Title: The Effect of Electrical Fields from High-Voltage Transmission Line on Cognitive Changes in Male Rhesus Macaque Monkeys: A Biological and Anatomical Study Using MRI Case Report Study

Authors: Hamed aliyari[1], Hedayat sahraei [2], Sahar Golabi [3], Mohammad Bagher Menhaj[1], Masoomeh Kazemi[2], Seyed Hossein Hosseinian[1,*]

1. *Center for Human-Engaged Computing Kochi University of Technology Kochi, Japan.*
2. *Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
3. *Abadan Faculty of Medical Sciences, Abadan, Iran.*

*Corresponding Author: Email: hosseinian2001@yahoo.com

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Abstract

Living near high-voltage power lines and exposure to high-frequency electromagnetic fields (EMFs) is a potential serious hazard to animal and human health. The present study was carried out to evaluate the effect of high-frequency EMFs from simulated high-voltage electric towers on cognitive, anatomical, and biological changes in male *Macaque.*

In this study, two *Rhesus Macaque* were recruited, one experimental and one control. The experimental subject was exposed to EMFs from simulated 3kV/m electric towers with a given protocol, and the control subject was tested without irradiation (4h a day, for 30 days). All required tests were performed before and after the intervention on both experimental and control monkeys. The anatomical alternation of the prefrontal area (PFA) was measured by MRI images. All tests were performed on irradiated and control animals before and after the intervention, and results were compared between irradiated and control animals.

the findings of the present study indicated increased white blood cell counts, after high-frequency EMFs irradiation. Also, the red blood cell counts showed a decreasing trend after irradiation. The plasma levels of adrenaline increased after irradiation. Besides, the blood glucose levels increased after irradiation. The PFA was different before and after the irradiation. Moreover, some behavioral disorders such as fatigue, drowsiness, anorexia and insomnia were observed after irradiation.

Results from biological tests and MRI showed elevated risk of immunodeficiency disorders, weakness, and behavioral disorders. A warning is given to people who live or work near high-voltage electric towers with high-frequency EMFs.

***Keywords*:** MRI; Rhesus macaque; EMFs; High-voltage transmission line; Adrenaline hormone.

Introduction

Nowadays, exposure to high-frequency electromagnetic fields (EMFs) is a potential serious hazard to animal and human health (1, 2). According to literature, people who are exposed to EMFs, are more likely to develop any type of blood cancer, abortion due to poor fetal implantation, and neurological disorders like stress, depression, and insomnia due to degradation in neuronal function and dysfunction by reactive oxygen species (ROS) molecule (3, 4). The ROS may harm the function of hippocampus neurons and is a major contributor to mental illnesses like Alzheimer's disease (5-7). Electric fields and currents exist in the body of all living organisms that act as a contributor to physiological control mechanisms such as the nervous system, cell death, growth, tissue growth and repair (8, 9). The EMFs are associated with many symptoms such as dizziness, tinnitus, weakness and fatigue, blurred vision, and drowsiness during work, as well as the emergence of unknown diseases, altered blood composition, impaired musculoskeletal system, and immune system dysfunction among animals and humans (10-13). Impaired secretion of hormones may lead to behavioral and cognitive disorders (1). Accordingly, EMFs adversely affect the secretion of protein and amine hormones by activating to G-protein coupled receptors and membrane enzymes (10, 11). The ACTH secreted from the anterior pituitary, acts to stimulate the adrenal cortex hormones and regulates the secretion of cortisol and adrenaline(10, 14). Results from many studies indicated themes from simulated high voltage towers, reduce melatonin secretion resulting in sleep, attention, learning and memory disturbances, and development of mental illness such as depression in *Rhesus* monkey (1, 7, 10, 11, 14-21).

Increased secretion of adrenaline, increases glucose release from the liver leading to increased blood glucose levels. Thus, the required energy for muscle and nerves is supplied (11, 15, 16, 22). Secretion of adrenaline increases due to stress and abnormal conditions. Producing more adrenaline, in turn, helps to increase the amount of energy to adapt to new conditions. Besides, further production of adrenalin may deplete sugar supplies (23, 24). In response to environmental factors in stress conditions, the production of T cell lymphocytes increases (25-27). The number of lymphocytes is 70% in apes and 30% in human blood. Environmental factors and stressors can increase white blood cells (WBC), or leukocytes. The abnormal increase in WBC counts may cause immune deficiency, cognitive impairment, infectious diseases, bone marrow failure, and leukemia (28). Several studies have shown that the EMFs generated from the simulated high voltage towers reduce the area of the hippocampus and the amygdala in *Rhesus* monkeys (29, 30).

