Shielding, but not zeroing of the ambient magnetic field reduces stress-induced analgesia in mice

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Magnetic field exposure was consistently found to affect pain inhibition (i.e. analgesia). Recently, we showed that an extreme reduction of the ambient magnetic and electric environment, by μ -metal shielding, also affected stress-induced analgesia (SIA) in C57 mice. Using CD1 mice, we report here the same findings from replication studies performed independently in Pisa, Italy and London, ON, Canada. Also, neither selective vector nulling of the static component of the ambient magnetic field with Helmholtz coils, nor copper shielding of only the ambient electric field, affected SIA in mice. We further show that a pre-stress exposure to the μ -metal box is necessary for the anti-analgesic effects to occur. The differential effects of the two near-zero magnetic conditions may depend on the elimination (obtained only by μ -metal shielding) of the extremely weak time-varying component of the magnetic environment. This would provide the first direct and repeatable evidence for a behavioural and physiological effect of very weak time-varying magnetic fields, suggesting the existence of a very sensitive magnetic discrimination in the endogenous mechanisms that underlie SIA. This has important implications for other reported effects of exposures to very weak magnetic fields and for the theoretical work that considers the mechanisms underlying the biological detection of weak magnetic fields.

Keywords: near-zero magnetic fields; nociception; stress-induced analgesia

1. INTRODUCTION

One biological function that has been consistently shown to be affected by exposure to magnetic fields is nociceptive or pain sensitivity. Analgesia (i.e. reduced pain sensitivity) has been frequently shown to be reduced by magnetic fields in various species of animals. Exposure to oscillating, extremely low frequency (ELF) magnetic fields reduced exogenous opiate (e.g. morphine) and endogenous opioid (e.g. stress-induced) mediated analgesia in mice (Kavaliers *et al.* 1984; Betancur *et al.* 1994; Del Seppia *et al.* 2000), land snails (Kavaliers *et al.* 1990, 1984; Kavaliers & Ossenkopp 1991, 1994) and homing pigeons (Del Seppia *et al.* 1995). Also, ELF magnetic fields induced modifications in pain perception thresholds (Papi *et al.* 1995) and pain-related somatosensory evoked potentials in humans (Sartucci *et al.* 1997).

The results of these studies strongly suggest that nociceptive responses are sensitive to various magnetic fields. Ossenkopp *et al.* (1983) showed that variations in the natural earth magnetic field (i.e. geomagnetic field) also affected pain sensitivity in mice, with greater nociceptive sensitivity and lower morphine-induced analgesia during a geomagnetic storm. This suggests that nociceptive responses may be sensitive to subtle variations in ambient

magnetic fields. This leads us to question what effects the absence of the geomagnetic field may have on nociceptive sensitivity and pain sensation.

The geomagnetic field can be eliminated by two different means (reviewed in Wiltshko & Wiltshko 1995): either through (i) shielding by using a magnetic-opaque material (e.g. μ -metal), or (ii) by vector nulling the local natural magnetic field with a coil system by running a current that generates a field equal and opposite to the local geomagnetic field (zeroed field). Both of these approaches have been used in studies where these near-zero magnetic conditions were either investigated directly (e.g. Kopanav *et al.* 1979; Asashima *et al.* 1991) or used as the 'sham' control group (e.g. Dawson *et al.* (1998), Rojavin *et al.* (1998) and Weiss *et al.* (1992) used μ -metal shielding, while Vorobyov *et al.* (1998) and Thomas *et al.* (1997a,b, 1998) used a vector-nulled magnetic field). It should be noted that the μ -metal box attenuates both the ambient static as well as the ambient time-varying ELF magnetic fields, while the vector-nulling (zeroing) approach zeroes only the static component. Also, the μ -metal, but not the vector nulling, shields the ambient electric fields.

We have recently shown that a brief exposure to either a regular 37 Hz sinusoidal or an irregularly varying ELF (<1 Hz) magnetic field increased basal nociceptive sensitivity in male C57 mice. Also, exposure to a hypogeomagnetic environment, obtained by shielding the ambient, laboratory-environment, magnetic fields with μ -metal, increased nociceptive sensitivity in laboratory mice in a

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manner similar to that caused by exposure to ELF magnetic fields (Del Seppia *et al.* 2000).

