependymal cells are low columnar or cuboidal epithelial-like cells that line the ventricles of the brain and the central canal of the spinal cord **Mescher 2012**. They have a fairly round nucleus with finely stippled chromatin pattern and inconspicuous nucleolus. Their apical surfaces are covered by microvilli and most of the cells have a central cluster of long cilia (**Del Bigio 2009; Gartner and Hiatt 2013)**. Their function related mainly to production, absorption, movement and monitoring the content of CSF (cerebrospinal fluid) and form a barrier responsible for the selective movement of fluids and molecules between the CSF and the central nervous system **(Meschr 2012; Gartner** and **Hiatt 2013; Muthusamy et al., 2015)**. Also provide trophic support and possibly metabolic support for progenitor cells and play an important immunological role during infection particularly, with viruses (**Emery** and **Staschak 1972; Del Bigio 2009)**. There are observations suggested that defects in ependymal adhesion or interference with the function of cilia can cause hydrocephalus through several different mechanisms (**Zariwala et al., 2007)**.

2. Material and methods

A- Animals and experimental design: Twenty male and female Wistar rats, 2 months old, each weighing 200-250 gm., at the beginning of the experiment were kept under hygienic conditions in a well-ventilated room, with free access to food and water, with a constant twelve hour light/dark cycle. The rats were randomly divided into two groups (ten animals each) and group housed (five rats of the same sex per cage). Group (1) kept in an EMF-free location. Group (2) was exposed to 900-MHz EMF for two hours/day for 60 consecutive days. The EMF was induced by 900-MHz continuous wave electromagnetic energy generator manufactured at the department of

electronics and communications engineering, faculty of engineering, Zagazig university, Zagazig, Egypt.

B- Tissue preparation: The rats were anesthetized by 4% chloral hydrate intraperitoneally and transcardial perfusion was applied with a warm (37 °C) solution of 1% Paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) according to the technique described by (**Peters 1970)**. After perfusion, all spinal cord segments were taken out. Samples from each segment were fixed in Karnovsky's fixative, and processed for paraffin and plastic sections for light and electron microscopy and examined in Zeiss 10 CA TEM (transmission electron microscope). Some samples were processed for frozen technique and others were processed for scanning electron microscopy and examined in Zeiss EV040 SEM according to (**Bozzola and Russell, 1998; Bancroft and Gamble 2007)**.

3. Results

Rats of the both groups were apparently normal and showed no behavioral changes, but histological examination revealed many histological alterations in ependymal cells of the 900-MHz EMF group in comparison to rats of the control group**.** These alterations could be summarized as follows:

A- Change in cell height, staining affinity, cellular detachment, and contour and contents of central canals:

Ependymal cells of the spinal cord in rats of the group (1) appeared as a single layer of cuboidal to columnar cells with a relatively dark basophilic round to oval nuclei with prominent nucleoli. The central canals had an irregular contour and contained eosinophilic fluid-like material (fig.1 a). In group (2) the cells were mostly cuboidal and the nuclei appeared more basophilic. The central canals in a few specimens appeared dilated and contained; large eosinophilic oval to spheroid plaques with basophilic tint at the periphery of the plaques and eosinophilic debris, particularly at the periphery of the cavity of the central canal (fig.1 b). In paraffin section stained with Nile blue sulfate Methyl green the ependymal cells of rats in the group (1) appeared mostly columnar with light green homogenous cytoplasm. In spite these cells don't rest on a basement membrane, the subependymal region appeared more basophilic than the other adjacent neuropil (fig.1 c). In group (2) the cells were less in height, obviously few light green stained cells detached into the lumen of the central canals which were dilated and naked parts of neuropil exposed directly to the central canal lumen in some animals (fig.1 d). The cellular detachment were very clear in the thionin-stained sections (fig. 1 e). In addition to ependymal cell detachment two animals in the group (2) showed discontinuity of ependymal cell lining with a presence of clusters of a subependymal glial cells (fig. 1. f).

