Title: Environmental Effect of HV Towers at the Cerebellum and Cognitive Impairments in the Monkey

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Abstract

Today, living or working of people in the vicinity and even under the high-voltage lines is a pernicious environmental hazard to humans. The male rhesus monkey is used to investigate the effects of fields produced by high-voltage towers. In this study, the function and level of effect in rhesus monkeys' brain have been investigated in cerebellum's cognitive, biological and structural perspective. Two monkeys have been used, one as a control and the second under test. The monkey under test was subjected to a simulated HV electrical field of 3 kV/m for 4 hours a day for a one month. Behavioral tests were performed using a device designed and built for this purpose. Concentration analysis of adrenocorticotropic hormones (ACTH) and inspection of glucocorticoid receptor gene's (GR) expression were performed by the RT-PCR method. Changes in cerebellar anatomy with MRI images were examined. All tests were performed before and after the test period and were compared with the control monkey. Cognitive tests showed a significant reduction for the monkey that was exposed to a high-voltage electrical field in the first week after field imposition compared with the same time before. Also, the expression of the GR gene was decreased and the concentration of ACTH hormone in plasma was increased. Surveying the level of cerebral MRI images did not show any difference, but hemorrhage was evident in a part of the cerebellum. The results of cognitive, biological and MRI tests in the tested monkey showed a decrease in the visual learning and memory indices.

Keyword: Cerebellum, Visual Learning, Visual Memory, Rhesus Monkey, High-voltage Towers, Electrical Field.

Introduction

Studies have shown that the frequency of electromagnetic waves that are propagating in high-voltage towers in residential areas can cause problems in blood and brain and even disability for residents living on the margins of the affecting magnetic fields [1-7].

The electromagnetic field of high-voltage towers threatens the environment as a harmful environmental hazard, so as electromagnetic fields have an inadequate effect on animals and cell division. They also cause biological effects, including changes in cell and tissue functions and changes in human marrow activity, as well as heart rate [8]. The electromagnetic fields activate the ROS (Reactive oxygen species) molecule. It is a reactive oxygen molecule that can produce peroxides and hydrogen oxides and then cause toxicity inside the neuron. The impact of electromagnetic fields (EMF) on the production of free radicals by ROS in neurons makes toxicity within them and degrades the function and structure of the neurons. The results of these abnormal changes cause neurological diseases, including Huntington, Alzheimer, and depression [9, 10]. Alzheimer disease is a severe defect in the function and structure of the hippocampal neurons which is accelerated by the ROS molecule [11]. Another neurological disorder caused by high-voltage tower's fields that is increasing day by day is the Alzheimer. Alzheimer is a neurological disorder that occurs in people of high age, which weakens concentration and impairs remembrance of memorabilia and memory. Research shows that workers who are more exposed to the electromagnetic field are more likely to afflict with this [11-13].

In addition, studies on experimental animals and domestic animals, as well as humans have shown that wavelength, duration of exposure and distance play a role in cell proliferation, so as interferes with cell proliferation at the DNA replication and also increases the incidence of congenital defects, reproductive disorders and various mutations [1, 2, 14-16]. One study involved close to 30000 matched case-control pairs of children living in the United Kingdom. It was found that children living in homes as far as 600 m from power lines had an elevated risk of leukemia. An increased risk of 69% for leukemia was found for children living within 200 m of power lines while an increased risk of 23% was found for children living within 200 to 600 m of the lines[4].This disorder was proportional to the duration of the proximity to the electromagnetic field and the wave type[3, 5]. According to the research, the most important effects of the high-voltage towers' field on children who live near high-voltage lines have doubled the risk of leukemia [16-19].

There are electrical current and internal electrical fields within all living organisms that are involved in complex mechanisms of physiological control, such as impairment neuromuscular systems, cellular death and activity and the development and repair of tissues. The synthetic properties of their possible effects on biological systems were also investigated. As a result of research, electromagnetic fields (EMF) [2, 7] initially causes dizziness, tinnitus, weakness and fatigue, blurred vision and sleepiness during work, as well as the emergence of unknown diseases, changes in blood composition, impairment in neuromuscular system, genetic transformation, the incidence of cancers such as lymphoma, leukemia, brain tumors, salivary gland cancer, and fertility disorder in men and women [2, 7, 20, 21].

