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Magnetic Field Effects On The Neuroprocessing Of Pain

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Medical Biophysics

MAGNETIC FIELD EFFECTS ON THE NEUROPROCESSING OF PAIN

(Thesis format: Integrated Article)

By

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Abstract

Magnetic fields can affect behaviour in a variety of ways, in a manner that is dependent on the particulars of the magnetic field exposure. A specific pulsed magnetic field with analgesic properties was investigated using functional magnetic resonance imaging with acute thermal pain. The functional activation of pain was significantly different pre/post exposure vs. a sham condition within areas of the brain associated with the affective component of pain, in particular the anterior cingulate and the right insula. Sleep was found to be a significant confound with a 45-minute exposure. This was the first time fMRI has been used as a tool to investigate bioelectromagnetics effects, and demonstrates that an MR system can be used for both image acquisition and exposure. This technique will have applications to functional tasks beyond the acute thermal pain tested here.

Keywords

Bioelectromagnetics, functional magnetic resonance imaging, blood oxygenation level dependent (BOLD) signal, acute pain, pulsed magnetic field, electroencephalography (EEG).

Co-Authorship Statement

John Robertson: Performed all manuscript writing, all MRI acquisitions, most data analysis and subject recruitment. Approved all manuscripts.

Julie Weller: Assisted with subject recruitment and data analysis for chapter 3, and approved chapter 3 manuscript.

Nicole Juen: Assisted with subject recruitment, EEG setup (chapter 5), MRI acquisitions, and data analysis (chapter 3 and 5), and approved chapter 3 and 5 manuscripts.

Jodi Miller: Assisted with subject recruitment, EEG setup, and MRI acquisitions (chapter 5), and approved chapter 5 manuscript.

Julien Modolo: Assisted with EEG analysis tool creation in MATlab and EEG analysis study design (chapter 5). Approved chapter 5 manuscript.

Jean Théberge: Created the exposure sequence allowing the pulsed magnetic field to be introduced to the MRI environment via the gradient coils, and also helped create MRI acquisition protocol for all chapters. Approved all manuscripts.

Dick Drost: Trained JR on MRI use, helped create MRI acquisition protocol and study design (chapter 2 and 3) and approved chapter 2 and 3 manuscripts.

Alex Thomas: Supervisor, invented the specific pulsed magnetic field sequences for analgesia, helped create experimental protocol, advised on statistical analysis; edited, reviewed, and approved all manuscripts.

Frank Prato: Helped create experimental protocol; edited, reviewed, and approved all manuscripts.

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List of Abbreviations

ICNIRP	International Commission on Non-Ionizing Radiation Protection
fMRI	Functional Magnetic Resonance Imaging
BOLD	Blood Oxygenation Level Dependent [Signal]
ASL	Arterial Spin Labeling
MF	Magnetic Field
ELF	Extremely Low Frequency (<3 kHz)
RF	Radiofrequency (3 kHz – 300 GHz)
TMS	Transcranial Magnetic Stimulation
CNP	Complex Neuroelectromagnetic Pulse
MRI	Magnetic Resonance Imaging
EEG	Electroencephalography / Electroencephalogram
PEMF	Pulsed ElectroMagnetic Field

General Introduction

Bioelectromagnetics, the study of how electromagnetic fields interact with biological systems including human behaviour, is one of the exciting frontiers of science today. Not only is it scientifically interesting and challenging for its own sake, but it also offers the potential for novel future therapeutic applications.

One such application is the use of pulsed magnetic fields to produce analgesia. A specific pulsed magnetic field known as the complex neuroelectromagnetic pulse or CNP was developed here in Canada and tested in animals, healthy volunteers, and volunteers with various chronic pain conditions, and found to have a significant analgesic effect. This effect is still present with head-only exposures, indicating that changes to neuroprocessing are being made.

This thesis examines changes in pain-related processing as measured by functional magnetic resonance imaging that follow pulsed magnetic field exposures.

In Chapter 1, the previous research in the field will be reviewed, including especially how magnetic fields can affect nociception and the potential confounds that the magnetic fields of the MRI system can pose.

In Chapter 2, the experimental results from a study where the MRI system itself was modified to produce a specific pulsed magnetic field and

then measure the effect on brain activation will be reported. A noxious thermal stimulus was delivered before and after the exposure, and the changes in fMRI activation were measured. We found that the areas of the brain associated with the affective component of pain had changes in pain-related processing after exposure, and that these differences could be observed with a small subject pool.

In Chapter 3, the results from an expansion of the first study will be reported. There, additional magnetic field strengths were tested to determine if there was a dose-response in the changes in pain-related activation.

In Chapter 4, the experiment is repeated with a 45-minute exposure, which serendipitously lead to the discovery of sleep as a potential confound in acute pain ratings.

In Chapter 5, the experiment is further refined with our 3 T hybrid MRI-EEG system, allowing us to not only examine the pain-related fMRI changes, but also any potential changes in resting EEG. We also included a 60 Hz sinusoidal magnetic field exposure to compare against the results observed with the pulsed magnetic field.

Within the appendices, Appendix E reprints an invited perspectives paper published in *The Environmentalist* describing the evolution and utility of hybrid imaging for investigating bioelectromagnetics. Appendix B provides additional experimental details and responses to the reviewers of Chapter 2 that was originally published as an online supplement to that paper.

Appendix C provides some more detail on the method of introducing a specific pulsed magnetic field to the MRI environment. Appendix D provides additional experimental details for the work on the hybrid MRI-EEG system.

Chapter 1: Introduction and Literature Review

1.1 Introduction to Bioelectromagnetics

Magnetic fields are fundamental forces of nature that can exert a force on moving electric charges. Moving electric charges themselves also create a magnetic field. The strength of a magnetic field is measured in Tesla (T), though outside of an MRI the fields encountered in everyday life (and most experiments described below) are more practically measured in μT. In the symmetry of physics, time-varying magnetic fields create electric fields, and this is one mechanism by which magnetic fields can influence the body electric. Electromagnetic fields can also cause heating of biological tissue: thermal effects are one of the classical interaction mechanisms, and the basis for many safety standards (particularly at radiofrequencies). For static and extremely low frequency magnetic fields (<3 kHz), the interaction with human and animal tissue is almost exclusively non-thermal. Indeed, unlike the nickel in your pocket, biological tissues generally interact weakly with magnetic fields, if at all, a fact that underlies the controversial nature of the study of bioelectromagnetics.

The Earth has a magnetic field of its own, which is primarily static in nature, with small time-varying components as well. The field strength of the Earth's magnetic field (geomagnetic field) varies across the surface of the earth, generally stronger at the poles and weakest at the equator, with London, Ontario at about 50 μT. The inclination of the field also varies, often

in subtle ways, across different regions, with the field near the poles nearly vertical, and near the equator nearly horizontal.

A variety of organisms have evolved ways of detecting the geomagnetic field, and exploiting it for various behaviours. Perhaps best known are the animal navigation behaviours, studied extensively in various bird species, but also in reptiles, sea turtles, and some mammals (Thalau *et al.*, 2006; Wiltschko and Wiltschko, 2006; Johnsen and Lohmann, 2008; Stapput *et al.*, 2008). Several different biological compasses have been proposed (Thalau *et al.*, 2006), with different underlying mechanisms for the biophysical detection of the magnetic field, leading to different properties (sensitivity to polarity vs. inclination, light-dependent or not, etc.).

Even bacteria can utilize the geomagnetic field for orientation, with strings of magnetite particles forming magnetosomes, which serve to align the bacteria with the prevailing field (Blakemore, 1975; Komeili, 2007).

In addition to the detection of magnetic fields by biological compass systems, the interaction of magnetic fields with biological systems is a wider field of study known as bioelectromagnetics. Some aspects of bioelectromagnetics is focused on the potential deleterious effects of exposure to the magnetic fields produced as a consequence of our modern industrial, electrified lives. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) has summarized some of the research in this area, and

produced exposure guidelines for workers and the general public (ICNIRP 2010).

Other aspects of bioelectromagnetics approach the matter from the therapeutic point of view (Robertson *et al.*, 2007; Shupak *et al.*, 2003), exploring how bioelectromagnetics interactions can be exploited as a tool, for example to improve health care options.

The approaches to the questions are not entirely exclusive, for example early work in the bioelectromagnetics lab in London, Ontario questioned what risks were associated with MRI scans (Prato *et al.*, 1992, 2010), however as an effect on nociception and analgesia was found – areas of immediate clinical relevancy – work gradually evolved to investigations that could potentially lead to novel treatments in the future. Though the results are of equal scientific interest no matter the motivation, the applications of these experiments did lead to several patents (Thomas *et al.*, 1999 US Patent #6,234,953; Thomas *et al.*, 2002[a] US Patent #7,280,861; Thomas *et al.*, 2002[b] US Patent #7,297,100) and a spin-off company being founded.

1.1.1 Interaction Mechanisms

Before any physiological or behavioural change can take place within a biological system, the magnetic field must be detected in some manner. Several detection mechanisms have been proposed, with each possessing its own set of properties in terms of sensitivity to different applied magnetic fields.

Many cell types, in particular the neural cells of animals and humans, utilize electric fields for signaling. Time-changing magnetic fields create an electric field in tissues via induction, and these induced currents can conceivably influence physiological and behavioural processes. This induced current mechanism is well understood classically, and the strength of the induced electric field depends on the frequency of the time changing magnetic field (the faster the MF varies, the stronger the induced current) and the MF intensity (higher MF leads to higher induced electric field). This interaction mechanism has very good support when the fields involved are large relative to the endogenous electric fields, and underlies nerve stimulation observed both as a side effect of some very strong MRI sequences (peripheral nerve stimulation, see Schaefer *et al.,* 2000), as well as with the use of Transcranial Magnetic Stimulation (Barker *et al*., 1985; see 1.2.1 for more on TMS).

When the induced currents are weak relative to the endogenous fields, or for neurons in particular, weak relative to the action potential threshold, there is more controversy as to the ability of time-varying MFs to influence biological processes, such as neuroprocessing. However, networks of neurons are more sensitive to weak fields than isolated neurons (Francis *et al*., 2003) so even sub-threshold signals may be relevant.

It is our hypothesis that the induced current mechanism is the initial biophysical detection mechanism that underlies the effects on neuroprocessing discovered in the experiments that will be described in

chapters 2-5. See section 1.2.3 below for more information on some previous work in our lab that would help support that hypothesis.

Other mechanisms for the interaction underlying bioelectromagnetics effects have been proposed. Magnetic particles, or biological magnetite, present within certain tissues possessing an inherent magnetic dipole that can align with the external field have been discovered in certain species, including mole rats (Thalau *et al.*, 2006), birds (Wiltschko and Wiltschko, 2006) and magnetotactic bacteria (Komeili, 2007). The alignment of, or torque upon these biological magnetite particles by an external static field, such as the geomagnetic field, is easily understood classically (Johnsen and Lohmann, 2008). In addition, time-varying fields may affect these magnetic domains in certain configurations. In particular, when the time-varying field is perpendicular to the static MF. However typical ELF field strengths have been suggested to be too weak to overcome thermal noise (Adair, 1994).

