



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 (H₂O₂) levels and decrease in catalase (CAT) and superoxide dismutase (SOD) activities) at 28 but not 43 days old, also the exposure affected acetylcholinesterase 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; H₂O₂, 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, sulfhydryl; SOD, superoxide dismutase; WHO, World Health Organization; WIAD, wireless internet access device; WiFi, Wireless Fidelity

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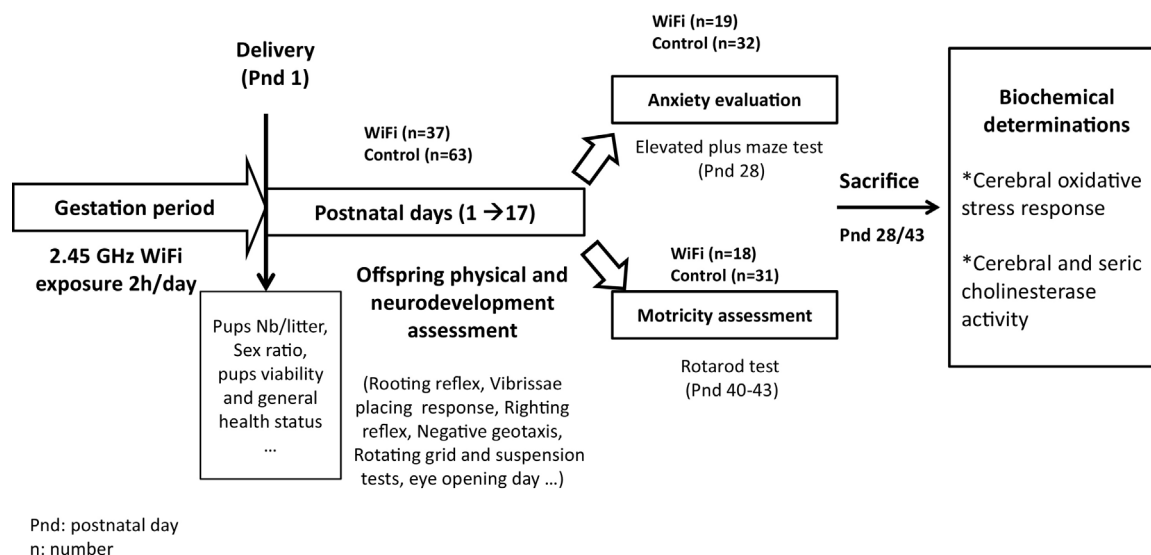


Fig. 1. Experimental design: Treatment and behavioural testing protocols.

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 (Orendáčová et al., 2009; Orendáčová et al., 2010; Aït-Aïssa et al., 2012; Pouletier 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 spermatozooids. 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.

2.4.3. Righting reflex

When a neonate is placed in the supine position, it immediately turns over in a righting reflex to restore its normal prone position. The pups were placed supine on a plane surface and then no longer restrained. The time needed to recover a normal prone position, the four paws being in contact with the plane surface, was noted in two consecutive trials with a cutoff measure of 60 s. The test was performed each 24 h from birth till day 6.

2.4.4. Negative geotaxis

Negative geotaxis is a postural reaction bringing the animal in the upright position when it was placed downwards. In our experiment, the pups were placed on a 20° tilted plane, their snout being downwards. The animals had to turn for 180° in order to bring their snout upwards. The time needed by the animal to achieve a 180° rotation was noted during a trial of 180 s. The test was performed each 24 h from birth till day 8.

2.4.5. Rotating grid

This test measures the ability of a pup to cling to a wire tray which stimulates its palm when it is turned over in a state of progressive imbalance. The rotating grid, placed at a height of 10 cm from the floor, measured 10 cm x 7 cm and rotated 360° at a speed of 1 rpm, whose meshes have 4 mm on side, and the wire a diameter of 1 mm. During the rotating grid test, the rat was placed horizontally on the grid, care being taken that the body was in a stable position before turning on the motor. While the grid rotated 360°, the angle of rotation reached before the animal fell was measured. The test was performed each 24 h from birth till day 10.

2.4.6. Suspension test

We used this test before the opening of the eyes. The horizontal wire (diameter: 1 mm, length: 25 cm) was suspended between two wooden poles at a height of 20 cm from a cushioned floor, to protect the rat from injury during falls. During the wire suspension test, the front paws of the rat were placed on the horizontal wire. The time taken before the animal fell was measured (cut-off: 60 s). The test was performed each 24 h from birth till day 17.

2.4.7. Eye opening

The age when both eyes were fully opened was noted.

2.5. Post-weaning tests

For post weaning tests, the offspring was distributed into two groups to undergo elevated plus maze and rotarod tests, respectively.