So, the purpose of this study was to investigate the effect of high-frequency EMFs from simulated high-voltage electric towers on cognitive, anatomical, biological, biochemical, and hormonal changes in male *Rhesus Macaque* monkeys.

Methods

Animals

Two *Rhesus* monkeys (*Macaca Mulatta*) aged 4-5 years, with an average weight of 4kg were recruited. All ethical standards regarding animal confinement, transport, location, and maintenance were observed under the international laws and regulations (IR.BUMS.REC.1394.112).

Experimentation protocol and simulation of high-frequency EMFs

First, pre-tests were conducted on study subjects. Of the two selected monkeys, one subject was irradiated by 3kV/m high-voltage EMF, 4h per day, for 30 days (10, 18, 31), and one subject was maintained in a non-irradiated environment and tested without irradiation (10, 18, 31). The monkeys kept in Teflon cages that, in addition to having special conditions for keeping the animal, such as strength, portability, and washability, had special properties for conducting scientific experiments (with the highest transfer properties and lowest resistance)(31, 32). High voltage EMF (3kV/m) produced which was simulated by a team of experts at Amir Kabir University, Tehran, Iran. Two metal plates (2*2m) were placed in the upper part and one in the lower part of the cage with a 2m distance, and a 6kV EMF was released to produce a uniform 3kV EMF inside the cage (1*1*1m) (7, 10, 11).

Behavioral tests

In this study, the monkeys were vaccinated against tetanus, pertussis, diphtheria, and polio before entering the laboratory. After entering the laboratory, it took three months to adapt to the environment and to adapt to the researchers. After this period, two perfectly healthy monkeys were included in the study. The monkey that was subjected to high-pressure surges was the freshest, smartest, and most active monkey in the laboratory. For this purpose, all observable behavior in subjects such as sleeping patterns, sedentary and natural appetite, drowsiness, and depression were observed and recorded before, during, and after intervention by video cameras (21, 33-35).

Biological test

Biological tests were performed on irradiated and control subjects before and after the intervention and during the recovery period and obtained data were then analyzed. According to experimentation protocol, a fasting period of 15 hours was required for monkeys. For biological analysis, a total of 10 c.c. of blood was taken in three stages, pre-, post-irradiated, and the recovery from the femoral artery of the animals, of which 5 c.c. was used to evaluate changes in the levels of adrenaline using the primate custom positive selection kit (MyBioSource, USA) at three stages. Another 5 c.c. of blood samples were used to measure fasting blood sugar, WBC counts, and RBC count sat three stages (7, 10, 17, 30, 36).

The anatomical alternation of the PFA was measured by MRI images and DICOM LiteBox files before and after the intervention (7, 37).

Recovery phase

Both of two selected monkeys, one subject who was irradiated and one subject who was maintained in a non-irradiated environment, staying in the same previous place and situation but both of them without irradiation for month (31).

Results

The behavioral results of the study showed that in the tested monkey exposed to the electric field of the simulated high-pressure rig, the behavioral-cognitive indices change over time. From the first day to the 30th day of the waving, cognitive changes could be seen with the naked eye (recording behavior by camcorders) .Our observations were from: monkeys under high-pressure electric field showed states of impatience, lethargy, anorexia, sleep disturbance, inactivity, and state of depression.

Behavioral tests investigated observable cognitive changes in the study subjects including drowsiness, loss of appetite, sleep deprivation, fatigue, inactivity, and depression (recorded by video cameras). Behavioral results showed an increasing trend in the mentioned criteria during the thirty days of irradiation. The behavioral analysis was performed one month before, during, and after irradiation (recovery phase). Behavioral tests were also performed on the control animal at three stages without irradiation, and no difference was observed in the control monkey.

The serum levels of adrenaline were shown to elevate after high-frequency EMFs irradiation compared with the control monkey. In addition, the adrenaline levels during the recovery phase were declining to the pre-irradiation phase. No difference was observed at three phases in the control monkey (figure 1).

The levels of fast blood glucose were shown to increase after high-frequency EMFs irradiation compared with the control monkey. Also, the blood glucose levels during the recovery phase were declining to the pre-irradiation phase. No difference was observed at three phases in the control monkey (figure 2).

The WBC counts showed an increasing trend after high-frequency EMFs irradiation compared with the control monkey (figure 3).