B- Change in ciliary tufts, microvilli number, basal process and cytoplasmic organelles:

The ependymal cell cytoplasm in rats of the group (1) appeared granular and basophilic with oval nuclei that had clumped peripheral chromatin. The apical surfaces had obvious ciliary tufts, and the basal surface of each cell projects a cylinder process into subependymal neuropil (Figs.1 g). In group (2) the cells had more basophilic cytoplasms and the apical surfaces showed less ciliary tufts and some cells became devoid from cellular processes (fig.1 h). The basal cell process became shortly extended into the subependymal neuropil in few cells. Scanning electron micrographs of the central canal ependyma of the control group clearly reflected densely packed ciliary tufts with clear appearance of individual cilia (fig.2 a), while in group (2) the scanning electron micrographs showed sparsely ciliated regions with abnormal clumping of the cilia and inability to distinguish individual cilium inside each tuft (fig. 2 b). Transmission electron micrograph of the spinal cord ependymal cells of the group (1) showed deeply indented nuclei with peripheral clumped chromatin, supranuclear Golgi apparatus, and very crowded apical pole with mitochondria and ciliary microtubules triplets and rootlets. The cilia extended into the central canal lumen in tufts, which blended in acute angels. The cells revealed very clear cell junctions of zonula adherent type (fig.2 c&d). Group 2 showed less crowded apical cell cytoplasm with fewer cilia microtubules triplets' and rootlets and fewer mitochondria. The cilia founded in a fewer number inside the tuft remnants that run into the central canal. Fragmentation along the individual cilium length was noticed. Precipitated granular material covering most of the apical ependymal external cell surfaces together with the presence of sub plasmalemmae electron dense area were noticed. In addition, numerous cytoplasmic vacuoles were observed. Some of them appeared electron lucent and coalesced together and the others were individually scattered and contained granular material (fig.2 e&f). The adherent junctions between the cells appeared less electron dense than the control group. There were no sex-related differences between males and females in the examined animals in both groups.