2.5.1. Elevated plus-maze

The aim of this test was to identify more general effects of WiFi exposure on anxiety-like behaviours in rat progeny on postnatal day 28. The test and procedure were as following described (Frye et al., 2000; Maaroufi et al., 2009). The maze was made of clear painted wood, the arms were 50 cm long and 10 cm width and the apparatus was elevated at a height of 50 cm. The closed arms were surrounded by a 50 cm wall while open arms had 0.5 cm edges. Time and number of entries in the different parts of the maze and numbers of rears were recorded as standard measures. A four-paw criterion validated an entrance into one arm and the animal was considered out of the arm when two paws had left the arm. Ethological measures included protected (i.e. closed arm) stretched attend postures (pSAPs), closed arm returns (the rat enters the central part with two paws and then goes back into the same closed arm), head scans (the rat points its head into an open arm with its body still in the centre or in a closed arm of the maze) and head dipping (Maaroufi et al., 2009).

2.5.2. Rotarod

Acquisition of the rotarod task was evaluated on postnatal days 40–43. The rotarod consisted of a wooden beam (diameter 5 cm, length 50 cm), covered by a layer of sticky plaster to ensure firm gripping, which turned around its longitudinal axis at a speed of 30 rpm. The floor under the beam (90 cm high) was covered with cushions to avoid any injury in case of falling. The rats were gently placed on the rotarod with their body axis perpendicular to the beam and in such an orientation that forward locomotion was necessary to avoid falling. They were given two trials of 3 min per day, for 4 days, with an inter trial interval of at least 2 min. Total time spent on the rod before falling as well as walking and suspension time (when the rats clung to the rod and were passively rotated) were recorded. Latencies before falling and time spent walking reflect motor abilities (Maaroufi et al., 2013).

2.6. Serum collection and brain homogenization

The offspring was slaughtered by decapitation at the age of 28 and 43 days following elevated plus maze and rotarod tests, respectively. Trunk blood was collected and centrifuged at 3000 rpm at 4 °C for 15 min in order to get the serum in supernatant fraction.

For tissue processing, the brain was immediately removed from skull and rinsed with sterile physiological saline solution. Only 0.4 g of each brain were homogenized in 4 ml of 50 mM phosphate buffered saline (PBS) (pH 7.4) and centrifuged at 10000 rpm for 10 min at 4 °C. Then, supernatant was aliquoted and stored frozen, as well as serum, at –80 °C until subsequent biochemical measurements (oxidative stress markers in brain and cholinesterase activity in brain and sera).

2.7. Biochemical determinations

2.7.1. Oxidative stress parameters in brain

Lipid peroxidation was determined by malondialdehyde (MDA) measurement according to the double heating method (Draper and Hadley, 1990). Brain H₂O₂ level was assessed according to Dineon et al. (1975). The activity of superoxide dismutase (SOD) was assessed using modified epinephrine assays (Misra and Fridovich, 1972). The catalase (CAT) activity was assayed by measuring the initial rate of H₂O₂ disappearance at 240 nm (Aebi, 1984). Thiol groups (–SH) concentration was measured according to Hu et Dillard method (Hu and Dillard, 1994). Finally, the protein content in brain supernatant was determined by the method of Bradford (Bradford, 1976) using bovine serum albumin (BSA) as standard.

2.7.2. Cholinesterase activities assays

Cholinesterase activities were determined at 25 °C in 0.1 M phosphate buffer (pH = 7.4) with 0.3 mM DTNB and 1.0 mM acetylcholine or butyrylcholine according to Ellman method (Ellman et al., 1961). Acetylcholinesterase (E.C.3.1.1.7) activity was assessed at brain level whereas butyrylcholinesterase (E.C.3.1.1.8) activity was determined in both brain and serum.

2.8. Statistical analysis

The data are expressed as means (± S.E.M.) or as percentages. Statistical analysis were performed by using Khi 2 test (for rooting reflex and vibrissae placing response) and non parametric Mann Whitney test (for the rest of tests) of StatView® software to compare between the means of the different parameters of the control and experimental groups. Results were considered statistically significant when $p \leq 0.05$; highly significant when $p < 0.001$.

Table 1
Delivery data and offspring development after maternal exposure to WiFi signal.

	Dams number	Delivery data						Sex ratio (%)	
		Male offspring number	Female offspring number	Offspring total number	Dead pups number	Alive pups number	Physical abnormalities	Male offspring	Female offspring
Control groups	5	36	27	63	0	63	0	57.14	42.86
WiFi exposed groups	5	21	16	37	0	37	0	56.76	43.24

3. Results

3.1. Delivery data and offspring development

Control and treated dams gave birth on the 21st day of pregnancy. *In-utero* 2.45 GHz WiFi signal irradiation did not adversely affect gestation (no abortion) or pups viability and physical development. Sex ratio, expressed as the percentage of males and females among the total number of pups, was similar in both control and treated group. Only total number of treated dams' pups was reduced by half when compared to control dams' offspring (Table 1).

