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Effects of extremely low-frequency electromagnetic fields on morphine-induced conditioned place preferences in rats

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Abstract

In the present study, we examined the effects of extremely low-frequency (ELF) electromagnetic fields on morphine-induced conditioned place preferences in rats. During the conditioning phase (12 days), three groups of rats were placed in a sensory cue-defined environment paired with morphine (10 mg/kg, i.p.) following exposure to either 20 Hz (1.80 mT) or 50 Hz (2.20 mT) or sham electromagnetic fields for 60 min/day, respectively, and were placed in another sensory cue-defined environment paired with physiological saline (1 ml/kg, i.p.) without exposure to electromagnetic fields. After finishing 12 days of conditioning, preference tests for the morphine-paired place were performed during a 10-day withdrawal period. The exposure to electromagnetic fields substantially potentiated morphine-induced place preferences in rodents, suggesting that ELF electromagnetic fields can increase the propensity for morphine-induced conditioned behaviors.

Keywords: Electromagnetic field; Morphine; μ-Opioid receptors; CPP; Rats

In humans, evidence is accumulating that exposure to extremely low-frequency (0–300 Hz) magnetic fields affects cognitive functioning [8,10,15,20], but may pose possible health risks. Previous studies showed that pre-exposure to 60 Hz electromagnetic fields with an intensity of 2 mT increased significantly the lethal effects of cocaine [1]. In addition, there is now substantial evidence that endogenous opioid systems involved in the modulation of behavioral functions are activated by electromagnetic fields [12–14]. Other research has indicated that exposure to electromagnetic fields resulted in alterations in the numbers of μ-opioid receptors in the brain [16,24]. A vast amount of evidence suggests that the positive rewarding effects and dependence on morphine are mediated predominately via the action of morphine on μ-opioid receptors [11,18,21]. For example, administration of morphine during exposure to a sensory cue-defined environment results in preferences for the previously non-preferred side of a test box, i.e., conditioned place preference (CPP) [3]. The CPP paradigm has been proposed as an animal model for testing drug dependence, since it involves a drug-associated conditioned cue [2]. Based on these previous studies, we wanted to know whether repeated acute exposures to electromagnetic fields would affect morphine-induced conditioned place preferences in rats.

Male Sprague–Dawley rats, obtained from the Kunming Medical School, weighing 250–280 g at the beginning of the experiment, were used in this research. Rats were housed four per cage in a 12 h light–12 h dark normal cycle (light 07:00–19:00 h) with food and water available at all times. The room temperature was maintained at 22 ± 1 °C. All experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

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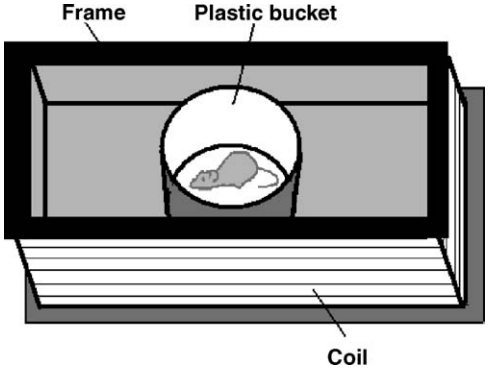

Fig. 1. Schematic diagram of electromagnetic field system and the exposure area. Note that the coils were wrapped horizontally around a plastic frame and the space inside the coils was the exposure area. During exposure, rats were placed in a plastic bucket.

The electromagnetic field was generated by a single coil of four layers, each having 250 turns. Each layer was wrapped horizontally above the previous layer around a 70 cm × 40 cm × 43 cm plastic frame. The coil was connected to a waveform generator for modulating the frequency and intensity of the electromagnetic field. By varying the input current to the coil, the flux density of electromagnetic fields in exposure area can be adjusted from the ambient level to the maximum coil-designed electromagnetic field strength of 14 mT.

The exposure area (60 cm × 30 cm × 43 cm) was inside the coil. During exposure, rats were placed in a non-metallic plastic bucket (diameter 25 cm, height 25 cm) which was mechanically isolated from the magnet and rested on a free-standing wood table to ensure that the subjects were exposed to the middle of the exposure area and thus to a constant electromagnetic flux. The variation of the electromagnetic fields in the plastic bucket as determined by actual measurement was ±4.5% of the mean. The electromagnetic field system and the exposure area are shown in Fig. 1.

