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Title: The Effect of 12 Hz Extremely Low-Frequency Electromagnetic Fields on Visual Memory of Male Macaque Monkeys

Short title: The Effect of ELF on Visual Memory

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Abstract

Introduction: Today, humans leave in a world surrounded by electromagnetic fields. Numerous studies have been carried out to discover the biological, physiological, and behavioral effects of electromagnetic fields on humans and animals. Given the biological similarities between monkeys and humans, the goal of the present research was to examine Visual Memory (VM), hormonal, genomic, and anatomic changes, and changes of the amygdala function in the male rhesus macaques who exposed to extremely low-frequency magnetic fields (ELF/MF).

Materials and methods: Four male Rhesus Macaques (Macaca mulatta) were used. For the behavioral tests the animals were needed to be fasting for 17 hours, and for the behavioral tests such as visual memory, cooperation of the animal was necessary. Using the radiation protocol, two of the monkeys were exposed to 12-Hz electromagnetic fields with a magnitude of 0.7 μT (electromagnetic radiation) four hours a day for a month. Before and after the exposure, a visual memory test was conducted using a coated device (visible reward) on a movable stand. Ten mL of blood was obtained from the femoral artery of the monkeys and half of it was used to examine cortisol serum levels using MyBioSource kits (made in the USA). The other half of blood samples were used to extract lymphocytes for assaying expressions of Glucocorticoid Receptor (GR) genes before and after radiation using the PCR method. Anatomic studies of the amygdala were carried out based on pre- and post-radiation Magnetic Resonance Imaging (MRI).

Findings: Research results indicated that visual memory in male primates increased significantly at the 12Hz frequency. Hormonal analysis at the 12Hz frequency showed a decrease in cortisol serum levels. However, visual memory and serum cortisol levels did not change considerably in male primates in the control group. There was no considerable amygdala volumetric difference at the 12 Hz frequency. The expression of the GR genes decreased at 12Hz compared to the control group.

Conclusion: In short, these results indicated that ELF may have a beneficial value for memory enhancement as indicated by the fact that exposure to the 12 HZ ELF can enhance visual memory. This may be due to a decrease in plasma cortisol, and/or expression of GR genes. Moreover, direct involvement of the amygdala in this regard cannot be recommended.

Key Words: ELF; Cortisol; Glucocorticoid receptor; Visual memory; Rhesus monkey
Highlights
The effects of Extremely Low-Frequency Electromagnetic Fields (ELF/EMF) of 12 Hz on monkeys. The results showed reduction in the serum cortisol levels. The expression of GR genes also was decreased. The amygdala anatomical area changes were not significant in the experimental group. In the experimental group, visual memory (delay of 30- and 60-seconds evaluation) improved at a frequency of 12 Hz.

Language Summary
Extremely Low-Frequency Electromagnetic Fields are one of the most important factors affecting the humans. This study aimed to determine the fields of 12 Hz frequency on the visual memory changes of male monkeys. The importance of research is due to the cognitive similarity of the monkey to humans. The findings of the research can be attributed to humans. Behavioral, hormonal, genetic, and anatomical studies indicated improvement in visual memory (test monkeys versus control monkeys). This study demonstrates the effect of the 12-Hz frequency on the monkey's visual memory. Researchers can use frequency 12- Hz on other cognitive indices.
1. Introduction

