

# Studying the physiology of the increased risk of electromagnetic fields

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**Studying the physiology of the increased risk of electromagnetic fields (EMF) in the central nervous system (CNS) and observing the role of treatment and behaviour regulation in patients**

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**Abstract**

The state of anxiety in rodents can be measured through the two-compartment box test, where the time the mouse remains immobile in the white compartment and the time it remains in the dark side indicate its anxiety. The hippocampus is an important site in the CNS that is involved in the formation of memories, and also plays a general role in information processing and subsequent behaviour regulation.

**Introduction**

The effects on human health that will result from the constant rise in electromagnetic radiation (EMR) exposure caused by the widespread use of wireless connection (WiFi: wireless fidelity) network technologies have started to draw attention by being more thoroughly studied in both social and scientific environments today (Akdag *et al.* 2016). Due to the fact that using the Internet has become a necessity in modern life, there is increased risk of electromagnetic fields (EMF) exposure from a variety of radiation sources, including WiFi, laptop and desktop computers, connected office equipment, and devices used at home, at work, in public places, and in schools (Hardell 2017). In the part of the electromagnetic spectrum known as non-ionizing radiations, which lack the energy to produce electromagnetic ionisation, are radiofrequency (RF: radio frequency) waves and microwaves (MW: microwaves). Mobile phones, WiFi-enabled telecommunications networks, military radar systems, and satellite communications all emit radiations known as radiofrequency electromagnetic radiations (RF-EMR), which cover a large portion of the electromagnetic spectrum in the frequency range of 3 kHz to 300 GHz. Although RF-

EMR interacts with several biological creatures and affects physiological systems in both thermal and non-thermal ways, its mode of action is still not completely understood (Verma *et al.* 2019). A subset of RF-EMRs called microwave electromagnetic radiations (MW-EMR) have a high frequency range (300 MHz to 300 GHz) and can have an impact on our health depending on their frequency and power. Microwave ovens, short-range wireless connections, Bluetooth in mobile phones, cable-displacer devices such wireless printers, keyboards, and mouse for PCs, and microwave ovens all use these frequencies. So, in daily life, high frequency (2.45 GHz) microwave radiation is used by current cell phones, laptop computers, WiFi technology, and microwave ovens. Lipids, proteins, and nucleic acids, which are biological macromolecules of living cells that transport information in non-thermal (non-thermal) ways, interact with MW-EMRs.

### **Material and Methods**

Within the scope of the paper, the experimental groups were formed from 35 healthy adult male Wistar Albino rats, 12-16 weeks old and weighing  $230 \pm 15$  g. Experiments on rats were carried out in the laboratory. Within the scope of the study, 35 rats were designed to form 5 groups. RF radiation application times. The first group is the control group and no application was made. RF radiation exposure was applied with a ESIME Zacatenco radiation equipment, field levels were measured with EMR 300. At the end of the 30-day experiment period, one of the testicular tissues belonging to the groups was preserved for oxidative damage examination, and the other testicular tissue for histological examination.

Paraffin blocks were obtained after the routine follow-up procedures were fixed in 10% neutral formaldehyde solution for at least 72 hours, and 4–5-micron thick sections were taken from the prepared paraffin blocks. Testicular tissues of the groups were examined by immunohistochemical methods. Immunohistochemical uptakes were evaluated for each antibody and for each group, and data were generated using Kruskal-Wallis and Mann-Whitney analysis to determine whether there was a significant difference in uptake intensities between groups. Parameters for oxidative damage were investigated in testicular tissue.

The radiation was carried out in the Electromagnetic Compatibility Laboratory, the equipment used to irradiate the rats was a GTEM (Transversal Electromagnetic in Giga Hertz). Cell, an amplifier and a signal generator.

The radiation batch (UHF) was subjected to a frequency of 860 MHz and a power of 0.5 W in the radiation equipment, for 4 hours/day for 35 days in winter and in summer, a second batch was subjected to this same radiation only for 15 days, taking care that the temperature was between 23-25°C and a humidity of 70%, without access to food or water.

A group of rats was used to submit it only to isolation for 4 hours a day without being exposed to radiation and a group of control rats which did not receive treatment.