Another effect of electromagnetic fields is a change in the normal secretion of neuroendocrine hormones, including melatonin, ACTH, cortisol, and epinephrine which can cause behavioral and cognitive impairment in person. The mechanism of EMF effect on the secretion of protein and amine hormones by activating the mechanisms of G receptor proteins and by activating membrane enzymes (PLC or AC) increases the production of the cAMP or DAG or activity of PKA or PKC which then causes phosphorylation of proteins with serine or threonine and actually causes changes in the transcription of genes [20, 22-24]. The ACTH peptide hormone secretes from the anterior pituitary and affects the cortical part of the adrenal gland positively. This hormone also increases the secretion of cortisol and aldosterone. The ACTH level drops by raising cortisol levels and vice versa. In fact, ACTH hormone acts as the regulator of hormones in cortical and central part of the adrenal gland [25-27].

Glucocorticoid receptor (GR) is an active transcription factor of flowing glucocorticoids and interferes with their effects on various biological functions in the body. These receptors play an important role in neurons and glands. Gene expression of GR receptors plays an important role in learning and memory, especially in hippocampal cells. The stress system activates the GR receptors and thus restores memory to the hippocampus of the rat. The expression of GR receptors in the peripheral blood lymphocytes is a good marker of the expression of these GR receptors in the hippocampal and prefrontal neurons and other neurons in the nervous system [28].

The importance of learning and how to enhance and improve this crucial cognitive process is in promoting human performance. Learning is from the most important activities of the neurology that is a relatively stable change in the person's feelings, thoughts and behaviors which has been made based on the previously recorded memory in the person [29, 30]. Actually, learning can be defined with two bases of memory and attention [31, 32]. Learning and memory mean communication between different areas of the brain, especially the hippocampal regions (the main position of memory formation), the prefrontal cortex (the main position of the focus and thought), the cerebellum (position of voluntary movement of the body) and visual paths [33, 34]. The cerebellum plays important roles in regulating body's movements, as well as in learning and thought. Stimulants within the neural fibers transform from the cerebellum to the rest of the brain, including the sensory cortex and visual system. These actions help in maintaining balance, converting thought to action and coordinating of movements [35]. According to studies, cerebellum also plays an important role in attention and spatial understanding [35-37]. On the other hand, injury or impairment of cerebellum impairs the coordination of the sensory and motor parts of the brain and disrupts the learning outcomes [38, 39].

The purpose of this study was to investigate the effects of high-voltage tower's field on cognitive and behavioral functions of the brain, including learning index and visual memory in a male rhesus monkey.

Materials and methods

Animals

The researchers have studied the human's and Mucaca monkeys' genes and found that 93% of gene sequences in the human and the monkey are common. Mucaca monkey is the closest to humans in cognitive aspect after the chimpanzee [1, 40]. Accordingly, this study was performed on the Mucaca monkey.

Two adult male rhesus monkeys (a control and one experiment)[1, 2, 7, 21, 41, 42], aged 4-5 years with an average weight of 4 kg were included after being fitted with the environment (12 months). The animal room was standard in terms of light, temperature, and humidity and has a condition of 12 hours of the day and 12 hours of the night [1, 2, 7, 21, 41, 42]. All ethical standards were observed in accordance with international law with regards to the transportation, location, and method of keeping animals (Baqyiatallah Medical University Medical Ethics Committee number 112-1394).

Protocols and simulation of high-voltage field

According to the protocol, after testing the pretest period, a monkey was exposed four hours a day to a simulated high-voltage electrical field of 3 kV/m for one month and the other monkey was kept in an environment without the field as a control sample [1].

The applied high-voltage electrical field was a field of 3 kV/m (Effects of exposure to a 400-kV, 50-Hz transmission line on human that simulated) that was simulated in the experimental environment of the Amirkabir University of Technology (Tehran Polytechnic) The High Voltage Laboratory (HVL); so that two metal plates (2*2 m), one below the monkey's cage and the other above were placed by a crane, the distance between two plates was 2 meters and a voltage of 6 kV was applied on them to create a uniform field of 3 kV/m on the monkey's cage(Teflon) (1*1*1 m) (Fig. 1) [1].

Figure 1. The presence of monkey's cage under a uniform electrical field of 3 kV/m.

