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Non-Thermal Extremely Low Frequency Magnetic Field Effects on Opioid Related Behaviors: Snails to Humans, Mechanisms to Therapy

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In 1984, it was initially discovered in mice that an extremely low frequency magnetic field (ELF-MF) could attenuate opiate induced analgesia. In the past 30 years, we defined some of ELF-MF exposure and subject state conditions that can both increase and decrease nociception in snails and mice and can induce analgesia in humans. In our search for mechanisms and our desire to translate our findings to the treatment of chronic pain in humans, we pioneered the use of electroencephalography and magnetic resonance imaging to monitor effects during exposure. We have contributed to an understanding of the phenomena but a considerable amount remains to be done by us and those who have undertaken corroboratory and complimentary work. As the recipient of the 2013 d'Arsonval Award, I was invited to prepare an article for Bioelectromagnetics that highlights research findings that led to the award. Here, I have focused on our main findings associated with the effects of nociception of exposure to ELF-MF. To enrich the value of this contribution, I have put our research into the context of work of others. Further, I have suggested future directions of research and the potential for linkages and synergies associated with the extensive literature on animal orientation. Hence, it needs to be acknowledged that this is a report of our contributions and not intended as a balanced review. Bioelectromagnetics. 36:333–348, 2015. © 2015 Wiley Periodicals, Inc.

Key words: magnetoreception; pulsed magnetic field therapy; nociception; analgesia; animal orientation and navigation

INTRODUCTION

A true highlight of my career in bioelectromagnetics was to be awarded the d’Arsonval Medal at the BioEM 2013 meeting in Thessaloniki, Greece. Traditionally, this award is accompanied with a request from the journal’s editor to prepare a report for the Bioelectromagnetics Society that focuses on the work for which the d’Arsonval Medal was given. As such I focused, using a chronological approach, on work done in our laboratory at the Lawson Health Research Institute (formerly Lawson Research Institute, London, Ontario, Canada). This approach resulted in a somewhat solipsistic document; however, I took the opportunity to make significant references to the seminal work done by others that influenced and helped direct our work and suggest new directions to take. It will be that I have left out other significant contributions and for that I apologize. However, only about 30% of the cited literature are those in which I am listed as an author. I also must state that listing myself as the only author in this review should not be taken to mean that this body of work is only attributable to me. Yes, I inspired and funded much of the work but none of it would have been undertaken without local, Canadian, and international collaborators for which I will be forever grateful. Nevertheless any omissions or errors are solely attributable to myself.

Bawin et al. [1975] and Bawin and Adey [1976] provided important initial findings that non-thermal

Conflicts of interest: None.

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extremely low frequency magnetic fields (ELF-MF) could have effects but the effects were dependent on exposure conditions such as frequency and amplitude of exposure. Importantly, Blackman et al. [1991] later confirmed these results showing that calcium-ion efflux from chick embryo brain tissue could be modulated by such ELF-MF exposures but care had to be taken in tissue preparation such as temperature. This hinted at a mechanism sensitive to frequencies that either related to the initial transduction mechanism or to a preference of the biological target to respond. Theories were proposed by Liboff, Lednev, Blanchard, and Binhi wherein the initial transduction event was only sensitive to specific field combinations [Smith et al., 1987; Lednev, 1991; Blanchard and Blackman, 1994; Binhi, 2000]. Others proposed that such an interpretation was too simplistic and coupling of the output of the initial transducer to the physiological/behavioral observable could be the source of the field characteristic dependence [Walleczek and Eichwald, 2000].

These proposed initial transduction mechanisms came under scrutiny and were criticized mostly based on signal to noise arguments that endogenous noise of warm and wet biological systems made it impossible for the relatively weak energy in ELF-MF to be detected [Adair, 1992, 1994, 1998, 1999]. These largely theoretical arguments may have negatively impacted funding as mechanism driven proposals were difficult to be convincing given that effects were often small and difficult to reproduce across different laboratories. It is my view that the well-established and extensive literature associated with using the earth’s geomagnetic field (GMF) as a navigational cue provides both possible explanatory mechanisms and robust reproducible effects that should be examined as relevant to the non-orientation biological effects reported in the bioelectromagnetics literature [Johnsen and Lohmann, 2008; Gauger et al., 2011; Hogben et al., 2012; Hore, 2012].