After the sections taken from the experimental groups were kept in an oven at 60 °C for 30 minutes, they were removed from xylol for 2x15 minutes and cleared of paraffin. Then, the slides were passed through decreasing series of alcohol (100%, 96%, 80%, 70%, 50%) and air-dried. After 10 minutes of washing in running water, they were stained in Harris Hematoxylin for 10 minutes and washed in running water for 10 minutes. It was dipped in 70% alcohol+2-3 drops of glacial acetic acid mixture and washed again in running water for 10 minutes. After the slides were kept in Eosin for 15 minutes and washed in running water for another 10 minutes, they were passed through a series of increasing grades of alcohol (50%, 70%, 80%, 96%, 100%), taken in xylol for 2x15 minutes and closed with entellan. In order to generate statistical data; 6 tubules were selected randomly for each subject, and the seminiferous tubule length was measured in 6 different regions in each tubule, and the data were recorded.

Glutathione (GSH) in tissue was studied with the spectrophotometric method with the ready kit. Solutions used: 0.3 M NaH<sub>2</sub>PO<sub>4</sub> DTNB-dithio nitro benzoic acid (0.4 mg/mL 1% sodium citrate). Tissue samples were weighed and homogenized with a homogenizer in cold TCA (1 g tissue+9 mL 10% TCA) on ice. Then, the homogenate was centrifuged at 4,000 rpm for 15 minutes at +4 °C, and the supernatant was taken and centrifuged again at 4,000 rpm for 8 minutes. 2 volumes of supernatant were mixed with 8 volumes of NaH<sub>2</sub>PO<sub>4</sub> and 1 volume of DTNB solution. After waiting 5-10 minutes at room temperature, the absorbance of the mixture was read in the spectrophotometer at a

wavelength of 412 nm versus the blank, and tissue GSH levels were calculated per  $\mu\text{mol/g}$  tissue.

## Results and Discussion

### Results

#### Body weight

Figure 1 shows a trend of weight increase in each of the groups throughout the treatment to which they were subjected due to their natural growth, observing that none of the treatments caused weight loss in the rodents.

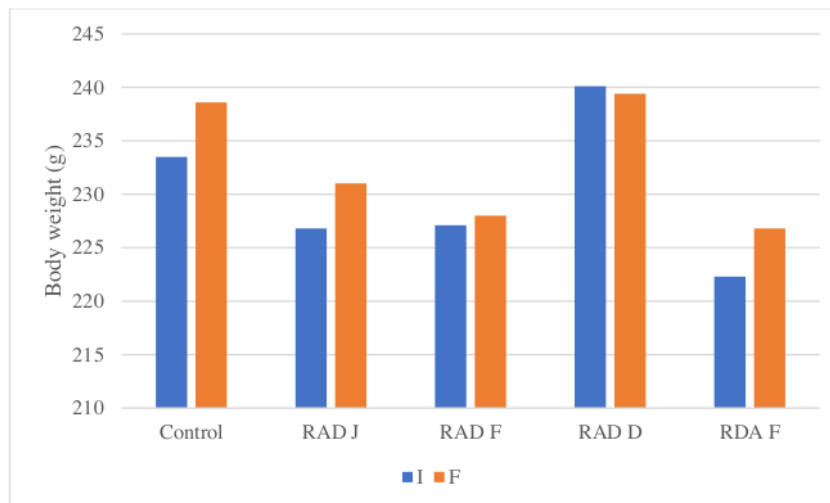


Figure 1. Body weight of treated rats. Where 'I' is the initial weight of the treatment and F is the final weight of the rats before being sacrificed. "One-way ANOVA",  $*p \leq 0.05$ : \*\*significant difference between the RDA F group compared to the Control group.

#### Locomotor activity in the open field

The results of the locomotor activity in the open field are presented in figures 2 and 3, where it is observed that no significant difference was found between the treated groups with respect to the control group. However, there is a tendency to increase the number of frames covered in the RAD D group with respect to the control group. Figure 10 shows

that there is a tendency to increase the locomotor and exploratory activity at two minutes, decreasing it at 3 minutes due to a coupling to the novel environment.

