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Thermal analgesic effects from weak, complex magnetic fields and pharmacological interactions

L.J. Martin, S.A. Koren, M.A. Persinger*

Behavioral Neuroscience Program, Behavioral Neuroscience Laboratory, Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

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Abstract

In several experiments, robust analgesia (equivalent to about 4 mg/kg of morphine) in male rats to thermal stimuli following exposures to weak (1 μT) complex magnetic fields was explored. The analgesia occurred when patterns of magnetic fields with burst-firing-like configurations were presented for 30 min once every approximately 4 s. The analgesic effects were intensity dependent. A different frequency-modulated pattern produced analgesia more quickly. The analgesic effects following exposure to the burst-firing magnetic fields were augmented conspicuously by preinjections of morphine (4 mg/kg) or agmatine (10 mg/kg), but blocked by naloxone (1 mg/kg). The results of these experiments suggest that rational design of the temporal structure of weak magnetic fields may be a novel, inexpensive, and reliable technique for elevating thresholds to some classes of painful stimuli.

Keywords: Complex magnetic fields; Analgesia; Temporal patterns; Agmatine; Morphine; Rat; Hotplate

1. Introduction

The development of analgesia by the administration of specific chemical compounds has been one of the most important contributions of pharmacological research to the reduction of pain and suffering. Pharmacological approaches to the alleviation of pain, often inferred by the elevation in response latencies to nociceptive stimuli, have pursued the molecular compatibilities of the ligand and the receptor. The implicit assumption of this approach is that the spatial arrangement of the potentially analgesic molecule ultimately determines its functional consequence.

Potential responsiveness to magnetism is a property that all materials possess as a result of the motion of their electrons. During the last decade, we have been testing the validity of the assumption that the specificity of intervention afforded by a pharmacological agent's spatial (molecular) structure can be duplicated by the appropriate complexity of a magnetic field's temporal structure. The temporal pattern for magnetic fields, like the complexity of molecular structure for chemicals or the complexity of sonic patterns for the specificity of "voice prints," is considered more important than the monotonic dimensions of intensity, concentration, or "loudness" to produce specific effects.

Most researchers who have studied the effects of magnetic fields upon biological systems have used temporally simple patterns, such as sine waves or square waves. We consider the use of these stimuli as equivalent to injecting the simply structured water molecule rather than a more complex molecule to produce specific effects. Both water and magnetic waves, whose peak-to-peak durations are similar and whose rise times and fall times reflect geometric simplicity, can be effective, often in a diffuse or nonspecific manner, when very large amounts or intensities are employed.

We have found that rational design of complex patterns of magnetic fields that imitate natural processes can duplicate their effects. McKay et al. (2000) exposed rats for 30 min to waveforms that had been modelled after salient electrophysiological patterns typically generated within the hippocampus or amygdala or to 7- or 20-Hz sine waves. Although the four types of magnetic fields were intensity matched (0.5–1 μT), the rats exposed to the magnetic fields imitating electrophysiological patterns displayed a marked

* Corresponding author. Tel.: +1-705-675-4824; fax: +1-705-671-3844.
E-mail address: mpersinger@laurentian.ca (M.A. Persinger).

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attenuation of contextual freezing behavior. Rats that had been exposed to the sine-wave fields did not differ significantly from those exposed to the sham fields.

In 1994, Fleming, Koren, and Persinger reported that whole-body exposure of rats once every 4 s for 20 min to a burst-firing magnetic field with intensities around 1 μT displayed elevated nociceptive thresholds to electric current delivered to the rats' footpads. The analgesic effect was still apparent 20 min after the removal of the magnetic field and was equivalent to the analgesia produced by 4 mg/kg of morphine. An elevation of nociceptive thresholds to thermal stimuli was apparent after 30 min of exposure to this pattern of magnetic stimulation (Ryczko and Persinger, 2002). Preadministration of naloxone attenuated the analgesic response.

However, other patterns presented at similar intensities did not evoke analgesia (Dixon and Persinger, 2001) by themselves, but eliminated the analgesic effects of either L-NAME (a nitric oxide synthase inhibitor) or morphine when each was injected separately. When both drugs were administered simultaneously, this specific magnetic field pattern did not reduce the analgesia. In the present series of experiments, we show how exposure to complex magnetic fields with intensities in the order of 1 μT (10 mG) can reliably and robustly elevate the response latency of rats to thermal stimuli. In every experiment, these treatments accommodated between 40% and 80% of the variance of our quantitative inference of analgesia.

2. Method

2.1. Subjects

Naive male ($N = 222$) albino Wistar rats, between 4 and 14 months old, were employed as subjects. They had been obtained from Charles River Breeders (Quebec) and had been habituated to a 12:12 L/D cycle (onset between 0730 and 0800 h) within temperature-controlled rooms (20–22 °C) for at least 1 month before the initiation of the experiments.

2.2. General procedure

All procedures had been reviewed by the university's Animal Care Committee. All rats were treated according to the guidelines of the Canadian Council on Animal Care. Rats were monitored daily by experienced animal care technicians. Purina rat chow and water were available ad libitum. The rats were maintained, usually three per cage, in standard wire cages (40 × 24 × 18 cm high) in racks or plastic cages (42 × 23 × 20 cm high) containing one-quarter inch corn cob or spruce wood shavings.

All experiments involved essentially the same procedure. Nociceptive thresholds were tested by removing each rat from its home cage and placing the rat on an Omnitech thermal plate that was maintained at 55 °C. The plate was enclosed within a plastic box (18 cm high) so that the rat could not escape. When the rat was placed on the surface (26 × 26 cm) of the plate, a foot pedal was depressed to initiate the electronic timing. Once the rat was observed to lick one of the hind feet twice in any combination, the rat was removed immediately from the apparatus. The elapsed time was automatically recorded. If the rat had not displayed the criterion response by 60 s, it was removed.

This first placement on the hotplate was called Trial 1 or the baseline trial. Immediately after the baseline trial, the rat was placed within the experimental apparatus that generated the magnetic or sham fields (see Section 2.3). All exposures were 30 min in duration. The rats were tested immediately for thermal response times on the same apparatus (Trial 2). The rats were then returned to their home cages for an additional 30 min. After the interval (60 min since the onset of a treatment and 30 min after the cessation of the treatment), the rats' response latencies were measured for the third time (Trial 3). This procedure was repeated over two successive days.