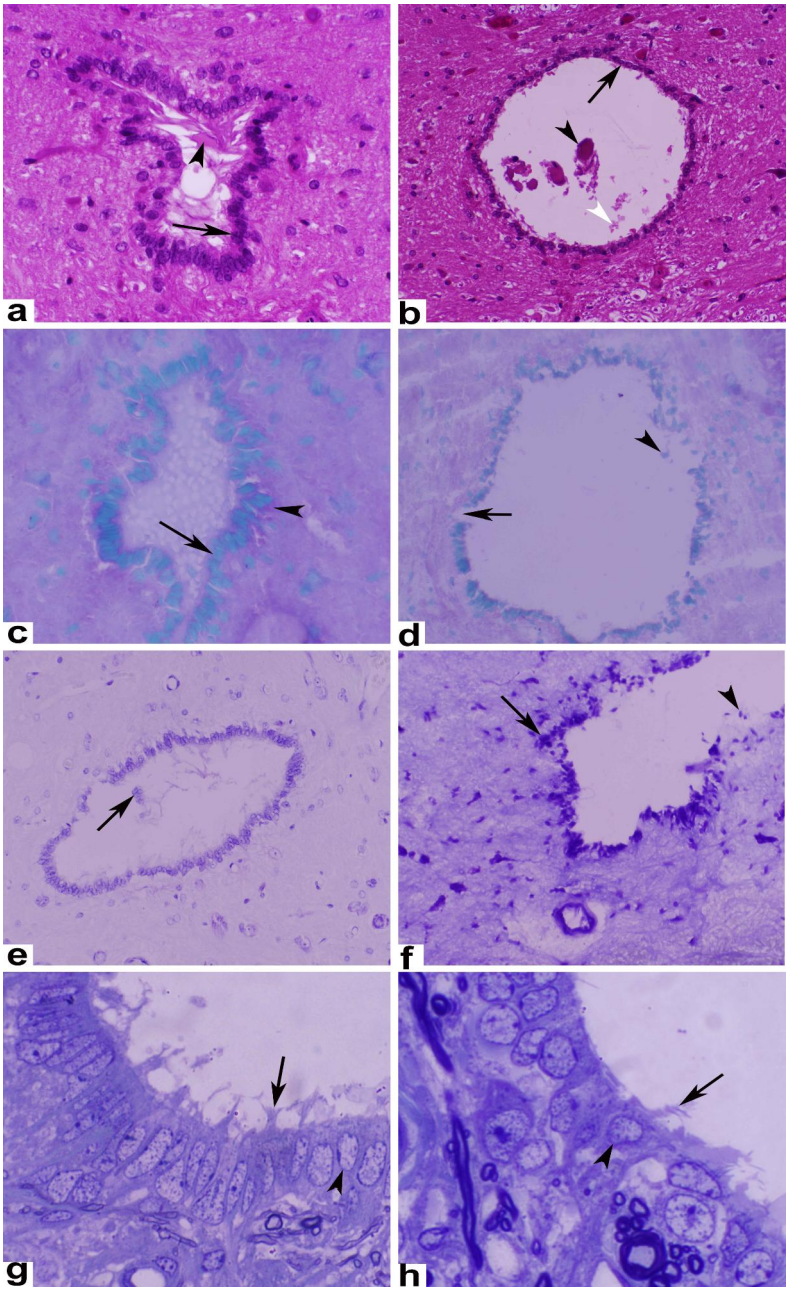

Fig. (1): a- Spinal cord L3 (GP. 1), showing the central canal with irregular contour and lined by single layer of cuboidal to columnar ependymal cells (arrow) with the presence of eosinophilic material in the lumen (arrowhead) (H&E, X40).
b- Spinal cord L3 (GP. 2), showing the dilated central canal lined by single layer of cuboidal ependymal cells (arrow) with the presence of eosinophilic plaques (black arrowhead) and debris in the lumen (white arrowhead) (H&E, X20).
c- Spinal cord L4 (GP. 1), frozen section showing light blue columnar ependymal cellular nuclei (arrow) with a deep basophilic subependymal region (arrowhead) (Nile blue sulfate-methyl green, X20).
d- Spinal cord L4 (GP. 2), frozen section showing discontinued light blue cuboidal ependymal cellular nuclei leaving naked parts of neuropil (arrow) with the presence of few detached cells in the dilated lumen (arrowhead). (Nile blue sulphate-methyl green X20).
e- Spinal cord C1 (GP. 2), showing detached ependymal cells in the lumen of central canal (arrow) (Thionin violet. X10).
f- Spinal cord T4 (GP. 2), showing detached ependymal cells (arrowhead) with discontinuity of ependymal cell lining and presence of clusters of subependymal glial cells (arrow) (Thionin, X20).
g- Spinal cord T3 (GP.1), showing ependymal cells having columnar nuclei with clumped peripheral chromatin (arrowhead) and prominent ciliary tufts at the apical surfaces (arrow) (toluidine blue. X100).
h- Spinal cord T3 (GP. 2), showing ependymal cells having cuboidal nuclei (arrowhead) with clumped peripheral chromatin and few ciliary tufts at the apical surfaces (arrow) (toluidine blue. X100).

