Advances in Research

22(3): 51-64, 2021; Article no.AIR.70025
ISSN: 2348-0394, NLM ID: 101666096

Histological and Histochemical Study of Radiofrequency Radiation effects on the Hippocampus during the Pre- and Postnatal Stages of Development

Joshua Oladele Owolabi[1*] and Olayinka Stephen Ilesanmi[2]

[1]Texila American University, Guyana, University of Global Health Equity, Rwanda, Babcock University, Nigeria.
[2]Department of Community Medicine, College of Medicine, University of Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2021/v22i330304
Editor(s):
(1) Prof. Pradip K. Bhowmik, University of Nevada Las Vegas, USA.
Reviewers:
(1) Arnab Banerjee, Serampore College, India.
(2) S. Rakoth Kandan, Christ University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/70025

Original Research Article

Received 01 May 2021
Accepted 06 July 2021
Published 10 July 2021

ABSTRACT

Background: The research was designed to model the exposure to radiofrequency radiation (RFR) by habitual users of RFR-enabled devices and to observe possible aberrations in tissues that are attributable to exposures. The RFR exposure regimen modelled cases of continuous, and intermittent exposures in human conditions, using Wistar rats. The primary objective of the study was to study intrauterine and postnatal exposure to RFR and study its effects on specific brain structural and functional attributes.
Materials and Methods: Experimental Wistar rats were housed in facilities that enabled exposure to specific type of RFR source (the 4G RFR-emitting internet router) and for specific durations which included 21 days of pregnancy and 35 post-natal days, marking the point of puberty. Following exposure, animals were sacrificed to excise brain tissues for histological analysis using the haematoxylin and eosin technique, histochemical analysis using the Nissl technique, and immunohistochemical techniques including the IBA 1 and Caspase 3 techniques for inflammation

**Corresponding author: E-mail: joshuaowolabi01@gmail.com, jowolabi@ughe.org;*

and potential apoptosis. Representative histological and histochemical photomicrographs were analysed using principles of qualitative histology and histochemistry.

Results and Conclusion: Findings from the current research showed that RFR-exposure did not produce teratogenic or neurodegenerative effects within the hippocampus. This was evident from the study of the hippocampus' histoarchitectural organisation, morphologies of the cells as well as their spatial distribution. Functional integrity of cells in the different regions of the hippocampal formation, namely the CA 1-4 areas as well as the dentate gyrus also showed that Nissl substance expression, which is a marker of neuron functional integrity, was relatively normally expressed across the experimental animals. This experimental modelling of human habitual exposure to RFR showed no evidence of prenatal teratogenic effects or postnatally induced extensive neurodegeneration up until puberty. However, it would be very important to indicate that RFR-exposure enhanced apoptotic potentials via the Caspase-3 pathway. The implications of this effect on later life mental health and neurological attributes will require further investigation

Keywords: Brain; hippocampus; Dentate gyrus; Cornu Ammonis; memory; radiofrequency radiation.

1. BACKGROUND

Radiofrequency radiation (RFR) had been reported in certain instances to have affected human health. It is clear that quality and reliable data will be required with respect to the nature of RFR effects on human health. This should be a matter of utmost importance and urgency as the world is increasingly embracing technology and deploying gadgets that use and emit RFR at an unprecedented level, dose, and intensity. Reliable data should come from diverse and complementary sources including the human epidemiological data, supplementary data, and case evidence among others. Very importantly, there should be quality evidence synthesis in the form of systematic review and meta-analysis. This study aimed to investigate the effects of RFR on the development of the brain, the structure of the brain hippocampus and the functional attributes.

2. REVIEW OF LITERATURE

Potential negative effects of RFR have been reported- including the increased risk of neurodegenerative diseases [1]. Alarm has been raised in different quarters on the potential negative effects that RFR exposure might have on brain development and mental functions which altogether could have significant effects on mental health. RFR had also been reported to affect sense organs including auditory mechanisms in experimental models [2], cognition and associated brain attributes in teenagers [3]. Furthermore, RFR has also been linked to other brain health aberrations, including epilepsy [4]. Much is obviously unknown about the mechanisms of the effects of RFR and its extent. On the other hand, a number of counter claims have been made to allay fears and state that the level and doses of exposures from the basic or routine daily use of radiofrequency in phones and other wireless devices might not be harmful for the brain. Interestingly, experimental exposure of cultured cells to RFR caused whole cells morphological aberrations, cellular DNA damage, cell cycle arrest, oxidative stress, and reactive oxygen species formation [5], suggesting the need to investigate this subject further. The hippocampus is a specialised structure in the temporal lobe of the brain which primary functions include memories consolidation and other roles that are related to learning, cognition and behaviour [6,7,8].

Radio-frequency radiation (RFR) belongs to the electromagnetic waves' spectrum. This would further imply that they could be natural or artificial because the earth has its natural electromagnetic fields while a number of gadgets are enabled by electromagnetic fields, hence might serve as artificial sources. To put things in perspective, electromagnetic field radiation is tagged RFR when the frequencies of the waves range between ~500 kilohertz (i.e. 500 kHz = 500,000 waves per second) to 2,000 megahertz (i.e. 2,000 MHz = two billion waves per second) [9]. Humans on the planet earth are exposed to certain natural RFR radiations which mainly include the sun, the atmosphere such as during lightning, and the earth electromagnetic field. Major artificial or man-made sources of RFR include the broadcasting radio and television signals, wireless phones transmitting signals-phones, cell phone towers, satellite sources etc., radar, Wi-Fi devices, Bluetooth® devices, and smart meters and scanners e.g., millimetre wave scanners such as full body scanners for security screening [10]. Valberg [9] would further illustrate

the nature of RFR by illustrating that power-line electric and magnetic fields oscillate at 60 Hz, hence on the spectrum, they are much below the frequency range of RFR. On the other hand, infra- red, light, and X-rays belong to the spectrum of electromagnetic radiation with frequencies that are much higher that the RFR band.

Relatively long before now, there were indications that RFR might influence neural activities, hence causing neurological disturbances [11,12]. This was considered to be an indication of what other effects RFR might have on body functions. While there are several discrete reports on the effects and potential risks. Singh and Kapo [13] stated that these data would not provide conclusive evidence on exact effects but would rather recommend quality precautionary measures. The implications of this position would be that there is always evidence that suggest risks and there is a need to carefully and objectively consider them through thorough research and careful evidence extrapolations. It might be important to start with the question of whether RFR could affect prenatal or embryonic tissues to elicit its effects. The answer is yes, and the brain tissue is also arguably the most vulnerable. Thermal effects of RFR reported included teratogenic effects on the neurons and/or foetuses. Early experimental observations showed that RFR had specific effects on the central nervous system, and these could imply teratogenesis [14]. Quality evidence exists from literature that RFR could impact the quality of cognitive functions in humans even with exposures that lasted for only minutes [15,16]. Animal studies showed that the effects of RFR might include impairment of cognitive functions [17]. RFR effects have also been linked to hyperactivity in animals or what was described as hyperactivity-like behaviour [18,19].