However, before any definitive conclusions regarding the possible effects of exposure to near-zero static ELF magnetic fields can be drawn, the generality of these effects on nociceptive sensitivity needs to be examined. First, as there are significant strain differences in basal nociceptive sensitivity and in response to various treatments (Mogil *et al.* 1999a,b), the findings obtained with the inbred C57 strain of mice need to be confirmed with outbred strains. Second, as the geomagnetic field, and superimposed laboratory magnetic fields, vary in space and time (reviewed in Olcese *et al.* (1988) and Wiltshko & Wiltshko (1995)), and this can influence nociceptive sensitivity (Ossenkopp *et al.* 1983), the effects of elimination of the ambient magnetic field at different geographical locations need to be investigated. Third, examination of the effects of different modes and duration of elimination of the geomagnetic field (i.e. shielding versus zeroing) needs to be performed.

Here we present: (i) a repetition in Pisa, Italy (with Swiss CD1 mice) of the original study carried out with C57 mice (Del Seppia *et al.* 2000) examining the effects on stress-induced analgesia (SIA) of exposure in a μ -metal box; (ii) a replication of the Italian study in a different geographical location (London, ON, Canada), including a direct comparison of the effects of shielding the geomagnetic field with μ -metal or zeroing the static component of the ambient magnetic field with Helmholtz coils; (iii) a full replication of the previous experiments with the μ -metal shielding and examinations of the effects of just eliminating the electric field using a copper box; and (iv) an examination of the effects of different durations of the exposure to the μ -metal shielding on nociceptive responses of mice.

2. MATERIAL AND METHODS

(a) *Animals*

Adult male and female Swiss CD1 mice (Charles Rivers, Italy for experiment 1; Charles Rivers, Canada for experiments 2, 3 and 4), two to four months old and weighing 25–35 g were used. The Swiss CD1 mice are an outbred strain commonly used in laboratories throughout the world. Because they are outbred, their genotype presents a high degree of variability and genetic differences may be expected between mice purchased from two geographically distant branches of the same provider. Detailed information about the genetic background of the strain of mice used in the present experiments is available on the Charles Rivers Web site: <http://www.criver.com/>. Mice were housed in same-sex groups of six in polyethylene cages (38 cm $\times$ 25 cm $\times$ 15 cm) under a 12 L:12 D cycle (light: 07.00–19.00) at $21 \pm 2^\circ\text{C}$. Food and water were freely available. The animals were held, and all of the experiments were conducted, according to the European Union and Canadian guidelines for laboratory animal care.

(b) *Assessment of nociception*

Nociception was measured as the latency of a foot lifting/lick to an aversive thermal stimulus (the 'hot-plate test': model DS37 (Ugo Basile, Comerio, Italy) in experiment 1; model HP (AccuScan Instruments Inc., Columbus, OH, USA) in experiments 2, 3 and 4), at $50 \pm 0.5^\circ\text{C}$. After displaying a nociceptive

response mice were quickly removed from the heated surface. As the maximum latency observed was 45 s, all mice were removed from the heated surface before the cut-off time of 60 s.

(c) *Magnetic exposure conditions*

The various magnetic fields used in the experiments are shown in table 1. Magnetic fields in the Italian study (Pisa) were measured by a gaussmeter (GM1A, probe PM-85, Applied Magnetics Laboratory, Baltimore, USA) and in the Canadian studies (London, ON, Canada) by a 3D fluxgate magnetometer (MAG-03MS 1000, Bartington Instruments Ltd, Oxford, UK).

The ' μ -metal box I' used in experiments 1 and 2 was cubic (27.5 cm per side) and made of 2 mm thick μ -metal (Inform System, Milano, Italy), an alloy made of iron and nickel. The box had its floor covered with plywood and was ventilated through 30 small holes (8 mm diameter) in the upper part of two walls, rendering the inside of the box almost dark. A control wooden box, identical in shape to the μ -metal box I and into which the mice were placed directly, was used.

The ' μ -metal box II' (33 cm $\times$ 38 cm $\times$ 20 cm) used in experiments 2, 3 and 4 was made of 1 mm thick μ -metal (Magnetic Shield Corp., Bensenville, IL, USA). The box had one hole (2.5 cm diameter) at each of the four corners (1 cm off the sides) of both the base and the top surfaces. A μ -metal cylinder (2.5 cm high) surrounded each hole, shielding the ambient magnetic field (table 1) and, partially, the light. During the experiment the box was placed on top of a pedestal that held it 4.5 cm off the surface, thus allowing air circulation through the top and bottom holes. Individual mice were placed in a 26 cm $\times$ 16 cm $\times$ 12 cm clean translucent polyethylene cage, covered by a clear Plexiglas top with 15 ventilation holes (diameter of 8 mm) that was inserted in the μ -metal box II. To reduce possible light reflection, in experiment 3 the inside of the μ -metal box II was laminated with 5 mm thick black Plexiglas. A cardboard box identical to the μ -metal box II was used as the control condition (darkened box).

