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Effects of Short-Term Exposure to (1mT, 50 Hz) Electromagnetic Fields on Calcium Concentration in Different Brain Regions of Mice: The Role of Calcium Channel Blocker

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Objective: To evaluate the effect of short-term exposure to 50 Hz (1 mT) extremely low frequency electromagnetic fields (ELF EMFs) on the Ca^{2+} concentration in 4 different regions of the mice brain (cortex, cerebellum, hippocampus and brainstem).

Setting: University of Bahrain, Arabian Gulf University.

Design: Prospective Randomized Controlled Study.

Method: Adult BALB/c male mice were exposed to 50 Hz (1 mT) ELF-EMFs for 2 hours/day for five consecutive days and were treated orally with the calcium channel blocker Amlodipine. Calcium was extracted from the mice brain tissues and the concentration of Ca^{2+} was determined using atomic absorption spectroscopy.

Result: The effect of ELF EMFs exposure on the Ca^{2+} concentrations varied in different regions of the brain, with a significant increase ($P<0.05$) only in the hippocampus and the brainstem. This increase occurred during short-term exposure to ELF EMFs and the Ca^{2+} concentrations started to decrease during the interval of no exposure.

Conclusion: The rise in Ca^{2+} concentration due to ELF EMFs exposure did not occur in mice treated with the calcium channel blocker Amlodipine. The increase in Ca^{2+} concentrations could have involved activation of the voltage-gated calcium channels (VGCCs) by ELF EMFs.

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Electromagnetic field (EMF) is defined as a force field that is associated with an electric current which contains electromagnetic energy[1,2]. The power lines and electronic appliances produce extremely low-frequency electromagnetic fields (ELF-EMFs) which emits 50 Hz frequency during the production, distribution and use of electricity[3-5]. The electromagnetic fields (EMFs) emitted from the electric currents in residential and commercial power lines and in home appliances such as televisions, computers, and kitchen stoves have the frequencies of about 50-60 Hz and therefore classified as ELF EMFs[3,6,7].

The increased exposure to EMFs has resulted in widespread public concern regarding the adverse effects of such fields on animal behavior and possible effects on human health[4,5,7]. Initial studies on the human exposure to ELF-EMFs have related such exposure to the increased risk of cancer, brain tumor, and neurodegenerative diseases[4,8,9].

Recent studies focused on the effects of ELF-EMFs exposure on the activities of the nervous system[4]. Some studies have addressed the possible influence of EMF exposure on learning and memory[3,4,10]. Researchers have focused on the cellular basis of ELF-EMFs effects and have suggested that ELF-EMFs exposure could induce electric fields in the body which would interact with the cell membrane, mainly voltage-gated ion channels of the nervous tissues and excite them[5,11,12].

The expression of voltage-gated Ca^{2+} channels (VGCCs) has been found to be enhanced in cells exposed to 50 Hz EMF resulting in an increase in VGCC numbers leading to increased intracellular Ca^{2+} concentrations[5,11,13]. For this reason, recent studies have focused on the effect of ELF-EMFs exposure on intracellular calcium ion (Ca^{2+}) concentration in neurons. It has been reported that an increase in Ca^{2+} levels in neurons is associated with changes in synaptic plasticity which leads to the decline in learning and memory[9,14-16]. Many studies have focused on the relationship between ELF-EMFs and the increased intracellular Ca^{2+} levels. Balassa et al and Manikonda et al had reported an increase in intracellular Ca^{2+} concentrations in hippocampal neural tissues of rat brain exposed to 50 Hz EMFs, as well as changes in Ca^{2+} signalling[5,9]. Therefore, the possibility that calcium channel blockers (CCB) might exert neuroprotective effects in exposed animals has been of some interest. El-Swefy et al reported that treating magnetic field-exposed rats by CCB led to a significant decrease in intracellular Ca^{2+} levels in brain tissues[17]. Would CCB play the role of neuroprotector against possible effects of ELF-EMF on motor coordination and motor learning abilities?

El-Swefy et al considered EMF as a stressor agent on the cells that result in an increase in the intracellular Ca^{2+} concentrations. Moreover, the addition of a calcium channel blocker has proven to have a protective effect against the damaging hazard of EMF[17]. Several studies have also suggested that since ELF-EMF exposure causes changes in Ca^{2+} levels in the cells. The disturbance of many neuronal processes that depends on Ca^{2+} signaling might lead to pathophysiological effects on the nervous system[5,18].

The aim of this study is to evaluate the effect of short-term exposure to 50 Hz (1 mT) ELF-EMFs on the calcium concentration in four main regions of the mice brain (cortex, cerebellum, hippocampus and brainstem).