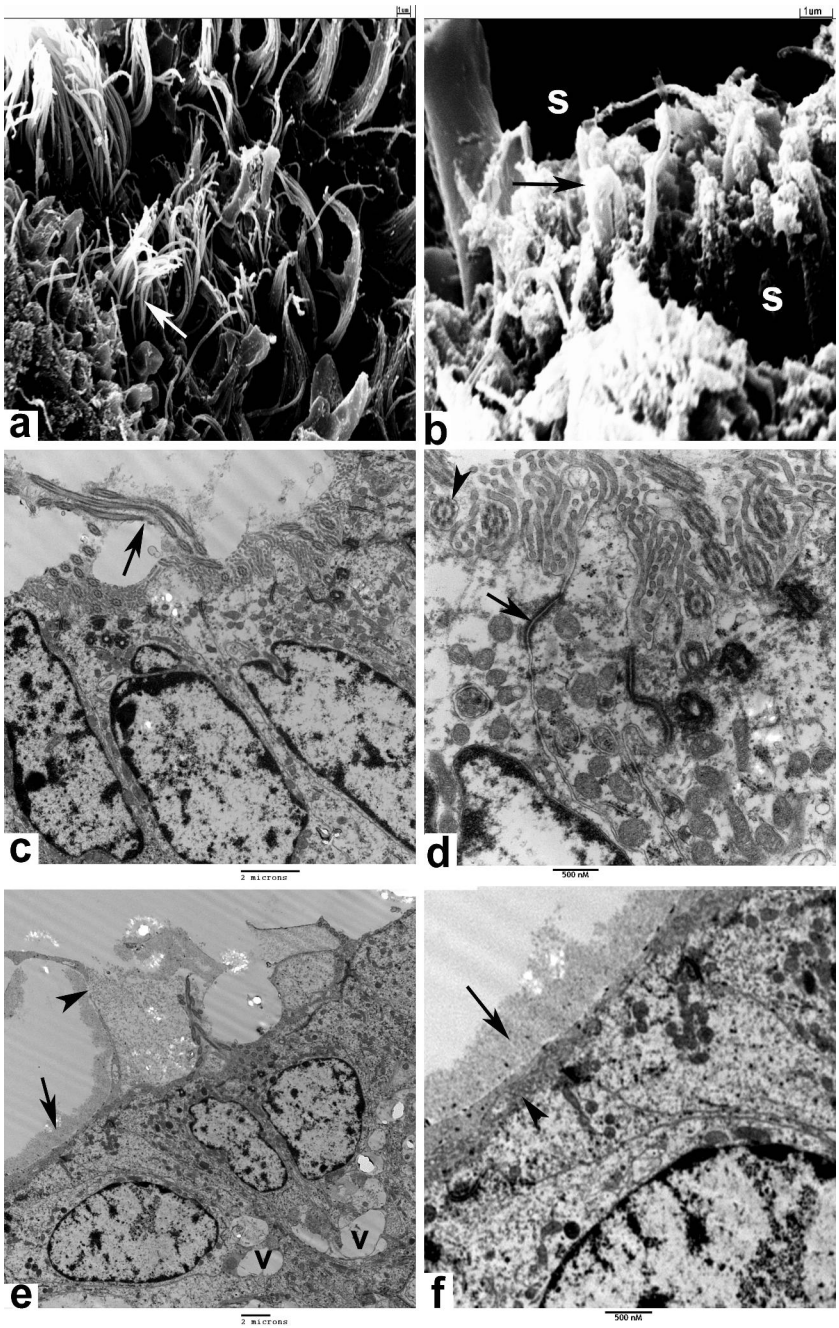

Fig. (2): a- Scanning electron micrograph of the surface of ependymal cells of group (1) C4 showing densely packed ciliary tufts with a clear appearance of individual cilia (arrow)).

b- Scanning electron micrograph of the surface of ependymal cells of group (2) C4 showing sparsely ciliated regions (s) with abnormal clumping of the cilia and inability to distinguish individual cilium inside each tuft (arrow).

c- Transmission electron micrograph of the spinal cord ependymal cells of the group (1) showing deeply indented nuclei with peripheral clumped chromatin, and very crowded cytoplasm. The cilia extended into the central canal lumen in tufts blended in acute angels. (arrow) (E/M, X14400).

d- High magnification to fig. (2 c) showing cell junctions of zonula adherent type (arrow) and very crowded cytoplasmic apical pole with mitochondria, endoplasmic reticulum and ciliary microtubules triplets (arrowhead) and rootlets (E/M. X45200).

e- Transmission electron micrograph of the spinal cord ependymal cells of the group (2) showing few cilia with ciliary fragmentation (arrowhead), precipitated granular material at the apical ependymal cell surfaces (arrow), sub plasmalemmae electron dense area and numerous granular and non-granular cytoplasmic vacuoles (V). (E/M. X7240).

f- High magnification to fig. (2 e) showing precipitated granular material at the apical ependymal cell surfaces (arrow), sub plasmalemmae electron dense area (arrowhead), less crowded apical cell cytoplasm with fewer cilia microtubules triplets' and rootlets and fewer mitochondria. (E/M. X22600).