The RBC counts was shown to decrease after high-frequency EMFs irradiation compared with the control monkey. Besides, the RBC counts during recovery phase was increasing to the pre-irradiation phase. No difference was observed at three phases in the control monkey (figure 4).

The PFA decreased after irradiation in the experimental monkey compared with the control monkey. Also, the PFA during the recovery phase was increasing to the pre-irradiation phase. No anatomical difference was observed in the control monkey (figure 5, 6).

Discussion

Today, industrialization remade much of people's life. Rapidly growing industrialized cities increased growth of power lines and proximity of overhead electric towers to residential and occupational places increased the risk of EMFs harms as a global public concern. According to the literature, the EMFs generated by high-pressure electric towers are a serious threat to the environment of animals and humans. This destructive environmental factor adversely affects health and lifestyle, and causes cognitive and behavioral changes in animals and humans (1, 10, 15, 25, 28).

Based on our results, the cognitive and behavioral elements were different after exposure to EMFs from simulated high-pressure towers in irradiated subject. Behavioral analysis of experimental and control monkeys revealed that homogeneous behavioral indices such as refreshment, sedentary, and natural appetite were clearly observed in control monkey, whereas experimental monkey showed drowsiness, loss of appetite, sleep deprivation, fatigue and depression after EMFs irradiation. These behavioral and cognitive changes in the tested monkey from the first day to the last day of the waving showed an increasing trend. On the other hand, from the day 30 onwards, with the interruption of the waving, cognitive and behavioral changes returned to the initial state before the waving. It indicates the reversible effects of the fields caused by high-pressure rigs. According to research, people who live near high-pressure towers develop cognitive impairments(38). The results of this study showed that exposure to high-frequency EMFs reduced melatonin secretion leading to sleep deprivation and immune deficiency in male *Rhesus* monkeys. sleep deprivation and immune deficiency are major contributors to depression (23, 39, 40). Besides, high-frequency EMFs have been shown to reduce gene expression of the NMDA receptor and subsequently reduce plasma levels of sodium and potassium. Sodium and potassium are the most important biological ions that cause irritability of the neurons and transmissions of nerve impulses. It is fundamental to note that sodium, potassium, and NMDA receptor gene are important elements in learning and memory processes (41-45). Exposure to extremely low-frequency EMFs (ELF-EMF 12Hz) has been shown to increase melatonin secretion. The MT1 (or Mel_{1A}) and MT2 (or Mel_{1B}) are two major receptors of melatonin which are a G protein-coupled receptor (GPCR). When receptors of melatonin bind to melatonin, the PKA or PKC enzymes are activated (10, 30). Also, ELF-EMFs at a frequency of 12Hz increase gene expression of NMDA, and subsequently improve visual memory of male *Rhesus* monkey. the ELF EMFs stimulate glutamate on NMDA receptors and inhibits magnesium from the NMDA membrane receptor, resulting in more sodium outside and more potassium inside the cell (10, 46, 47). The highest density of the NMDA receptor gene is found in the hippocampus, amygdala, and prefrontal cortex in the central nervous system which plays an important role in learning and memory processes (1, 7, 15, 30, 41, 43). The findings of the present study also reveal that the plasma concentration of adrenaline increased after high-frequency EMF irradiation in experimental monkey. Adrenaline is a catecholamine secreted from the central part of the adrenal gland,

and when cortisol levels are low, adrenaline secreted in response to anxiety and helps to chill and cope with tragedies. Increased levels of adrenaline among study subjects may be due to impaired behaviors in this study. The activity of adrenaline hormone is dependent on GPCR and activity of adenylate cyclase and increased cAMP as a secondary messenger activates adrenaline (13, 48).

Hormones are chemicals released for the development and enhancement of cognitive capabilities, and any stimulus, environmental factor, behavioral and cognitive impairments influence their secretion. Evidence shows that stress coping strategies in male *Rhesus* monkeys are different, and the cortisol and adrenaline secretion vary depending on the personal characteristics of the animals (11, 15, 49). Biochemical studies have shown that the blood glucose increased after exposure to simulated high-frequency EMFs. Adrenaline (epinephrine) is shown to elevate blood glucose which is consistent with the findings of this study. moreover, results from hematological studies indicated a reduction in RBC counts among irradiated monkeys after exposure to high-frequency EMFs. Reduced RBC counts (anemia) is associated with drowsiness, fatigue, immune deficiency, and depression in irradiated animals, which is consistent with results of behavioral tests in subjects irradiated to high-frequency EMFs. Previous studies have shown that exposure to high-voltage electric fields was associated with sleep and depression in male *Rhesus* monkeys (7, 23). Results from hematological studies also showed an increase in the WBC counts after irradiation. Increased WBC counts as an infectious agent may impair normal immune function. The results from behavioral tests indicate a weakened immune system, drowsiness, and inactivity amongst subjects exposed to simulated high-frequency EMFs which can be attributed to the immune deficiency of the male *Rhesus* monkey.

