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Effects of chronic single – and dual–band radiofrequency exposure on spatial learning and memory retention in growing male rats

Efectos de la exposición crónica a radiofrecuencias de banda simple y doble sobre el aprendizaje espacial y la retención de la memoria en ratas macho en desarrollo

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ABSTRACT

Radiofrequency electromagnetic fields have become increasingly present in daily life due to the widespread use of wireless technologies. However, their long–term effects on cognitive functions remain insufficiently understood, particularly under repeated multi–frequency exposure conditions. This study investigated the effects of chronic exposure to 800 MHz, 900 MHz, and sequential 800/900 MHz RF fields on spatial learning and memory in growing male Wistar rats. Animals were divided into four groups: sham–exposed, 800 MHz, 900 MHz, and sequential 800/900 MHz exposure. Exposures were administered 6 days per week for 4 months. Spatial learning and memory were assessed using the Morris water maze. During the acquisition phase, rats underwent four trials per day for five consecutive days, and escape latency was recorded. Memory retention was evaluated during the probe trial by measuring time spent in the target quadrant and platform–site crossings. Escape latency significantly decreased across training days in all groups, indicating preserved acquisition learning. No significant effect of exposure was observed during the acquisition phase. In contrast, probe trial analysis revealed a significant reduction in target–quadrant time in the sequential 800/900 MHz group compared with sham–exposed and 800 MHz animals, whereas platform–site crossings were not significantly affected. Overall, chronic radiofrequency exposure did not impair spatial learning acquisition, but sequential exposure to 800 and 900 MHz was associated with reduced spatial memory retention.

Key words: Radiofrequencies; Radiofrequency electromagnetic fields exposure; Behavioral testing; Spatial learning; Morris water maze

RESUMEN

Los campos electromagnéticos de radiofrecuencia están cada vez más presentes en la vida cotidiana debido al amplio uso de las tecnologías inalámbricas. Sin embargo, sus efectos a largo plazo sobre las funciones cognitivas aún no se comprenden completamente, especialmente en condiciones de exposición repetida a múltiples frecuencias. El presente estudio investigó los efectos de la exposición crónica a campos electromagnéticos de radiofrecuencia de 800 MHz, 900 MHz y exposición secuencial de 800/900 MHz sobre el aprendizaje espacial y la memoria en ratas macho Wistar en crecimiento. Los animales fueron divididos en cuatro grupos: control simulado, 800 MHz, 900 MHz y exposición secuencial 800/900 MHz. Las exposiciones se realizaron 6 días por semana durante 4 meses. El aprendizaje espacial y la memoria fueron evaluados mediante el laberinto acuático de Morris. Durante la fase de adquisición, las ratas realizaron cuatro ensayos por día durante cinco días consecutivos y se registró la latencia de escape. La retención de la memoria se evaluó durante la prueba de sonda mediante la medición del tiempo pasado en el cuadrante objetivo y el número de cruces sobre la ubicación previa de la plataforma. La latencia de escape disminuyó significativamente a lo largo de los días de entrenamiento en todos los grupos, lo que indica una preservación del aprendizaje de adquisición. No se observó un efecto significativo de la exposición durante la fase de adquisición. En contraste, el análisis de la prueba de sonda mostró una reducción significativa del tiempo pasado en el cuadrante objetivo en el grupo de exposición secuencial 800/900 MHz en comparación con los animales control y los expuestos a 800 MHz, mientras que el número de cruces de la plataforma no mostró diferencias significativas. En conjunto, la exposición crónica a radiofrecuencia no alteró el aprendizaje espacial durante la adquisición, pero la exposición secuencial a 800 y 900 MHz se asoció con una disminución de la retención de la memoria espacial.