4. Discussion

The biological effects of EMFs on body systems received great interest during the last few decades due to the change in the lifestyles and increased urbanization, but till now we do have neither sharp safety levels nor clear-cut positive or negative effects for that kind of radiation. Also till now we have not standardized protocols for testing the biological effects of EMFs on body systems in experimental animals. This is due to the Published reports about the biological effects of EMFs are contradictory (**Demsia et al., 2004)**. The present study focuses on ependymal cellular alterations induced by 900-MHz EMF in rat's spinal cords. The choice of 900-MHz frequency was based on the fact that at the present, most of the cell phones in Europe and the Middle East generally work at a frequency of 900-MHz in the GSM systems (**Koyu et al., 2005; Panagopoulos et al., 2007)**. Based on the present findings the spinal cord ependymal cell response to 900-MHz EMF was very clear. This response represented by a kind of cellular atrophy besides cellular detachment and abnormal dilatation of the lumens of central canals. The changes in cilia, microvilli and contents of the central canal could be related either to alterations in the flow or composition of CSF or alterations in the ependymal cells itself (**De Santi et al., 1990; Del Bigio 1993; Paez et al., 2007; Zariwala et al., 2007; Del Bigio 2009)**. The detachment of ependymal cells into the CSF was due to disruption of the binding of ependymal cells to each other's or to the substrate (**Batiz et al., 2006)**. The clustered glial cells in the subependymal region suggested that these cells subjected to chronic stress where ependymal cells are absent (**Cavanagh 1999).** The question is; How EMFs induce such cell adaptations? Previous studies suggested that exposure to weak EMFs influence cell processes because EMFs represent environmental stress to which a cell can adapt without catastrophic consequences (**Goodman et al., 1995)**. Other studies reported that EMFs induce cellular chemical changes (changes in ionic and molecular currents, lifetimes of free radicals and the orientation of molecules) followed by biological changes in the cellular organelles and DNA (**Li and Chow 2001; Lai and Singh, 2004; Hardell and Sage 2008)**.

5. Conclusion:

The results in this study emphasize that 900-MHz EMF induced ependymal cellular adaptations. Therefore, until the ongoing researches reach to introduce cost-effectively, applicable measures to limit EMF exposure it is advisable to avoid exposure to EMFs as much as possible and the recommended health and protective precautions should always be applied.

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References

1. Bancroft, G. D., Gamble, M., 2007. Theory and Practice of Histological Techniques. 6th Ed., Churchill Livingstone Edinburgh, London, Melbourne and New York.
2. Bartsch, H., Bartsch, C., Seebald, E., Deerberg, F., Dietz, K., Vollrath, L., Mecke, D., 2002. Chronic exposure to a GSM-like signal (mobile phone) does not stimulate the development of DMBA induced mammary tumors in rats: results of three consecutive studies. Radiat. Res. 157, 183–190.
3. Batiz, L. F., Oliver, C., Alvarez, M., Rodriguez, S., Rodriguez, E. M., 2006. Molecular mechanisms underlying neuroepithelial/ ependymal denudation in the hydrocephalic hyh mutant: spatial and temporal expression of alpha-SNAP and N-cadherin. CSF Res 3 (Suppl 1): S16.
4. Bortkiewicz, A., 2001. A study on the biological effects of exposure mobile-phone frequency EMF. Med. Pr. 52, 101–106.
5. Bozzola, J. J., Russell, L. D., 1998. Electron Microscopy. Principles and Techniques for Biologists. 2nd Ed. John and Bartlett Publisher. Boston, Toronto, London, and Singapore. pp. 148-200.
6. Cavanagh, J. B., 1999. Corpora-amylacea and the family of polyglucosan diseases. Brain Res Brain Res Rev 29, 265–295.
7. Cox, D.R., 2003. Communication of risk: health hazards from mobile phones. J. R. Stat. Soc.: Ser. A (Stat. Soc.) 166, 241–245.
8. De Santi, M. M., Magni, A., Valletta, E.A., Gardi, C., Lungarella, G., 1990. Hydrocephalus, bronchiectasis, and ciliary aplasia. Arch Dis Child 65, 543–544.
9. Del Bigio, M. R., 1993. Neuropathological changes caused by hydrocephalus. Acta Neuropathol 85, 573–585.
10. Del Bigio, M. R., 2009. Ependymal cells: biology and pathology. Acta Neuropathol. DOI 10.1007/s00401-009-0624-y.
11. Demsia, G., Vlastos, D., Matthopoulos, D. P., 2004. Effect of 910-MHz Electromagnetic Field on Rat Bone Marrow The Scientific World Journal, 4(S2), 48–54.

