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Scientific Report

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). To conduct the study, four monkeys were selected, of whom two monkeys were irradiated by 12-Hz ELF-EMFs with a magnitude of 0.7 microtesla, and two monkeys were tested without irradiation (control group). A blood sample was taken in three stages, namely pre- and post-irradiated and the recovery phases. Changes in the plasma levels of sodium, potassium, and adrenocorticotropic hormone (ACTH) were evaluated. Moreover, gene expression of N-methyl-D-aspartate (NMDA) receptors was assessed. The anatomical change of the brain's prefrontal area was measured by magnetic resonance imaging and Digital Imaging and Communications in Medicine LiteBox file. The abilities of VL, VM, and VWM significantly improved after the irradiation. Furthermore, the expression of the NMDA receptors gene and the plasma levels of sodium, potassium, and ACTH significantly enhanced after the irradiation. However, the prefrontal area was not significantly affected by the irradiation. No significant differences were observed in any of the studied factors in the control group. Our findings suggested that ELF-EMFs irradiation at 12 Hz positively affected VL and VWM. Consequently, 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

1. 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. The results of numerous studies conducted on animals and humans have revealed the harmful effects of EMFs on organisms, especially on their cognitive performance of the central nervous system (CNS) (1-3). Despite the difficulty of performing experimental tests on monkeys, the findings of animal studies on monkeys are valuable because *Macaca mulatta* monkeys are cognitively the most closely related animals to humans whose 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 related to humans genome (4-6). Electromagnetic fields can also have negative effects, among which is their role as molecule activators of reactive oxygen species (ROS). Reactive oxygen species 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, such as Huntington's disease, Alzheimer's disease, and depression (7-10). Alzheimer's disease is caused by the defective structure and function of the hippocampal neurons, which can be facilitated by ROS molecules (10, 11).

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 (12, 13). The findings of previous studies have 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 (14, 15).

Another adverse effect of EMFs is the changes in the normal secretion of neuroendocrine hormones, including melatonin, adrenocorticotropic hormone (ACTH), cortisol, and epinephrine. Impaired secretion of these hormones may lead to behavioral and cognitive disorders (16, 17). In this respect, 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 adenosine monophosphate (cAMP) or diacylglycerol (by activating protein kinase A [PKA] or protein kinase C). This causes the phosphorylation of proteins that have serine or threonine endings, resulting in alternations in gene transcription (18-20). The ACTH secreted from the anterior pituitary stimulates 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. Adrenocorticotropic hormone acts to maintain the size and function of the adrenal gland (21, 22).

Understanding the learning process and ways to improve it plays a key role in promoting skillful practices and human 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 (23, 24). Learning can be characterized by two basic elements, attention, and concentration (24). Learning and memory are defined as the communication between different areas of the brain, especially the hippocampal regions and the prefrontal cortex (25, 26). The reason for examining the effects of EMFs at a frequency of 12 Hz is due to their alignment to the peak frequency of alpha brainwaves. The frequency range for alpha is originally between 8 and 12 Hz. Alpha brainwaves have been shown to play an important role in improving cognitive indicators (27, 28). Therefore, this study aimed to investigate the effect of extremely low-frequency (ELF)-EMFs on the behavioral and cognitive functions of the brain, including visual learning, andvisual memory, Visual working memory.

2. Materials and Methods

2.1. Animals and Extremely Low-Frequency Exposure Protocol

A total of four rhesus monkeys (*Macaca mulatta*) aged 4-5 years old, with an average weight of 4 kg were included in the study. All four monkeys were entered into the study after passing compatibility tests. The light, temperature, and humidity of the animal holding room were standard, with diurnal lighting on a 12:12-h light-dark cycle. All ethical considerations regarding animal confinement, transport, location, and maintenance were observed under the international law and the ethics code of 12345.

Of the four assigned monkeys, two monkeys were irradiated by 0.7 (μT) ELF-EMFs either 12-Hz ELF-

EMFs, 4 h a day for 30 days, and two monkeys were maintained in a non-irradiated environment and were 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 0.5 m with each side of length at the time of irradiation. The signal generator was able to adjust the intensity and frequency (at the range of 1-300 Hz) of waveforms and EMF.

Figure 1. Scheme of the animal holding room with a full-face shield and the animal cage made of Teflon

2.2. 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 h was required for monkeys. For biological analysis, a total of 10 cc of blood was taken in three stages, namely pre- and post-irradiated and the recovery from the femoral artery of the animals, of which 5 cc was used to evaluate changes in the level of serum sodium, potassium, and ACTH using the primate custom positive selection kit (MyBioSource, Enzyme-Linked Immunosorbent Assay Kits) in three stages (29). Another 5 cc of blood sample was used to extract lymphocytes to detect gene expression of NMDA receptors using the real-time polymerase chain reaction method in three stages (30-32).