The use of electronic devices in industrial processes, research projects, energy generation and distribution, new transportation technologies, consumer goods, and medical applications have increased the exposure of humans and animals to electromagnetic fields. Based on the research of electromagnetic fields and electric fields, high-pressure towers are known to be one of the most important factors. These fields have different effects on animals and humans (Aliyari et al., 2018a; Kazemi et al., 2017). During the past decade, numerous studies have been carried out on the effects of low-frequency electromagnetic radiations on the function of different parts of the nervous system and memory in humans and animals. Although important experiments have been conducted on humans to examine the effect of electromagnetic fields, more precise and comprehensive research has been undertaken on animal models. An important animal model for scientific research is Macaca mulatta, which is known as the rhesus macaque (monkey) (Mitchell and Leopold, 2015; de Lorge and Grissett, 1977; Fabbri-Destro and Rizzolatti, 2008). Cognitive-behavioral investigation is among the modern studies on this animal model. Moreover, since 98% of human and rhesus macaque genes are similar, researchers used the animal model to conduct various cognitive tests such as visual memory tests (Fang et al., 2011; Kanthaswamy et al., 2013; Baharara and Zahedifar, 2012b). Electromagnetic fields might leave different effects on organisms, while extremely low-frequency electromagnetic fields lead to neuron stimulations. The effects of magnetic fields at low frequencies (ELF MF) on the activity of the human brain, has long been preoccupied in the minds of many researchers (Marino and Becker, 1977; Kula et al., 2002; Sobczak et al., 2002; Al-Akhras et al., 2006; Zare et al., 2005). Visual memory is among the important functions of the nervous system, which is severely influenced by environmental factors. Two of the environmental factors affecting this function are stress and anxiety that can lead to visual memory dysfunction. Magnetic fields affect the activity of brain cells and brain waves as proved in extensive research on human and animal models. ELF-EMF with different frequencies and magnitudes reduce or increase the strength of different brain frequencies. However, the magnitude and frequency of magnetic fields that cause a decrease or increase in waves are not known yet, and it is only found that people’s response depends on the duration of exposure to the field and effects of previous fields, and thus the sensitivity of humans to magnetic fields differs from person to person and requires extensive research (Cvetkovic and Cosic, 2009; Cvetkovic et al., 2008; Cook et al., 2002).
The amygdala plays a key role in response to fear. Save amygdala and memory consolidation in other areas of the brain are affected. Hippocampus and amygdala glucocorticoid receptors play an important role in the acquisition and consolidation of spatial and emotional information (Lynch, 2004; Mehrdad et al., 2008; Otmakhova et al., 2013).

There are contradictory findings about the effects of extremely low-frequency electromagnetic fields on the cortisol hormone. For example, with an extremely low-frequency (ELF) field with a magnitude of 0.13 μT the adrenal gland tissue (serum cortisol) decreased in guinea pigs at a frequency of 5Hz. Moreover, cortisol variations led to variations of sensory thresholds, memory, intelligence, and concentration. These effects manifest in the form of depression, fatigue, and rarely psychosis as well as changes in hearing, sense of taste, and olfaction due to a shortage of cortisol (Hampton et al., 2005; Phillips et al., 2006; Cook et al., 2002; Baharara and Zahedifar, 2012b).

The quantitative RT-PCR (reverse transcription-polymerase chain reaction) is a common method for this purpose. This technique is widely used with microarray to measure the expression of genes. Beta-actin is used as a local standard for the expression of target genes including the GR-receptors (Al-Bader and Al-Sarraf, 2005; Gantasala et al., 2013). The glucocorticoid receptor (GR) is a circulating active glucocorticoid transcription factor, which mediates its effects on different biological functions in the body. These receptors play an important role in nerve cells and glands. Expression of GR-receptors crucially affects learning and memory, especially in hippocampus cells. The stress system activates GR-receptors and memory re-consolidation in rat hippocampus (Conrad, 2005; Heijtz et al., 2010; Patel et al., 2008; Sandi, 1998). The monkey's memory was examined by behavioral-cognitive, hormonal, and genomic analysis.
2. Materials and Methods

2.1. Animals

Four adult male rhesus macaques (Macaca mulatta) 4-5 years of age (W: 4kg) were used. In this research, two monkeys were placed in the 12-Hz electromagnetic field. Two other monkeys were not in the field of electromagnetic fields, as control. The animals received all needed vaccines (hepatitis-B, HIV, and herpes). The monkeys were kept at the animal room laboratory of Neuroscience Research Center, Baqiyatallah University of Medical Sciences for 150 days for adaptation. The animal room condition was as follows: 12/12 hours dark and light with constant temperature (24±2°C) and adequate food and water (meal at 8, 12, and 16 O’clock and water was provided with scaled water nipples container in 1000 ml volume specifically designed for monkeys). All of the experiments were conducted according to the Baqiyatallah Medical University Medical Ethics Committee number 112-1394.