Biological test

Behavioral studies before and after applying the field were examined on both tested and control monkeys. The monkey should be fast for 17 hours to do behavioral tests. An amount of 10 ml blood was taken from the femoral area of each monkey for biological tests. The 5 ml was used to measure and compare the changes in serum hormonal concentrations using the special ACHD primates' kit, prepared from the My Biosource Company, USA, and ELISA device; and another 5 ml was used to assess NMDA receptor gene's expression from peripheral blood lymphocyte cells by the RT-PCR method. Lymphocyte isolation was performed using a Ficoll solution by a centrifuge device. Measurement of hormonal concentration was performed in three steps (before applying the field, after that and recovery stage) [1].

The volumetric measurements of MRI images of cerebellar anatomy in samples were performed before applying the field and after that by DICOM Lite Box software [43].

Primers Sequences Glucocorticoid Receptor (GR):

- Forward primer: AGGAAAAGCCATTGTCAAGAGG;
- Reverse primer: CCTCTACAGGACAAACTGATAG.

PCR-GR-Receptors 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 Ficole 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 expression of GR-receptor genes using the PCR technique. Using peripheral blood lymphocytes expressed receptors GR were analyzed by PCR(Polymerase Chain Reaction). 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, 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) in accordance with the manufacturer's guidline. 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, Bioneer kit (Takara, Japan) was applied. priefly, 100 ng of each RNA sample were 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 were 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 the rmocycler (Techne, UK) containing 1.5 μl cDNA, 0.2 mM of the deoxynucleos idetriphos-phates (dNTPs), 2.5 mM $MgCl_2$, 10 pmol of each primers and 1.5 U of Taq DNA Polymerase (Cinnagen, Iran). PCR program was 6 min initial denating 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 etidium bromide and visualized under UV gel document. Finally, the density of each Product band was measured by Image J software.

Behavioral test

Visual Learning

To measure the visual learning test, a container designed for this purpose was used. This container has a spring-hinged opening that can only be opened in one direction so that the animal should hold the lid of the container with one hand and get the reward with another hand in order to reap the reward inside the container (peanuts) (Fig. 2). The protocol was so that 10 repetitions a day for a week; and after the test period, it was the same except for the container door's direction [44].

Figure 2. A Plexiglass transparent container designed to perform a visual learning test and the cage that is prepared by Teflon.

Visual Memory

The visual memory behavior recorder consisted of two dishes (each one with an opening in one direction only) and was non-transparent (reward was inside the container, invisible to the primate). Two coated containers were located on a movable base [45, 46]. This test was performed in 2 phases of 30 seconds and 60 seconds (Figure 3).

The first phase of the test: The visual memory behavior recorder device was positioned in front of the primate eyes, the favorite reward of animal (peanut) was placed in one of the dishes randomly, and after 30 seconds the dishes were given to the animal. The animal was allowed to effort reaching the reward only for one time. Therefore, he should pay an attention and focus, because he may deprive of gaining reward if making a mistake in the first attempt. The test was performed 10 times a day.

The second phase of the test: This phase was exactly the same as the first phase, except that the dishes were given with a 60 seconds delay.

The protocol of these tests was performed one month before and one month after the test and was compared with the control sample and with each other[1].

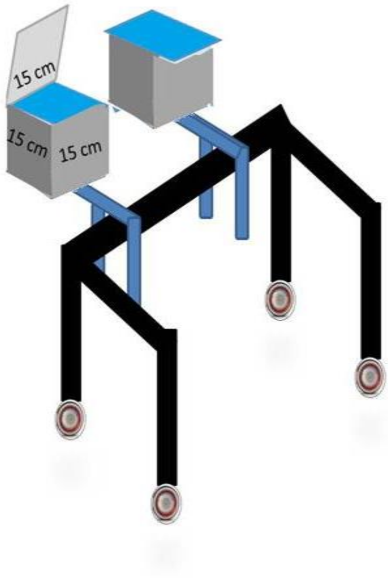

Figure 3. The visual memory behavior device shape test (Delay Response Task)

Results

The results of behavioral study indicated a decrease in the performance of visual learning tests in the tested monkey under high-voltage field compared to the pre-test period, while the control sample had no significant change (Fig. 4)