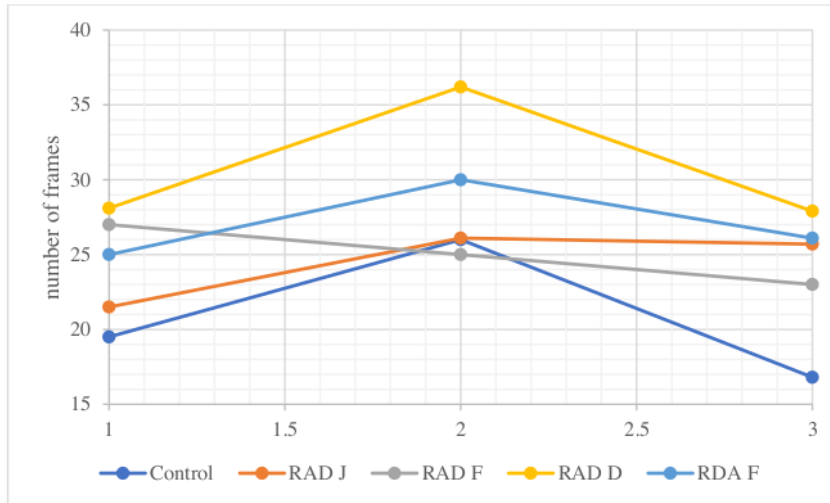


Figure 2. Number of frames that rats crossed every minute for 3 minutes in the open field test after irradiation with a UHF frequency of 860 MHz or isolation ("One-way ANOVA" (\* $p \leq 0.05$ )).

Figure 3 shows an increasing trend in the # of cumulative crosses of all groups with respect to TG, highlighting that the RAD D group presented greater locomotor and exploratory activity in an unknown environment for rodents.

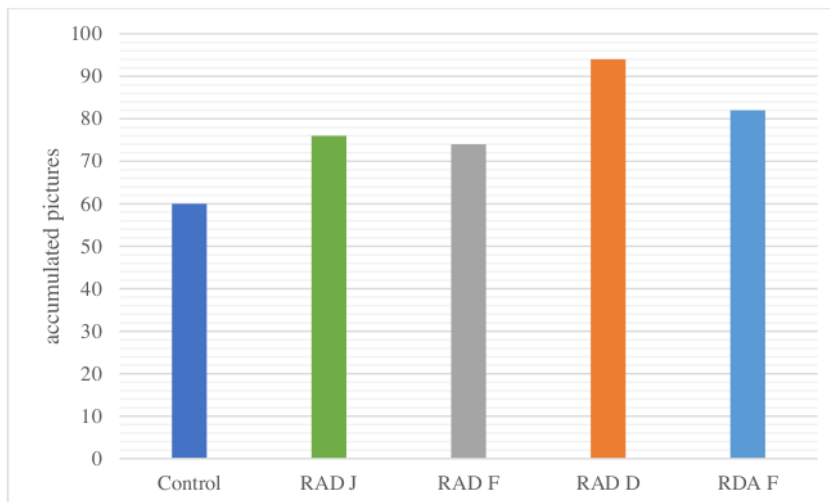


Figure 3. Cumulative number of frames in 3 minutes crossed by rats in the open field test after irradiation with a UHF frequency of 860 MHz or isolation ("one-way ANOVA" (\* $p \leq 0.05$ )).

#### Anxiety test (two-compartment box)

Figures 4, 5 show the results of the experiment in the two-compartment box (light/dark) with male rats subjected to 860 MHz radiation. A significant difference was found in the immobility time, in the number of total transitions and in the residence time with respect to the control. Figure 4 showed a significant difference between the RAD J group and the control group, so there is a tendency to spend less time exploring the target compartment, showing that the RAD F and RAD D groups have a tendency to explore an environment new.

