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Thermal Analgesic Effects from Weak, Complex Magnetic Fields: Critical Parameters

L. J. MARTIN*, S. A. KOREN, AND M. A. PERSINGER

Behavioural Neuroscience Laboratory, Laurentian University, Ontario, Canada

Magnetic applications have been used to treat painful stimuli in various contexts. Magnetic fields that mimic electrophysiological patterns have been referred to as complex magnetic fields. In 7 different experiments, the optimal parameters for producing a robust analgesia (equivalent to about 4 mg/kg of morphine) in male rats to thermal stimuli following exposures to weak (1 microTesla (μT)) complex magnetic fields were explored. Thermal nociceptive thresholds for male Wistar rats were examined after exposure to the experimental treatment. Two different complex magnetic patterns were investigated for their potential analgesic effects. Rats were exposed (whole body) to a magnetic field treatment for a pre-defined duration usually lasting 30 min unless otherwise specified. The parameters evaluated for eliciting analgesia included the optimal time delay between successive presentations of the magnetic field, total length of magnetic field exposure, and the duration the specific values that generated the total pattern were activated. Maximum analgesia occurred when patterns of magnetic fields with burst-firing-like configurations lasting 690 msec were presented once every approximately 4 sec for 30 min rather than 60 min. Rats with histories of epilepsy and brain damage showed maintained elevation of nociceptive thresholds for at least one week after a single exposure to these magnetic fields. A different frequency-modulated pattern also produced an analgesic response that lasted for 4 h following an exposure of 30 min but not 2 h. A constantly generated pattern derived from a chaos (May algorithm) function produced similar levels of analgesia. The results of these experiments suggest that rational designs of the temporal structures of weak magnetic fields may be a novel, inexpensive, and, reliable technique for producing analgesia to some classes of painful stimuli.

Keywords Analgesia; Magnetic stimuli; Rat; Thermal stimuli.

*Present address: Institute of Medical Science, Medical Science Building Room 3318, University of Toronto, Toronto, Ontario M5S 1A8, Canada; E-mail: loren.martin@utoronto.ca

Address correspondence to M. A. Persinger, Behavioural Neuroscience Laboratory, Laurentian University, Sudbury, Ontario P3E 2C6, Canada; E-mail: mpersinger@laurentian.ca

Introduction

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 and its interactions with specific classes of receptors ultimately determines its functional consequence.

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 patterns for magnetic fields are considered more important than the monotonic dimensions of intensity to produce specific effects. The idea is similar to the fact that most of the information within a sentence is determined by its structure and content rather than how loud it is spoken. In other words, if a magnetic pattern mimics an electrophysiological or naturally occurring process it may be more efficacious and have more biological relevance than a simply structured pattern.

We have found that rational design of complex nonsinusoidal asymmetrically-shaped patterns of magnetic fields (referred to as complex magnetic fields here in) that imitate natural processes can duplicate their effects more than the traditional employment of sine waves or square waves. McKay and colleagues [1] exposed rats for 30 min to waveforms that had been modeled after salient electrophysiological patterns typically generated within the hippocampus or amygdala or to intensity-matched (0.5 to 1 μT) 7-Hz or 20-Hz sine waves. The rats that had been exposed to the magnetic fields imitating electrophysiological patterns displayed a marked attenuation of contextual freezing behavior while the rats that had been exposed to the symmetrical fields did not differ significantly from those exposed to the sham-fields.

In 1994, Fleming et al. [2] 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.

We had selected the 4 s interstimulus interval on the basis of the elicitation of analgesia by presentation of foot-shock once every 4 s as opposed to continuous application reported by Lewis et al. [3]. Our working hypothesis has been that whole-body exposure to an appropriately configured magnetic field presented once every 4 s can induce effects directly within the brain. They would be comparable to those invoked by normal sensory transduction through the foot pads.

In the present series of experiments, we show how this burst-firing pattern (which also has been shown to reduce depression and pain in clinical patients subsequent to mechanical head trauma [4, 5]) with intensities in the order of 1 μT (10 mGauss) 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 the quantitative inference of analgesia.

Methods

Subjects

Naive male ($n = 146$) albino Wistar rats, between 4 months 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 h to 0800 h) within temperature-controlled rooms (20–22°C) for at least one month before the initiation of the experiments.

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. Details of the housing procedures have been published elsewhere [6].

All 7 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 cm × 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 either placed within the experimental apparatus that generated the magnetic fields or placed within the experimental apparatus but no magnetic fields were generated. With the exception of Experiments 1 and 2, all exposures were 30 min in duration. After the 30 min of exposure, the rats were tested immediately for thermal response times on the same apparatus (Trial 2). For all but 2 experiments (Experiments 1 and 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). For most experiments this procedure was repeated over 2 successive days. All rats were tested between 0800 h and 1200 h.

For all experiments, the 30 min exposures to the magnetic fields or sham-field conditions occurred within a plastic 25 × 25 × 25 cm (deep) plastic cage. A pair of solenoids generated the magnetic fields through the cage [2]. 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. We previously have determined that the fidelity of the patterns generated through DAC systems is highly reliable, in that the electrical signal driven by our computer output is highly correlated with the measured magnetic field ($r = 0.9$ to 0.98) [7].

All experiments involved between 4 and 9 rats per group. This small number of subjects per group was possible because the effect size (eta-squared (η^2) or partial omega-squared estimate), or the explained variance, ranged robustly between about 40 and 80%. Consequently the numbers of rats per experiment were minimized according to the guidelines for the Canadian Council on Animal Care. The range in numbers of rats per group (4–9) also was determined by the numbers of rats of similar age at a given time available for an experiment.

