DOI: 10.4149/BLL_2012_117

Bratisl Lek Listy 2012; 113 (9)

521 – 524

EXPERIMENTAL STUDY

The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats

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Abstract: *Purpose:* The purpose of this study was to investigate the behavioral changes induced by 50 Hz, 10 mT flux density Sinusoidal Magnetic Field (MF).
Material and methods: Seventy-six young adult male Wistar albino rats were used in the study. They were separated into two groups: control group (C) n=38; MF group n=38. C animals were left under the same conditions with the MF group for 21 days but with prevented or avoided exposure to MF. Anxiety and stress-related behavioral changes were investigated by elevated plus-maze and hole-board systems. Just before being tested in the maze, each animal was tested by means of the hole-board method in order to separate the directed exploration behavior and locomotion activity changes from anxiety-related behavior.
Results: In the hole-board system parameters there were no statistically significant differences between the two groups. There was a statistically significant difference between MF and C groups when the ratio of time spent on open arms to the total time spent on all arms was evaluated (0.12±0.08 and 0.34±0.18 respectively and $p<0.01$).
Conclusion: Our results suggest that after 21 days, a continuous exposure to extremely low frequency of magnetic field (50 Hz, 10 mT) has no significant effect on activity and exploration activity but significantly induces stress and anxiety-related behavior in rats *(Tab. 2, Fig. 9, Ref. 19)*. Full Text in PDF *www.elis.sk.*
Key words: magnetic field, anxiety, elevated plus-maze, hole board, stress, behavior, rat.

Static, sinusoidal and specific pulsed magnetic fields have been shown to alter the animal and human behaviors such as directional orientation, learning, pain perception (nociception) and anxiety-related behaviors (1 – 6).

Extremely low frequency (0.1 – 100 Hz) of sinusoidal magnetic fields (MF) of 50 or 60 Hertz generated predominantly by power lines, appliances and video display terminals could endanger health by producing a variety of effects in biological systems. Appliances can generate fields higher than those of transmission lines but in most cases they fall off rapidly. However electric blankets expose the users for eight hours at a time and electric hair dryers and shavers are operated close to the body. Power frequency fields carry too little energy in each quantum to break the chemical and molecular bonds, so they are not accepted as a sort of biological damage such as caused by X-rays, but on the other hand they are supposed to induce currents and create potential differences in biological structures.

Although the present knowledge is fragmentary and the coherent theory to explain the observations seems far off, continuously present power frequency fields in our modern environment bring about potential health effects which are a matter of serious scientific and public health policy concern.

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That concern is focused on cancer and developmental abnormalities and to a lesser extent on endocrine and nervous system and psychiatric problems including anxiety disorders and depression (7 – 9).

The general aim of our studies is to assess the psychological and psychophysiological effects of low-intensity 50 Hz MF exposure. In addition, we investigated the effect of extremely low frequency of magnetic field (50 Hz, 10 mT) on the behavior of rats under experimental conditions.

Materials and methods

Seventy-six young male adult Wistar Albino rats, weighing 150±50 grams were used in the study. They were kept in a room at 23±2 °C with a 12-hour light cycle.

Rats were allowed free access to standard laboratory food and tap water *ad libitum*. In order to minimize the handling stress, one week before the experiment, each animal was handled each day for 5 minutes.

Rats were separated in two groups:

I. Control group (C); n=38,

II. Group exposed to magnetic field (MF); n=38.

The animals in C group were kept under the same experimental conditions with MF group for 21 days except for the effect of extremely low frequency of electromagnetic field.