Animal orientation to the earth’s GMF is well established in many different animal phyla including mollusks [Kavaliers et al., 1990], fish [Molteno and Kennedy, 2009; Takebe et al., 2012], bacteria [Blakemore, 1975], insects [Kirschvink 1981; Geogar et al., 2010], rodents [Kavaliers et al., 1984] news [Phillips, 1986], birds [Wiltischko and Wiltischko, 1972, 2012; Ritz et al., 2004], and ungulates [Burda et al., 2009] to name a few. Mechanisms involved have been extensively explored. It has been shown that some animals detect earth’s magnetic field through the torque on an array of magnetite particles and this has been demonstrated as consistent with classical mechanics laws [Polk 1994; Wu and Dickman, 2012]. However, as Figure 1 outlines, particles of magnetite have, to date, failed to convincingly explain the coupling between geomagnetic sensitivity, short wavelength light, and radiofrequency (RF) interference. When these relationships are reported there is a tendency in this literature to speculate that data supports a Radical Pair Mechanism as initial transduction mechanism. Ritz et al. [2004] demonstrated that orientation in birds associated with detection of GMFs could be disrupted by RF irradiation at intensities far below those that would elicit a thermal response. This result, as much as any other experiment, demonstrated that theoretical objections of “warm and wet” can be overcome.

Animal orientation literature has traditionally been limited to biological effects of the static component of the GMFs and static fields in laboratory investigations designed to test behavioral orientation responses when static field direction is manipulated. This “state-of-mind” was predicated by the belief that since the ELF-MF signal generated by natural and anthropogenic activity were much smaller in amplitude than static GMF, such disturbances were too weak to be detected by biological organisms.

More recent animal orientation literature has demonstrated exposure to extremely low frequency (ELF) fields can disrupt orientation to static GMF (Table 1). Burda et al. [2009] showed that 50 Hz fields of about 0.1 μT (more than two orders of magnitude lower than the static GMFs) can disrupt orientation of ruminants to the GMF. As outlined in Table 1, this is not an isolated finding. A number of reports found that ELF magnetic fields in the 1 μT range can disrupt orientation to GMF. Surprisingly, Engels et al. [2014] showed that anthropogenic electromagnetic noise, as weak as 100 nT and as integrated between 2 kHz and 5 MHz, disrupts magnetic compass orientation in migratory European robins. In summary, there is growing evidence from animal orientation literature that ELF-MF, rather than just static magnetic fields, may affect animal orientation. Hence, these observed effects and their potential explanation may have relevance to non-orientation effects of ELF-MF. That is, much of the ELF-MF bioelectromagnetics literature primarily focuses on non-orientation effects [e.g., nociception; see review by Del Seppia et al., 2007], which we speculate may have the same initial transduction mechanism purported to be associated with animal orientation.

This report aims to identify our historical progression of contributions within the wider context of the literature of the time. Specifically, with respect to our contribution on effects of ELF-MF exposure on opioid related behaviors: (i) initial discoveries of the
phenomena; (ii) testing of proposed mechanisms under ambient magnetic and electric field conditions; (iii) similarity with animal orientation of effects associated with simultaneous light exposure and ELF-MF exposure under conditions of ELF magnetic field shielding; (iv) experience with a pulsed field to induce anti-nociception in animals and analgesia in humans; and (v) consideration of important experiments still to be undertaken.

Note that our focus on a measured outcome has been on nociception in animals and the related phenomena of analgesia in humans. Surprisingly, although nociception is a generalized response to almost any adverse stimulus, its response to ELF-MF

Fig. 1. Although somewhat simplistic, assignment to a mechanism of observed phenomena in animal orientation literature is related to the ability of the animal to sense magnetic field polarity. If the animal’s orientation is reversed when polarity of the ambient magnetic field is artificially inverted, then speculation is that the animal’s sensor must include permanently magnetized particles. However, if no biological effect occurs (i.e., orientation does not rotate and align with the changed directions of the polarity of the static magnetic field) then a mechanism, which cannot distinguish between magnetic field polarity, such as a RPM, which is only dependent on magnetic field amplitude but not its direction, is supported.