The radical pair mechanism has also been shown to underly some of the navigation behaviours in birds (Wiltschko and Wiltschko, 2006). The radical pair mechanism utilizes chemical reactions that produce free radicals, that is, molecules with unpaired electrons. In an external magnetic field, these electrons will align with or against the field (spin-up, or spin-down), which represent different energy states. In order for two radicals to recombine, the electrons must be in complementary spin states (one spin-up, one spin-down). Different applied magnetic fields change the energy levels and thus the

recombination rates. These reactions occur on very short timescales, so ELF time-varying magnetic fields appear static to any given recombination (Gauger *et al*., 2011). Generally only the field strength of time-varying fields matter, though it is quite possible that the modulation of the reaction rates with the frequency of the ELF fields may modulate the concentration of reaction products at the same frequency, which may lead to a downstream biological effect, even though the initial detection mechanism itself is insensitive to frequency.

Ion resonance mechanisms, where the kinetics of an ion-protein complex may be altered by combinations of static and time-varying fields have also been explored. The mechanism depends on the charge-to-mass ratio of the ion, and combinations of static and time-varying magnetic fields (Lednev, 1991).

1.2 Background to Magnetic Field Therapies

1.2.1 Magnetic field treatments

Magnetic field-based treatments have been proposed since antiquity, with mixed success.

The use of static magnets for the treatment of pain has a long and storied history. The evidence for the efficacy of such treatments has been equivocal (Eccles, 2005, Shupak *et al*., 2003), with many studies criticized for lack of detail, control, or poor dosimetry (Colbert *et al*., 2008).

Time-varying magnetic field applications are more varied, with stronger evidence in their favour. The strongest time-varying magnetic fields are found in Transcranial Magnetic Stimulation (TMS) where the induced currents from magnetic pulses of 10^4 T/s and above can deterministically stimulate a region of brain tissue, generally close to the skull. The depolarization – and longer-lasting changes in excitability – from the stimulation have been used as research tools for many years, and the use of TMS for the treatment of various psychological disorders has been thoroughly investigated (see Wassermann and Lisanby, 2001 for review).

Weaker pulsed magnetic fields have been used in bone stimulators, an FDA-approved treatment that involves the local application of the stimulator to induce an electric field within the area of the break. It is hypothesized that these fields can help activate the healing process in non-union fractures, where the break remains unhealed after several months (Bassett *et al.*, 1977; Bassett, 1993; Shupak *et al.*, 2003).

1.2.2 Magnetic fields effects on pain

Some of the most reproduced studies in bioelectromagnetics treatments are effects on opioid analgesia systems. Del Seppia *et al*. (2007) reviewed a number of experiments on nociception, concluding that: "The effects on pain, nociception and opiate-mediated analgesia constitute one of the most reproducible and reliable effects of EMFs [electromagnetic fields]."

Though individual studies and their effects have been reproducible,

there is nonetheless a great deal of variety in the literature. Many studies find that magnetic field exposures heighten nociception, or dampen the analgesic effect of pharmaceuticals or stress, while others find an analgesic response. Vastly different exposure paradigms, from pulsed magnetic fields to sinusoids, static fields, and even the removal of the background fields have been found to influence nociception in various ways.

The method of exposure can also play a role. Some studies (e.g., Heden and Pilla, 2008) utilized localized exposures, usually in an effort to treat the cause of the pain rather than the behavioural experience of pain itself. This approach however would not help in many cases of chronic pain where there is no tissue injury causing the pain, or where the injury has long since healed, yet the pain remains. In patients with shoulder periarthritis – a chronic pain condition that does feature local inflammation – a localized 1 mT, 100 Hz magnetic field (as well as a complex modulated field) had a profound effect on pain ratings after daily repeated exposures for 15 days, with many patients reporting zero pain (Battisti *et al*., 2009; Rigato *et al*., 2002). However, it appears as though these studies were only single-blind.

Small animal studies, such as those involving snails, mice, and rats, by their nature tend to feature whole-body exposures, so it is difficult to determine where the site of action lies.

Using a whole-body pulsed field exposure, Papi *et al*. (1995) found decreased electrical pain thresholds in human volunteers. Also with regular

(twice daily), double-blind exposures to a whole-body pulsed magnetic field, Sutbeyaz *et al*. (2006, 2009) found a significant effect on the self-reported pain scores of osteoarthritis (2006) and fibromyalgia (2009) patients, with no changes in their drug regimens.

Shielding studies utilize a fabricated laminated alloy material known as mu metal that possesses a very high magnetic permeability, giving it the ability to shunt magnetic fields away from the inside of an enclosure – creating a shield. Both static and time-varying fields are attenuated within the enclosure. Del Seppia *et al.* (2000) found that a 2-hour exposure to the shielded environment attenuated stress-induced analgesia in mice. This was specific to the shielding of both static and time-varying fields, as zeroing of the static magnetic field did not affect stress-induced analgesia (Choleris *et al*., 2002).

Furthering the work, Prato *et al*. (2005) found that daily shielding produced an analgesic response following 5 consecutive days of exposure to the hypogeomagnetic environment. Subsequent experiments also demonstrated that the effect was likely related to opioid analgesia, as the shielding-induced analgesia was naloxone-reversible, and could augment morphine-analgesia; and that the effect was light-dependent (Koziak *et al*., 2005).

Acute exposure to time-varying fields also has various effects, often to reduce analgesia produced by stress or pharmaceuticals. Looking at the time-

varying fields of MRI systems (also described further in section 1.4.2), researchers at the University of Western Ontario found a reduction in morphine- (Ossenkopp *et al.*, 1985, Prato *et al.*, 1992) and fentanyl-induced analgesia (Teskey *et al.*, 1988) in mice. Del Seppia *et al.* (2000) saw an attenuation of stress-induced analgesia after a 2-hour exposure to a 37 Hz magnetic field. A 60 Hz magnetic field eliminated the normal nighttime change in sensitivity to a noxious stimulus, and at higher field strengths (2 mT) created daytime hyperalgesia (Jeong *et al.*, 2000). Moreover, in mice kept in constant darkness for 5 days, a 12 h/day magnetic field exposure partially restored the diurnal difference in response latencies seen when mice were kept under a normal 12 h light/12 h dark cycle (Choi *et al.*, 2003).

Refining the early work using MRI-based magnetic fields, the London, Ontario group chose to create a set of nested Helmholtz coils that could deliver simpler magnetic fields (i.e., sinusoids) to further investigate bioelectromagnetics mechanisms without the confounds that the series of magnetic fields of an MRI system present. Work with snails indicated that the mechanism of ELF inhibitory effects on opioid-analgesia was at least partially based on a parametric resonance model (Prato *et al.*, 1996) and had light-dependent components (Prato *et al.*, 1997).

Further exploration of the field combinations for the parametric resonance model experiments in snails yielded magnetic field exposures that could increase or decrease the response to a noxious stimulus (Prato *et al.*,

2000). That is, not only could magnetic fields counteract the effect of analgesic compounds, they could also serve as analgesic agents themselves.

1.2.3 Specific pulsed magnetic field

Knowing that certain magnetic field configurations could interfere with the analgesic effect of opioids (Prato *et al.*, 1996, 1997, 2000), Dr. Thomas endeavored to find a magnetic field exposure that could instead interfere with the affective processing of pain, which would produce a useful analgesia-like response. Working with an induced current mechanism hypothesis and biologically relevant pulseforms, a specific pulsed magnetic field known as the "complex neuroelectromagnetic pulse" (CNP) was developed (Thomas *et al.*, 1997[a], 1999). This pulsed field design has been shown to reduce nociception in land snails, with partial reversal by naloxone, in contrast to a differently pulsed magnetic field (Thomas *et al.*, 1997[a]). The anti-nociceptive effect was also reduced by δ-opioid antagonists, but not κ-opioid antagonists (Thomas *et al.*, 1997[b]).

The analgesic-like effect of the CNP has been well-studied in snails, including the development of tolerance over multiple days, the time-course of the anti-nociception, and the interaction with opioids (Thomas *et al.*, 1998). In snails, a 15- or 30- minute exposure produced a maximum response within 15 minutes post-exposure, with a significant reduction by 60 minutes post-exposure. Repeating the exposure for 9 consecutive days substantially reduced (but did not eliminate) the effect by day 9. This repeated exposure

also reduced the analgesic response of a δ-opioid agonist, indicating cross-tolerance.

The CNP was also tested in mice (Shupak *et al.*, 2004[a]) where a significant analgesic effect was seen. Interestingly, CNP exposure reduced the effect of morphine on open-field behaviours, indicating that though the analgesic-like effect may be partially opioid-like, it is not the same as activating the (μ) opioid system.

In human volunteers, the pulsed magnetic field exposure significantly increased pain thresholds without affecting sensory thresholds (Shupak *et al.*, 2004[b]).

Head-only exposures were then tested with volunteers with several chronic pain conditions, and the CNP was found to significantly decrease pain scores in those subjects with rheumatoid arthritis (Shupak *et al.*, 2006). A daily repeated exposure trial was then conducted with chronic pain patients where a portable device, delivering head-only exposures, was used within the subjects' own homes. A non-significant tendency towards decreasing pain scores was observed in patients with fibromyalgia (Thomas *et al.*, 2007).

Though these studies with chronic pain conditions warrant further research, the use of head-only exposures does give weight to the hypothesis of a biologically-relevant signal acting via induced currents within the brain.

1.2.4 Other magnetic field effects

The CNP has also been investigated in humans for potential effects on other behavioural endpoints aside from pain.

Standing balance was investigated in healthy volunteers, with a significant improvement in both eyes open and eyes closed balance (Thomas *et al.,* 2001[a]). With the addition of patients with rheumatoid arthritis and fibromyalgia, a (non-significant) differential effect was observed, with rheumatoid arthritis patients experiencing an improvement of eyes-closed standing balance with CNP exposure, while fibromyalgia patients did not (Thomas *et al*., 2001[b]). This differential response between conditions lead to a patent application for the diagnostic use of pulsed magnetic fields (Thomas *et al*., 2002[a]).

Several studies on the effects of pulsed magnetic fields on the human electroencephalogram (EEG) have been conducted by Charles Cook (Cook *et al*., 2004, 2005). The alpha frequency band (8-12 Hz) has been most thoroughly examined, with effects seen in the resting EEG within minutes of exposure. Interestingly, these studies indicate that the effects of pulsed magnetic fields can be very pattern-dependent, with two slightly different CNPs, differing only in the time between repetitions of the pattern (1200 vs 5000 ms) producing opposite effects in the alpha response (Cook *et al*., 2009).

1.3 Introduction to MRI and EEG

1.3.1. Functional MRI

The availability of MRI facilities dedicated for research here at the Lawson Health Research Institute allowed us to plan a functional MRI study to examine whether the changes in pain perception were related to specific changes in the processing of pain, and also to provide an objective measure of those changes.

Functional MRI (fMRI) is an imaging technique that allows us as researchers to obtain information about changes in brain blood flow and oxygenation. It's based on the Blood Oxygenation Level Dependent (BOLD) signal phenomenon. Hemoglobin contains at its core an iron atom, and the magnetic field properties of that molecule vary depending on whether or not hemoglobin is carrying oxygen. That is, oxygenated hemoglobin (oxyhemoglobin) is diamagnetic (weakly opposes the magnetic field of the MRI), whereas deoxygenated hemoglobin (deoxyhemoglobin) is paramagnetic (has a magnetic moment that aligns with the main field of the MRI, and that affects the susceptibility of nearby protons in the MRI).

With certain MRI sequences a higher signal level is obtained with higher concentrations of oxyhemoglobin (vs. deoxyhemoglobin), as the local magnetic field is more homogeneous.