Palabras clave: Campos electromagnéticos de radiofrecuencia; Exposición a radiofrecuencia; Aprendizaje espacial; Retención de la memoria; Laberinto acuático de Morris

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INTRODUCTION

Nowadays, human beings and animals are continuously surrounded by radiofrequency electromagnetic fields (RF–EMFs) from multiple sources including mobile phones, Wi–Fi signals, and antennas [1, 2]. Consequently, increasing attention has been paid to "electromagnetic pollution" as a potential environmental stressor, particularly in young organisms that may experience early and repeated exposure [2, 3].

Experimental studies indicate that RF–EMF exposure may affect biological systems, although reported findings remain inconsistent depending on the experimental model and exposure conditions [3]. Previous investigations have described alterations in oxidative status, tissue biomarkers, physiological responses, and neurobehavioral functions following RF–EMF exposure [4, 5, 6]. However, variations in dosimetry, exposure duration, and experimental protocols complicate the interpretation of these effects and highlight the need for standardized approaches [7].

The nervous system is considered one of the main targets of RF–EMF exposure because cognitive processes such as learning and memory depend on synaptic plasticity and are particularly sensitive to oxidative stress [1, 3]. Among the available behavioral approaches, the Morris water maze (MWM) is widely recognized as a reliable method for assessing spatial learning and memory in rodents [8, 9, 10]. Nevertheless, studies investigating RF–EMF effects on cognition have produced heterogeneous results, with some reporting behavioral impairments while others found no significant alterations [11].

Another issue is ecological relevance. In everyday life, RF–EMF exposure rarely comes from a single source or a single frequency band. Instead, organisms are more likely to encounter different signals over time. For this reason, including more than one frequency band within the same experimental design may offer a more realistic approximation of actual exposure conditions [2, 7].

In this context, the present study assessed the effects of whole–body RF–EMF exposure on behavioral outcomes in growing male albino Wistar rats. Spatial learning and memory were examined in the MWM after chronic exposure to 800 MHz, 900 MHz, or sequential dual–band exposure (800 MHz followed by 900 MHz). Since the exposure system relied on a single RF–EMF generator, the two frequencies could not be delivered simultaneously. A sequential exposure design was therefore adopted to model repeated multi–band exposure under controlled conditions.

MATERIAL AND METHODS

Animals

Forty (40) Male albino–Wistar rats (*Rattus norvegicus*) were obtained from Pasteur Institute, animal facility (Algeria). The animals were kept in standard cages under controlled room temperature and natural photoperiod (5 rats per cage). Tap water and food pellets were provided *ad libitum*. After 15 days (d) of acclimation, animals were randomly assigned to four groups (n = 10 each). At the start of the exposure phase, body weight averaged about 145 g (range: 110–169 g).

Radiofrequency exposure system

To conduct the experiment, RF–EMF exposure was generated using a signal generator (Eurotek SG1000, 1 GHz, Italy), a power supply (Mean Well NES–100–24, China), a RF amplifier and antenna (Siemens S30861–Q2253–X–14/01, Germany), and eight non–metallic cages (40 × 28 × 17 cm, L × W × H), each housing five animals (FIG. 1). System performance was verified before the first exposure session.

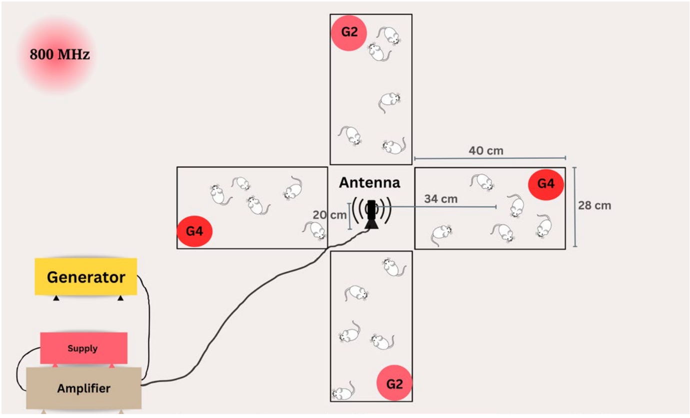

FIGURE 1. Schematic illustration of the RF–EMF exposure system. G 2 and G 4 were exposed to 800 MHz for 3 h·d^{-1}, whereas G 3 and G 4 were subsequently exposed to 900 MHz for an additional 3 h·d^{-1}

