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ARTICLE

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The effect of Wi-Fi electromagnetic waves on neuronal response properties in rat barrel cortex

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ABSTRACT

There is a growing number of studies on the possible biological effects of Wi-Fi radiations on nervous system. In this study we investigated the effect of Wi-Fi exposure on single neuron responses to natural stimuli by using whisker to barrel pathway. This study was done on 29 male Wistar rats. Neuronal spontaneous activity and ON and OFF responses to displacement of principal whisker (PW), adjacent whisker (AW) and combination of PW-AW stimulation (as natural stimuli) were recorded in barrel cortex of anaesthetised rats. A D-link Wi-Fi device was used for 1 h exposure to 2.4 GHz microwaves in data mode (18.2 dBm and 44% for power and duty cycle). A condition test ratio (CTR) was calculated for assessing neuronal integrative properties. Wi-Fi radiations decreased CTR for ON responses. However, neuronal spontaneous activity and ON and OFF responses were not significantly changed following exposure to Wi-Fi signals. The results of this study demonstrated that exposure to Wi-Fi radiation could modulate integrative responses to natural stimuli in barrel cortex.

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Introduction

We are surrounded by lots of nonionizing electromagnetic fields (EMFs) with different frequencies and intensities. Wi-Fi (Wireless fidelity) signals and signals emitted from mobile devices are most important sources of electromagnetic radiation (Celikozlu et al. 2012; Banaceur et al. 2013).

There are controversies about the biological effects of Wi-Fi radiations on nervous system. It was reported that a long term exposure to Wi-Fi signals induced anxious behaviours (Othman et al. 2017) and also impaired learning assessed by spontaneous object recognition (SOR) test (Hassanshahi et al. 2017) in rats. In human, Papageorgiou et al., reported a gender difference in the effect of Wi-Fi radiation on event-related potential (ERP) during an auditory Hayling task: the amplitude of the P300 ERP component decreased for males and increased for females during cognitive task performance in genuine Wi-Fi, but not in sham exposure (Papageorgiou et al. 2011). Also, it was reported that RF-EMF exposure can increase reaction time in young university students (Ayoobi et al. 2017). It seems that specific protocols of RF-EMF can modulate EEG signals (Shafiei and Firoozabadi 2014; Shafiei et al. 2014).

On the other hand, some reports demonstrated little effects of Wi-Fi signals on brain function. Zentai et al., reported that 60 min exposure to Wi-Fi signals didn't alter human oscillatory brain function (assessed by EEG measurement) or sustained attention (measured by reaction time) (Zentai et al. 2015).

Recording electrical potentials is a valuable method for studying neuronal functions in somatic, motor and other areas of nervous system. In this study, we compared response properties of barrel cortex neurons (part of the primary somatosensory cortex processing sensory information from facial whiskers in rats and other rodents (von Heimendahl et al. 2007)) to natural stimuli after acute exposure to Wi-Fi signals. The whisker pathway in rodent is a suitable model for investigation of neuronal responses to natural environmental stimuli (Arabzadeh et al. 2005).

Materials and methods

Animals

29 male Wistar rats weighing (180–220 g) were used in this study. The animals were allocated into one group and used for one experiment. Rats were purchased from animal house of Rafsanjan University of Medical Sciences. They were kept in standard cages (3–4/cage). The room temperature of 22 ± 2 °C and a 12 h dark and light cycle was set for animals. Animals had free access to water and food. All procedures were performed according to the ethical guidelines of Rafsanjan University of Medical Sciences (RUMS) and the European communities Council Directive of 24 November 1986 (86/609/EEC).

Wi-Fi device

The properties of Wi-Fi device was described in our previous study, in brief; the device name is D-LINK from England.

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It generate 2.4–2.4835 GHz radio frequency signals using two 5 dB external antennas. The Wi-Fi device has been measured and calibrated inside a shielded pyramidal anechoic chamber. RF output power and duty cycle were measured by spectrum analyser (Agilent, Santa Clara, California USA). The power and duty cycle for this device was 18.2 dBm and 44%, respectively in data mode (Hassanshahi et al. 2017).