Neural activity, such as when processing a task, represents a metabolic demand, which in turn causes an increase in blood perfusion to the area of

active brain tissue. This is known as the hemodynamic response, and there is a well-known delay between the onset of the neural activity and the response of the cerebral blood flow. Likewise, the increased blood flow typically persists for a few seconds following the cessation of the stimulus and neural processing. The hemodynamic response leads to an overshoot in oxygenated blood to the tissue, that is, more oxyhemoglobin is present during activation than during rest, leading to an increased BOLD MRI signal (with certain MRI acquisition sequences). The BOLD signal is the result of a complex interaction between neural activity, metabolic demand & by-products, blood flow, blood volume, and blood oxygenation (Detre and Wang, 2002).

A function estimating the shape of a typical hemodynamic response in the visual cortex was published by Friston *et al.* (1998), and has subsequently been found to be an acceptable approximation for the blood flow responses in other areas of the brain across a wide variety of tasks, and has become known as the "canonical hemodynamic response function". All experiments described later use the canonical hemodynamic response function in the analysis.

The BOLD signal increase with activation is typically small, around 1% of the total MRI signal. Thus fMRI is a subtraction technique, where sets of images are taken during a task, and during a rest state, and the signal over time is found. This BOLD signal change is then compared to a predicted signal change, which is obtained by convolving the stimulus pattern over time (for these experiments, the thermal stimulus turning on and off) with

the hemodynamic response function. If the change in the BOLD signal level over time within a brain area correlates well with the predicted change, above some statistical threshold, the area is said to be “activated”. If there is a negative correlation (less BOLD signal, i.e. more deoxyhemoglobin during the stimulus) then the area is said to be “deactivated”. Both inhibitory and excitatory neural firing present metabolic demands however, so “activation” as measured by fMRI may represent inhibitory neural processes.

1.3.2 EEG

Electroencephalography (EEG) involves the use of electrodes on the surface of the scalp to detect the electrical activity of the living brain. For this electrical activity to be detectable on the scalp, postsynaptic potentials of many neurons must sum together, which involves the synchronous activity of layers of parallel pyramidal neurons (Fisch, 1999).

Rhythmic activity is a normal observation in the EEG. This EEG behaviour is classified into frequency bands: alpha (8-13 Hz), beta (13-30 Hz), gamma (30+ Hz), delta (<4 Hz), theta (4-8 Hz). Different areas of the brain exhibit different dominant rhythms, and the EEG frequency spectrum changes depending on the processing/mental state that is occurring. The alpha rhythm is the dominant one observed over the posterior area of the cortex during resting wakefulness. It is strongest with the eyes closed, and can be suppressed by sudden attention to visual stimuli (Fisch, 1999).

Within bioelectromagnetics studies using EEG as an observable, the most commonly reported effects are within the alpha frequency band (for reviews, see: Cook *et al.*, 2002, 2006), although many other effects have also been investigated. With a specific pulsed magnetic field, occipital alpha activity initially increased post-exposure, then reversed and decreased relative to sham after several minutes (Cook *et al.*, 2004). This effect on occipital alpha was dependent on the specifics of the pulseform, as a subtle change to the repetition timing altered the effect (Cook *et al.*, 2009). Ghione *et al.* (2005) found an increase in occipital alpha activity following a 90-minute, 80 μT 50 Hz exposure, but not after 40 μT.

1.4 Confounds – MRI effects on cognition

When performing bioelectromagnetics studies within an environment that has very strong magnetic fields present to produce the MRI images, we must always keep the issue of confounds present in our minds. An MRI produces three types of electromagnetic fields for its operation, including a very strong static magnetic field (1.5 T or 3.0 T for the experiments described in chapters 2-5), a time-varying magnetic field in the form of the gradients, with field strengths of mT/m varying on the order of kHz, and radiofrequency fields (64 or 128 MHz).

There are well-known deterministic effects of the fields from an MRI system at certain combinations of field strengths and frequencies, such as peripheral nerve stimulation (Schaefer *et al.*, 2000) or RF heating (Shellock,

2000). Safety limits prevent these deterministic effects from occurring during a typical scan. Stochastic effects (that is, random, non-deterministic) may also result from exposure to the fields in play, and several methods of investigating these effects are discussed in more detail below.

1.4.1 Physiological Effects

Cardiovascular parameters, including systolic and diastolic blood pressures and heart rate have not been affected by MRI scans in previous studies (Atkinson *et al*. 2007), with the exception of a small effect on systolic blood pressure in one study at 8 T (Chakeres and de Vocht, 2005).

The effects of an MRI scan on stress hormone (cortisol, DHEA, and testosterone) levels were examined in children (ages 9-14) by Eatough *et al*. (2009). They found that with an actual MRI scan at 3 T, all three hormones were elevated, but in a simulated scan (including a mockup of a scanner and acoustic noise, but no magnetic fields) these hormones were stable or for cortisol, even below initial levels. Though these hormonal changes may be due to the magnetic fields of the MRI system, the study was not blinded, and there may be additional stress from the actual MRI system (e.g., vibration) that could account for the difference as well.

1.4.2 Animal Behaviour

In a series of experiments with snails, mice, rats, and humans, the Bioelectromagnetics group at the University of Western Ontario and Lawson

Health Research Institute (where the work described in this dissertation was also conducted) investigated the effects of MRI-related magnetic fields on various behaviours with a particular focus on opioid-related nociception.

Ossenkopp *et al*. (1985) found a reduction in morphine analgesia in mice exposed to a 0.15 T MRI procedure, and that the effect also had a day/night dependence, with a stronger reduction in analgesia at night. Similarly, Teskey *et al*. (1988) found a significant reduction in fentanyl-induced analgesia in mice exposed to a similar 0.15 T MRI procedure.

Prato *et al*. (1992) found that in mice, analgesia from an injection of morphine was significantly attenuated by exposure to an imaging sequence in a 0.15 T MRI system. The effect was observed with just the exposure to the time-varying gradient fields, indicating that those are the fields to be most focused on; the radiofrequency fields did have a lesser impact on the attenuation of analgesia, while the static field had no effect (Prato *et al*., 1987). Pre-exposure to the imaging fields had a larger effect on attenuating the morphine analgesia than exposure after injection, which is a relevant concern for the study design used in the experiments to follow, where the specific pulsed magnetic field whose analgesic properties are under investigation was applied after the first round of exposure to the gradient fields for functional imaging.

In a T-maze experiment, rats displayed a significant aversion to entering the arm of the maze that was within a 4 T MRI system, whereas the aversion

was not present at 1.5 T (Weiss *et al.*, 1992). This response was dependent on the vestibular system, as rats with surgically damaged inner ears did not respond to the high static fields (Houpt *et al.*, 2007). Conditioned taste aversion (CTA) can also result from the exposure to a 14.1 T system in rats, and this CTA is unrelated to the speed of insertion and removal (Houpt *et al.*, 2011). The aversion in rats is likely related to the experience of vertigo commonly reported by humans near high static field MRIs (Schenck *et al.*, 1992). Open-field behavioural testing following a 0.15 T MRI found a non-significant decrease in the exposed group relative to the sham, and a significant decrease as compared to a control group (the sham group, with exposure to loud sounds but not the magnetic fields, was in-between the exposed and control groups) after one exposure, but no significant differences after five consecutive days of exposure (Ossenkopp *et al.*, 1986). No effect of exposure was seen on spatial memory performance with an 8-arm maze either (Innis *et al.*, 1986).

In a normal, healthy animal, there is little evidence that behavioural effects of the magnetic fields of an MRI that persist beyond the direct exposure, though few studies have been published (Sweetland *et al.,* 1987). There is evidence that transient effects, or when in a non-homeostatic state such as when opioids are administered, the magnetic fields – in particular the time-changing gradient fields – can affect biological responses, such as nociception and analgesia. This is a particular concern for the studies that

are described in chapters 2-5 as the pain testing (both functional imaging and verbal scores) is conducted within the magnet, simultaneously with the time-varying imaging fields.

1.4.3 Human Behaviour

Passing through a strong static magnetic field with a spatial gradient, as when entering an MRI system, can produce various temporary symptoms in human volunteers. Vertigo, lightheadedness, nausea, and a metallic taste are the most commonly reported (Chakeres and de Vocht 2005), and incidence and severity typically scales with field strength and speed of ingress into the bore (e.g., Schenck *et al*., 1992, Atkinson *et al*., 2007, Weintraub *et al*., 2007).

Other cognitive effects of exposure to MRI-related fields have been examined in human volunteers. Sweetland *et al*. (1987) did not find a significant effect of 0.15 T MRI on various cognitive tests. Atkinson *et al*. (2007) did not find any effect on short-term memory, attention, or fatigue tests in a small study using 25 volunteers. The volunteers underwent a sodium imaging scan at 9.4 T, so the bulk of their head exposure took place within the homogeneous field region at the centre of the bore, with the subjects stationary.

In the fringe fields of a 1.5, 3 T (de Vocht *et al*., 2006), and 7 T MRI, de Vocht *et al*. (2007) found that performance in visual tracking tasks was impaired after subjects moved their heads within the magnetic field, and also

an impairment of tasks requiring hand-eye coordination (non-significant in 2007). In 2006, the same group (de Vocht *et al.*, 2006) found an effect on a working memory test that was not replicated in the 2007 study (de Vocht *et al.*, 2007). The authors also suggest that one important difference in their studies vs those of other groups (e.g., Atkinson *et al.*, 2007, Innis *et al.*, 1986) was that testing took place during exposure. If that was a critical difference, then it would suggest that any cognitive effects are short-lived, fading by the time the subject leaves the field and reaches the testing location for the study.

On the other hand, Rohan *et al.* (2004) found that exposure to an MRI procedure could influence mood in patients with bipolar disorder, and anecdotally reported that the effects could last several days or more (private communication). The effect was specific to the MRI sequence they were using (echo planar magnetic resonance spectroscopic imaging), as a different active MRI sequence (spoiled gradient echo scan) had a significantly different (null) effect.

1.4.4 Other Confounds

The MRI system presents experimental confounds beyond the electromagnetic fields. Most notably, active scans (especially fMRI sequences) produce acoustic noise, enough to require the use of ear plugs, and acoustic noise may influence the activation of other functional tasks (Burke *et al.*, 2000). Confinement within the bore, and also restraint of the participant's

head to reduce motion for scanning can also have psychological impacts. For example, Brockway and Bream (1992) initially found some declines in performing memory tasks after a 1.5 T MRI, but in follow-up experiments including a sham scanner that simulated the acoustic noise and confinement, did not see group differences vs the active MRI. They then attributed the earlier cognitive effects to the psychological influence of the scanner environment and not the static magnetic field or radiofrequency exposure. Gutchess and Park (2006) likewise found impairment in a memory task during an fMRI scan, but did not control for magnetic field exposure, so the effect is likely due to the confinement and noise. Burke *et al.* (2000) found that in rats the area of activation from electrical stimulation as measured by a BOLD-fMRI study was modulated by the acoustic noise of the scanner.

Also, with the use of the canonical hemodynamic response function (Friston *et al.*, 1998), we are also making the assumption that the pulsed magnetic fields introduced as part of the studies do not affect the hemodynamic response of our volunteers. The evidence for an effect of magnetic fields on blood perfusion is mixed, with many studies suggesting a biphasic effect that depends on the initial state of the system (McKay *et al.*, 2007). The specific pulsed magnetic field investigated later in this work was investigated by McKay *et al* (2010), and was not found to influence acetylcholine-perturbed blood flow in skeletal muscle in rats.