TABLE 1. Partial Literature Review of Thresholds for ELF-MF Effects

<table>
<thead>
<tr>
<th>#</th>
<th>Report</th>
<th>Animal</th>
<th>Hz</th>
<th>Amplitude</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prato et al. [2013]</td>
<td>Laboratory mice</td>
<td>30</td>
<td>0.033 μT</td>
<td>Nociception</td>
</tr>
<tr>
<td>2</td>
<td>Burger et al. [2010]</td>
<td>Ansell’s mole-rats</td>
<td>0.5</td>
<td>1 μT</td>
<td>C-Fos</td>
</tr>
<tr>
<td>3</td>
<td>Ossenkopp et al. [1983]</td>
<td>Laboratory mice</td>
<td>~10</td>
<td>0.1 μT</td>
<td>Nociception</td>
</tr>
<tr>
<td>4</td>
<td>Walker and Bitterman [1989]</td>
<td>Honeybees</td>
<td>0</td>
<td>0.3/0.03 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>5</td>
<td>Kirschvink et al. [1997]</td>
<td>Honeybees</td>
<td>10</td>
<td>4.3 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>6</td>
<td>Kirschvink et al. [1997]</td>
<td>Honeybees</td>
<td>60</td>
<td>430 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>7</td>
<td>Burda et al. [2009]</td>
<td>Ruminants</td>
<td>50</td>
<td>~0.1 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>8</td>
<td>Nishimura et al. [2010]</td>
<td>Lizard</td>
<td>6/8</td>
<td>2.6 μT</td>
<td>Tail lifting</td>
</tr>
<tr>
<td>9</td>
<td>Oliveriusova et al. [2012]</td>
<td>Mole-rat</td>
<td>0</td>
<td>47 μT</td>
<td>Orientation (increased noise when only static is rotated)</td>
</tr>
<tr>
<td>10</td>
<td>Larkin and Sutherland [1977]</td>
<td>Migrating birds</td>
<td>72/80</td>
<td>0.1 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>11</td>
<td>Beason [2005]</td>
<td>Pigeons</td>
<td>~1</td>
<td>0.01 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>12</td>
<td>Winklhofer et al. [2013]</td>
<td>European robin</td>
<td>0</td>
<td>4 μT</td>
<td>Orientation</td>
</tr>
<tr>
<td>13</td>
<td>Mora et al. [2014]</td>
<td>Pigeons</td>
<td>~1</td>
<td>50 μT</td>
<td>Orientation</td>
</tr>
</tbody>
</table>
has become one of the most reproducible observations [Del Seppia et al., 2007]. It is a curiosity that in our quest to understand the initial biophysical transduction mechanism of magnetoreception, which we expect is very specific, we have employed a very non-specific outcome. Magnetoreception is a sense and like other senses (e.g., vision) probably depends on a very specific biophysical mechanism. However, like other senses, awareness of exposure results in an almost infinite number of behavioral responses as they are modulated by both conscious and unconscious processes. Therefore, success of nociception as an end point is dependent on making the outcome specific to the exposure, which is dependent on suppression of other competing stimuli. As we discovered, how this was achieved held a number of surprises that were far from apparent at the time.

CONTRIBUTIONS MADE

Initial Phenomena Discovery

In 1984, Kavaliers, Ossenkopp, and Hirst published a seminal paper that magnetic fields from two counter rotating horseshoe magnets could abolish morphine induced analgesia in mice [Kavaliers et al., 1984]. At this time my laboratory was introducing magnetic resonance imaging (MRI) to Canada and a decision was made to partner with Drs. Kavaliers, Ossenkopp, and Innis to investigate effects of MRI imaging exposure on various animal behaviors, including opioid behaviors in mice. We found that exposure to a full MRI procedure (albeit at much lower static and ELF magnetic fields than those used today) attenuated opioid induced analgesia [Ossenkopp et al., 1985]. In follow-up experiments, we found that MRI static field (0.15 T) had no effect, ELF modulated RF field (6.25 MHz carrier) had an intermediate effect and ELF magnetic field produced by the MRI gradient had the greatest effect [Prato et al., 1987]. We later confirmed that similar results occurred when MRI exposure was performed at 1.5 T; currently the most common field strength used in clinical imaging [Prato et al., 1992]. Of note, we also investigated MRI effects on human cognition [Sweetland et al., 1987], and in rats with respect to open-field behavior, passive avoidance learning and spatial memory [Innis et al., 1986; Ossenkopp et al., 1986].

In the mid-1980s Kavaliers and Ossenkopp [1985a, 1985b] and Kavaliers et al. [1990] established that the effect of attenuation of opioid induced analgesia occurred in a number of mouse species. In 1991, a personal communication with Dr. Valery Lednev suggested we use exposure conditions much simpler than the complex fields associated with MRI so that some initial transduction theories such as those of Drs. Lednev [1991] and Liboff [Smith et al., 1987] could be tested. Further, Kavaliers et al. [1990] demonstrated that effects seen in mice were also seen in the land snail cepaea nemoralis. The fact that land snails could be used rather than mice made experiments less expensive, and as 10–15 animals could be exposed at the same time it allowed testing of many different exposures over a relatively short period of time.

