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Effects of exposure to extremely low-frequency magnetic field of 2 G intensity on memory and corticosterone level in rats

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Abstract

In the present study, we examined the effects of chronic exposure (1 and 2 weeks) to an extremely low-frequency magnetic field (ELFMF) of 2 G intensity on memory in rats using an object recognition task. Comparable groups of rats were exposed for 1, 2 or 4 weeks to ELFMF and the following day blood samples were collected from each rat for the measurement of corticosterone level. Our results demonstrate that exposure to ELFMF induces a significant increase in the level of corticosterone in blood plasma and is associated with impairment in discrimination between familiar and novel objects.

Keywords: Magnetic field; ELFMF; Stress; Corticosterone; Memory; Object recognition; Rats

1. Introduction

Extremely low-frequency magnetic fields (ELFMF), such as those originating from residentially proximate power lines, household electrical wiring and medical devices, have been reported to produce a variety of biological effects [6,14,20,33,37,59,66,68], interfere with the activity of the brain [1,5,13,15,17,18,22,27,31] and may generate behavioral and cognitive disturbances [16,21,25,26,29,32,35,38,43,44,55,57,61]. Frequent and/or prolonged exposure to these devices of an ever-growing number of people of different ages raises some concerns about the effects of ELFMF on human health. Some efforts have been made recently to investigate the incidence of ELFMF on human and animal physiology and behavior. The results of these studies are mostly inconsistent and contradictory [3,5,17,19,28,40,42,44,45,47,55–58,64,65].

Some reports suggest that ELFMF may act on the hypothalamic–pituitary–adrenocortical axis activity and alter the plasma corticosterone level [7,24,52,67]. Such effects may interfere with memory performance as there is evidence suggesting impairing effects of stress-induced corticosterone release on object recognition in rats [2,41].

In the present study, we examined the effects of chronic exposure to ELFMF of 2 G intensity on memory in rats and on their plasma corticosterone level. The object recognition task, based on spontaneous exploratory activity of rats, was used as an appropriate model for assessing the effect of ELFMF on memory [10,11]. In this task, normal rats spend more time exploring a novel object than they spend exploring a familiar object; this reflects their memory of the familiar object. This task does not involve positive or negative reinforcement or the learning of a rule and has clear parallels with human recognition memory.

2. Methods

2.1. Animals

Sixty-seven male Wistar rats (200–250 g, Faculty of Veterinary, Moshtohor, Egypt) were used in this experiment. After 3 days of habituation to the laboratory environment, the animals were housed in either a magnetic field chamber

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or a similar chamber without a magnetic field. They were kept in pairs in an opaque plastic cage (width 31 × length 47 × height 21 cm). The colony room was held under a 12-h light/12-h dark cycle (light 0700–1900 h at 180 lx) and at 23 ± 1 °C. They had ad libitum access to food and water. During their stay in the respective housing conditions, they were removed daily from their cages for cleaning the cages and renewing their food and water supply. The experimenter also handled the animals for about 3 min each day.

2.2. Apparatus

2.2.1. Object recognition

The apparatus consists of an open box (width 100 × length 100 × height 50 cm) made of wood, painted mat gray inside. The floor was covered with wood chip bedding (≤ 1-cm depth), which was moved around between trials/days to prevent build up of odor in certain sites. The objects to be discriminated were available in triplicate copies and were made of a biologically neutral material such as glass, plastic or metal. The objects were weighted so that the animals could not move them around in the arena. They are not known to have any ethological significance for the rats and they had never been associated with a reinforcer.

2.2.2. Magnetic field chamber

Consisted of a parallel double walled cylindrical cage made from copper plate (2-mm thick) and was 114-cm internal diameter, 140-cm external diameter and 152-cm long. The two cylinders were sealed at each end with copper to permit water flow between the two layers. Four coils of 270 turns each from electrically insulated 2.2-mm copper wire were wound around the outer cylinder at equal distance. The four coils were connected in parallel to minimize the total impedance of the wire and allow a homogenous magnetic field within the chamber volume. The cylinder was grounded. A mesh from copper was used to cover both ends of the cylinder. The coils were connected to a Variac fed from the mains (220 V and 50 Hz). The magnetic field inside the chamber was measured at different locations using a hand-held Gauss/Tesla Meter Model 4048. A probe T-4048.001 (USA) of ±2% accuracy was used to calibrate the magnetic field. The field strength can be varied by means of Variac up to 2.5 mT inside the homogenous zone without an increase in the chamber temperature (±0.5 °C).