Recording and whisker movement devices

As described in our previous studies (Mohammadi et al. 2017; Salari et al. 2017; Soltani et al. 2018), tungsten microelectrodes (FHC, Bowdoinham, ME, US) were used to record single unit action potentials from barrel cortex neurons in rats. The electrode was located in barrel cortex by a micromanipulator (WPI Company). Recorded data was amplified and digitised by using Electromodule hardware (Science Beam Inst Tehran, Iran). The obtained data were analysed by eSorter software offline (Science Beam Inst Tehran, Iran).

Electromodule system also controlled whiskers movement as follows. A thin glass tube with an internal diameter of 0.69 mm was attached to the centre of each loudspeaker and the rat whisker was placed into the other end of the tube. The whiskers were moved through Electromodule system by applying appropriate voltages to the speakers.

Extracellular recording

After induction of anaesthesia in rat (by 1.5 g/kg/i.p urethane) it was fixed in the stereotactic apparatus. Then a craniotomy (4 mm) of the right cortex was performed and the microelectrode entered into the barrel cortex. The microelectrode was firstly placed at a depth of 500 to 800 micrometers from the skull surface to make sure it was in the barrel cortex and also for easily detection of principal whisker. The whiskers, were stimulated and the neuronal response delay was measured. If the response latency was below 10 ms, it was then considered as the principal barrel for that whisker. At the next step, the cortex was covered with 3% agar solution in saline and the microelectrode was transferred to a depth of 200–400 micrometers and all other recordings were made in this depth which were corresponded to layers II and III of barrel cortex. The closest caudal whisker to the principal whisker (PW) was considered as the adjacent whisker (AW) and like the principal whisker, it was placed inside another thin tube attached to the speaker. Other rat whiskers were shortened to avoid contact with the tube (Mohammadi et al. 2017).

Displacement of whiskers

The whiskers were moved through the speakers with the following specifications; the time took to displace whisker to the new position was 5 ms. The duration of staying in the new position was 200 ms. The displacement range was 500 micrometer. The whisker return time from the new status to the initial position was 5 ms. This procedure was repeated for 50 times (the inter-stimulus interval was 1000 ms). The amplitude of whisker displacement was calibrated with a graduated microscope (Motaghi et al. 2006).

Following the displacement of whiskers, two types of neuronal responses (known as ON and OFF responses) were recorded. The ON response was recorded when the whisker moves from initial position to the new state and the OFF response was recorded when whisker moves back to its original state. To assess excitatory and integrative receptive fields the PW and AW were displaced alone or in combination respectively.

Recording procedure

After 1 h recovery, the neuronal spontaneous activity (for 600 sec) (there was no whiskers deflections) was recorded. Then we recorded the neuronal responses to deflection of PW, AW and combination of AW-PW deflection (AW was deflected 30 ms prior to PW deflection). Then the Wi-Fi device was turned on for 1 h (in this period no recording was done) and a file (same size and content for all experiments) was transferred between two computers through Wi-Fi device. It was reported that transferring data through Wi-Fi devices produce more exposure to EMF radiations (Joseph et al. 2013).

The device was placed at a distance of 25 cm from the head of the animal. Then the same procedure as previous application of Wi-Fi radiations was repeated. The depth of electrode didn't change throughout the recording.

Data analysis

The obtained data from neuronal spontaneous activity (before Wi-Fi application) was transferred to esorter software and single units were isolated by template analysis method. Then the obtained templates were saved and used to analyse the data that obtained from neuronal spontaneous activity after induction of Wi-Fi radiations. By this way we assumed that same units' responses were analysed before and after induction of Wi-Fi waves. For analysis of neuronal responses to whiskers deflection, same procedure was performed as of spontaneous activity. However the templates might be different from those obtained during analysis of spontaneous activity.