Finally, maintaining double-blinding can be an issue when adapting clinical diagnostic equipment such as MRI scanners to experimental intervention applications. In the case of the experiments that follow, the pre-exposure testing was performed double-blind, however the experimenter (J.A.R.) became unblinded at the exposure stage. A similar difficultly was experienced by de Vocht *et al*. (2007) resulting in their experiments merely being single-blind, where the subject is unaware of his or her exposure condition, but the experimenter is not blinded.

1.5 Discussion

The interaction of magnetic fields with human and animal behaviour is a fascinating, fast developing area of science. Effects on analgesia are both some of the most reproducible, as well as the most therapeutically interesting. Work in our group has demonstrated that the site of action for the analgesic effect of pulsed magnetic fields is likely the brain, and so it would be of interest to use fMRI to examine how the neuroprocessing of pain is altered by these pulsed magnetic fields.

This is an undertaking not without its challenges, as there is evidence that the magnetic fields of the MRI system may themselves alter nociception, or reduce antinociception produced by other agents.

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Chapter 2: Low Frequency Pulsed Electromagnetic Field Exposure Can Alter Neuroprocessing in Humans

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Low Frequency Pulsed Electromagnetic Field Exposure Can Alter Neuroprocessing in Humans

By: John A. Robertson, Jean Théberge, Julie Weller, Dick J. Drost, Frank S. Prato, Alex W. Thomas

2.1 Introduction

Current research demonstrates that magnetic fields affect various aspects of animal behaviour. The influence that magnetoreception can have on the orientation and migration of various species has been widely reported (Johnsen and Lohmann, 2008; Stapput *et al.*, 2008), and there is also considerable evidence that magnetic fields, in particular low frequency

magnetic fields, influence nociception in animals and humans (Del Seppia *et al*., 2007). The initial biophysical detection mechanism of magnetoreception remains controversial but three candidate general mechanisms exist: a) detection by magnetic dipoles within cells and tissue; b) detection of an induced current; c) and detection via the different chemical reaction rates when the electron spins of free radicals are affected by a magnetic field. Evidence to date suggests that the effect on animal orientation is mediated by tissue dipoles and/or the free radical mechanism (Johnsen and Lohmann, 2008) while the evidence for antinociceptive effects may depend on several mechanisms (Del Seppia *et al*., 2007; Prato *et al*., 2009; Prato *et al*., 2000; Thomas *et al*., 1997). Within the strong static field of an MRI (1.5 T) mechanisms a) and c) are not likely candidates for the explanation of pulsed electromagnetic field effects – see Appendix B for further discussion on interaction mechanisms. Here we test for the induced current mechanism for magnetoreception in humans using fMRI wherein we hypothesize that the application of low frequency time varying magnetic fields induces currents affecting neural firing in the central nervous system. Though the induced currents are very weak, networks of neurons are more sensitive to weak fields than isolated neurons (Francis *et al*., 2003).

It has been shown that analgesia can be induced by repeated exposures to a simple sinusoidal magnetic field repeated daily (Kavaliers and Ossenkopp, 1993) and by exposure to an extremely low frequency (ELF)

pulsed electromagnetic field (PEMF) (Thomas *et al*., 1997). It has been further reported that this pulsed magnetic field can induce analgesia in humans (Shupak *et al*., 2004; Thomas *et al*., 2007). Moreover, in humans the effect is specific to nociception and does not affect thermal sensory thresholds (Shupak *et al*., 2004), and is effective on both acute and chronic pain (Thomas *et al*., 2007). The analgesic effect appears to operate via the central nervous system, as suggested by the effectiveness of localized head-only exposures, and reports of pulsed magnetic field exposures affecting EEG measurements (Cook *et al*., 2005).

Aside from nociception, research into how magnetic fields can affect biological systems is increasingly showing that pulsed magnetic fields can have subtle neuromodulatory effects. Capone *et al*. (2009) found that a 75 Hz pulsed electromagnetic field altered a transcranial magnetic stimulation measure in human volunteers. Rohan *et al*. (2004) describes the temporary beneficial effects of exposure to a pulsed MRI gradient MF sequence while patients with bipolar disorder were undergoing a MR spectroscopy examination. The exposure was caused by the switching magnetic field gradients needed to generate the MRI images. This report was seminal as it suggested that the MRI gradients could be used to induce electric currents with neuromodulatory effects, and that magnetoreception in humans was not confounded by exposure to the strong static field from MRI. Hence we programmed the gradient system of a 1.5T clinical MRI system to deliver an

analgesia-inducing PEMF and to monitor, using blood oxygenation level dependent (BOLD)-fMRI, the effect of that exposure on the neural processing related to pain perception in healthy volunteers.

Functional Magnetic Resonance Imaging (fMRI) can determine the localized relative changes in neural activation of regions of the brain based on changes in blood oxygenation and blood flow that occur in response to the altered metabolic demand of activated neurons. Processing of pain involves coordinated activation across many sites in the brain, including the cingulate cortex, the somatosensory areas, the insula, and other parts of the limbic system (Apkarian *et al*., 2005). Neuromodulation is potentially a very important factor in the fMRI signal according to a recent review by Logothetis (2008), and BOLD-fMRI has been used to determine subtle changes in the experience of pain in previous studies (Wager *et al*., 2004).

2.2 Methods

Right-handed healthy adult subjects aged 18-60 were recruited to participate in a functional magnetic resonance imaging study. Exclusion criteria included claustrophobia, nerve damage to the hand, analgesic use on the day of the study, alcohol use on the day of the study, and the inability to lie still for an hour, as well as any other MRI exclusion criteria (e.g.: cardiac pacemakers). Subjects were blinded to their condition of sham vs pulsed magnetic field exposure.

Subjects were given acute thermal pain with a Medoc TSA-II (Medoc, Israel). A 1.6 x 1.6 cm Peltier thermode probe was attached to the hypothenar region of the right hand and heated under computer control (heat stayed on for 21 seconds, off for 24 seconds, with 3 second ramps in between). Each subject underwent a test prior to the fMRI to determine their individual pain tolerance. The target temperature was adjusted individually to attain a subjective pain rating of at least 7/10 on a verbal analog scale (1-10). Subjects were asked to confirm that they could tolerate that level of pain without moving when in the scanner. Actual temperatures varied between 48 and 51°C, depending on the subject.

After informed consent and thermal pain pre testing, subjects were placed in the MRI system, told to hold still and keep their eyes closed during the functional imaging, and that they would have a 50-50 chance of receiving a pulsed magnetic field exposure that may have analgesic effects. Single-shot echo-planar BOLD images were acquired (16, 5 mm-thick oblique slices, 64 x 64 resolution, 192 mm FOV, 3 s TR, 50 ms TE). Slices were primarily transversal, inclined when viewed sagittally so that the frontal sinuses were not included in the imaging volume.

fMRI images were acquired on a Siemens Avanto 1.5 T MRI while the thermal pain cycled on and off, 10 times for each round of functional imaging. Immediately after each round the subjects were asked to rate their subjective pain verbally over the intercom. The subjects then had a 15 minute "rest"

period within the MRI system during which time they were not allowed to move and were exposed to the PEMF, or a sham condition. Subjects' heads were gently restrained using the adjustable foam pads included with the Siemens Avanto head coil. The functional imaging and pain protocol was then repeated to obtain "post-exposure" data, following which T1-weighted anatomical images were obtained (3D MPRAGE sequence, 1 mm isovoxel resolution, 192 slices, 256x256 mm FOV).

The pulsed magnetic field exposure was done within the MRI system by programming the Z-gradient coils (the gradient along the bore of the magnet). The peak gradient strength was 2 mT/m, and the patient table was offset 10 cm cranially from the isocentre so that the field at the brow level was set to be 200 μT, the same field strength used in whole-body exposures (non-MRI) within our lab in the past with Helmholtz coils (Shupak *et al.*, 2004); see figure 2.1 for the waveform of the complex pulsed electromagnetic field, and Appendix B for more detail on the table offset. The peak rate of change of the applied PEMF is 0.4 T/s (with a gradient slew rate of 4 mT/m/ms). The functional imaging portion of the scan used a pulsed gradient waveform with a peak rate of change of magnetic field within the imaging volume of 8 T/s (a gradient slew rate of 160 mT/m/ms); see Appendix B for more detail on the waveform of the fMRI sequence.

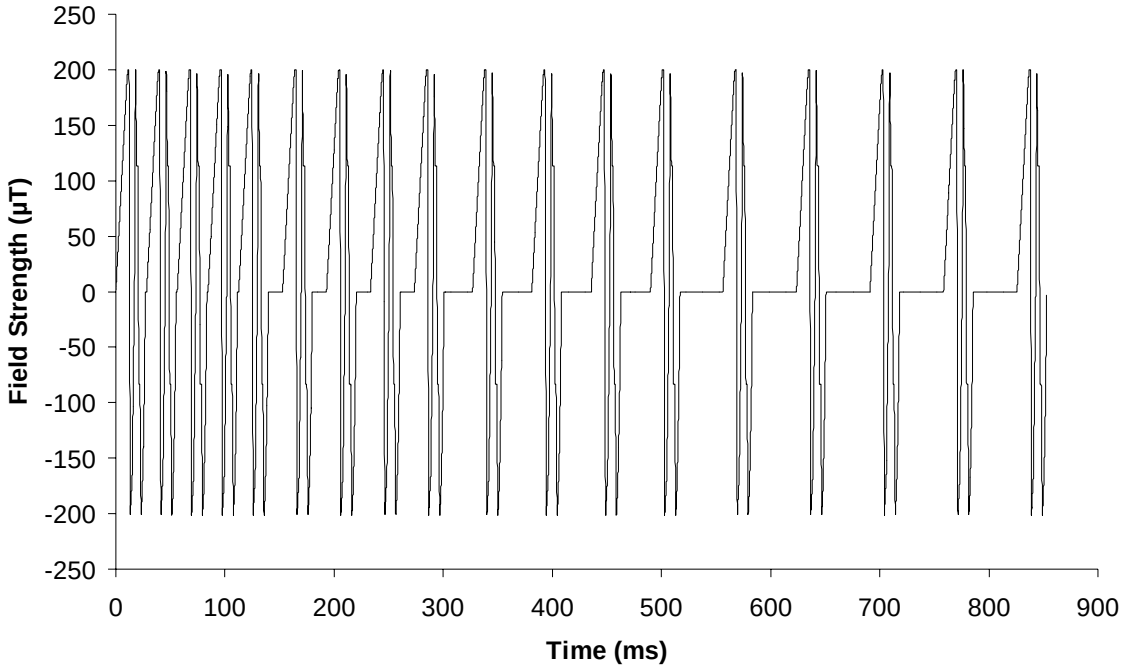

Figure 2.1: PEMF Waveform.

The PEMF used in this and previous studies. This 853 ms long base pattern was repeated 4 times with varying delays between each repetition (110 ms, 220 ms, 330 ms, with 1200 ms at the end) for an overall period of 5272 ms. The field strength refers to the field strength at the brow, due to the gradient it would be stronger at the top of the head, weaker towards the bottom.

The PEMF exposure did produce some acoustic noise within the MRI, however the scanner is a noisy environment, and we were unable to detect the difference in noise levels above background with either a RadioShack sound level meter (model 33 2055, using an acrylic tube to help direct the sound from the centre of the bore to the meter held safely outside the main field at the foot of the bed) or with a piezoelectric microphone (Bruel & Kjaer type 2801, Denmark); for comparison the sound of the functional imaging sequence was measured as having 5X the background sound pressure on the piezoelectric microphone (an increase of), and an increase of 13 dB on the RadioShack sound level meter. That subjects were not able to determine which condition they were in was confirmed by a chi-squared test on their believed condition ($p>0.05$).