Two magnetic field chambers were used for this experiment. Six animal cages were located inside each chamber. Thus, with two rats per cage, twelve rats can be housed in the magnetic chamber at a time. The cages were positioned at each end of the chamber leaving an empty space in the middle that was not accessible to the ambient room light. The illumination of the boxes inside the chamber was about 41 lx and the temperature was 23 ± 1 °C. The magnetic field at the location of the cages was 2 G for the treatment groups and 0.01 G for controls.

2.3. Treatments

Rats were exposed to ELFMF of 2 G intensity while maintained in their cages inside the magnetic field chamber. One group of rats ($n = 9$) was used as control and was not exposed to ELFMF. They were housed for 2 weeks in a magnetic chamber that was turned off. After that, they were tested immediately in the object recognition task. A second group of rats ($n = 9$) was exposed to ELFMF for 1 week then tested immediately in the object recognition task. A third group ($n = 9$) was exposed to ELFMF for 2 weeks then tested immediately in the object recognition task. Since the sample size is 9, one animal from each group was housed alone.

Comparable groups of rats were also exposed for 1 ($n = 10$), 2 ($n = 10$) and 4 ($n = 10$) weeks to ELFMF, then blood samples were collected from each rat for the measurement of corticosterone level. Control rats ($n = 10$) were maintained for 4 weeks in a cylinder magnetic chamber without exposure to ELFMF. These four groups were not tested in the object recognition task. Blood samples were collected from the tail vein from each rat for the measurement of plasma corticosterone levels [8]. During blood sampling, the animals were lightly restrained and a lateral tail vein was punctured with the corner of a razor blade and 200 μl of blood was collected in a heparinized capillary tube. Plasma, obtained after centrifugation, was stored at −80 °C until assay. Plasma corticosterone was measured by radioimmunoassay (RIA kit, ICN Biomedicals, Los Angeles, CA) using a highly specific corticosterone antiserum with a detection threshold of 0.1 μg/100 ml.

2.4. Behavioral testing

2.4.1. Habituation

Rats were habituated to the open box before the start of the object recognition task proper for 5 min each day for 5 days. They were removed from the magnetic field chamber for that period of habituation. Each rat was allowed to explore the box and a junk light object (always the same object for all rats and for every day of habituation).

2.4.2. Object recognition test

The test was run in one session a day with 48 h interval between sessions. Each rat was tested in two sessions. A session consists of a sample phase (3-min duration) and a choice phase (3-min duration) with 15-min retention interval between the two phases. The objects used during the memory test were in triplicate. Rats were exposed to two identical objects during the sample phase and to two different objects in the choice phase, one seen previously (and therefore familiar) and one new (and therefore novel) object. The familiar object consisted of a third copy of the objects presented in the sample phase (Fig. 1). The position of the familiar object is selected randomly from the two locations previously occupied by the sample objects, and therefore the

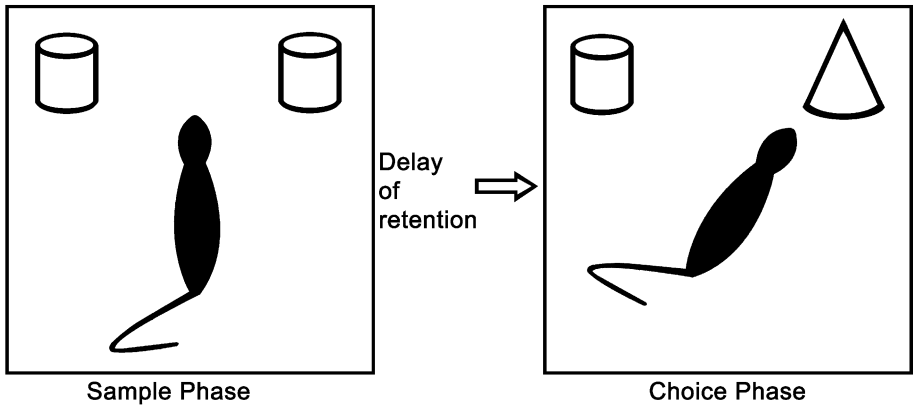

Fig. 1. Schematic representation of the testing procedure in the object recognition task.

novel object occupied the vacant position. Different sets of objects were used in Sessions 1 and 2.