The anatomical assessment by MRI is a common and non-invasive technique used by neurologists to examine neurological disorders such as Alzheimer's disease. Cerebral atrophy in MRI of the hippocampus represents the presence of Alzheimer's disease. According to the results from anthropometric measurements of PFA by MRI through DICOM LiteBox, the irradiated subject showed a decreasing trend in PFA after the irradiation compared with the control monkey (37).

The frontal lobes are involved in behavior and personality. The smallest anatomical changes in the PFA can rapidly alter behavior and personality and cause behavioral and cognitive impairments. The frontal lobe is the most important part of the brain that plays a major role in cognition and behavior. Disruption of it disrupts natural behaviors and cognitive indicators(32). Previous studies have shown that high-frequency EMFs can cause anatomical changes in the hippocampus and amygdale MRI of male *Rhesus* monkey (50, 51). Kazemi et al 2018, argued that no changes were observed in hippocampus MRI of male *Rhesus* monkey after exposure to low-frequency EMFs at a frequency of 12Hz (10).

It is important to note that the recovery phase is a period in which the subject is recovered to the pre-irradiation level. Although the effect of high-frequency EMFs is temporary, the subject was irradiated four hours a day for four months. Besides, people living near high-voltage power lines are constantly exposed to high-frequency EMFs. Thus, further research is required to precisely examine various pathological conditions.

Conclusion

The findings of the present study suggest the observable behavioral disorders such as fatigue, anorexia, sleep deprivation, and depression in the experimental subject exposed to the electric field from simulated high-pressure towers were consistent with the results of other tests. The increase in adrenalin concentration increases glucose levels. Increased WBC counts cause immune deficiency. decreased RBC counts lead to anemia, fatigue, and drowsiness in experimental monkey. All were matched with the monkey's behavior. It can be concluded that the EMFs from high-pressure electric towers are a serious hazard to cognitive abilities and can lead to mental disorders in humans and animals.

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Conflict of Interests:

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

Ethical Approval:

All ethical standards were met based on the international IR.BUMS.REC.1394.112

Figure 1: Adrenaline plasma levels (Pre-test, Post-test, Recovery)

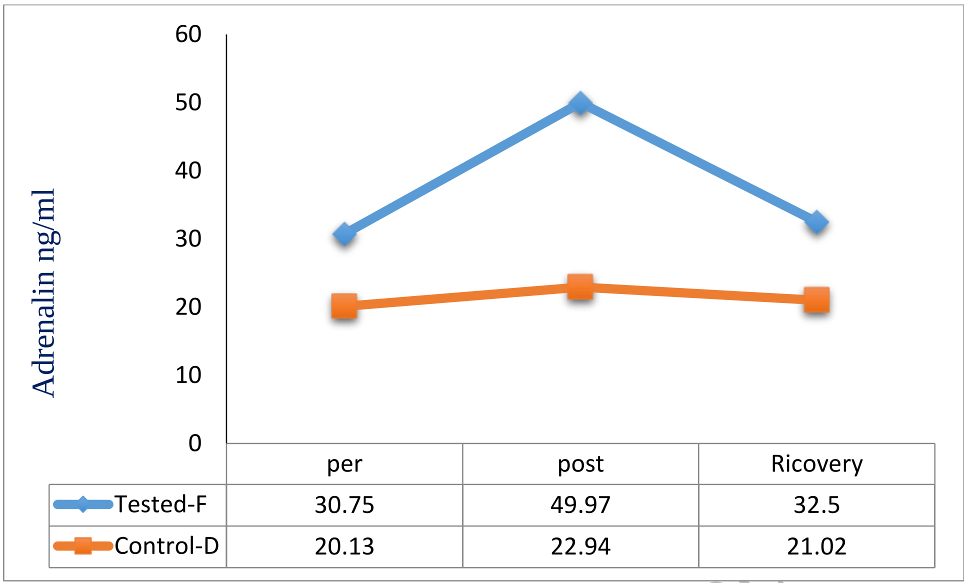

Figure 1. The serum levels of adrenaline were shown to elevate after irradiation. The adrenaline levels during the recovery phase were declining to the pre-irradiation phase

