# Protective Effect of Pretreatment with Calcium Channel Blockers on Motor Coordination Deficit of Mice due to Exposure to Extremely Low-Frequency Electromagnetic Fields

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**Background: The mechanisms of ELF-EMFs action on the brain are still poorly understood. One of the possible explanations of these effects could be due to the increase in the intracellular $Ca^{2+}$. This increase in $Ca^{2+}$ may lead to a decrease in cholinergic activity. These changes in brain activities may lead to changes in motor coordination and motor learning and the possibility that calcium channel blockers might exert neuroprotective effects.**

**Objective: To evaluate the protective effect of calcium channel blocker (Amlodipine) on deficiency in motor learning abilities of exposed mice to extremely low-frequency electromagnetic Fields ELF-EMFs (1 mT, 50 Hz).**

**Setting: Animal House, Arabian Gulf University, Kingdom of Bahrain.**

**Design: An Experimental Animal Study.**

**Method: Mice were divided randomly into four groups: Group I: (Control group) 8 mice; Group II: 8 mice exposed to ELF-EMF (2 h/day) immediately before each session of training; Group III: 8 mice exposed to ELF-EMF and treated previously by calcium channel blockers (3mg/kg/day) for four weeks; Group IV: 8 mice exposed to ELF-EMF but left to rest for two weeks with no electromagnetic field exposure, then trained. Rotarod experiments were performed on the four groups. The effect of rotation speed (45,50,55,60 rpm) was tested on a 5-day course.**

**Result: Result revealed statistically significant enhancement in the motor coordination performance in Group II, III and IV, over the five days of training, but showed an appreciable deficit in motor learning abilities for Group II. The results from Group III revealed that learning abilities for exposed mice were improved by giving the mice a therapeutic dose treatment of calcium channel blockers Amlodipine. These results indicate that the deficiency in motor learning abilities effects is partially due to the increase of the calcium levels in specific parts of mice brains, namely; hippocampus and brainstem, which was supported by an earlier study.**

**Conclusion: Treating the mice with calcium channel blocker might prevent deficiency in motor learning abilities of exposed mice to ELF-EMF.**

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The mechanism of ELF-EMFs action on the brain is still not fully understood. One of the possible explanations of these effects could be due to the increase in the intracellular $Ca^{2+}$. ELF-EMF was found to enhance the expression of voltage-gated $Ca^{2+}$ channels on the plasma membrane of the exposed cells. The consequent increase in $Ca^{2+}$ influx is likely to be responsible for the EF-induced modulation of neuronal cell proliferation and apoptosis[1]. This increase in $Ca^{2+}$ may lead to a decrease of cholinergic activity in the frontal cortex and hippocampus of the adult rats upon exposure to magnetic field[2].

Rotarod was popularized to test for neurological deficits and still used in biochemical research in rats and mice[3-5]. This method was used to investigate the potential effects of

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magnetic field on the prenatal development of mice, where pregnant mice are exposed to a (50 Hz, 20 mT) magnetic field, which showed that exposed animals remained on a rotarod for less time as juveniles[6]. A similar experiment was performed by our research group to investigate the possible effect of an extremely low-frequency magnetic field following a continuous 7-day exposure[7]. The study revealed a pronounced deficit in the learning abilities of the prenatally exposed groups, but no pronounced effect on the neonatal exposed group.

Mice have been chosen for this study since the rodent brain's proliferation, differentiation and re-organization resembles closely the human's brain functions[8].

The magnetic field strength (1 mT) used in our study is within the limits contained in the guidelines of the International Commission on Non-Ionizing Radiation Protection ICNIRP for maximum levels of magnetic-field exposure for occupational situations are 1 mT[9].

The aim of this study is to evaluate the protective effect of calcium channel blocker (Amlodipine) on deficiency in motor learning abilities of exposed mice to extremely low-frequency electromagnetic Fields ELF-EMFs (1 mT, 50 Hz).

