Effect Of Wifi Signal Exposure In Utero And Early Life On Neurodevelopment And Behaviors Of Rats

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Research Article

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Abstract

Objectives: To examine the long-term effects of prenatal and early life WIFI signal exposure on neurodevelopment and behaviors as well as biochemical alterations of Wista rats.

Methods: On the first day of pregnancy (E0), expectant rats were allocated into two groups: the control group (n=12) and the WiFi-exposed group (WiFi group, n=12). WiFi group was exposed to turn on WiFi for 24h/day from E0 to postnatal day (PND) 42. The control group was exposed to turn off WiFi at the same time. On PND7-42, we evaluated the development and behavior of the offspring, including body weight, pain threshold, and swimming ability, spatial learning and memory among others. Also, levels of proteins involved in apoptosis were analyzed histologically in the hippocampus in response to oxidative stress.

Results: The results showed that WiFi signal exposure in utero and early life (1) increased the body weight of WiFi+M group, (2) no change in neuro-behavioral development were observed in WiFi group, (3) increased learning and memory function in WiFi+M group, (4) enhanced comparative levels of BDNF and p-CREB proteins in the hippocampus of WiFi+M group.

Significance: Prenatal WiFi exposure has no effects on hippocampal CA1 neurons, oxidative equilibrium in brain and neurodevelopment of rats. Some effects of prenatal WiFi exposure are sex-dependent. Prenatal WiFi exposure increased the body weight, improved the spatial memory and learning function and induced behavioral hyperactivity of male rats.

1. Introduction

As the economy and society develop, Wireless Fidelity (WiFi) communication services are extensively used in household, industrial, military, medical, and scientific setting in recent years. The increase in exposure to the WiFi wireless communication signal has posed major concerns with regards to its effects on human health (Othman et al., 2017a, At-Assa et al., 2013).

Potentially harmful effects of WiFi exposure have been studied on various tissues and body systems (Redmayne et al., 2013). Studies have suggested that continuous RF radiation exposure could have an effect on human health and lead to disorders like headaches, cancer, anemia, among other health problems (Othman et al., 2017b, Redmayne et al., 2013, Pallarés et al., 2013, Aït-Aïssa et al., 2012). One research revealed that in rats, WiFi radiation (2.45 GHz) can cause a reduction in sperm parameters and an upsurge in apoptosis-positive cells in the seminiferous tubules (Mortazavi et al., 2013, Le Quément C, 2012) Saili et aldemonstrated that acute WiFi signal exposure (2.45 GHz) can affect the cardiovascular system (heart rhythm and blood pressure) in mature male rabbits. Review studies raised a degree of scientific uncertainty of the risk of radiofrequency (RF) transmissions to human health and suggested taking precautions, especially in children. Among several possible biological targets, the effect of WiFi signal on the nervous system has received a special focus because of its immense cellular diversity, electrical nature and organizational complexity (Shokri et al., 2015). The memory performance for a year was inversely related to the collective duration of wireless phone use and more significantly to RF-EMF

dose (Schoeni et al., 2015). The implication of this is that RF-EMF exposure influences memory function (Zhijian et al., 2013). Other studies have reported some beneficiary effects of microwave irradiation. They announced beneficial cognitive effects of RF radiation in Alzheimer's disease (Mortazavi et al., 2013, Banaceur et al., 2013), But the evidence for this remains indistinct.

Currently, the effects of 2.4 GHz WiFi signal exposure on the nervous system have mostly been studied in adult animals, and few in utero and postnatal exposure studies have been carried out. The embryonic period is considered a critical phase in the development and the nervous system and immature brain plasticity towards environmental factors recognized them as possible RF field targets. However, the neurological effects of WiFi exposure early in life, particularly during pregnancy have not been extensively studied (Ö et al., 2016, Altunkaynak et al., 2015). There is limited data with regards to the long-term WiFi effects on the physiology of the brain. Whether WiFi exposure has positive, negative or no effects on neurodevelopment and behaviors is not clear.

Given the progressive increase in the 2.45 GHz wireless networks, concerns should be raised concerning the effects of continuous and long-term whole-body exposure to these high WiFi network frequencies (Shokri et al., 2015). Therefore, we explored the long-term effects of prenatal and early life exposure to 2.54 GHz WiFi signals on neurodevelopment and behaviors as well as biochemical alterations of Wista rats.

