ORIGINAL ARTICLE

Neuroanatomical Alterations in the Rat Auditory Cortex Induced by Fourth and Fifth Generation Wireless Radiation Exposure: A Laboratory-Based Experimental Study

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ABSTRACT

Objective: To compare the effects of Fourth and Fifth Generation 2100 MHz and 3500 MHz) radiofrequency electromagnetic radiation (RF-EMR) on cortical layer organization and granule cell necrosis in the auditory cortex of rats.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: The study was conducted at the Anatomy Department, Islamic International Medical College, Rawalpindi, Pakistan from November 2023 to October 2024.

Methods: Thirty adult male Sprague-Dawley rats were randomly divided into three groups (n = 10 per group). Group A served as the control with no RF-EMR exposure. Group B was exposed to 2100 MHz Fourth Generation (4G), and Group C to 3500 MHz Fifth Generation (5G) RF-EMR using an ADALM-PLUTO module. All exposures were conducted for 5 hours daily over 4 weeks. Following exposure, the rats were sacrificed, and their auditory cortices were subjected to histological analysis using Hematoxylin-Eosin staining. Cortical layer organization and granule cell necrosis were evaluated. Necrosis was graded from 0 (no necrosis) to 4 (severe necrosis). Data was analyzed using SPSS version 27, with a *P*-value ≤ 0.05 considered statistically significant.

Results: All groups showed regular cortical layering with distinct borders. Granule cell necrosis was absent in controls, minimal in 4G (Group B), and more severe in 5G (Group C), where 10% showed severe necrosis. Necrosis grades differed significantly among groups (*P* = 0.006), with 5G showing the highest neurotoxicity.

Conclusion: Compared to 4 G exposure, 5G radiofrequency electromagnetic radiation results in significant granule cell necrosis in the auditory cortex, suggesting that higher-frequency electromagnetic radiation may induce greater neurotoxic effects. These findings highlight the potential risks of prolonged exposure to 5G radiation, particularly regarding neuronal integrity and brain health.

Keywords: *Auditory Cortex, Electromagnetic Radiation, Histology, Oxidative Stress.*

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Introduction

Mobile phones have become essential tools in modern life, offering unparalleled convenience and connectivity. However, concerns have emerged about the potential health effects of the radiofrequency electromagnetic radiation (RF EMR) they emit. This is particularly relevant to the brain's auditory cortex, which is directly exposed during phone use.[1,2] The auditory cortex plays a critical role in sound processing, learning, prediction, and auditory decision-making. It is intricately connected to regions involved in memory and cognition, such as the prefrontal cortex, hippocampus, and fornix.[3]

In humans, the primary (A1) and secondary (A2) auditory areas are located in Heschl's gyrus within the temporal lobe. These regions are histologically characterized by six distinct layers, predominantly composed of pyramidal and granule cells, and feature dense myelination with high levels of acetylcholine esterase, cytochrome oxidase, and parvalbumin.[4] While these structures are conserved across mammals, their specific anatomical locations vary, such as in rats, where A1 is situated in the middle temporal area flanked by dorsal and ventral secondary auditory areas.[4]

Mobile phones emit RF EMR, a subset of non-ionizing radiation with frequencies ranging from 30 kHz to 300 GHz. Most mobile phones operate between 800-2000 MHz, with 5G technology expanding this range to 450-3800 MHz. RF EMR exposure is measured by frequency and Specific Absorption Rate (SAR), with a limit of 2 W/kg established by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) in many countries.[5-7]

With its high water content, oxygen utilization, and polyunsaturated fatty acid content, the brain is particularly susceptible to RF EMR's thermal and non-thermal effects. While the body can tolerate minor temperature increases, significant heating can cause tissue damage.[8,9] Non-thermal effects include membrane permeability changes, causing disruption of the electrochemical gradient across the membrane, leading to oxidative stress due to overproduction of reactive oxygen species (ROS).[10] Oxidative stress causes lipid peroxidation, apoptosis, and structural brain damage.

An increasing number of studies have highlighted the detrimental effects of RF EMR exposure on various organs, as demonstrated by histological, biochemical, and immunohistochemical analyses.[8] Anjali Sharma (2021) confirmed hepatic and neuronal injury in rats exposed to EMR, and the potency of damage was more prominent in the brain.[5] Abdalla Ahmed et al. (2022) observed a decrease in the number of pyramidal cells in rat's hippocampus after exposure to RF-EMR.[10]

Despite these findings, a significant knowledge gap persists in our understanding of the contrasting impacts of current 4G electromagnetic radiation (EMR) exposure and the anticipated future exposure to 5G on the auditory cortex of the brain, responsible for the sense of hearing. Hearing loss is a significant public health concern, affecting approximately 20% of the global population according to the World Health Organization (WHO). This study intends to determine the potential differences in the underlying mechanisms by which 4G and 5G RF-EMR exposure may influence the histomorphology of the rat's Auditory cortex.