The anatomical changes of the prefrontal area were measured by magnetic resonance imaging (MRI) and Digital Imaging and Communications in Medicine LiteBox file before and after the intervention (33, 34). This was a descriptive study and no statistical analysis was performed due to the small sample size.

2.2.1. 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. The well had only one open side; therefore, the animal could 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 (3, 35).

2.2.2. 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 plate; as a result, the subject could not see where the reward was hidden. The two opaque plates wells (Figure 2) were placed on an adjustable base (36, 37). The tests were performed for outage lengths of 30 and 60 sec as follow:

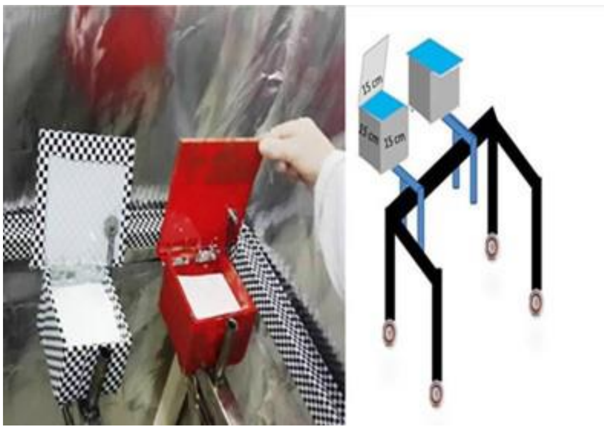

Figure 2. Opaque wells placed on an adjustable base designed to test the visual working memory

2.2.2.1. Visual Memory Test

The device was placed in front of the monkey and the reward was randomly placed in one of the wells. The subject needed to pay attention and remember the spatial information and use this information to make the correct choice. Subsequently, a curtain appeared in front of the monkey's eyes and after a delay period of 30 sec, 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 was deprived of the reward. The test was performed 10 times a day (3).

2.2.2.2. Visual Working Memory Test

This test was completely similar to the visual memory test with a delay period of 60 sec as the curtain was pulled out after 60 sec and the well was placed on an adjustable base. This trial was conducted 10 times a day. The protocol included 4 weeks with 10 trials a day before and after the irradiation (3). Eventually, the results were compared with the control group.

3. Results

The visual learning significantly improved after the intervention in the irradiated group (Figure 3). Moreover, the results showed that visual memory and visual working memory were improved after the intervention in the irradiated group (Figures 4 and 5, respectively). Additionally, plasma levels of ACTH significantly increased after the intervention in the irradiated group (Figure 6). Furthermore, plasma levels of sodium and potassium significantly boosted after the intervention in the irradiated group (Figures 7 and 8, respectively). The gene expression of NMDA receptors was also 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 was defined as returning to the previous stage of irradiation.

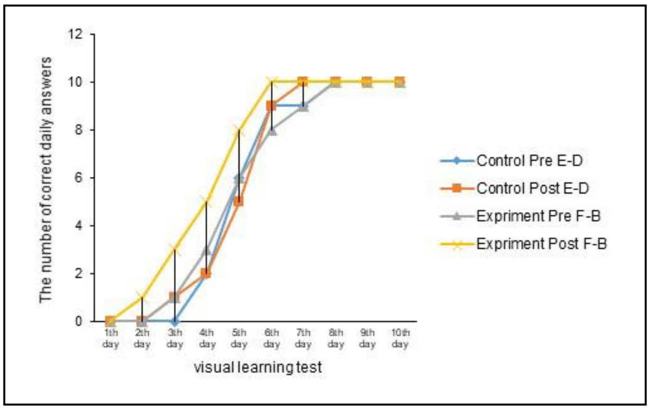

Figure 3. Results of visual learning tests before and after the intervention in irradiated group (F-B) and control group (E-D) without irradiation

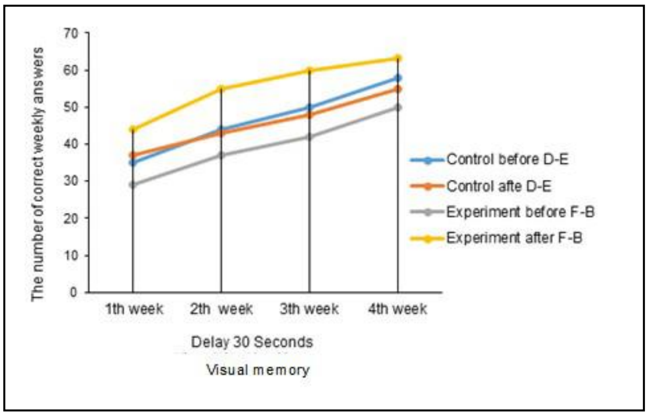

Figure 4. Pre- and post-test results of visual memory in the irradiated group (F-B), compared to the control group (E-D) at a delay period of 30 sec