2. Materials And Methods

2.1. Animals

Adult Wistar rats (both sexes) were procured from Harbin Medical University (Harbin, China). Both female (weight 240–280g) and male (weight 280–320g) rats were used for the experiments. The feeding method and first day of pregnancy (E0) determination were conducted as described previously. Approval for animal procedures was provided by the Experimental Animal Ethics Committee, Daqing Campus of Harbin Medical University.

2.2. Experimental design

On E0, the gravid rats were separated into 2 groups: the control group (n = 12) and the WiFi-exposed group (WiFi group, n = 12). Separate housing was provided for every pregnant rat. WiFi group was exposed to turn on Wi-Fi for 24h/day for 9 weeks. The Control group was exposed to turn off Wi-Fi for the same time. WiFi device was (802-16e 2005 WiMAX Indoor CPE antennae, model number: WIXFMM-130, China) with a frequency of 2,450MHz (2.45 GHz). The duration of radiation was 24h/day in a 30-cm distance from the antenna to the cages. We test the average electric field intensity is 2.1 v/m, the average power density is 82.32 mv/m^2, average magnetic field intensity is 14.31 mA/m, and there are no differences between the inside and outside of the plastic cage.

The litters born from the control and WiFi group rats were included in the control and WiFi groups, correspondingly. On the PND21, the offspring were weaned then separated into various cages based on sex. We carried out the following experiments on the offsprings.

2.3 Developmental and behavioral assessments

2.3.1 Postnatal growth

Weight increase was observed on PNDs 7, 14, 21 and 28, 35, 42.

2.3.2 Neuro-behavioral development

The pups were tested for behavioral development as described by Schneider and Przewlocki(Schneider and Przewłocki, 2005). This test was performed on each group containing eight animals.

Swimming performance: swimming test was used to evaluate motor development and integration of a coordinated series of reflex responses on PNDs 8, 10, 12, and 14.

Negative geotaxis: The test evaluated, motor development, vestibular function, and activity. These behaviors were assessed on PNDs 8, 10, 12, and 14.

Pain threshold: Pain threshold was determined by the hot plate method on PNDs 9, 11, 13, and 15.

2.3.3. Self-grooming test

Self-grooming test was conducted to assess the repetitive and stereotyped behavior of each rat. The tests were performed as previously described (Kim et al., 2017). Briefly, the rats were individually put into standardized cages under light (40 lux). The cages were empty to avoid digging in the bedding, which is considered as a competing behavior. The rats were familiarized to the cages for five minutes, then timed for ten minutes. An experienced investigator scored the total time spent in grooming during the period.

2.3.4. Open field test

In the present study, open field test was conducted on PND 30 to evaluate the rats' locomotor activity. The test was performed as previously described. Prior to the test, the rats were familiarized with the test box for five minutes. Subsequently, the cumulative distance traveled by each rat plus the movement duration (within a ten-minute session) was recorded using the auto-tracking camera system (YH-OF, YiHong, China).

2.3.5 Morris water maze

This was carried out to determine the spatial learning and memory of rats, as detailed before(Wu et al., 2017). The rats were first trained for 4 consecutive days (i.e., from PND 36 to 39). After the training, an 8cm diameter platform was put at the center of the third quadrant of the water maze, then hidden 1.5cm underneath the surface of the water. Each rat was given four trials (60 s each) daily to locate the platform. The time taken to locate the platform (escape latency) was regarded as index of performance.

On day 5, the platform was eliminated, and the number of times that a rat passed through the circular area (diameter, 10cm) that previously contained the platform within 60s was recorded. And this was taken as the index of spatial memory.

2.4. Histological tests

2.4.1 Tissue preparation

Histological tissue preparation was conducted according to the methods described by Wu et al (Wu et al., 2018). Briefly, on PND 43, rat tissues from each group were perfused in ice-cold saline (0.9%) then in 4%-paraformaldehyde. Subsequently, the brains were removed, then post-fixed using 4% paraformaldehyde, then cryopreserved at 4°C in a 30% w/v sucrose solution. Next, frozen coronal sections (50 μm thick) were generated on slides for staining and immunohistochemistry. All the sections were preserved at − 80°C for further studies.

2.4.2 Nissl staining

The sections were washed with distilled water and immersed for 15 minutes in crystal violet (0.1%) at room temperature (RT), then washed again with distilled water. Subsequently, the sections were dehydrated, then sealed with neutral balsam before taking photomicrographs using a light microscope.