Figure 2: Fast Blood Sugar levels (Pre-test, Post-test, Recovery)

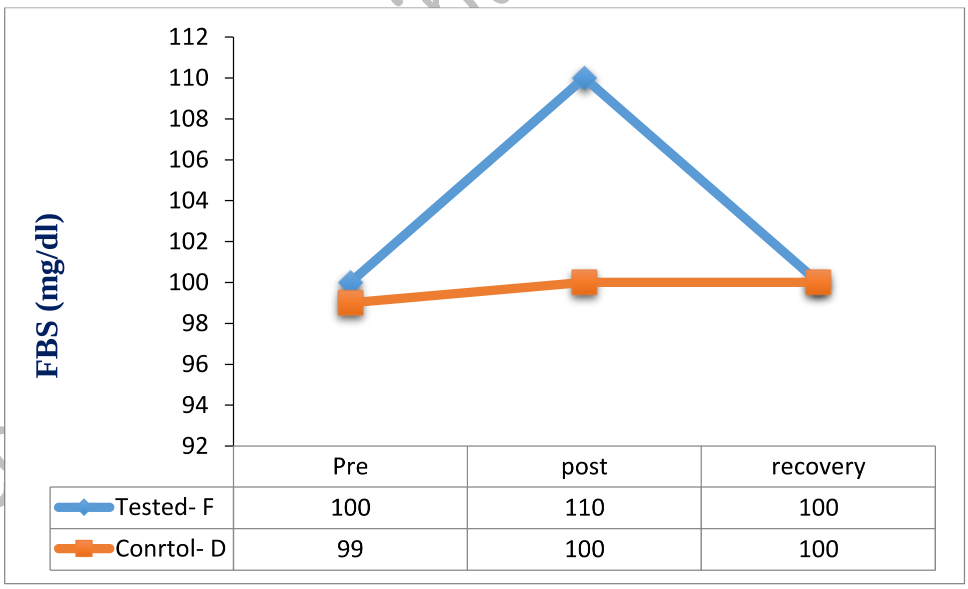

Figure 2. The increasing trend of fast blood glucose levels after irradiation. The fast blood glucose levels during the recovery phase were declining to the pre-irradiation phase

Figure 3: WBC counts (Pre-test, Post-test, Recovery)

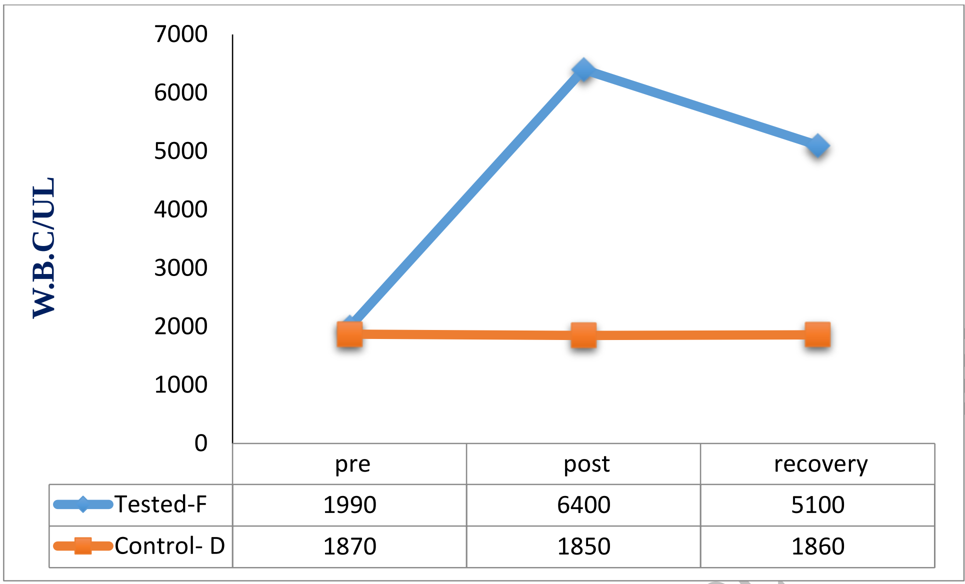

Figure 3. The increasing trend of WBC counts after irradiation. The WBC counts during the recovery phase was declining to the pre-irradiation phase

Figure 4: RBC counts (Pre-test, Post-test, Recovery)