Lai [20] emphasised that most animal studies showed that RFR had effects on behavioural parameters, however, many human studies had reported that RFR had no such effects. The author had attributed such variations to either the variations in the biological milieu of human versus experimental animals or the variations in the experimental regimens of exposure versus the human real exposure patterns. There is merit in such arguments. However, it should be noted that modelled experimentation has remained inseparable and indispensable to biomedical sciences and as such, in the past have given highly reliable data. What one might advocate, for, going forward, is the need to carefully and accurately model experimental studies after patterns of human exposure and to carefully measure the effects in manners that could provide accurate extrapolations. One thing that might not be divorceable from the crisis of lack of consensus on the nature of RFR effects is the significant political, and economical vested interests, that often show in how stakeholders in the world of business and in government often choose to select what might constitute their body of evidence.

It is also important to consider RFR effects on brain development in line with the roles of exposure duration and dose. For instance, both human data and modelled experimental data have shown that RFR exposure could impair fertility and reproduction both in the male and in the female [21]. These human and experimental data are also supported by a collection of epidemiological data on male reproductive health. While the current study is not about reproductive health but the pre- and the post-natal development and functions of the brain structures, it is an indicator that certain effects of RFR could be observable right from the stage of gametogenesis. It might also be a pointer to the fact that effects of RFR on the developing foetus are relatively possible during the stages of development.

3. MATERIALS AND METHODS

3.1 Research Design: Animal Models of Habitual Exposures to RFR

Wistar rats were used as models for investigating the effects of pre- and postnatal exposure to radiofrequency radiation on brain development, structural and functional integrity as well as specific behavioural attributes. Pregnant experimental Wistar rats were kept in customized animal holding facilities with controlled exposure to radiofrequency [RFR] radiation throughout the duration of pregnancy. Exposure lasted for the duration of pregnancy and up to the puberty (Day 35 of postnatal life). Animals were allowed to live and move freely within the enclosed facility with RFR device installed to give the required range of exposure dosage per day. The continuous exposure implied that exposure source was not turned off at any time throughout the duration of the experiment. The intermittent exposure regimen implied that exposure was turned off and on based on the research design to eventually give half exposure duration per assigned duration and consequently, the entire

duration of the experiment. To analyse the potential effects of RFR on brain and its development and structure; the effect of the 4G RFR on hippocampal development, structure, and functional neurochemistry was considered.

3.2 Experimental Groups for Exposure

Each Group-Community served as a model for a defined scenario for the use of RFR-enabled devices, hence exposure. The animals were held in the enclosed facility that represents this modelled exposure while the devices were being used.

Group 1: This served as the control group without exposure

Group 2: 6 Hour continuous exposure
This served as the experimental group with low-intermittent exposure

Group 3: 6 Hour intermittent exposure
This served as the experimental group with low-continuous exposure

Group 4: 12 Hour continuous exposure
This served as the experimental group with moderate-intermittent exposure

Group 5: 12 Hour intermittent exposure
This served as the experimental group with moderate- continuous exposure

Group 6: 24 Hour continuous exposure
This served as the experimental group with high-intermittent exposure

Group 7: 24 Hour intermittent exposure
This served as the experimental group with high-continuous exposure

Data collection from the experimentation included qualitative and quantitative data. Qualitative data included photomicrographs of the brain hippocampus which were analysed for cell morphology, spatial distribution, and expressions of specific proteins including IBA1 for assaying neuroinflammation and Caspase 3 for assaying apoptosis.

3.3 Histology: Haematoxylin and Eosin [H & E] [22,23]

Formalin-fixed paraffin-embedded [FFPE] tissues were sectioned following standard tissue processing protocol. Tissue sections were be mounted on histological slides and de-waxed. The sections were stained with eosin and counterstained with haematoxylin.

3.4 Nissl Stain Technique [24]

Sections of FFPE tissues were mounte on glass and demonstrated using the Cresyl fast violet technique to observe Nissl bodies which are ribosome-endoplasmic reticulum conjugates that that are indicative of neuronal intracellular protein synthesising activities.

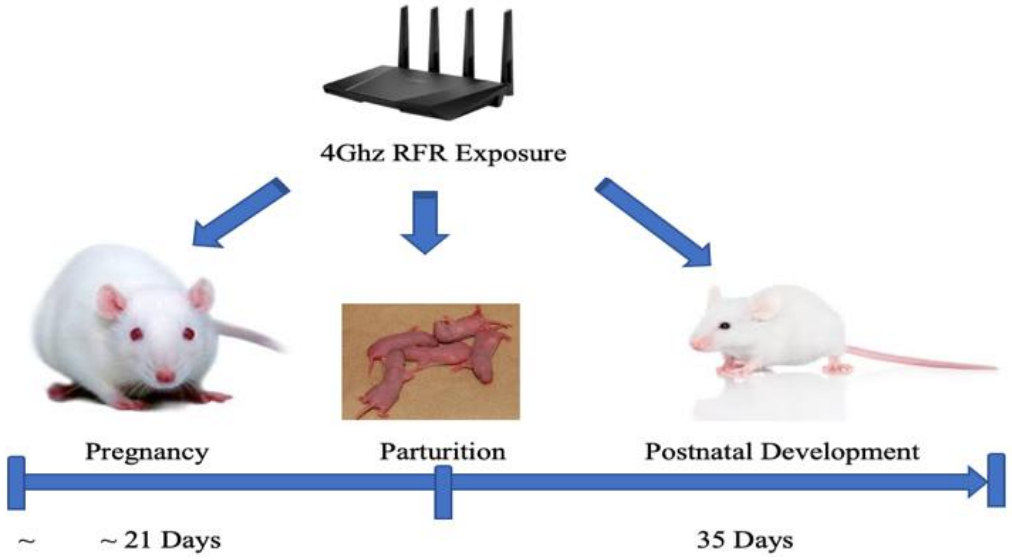

Fig. 1. Schematic illustration of RFR exposure during experiment

3.5 Histochemistry: IBA1 and Caspase 3

Samples that were used for the histochemistry of IBA1 [ionized calcium binding adaptor molecule 1] and Caspase 3 were fixed in buffered formal saline for at least 48 hours. Following this, the tissues were processed following histological principles. Sections of about 10µ were made with the rotatory microtome. The sections were mounted on the histological slides. Appropriate primary antibodies followed by secondary antibodies will be used to demonstrate IBA1 and Caspase 3 respectively. *IBA 1 and Caspase-3* Activity Colorimetric *Assay Kit*s were used.

