

*Research Article*

## The possible effects of electromagnetic waves of Wi-Fi router on the hippocampus of male rats

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### Abstract

**Background:** Nowadays, there is increasing in incidence rates of using the electromagnetic field in daily life at work and at home, and it considers one of the environmental stimuli affects different cells and organs in the body. There are different sources of electromagnetic waves in our environment, including wireless communications, radars, satellites, TV, and radio antennas. Wi-Fi networks are among the most common inducers of the electromagnetic field. It is a cheap common technology that exposes its users to a spectrum of short wavelength electromagnetic waves (2.45 GHz). It may affect cell function via non-thermal effects, and its exposure can increase reactive oxygen species (ROS) formation and decrease cognitive function. **Methods:** In this study, 10 male albino rats were exposed to 2.45 GHz Wi-Fi router radiation /12 hours per day per week for 6 weeks. **Results:** revealed that the Wi-Fi router radiation caused remarkable degeneration of hippocampal pyramidal and granular neurons and an increase in the aggregation of the intraneuronal neurofibrillary tangles (NFTs) in the neuronal cell body. **In conclusion,** electromagnetic radiation exposure can cause hippocampal dysfunction through the neuronal damage effect.

**Key words;** EMR, Hippocampus, Wi-Fi router

### Introduction

The Hippocampus, the region of the brain that is associated primarily with memory. Its shape resembles that of a sea horse. The hippocampus, a part of the limb system, which is particularly important in regulating emotional responses. It is involved in storing long-term memories and in making those memories resistant to disremember. It plays an important role in spatial processing and navigation<sup>(1)</sup>.

Nowadays, there is increasing in incidence rates of using the electromagnetic field in daily life at work and at home, and it is considered one of the environmental stimuli affects different cells and organs in the body. There are different sources of electromagnetic waves in the environment, including wireless communications, radars, satellites, TV, and radio antennas<sup>(2)</sup>.

It is also widely used in medical instruments and the hospitals. Wi-Fi networks are among the

most common inducers of the electromagnetic field. It is a cheap common technology that exposes its users to a spectrum of short wavelength electromagnetic waves (2.45 GHz). We are exposed to different sources of low energy electromagnetic field every day<sup>(3)</sup>.

Exposure to radiofrequency electromagnetic radiation (RF-EMR) induces an imbalance in the oxidant/antioxidant defence system in the brain indicating that the internal environment of each brain cell was getting disturbed by the insult from RF-EMR<sup>(4)</sup>

One of these EMR is Wi-Fi which may affect cell function via non-thermal effects, and its exposure can increase reactive oxygen species (ROS) formation and decrease cognitive function<sup>(5)</sup> (Belpomme et al., 2018). Recent studies revealed that exposure to radiation emitted from Wi-Fi technology has many health hazards on different body organs such as; brain, liver,

kidney, heart, pancreas, reproductive system, blood. This radiation carries also risk of cancer<sup>(6)</sup>

## Material and Methods

### Animals:

This study was conducted in Department of Histology and Cell Biology, Faculty of Medicine, Minia University, Egypt. This work was carried on twenty adult male albino rats with body weight 150-250 gm, age 6-8 weeks and pathogenically free. Animals were obtained from the study animal house of Minia University laboratory animals growing center of the Faculty of Agriculture.

Rats were housed in hygienic plastic cages in a clean, well-ventilated room and were given free access to food (a standard diet of commercial rat chow) and water with normal light and dark cycles. Rats were maintained at a laboratory temperature ranged from 24-30°C and exposed to 12 hours light and 12 hours dark cycle. Rats were left to acclimatize to the environment for 2 weeks prior to inclusion in the experiment. All aspects of animal care and treatment were carried out according to the local guidelines of the ethical committee of the Faculty of Medicine of Minia University.

### Wi Fi exposure system:

The Wi-Fi signal was picked up directly by a commercial Access Point for use indoors (ZTE - ZXHN H108N Router with 802.11 g mode and WPA2 network protection)<sup>(7)</sup>. The device supports wireless networking speeds of up to 150 Mbps (Turbo mode) on the popular 2.45 GHz public frequency.

Exposed group was placed in cages surrounded by aluminium foil to concentrate the radiation and the cages were placed at a distance of 25 cm from the router device<sup>(8)</sup>, animals of the exposed group were exposed to Wi-Fi radiations from 2<sup>nd</sup> week of experiment daily for 12 hours per day for 4 weeks while the control group was isolated in a separate room away from any source of radiations (0 Hz).

