



A Study of RFR Effects on the Brain Neurochemistry during Pre- and Postnatal Brain Development

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The research was designed to model animals which mimic the exposure to the radiofrequency radiation [RFR] by habitual users of RFR-enabled devices and to observe possible aberrations in behaviours that are attributable to exposures. The research was designed to model cases of continuous, and intermittent exposures in human conditions, using Wistar rats. The primary objective of this study was to study intrauterine and postnatal exposure and study the effects brain structures, functions and behaviours in Wistar rats.

Materials and Methods: The experiment started with 42 pregnant rats that were exposed to RFR [4G] to observe the possibilities of RFR-induced teratogenic effects. Thereafter, half of the offspring were sacrificed for their brains to be studied at birth. The other half were exposed to RFR to observe postnatal effects of RFR radiation until puberty. The exposure regimen was the same for the mothers and the offspring in each group. What varied was the duration of exposure per day

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being 6 hours, 24 hours and 24 hours. Experimental Wistar rats were housed in facilities that enabled exposure to specific dose (4G) of radiations during pregnancy (~21 days) and during post-natal days until day 35, marking approximately the point of puberty. Following the dissection of the rat, brain tissue samples were homogenized in buffered phosphate saline [PBS]. The homogenates were centrifuged, and the supernatant was assayed for each neurotransmitter of interest. The values from each sample were recorded for the sample. The mean values for samples that constituted a group was calculated and statistically analyzed. ELISA biochemical assay kits were used.

Results and Conclusion: RFR-exposure caused changes in neurotransmitters and enzyme neurochemistry. Cytochrome C oxidase enzyme and neurotransmitters especially dopamine, gamma-amino butyric acid, glutamine and serotonin and their activities levels were significantly altered especially with prolonged duration of RFR exposure. These findings would altogether suggest that radiofrequency radiation exposure might change brain neurochemistry permanently following exposure either during the intrauterine or the postnatal stage of life. The implications of these changes on later life mental health and neurological attributes will require further investigation

Keywords: Brain; development; neurotransmitters; enzyme; radiofrequency radiation.

1. BACKGROUND

Man-made radiofrequency radiation (RFR) might have polluted the natural electromagnetic environment of the planet earth. This effect is one of the possibly unanticipated or unappreciated fallout of technological advancement especially with the use of the internet more than ever before. Humans are now more exposed to electromagnetic radiation, possibly more than ever before because of the introduction of artificial electromagnetic radiation sources. It is clear that the new release of electromagnetic radiation into the environment through manmade sources is closely linked to industrialization and technological advancement and that could mean that the dose or intensity could greatly vary from place to place with exposure being possibly linked to the level of technological advancement in a particular environment. Hence, electromagnetic radiation in the form of radiofrequency radiation is becoming a subject of occupational concern as well as a health concern. It is known that the main sources of technology-related radio frequency radiation include radio waves and microwaves (100 kHz–300 GHz), and power frequency EMF (50, 60 Hz).

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 and an unprecedented level, dose, and intensity. Reliable data should come from diverse and

complementary sources including the human epidemiological data, experimental data, and case evidence among others. Very importantly, there should be quality evidence synthesis in the form of systematic review and meta-analysis among others.

2. LITERATURE REVIEW

RFR-producing devices are extensively used in the industry, telecommunication, medicine, and in everyday life in gadgets and devices such as microwave ovens, mobile phones and similar WIFI-enabled or internet connected devices [1]. RFR is arguably becoming increasingly popular in terms of its use to enable gadgets that are connected to or operated via the internet. The use of RFR gadgets will no doubt remain increasingly popular in years to come with the demonstrated need for humanity to increasingly enhance the capability of the internet as demonstrated in the development of the internet of things [2] and development of higher-capacity generation of WIFI gadgets in the forms of the 4G and the 5G and any other ones to come. It is over worthy of note that the specific levels of safety with RFR sources and doses are still not yet clearly established either because of obvious lacunae in the existing body of knowledge or the heterogeneity in the types, sources and quality of existing results for extrapolations to inform policies and practices [3]. It is however worthy of mention that RFR is already linked certain to increased risks of cancer types including glioma and acoustic neuroma [4]. The specific mention of glioma and acoustic neuroma would further indicate that RFR could have effects on human

mental health beyond cancers. It is clear that the world is now bathing in significantly increased ambient RFR due to the addition or contribution of manmade sources of RFR to the original earth's natural electromagnetic fields [5,6]. The implications of this on mental health should be critically considered and thoroughly investigated.