3.2. Pup body weight evolution

There is no statistically significant differences in offspring body weight gain between radiated and control groups along the first 17 days old.

3.3. Pups neurodevelopment

3.3.1. Rooting reflex

For the first 12 postnatal days, prenatally WiFi-exposed pups responded significantly ($p < 0.05$), less than control pups when their facial regions were stimulated (Fig. 2). Thus, the treatment affected pups motivation to have a feed. Then, from the postnatal day 13, rooting reflex decreased in both treated and control groups but significantly later in prenatally exposed pups than control ones. *In-utero* WiFi exposure seemed to affect pups distinction between experimenter stimulation and mother teat.

3.3.2. Vibrissae placing response

Both *in-utero* WiFi irradiated and control pups exhibited vibrissae placing response improvement with age at a no-significant difference

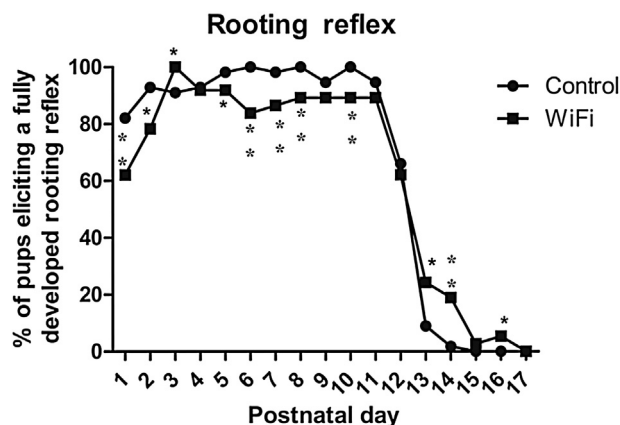


Fig. 2. Effect of in utero Wifi exposure on rooting reflex maturation. Animals were prenatally exposed or not to a 2.4 GHz WiFi signal during gestation and the test was performed each 24 h till day 17. Data are given as mean $\pm$ SEM. * ($p < 0.05$) and ** ($p < 0.001$) compared to control group.

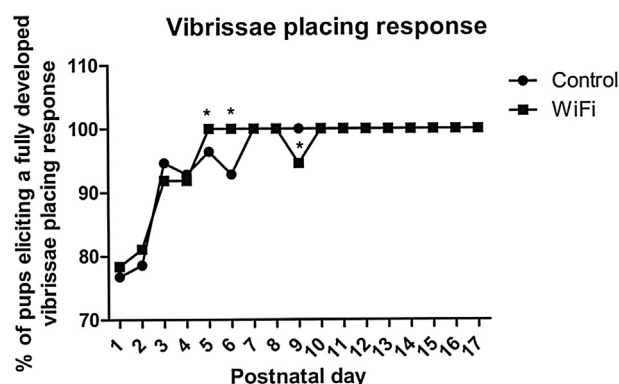


Fig. 3. Effect of in utero WiFi exposure on vibrissae placing response in rats pups. Animals were prenatally exposed or not to a 2.4 GHz WiFi signal during gestation and the test was performed each 24 h till day 17. Data are given as percentage. * ($p < 0.05$) compared to control group.

except some ones (on postnatal days 5, 6 and 9) likely due to chance. Therefore, prenatal WiFi exposure did affect neither offspring vibrissae sensitivity nor motor coordination of the anterior trunk part (Fig. 3).

3.3.3. Righting reflex

Time required for a fully developed righting reflex showed a decrease with age in both treated and control groups. However, throughout test period, prenatally WiFi exposed pups spent significant ($p < 0.05$, $p < 0.001$) more time to turn over to restore their normal prone position when they were placed in a supine position contrary to control (Fig. 4).

Moreover, the average age to fully achieve this reflex was the 4th postnatal day within 20 s for the control mothers' offspring which is in agreement with Gelly investigation (Gelly, 1979). Yet, it is not the case with pups of WiFi-treated mothers (more than 30 s). Vestibular structures, cervical joint receptors and muscle receptors involved in

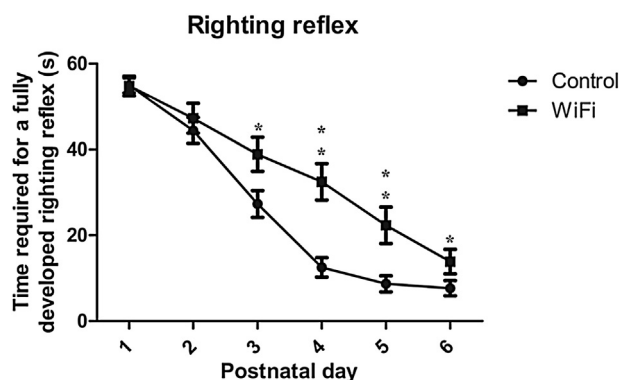


Fig. 4. Effect of in utero exposure to WiFi on righting reflex in rats offspring. Animals were prenatally exposed or not to a 2.4 GHz WiFi signal during gestation and the test was performed each 24 h till day 6. Data are given as mean $\pm$ SEM. * ($p < 0.05$) and ** ($p < 0.001$) compared to control group.