4. RESULTS

4.1 Histology: RFR Effects on Hippocampal Formation Structural and Functional Integrity

The current study considered the effect of RFR on the structure of the hippocampal formation using the haematoxylin and eosin technique to demonstrate the structure of the entire hippocampal formation and its subunits that include the dentate gyrus and its Cornu Ammonis [CA], areas [CA 1-4]. Representative photomicrographs of the hippocampal formation for each group as well as the subsections- dentate gyrus and CA1-4, were carefully considered. It should be noted that different aspects of the hippocampal formation have varying degrees of vulnerability to agents of neurotoxicity and teratogenicity. This might be partly attributable to the functional roles and connections that are associated with each CA region. These factors explain why each of these regions was specifically analysed. Representative photomicrographs of all the experimental animal groups [Groups 2-7] showed that the hippocampal structural integrity was generally preserved both in their general outline and the subunits, relative to the control, Group 1 [See Figs. 2 and 3]. Also, representative photomicrographs in Figs. 4 and6 showed the distribution of Nissl substance in the hippocampus of the experimental animal, Groups 1-7. The entire photomicrographs and its subunits- dentate gyrus [DG] and Cornu Ammonis [CA], including the CA 1-3 regions – were considered. The hippocampal formation and its subunits showed no differential aberrations in Nissl substances expression that could be attributable to the effect of RFR exposure [See Figs. 4 and 5].

4.2 IBA 1 Expression

Photomicrographs of tissues showing IBA 1 expression in the dentate gyrus of the experimental animals show mild evidence of enhanced IBA 1 expression only when exposure was persistent and for longer hours; however, these would not indicate any marked aberrations in the expression of IBA 1 in the dentatae gyrus of the experimental animals; either when the exposed groups are compared with the Control or when the groups are compared against one another based on the duration of exposure. This would indicate that there was no marked acute neuroinflammation at birth on the basis of the RFR in the groups that is also indicated by the IBA 1 expression, which in turn is a marker of microglial activations that might serve as a marker of neuroinflammation. The expression of the IBA 1 in the brain of experimental animals that were sacrificed at puberty was analysed to observe neuroinflammations that could be attributable to the effects of RFR radiation as marked by the expression of IBA 1 in the cells. Observations of experimental Groups 2-7, relative both to the Control [Group 1] and one another did not show significant aberrations in the patterns of IBA 1 expression across the groups. The implication is that there were not marked aberrations that could serve as an indication of RFR induced acute neuroinflammation [at puberty] and, consequently microglial activation in the experiential groups because of their exposure to the RFR during development.

5. IMMUNOHISTOCHEMISTRY OF THE DENTATE GYRUS: ANALYSIS OF RESULTS

5.1 Prenatal Caspase 3 Expression

The prenatal exposure of the experimental rats to the RFR and the potential effects on cell death as mediated by the Caspase 3 was studied using the Caspase 3 immunohistochemistry technique. There were no marked aberrations in the expression of Caspase 3 across the experimental animal groups at birth as observed in the analysed photomicrographs. This would imply that during intrauterine life stage till birth, the exposure might not be significantly interfering with the apoptotic pathways as mediated by the Caspase 3. The expression of Caspase 3 across the experimental animal groups 1-7 dentate gyrus was again studied at puberty. Groups 4, 6 and 7 showed enhanced expression of the Caspase 3 in their dentate gyri. This is considered a marker of enhanced potential apoptotic process. Noting that Caspase 3 is typically expressed prior to cell death as an

indicator of enhanced apoptotic potential; the implication would be that the potential of apoptosis in the dentate gyrus of the exposed experimental animal was markedly enhanced. Furthermore, it would be important to note that both groups 6 and 7 were exposed to RFR for longer hours [24 hours] with F being on a daily basis and 7 being on a daily intermittent [see red arrows]. The marked enhanced expression of Caspase 3 in these groups would indicate that prolonged RFR had significant effects on the cellular apoptotic pathway, capable of causing induced and enhanced cell death. This could also serve as the basis for the marked expression of Caspase 3 in the Group 4 that was constantly exposed. In addition, Group 6, with uninterrupted daily RFR exposure had relatively enhanced Caspase 3 expression when compared with Group 7 with exposure on alternate days. Altogether, these observations would indicate that the effects were dose dependent and prolonged exposure would cause more significant effects that might lead to induced apoptotic potentials.

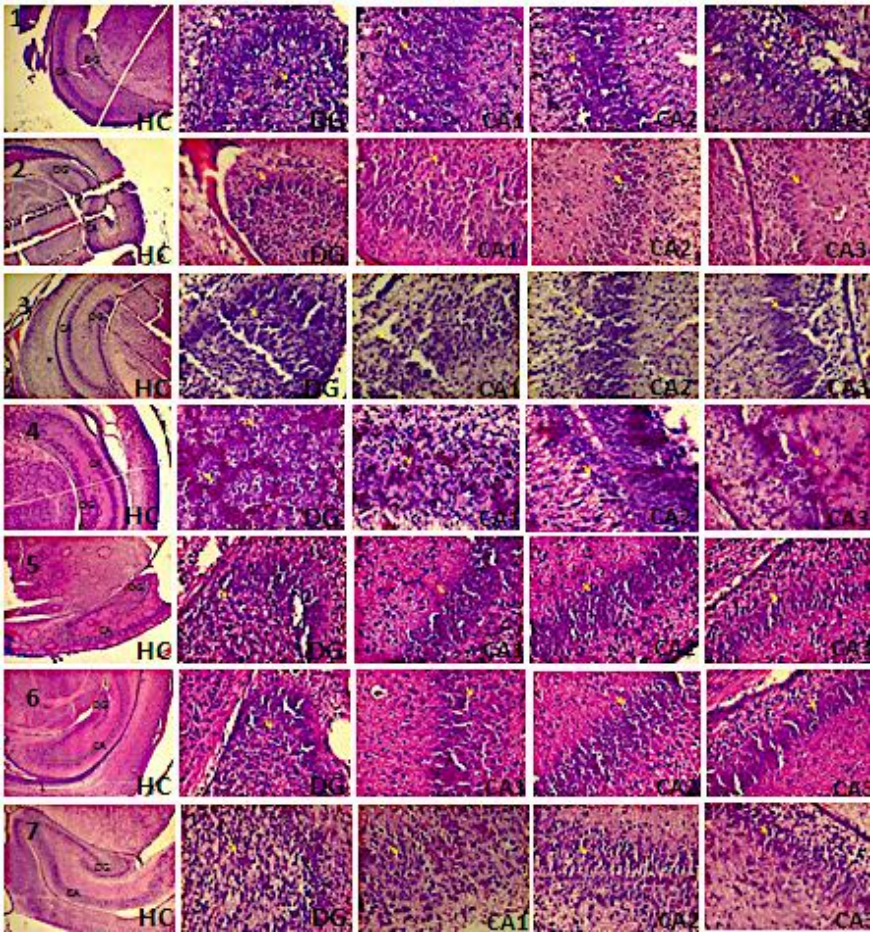

Fig. 2. Photomicrographs of the experimental animals' hippocampi and the subdivisions at birth [Postnatal D0] following exposure to regimented durations of RFR [Groups 2-7]; and compared with the Controls [Group 1]. In each Group [in the rows]: HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1; CA2= Cornu Ammonis2; CA3= Cornu Ammonis 3. Photomicrographs present no seriously deleterious effects attributable to RFR exposure in terms of cell morphology and spatial distribution or general histoarchitecture of the tissues. [H&E, x400]