## METHOD

The procedure of the experiment is similar to the one explained in our earlier studies[7,10]. The electromagnetic fields were delivered by a Helmholtz coil pair 40 cm in diameter and each coil has 154 turns, and the two coils are separated at a distance 20 cm. The animal cage was made of plastic and placed inside the coil system. The coils were powered with a power generator of standard signals, providing stability of voltage. A sinusoidal current (50 Hz) was passed through the coils, producing a magnetic field. The magnetic field intensity was not uniform and in the range of 0.8-1 mT, depending on the spatial positioning. The measurements were taken across the space between the coils showing the highest value of magnetic field intensity of (1 mT) at the center and the lowest value (0.8 mT) at the edge of the cage. The plastic (non-magnetic) cage was placed at the center of the coil system. The magnetic field intensity was measured by a Digital Tesla Meter probe (PHYWE Systeme GmbH & Co. KG, Germany) with a sensitivity of microTesla. Magnetic force lines were parallel to the horizontal component of the local geomagnetic field. The background MF 50 Hz did not exceed 1 nT as measured with using Multidetector II (Gewerbegebiet Aaronia AG, Germany) with a sensitivity of 1 nT.

Animals were divided into four groups (8 mice in each group) and each group has been placed in two different cages, four in each cage and each cage was placed inside the coil system. All were subjected to the same experimental procedure as the ELF-EMF exposed ones. All studied animals were males and in their adulthood stage (12-13 weeks old, 20-30 gm body mass).

In Group I (control), the two cages were subjected to the same experimental procedure as the ELF-EMF exposed ones, but the source of the electromagnetic field was not activated. In this case, the value of the average magnetic field throughout the experiment was at the level of the natural environment. For Group II, the magnetic field was activated for 2 hours daily for five consecutive days. Training at rotarod was performed immediately after field exposure. For Group IV, the mice were exposed to the magnetic field for five consecutive days two hours daily then trained after two weeks. The following conditions were controlled: temperature 23 ± 2 °C, relative humidity 60–70% and 12-hr dark/12-hr light cycles. No food or water restrictions. The plastic cage's dimensions were 23 cm width × 38 cm length × 20 cm height. Breathing and movements were not restricted and each cage was placed in the center of the Helmholtz coil pair. Each cage housed 4 mice and exposed in the coils at one time.

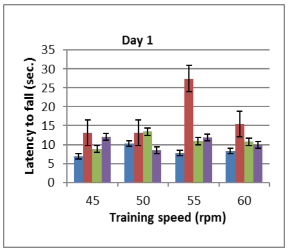
Mice in Group III were treated orally by nasogastric tube with calcium channel blocker (amlodipine) 3 mg/kg/day of total body mass which is marketed as Norvasc (oral 5 mg tablets; Pfizer Products Inc.). At this dose level, no expected pathological changes in the mice systems should be noticed, as was indicated by the product monograph published by Pfizer Products[11]. The doses were administrated daily for four consecutive weeks for a total of twenty-eight doses, before and during the field exposure, in which the magnetic field was activated for 2 hours daily for five consecutive days.

The rotarod is used to assess motor coordination and balance. Up to 4 habituation sessions and 5 test sessions were recorded per animal. The rotarod apparatus consists of a rotating drum with a grooved surface for gripping. The rotation can be set at a constant speed. In the habituation session, mice are initially placed on the stationary drum for 1 min and on the rotating drum (4 rpm lowest speed) for 3 sessions lasting one minute, each with 10 minutes between each session. For test sessions, each mouse was first trained by setting the rotating drum to the different test speeds (45, 50, 55 and 60 rpm which corresponds to 4.5 m/min, 5 m/min, 5.5 m/min and 6 m/min respectively). The actual test session started 30 minutes after the training by placing the animal on the rotating rod with specific velocity and recording the time till it falls down from the rod. The test is repeated with every rod velocity setting. The time between each drum velocity test session and the other is 15 minutes. After different sessions with different speeds, the trial is ended and the mouse returned to its home cage. The test is repeated for 5 consecutive days.

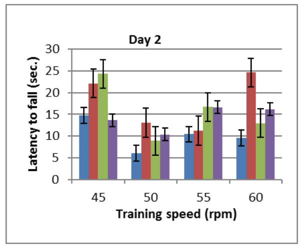
Excel 2007 was used for all statistical analyses. We performed one way ANOVA, to compare the data taken from each group with the data of the control. Differences were considered significant at $P < 0.05$ and 0.01.

## RESULT

The rotation speed of the rotarod has an effect on the learning ability of the mice, see figure 1 (A-E). A faster-rotating rod should lead to decreased latencies before falling. The average performance of the mice for each day of training as a function of the rotating speed of the rod in each group was compared to the average performance of the mice in the control group. The data showed a significant increase in the latencies for the ELF-EMF exposed group (Group II) compared to the performance of the controlled mice (Group I), for the first four days of training. Group III (exposed and treated with calcium blocker) revealed a significant increase in the latencies to fall in days 2 and 4 and no change in the average performance was noticed for the previously exposed mice (Group IV) for the first two days but a significant increase was noticed for the other three days.