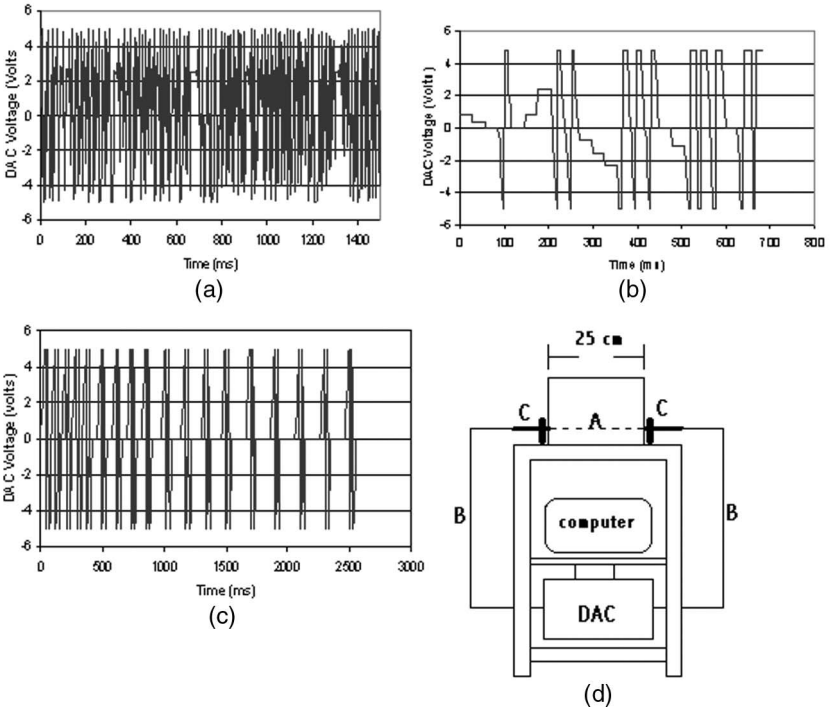

Figure 1. The patterns of the magnetic fields employed in this study. a) May algorithm with an x value of .6 and a lambda $r = 4$. b) The burst-firing pattern. All point durations = 3 msec. c) For comparison the frequency-modulated (Thomas pulse) that also has been successful to produce analgesia is shown. d) A schematic of the equipment used to generate the magnetic patterns [A]: corresponds to the exposure area that contained the rat during magnetic field exposure; the dashed line represents the approximate level of the head of the rat in relation to the solenoids during exposure. [B]: is the electrical connection between the solenoid complex and the DAC box. [C]: is the solenoid/iron core nail complex which propagated the magnetic field.

Experiment 1: Optimal Duration of Exposure. Although an exposure of 30 min to the magnetic fields was selected because of convenience and the efficacy for reducing subjective pain and depression in human patients [4], there was the possibility that longer exposures might be more effective. In this study, rats were exposed to the burst-firing magnetic field (see Fig. 1) once every 4 s for either 30 min ($n = 5$) or 60 min ($n = 5$). All rats, including those exposed to the sham-fields ($n = 5$), remained in the exposure chamber during the period between 30 min and 60 min after the onset of the trial instead of the usual procedure of being returned to the home cage. Rats exposed to the magnetic pattern for only 30 min had the magnetic field turned off during their second exposure to the chamber, while the 60 min group were still exposed to the magnetic pattern.

Experiment 2: Frequency-Modulated Exposure Times. There was a possibility that different exposure times to a different frequency modulated-pulse would also result in a different analgesic response as observed in Experiment 1. Based on the results of the different exposure times for the burst-firing pulse, rats were randomly

selected to be exposed to a frequency-modulated pattern for 30 min, 2 h or 4 h. This pattern, also called the Thomas pulse [8], has been shown to induce analgesia in snails [9–11], and mice [12]. One sweep of the frequency-modulated pattern lasted approximately 2.5 s and was presented every 3 msec. A group of rats were also exposed to sham-field conditions. There were 4 rats per group. All rats were housed within the magnetic field exposure chamber for a total of 4 h. The magnetic field was disconnected at the appropriate time for rats exposed for 30 min and 2 h. All rats were tested on the hotplate for baseline measurements, immediately after removal from the magnetic field exposure chamber (i.e., 4 h after baseline) and 30 min after the cessation of the magnetic field treatment (i.e., 4.5 h after baseline). Testing was conducted over one day instead of 2 consecutive days, with each rat being exposed to the treatment apparatus for 4 h.

Experiment 3: How Long Does the Analgesia Persist? If the optimal duration of a single exposure was about 30 min, what is the duration of the persistence of the effect? To begin testing the persistence, rats were exposed to the burst-firing field for 30 min ($n = 6$) or to the sham-field condition ($n = 6$). One group of field-exposed rats was tested for analgesia 30 min after the initiation of the exposure and then 60 min later (as usual). Another group of rats was tested for analgesia 30 min after the initiation of the treatment and again (instead of 30 min later) 4 h later ($n = 6$). During the intervening period after the exposure to the treatment all rats were maintained in their home cages. Testing occurred on 2 consecutive days. On each day, 3 hotplate latency trials were recorded (i.e., (Trial 1) baseline, (Trials 2 and 3) conducted after magnetic field exposure).

Experiment 4: Optimal Timing of the Pulsing Sequence. In the original Fleming et al. [2], study the burst-firing magnetic pattern was presented once every 4 s based on the increased analgesia resulting from intermittent foot shocks [3]. The present experiment was designed to discern if this time delay between successive presentations of the magnetic pattern (i.e., pulsing sequence) was optimal. This time delay also has been referred to as inter-stimulus interval (I.S.I.)

Rats were assigned to one of 7 treatments ($n = 6$/group). Those exposed to the burst-firing magnetic fields were administered these fields for 30 min. One sweep of the burst-firing magnetic pattern lasted 690 msec. Although, the delay between the successive sweeps of the magnetic pattern was either: 4 msec, 400 msec, 4000 msec, 8000 msec, or 15,000 msec (15 s). Stated alternatively, the duty cycle or percentage of time the magnetic field is on during the 30 min application would correspond to 99.4%, 63.3%, 14.7%, 7.9%, and 4.4% respectively. In addition to the usual sham-field group, we employed a colony control group. After baseline measures, the rats from this group remained in their cages except during the two trials to test thermal threshold.

Experiment 5: What is the Optimal Activation Time for a Pulsed Magnetic Pattern? Our method of generating complex magnetic fields involves converting a number between 0 and 255 to a specific voltage. The magnetic patterns used in our lab consist of a predefined set of points with each point being programmable to any value between 1 and several 100 s of msec (the very long durations can be employed to simulate the much slower geomagnetic activity). In both human and rat studies [13], we had found that, when each of the points that composed a specific pulsed

magnetic pattern were presented for 3 msec in length, this resulted in maximal behavioral or physiological effects. However, empirical verification was required for the analgesic effects.

In this experiment groups of rats were exposed to the sham condition ($n = 9$) or to the burst-firing magnetic field once every 4 s for 30 min. There were 3 groups of field-exposed rats that received the points of the burst-firing field for durations of 1 msec ($n = 4$), 3 msec ($n = 4$), 10 msec ($n = 4$), or 20 msec ($n = 5$). In other words, since the burst-firing pattern consists of 230 points, the rats received presentations of the burst-firing which were separated by 4 s for either 230 msec, 690 msec, 2.3 seconds, or 4.6 s with a total magnetic field exposure time of 30 min. Because the duration for each point also determined the numbers of changes per unit time, there were, as predicted by Faraday's Law, differences in intensities within the exposure area. For example, near the center of the cage, the peak strengths for the continuously presented pattern were 11.6 mG, 7.3 mG, 3.8 mG, and 1.2 mG (or 1.1, 0.7, 0.4, and 0.1 μTesla) for the 1 msec, 3 msec, 10 msec, and 20 msec point durations, respectively. The net intensities were verified independently with a MEDA FM-300 magnetometer. The rats were tested for analgesia at the standard times. There also were concerns regarding the electrical properties of the coils and characteristics of the equipment generating the signal. We have stated in a previous publication that the latency or rise time between successive 3 msec points was determined by direct measurement to be 110 μs for the solenoids. Hence, although the duration each value between 0 and 255 remained at that voltage was 1, 3, 10, or 20 msec, the time required to achieve this value was only 110 μs. The empirical values also matched the theoretical values from the specifications for the computer chips. The converter was accessed through a parallel port (output) from the computer and the typical port latency was about 100 μs as reported previously [7].