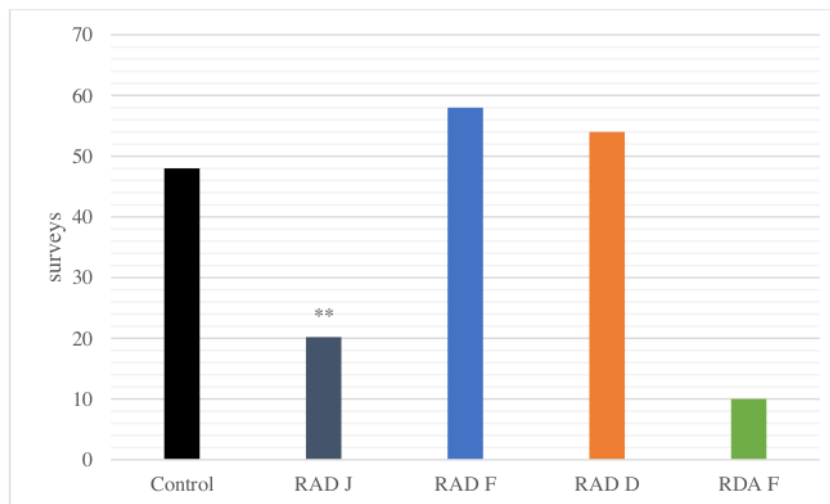


Figure 4. Exploratory surveys performed by rats in the white compartment in the anxiety test in the two-compartment box after irradiating them with a UHF frequency of 860 MHz. (One-way ANOVA, (\* $p \leq 0.05$ )). \*\* Significant difference between the RAD J group compared to the RAD F group.

In Figure 5, the RAD F and RAD D groups show a tendency to make greater transitions from the white to the black compartment and vice versa compared to the TG group,

which represents greater exploratory behavior, with the RAD J and RDA F groups showing the opposite behavior.

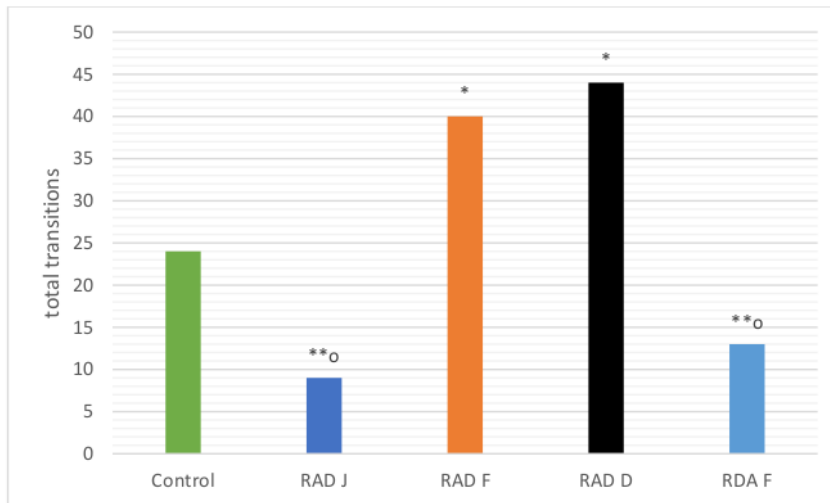


Figure 5. Number of total transitions that rats made from the black to the white compartment and vice versa in the anxiety test in the two-compartment box after irradiating them with a UHF frequency of 860 MHz. (One-way ANOVA ( $*p \leq 0.05$ )). \* Significant difference between the RAD F and RAD D group compared to the Control group. \*\* Significant difference between the RAD J and RDA F group compared to the RAD F group. ° Significant difference between the RAD J and RDA F group compared to the RAD D group.

#### Depression test (forced swim)

Figures 6, 7 and 8 show the behavior of male rats in the forced swim test; A significant difference was found in the swimming time of the rats irradiated in June and those irradiated for 15 days with respect to the control. Figure 6 shows an increase in swimming time in the RAD F and RDA F groups, and a decrease in swimming time in the RAD J groups with respect to TG.