Experimental design

During exposure, animals were placed in a secondary chamber maintained under the same environmental conditions as the main housing room. Briefly, animals were divided into four groups (G):

- G 1: Sham–exposed controls
- G 2: 800 MHz–exposed group (3 h·d^{-1})
- G 3: 900 MHz–exposed group (3 h·d^{-1})
- G 4: Sequential dual–frequency exposure group (800 MHz for 3 h·d^{-1} followed by 900 MHz for 3 h·d^{-1}; total: 6 h·d^{-1})

Animals were exposed 6 d per week for 4 months. The fourth group was included to evaluate the effects of repeated exposure to two RF frequency bands within the same experimental protocol. This design was intended to better reflect real–life exposure conditions, where organisms are typically exposed to multiple RF sources rather than a single signal. Because the exposure system used a single RF–EMF generator, simultaneous exposure to both frequencies was not possible. Animals in this group were therefore exposed sequentially to 800 MHz followed by 900 MHz under identical experimental conditions.

Spatial learning and memory were evaluated using the MWM on d 110 of the protocol (FIG. 2).

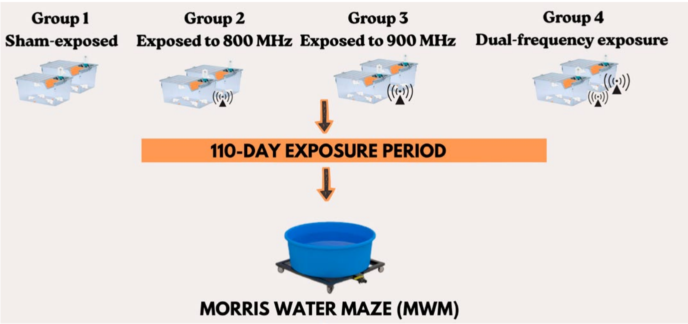

FIGURE 2. Experimental design of chronic RF–EMF exposure

Morris water maze task

The MWM is a widely used behavioral test for evaluating spatial learning and memory in rodents. The task is based on the animal's ability to use distal visual cues to locate a hidden submerged platform in a circular pool. Spatial learning is assessed during repeated acquisition trials, whereas memory retention is evaluated in a probe trial after platform removal [10, 12].

In this test a blue circular tank (140 cm diameter, 50 cm height) filled with dark water, to a depth of 25 cm, was used. The maze was placed in a room with multiple visual cues for spatial orientation. A transparent escape platform (10×10×22 cm) was submerged 3 cm below the water surface (FIG. 3) [13, 14].

Rats underwent four acquisition trials per d for five d. For each trial, animals were released from one of four randomized starting positions while the hidden platform remained fixed. Each trial lasted a maximum of 60 second (s), and escape latency was recorded. Rats that failed to locate the platform within the allotted time were gently guided to it and allowed to remain there for 10 s.

A probe trial was performed on d 5. In this test phase, the platform was removed and rats were allowed to swim freely for 60 s. Spatial memory retention was assessed by measuring the

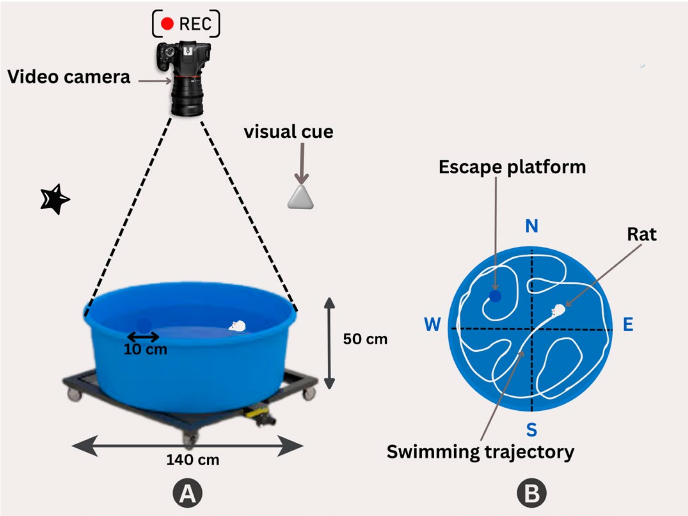

FIGURE 3. Morris water maze (MWM) apparatus: 3D representation (A), top view (B)

time spent in the target quadrant and the number of crossings over the platform location.