The advancements that the internet and technology has brought about have significantly contributed to increased exposure to man-made RFR sources. It is reported that the use of the mobile phones has greatly increased only in the last 20 years [1]; and the effects of such unprecedented rise in the use of mobile phones, with many emitting RFR might not be known in the short term. Without causing panic or fears out of the afforested reality, the implication is that quality research is required on this subject. It is also interesting to note that amongst the sources of RFR radiation that have been linked to cancers, the mobile phone emitted RFR has become quite notable [7,8,9]. This might not be unconnected with the fact that the habitual use of the mobile phone might cause greater proximity to the source of radiation as well as increased frequency and duration of exposure. However, it is also becoming clear that other sources of RFR such as handheld devices and similar gadgets including iPads, notebooks and personal computers such as laptop use are becoming increasingly popular. The need to work remotely over the internet, using WIFI-enabled devices is a major change that has occurred in several walks of life and workplaces between the year 2020-21 and possibly beyond simply because of the changes induced by the COVID-19. In fact, several schools operated online than these required using WIFI-enabled devices for unprecedented long hours.

The implication of the currently available evidence is also the potential teratogenicity of radiofrequency radiation, an aspect that has only there recurrently raised concerns but remains relatively poorly explored. In this particular instance, it is critical to explore the nature, and the interactions of radiofrequency radiation with the developing brain. When nervous system cells and tissues are exposed to interact with developing brain cells and tissues in doses that could interfere with development, there should be adequate investigations as to whether such exposures that could impact the quality of mental health and neurological function during the postnatal life stages. It is therefore clear that significant gaps in knowledge exist on the subject

of RFR and its actual effects on health. Quality quality research would be required to address this subject in a holistic way to provide data on technological, behavioural, policies, standard practices and regulations matters (Federal Communication Commission), [10,11].

The highlighted literature sources among several others of their kind would point out two major things. One is the fact that there is a strong link between radiofrequency radiation exposure and certain health problems. It is also clear that the strongest of such known links is the RFR potential to trigger cancer or tumour development. The concern here however is that the existing evidence is currently inadequate to take clearly informed positions and to develop policies and practices that are largely evidence-based. This would therefore imply that significant research investment in terms of effort and resources is required to address this subject. This subject should therefore be a very important subject of research and scientific interest and discuss.

2.1 Aim

The aim of this study was to model RFR exposure and observe possible effects of exposure during the intrauterine and the postnatal stages of life on selected neurotransmitters and enzyme towards appreciating potential effects of RFR on vital neurochemistry prior to birth and until puberty.

3. MATERIALS AND METHODS

Experimental Wistar rats were housed in facilities that enable exposure to specific dose (4G) of radiations and for specific durations- 21 days of pregnancy and 35 post-natal days, marking the point of puberty. The experiment started with 42 pregnant rats that were exposed to RFR to observe the possibilities of RFR-induced teratogenic effects. Thereafter, half of the offspring were sacrificed for their brains to be studied at birth. The other half were exposed to RFR to observe postnatal effects of RFR radiation until puberty. The exposure regimen was the same for the mothers and the offspring in each group. Also, emission was the same, from 4G RFR-emitting devices; what varied however was the duration per day and whether emission and exposure was continuous or intermittent. Table 1 shows the animals grouping and the exposure regimen. Following, exposure regiments, experimental animals were sacrificed,

the brain tissues were excised and homogenised. The homogenates were centrifuged to obtain the supernatants. From the sample supernatants, specific enzymes and neurotransmitters assays were carried out. Quantitative data on various parameters of interest were analysed using the GraphPad Prism statistical package, version 8, and group means for parameters were compared and analysed using the McNemar test ($p \leq 0.05$). Quantitative data are presented as tables.