The measured parameters were classified as neuronal spontaneous activity, neuronal ON and OFF responses to PW and AW deflection and a conditioned test ratio (CTR). CTR measures the integrative properties of neuronal responses and calculate as follows. Magnitude of responses to deflection of PW and AW (when they deflected alone)/magnitude of responses to deflection of PW and AW when they deflected in paired combination. CTR < 1 indicates the inhibitory effect of AW displacement on neuronal responses to displacement of PW. CTR > 1 indicates the excitatory effect of AW displacement on neuronal responses to displacement of PW (Armstrong-James and Callahan 1991).

Statistical analysis was performed using Excel and SPSS softwares. All data were expressed as mean ± standard error

of mean (SEM). Paired t-test was used to compare the data before and after induction of Wi-Fi radiation. In all comparisons $p < 0.05$ was considered as significant level.

Result

The effect of Wi-Fi radiation on spontaneous activity of neurons

The neuronal spontaneous activity was recorded for 600 s. The post Wi-Fi exposure recording was also repeated. Figure 1 shows the cumulative histogram of spontaneous responses pre-and post-exposure to Wi-Fi signals. The observed rising in spontaneous activity following Wi-Fi exposure was not significant (15.5 ± 1.3 Hz vs 17.4 ± 1.5 Hz, paired t-test, $t_{62} = -1.5$, $p = 0.13$).

The effect of Wi-Fi radiation on excitatory receptive fields

Response to PW deflection

Figure 2 illustrates how the response of barrel cortex neurons to PW stimulation was modulated following Wi-Fi radiations. Neuronal ON and OFF response magnitude to PW deflection were not affected following exposure to Wi-Fi radiations (ON before 4.6 ± 0.35 vs ON after Wi-Fi 4.7 ± 0.4, $t_{21} = -0.7$, $p = 0.45$) (OFF before 3.5 ± 0.44 vs OFF after Wi-Fi 4.5 ± 0.6, $t_26 = -1.7$, $p = 0.08$).

Response to AW deflection

Wi-Fi radiations had no significant effects on neuronal ON and OFF response magnitude to AW deflection (ON before 1.1 ± 0.2 vs ON after Wi-Fi 1.3 ± 0.3, $t_{22} = -0.6$, $p = 0.5$) (OFF