Testing of Proposed Initial Transduction Mechanism

With collaborators Martin Kavaliers, Peter Ossenkopp, and then Ph.D. student Jeff Carson, we received a grant from the University of Western Ontario (London, Ontario, Canada) to build a three nested square Helmholtz coil-like exposure system, which gave us flexibility to superimpose time varying magnetic fields in the ELF frequency range with static fields (exposure system described in Prato et al. [1995]). Our first results exploring potential mechanisms (i.e., evidence that an induced current mechanism was inconsistent with our results and hinted that a window/resonance mechanism similar to that of Lednev’s was supported) were published in 1995 [Prato et al., 1995]. A series of extensive experiments, which included thousands of land snails, were then performed showing consistency of effects with predictions of Lednev’s “parametric resonance model” and not supportive of an induced current, free radical or magnetite model for initial transductions of ELF-MF. However, potential that a small effect was related to an induced current mechanism could not be excluded.

In 1994, a seminal paper published by Dr. Alleva’s group showed that a inhomogeneous static magnetic field of 3–4 mT could attenuate opioid induced analgesia only under conditions of simultaneous white light exposure but not under dark conditions or simultaneous red light exposure [Betancur et al., 1994]. This finding suggested to us a number of experiments, which we carried out showing ELF-MF exposure effects on opioid induced analgesia, were also modified by simultaneous light exposure. Table 2 summarizes our results and Figures 2 and 3 (taken with permission from Prato et al. [1996]) demonstrates three outstanding features of ELF-MF effects not explainable by the current form of resonance models such as those of Lednev, Liboff, Blanchard, and Binhi.

First and foremost is the effect of simultaneous light exposure. Light exposure effects have not been
incorporated into any resonance models but certainly a mainstay of the radical pair mechanism (RPM). We have some evidence that light could be influencing a post detection effect and not part of the initial transduction [Prato et al., 1998] and also possibly modulating nitric oxide synthase [Kavaliers et al., 1998; Kavaliers and Prato, 1999]. The relationship between nitric oxide and ELF-MF effects has also been implicated in mice [Jeong et al., 2006] and potentially in humans [Nelson et al., 2013].

The second result not explained by then existing theories was related to effects observed when static

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Induced current</th>
<th>Free radical</th>
<th>Magnetite</th>
<th>Parametric resonance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation of $B_{ac}$ at 60 Hz</td>
<td>x</td>
<td>—</td>
<td>—</td>
<td>√</td>
<td>Prato et al. [1995]</td>
</tr>
<tr>
<td>Variation of frequency for $B_{ac}$ and $B_{DC}$</td>
<td>x</td>
<td>x</td>
<td>—</td>
<td>√</td>
<td>Prato et al. [1995]</td>
</tr>
<tr>
<td>Variation of angle between $B_{ac}$ and $B_{DC}$</td>
<td>x</td>
<td>—</td>
<td>x</td>
<td>√</td>
<td>Prato et al. [1996b]</td>
</tr>
<tr>
<td>Variation $B_{AC}$ and $B_{DC}$ at 30 Hz</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>√</td>
<td>Prato et al. [2000]</td>
</tr>
<tr>
<td>Variation of angle between $B_{AC}$ and $B_{DC}$ in light and dark</td>
<td>—</td>
<td>—</td>
<td>x</td>
<td>—</td>
<td>Prato et al. [1996a]</td>
</tr>
<tr>
<td>Investigation of light/dark effects at 30, 60, 120 Hz</td>
<td>x</td>
<td>x</td>
<td>0</td>
<td>√</td>
<td>Prato et al. [1997]</td>
</tr>
<tr>
<td>Investigation of light/dark effects during day/night</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>√</td>
<td>Prato et al. [1998]</td>
</tr>
</tbody>
</table>

Fig. 2. Thermal (40°C) response latencies of snails injected with the enkephalinase inhibitor SCH 34826 (1.0 μg/1.0 μl saline) and exposed for 15 min to either a 60 Hz (exposed) or a sham 60 Hz (sham) magnetic field in either presence of light (light) or absence of light (dark). In the 60 Hz exposure, the angle between vertical 60 Hz magnetic field and 78.1 μT static magnetic field was 67.5°. Sample sizes are given in the figure. Vertical lines denote a standard error of the mean. Reprinted from Prato et al. [1996a] with permission from Royal Society Publishing.
magnetic field and ELF-MF were not parallel. None of the window/resonance models deal with this phenomenon; however, we proposed a simple projection approach, suggested in a private discussion with Dr. Lednev, which has some experimental support [Prato et al., 1996a,b].

The third result not incorporated into the window/resonance models is demonstrated in Figures 2 and 3, which show a “residual” effect of ELF-MF, independent of angle and light conditions. Note, for these exposures, where most of the observed effect is a function of the cosine of angle between ELF-MF and static field (Fig. 3), possibility of an induced current effect was kept constant as both amplitude and direction of the ELF portion of exposure was vertical (60 Hz, peak amplitude of 299 μT). Therefore, this residual effect is consistent with an induced current transduction mechanism.