The magnetic fields were generated by eight serially connected copper solenoid coils, each having 560 turns. The cores of the coils were filled with soft iron rods that were tightened to increase the

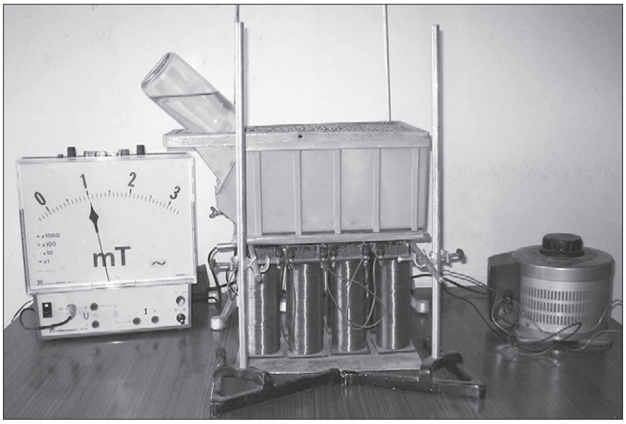

Fig. 1. Application of The magnetic field.

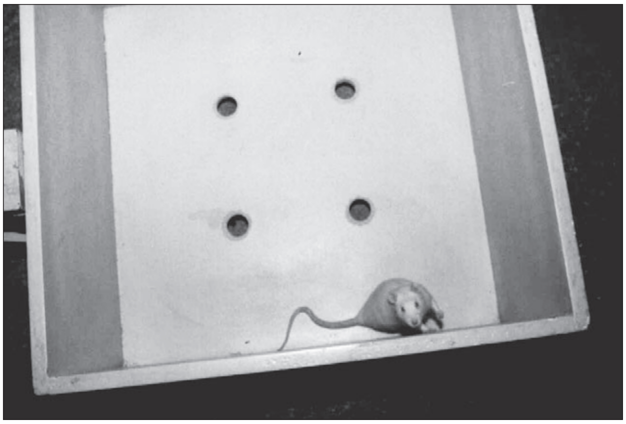

Fig. 2. Holeboard apparatus.

magnetic flux intensity. Plastic cages were used so that the strength and distribution of magnetic field would not be affected. The coils were placed vertically and the cages were held 1.2 cm above the coils to hinder them from probable vibration. To protect the cages from heat of the coils, 1-cm thick wooden plates were placed between the coils and the cages (Fig. 1). The coils were connected to 220 V, 50 Hz sinusoidal city electric system. When the current passed through the coils, the magnetic flux intensities were measured at six different places inside the cages in order not to exceed 10 militesla. The magnetic field was measured by Leybold Heraus 54050 model Hall-effect teslameter.

The rats were investigated for behavioral effects of MF by means of the elevated plus maze, which is one of the most validated methods of measuring the anxiety (10 – 12).

Elevated plus-maze test

The test is based on a procedure used by Montgomery in 1958, in which he showed that an exposure to elevated (open) maze alley evoked an approach-avoidance conflict that was considerably stronger than the one evoked by an exposure to enclosed maze alley.

The elevated plus-maze consists of four wooden arms connected by a central square. All arms are horizontal and at 90° angle from each other, making the shape of a plus sign. The maze is raised to the height of 50 cm from the ground. Two of the opposite arms have high wooden walls whereas the other two opposite arms do not (Fig. 2).

These arms are therefore referred to as enclosed and open arms, respectively. The central square is not enclosed and the rat is initially placed in this area facing an enclosed arm. The rat has free access to all four arms for a five-minute period. During this time the number of entries into the arms and the time spent on each type of arm are measured. The rat is counted as being on an arm only when all its four paws are on it. The rats typically spend 30 – 60 seconds on the open arms and about three minutes in the enclosed arms. The remaining time is spent with one or more paws in the central square.

An anxiolytic effect is defined as an increase in the percentage of entries onto the open arms out of the total number of entries. Similarly an anxiogenic effect is therefore a decrease in the percentage of entries and percentage time spent on the open arms. This definition therefore takes into account any non-specific effects on motor activity by including the data for the enclosed arms. For validation of this test see (12).

The rat is placed in the centre of the maze which is facing one of the open arms and scored by two observers for five minutes.

- Number of entries into open arms (all four paws on one arm),
- Number of entries into closed arms,
- Time spent on open arms,
- Time spent in closed arms.