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment; HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1 region; CA2= Cornu Ammonis 2 region; CA3= Cornu Ammonis 3 region. Yellow arrows indicate neurons

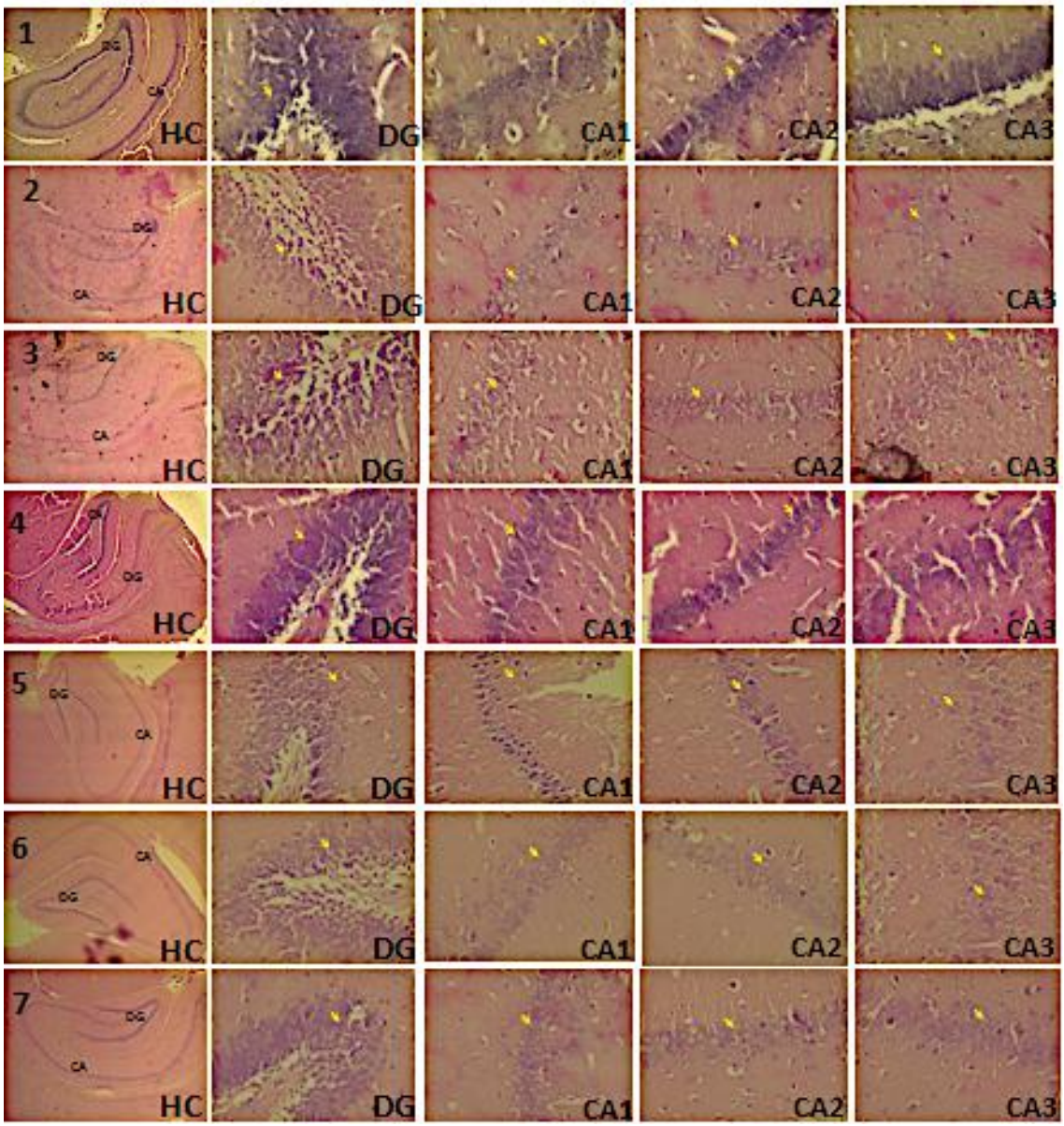

Fig. 3. Photomicrographs of the experimental animals' hippocampi and the subdivisions at puberty [Postnatal D35] following exposure to regimented durations of RFR [Groups 2-7]; and compared with the Controls [Group 1]. In each Group: HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1; CA2= Cornu Ammonis2; CA3= Cornu Ammonis 3. Photomicrographs reveal no evidence of seriously deleterious effects of exposure in terms of cell morphology and spatial distribution or general histoarchitecture of the tissues. [H&E, x400]

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment; HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1 region; CA2= Cornu Ammonis 2 region; CA3= Cornu Ammonis 3 region. Yellow arrows indicate neurons