2.5. Performance measures and analyses

The basic measure was the time spent by rats in exploring objects during the sample phase and during the choice phase. Exploration of an object was defined as directing the nose to the object at a distance ≤ 2 cm and/or touching it with the nose; conversely, turning around or sitting on the object was not considered as exploratory behaviour.

Comparisons focused on the time spent by rats in exploring objects during the sample and choice phases. Analyses of variance and post-hoc comparisons (Student–Newman–Keuls) were performed on the following measures:

- e_1: the total time spent exploring the two identical objects in the sample phase;
- e_2: the total time spent exploring the two objects in the choice phase;

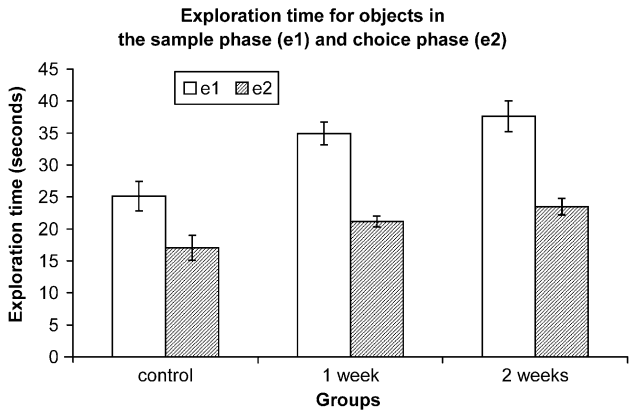

Fig. 2. Time spent by rats exploring both objects in the sample phase (e_1) and the choice phase (e_2). In the sample phase, both the 1- and 2-week ELFMF-treated rats explored significantly more than control ($P<.004$), whereas in the choice phase only rats exposed for 2 weeks to ELFMF explored more than control ($P<.01$).

- d_1: the discrimination index, which is the difference in time spent exploring the two objects in the choice phase (e.g., time spent for novel object minus time spent for familiar object);
- d_2: the discrimination ratio, which is the difference in exploration time between novel and familiar objects (d_1) divided by the total time spent exploring the two objects in the choice phase (e.g., novel − familiar/novel + familiar). This ratio makes it possible to adjust for any differences in the total amount of exploration time.

In addition, a paired comparison Student's t test was performed within each group to examine whether there was any difference between the times spent for novel object and familiar object in each testing session.

3. Results

3.1. Overall time spent exploring objects in the sample phase and in the choice phase

There was a significant difference between groups in both phases of the task [$F(2,24)=9.08$, $P<.001$ for e_1 and $F(2,24)=5.11$, $P<.01$ for e_2]. In the sample phase, both the 1- and 2-week ELFMF-treated rats explored the two sample objects significantly more than control animals ($P<.004$),

Table 1
Mean discrimination (±S.E.M.) performance of rats in the object recognition task and results from the paired comparisons (two-tailed Student's t test) between the time spent for novel objects and the time spent for familiar objects

	Control	1 week	2 weeks
Session 1	8.44 ± 2.72	7.67 ± 2.09	2.11 ± 2.56
	$t_8=3.10$, $P<.01$	$t_8=3.66$, $P<.006$	$t_8=0.83$, $P>.10$
Session 2	13.22 ± 3.18	6.22 ± 2.40	3.56 ± 2.06
	$t_8=4.10$, $P<.003$	$t_8=2.59$, $P<.03$	$t_8=1.73$, $P>.10$

whereas in the choice phase only the rats treated for 2 weeks with ELFMF explored more than control ($P < .01$).

There was no significant difference between results from the two sessions on both e_1 and e_2 ($P > .10$). Fig. 2 represents pooled data from two testing sessions.

