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Exposure to radio-frequency electromagnetic waves alters acetylcholinesterase gene expression, exploratory and motor coordination-linked behaviour in male rats

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ABSTRACT

Humans in modern society are exposed to an ever-increasing number of electromagnetic fields (EMFs) and some studies have demonstrated that these waves can alter brain function but the mechanism still remains unclear. Hence, this study sought to investigate the effect of 2.5 Ghz band radio-frequency electromagnetic waves (RF-EMF) exposure on cerebral cortex acetylcholinesterase (AChE) activity and their mRNA expression level as well as locomotor function and anxiety-linked behaviour in male rats. Animals were divided into four groups namely; group 1 was control (without exposure), group 2–4 were exposed to 2.5 Ghz radiofrequency waves from an installed WI-FI device for a period of 4, 6 and 8 weeks respectively. The results revealed that WiFi exposure caused a significant increase in anxiety level and affect locomotor function. Furthermore, there was a significant decrease in AChE activity with a concomitant increase in AChE mRNA expression level in WiFi exposed rats when compared with control. In conclusions, these data showed that long term exposure to WiFi may lead to adverse effects such as neurodegenerative diseases as observed by a significant alteration on AChE gene expression and some neurobehavioral parameters associated with brain damage.

1. Introduction

The use of wireless technologies such as Wireless Fidelity (Wi-Fi) communication devices have been growing tremendously over the past years in houses, workplaces, public areas, schools among others. However, rapid development of wireless technologies has steadily increased the environmental electromagnetic field (EMF) levels. Public and scientific awareness that was previously focused on the adverse health effects of EMF emitted from mobile phones has shifted to the biological hazards of wireless equipment such as Wi-Fi because the health effects of such equipment are still unclear [33]. The Council of Europe recommends restrictions on the use of mobile phones and internet access in all schools across the continent to protect young children from potentially harmful radiation [18,24].

Therefore, understanding the relationship between electromagnetic fields and health diseases such as neurological disorder is very important for public especially for young children whom utilize wireless internet very frequently during adolescent years. In addition, uncontrolled wireless internet usage can turn into a habit and may continue throughout ones life being unaware of potential harmful effects of electromagnetic fields [15,2].

Acetylcholine (ACh) is a neurotransmitter with an important role in many functions of both the peripheral and central nervous systems acting in the learning and memory processes as well as locomotor control and cerebral blood flow [8,19,11]. The proximal promoter of the AChE gene includes; among others, consensus motifs for the leukemia-associated factorAML1/Runx1 [17,22], and *c-fos*, a transcription factor known to regulate AChE gene expression under stress [17]. Exposure to radiofrequency can happen from a multitude of sources such as smart devices, phones, office gadgets connected to Wi-Fi, desktops, laptops etc which has been found to cause a wide range of biochemical and physiological dysfunctions such as anxiety, obesity, reduced cognitive function etc. It has been shown that the AChE activity is implicated in cell proliferation and neurite outgrowth [7]. AChE is

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expressed in brain tumors including meningiomas, astrocytomas andglioblastomas [26,4]. Research studies have revealed that AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of central nervous system disorders [7]. Interestingly, AChE levels are found to be very low in all types of normal glia, but increased in astrocytic tumors [30,16]. This hinted the possibility that tumor-specific transcription factors regulate AChE gene expression in astrocytomas. Thus, this enzyme is a target for the emerging therapeutic strategies to treat neurological disorders such as Alzheimer's disease (AD), Parkison disease, hungtinton's disease etc [29].

Due to the significance of AChE in neurological disease and the health implications of WiFi exposure in humans, it is expedient to determine the effect of long-term exposure of this radiation on neurological function. Hence, this study sought to investigate the effect of 2.5 Ghz band radio-frequency electromagnetic waves (RF-EMF) exposure on cerebral cortex acetylcholinesterase (AChE) activity and their mRNA expression level as well as locomotor function and anxiety-linked behaviour in male rats.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents such as: $MgCl_2$, TRIzol Reagent, Taq DNA polymerase (Invitrogen), 5,5′-dithiobisnitrobenzoicacid, acetylthiocholine iodide were obtained from Sigma-Aldrich (USA), while others used were all of analytical grade. The water used was glass-distilled.

2.2. Animal care

Twenty-four (24) male albino rats (four weeks old and nearly of the same weight) were obtained from the animal breeding unit at College of Medicine, Afe-Babalola University. They were acclimatized for a period of two weeks before subjecting them to experimentation. The animals were provided with standard pellet diet (obtained from ABUAD farm) and were given water *ad libitum*. The handling and use of the animals were in accordance with NIH guildline for the care and use of Laboratory animals. This study was approved by the animal ethical committee of Afe-Babalola University, Ado-Ekiti, Nigeria.