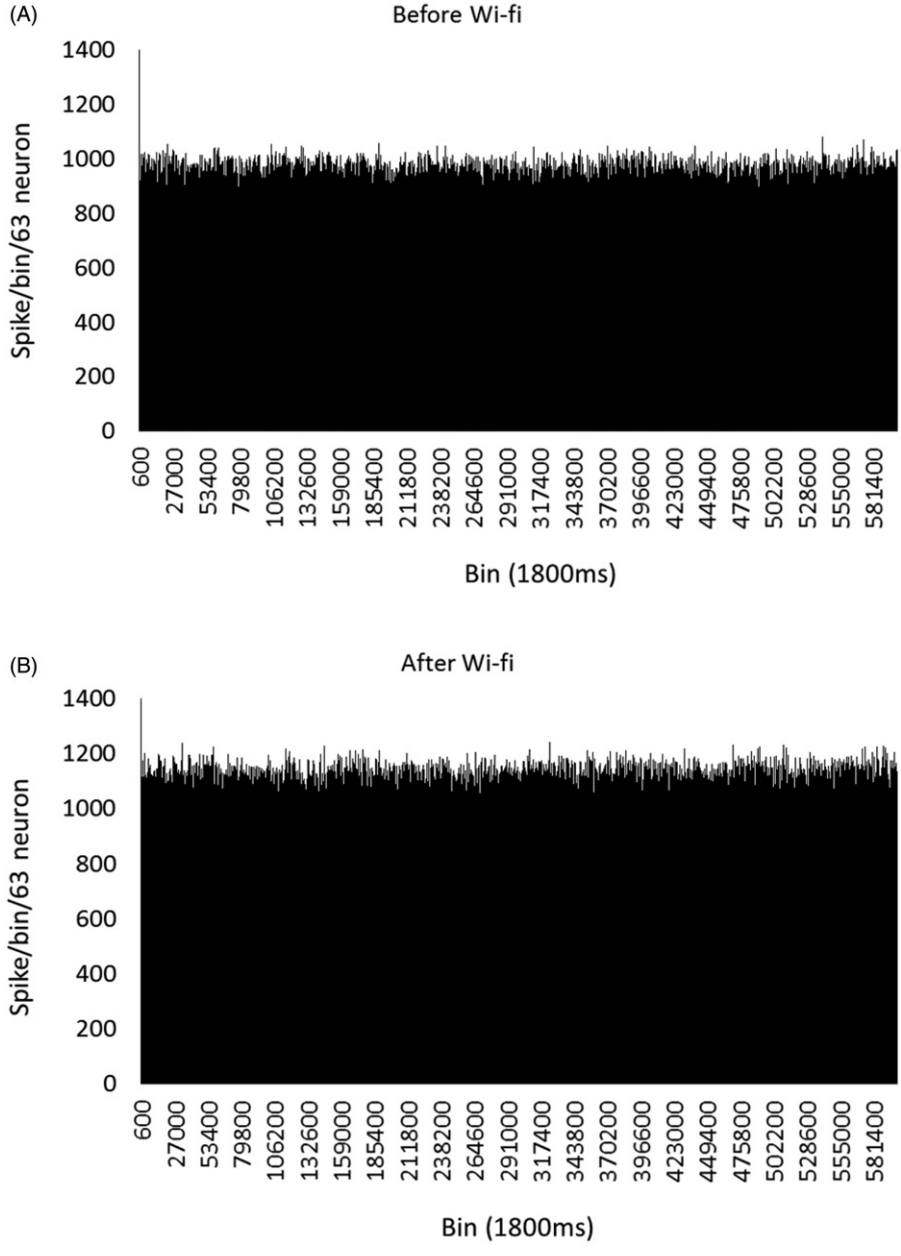


Figure 1. Cumulative histogram of 63 neuronal spontaneous activity before (A) and after (B) the exposure to Wi-Fi radiation. Bin size is 1800 ms.

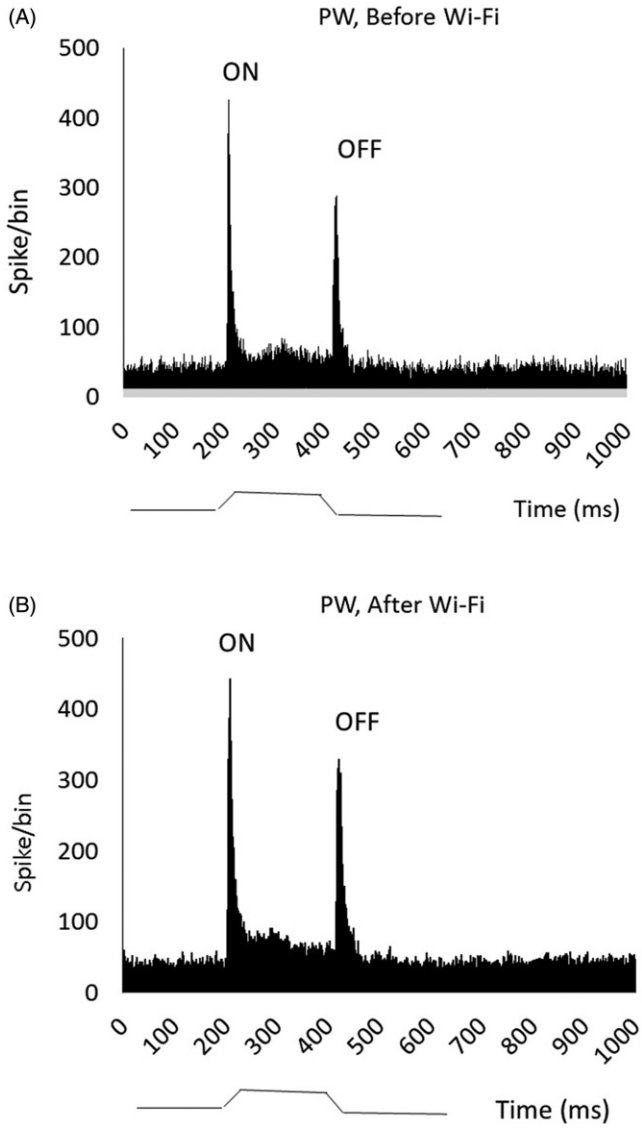


Figure 2. Cumulative peristimulus time histogram of neuronal ON and OFF responses to displacement of principal whisker. A and B response were recorded before and after induction of Wi-Fi waves respectively. Insets shows stimulation pattern.