Functional image processing was done with Brain Voyager (Brain Innovation B.V., the Netherlands) v1.9.9. Individual datasets were preprocessed with temporal filtering (with a high pass filter that had a cut-off frequency of 3 cycles/scan), 3D motion correction, spatial smoothing (Gaussian 8 mm FWHM) and then spatially normalized to Talairach space to be combined for a General Linear Model (GLM) group analysis. For the sake of analysis, the "pain" condition was defined to be when the heat was on at target temperature; all other images (baseline and the ramps) were taken to be part of the "rest" condition. Default hemodynamic response curves were used. An average of all Talairach anatomicals was created to display the

results of the GLM analysis. The default False Discovery Rate (FDR) method was used to balance images to $q<0.05$. The FDR is an algorithm that accounts for multiple comparisons within fMRI analysis that is less stringent than a Bonferroni correction. All images are presented in the radiological convention (left-is-right).

Based on the initial results seen from the separate-group analysis within Brain Voyager and on the *a priori* knowledge of brain regions associated with pain processing, 1 cm^3 cubic regions of interest were chosen and the beta weights exported for analysis in SPSS to explore potential interactions. An alpha level of $p<0.05$ was selected for statistical significance, with no corrections made for testing multiple ROIs (8 total: anterior, dorsal-medial, posterior cingulate; ipsilateral/right and contralateral/left insula and hippocampus/caudate; thalamus).

All procedures were approved by the University of Western Ontario Human Ethics Review Board (protocol #10059).

2.3 Results

Thirty-one subjects have been included in the analysis (17 sham, 14 PEMF, see Table 2.1 for summary subject information). Differences were observed within groups over time, as well as between groups in functionally relevant areas: the anterior cingulate, the insula, and the hippocampus/caudate (Figure 2.2, 2.3, and 2.4). For each figure, the blue/green false colours indicate that there was less activation in the post-

exposure (PEMF or sham) compared to the pre-exposure fMRI, and the yellow/red colours indicate more activation during the painful stimulus after exposure compared to pre-exposure.

Guided by these visual results, the data from 1 cm^3 volumes were extracted and analyzed within SPSS to obtain a full model. Significant interactions were found for the anterior cingulate, ipsilateral (right) insula, and the bilateral hippocampus/caudate region (see Table 2.2 for details). The analyzed beta weights (a measure of the strength of the correlation between the BOLD signal measured and the pain on/off predictor) are plotted in Figure 2.5 to demonstrate that the interaction is due to a decrease in activity following the 200 μT pulsed magnetic field exposure. See Appendix B for additional information about main effects of time as well as event related average BOLD signal time courses.

	Average age	Gender	Temperature set-point	Guessed in sham condition	Pre pain rating	Post pain rating
PEMF	32.2 ± 2.7	6 F / 8 M	49.6 ± 0.2	57%	8.14±0.2	7.57±0.4
Sham	27.6 ± 1.5	9 F / 8 M	49.6 ± 0.2	71%	8.79±0.2	8.54±0.3

Table 2.1: Summary of subject vitals for each group ± SEM.

The differences in both pre- and post-exposure pain ratings were significant between groups (pre: $F_{1,29}=5.2$, $p<0.05$, post: $F_{1,29}=4.9$, $p<0.05$), but the interaction was not.

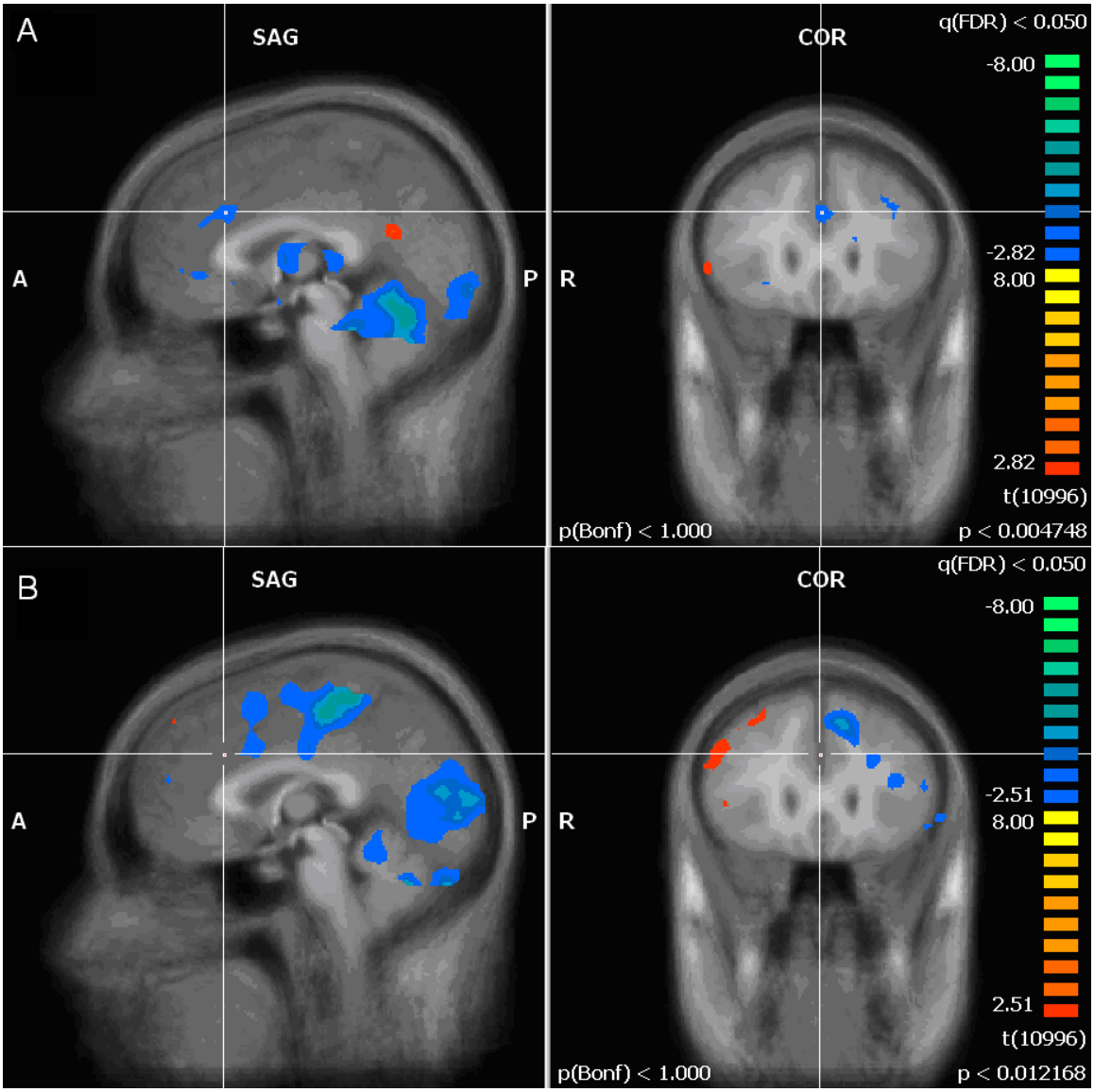

Figure 2.2: Anterior cingulate.

A – PEMF Post-Pre condition. This is the statistical difference between the activation seen with pain after exposure minus that seen before. There was a significant decrease in activity after pulsed PEMF exposure compared to before exposure in the anterior cingulate.

B – Sham Post-Pre condition. This image indicates that there was no change in activity within this region in the sham group over time.

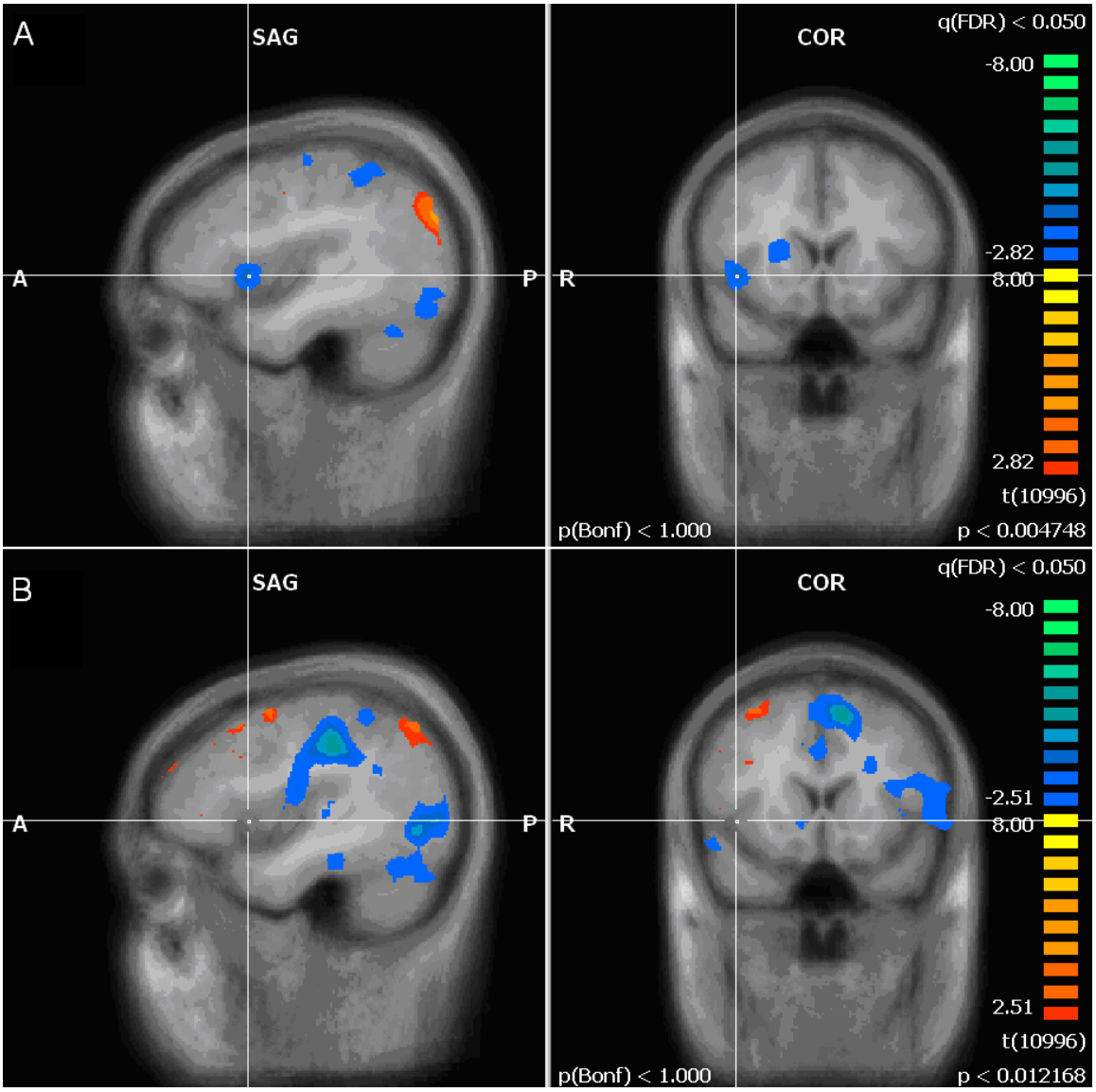

Figure 2.3: Ipsilateral insula.

A – PEMF Post-Pre. This image shows a significant decrease of activity within the right insula following PEMF exposure as compared to pre-exposure.

B – Sham Post-Pre. No difference is seen due to time alone in the sham condition.

