

## Long-term exposure to low frequency electro-magnetic fields of 50- and 217-Hz leads to learning and memory deficits in mice

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### ABSTRACT

Electromagnetic field (EMF) radiation affects cellular and brain chemistry and function, resulting in deleterious effects such as free radicals formation, impaired DNA repair, reduced melatonin and blood brain barrier protection, and defects on learning and memory and other higher brain functions. In this paper the effects of low frequency EMF of 50- and 217 Hz, ranges often associated with common electronic devices such as televisions and cell phones were examined on learning and memory in adult male mice. Five groups (n=10 mice/group) of mice (1 control and 4 experimental) were initially trained for the *passive avoidance* (PA) test. They were then placed in devices creating EMF radiation with varying intensities (0.5 to 2 milli-Tesla, mT) and frequencies (50- and 217-Hz) for 2-weeks (16 hrs/day). Control mice received no radiation. Learning and memory was tested by the PA test and evaluated based on the following parameters: mean step through latency (STL), number of crossing (Cr#) and time in dark compartment (TDC). Results showed significant deficiencies in learning and memory in the EM-exposed mice compared to controls: mean STL decreased significantly ( $p < 0.001$ ) in the 50 Hz group (1 and 1.5 mT intensities). In the 217 Hz group, STL also decreased in the 0.5 and 2 mT groups ( $p < 0.05$ ). There was a notable increase in mean Cr# for both groups and TDC for 50 Hz group. Results confirm that long-term exposure to EMF radiation of 50 and 217 Hz, imparts significant harmful changes on memory and learning, reiterating the need for preventive measures against such exposures.

**Keywords:** memory; low frequency electromagnetic fields; passive avoidance learning

### INTRODUCTION

Memory and learning are of great importance in human life and activities. Many factors are known to have positive and negative effects on memory formation and recall. Earlier findings demonstrated that electromagnetic fields (EMF) disturb spatial orientation and recognition in experimental animals [1, 2, 3]. These studies were confirmed by more recent studies that illustrate deficits in other types of learning and memory [4, 5, 6] including concomitant alterations in hippocampal CA3 region [5]. Other investigations have either not found deficits in cognitive functions or reported positive and useful effects of low frequency, short-term EMF on memory in some animal species [7, 8]. Thus effects of low

frequency (0-300 Hz) EMF on cognitive processes have either not been adequately studied or are controversial at best. Given the multitude of electronic devices used in everyday modern life, especially the marked overuse of cell phones along with the reported possible harmful effects of EMF radiation on body tissues and health, further study of the biologic, neurobiological and other effects on health appears to be critical and necessary to investigate.

Electronic devices, electric power-lines and cell phones used in human environments produce EMF of 50 and 217 Hz. The aim of this paper is to artificially generate the latter EMF radiation in varying intensities of 0.5-2 *milliTesla* (mT), in order to investigate the effects of long-term

exposure on the central nervous system in experimental animals to said devices. In particular, we focus on learning and memory in mice using the well-known passive-avoidance learning (PA) testing system and the associated quantitative parameters: *step through latency* (STL), *time in dark compartment* (TDC) and the *number of crossings* (Cr#). It is anticipated that the results of this investigation will help elucidate the effects of EMF on learning and memory and help increase our understanding of the consequences of such effects. We hope this information will allow for the development of better methods to provide protection for the human body and brain from harmful exposure to these particular types and levels of radiation.

## MATERIALS AND METHODS

### *Equipment and devices*

Experimental animals were exposed to various EMF's of varying frequencies and intensities produced by an EMF generator apparatuses consisting of 2 Helmholtz coils, 60 cm in diameter, and a pulse generator device with differential frequencies and intensities. The devices used in this study were manufactured locally and tested for delivery of appropriate EMF, frequency and intensity by local engineers at Shahid Beheshti University.(after calibration for intensity and frequency ,The apparatus evaluated and certified for accuracy) .The Experimental mice were carefully exposed to these devices under the specific conditions detailed below.

### *Animals*

Animals used in this study were healthy adult (5 weeks) male NMRI mice obtained from the animal colony of the Neuroscience Research Center in Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### *Bioethics of animal care*

This project was performed in the research laboratories of Shahid Beheshti University of Medical Sciences and adhered to the standards and specifications of international conventions on animal testing and care required in experimental studies. The project was approved by the Shahid Beheshti University of Medical Sciences

Committee on Bioethics and Animal Care.