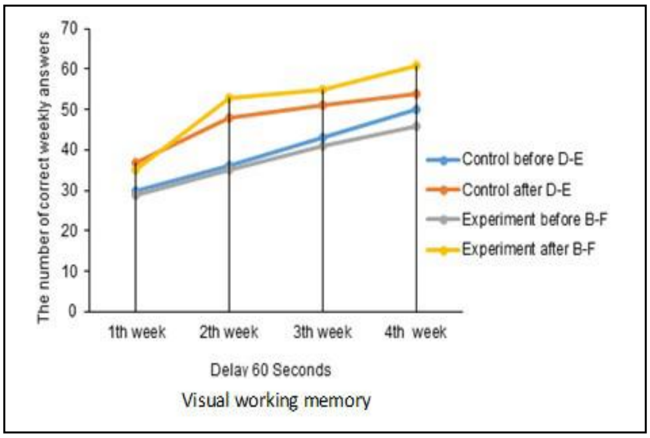

Figure 5. Pre- and post-test results of visual working memory in the irradiated group (F-B), compared to the control group (E-D) at a delay period of 60 sec

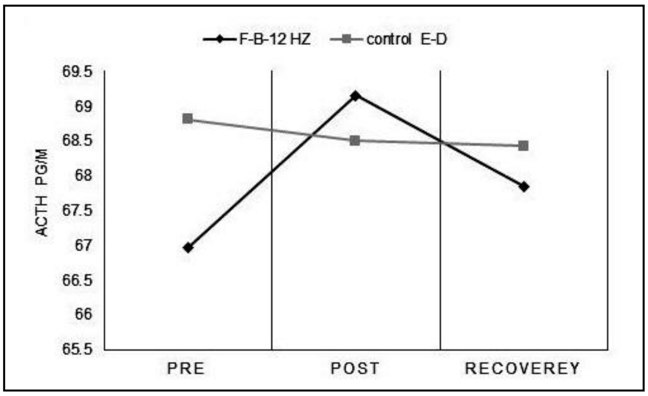

Figure 6. Changes in adrenocorticotropic hormone in the irradiated (F-B) and control (E-D) groups after the intervention

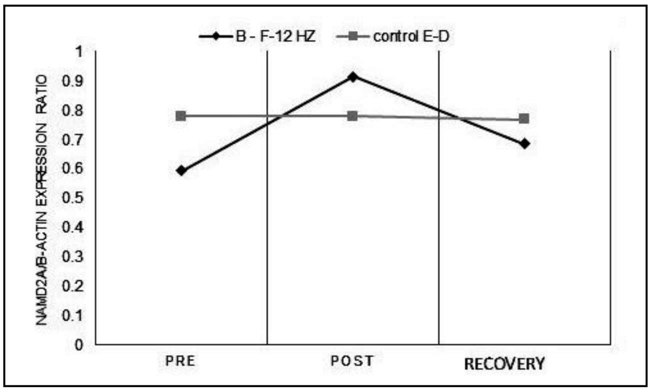

Figure 9. Changes in gene expression of NMDA receptors in the irradiated (and control (E-D) groups after intervention

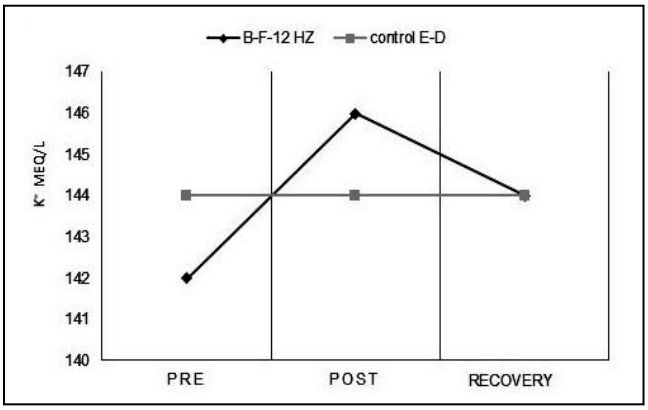

Figure 7. Changes in plasma levels of sodium in the irradiated (and control [E-D]) groups after the intervention

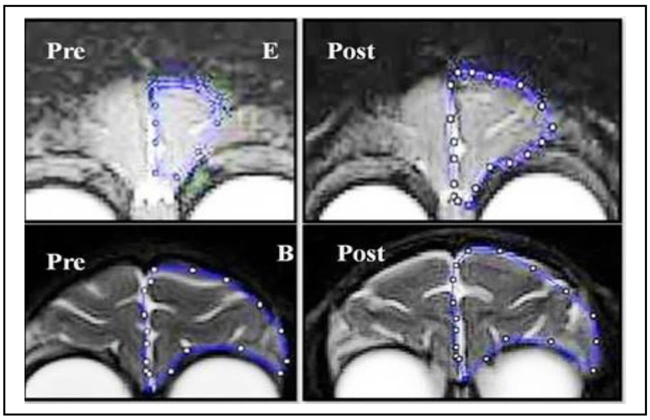

Figure 10. Anatomical change of the pre-frontal area by magnetic resonance imaging (left and right prefrontal areas with coronal slice) in the irradiated (and control [E-D]) groups after the intervention