For all experiments, the 30-min exposures to the magnetic fields or sham-field conditions occurred within a plastic cage (25 × 25 × 25 cm deep). A pair of solenoids generated the magnetic fields through the cage (Fleming et al., 1994). In Experiment 3, a Helmholtz coil was also used. The rat was placed within a 41 × 30 × 31 cm plastic cage that fits exactly within the coil (see Section 2.3 for details). If the rats were exposed to the sham field, then all equipment was operating except the cable connecting the solenoids or the coil to the computer generating the fields was not connected or the program was not initiated.

All experiments involved between four and six rats per group. This small number of subjects per group was possible because the effect size (η^2 or partial ω^2 estimate), or the explained variance, ranged robustly between about 40% and 80%. Consequently, the number of rats per experiment was minimized according to the guidelines for the Canadian Council on Animal Care.

2.2.1. Experiment 1: Electrical vs. thermal analgesia

Fleming et al. (1994) had found that 20 min of exposure to the burst-firing magnetic field (Fig. 1a) once every 4 s produced elevated thresholds for the response to electrical stimulation of the footpads. The change was equivalent to the consequences of a subcutaneous injection of 4 mg/kg of morphine. To discern if the analgesic effect of this field upon thresholds for thermal nociception was similar, rats were exposed to one of six different conditions before analgesic thresholds for either the thermal stimuli or electrical current were tested.

Following Trial 1 (baseline), rats were randomly assigned to the following groups: (1) sham condition, (2) 5 min of field followed by 25 min of sham field, (3) 25 min of sham field followed by 5 min of magnetic field, (4) 15 min of magnetic field followed by 15 min of sham field, (5) 15 min of sham

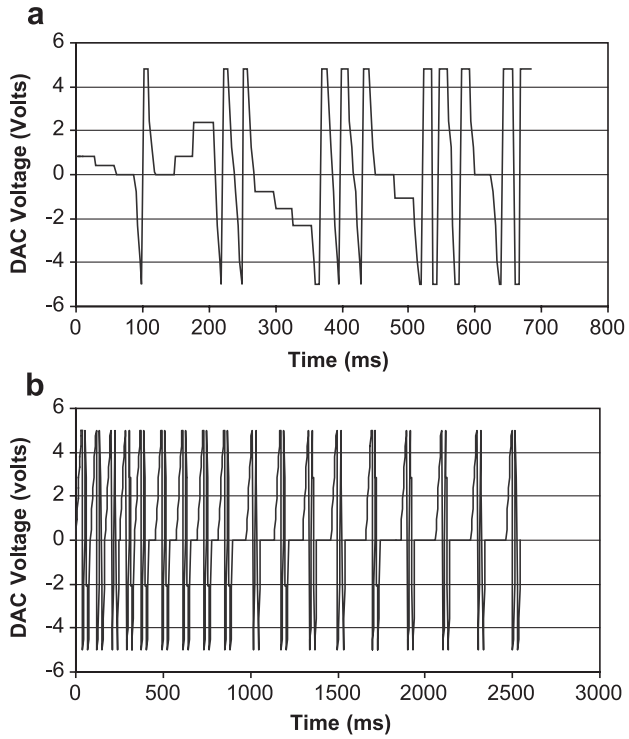

Fig. 1. Temporal structure of the two patterns of magnetic fields employed in this study. The vertical axis refers to the voltage values converted from numbers between 0 and 255 (127 = 0 V). The horizontal axis reflects the temporal organization of the pattern created by the series of numbers. The duration of each number or point was 3 ms. (a) Burst-firing pattern, (b) frequency-modulated pattern.

field followed by 15 min of magnetic field, or (6) 30 min of magnetic field. They were then tested for either (1) thermal analgesia on the hotplate or (2) electrical analgesia, immediately after the end of this period (Trial 2) and again 30 min later (Trial 3) after they had been replaced in their home cages for 30 min.

Threshold for electrical stimulation to the footpad involved the same equipment as employed by Fleming et al. (1994). The rats were placed in a plastic chamber that contained a metal grid floor. The intensity of the electric current delivered by an A-615-C Master Shocker (Lafayette Instrument, Lafayette, IN) through the grid was slowly increased from 0 until the rat displayed a clear flinch. This movement was qualitatively distinctive. The intensity (mA) was then determined and the rat was immediately removed.

2.2.2. Experiment 2: Intensity dependence of analgesia

Dose dependence is considered one of the most powerful indicators of a systematic relationship between the amount of a substance and its consequences. In this experiment, rats were exposed for 30 min to the burst-firing magnetic field presented once every 4 s. In addition to the sham-field group, there were three treatments. Rats were exposed to maximum intensities of 3 μT (the usual), 0.3 μT, or about 30 nT. The minimal values were about one-tenth of the maximum value.

2.2.3. Experiment 3: Spatial parameters of field

The geometry of the application of any force often determines its consequences. We had employed the spatially heterogeneous field for analgesia because the more spatially focal application was similar to the application geometry of the device by which we applied these fields across the brains of patients (Baker-Price and Persinger, 1996, 2003). In the present experiment, rats were exposed to the usual field parameters [30 min of burst firing with 4 s interstimulus interval (ISI), 2.5 μT (25 mG), heterogeneous field] or to the same field condition within a Helmholtz coil. The burst-firing field was applied with average intensities of either 0.5 μT (5 mG), 1 μT (10 mG), or 2.5 μT (25 mG).

These strengths were relatively homogeneous throughout the exposure area for the rat. We selected these increments because they overlapped with the major portions of the exposure intensities within the heterogenous (solenoid) fields. The results of these homogeneous intensities might allow us to begin to discern if the magnitude of the change time-varying component of the field or the spatial gradient within which the rat was exposed was a primary factor in the production of the analgesia.