2.2. ELF Exposure Procedure

The ELF equipment consists of a wave generator that can generate different frequencies from 1 to 300 HZ, made by Dr. Jafargholi laboratory, Amir Kabir University of Technology, Tehran, Iran. This generator can produce a magnetic field with a magnitude of 0.7 µT in 160 cm diameter circle field. The ELF exposure was conducted for each primate as follows: each primate was transferred to the shielded room in a 1×1×1 m Teflon cage. The cage was standing in 50 cm distance from the ELF equipment and the wave generator became ON. The exposure was lasted 4 h/daily for four weeks each animal. The results of the present study are descriptive

2.3- Visual Memory Response Task

A device for recording (VM) behavior (visible) was designed for this test. The two coated dishes were on a movable stand (Cook et al., 2002; Richter-Levin and Akirav, 2000; Kazemi et al., 2017). The animals have entered the test after 17 hours of fasting. The primates were transferred to the behavioral test room separately, and the test was carried out in 2 phases (four weeks, ten times a day)

**Phase I:** The visual memory behavior recorder was placed before the primates, and the primate’s favorite reward (peanut) was randomly put in one of the dishes. After a delay of 30 seconds of
delay, the dish was presented to the animal on a movable stand. The animal was allowed to make only one attempt to pick the reward from one of the two dishes. In other words, the animal had to remember the dish containing the reward, and if it opened the wrong container, it would be deprived of the reward. This test was carried out three times a day for delay 30 seconds before the eyes of the primate. This method was repeated in all of the four phases of the visual memory behavior function test.

Phase II: To record visual memory behavior with a visible reward, the peanut was put in one of the coated dishes before the eyes of the animal, and the dish was presented to the animal following delay 60 seconds of delay on a movable stand. The second phase was repeated three times a day. (Constantinidis and Procyk, 2004; Kazemi et al., 2017).

2.4- MRI

The last phase of the tests consisted of magnetic resonance imaging (MRI) of primates’ brains. To this end, the first arrangements were made with Imam Khomeini MRI Center. The primates had to be fasting for 9 hours prior to the MRI. Moreover, the primates had been anesthetized with ketamine (10-20 mg/kg) prior to the MRI, and their brains were imaged using a 3T MRI device in the T1 and T2 phases with 3-mm sections of the axial, sagittal, and coronal regions (for better anatomic interpretation of the regions of concern). In the image interpretation phase, volumetric assessments of a special part of the memory including the right amygdala were analyzed in Image J (Shanthi and Singh). In the interpretation of MRI images of the primates, the T2 technique was used.

2.5- Hormonal Assays

To obtain the blood samples, the primates were put in the stabilized state of consciousness, and 10cc of blood was obtained from the femoral region (popliteal artery). Blood samples were obtained to measure neurotransmitter, hormonal and genomic factors before and after radiation and recovery phases. The blood samples were divided into two parts. The first part was centrifuged at 3000RPM for 5 minutes at a temperature of 4°C and the serum content was isolated. The serums isolated from blood samples were assayed to measure and compare variations of serum concentrations of hormones using diagnostic kits for cortisol (purchased from MyBioSource, USA,
with the 0.04-2000 ml/ng standard and unit range), and Variations of hormone concentrations were measured in the pre-radiation, post-radiation, and recovery phases.

