Improvement of Cognitive Indicators in Male Monkeys Exposed to Extremely Low-Frequency Electromagnetic Fields

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Abstract

Today, the production of Extremely Low-Frequency Electromagnetic Fields (ELF-EMFs) has significantly increased. This study aimed to investigate the effect of the ELF-EMFs on the structure and function of the brain in male Rhesus monkeys in terms of visual learning (VL), visual memory (VM), and visual working memory (VWM). Four monkeys were selected, of whom two monkeys irradiated by 0.7 Microtesla ELF-EMFs, 12 Hz, and two monkeys tested without irradiation (control group). A blood sample was taken in three stages, pre-, post-irradiated, and the recovery phases. Changes in the plasma levels of sodium, potassium, and adrenocorticotropic hormone (ACTH) were evaluated. Also, gene expression of N-methyl-D-aspartate (NMDA) receptors was assessed. The anatomical change of the brain’s prefrontal area was assessed by magnetic resonance imaging (MRI) and Digital Imaging and Communications in Medicine (DICOM) LiteBox file. The visual learning, visual memory, and visual working memory abilities significantly improved after the irradiation. Also, expression of the NMDA receptors gene and the plasma levels of sodium, potassium, and ACTH significantly increased after the irradiation. However, the Prefrontal area was not significantly affected by irradiation. No significant differences were observed in any of the studied factors in the control group. Our findings suggest that ELF-EMFs irradiation at 12 Hz, positively affected visual...
learning and visual working memory. Thus, 12 Hz ELF-EMFs irradiations can be widely applied to improve cognitive abilities in monkeys.

**Keywords:** Adrenocorticotropic Hormone, Extremely Low-Frequency Electromagnetic Fields, *Rhesus* Monkey, Visual Learning, Visual Memory, Visual Working Memory

**INTRODUCTION**

Environmental factors influence the biology of living organisms in different ways. Nowadays, one of the most important biotic health-threatening factors is electromagnetic fields (EMFs). The production of EMFs is expanding as technology advances. Many animal and human studies have revealed the harmful effects of EMFs on organisms, especially on their cognitive performance of the central nervous system (CNS) (Kheifets et al., 2005; Ahlbom et al., 2008; Kazemi et al., 2017). Despite the difficulty of performing experimental tests on monkeys, the results of animal studies on monkeys are valuable because *Macaca Mulatta* monkeys cognitively the most closely related to humans, and results can be generalized to humans. In 2007, researchers examined the human and *Rhesus* monkey genome sequences and found that 0.98% of the human genome was sequenced with *Macaca Mulatta*. The *Rhesus* genome is the second non-human primate after Chimpanzees to be the most closely related to humans (Gao et al., 2002; Marques et al., 2005; Fang et al., 2011). EMFs can also have negative effects. Among them is their role as molecule activators of reactive oxygen species (ROS). The ROS is capable of generating peroxides, hydroxides, and free radicals, resulting in toxicity inside the nerve cells. The creation of toxicity within the neurons may harm the structure and function of neurons leading to diseases like Huntington, Alzheimer's Disease, and Depression (Adey, 1988; Lipton, 2005; Cifra et al., 2011; Kıvrak et al., 2017). Alzheimer's Disease is caused by defective structure and function of the hippocampal neurons, which can be facilitated by ROS molecules (Markesbery, 1997; Kıvrak et al., 2017).

The gene expression of N-methyl-D-aspartate (NMDA) receptors plays a crucial role in the processes of learning and memory. The highest density of the NMDA receptors gene is found in the hippocampus, amygdala, and prefrontal cortex (Moosmang et al., 2005; Dalmau et al., 2007).
Previous studies indicated that the gene expression of NMDA receptors in peripheral blood lymphocytes is an important marker of the expression of this gene in the CNS (Lacreuse et al., 2000; Baumann and Pham-Dinh, 2001).