METHOD

Adult BALB/c male mice 12-13 weeks old, 20-30 g body weight were used. Mice were kept in 25^{o}C, 50% humidity and 12 hours light/dark. They were allowed free access to rodent

chow diet (commercial pellet food) and tap water. Mice were assigned to four groups (8 mice per group). In group 1, mice were not exposed to ELF-EMFs which represent the control group; mice in group 2 were exposed to 50 Hz (1 mT) ELF-EMFs for 2 hours/day for five consecutive days; in group 3, mice were exposed to 50 Hz (1 mT) ELF EMFs for 2 hours/day for five consecutive days; the brain tissues were collected after 2 weeks with no exposure in those 2 weeks interval. Group 4 were treated by nasogastric tube with the calcium channel blocker (CCB) Amlodipine 3 mg/kg/daily/orally for four consecutive weeks before and during exposure to 50 Hz (1 mT) ELF-EMFs (2 hours/day for five consecutive days); the brain tissues were collected at the end of the four-week period.

The magnetic field strength (1 mT) used was within the limits contained in current occupational and public environment magnetic field exposure guidelines. The intensity of ELF-EMFs (1 mT) used was within the limits of guidelines for occupational and public exposure to magnetic field[19]. Animals were decapitated after the inhalation of isoflurane as an anesthetic agent.

All experiments were conducted according to the guidelines of the Arabian Gulf University Committee (AGUC) for welfare of experimental animals.

The procedure was similar to the one used in earlier studies[10,20]. The electromagnetic fields were delivered by a Helmholtz coil pair 40 cm in diameter and each coil has 154 turns, and the two coils were separated by a distance of 20 cm. The animal cage was placed inside the coil system. The coils were powered by a generator of standard signals, providing stability of voltage. A sinusoidal current (50 Hz) was passed through the magnet, producing relative homogenous alternating MF with an average magnetic field (B) of 1 mT, measured by a PHYWE Digital Tesla Meter probe with sensitivity of microtesla. Magnetic force lines were parallel to the horizontal component of the local geomagnetic field. The background MF of 50 Hz did not exceed 1 nT as measured using Multidetector II with sensitivity of 1 nT.

The animals were decapitated, and their brains were immediately removed. The two hemispheres were isolated and dissected into four regions including the cortex, cerebellum, hippocampus and brainstem. After dissection, the brain tissues were frozen at -20^{o}C.

Brain calcium was determined following the method of Ferko et al as adapted by Korf et al with some modifications[21,22]. Frozen brain tissues were weighed, and 30-50 mg were digested with 500 µl concentrated HNO_3 and left for 16 hours at room temperature. The samples were then heated in a water bath at 45°C for 1 hour and at 80°C for 1 hour until all tissues were dissolved. The digested samples were then placed in a muffle furnace at 550°C overnight. After cooling, 2 mL of 0.6 M HCl containing 1% of $La(NO_3)_3$ were added to the samples and the Ca^{2+} concentrations were determined using atomic absorption spectroscopy. Six standard solutions containing 6.25, 12.5, 25, 50, 75 and 100 µg g^{-1} of Ca^{2+} ions were prepared from $CaCO_3$ stock solution (500 µg g^{-1} Ca^{2+} ions). Samples with known amount of calcium (45 µg g^{-1}) were also prepared and analyzed in every run following the same procedure.

The statistical analysis was performed using the statistical package from Microsoft Excel 2007. Results are presented as mean values and standard deviation (mean±SD). The mean values of each measured parameter were statistically analyzed using one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test (Post Hoc analysis) using SPSS version 21.0 for Windows. $P \leq 0.05$ was considered statistically significant.

RESULT

The Ca^{2+} concentrations of mice brain tissues of all groups studied are summarized in table 1 and figure 1. A significant difference in the Ca^{2+} concentrations was recorded between the four brain regions within each study group, $P \leq 0.05$. However, no significant difference in Ca^{2+} concentrations was observed in the cortex (P=0.382) and cerebellum (P=0.250) samples between the various groups.

Table 1: Effects of 50 Hz ELF EMFs Exposure on Ca^{2+} Concentrations (µg g^{-1} wet weight) in Mice Brain Tissues (Mean±SD)

	Groups			
Brain Sample	**1 Control** (n=8)	**2 EMF only** (n=8)	**3 EMF late effect** (n=8)	**4 EMF + Rx** (n=8)
Cortex	19.17±1.72[a]	21.01±1.05[a]	20.51±2.17[a]	19.13±2.99[a]
Cerebellum	29.23±2.13[a]	27.12±2.54[a]	34.22±4.92 [a]	32.36±3.40 [a]
Hippocampus	13.57±1.54[a]	63.01±1.97	29.56±1.84	12.22±2.03[a]
Brainstem	17.83±1.91[a]	31.36±2.83	19.99±1.93[a]	16.84±2.53[a]

Figures in the same row having same superscript are not significantly different at $P \leq 0.05$.