1 (A)

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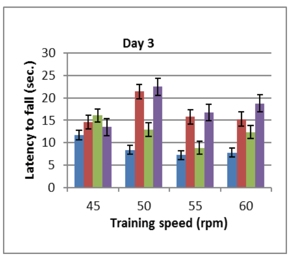


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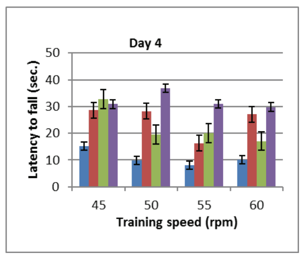


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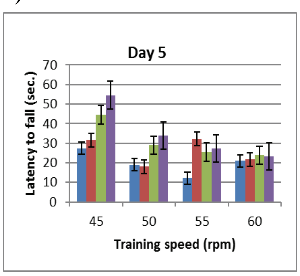
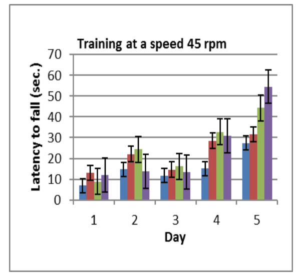



1 (E)

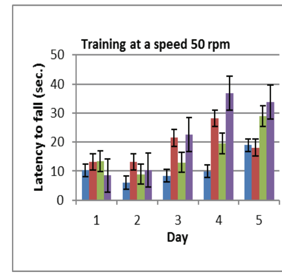
Group I Group II Group III Group IV

**Figure 1 (A-E): Rotarod performance in adult mice. Group I: The Control Animals. Group II: exposed to (1mT, 50 Hz) ELF-EMFs for 2 hours daily for five consecutive days and training was performed after each session of exposure. Group III: exposed to 1mT, 50 Hz while they were treated orally with calcium channel blocker. Group IV: exposed to EMF in the same way as for Group II but left to rest for two weeks and then rotarod training was performed on a 5-day training course. Values represent mean ± standard error of the mean, S.E.M. for N = 8.**

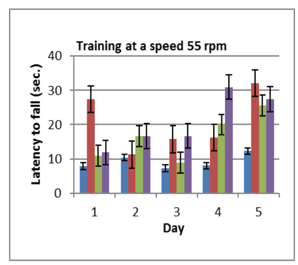
The time the animals could stay on the rotating rod at speeds of 45 rpm (corresponds to 4.5 m/min), 50 rpm (5m/min), 55 rpm (5.5 m/min) and 60 rpm (6 m/min) were recorded. The data for groups II, III and IV were compared to the control group (Group I), these data represent the learning memory for the mice, see figure 2. The results for ELF-EMF exposed (Group II) compared to the control (Group I) revealed no significant exposure effect during the 5-day trails effect for testing speed 45 rpm. However, with speed of 50 rpm (which corresponds to 5 m/minutes) the duration the control mice could stay on the rod was statistically shorter than the time recorded by the exposed animals. Similar results were obtained with even higher rod speed rotations; namely 55 rpm which corresponds to 5.5 m/minutes and for 60 rpm or 6 m/minutes. Group III (exposed and treated with calcium blocker) did not show significant effect compared to the control, for testing speed of 45 rpm and 50 rpm, while the significant effect was observed with higher speeds of 55 rpm and 60 rpm. Results from group IV (previously exposed animals) showed no significant difference compared to the control group at a speed of 45 rpm. The significant effect was recorded for higher speeds of 50 rpm, 55 rpm, and for 60 rpm.



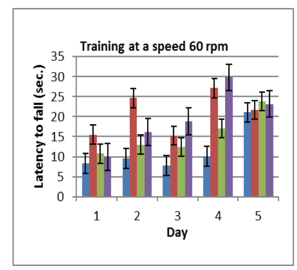

2 (A)




2 (B)




2 (C)




2 (D)

Group I Group II Group III Group IV

**Figure 2 (A-D): Rotarod performance in adult mice. Group I: the control animals. Group II: exposed to 1mT, 50 Hz ELF-EMFs for 2 hours daily for five consecutive days and training was performed after each session of exposure. Group III: exposed to 1mT, 50 Hz while they were treated orally with calcium channel blocker. Group IV: exposed to EMF in the same way as for Group II but left to rest for two weeks with no electromagnetic field exposure, and then rotarod training was performed on a 5-day training course. Values represent means ± standard error of the mean, S.E.M. for N = 8.**