### Experimental design:

Twenty adult male healthy Sprague–Dawley albino rats (6–8 weeks) weighing 150-250g were

used in this study. Rats were divided randomly into two different groups of 10 animals each:

#### **1) The control group (group I):**

This group was formed of 10 rats and these rats were isolated in a separate room away from any radiation source and were evaluated after four weeks.

#### **(2) The Wi-Fi exposed group (group II):**

This group was formed of 10 rats and were exposed to radiations emitted from the Wi-Fi router device daily for 12 hours/day for 4 weeks.

### **Rat scarification and tissue harvestings:**

Rats were sacrificed at the end of the experiment by decapitation under light halothane anaesthesia and were fixed by intracardiac perfusion of 5cm 4% paraformaldehyde technique<sup>(9)</sup> to prevent brain tissues fragmentation and preserve the normal brain tissues architecture. Brains were removed and hippocampi were rapidly carefully dissected out to be assigned for different histological procedure. Specimens fixed in 10% formal saline for 48 hours, then washed by tap water and processed for paraffin embedding, sectioning and staining for morphological and morphometric studies using light microscopy.

#### **A) Histological study:**

##### **I- For light microscopic examination:**

###### **1) The Paraffin Technique**

The hippocampal specimens buffered. After proper fixation, the samples were dehydrated in a graded alcohol series, cleared in xylene and embedded in paraffin wax then cut by a microtome. Five-micrometer sections were mounted on glass slides for further staining<sup>(10)</sup>.

###### **a) Staining with Hematoxylin and Eosin (H&E):**

Some sections were mounted on glass slides, deparaffinized and emersed in hematoxylin stain for 7 minutes, washed well in running tap water, then emersed in eosin for 3 minutes and the surplus stain was washed off in the water. The sections were dehydrated in alcohol, cleared by xylene, and then cover-slipped to be viewed by light microscopy for the general histological analysis study<sup>(10)</sup>

###### **b) Staining with Gallyas-Braak silver stain:**

Gallyas method is one of the most “sensitive” silver-staining that clearly labels neurofibrillary tangles (NFTs), as well as neuronal damage<sup>(11)</sup>

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## Results

### The Histological study:

#### ☉ Histological results of haematoxylin and eosin (H&E):

##### ➤ Control group:

Higher power examination of CA3 area showed it was formed of large, normal pyramidal loosely packed neurons. Each neuron contains a single, rounded central large, vesicular nucleus with prominent nucleoli (Figure 1A). notice the normal pink neuropil background which was formed of neuronal fibres.

##### ➤ The Wi Fi group:

This group showed distinguished histological changes in the form of heterogeneity of pyramidal neurons in CA3: mostly of the pyramidal neurons appear degenerated, lose their normal arrangement and were widely separated with shrunken cell bodies, pyknotic nuclei and perineural space and few pyramidal cells appear

normal with basophilic cytoplasm and large central rounded vesicular nuclei (Figure 1B)

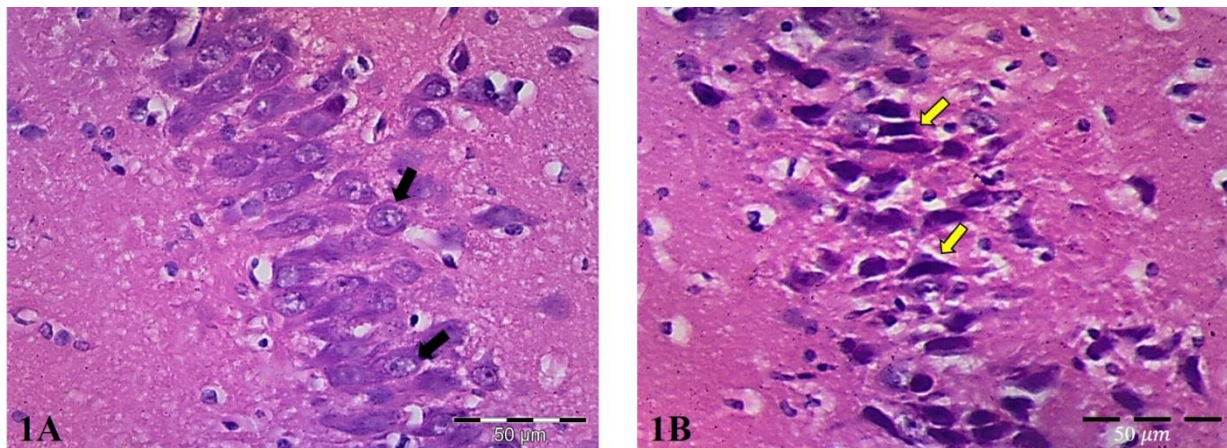
#### ☉ Histological results of Gallyas-Braak silver stain:

##### ➤ Control group

The examination of CA3 area showed the normal morphological structure of pyramidal cells with observation of their arrangement, each pyramidal cell appeared normal with a single, rounded central large, vesicular nucleus with prominent nucleoli (Figure 2A).

##### ➤ The Wi Fi group:

In this group, the pyramidal cells lost their normal architecture and normal arrangements. The pyramidal cells appeared darkly stained and destructed cells with shrunken cell bodies, pyknotic nuclei and surrounded by dark stained processing (Figure 2B).

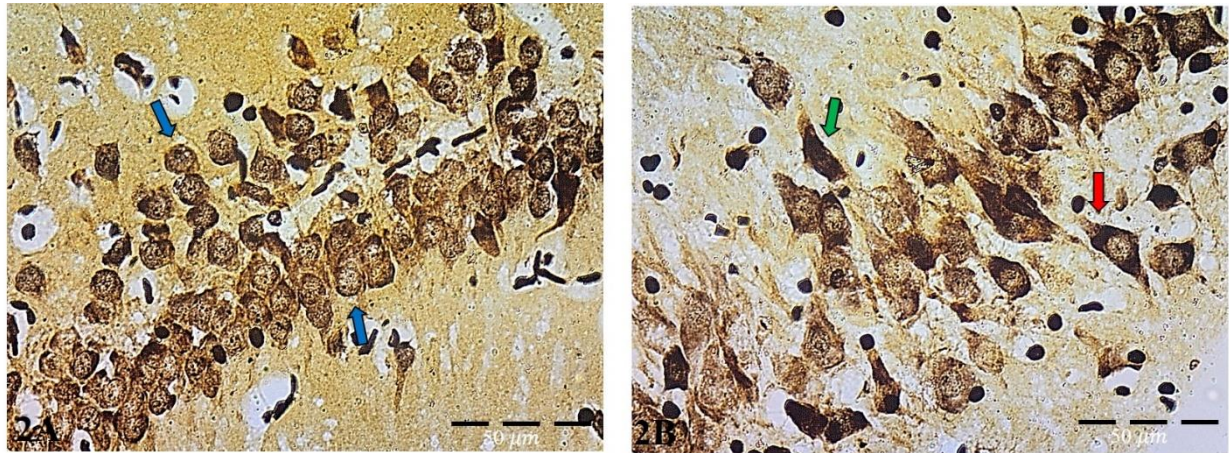


**Figure 1:** Photomicrographs of the rat hippocampus showing Cornu Ammonis (from CA3) area:

**A)** represent control group, pyramidal cells appear normal with a single, rounded central large, vesicular nucleus with prominent nucleoli (black arrows) and normal neuropil.

**B)** represent Wi-Fi group with destructed hippocampal architecture, the pyramidal cells appear shrunken with pyknotic nuclei (yellow arrows) and highly vacuolated neuropil can be seen.

(H&E X 400) Scale bar: 50µm



**Figure 2:** Photomicrographs of the rat hippocampus stained with Gallyas-Braak silver stain showing Cornu Ammonis (from CA3) area:

**A)** represent control group with normal hippocampal architecture, pyramidal cells appear normal with a single, rounded central large, vesicular nucleus (blue arrows).

**B)** represent Wi-Fi group with destructed hippocampal architecture, the loosely packed pyramidal cells appear shrunken with pyknotic nuclei (green arrow) and some pyramidal cells surrounded by dark stained processing containing NFTs (red arrow).