2.3. Electromagnetic field exposure

A signal device, which emits Wi-Fi signals at approximately 2.5 GHz frequency band, was used to represent the exposure system. The design and methodology for exposure to radiation was according to [10]. The radiation-generating device was tested at the laboratory of the Physics and Electronics Engineering Department and was able to create electric field densities from 0.1–45.5 V/m while the maximum output power was 2 W. The electric field density was set at 11 V/m. Rats in the control and exposure groups were placed in a Plexiglas cage (55 × 32 × 20 cm). For the RF exposure group, a shield was used to ensure that no other EMF/RF exposure sources from external environment cause interferences. Rats were free to move with no restriction in the cage during the study. The rats in the control and exposure groups lived in the cage under normal circumstances. Rats in the exposure group were subject to 2.5 GHz RF radiation 24 h/d for 4, 6 and 8 weeks respectively. Rats in both groups were kept 50 cm far away from the antenna of the generator. The same experimental conditions were applied to the rats in the control group, except the irradiation. Electromagnetic power density and the electrical field inside the Plexiglas cage were measured by field probe EMR 300 (data not shown).

2.4. Experimental design

After acclimatization, the animals were divided into four groups (n = 6) where the first group (control group) had no exposure while groups 2–4 were exposed to 2.5 Ghz radiofrequency waves from an installed Wi-Fi device at intervals of 4, 6 and 8 weeks with free access to food and water *ad libitum*. After the treatment period, animals were fasted overnight and euthanized using light ether anesthesia. In this study, the cerebral cortex was isolated from the whole brain and analyzed for key enzyme of the cholinergic system.

2.5. Behavioral study

On the final day of the exposure, animals were subjected to neurobehavioral study to assess the locomotor activity and anxiety level of the experimental animals.

2.5.1. Open field test

The analysis of locomotor activity of rats was measured by the open-field test (OFT). The animals were placed in an open field container (4 × 4 × 40 cm dimensions), with its floor divided to 16 equal-sized squares. A video camera was placed on the top of the apparatus at 120 cm heights for video typing. Each animal was placed in the apparatus and after 5 min for habituation; its activity was recorded for 10 min. The types then were analyzed for locomotion (number of line crossing) by an independent trained observer without the knowledge of the experiment.

2.5.2. Rotarod test

The animals were placed on the rotating rod (Rotarod) at 34 rpm to measure passive rotation and time it takes for animal to fall off the rotating rod which is used as an indicator of motor coordination level in animal model.

2.6. Biochemical evaluation

2.6.1. Determination of acetylcholinesterase (AChE) activity

The AChE enzymatic assay was determined using a modification of the spectrophotometric method as previously described by [22]. The reaction medium (2 mL final volume) contained 100 mmol/L of K^+-phosphate buffer, pH 7.5, and 1 mmol/L of 5,5′-dithiobisnitrobenzoic acid. The method is based on the formation of the yellow anion, 5,5′dithio-bis-acid-nitrobenzoic, measured by absorbance at 412 nm during 2 min incubation at 25 °C. The enzyme (40–50 μg of protein) was pre-incubated for 2 min. The reaction was initiated by adding 0.8 mmol/L of acetylthiocholine iodide. All samples were run in triplicate, and enzyme activity was expressed in μmol./mg protein.

2.6.2. Real-time reverse transcription polymerase chain reaction (RT-qPCR)

The analysis of AChE mRNA expression was carried out by a two-step quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay. Total RNA of the cerebral cortices of experimental animals were extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. The total RNAs extracted were quantified using NanoDrop 2000 spectrophotometer and the ratio of OD28/OD280 of all extracted RNA samples was between 1.8 and 2.0. For reverse transcription (RT), first strand complementary DNA (cDNA) was synthesized from RNA by using a cDNA synthesis kit (Maxima H Minus First Strand cDNA Synthesis Kit) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction was performed in 20 μL reaction volumes containing 2 μL RT product (cDNAs) as template, 1X PCR buffer, 25 μM dNTPs, 0.2 μM of each primer (Table 1), 1.5 mM $MgCl_2$, 0.1X SYBR Green I (molecular probes), and 1U Taq DNA polymerase (Invitrogen).The thermal cycle was carried out using a Step One Plus real-time PCR system (Applied Biosystems, NY) according to the following protocol: activation of the Taq DNA polymerase at 95 °C for 5 min, followed by 40 cycles of 15 s at 95 °C, 15 s at 8 °C, and 25 s at 72 °C. Threshold and baselines were