12. Emery, J. L., Staschak, M.C., 1972. The size and form of the cerebral aqueduct in children. Brain 95, 591–598.
13. Gartner, L. P., Hiatt, J. L., 2013. Color Atlas and Text of Histology 6th Ed. Lippincott Williams and Wilkins.
14. Goodman, E.M., Greenebaum, B., Marront, M. T., 1995. Effects of Electromagnetic Fields on Molecules and Cells. International Review of Cytology 158, 279-338.
15. Hardell, L., Sage, C., 2008. Biological effects from electromagnetic field exposure and public exposure standards Biomedicine & Pharmacotherapy 62, 104-109.
16. Huber, R., Schuderer, J., Graf, T., Jutz, K., Borbely, A., Kuster, N., Achermann, P., 2003. Radio Frequency Electromagnetic Field Exposure in Humans: Estimation of SAR Distribution in the Brain, Effects on Sleep and Heart Rate Bioelectromagnetics 24, 262-276.
17. Knave, B., 2001. Electromagnetic fields and health outcomes. Ann. Acad. Med. Singapore 30, 489–493.
18. Koyu, A., Cesur, G., Ozguner, F., Akdogan, M., Mollaoglu, H., Ozen S., 2005. Effects of 900MHz electromagnetic field on TSH and thyroid hormones in rats. Toxicology Letters 157, 257–262.
19. Lai, H., Singh, N. P., 2004. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect;112(6), 687-694.
20. Leszczynski, D., Joenvaara, S., Reivinen, J., Kuokka, R., 2002. Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cell: Molecular mechanism for cancer and blood-brain barrier related effects. Differentiation 70, 120–129.
21. Li, S.H., Chow, K. C., 2001. Magnetic field exposure induces DNA degradation. Biochem. Biophys. Res. Commun. 280, 1385–1388.
22. Mescher, A. L., 2012. Junqueira's Basic Histology: Text and Atlas, 13th Ed. The McGraw-Hill Companies.
23. Muthusamy, N., Sommerville, L. J., Moeser, A. J., Stumpo, D. J., Sannes, P., Adler, K., Blackshear, P. J., Weimer, J. M., Ghashghaei, H.T., 2015. MARCKS-dependent mucin clearance and lipid metabolism in ependymal cells are required for maintenance of forebrain homeostasis during aging. Aging Cell 14, pp. 764–773.
24. Odaci, E., Bas, O., Kaplan, S., 2008. Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain research 1238, 224-229.
25. Paez, P., Batiz, L. F., Roales-Bujan, R., Rodriguez-Perez, L. M., Rodriguez, S., Jimenez, A. J., Rodriguez, E. M., Perez-Figares, J. M., 2007. Patterned neuropathologic events occurring in hyh congenital hydrocephalic mutant mice. J Neuropathol Exp Neurol 66, 1082–1092.
26. Panagopoulos, D. J., Chavdoula, E.D., Nezis, I.P., Margaritis, L.H., 2007. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat. Res. 626, 69–78.
27. Peters, A., 1970. The fixation of central nervous tissue and the analysis of electron micrographs of the neuropil, with special reference to the cerebral cortex. In: Contemporary Research Methods in Neuroanatomy. Springer-Verlag, New York, Heidelberg, and Berlin. pp 56-75.
28. Polk, C., Postow E., 1995. handbook of biological effects of electromagnetic fields. CRC press.
29. Rothman, K, J., 2000. Epidemiological evidence on health risk of cellular telephones. Lancet 356, 1837–1840.
30. Somosy, Z., 2000. Radiation response of cell organelles. Micron 31, 165–181.
31. Tattersalla, E. H., Scott, I. R., Wood, S. J., Nettella, J. J., Bevir, M. K., Wang, Z., Somasiri, N. P., Chen, X., 2001. Effects of low intensity radiofrequency electromagnetic fields on electrical activity in rat hippocampal slices. Brain Research 904, 43–53.
32. WHO 2003. International Journal of Hyperthermia, Vol. 19, No. 3, pp. 215-384.
33. Zariwala, M. A., Knowles, M. R., Omran, H., 2007. Genetic defects in ciliary structure and function. Annu Rev Physiol 69, 423–450.

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