### *Experimental design*

Ninety adult male mice were divided into 3 major groups: one control (N=10) and 2 experimental (N=40 per group). The first experimental group was divided into 4 "intensity" subgroups (N=10/group) of varying exposure intensities (0.5, 1.0, 1.5, 2.0 mT), each of which were exposed to EMF radiation of 50 Hz for 16 hrs per day for 2 weeks (15 days). The second experimental group was divided in the same manner of subgroups as was done in group 1, both in terms of intensity subgroups and animal number (N=10) with the exception that they were exposed to EMF radiation of 217 Hz, for 16 hrs per day for 15 days. The third group (N=10 mice) served as the non-exposed control group. This group was placed in the same equipment as were groups 1 and 2 but the devices were not allowed to run, therefore, producing no electromagnetic radiation.

### *Testing for learning and memory*

In our experiments, each PA test had 3 sections: adaptation, acquisition (producing shock) and recall. Prior to experimentation, all animals were pre-trained to adapt to the PA testing system, in order to assess the status of animal learning and memory functions following EMF exposure. The PA testing system allows for the quantitative measurement of memory recall used in the learning and memory of learned tasks, once an animal has learned how to avoid danger. To do so, each animal was placed in an *I-maze apparatus* to measure memory. Under these conditions, animals learned that if they entered the dark chamber of the apparatus, they received a shock. By employing this method, memory was tested one day later by the amount of time delay they showed before stepping into the dark chamber (STL, step through latency).

The animal's behavior was initially monitored for 600 sec and the time delay to step into the dark chamber was measured (STL = step through latency) as an index of memory. Average delay time (STL) was computed for each experimental subgroup and compared to the control group average. In addition to STL, two other quantitative parameters of the PA system were tested: TDC (*time in dark compartment*) and the

Cr# (number of crossing between the light and dark chambers). After completion of the experimental exposures to EMF (no exposure for the control group), each animal from the control and experimental groups was individually tested for learning and memory function via the PA testing system and its associated quantitative parameters (see above).

#### Statistical analysis

To determine the statistical significance of differences the means of STL and TDC between the control and experimental groups, the test of "one way analysis of variance" (ANOVA) was employed that they followed by Dunnett's test for multiple comparison, as a post hoc test. The significance level was set at  $P < 0.05$ . For Cr#, statistical significance between the control and experimental groups was identified by Kruskal-Wallis test with post-hoc Mann-Whitney U tests with Bonferroni correction. A p-value of less than 0.05 was considered to indicate statistical significance, except when Bonferroni's correction for Mann-Whitney U tests was applied. In this situation, a p-value of less than 0.005 was considered to indicate statistical significance.

## RESULTS

The results of this study revealed noteworthy differences between the learning and memory performance of the experimental and control groups. However, the intensity and direction of the effects was highly dependent on frequency of EMF radiation fields as well as on the concentration of the radiation.

Table 1 shows in the 50 Hz exposed group that there is the statistical significant difference of STL, TDC and Cr# between the control and experimental groups ( $p < 0.001$ ). In the 217 Hz exposed group, it has not observed any significant difference of TDC between the control and experimental groups, but there is the statistical significant difference of STL and Cr# between the control and experimental groups ( $p = 0.024$  and  $p = 0.018$ , respectively).

Figure 1 reveals that the mean STL values in the 50 and 217 Hz exposed group. In the 50 Hz exposed group (representing most electronic home devices and electric cables), according to

the results of Dunnett's test, the significant difference was observed between 1 mT field subgroup and control group (105.6 and 464.8,  $p < 0.001$ ) and 1.5 mT field subgroup and control group (155.6 and 464.8,  $p = 0.002$ ). In the 217 Hz exposed group (representing cell phone Hz fields in Iran), according to the results of Dunnett's test, the significant difference was observed between 2 mT field subgroup and control group (186.2 and 464.8,  $p = 0.028$ ) and 0.5 mT field subgroup and control group (141.9 and 464.8,  $p = 0.011$ ).