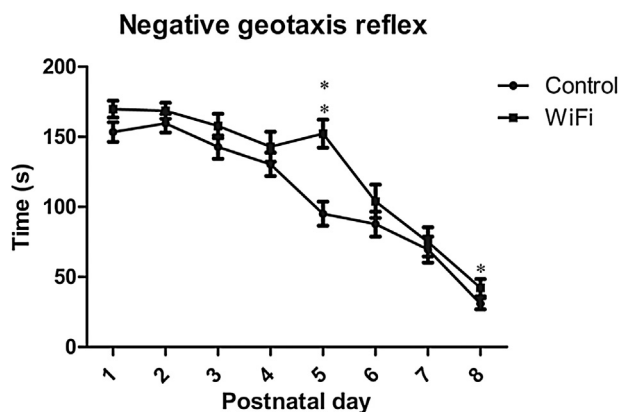


Fig. 5. Effect of in utero Wifi exposure on negative response geotaxis. Animals were exposed or not to a 2.4 GHz Wifi signal during gestation and the test was performed each 24 h till day 8. Data are expressed as mean $\pm$ S.E.M. **($p < 0.01$) and ***($p < 0.001$) compared to control group.

this reflex seemed to be affected by Wifi exposure.

3.3.4. Negative geotaxis

Time taken by control as well as Wifi-treated pups to elicit a fully developed negative geotaxis reflex showed a decrease throughout test period, reflecting performance improvement. Nevertheless, this reflex was delayed in pups prenatally treated with Wifi as compared to control group, with a significant and highly significant differences at 5th ($p < 0.001$) and 8th ($p < 0.05$) PNDs respectively (Fig. 5).

Control pups achieved 180° rotation in less than 30 s at an average age of 8 days which is in agreement with Gelly's study (Gelly, 1979). However, treated pups, made significantly ($p < 0.05$) more time showing affected motor system maturation, particularly anterior paw coordination.

3.3.5. Rotating grid

As shown in Fig. 6, both control and treated pups exhibited enhanced ability to secure on the grid with age. However, throughout test period, the angle reached before pups falling was greatly lower ($p < 0.05$, $p < 0.001$) in Wifi-treated groups of animals than in controls, sign of altered muscle strength.

3.3.6. Suspension test

For the first 14 postnatal days, prenatally Wifi-exposed pups fell significantly ($p < 0.05$, $p < 0.001$) earlier than control groups (Fig. 7). Therefore, Wifi signal decreased muscle strength of pups still blind. Starting from 15th postnatal day, treated pups made more time to

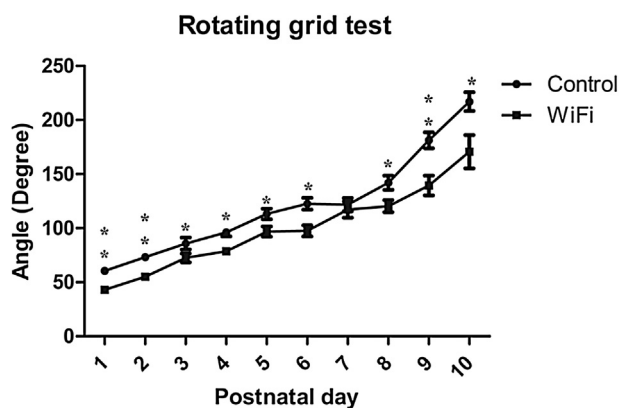


Fig. 6. Effect of in utero Wifi exposure on the angle reached before falling on the rotating grid. Animals were exposed or not to a 2.4 GHz Wifi signal during gestation and the test was performed each 24 h till day 10. Data are expressed as mean $\pm$ S.E.M. *($p < 0.05$) and **($p < 0.001$) compared to control group.

Suspension test

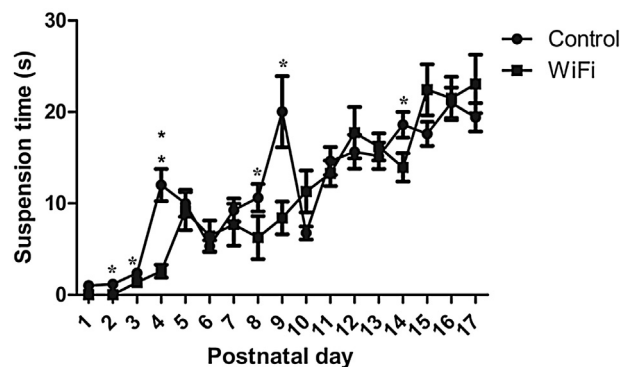


Fig. 7. Effect of in utero Wifi exposure on latencies before falling from horizontal wire. Animals were exposed or not to a 2.4 GHz Wifi signal during gestation and the test was performed each 24 h till day 17. Data are expressed as mean $\pm$ S.E.M. *($p < 0.05$) and **($p < 0.001$) compared to control group.

fall from the horizontal wire than in control group but the difference did not reach significance. Prenatal Wifi exposure is likely to emphasize fear in eye-opened pups.