Methods

A lab-based experimental study was conducted in collaboration with the National Institute of Health (NIH) and the Anatomy Department of Islamic International Medical College, Rawalpindi, Pakistan after approval by the Ethics Review Committee of the college vide letter no: Riphah/IIMC/IRC/23/30108, held on dated: 1st September 2023. The study lasted one year from November 2023 to October 2024.

Thirty adult male Sprague-Dawley rats, each weighing 200-250 g and free of visible abnormalities, were conveniently selected for the study. The rats were randomly divided into experimental and control groups and housed in the Animal House at NIH, Chak Shehzad, Islamabad. They were kept in standard conditions, including a temperature of 22 ± 0.5°C, 50% humidity, and a 12-hour light/dark cycle in an air-conditioned room. The rats were given unlimited access to food and water for seven days to acclimate. During the experiment, they were fed rat pellets and provided with water ad libitum.

Control group (Group A)

Ten adult male rats were kept on a standard diet orally for up to four weeks throughout the experiment. Rats in the control group were not exposed to electromagnetic radiation.

Experimental group (Group B)

Ten adult male rats were exposed to 2100 MHz(4G) EMF for 5h/day for 4 weeks.

Experimental group (Group C)

Ten adult male rats were exposed to 3500 MHz(5G) EMF for 5h/day for 4 weeks.

The electromagnetic radiation exposure system utilized the ADALM-PLUTO (PlutoSDR) module with an AD9363 transceiver, operating at 2100 MHz and 3500 MHz frequencies with a 20 MHz bandwidth.[11] A 10 cm 2276 Trainer antenna replaced the original to enhance performance and allow precise adjustments. Rats were housed in 40 × 40 × 60 cm cages and exposed to electromagnetic radiation at far-field distances of 14 cm (2100 MHz) and 24 cm (3500 MHz). MATLAB software controlled the setup, ensuring 0 dB antenna gain for standardized exposure and a controlled experimental environment.

After 4 weeks, the rats were dissected, and brain samples were fixed in 10% formalin. Sectioning was done at NTP-7 level 4, identified on the ventral surface by the interpeduncular nucleus.[12,13] Two coronal sections of 5 µm were taken using a microtome. These sections were then mounted on glass slides. Subsequently, the slides were examined under a light microscope and stained using the Hematoxylin-Eosin technique. Images of the stained samples were captured with a camera for detailed analysis.[14]

Images were shifted to Image J software, and qualitative analysis was done. Cortical layer organization and necrosis were observed in the granule cells across all four cortical zones, in each of the two coronal sections of the auditory cortex, starting from the rhinal sulcus. The necrotic granule cells were identified by their "red dead" appearance, characterized by hyper-eosinophilic cytoplasm.[15] These features confirm that the affected neurons underwent cell death via necrosis, as indicated by the distinct structural changes in the cytoplasm and nucleus. Their open-faced nuclei and prominent nucleoli identify normal granule cells.[16] For qualitative analysis, the following criterion was used (Table-1).[17]

Table-1: Grading Criterion used for qualitative analysis

Parameter	Grade 0 Negligible	Grade 1 Minimal	Grade 2 Mild	Grade 3 Moderate	Grade 4 Severe
Differentiation of Cortical Layers	All 6 layers are preserved in 8 HPF/rat	All 6 layers are preserved in 6 HPF/rat	All 6 layers are preserved in 4 HPF/rat	All 6 layers are preserved in 2 HPF/rat	All 6 layers are not preserved in 8 HPF/rat
Granule cell necrosis	No necrosis Seen in any zone/rat	Necrosis seen in 1 or 2 zones/rat	Necrosis seen in 3 or 4 zones/rat	Necrosis seen in 5 or 6 zones/rat	Necrosis seen in 7 or 8 zones/rat

Data analysis was done using SPSS version 27, and the Chi-square test was used for qualitative analysis. The significance level of $P \leq 0.05$ was used to determine statistical significance.

Results

Rats have six histological layers in their auditory cortex, which is koniocortical in nature. However, unlike humans, layers 2 and 3 are merged. All the rats in control group A and experimental group B and C showed well preserved cytoarchitectural organization of cortical layer. (Figure-1and 2)

The analysis of granule cell necrosis revealed significant differences among the groups. No granule cell necrosis was observed in Group A. In contrast, Group B displayed a more varied distribution of necrosis, with 10% (n=1) of rats exhibiting mild