2.2.4. Experiment 4: Comparison of two complex patterns

Thomas et al. (1998) reported that a frequency-modulated pattern induced analgesia in snails. This pattern, also developed in our laboratory and previously described as the Thomas pulse, is composed of 849 points (again, each value ranged between 0 and 255, i.e., 256 increments). When the points in this pattern were presented for 3 ms and the ISI was 3 ms (effectively a continuous presentation) for 30 min to rats, significant analgesia was displayed. In the present study, we directly compared the efficacy of this frequency-modulated pattern with our typical burst-firing pattern (ISI = 4 s) whose point durations were also 3 ms.

Within a few minutes after the third testing on the hotplate on the two successive days, each rat's ambulatory activity was measured in an open field (Persinger, 1969). The number of squares traversed, number of fecal boluses, urine spots, and episodes of grooming and rearing were measured during a single 2-min period. The purpose of these measurements was to discern if the increased response latencies to thermal stimuli following exposure to the magnetic fields, that we were inferring to be analgesic, might have been confounded by a nonspecific lethargy or motor incapacity.

2.2.5. Experiment 5: Verifying the specificity of Experiment 4

The results of the previous experiment suggested that the analgesic response occurred more quickly when the rats were exposed to the frequency-modulated field relative to the burst-firing pattern. However, the former was presented continuously while the latter was presented once every 4 s. The possibility existed that the quicker response to the frequency-modulated field was an artifact of the ISI. To test this hypothesis, rats were exposed to either the

burst-firing or the frequency-modulated field that was presented either continuously or once every 4 s (four treatment groups plus sham group). All point durations were 3 ms.

2.2.6. Experiment 6: Interactions between two types of magnetic fields and morphine or agmatine

Our working hypothesis has been that the appropriate temporal patterns of magnetic fields can simulate the effects of the spatial structure of chemicals. In the present study, morphine, a well-known opiate agonist, and agmatine were selected to potentially interact with the effects of the frequency-modulated and burst-firing fields. We reasoned that because the two patterns of magnetic fields resulted in different latencies of analgesia, these two patterns might interact differently with known analgesics. Morphine was selected as a classical opiate analgesic. If the burst-firing magnetic field was mediating its effects through the opiate systems, then a summative effect would occur. We (Fleming et al., 1994) had shown that exposure to the burst-firing field produced analgesia that was comparable to 4 mg/kg of morphine.

Agmatine was selected because it has been shown to enhance morphine analgesia and prevent tolerance (Kolesnikov et al., 1996; Yesilyurt and Uzbay, 2001) involved with mu-opiod receptors and D-Pen2, D-Pen5 enkephalin, mediated by sigma-opiate receptors, in a dose-dependent manner. Like many imidazoline drugs, this product of the decarboxylation of L-arginine is sequestered with moderate affinity to all subclasses of alpha-2 receptors. We assumed that if our magnetic fields were influencing opiate receptors, then coadministration of agmatine and these magnetic fields might enhance analgesia. Colocalization of the alpha-2 noradrenergic receptor and the mu-opiate receptor has been reported by Van Bockstaele and Commons (2001).

Rats were assigned to one of nine different groups. Immediately after the baseline nociceptive threshold was obtained, rats were subcutaneously injected with either morphine sulphate (4 mg/kg), agmatine (10 mg/kg), or physiological saline. They were then randomly assigned to one of three treatments: burst-firing magnetic field, frequency-modulated magnetic field, or sham-field conditions. The rats were exposed to the magnetic field or sham-field conditions for 30 min before the first and second (30 min later) thresholds were measured.

2.2.7. Experiment 7: Naloxone blocking of morphine and burst-firing magnetic field analgesia

Rats were injected with either isotonic saline (1 cc/kg) or morphine (4 mg/kg) and then immediately after with either saline (1 cc/kg) or naloxone (1 mg/kg), immediately after baseline measurements from the hotplate had been recorded. The rats were then exposed to the burst-firing magnetic field or the sham-field condition for 30 min before the usual threshold measurements were obtained. The treatments were as follows: saline + saline + sham field, saline + saline + magnetic field, morphine + naloxone + sham field, saline + naloxone + magnetic field, and saline + naloxone + sham field.

2.2.8. Statistical analyses

Except for Experiment 1 where percentage changes from baseline were employed to allow comparisons of nociception to thermal and electrical stimuli, all dependent measures involved the *net* differences in seconds in response latency between Trial 2 and the baseline and between Trial 3 and the baseline. We measured (1) the actual response time (in seconds) to display the criterion response for each of the three trials [baseline or Trial 1, Trial 2 (30 min later), or Trial 3 (60 min later)], (2) the net (subtracted) difference between the latency to respond on Trial 2 and Trial 3 compared to the baseline, and (3) the relative difference in latencies to respond, defined as the values for (Trial 2 − Trial 1)/Trial 1 and (Trial 3 − Trial 1)/Trial 1. In general, there have been no substantial differences between groups of rats for baseline latencies. The net differences (in seconds) between Trial 2 and Trial 1 and between Trial 3 and Trial 1 have shown the most systematic and comparable values between and within experiments.

Because the basic procedures were similar, all analyses involved at least a three-way analysis of variance with two (within subject) repeated measures (trials, days) and one between level (treatment, i.e., sham-field vs. magnetic-field variations). Post hoc tests for main effects involved Tukey's set at $P < .05$. Post hoc tests for mixed interactions between within-subject and between-subject measures involved the appropriate combinations of paired t tests and Tukey's, both set at $P < .05$. All analysis involved SPSS software on a Vax 4000 computer.

2.3. Apparatus

2.3.1. Exposure systems

The primary exposure system, the $25 \times 25 \times 25$ cm plastic chamber within which the subjects were exposed to the field, has been described elsewhere (Fleming et al., 1994). Essentially, two large nails (25 cm long, 1 cm diameter, about 140 g) wrapped with 1050 turns of 20-gauge insulated wire, were apposed to the opposite sides of the plastic exposure container along the horizontal plane. The linear axis of the nails was approximately 4.5 cm above the surface of the plastic floor that was covered by one-quarter inch corn cob bedding. The plane of the two poles of the solenoids was approximately the level of the rat's head. The peak strength 2 cm from either pole within the bisector between the two poles was 2.5 μT. At radii of 4, 6, 8, or 10 cm from each pole, the strengths were 1.7, 1.0, 0.5, and 0.3 μT, respectively.