It is important to consider the difference between ELF-MF and static field effects on opioid induced analgesia/anti-nociception. Throughout, we have focused on effects of time changing magnetic fields and have side-stepped the issue of effects from static fields, which could be relatively homogenous or non-homogenous. Our experience has been somewhat inconsistent. In snails, we reported very small increases in anti-nociception observes as static fields were increased from 40 to 60 μT for light exposures while a decrease in anti-nociception for dark exposures during the day [Prato et al., 1997]. However, in mice we reported no effect from exposure to a very homogeneous 1,500 mT [Prato et al., 1987]. The paper by Betancur et al. [1994] on effects of simultaneous light exposure used a non-uniform static field. The extensive work of Laszlo reported effects of both homogenous and non-homogenous magnetic fields [Laszlo et al., 2007; Sándor et al., 2007; Laszlo and Hernadi, 2012; Kiss et al., 2013; Hernadi and Laszlo 2014]. Of interest is data and speculation by this group that it is not the average amplitude of static magnetic field but its gradient that is responsible for inducing analgesia [Kiss et al., 2013]. Nevertheless, in the same article, Kiss et al. summarized their experimental data showing induction of analgesia as a function of static magnetic field strength with a saturation of effects at 200 mT. If it is predominantly the gradient rather than absolute value of the static magnetic field, this would be consistent with our negative results when mice were placed in a very homogenous magnetic field (i.e., within the center of an MRI magnet) [Prato et al., 1987].

Our final work using Lednev’s “prescribed” fields [Prato et al., 2000] demonstrated it was possible to induce anti-nociception directly using only ELF-MF. Although this was an important finding supporting Lednev’s model the analgesic induction was too small an effect for translation to patient care. At this point two important discoveries were made. We showed that induction of analgesia could be very significantly increased if a pulsed magnetic field was used rather than a sinusoidal field [Thomas et al., 1997]. As this could have significant clinical value to treat pain it was aggressively pursued and is discussed below. The other discovery was made by Del Seppia et al. [2000]. They showed that when mice were placed in a magnetic field shielded environment, stressed induced analgesia was reduced. We next discuss how this effect of ambient magnetic field shielding was used to further investigate the initial transduction mechanism.

**Further Mechanistic Studies Under Ambient Magnetic Field Shielded Conditions**

The seminal work of Del Seppia et al. [2000] was repeated and extended in our laboratory by Elena

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Choleris [Choleris et al., 2001]. There were two important findings in this study. First, the effect could not be replicated by simply nulling the static ambient magnetic field using Helmholtz coils and, second, there was no effect if only ambient electric field was shielded. This suggested a remarkable possibility: animals could sense ambient ELF-MF of the order of less than 0.2 μT at 50 and 60 Hz. However, the procedure involved in the generation of stress induced analgesia was complicated and attenuation by shielding was a small effect. Hence, we investigated if we could induce anti-nociception/analgesia with repeated 1h exposures to shielded environments. This approach was suggested by some earlier work of Kavaliers and Ossenkopp [1993], who had demonstrated that repeated exposures to 60 Hz MF could induce nociception whereas a single exposure reduced opioid induced anti-nociception. As we predicted, a very large increase in nociception was observed as shown in Figure 4 [Prato et al., 2005]. Note that the effect was eliminated by the opioid antagonist naloxone and ambient electric field shielding did not produce an effect. As this effect was robust [Prato et al., 2005] and did not require opioid manipulation (no injection needed) it was ideal to investigate the perturbing effects of introducing into the mu-metal box, that is, shielded environment, ELF-MF, and light of different wavelengths and intensity.

We found that induction of analgesia (Fig. 4) could be abolished by introducing light of short wavelengths of similar intensity needed for bird orientation [Prato et al., 2009]. We further discovered that the effect could be attenuated or abolished by introduction into the μ-metal box of ELF-MF depending on amplitude (Fig. 5) and frequency of ELF-MF [Prato et al., 2011, 2013a].