3.2. Object recognition performance

The discrimination between familiar and novel objects after 15-min delay of retention is impaired in rats treated for 2 weeks with ELFMF. The time they spent exploring the novel object is not significantly different from the time they spent exploring the familiar one in both testing sessions ($P > .10$). Control and rats treated with ELFMF for 1 week were able to discriminate between objects in both testing sessions (see Table 1).

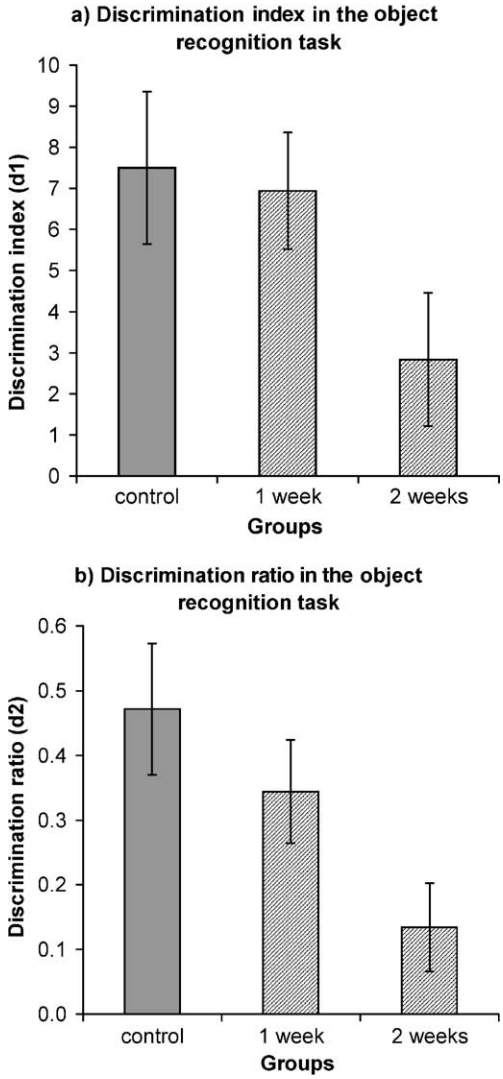

Fig. 3. Mean value (±S.E.M.) of the discrimination index (a) and discrimination ratio (b) in the object recognition task. There is a significant difference between groups for d_2 [$F(2,24) = 4.09$, $P < .03$], but not for d_1 [$F(2,24) = 2.42$, $P > .10$]. d_2 in rats treated with ELFMF for 2 weeks is lower than that of control rats ($P < .02$).

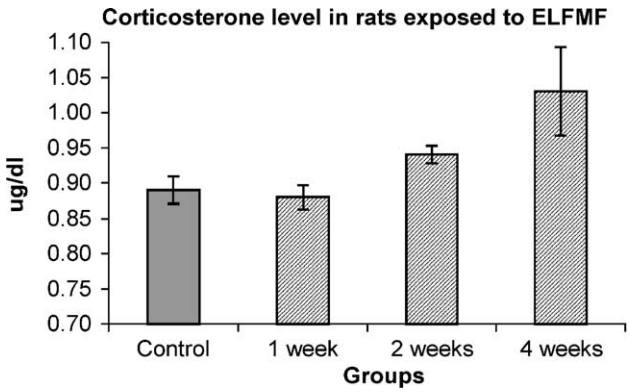

Fig. 4. The corticosterone concentrations (mean ± S.E.M.) measured after 1, 2 and 4 weeks in rats chronically exposed to ELFMF. There is a significant effect between groups [$F(3,36) = 3.94$, $P < .02$]. Two and 4 weeks ELFMF-treated groups are significantly different from control and 1 week ELFMF-treated group ($P < .05$).

A one-way ANOVA revealed significant differences between groups for the discrimination ratio (d_2) [$F(2,24) = 4.09$, $P < .03$], but not for the discrimination index [$F(2,24) = 2.42$, $P > .10$]. The performance of rats treated with ELFMF for 2 weeks was significantly higher than that of control ($P < .02$).

There was no significant difference between results from the two sessions on both discrimination index and discrimination ratio ($P > .10$). Fig. 3a and b represents pooled data from two testing sessions.

