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Postnatal development and behavior effects of *in-utero* exposure of rats to radiofrequency waves emitted from conventional WiFi devices

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ABSTRACT

The present work investigated the effects of prenatal exposure to radiofrequency waves of conventional WiFi devices on postnatal development and behavior of rat offspring. Ten Wistar albino pregnant rats were randomly assigned to two groups (n = 5). The experimental group was exposed to a 2.45 GHz WiFi signal for 2 h a day throughout gestation period. Control females were subjected to the same conditions as treated group without applying WiFi radiations.

After delivery, the offspring was tested for physical and neurodevelopment during its 17 postnatal days (PND), then for anxiety (PND 28) and motricity (PND 40-43), as well as for cerebral oxidative stress response and cholinesterase activity in brain and serum (PND 28 and 43). Our main results showed that the *in-utero* WiFi exposure impaired offspring neurodevelopment during the first seventeen postnatal days without altering emotional and motor behavior at adult age. Besides, prenatal WiFi exposure induced cerebral oxidative stress imbalance (increase in malondialdehyde level (MDA) and hydrogen peroxide (H_2O_2) levels and decrease in catalase (CAT) and superoxide dismutase (SOD) activities) at 28 but not 43 days old, also the exposure affected acethylcolinesterase activity at both cerebral and seric levels. Thus, the current study revealed that maternal exposure to WiFi radiofrequencies led to various adverse neurological effects in the offspring by affecting neurodevelopment, cerebral stress equilibrium and cholinesterase activity.

1. Introduction

Due to the tremendous proliferation of man–made electromagnetic sources marking the second half of the 20th century, the world underwent an electromagnetic revolution which had drastically increased the natural electromagnetic background which probably cause many health issues.

Although authorities on the subject had set safety rules to protect the community against radiofrequency fields (RF) exposure (Litvak et al., 2002; Leszczynski and Xu, 2010), these non-ionizing radiations (frequency ranging from 30 kHz to 300 MHz), remain a worrying part of the electromagnetic spectrum due to their daily uses accompanied to the recent classification as 2B agents ('possibly carcinogenic to humans') by the International Agency for Research in Cancer (IARC) (Baan et al., 2011).

Whereas in the past decade, microwave ovens and radar equipment were the main topic of most studies, it is now wireless technologies, especially Wireless Fidelity (WiFi) devices that attract special attention. Indeed, this emerging technology, operating at 2.45 GHz, has recently seen a large scale-expansion and become ubiquitous in daily life (Brunel, 2004; Dasdag et al., 2015) which raised public and scientific concern regarding its health hazardous effects. The environmental wireless 802.11.g device (also called WiFi device, or wireless internet access device or WIAD) has in general higher frequency ranges and longer exposure times than wireless phones (Viel et al., 2009).

Prenatal life is a critical development stage given the high sensitivity of developing tissues to noxious environmental agents (Dietert and Piepenbrink, 2008), especially RF radiations. Indeed, tissues absorption

Abbreviations: DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase; GSM, Global System of Mobile; H_2O_2, hydrogen peroxide; IARC, International Agency for Research in cancer; MDA, malondialdehyde level; PBS, phosphate buffered saline; PND, postnatal days; pSAPs, protectedstretched attend postures; RF, radiofrequency fields; ROS, reactive oxygen species; rpm, revolution per minute; S.E.M, standard error of the mean; SAR, specific absorption rate; −SH, sulfydryl; SOD, superoxide dismutase; WHO, World Health Organization; WIAD, wireless internet access device; WiFi, Wireless Fidelity

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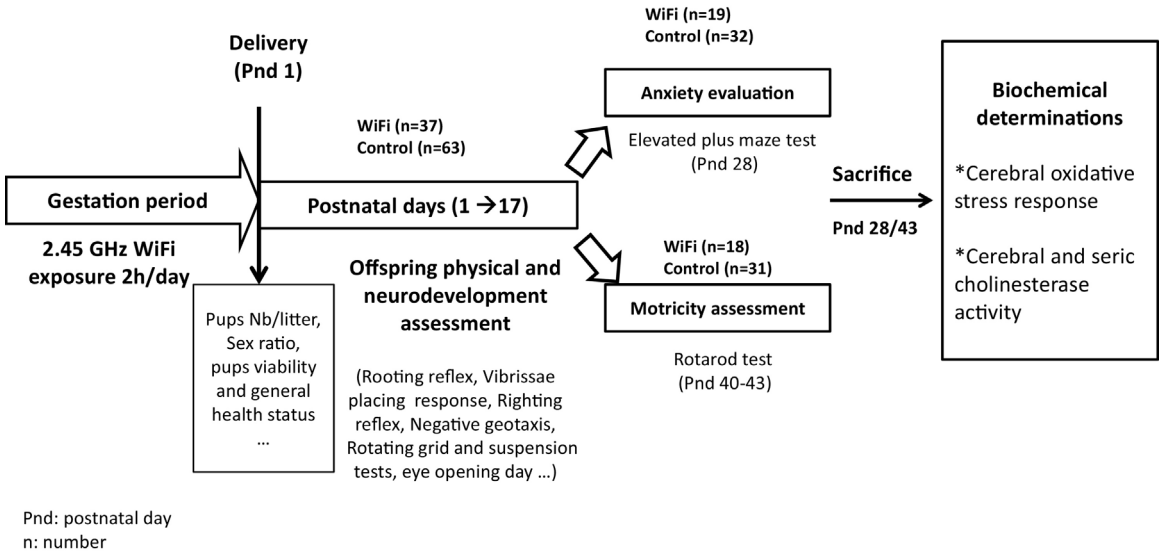