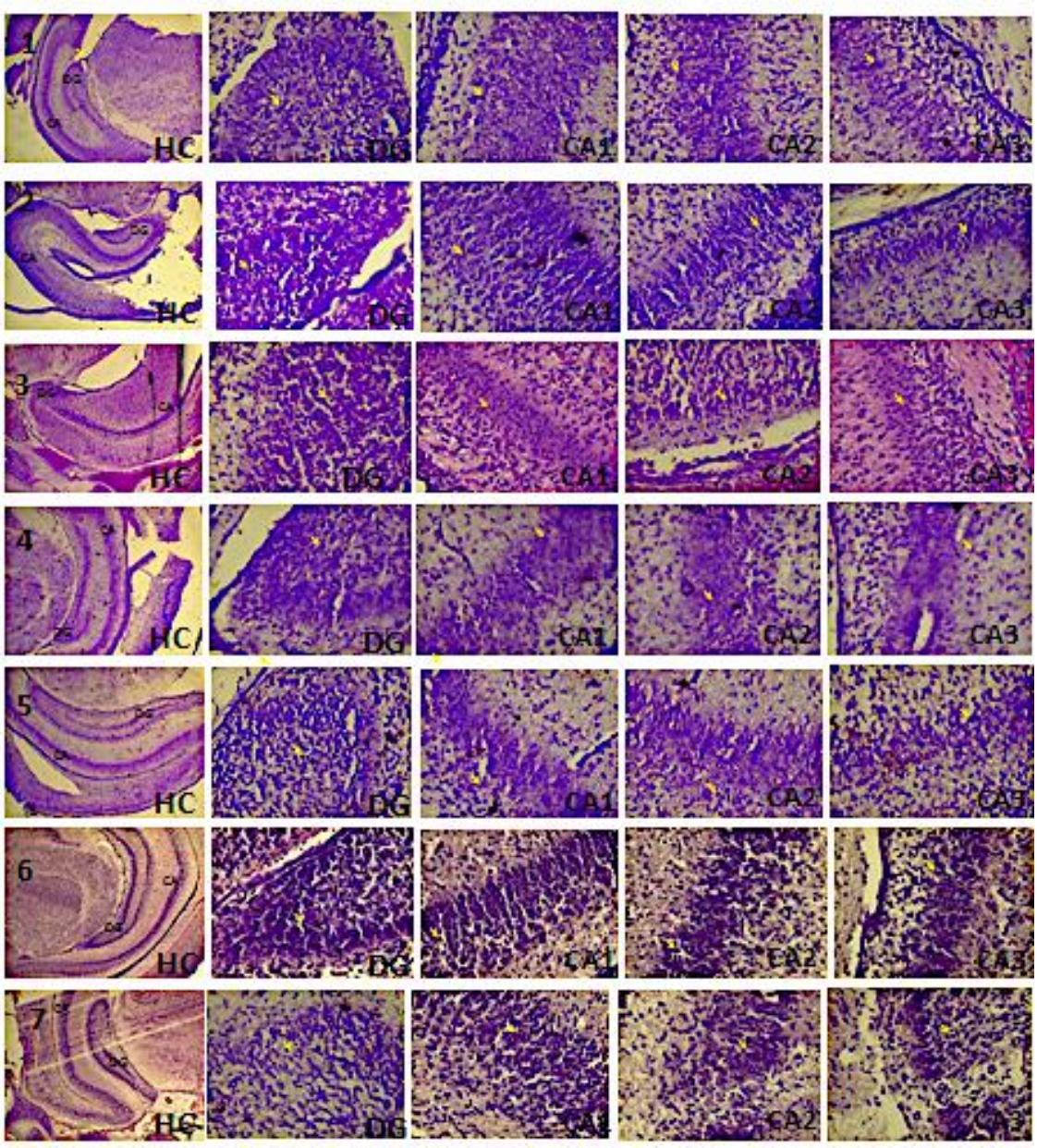

Fig. 4. Photomicrographs of the experimental animals' hippocampi and the subdivisions at birth [Postnatal D0] following exposure to regimented durations of RFR [Groups 2-7]; and compared with the Controls [Group 1]. In each Group [in the rows]: HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1; CA2= Cornu Ammonis2; CA3= Cornu Ammonis 3. Photomicrographs reveal no seriously deleterious effects of exposure in terms of cell morphology and distribution of Nissl substance as a marker of functional integrity. [CFV, x400]

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment; HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1 region; CA2= Cornu Ammonis 2 region; CA3= Cornu Ammonis 3 region. Yellow arrows indicate neurons

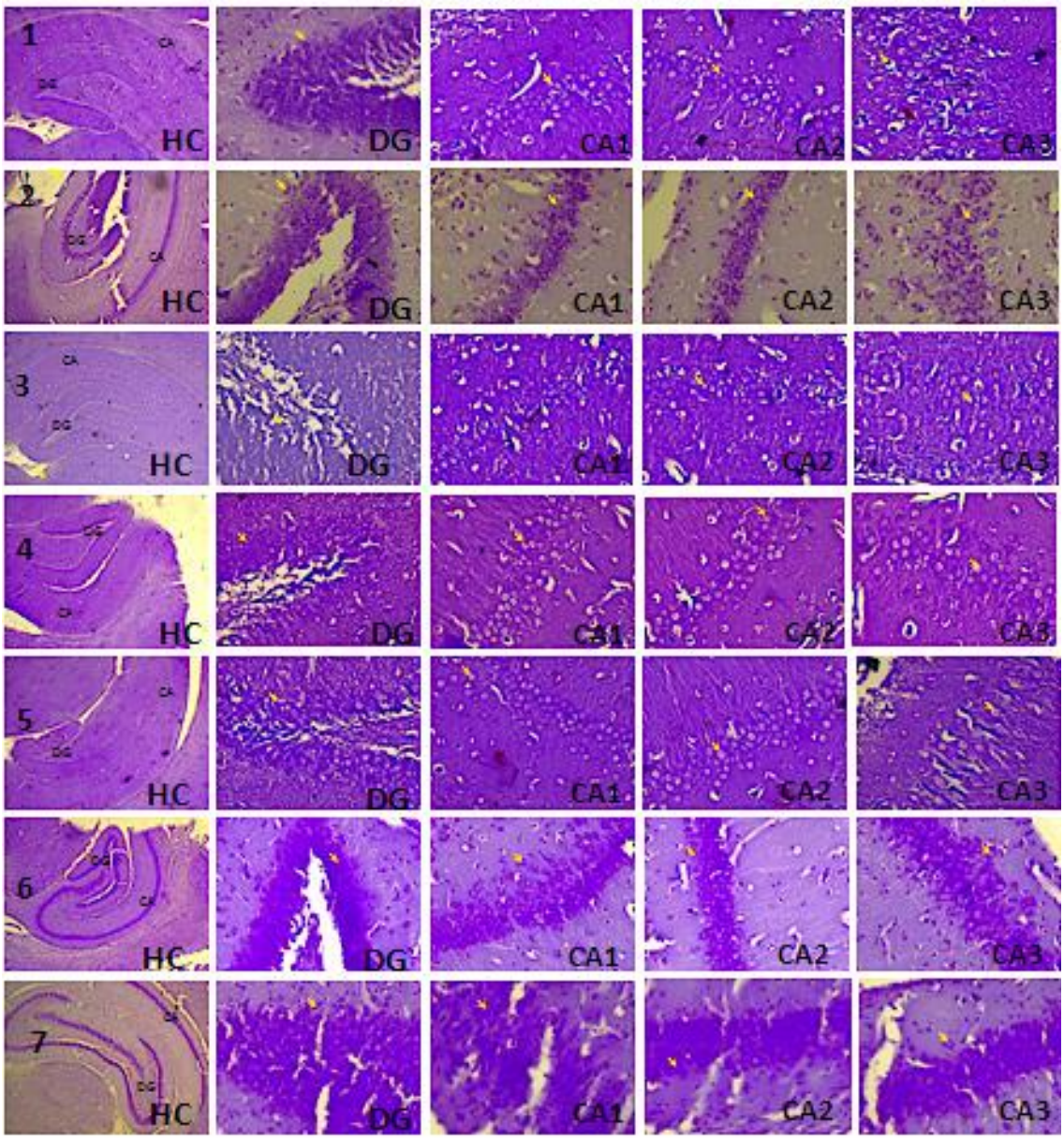

Fig. 5. Photomicrographs of the experimental animals' hippocampi and the subdivisions at puberty [Postnatal D35] following exposure to regimented durations of RFR [Groups 2-7]; and compared with the Controls [Group 1]. In each Group [in the rows]: HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1; CA2= Cornu Ammonis2; CA3= Cornu Ammonis 3. Photomicrographs reveal no seriously deleterious effects of exposure in terms of cell morphology and Nissl substance as a marker of functional integrity during the pre-pubertal postnatal stage of life. [CFV, x400]