2.4.3. NeuN immunohistochemistry

NeuN immunohistochemistry was carried out as described by our previous report (Wu et al., 2017)to assess mature neurons. Briefly, the sections were incubated with the primary antibody mouse anti-NeuN (1:50, Chemicon International, USA) overnight at 4°C. After rinsing, the sections were incubated with suitable biotinylated antibodies (1:200) for 15 min at 37°C then subjected to 3.3-diaminobenzidine (DAB) exposure for about 2 minutes. Sections were subsequently put into tap water to stop the DAB reaction. Following several washing and dehydration steps, the sections were put in coverslips then visualized using a light microscope and photographed. The control sections were incubated with 10% normal goat plasma as opposed to the primary antibody. All the subsequent sections were incubated as illustrated above. No positive immunoreaction was observed.

2.5. Measurement of superoxide dismutase (SOD), malondialdehyde (MDA)

The contents and enzymatic activity of SOD in the brain of rats from every group were assessed using various commercial assay kits as per the methods described by the manufacturer (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). The level of MDA was expressed in nmol/mg protein. The SOD activity was identified as U/mg protein.

2.6. Western blot assay

This assay was conducted as per the methods described by Wu et al.(Wu et al., 2017). The experiment involved using the hippocampal tissues from different groups. An aliquot of hippocampal tissue was

homogenized in ice-cold RIPA lysis buffer. Then. 30μg protein samples were resolved using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel) electrophoresis and transferred to nitrocellulose membranes. After blocking for 1 hour using 5% skim milk, the membranes were incubated overnight at 4◦C together with the following antibodies: anti-BDNF (1:1000; Cell Signal, USA), anti-CREB (1:500; Cell Signal, USA), anti-Bcl-2 (1:1000; Cell Signal, USA), anti-Caspase3 (1:500; Cell Signal, USA) and mouse anti-GAPDH (1:1000, Zhongshan Jinqiao Biotechnology, China). The next day, the membranes were incubated for 1 hour at RT together with the HRP-labeled goat anti-rabbit/ anti-mouse secondary antibody (1:2000, Santa Cruz, USA). HRP signals were detected using a chemiluminescence imaging machine (Image Quant LAS 4000 minis; Applygen Technologies Inc., China). A scanner was used to digitized the images before they were analyzed by Quantity One software (Bio-Rad Laboratories, USA).

2.7 Statistical analysis

All data were shown as the mean ± SEM. The analyses were completed in SPSS version 18.0 (Beijing Stats Data Mining Co., Ltd.). Statistical analysis was conducted using a two-way analysis of variance (ANOVA) to compare two levels of prenatal status (WiFi versus control) and sex (males versus females) and the means were separated by Bonferroni's post-tests.

3. Results

3.1. Effects of WIFI signals on bodyweight and neuro-behavioral development of offspring

The body weight of offspring was monitored for 6 weeks after birth. As showed in Fig. 1A, the mean body weight of male rats in the WiFi group (WiFi + M) significantly increased compared with male rats in the control group (Con + M) at the P28, P35 and P42 ($p < 0.01$). However, no considerable difference in the mean body weight was found during the entire exposure period between female rats in the WiFi group (WiFi + F) and female rats in the control group (Con + F).

The result of the neuro-behavioral development test indicated no significant differences in the WiFi group relative to the Control group in the following aspects: swimming performance (Fig. 1B), pain threshold (Fig. 1C) and negative geotaxis (Fig. 1D).

3.2. Effects of WiFi signals on the stereotyped and repetitive behaviors of offspring

Self-grooming was used to observe the stereotyped and repetitive behaviors of each group within 10 minutes. No remarkable changes in total times (Fig. 1E) and duration of self-grooming (Fig. 1F) were found between the WiFi group and the Control group.

The results of open field tests showed that for the WiFi + M group, the distance spontaneously traveled was considerably longer than that of the Con + M group ($p < 0.05$), while the WiFi + F group did not differ

significantly from the Con + F group (Fig. 1G). Furthermore, the results of the velocity of movement showed no significant changes between the groups (Fig. 1H).

3.3. Effects of WiFi signals on spatial learning and memory function of offspring

The spatial learning and reference memory of each group were assessed using the Morris water maze test.

In the place navigation test, the escape latency was remarkably shortened in the offspring of the WiFi + M group relative to those of the control rats, especially during sessions 3 ($p<0.01$) and 4 ($p<0.01$). Yet, no remarkable differences were found between the WiFi + F and Con + F group as showed in Fig. 2A and 2B.

Concerning the probe test, the frequency of passing (passing times) of rats in the WiFi + M group showed a marked elevation relative to the Con + M group ($p < 0.01$). While rats in the WiFi + F group exhibited no marked differences relative to those in the Con + F group (Fig. 2C and 2D).