We also tested the effects of MF on motor activity and exploratory behavior in the hole-board system, which allows the separation of directed exploration behavior and locomotion activity changes from anxiety-related behavior (13, 14).

Holeboard test

The hole board was a wooden box (60 x 60 x 35 cm) with four holes of 4 cm in diameter symmetrically spaced in the floor. The ground was divided into 9 squares, thus allowing the counting of squares visited during a period of time (Fig. 3).

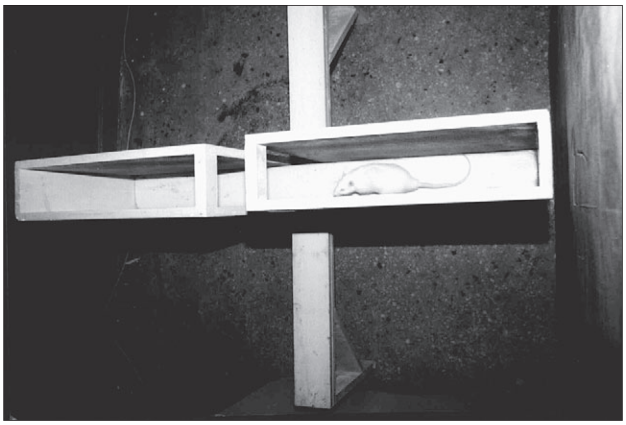

Fig. 3. Elevated plus-maze test contruction with four wooden arms.

Tab. 1. The parameters of the hole-board test.

Parameter	Control group (n=38)	Experimental group (MF) (n=38)	
Number of head dips	8.26±4.53	9.48±5.36	p>0.05
Time of head dipping activities (sec)	21.48±9.76	19.62±9.53	p>0.05
Number of squares visited	38.73±16.24	35.46±11.73	p>0.05
Number of grooming activities	5.32±2.41	6.43±3.17	p>0.05
Number of rearing activities	25.83±9.83	27.62±10.79	p>0.05
Time of moving (sec)	143.17±107.74	136.86±113.34	p>0.05

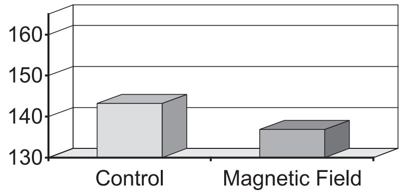

Fig. 4. The moving time of the rats in the hole board (n=38; p>0.05).

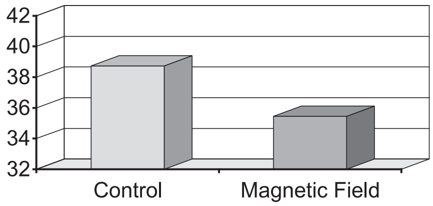

Fig. 5. The number of squares visited in the hole board (n=38; p>0.05).

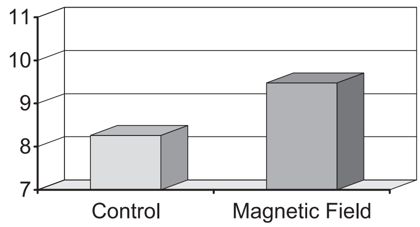

Fig. 6. Numbers of head dips in the hole board (n=38; p>0.05).

For testing, the animal was taken from its home cage and placed on the hole board for five minutes. The number of head dips and time spent head dipping (exploration activity), number of squares visited (with all four paws on one square (locomotion activity) were counted.

After the hole-board test, the animal was placed in the center of the plus maze with its head facing an open arm, and observed for five minutes. It was accepted to be on a certain arm when all its four paws were on that arm. During the test, open arm entries and closed arm entries were counted and total time spent on open arms and total time spent in closed arms were measured. Indeed it has been shown that anxiolytic drugs increase the ratio of open arm entries to the total number of arm entries as well as increase the time spent on open arms whereas anxiogenic drugs have an opposite effect (15).