3.3.7. Eye opening

In treated group (15.81 ± 0.10), pups eyes were opened one day before control group (16.4 ± 0.14).

3.3.8. Elevated plus maze test

Maternal Wifi radiations exposure did not influence emotional behavior of the progeny since no significant effect was observed in the number of entries and time spent in the open arms of the maze ($p > 0.05$) (Table 2).

3.3.9. Rotarod performance test

Rotarod test showed no significant effect on the motricity coordination of the progeny due to Wifi irradiation during pregnancy. Indeed, there was no significant effect ($p > 0.05$) on the total time on the rod as well as walking time in Wifi treated rats compared to their controls (Table 3).

3.4. Oxidative stress response, acetylcholinesterase and butyrylcholinesterase activities

Table 4 showed that repeated Wi-Fi exposure during gestation induced cerebral oxidative stress response in offspring on postnatal

Table 2

Behaviours of control and exposed rats in the elevated plus maze. Animals were prenatally exposed or not to a 2.4 GHz Wifi signal during gestation and the test were performed at 28 days of age. Data are expressed as mean $\pm$ S.E.M. *($p < 0.05$) compared to control group. SAP: Stretched attend postures, s: second.

	Control offspring	Treated offspring
Time in the centre (s)	74.66 $\pm$ 7.12	75.01 $\pm$ 5.13
Time in the open arms (s)	56.5 $\pm$ 7.51	82.23 $\pm$ 11.06
Time in the closed arms (s)	168.84 $\pm$ 11.29	142.76 $\pm$ 13.54
Number of open arms entries	4.96 $\pm$ 0.53	6.31 $\pm$ 0.91
Number of closed arms entries	6.71 $\pm$ 0.45	8.31* $\pm$ 0.67
Number of closed arms reentries	0.54 $\pm$ 0.15	1.31 $\pm$ 0.34
Number of rears	12.63 $\pm$ 1.13	14.94 $\pm$ 1.23
Number of head drips	17 $\pm$ 1.52	23.31* $\pm$ 2.58
Number of SAPs	5.67 $\pm$ 0.44	4.44 $\pm$ 0.79
Stretching into the open arms without entering	10.79 $\pm$ 0.84	13.13* $\pm$ 0.77
Open arms' time (%)	26.14 $\pm$ 3.62	37.37 $\pm$ 5.17
Number of open arms' entries (%)	39.78 $\pm$ 3.34	41.48 $\pm$ 2.78
Total activity	11.67 $\pm$ 0.82	14.63 $\pm$ 1.37

Table 3
Motor behavior of control and treated rat offspring in rotarod test. Animals were prenatally exposed or not to a 2.4 GHz WiFi signal during gestation. The test was performed from 40 to 43 days of age. Data are expressed as mean $\pm$ S.E.M.

	D1			D2			D3			D4		
	Tt(s)	Tr(s)	Ts(s)	Tt(s)	Tr(s)	Ts(s)	Tt(s)	Tr(s)	Ts(s)	Tt(s)	Tr(s)	Ts(s)
Control offspring	60.46 $\pm$ 10.22	48.74 $\pm$ 9.79	11.73 $\pm$ 0.97	114.99 $\pm$ 11.40	106.13 $\pm$ 11.66	8.86 $\pm$ 1.37	130.36 $\pm$ 11.16	120.58 $\pm$ 11.45	9.77 $\pm$ 1.58	140.10 $\pm$ 10.18	129.59 $\pm$ 10.59	10.51 $\pm$ 2.21
WiFi- treated offspring	34.57 $\pm$ 7.98	25.42 $\pm$ 7.88	9.15 $\pm$ 0.88	86.57 $\pm$ 13.57	77.48 $\pm$ 13.92	9.10 $\pm$ 1.11	124.73 $\pm$ 14.76	117.54 $\pm$ 15.09	7.18 $\pm$ 1.19	124.91 $\pm$ 13.40	114.02 $\pm$ 14.44	10.88 $\pm$ 2.04

D: Day. Tt: Total time on the rod; Tr: Turning time; Ts: suspension time; s: second.

day 28 by significantly increasing MDA and H_2O_2 levels ($p < 0.001$) and significantly decreasing SOD and CAT activities ($p < 0.05$) compared with control offspring. However, WiFi exposure did not affect SH levels ($p > 0.05$).

However, the oxidative stress response seems to be reversible on postnatal day 43. Indeed, there was no significant difference between control and treated offspring neither in prooxidant factors (except H_2O_2 level), all antioxidant enzymes activities were restored to near their control levels.