The second apparatus, employed only in Experiment 3, was a $41 \times 30.5 \times 31$ cm Helmholtz coil. It was wrapped with AWG 20 wire so that the resistance was 30 Ω. The subjects were maintained within the area by a plastic

container whose dimensions were 21 cm (length) × 28 cm (width) × 27.5 cm (height). The floor of this chamber also contained one-quarter inch corncob for bedding. The maximum range of the strength of the magnetic fields for every 2 cm within the exposure volume ranged between 2.3 and 3.0 μT. The measurements were completed with a Metex N380 meter coupled to a magnetic sensor (140-3-60-1499, Electric Field Measurements, Rt. 183 W. Stockbridge, MA 01266) that reflected the root mean square of the intensity. The net intensities were verified independently with a MEDA FM-300 magnetometer.

To visualize these values, the intensities for each of the 2-cm positions within the volumes for each field geometry (solenoids vs. Helmholtz coil) were plotted for increments of 10 mG for each plane of the vertical axis (Wells, 1994). Fig. 2 shows the mosaic and classical contours for the solenoids (Fig. 2a and c) and the Helmholtz coil (Fig. 2b and d). The mosaics (a and b) showed the relative homogeneity of the strength of the magnetic field within the volume of exposure for the Helmholtz coil (b) and the marked spatial gradient between the two solenoids (a). Our studies have focused upon the solenoids (spatially heterogenous fields) because they have been more consistently associated with analgesic effects (Martin and Persinger, 2003).

2.3.2. Magnetic field generating equipment

The patterns of the magnetic field were generated by first creating a file that contained a column of numbers. Each number ranged between 0 and 255 such that any value below 127 was negative polarity and any value above 127 was positive polarity. When the sequential order of the numbers was displayed along the horizontal axis and the values between 0 and 255 were displayed along the vertical axis, the shape or pattern was clearly visualized. The two primary patterns employed in the present study, the burst-firing and frequency-modulated patterns, are shown in Fig. 1.

The software, named Complex (© Koren, 1993–2001), transformed each of the numbers within a file to voltages ranging between +5 V (the number 255) and −5 V (the number 0). The number 127 was 0 V. The duration (ms) that the specific voltage was generated was the point duration. In the present study, the point durations were always 3 ms. This means that the voltage associated with a given number between 0 and 255 was constant for 3 ms before the next number was accessed and the next voltage was generated.

The latency (rise time) between successive 3-ms points was determined by direct measurement to be 200 μs for the coil and 110 μs for the solenoids. Hence, although the

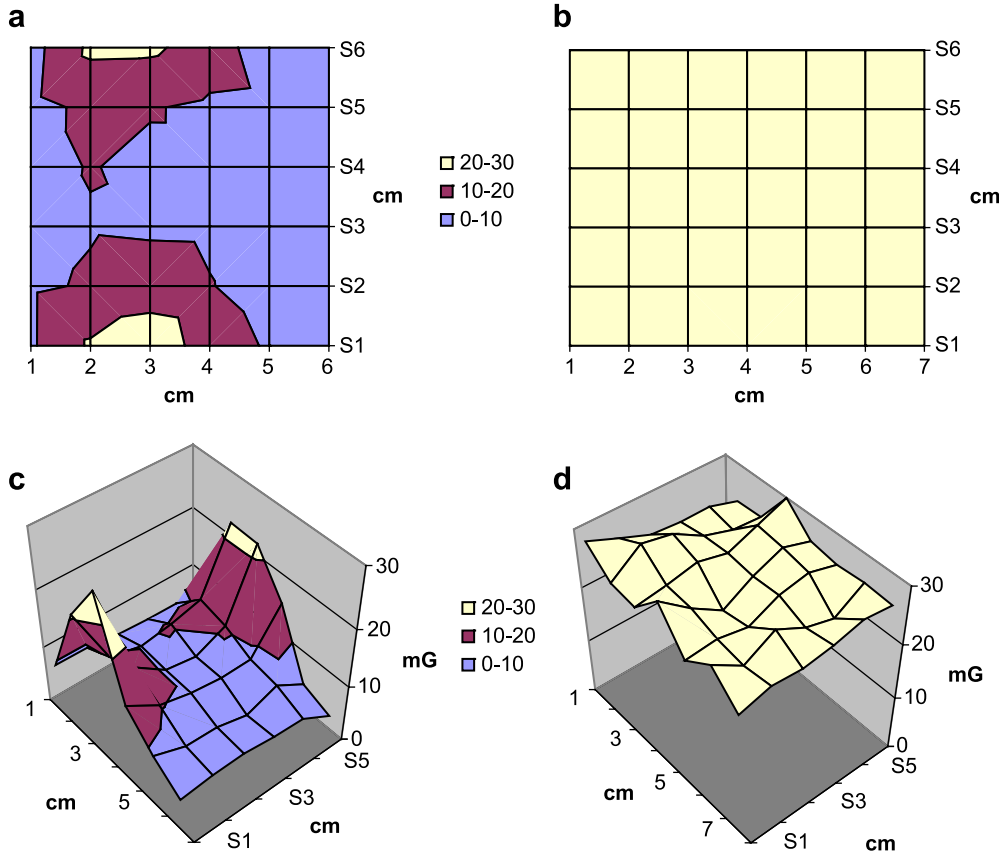

Fig. 2. Spatial distributions of increments of intensities of the magnetic fields (in mG) within the field of the solenoids (a,c) and the Helmholtz coil (b,d) at a vertical distance of 4 cm above the surface upon which the rat was placed. Shades of grey indicate ranges of intensity: white = 20–30 mG (2–3 μT), dark grey = 10–20 mG (1–2 μT), light grey = 0–10 mG (0–1 μT). In the online version, different colours indicate ranges of intensities: yellow = 20–30 mG (2–3 μT), red = 10–20 mG (1–2 μT), blue = 0–10 mG (0–1 μT).

duration each value between 0 and 255 remained at that voltage was 3 ms, the time required to achieve this value was only 200 μs for the coil and 110 μs for the solenoids. The empirical values matched the theoretical values from the specifications for the computer chips.