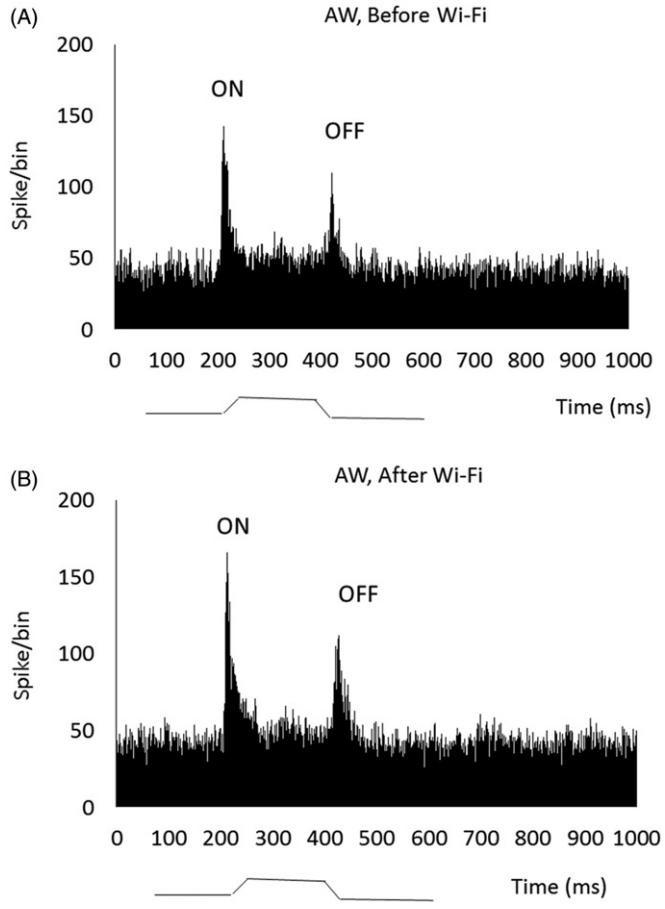


Figure 3. Cumulative peristimulus time histogram of neuronal ON and OFF responses to displacement of adjacent whisker. A and B response were recorded before and after induction of Wi-Fi waves respectively. Insets shows stimulation pattern.

before 0.05 ± 0.2 vs OFF after Wi-Fi 0.3 ± 0.1, $t_{18} = -1.1$, $p = 0.2$) (Figure 3).

The effect of Wi-Fi radiation on response integration

To investigate whiskers interactions, a conditioning-testing paradigm was used in which AW deflected 30 ms before PW. The strengths of these interactions were quantified by applying a condition-test ratio (CTR) measurement (see methods). CTR for ON responses were decreased following exposure to Wi-Fi radiations (0.6 ± 0.2 vs 0.4 ± 0.2, $t_{21} = 2.4$, $p = 0.024$). However, CTR for OFF responses were not affected following Wi-Fi radiations (0.5 ± 0.3 vs 0.6 ± 0.4, $t_{20} = -1.5$, $p = 0.12$) (Figure 4).

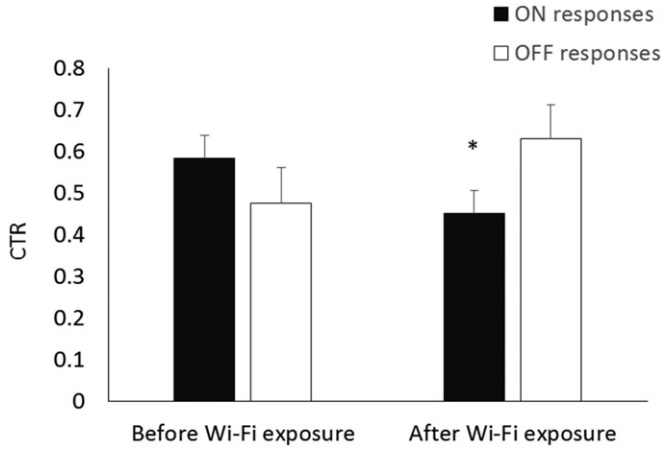


Figure 4. CTR for ON and OFF responses before and after exposure to Wi-Fi radiations. *Significant vs before exposure to Wi-Fi. The value of CTR > 1 indicates facilitation, while <1 means inhibitory effect of the first whisker deflection on response to second whisker deflection. Error bars represent S.E.M.

