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Original Research

Comparison of Analgesic Effects Induced by Different Strengths of Extremely Low-Frequency Electromagnetic Fields

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ABSTRACT

Objective: Our aim was to compare the results of the most commonly used analgesic measurement techniques and to determine the time and intensity at which the analgesic effects of the magnetic field (MF) are most effective.

Methods: This study compared the analgesic effect of MF strengths (1, 5, and 10 mT) in 30 adults, male Wistar albino rats weighing 200-250 g. The analgesic effects were measured using tail-flick (TF) and hot-plate (HP) tests. To determine the optimum MF strength, rats were assigned into four groups: sham group and exposed to 1, 5, and 10 mT MF groups. Rats were placed in a solenoid, and MF of 50 Hz for 165 min was administered daily for 15 days. All four groups were kept in the solenoid for 165 min/15 days and exposed to MF. However, the analgesic effect was measured only on day 0, 4, 7, 11, and 15 using TF and HP tests. The latencies of analgesia were converted to a percentage of maximal antinociceptive effects (% MPE).

Results: When the maximum analgesic effect of the 5 mT MF was determined on the seventh day, the% MPEs were 5.37±0.51, 13.66±1.27, 25.89±3.00, and 25.37±2.41 in the sham, 1 mT, 5 mT, and 10 mT groups, respectively. The optimum effect was observed with 5 mT MF on the seventh day and with 90 min in the solenoid.

Conclusion: We didn't find any differences between the analgesic responses to the TF and HP tests.

Keywords: Analgesic effects, extremely low-frequency magnetic fields, tail-flick test, hot-plate test, Wistar albino rats

INTRODUCTION

It is known that extremely low-frequency magnetic fields (ELF-MFs) can modify human and animal behaviors, such as orientation, learning, nociception, and anxiety-related behaviors (1-3). The International Association for the Study of Pain defines pain in humans as "an unpleasant, sensory, and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (4, 5). Recently, a majority of studies have particularly focused on the mechanism of magnetic antinociception. A previous study in this laboratory showed that ELF-MFs affected the acute and chronic effects of pain in experimental animals (6). We measured thermonociceptive sensitivity in diabetic (treated with insulin) rats under repeated exposure of ELF-MFs for 30 days. Other studies have shown that land snail and mice inducted analgesia through repeated, daily exposure to oscillating fields or to specific pulsed MFs (7, 8). Various tests have been suggested to assess MF antinociception in animals. A majority of tests measure a specific group of nociceptors or particular sites of the central nervous system for nociceptive processes. Therefore, a difference in the response may be observed due to different testing methods. In our opinion, the two most popular nociceptive tests, namely rodent tail-flick (TF) and hot plate (HP) have not been sufficiently compared with literature, and the intensity of MF, which affects the most effective analgesia, has not been studied in either of the MF intensities. Hence, our aim was to compare the results of the most commonly used analgesic test measurement techniques and to determine the time and intensity at which the analgesic effects of MF are most effective.

METHODS

Animals and Electromagnetic Field Exposure Conditions

The experimental procedures applied in this study were confirmed by the Institutional Review and Animal Use Committee of the Cumhuriyet University School of Medicine, and the study was organized and designed by following the guidelines for

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the care and use of laboratory animals for research. Thirty adult male Wistar albino rats weighing 200-250 g were tested. Rats were maintained at 25°C±1°C and under a 12-h light/dark cycle. All experiments were performed during the light cycle (10:00-14:00).

The electromagnetic field (EMF) exposure system consists of a power supply, solenoid with plexiglass cage, and timer (Figure 1). Before the EMF exposure, all rats were accustomed to their environment for 1 week. The MF groups were left in a solenoid with an MF of 50 Hz, and strengths of 1, 5, and 10 mT were applied daily for 165 min. They were exposed to two non-stop pulses of 30 min with 15 min intervals. The animals were subjected to repeated exposures of alternating 50 Hz EMF for 15 days, which was performed in three different MFs. The EMF that was generated in a specially designed solenoid (500 mm in length and 210 mm in diameter, 1400 turns of insulated 1.4 mm copper wire). An electrical current (50 Hz, respectively 25, 120, and 220 V) was passed through the device (with a time relay) at strengths 1, 5, and 10 mT. The animals were exposed to EMF with an alternating current for four, 30-min implementations deactivated by 15-min intervals; thus, the entire EMF sessions were carried out during the same time period (9.00-11.00 a.m.) and lasted 165 min daily. The EMF intensity in the solenoid was measured using a digital tesla meter with an axial probe (PHYWE 8010 Model Digital Teslameter; Figure 2 a-c). The solenoid was always retained in the north-south direction, and its temperature was maintained at 22.0°C±2°C. The plexiglass rat cage (dimensions, 40×17×13 cm) was placed in the solenoid. Four rats were simultaneously placed in the cage to be exposed to EMF. The control group rats were also placed in the animal cage, but they were not exposed to EMF. The plexiglass cage was designed to supply food and water for rats.