The 'copper box' used in experiment 3 was identical in size to the μ -metal box II and was made of copper-clad (0.034 mm thick) Fiberglas Epoxy Resin board (2 mm thick) (FR4 Board, Injectorall Electronics, New York, USA). The Fiberglas control box was made identical to the copper box without the copper coating. The inside of the copper and the control boxes was laminated with black Plexiglas (5 mm thick) to reduce light reflection. The mice were exposed in the polyethylene cage as in experiment 2, which was placed inside the boxes. Table 2 shows the magnetic field attenuation of the μ -metal box and the copper box at various field frequencies.

The 'vector-zeroed field' in experiments 2 and 3 was generated by three mutually orthogonally nested Helmholtz coils (diameters: 2.0 m (horizontal north/south), 1.75 m (horizontal east/west) and 1.5 m vertical) and each consisted of 10 turns of 8 gauge stranded conductor wound on Lexan frames (Parker Plastics, London, ON, Canada). Each Helmholtz pair was driven by a Techron constant-current amplifier (CCA) (model 7780EI6, Crown International, Elkhart, IN, USA) via digital to analogue (D/A) converter. A 9 pole Bessel low-pass filter (LPF, 1 kHz corner frequency) before the amplifier input removed high-frequency noise from the D/A converter signal (Thomas *et al.* 2001). The magnetic field was monitored with a 3D fluxgate magnetometer through an analogue to digital (A/D) converter (MAG-03MS 1000 $\mu\text{T} \pm 50\text{ nT}$ offset, 12 pT_{rms} resolution; Bartington Instruments Ltd, Oxford, UK).

Table 1. Characteristics of the magnetic field exposures in the three experiments.

(Vertical (B_V), horizontal (B_H) and total (B_T) values of the magnetic vectors (μT) are reported. The magnetic fields in the two μ -metal boxes (rows 1, 3 and 6), the coils generating the zeroed field (rows 4 and 7), the copper box (row 8) and the respective control groups (rows 2, 5 and 9) are shown.)

	static (DC) magnetic field (μT)			time-varying (60 Hz) magnetic field (μT)		
	B_H	B_V	B_T	B_H	B_V	B_T
μ -metal box I (experiments 1 and 2)	0.04	0.05	0.07	—	—	—
wooden box (experiment 1)	23.4	39.9	46.2	—	—	—
μ -metal box II (experiment 2)	0.07	0.06	0.09	—	—	—
zeroed field (experiment 2)	0.03	0.02	0.04	—	—	—
darkened box (experiment 2)	11.6	50.0	51.3	—	—	—
μ -metal box II (experiment 3)	0.24	0.25	0.34	< 0.001	< 0.001	< 0.001
zeroed field (experiment 3)	0.07	0.08	0.10	0.08	0.06	0.10
copper box (experiment 3)	13.37	51.06	52.78	0.10	0.05	0.12
control box (experiment 3)	14.72	39.54	42.19	0.15	0.07	0.17

Table 2. Magnetic field attenuation measured at various frequencies (left column) with a Bartington flux gate magnetometer within the copper and μ -metal boxes relative to air.

frequency (Hz)	attenuation	
	copper box	μ -metal box
0.00×10^1	1.000×10^0	$8.000\ 0000 \times 10^{-3}$
5.80×10^1	9.980×10^{-1}	$7.230\ 6580 \times 10^{-3}$
1.25×10^2	9.900×10^{-1}	$1.749\ 1609 \times 10^{-2}$
2.50×10^2	9.500×10^{-1}	$3.221\ 6495 \times 10^{-2}$
3.03×10^2	9.300×10^{-1}	$3.163\ 5558 \times 10^{-2}$
5.88×10^2	7.700×10^{-1}	$8.264\ 4628 \times 10^{-2}$
1.25×10^3	4.200×10^{-1}	$8.628\ 1277 \times 10^{-2}$
2.50×10^3	1.413×10^{-1}	$1.162\ 5203 \times 10^{-1}$

(d) Experimental procedures

Four experiments were run; one in Pisa, Italy ($43^\circ 41' \text{ N}$, $10^\circ 23' \text{ E}$) and three in London, Ontario, Canada ($43^\circ 02' \text{ N}$, $81^\circ 09' \text{ W}$). The local ambient magnetic fields, measured in the control conditions, are shown in table 1 and reveal that at the two locations the differences in the horizontal (B_H), vertical (B_V) and total (B_T) ambient magnetic field were: experiments 1–2, $B_H = 11.8 \mu\text{T}$, $B_V = 10.1 \mu\text{T}$, $B_T = 5.1 \mu\text{T}$; and experiments 1–3, $B_H = 8.68 \mu\text{T}$, $B_V = 0.36 \mu\text{T}$, $B_T = 4.01 \mu\text{T}$. All of the experiments were run during the mid-light phase in an illuminated room and all of the boxes were cleaned with alcohol between different experimental mice.