Experiment 6: Are There Permanent Changes in Nociceptive Threshold? The typical procedure has been to expose rats on 2 successive days to the treatments. In general the analgesic effects on the second day are (about 20%) less then those on the first day. All of these studies had involved normal rats. In our human studies, patients who have sustained brain injuries (primarily from motor vehicle incidents) reported greater analgesic effects compared to normal volunteers. These antinociceptive effects often persisted for more than a week after a single treatment [4, 5].

To discern if similar long-term elevations in pain threshold would be displayed by animals with acquired brain injuries, we exposed rats to the magnetic field treatment in which brain damage had been produced by the consequences of limbic seizures [13–16]. The seizures had been induced by a single subcutaneous injection of lithium (3 mEq/kg) followed, 4 h later, by a single subcutaneous injection of pilocarpine (30 mg/kg). Within a few minutes of the onset of the overt seizures (about 30 min after the injection of the pilocarpine), the rats were injected with either ketamine (100 mg/kg) or acepromazine (25 mg/kg) to promote survival [15, 16]. The rats had been maintained in single cages to prevent agonistic behaviors. Age-matched controls had been separated and maintained similarly.

In Part A of this experiment, brain-damaged rats were exposed to the sham-field ($n = 4$) or burst-firing magnetic field for 30 min and then tested immediately or 30 min later ($n = 7$). In Part B of this experiment, normal rats were exposed to the burst-firing ($n = 7$) or sham-fields ($n = 4$) for 30 min and tested immediately or

30 min later. However, instead of being tested the following day, all rats were tested after *one week* had elapsed.

Experiment 7: The Analgesia Induced by Changing Chaos Patterns. The previous experiments involved exposures to complex but fixed-pattern magnetic fields. In the present experiment, we examined the effects of the enhanced complexity and irregularity of the pattern. Our theoretical work has suggested that some quantitative value for chaos and entropy contained within a pattern of the magnetic field may be fundamental to its effects on biological systems.

The May algorithm ($\times\text{prime} = rx(1 - x)$) was generated with the initial values of $x = 0.6$ and lambda $= r = 4$ [17, 18]. With these parameters a different chaotic pattern was presented with each of the 330 presentations of the field during the 30 min of the exposure period with 4 s separating each sweep. Alternatively stated, the magnetic pattern generated from the algorithm was always different from the magnetic pattern that preceded it during the pulsing sequence. We specified each point-duration to 3 msec and limited the number of points in each sequence to 500. An example of the pattern generated during the first generation (sweep) is shown in Fig. 1b.

One group of rats was exposed to the successively unique May patterns (once every 4 s for 30 min while another group was exposed to the fixed, burst-firing pattern once every 4 s for 30 min. A third group was exposed to the sham-field. Because of the memory requirements for generating the May algorithms a 486 computer was used instead of the usual IBM XT. There were 4 rats per group.

Apparatus

Exposure Systems

The primary exposure system was the 25 × 25 × 25 cm plastic chamber within which the subjects were exposed to the field. Two large nails (each 25 cm length, 1 cm diameter) wrapped with 1,050 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 2 poles of the solenoids was approximately the level of the head of an adult rat when walking on the surface. The solenoids were connected so that the field was generated between the 2 nails ("poles").

The measurements were completed with a Metex N380 meter coupled to a magnetic sensory (Electric Field Measurements, Rt. 183 W. Stockbridge, MA, USA) power frequency meter. The peak strength 2 cm from either pole within the bisector between the 2 poles was 2.5 μT. At radii of 4 cm, 6 cm, 8 cm, or 10 cm from each pole the strengths were 1.7 μT, 1.0 μT, 0.5 μT, and 0.3 μT, respectively. Along the edges of the cage the values ranged between .15 and .35 μT (150 to 350 μT). Within the vertical axis the mean intensities for each of six planes separated by 2 cm was 0.9, 1.0, 0.5, 0.4, 0.2, and 0.1 μT.

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 column of numbers that generate the fields in our studies have ranged between 150 to 10,000.

The software, named Complex [19–21] transformed each of the numbers within a number file to voltages ranging between +5 V (the number 255) and −5 V (the number 0). The number 127 was 0 V. The duration, in msec, that the specific voltage was generated was the point (or "pixel") duration. In the present study the point duration was always 3 msec except in Experiment 4. This means that the voltage associated with a given number between 0 and 255 was constant for 3 msec before the next number was accessed and the next voltage was generated.

Statistical Analyses

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 *normal* 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.

Consequently, except for Experiment 5 where absolute responses were required by the experimental design, all dependent measures involved the net differences in seconds in response latency between Trial 2 and the baseline and Trial 3 and the baseline. The absolute response latencies to respond were analyzed in Experiment 5 because the persistence of the treatment effect over time (one week) was anticipated.

Because the basic procedures were similar, all analyses involved at least a 3-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.

Results

Experiment 1

The means and S.E.M. for the net differences in latency to respond for rats that had been exposed to the sham-field condition or to the field for either 30 min or 60 min before testing for analgesia are shown in Fig. 2. Analysis of variance showed a statistically significant difference [$F(2, 12) = 92.66$, $p < .001$; $\eta^2 = 94\%$] between

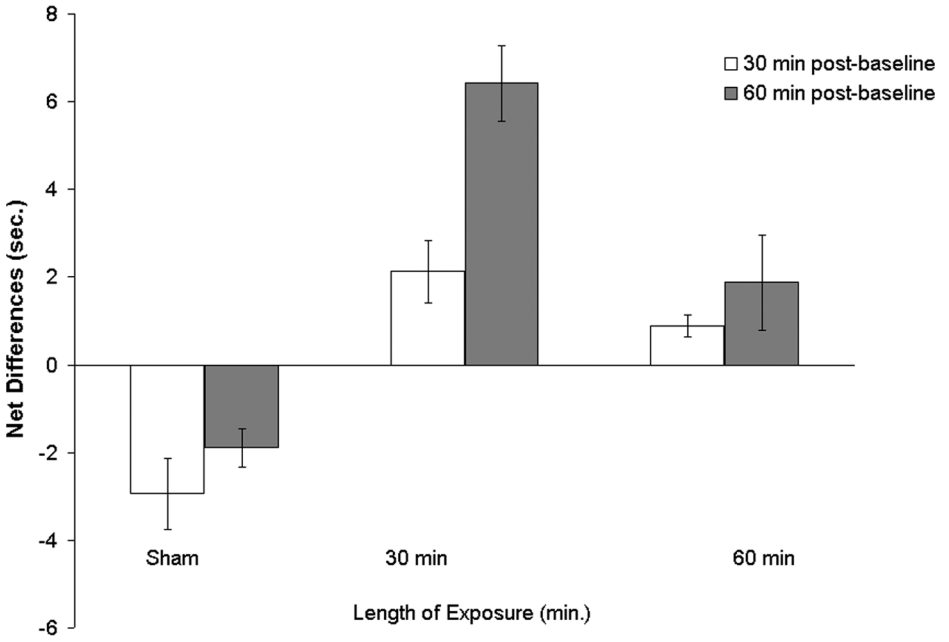

Figure 2. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli in Experiment 1 after baseline for rats exposed to the burst-firing field once every 4 s for either 30 min or 60 min.

the 3 treatments. Post hoc analysis indicated that rats exposed for either 30 min or 60 min to field displayed longer latencies for both trials compared to the sham-field-exposed group.