Figure 1. Schematic representation of placement of the rats in the solenoid with the pulsed MF. At the bottom, the schematic of MF that the rats are exposed to for a total of 165 min with 30 min of exposure and 15 min of silencing is indicated. a) MF experimental setup at 1 mT; b) MF experimental setup at 5 mT; c) MF experimental setup at 10 mT

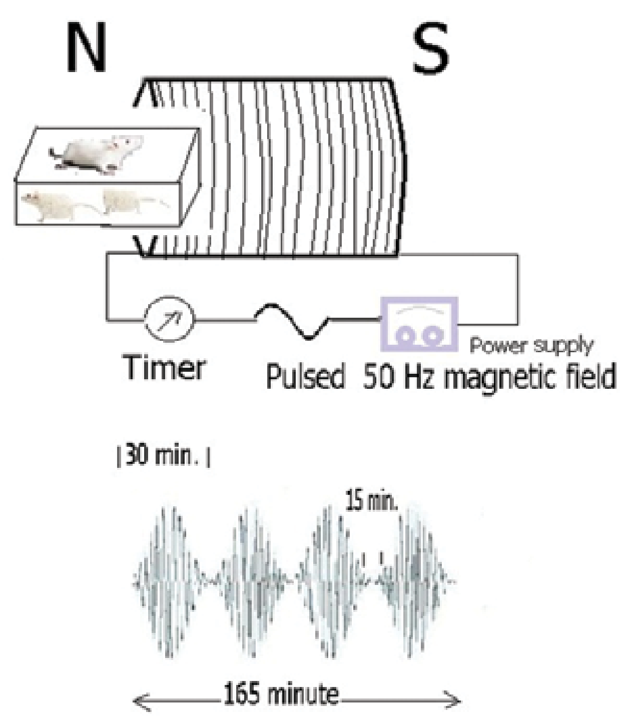
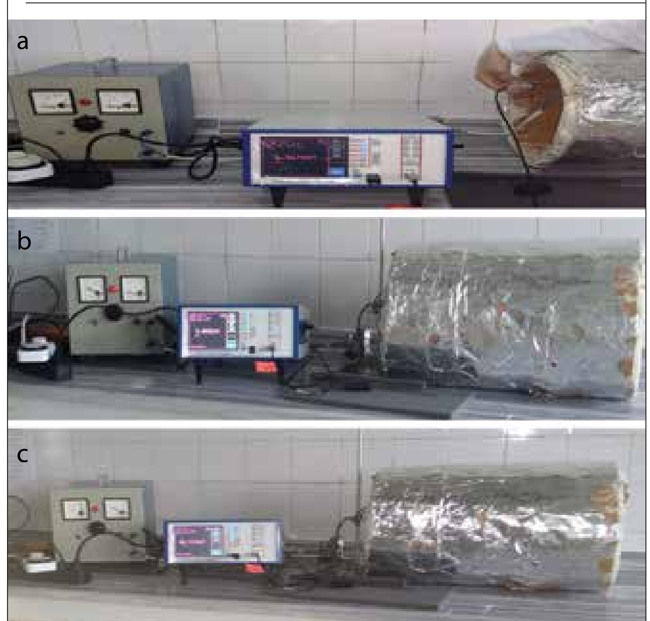


Figure 2. a-c. Experimental set up (a, b, c) for MF strengths (1, 5, and 10 mT). The solenoid was powered by a power supply



Experimental Protocols

First, the following procedure was established to determine MF strength and the day on which the most effective analgesia was induced. Rats were randomly delivered to one of four groups: sham (the control group, placed in the solenoid but not exposed to MF) and exposed to a 1, 5, and 10 mT MF. The antinociceptive effects of three different EMF strengths (1, 5, and 10 mT) were evaluated at 30-min intervals (0, 30, 60, 90, and 120 min) using TF and HP test in rats (n=6). Initially, the maximum analgesic effect of EMF was detected in 15 days.