Biochemical Analysis of neurotransmitters in the brain tissue homogenates included GABA, Serotonin, Acetylcholine, Dopamine, Glutamate. Generally, tissue samples were first rinsed in ice-cold phosphate buffer saline (0.01 mol/l, ph 7.0-7.2) to thoroughly remove all forms of blood and also weighed before homogenization. The samples were homogenized with an automated homogenizer. Neurotransmitters' activities or functional levels in the brain were presented in value unit per tissue volume. The mean values of neurotransmitters activities were presented on tables. Animals in the current study were handled following these guidelines and standard recommendations of the National Research Council Guide for the Care and Use of Laboratory Animals [12]. Ethical approval for this body of work had been sought and obtained from the Babcock University Health Research Ethical Committee (BUHREC); with an ethical clearance BUHREC NO: 814/18.

3.1 Gamma-Amino Butyric Acid (GABA) Neurotransmitter [13]

The tissue samples were first rinsed in ice-cold phosphate buffer saline (0.01 mol/l, ph 7.0-7.2) to thoroughly remove all forms of blood and also weighed before homogenization. The tissues were minced into small pieces and homogenized in 5-10ml of pbs using a glass homogenizer on ice. The resulting suspension was sonicated with an ultrasonic cell disrupter or subjected to 2 freeze-thaw cycles to break the cell membrane further. After that, the homogenates were centrifuged for ten (10) minutes at 2,000g. The supernatant was removed and assayed immediately. Following the standard assay procedure, reading was done at 450nm immediately.

3.2 Glutamate Assay

To attain the glutamate standards for colorimetric detection, 10ml of the 0.1m glutamate standard was diluted with 990 μ l of the glutamate assay buffer to prepare a 1mm standard solution. This was followed by adding 0, 2, 4, 6, 8, and 10 μ l of the 1mm standard solution into a 96 well plate, generating 0 (blank), and 2, 4, 6, 8, and 10 nmol/well standards. Glutamate assay buffer was thereafter added to each well to bring the volume to 50 μ l. Following the standard assay procedure, the absorbance was measured at 450nm (a450).

Table 1. A table presenting the experimental animals grouping and he regimens of RFR exposure as well as the rationale of grouping and exposure

Group	Duration of RFR Exposure/day	Description	Rationale
A	0	Control	This served as the standard reference group without exposure
B	6 hours	6-hour daily intermittent exposure to RFR.	This served as the experimental group with short-duration intermittent exposure
C	6 hours	6-hour daily continuous exposure to RFR.	This served as the experimental group with short-duration continuous exposure
D	12 hours	12-hour daily intermittent exposure to RFR.	This served as the experimental group with medium-duration intermittent exposure
E	12 hours	12-hour daily continuous exposure to RFR.	This served as the experimental group with medium-duration continuous exposure
F	24 hours	24-hour daily intermittent exposure to RFR.	This served as the experimental group with long-duration intermittent exposure
G	24 hours	24-hour daily continuous exposure to RFR.	This served as the experimental group with long-duration continuous exposure

The table presents information on the pattern of experimental animals' exposure to the 4G device daily and the rationale for the exposure regimen. In each group, exposure lasted throughout pregnancy [i.e., pregnant rats were exposed to RFR to study potential teratogenic effects] and this continued until puberty [i.e. to observe the nature of continual exposure until puberty]

3.3 Dopamine Assay

This assay employed the competitive inhibition enzyme immunoassay technique. Monoclonal antibody specific to dopamine was pre-coated onto a micro plate. Competitive inhibition reaction was launched between biotin-labeled dopamine and unlabeled dopamine (standards and samples) with the pre-coated antibody specific to dopamine. The unbound conjugate was washed off after incubation. Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After adding the substrate solution, the intensity of color developed was reverse proportional to the concentration of dopamine in the sample. After the step-by-step procedure, 50 μ L of stop solution was added and absorbance was read immediately at 450nm [14].

3.4 Serotonin

Acylated standards, 100 μ L was pipetted, plus controls and samples into the appropriate wells of the 5Ht/5-HIAA Microtiter Strips followed by pipetting 25 μ L of the 5Ht antiserum into all wells. Plates were covered with adhesive foil and incubated for 15 -20 hours at 2 – 8 °C. Standard serotonin ELISA protocol was followed all through. The absorbance of the solution in the wells was read within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

3.5 Cytochrome C oxidase

Following the homogenization of tissue samples in 5-10ml of pbs using a glass homogenizer on ice, followed by centrifuging and collection of the supernatant, the homogenate was assayed for the enzyme following a standardised spectroscopic method for the determination of cytochrome C oxidase content in the tissues [15].