The observation that the effect of light was similar in intensity and wavelength to effects on animal orientation, particularly in bird and newt, suggests a RPM mechanism. Note, for much of the literature associated with bird orientation, there is evidence that both a magnetite mechanism and a RPM are supported as present simultaneously in the same animal as particularly demonstrated in birds [Wiltschko and Wiltschko, 2012]. Here, however, we are focused on orientation work that focused on the RPM mechanism based on a shortwave length light dependence and elimination of the effect from specific RF exposures. To our surprise and consternation, the effect of introduced ELF-MF initially scaled as an induced current mechanism [Prato et al., 2011]. Next, we decided to try to establish a threshold for ELF-MF perturbation and in one publication suggested that if the product of amplitude and frequency exceeded 1,000 nT-Hz, ELF-MF could be detected in a static and ELF-MF shielded environment [Prato et al., 2011]. In an extension, we confirmed that threshold must be below 33 nT at 30 Hz and that an introduced static field similar in strength to ambient GMF had a very small effect. We speculated that this effect of introduced static field may be caused by a 7% gradient associated with this static field. In essence, mice are not detecting average static field at 44 μT but are instead detecting an induced ELF-MF as they travel through the magnetic field gradient. This is potentially an important observation as it would suggest that animals with magnetoreception may detect changes in GMF as extremely low frequencies generated by moving through GMF gradients. This interpretation would be consistent with data summarized in Table 1 associated with animal orientation. This is also consistent with reports that suggest it was not static magnetic field but its gradient that was more important for induction of analgesia [Kiss et al., 2013]. Also it has recently been shown that pigeons can detect changes in a static magnetic field inclination, which would correspond to an induced ELF if magnetic field detector was at a fixed inclination [Mora et al., 2014]. Currently, we are attempting to investigate this possibility by introducing static magnetic field of differing gradient strengths into the shielded environment. To date, results have not been sufficiently conclusive and may require monitoring mouse activity within the gradient field to estimate induced ELF for each mouse.

In summary, these shielded experiments were very informative but somewhat difficult to understand.
given opposite effects seen from light, which eliminates the effect in a shielded environment but allows it to occur for experiments performed under ambient conditions for both opioid related behaviors and for animal orientation in non-shielded environments. Our working assumption has been that mice undergo stress induced analgesia when placed in the shielded environment due to an acute loss of sensing of ambient magnetic fields. An introduced ELF-MF blunts the ability to sense the field loss as presumably occurs under exposure conditions when animals are in non-shielded environments or just experience loss of anxiety as the introduced ELF-MF field, which is now sensed as familiar to that outside of the shielded box. In orientation literature, there is evidence that short wavelength light of sufficient intensity allows calibration/recalibration of the relationship between GMF and orientation. As we have done all experiments during the day, it is possible that introduction of the correct light exposure allows mice to recalibrate and adjust to this shielded environment. Note that an important outcome with respect to mechanisms is that these results demonstrate that animals can detect anthropogenic ELF-MF. Typically, anthropogenic fields are too weak to be explained by free radical mechanism (FRM) [Mouritsen and Hore, 2012] or by arrays of magnetic particles [Wu and Dickman, 2012] as these mechanisms are presently proposed. An exception to this general statement on FRM is the recent proposal by Barnes and Greenebaum [2015], which suggests that free radical effects are theoretically possible at such low magnetic field amplitudes if nuclear spin transitions affect electronic spin transitions.

**Induction of Analgesia With Pulsed Fields Below 500 Hz**

Dr. A.W. Thomas, then a graduate student, suggested that a pulsed field with frequency content below approximately 500 Hz (see Fig. 6) would interact more efficiently with a biological system than would a sinusoidal ELF-MF. Over the course of the next decade he demonstrated that such a field could induce analgesia in snails (see Fig. 7) [Thomas et al., 1997], and mice [Shupak et al., 2004a], and benefit patients in chronic pain [Thomas et al., 2007]. We found evidence that the application of this pulsed field did not affect thermal sensory thresholds whereas pain thresholds were significantly increased [Shupak et al., 2004b]. Work was also carried out on the effects of this pulsed field on balance demonstrating improvement in postural sway of healthy volunteers [Thomas et al., 2001a] and potentially in patients with rheumatoid arthritis and fibromyalgia [Thomas et al., 2001b]. It is of some interest that effects of pulsed magnetic fields on postural sway were dependent on light

![Fig. 5. Pre-(open circles) and post-(closed circles) exposures to a 30 Hz magnetic field with different amplitudes. The sham was when mice were exposed in a fiberglass box, which did not attenuate ambient ELF-MF. All other exposures were carried out inside a μ-metal box, which shielded static and ELF magnetic fields. Magnetic field designation in nT corresponds to the peak amplitude of the 30 Hz magnetic field. Error bars correspond to one standard error of the mean. Reprinted from Prato et al. [2011] with permission from Wiley.](image-url)
intensity levels [Prato et al., 2001]. This was consistent with the fact that animal orientation and opioid related behavioral effects are modulated by simultaneous exposure to light.

Dr. Thomas and then graduate student Charles Cook examined the impact of the pulsed magnetic field on the human resting electroencephalography (EEG) [Cook et al., 2004, 2005, 2009]. They found effects after and during a 15 min pulsed magnetic field exposure. The significantly higher occipital alpha activity seen is consistent with other experiments examining EEG effects of responses to ELF-MF and ELF modulated RF fields [Cook et al., 2006].