Fig. 1. Experimental design: Treatment and behavioural testing protocols.

rate of electromagnetic radiations depends on dielectric properties and organ conductivity. Since body water content is markedly important during gestation, pregnant mothers and their fetuses are hypersensitive to electromagnetic radiations (Narayanan et al., 2013).

Amongst many WiFi signal biological targets, nervous system has received the greatest attention given its great cellular diversity, organizational complexity and electrical nature (Altunkaynak et al., 2016; Kaplan et al., 2016). Whether 2.4 GHz Wi-Fi signal exposure may impact nervous system has been investigated mainly in adult animals (Cassel et al., 2004; Crouzier et al., 2007). Nevertheless, the neurological effects associated with WiFi exposure occurring early in life or even during gestation period still not well studied (Orendácová et al., 2009; Orendáčová et al., 2010; Aït-Aïssa et al., 2012; Poulletier de Gannes et al., 2012; Aït-Aïssa et al., 2013).

The current investigation was undertaken to assess, in rats, the non-thermal effects of daily two-hour *in utero* exposure to a 2.45 GHz Wi-Fi signal till delivery on offspring neurodevelopment, emotional and motor behavior as well as cerebral oxidative stress response and cholinesterase activity.

2. Materials and methods

2.1. Animals

Animals were used along this study in accordance with the Tunisian Code of Practice for the Care and Use of Animals for Scientific purposes and the protocol was approved by the Ethic Committee of Carthage University, Tunisia. All measures were taken to minimize animal pain and discomfort.

Albino Wistar rats (SIPHAT, Ben Arous, Tunisia) were housed in the animal facility (Faculty of Sciences of Bizerte) in standard cages under controlled physical conditions and 12:12-h light–dark cycle, with free access to food (standard rat pellet) and tap water *ad libitum* (except during WiFi exposure). Animals were kept some days before the start of the experiment for acclimatization.

Primiparous females were met with unexposed male rats of the same strain (2:1) and vaginal smear was microscopically examined each evening. The day 1 of pregnancy was confirmed by the presence of both typical oestrous stage vaginal cells and spermatozoids. Pregnant dams were then randomly assigned to two sets: control and experimental groups with 5 rats per each.

2.2. Exposure system

The WiFi signal was picked up directly by a commercial Access Point for use indoors (D-Link DWL-3200 AP with 802.11 g mode and WPA2 net-work protection) as previously described (Saili et al., 2015; Othman et al., 2017). The device supports wireless networking speeds of up to 108Mbps (Turbo mode) on the popular 2.4 GHz public frequency.

Exposed group was placed at a distance of 25 cm from the Antennas of WiFi modem (Saili et al., 2015) and received WiFi radiations for 2 h a day along gestation while the control set was subjected to the same conditions without applying Wi-Fi signal (0 Hz).

2.3. Offspring development

On delivery, the number of pups per litter, sex ratio (expressed as the percentage of males and females among the total number of pups) and mortality at birth were noted. The day after, pups were identified with tail-marks and weighed daily for 17 days.

2.4. Observation of physical and reflex development of rat pups

Sensorimotor development of all pups was tested daily according to the method as previously described (Fox, 1965; Wayner, 1976) between 10 am and 12 am along the first 17 days of age. Tests of the functional development of offspring included response to rooting reflex, vibrissae placing response, righting reflex, negative geotaxis, suspension test and rotating grid (Fig. 1). The age when both eyes were fully opened was noted. The details of these tests have been previously described in (Chehimi et al., 2012).

2.4.1. Rooting reflex

The experimenter induced the pup to crawl forwards, pushing its head in a rooting fashion when he bilaterally stimulated the pup's snout between its thumb and its forefinger. The number of pups producing this reflex was noted every day from day 1 to day 17.

2.4.2. Vibrissae placing response

When a pup is handled by its tail and its vibrissae are touched with a pencil, it raises its head and extends its forelimbs in order to grasp the object. The number of animals eliciting this reflex was noted every day from day 1 to day 17.