The anxiety score was computed according to the formula:

Anxiety score = (number of open arm entries) ÷ (total number of arms entries)

Also, the ration of total time spent on open arms to total time spent on all arms was evaluated as an anxiety measure.

Unpaired "t" test was used to evaluate the locomotion activity, exploration activity and anxiety measures (PC/OXSTAT). Data were expressed as mean and standard deviation of mean.

Results

In the hole-board test there was no statistically significant difference between the two groups ($p > 0.05$) when locomotion activity scores (number of squares visited) and exploration activity (number and time of head dipping) were evaluated (Tab. 1). The graphical representation of hole-board tests are shown in Figures 4 – 6.

When anxiety scores were compared there was a statistically significant difference between the C group and experimental MF group (Tab. 2). Also there was a statistically significant difference between MF and C groups when the ratio of time spent on open arms to the total time spent on all arms was evaluated (0.12 ± 0.08 and 0.34 ± 0.18 respectively and $p < 0.01$). The graphical representation of Anxiety Scores is shown in Figures 7 – 9.

Discussion

In general, the biologic effects reported in many studies have not confirmed any particular pathologic effect even after prolonged exposures to high-strength fields (100 kV/m) or high-intensity magnetic fields (10 mT) (16).

Tab. 2. The results of Anxiety Scores (Elevated Maze Test Application total time is 300 sec).

Parameter	Control group (n=38)	Experimental group (MF) (n=38)	
Time spent on open arm (sec)	105.34±108.19	37.61±31.46	p<0.01
	195.28±107.34	258.15±144.36	p<0.01
Time spent on open arm (%)	36.24±23.14	14.56±10.26	p<0.01
Time spent on closed arm (%)	63.72±34.46	83.48±45.23	p=0.28
Number of entries into closed arm	3.2±1.4	1.8±0.82	p<0.01
Anxiety Scores	0.34±0.18	0.12±0.08	p<0.01

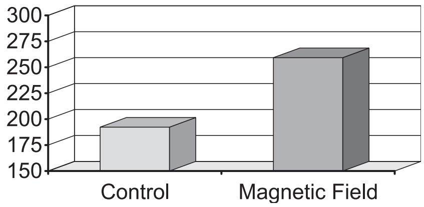

Fig. 7. Time spent in the closed arms in the elevated maze test (n=38; p<0.01).

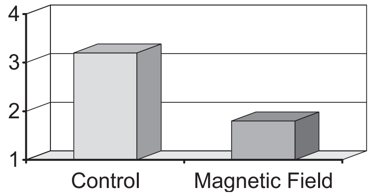

Fig. 8. The numbers of entries into the closed arms (n=38; p<0.01).

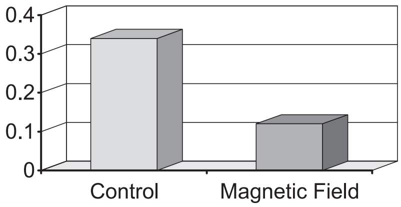

Fig. 9. Anxiety score in the elevated maze test (n=38; p<0.01).

An early behavioral study of Persinger has been repeated by Rudolph et al and Thomas et al who found that an exposure of rats to weak oscillating MF (typically of 50 Hz) modifies their open field response in particular by increasing their rearing activity which is involved in exploration and also stress-related anxious behavior (17, 18).

After such studies, the effects of electromagnetic fields on psychiatric problems including anxiety disorder and chronic depression have become a matter of concern. Studies on behavior of mice exposed to strong fields according to different protocols (field from 1 to 8.5 mTesla, continuous or intermittent exposure) showed stress-like effects on open field task, sometimes accompanied by reduced body weight (9, 19).

Our results suggest that after 21 days, a continuous exposure to extremely low-frequency magnetic field (50 Hz, 10 mT) has no significant effect on activity and exploration activity but significantly induces stress and anxiety-related behavior in rats.

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Received November 26, 2011.
Accepted February 21, 2012.