The interaction between trial and treatment was statistically significant. Post hoc analysis indicated that the primary source of this interaction was due to the diminished analgesia after 60 min exposure for the rats tested at that end of this period compared to the rats that had received only 30 min of treatment and were tested again after 30 min after no treatment.

Experiment 2

The greatest analgesic response was acquired when rats were exposed to the frequency-modulated pulse for 30 min and tested 4 h later compared to sham-exposed rats [$F(3, 8) = 4.37$, $p < 0.05$, $\eta^2 = 62\%$]. The groups exposed to the same magnetic field for either 2 h or 4 h did not differ significantly from each other or from the sham group. The means and SEMs for thermal response times for rats exposed to the frequency modulated pulse for 30 min, 2 h or 4 h is presented in Fig. 3.

Experiment 3

The means and S.E.M. for the net differences to respond after baseline following 30 min of treatment or 4 h later for the rats exposed to the burst firing magnetic field pattern or to the sham-field condition for the first and second days of testing are shown in Fig. 4. Analysis of variance showed a significant difference between the

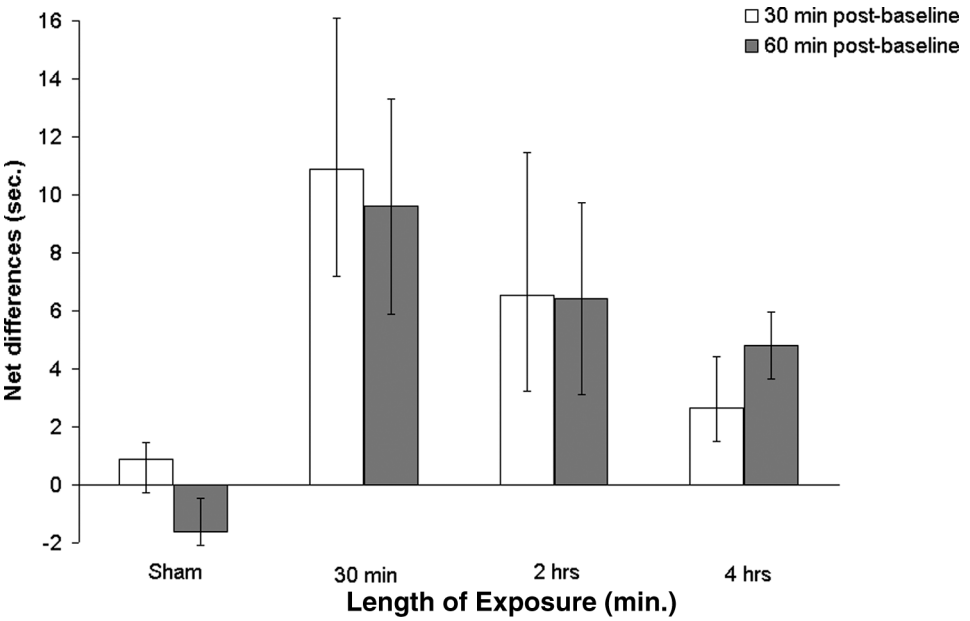

Figure 3. The thermal response times of rats exposed to the frequency modulated pattern in Experiment 2 are displayed. Rats exposed for 30 min and tested 4 h later displayed the greatest analgesia compared to sham rats on the second net trial. Rats exposed for 2 h and 4 h did not differ significantly from any group. Error bars denote SEM.

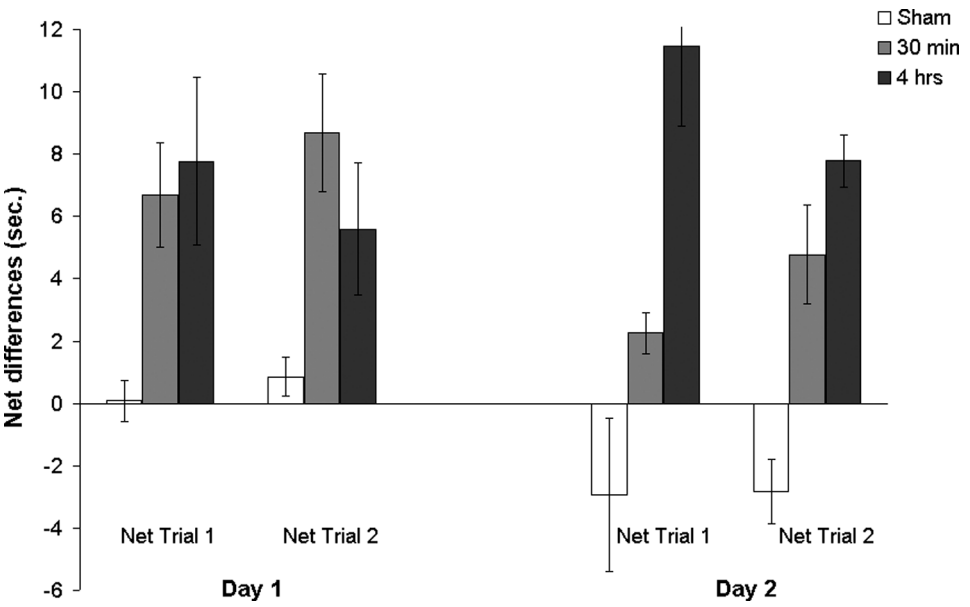

Figure 4. Means and S.E.M. for the net differences in time (in seconds) to respond either 30 min or 4 h after 30 min exposure to the burst-firing field in Experiment 3. The results for 2 successive days are shown.

groups [($F(2, 15) = 15.35$, $p < .001$; $\eta^2 = 67\%$]. Post hoc analysis indicated that the rats exposed to the magnetic field and tested either immediately after the 30 min of exposure or 4 h later (3.5 h of no exposure) displayed more analgesia than the sham-field-exposed rats.

There was a statistically significant interaction between the treatments and the days of testing [$F(2, 15) = 6.44$, $p = .01$; $\eta^2 = 46\%$]. Post hoc tests revealed the source of the interaction was due to the significantly greater analgesia on the *second* test day after 4 h compared to the first day while this difference was not significant for either the sham-field group or the group tested 30 min after the beginning of the exposure.

Experiment 4

The means and S.E.M. for the net differences to respond for the various treatments are shown in Fig. 5. One way analysis of variance demonstrated statistically significant differences between the groups [$F(6, 21) = 6.92$, $p < .001$; $\eta^2 = 66\%$]. Post hoc analyses for the main effects showed that when the magnetic pattern was pulsed every 0.4 s and 4 s, the rats displayed significantly longer latencies than those exposed to .004 s, 8 s or 15 s intervals between the burst presentations or to the sham-field or colony-room controls.