As can be seen in Fig. 8, cerebral acetylcholinesterase activity was significantly increased on postnatal days 28 and 43 in exposed offspring. Seric butyrylcholinesterase activity was decreased in 43 day aged exposed progeny. Such alteration in cholinesterase activities may affect corresponding neurotransmitters action and then, behavior.

4. Discussion

The present study was aimed at examining the effects of the in utero exposure to WiFi signal (2 h per day throughout gestation period) on offspring neurodevelopment, emotional and motor behavior as well as cerebral oxidative stress response and cholinesterase activity. The main results revealed that prenatal WiFi exposure did not affect delivery endpoints and pups physical development. Behaviorally, exposed offspring exhibited neurodevelopment impairments at the first 17 PNDs but no alteration in the emotional and motor behavior at 28 and 40 till 43 days old. Furthermore, gestational WiFi irradiation disrupted cerebral oxidative stress equilibrium only on PND 28, raised cerebral acetylcholinesterase activity on PNDs 28 and 43 and decreased seric butyrylcholinesterase activity on PND 43.

The current results showed no deleterious effects of prenatal exposure to WiFi radiations on gestation delivery outcomes and physical post development of pups. These findings are in agreement with the study of Pouletier de Gannes et al. (2012) reporting no signs of teratology during pre- or postnatal development following the *in-utero* exposure to a 2.45 GHz Wi-Fi signal even at a high specific absorption rate (SAR) of 4 W/kg. Likewise, according to Ogawa et al. (2009), gestational exposure to wideband code division multiple access (W-CDMA) signal at a 1.95 GHz did not induce embryotoxicity or affect offspring viability, sex ratio and physical development in rat.

However, our data are in contrast with other previous investigations proving negative impact of gestational radiofrequencies exposure. Indeed, prenatal exposure to 2.45 GHz signal triggered postnatal growth restriction in rat (Dasdag et al., 2012; Sangun et al., 2014). Detrimental effects on pre- and post development were also linked to 2.45 GHz signal when irradiation occurred only before gestation (mating period) (Singh et al., 2009) or both before and during pregnancy (Shahin et al., 2013). Moreover, intrauterine exposure to Global System of Mobile (GSM) radiations at a frequency varying from 850 to 1 800 MHz was shown to be harmful for pregnant dams and their newborns in term of embryogenesis pre and post development in rabbit (Budak et al., 2009), rat (Alchalabi et al., 2016), mouse (Yang et al., 2013) and avian species (Al-Qudsi and Azzouz, 2012; Tsybulin et al., 2013). This conflict in results regarding the effects related to radiofrequencies exposure could be explained by the variety of experimental designs and methods as frequency, power density, radiation sources, head only/whole body exposure, exposure duration and used testing apparatus and methods.

For pups' neurodevelopment, we noticed sensorimotor impairments in all performed tests except vibrissae placing response. Such neurodevelopment disorders could be due to several RF-induced effects at cellular, biochemical and electrophysiological levels. Indeed, prenatally exposed rodent offspring to RF radiation exhibited apoptosis and neurodegeneration of Purkinje neurons and granule cells in the cerebellum (Kokturk et al., 2013), loss of pyramidal cells and glia fibrillary acidic protein (GFAP) overexpression in the hippocampus (Stasinopoulou et al., 2016), a decrease in neuronal excitability of

Table 4

Effect of in utero Wifi exposure on pro- and anti-oxidant status on cerebral level in rats offspring. Animals were prenatally exposed or not to a 2.4 GHz Wifi signal throughout gestation period and brains were harvested at the age of 28 and 45 days old. Data are expressed as mean $\pm$ S.E.M. *($p < 0.05$) and ** ($p < 0.001$) compared to control group.

Postnatal day		28		43	
		Control offspring	Treated offspring	Control offspring	Treated offspring
Pro-oxidant status	MDA level (nmol/mg proteins)	0.15 $\pm$ 0.05	0.80** $\pm$ 0.09	0.27 $\pm$ 0.06	0.29 $\pm$ 0.06
	H2O2 level (nmol/min/mg proteins)	0.003 $\pm$ 8×10^{-4}	0.022* $\pm$ 5×10^{-3}	0.003 $\pm$ 4×10^{-4}	0.038** $\pm$ 6×10^{-3}
Anti-oxidant status	SOD concentration (U/ml)	6.79 $\pm$ 1.23	2.43* $\pm$ 0.73	7.18 $\pm$ 1.45	7.14 $\pm$ 1.98
	CAT activity (nmol/min/mg proteins)	0.05 $\pm$ 6×10^{-4}	0.04* $\pm$ 3×10^{-3}	0.05 $\pm$ 2×10^{-3}	0.04 $\pm$ 2×10^{-3}
	SH level (mM)	0.41 $\pm$ 0.02	0.41 $\pm$ 0.02	0.54 $\pm$ 0.08	0.35 $\pm$ 0.04

MDA: Malondialdehyde, H₂O₂: Hydrogen peroxide, SH: Thiol, SOD: Superoxide dismutase, CAT: Catalase, U: Unit.