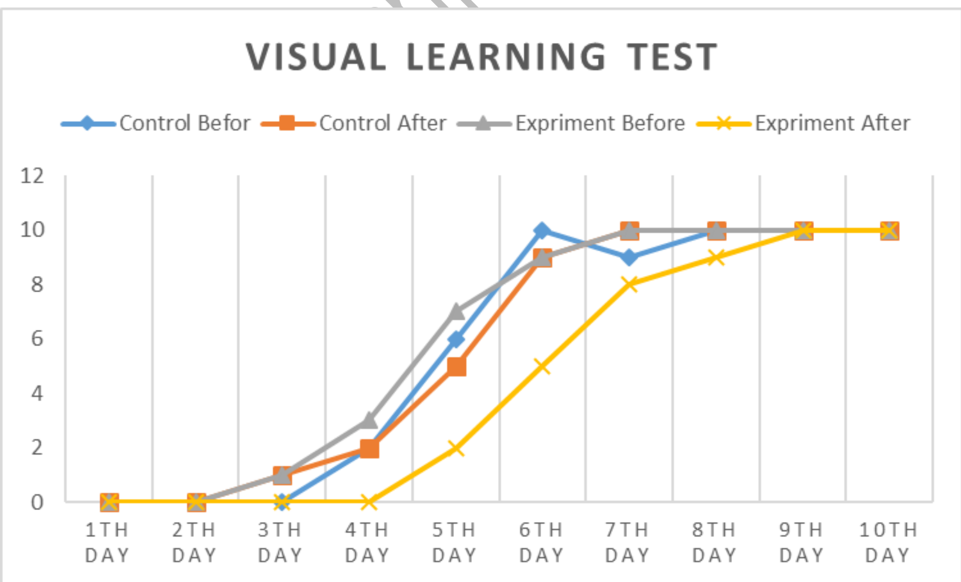

Figure 4. The visual learning test

In addition, the monkey under test has been associated with reduced performance in terms of visual memory in the condition of high-voltage field compared to the previous condition, while there was no significant change in the control sample (Fig 5 and 6).

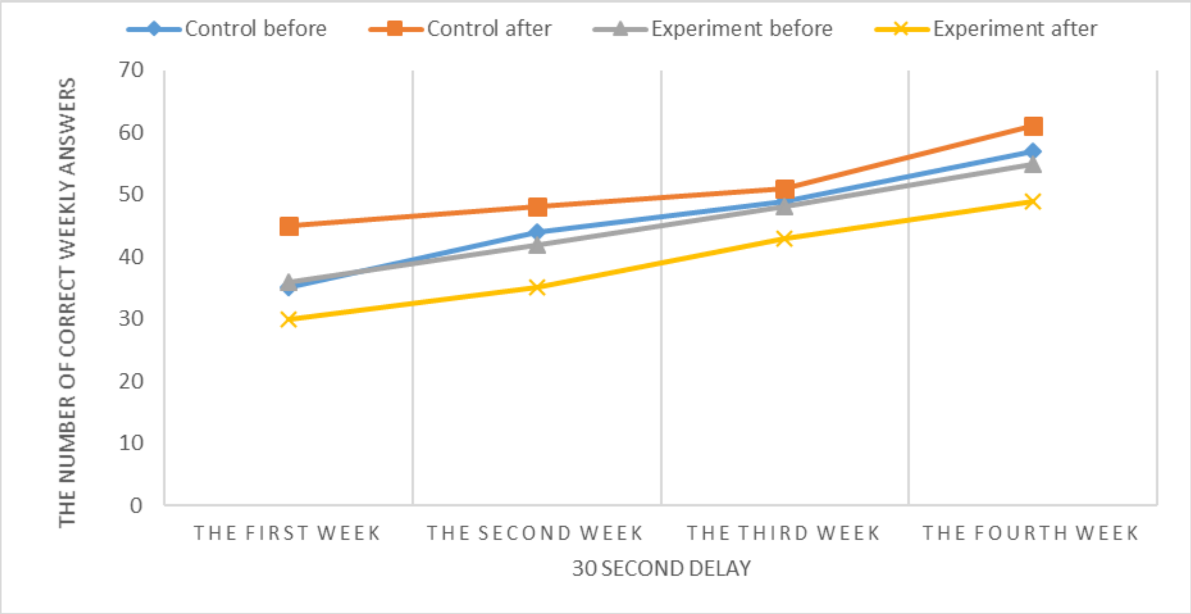

Figure 5. The visual memory test at a delay of 30 seconds.