Antinociception Tests

TF test

Nociceptive responses in all groups were measured using the radiant-heat TF test. This test is generally used to specify sensory functions under different strengths of MF since it is comparable with certain quantitative sensory tests used in clinics. The TF test mostly projects the activity of simple spinal reflex arcs. It ensures information on peripheral nerves and spinal functions in relative isolation from higher nociceptive processing and cognitive systems (9). The nociception response was measured using a tail-flick apparatus (May TF 0703 Tail-Flick Unit, Commat, Turkey). Animals were individually placed on a plate with the temperature adjusted to 51°C±1°C. The cut-off time was 15 s to prevent damage to their tails (10). The analgesia response measured on the HPis considered originating from a combination of central and peripheral nervous system mechanisms (11). Animals were

Figure 3. Analgesic effects of three different EMF strengths (1, 5, and 10 mT) on rats measured using the TF (a) and HP tests (b). The maximal analgesic effect of MF was observed on day 7 in all groups of rats: ELF-EMF (1,5, and 10 mT). The analgesic activity of MF of 1, 5, and 10 mT were significantly higher than control group rats (p<0.01). Each point represents the mean±SEM of % MPE for six rats. HP, hot plate; TF, tail-flick; SEM, standard error means; ELF-EMF, extremely low-frequency electromagnetic fields; % MPE, the percentage of maximal antinociceptive effects. *p<0.01 compared to the control, **p<0.05 compared to 1 mT group

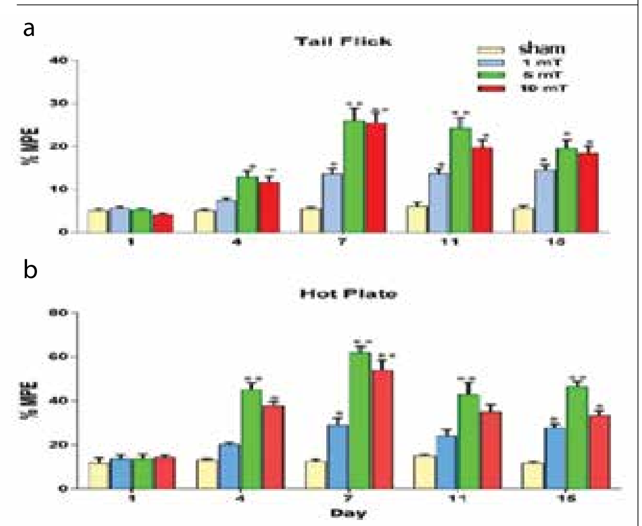
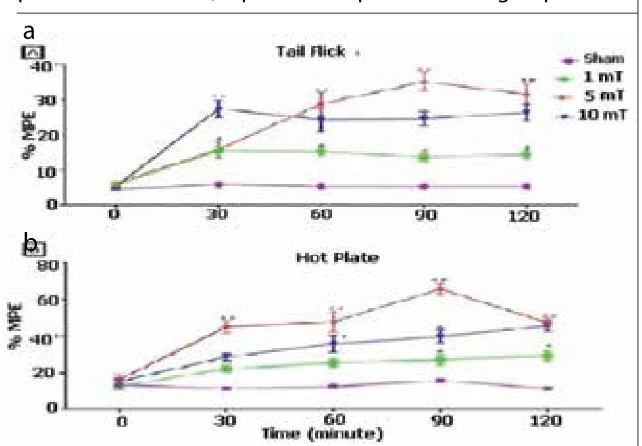


Figure 4. Time-dependent change of EMF analgesic effects. The effect of EMF in the TF (a) and HP tests (b). The maximal analgesic effect was determined in the 5 mT group and at 90 min measurement for three different MF strengths on the seventh day. Each point represents the mean±SEM of % MPE for six rats. HP, hot plate; TF, tail-flick; SEM, standard error mean; ELF-EMF, extremely low-frequency electromagnetic fields. *p<0.01 compared to the control, **p<0.05 compared to 1 mT group



individually placed on anHP (May AHP 0603 Analgesic Hot-plate Commat, Turkey) with the temperature adjusted to 54°C±3°C. The latency to the first sign of paw licking or jumping to avoid the heat is treated as an index of the pain threshold; the cut-off time to avoid damage to the paw was 30 s (12).

Data Analysis

To calculate the percent of maximal antinociceptive effects (% MPE), the TF and HP latencies (in seconds) were converted to percent antinociceptive effect using the following equation:

% MPE=[(postdrug latency–baseline latency)/(cut-off value-baseline latency)]×100.

Statistical Analysis

Group sizes were based on the following equation: N=2+C(S/d)2, where N is the group size, C is the constant obtained according to α and β, S is standard deviation, and d is effect size. The statistical power was assumed to be 1, and α was 0.05. All results are expressed as a mean ± standard error of the mean (SEM). The effect of antinociception was measured and the mean of % MPEs in all groups was computed. Data were analyzed using the analysis of variance followed by Tukey test. All statistical tests were performed using the SPSS (Statistical Package for the Social Sciences) software version 22.0 (IBM Corp.; Armonk, NY, USA). A significant difference was defined as p<0.05 in comparison to the sham group.