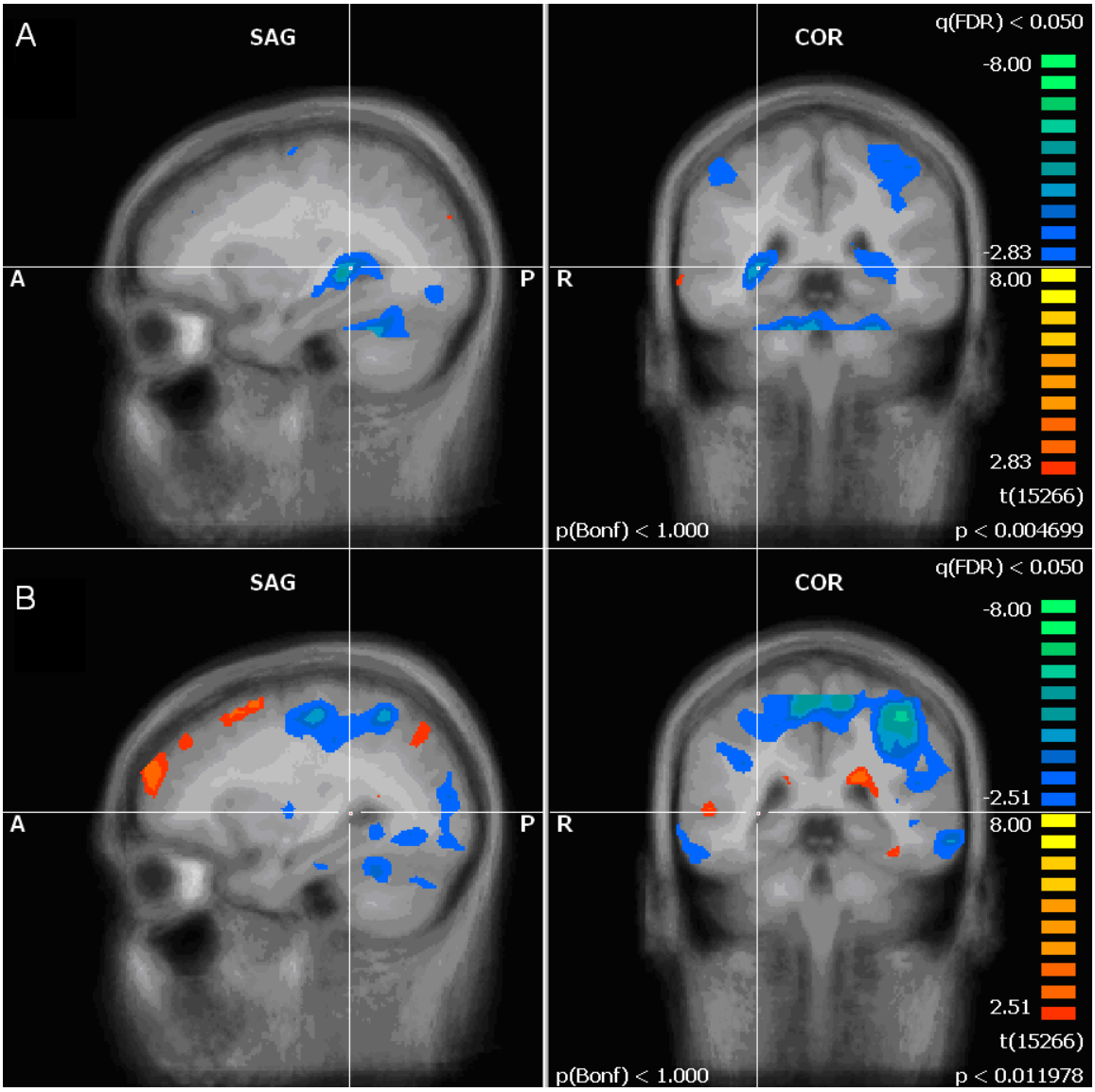

Figure 2.4: Hippocampus/Caudate.

A – PEMF Post-Pre. This image shows a significant decrease of activity within the hippocampus/caudate area following PEMF exposure as compared to pre-exposure. Due to the relatively poor spatial resolution it is difficult to say exactly which structure(s) this activity is originating from.

B – Sham Post-Pre. No difference is seen due to time alone in the sham condition.

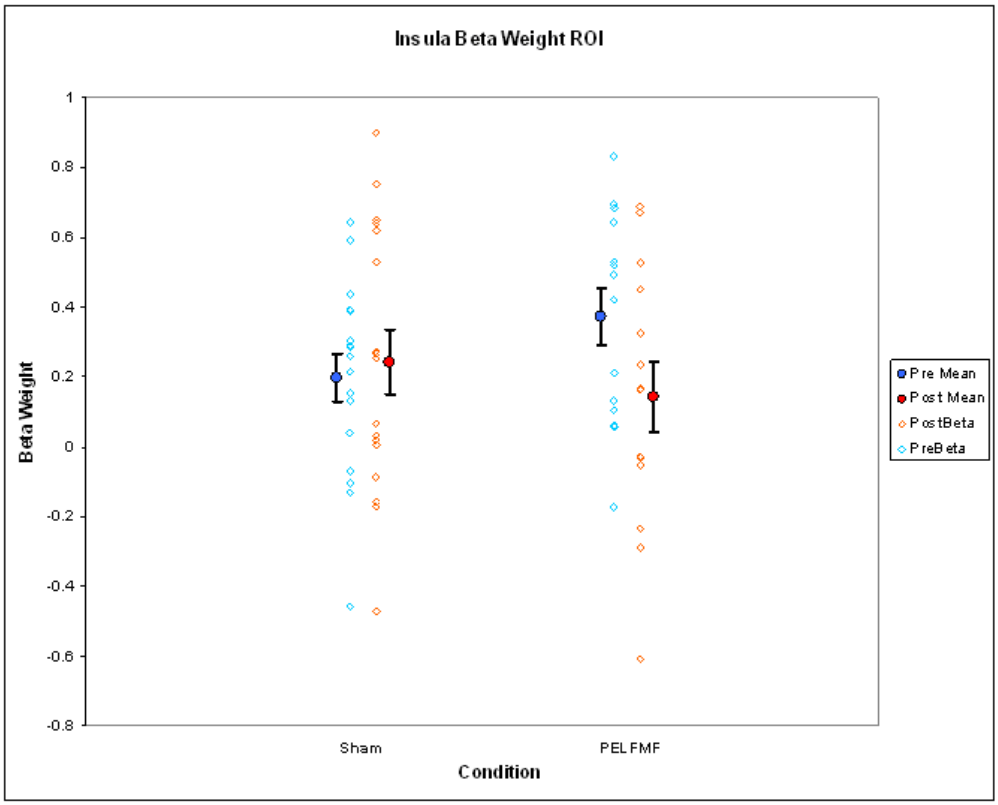

Figure 2.5: Beta weights from ipsilateral insula.

The means before (large blue circle) and after (large red circle) sham or pulsed magnetic field exposure (PEMF) are plotted, with error bars as +/- SEM. There is a relative decrease following PEMF exposure, giving rise to a significant interaction. Individual beta scores are plotted as faint open symbols for pre (blue) and post (orange) exposure to demonstrate the range of individual variability. The other interactions found likewise appear to be driven largely by a decrease in activity following PEMF exposure.

Region	Interaction F	Interaction p	Partial eta-squared	Observed power
Anterior cingulate	$F_{1,29} = 6.834$	$p < 0.05$	0.19	0.72
Insula (ipsilateral)	$F_{1,29} = 5.204$	$p < 0.05$	0.15	0.60
Hippocampus/Caudate (ipsilateral)	$F_{1,29} = 13.803$	$p < 0.01$	0.32	0.94
Hippocampus/Caudate (contralateral)	$F_{1,29} = 6.055$	$p < 0.05$	0.17	0.66

Table 2.2: Summary of significant interactions found.

2.4 Discussion

The anterior cingulate, insula, and hippocampus/caudate are classically associated with the integration of the affective components of pain, and a decrease in activation here corresponds well with our hypothesis that this PEMF influences pain processing in central structures. Functional changes were largely detected only in central structures, which could have been anticipated by the previous work indicating that pain, but not sensory thresholds were affected (Shupak *et al.*, 2004). Some changes in the somatosensory areas were observed over time, but there was no significant interaction with magnetic field exposure.

These fMRI effects were seen after a 15 minute exposure, consistent with effects seen in humans on EEG from similar length exposures (Cook *et al.*, 2005) and antinociception seen in snails and rodents also after 15 minute exposures (Thomas *et al.*, 1997). However in previous human studies investigating subjective relief from both acute and chronic pain, longer periods of exposure were used (30 min, Shupak *et al.*, 2004; 40 min, Thomas *et al.*, 2007). Here the short 15 minute exposure did not lead to subjective effectiveness, yet the pulsed magnetic field exposure did induce significant changes in functional activity. It is possible that the effect of the PEMF on nociception is altered by the interactions of the strong static field (1.5 Tesla) (Laszlo and Gyires, 2009) and the time-varying fields associated with the imaging procedures (Prato *et al.*, 1992).

It is interesting to note that neuromodulation occurred in the environment of the MRI. The effects of the 1.5 T static magnetic field should have interfered with any free radical mechanism, which depends largely on the sum of the low-frequency and static field strengths (within the MRI this sum would be much larger than the experiments outside the MRI) (See NIEHS report, 1998, §4.8.3.5). The torque on iron particles such as magnetite produced by the ELF MF would have been very small compared to the torque produced by the 1.5T static field of the MRI main magnet. There was no subjective change in pain ratings, but the ELF MF produced during the fMRI procedure could have induced an increase in pain sensitivity countering the analgesic effect as has been previously reported (Prato *et al*., 1992; Prato *et al*., 1987). Hence the hypothesis that the effect is produced by induced currents is not contradicted and suggests that the induction of analgesia, at least in humans, may depend on a different mechanism than that for animal orientation and homing. This is not surprising given current evidence that magnetoreception even in birds may be achieved by more than one mechanism with one dependent on light exposure (free radical mechanism) and one independent of light exposure (magnetite) (Johnsen and Lohmann, 2008; Stapput *et al*., 2008). Even within the induced current paradigm, different pulse designs may differentially influence behaviour (Thomas *et al*., 1997). Using the gradient fields to produce a biological effect may become an important technique in the future, particularly if a "magnetic contrast" can

be developed, such as a pulsed magnetic field that differentially affects fMRI processing between healthy and patient populations for a certain disease/disorder.

2.5 Acknowledgements

The authors would like to thank Dr. Derek Mitchell, Dr. Dwight Moulin, Dr. Alexandre Legros, Dr. Keith St. Lawrence, and Mr. Lynn Keenliside for their assistance with this project. This project was funded by a grant from the Canadian Natural Sciences and Engineering Research Council and the Canadian Institutes of Health Research.

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Chapter 3: Evidence for a dose-dependent effect of pulsed magnetic fields on pain processing

Note: a version of this chapter has been published in the journal *Neuroscience Letters.*

Evidence for a dose-dependent effect of pulsed magnetic fields on pain processing

By: John A. Robertson, Nicole Juen, Jean Théberge, Julie Weller, Dick J. Drost, Frank S. Prato, Alex W. Thomas

3.1 Introduction

Extremely Low Frequency (ELF) Magnetic fields (MF) have been found to have differential biological effects on nociception in a variety of organisms, and the effect on nociception is one of the most robust effects of time-varying magnetic field exposure studied. Increases or decreases in nociceptive sensitivity can be produced depending on the type and duration of magnetic

field exposure, and there may be several different mechanisms of magnetic field interaction underlying effects on nociception (Del Seppia *et al.*, 2007; Prato *et al.*, 2000; Prato *et al.*, 2009; Thomas *et al.*, 1997).