BDNF/CREB signaling pathway is considered to play significant functions in learning and memory processes. Thus, we evaluated the changes in BDNF protein levels and the ratio p-CREB/CREB in the hippocampal tissues of different groups of rats by Western blot analysis.

The BDNF expression in the rats' hippocampal tissues in each group showed that the expression of BDNF was remarkably elevated in relation to rats in the WiFi + M group and the Con + M group ($p < 0.01$), and these effects were comparable between the WiFi + F group and the Con + F group (Fig. 2E).

Results of p-CREB expression in the rats' hippocampal tissues showed that the ratio of p-CREB to total CERB was significantly increased between the WiFi + M group and the Con + M group ($p < 0.01$). These effects were comparable between the WiFi + F group and the Con + F group (Fig. 2F).

3.4 Effects of WiFi signals on neurons in the hippocampus of offspring

We examined whether WiFi signals could affect neurons in the hippocampus of WiFi -exposed rats. We conducted a histological study through Nissl staining to examine neuronal changes in the CA1 region of the hippocampus. Regarding Nissl staining of the control group, a clear neuronal cell outline with a compact structure and abundant cytoplasm and a cell body was observed (Fig. 3A). Neuronal degeneration and loss were not detected in the WiFi group. No remarkable differences were detected with regards to the number of surviving cells in the CA1 region of the hippocampus between the WiFi group and the control group (Fig. 3B).

Similarly, NeuN immunohistochemistry also revealed no differences in mature neurons in the pyramidal cells of the hippocampal CA1 region in the WiFi-treated rats (Fig. 3C). The number of positive cells (dark

brown indicates NeuN-positive cells) in the hippocampal CA1 was no obvious change in the WiFi group relative to the control group (Fig. 3D).

Bax and caspase-3 are key proteins related to neuron damage. To examine the effect of WiFi on hippocampal neurons at the molecular level, the expression of Bax and caspase-3 was tested. The results showed no significant changes in the expression of caspase-3 and Bax proteins between the groups (Fig. 3E and 3F).

3.4 Effects of WiFi signals on brain oxidative stress response of offspring

To further study the effect of WiFi on the brain of offspring. MDA content SOD antioxidant enzyme activity in the rats' hippocampal tissues in each group was measured. MDA level is an important indicator of lipid peroxidation. No statistically significant alteration was found in MDA content in the hippocampal tissues of WiFi rats compared with the Control group (Fig. 4B). Besides, we also tested the activity of the antioxidant enzyme. Figure 4A showed that compared with the Con group, SOD activity in the hippocampus of the WiFi group was not significantly changed.

4. Discussion

Internet access is currently considered a must have in daily routines and therefore has been installed in almost all communication gadgets (Zhi et al., 2018 , Nazıroğlu et al., 2013) . Consequently, continuous exposure to Wi-Fi has become a very common risk factor for poor health (Foster and Moulder, 2015 , At-Assa et al., 2013 , Aït-Aïssa et al., 2012).

The present study investigated the effects of a 2.54 GHz WIFI signal exposure during prenatal and early life (24 h/day for 9 consecutive weeks) on rat neurodevelopment, behaviors and cognition as well as biochemical indexes alterations.

Our study found that WiFi exposure did not affect offspring physical and functional development. These results agree with a study by Poulletier et al. , 2012. Behaviorally, exposed offspring exhibited no alteration in motor and emotional behavior. Contrarily, some studies have revealed that exposure to WiFi radio frequencies during pregnancy could affect neurological functions of offspring (Othman et al., 2017b , Othman et al., 2017a) . However, this could have been due to the high radiation dosage tested in these studies. Herein, we revealed that in most tests, the effect of WiFi treatment was dependent on the sex of the offspring, and this was consistent with the findings of Zhang et al; however, its mechanism of action remains unclear.

EMF exposure has been shown to have contradictory effects on the cognitive functions of animals including humans. Dubreuil et al. revealed that RF expose can reduce performance in rodents, particularly in tasks that require spatial memory (Dubreuil et al., 2003) . Contrarily, Kumin et al. demonstrated that exposure to a signal of 900 MHz frequency for five weeks (i.e., 2 hours a day, five days per week, SAR 3

W/kg) can significantly improve memory and learning abilities of young rats (Kumlin et al., 2007). Herein, we observed that prenatal WiFi exposure can enhance cognitive ability. Several studies have shown that protein synthesis occurs in neuronal dendrites and might be the cellular basis of memory and learning. Currently, it's not known whether microwave radiation affects protein synthesis, particularly in the brain. In this study, we found that prenatal WiFi exposure can increase the expression of BDNF and phosphorylated CREB proteins. However, the detailed mechanism still needs to study further.