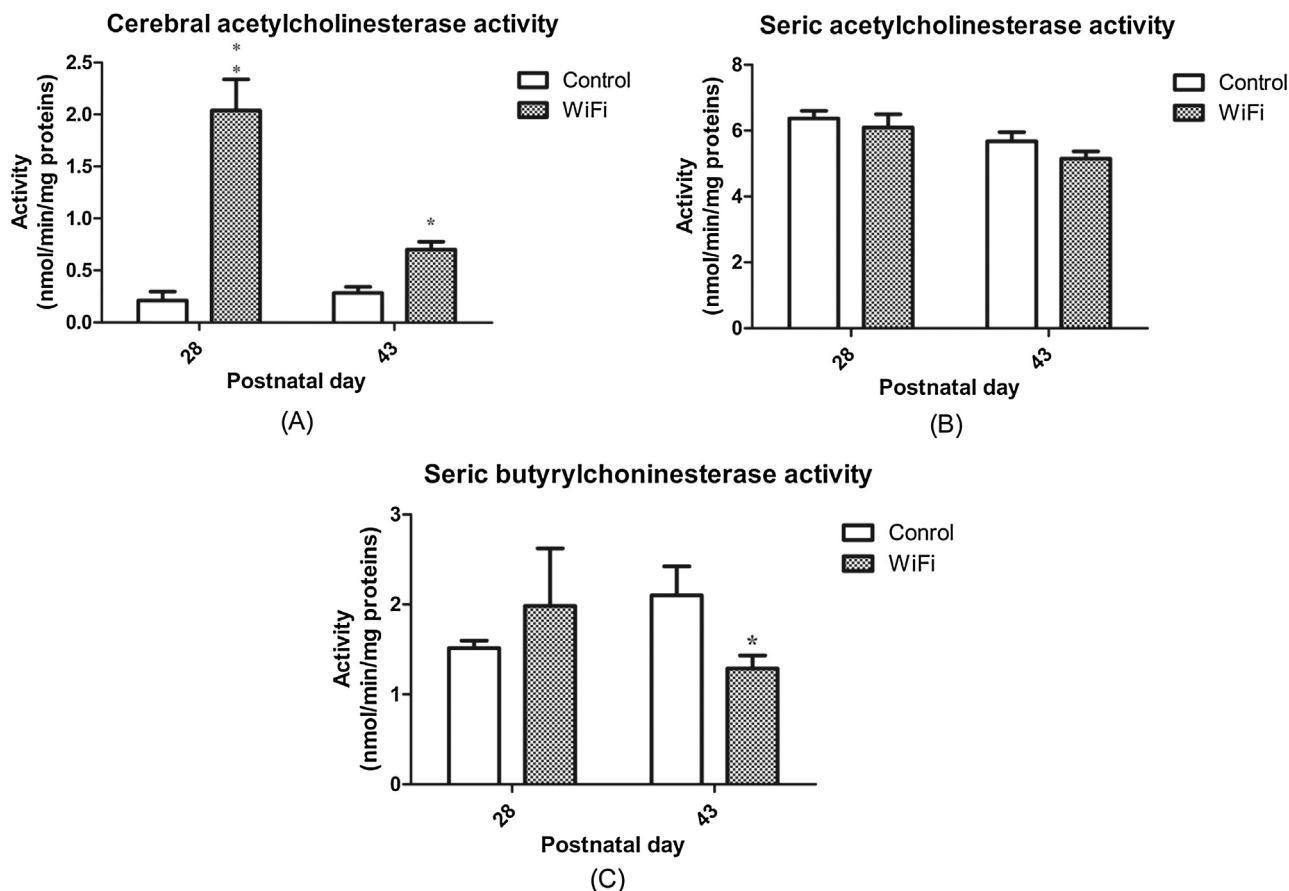


Fig. 8. Effects of in utero 2.45 GHz WiFi signal exposure on cerebral (A) and seric (B) acetylcholinesterase activity and seric butyrylcholinesterase activity (C) in rat offspring at the age of 28 days. Data are given as mean $\pm$ S.E.M. *($p < 0.05$) **($p < 0.001$) compared to control group.

Purkinje neurons (Haghani et al., 2013) and an impairment of glutamatergic synaptic transmission onto pyramidal cells in the pre-frontal cortex (Aldad et al., 2012). Furthermore, in vitro investigation showed that RF exposure inhibited neurite outgrowth in embryonic neural stem cells differentiated neurons (Chen et al., 2014). Moreover, Fragopoulou et al. (2012) reported a transient ossification defect in mouse foetuses following mild exposure to GSM 900 MHz radiations during pregnancy, which may explain, at least in part, the neurodevelopmental alteration found in our study.