2.6- Glucocorticoid- Receptor Assays

The second part of blood samples was used for cellular and molecular assays. To this end, after collecting the blood samples, blood lymphocytes were isolated using the Ficoll solution in a centrifuge that was used for 5 minutes at 1500RPM in the beginning followed by another 15 minutes at 2500 RPM. The isolated lymphocytes were tested to determine the expression of GR-receptor genes using the PCR technique. Using peripheral blood lymphocytes expressed receptors GR were analyzed by PCR. To assess the impact of the expression visual learning and visual memory of Glucocorticoid receptor (GR) genes involved in mature and immature monkey, the semi-quantitative reverse transcriptase-polymerase chain reaction (semi-RT-PCR) was utilized. As described earlier, the peripheral blood sample was collected from each animal in related time and the total mRNA was purified by the RNX-Plus kit (Cinna gen, Iran) under the manufacturer's guideline. The quantity and quality of each isolated RNA was evaluated using the nanodrop spectrophotometer (Thermos, USA) and agarose gel electrophoresis, respectively. After that, to synthesize cDNA from each sample, the Bioneer kit (Takara, Japan) was applied. briefly, 100 ng of each RNA sample was converted to cDNA by the master mix containing M-MLV reverse transcriptase, random hexamers, oligo dT, and related buffer. Finally, the GR - 2A gene expression was detected using PCR and related specific primer set (table 1). The mRNA expression of β-actin was surveyed as an internal control. All PCR reactions were performed in a thermocycler (Techne, UK) containing 1.5 μl cDNA, 0.2 mM of the deoxynucleic ide triphosphates (dNTPs), 2.5 mM MgCl2, 10 pmol of each primer and 1.5 U of Taq DNA Polymerase (Cinnagen, Iran). PCR program was 6 min initial denaturation at 94°C, 35 cycles of 45 s at 95°C, 45 s at 58°C for GR. To measure the density of amplicons, each PCR product was run on 2% agarose gel electrophoresis, stained by ethidium bromide, and visualized under UV gel document. Finally, the density of each Product band was measured by Image J software(Hillmann et al., 2000; Hosseini et al., 2015).
3. Results

Results of examining shows the percentage of correct answers than after irradiation the effects of ELF waves on visual memory and visual memory function of the primates revealed that in the B-F experimental monkeys (which was exposed to the 0.7 $\mu T$ field at 12Hz) visual memory (with a visible reward) changed considerably following the radiation. However, in the D-E control monkeys, visual memory did not change considerably (with a visible reward) (Fig.1, 2, 3). Average number of weekly replies in the control group and experiment12Hz/group.

Results of anatomical assays at the 12 HZ frequencies using the 0.7 $\mu T$ field surface analysis (pre and post-radiation) of the left amygdala of a primate considering anatomy in the Sagittal section of amygdala using Image J software (locations are indicated by the pointer) (Fig. 4,5, Table1). Average of two monkeys’ control and experiment.

Variations of cortisol under the impact of the 12Hz and the 0.7 $\mu T$ field in the B-F experimental monkeys decreased considerably following the radiation. Besides, changes of cortisol in the D-E control monkeys showed an inconsiderable following. The recovery of both primates involved restoration of their states to the pre-radiation phase (Fig6) (Average of two monkeys’ control and experiment.

Effects of radiation with the 12Hz and the 0.7 $\mu T$ field on the expression of glucocorticoid (GR) in B-F experimental monkeys showed that changes in expression of GR under the effect of the 12Hz wave and a field with a magnitude of 0.7 $\mu T$ in the B-F monkeys decreased following the radiation. In addition, variations of the GR gene in the D-E monkeys showed an inconsiderable. The recovery of both primates involved the restoration of their states to the pre-radiation phase (Fig 7). Average of two monkeys’ control and experiment.
4. Discussion

With the increased use of generators of electromagnetic fields in modern industrial societies study of the biological effect of extremely low-frequency electromagnetic fields (as important factors influencing environments) with different magnitudes has grabbed the attention of researchers in the past decades. The effects of low-frequency (12 Hz) electromagnetic waves with the 0.7 $\mu T$ magnetic field magnitude on cognitive changes in primates were studied. Results of studying the effects of ELF-MF waves on animal and human models are contradictory. According to research literature, the effects of electromagnetic fields depend on wave properties and radiation length as well as biological and physiological properties of humans and animals (Baharara and Zahedifar, 2012a; Wingenfeld and Wolf, 2015). Also, magnetic fields with a frequency of 50 Hz impair memory and learning. Results of research by other researchers have also revealed that ELF waves with 10 and 30 Hz frequencies and field magnitude of 2 $\mu T$ improved memory and learning in the experimental group rats significantly as compared to the control group (An et al., 2015; Casile, 2013; D’Angelo et al., 2015; Rimbach et al., 2014; Sakhnini et al., 2014). In this research, the effects of ELF on the most important memory cognitive factor were studied at two different frequencies which matched the delta (1 Hz) and alpha (12 Hz) brain wave frequencies. As we know, visual information reaching the occipital cortex, directly and indirectly, flows to the hippocampus, which plays a major role in learning processes and memory consolidation (especially spatial memory). Mammals use environmental signals and visual signals to learn and memorize a position in the space or their physical position in the space or the environment (Sakhnini et al., 2014; Kiorpes and Movshon, 2004).