Recently, we reported that exposure to a specific pulsed electromagnetic field (PEMF) could alter the processing of acute thermal pain as measured by fMRI (Robertson *et al.*, 2010). The pulsed, extremely low frequency fields were introduced within the MRI between functional imaging sessions by the Z-gradient coils of the 1.5 T scanner.

We hypothesized that the initial biophysical transduction step may be in part depend on an induced current mechanism. One piece of evidence that might support this theory would be the presence of a dose-dependent response, which would not necessarily be present for a resonance-based mechanism. For low frequencies, a radical-pair based mechanism depends only on the strength of the magnetic field applied. An effect dependent on a radical-pair transduction mechanism may also show a dose-response, however we view that as unlikely given the very strong static field (1.5 T) present in the imager, as it is unlikely that there would be a window of effect between 1.5000 T and 1.5002 T. Beyond the initial transduction step however, any dose-response could be masked or accentuated by the biological response (i.e. changes to the neural network).

To search for a dose-dependent response we recruited additional subjects to participate in the study that has been previously reported (Robertson *et al.*, 2010).

3.2 Methods

Please see Robertson *et al.* (2010) for the full details of the initial study and image analysis. Briefly, right-handed healthy adult subjects aged 18-60 were recruited to participate in a functional magnetic resonance imaging study. Subjects were blinded to their condition of sham vs pulsed magnetic field exposure. The data from 47 subjects have been included in the analysis; the data from the sham and 200 μT exposed subjects (17 sham, 14 at 200 μT PEMF) has been previously presented (Robertson *et al.,* 2010), and data from subjects exposed to 100 μT PEMF (N=6) and 1000 μT PEMF (N=10) has been added to examine a potential dose response. See table 3.1 for descriptives on the subjects and subjective pain scores.

Condition	Average age	Gender	Temperature set-point	Guessed in sham condition	Pre pain rating	Post pain rating
Sham	27.6 ± 1.5	9 F / 8 M	49.6 ± 0.2	71%	8.8±0.2	8.5±0.3
100 μT	23.0± 1.4	3 F / 3 M	49.8 ± 0.2	50%	8.5±0.4	8.2±0.6
200 μT	32.2 ± 2.7	6 F / 8 M	49.6 ± 0.2	57%	8.1±0.2	7.6±0.4
1000 μT	27.1 ± 3.1	6 F/ 4 M	49.5 ± 0.2	30%	8.2±0.4	8.0±0.4

Table 3.1: Descriptive statistics, +/- SEM.

Subjects were given acute thermal pain with a Medoc TSA-II (Medoc, Israel). A 1.6 x 1.6 cm Peltier thermode probe was attached to the hypothenar region of the right hand and heated under computer control (heat stayed on for 21 seconds, off for 24 seconds, with 3 second ramps in between). The target temperature was adjusted individually to attain a subjective pain rating of at least 7/10 on a verbal analog scale (1-10) prior to the scans. Subjects were asked to confirm that they could tolerate that level of pain without moving when in the scanner. Actual temperatures varied between 48 and 51°C, depending on the subject.

After informed consent and thermal pain pre testing, subjects were placed in the MRI system, told to hold still and keep their eyes closed during the functional imaging, and that they would have a 50-50 chance of receiving a pulsed magnetic field exposure that 'may have analgesic effects.' Data was acquired in the form of single-shot, echo planar BOLD images (16, 5 mm-thick oblique slices, 64 x 64 resolution, 192 mm field view, 3 s repetition time, 50 ms echo time).

Functional MRI images were acquired on a Siemens Avanto 1.5 T MRI (Siemens, Erlangen Germany) while the thermal pain cycled on and off, 10 times for each round of functional imaging. Immediately after each round the subjects were asked to rate their subjective pain verbally over the intercom. The subjects then had a 15 minute 'rest' period within the MRI system during which time they were not allowed to move and were randomly exposed to

either the PEMF, or a sham condition. Subjects' heads were gently restrained using the adjustable foam pads included with the Siemens Avanto head coil. The functional imaging and pain protocol was then repeated to obtain 'post-exposure' data, following which T1-weighted anatomical images were obtained (3D MPRAGE sequence, 1 mm isovoxel resolution, 192 slices, 256x256 mm FOV).

The pulsed magnetic field exposure was done within the MRI system by programming the Z-gradient coils (the gradient along the bore of the magnet). The peak field strength at the brow level was set to either sham, 100, 200, or 1000 μT. The patient table was offset 10 cm cranially from the isocentre, and the peak gradient strength was 1, 2, or 10 mT/m (respectively) to generate each magnetic field exposure condition (Robertson *et al.*, 2010). The timing of the pulsed field pattern was the same for each condition, only the field strength was scaled. The peak rate of change of the applied PEMF was 0.2, 0.4, or 2 T/s (with a gradient slew rate of 2, 4, or 20 mT/m/ms) at the centre of the head. For comparison, the functional imaging portion of the scan used a pulsed gradient waveform with a peak rate of change of magnetic field within the imaging volume of 8 T/s (a gradient slew rate of 160 mT/m/ms), where the top of the brain/imaging volume would be approx 5 cm offset from isocentre.

The PEMF exposure did produce some acoustic noise within the MRI, however the scanner is a noisy environment, and we were unable to detect the difference in noise levels above background with a RadioShack sound

level meter (Model 33 2055), using an acrylic tube to help direct the sound from the centre of the bore to the meter held safely outside the main field at the foot of the bed; for comparison the sound of the functional imaging sequence was measured as having an increase of 13 dB on the sound level meter. It is expected that 1000 μT exposure level would produce more acoustic noise than the 200 μT exposure. Subjects were asked which condition they believed they were in and were unable to discern their respective exposure condition. This was confirmed by a chi-squared test on their stated condition: all groups were not significantly different from a predicted 50% response rate ($p>0.05$), however if the sham group's response rate was selected as the predicted distribution (70% believed they were in the sham exposure), then the frequency of responses in the 1000 μT exposure group was significantly different than expected (30% responding that they were in the sham group, $p<0.05$).

Functional image processing was done with Brain Voyager (Brain Innovation B.V., the Netherlands) v1.9.9. Individual datasets were preprocessed with temporal filtering (with a high pass filter that had a cut-off frequency of 3 cycles/scan), 3D motion correction, spatial smoothing (Gaussian 8 mm FWHM) and then spatially normalized to Talairach space to be combined for a General Linear Model (GLM) group analysis. For the sake of analysis, the "pain" condition was defined to be when the heat was on at target temperature; all other images (baseline and the ramps) were taken to

be part of the "rest" condition. Default haemodynamic response curves were used for convolution of the on/off predictor.

Regions-of-interest (ROIs) that were found to have significant interactions in the previous study (Robertson *et al.*, 2010 or Chapter 2) were used to analyze the additional subject data, namely the anterior cingulate cortex, ipsilateral (right) insula, and both left and right hippocampus/caudate nucleus areas. The pre-exposure data was subtracted from the post-exposure data to get a difference over time. The correlation between this difference over time and the magnetic field intensity (with sham considered to be 0) was then determined. No correction for multiple comparisons (4 ROIs total) was made.

All procedures were approved by the University of Western Ontario Human Ethics Review Board (protocol #10059).

3.3 Results

Significant correlations between the differences between pre- and post-exposure brain activation, as measured by the "beta weight," and field strength were found for the anterior cingulate ($p < 0.05$, $r^2 = 0.095$) (Figure 3.1) and insula ($p < 0.05$, $r^2 = 0.101$) (Figure 3.2), but not for either left or right hippocampus/caudate area (not shown). The anterior cingulate region of interest is shown in figure 3.3 with the differences between pre- and post-exposure activation displayed for each condition.

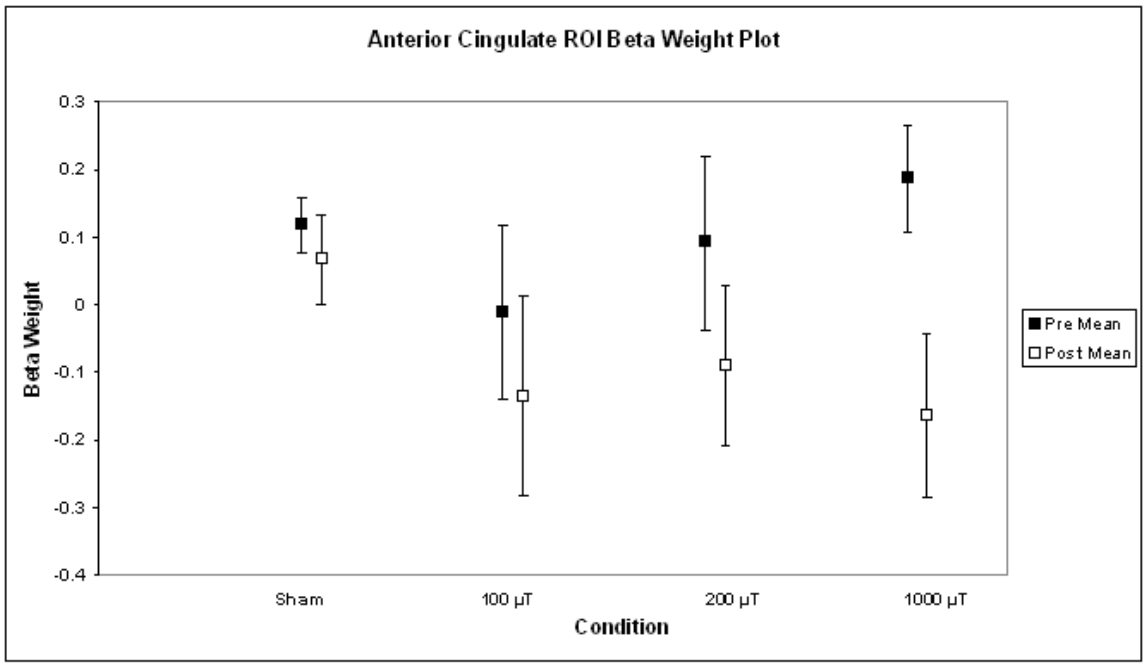

Figure 3.1: Anterior cingulate.

The means for pain activation in the anterior cingulate before (black symbol) and after (white symbol) sham or pulsed extremely low frequency magnetic field exposure (PEMF) are plotted, with error bars as +/- SEM.