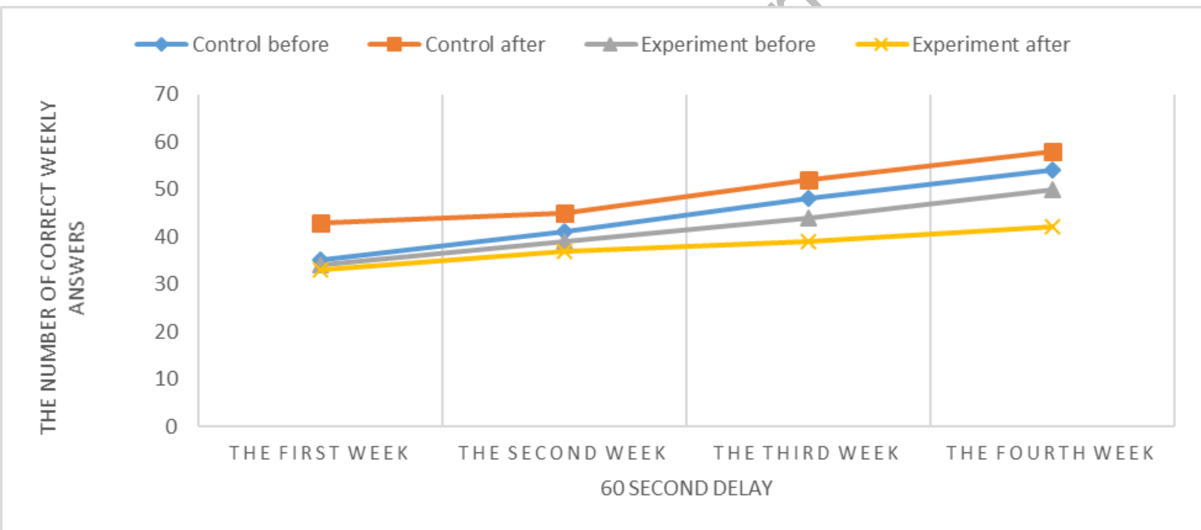

Figure 6. The visual memory test at a delay of 60 seconds.

The results of the ACTH hormone test in the monkey under test showed a significant increase; while the control sample had no significant change. It was worth noting that within one month of recovery, this hormone was still decreasing (return to baseline) in the test monkey (Fig. 7).

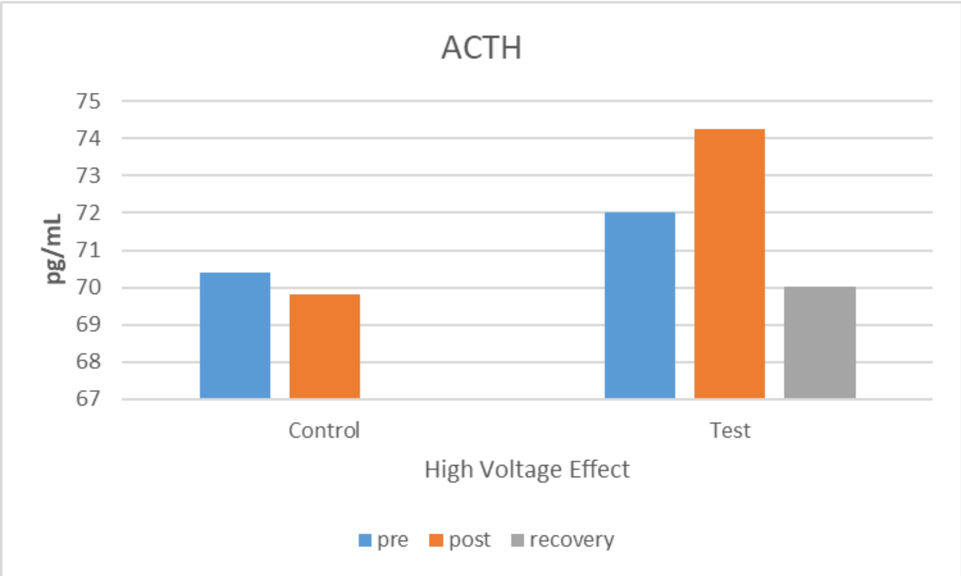

Figure 7. The ACTH hormone changes in the test sample before and after applying the field and the control sample before and after applying the field.

The results of GR receptor gene's expression in the monkey under test showed a significant reduction in the level. It must be noted that during a one-month recovery period, it was returning to the baseline state. On the other hand, no significant changes were observed in the control sample (Fig. 8).