All experiments were conducted under identical environmental conditions, including constant lighting and a water temperature of 22 ± 1°C [8, 12].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27. Data were checked for normality and homogeneity of variance before analysis. Results are presented as mean ± SEM, and statistical significance was set at $P<0.05$.

Escape latency during the acquisition phase was analyzed using a linear mixed–effects model (LMM), with "Exposure" as the between–subject factor and "Day" as the repeated factor. For the probe trial, time spent in the target quadrant and platform–site crossings were compared among groups using one–way ANOVA followed by Tukey's post hoc test when appropriate. Welch and Brown–Forsythe tests were also used to confirm result consistency.

RESULTS AND DISCUSSION

Acquisition phase

During this phase, spatial learning was evaluated by measuring escape latency to reach the hidden platform over five consecutive days, with four trials conducted per day. Escape latency data were analyzed using an LMM, with "Exposure" as the between–subject factor, "Day" as the repeated within–subject factor, and animal identity included as a random effect.

The analysis revealed a significant main effect of Day (F (4, 73.651) = 33.442, $P<0.001$), indicating that escape latency changed significantly across training days and confirming progressive spatial learning in all groups. In contrast, the main effect of Exposure was not significant (F (3, 21.036) = 1.423, $P = 0.264$), indicating that overall acquisition performance did not differ significantly among exposure conditions. Likewise, the Exposure × Day interaction was not significant (F (12, 67.749) = 1.276, $P = 0.253$), suggesting that the pattern of acquisition across days was comparable among groups (TABLE I).

TABLE I
Linear mixed–effects model analysis of escape latency during the acquisition phase of the Morris water maze

Effect	Numerator df	Denominator df	F	*P*–value
Exposure	3	21.036	1.423	0.264
Day	4	73.651	33.442	< 0.001***
Exposure × Day	12	67.749	1.276	0.253

Note: *** $P<0.001$. Morris water maze: MWM

Overall, these findings indicate that all groups acquired the task over time, with no evidence that RF–EMF exposure significantly altered either overall escape latency or the temporal trajectory of spatial learning during the acquisition phase (TABLE II, FIG. 4).

TABLE II
Mean escape latency (± SEM, s) across the 5 acquisition days in the Morris water maze

Day	Sham–exposed	800 MHz	900 MHz	Sequential 800+900 MHz
1	23.72 ± 2.14	34.21 ± 8.10	25.72 ± 3.05	39.62 ± 6.84
2	13.19 ± 2.25	15.93 ± 1.89	22.12 ± 4.18	16.87 ± 4.12
3	8.66 ± 0.82	14.14 ± 2.86	9.16 ± 0.70	10.21 ± 0.57
4	11.16 ± 1.13	9.18 ± 2.44	10.12 ± 1.73	7.88 ± 0.60
5	7.25 ± 1.14	11.50 ± 2.17	8.62 ± 1.30	7.62 ± 0.88