The numbers were transformed to the specific voltage by a custom-made (by the second author) digital-to-analogue converters (DACs). The converter was accessed through a parallel port (output) from the computer; the typical port latency was about 100 μs. Unless otherwise specified, all of the different computers that generated the values producing the magnetic fields in the present studies were IBM XTs. The fidelity of the patterns generated through DAC systems have been verified by placing small reed switches within the exposure area. The solenoids then served as sensors (instead of the typical electrodes) to a Beckman electroencephalograph. Direct strip chart recording of the induced pattern within the solenoids were recorded. The correlation (r) between the successive changes in the height of the signature recorded by the strip chart and the values within the DAC (number) files from which the pattern was generated ranged between .90 and .98.

3. Results

To minimize redundancy, only the results of the multilevel analysis of variance that demonstrated the differences in the response latencies (our inference of analgesia) between the magnetic field treatments and the sham-field treatments or between the drugs and the treatments are presented. In general, there were always statistically significant differences between the 2 days of treatment because of the reduced response latency for all groups on the second day. However, the main effects (both days combined) for magnetic field treatments were also statistically significant and accommodated between 30% and 60% of the variance in the response latencies. Unless there were significant interactions between trials and/or days and the treatments, these values are not presented.

3.1. Experiment 1

The means and S.E.M. for the *percentage* increase in analgesia from the baseline for rats tested on the hotplate (thermal nociception) or the electrical grid (electric current nociception) as a function of the duration of exposure to the sham field or burst-firing magnetic field are shown in Fig. 3. The most powerful result of the four-way analysis of variance with two between-subject factors (type of nociception and durations of exposure to sham/magnetic field) and two within-subject factors (trials per day and days) was the statistically significant [$F(1,48) = 13.44$, $P < .001$; $\eta^2 = 25\%$] interaction between the response to the type of nociceptive stimuli (thermal vs. electrical) and the trials.

Post hoc analysis showed that the major source of this interaction was due to the greater percentage increase in latency to respond 60 min after the beginning of the treatment for the rats tested for thermal nociception after 30 min of exposure to the burst-firing magnetic field. For comparison with later experiments, the M and S.E.M. (in parentheses) for the net changes in response latencies for the rats placed on the hotplate after 30 min of exposure were 5 (1.5) s; 30 min later, these values were 9.2 (2.1) s.

3.2. Experiment 2

The means and S.E.M. for the latencies to respond immediately after 30 min of exposure or 30 min later to the various intensity fields are shown in Fig. 4. The results showed a significant difference between groups exposed to the different intensities [$F(3,20) = 6.28$, $P < .01$; $\eta^2 = 49\%$]. Post hoc analysis indicated that the groups exposed to our usual intensities (high) displayed significantly more analge-

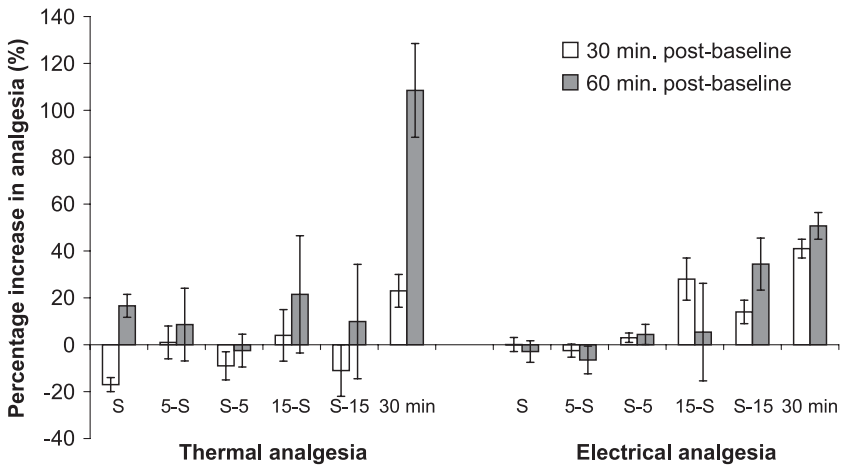

Fig. 3. Means and S.E.M. for the percentage increase in response latency to thermal and electrical stimuli to the footpads as a function of various temporal combinations of sham field and the burst-firing field ($n = 4–6$/group). Values whose S.E.M.'s do not overlap are significantly different ($P < .05$).

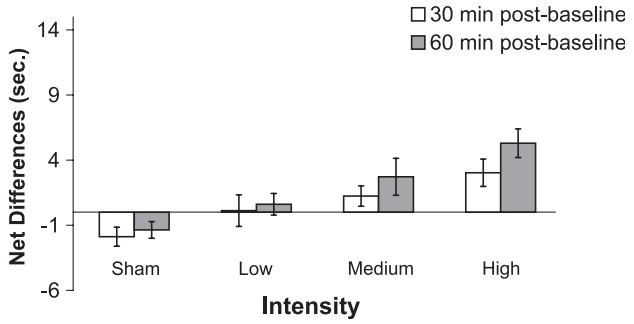

Fig. 4. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli relative to baseline for rats exposed to the burst-firing magnetic field once every 4 s at various intensities within the solenoids ($n=4-6$/group). Values whose S.E.M.'s do not overlap are significantly different ($P<.05$).

sia than those exposed to the low-intensity or sham-field conditions.

3.3. Experiment 3

The means and S.E.M. for the response latencies for rats exposed to the sham field or to the burst-firing field or within the Helmholtz coil with intensities of either 0.5, 1, or 2 μT, or to the two solenoid (indicated as "nails") fields or its sham conditions are shown in Fig. 5. The results showed statistically significant group differences [$F(5,18)=3.43$, $P<.05$; $\eta^2=49\%$].

Post hoc analyses indicated that the source of this difference was primarily due to the increased analgesia for the rats exposed to the magnetic fields compared to rats exposed to the sham-field condition for the solenoids. The groups exposed to each of the more homogeneous strength fields of 0.5-, 1.0-, and 2.5-μT fields within the Helmholtz coil and the group exposed to the more heterogenous range of 0.5- to 2.5-μT fields generated by the solenoids exhibited significantly stronger analgesia 30 min after removal of the field (60 min postbaseline) relative to the sham fields for either the solenoids or the Helmholtz coil.