Figure 2 shows that the mean Cr# values in the 50 and 217 Hz exposed group. In the 50 Hz exposed group, according to the results of Mann-whitney test, the significant difference was observed between 1 mT field subgroup and control group (7.1 and 1.8,  $p = 0.001$ ). In the 217 Hz exposed group, according to the results of Mann-whitney test, the significant difference was observed between 2 mT field subgroup and control group (6.7 and 1.8,  $p = 0.002$ ). Figure 3 reveals that the mean TDC values in the 50 and 217 Hz exposed group. In the 50 Hz exposed group, according to the results of Dunnett's test, the significant difference was observed between 1 mT field subgroup and control group (127.6 and 24,  $p < 0.001$ ). In the 217 Hz exposed group, there was not any significant relationship between TDC and varying exposure intensities based on one-way ANOVA.

Table 1. differences of STL, TDC and Cr# between the control and experimental groups in the 50 and 217Hz exposed groups.

Hz		Test	Statistics	p-value
217	TDC	ANOVA	1.296 <sup>£</sup>	0.27 <sup>ns</sup>
	STL	ANOVA	3.109 <sup>£</sup>	0.024 <sup>*</sup>
	Cr#	Kruskal-Wallis	11.977 <sup>£</sup>	0.018 <sup>*</sup>
50	TDC	ANOVA	10.146 <sup>£</sup>	<0.001 <sup>**</sup>
	STL	ANOVA	10.913 <sup>£</sup>	<0.001 <sup>**</sup>
	Cr#	Kruskal-Wallis	28.129 <sup>£</sup>	<0.001 <sup>**</sup>