Morris water maze: MWM

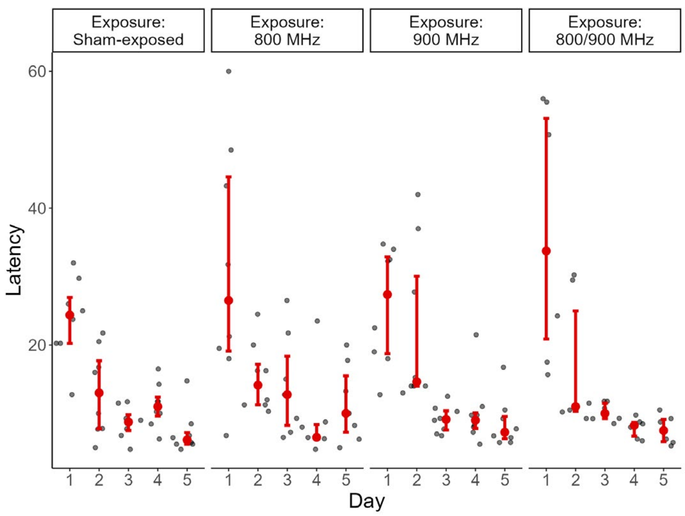

FIGURE 4. Escape latency during the acquisition phase of the Morris water maze. Gray dots represent individual values, and red symbols indicate group means ± SEM

The present study indicates that chronic RF exposure did not significantly alter acquisition learning in the MWM. Escape latency decreased across training days in all groups, while neither the main effect of "Exposure" nor the "Exposure × Day" interaction reached statistical significance. This suggests that spatial learning remained globally preserved under the present exposure conditions.

The findings are consistent with previous studies showing no clear cognitive or behavioral effects after 900 MHz RF–EMF exposure in rats. Dubreuil *et al.* [15], for instance, did not observe behavioral changes after head–only exposure to 900 MHz electromagnetic fields. Similar results were also reported by Ammari *et al.* [11] and Klose *et al.* [16] using different behavioral tests, including the MWM, radial–arm maze, and open–field test.

Probe trial

To assess spatial memory retention after completion of the acquisition phase, the time spent in the target quadrant during the probe trial was compared among exposure groups. One–way ANOVA revealed a significant overall group effect (F (3,25) = 7.860, *P* = 0.001), indicating that retention performance differed significantly across experimental conditions. Post hoc Tukey comparisons showed that rats in the sequential 800/900 MHz group spent significantly less time in the target quadrant than sham–exposed animals (*P*<0.001) and the 800 MHz group (*P* = 0.032). No other pairwise comparison reached statistical significance.

These findings suggest that sequential dual–frequency RF exposure was associated with impaired spatial memory retention, whereas single–frequency exposure did not produce a statistically significant deficit under the present conditions (TABLE III, FIG. 5).

TABLE III
Probe trial performance in Morris water maze (Mean ± SEM)

Exposure group	n	Time spent in the target quadrant (s)	Platform–site crossings
Sham–exposed	8	32.74 ± 1.67	7.25 ± 0.59
800 MHz	7	27.82 ± 2.67	6.29 ± 1.19
900 MHz	8	25.70 ± 1.70	8.00 ± 0.71
Sequential 800/900 MHz	6	19.26 ± 1.44	5.83 ± 0.54

Note. For time spent in the target quadrant, one–way ANOVA showed a significant overall group effect (F (3,25) = 7.860, *P* = 0.001). Tukey's post hoc test indicated significant differences between sham–exposed vs sequential 800/900 MHz (*P*<0.001) and 800 MHz vs sequential 800/900 MHz (*P* = 0.032). For platform–site crossings, the overall ANOVA was not significant (F (3,25) = 1.447, *P* = 0.253). Morris water maze: MWM

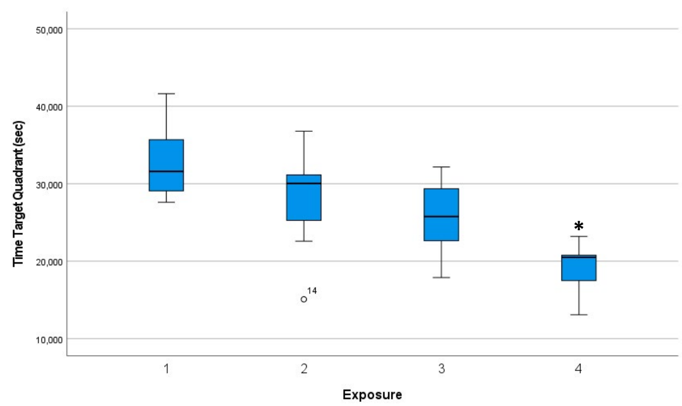

FIGURE 5. Time spent in the target quadrant during the probe trial in sham–exposed (1) and RF–exposed rats (2, 3 and 4). The sequential 800/900 MHz group showed a lower target–quadrant time than the sham–exposed and 800 MHz groups