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment; HC= Hippocampus; DG= dentate gyrus; CA1= Cornu Ammonis 1 region; CA2= Cornu Ammonis 2 region; CA3= Cornu Ammonis 3 region. Yellow arrows indicate neurons

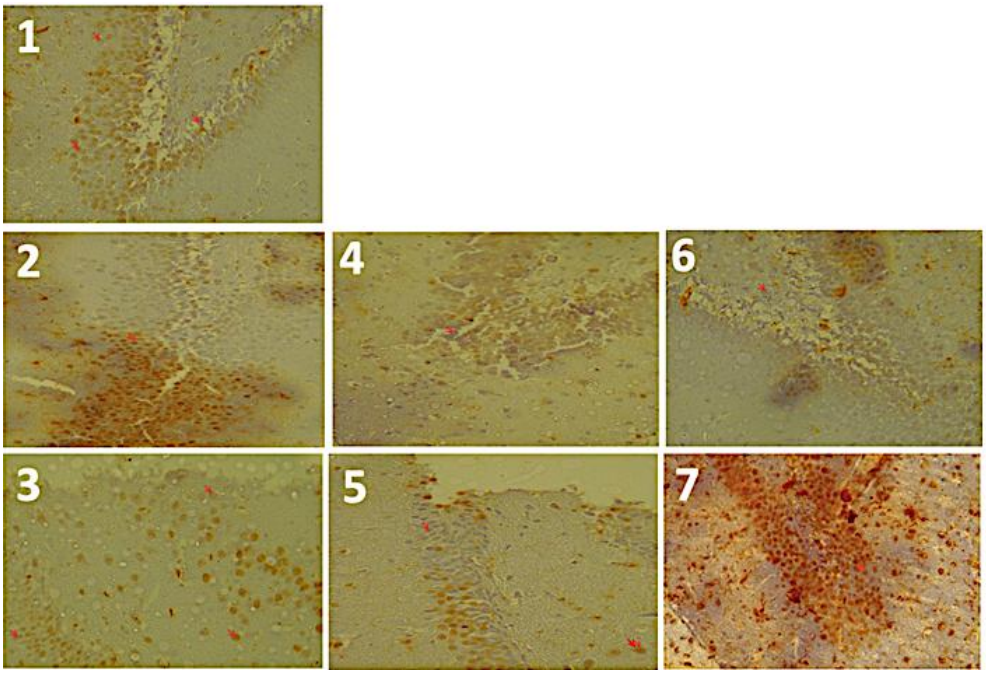

Fig. 6. Post-natal Photomicrographs of Dentate Gyrus of experimental Animals Groups A-G (IBA1). Longer hour and daily exposure caused enhanced IBA1 expression that is suggestive of mild microglial activation [2 and 7]

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment. Red arrows indicate neurons

6. DISCUSSION

Pre- or postnatal RFR exposure did not significantly alter hippocampal structural integrity at birth or at puberty. Analysis of the photomicrographs showed that the pre- or postnatal RFR exposure did not significantly alter hippocampal structural integrity at birth or at puberty. The inference from these observations would be that RFR did not cause extensive neurodegeneration or significant teratogenic effect that could have impaired proper development of the entire hippocampus or any of its sub-regions. Certain previous observations have suggested that RFR exposure might cause autophagy of brain cells [19]; which would manifest as neurodegeneration. The current findings, however, did not have any specific and strong evidence to suggest that RFR caused neurodegeneration at the experimental dose and duration of exposure, which typically mimicked human pattern of exposure.

Pre- or postnatal RFR exposure did not significantly alter Nissl substance expression as a marker of neuronal functional integrity through protein synthesis at birth or at puberty. The Cresyl fast violet technique was used to demonstrate the hippocampal formation to observe the expression of Nissl substance within its cells. Noting that Nissl substance [or Nissl bodies] is a conjugate of the rough endoplasmic reticulum and ribosome, it is therefore used as a marker of functional neuronal activity with respect to protein synthesis. In the current study, the distribution of Nissl substance in the hippocampal formation across the experimental animal groups whether in the hippocampal formation or in any of its subunits including the dentate gyrus and a Cornu Ammonis [CA], including the CA 1-4 areas showed no differential aberrations in Nissl substances expression that was attributable to the effect of RFR exposure. The implication of these would be that RFR exposure either during the pre- or postnatal life stages did not alter protein synthesis activities within hippocampal formation neurons for which Nissl substance distribution serves as a reliable marker. Unlike the findings of Tan et al. [25], the current study did not record significant alterations in Nissl substance expression in hippocampal cells.