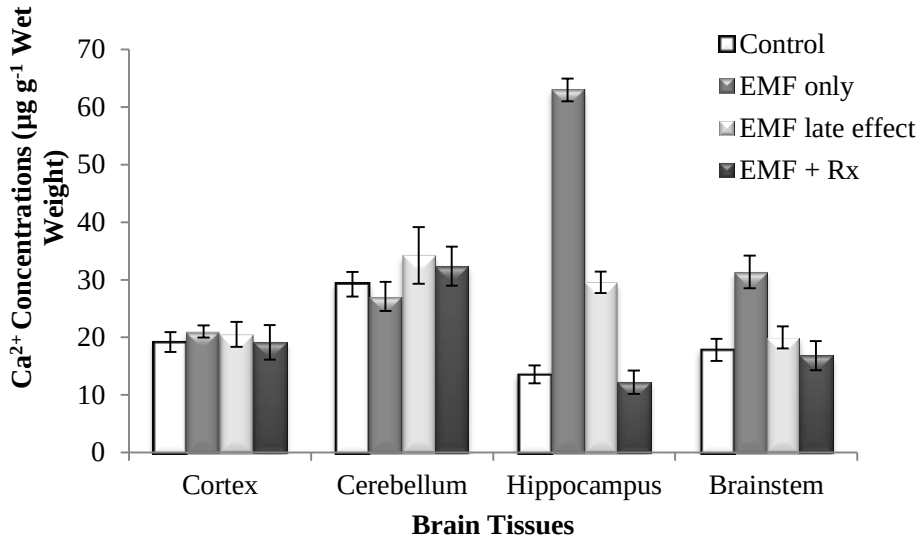

Figure 1: Effects of 50 Hz (1 mT) ELF-EMFs Exposure on Ca^{2+} Concentration (µg.g^{-1} wet weight) in Mice Brain Tissues (Mean±SD)

The highest Ca^{2+} concentration (63.01±1.97 µg g^{-1} wet weight) was recorded in the hippocampus samples of group 2 which were exposed to 50 Hz (1 mT) ELF-EMFs for 2 hours/daily for 5 consecutive days. The lowest Ca^{2+} concentration (12.22±2.03 µg.g^{-1} wet weight) was registered in the hippocampus samples of group 4 which were treated with the CCB Amlodipine daily for 4 consecutive weeks, before and during the exposure to ELF-EMFs. The brainstem Ca^{2+} concentrations were significantly elevated in group 2 which were exposed to 50 Hz (1 mT) ELF-EMFs for 2 hours/daily for 5 consecutive days; while no significant difference was recorded in the brainstem Ca^{2+} concentrations among groups 1, 3 and 4.

DISCUSSION

Several studies have reported the possible effects of ELF-EMFs on learning and memory consolidation, attention, perception, and neurobehavioral functions[3,4,23,24]. The present study reveals that short-term exposure to ELF-EMFs seems to play a role in the Ca^{2+} levels in different parts of the brain. This was evident by the significant increase in the hippocampus and brainstem tissues of the mice exposed for a short time (2 hours/day for 5 consecutive days), whereas no significant elevation in Ca^{2+} levels in cortex and cerebellum tissues was observed. The increase in Ca^{2+} concentrations in the hippocampus in the present study is consistent with the results of Manikonda et al in which the hippocampus Ca^{2+} levels also elevated in response to exposure to 50 Hz (50 and 100 μT) EMF. However, the authors have not determined the Ca^{2+} concentrations in the other brain regions[9]. In addition, Balassa et al have indicated significant effects of EMF exposure on hippocampus as well as on the cortex of mice exposed to 50Hz (0.5 and 0.3 mT) ELF-EMFs during fetal and newborn period; the present study did not show any significant effect of ELF EMFs on the cortex Ca^{2+} concentrations in adult mice[5].

In addition, Ca^{2+} levels returned to the basal level in the control samples in brainstem tissues of the mice subjected to the same exposure protocol but brain tissues were collected after two weeks of no exposure; the hippocampus tissues Ca^{2+} levels significantly decreased but remained elevated in comparison to the control group. This means that the increase in the Ca^{2+} concentration, as a result of exposure to ELF EMFs, was not permanent since the concentration of Ca^{2+}subsided during the two weeks interval of no exposure.