3.3. ELFMF on corticosterone level

Exposure to ELFMF was associated with a significant increase in the plasma corticosterone level [$F(3,36) = 3.94$, $P < .02$]. Two and 4 weeks exposure to ELFMF produced a significant increase in corticosterone level compared to the effect of 1 week or no exposure to ELFMF ($P < .05$). These data are shown in Fig. 4.

4. Discussion

Our results demonstrate that chronic exposure to ELFMF produces a significant increase in the plasma level of corticosterone and impairs the discrimination between familiar and novel objects after a 15-min retention interval. These results add to the growing evidence of a possible health hazard from constant use of some electric and electronic appliances in everyday life.

Our results are in contrast to those recently reported by Sienkiewicz et al. [54] who found no effect of ELFMF exposure on object discrimination. However, in this study, the exposure of ELFMF was acute and of short duration (45 min, once only), whereas in our study rats were exposed chronically for 24 h/day for 1, 2 or 4 weeks. Furthermore, in Sienkiewicz et al.'s study [54], the ELFMF exposure was given during the consolidation phase, while in our study the

exposure was administered before the sample phase of the task. The rationale for our chronic exposure design is that acute exposure to ELFMF is unlikely to have significant and maintained effects on memory performance. The chronic exposure design avoids the inconsistent effects of ELFMF on performance that are only transient or varies with time. Furthermore, in everyday life, some people are exposed to ELFMF almost continuously though at variable and unpredictable frequencies.

The level of exploration of ELFMF treated rats was significantly higher during the sample phase in both groups and also remained higher in the group treated for 2 weeks with ELFMF in the choice phase. This higher level of exploration did not contribute to a better discrimination between objects as we usually see with medial septum and fornix lesioned rats [9,12]. One would expect that longer exploration of the objects during the sample phase would permit better encoding of the objects and facilitate recognition, but it is also possible that this high level of exploration reflects hyperactivity only.

In the present study, chronic exposure to ELMF also appears to affect the level of corticosterone in plasma. This supports the results of several studies pointing to the effects of ELFMF on the endocrine and immune system [33,39,64,65]. The increase in plasma corticosterone level might be the result of stress generated by such continuous exposure to electromagnetic radiation. It has been demonstrated that a stress caused from the presentation of predator odors [41] or chronic restraint [2] impairs object recognition in rats. It has also been reported that an elevation of plasma corticosterone levels is often associated with stress [4,34,36,60] though not always associated with impairment of memory performance [4,46,53]. It is possible that the elevation of plasma corticosterone following chronic exposure to ELFM may underlie the increased hyperactivity observed in the sample phase as well as the impairment in object recognition. This conclusion remains, however, speculative as it is based on two separate sets of experiments using different batch of animals.

Unequivocal conclusions cannot be drawn without further investigation of the effect of ELFMF in much improved testing conditions. For example, our rats were maintained in cylindrical chambers for a long period of time. Though the cylinders were open at both ends leaving access to air and light, the luminance was very low (41 lx). The changes in corticosterone level and cognitive performance may not be a direct consequence of ELFMF alone but the result of an interaction between this factor and the housing conditions [62]. It would be of interest to examine the effect of ELFMF on rats maintained in their home cage under standard laboratory conditions or in a seminaturalistic environment.

While there is increased number of reports on the biological effects of ELFMF, only few have been devoted to behavior and cognition. Results from these reports are inconsistent and sometimes contradictory [3,5,17,19,28,40,42,44,45,47,64,65]. This is probably due to the lack of standard parameters in the dosage and duration of exposure to magnetic fields [30,38]. In contrast to the worrying reports on the deleterious effects of ELFMF on human health, other studies suggested beneficial effects of low magnetic or electromagnetic fields in certain conditions [23,24,47–51,63].

The present results demonstrate the possible cognitive and biological effects of exposure to ELFMF and raise attention to the possible health hazard associated with domestic electric devices. Further studies are underway to examine the effect of ELFMF at different intensities and in comparable tasks that involve spatial memory and visual attention. Other physiological parameters in addition to the plasma corticosterone level will be assessed.

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