The brain has been shown to be more prone to oxidative injury during development in early years of life (Ö et al., 2016). Besides, oxidative stress can be activated via several mechanisms, such as electromagnetic radiation, thus resulting in molecular impairment. The oxidative stress response due to exposure to WiFi signals has been previously investigated in an animal model. It is noteworthy that previous studies that investigated the harmful effects of RF-EMR have reported inconsistent findings. Ozben T 2007 and Kasra Kamali1 2018 implicated microwave radiation (Shokri et al., 2015 , Foster and Moulder, 2015 , At-Assa et al., 2013 , Aït-Aïssa et al., 2012) in apoptosis through their ability to trigger lipid peroxidation of cell membranes and as a result yield apoptosis signal (Kamali et al., 2018 , Ozben, 2010 , Dasdag et al., 2008). However, other studies have indicated that EMR has no considerable impact on the antioxidant defense system because of unaltered oxidative stress markers such as MDA (At-Assa et al., 2013). In the present study, WiFi exposure didn't induce brain oxidative stress response in offspring, suggesting that the possible damaging impacts of such radiations could be dependent on the exposure duration, dose, age, and body posture (Peter and Richard, 2010). Contradictory results of the mentioned research could be because of differences in study methods, especially in the duration of exposure and dose of WiFi signal.

In conclusion, the findings of this study indicate that prenatal WiFi exposure does not affect the offspring's hippocampal neurons, oxidative equilibrium in brain and neurodevelopment and emotional responses. Notably, some effects of WiFi exposure are sex-dependent. Prenatal WiFi exposure increased the body weight, improved the spatial memory and learning function and induced behavioral hyperactivity of male rats.However, there is a need to conduct further studies, especially on biochemical and neuro molecular mechanisms underlying such effects.

Declarations

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Competing interests

The authors declare that they have no competing interests

Authors' contributions

All authors contributed to the study conception and design. H.W., D. M. and P. W. Designed the study; B. S., Y. M., P. C., J. W., and B. Z., Performed the experiment; P. R., J. W., B. Z., and Y.C. Analyzed data; H. W. and D. M. Wrote and revised manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals (NIH Publication No. 8023, revised 1978) and approved by the Ethics Committee of Daqing Campus, Harbin Medical University.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