The 2-way analysis of variance with one level repeated (30 min vs. 60 min post-baseline) and one between subject level (treatment) demonstrated a statistically

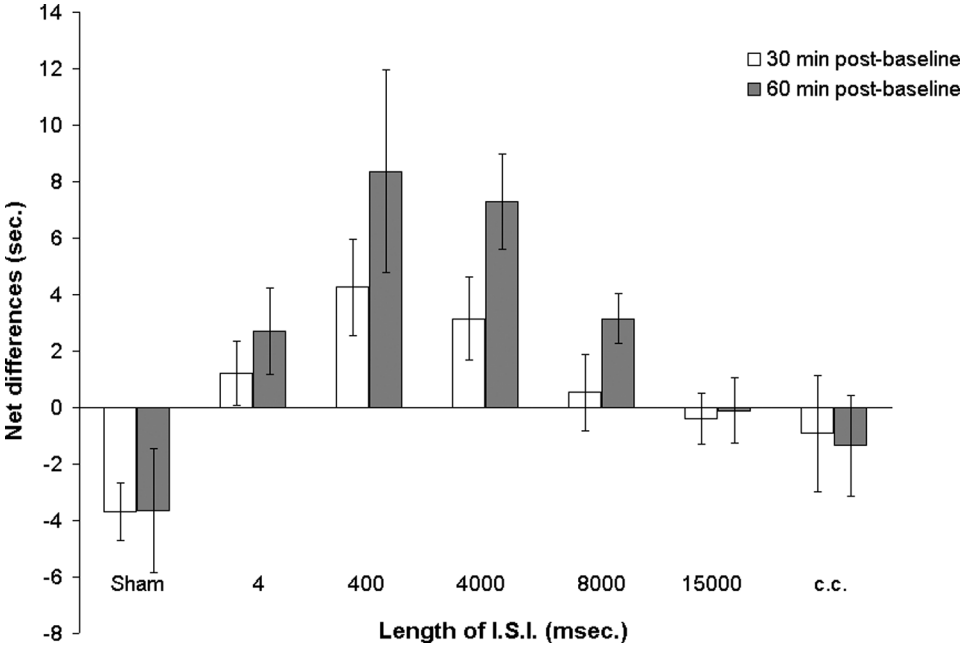

Figure 5. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli relative to baseline for rats exposed for 30 min to the burst-firing field presented with various inter-stimulus intervals between 4 and 15000 msec in experiment 4. cc refers to colony controls.

significant interaction. Post hoc analysis showed that the major source of this interaction was the significantly greater latency 30 min after the end of the 30 min treatment for those rats exposed to a magnetic field pulsed every 4 s compared to the sham-field, colony-room controls, or those groups exposed to the .004 s, 8 s, or 15 s, ISIs.

Experiment 5

The means and S.E.M. for the net differences in latencies to respond 30 min and 60 min after the baseline measurements for groups of rats that had been exposed to the sham-field or to the burst-firing magnetic field whose point durations for the pattern generating the field were either 1 msec, 3 msec, 10 msec, or 20 msec are shown in Fig. 6. There were statistically significant differences between the point durations [$F(4, 21) = 5.91$, $p < .01$; $\eta^2 = 53\%$]. Weak, but statistically significant, interactions between the point durations and the time of testing, 30 min and 60 min after initial exposure, [$F(4, 21) = 2.76$, $p < .05$; $\eta^2 = 35\%$] and the point durations and days [$F(4, 21) = 2.94$, $p < .05$; $\eta^2 = 36\%$] also were found.

Post hoc analyses indicted that the source of the interaction was due to the significantly elevated analgesia after 30 min for the group exposed to the pattern with 3 msec point durations (i.e., each pulse lasted 690 msec) compared to the sham-field group and the group exposed to the same magnetic field pattern with either 1 msec or 20 msec point durations. After 30 min post exposure (60 min) all of the field-exposed groups, except those exposed to the 20 msec point durations, displayed

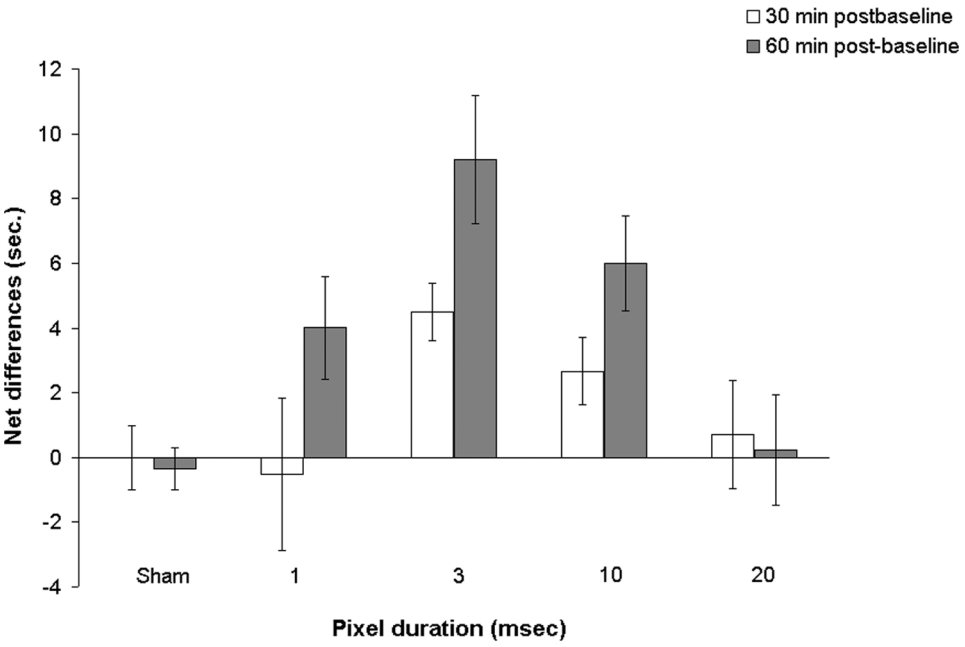

Figure 6. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli for rats exposed to the sham-field or to the burst-firing field (presented once every 4 s) whose point durations were either 1 msec, 3 msec, 10 msec, or 20 msec in Experiment 5.

greater response latencies than the sham-field group. The group exposed to the 3 msec point durations exhibited significantly longer latencies than the group exposed to the field with 1 msec point durations (i.e., each pulse lasting 230 msec).

Experiment 6

Part A: The means and S.E.M. for the net difference in response times for the rats with a history of brain damage from induced seizures are shown in Fig. 7. Compared to the seized rats exposed to the sham-field conditions, the seized rats exposed to the burst-firing field showed an increased analgesic response on the first day only. This was not evident (not shown) on the second day.