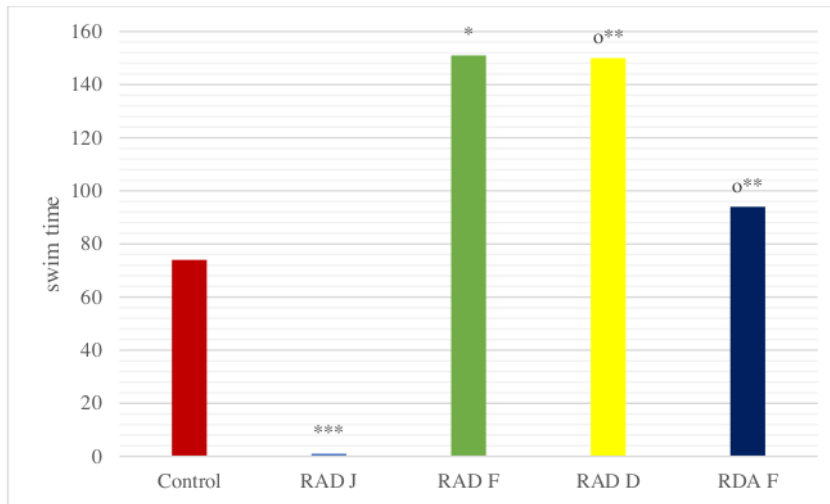


Figure 6. Swimming time of the rats in the forced swimming test after irradiating them with a UHF frequency of 860 MHz. (One-way ANOVA ( $*p < 0.05$ )). \* Significant difference between the RAD J and RAD F group compared to the Control group. \*\* Significant difference between the RAD J, RAD D and RDA F group compared to the RAD F group. ° Significant difference between the RDA F and RAD D group compared to the RAD J group.

Figure 7 shows an increase in the immobility time in the RAD J and RDA F groups, and a decrease in the immobility time in the RAD F and FRD15 groups with respect to TG. The time of immobility is analogous to depression since there is no struggle to get out of a stressful environment, so the RAD J groups that present a significant difference have a tendency to present a state of depression.

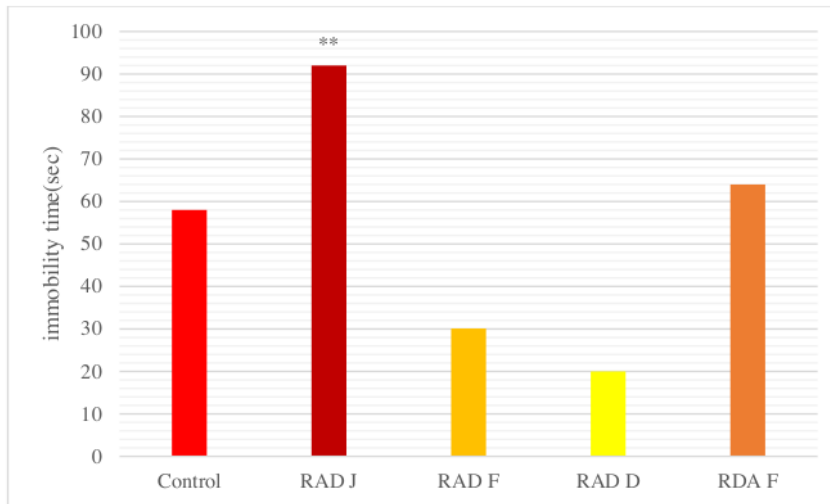


Figure 7. Immobility time in the forced swim test of rats after irradiation with a UHF frequency of 860 MHZ. (One-way ANOVA ( $*p \leq 0.05$ )). \*\* Significant difference between the RAD J group compared to the RAD D group.

Figure 8 shows an increase in the scaling time in the RAD J and RAD D groups, and a decrease in the scaling time in the RAD F and RDA F groups with respect to TG. The escalation time represents the fight time to get out of a stressful environment, so the RAD J and RAD D groups presented greater fight.

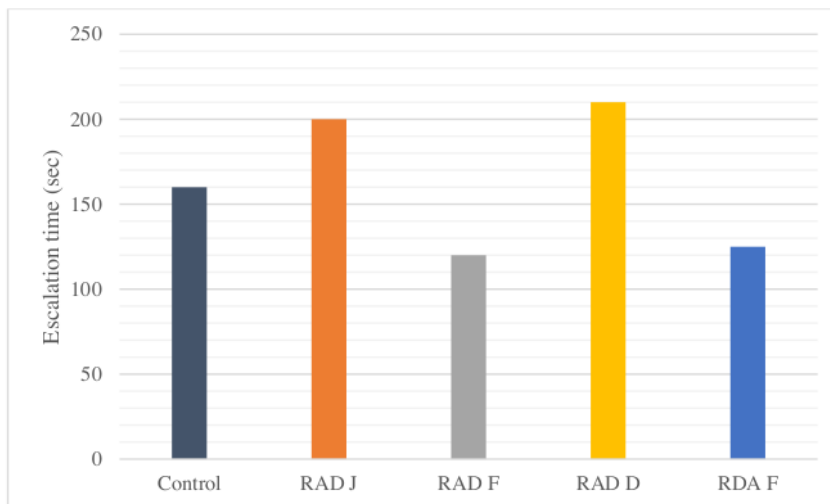


Figure 8. Escalation time in the forced swim test of rats after irradiation with a UHF frequency of 860 (one-way ANOVA, (\* $p \leq 0.05$ )).