Another adverse effect of EMFs is changes in the normal secretion of neuroendocrine hormones including melatonin, ACTH, cortisol, and epinephrine. Impaired secretion of these hormones may lead to behavioral and cognitive disorders (Rodriguez et al., 2004; Hong et al., 2011). As such, EMFs adversely affect the secretion of protein and amine hormones by activating G-protein-coupled receptors and membrane enzymes (phospholipase C or adenylyl cyclase), leading to increased production of cyclic-AMP (cAMP) or diacylglycerol (DAG) (by activating protein kinase A or protein kinase C). This causes the phosphorylation of proteins that have serine or threonine endings, resulting in alternations in gene transcription (McCann and Welsh, 1990; Kwok et al., 2005; Haddad et al., 2007). The ACTH secreted from the anterior pituitary, works to stimulate the adrenal cortex and increases the secretion of cortisol and aldosterone. For example, decreased cortisol production results in increased ACTH secretion in an attempt to restore cortisol levels. ACTH acts to maintain the size and function of the adrenal gland (Smith and Vale, 2006; Papadimitriou and Priftis, 2009). The process of learning and ways to improve it play a key role in improving human performance related to skills and knowledge. Learning is one of the most important neurological activities that involves a fairly stable change in feelings, thoughts, and behaviors obtained through memories of the individual (Roelfsema et al., 2010; Duhigg, 2012). Learning can be characterized by two basic elements, attention, and concentration (Roelfsema et al., 2010). Learning and memory is defined as the communication between different areas of the brain, especially the hippocampal regions and the prefrontal cortex (Sotres-Bayon et al., 2006; Olson and Colby, 2014). The reason for examining the effects of EMFs at a frequency of 12 Hz is due to its alignment to the peak frequency of alpha brainwaves. The frequency range for alpha is originally between 8-12 Hz. Alpha brainwaves have been shown to play an important role in improving cognitive indicators (Klimesch, 1999; Ward, 2003). So, this study aimed to investigate the effect of extremely low-frequency electromagnetic fields on the behavioral and cognitive functions of the brain including visual learning, visual memory, and visual

MATERIAL AND METHODS
Animals and ELF exposure protocol

A total of four Rhesus monkeys (*Macaca Mulatta*) aged four-five years, with an average weight of 4kg were recruited. All four monkeys entered the study after passing compatibility tests. The light, temperature, and humidity of the animal holding room was standard, with diurnal lighting on a 12:12-h light: dark cycle. All ethical standards regarding animal confinement, transport, location and, maintenance was observed under the international law and the ethics code of 12345.

Of the four assigned monkeys, two monkeys irradiated by 0.7 (μT) ELF-EMFs either 12 Hz, 4h a day, for 30 days, and two monkeys maintained in a non-irradiated environment and tested without exposure to ELF-EMFs. The animal holding room was equipped with a full-face shield. The animal cage was made of Teflon and sized 80 * 1 * 1 m (Figure 1) to allow the currents generated from EMFs to fully pass through the cage. The distance between the signal generator with an antenna (designed by a team of experts at Amir Kabir University, Tehran, Iran) and the two cages containing primates, was with each side of length 0.5 meter at the time of irradiation. The signal generator was able to adjust the intensity of waveforms, frequency (range from 1 to 300 Hz), and electromagnetic field.

Behavioral tests and biological analysis

Behavioral tests were performed on irradiated and control monkeys before and after the intervention. According to experimentation protocol, a fasting period of 17 hours was required for monkeys. For biological analysis, a total of ten c.c. of blood was taken in three stages, pre-, post-irradiated, and the recovery from the femoral artery of the animals, of which five c.c. was used to evaluate changes in the level of serum sodium, potassium, and ACTH using the primate custom positive selection kit (at MyBioSource, ELISA Kits) in three stages (Tekieh et al., 2017). Another five c.c. of blood sample was used to extract lymphocytes to detect gene expression of NMDA receptors using the Real-Time PCR method in three stages (Hillmann et al., 2000; Xuan et al., 2010; Hosseini et al., 2015).

The anatomical changes of the prefrontal area was measured by MRI and DICOM LiteBox file before and after the intervention (McAuliffe et al., 2001; El-Kafrawy and Barsoum, 2012). This was a descriptive study and no statistical analysis was performed due to the small sample size.
**Visual learning test**

To perform the visual learning test, a Plexiglas well was designed, so that animals were able to see the food inside the well. The dish was provided to the animals at 10 cm from the front of the cage. It well has only one open side, so that the animal can hold the peanut well with one hand to reap the reward (peanut) within the well and hold it with the other hand. The protocol was performed for 10 days, 10 times a day before and after the irradiation. The same protocol was carried out after the irradiation with the opening on the opposite side of the well. The control samples were tested and compared without irradiation (Lyons et al., 2010; Kazemi et al., 2017).

**Memory tests**

To perform the memory test, a device was designed to record the visual memory and visual working memory of the animal, with two opaque wells each with an opening in one side plates so that the subject cannot see where the reward is hidden. The two opaque plates wells (Figure 2) were placed on an adjustable base (Tsujimoto and Postle, 2012; Darusman et al., 2014). The tests were performed for outage lengths of 30 and 60 seconds as follow:

**Visual memory test**

The device is placed in front of the monkey and the reward is randomly placed in one of the wells. The subject needs to pay attention and remember the spatial information and use this information to make the correct choice. Then a curtain appeared in front of the monkey’s eyes and after a delay period of 30 seconds, the curtain was pulled off and the well was provided to the monkey on an adjustable base. The subject was allowed to choose and open one of the two wells only once. If the first attempt was wrong, the monkey is deprived of the reward. The test was performed 10 times a day (Kazemi et al., 2017).