Concerning progeny behavior at adult age, our study held that prenatal WiFi exposure did not affect emotional behaviour on PND 28, nor motor coordination at 40–43 days of age. We suggest (one of) two reasons to explain this finding. Already detected neurodevelopment disorders might be not persisting into adulthood. In this vein, Bornhausen and Scheingraber (2000) demonstrated that continuous exposure of pregnant rats to GSM 900 MHz did not induce any measurable cognitive deficits in the offspring tested at the adult stage.

On the other hand, adult offspring might display behavior disorders that could not be noticed in our investigation due to either the chosen behavioral tests or the non distinction between genders while testing. Indeed, mouse fetal exposure to GSM 800–1900 MHz radiations elicited memory impairment, hyperactivity and anxiety decrease at adulthood (Aldad et al., 2012). In addition, the exposure of mice to 9.417 GHz microwaves during pregnancy increased anxiety and decreased depression-related behavior in both male and female offspring and impaired learning and memory in only males tested from 5 weeks old (Zhang et al., 2015).

Prenatal WiFi exposure impact on neurodevelopment as well as behavior could be due to an effect at biochemical level. Biochemical analysis in the offspring brain on PND 28 showed that prenatal exposure to WiFi 2.45 GHz signal induced numerous indicators of oxidative stress response. This included a decrement of the activity levels of antioxidants such as CAT and SOD and an increase in the hydrogen peroxide level as well as lipid peroxidation biomarker. This

redox imbalance showed to be reversible on PND 43 probably due to the defending role of thiol groups which were not affected by RF prenatal exposure neither on PND 28 nor 43. Besides, the delay after the treatment (43 days) may perhaps explain the oxidative equilibrium recovery.

These results are in agreement with previous studies concluding a causative link between radiofrequencies emitted from wireless technologies and oxidative brain damage see reviews (Dasdag et al., 2015; Kaplan et al., 2016). According to Celik et al. (2016), 2.45 GHz Wi-Fi signal exposure from pregnancy to 3 weeks old (1 h/day for 5 days/week) augmented lipid peroxidation levels and decreased glutathione peroxidase (GSH-Px), Glutathione (GSH) activity as well as antioxidant vitamin concentrations (vitamin A, E and C) and beta-carotene in the brain and the liver of developing rats. In this vein, the 900 MHz and 1800 MHz radiations exposure during pregnancy and neonatal development caused oxidative brain and liver injury in growing rats. Gestational exposure to GSM 1800 MHz induced oxidative stress response in pregnant dams of rat (Alchalabi et al., 2016).

Intra-uterine stage is very sensitive to electromagnetic radiations. Indeed, apart from the prominent water content in cells, the fetus is contained within the amniotic fluid. Thus, once exposed to radiations, water decomposition occurs releasing ROS that yield oxidative injury particularly in the brain of newborns (Naziroğlu and Gümrall, 2009; Selaković et al., 2013). Indeed, the brain is lowly protected, especially against radiations; given skull thinness during development (Cetin et al., 2014), high oxygen consumption, paucity of antioxidant system (Halliwell, 2006) and the important level of polyunsaturated fatty acids that are amongst the main targets of ROS (Ozmen et al., 2007).

As for cholinesterase activity, prenatal exposure to 2.45 GHz Wi-Fi signal increased cerebral acetylcholinesterase activity on both PNDs 28 and 43 and diminished seric butyrylcholinesterase activity at 43 days of old. Previously, it has been demonstrated that exposure to radiofrequencies induced changes in different cholinergic parameters such as acetylcholine concentration (Modak et al., 1981) and release (Testylier et al., 2002), choline uptake (Lai et al., 1987; Lai et al., 1989) and acetylcholinesterase activity (Dutta et al., 1992; Ahmed et al., 2006; Valbonesi et al., 2016). Such alteration of neurotransmitter system may influence behavior. Indeed, the hippocampus was revealed to be injured by long-term microwave exposure resulting in cognitive function impairment as a consequence of neurotransmitter disruption (Zhao et al., 2012).

5. Conclusion

In summary, the present investigation revealed that maternal exposure to radiofrequency (Wi-Fi 2.45 GHz signal) impaired the neurodevelopment of rat offspring aged 1–17 days but had no adverse effects on its behavior (emotional and motor behavior) on postnatal days 28 and 43. Furthermore, gestational Wi-Fi exposure induced oxidative damage in brain tissue of the progeny on postnatal day 28 and affected cholinesterase activity at both cerebral and seric levels. Ultimately, the study held that Wi-Fi signal is not completely safe at home near the animal or human body. Furthermore, human being and animal studies are needed to answer the question of whether radiofrequency waves emitted from Wi-Fi devices placed so close have long term bioeffects.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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