Dr. Thomas and then graduate student John Robertson examined effects of processing of acute thermal pain of human exposure to pulsed magnetic fields. Brain activation was visualized using functional magnetic resonance imaging (fMRI). This work strongly suggested that low-frequency pulsed EMF exposure can alter neuroprocessing in humans [Robertson et al., 2010a]. Significant interactions were found between pre- and post-exposure activation between sham and exposed groups for the ipsilateral (right) insula, anterior cingulate, and bilateral hippocampus/caudate. A second publication showed size of the effect in the anterior cingulate scaled with amplitude of pulsed fields [Robertson et al., 2010b] as shown in Figure 8.

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Mechanistically this fMRI data is both intriguing and confounding. A dose response related to amplitude increases induced currents so an induced current response is somewhat supported. However, as when we started out in the 1980s with MRI, the situation is confounded by other fields present including time changing magnetic fields associated with the MRI gradient, static fields in 1.5 to 3.0 T range and ELF modulated RF. Nevertheless, this is a very important result showing that relatively weak pulsed magnetic fields can affect neuroprocessing and possibly neuroplasticity.

Our one big disappointment was the suspension of a private company funded clinical trial in patients with fibromyalgia when interim analysis showed discouraging results. Currently, the technology is owned by Baylis Therapeutics (Mississauga, Canada).

**Future Directions**

There is still considerable work to be done to explore the possibility that mechanisms associated with modulation of opioid related behaviors from ELF-MF exposures are similar to those associated with magnetoreception in animal orientation.

One of the more fundamental needs is to establish the threshold of magnetoreception. We have shown it is of the order of 1,000 nT-Hz although others have to confirm this finding, and we and others should investigate the frequency dependence of this threshold. Given that current main line theories for mechanisms in magnetic field navigation are having

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difficulty explaining effects at magnetic fields 100 to 1,000× greater, is almost impossible to realistically consider mechanisms such as FRM, arrays of magnetite particles or induced currents if threshold is as low as 1,000 nT-Hz.

Binhi [2008] proposed a hybrid initial transduction mechanism that combines FRM and particles of magnetite. Figure 9 summarizes how to incorporate super paramagnetic iron oxide (SPIO) particles as part of initial transduction mechanism. These are particles, which like magnetosomes found in bacteria, are made of magnetite, but unlike magnetosomes are not large enough to hold a permanent magnetization, that is, hold a single magnetic field domain. Hence, their magnetic moments are unbound by particle geometry, which as proposed by Binhi [2008] and also briefly suggested in a review by Mouritsen and Hore [2012], might allow stochastic resonance to “imprint” weak ELF-MF. This could amplify applied ELF-MF by orders of magnitude. These “imprints” would generate magnetic fields within 100 nm of SPIO particles that would exceed 10 mT [Binhi, 2008]. Within such proximity to SPIO particles, the radical pair mechanism becomes viable as it may no longer violate accepted coherence times [Gauger et al., 2011]. Further, it helps explain the broad RF resonance that was observed [Ritz et al., 2004; Engels et al., 2014] and not been explained in bird orientation literature.

![Figure 9. A proposed hybrid approach to initial transduction mechanism involving super paramagnetic iron oxide (SPIO) particles as initial transduction site for biological effects from weak ELF-MF exposures. Within the proximity of the SPIO particle, weak ELF-MF are amplified above thermal noise such that the Radical Pair Mechanism and the Induced Current mechanism become viable concepts. This figure has been developed largely from discussion in work published by Binhi [2008] and in discussions with Vladimir Binhi that led to the presentation of a similar diagram at the 2013 BioEM meeting in Thessaloniki, Greece.](image_url)

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In a uniform static magnetic field, RF resonance frequency that would perturb the FRM is fixed and proportional to the local field. If, however, response is to an ELF-MF amplified by a variable amount depending on proximity to the SPIO particle, then a broad RF resonance would be expected, which is what was observed [Ritz et al., 2004]. Note that within the vicinity of these particles, amplified dB/dt might be sufficient to also induce free radicals directly. If this latter situation occurred, it could explain why very weak ELF-MF could also scale as amplitude frequency product explaining why at times effects scale as an induced current mechanism [Prato et al., 2011]. There is a need for theoretical work to evaluate this concept. In addition, there is a need to perform experiments measuring the relationship between free radicals and SPIO concentrations under various ELF-MF exposures using both cells and cell free systems.