Cognitive functions (such as attention) of visual memory increase processing speed and potentize relationships between different brain parts. Improvement of communications between different brain members, especially the hippocampus (the origin of memory), the prefrontal cortex (the origin of concentration and thinking), the cerebellum (controls conscious body movements), and optic tracts, is another outcome (Murphy et al., 1996; Kalin et al., 2004). Every learning process starts with attention and develops with the activation of the working memory. Without adequate information, the person experiences learning impairments. Attention is one of the important sublime mental activities and is one of the main dimensions of the cognitive structure. It plays a significant role in the structure of intelligence, memory, and perception (Basile and Hampton,
Results of cognitive tests show the percentage of correct answers than after irradiation including visual memory (visible) before and after radiation in the B-F primates at the 12-frequency showed that visual memory (visible) increased significantly after 30 and 60 seconds at the 12Hz frequency as compared to the pre-radiation state. Besides, the behavior displayed by the B-F monkeys at the 12Hz frequency was interesting, because the B-F monkeys was highly restless, reckless and noisy and everybody was complaining about its noisiness. However, it was very calm and careful after being radiated at the 12Hz frequency. The visual memory of this monkeys did not change significantly in control groups, and no significant difference was also observed in its behavior. In cognitive tests, attention and concentration play substantial roles in vision, and activity of mirror neurons is the result of careful observation. Mirror neurons are located in the F5 prefrontal region (Caggiano et al., 2013; Fabbri-Destro and Rizzolatti, 2008; Tallon-Baudry et al., 2004).

Research results indicated that in different phases of memorization numerous structures are involved in the brain. Therefore, there is no special memory center in the brain, but the role of the frontal lobe, hippocampus, and amygdala in memory is more significant. Studies on vision or the encoding phase are mostly centered on the left hemisphere, which is more involved in the retrieval of information (McLeod, 2007; Keller and Roberts, 2009; Newman et al., 2009; Tae et al., 2008). Amygdala substantially contributes to spatial memory and emotional learning, while GR receptors of the amygdala and hippocampus are greatly involved in the consolidation of spatial and emotional information (Chou et al., 2002; Sandi, 1998). Afterward, the amygdala was studied using MRI and the T2 technique based on sagittal sections of the D-E and B-F monkeys. Results of volumetric assessments of the left amygdala in the B-F monkeys (12Hz frequency) and the D-E (control groups) did not show a drastic change in the post-radiation phase as compared to the pre-radiation phase.

In humans/primates and rats, cortisol and corticosterone significantly contribute to the regulation of cognitive processes, respectively (Kazemi et al., 2017; Aliyari et al., 2018b; Tekieh et al., 2017). Other reports suggest that rats in stressful environments show an increase in plasma corticosteroid concentration and eventually learning and memory impairments. Also, exposure of rats to radio waves increases plasma corticosterone levels and results in spatial memory impairment (Akirav et al., 2004; Baker and Kim, 2002). Research results suggest that under the effect of ELF waves at
the 12 Hz frequency on the serum level of corticosterone, the level of this hormone decreased in rats (Mahdavi et al., 2014). Concerning levels of cortisol in the B-F monkeys at the 12Hz frequency and the D-E control monkeys revealed that variations of cortisol in the B-F monkeys (12Hz frequency) decreased following radiation. This finding is also in line with findings reported by other researchers. However, Changes in cortisol in control monkeys did not change. Restoration of hormonal changes to the pre-radiation state in the recovery phase is reflective of unstable changes, which can be both positive and negative. If the aim of radiation is to potentate, this result is a negative outcome, because it is unstable. However, if radiation is meant to destruct, it is a positive outcome as it is not stable and is transient. It is worth stating that cortisol is the most important hormone secreted by the adrenal cortex in humans in response to stress factors. When the brain considers a factor to be a threat factor (stress factor), the amygdala is activated and stimulates the hypothalamic par ventricular nucleus and the CRH hormone is discharged by the neurons of this nucleus into the blood (Balbo et al., 2010; Sandi, 1998).