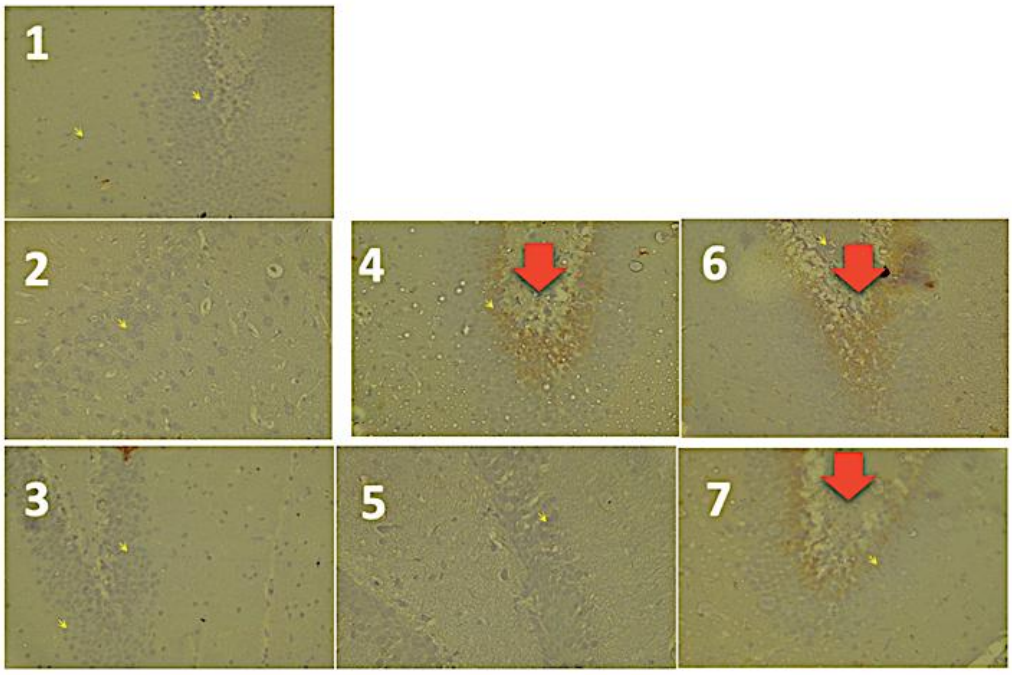

Fig. 7. Postnatal Photomicrographs of Dentate Gyrus (CASPASE3); GROUPS 1-7. The enhanced Caspase 3 expression in the dentate gyrus of the Groups 4, 6 and 7 relative to Control and other groups; as a marker of enhanced apoptotic potential [Red arrows]. Yellow arrows indicate neurons

Group 1- Control animals; Group 2= animals exposed to continuous RFR for 6 hours daily during experiment; Group 3= animals exposed to intermittent RFR for 6 hours daily during experiment; Group 4= animals exposed to continuous RFR for 12 hours daily during experiment; Group 5= animals exposed to intermittent RFR for 12 hours daily during experiment; Group 6= animals exposed to continuous RFR for 24 hours daily during experiment; Group 7= animals exposed to intermittent RFR for 24 hours daily during experiment

Immunohistochemistry results showed no evidence of significant teratogenicity effects or neuroinflammation but enhanced apoptotic potential in dentate gyrus cells following postnatal RFR exposure for 12-24 hours. Selected immunohistochemistry methods including IBA1, and Caspase 3 were used to demonstrate hippocampal formation cells. Caspase 3 was used to demonstrate potential apoptosis while IBA1 was used to demonstrate neuroinflammation within the hippocampal formation. Representative photomicrographs of the experimental animals' hippocampal formation demonstrating IBA1 were carefully studied for IBA1 expression for potential microglial reaction in response to neuroinflammation. There was no significant microglia reaction that could have served as a marker of extensive neuroinflammation of pathological significance. On the other hand, Caspase 3 is of importance because it does not just demonstrate the occurrence of cell death within the hippocampal formation structures, but potentially enhanced risk of cell death via the Caspase-3 pathway.

In the current study, Caspase-3 expression in the hippocampal formation of the animals that were exposed to radiofrequency radiation at birth was relatively normal in almost every group when compared with the control. However, the hippocampi of the experimental animals whose brains were exposed especially to the longer duration of radiofrequency radiation during the postnatal life stages at puberty had enhanced expression of caspase-3 at puberty. This would be of interest as it would be a marker of enhanced risk of neurodegeneration of the dentate gyrus cells of the hippocampal formation in these groups. Furthermore, it would be worthy of note that the dentate gyrus is the site of adult neurogenesis within the hippocampal formation. Therefore, the enhanced risk of apoptosis or neurodegeneration in this case might alter the pattern of normal neurogenesis within the

dentate gyrus. The inference from this collection of observations and experimental evidence would be that RFR might be a risk factor for neurodegeneration or impaired neurogenesis. Exposure of the hippocampal formation to RFR during early postnatal life up until puberty for a relatively long duration- and in this case between 12 and 24 hours- on a daily basis did affect normal hippocampal neurogenesis. This should be of great research interest to stakeholders in the fields of neuroscience, mental health, and neuroepidemiology.

It is known that RFR, and other electromagnetic waves could increase the permeability of the blood brain barrier. Prolonged RFR exposure also increases ROS formation [26]. There is evidence that RFR might induce oxidative stress which in turn is linked to an increased risk of neurodegeneration [27,28]. In fact, certain studies had indicated that RFR from mobile phones could upset the oxidant-to-antioxidant balance within the brain [29,30]. These previous findings point to the fact that RFR exposure could increase apoptotic potentials in brain cells. This is in line with the findings of the current study. This study has further expanded the frontiers of knowledge on mechanism by which RFR might induce neurodegeneration by identifying the Caspase-3 as a player, hence implicating the Caspase-3 apoptotic pathway. The Caspase 3 pathway has been implicated in the potential apoptotic effects of RFR exposure [31]. This study further confirmed such previous observations.

7. CONCLUSION

In conclusion, this study showed that the exposure of organisms to RFR during the prenatal stage of development did not cause extensive observable teratogenic effects in the hippocampus. Similarly, postnatal RFR exposure did not produce an extensive observable neurodegenerative effect in the hippocampal structure. However, postnatal exposure to RFR for 12-24 hours increased apoptotic potential of the dentate gyrus cells via the Caspase-3 Pathway. It is therefore strongly recommended that significant research investment be undertaken to understand the specific effects of RFR on brain development, as well as structural and molecular characterisation using diverse research methods and approaches, especially by aligning and combining experimental neuroscience and epidemiological methods. This has become important as the world is increasingly embracing technologies and innovations, many of which currently use RFR, and with a trend that predicts monumental increase in RFR generation and exposure in the years to come with advancements in the RFR-enabled devices.

ETHICAL APPROVAL

Animals in the current study were handled following these guidelines and standard recommendations:

- IACUC Institutional Animal Care and Use Committee OLaw and *Guide for the Care and Use of Laboratory Animals* regulations
- The National Research Council Guide for the Care and Use of Laboratory Animals, 2011[32].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dasdag S, Akdag MZ, Erdal ME, Erdal N, Ay OI, Ay ME, Yilmaz SG, Tasdelen B and Yegin K. Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue, International Journal of Radiation Biology. 2015;91(7): 555-561, DOI: 10.3109/09553002.2015.1028599
2. Hidisoglu E, Deniz Kantar-Gok, Sukru Ozen, Piraye Yargicoglu. Short-term 2.1 GHz radiofrequency radiation treatment induces significant changes on the auditory evoked potentials in adult rats. International Journal of Radiation Biology. 2018;0(0):1-14.
3. Bamdad K, Shakiba A, Esmaeili M Consequences of 2.4-2.48 Ghz non-ionizing radiation of Wi-Fi router devices on the information processing speed in adolescents. J Psychol Cognition. 2018; 3(1):1-5.
4. Ghazizadeh V and Nazıroğlu M Electromagnetic radiation (Wi-Fi) and epilepsy induce calcium entry and apoptosis through activation of TRPV1 channel in hippocampus and dorsal root ganglion of rats. Metab Brain Dis. 2014; 29(3):787- 799.
5. Manna D and Ghosh R Effect of radiofrequency radiation in cultured