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Figures

Fig 1

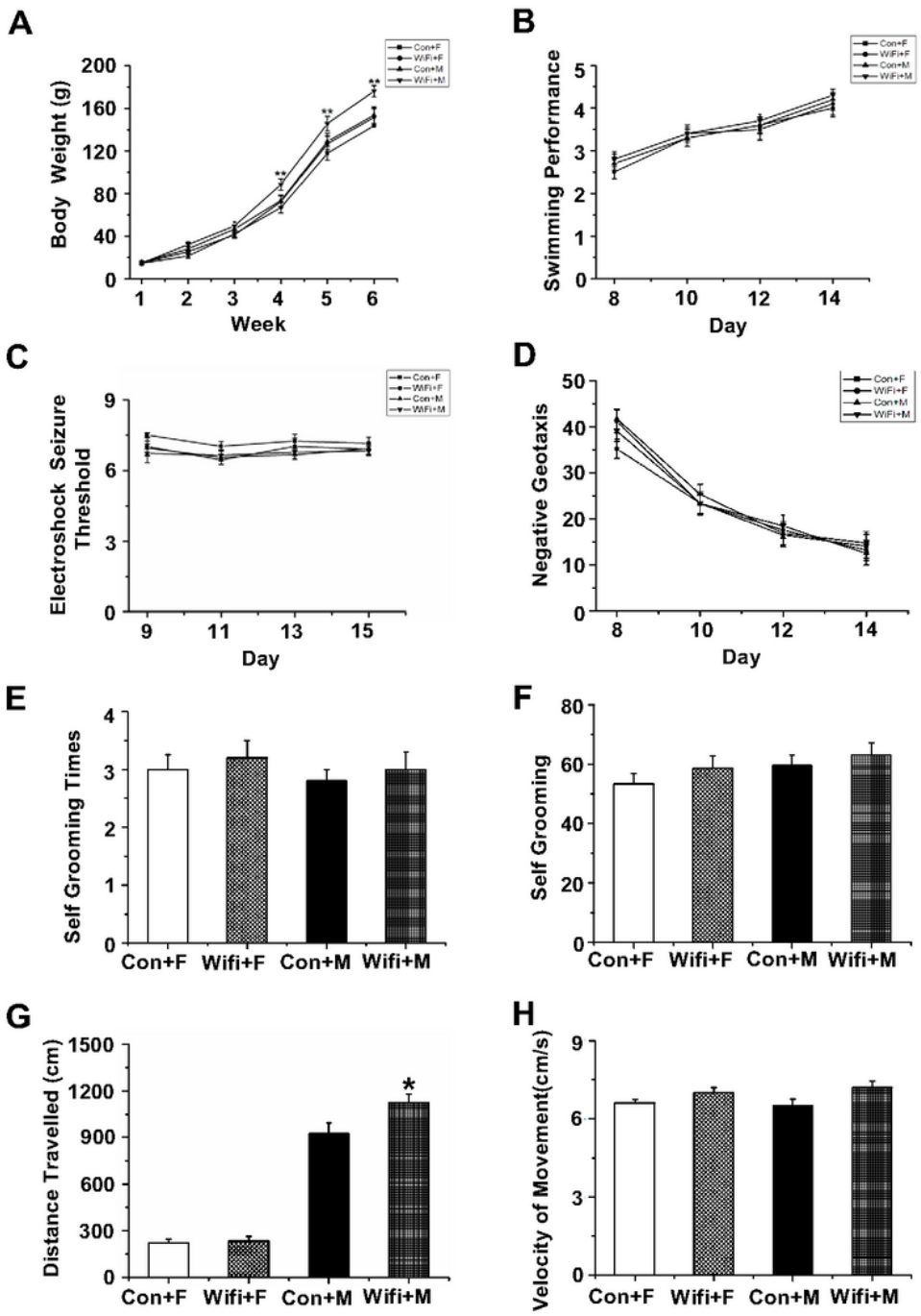

Figure 1

Effects of WiFi signals on bodyweight, neurodevelopment and behaviors of offspring in different group

(A) The result of body weight tests of offspring in each group. (B) The result of swimming ability tests of offspring in each group. (C) The result of the pain threshold tests of offspring in each group. (D) The result of geotaxis test of offspring in each group. (E-F) The result of self groom test of offspring in each

group. (G-H) The result of open field tests of offspring in each group. All data are expressed as mean ± SEM. (n = 8). *P < 0.05, Wifi+M vs. Control+M; **P < 0.01, Wifi+M vs. Control+M.