4. RESULTS

4.1 Neurochemistry of Selected Enzyme- Cytochrome C oxidase

The effect of experimental animals' exposure to radiofrequency radiation during the intrauterine stage of life could be observed in measurable changes in the level of the enzyme cytochrome C oxidase activity within the brain tissues. When compared with the Control, [Group A], Groups D, E, F and G levels of cytochrome C oxidase

activities were significantly higher [Table 2]. It was also observable that changes [increase] in cytochrome C oxidase activities levels were recorded based on the overall duration of exposure. There was slight but no statistically different changes when the exposure was continuous for the duration or intermittent. What this might imply is that the overall duration or longevity of exposure time was more important in terms of effects than whether the RFR source had continuous or intermittent release of RFR emissions. It is important to critically consider the potential of having a sustained elevated activity levels for Cytochrome C oxidase in these experimental groups.

Brain neurochemistry with specific emphasis on the activities level of the Cytochrome C oxidase present patterns of aberrations that could be attributed to the effects of RFR exposure. Cytochrome C oxidase activities level in the experimental animals' brains at puberty showed that the activity levels of this enzyme were generally elevated across all exposed groups, that is Groups B to G [Table 3]. The implications of this would be that following prenatal exposure, in which case activity level of this enzyme was markedly elevated at birth, this elevation had been sustained from birth up to puberty stage. This would further show a sustained shift in brain neurochemistry activity, which was measurable at puberty suggesting that this shift in brain neurochemistry might be sustained until latter stages of life. It would further imply that radiofrequency radiation exposure could alter brain neurochemistry in a sustained manner, and these might include energy metabolism as well as other functional activities of the brain that are related to the functions of cytochrome C oxidase in particular.

When groups were compared on the basis of whether intermittent or continuous exposure to radiofrequency radiation caused any measurable differential effects on brain neurochemistry with respect to the functional activity levels of cytochrome C oxidase would have, significance increase was only measurable between the brains Groups D and E in which case intermittent exposure for 12 hours was matched with continuous exposure for 12 hours on daily basis. This might also point to the fact that increased exposure rather than intermittent might have influence on the level of change in brain neurochemistry. The fundamental inference from these observations, however, remains that both intermittent and sustained or continuous

exposure to radio frequency radiation within a given period of time could have effects that could cause a shift in brain neurochemistry by increasing the activity of cytochrome C oxidase, influencing brain metabolism and other related functional activities within the brain.

4.2 Neurochemistry of Selected Neurotransmitters

Radiofrequency radiation exposure of animals during the pre-natal stage of development had measurable effect on neurotransmitters activities within the brain as there was general change in form of increase in the levels of neurotransmitter activities in the experimental animals' brains that were exposed to radiofrequency radiation. Increases in neurotransmitters activities in certain instances were statistically significant including Groups B, C, D, F, and G.

4.2.1 Dopamine activities in the brain at birth

The assay and evaluation of changes in dopamine neurotransmitter activities in experimental groups B-G relative to the control [A] showed that there was general increase in the level of dopamine activities across the groups when compared with the control. This is generally attributable to the effect of the exposure. These changes in dopamine activity levels were however statistically significant in Groups D, E, F, and G. In the matched groups, that included B and C; D and E; F and G, in which case similar durations of exposure were used both continuously and intermittently, no significant variations in the matched or paired groups were observable [Table 4]. The general inference from these observations therefore was that exposure to either continuous or intermittent radiofrequency radiation for 12 to 24 hours during intrauterine life elevated the level of dopamine activities significantly whether the exposure was continuous or intermittent.

4.2.2 GABA activities in the brain at birth

GABA activities in the brain at birth was generally elevated with only Groups E, F and G being statistically significant. This would indicate that the although the activity level of GABA neurotransmitter in the experimental animals' brains were generally elevated at birth, only prolonged [12-24 hours] duration exposure produced effects that significantly elevated the activities of GABA neurotransmitter in the brain. When the matched groups were compared, there

were no statistically significant changes in the level of GABA activities in the brain, showing that there was no differential effects in terms of intermittent and continuous exposure [Table 4].