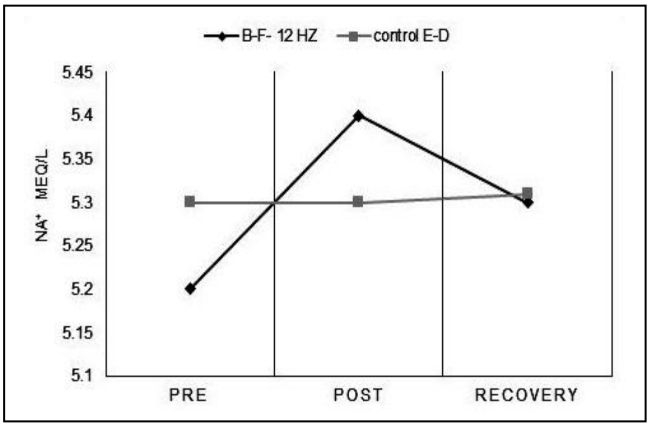

Figure 8. Changes in plasma levels of potassium in the irradiated (and control [E-D]) groups after the intervention

Table1. Anatomical change of the pre-frontal area by magnetic resonance imaging (left and right prefrontal areas with coronal slice) in the irradiated (and control [E-D]) groups after the intervention

Monkey	Left Prefrontal $(mm)^3$		Right Prefrontal $(mm)^3$	
	Before	After	Before	After
B (12 Hz)	5902.5	5913	5989.5	5962.1
E-Control	6190.5	6108.5	6211.5	6232.5

4. 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 (38, 39). One of today's research challenges is how to improve cognitive skills, such as learning and memory. The improvement of these skills can be effective in the quality and lifestyle of individuals based on previously recorded memory (3, 40).

The findings of this study showed that 12-Hz ELF-EMFs irradiation with a magnitude of 0.7 microtesla significantly influenced cognitive and behavioral elements in monkeys. The included monkeys were noisy, violent, and inattentive before the intervention; nevertheless, the behavioral characteristics of the monkeys changed after the irradiation and they were calm and attentive as they demonstrated cooperation in behavioral tests. In addition, cognitive tests revealed higher scores of visual learning in the irradiated group than in the control group. A major element of learning is attention and concentration (24), which was significantly improved in irradiated monkeys. Moreover, the visual working memory of the monkeys was shown higher in the irradiated group, compared with the control group. The findings of the previous studies have shown that learning and recall are the basis of memory (41). 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. Adrenocorticotropic hormone acts as a regulator of cortisol, which plays an important role in memory and learning function in the CNS (22). 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. Electromagnetic fields exposure may cause abnormalities in cortisol secretion and an increase in oxidative stress, and subsequently, the production of free radicals, resulting in neurodegenerative diseases by disrupting normal neuronal function. Consequently, abnormalities in cortisol secretion result in neurological and cognitive impairments (42). Additionally, ELF-EMFs exposure may increase membrane excitability and enhance 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 (19, 43). The findings of the present study indicated an increase in the plasma levels of ACTH, which could 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 (12, 44). The results of the present study showed the increased expression of NMDA receptors after the intervention in irradiated monkeys, compared to 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 (12, 45), 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. Extremely low-frequency 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 an increase in the gene expression of the NMDA receptors is associated with increased plasma levels of sodium, potassium, and Ca^{++} (45, 46). The effect of Ca^{++} was not examined in the present study, and it is strongly suggested 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 (47, 48), 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 (33, 34, 49). 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 in comparison to 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 12 Hz is introduced as a reference frequency in ELF-EMFs spectrum for effective enhancement of cognitive abilities, it is required to provide a strategy for unstable alternations in the recovery stage; therefore, stable results in the recovery stage can be gained.

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

Authors' Contribution

Study concept and design: H. A., H. S. and M. K.
Acquisition of data: H. A., N. K., E. T., H. T., M. S., H. Gh., Gh. M., M. S., Z. B. and H. M. H.
Analysis and interpretation of data: H. A., M. K., S. G. and H. S.
Drafting of the manuscript: H. A. and S. G.
Critical revision of the manuscript for important intellectual content: S. G.
Statistical analysis: H. A., M. K. and S. G.
Administrative, technical, and material support: M. K.

Ethics

This research was conducted with the approval of the Neuroscience Research Center, Baqiyatallah University of Medical Science, Tehran, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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