Fig 2

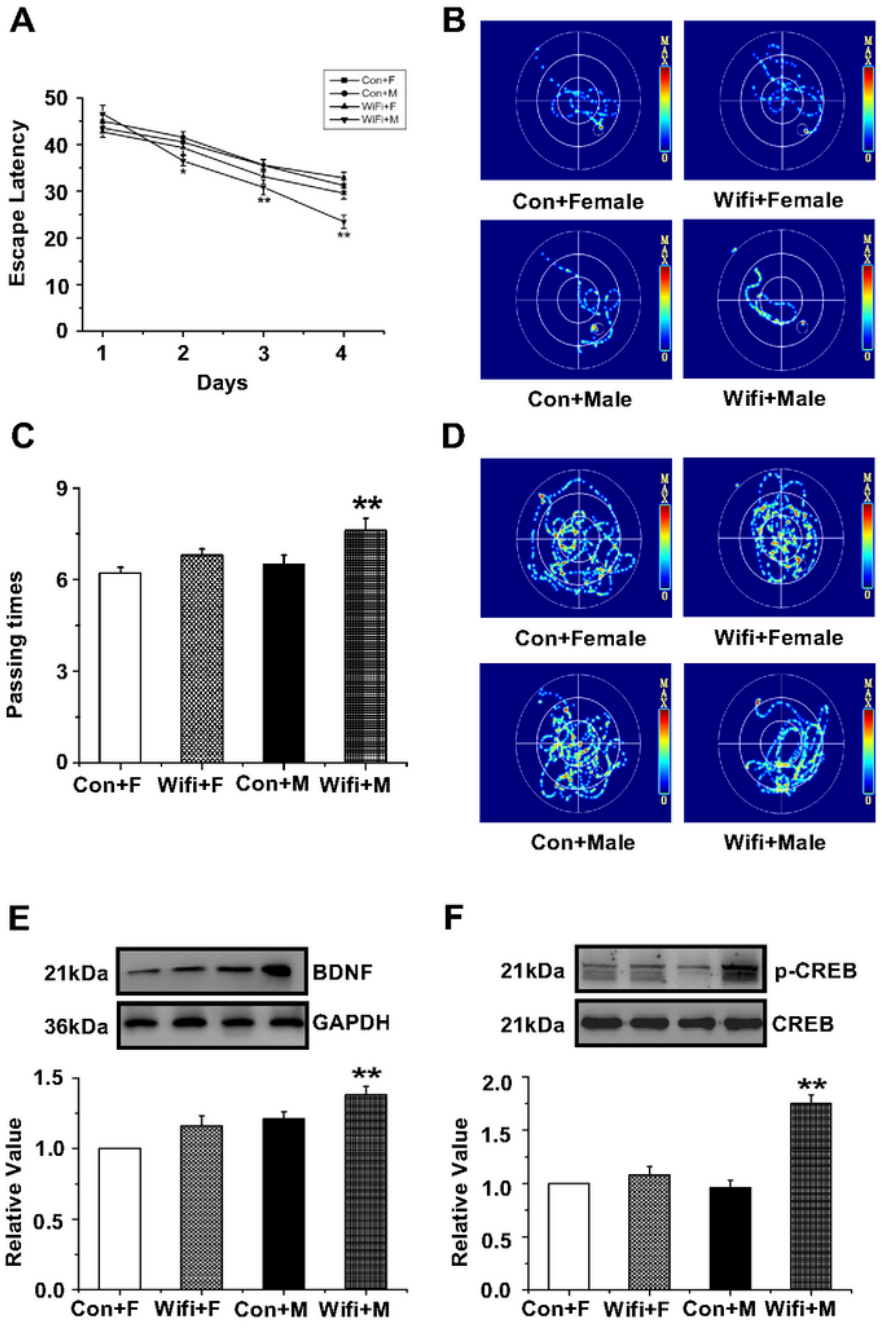

Figure 2

Effects of WiFi signals on spatial learning and memory function of offspring The Morris water maze was used to assess spatial learning and reference memory. (A) Representative traces of escapes latency (B) Represented the average of four trials of the latency testing session of all offspring in each group. (C) Representative traces of passing times through the circular area. (D) Represented the number of passing times of all offspring in each group. (E-F)The expression of BDNF protein levels and the ratio p-CREB/CREB in the hippocampal tissues of different groups of offspring by Western blot analysis. All data are expressed as mean ± SEM. (n = 8). **P < 0.01, Wifi+M vs. Control+M.