RESULTS

Analgesic Effects of Different Strengths of MF

Based on the TF and HP tests, we determined the strength and duration of MF that produced the most effective analgesia. The analgesic activity of MF 1 mT (TF: 13.66±127 and HP: 28.95±3.10), 5 mT (TF: 25.89±3.00 and HP: 61.73±2.95), and 10 mT (TF: 25.37±2.41 and HP: 53.85± 4.62) groups were significantly higher than control group rats ($F_{3,20}$=23.13, p<0.05 for TF and $F_{3,20}$=50.46, p<0.01 for HP; Figure 3). The maximal analgesic effect was determined at 5 mT group and 90-minute measurements (TF: 35.13 ± 2.63 and HP: 65.73 ± 2.92) for three different MF strengths ($F_{3,20}$= 555.51 for TF and $F_{3,20}$=766.03 for HP, p<0.001; Figure 4).

For the TF test, % MPE values were significantly high in all groups on day 7 ($F_{3,20}$=23.43, p<0.05) in comparison to the sham group. Tail -flick latencies decreased significantly on days 11 and 15 (p<0.05; Figure 3 a).

For the HP test, % MPE values increased significantly in all groups on day 7. Day in HP test ($F_{3,20}$=50.26, p<0.05). Figure 3 b represents that HP latencies decreased on days 11 and 15.

DISCUSSION

Numerous studies have shown that EMF reduces pain. The differences among these investigations are MF intensities and application periods. The inhibitory effects of EMFs on pain have been demonstrated in a variety of studies (13, 14). Consistent with these findings, 15-30 min acute exposures to EMFs block the elevated pain responses in snails (15). Our results suggest that four times 30-min acute exposures to EMFs enhance the analgesic activity measured using theTF and HP tests in rats.

This study investigated pulsed MFs of three strengths to identify a potential dose-response relationship based on theTF and HP tests. There were significant changes in pain processing activity when exposed to a 5mT MF.

The effect peaked on day 7 and then decreased to control levels. These results indicated that the magnetic antinociception reached a maximum on days 6-7. From day 11 onward, the TF and HP tests began to show a progressive decline until they were distinct from the responses of the sham group. According to Tiffany and Maude-Griffin, this may be because of opioid tolerance, which is a typical effect of repeated administrations of opiates (16). There is no data on agonist-antagonist opiate receptors. Similar findings were observed in selected brain regions in rats after chronic MF exposure (-100 mT, 50 Hz, 8 h per a day, for 8 months) (17). This raises the possibility that MFs have a direct effect on opioid receptor number, binding activity, or functional activity. In agreement with this, showed that four a 4-day magnetic exposure increased the levels of beta-endorphin and substance P in the hypothalamus of rats (18). It seems possible to increase the antinociceptive effect on opiates by increasing the effect of the MF on the seventh day.

No difference was found between the results of the TF and HP tests. Hence, the MF uses pathways between the sensory, central, and peripheral nervous system similar to that used for creating analgesia. Langerman et al. (19) in 1995 created a design to compare the TF and HP tests for (a) evaluating the tolerance of morphine and (b) assessing the influence of repeated testing on morphine antinociception. The TF and HP responses were dissimilar for morphine infusion. This may be attributed to the differential effects of morphine on spinal and supraspinal sites (19).

We have noted a widespread expectation that a greater MF strength will increase the analgesic effect. However, the analgesic effect in our study peaked at 5 mT and decreased at 10 mT (Figure 3). Hence, we propose the concept of an effective MF dose. Robertson et al. (20) showed significant correlations between different MF strengths and a change in the BOLD activity in the anterior cingulate and ipsilateral insula, indicating that there is either a dose-response or a threshold effect of EMFs. They used EMF strengths of 100, 200, and 1000 μT and found significant increases in the analgesic effect at 100 and 200 μT but a decrease at 1000 μT. Similarly, our study showed an increasing analgesic effect with MFs of 1 and 5 mT and a decrease at 10 mT, which may be due to habituation. However, further research is needed to evaluate the cause of decreasing analgesic effect at higher MF strengths.

Ethics Committee Approval: Ethics committee approval was received for this study from Institutional Review and Animal Use Committee of the Cumhuriyet University School of Medicine (Approval Date: 19.06.2014; Approval No: 65202830/125).

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Conflict of Interest: The authors have no conflicts of interest to declare.

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