In a very recent publication [Barnes and Greenebaum, 2015], there is an intriguing suggestion that sensitivity to exposure to weak ELF-MF and weak RF may be associated with interaction between nuclear spins and electronic spins. The involvement of nuclear spins is attractive since it resolves two issues associated with the FRM that only considers electron spins: short coherence times and resonances in the ELF range. In general, nuclear transitions have been ignored as by themselves they should be isolated and uncoupled from biochemical phenomena. However, in nuclear magnetic resonance it is well known (e.g., basis of magnetic resonance imaging) that biochemical processes can affect the population of nuclear spin states. What has been ignored is the possibility that changing nuclear spin states could affect electron spin transition in radical pairs (e.g., singlet to triplet transitions). Barnes and Greenebaum point out this possibility supported by a significant number of reports albeit in isolated relatively simple molecules. As these authors point out there is need for additional experimentations under carefully controlled experimental conditions that avoid organism stressors that could change free radical concentrations. Given that this proposal predicts many possible resonances from RF to ELF, of which some could be at very weak field amplitudes, we suggest that such experiments should be undertaken in an environment shielded from anthropogenic sources since varying anthropogenic background could severely confound results.

A major implication of our work in mice under shielded conditions is that the threshold for effects in humans can only be found if investigations are under magnetic field shielded environments. As we have reported in mice (see Further Mechanistic Studies Under Ambient Magnetic Field Shielded Conditions Section) and others have reported with respect to bird orientation [Engels et al., 2014] thresholds may be below ambient ELF-MF and RF in locations where there are anthropogenic sources. Hence, inconsistencies of effects of ELF-MF on EEG [Cook et al., 2006] could be related to effects of experiments being undertaken by different groups under different ambient ELF-MF. Note, it has been shown that RF can also affect nociceptive responses in snails [Nittby et al., 2012], which further suggests a common mechanism as RF perturbs animal orientation [Ritz et al., 2004; Engels et al., 2014]. The possibility that inconsistencies of effects of RF on EEG [Cook et al., 2006] are related to differences in ambient fields strongly suggests that effects of ELF-MF and RF on EEG should be examined under shielded conditions (i.e., to remove the potential for confounding effect due to fields.)

If ELF-MF can affect neural processing, the impact of such fields associated with MRI should be investigated. fMRI is used extensively in neurological research and potential confounds on results should be investigated.

There are reports that DNA damage may occur from exposure to MRI [Yildiz et al., 2011; Fiechter et al., 2013]. As MRI fields exceed GMFs and anthropogenic ELF-MF by more than 10,000 x for the static field and 100 x for the ELF-MF, looking for an increase in DNA damage could be an indirect test of the SPIO hypothesis. If such high fields do cause an increase in free radicals and resulting DNA damage, it supports the concept that high fields in proximity to SPIO particles could also increase free radical concentrations. The advent of combined positron emission tomography (PET)/MRI systems provides another opportunity. Ionizing radiation of PET produces free radicals, but in the MRI, strong magnetic fields could increase the number of free radicals that do not recombine by increasing transitions from singlet state to triplet state. It would be a paradoxical outcome if this increase in free radicals in a PET/MRI exposure was greater than that observed from a PET/CT exposure. The concept that MRI is intrinsically safer than CT due to absence of ionizing radiation may not be true under all conditions.

As we stated earlier, the seminal work of Ritz et al. [2004] showed that biological effects (orientation to a static field disrupted) can occur from very weak RF exposure. One major experiment still needed in the ELF/nociception arena is to determine if such RF exposures affect opioid related behaviors. The work by Nittby et al. [2012] suggests this is the case. Although a bit of an engineering challenge, it would be possible to introduce a polarized RF field within a
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shocked enclosure and determine if the increase in nociception (Fig. 4) was attenuated/abolished by RF exposure.

**SUMMARY**

Scientific knowledge continues to expand at an ever increasing rate driven by testing new ideas and development of new research tools that allow us to investigate where we could not before [Dyson, 2012]. For example, advances in molecular biology have allowed the establishment that human cryptochrome exhibits light dependent magneto sensitivity by testing the human fluoroprotein cryptochrome using a transgenic approach in the magnetoreception system of Drosophila [Foley et al., 2011]. These advances have also allowed us to determine if effects occur during exposure, with an excellent example coming from the laboratory of Dr. Jeffrey Carson demonstrating that a 100 mT static magnetic field had no effect during or immediately after exposure on heat shock protein 70 using a hsp 70/luc reporter system [Belton et al., 2011].

It is the hypothesis of this review that greater convergence of research between animal orientation researchers and non-orientation research carried on by our Bioelectromagnetics Society members will result in a greater understanding of fundamental mechanisms of magnetoreception. A heightened dialogue between these two groups, coupled with access to new tools and new ideas that will arise, will invigorate our research.

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