It is worth stating that glucocorticoid and glutamate systems of the brain are among the most important parts of the nervous and hormonal systems of the body, which are influenced by environmental conditions. Since the expression of gene and receptor proteins of these two systems in the peripheral blood lymphocytes are similar to the nervous system and change under the effect of ELF waves, these two properly manifest the performance of the aforementioned two systems. The expression of GR genes was tested using the PCR technique. White blood cells in monkeys are mostly formed of lymphocytes and GRs in lymphocyte cells are similar to the GRs in neurons of the central nervous system. Therefore, frequency (%) of lymphocytes in the blood of monkeys allows for examination of expression of GR genes as well as examination of the effects of ELF waves on the two receptor genes. Expression of the GR genes is completely associated with cognitive behavior including memorization and learning (Conrad, 2005; Heijtz et al., 2010; Magee et al., 2002; Vugmeyster et al., 2004). Beta-actin is widely used as a local standard in biological research regardless of animal type and tissue type. GR genes beta-actin was used as an internal control (Al-Bader and Al-Sarraf, 2005; Gantasala et al., 2013). One of the most important functions of stress hormones is excessive activity of the brain glutamate system, which is followed by extensive destruction of different brain regions. Therefore, researchers believe that a decrease in memory following chronic stress is caused by the effects of stress hormones on the increased

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activity of the glutamate system. Previous research also suggested that glucocorticoids affect many cognitive processes. For example, acute administration of compounds such as corticosterone increases long-term memory consolidation in a dose-dependent manner (Phillips et al., 2006; Sandi, 1998; Pipa et al., 2009). The effect of ELF waves on the expression of the GR gene in the B-F monkeys (12Hz) was studied with the 0.7 μT field. Results of investigations showed that GR gene expression in the B-F primate (12Hz frequency) decreased slightly following the radiation and GR gene expression in the control group did not change. Besides, the recovery involved restoration to the pre-radiation state. One of the mechanisms of action of glucocorticoids is the alteration of gene expression following activation of intracellular receptors. The GR is widely scattered in the brains of reptiles, and its concentration is higher in the hippocampus, par ventricular nucleus, and hypothalamus. Recent research indicated that the effects of glucocorticoids on memory processes are efficacious. These quick effects are seemingly caused by the interaction of glucocorticoids with several neurotransmitters in the brain. Hence, effects of glucocorticoids on the memory and memory consolidation may be exerted via excitatory amino acids and their receptors (Finsterwald and Alberini, 2014; Conrad, 2005; Patel et al., 2008). On the other hand, the role of calcium ions and calcium channels in memory is also confirmed, because glucocorticoids increase the concentration of calcium ions and activation of calcium channels. Therefore, one of the mechanisms involved in the influence of glucocorticoids on memory may be the increase in the activity of calcium channels. ELF-MF fields, like environmental factors, probably have inhibitory effects on the expression of the GR gene. ELF-MF waves impair glucocorticoid receptors. Stress, fear, and anxiety leave negative impacts on cognition and function of the central nervous system. Moreover, cortisol (the stress system) and the adrenal sympathetic system (the emotion system) can also have drastic effects (Lewczuk et al., 2014; Sandi, 2011; Touitou and Selmaoui, 2012; Mahdavi et al., 2014). The prefrontal cortex receives sensory inputs from the limbic system, and after information and experience are processed the resulting data is stored in the form of memory in certain regions. The processed data is stored within several seconds in the form of information in the short-term memory and is transferred to the long-term memory in a more stable form and is eventually transferred to the hippocampus. Similar to the present research, visual memory were tested for 30 seconds and 60 seconds after exposure to ELF-MF, respectively (Kandel et al., 2014; Mayford et al., 2012; Pipa et al., 2009) Based on cortisol assessment research, it is directly related to the cognitive behavior of the monkey. Abnormal
increasing and decreasing in cortisol levels, leads to cognitive changes in the monkey relative to the effective dose. In 2018, Hamed Aliyari et al showed that the electric field of the high-voltage fields' tower caused an excessive reduction in cortisol levels compared to basal cortisol, which was a diced monkey of cognitive impairiment (weakening of visual working memory) and depression. But in this study, the cortisol concentration was lower than basal level that improved cognitive index (visual memory). Electromagnetic magnitude reduced the concentration of cortisol (effective dose). An effective dose, any stimulus that changes the concentration of cortisol, resulting in cognitive changes in humans and animals(Tekieh et al., 2017; Aliyari et al., 2018a). Reduced expression of the GR genes enhances the cognitive memory of the visual memory in the monkey. Results of radiation at the 12Hz frequency with the 0.7 μT field showed a decrease in expression of the GR gene. The decrease in expression of GR gene and cortisol concentration improved visual memory in the B-F monkeys following exposure to an electromagnetic field at the 12Hz frequency. The result of cognitive behavior of B-F monkeys showed that they were more relaxed, more vivacious, more carefully. So, it can be said that all of the extremely low-frequency electromagnetic fields are not harmful because their harm depends on frequency, field magnitude, or radiation lengths as well as other factors. Perhaps further research may result in the identification of other useful frequencies such as the frequencies used in neurofeedback. However, the attainment of this goal calls for more fundamental studies by interested researchers.