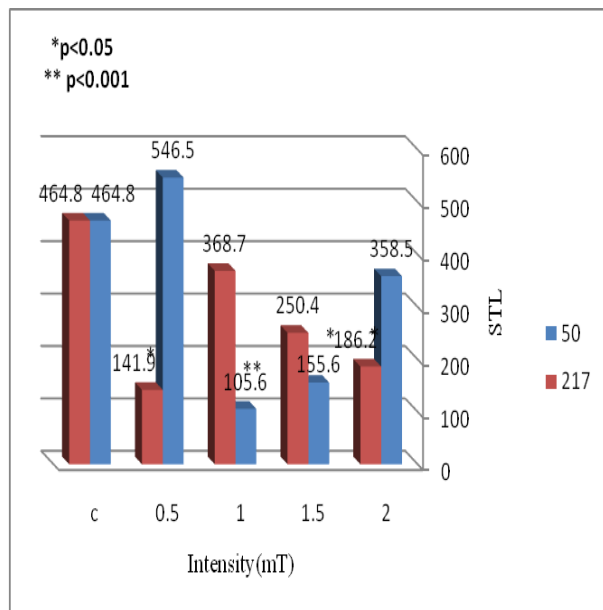
<sup>£</sup> Chi-square statistics

<sup>£</sup> F statistics

\*  $p < 0.05$

\*\*  $p < 0.01$

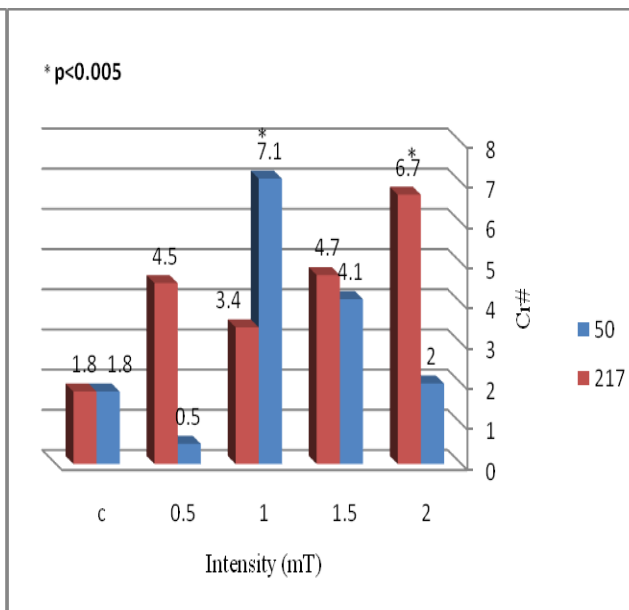
<sup>ns</sup> not significant



**Figure 1.** Effects of EM radiation with 50 and 217 Hz frequency and intensities of 0.5, 1, 1.5 and 2 mT on performance of adult mice in the passive avoidance test of memory, specifically the time delay in stepping into dark chamber (STL, step through latency). Note that the STL in the experimental subgroup of 1 and 1.5 mT (with 50 Hz frequency) decreased significantly ( $p < 0.001$ ) compared to control animals (ANOVA, Dunnett's test) with 217 Hz frequency although all four experimental subgroups showed decreases in their mean STL values, only those of the 0.5 and 2.0 mT subgroups showed significant decreases ( $p < 0.05$ ) respectively, compared to control animals (ANOVA, Dunnett's test).

## DISCUSSION

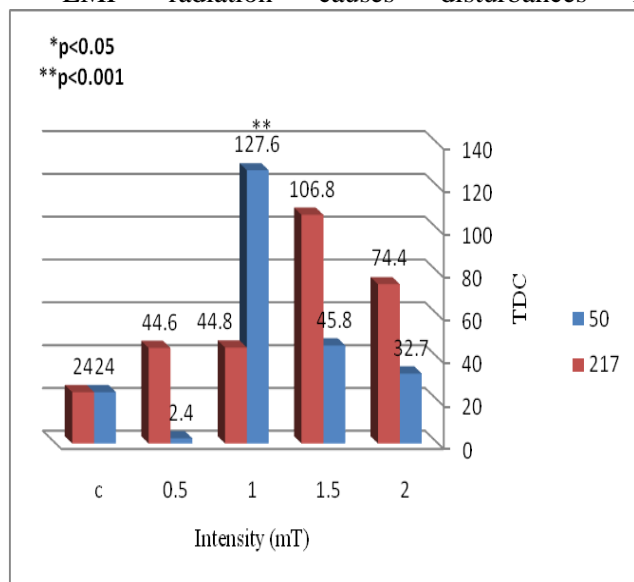
The results of this study delineate that certain injurious effects on memory function does occur in mice as a result of exposure to low frequency EMF, confirming many previously conducted studies in mice [1-3]. The results also support more recent studies suggesting deficits in learning and memory in mice and rats [4-6]. However, there have been other studies that did not find deficits in cognitive function in other animal species; in some other cases, investigators have actually reported *positive* effects of low frequency, short-term EMF on memory [7, 8]. Our findings of the harmful effects on memory, particularly in the 50- and 217 Hz frequency



**Figure 2.** Effects of EM radiation with 50 and 217 Hz frequency and intensities of 0.5, 1, 1.5 and 2 mT on performance of adult mice in the passive avoidance test of memory, specifically the number of crossings (Cr#) between the dark and light chambers. Note that with 50 Hz frequency only the 1.0 mT intensity subgroup animals showed highly significant increases in mean Cr# values, ( $p < 0.005$ ) respectively compared to control unexposed mice (Mann-whitney test) with 217 Hz frequency all four intensity experimental subgroups showed increases in this parameter but the mean values of Cr# of 2.0 mT subgroups showed significant increases ( $p < 0.005$ ) respectively, compared to control animals (Mann-whitney test).

ranges, are alarming in view of the fact that low frequency radiation ranges are associated with many electronic apparatuses regularly used by humans, such as televisions and in particular cell phones. This issue together with the excessive use of such devices, not just in modern urban environments but also in rural and less developed areas of the world, stirs immense cause for concern. To elucidate the background of these techno-social matters and determine the biologic foundations of these effects, we will review some of the most important findings and what these findings reveal. Many investigations have indicated that EMF of low Hz, similar to those that we utilized in our study, which are similar to

those associated with the use of home and office electronic devices, appears to have considerable harmful biologic effects. At the cellular level, EMF radiation causes disturbances in



**Figure 3.** Effects of EM radiation with 50 and 217 Hz frequency and intensities of 0.5, 1, 1.5 and 2 mT on performance of adult mice in the passive avoidance test of memory, specifically the amount of time spent in the dark chamber (TDC). Note that with 50 Hz frequency only the 1.0 mT intensity subgroup of experimental mice showed highly significant increases in the mean values of TDC ( $p < 0.001$ ) respectively compared to non-exposed control animals (Dunnett's test).