The general experimental procedure for all of the experiments except part of experiment 4 (details later) consisted of the following steps.

- Nociceptive responses of mice were recorded prior to any experimental exposure 'pre-nociceptive responses'.
- Mice were maintained under various magnetic exposure conditions for 90 min.
- Mice were immobilized for 30 min inside translucent polypropylene 50 ml centrifuge tubes. While being restrained, stressed mice were kept under the same experimental magnetic exposure condition as in step (ii).
- Measurement of the nociceptive responses of mice after restraint stress was performed in a manner identical to that in step (i), 'post-nociceptive response'.

In experiment 1 (Pisa, Italy), two groups of male and female CD1 mice were used. Mice of the first group ($n = 12$) during step (ii) of the study were individually placed inside the μ -metal box I. A second control group of mice ($n = 13$) was placed in the control wooden box identical to the μ -metal box I and were exposed to the local ambient magnetic field (table 1).

In experiment 2 (London, ON, Canada) the study was repeated with the two μ -metal boxes following a similar procedure with a total of five groups of male CD1 mice: group 1, wooden box, $n = 25$; group 2, μ -metal box I, $n = 41$; group 3, darkened polyethylene box (control for group 4), $n = 15$; group 4, μ -metal box II, $n = 15$; group 5, vector-zeroed field, $n = 30$. In this last group, the mice were placed at the centre of three orthogonally nested Helmholtz coils (details of coils and boxes in § 2c) that generated a magnetic field that vector nulled the local ambient magnetic field and were held in the same box used for group 1.

Experiment 3 (London, ON, Canada) examined whether the effects of the μ -metal box were due to its shielding of the ambient electric field together with the magnetic field. Experiment 3 included four groups of mice: group 1, control box, $n = 20$; group 2, μ -metal box II, $n = 21$; group 3, zeroed field, $n = 20$; group 4, copper box, $n = 21$. The latter shielded the ambient electric field whilst leaving the magnetic field unaltered (less than 5% attenuation of the magnetic field for frequencies below 300 Hz as shown in table 2).

In experiment 4 (London, ON, Canada) the effects of the

duration of exposure to the μ -metal box shielding of the geomagnetic field on the nociceptive sensitivity were examined. A total of four groups of mice were run. Two groups (μ -metal box II, $n = 15$, and control, $n = 15$) underwent the same general experimental procedure as described above (2 h of total exposure to the magnetic condition) while two other groups of mice underwent the same procedure except for step (ii). Immediately after the pretest for nociceptive sensitivity, mice were restrained in the tube and individually placed in the polyethylene cage, which was then introduced either in the μ -metal box II ($n = 15$) or in the darkened polyethylene box (control, $n = 14$) for 30 min. At the end of the exposure the post-nociceptive responses of the mice were determined. The total exposure time of these two groups of mice was 30 min instead of 2 h.

(e) Statistical analyses

Response latencies were analysed with a mixed-design repeated-measure analysis of variance (ANOVA) with an exposure condition (two levels in experiment 1: μ -metal box I, wooden box; five levels in experiment 2: μ -metal box I, wooden box, zeroed field, darkened polyethylene box, μ -metal box II; four levels in experiment 3: control box; μ -metal box II, zeroed field, copper box) as the independent factor and latency (two levels: pre and post) as the repeated measure within subject-dependent variable. Data from experiment 4 were analysed with a 2×1 repeated measure ANOVA with exposure condition (two levels: control and μ -metal II box) and duration of treatment (two levels: 2 h, 30 min) as the two between-factor variables and latency (two levels: pre and post) as the repeated measure within subject-dependent variable. Mean comparisons between the various groups of treatment/exposure were planned *a priori* in the ANOVA model. Also, in all of the experiments for each group a 0×1 repeated-measure ANOVA was used to compare the pre- and post-latencies. All of the analyses were run with the SUPERANOVA (Abacus Concepts, Inc., Berkeley, CA, USA) statistical package with a level of significance of $p < 0.05$.

3. RESULTS

(a) Experiment 1 (Pisa, Italy)

While exposure to the wooden control box had no significant effects on nociceptive sensitivity, a 2 h stay in the μ -metal box significantly decreased the response latencies, which was indicative of a significant increase in nociceptive sensitivity (figure 1). There was a significant main effect of the repeated measure, latency ($F_{1,23} = 6.49$, $p = 0.018$) and a trend of the interaction of latency $\times$ exposure condition ($F_{1,23} = 3.07$, $p = 0.093$). While there was no difference between the pre- and post-nociceptive responses ($F_{1,12} = 0.342$, n.s.) of the mice held in the wooden box, there was a significant reduction from the pre- to the post-latency ($F_{1,11} = 8.544$, $p = 0.014$) of the mice held in the μ -metal box. The post-latencies of the mice kept in the μ -metal box were significantly lower than those of the mice kept in the wooden-box ($F_{1,23} = 4.855$, $p = 0.038$).

(b) Experiment 2 (London, ON, Canada)

While exposure to both μ -metal boxes significantly reduced nociceptive sensitivity, exposure to a zeroed field had no evident effect (figure 2). The overall ANOVA revealed significant main effects of latency ($F_{1,121} = 18.17$, $p = 0.0001$) and exposure ($F_{4,121} = 5.038$, $p = 0.0009$) and

Figure 1. Mean ($\pm$ s.e.m.) latency (s) of foot-lifting responses to an aversive thermal stimulus of the two groups of Swiss CD1 mice used in experiment 1 before (pre) and after (post) the experimental treatment. Mice were restraint stressed for 30 min after having been kept for 90 min in a wooden box (ambient magnetic field) or in the μ -metal box I in Pisa, Italy. (See § 2d for details.)

Figure 2. Mean ($\pm$ s.e.m.) latency (s) of foot-lifting responses to an aversive thermal stimulus of the two groups of Swiss CD1 mice used in experiment 2 before (pre) and after (post) the experimental treatment. Mice were restraint stressed for 30 min after having been kept for 90 min in: a wooden box (ambient magnetic field); the μ -metal box I; a wooden box placed at the centre of a Helmholtz coils system that vector nulled the geomagnetic field (zeroed field); the darkened control box (ambient magnetic field); or the μ -metal box II in London, ON, Canada. (See § 2d for details.)

of their interaction ($F_{4,121} = 9.594$, $p = 0.0001$). Mean comparisons showed that the post-latencies of the μ -metal groups were lower than those of the wood (μ -metal box I: $F_{1,65} = 11.47$, $p = 0.0010$) and control darkened box (μ -metal box II: $F_{1,29} = 18.80$, $p = 0.0001$) and the zeroed field (μ -metal box I: $F_{1,70} = 17.44$, $p = 0.0001$; μ -metal box II: $F_{1,44} = 28.54$, $p = 0.0001$) groups. The post-latency was higher than the respective pre-latency in the wood ($F_{1,24} = 19.68$, $p = 0.0002$) and zeroed-field ($F_{1,29} = 16.73$, $p = 0.0003$) groups, which was indicative of the induction of restraint SIA. This analgesic response was not evident

Figure 3. Mean ($\pm$ s.e.m.) latency (s) of foot-lifting responses to an aversive thermal stimulus of the two groups of Swiss CD1 mice used in experiment 2 before (pre) and after (post) the experimental treatment. Mice were restraint stressed for 30 min after having been kept for 90 min in: a darkened control box (ambient magnetic field); the μ -metal box II; the control box placed at the centre of a Helmholtz coils system that vector nulled the geomagnetic field (zeroed field); or the copper box in London, ON, Canada. (See text for details.)

in the mice held in the μ -metal box I ($F_{1,74} = 0.67$, n.s.), while the mice exposed to the μ -metal box II ($F_{1,14} = 12.95$, $p = 0.0029$) showed a significant reduction in their thermal responses.

(c) Experiment 3 (London, ON, Canada)

Elimination of the electric field (copper box) did not reduce SIA (figure 3). The overall ANOVA revealed significant main effects of latency ($F_{1,78} = 55.57$, $p = 0.0001$) and exposure ($F_{3,78} = 3.70$, $p = 0.015$) and of their interaction ($F_{3,78} = 8.90$, $p = 0.0001$). Mean comparisons showed that the post-latency of the μ -metal box II group was lower than that of the control darkened box ($F_{1,40} = 14.93$, $p = 0.0002$), the zeroed field ($F_{1,40} = 15.74$, $p = 0.0002$) and also the copper box ($F_{1,41} = 5.02$, $p = 0.0279$) groups. The post-latency was higher than the respective pre-latency in the control ($F_{1,19} = 28.14$, $p = 0.0001$), zeroed field ($F_{1,19} = 19.47$, $p = 0.0003$) and copper box ($F_{1,20} = 17.90$, $p = 0.0004$) groups, which was indicative of the induction of restraint SIA. This analgesic response was not evident in the mice held in the μ -metal box II ($F_{1,20} = 1.01$, n.s.).

(d) Experiment 4 (London, ON, Canada)

The results of experiment 4 repeated the inhibitory effects of a 2 h stay in the μ -metal box on nociceptive sensitivity and analgesia (figure 4) and further showed that a 30 min exposure to the μ -metal box had no significant effect on response latency. There was a significant main effect of latency ($F_{1,74} = 18.93$, $p = 0.0001$) and a trend for exposure condition ($F_{2,74} = 3.78$, $p = 0.057$). The binary interactions of exposure condition $\times$ exposure duration ($F_{1,55} = 4.12$, $p = 0.047$) and latency $\times$ exposure duration ($F_{1,55} = 18.31$, $p = 0.0001$) were significant, while the interaction latency $\times$ exposure condition showed a statisti-

Figure 4. Mean ($\pm$ s.e.m.) latency (s) of foot-lifting responses to an aversive thermal stimulus of the four groups of Swiss CD1 mice used in experiment 3 before (pre) and after (post) the experimental treatment. Two groups of mice were restraint stressed for 30 min after having been kept for 90 min in a wooden box (ambient magnetic field) or in a μ -metal box (2 h total exposure). Two different groups of mice were restraint stressed for 30 min without being pre-exposed to the control (ambient magnetic field) or μ -metal box experimental condition (30 min total exposure). Experiment 3 was run in London, ON, Canada. (See text for details.)

cal trend ($F_{1,55} = 3.68$, $p = 0.060$). As well, the three-way interaction latency $\times$ exposure condition $\times$ exposure duration was significant ($F_{1,55} = 13.91$, $p = 0.0005$). The 2 h exposure and restraint stress induced a significant increase (post higher than pre) in the response latencies of the control mice ($F_{1,14} = 10.72$, $p = 0.0055$), which was indicative of the induction of analgesia, while in the μ -metal group, the post-latencies were lower than the pre-latencies ($F_{1,14} = 12.95$, $p = 0.0029$). In the mice that were exposed for 30 min there was also a significant analgesic response both in the control ($F_{1,13} = 13.34$, $p = 0.0029$) and in the μ -metal group ($F_{1,13} = 15.65$, $p = 0.0014$). The post-latencies of the mice exposed to the μ -metal box for 2 h were lower than either those of their respective control mice ($F_{1,14} = 16.57$, $p = 0.0002$) or those of mice that were exposed to the μ -metal box for only 30 min ($F_{1,14} = 19.43$, $p = 0.0001$). The post-latencies of the μ -metal and control groups of mice exposed for a total of 30 min were similar ($F_{1,14} = 4.7$, n.s.). Also, the analgesic responses displayed by the control groups that stayed in the darkened polyethylene box for a total of either 2 h or 30 min were similar ($F_{1,14} = 0.13$, n.s.). Together, the results of experiment 4 confirm those of experiment 2 obtained with the μ -metal box shipped to Canada from Italy, showing that a 2 h stay in the μ -metal box eliminated SIA. Moreover, results of experiment 4 show that a short (30 min) stay in the μ -metal box is not sufficient for the effects of ambient magnetic field shielding on SIA to occur.

In all of the analyses there were no significant differences between the pre-exposure nociceptive response values of the various groups.

4. DISCUSSION

The present series of experiments repeatedly showed that a 2 h stay in a μ -metal box (I and II) resulted in a significant decrease in response latencies, which indicated a significant increase in nociceptive sensitivity and reduction in SIA. The results of two distinct experiments showed that the inhibitory effect of the near-zero magnetic field on SIA depended on the mode of production. SIA was significantly reduced in the CD1 mice by exposure to a μ -metal box but not to a zeroed (vector-nulled) magnetic field. The effect of the μ -metal box was specific to the shielding of the ambient magnetic field, as a 2 h exposure within a copper box, which shields the electric field, did not affect SIA. In addition, a prestress exposure to the μ -metal box is necessary for the inhibitory effects on SIA to occur. The suppression of SIA observed in the μ -metal box condition is due to the specific treatment, and is not the result of exposure to just a novel environmental condition. Mice kept in an identical condition (i.e. degree of novelty, illumination and environment characteristics) in the control groups of all of the four experiments showed significantly higher SIA.

The elimination of the analgesic response by exposure to the μ -metal box observed in the present study confirms and extends previous results with C57 mice (Del Seppia *et al.* 2000). Here the inhibitory effects of the exposure in the μ -metal box on SIA were replicated with another strain of mice (CD1), in two different geographical locations (Pisa, Italy and London, ON, Canada), by different experimenters and with two different μ -metal boxes (with an analogous attenuation factor). The absence of SIA in the control mice of experiment 1 (figure 1) may be ascribed to differences in housing and handling procedures as well as to possible genetic differences between the Swiss CD1 mice purchased in Italy and Canada (see Cirulli *et al.* 2000). Independently from differences in the response to restraint stress, the reduction of the nociceptive responses induced by μ -metal shielding of the ambient magnetic field was observed in both locations. This effect was somewhat greater in experiment 4 where exposure to the μ -metal box reduced nociceptive responses of the mice to levels significantly lower than the pre-measure responses. These differences in response to the same experimental manipulation may be ascribed to genetic differences in the subpopulations of mice belonging to an outbred strain. Overall, the SIA-reducing effect elicited by μ -metal elimination of the ambient magnetic field appears to be a highly reproducible and robust phenomenon.

The inhibitory effects on SIA induced by the μ -metal exposure is similar to that observed following exposure to various exogenous magnetic fields in mice and other species (Ossenkopp *et al.* 1985; Kavaliers & Ossenkopp 1986, 1987, 1988a,b, 1991; Ossenkopp & Kavaliers 1987; Betancur *et al.* 1994; Ghione *et al.* 1994; Del Seppia *et al.* 1995, 2000; Papi *et al.* 1995; Prato *et al.* 1995, 1996a,b; Sartucci *et al.* 1997). Those studies showed that the effect of artificial magnetic fields on SIA is similar to that obtained after administration of the prototypic opiate antagonist naloxone, supporting an inhibitory effect of various magnetic fields on opioid-mediated responses. Exposures to exogenous ELF magnetic fields (e.g. Papi *et*

al. 1992; Luschi *et al.* 1996) and to a near-zero magnetic field created by μ -metal shielding of the geomagnetic field have similar inhibitory effects on opioid functions (Del Seppia *et al.* 2000). In both the present study involving a decreased magnetic-field-exposure condition, and previous studies using artificial (greater than normal) magnetic fields, the experimental procedures involved the exposure to magnetic-field conditions that were different from those that the animal experienced during its normal life, and outside the range in which the species has evolved. This suggests that, as for other environmental factors (such as temperature or gravity), decreases as well as increases in normal ambient magnetic-field conditions can induce alterations in physiology and/or in behaviour. Taken together, these data indicate that exposure to an abnormal magnetic-field condition has an inhibitory influence on the endogenous opioid system, an important and phylogenetically conserved mechanism involved in stress compensation (Akil *et al.* 1984).

Surprisingly few studies have examined the biological effects of near-zero magnetic environments. Several studies that have utilized shielding or active zeroing of the geomagnetic field as a control group for investigation of various biological effects of exogenous magnetic fields, do not allow an evaluation of the effects of the near-zero magnetic condition *per se* because they do not include a group of animals exposed to the ambient magnetic field (e.g. Michael 1977; Weiss *et al.* 1992; Dawson *et al.* 1998; Rojavin *et al.* 1998; Vorobyov *et al.* 1998; Thomas *et al.* 1997a,b, 1998). Few studies have examined the biological effects of shielding the ambient magnetic field (Kopanev *et al.* 1979; Chew & Brown 1989; Asashima *et al.* 1991; Tipping *et al.* 1999). Various biological effects have also been reported with the near-zero magnetic environment being created by vector nulling of the ambient magnetic field with energized coil systems (Miro *et al.* 1970; Bliss Heppner 1976; Ioalè & Teyssèdre 1989; Lednev *et al.* 1996; Thomas *et al.* 1997b; Mouritsen 1998; Morariu *et al.* 2000). All of these studies involved different species and biological responses, leading to incongruent findings that do not allow any overall conclusions. The present results, showing differential effects of two modes of near-elimination of the ambient magnetic field on the same biological response, suggest a means of resolving these discrepant findings.

The present study is, to our knowledge, the first that directly compares the effects of two modalities of creation of a near-zero magnetic field condition on a biological function, nociception, that has been repeatedly found to be affected by alterations of the magnetic environment (Kavaliers *et al.* 1984; Betancur *et al.* 1994; Del Seppia *et al.* 2000). The reason for the differences between the inhibitory effects on SIA obtained with μ -metal shielding and the absence of an effect observed with vector nulling is, at present, unclear. The values of the static magnetic fields measured in the two conditions in experiment 2 were both very close to zero and very similar, though not identical, with the difference being in the order of 0.01–0.03 μ T (table 1). The different responses of mice to the two near-zero magnetic exposures may reflect these minor differences in the static magnetic field present during exposure.

Alternatively, mice may be responding to slight tem-

poral variations in the geomagnetic field or to the ELF magnetic fields present in the ambient environment caused by electrical power distribution and use. As a result of interactions between the solar electromagnetic radiation and the geomagnetic field, the local natural magnetic field varies both in space and in time (reviewed in Burch (2001)). At northern latitudes where our experiments were run, short-term, temporal variations of the geomagnetic field are around $0.07 \mu\text{T}$ (reviewed in Wiltshko & Wiltshko (1995)). While the μ -metal box attenuated all of the ambient ELF magnetic fields, the Helmholtz coils, through vector nulling in the three dimensions, eliminated only the static component of the ambient magnetic field, leaving the temporal fluctuations unaltered (in our experiment 3 these were $0.17 \mu\text{T}$, table 1). The nociceptive responses of mice may respond differently to an almost total absence of magnetic field (μ -metal box) and to the residual time-varying component of the geomagnetic field that is not zeroed by the Helmholtz coils. Both of these hypotheses suggest that mice are sensitive to extremely small, either static or time-varying, differences between the two near-zero magnetic environments.

Alternatively, vector nulling, in the zeroed-field condition, may be different from shielding of the magnetic field, as in the latter the magnetic vector potential may not be eliminated (Aharonov & Bohm 1959). This implies that the biophysical magnetic-field-detection mechanism was sensitive to the magnetic vector potential rather than directly to the magnetic field. However all magnetic-field-detection mechanisms (Walker *et al.* 1997; Lohmann & Johnsen 2000) explored to date do not possess such differential sensitivity.

A variety of different mechanisms, including induced electric currents, magnetite, radical pair recombination and resonance interactions (e.g. parametric resonance model), have been proposed to explain magnetic-field detection and effects (Kirschvink & Walker 1985; Lednev 1991, 1996; Adair 1994; Prato *et al.* 1995, 2000; Brocklehurst & McLauchlan 1996; Lednev *et al.* 1996). Through judicious manipulation of both the static and ELF magnetic field exposure conditions, evidence has accumulated suggesting that magnetic-field effects are likely to follow a parametric resonance-related mechanism (Prato *et al.* 1995, 1996a,b, 2000) rather than one being dependent on either induced currents, radical pairs or magnetite. This does not, however, preclude a possible role for other mechanisms. Whether these effects of magnetic fields occur in various locations throughout an organism or there exists a specific organ for the detection of magnetic fields is not known. Also, the existence and location of a specific magnetic-field receptor in animals, has been, and still is, a matter of controversy (reviewed in Papi (1992)). Magnetic fields have been proposed to alter the properties and stability of biological membranes, their transport characteristics, and the intra- and extracellular distribution and flux of calcium ions. Results of a number of investigations have shown that the inhibitory effects of magnetic fields on opioid analgesia in snails involve alterations in the functioning of calcium and/or flux of calcium ions (Kavaliers & Ossenkopp 1987, 1991) consistent with a resonance model (Prato *et al.* 1995), as well as alterations in protein kinase C-associated and nitric oxide and nitric oxide synthase-related transduction mechanisms

(Kavaliers *et al.* 1998; Kavaliers & Prato 1999; Prato *et al.* 2000). The absence of magnetic field influencing biological systems following similar mechanisms remains to be determined, although Lednev *et al.* (1996) suggested that effects seen at near-zero (vector-nulled) magnetic field conditions are related to the parametric resonance model.

In summary, the results of the present study show that a reduction in SIA can be obtained by exposing mice for 2 h to a near-zero magnetic field created by μ -metal shielding but not when the near-zero condition is obtained by vector nulling the ambient magnetic field with Helmholtz coils. This suggests that the two conditions are not equivalent and that care should be taken when selecting the near-zero magnetic conditions, particularly when obtained with μ -metal shielding, as the 'control' group in studies on the biological effects of magnetic fields. Furthermore, our data suggest that mice are sensitive to variations in the ambient ELF magnetic field possibly as low as $0.01 \mu\text{T}$. Theoretical models of the interactions of magnetic fields with biological systems (reviewed in Walker *et al.* (1997) and Lohmann & Johnsen (2000)) predict that these fields are too weak to be detected (Weaver & Astumian 1990; Adair 1994, 1998; Polk 1994). The present results suggest that such models need to be expanded to include low-intensity (*ca.* $0.1 \mu\text{T}$) static and time-varying magnetic fields.

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