**Conflict of Interests:**
The authors have no potential conflict of interests pertaining to this journal submission.

**Acknowledgment:**
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**Ethical Approval:**
All ethical standards were met based on the international ir.bmsu.rec.1394.112
Figures and Tables

Figure 1: Visual memory Response Task.
It shows the design of the two Plexiglass coated dishes (containing invisible reward). The lid of the dish opens only in one direction. The dish is placed on a movable stand and can be moved quickly.

Figure 2: Visual memory before radiation and after radiation under the effect of the 12Hz wavelengths and the 0.7 μT electromagnetic field.
The figure shows the percentage of correct answers after irradiation. The VM response task (visible reward) of the D-E control and B-F monkeys in the phases 1 (delay 30 seconds).

Figure 3: Visual memory before radiation and after radiation under the effect of the 12Hz wavelengths and the 0.7 μT electromagnetic field.
The figure shows the percentage of correct answers after irradiation. The VM response task (visible reward) of the D-E control and B-F experimental monkeys in phase 2 (delay 60 seconds).

Figure 4: Amygdala area sagittal section, pre and Post radiation (cod D)
Surface analysis (pre- and post-radiation) of the left amygdala of the control monkeys, using Image J software.

Figure 5: Amygdala area sagittal section, pre and Post radiation (cod B)
Surface analysis (pre- and post-radiation) of the left amygdala of the experimental monkeys, using Image J software.

Table 1: Amygdala area sagittal section, pre and Post radiation (control and experiment)
With the coronal section, shows the D-E and B-F monkey's amygdala area with the sagittal section resulted from type T2 MRI before radiation and after radiation.
Figure 6: Indicates the level of plasma cortisol in the control and experimental group.

It shows variations of cortisol in B-F monkeys before and after radiation with the 12Hz wavelengths and the 0.7 μT field magnitude.

Figure 7: Indicates the expression of the GR receptor gene in the control and experimental group.

It shows changes of expression of the GR receptor in the D-E control and B-F experimental monkeys before radiation and after radiation with the 12Hz wavelengths and the 0.7 μT field.
Fig. 1.
Fig. 2

![Graph showing the number of correct weekly answers over four weeks with different delays and conditions.](image)

**Delay 30 seconds**

Fig. 3

![Graph showing the number of correct weekly answers over four weeks with different delays and conditions.](image)

**Delay 60 seconds**
Fig. 4.
### Table 1

<table>
<thead>
<tr>
<th>Monkey Codes</th>
<th>Amygdala Area (mm$^3$) Pre-Radiation</th>
<th>Amygdala Area (mm$^3$) Post Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>12Hz B-F</td>
<td>10.998</td>
<td>10.128</td>
</tr>
<tr>
<td>(control) D-E</td>
<td>13.581</td>
<td>13.683</td>
</tr>
</tbody>
</table>

Fig. 6
Fig. 7

![Graph showing GR/β active expression ratio over time.](image)

- **Per**, **Post**, and **Recovery** stages are marked on the x-axis.
- The y-axis is labeled as **GR/β active expression ratio**.
- Two lines represent different conditions:
  - B,F-12 HZ
  - D-E-control
References:


