University of Bahrain
Journal of the Association of Arab Universities for Basic and Applied Sciences
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آثار تعرض الفئران قبل الولادة وحديثي الولادة للمجالات الكهرومغناطيسية بتردد 50 هيرتز على أدائهم عند استخدام متاهة موريس المائية

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الملخص:

المجالات المغناطيسية ذات التردد المنخفض للغاية (ELF –MF)، كالتي تنشأ عن خطوط الكهرباء في المناطق السكنية والمناطق المهنية، وتلك التي تنتج من الأجهزة المنزلية والطبية، قد تكررالحديث عنها بأنها يمكن أن تؤدي إلى مجموعة متنوعة من الآثار البيولوجية، والتي قد تحفز عدد من التغييرات في السلوك عند أنواع مختلفة من الكائنات الحية، مثل الحشرات والقوارض. ان الغرض من هذا البحث هو دراسة التأثيرات المحتمله للمجالات المغناطيسية ذات التردد المنخفض للغاية:(ELF–MF)(50 Hz,1mT) على الذاكرة المكانية والتعلم الوظيفي عند الفئران (قبل الولادة وحديثي الولادة) عقب التعرض المستمر لمثل هذه المجالات لمدة 7 أيام، وذلك باستخدام متاهة موريس المائية. وقد تم اختيار فئران غير ناضجة لهذه الدراسة حيث أن الخلايا العصبية في أدمغة هذه القوارض غير الناضجة لا تزال لديها القدرة على الانقسام، والتمايز وإعادة التنظيم، وبذلك تشبه الدماغ النامي للطفل البشري. وبالتالي تمثل أدمغة الفئران غير الناضجة نموذج حيواني حساس ومفيد، لدراسة الآثار البيولوجية المحتملة لELF –MF. تقدم نتائجنا أدلة مقنعة على أن تعرض الفئران غير الناضجة لفترات طويلة الى ال MF، يسبب عجز ملموس على المدى الطويل في قدرات التعلم.

Journal of the Association of Arab Universities for Basic and Applied Sciences (2014) **15**, 1–5

University of Bahrain
Journal of the Association of Arab Universities for Basic and Applied Sciences
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ORIGINAL ARTICLE

Effects of exposure to 50 Hz electromagnetic fields on Morris water-maze performance of prenatal and neonatal mice

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Received 14 May 2012; revised 1 April 2013; accepted 12 May 2013
Available online 6 June 2013

KEYWORDS
Low-frequency magnetic field;
Immature rodent brain;
Biological effects;
Learning abilities

Abstract Extremely low-frequency magnetic fields (ELF-MF), such as those originating from residential and occupational power lines, household appliances and medical devices, have been reported repeatedly to produce a variety of biological effects, which may induce a number of changes in behavioral differences of different living species, like insects and rodents. The purpose of the present study is to investigate the possible effect of an extremely low-frequency magnetic field ELF-MF (50 Hz, 1 mT) on spatial learning and memory functions in mice (prenatal and neonatal exposed mice) following a continuous 7-day exposure, using Morris water-maze. Immature mice have been chosen for this study since; the immature rodent brain still has the capacity to undergo proliferation, differentiation and re-organization and more closely resembles the developing brain of a human child. Thus, the immature rodent brain may provide a sensitive and useful animal model to study the possible biological effects of ELF-MF. Our results provide convincing evidence that long time MF exposure to immature mice; causes appreciable long term deficit in learning abilities.

1. Introduction

Extremely low-frequency electromagnetic fields (ELF-MF), such as those originating from residential and occupational power lines, household appliances and medical devices, have been reported repeatedly to produce a variety of biological effects, which may induce a number of changes in the behavior of different living species, like insects and rodents (Kirschvink, 1997; Lai, 1996). It has also been reported that ELF-MF affects human cognition and electrophysiology (Cook et al., 2002). ELF-MF may interfere with the activity of the brain (Bawin et al., 1996; Bruner and Harvey, 1998; Gona et al., 1993; Gundersen et al., 1986; Jenrow et al., 1998), and may generate behavioral and cognitive disturbances (Lai et al., 1993; Cuccurazzu et al., 2010; Mostafa et al., 2002; Gerth

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Peer review under responsibility of University of Bahrain.

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http://dx.doi.org/10.1016/j.jaubas.2013.05.004

et al., 1983; Lai, 1996; Lai et al., 1998; Levine et al. 1995; Lyskov et al. 1996). These pronounced effects are found to interfere with memory performance (Mostafa et al., 2002; Lai et al., 1998). Most of these tests have been made on upon exposure of adult animals, and very few works have been done on learning activities upon exposure of prenatal or neonatal animals.

One of the most commonly used tests to study the spatial learning and memory functions is the Morris water-maze (MWM) (Morris et al., 1982). Extensive evidence has been reported of its validity as a measure of hippocampally dependent spatial navigation and reference memory (Morris, 1993), its specificity as a measure of place learning, and its relative immunity to motivational differences across a range of experimental treatment effects that are secondary to the central purpose of the task (genetic, pharmacological, nutritional, toxicological and lesion). This method has been used repeatedly to study the effects of different drugs (Geert-Jan et al., 1998; Kamphuis et al., 2003; Kamal et al., 2000) and the effects of ELF-MF (Lai et al., 1998) on learning abilities.

The purpose of the present study is to investigate the possible effect of an extremely low-frequency electromagnetic field ELF-EMF (50 Hz, 1 mT) on spatial learning and memory functions in adult mice (prenatal and neonatal exposed mice) following a continuous 7-day exposure, using Morris water-maze.

In this behavioral model, mice are required to locate a submerged platform in a circular pool containing opaque water. Immature mice have been chosen for this study since; the immature rodent brain still has the capacity to undergo proliferation, differentiation and re-organization and more closely resembles the developing brain of a human child (Katyal and McKinnon, 2008). Thus, the immature rodent brain may provide a sensitive and useful animal model to study the possible biological effects of ELF-EMF.

2. Material and methods

2.1. Electromagnetic fields

The coils: The procedure of the experiment is similar to our earlier study (Sakhnini et al., 2012). 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 by a distance of 20 cm. The animal cage was placed inside the coil system. The coils were energized with a generator of standard signals, providing stability of voltage and frequency with a precision of significant digits (Jelenkovic et al., 2006). 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 (PHYWE Systeme GmbH & Co. KG, Germany) with sensitivity of micro Tesla. 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 a Multidetector II (Gewerbegebiet Aaronia AG, Germany) with sensitivity of 1 nT.

2.2. Animals

Animals (BALB/c female mice, Arabian Gulf University, Manama, Bahrain) are divided into three groups. Sham-exposed animals 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 all 7 days was at the level of the natural environment (< 1 nT).

For group one, two pregnant female mice had been placed in two different cages. As the pups were born the electromagnetic field was activated and kept for 7 days of continuous exposure. While for group 2 two pregnant female mice had been placed in two different cages and the magnetic field was activated for 7 days at the end of the pregnancy period. The mice of these two groups and those of group 3 (sham-exposed) were allowed to grow, and water maze experiments were performed when the mice reached adulthood (12–13 weeks old, 20–30 gm body weight). All water maze experiments were performed blindly. The animals were maintained under controlled conditions: temperature 23 ± 2 °C, relative humidity 60–70%, and 12-h dark/12-h light cycles. Food and water were not restricted. To avoid contact with metallic parts of the system, plastic cages with standard dimensions for mice (23 cm width × 38 cm length × 20 cm height) were used. The cages did not restrict breathing and movements.

2.3. Morris water maze

The Morris water maze was performed as described previously (Kamal et al., 2000; Hensbroek et al., 2003). All experiments were conducted in accordance to the guidelines of the Arabian Gulf University committee for welfare of experimental animals. The maze consisted of a circular pool (diameter 140 cm, 50 cm height) filled to a depth of 30 cm with water (25 °C) which was placed in a darkened room, illuminated by sparse red light. The platform was circular (diameter 10 cm) and made of perforated Plexiglas that provided the mice with extra grip. The platform was placed 1 cm below the surface of the water in the center of a quadrant. Starting positions changed in every trial in a pseudo-random fashion. A trial ended when a mouse was 10 s on the platform or when 120 s had passed. Mice that failed to find the platform within 120 s were directed while in water toward the platform, so that they climbed the platform by themselves. After the mice had been 20 s on the platform they were retrieved. The animals were given 5 acquisition trials per day on 5 consecutive days. Mice location was sampled every 0.1 s using a video computer system (Stoelting Co. USA). Swimming speed, latency and distance swam to reach the platform were measured.

2.4. Statistics

SPSS (20, 2012 issue) was used for all statistical analyses. Data were analyzed using ANOVA. Between-groups or between-groups with repeated measures comparisons were used where appropriate. When significant interactions were present, follow-up analyses were performed by separate one-way ANOVAs between variables where Dunetts two-sided test was performed to determine which group is different from the control. Differences were considered significant at $P < 0.05$ and 0.01.

3. Results

As a memory formation function, we tested short-term memory for specific spatial location using the Morris-water maze.

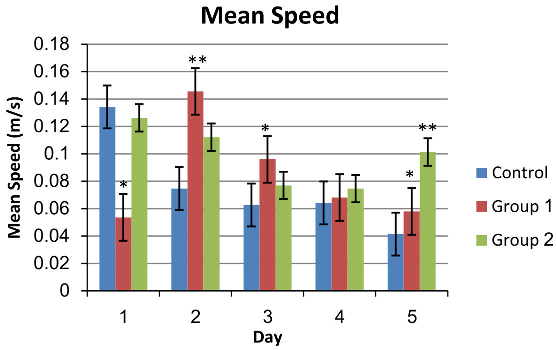

Figure 1 Morris water maze learning in adult animals. The mean speed (m/s) to reach the platform during 5 consecutive days of training. Group 1 consists of mice that were exposed to 1 mT, 50 Hz EMFs for 7 days after their birth. Group 2 mice were exposed to 1 mT, 50 Hz EMFs for 7 days before they were born. The mice of these two groups and those of sham-exposed controls were allowed to grow, and water maze experiments were performed when the mice reached adulthood (12–13 weeks old, 20–30 gm body weight). The behavior of the mice in groups 1 and 2 was compared to that of the sham-exposed controls in each day. Values represent means ± standard error of the mean, S.E.M. for $N = 8$ for control, $N = 17$ for group 1, and $N = 13$ for group 2. $^{*}p < 0.05$ and $^{**}p < 0.01$.

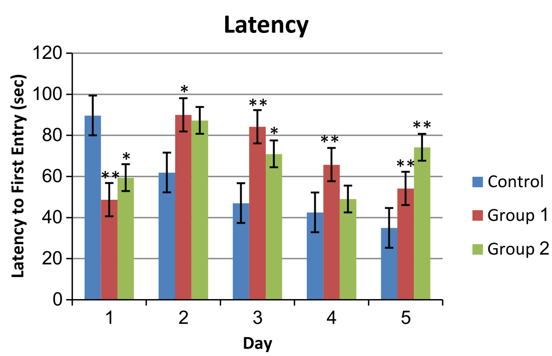

Figure 2 Morris water maze learning in adult animals. The latency to first entry to reach the hidden platform during 5 consecutive days of training. Group 1 consists of mice that were exposed to 1 mT, 50 Hz EMFs for 7 days after their birth. Group 2 the mice were exposed to 1 mT, 50 Hz EMFs for 7 days before they were born. The mice of these two groups and those of sham-exposed controls were allowed to grow, and water maze experiments were performed when the mice reached adulthood (12–13 weeks old, 20–30 gm body weight). The behavior of the mice in groups 1 and 2 was compared to that of the sham-exposed controls in each day. Values represent means ± standard error of the mean, S.E.M. for $N = 8$ for control, $N = 17$ for Group 1, and $N = 13$ for group 2. $^{*}p < 0.05$ and $^{**}p < 0.01$.

Fig. 1 shows the mean swim speed of the three groups of mice during training. ANOVA results (one way, single factor) showed significant differences in-between the groups as follows: in day-1 of testing ($F[2,182] = 31.88$, $P < 0.01$), compared for the sham-exposed control group in day-2 of testing ($F[1,182] = 23.27$, $P < 0.01$). In the successive day-3 ($F[1,182] = 5.32$, $P < 0.01$), day-4 ($F[1,182] = 0.34$, $P < 0.01$) and day-5 ($F[1,182] = 16.48$, $P < 0.01$). The previous data statistical analysis showed a significant increase in the mean speed for both exposed groups compared to the sham-exposed control. In the control mice the latency and distance swum to reach the platform decreased gradually during the 5 days of training, (Figs. 2 and 3). Data analysis showed a significant increase in the average latency for both exposed groups compared to the sham-exposed control: day-1 ($F[1,182] = 19.26$, $P < 0.01$), day-2 ($F[1,182] = 9.52$, $P < 0.01$), day-3 ($F[1,182] = 14.96$, $P < 0.01$), day-4 ($F[1,182] = 6.13$, $P < 0.01$) and day-5($F[1,182] = 9.55$, $P < 0.01$). Moreover a significant increase in the average distance to first entry was also found: day-1 ($F[1,182] = 44.26$, $P < 0.01$), day-2 ($F[1,182] = 47.89$, $P < 0.01$), day-3 ($F[1,182] = 16.19$, $P < 0.01$), day-4 ($F[1,182] = 2.78$, $P < 0.01$)and day-5($F[1,182] = 16.27$, $P < 0.01$).

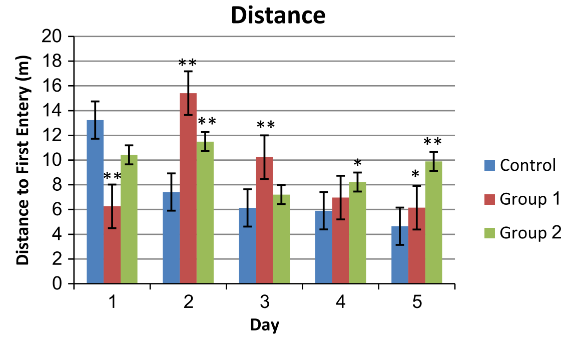

Figure 3 Morris water maze learning in adult animals. The distance to first entry to reach the hidden platform during 5 consecutive days of training. Group 1 consists of mice that were exposed to 1 mT, 50 Hz EMFs for 7 days after their birth. Group 2 mice were exposed to 1 mT, 50 Hz EMFs for 7 days before they were born. The mice of these two groups and those of sham-exposed controls were allowed to grow, and water maze experiments were performed when the mice reached adulthood (12–13 weeks old, 20–30 gm body weight). The behavior of the mice in groups 1 and 2 was compared to that of the sham-exposed controls in each day. Values represent means ± standard error of the mean, S.E.M. for $N = 8$ for control, $N = 17$ for Group 1, and $N = 13$ for group 2. $^{*}p < 0.05$ and $^{**}p < 0.01$.

From the second day of training the sham-exposed control mice reached the platform with significantly shorter latency, and distance compared to the exposed groups. This better performance in the control group could be seen also in the 3rd, 4th and 5th days.

4. Discussion

The current study was undertaken to investigate whether 50 Hz EMF can elicit a deficit, in spatial learning and memory functions of prenatal and neonatal mice. Two groups of mice were subjected to the ELF-EMFs, in addition to a sham exposed group. From the second day of training the sham-exposed control mice reached the platform with significantly shorter latency, and distance compared to the exposed groups. This better performance in the control group could be seen also in the 3rd, 4th and 5th days. Earlier reports showed a similar behavior for control animals, (Kamal et al., 2000;

Notenboom et al., 2010). The first group consists of mice that were exposed to 1 mT, 50 Hz EMFs for 7 days after their birth whereas in the second group the mice were exposed to 1 mT, 50 Hz EMFs for 7 days before they were born. After the exposure the mices' spatial learning and memory functions were assessed using the Morris water maze test. Data from these experiments show that prenatal and neonatal exposure to (1 mT 50 Hz) electromagnetic fields significantly affect the mices' rate of learning, when compared to sham-exposed controls. Although swim average speed was enhanced by EMF exposure especially in the first days, the latency and distance to reach the platform were increased compared to the control group, meaning that the exposed animals had difficulty in learning the test and/or retaining the spatial memory and that their failure to reach the disk with equal efficiency to the control animals was not due to differences in swim velocity, (Fig. 1). Exposed-animals navigation strategy showed that their latency and distance to reach the platform were not significantly enhanced when compared to the control animals, (Figs. 2 and 3). A somehow similar performance deficit was also reported in adult rats exposed to 50 Hz magnetic field (Lai et al., 1998). We showed in earlier studies on diabetic rats tested in the Morris water maze that this behavior of increased latency and distance to reach the platform, is due to alterations in the hippocampal synaptic plasticity (Kamal et al., 2000). This synaptic activity is regulated by the cholinergic system that acts synergically with glutamatergic transmission (Jerusalinky et al., 1997). In general, the cholinergic system is thought to play an important role in hippocampal-dependent learning and memory. Hippocampal cells possess several varieties of long-lasting synaptic plasticity. All forms of synaptic plasticity are induced by afferent activation, all involve Ca^{2+} influx, all can be blocked by Ca^{2+} chelators, and all activate Ca^{2+}-dependent mechanisms (Teyler et al., 1994).

In this study we have seen the effect of ELF-MF and stated the possible reasons behind it, but to explain the exact mechanism of interaction would be a difficult task. As the exact mechanism by which magnetic fields affect biological systems is not yet well understood the problem arises of explaining, how the low-energy influences of weak magnetic fields can compete with the thermal and electrical noise of cells at normal temperature using the theoretical studies.

A possible explanation of the inhibitory effects of magnetic fields could be due to the increase in the intracellular Ca^{2+}. This increase of intracellular calcium ion concentration Ca^{2+} is a result of excessive or persistent activation of glutamate-gated ion channels that may cause neuronal degeneration (Coyle and Puttfarcken, 1993; Lisi et al., 2005). ELF MF was found to directly enhance the expression of voltage-gated Ca^{2+} channels on plasma membrane of the exposed cells. The 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 acute magnetic field (Jelenkovic et al., 2006). These effects may be attributed to the action of both electric and magnetic components of the electromagnetic wave. From a theoretical analysis (King and Wu, 1998), it has been demonstrated that, for perfectly spherical cells, the electric component of an EMF is effectively shielded by the cell membrane, in contrast for a cylindrical cell (which is long compared to its radius), it is also shown that the induced electric field is the same as the axial field outside the cell. In this case, the cell membrane has no shielding effect. Then, since the neuron cells are far from being spherical, the effect of the electric field is inevitable. Calcium overload generates oxidative stress and causes neurodegeneration associated with necrosis (Singh et al., 2003). In a previous study on streptozotocine-induced diabetic rats we showed that disturbed behavioral performance of animals in learning and memory testing was associated with disturbed Ca^{2+} homeostasis affecting the basic hippocampal cellular functions, (Kamal et al., 2003). However, linking between complex animal behaviors as learning and memory to the very basic cell metabolism and ion channels is very perspective and difficult. Very extensive and complex work is needed in this field to explain the relation between cellular level mechanisms and the complex higher levels of brain function as cognition.

Our results demonstrated that the exposure of prenatal and neonatal mice to magnetic field may cause subsequent or later effects on brain activities during adulthood. These results are in context with other reported results (Yang et al., 2006) where prenatal stress causes cognitive deficits and increases vulnerability to affective disorders in children and adolescents. Furthermore, prenatal stress enhanced the effects of acute stress on the hippocampal impaired long-term potentiation and long-term depression. This will result as impaired spatial learning and memory in the Morris water maze in the young rat offspring 5 (weeks old).

In conclusion, our results show that long time MF exposure to immature mice; causes appreciable long term deficit in learning abilities. This has been supported by our previous work, as we have also found that magnetic field subacute exposure causes a deficit in motor coordination in the prenatal mice (Sakhnini et al., 2012). The mechanism of the interaction between the non-ionizing electromagnetic radiation and the biological target, still remains to be understood, so experiments in our laboratories are in progress to define such mechanism.

Acknowledgment

The authors wish to acknowledge the help of Dr. Maryam Almannaie, from Department of Mathematics for the statistics calculations.

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