Part B: The means and S.E.M. for the absolute time to respond to the thermal stimuli during baseline and after 30 min or 60 min since the beginning of the 30-min exposure to the burst-firing field are shown in Figs. 8 and 9. Data for the rats in which brain damage had been induced by lithium/pilocarpine-induced seizures about 6 months previously and age-matched normal rats are presented separately. The results show the changes following the first treatment and the second treatment one *week* (vs. the usually one day) later.

The most significant and powerful results from the three way analysis of variance with 2 levels repeated (sham-field first week vs. sham-field second week; baseline, +30 min, +60 min after beginning of treatment) and one between subject level was the interaction between the application of the field either on the first or second week, the trial, and whether or not the rats had been seized or not [$F(1, 20) = 357.08$, $p < .001$; $\eta^2 = 41\%$]. Post hoc analysis with Tukey's and

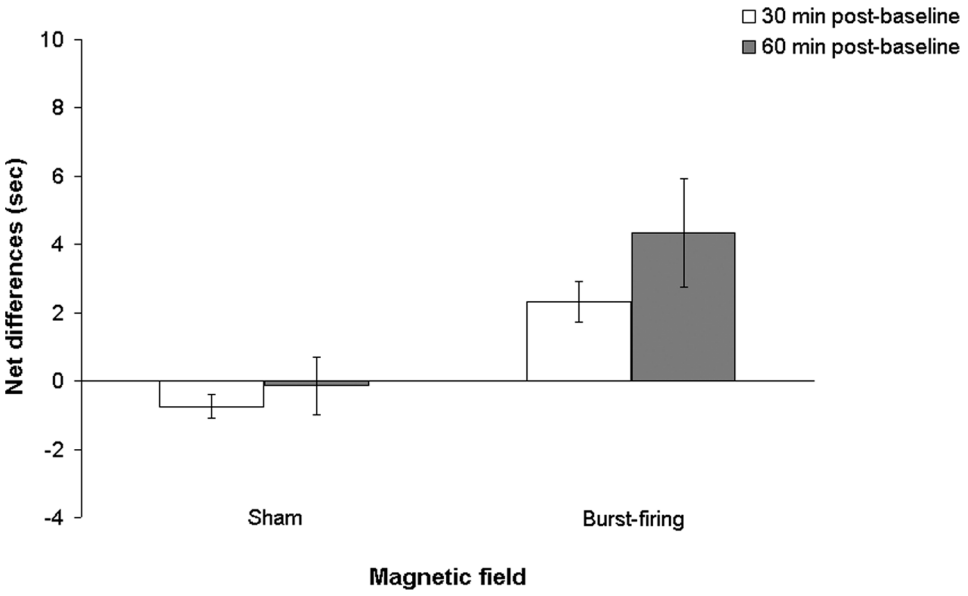

Figure 7. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli relative to baseline for rats exposed to the burst-firing field for rats with histories of seizure-induced brain damage in Experiment 6.

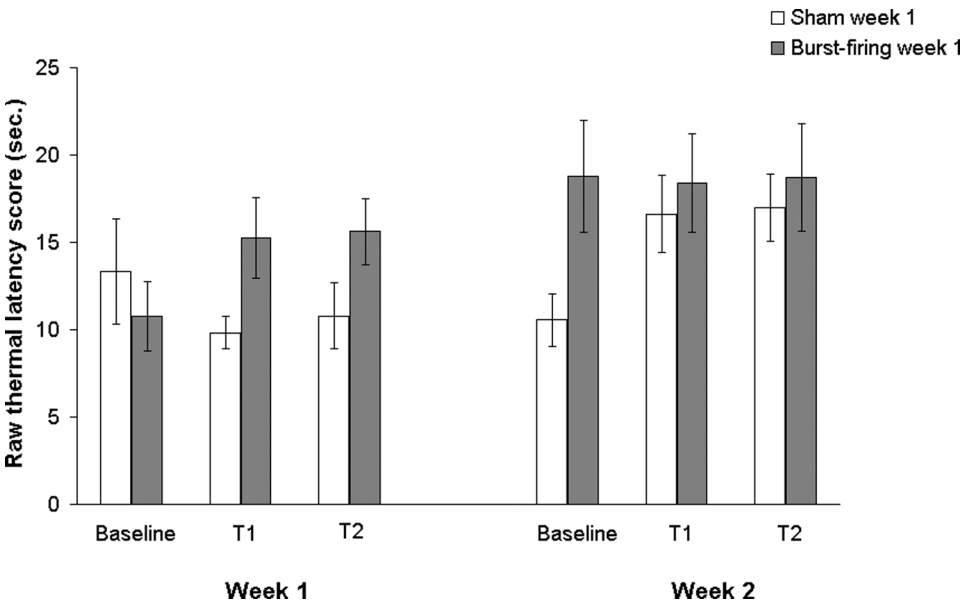

Figure 8. Means and S.E.M. for latency to respond during baseline and 30 min (T1) and 60 min (T2) after the initiation of the 30 min exposure to a burst-firing field or sham during the first week and then to the opposite condition one week later for rats with histories of seizure-induced brain damage in Experiment 6.

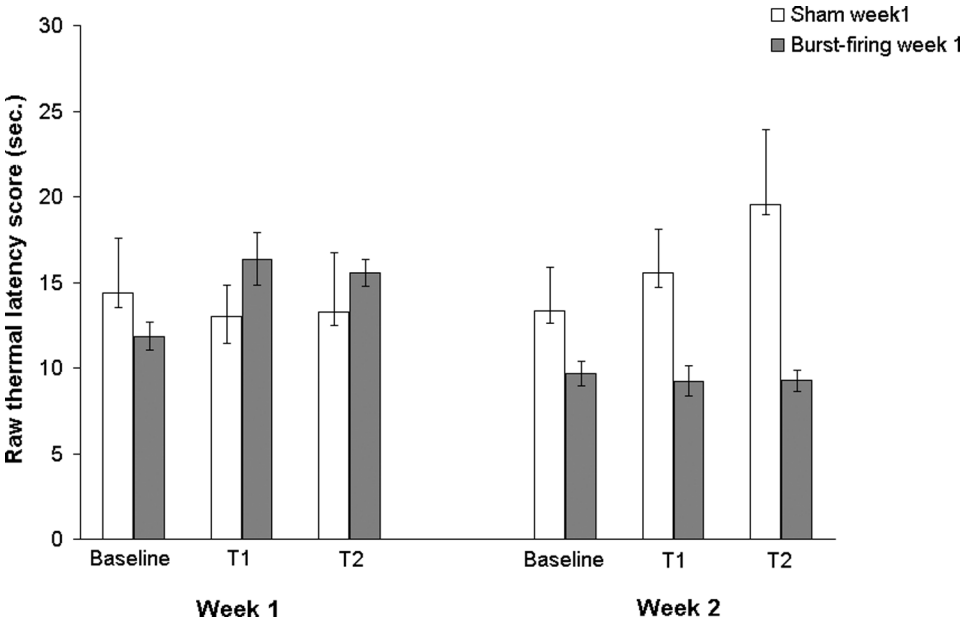

Figure 9. Means and S.E.M. for latency to respond during baseline and 30 min. (T1) and 60 min (T2) after the initiation of the 30 min exposure to a burst-firing field or sham field during the first week and then to the opposite condition one week later for normal rats in Experiment 6.