With the 217 Hz frequency all four of the experimental intensity subgroups showed increases but there was not any significant relationship between TDC and varying exposure intensities based on one-way ANOVA.

Indeed a recent study by Chen et al. (2011) found that exposure of mice to EMP (electromagnetic pulses) significantly decreased associative learning in mice; interestingly administration of antioxidants acted as an effective protective agent from EMP exposure, indicating increases in oxidative stress and damage in the brain by EMP exposure [6]. At the tissue and whole body levels, long-term exposure to such fields has even led to the induction of cancers including leukemias and lymphomas in rodents [10, 21]. Also reported are significant increases in body weight and growth of rat pups [22]. Exposure to pulsatile radiation has effects on bone cells [23], including changes in shape and differentiation of osteoblasts [17].

proliferation of oral cells [18], cellular DNA damage [19], cellular differentiation [17,18], programmed cell death (apoptosis) [20] and formation of free radicals in cells [21].

Other investigations have shown a positive influence of exposure to EMV on the brain and cognitive effects. Thus 20 min of exposure to an EMF of 4  $\mu$ T intensity improved spatial memory and learning [9]. Similarly, low frequency EMF exposure increased animal mobility by increasing the activity of brain dopamine receptors [11]. A report by Mostafa et al. [12] showed that low frequency EMF with 2 G intensity caused changes in memory and plasma corticosterone levels. Also Shupak et al. [13] found that low frequency EMF radiation can help overcome addiction to morphine painlessly.

EM fields produced by cell phones exert effects on the central nervous system, influencing EEG and other bioelectric potentials originating in the brain [14]. In 2008, Fu and collaborators showed that low frequency EMF significantly alters spatial memory in mice [15]. This is confirmed by similar studies showing that exposure to 50 Hz EMF has deleterious effects on spatial memory [1, 24]. Indeed chronic exposure to EMF of 60 Hz decreases memory [3]. In particular, spatial memory is deleteriously altered following 10 days of exposure to such fields. On the other hand, a 2001 report showed that subjecting rats to EMF of 50 Hz for 45 minutes with various intensities had no discernible effect on object recognition and memory [16]. Similarly, according to Dubreuil et al. (2003), placing heads of mice in EMF of GSM900 cell phones had no effect on spatial memory, measured by various methods [25].

In contrast, Hardell et al. (2008) reported on a meta-analysis of 10-year-long study on the effects of cell phone users, and found a significant relationship with brain tumors [26], confirming an earlier 1999 study and review which had concluded that low frequency EMF radiation, associated with cell phones, was considered a risk factor for brain tumors and other brain pathologies [27]. On the other hand, some studies have indicated that EMF of 50 Hz has no significant effects on spatial memory [28] and that short-term immediate exposure can actually

increase spatial memory in deer mice and meadow voles [7, 8].

Thus the deleterious effects of exposure to low frequency (ELF:0-300) EMF on cognitive functions may appear to be somewhat controversial. However, it has been indicated that the differences in results may be due to the implementation of different behavioral methods, use of different EMF intensities, the threshold intensity and other differences in the methods of exposing animals to such radiation fields.

Other factors such as rewards (e.g. giving a positive reinforcement) and punishments (negative reinforcement of animals, e.g. falling in water) may also affect the direction of observed effects on memory [15]. One study that illustrates this has shown that twelve days of exposing mice to 20 and 50 Hz fields increased the preference for morphine-conditioned placement. This may be due to the specific factors relating to the use of morphine as reinforcement [13].

## CONCLUSION

The present study confirms a large number of investigations reviewed here claiming that exposure to low frequency EMF radiation associated with cell phones and televisions does have a harmful effect on learning and memory.

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With the ever increasing use of cell phones, televisions and other electronic devices, the possibility of exposure to low frequency EMF radiation and the resulting harmful effects is increasing. Specifically, the various effects on the brain including: changes in its electrical activity, energy metabolism, molecular genetic effects, neurotransmitter balance, cognitive functions, sleep patterns and the development of brain disorders such as tumors has been recognized. In addition, health risks associated with the increased use of cell phones in public places appears to be escalating as people are unknowingly, indirectly and inadvertently being exposed to this harmful radiation just by being present in crowded public places [29].

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