## Discussion

Although these experiments measure behavioral responses, the locomotor activity test in the open field allows us to evaluate the alertness and walking ability of rats, since their natural instinct is to explore new places (Carbajal et al., 2007). No significant differences were found in this test, so radiation and isolation do not affect locomotor activity of rodents or exploration behavior in a novel environment.

In this test, a significant increase was found in these variables in the animals that received radiation with respect to the control, which indicates a state of anxiety generated by the radiation and indirectly supports Gray's theory, which states that fear and frustration are emotional responses that involve the same neural circuits that are activated by electrical stimulation of the amygdala and produce behaviors related to fear and anxiety (Kamenetzky et al., 2011).

In addition, in situations of stress, a cascade of physiological responses is activated in the body that help it to adapt. Isolation causes important changes in behavior, it is characterized by an increase in spontaneous motor activity as well as motor responses to adapt to a new environment and a greater secretion of glucocorticoids (Moragrega, 2005), the sympathetic nervous system is also activated inducing the secretion of catecholamines in the adrenal medulla and thus producing the effects on cardiovascular function and metabolism; on the other hand, corticotropin-releasing hormone is released from the hypothalamus, which activates the pituitary gland to release adrenocorticotropin hormone (ACTH) and finally, it acts on the adrenal cortex to release glucocorticoids (GC), whose effects they are aimed precisely at increasing the availability of energy in certain parts of the body (Martínez, 2007), this response corresponds to the activation of the hypothalamus - pituitary - adrenal glands axis.

On the other hand, the rats irradiated in February (winter) as well as those that were exposed to radiation for 15 days show a greater exploratory behavior due to the increase

in the number of transitions compared to the control. An intense exposure to electromagnetic radiation can cause the release of corticosteroids into the bloodstream, probably due to thermal stimulation of the pituitary gland, so that any change in temperature over a specific region of the brain can be a sufficient cause to trigger a stress response, which goes to release ACTH. Glucocorticoids released into the circulation promote the mobilization of stored energy and potentiate the many effects mediated by the sympathetic nervous system (Redolar, 2011), which would lead to increased activity in rats. Just as cortisol levels that vary between day and night are involved, this cortisol variation is a complex combination of rapid oscillations that last a few hours in a circadian manner (Zavala, 2015); these circadian cycles serve to synchronize behavior and bodily changes to changes in the environment (Pérez, 1998), thus making rodents more active at night and reaching maximum levels of corticosterone during the afternoon, just before waking up (Zavala, 2015).

Regarding the state of depression of the rats evaluated with the forced swimming test, there was a significant decrease in swimming time in the group irradiated in summer with respect to the group irradiated in winter and with the control group; which indicates that the group irradiated in summer presented depression, while those in winter did not show this behavior. According to the interpretation of active behaviours proposed by Detke in 1995 (Martínez et al., 2012), it is possible that in males, the activation of both serotonergic and noradrenergic neurotransmission systems occurs, which are the involved in depression. However, both batches received the same treatment, only varying the season of the year, so that the light conditions during the different seasons of the year influence the behavior of the rodents, since in summer there are 16 hours of light and 8 hours of darkness, rodents develop less depressive-type behaviors, while in an environment with 8 hours of light and 16 hours of darkness (Vanderbilt, 2015) that is, with fewer hours of light, as happens during winter, it causes depressive phenomena and causes an increase in corticosterone levels (Tara et al., 2012), therefore a greater activity by rodents is presented in the results obtained, the opposite happening with summer rats.

According to a study conducted by Navid S. et al. (2017) on neonatal mice, when melatonin was applied to spermatogonial stem cells of mice in vitro, colonization of cells

that received melatonin was found to be significantly higher than in the control group that did not. In another study conducted in China, it was stated that melatonin stimulated the production of GDNF in Sertoli cells and provided goat spermatogonial stem cell proliferation (Niu B, 2016).

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