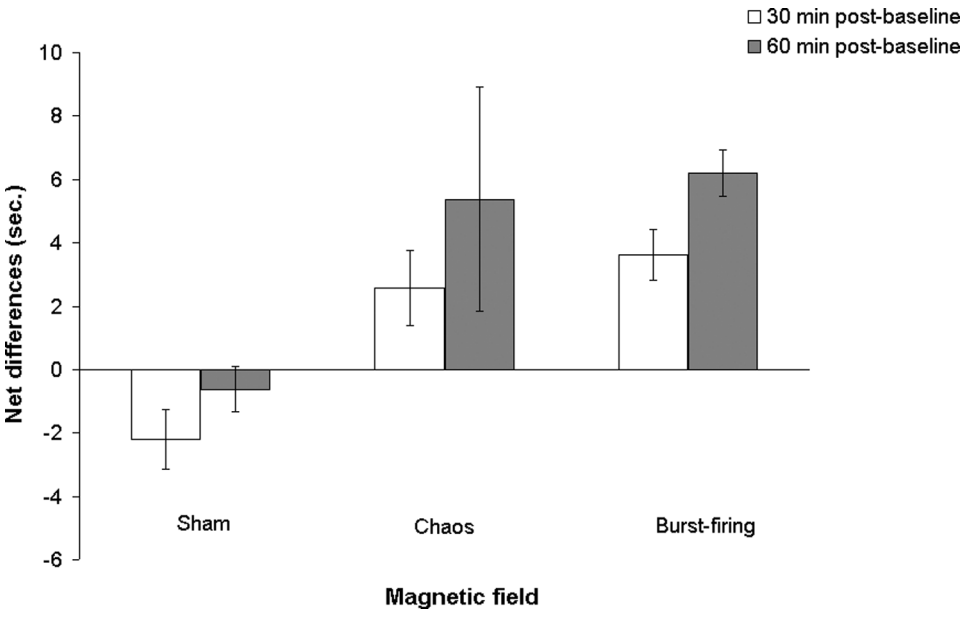

Figure 10. Means and S.E.M. for the net differences in time (in seconds) to respond to thermal stimuli after a 30 min exposure to either a sham-field or to either the burst-firing field or to the "chaos" field generated by the May algorithm in Experiment 7.

correlated *t*-tests indicated that the primary source of the interaction was due to the maintained elevation of the threshold for only the seized rats that had been exposed to the burst-firing magnetic field on the first week compared to the normal rats.

Superimposed on this effect was the usual elevation of nociceptive threshold for both groups of rats 30 min after they had received the burst-firing field for 30 min. This effect was still present 30 min after the cessation of the treatment. The seized rats that had received the treatment during the first week did not show a greater elevation in threshold following the 30 min of treatment.

Experiment 7

The means and S.E.M. for the net differences in the latency to respond compared to baseline for rats exposed to the chaos-patterned field, burst-firing field, or sham-field on Day 1, 30 min and 60 min after the initiation of the 30 min treatment, are shown in Fig. 10. There was a statistically significant difference between the groups [$F(2, 9) = 8.72$, $p < .01$; $\eta^2 = 66\%$]. Post hoc analysis showed that the groups that were exposed to either the burst-firing or chaos-patterned field showed more analgesia compared to the sham-field group. There were no significant differences in analgesia between the 2 field-exposed groups.

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 4 s. This analgesic effect was still apparent 3.5 h after the cessation of the treatment. The absolute values for the magnitude of the increases in response latency from baseline values across experiments were consistently between 5 s and 15 s for the magnetic-field exposed rats and about 0 s for the sham-field-exposed rats. This difference in latency is equivalent to rats injected with 4 mg/kg of morphine compared to saline injected controls.

The optimal temporal configurations of the applied magnetic fields explained about 50% of the variance in these response latencies. That simply the number of presentations of the fields during the exposure was not the critical factor to evoke the maximum analgesia was shown in Experiment 4. A time delay of 4 msec between successive presentations of the burst-firing field was less effective than delays of 400 and 4000 msec between field presentations. There also appeared to be an optimal duration of exposure to produce maximum analgesia for both the burst-firing and frequency-modulated patterns. This duration was 30 min. Longer exposures reduced the magnitude of the analgesic effect. Interestingly, many clinical treatments such as massage therapy and acupuncture treatments are often administered for 30 min.

The duration for which each point was maintained at a particular voltage may be an important variable for the production of the analgesic effect. As shown in Experiment 5, point durations of 3 msec were more effective than durations of 1 msec: 1) the 1 msec durations were associated with stronger magnetic fields within the exposure chamber (because of the greater numbers of changes in points per unit time); and 2) the analgesic effects were marginal compared to the same pattern with points presented for 3 msec, the results of this experiment indicated there must be a factor other than simple intensity or dB/dt (change in field strength per unit time) that produces the phenomenon. The greater efficacy for computer-generated magnetic fields with point durations of 3 msec compared to 1 msec to induce overt seizures in rats [13] and to encourage neuroelectrical phenomena such as the "sense of a presence" in humans beings [22], suggests that a critical time is required for the stable maintenance of a given intensity of the applied field to either entrain or resonate with the relevant neuronal activity.

The analgesic effects of the burst-firing magnetic field and the one generated by the May algorithm were comparable. Whereas the rats exposed to the burst-firing field received the same complex structure approximately 380 times during 30 min of exposure, the rats exposed to the continuously generated May algorithm received 330 different structures during the 30 min exposures. One explanation for this similarity of effects for the burst-firing field and a "chaotic" pattern may be some intrinsic "prototypic" structure within both shapes. The burst-firing pattern had been extracted from an exemplary firing of an amygdaloid neuron. It may be possible that any pattern that shares characteristics with a "class" of patterns may evoke similar responses. This effect would be similar for the capacity for large numbers of different pharmacological agents, with quite variant molecular structures, to evoke very similar pharmacokinetic responses, assuming the specific configuration for the ligand-receptor binding is present. Alternatively, we have been under the assumption that whole-body exposure to an appropriately configured magnetic field presented once every 4 s can induce effects directly within the brain based on the results of Lewis et al. [3]. It is interesting to note that, in the article by Lewis and colleagues [3], continuous foot shock also increased tail flick latencies but, unlike intermittent foot shock, these latencies were not reversible by naloxone

or dexamethasone. This concept may apply to the similar level of analgesia attained with exposure to 2 seemingly different magnetic patterns with the outcome or final response appearing to be similar but having different underlying mechanisms.