Fig 3

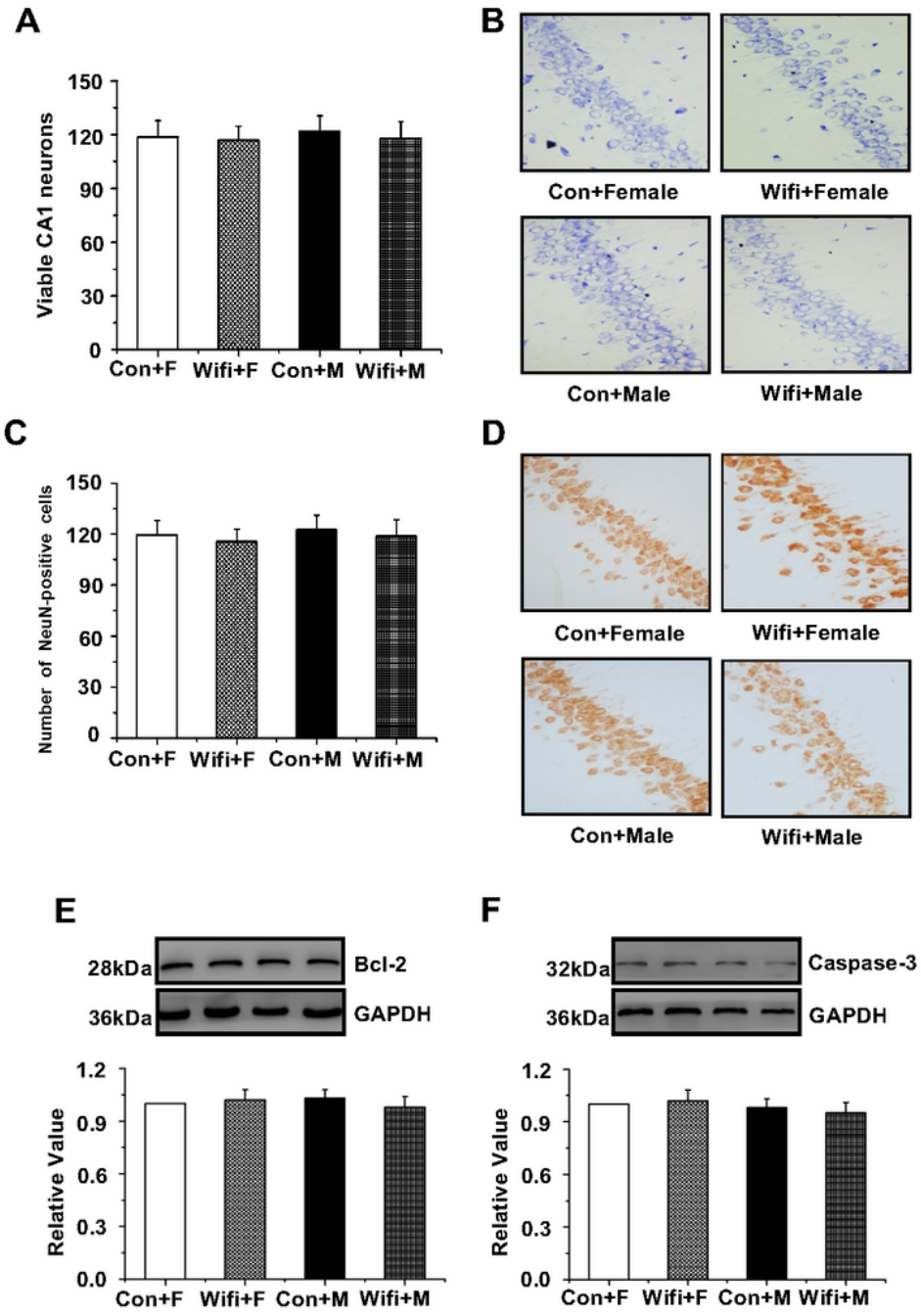

Figure 3

Effects of WiFi signals on neurons in the hippocampus of offspring in differnt group (A) Representative images of Nissl staining of hippocampal CA1 region. (B) Statistical analysis of the surviving neurons in each group. (C) Representative images of the NeuN immunohistochemistry in hippocampal CA1 region. (D) Statistical analysis of the the NeuN positive cells in each group. (E-F)The expression of apoptosis-

related proteins of hippocampus of offsprings in each group, E show representative Western blots for Bcl-2 and F show representative Western blots for caspase-3. All data are expressed as mean ± SEM. (n = 6).

Fig 4

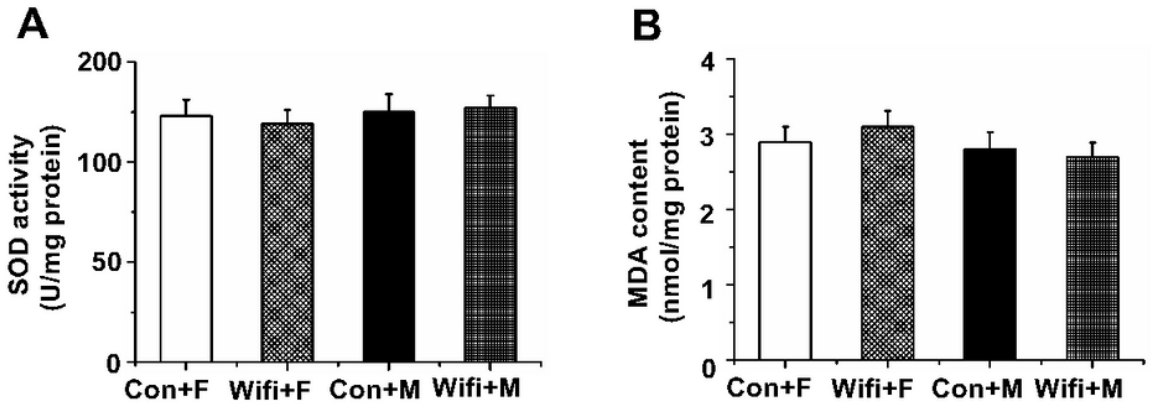

Figure 4

Effects of WiFi signals on brain oxidative stress response of offspring in differnt group (A) SOD activity in hippocampus. (B) MDA contents in hippocampus. All data are expressed as mean ± SEM. (n = 6).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- highlights.doc