The room in which the rats were exposed to the electromagnetic fields was different to that used for CPP testing or housing. Twenty-one rats (seven per group) were randomly divided into three experimental groups: Group 1 was for sham electromagnetic field exposure; Group 2 was exposed for 1 h/day for 12 days to a 20 Hz field at a flux density of 1.80 mT; and Group 3 was exposed for 1 h/day for 12 days to a 50 Hz electromagnetic field at a flux density of 2.20 mT. Sham-exposed rats were held in a similar but non-energized system. The experimental groups, the timing of magnetic fields manipulations and CPP procedure in experiments are illustrated in Table 1.

The three compartment CPP apparatus, based on the design of Carr and White [5], was made of wood with Plexiglas walls. Two of the compartments were identical in size (45 cm × 45 cm × 30 cm), but differed in shading (white or black) and floor texture (smooth or textured). The third 'choice' compartment (36 cm × 18 cm × 30 cm) was adjacent to the rear of the two big compartments, access to which was through guillotine doors. In this apparatus, rats show no consistent preference for either big compartment prior to conditioning [5]. There were three phases to the conditioning of place preference: the pre-conditioning (1–3 days), conditioning (4–15 days) and preference tests (CPP test 16–25 days). In the pre-conditioning phase, animals were placed in the center choice compartment with the guillotine doors removed to allow access to the entire apparatus. Three 15-min habituation sessions (1–3 days) were initially performed to reduce the stress associated with the novelty of experimental procedures and exposure to the apparatus. We used a counterbalanced design by compartment and conditioning sessions with morphine or saline treatment either in the morning or afternoon. During the subsequent conditioning phase (12 days), exposure or sham exposure to the electromagnetic field for 60 min/day was immediately followed by morphine (morphine hydrochloride, 10 mg/ml; 10 mg/kg, i.e., 1 ml/kg, i.p.) treatment and confinement to one compartment for 50 min. Thus, rats received physiological saline (1 ml/kg, i.p.) treatment without exposure or sham exposure to electromagnetic field on alternate conditioning sessions were confined to another compartment for 50 min. All to-be conditioned rats were injected with either saline or morphine hydrochloride in the morning and afternoon. The schedule (morphine, a.m.; saline, p.m.; or saline, a.m.; morphine, p.m.) of each rat was chosen at random before conditioning began, but once it was selected, it was fixed for the duration of the experiment. Morning and afternoon injections were at least 6 h apart. The order of the injection (morphine or saline, i.p.) and compartment (white or black) was counterbalanced across subjects.

Ten preference tests were performed as follows during 10-day withdrawal: rats were placed in the center choice compartment with doors open and were allowed free access for 15 min. The time spent in each compartment during a daily 900 s session was recorded. CPP was measured by the difference in time spent between the morphine-paired and saline-paired compartments. The amount of conditioned preference was measured in time spent in the morphine-paired place and are expressed as the means ± S.E.M.

Repeated-measure ANOVAs followed by Dunnett's post hoc tests was used to analyze data. The analyses treated

Table 1
The timing of magnetic field manipulations and CPP procedures

Group	*N*	Pre-conditioning	Expose to electromagnetic field	Conditioning	CPP-test (withdrawal)
Sham/morphine	7	15 min/day (1–3 days)	Sham 60 min/day (4–15 days)	Morphine + saline (4–15 days)	900 s/day (16–25 days)
20 Hz/morphine	7	15 min/day (1–3 days)	20 Hz 60 min/day (4–15 days)	Morphine + saline (4–15 days)	900 s/day (16–25 days)
50 Hz/morphine	7	15 min/day (1–3 days)	50 Hz 60 min/day (4–15 days)	Morphine + saline (4–15 days)	900 s/day (16–25 days)