4.2.3 Glutamate activities in the brain at birth

The glutamate neurotransmitter activities in the brains of experimental animals was assayed at birth to provide insight into potential alterations in the activities of this neurotransmitter due to the exposure of the brain to radiofrequency radiation during the intrauterine stage of development. Glutamate activity was generally elevated significantly relative to control in groups D, E, F and G. This would imply that exposure to radiofrequency radiation between 12 to 24 hours daily, whether intermittently or continuously altered functional neurochemistry of glutamate activity. Within the matched groups however [D and E; F and G], no significant variations were observed between the matched groups, hence, no attributable differential elevation in glutamate activity is attributable to whether exposure was continuous or intermittent exposure for durations between 12 to 24 hours daily as used in the current experiment [Table 4].

4.2.4 Serotonin activities in the brain at birth

The level of serotonin activity within the experimental animals' brains at birth was generally statistically significant across the groups. This would also imply that radiofrequency radiation exposure during the intrauterine stage of life elevated serotonin activity significantly in the experimental animal brains. In the matched groups, no differential statistical significance was observable in serotonin levels in experimental animals' brains [Table 4].

Radiofrequency radiation exposure of animals during the postnatal stage of development had measurable effect on neurotransmitter activities within the brain. There was general change in the form of increase in the levels of neurotransmitters activities in the experimental animals' brains that were exposed to radiofrequency radiation. Increase in neurotransmitters activities in certain instances were statistically significant.

4.2.5 Dopamine activities in the brain at puberty

Dopamine levels were significantly elevated in the brains across all experimental animal groups

when the neurotransmitter activities within the brain were assayed at puberty. The inference from this observation would be that the observable elevation in the activities of dopamine due to radiofrequency radiation exposure at birth was sustained up to puberty. Furthermore, it would indicate that the potentially established shift in this neurotransmitter chemistry within the brain as determined by radiofrequency radiation exposure prior to birth and postnatally. No differential statistically significant variation was observed between the matched groups [B and C; D and E; F and G] [Table 5].

4.2.6 GABA activities in the brain at puberty

At puberty, the GABA activities as assayed in the brains of the experimental animals showed that GABA activities levels were elevated in groups D, E, F, and G. This pattern was similar to what was observe in terms of the activities of GABA at birth, implying that longer duration of exposure, 12 to 24 hours, could cause elevated GABA activities in the brain up until the puberty stage while shorter exposure duration of 6 hours did not. No differential statistically significant variation was observed between the matched groups [B and C; D and E; F and G] [Table 5].

4.2.7 Glutamate activities in the brain at puberty

Glutamate activities in the brain were assayed at puberty Glutamate activity levels in the experimental animals' brains were significantly elevated across all the groups. These observations shared semblance with the elevations in glutamate activities as previously observed at birth in Groups D-G. It is worthy of note however, that there were no such elevations in Groups B-C at birth. No differential statistically

significant variation was observed between the matched groups [B and C; D and E; F and G] [Table 5].

4.2.8 Serotonin activities in the brain at puberty

Serotonin activities assay at puberty showed that its activity levels in the experimental animals' brains were significantly elevated in all the groups. These observations shared close semblance with the elevations in glutamate activities as previously observed at birth in all experimental groups that were exposed to RFR [Groups B-G]. No differential statistically significant variation was observed between the matched groups [B and C; D and E; F and G] [Table 5].

5. DISCUSSION

Radiofrequency radiation had been reported to have altered a number of brain neurotransmitter systems. These alterations in neurotransmitter systems might also have specific links to functional changes in neuronal or neurological activities. Specifically, there were reported changes in GABA receptors in association with radiofrequency radiation [16]. Effects of RFR have also been associated with synaptic structural changes [17]; so also changes and modifications in neurotransmitter release or binding properties of receptors [18]. Some of these changes in neurotransmitter systems as well as corresponding receptor-binding properties including synaptic structure changes have been linked to corresponding alterations in relevant enzymatic systems [19]. Such changes might explain the reason for alterations in the activities of cytochrome C oxidase alongside the recorded changes in neurotransmitter functional activities as observed in the current experiment.