Treatment of ELF-EMFs exposed mice with Amlodipine significantly reduced hippocampus and brainstem Ca^{2+} concentrations with no effect recorded in the cortex and cerebellum Ca^{2+} levels. This means that Amlodipine had blocked the calcium channels in the hippocampus and brainstem, thus protecting the cells against any possible damage. These results are consistent with the results of El-Swefy et al in which calcium channel blocker treatment has suppressed the effects of EMF exposure[17].

El-Swefy et al have examined both short-term and long-term effects of EMF exposure in rat brain and concluded that the cells consider EMF as a stressor agent. Short-term exposure elicits a cellular adaptation response with a transient increase in Ca^{2+} concentration. However, long-term exposure could be lethal due to prolonged increase in Ca^{2+} concentration which is accompanied by oxidative damage, pro-inflammatory reactions and apoptosis. In addition, blockade of the calcium ion channels in the cells by addition of Amlodipine protected the tissues from the damaging effects of EMF[17].

Many studies have associated excess Ca^{2+} in neurons with memory loss in aging and neurodegenerative diseases due to changes in synaptic plasticity and cell death[15,16,25,26]. Manikonda et al have reported that changes in Ca^{2+} signaling occurs due to increased Ca^{2+} concentrations in the hippocampus in rat brain exposed to 50 Hz EMF[9].

The increase in Ca^{2+} concentrations in various regions of the brain can be associated with the various effects of EMF on memory and neurobehavioral functions. Hippocampus is an important region involved in learning and memory. Various studies revealed that exposure to 50 Hz EMF led to decline in learning and memory[14-16]. A study by Jadidi et al has shown that exposure to 50 Hz, 8 mT EMF impaired hippocampus-dependent spatial memory consolidation in mice using water maze[3]. Similarly, Foroozandeh et al found damaging effects on learning in both male and female mice[4]. In addition, Sakhnini et al had found that

continuous exposure of immature mice to 50 Hz EMF has resulted in long-term deficits in spatial learning and memory[10].

Brainstem is also a very important region of the brain which is responsible for various functions, some of which are consciousness and alertness, sleep cycle, as well as motor coordination and learning[27,28]. Akerstedt et al reported sleep disturbance, and Trimmel et al reported reduction of attention and perception[23,29]. Exposure to 50 Hz (1 mT) EMFs has also been shown to affect the motor coordination of prenatally exposed mice using Rota-Rod experiments[20]. In this study, increased Ca^{2+} concentration in the brainstem was revealed in response to EMFs exposure. Motor learning involves the interaction between various areas of the brain including the cortex, basal ganglia, cerebellum as well as the brainstem[27,28].

Some studies found no effect of exposure to ELF-EMFs on learning and memory[24]. Different studies on the effect of ELF EMFs differ in the EMF exposure and experimental designs; therefore, their results are inconsistent and difficult to interpret[30]. However, the possibility of negative effects of ELF-EMFs on cognition cannot be excluded[24]. Therefore, the effect of 50 Hz ELF-EMFs exposure would depend on different parameters of the experimental design, such as intensity of EMF, duration of exposure (short-term or long-term), and age of the exposed animal (prenatal, newborn or adult)[5,17]. The effect of ELF-EMFS in the current study was different in different regions of the brain, with the hippocampus being most affected.

The present study revealed that CCB suppressed the increase in Ca^{2+} concentrations. This is consistent with many studies; Pall clearly indicated that EMF exposure has resulted in excessive activity of VGCCs in many cell types and some of these studies suggested specifically L-type VGCCs to be involved[18]. In addition, other studies have suggested that the increase in intracellular Ca^{2+} concentration occurs immediately after EMF exposure leading to Ca^{2+}/calmodulin-dependent increase in nitric oxide[18,31]. However, in order to explain the effects of EMF on neurobehavioral functions, a major research is required to link the basic mechanisms at cellular level with the complex functions of the brain at higher level[10].

In order to specifically determine the intracellular Ca^{2+} concentration, other techniques have to be employed, such as in situ hybridization, immunohistochemistry, and fluorescence techniques.

CONCLUSION

This study has shown that the effect of short-term exposure to 50 Hz (1 mT) ELF-EMFs was different in different regions of the brain. There was no significant effect of ELF-EMFs exposure on the Ca^{2+} concentration of the cortex and cerebellum while the hippocampus and the brainstem have shown a significant increase in their Ca^{2+} concentrations.

In order to explain the exact mechanism of effects of ELF EMFs on neurobehavioral functions, major research is recommended.

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