mammalian cells: A review. Electromagnetic Biology and Medicine, 2016;35(3): 265-301.
6. Wible CG. Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. Behavioral sciences (Basel, Switzerland). 2013;3(2):298–315. Available:https://doi.org/10.3390/bs3020298
7. Rubin RD, Watson PD, Duff MC and Cohen NJ. The role of the hippocampus in flexible cognition and social behavior. Frontiers in human neuroscience. 2014;8:742. Available:https://doi.org/10.3389/fnhum.2014.00742
8. Lee JK, Johnson EG, and Ghetti S. Hippocampal Development: Structure, Function and Implications. In: Hannula D, Duff M. (eds) The Hippocampus from Cells to Systems. Springer, Cham; 2017.
9. Valberg PA. Radio Frequency Radiation (RFR): The Nature of Exposure and Carcinogenic Potential. Cancer Causes & Control Springer The Harvard-Teikyo Program Special. 1997;8(3): 323-332.
10. ACS Radiofrequency (RF) Radiation. The American Cancer Society medical and editorial content team; 2020. Available:https://www.cancer.org/cancer/cancer-causes/radiation-exposure/radiofrequency-radiation.html Accessessed 3rd May 2021.
11. Czerski P, Ostrowski K, Shore ML, Silverman CH, Sues MJ, and Waldeskog B, eds. “Biological Effects and Health Hazard of Microwave Radiation: Proceedings of an International Symposium,” P. Czerski, et al., eds., Polish Medical Publisher, Warsaw;1974.
12. Lai H. Neurological Effects of Radiofrequency Electromagnetic Radiation. In: Lin J.C. (eds) Advances in Electromagnetic Fields in Living Systems. Advances in Electromagnetic Fields in Living Systems, vol 1. Springer, Boston, MA; 1994. Available:https://doi.org/10.1007/978-1-4615-2542-4_2
13. Singh S, and Kapoor N Health Implications of Electromagnetic Fields, Mechanisms of Action, and Research Needs", Advances in Biology, 2014, Article ID 198609, 24 pages; 2014. Available:https://doi.org/10.1155/2014/198609
14. O'Connor ME. Prenatal microwave exposure and behavior. Progress in Clinical and Biological Research. 1988;257:265-288.
15. Schmid MR, Loughran SP, Regel SJ, Murbach M, Bratic Grunauer A, Rusterholz T, Bersagliere A, Kuster N, Achermann P Sleep EEG alterations: effects of different pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 2012;21:50-58.
16. Wallace D, Eltiti S, Ridgewell A, Garner K, Russo R, Sepulveda F, Walker S, Quinlan T, Dudley S, Maung S, Deeble R, Fox E Cognitive and physiological responses in humans exposed to a TETRA base station signal in relation to perceived electromagnetic hypersensitivity. Bioelectromagnetics. 2012;33:23-39.
17. Deshmukh PS, Nasare N, Megha K, Banerjee BD, Ahmed RS, Singh D, Abegaonkar MP, Tripathi AK, Mediratta PK Cognitive impairment and neurogenotoxic effects in rats exposed to lowintensity microwave radiation. Int J Toxicol. 2015;34:284-290.
18. Kim JH, Yu DH, Kim HJ, Huh YH, Cho SW, Lee JK, Kim HG, Kim HR. Exposure to 835 MHz radiofrequency electromagnetic field induces autophagy in hippocampus but not in brain stem of mice. Toxicol Ind Health. 2017a;1: 748233717740066. DOI: 10.1177/0748233717740066.
19. Kim JH, Lee JK, Kim HG, Kim KB, Kim HR. Possible Effects of Radiofrequency Electromagnetic Field Exposure on Central Nerve System. Biomolecules & therapeutics, 2019;27(3):265–275. Available:https://doi.org/10.4062/biomolther.2018.152
20. Lai HA summary of recent literature on neurobiological effects of radiofrequency radiation. in “Mobile Communications and Public Health” Markov, M. (ed.), CRC Press, Boca Raton, FL, Chapter. 2018; 8:187-222
21. Singh R, Nath R, Mathur AK, Sharma, RS Effect of radiofrequency radiation on reproductive health. The Indian journal of medical research. 2018;148(Suppl):S92–S99. Available:https://doi.org/10.4103/ijmr.IJMR_1056_18
22. Luna L. Harris’ Methods for Staining Cellular Entities. Histopathologic Methods

and Color Atlas of Special Stains and Tissue Artifacts. American Histolabs. 1992;4(71):92.

23. Sheehan D and Hrapchak B. Theory and practice of Histotechnology, 2nd ed, , Battelle Press, Ohio. 1980; 262-264.
24. Caceci T. Histology, Lecture 29, Introduction to Special Stains Techniques, Nerve Tissue Staining, 2014;1-29. Available:www.histology.com, accessed 2nd March 2020.
25. Tan S, Wang H, Xu X, Zhao L, Zhang J, Dong J, Yao B, Wang H, Zhou H, Gao Y and Peng R. Study on dose-dependent, frequency-dependent, and accumulative effects of 1.5 GHz and 2.856 GHz microwave on cognitive functions in Wistar rats. Scientific reports. 2017;7(1):10781. Available:https://doi.org/10.1038/s41598-017-11420-9
26. Leszczynski D, Joenvaara S, Reivinen J, Kuokka R. Non thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer and blood–brain barrier-related effects. Differentiation. 2002;70:120–129.
27. Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8 GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res. 2006;602(1–2):135–142.
28. Varsha S, Santosh KM, Harish CP Oxidative stress in neurodegeneration. Adv Pharm Sci. 2011;20113–19.
29. Jelodar GA, Akbari A, Nazifi S The prophylactic effect of vitamin C on oxidative stress indexes in rat eyes following exposure to radiofrequency wave generated by a BTS antenna model. Int J Radiat Biol 2013;89(2):128–131.
30. Akbari A, Jelodar GA, Nazifi S. Vitamin C protects rat cerebellum and encephalon from oxidative stress following exposure to radiofrequency wave generated by BTS antenna mobile. Toxicol Mech Methods. 2014a ;24(5):347–352.
31. Liu B, Jian Z, Li Q, Li K, Wang Z, Liu L, Tang L, Yi X, Wang H, Li C, Gao T (2012). Baicalein protects human melanocytes from H2O2 induced apoptosis via inhibiting mitochondria-dependent caspase activation and the p38 MAPK pathway. Free Radical Bio Med. 2014a;53183–193.
32. National Research Council. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press; 2011. Available:https://doi.org/10.17226/12910.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/70025