**Visual working memory test**

This test is completely similar to the visual memory test, with a delay period of 60 seconds, as the curtain was pulled out after 60 seconds and the well was placed on an adjustable base. This trial was conducted 10 times a day. The protocol included four weeks with 10 trials a day before and after the irradiation (Kazemi et al., 2017). Eventually, results were compared with the control group.
RESULTS

The visual learning significantly improved after the intervention in the irradiated group (figure 3). Also, results showed that visual memory and visual working memory were improved after the intervention in the irradiated group (figure 4 and figure 5 respectively). Besides, plasma levels of ACTH significantly increased after the intervention in the irradiated group (figure 6). Furthermore, plasma levels of sodium and potassium significantly increased after the intervention in the irradiated group (figure 7 and figure 8 respectively). Also, the gene expression of NMDA receptors was significantly different after the intervention in the irradiated group (figure 9). However, the prefrontal area was not significantly different after the intervention (figure 10 and table 1). No significant differences were observed in any of the studied factors in the control group.

The recovery stage is defined as returning to the previous stage of irradiation.

DISCUSSION

Today, one of the environmental factors influencing the health of organisms is ELF/EMFs. Evidence shows that ELF/EMFs exposure, in addition to its adverse effect on health and lifestyle, might cause cognitive and behavioral changes in both animals and humans (Feychting et al., 2005; Thielens et al., 2018). One of today's research challenges is how to improve cognitive skills such as learning and memory. Improvement of these skills can be effective in the quality and lifestyle of individuals based on previously recorded memory (Devesa et al., 2016; Kazemi et al., 2017).

The findings of this study showed that 0.7 Microtesla ELF/EMFs 12Hz irradiation significantly influenced cognitive and behavioral elements in monkeys. The recruited monkeys were noisy, violent, and unattractive before the intervention but after the irradiation, the behavioral characteristics of the monkeys changed and they were calm and attentive, as they demonstrated cooperation in behavioral tests. Also, cognitive tests revealed higher scores of visual learning in the irradiated group than the control group. A major element of learning is attention and concentration (Roelfsema et al., 2010) which was significantly improved in irradiated monkeys. Also, the visual working memory of the monkeys was shown higher in the irradiated group compared with the control group. Findings of the previous studies showed that learning and recall are the basis of memory (Crowder, 2014). The cognitive elements, attention, learning, and memory gradually improved after the irradiation, and as a
result, visual working memory was significantly improved in irradiated monkeys compared to control monkeys. Hormonal tests also indicated increased levels of ACTH after the irradiation. ACTH acts as a regulator of cortisol, which plays an important role in memory and learning function in the CNS (Smith and Vale, 2006). An increased level of ACTH is associated with decreased cortisol secretion from the cortex of the adrenal gland. The calm condition of the monkeys was attributed to reduced levels of cortisol and increased attention and concentration. EMFs exposure may cause abnormalities in cortisol secretion and increase the oxidative stress and subsequently production of free radicals, resulting in neurodegenerative diseases by disrupting normal neuronal function. Thus, abnormalities in cortisol secretion result in neurological and cognitive impairments (Consales et al., 2012). Also, ELF/EMFs exposure may increase membrane excitability, and increase the permeability of the membrane through voltage-gated and ligand-gated receptors. The increased activity of G-protein-coupled receptors increases the level of ACTH by increasing cAMP and calcium ion as a secondary messenger leading to increased activity of PKA and genomic alternations in neurons (Grassi et al., 2004; Kwok et al., 2005). The present study indicated an increase in the plasma levels of ACTH, which can reduce cortisol levels. Hormonal changes may cause cognitive changes in monkeys. Glucocorticoid and NMDA receptors are important receptors affected by ELF/EMFs and play a crucial role in learning and memory. The highest density of the NMDA and glucocorticoid receptors gene has been found in the hippocampus, amygdala, and prefrontal cortex. Gene expression of these two receptors plays an important role in the learning and memory in both humans and primates (Moosmang et al., 2005; Trillo et al., 2013). The results of the present study showed the increased expression of NMDA receptors after the intervention in irradiated monkeys compared with control monkeys. Increased gene expression of NMDA receptors is associated with improved learning and memory functions. Given the important role of sodium and potassium in the transmission of neural signals (Moosmang et al., 2005; Varró et al., 2009), the plasma levels of these ions were examined before and after the intervention in both groups. The levels of these ions significantly increased in irradiated monkeys. ELF/EMFs stimulates glycine and glutamate site on NMDA receptors and blocks magnesium inhibitory effect on NMDA receptors, resulting in more sodium outside and more potassium inside the cell. Increased gene expression of the NMDA receptors also
increases calcium (Ca++) concentration which is another important ion in learning and memory processes. There is evidence that increasing the gene expression of the NMDA receptors is associated with increased plasma levels of sodium, potassium and Ca++ (Varró et al., 2009; Komaki et al., 2014). The effect of Ca++ was not examined in the present study, and we strongly suggest to include this important ion in future research. Given the fact that EMFs at the frequency of 12 Hz is aligned to the peak frequency of alpha brainwave and these waves play an important role in creativity, attention, mental, physical and balance abilities of individuals (Başar, 2012; Klimesch, 2012), it can be concluded that the 12 Hz EMFs may be overlapped with the 12 Hz alpha brainwaves and improved cognitive indicators. The anatomical assessment by MRI is a common and non-invasive technique used by neurologists to examine neurological disorders such as Alzheimer's Disease (McAuliffe et al., 2001; El-Kafrawy and Barsoum, 2012; Harach et al., 2015). According to the results from anthropometric measurements of the prefrontal area by MRI, the irradiated monkeys showed no changes in the prefrontal area after the irradiation and also compared with the control group. Results from biochemistry and genomic tests revealed that during the recovery phase, irradiated monkeys showed to be likely to recover to the pre-irradiation stage, and structural alterations were less likely to be observed in MRI. The anatomical changes could also be measured if these alternations revealed a stable trend. If we want to introduce 12 Hz as a reference frequency in ELF/EMFs spectrum for effective enhancement of cognitive abilities, we need to provide a strategy for unstable alternations in the recovery stage so that stable results in the recovery stage can be gained.