To further characterize probe trial performance, the number of platform–site crossings was also analyzed among exposure groups. One–way ANOVA showed no significant group effect (F (3,25) = 1.447, *P* = 0.253). This result was consistent with robust tests of equality of means, which were also non–significant (Welch, *P* = 0.152; Brown–Forsythe, *P* = 0.263). Although descriptive differences were observed, with the highest mean value in the 900 MHz group and the lowest in the sequential 800/900 MHz group, these variations did not reach statistical significance (TABLE III, FIG. 6).

Overall, the probe trial results indicate that the most prominent exposure–related effect concerned time spent in the target quadrant, whereas platform–site crossings appeared less sensitive in detecting between–group differences.

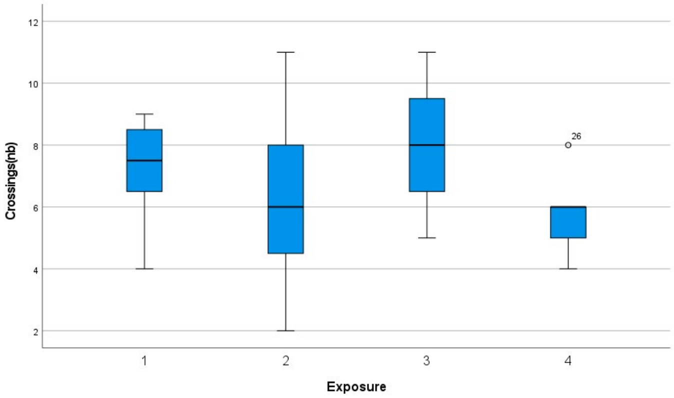

FIGURE 6. Number of platform–site crossings during the probe trial in sham–exposed and RF–EMF exposed rats. No significant between–group difference was detected for this parameter (° abberant value)

The probe trial showed a more selective effect, as the sequential 800/900 MHz group spent less time in the target quadrant than sham–exposed and 800 MHz animals, suggesting altered memory retention rather than impaired acquisition. Similar findings have been reported previously, including altered spatial memory after RF exposure in the Morris water maze [17]. However, results in the literature remain inconsistent and may depend on exposure conditions and behavioral endpoints [7, 18].

The poorer performance observed in the sequential exposure group should nevertheless be interpreted cautiously, since this group was exposed for a longer daily duration than the single–frequency groups. Therefore, it remains unclear whether the observed effect was related to dual–frequency exposure, longer exposure time, or both. In addition, the absence of significant differences in platform–site crossings suggests that the effect was relatively modest and endpoint–dependent.

CONCLUSION

In summary, the present findings indicate that chronic RF–EMF exposure did not significantly alter acquisition learning in the MWM, as all groups improved across training days and showed comparable learning trajectories. In contrast, the time spent in the target quadrant was significantly reduced in the sequential 800/900 MHz group during the probe trial, suggesting impaired spatial memory retention under this exposure condition. The absence of a significant difference in platform–site crossings indicates that not all memory indices were equally sensitive. Overall, these results support the view that RF–related cognitive effects, when present, may emerge more clearly at the level of memory retention than during the acquisition phase itself.

Declaration of competing interest

No competing interests

Declaration of generative AI and AI–assisted technologies

The authors utilized ChatGPT 5 and QuillBot to enhance the linguistic quality. All content was reviewed and edited as needed by the authors, who take full responsibility for the final version of the publication.

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