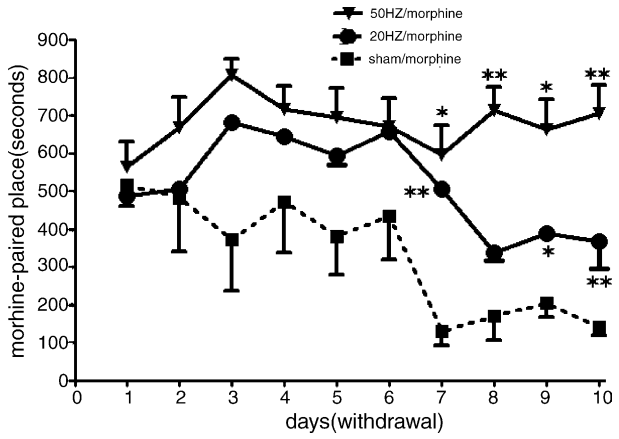

Fig. 2. Effects of exposure to electromagnetic fields on morphine-induced CPP. Exposure to electromagnetic fields groups potentiated preference for the morphine-paired compartment compared to sham group. ANOVA showed significant main effect of DAY ($F_{(9,\ 162)} = 5.70$; $p < 0.001$) and interaction of day by group ($F_{(18,162)} = 2.36$; $p = 0.002$). $^{**}p < 0.01$, $^{*}p < 0.05$ (see text for details of statistical analysis). Error bars are standard errors of the means.

DAY as a repeated measure and group as between-subjects factors. Statistical analysis was conducted using the SPSS 10.0 Statistical Package. Statistical significance was set at the probability level of $p < 0.05$.

Effects of exposure to electromagnetic fields on morphine-induced CPP in sham, 20 and 50 Hz during 10-day withdrawal are shown in Fig. 2. Regression analyses revealed that there was a decline in the amount of conditioned preference for all groups during the 10-day withdrawal period. Consistent with this, the overall ANOVAs for the electromagnetic fields groups and sham experiments revealed a significant main effect of DAY ($F_{(9,162)} = 5.70$; $p < 0.001$). The decline of CPP in the group experiencing the 50 Hz field was the slowest relative to other two groups, as judged by elevated daily mean values though Dunnett's test comparing the 50 and 20 Hz groups did not achieve significance ($p > 0.05$). However, both the 50 and 20 Hz groups showed potentiated CPP relative to the sham controls ($p < 0.05$). The 50 Hz electromagnetic fields significantly potentiated CPP compared with sham controls on day 7: $F_{(1,13)} = 9.49$; $p < 0.01$; day 8: $F_{(1,13)} = 11.05$; $p < 0.01$; day 9: $F_{(1,13)} = 7.67$; $p < 0.05$; and day 10: $F_{(1,13)} = 13.62$, $p < 0.001$, respectively. Similarly, 20 Hz electromagnetic fields potentiated CPP in the 20 Hz group as compared with controls on day 7 ($F_{(1,13)} = 19.9$; $p < 0.01$), day 9 ($F_{(1,13)} = 5.6$; $p < 0.05$) and day10 ($F_{(1,13)} = 10.0362$; $p < 0.01$).

Our study demonstrated for the first time that exposure to extremely low-frequency electromagnetic fields can potentiate and prolong morphine-induced CPP during withdrawal in rats, suggesting that exposure increases and prolongs the propensity for morphine-induced conditioned preferences.

Previous studies have proposed that exposure to electromagnetic fields increased the number of μ-opioid receptors and activated endogenous opioid systems in the brain [6,16,17,22–24]. Mu and delta-opiate receptors mediate positive reinforcement following morphine activation and the endogenous opioid system is also a major player in addiction [7]. Both pharmacological and genetic experimental manipulations of the opioid system demonstrate that endogenous opioids influence the reinforcing effects of many drugs of abuse [9]. A significant increase of opioid receptors generated by the electromagnetic field exposure augmenting morphine's rewarding effects could have potentiated morphine-induced CPP. Furthermore, some experimental studies have shown that 50 Hz electromagnetic field has no effect on the blood–brain barrier permeability in animals [19].

In conclusion, exposure to extremely low-frequency electromagnetic fields potentiates morphine-induced conditioned place preferences, adding to the literature on cognitive [8,10,15,20] and mood [4] changes arising from exposure to ELF electromagnetic fields. These results are consistent with previous evidence that exposure to electromagnetic fields result in alterations of the numbers of μ-opioid receptors and endogenous opioid system in the brain. However, it must be pointed out that the effect of electromagnetic field exposure on the density of opioid receptors and the location in the brain areas where they are activated needs to be further studied and characterized. This would allow us to gain insight into the possible mechanisms of interaction of electromagnetic fields and drug addiction.

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