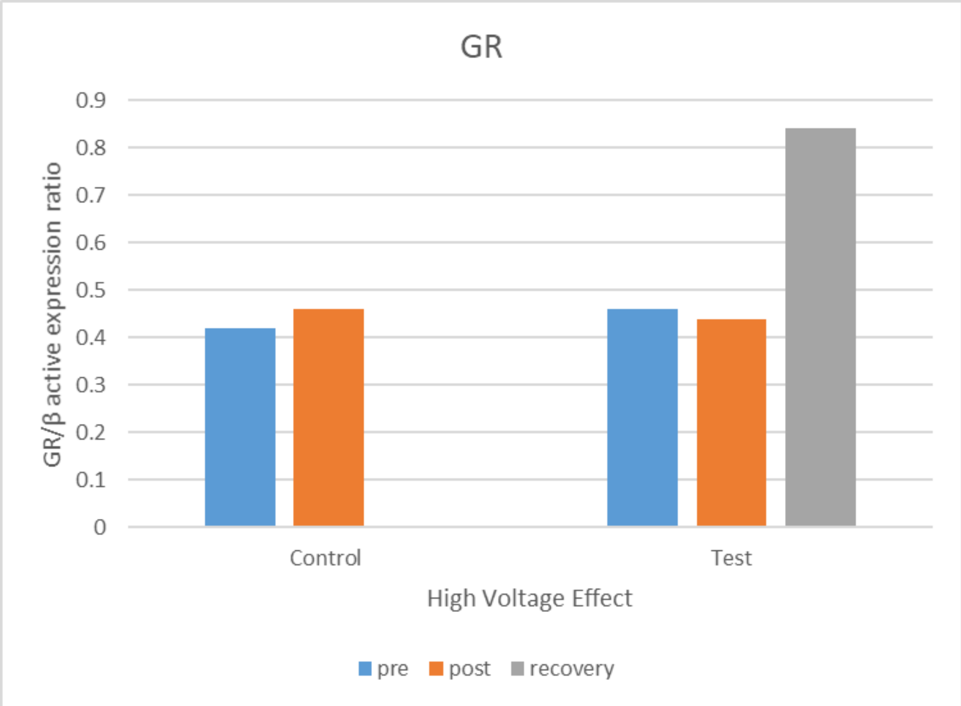

Figure 8. The changes in GR receptor gene's expression in the test sample before and after applying the field and the control sample before and after applying the field.

MRI examinations of the cerebellum have not shown any significant changes in volumetric variation in the sagittal incision (Fig. 9). It is worth noting in the clinical and histological examination of MRI for cerebellar assessment, the hemorrhage was observed in the left region of the cerebellum. This is while the MRI image did not show such a complication before applying the field. The clinical examination of the tested monkey exposing to a simulated high-voltage field showed that the tested cerebellum had a bleeding disorder in the left cerebellar part compared with the time before the test (Fig. 10).