If Fig. 1 is closely examined, then the periods of "no energy" would be very similar to the single "burst-firing" pattern we digitized from a single cell. This similarity has at least 2 implications. First, the apparent "chaotic" temporal structure of burst-firing neurons may contain intrinsic properties that are common to all cells with this capacity and this capacity is related to the May algorithm. Second, although thousands of neurons also may show their idiosyncratic signatures, they all share an essential pattern that evokes similar properties representative of the entire population. As an example, there are more than 6 billion *Homo sapiens* on the planet. Although each is different, some of the biochemical and electrical characteristics of any single person would be strongly representative of every one of the remaining members of the species.

Pharmacological research often employs "normal" rats to test the efficacy of drug treatments. However, many drugs are effective only when conditions are abnormal. For example, the antipyretic effects of acetylsalicylic acid are only evident if the person exhibits a fever. The same drug and the same dosage do not affect the temperature of a person without a fever. A single trial of medication of an antidepressant can produce favourable effects for depressed patients long after the drug is discontinued. The same antidepressant given to a euthymic individual does not elevate mood.

In general we have not observed "carry over" in analgesia between days in normal rats. The small decreases in response latency with repeated daily testing are more likely the consequences of learning than attenuated analgesia. However, rats with histories of significant multifocal brain damage associated with seizures induced by lithium and pilocarpine displayed persistent analgesia following only 30 min of exposure to the burst-firing field. The persistence was sufficient to be evident in the baseline response latencies one week later.

Previous reports [23] have indicated that damage to the clausturm in animals that have sustained multifocal brain damage, increased nociceptive thresholds to painful stimuli. The claustrum was damaged by inducing chronic epilepsy with therapeutic levels of lithium followed by pilocarpine to induce seizure [13–16]. This structure has been suggested to be a major component of a pathway that influences nociceptive thresholds [23, 24]. Although the biochemical bases for the persistence of the analgesia in brain damaged rats must be identified, the clinical relevance of this observation is obvious. The claustrum has been reported to be an important relay nucleus in the somatosensory pathway [24] and damage to this structure may result in disinhibitory efferent responses. The major destinations of efferent projections arising from the claustrum are the prefrontal, parietal, and limbic cortices [25], all of which have been shown to bind opioid-like molecules and to interact with nociceptive responses [26]. If the claustrum is the major regulator of the pathway that involves these cortical areas, then neuronal loss or reorganization within this structure may disinhibit the pathway and the effects of the magnetic stimuli may be augmented and/or prolonged. Since, the burst-firing pattern was modeled after a single burst-firing neuron in an epileptic patient, there also is the possibility that the central nucleus of the amygdala, which is relatively spared after the brain damage induced by lithium-pilocarpine seizure induction [13–15] is responsible in part for some of the observed effects.

Nitric oxide (NO) and nitric oxide synthase (NOS) have been shown to have binding affinity for receptors in the claustrum [27]. NO and NOS have been reported to exhibit antagonistic effects on opioid-induced antinociception and exposure to magnetic stimuli which typically decreases opioid induced analgesia did not reduce this analgesia when the NOS inhibitor L-name was administered [28]. In general, the analgesic affects of magnetic field treatments have been attributed to the opioid system [2, 9]. Thomas and colleagues [9] have argued that the analgesic effects induced by the same frequency-modulated field employed in our study affected the opioid systems, more specifically, the δ and μ receptors. Antagonists specific for these subtypes of opiate receptors reduced the analgesia induced by the magnetic field treatment. There also has been extensive literature demonstrating that various magnetic field treatments can decrease the analgesia induced by opiate compounds [28] and decrease stress induced analgesia [29]. Our findings are congruent with the antinociceptive observations reported by other researchers [2, 9–11]. In the studies that reported decreased analgesia as a result of magnetic field exposure, the magnetic fields were typically simple in structure and may have had no biological relevance. The magnetic fields used to decrease opiate or stress induced analgesia also were 100–200 times greater in intensity than the intensities employed in the experiments reported here. The intensity may be a critical feature to explain the differing effects; low intensity of the magnetic field may act as a stimulant and a high intensity of the magnetic stimuli may act as an inhibitor. We are not the first to apply this concept to magnetic stimuli [30]. However, this may be a possible explanation for the conflicting results concerning the analgesia in the magnetic field literature. This argument is congruent with the concept of hormesis and has been applied to many different systems (electrical and chemical) since it was introduced over 450 years ago by Aureolus Paracelsus.

There are 2 issues that may be directly relevant to the successful application of this procedure for at least some types of analgesia. First, all of our exposures and testing occurred during the first half of the photophase (between 0730 and about 1200h local time) for the test subjects. Unpublished research from our laboratory have shown that exposure and testing during the first half of the light cycle produces more analgesia than exposure and testing during the last half of the light cycle. As shown by oncological research, the time at which a treatment is given during the circadian cycle has been shown to be critical for its efficacy.

Secondly, isolation of the mechanism by which these weak, complex magnetic fields produce the analgesia at a molecular of even atomic level may facilitate the isolation of the critical temporal patterns that maximize the analgesia. Most physicists would argue that the electric field induced within the organism by the changing applied magnetic field must mediate the effects. Variations of Faraday's Law indicate that the potential difference V, generated by a μ Tesla fields changing approximately every 1 msec (the point durations) in a 0.01 m space (the approximate length of a rat) would be at most within the μV range. Assuming about 50 ohm/cm^2 for the resistance of extracellular fluid and even less resistance for the single lay of charged ions along neuronal membranes, the induced current would be within the high nA to low pA range.

As indicated by Becker [31] even DC currents in the range of nA/cm are sufficient to affect cell growth. If the mechanism for analgesia directly involves a variant of the change in strength of the applied magnetic field per time, then identification and control of the concurrent processes such as resonance-matching

and impedance matching between the temporal structure of the applied magnetic field and the intrinsic characteristics of the targeted biological system may be essential to optimize the analgesic effects. These calculations may also coincide with Becker's DC "current of injury" hypothesis which current in the pA range was found to be required for healing and regeneration [32].

These mechanisms would be required to explain the optimal duration of exposure to the patterned magnetic fields. We found maximum analgesia after about 30 min of exposure. Protracted exposure of 4 h diminished the analgesic effects, even though analgesic effects were still obvious 4 h after exposure for only 30 min. This dilution may reflect a Newtonian-type process reflected as the third law of motion in physics: "for every action there is an equal and opposite reaction." This principle is manifested as homeostasis in physiology, Le Chatellier's principle in chemistry, and reactive inhibition in learning. Interestingly, recent work by Rob Lafrenie (private communication, 17 November, 2003, Northeastern Ontario Regional Cancer Centre, Sudbury, Ontario), who exposed B16 (melanoma) cell lines to the frequency-modulated pattern continuously for either 1 h, 2 h, or 4 h, showed that MAP-Kinase and ERK-1 and ERK-2 pathways were not activated after 1 h of exposure, were partially activated after 2 h, and were clearly activated after 4 h of exposure. If this latency of response for signalling is generalizable, then the protracted presentation of even the most optimal temporal structure of magnetic field pattern may ultimately, loose their analgesic properties as counter processes become initiated.

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