3.4. Experiment 4

The means and S.E.M. for the latencies to respond for the groups exposed to the sham field, the frequency-modulated field, or the burst-firing field are shown in Fig. 6. Two-way analysis of variance again demonstrated a statistically significant difference between groups [$F(2,18)=22.77$, $P<.001$; $\eta^2=72\%$]. The groups exposed to the patterns of magnetic fields displayed greater analgesia compared to the sham-field exposed group. The interaction between the trials (30 vs. 60 min after initiation of treatment) and treatment was also statistically significant [$F(2,18)=3.92$, $P<.05$; partial $\eta^2=30\%$].

Post hoc analysis indicated that the primary source of the interaction was due to the greater latencies displayed by the group exposed to the frequency-modulated pattern within 30 min after the beginning of the exposure compared to the group exposed to the burst-firing field. However, the response latencies did not differ significantly between the two types of fields when more than 60 min had elapsed since the beginning of the exposures.

Two-way analysis of variance with one level repeated (days) and one between-subject level (sham field, burst-firing field, and frequency-modulated field) showed no statistically significant main effects or interactions between treatment and days for the numbers of squares traversed in the open field. There were also no significant differences for numbers of rearings, grooming, or episodes of urination.

A significant interaction was observed between treatment and days for numbers of fecal boluses in the open field. Post hoc analysis indicated that the rats exposed to the two fields defecated less during the first day only compared to the sham-field group. The group difference was not significant statistically for the second day. The means (S.E.M.) for the

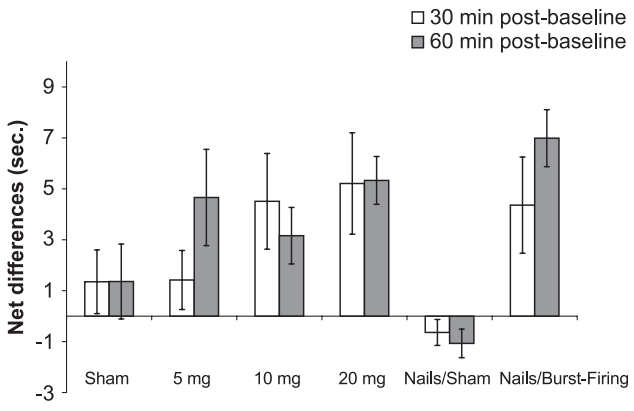

Fig. 5. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli relative to baseline for rats exposed to the burst-firing field once every 4 s at various intensities within the Helmholtz coil. Comparisons to rats exposed to the solenoids ("nails") are also shown ($n=4-6$/group). Values whose S.E.M.'s do not overlap are significantly different ($P<.05$).

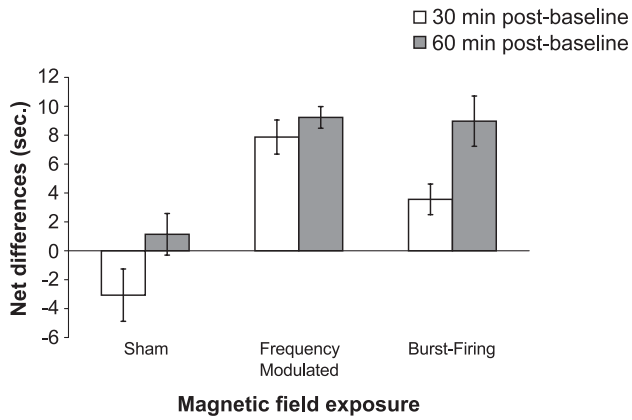

Fig. 6. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli relative to baseline for rats exposed to the frequency-modulated ("Thomas") pattern or burst-firing pattern ($n=4-6$/group). Values whose S.E.M.'s do not overlap are significantly different ($P<.05$).

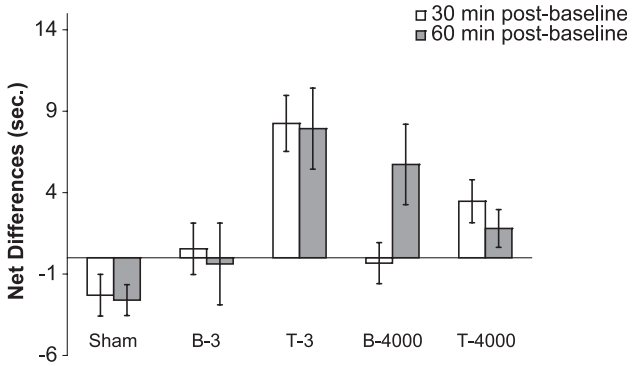

Fig. 7. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli after a 30-min exposure to either a sham field or to either the frequency-modulated (T) or burst-firing field (B) whose ISIs were either 3 or 4000 ms ($n = 4-6$/group). Values whose S.E.M.'s do not overlap are significantly different ($P < .05$).

three groups for numbers of fecal boluses on the first day were as follows: sham field 0.9 (0.4); frequency-modulated field 0.2 (0.2); and burst-firing field 0.3 (0.3). Kruskal–Wallis (nonparametric) analysis verified the group differences for the first day [$\chi^2 = 7.53$, $P = .02$].

3.5. Experiment 5

The means and S.E.M. for the net differences in responding compared to baseline for rats exposed either to the sham field, or to the burst-firing or to the frequency-modulated field presented either continuously or once every 4 s are shown in Fig. 7 for the first day (although the effect was evident but attenuated on the second day, as usual). There was a significant difference between the groups [$F(4,19) = 6.34$, $P < .01$; $\eta^2 = 57\%$]. Post hoc analysis showed that the primary sources of the group differences were due to the elevated analgesia for the group exposed continuously to the frequency-modulated pattern or to the burst-firing pattern once every 4 s compared to the groups exposed to the sham field and to the burst-firing field presented continuously.

The only statistically significant interaction [$F(4,19) = 2.96$, $P < .05$; $\eta^2 = 38\%$] between treatment, trials, and days was due primarily to the significant increase in latency to respond after 60 min had elapsed since the beginning of the treatment with the burst-firing field presented every 4 s compared to the frequency-modulated field presented continuously on the first day of treatment.