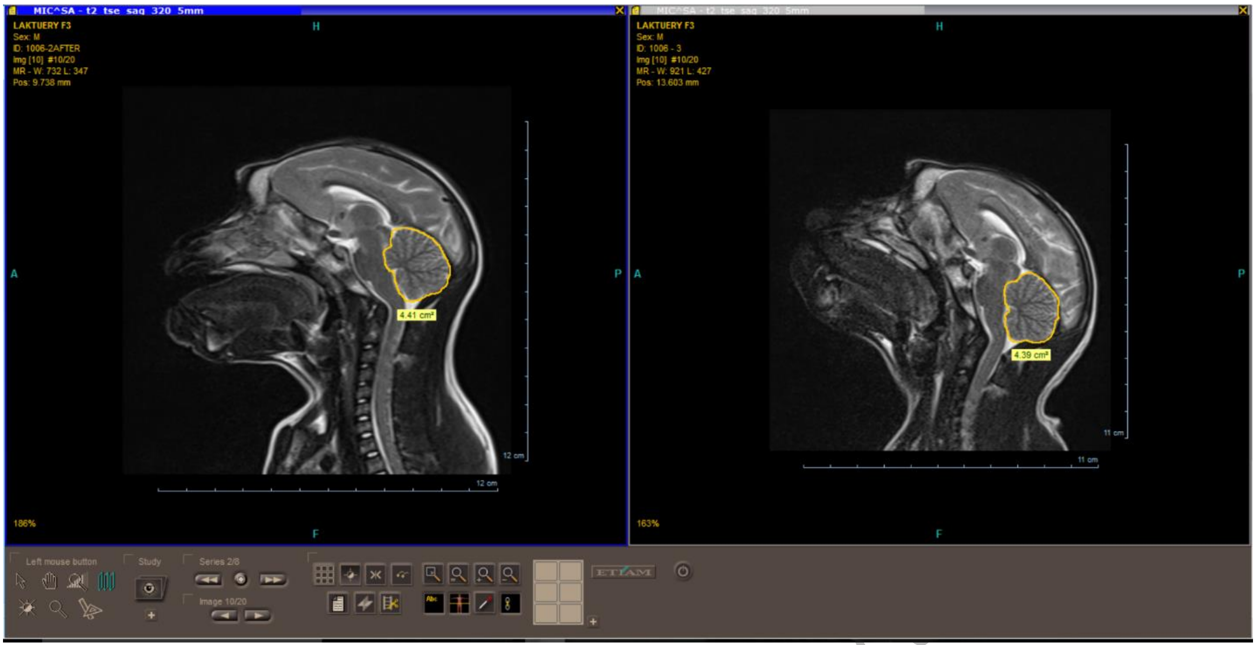

Figure 9. The anatomical size of the cerebellum computed with the DICOM Lite Box software with MRI images of the test sample.

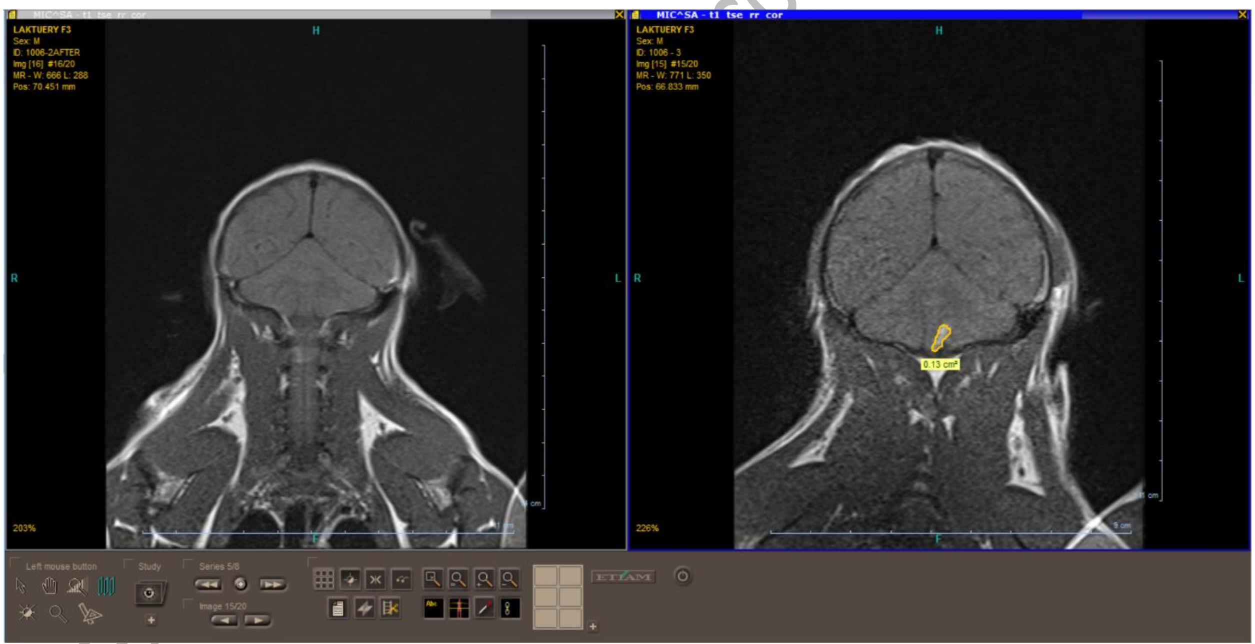

Figure 10. The incidence of hemorrhagic lesions in the cerebellum section of the tested monkey after the test period (The right photo is for after the test period).

Discussion

Nowadays, environmental hazardous factors threaten the environment, organisms, especially humans' safety[6]. One of these damaging environmental factors is the electromagnetic fields produced by high-voltage towers, so that industrialization of cities and expansion of electrical lines and the proximity of high-voltage towers with residential buildings have increased the risks of high-voltage fields[1, 6, 41]. Based on the research, high-voltage fields are not only influencing the health and lifestyle of people but also cause cognitive and behavioral changes in animals and humans [1, 6, 41, 49].