**Table 1: Linear Regression Analysis Showing Learning Abilities of the Mice at Different Groups, *P<0.05 and **P<0.01.**

| Training Speed | rpm 45 | rpm 50 | rpm 55 | rpm 60 |
|---|---|---|---|---|
| **Group I** | $^{**}R^2$=0.74 | $^{*}R^2$=0.47 | | $R^2$=0.56 |
| **Group II** | $R^2$=0.70 | $R^2$=0.39 | $R^2$=0.07 | $R^2$=0.19 |
| **Group III** | $^{**}R^2$=0.80 | $^{**}R^2$=0.72 | $R^2$=0.58 | $R^2$=0.80 |
| **Group IV** | $^{**}R^2$=0.80 | $^{**}R^2$=0.87 | $^{*}R^2$=0.79 | $^{**}R^2$=0.72 |

Linear regression analysis was used to analyze the learning abilities of the mice in different groups. The coefficient of determination $R^2$ and P were calculated, see table 1. The data showed that Group I (control) learning could be achieved through the five days of practice for most of the speeds. For Group II (ELF-EMF exposed), learning could not be achieved at any speed, while for Group III (exposed and Ca channel blocker treated) learning was achieved for speeds of 45 rpm and 50 rpm and could not be achieved for higher speeds. Mice in Group IV (previously exposed) showed better learning skills than those in Group III where learning could be achieved for most of the speeds.

## DISCUSSION

Animals with significantly lower abilities to stay on the rotating rods before they fall down have less motor coordination. ELF-EMF exposed mice (Group II) showed better motor coordination performance on day one, which is similar to earlier studies. It has been reported that the motor system can be modulated by exposure to ELF-EMF[12, 13].

Mice in groups II, III and IV during the 5-day trials for the exposed groups could stay for longer periods on the rod compared to group I (control). However, the data indicate that the mice in group II showed no significant learning ability at any speed, while mice in group 1 (control) showed a significant ability to learn across the five testing days. Our result is similar to another study which revealed that learning memory was impaired when male mice were exposed to ELF-MF of 2 mT for four hours daily[14].

Mice in group III (ELF-EMFs and Calcium channel blocker treated) showed significant motor learning abilities compared to group II, see table 1. This indicates that calcium channel blocker might play an important role in protecting the brain from the harmful effects of ELF-MFs. This may be due to two effects, one is that the calcium blocker (resveratrol) reduces the intracellular calcium levels in parts of the brain responsible for motor coordination and second is the beneficial effects of resveratrol are not only limited to its antioxidant and anti-inflammatory action but also include activation of sirtuin 1 (SIRT1) and vitagenes, which can prevent the deleterious effects triggered by oxidative stress[15]. However, this group showed enhanced motor coordination performance, which indicates that ELF-EMFs affect different activities in different ways, as calcium channel blocker could restore the learning abilities but did not reverse the enhancement in motor abilities, see figure 1.

Group IV showed the effect of exposure for short times to ELF-MFs, which could cause short time effect on motor learning, as significant learning could be retrieved after two weeks of resting, through the five-day training course, see table 1.

Our result suggests that the motor system could be modulated by ELF-EMFs, hence there may be differences in the underlying function of the brain regions involved in rotarod performance (motor coordination and motor learning abilities) among the exposed groups. Despite the increasing number of studies about the biological effects of ELF-EMFs, the exact mechanism of interaction of this exposure with brain functions is not yet well understood, but the effects are well documented. The exposure to electromagnetic fields, in general, can affect the central nervous, endocrine and immune systems, and consequently, it can influence neural functions such as motor control and cognitive abilities[16,17].

Overall, these results provide additional evidence that 50 Hz magnetic fields may cause reversible changes in the processing of motor learning abilities in mice, as the data from Group IV demonstrate. Figure 1 showed that the motor coordination performance for these mice is enhanced which indicates that although learning abilities are restored after two weeks, the enhancement of the motor coordination performance is not reversed.

## CONCLUSION

**Our results provide possible evidence that short term EMFs exposure causes deficits in motor learning abilities in adult mice, but enhances motor coordination performance for exposed mice, where mice could stay for longer time on rotarod. Learning abilities effects can be improved by therapeutic dose treatment of calcium channel blockers, and by stopping the ELF-EMFs exposure for two weeks.**

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**Author Contribution:** All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

**Potential Conflict of Interest:** None.

**Competing Interest:** None.

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