To evaluate the strength of the potential interaction between shape of the field (burst-firing vs. frequency-modulated) and their temporal pattern of presentation (every 3 ms or once every 4000 ms), a four-way analysis of variance (the two within-subject levels: trials and days; the two between-subject levels: ISI and field shape) was completed. The statistically significant interaction [$F(1,19) = 9.28$, $P < .01$; $\eta^2 = 32\%$] between the shape of the field and the ISI was due in large part to the greater analgesic effect of the frequency-modulated field presented continuously (3 ms) compared to the burst-firing field presented continuously while the reverse was evident when both fields were presented every 4000 s. The four-way interaction between pattern of field, ISI, trial, and day [$F(1,19) = 9.01$, $P = .01$; $\eta^2 = 32\%$] was due in large part to the elevated response latencies 60 min after the beginning of the exposure for the rats that had been exposed to the burst-firing field once every 4 s on the second day compared to all other groups (figure not shown).

3.6. Experiment 6

The means and S.E.M. for the differences in the latencies to respond for the rats given saline, agmatine, or morphine and then exposed to either the sham field, burst-firing field, or frequency-modulated field are shown in Table 1. A five-way analysis of variance with two levels repeated (two trials per day, 2 days) and three between levels (type of drug: saline, morphine, agmatine; type of field: sham, frequency-modulated, or burst-firing; block of experiment) showed a statistically significant difference in analgesia for the rats exposed to the magnetic fields [$F(2,18) = 5.81$, $P < .01$; $\eta^2 = 39\%$] and to the different drugs [$F(2,18) = 6.52$, $P < .01$; $\eta^2 = 42\%$].

There were no statistically significant differences between the five blocks [$F(4,18) = 1.83$, $P > .05$]. No statistically significant interactions occurred between the levels of the main effects. A statistically significant interaction between trials and magnetic field treatment [$F(2,18) = 3.36$, $P = .05$; $\eta^2 = 27\%$] was apparent although the main repeated measure between trials was not [$F(1,18) = 2.96$, $P > .05$]. There were also no significant differences between thresholds for the 2 days.

Post hoc analyses indicated that the rats exposed to either type of magnetic field displayed significantly longer latencies to respond than did the sham-field groups on 30 and 60 min later. Thirty minutes later, rats that had been administered with morphine exhibited greater analgesia than those administered with saline. Sixty minutes later, the rats that

Table 1
Means and standard errors of the mean for the net differences (in seconds) for latencies to respond relative to baseline for rats receiving either saline, agmatine, or morphine and exposed either to the sham field, frequency-modulated field (FM), or burst-firing field

Condition	Saline		Agmatine		Morphine	
	M	S.E.M.	*M*	S.E.M.	*M*	S.E.M.
Sham field						
+30 min	−2.0[a]	2.2	0.8	2.2	4.9[b]	0.7
+60 min	−2.1[a]	1.2	0.2	1.9	6.4[b]	0.8
FM magnetic field						
+30 min	6.5[a]	1.5	9.6	2.5	12.0[b]	2.6
+60 min	8.8[a]	1.6	10.5	1.8	12.6[b]	1.3
Burst magnetic field						
+30 min	2.8[a]	0.4	6.5[b]	0.7	12.2[c]	1.6
+60 min	6.6[a]	0.4	10.8[b]	1.1	13.4[c]	1.5

Superscripts refer to comparisons of values in rows: a vs. b, $P < .05$; b vs. c, $P < .05$; and a vs. c, $P < .01$.

were administered morphine exhibited greater analgesia than the groups that received either the saline or agmatine, which did not differ from each other.

Agmatine significantly increased the analgesic effects of the rats exposed to the frequency-modulated pattern after 30 min (first trial) compared to the rats that had received saline or agmatine and were then exposed to the sham field. Agmatine also potentiated the effects of the burst-firing field 30 and 60 min after the initiation of treatment compared to saline- or agmatine-sham-exposed rats.

The administration of this dosage of morphine also augmented the analgesic response to both magnetic fields. However, this augmentation was more evident for the burst-firing magnetic field 30 and 60 min after the initiation of the treatment. The most conspicuous effect was the acceleration of the intensity of the analgesic response during the exposure to burst-firing field such that it was similar to the effects of the frequency-modulated magnetic field. There were no significant differences between days for any of the levels except for an increased latency during the baseline on the second day for the rats that had received agmatine [$t(14) = 2.48$, $P < .05$]. There were no significant differences between the baseline measures for the 2 days for the rats treated with morphine or saline.

3.7. Experiment 7

The means and S.E.M. for the net differences in responding compared to baseline for the five treatments are shown in Table 2. Results of the analyses demonstrated statistically significant differences [$F(4,15) = 12.26$, $P < .001$; $\eta^2 = 0.78$ or 78% of the variance explained] between the treatments. Post hoc analysis indicated the rats that received the burst-firing field showed greater analgesia than the groups that received either the morphine or magnetic field after receiving naloxone or the sham field after receiving either saline or naloxone. These four groups did not differ significantly from each other. The mean and S.E.M. for the rats' weights were 650 and 15 g, respectively.

Table 2
Means and standard errors of the mean for the net differences (in seconds) for latencies to respond for rats receiving various combinations of the burst-firing magnetic field and/or mu-opiate related drugs ($n = 4$/group)

Condition	Day 1				Day 2			
	+30 min		+60 min		+30 min		+60 min	
	M	S.E.M.	*M*	S.E.M.	*M*	S.E.M.	*M*	S.E.M.
Saline + saline + saline	−1.3[a]	0.7	−1.0[a]	0.8	−1.8[a]	0.8	−2.0[a]	1.1
Saline + saline + burst	3.6[c]	1.1	6.7[c]	0.7	2.0[b]	1.1	6.8[b]	1.2
Morphine + nalox + sham	−2.2[a]	1.5	0.8[b]	0.3	1.3[b]	0.7	−0.1[a]	0.3
Saline + nalox + burst	−0.2[b]	0.3	0.6[b]	0.6	0.1[b]	1.2	0.6[a]	1.3
Saline + nalox + sham	−2.3[a]	0.8	−1.5[a]	0.7	−4.5	3.7	−1.8[a]	0.8

Superscripts refer to comparisons of values in columns a vs. b, $P < .05$ and b vs. c, $P < .05$.