Discussion

The purpose of this study was to investigate the effect of short time exposure to Wi-Fi 2.4 GHz radiations on neuronal response properties to natural stimuli in rat barrel cortex. The results demonstrated that Wi-Fi radiation could modulate integrative responses. However, excitatory receptive fields and spontaneous activity was not affected by Wi-Fi signals.

Our previous study demonstrated that multisensory encoding of information may be modulated following Wi-Fi radiations (Hassanshahi et al. 2017). This conclusion is more supported by the result of this study which demonstrated modulatory effects of Wi-Fi radiations on neuronal integrative properties.

There are growing number of reports demonstrating some effects of Wi-Fi radiations on nervous system. Nazirogl et al., reported that Wi-Fi radiations may increase Lipid peroxidation, cell viability and cytosolic Ca^{2+} values in DRG neurons (Naziroglu et al. 2012). It seems that Wi-Fi waves have oxidative toxic effects on the eye lens in animals (Tok et al. 2014) and the brain of developing rats (Celik et al. 2016). Recently, Dasdag et al., Reported a severe reduction in some brain miRNA expression (mir 107 and miR-106b-5p) due to Wi-Fi radiations (Dasdag et al. 2015). Ibitayo et al., reported brain DNA damage following Wi-Fi radiation in rats (Ibitayo et al. 2017). Another important finding is that voltage-gated calcium channels are modified by Wi-Fi radiations (Pall 2014). In a recent study, Pall et al., reported that these channels can cause cancer (including tumour promotion, tissue invasion and metastasis) via 15 mechanisms (Pall 2018).

Most of studies that reported little adverse effect of Wi-Fi waves were focussed on other body systems instead of nervous system. Akdag et al., examined the effects of long term exposure to Wi-Fi waves (one year) on DNA damage in the kidneys, liver, brain and testicles. They found significant effects of Wi-Fi only in testes (Akdag et al. 2016). Also it was shown that Wi-Fi waves has no effect on apoptosis and growth factors in human adipose-derived stem cells (Lee et al. 2014) and development of chicken embryos (mortality or malformations) (Woelders et al. 2017).

In our previous studies with similar design to the present one, we showed that sham or vehicle treatment had no significant effect on neuronal response properties in barrel cortex (Mohammadi et al. 2017; Salari et al. 2017; Soltani et al. 2018), so for this study we did not repeat sham experiments. In this study, we recorded from superficial layers in barrel cortex. It was reported that Synaptic computation and sensory processing are more predominant in superficial neocortical layers (Petersen and Crochet 2013). Also most of experience dependent plasticity's happening in superficial layers (Glazewski 1998). So we hypothesis that neurons in superficial layers may be more sensitive to Wi-Fi radiation. It is suggested to record from other layers in future studies. We measured impedance of the electrode with and without Wi-Fi radiations (in saline solutions, and the device was 20 cm far from electrode). The results demonstrated no change in electrode impedance assuming that electrical properties of electrodes were not affected by Wi-Fi radiations.

Conclusion

In conclusion; to our knowledge, this is the first study that investigated the effect of Wi-Fi signals on single neuron electrophysiological responses to natural stimuli. Results demonstrated modulatory effects for Wi-Fi radiations on neuronal responses to complicated natural stimuli (i.e., stimulation of two whiskers need more complicated neural responses that stimulation of single whisker). However, further studies with more complicated natural stimuli will give us a better interpretation about these modulatory effects of Wi-Fi radiations.

Disclosure statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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