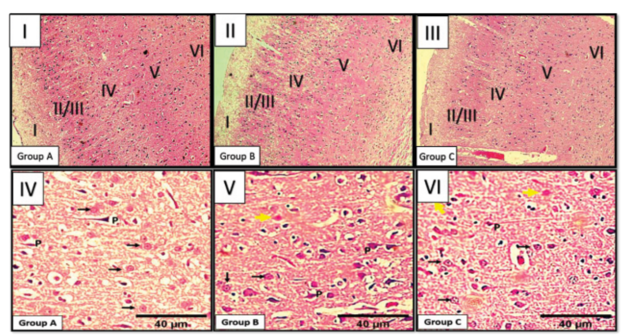

Fig.1: Photomicrograph of coronal section of auditory cortex showing organization of cortical layers in control group A(I) and experimental groups B & C (II,III). H&E stained. Magnification 40X. Photomicrograph showing granule cell necrosis in Groups B & C (V, VI) with intensely stained cytoplasm of necrotic cells (yellow arrow). (P) shows pyramidal cells, and black arrows show normal granule cells with open-faced nuclei with prominent nucleoli, stained. Magnification 200x

necrosis, 50% (n=5) experiencing minimal necrosis, and 40% (n=4) showing no necrosis, while no moderate or severe necrosis was recorded. Group C had the highest levels of necrosis: 10% (n=1) of rats showed severe necrosis, 20% (n=2) showed mild necrosis, 60% (n=6) exhibited minimal necrosis, and 10% (n=1) showed.

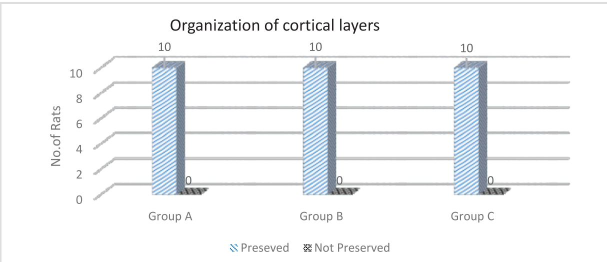

Fig.2: Bar graph showing no. of rats showing the preserved and non-preserved organization of cortical layers in groups A, B, and C

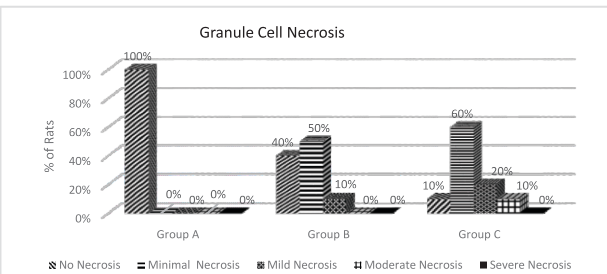

Fig.3: Bar graph showing the degree of Granule cell necrosis in groups A, B, and C

Discussion

This study explored the effects of 4G and 5G electromagnetic radiation on the orientation of cortical layers and granule cell necrosis in rats' auditory cortex. The aim was to assess how different levels of electromagnetic radiation exposure affect neuroanatomical organization, particularly neuronal integrity and associated inflammatory responses**.**

In our study, we permitted the rats to move freely within their cages.[18] This approach was intended to reduce anxiety associated with being confined. Rats in all groups displayed typical cortical layer organization, with distinct and well-defined borders between the layers. Unlike our findings, Angelene Chui et al. (2020) reported that oxidative stress impairs neurogenesis under conditions associated with increased oxidative stress.[16]

In this study, granule cell necrosis was observed in rats exposed to 4G and 5G electromagnetic radiation (EMR), with a more pronounced severity in the 5G group. This indicates that 5G EMR has a greater detrimental impact on granule cells compared to 4G. The necrosis is attributed to oxidative stress caused by reactive oxygen species (ROS), which arise due to the disturbance of ion channels in cell membranes, ultimately leading to cell death.[5,19,20] These findings align with previous research, such as Dong Liu et al., who reported granule cell necrosis following acute exposure to electromagnetic radiation, and SKM Belal, who observed cell necrosis in rat brains after exposure to 900 and 1800 MHz EMR.[21] These studies collectively support the notion that increased EMR exposure, particularly at higher frequencies like 5G, can exacerbate oxidative stress and necrotic damage in neural tissues.[22]

While the study utilized a standardized and consistent exposure protocol to ensure experimental control, it may not entirely reflect the variable and intermittent patterns of electromagnetic radiation exposure typically encountered in daily human mobile phone use. Differences in exposure duration, device usage habits, and environmental factors in real-life scenarios could influence outcomes and should be considered when interpreting the findings.

Conclusion

Histological analysis revealed consistent cortical layer structure across all groups. However, a significant increase in granule cell necrosis in the 5G group points to severe neurotoxic effects associated with neuroinflammation and cellular damage. Overall, the findings suggest that 5G radiation causes more pronounced neuroanatomical changes than 4G, indicating more substantial neurotoxic effects at higher frequencies. Additional studies are necessary to examine the long-term impact of 5G exposure on cognitive and auditory functions. Research should focus on human populations and include advanced techniques like electron microscopy. These studies are crucial for understanding potential health risks. Continued investigation will help clarify any long-term effects of 5G radiation.

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Author Contributions

AR: Conception and design of the work, writing original draft (methodology, investigation), data acquisition, curation, and statistical analysis, validation of data, interpretation, and write-up of results
SA: Conception and design of the work, revising, editing, and supervising for intellectual content
UU: Writing original draft (methodology, investigation)
SF: Data acquisition, curation, and statistical analysis
TQ: Revising, editing, and supervising for intellectual content
TF: Validation of data, interpretation, and write-up of results