One of today's research challenges is the importance of learning and how to promote and improve this cognitive process in promoting human performance[50-52]. Learning is from the most important neurological activities includes a relatively stable change in the senses, thought and behavior of an individual, which has been made in a person based on the previously recorded memory [1, 53].

The results of the study indicated that the tested monkey showed significant changes in terms of cognitive and behavioral elements as exposing to high-voltage field[6]. As the behavioral characteristics, the tested monkey was intelligent, with high attention, stirring and vitality, and active before the test, but there were significant changes after the test period[1]. After the test, the monkey turned into an unresponsive, lethargic, inactive, jadish and depressed one, even with a weight loss of about 1 kilogram. The results of the cognitive tests indicated a reduced correct response to the tests after the test period. The bases of learning are attention and concentration that were gradually diminished in the tested monkey at the time of placement under the field, as well, animal's visual learning decreased similarly. Research has shown that learning and memorizing are the basis of memory [54, 55]. The cognitive elements of attention, learning and memorizing impaired in the tested monkey resulting in decreased visual memory[1, 42]. Hormonal studies in the tested monkey indicated an increase in the ACTH level after the test[41]. The ACTH hormone acts as a regulator of cortisol hormones which plays an important role in cognitive indices in memory and learning in the central nervous system (CNS)[2, 7, 41, 42]. Increasing the ACTH hormone reduces the release of cortisol hormone from the cornea of the adrenal gland[27]. Abnormal changes in cortisol hormone secretion result in nervous disorders in an individual[41, 56-58]. Electromagnetic fields cause abnormal changes in cortisol secretion and increase the activity of the stress and oxidative pathway to produce free radicals, leading to neurological diseases by interfering with normal functioning of neurons [1, 17, 59]. An increase in ACTH by increasing the activity of membrane receptors of G proteins by means of increasing cAMP and calcium ion as a secondary messenger increase the activity of PKA and genomic changes in neurons [60]. The results of the study indicated an increase in ACTH, which can reduce normal cortisol level. These changes could disrupt the cognitive elements of the tested monkey; so that it can be argued that cortisol hormone changes played an important role to impair learning and memory, inactiveness and depression in the tested monkey, and the results clearly confirmed this. Cortisol, on the other hand, plays an important role in expressing the GR receptor gene. The GR, NMDA receptors (play an important role in memory and learning processes) are scattered throughout the CNS regions[7, 41]. The highest concentration of GR and NMDA receptor genes in CNS is in the hippocampus, prefrontal, amygdala, and cerebellum [22, 35, 38, 39]. These two receptor gene's expressions play an important role in memory learning of humans and primates. The results of the study showed that the expression of the GR receptor gene in the tested monkey was reduced[1]. The decrease in the expression of the GR receptor gene has also an important role in reducing learning and memory [61]. However, there were no significant changes in the control sample in none of the above-mentioned cases (cognitive, behavioral, gene, and hormonal factors).

Anatomical investigation of the brain by the MRI image is a common and non-invasive technique that neurologists might use this method for neurological disorders, including Alzheimer [62, 63]. Anatomical examination of the cerebellum by MRI imaging in the tested monkey showed no significant changes in the cerebellum. This volumetric study was measured by the DICOM Lite Box software. But the clinical examination of the tested monkey exposing to a simulated high-voltage field showed that the tested cerebellum had a bleeding disorder in the left cerebellar part compared with the time before the test [47, 48]. This complication was confirmed by a radiologist Dr. Hossein Ghanaati of Tehran University that could be another reason for decreased learning and memory of the tested monkey.

Conclusion

A general conclusion of this study deduced that cognitive tests (visual learning and visual memory) in the tested monkey showed a decrease in the correct functioning in case of applying a simulated high-voltage field. This disorder was assessed by the ACTH hormonal study and its increase in the tested sample. Also, with the genetic review of the GR receptor that was associated with a reduction in the expression of the GR receptor gene, given that this gene plays an important role in learning and memory, it is another confirmation of the disorder. Finally, whereas anatomical examination of the test sample had no changes in the volume of the cerebellum, but the hemorrhage in the cerebellum was observed in the clinical examination which can cause cognitive impairment (learning and visual memory).

According to the results of this study, it can be argued that the fields of high-voltage towers are as serious cognitive hazards for residents around the power lines which require further research in this context.

Conflict of Interests

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

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