Table 2. Results of Brain Tissues Neurochemistry at Birth: Cytochrome C oxidase activities in the Brain at Birth

	A	B	C	D	E	F	G
Cytochrome	0.005500	0.01033	0.01350	0.01883*	0.02083*	0.02617*	0.02883*
SEM	0.0008851	0.001282	0.001500	0.001759	0.001778	0.002104	0.002903

[*; P<0.05; *indicate statistical significance of Cytochrome C activities in an exposed group relative to control]

Table 3. Results of Brain Tissues Neurochemistry at puberty: Cytochrome C activities in the Brain at Puberty

	A	B	C	D	E	F	G
Cytochrome	0.005333	0.009833*	0.01383*	0.02211*^	0.02983*^	0.04000*	0.03417*
SEM	+0.0004820	0.0008333	0.001222	0.001275	0.001682	0.001932	0.002040

[*indicate statistical significance of Cytochrome C activities in an exposed group relative to control; ^indicate statistical significance of Cytochrome C activities in an exposed group relative to other groups; P<0.05]

Table 4. Results of Brain Tissues Neurochemistry at Birth: Neurotransmitters' Activities in the Brain at Birth

	A	B	C	D	E	F	G
Dopamine	0.1162	0.1207	0.1255	0.1315*	0.1370*	0.1433*	0.1483*
SEM	0.0008333	0.001308	0.001746	0.002232	0.003246	0.004047	0.003801
GABA	0.1142	0.1183	0.1220	0.1237	0.1335*	0.1393*	0.1443*
SEM	0.0008333	0.001202	0.001571	0.002445	0.003117	0.003221	0.003658
Glutamate	0.1202	0.1262	0.1317	0.1365*	0.1427*	0.1482*	0.1535*
SEM	0.001600	0.001470	0.001909	0.001979	0.002140	0.002713	0.002941
Serotonin	0.1230	0.1290*	0.1415*	0.1413*	0.1452*	0.1510*	0.1578*
SEM	0.0008944	0.001390	0.007813	0.002565	0.002561	0.002463	0.003177

[*indicate statistical significance of neurotransmitters activities in an exposed group relative to control]

Table 5. Results of Brain Tissues Neurochemistry at Puberty: Neurotransmitters' Activities in the Brain at Puberty

	A	B	C	D	E	F	G
Dopamine	0.1166	0.1235*	0.1297*	0.1391*	0.1482*	0.1557*	0.1520*
SEM	0.001411	0.002592	0.002431	0.001752	0.003270	0.002929	0.003120
GABA	0.1140	0.1187	0.1223	0.1307*	0.1407*	0.1488*	0.1443*
SEM	0.0007588	0.001745	0.001944	0.001664	0.003040	0.002971	0.003422
Glutamate	0.2096	0.2158*	0.2277*	0.2367*	0.2458*	0.2560*	0.2512*
SEM	0.004430	0.005805	0.001430	0.001285	0.001973	0.001506	0.002151
Serotonin	0.1377	0.1428*	0.1475*	0.1599*	0.1690*	0.1792*	0.1738*
SEM	0.001884	0.002574	0.002941	0.001998	0.003830	0.003936	0.004636

[*; $P < 0.05$] including Groups B, C, D, E, F and G

Results from the current study showed a relatively generalized positive shift in brain neurochemistry, affecting the major enzyme of interest as well as the specific neurotransmitters of interest which in this case is presented as a generalized increase in neurotransmitter activities. It is important to note that these generalised positive shifts in specific enzyme and neurotransmitters' neurochemistry might not be necessarily linkable to specific aberrations of pathophysiological importance in a particular neurotransmitter system. However, what is being established is a general shift in the functional chemistry of the central nervous system. It is also important to note that this positive shift is attributable to radiofrequency radiation exposure and the changes were largely statistically significant. Furthermore, this positive shift in brain neurochemistry was also observable generally at birth and in most instances was sustained up until puberty, pointing to the fact that the shift was rather non-transient but established. Therefore, it is logically from this body of evidence that the radiofrequency radiation exposure during intrauterine and postnatal life up until puberty had a generalized effect specific neurotransmitter system by shifting neurotransmitter system activities positively and in a sustained manner. Ahmed et al., [20] had

reported that EMR exposure at a frequency of 1800 MHz and a specific absorption rate of 0.843 W/kg induced significant changes in amino acid neurotransmitters in the experimental rat brains, with the effects relatively more severe in juvenile animals. The findings from the study of Ahmed et al., [20], which agrees to the current research finding, might also point to the relatively increased vulnerability of young and juvenile brains to RFR exposure.