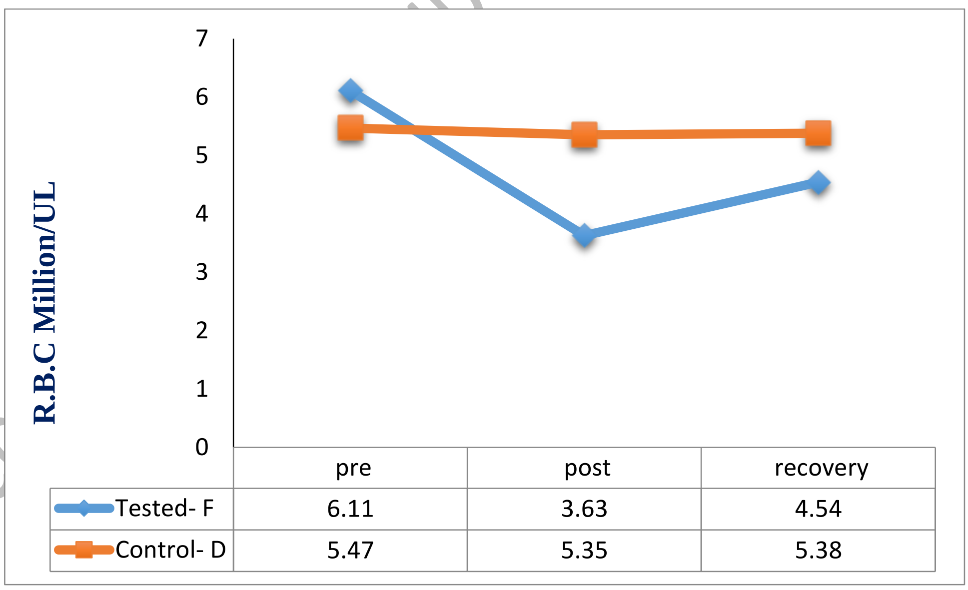

Figure 4. The RBC counts were shown to decrease after irradiation. The RBC counts during the recovery phase was elevating to the pre-irradiation phase

Figure 5 and 6: Pre-frontal area (PFA)

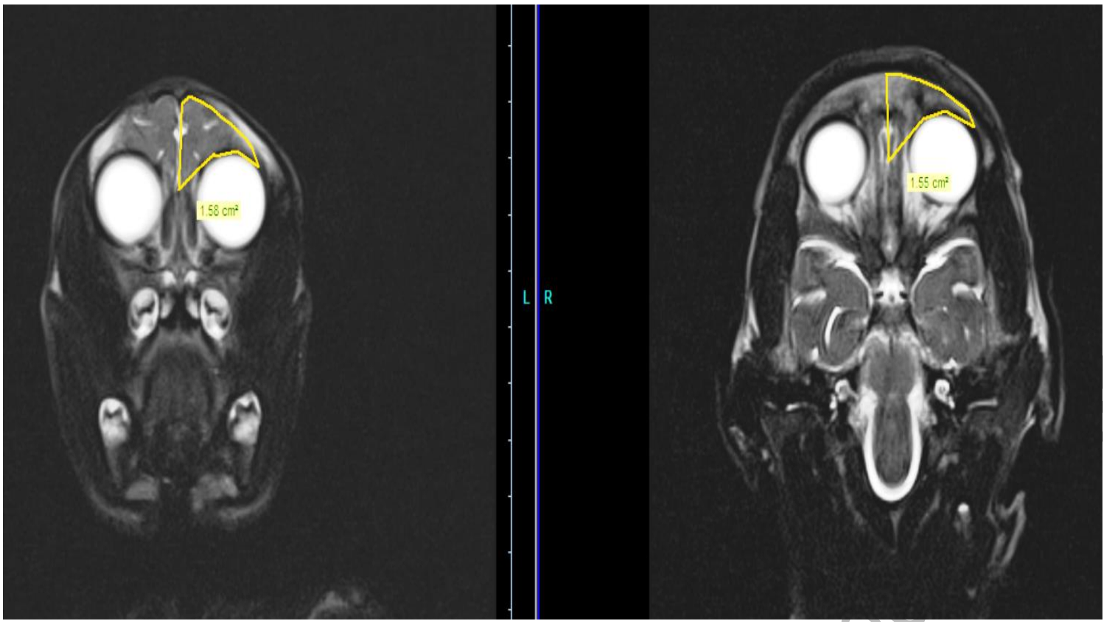

Figure 5, 6. The pre-frontal area were shown to decrease after irradiation. The pre-frontal area during the recovery phase was elevating to the pre-irradiation phase

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