The findings of the current study suggest that 0.7 (µT) ELF/EMFs irradiation at 12 Hz positively affects visual learning and visual working memory in Rhesus monkeys. Increased levels of ACTH, sodium, and potassium as well as increased gene expression of NMDA receptors supported these findings. Results of behavioral, hormonal, biochemical, and genomic tests revealed that 12 Hz ELF/EMFs irradiation can be widely applied to improve cognitive abilities in Rhesus monkeys.

Authors’ Contributions

HA, HS, MK: Conceptualization; HA, NK, ET, HT, MS, H Gh, GhM, MS, ZB, HMH: Data Curation; HA, MK, SG: Formal Analysis; MK: Funding acquisition; HA, MK, SG: Investigation; HA, MK,
Ethical Statements

This research was conducted by the approve of Neuroscience Research Center, Baqiyatallah Medical Science University.

Conflict of Interest

The authors have no potential conflict of interest pertaining to this journal submission.

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References


**Figure 1.** A scheme of the animal holding room with a full-face shield and the animal cage that made of Teflon

**Figure 2.** The opaque wells placed on an adjustable base designed to test the visual working memory
Figure 3. Results of visual learning tests before and after the intervention in irradiated group (F-B) and control group (E-D) without irradiation

![Graph showing visual learning test results](image)

Figure 4. Pre- and post-test results of visual memory in the irradiated group (F-B) compared with control group (E-D) at delay period of 30s

![Graph showing visual memory test results](image)
**Figure 5.** Pre- and post-test results of visual working memory in the irradiated group (F-B) compared with control group (E-D) at delay period of 60s

**Figure 6.** Changes in ACTH in the irradiated (F-B) and control (E-D) groups after intervention
**Figure 7.** Changes in plasma levels of sodium in the irradiated (and control (E-D) groups after intervention

![Graph showing changes in plasma levels of sodium](image)

**Figure 8.** Changes in plasma levels of potassium in the irradiated (and control (E-D) groups after intervention

![Graph showing changes in plasma levels of potassium](image)
Figure 9. Changes in gene expression of NMDA receptors in the irradiated (and control (E-D) groups after intervention

Figure 10. The anatomical change of the pre-frontal area by MRI (Prefrontal left and right areas with coronal slice) in the irradiated (and control (E-D) groups after intervention
**Table 1.** The anatomical change of the pre-frontal area by MRI (Prefrontal left and right areas with coronal slice) in the irradiated (and control (E-D) groups after intervention

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Left prefrontal (mm)^3</th>
<th>Right prefrontal (mm)^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>B (12 Hz)</td>
<td>5902.5</td>
<td>5913</td>
</tr>
<tr>
<td>E-Control</td>
<td>6190.5</td>
<td>6108.5</td>
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</tbody>
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