(Silver stain X 400) Scale bar: 50µm

**The morphometric results:**

➤ **Histopathological scoring of degenerated pyramidal neurons in CA:**

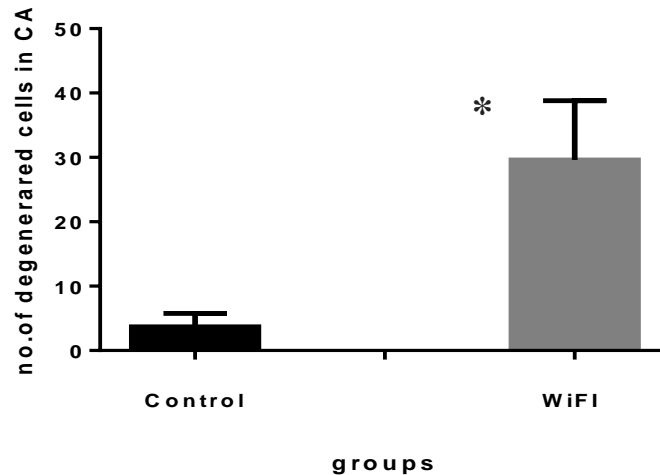
In Wi-Fi exposed group there was a significant increase in the mean number of the degenerated

pyramidal neurons in Cornu Ammonis (CA) by comparison to the control group (P=0.000). (Table 1, Histogram I).

**Table 1:** The mean numbers of the degenerated pyramidal neurons in the studied groups (n=10).

	Control	Wi-Fi	P value
	N=10	N=10	
Mean ± SEM	4.3 ± 0.85	31.8 ± 5.2	<0.0001*
<b>P value between each two groups</b>			
Control		< 0.0001*	
Wi-Fi			

*One Way ANOVA test for quantitative data between the four groups followed by t-test analysis between each two groups\*: Significant level at P value < 0.05*



**Histogram I: Counting degenerated pyramidal cells in Cornu Ammonis in the studied groups (n = 10).**

\* Significant vs: Control group.

### Discussion

Nowadays we live in a world that becomes trapped in an electromagnetic radiation (EMR) resulted from marked increase in usage of wireless devices. These Wi-Fi devices have become an important thing in every home, schools, universities, coffee shops, and all governmental institutions<sup>(12)</sup>. Many researches on the effect of exposure to wireless devices on different organs have been increased nowadays; however, the effect on the hippocampus is not clear. This study was conducted to figure out the histological changes in hippocampal tissues after exposure to radiation emitted from router device and its relation to the duration of exposure. This work tried to simulate the same conditions that most people exposed; Wi-Fi group were exposed to Wi-Fi radiation for 12 hours per day from 9 am to 9 pm, the same duration that the employees are exposed to during their work. The router device 2.45 GHz that was used is the commercial one that is used nowadays as done by<sup>(13)</sup> Ibrahim et al., (2019) in their study.

By using hematoxylin and eosin the control group showed normal histological structure of the hippocampal tissue. While the exposed groups to Wi-Fi radiation router device showed many hippocampal morphological changes in the form of marked degeneration of pyramidal and granular cells and highly vacuolation of neuropil of the exposed groups in compare with the control group this was explained in<sup>(14)</sup>

(Karimi et al., 2018) study which revealed that the hippocampal morphology showed that the neuronal density in the hippocampal CA3 area was significantly decreased by Wi-Fi radiation exposure.

Gallyas-Braak silver stain using, provided us by a clear vision about the effect of EMR on hippocampal different regions as increase the degeneration rate of hippocampal neurons specially the pyramidal neurons as previously described by (El-Kafoury et al., 2019)<sup>(15)</sup>. In the Wi-Fi group there was apparent increase in number of cells containing neurofibrillary tangles in their cell bodies as an indicator for cell degeneration appeared by silver stain. These results came with agreement of (Marson et al., 2021)<sup>(16)</sup> study.

The role of EMR in inducing oxidative stress has been also approved by The mechanism by which EMR induced damage can be simplified by production of reactive oxygen species as nitric oxide and significant decrease in total antioxidant as superoxide dismutase after EMF exposure<sup>(17)</sup>. These free radicals lead to damage of large cellular molecules such as lipids, proteins and nucleic acid and induce cell apoptosis.

### Conclusion

From this study, the exposure to Wi-Fi router devices can cause many structural changes in the

hippocampal tissue, These structural changes due to the effect of electromagnetic radiation in induction of apoptosis and oxidative stress. Interestingly, hazardous effects of Wi-Fi router devices were time dependent.

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