It should be noted that the work of Aboul Ezz et al. [21] had previously reported RFE exposure associated changes in neurotransmitters activities in various brain regions with significant changes in the hippocampus, the hypothalamus, midbrain and the medulla. The studied neurotransmitters in the referenced instance included dopamine (DA), norepinephrine (NE), and serotonin (5HT). Following the analysed results as obtained in their study, Aboul Ezz et al. [21] stated that EMR exposure (through mobile phones) resulted in significant alterations in the studied neurotransmitters activities and postulated that these changes in these monoamine neurotransmitters might account for certain reported adverse effects that are associated with EMR, especially memory, learning, and stress. While it has been reported

that radiofrequency radiation at both lower and higher doses can all produce effect on brain functions, brain health and mental health attributes. It is also known that duration of exposure also plays significant roles in the gravity of the effect produced [21,22]. While Aboul Ezz et al. [21] had highlighted the fact that the different brain regions could respond differently to RFR or EMR, it should also be emphasised that neurotransmitters systems connect specific brain regions functionally, and their alterations as such could imply that RFR effects on brain neurochemistry would affect the structures and regions that are connected by the neurotransmitters systems. Megha, Deshmukh and Ravi [23] had studied the implications of neurotransmitters aberrations on vital key regulating enzymes in rat brain. To correlate with human exposure scenarios, Buchner and Eger [24] had also reported that observed changes in vital neurotransmitters following EMR/RFR exposure from a base station were of clinical importance.

Another very important point to highlight from the current study is that pre-natal radiofrequency radiation exposure can also significantly alter neurotransmitter systems within the brain. It is worthy of note that specific neurotransmitters activities were significantly elevated which includes dopamine, GABA, glutamate and serotonin. The current findings are in line with the previous research report of Ismail et al., [25] which had reported that following exposure to radiofrequency radiation through mobile phones, specific monoamine neurotransmitters activities including histamine, dopamine, adrenaline, and noradrenaline were significantly elevated. The implication of the current findings in alignment with previous findings will therefore include the fact that attention should be paid to the potential teratogenicity of radiofrequency radiation at different doses, even if that could not be clearly established in the current study. It might require a multi-dimensional approach to such investigations including epidemiology, experimental and mixed methods research designs to be able to provide more reliable data and information as to the specific nature of the effects attributable to radiofrequency radiation. Another important thing that is yet to be clearly established from the body of data that is currently available is the long-term consequences of some of the reported changes in brain neurochemistry that has been attributable to radiofrequency radiation. This should be a subject of continual research interest.

While the current evidence is inadequate to determine the specific effects of radiofrequency radiation on health, especially mental health, it is important to evaluate the currently available pieces of evidence and consider what clue these would give as to what direction research attention might be focused as a matter of priority. If certain current pieces of evidence have linked radiofrequency radiation exposure to increase the risk of developing certain central nervous system tumors or cancer, this should serve as an indicator as to what effects radiofrequency radiation may have on functional neuronal mechanisms including neurotransmission, neurotransmitter activities and molecular activities which aberrations are usually linked to different disorders that are not necessarily in the forms of tumours or cancers. For example, aberrations in neurotransmitter systems alone could lead to significant mental health disorders. An example of such includes the link of serotonin system disorders to depression or depression-like disorders. While the serotonin neurotransmitter system is typically linked to a mental state or mood disorder, dopamine system definition is rather linked to a motor or movement disorder which is the case when low dopamine production is linked to substantia nigra cellular degeneration within the brainstem causing Parkinsonism- a major movement disorder. Quite specifically, Kim et al., [26] had linked decreased dopamine in striatum to observable difficulties in locomotion. These instances, therefore, illustrate the importance of neurotransmitter systems in sustaining mental health and neurological functions. By extension, the implication of this includes the fact that the effects of radiofrequency radiation need to be critically and extensively explored beyond their current links to tumour and cancer development.

6. CONCLUSION

RFR-exposure caused changes in neurotransmitters and enzyme neurochemistry. The activities levels of the cytochrome C oxidase enzyme and neurotransmitters, including dopamine, GABA, glutamate and serotonin were significantly altered especially with prolonged duration in RFR exposure. These findings would altogether show that radiofrequency radiation exposure could alter brain neurochemistry permanently following exposure either during the intrauterine or the postnatal stage of life. The implications of these changes on later life mental health and neurological attributes will require further investigation.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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