4. Discussion

The results of these experiments indicate that a reliable and robust analgesia to thermal stimuli delivered to the footpads can be evoked by at least 30 min of exposure to burst-firing magnetic fields presented once every approximately 4 s. The absolute values for the magnitude of the net differences in response latency across experiments were consistently between 5 and 15 s for the magnetic-field-exposed rats and about 0 s for the sham-field-exposed rats. The treatment explained about 50% of the variance in these response latencies.

In the present studies, we found that the presentation of a frequency-modulated magnetic field produced a different latency of analgesia than the burst-firing pattern. In most of our studies, the frequency-modulated pattern has produced its maximum effect after the rats have been exposed continuously for 30 min. The analgesia was maintained and did not increase during the subsequent 30 min of no field exposure.

On the other hand, the burst-firing field required an additional 30 min after the cessation of the 30-min exposure (the additional 30 min of no exposure) to produce a comparable level of analgesia. These different latencies to achieve maximum analgesia by different temporal patterns of magnetic fields might be analogous to the different latencies for different structures of analgesic molecules to achieve their effects.

Like the delivery of pharmacological compounds, there may be different optimal temporal patterns for the presentation of specific shapes of magnetic fields to produce the maximum analgesia. The burst-firing field did not produce analgesia when the ISI was the one optimal for the frequency-modulated field. The frequency-modulated field did not produce the maximum analgesia when it was presented at the ISI most optimal for the burst-firing field.

The rats exposed to the solenoids or spatially heterogeneous magnetic fields with maximum strengths of 250 nT (0.25 μT) exhibited an analgesic response that was significantly greater than the usual sham-field responses. Exposure to maximum intensities of 30 nT did not produce responses that differed significantly from the sham fields. Grossly, the effect was linear and dependent upon the intensity of the field. Because these values were the maximum strength of the field nearest the solenoids, this meant that a significant analgesic response for the rats exposed to the "medium" intensity field occurred with most of the values within the range of 25–250 nT but not below 3–30 nT. For comparison, the background,

nonspecific (probably 60 Hz) strength during the presentation of the sham fields was between 10 and 20 nT.

The potentiation of the analgesic effect of 4 mg/kg of morphine by both patterns (burst-firing presented once every 4 s and frequency-modulated presented continuously) was comparable in magnitude and similar to the singular effects of between 7 and 8 mg/kg of morphine (Dixon and Persinger, 2001). Stated alternatively, the 30 min of exposure to the magnetic fields was equivalent to the effects of about 4 mg/kg of morphine, as reported for other paradigms (Fleming et al., 1994). The typical latency (60 min after beginning of the exposures) of the maximum response to the burst-firing magnetic field treatments was not apparent when the rats had also received morphine. The analgesia was maximum after only 30 min.

The analgesic effects of the burst-firing magnetic field were eliminated by preinjection with naloxone. This dosage also eliminated the analgesia associated with morphine. Elimination or near elimination of the analgesic effects of burst-firing magnetic fields was also found by Ryczko and Persinger (2002). These results suggest that the fields may be affecting intracellular process stimulated by mu (opiate) receptors.

The smaller enhancement of analgesia in rats that received both the agmatine and either the burst-firing or the frequency-modulated pattern was maximum immediately after the 30 min of exposure. Agmatine was selected because it has been shown to enhance morphine analgesia (Kolesnikov et al., 1996; Yesilyurt and Uzbay, 2001). We reasoned that if the analgesic effects of the burst-firing magnetic field involved the same processes as those of morphine, then agmatine should enhance the analgesic effects. Our results would be consistent with, but not proof for, this hypothesis.

We cannot exclude the possibility that agmatine's moderate affinity for all subclasses of alpha-2 noradrenergic receptors and the colocalization of the alpha-2 receptors and the mu-opiate receptor (Van Bockstaele and Commons, 2001) may have resulted in potentiated analgesic effects by a different mechanism than for morphine. If alpha-2 noradrenergic and mu receptors involve different molecular configurations and pharmacokinetic values for binding and activation, then the analgesic effects from the magnetic field exposures may have been mediated through postreceptor processes, within the inner cell membrane or cytoplasm, which both types of receptors share.

The simplest biophysical mechanism to accommodate our results is a variation of Faraday's principle of induction. The potential difference or electric field V, generated by a 10^{-6} T (10 mG) magnetic field changing at 10^{-4} s (the rise time for each point duration composing the patterns) in a 0.1-m (10-cm) space (the approximate length of a rat) would be in the millivolt range. Assuming about 50 Ω/cm^2 for resistance of extracelluar fluid, the induced current would be within the nA range. As indicated by Becker (1965), even DC currents in the order 1 nA/cm are sufficient to affect cellular activity. Several calculations by Durand-Manterola et al. (2001) have indicated that natural electromagnetic phenomena whose amplitudes are within the range of microTesla (such as micropulsations and whistlers from distal lightning discharges) can induce electric currents, within the upper pA range, that can be larger than cellular sources.

The major argument against our hypothesis that temporal patterns of magnetic fields can simulate the spatial (molecular) structure of ligands is that the induction of the electric field (and presumably the current within intraorganismic conductive spaces) would occur almost immediately. Our results indicated that more than 15 min was required to produce the analgesia. The latency and magnitude of the effects elicited by exposures to the magnetic fields employed in this study are more compatible with interactions with intrinsic chemistry rather than the instantaneous creation of a "virtual electromagnetic ligand."

This latency may reflect a delayed chemical response, such as the release of endogenous opiates, that requires time to reach behavioral effectiveness. The observation that naloxone blocked the analgesic effect would be consistent with this explanation. If the changes in the shapes of the magnetic field over time (temporal structure) were similar to the molecular organizations of drugs (spatial structure), then one would expect that another set of variables would generate the equivalence of the pharmacokinetics of the magnetic field. At present, there is no obvious code or "Rosetta Stone" for the translation of electromagnetic patterns into molecular structures or vice versa.

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