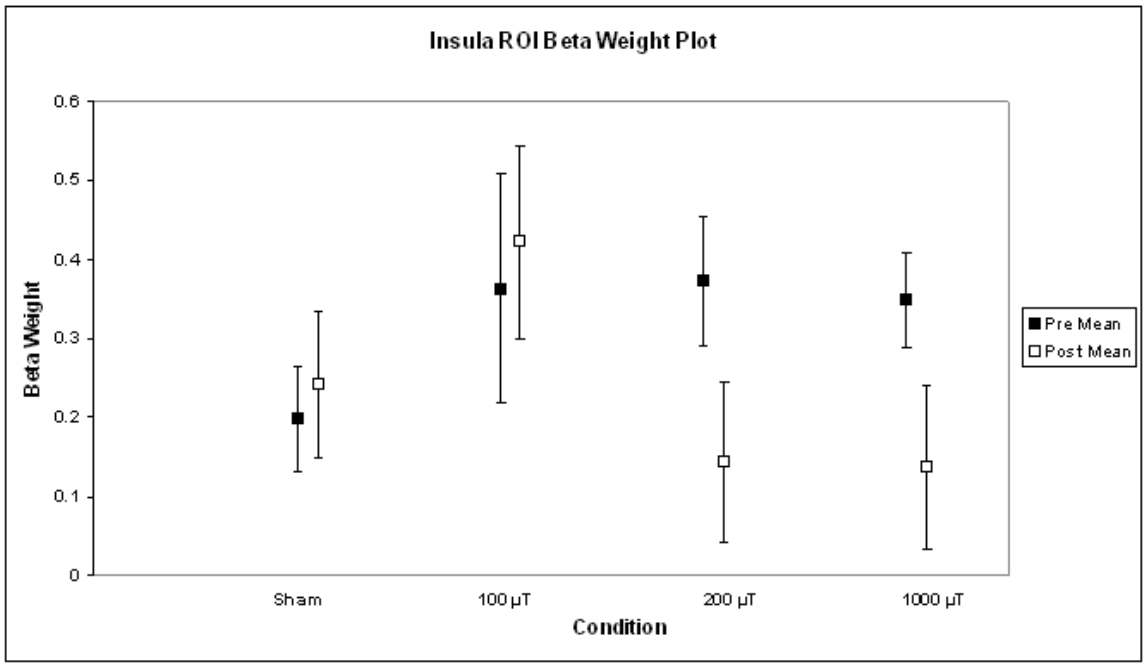

Figure 3.2: Insula.

The means for pain activation in the right insula before (black symbol) and after (white symbol) sham or pulsed extremely low frequency magnetic field exposure (PEMF) are plotted, with error bars as +/- SEM.

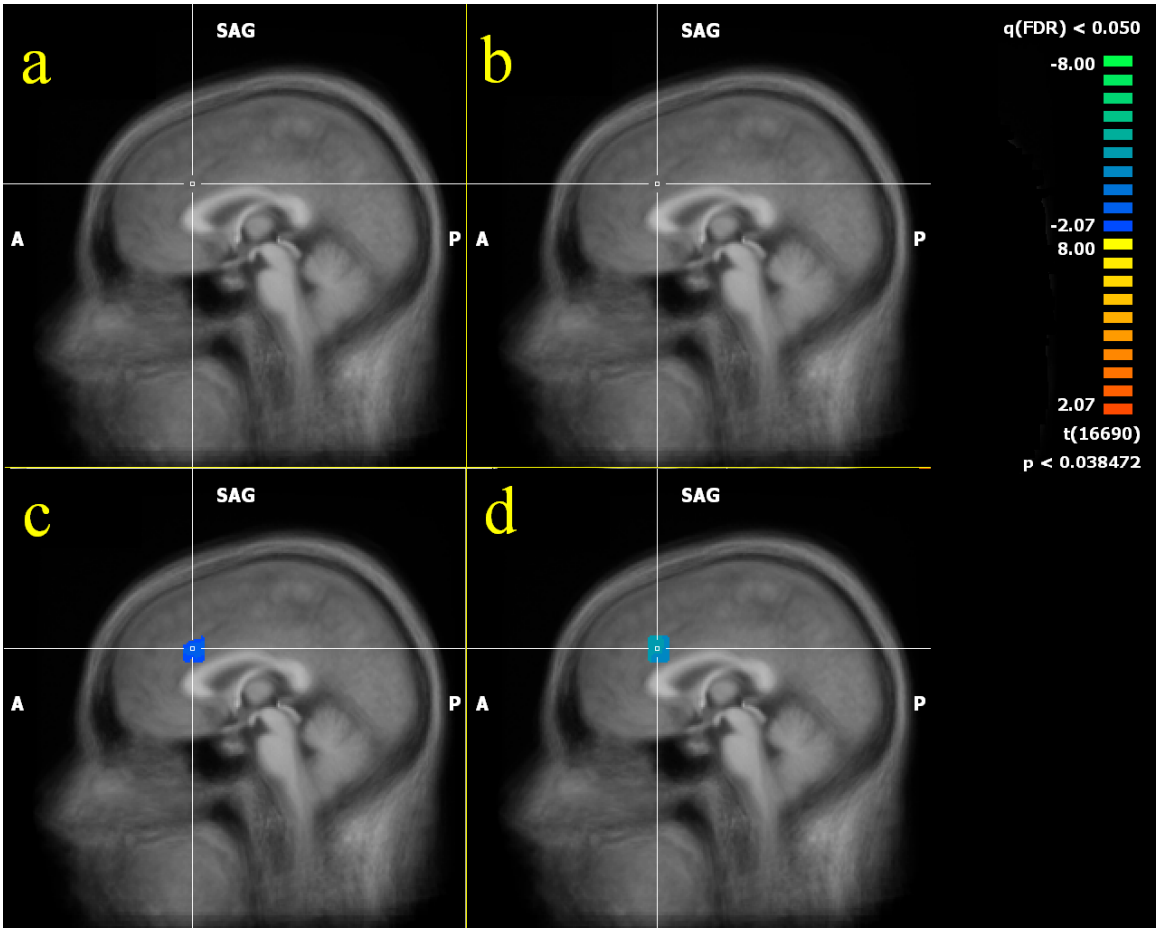

Figure 3.3: fMRI images.

The difference in activation between pre- and post-exposure within the anterior cingulate region of interest is shown for each exposure condition (a) sham, (b) 100 μT, (c) 200 μT, (d) 1000 μT.

3.4 Discussion

The present study investigated different PEMF intensities to identify a potential dose response relationship in regions of the brain that had previously been identified to have significant changes in pain processing activity when exposed to a 200 μT field. Significant correlations between the changes in neural activation and field strength were found for both the anterior cingulate cortex and insula, providing some evidence for a differing response at different field intensities, the beginnings of a dose-response effect. As the strength of PEMF exposure increased, less post-exposure processing of pain was evident in these regions. However, no such correlation was found for the hippocampus/caudate areas, indeed it appears as though there is a reversal of the putative analgesic effect in these areas at higher field strengths. There does appear to be some sort of differential dose response, however with the limitations of the present study it is difficult to say whether this represents a true quasi-linear dose-dependency, or if there is a threshold effect somewhere between 100 and 200 μT for the anterior cingulate and the insula; and the pattern seen for the hippocampus/caudate regions might suggest a window of response. It is unclear if the dose responses would be due to the initial transduction mechanism, or if the different properties/neural specialization of the brain structures in question could be responsible.

The current study was exploratory, as it was the first to investigate the effects of MF exposure on brain activation using varying field strengths. The use of the MRI hardware made changing the desired field strength very easy, however, one must be aware in this setup of the potential for acoustic noise confounds, which increase along with field strength.

Our results may lend support to the induced current model, as a dose-relationship response was found through significant correlations observed in the anterior cingulate and the insula. However, as seen here, not all brain areas exhibited this behaviour: this may be due to differences between brain structures, or the interaction of several mechanisms to produce the overall analgesic effect.

3.5 Acknowledgements

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Chapter 4: Magnetic Field Exposure Can Alter Pain-Related Brain Neuroprocessing in Humans

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Magnetic Field Exposure Can Alter Pain-Related Brain Neuroprocessing in Humans

By: John A. Robertson, Jean Théberge, Frank S. Prato, Alex W. Thomas.

4.1 Introduction

Pulsed, extremely low-frequency (ELF, from DC to 300 Hz) magnetic fields (MF) have been shown to affect pain sensitivity in snails, rodents, and humans (Del Seppia *et al.*, 2007; Prato *et al.*, 2000; Thomas *et al.*, 1997). ELF MF can have different effects on nociception depending on a variety of parameters of the magnetic field (and light) exposure, increasing or decreasing sensitivity to a noxious stimulus. Inhibition of nociception was found to be stronger with a specific pulsed electromagnetic field (PEMF) (Thomas *et al.*, 1997), leading to further studies with humans, both healthy controls and chronic pain patients (Shupak *et al.*, 2004, 2006). Head-only

exposures have been examined (Shupak *et al.*, 2006) providing evidence for a centrally-mediated mechanism.

To investigate the ways in which this magnetic field could affect pain neuroprocessing in healthy volunteers, a functional magnetic resonance imaging (fMRI) study was undertaken (Robertson *et al.*, 2010a). Acute thermal pain-related activation as measured by the beta weight was significantly different between pre- and post-exposure in some regions of interest (ROI), the anterior cingulate and right insula in particular, using a 15-minute, 200 μT exposure paradigm. No significant changes in subjective pain ratings were observed in these studies, which was a departure from previous work with these PEMF. There could be several reasons for this finding, including a potential anti-analgesic effect of the MRI, as previously seen in snails and mice (Prato *et al.*, 1992, 1987).

A pilot study adding additional subjects at other field strengths (100 μT and 1000 μT) provided initial evidence that there may be a dose-response relationship supportive of an induced current mechanism (Robertson *et al.*, 2010b). Thus, there is some doubt that the static magnetic field of the MRI environment confounds the experiment, as a strong static field should not affect an induced current mechanism. However, competing effects of increasing sensitivity due to the imaging (gradient) time-varying magnetic fields is possible, and there is evidence that these magnetic fields of the MRI environment may inhibit opioid-related analgesia (Prato *et al.*, 1987; Teskey

et al., 1988; Prato *et al.*, 1992; Laszlo and Gyires, 2009). It was hypothesized that one relevant difference between previous work and the fMRI studies (Robertson *et al.*, 2010a,b) was the short exposure period of 15 minutes. While studies with snails (Prato *et al.*, 2000, Thomas *et al.*, 1997) used 15 minute exposures, human studies have used 30 (Shupak *et al.*, 2004, 2006) or 40 (Thomas *et al.*, 2007) minute exposures. Thus, the current study was initiated using a similar acute thermal pain stimulus with functional imaging, but the exposure period was extended to 45 minutes.

4.2 Methods

Right-handed healthy adult subjects of both genders aged 18-60 were recruited to participate in a functional magnetic resonance imaging study. Exclusion criteria included claustrophobia, nerve damage to the hand, analgesic use on the day of the study, alcohol use on the day of the study, and the inability to lie still for an hour, as well as any other MRI exclusion criteria (e.g.: cardiac pacemakers). Subjects were blinded to their condition of sham vs pulsed magnetic field exposure, and assigned to an exposure condition randomly. The experimenter was blinded to the subject condition until after the pre-exposure scanning and subjective pain scoring, however, once the exposure condition (sham or PEMF) began, the experimenter became aware of the subject's condition.

Subjects were given acute thermal pain with a Medoc Pathway (Medoc, Israel). A 1.6 x 1.6 cm Peltier thermode probe was attached to the hypothenar

region of the right hand and heated under computer control (heat stayed on for 24 seconds, off for 30 seconds, with 3 second ramps in between; two, 1 second, 3 °C dips in temperature were included in the plateau). Each subject underwent an ascending method of limits test (Coren *et al.*, 1999; Medoc, 2007) prior to the fMRI to determine their individual pain tolerance. The target temperature was adjusted individually to attain a subjective pain rating of at least 7/10 on a verbal analog scale (1-10). Subjects were asked to confirm that they could tolerate that level of pain without moving when in the scanner. Actual temperatures varied between 47.5 and 50.0°C, depending on the subject, with a mean (±SEM) of 49.0°C ±0.1°C.

After informed consent and thermal pain pre-testing, subjects were placed in the MRI system, asked to hold still and keep their eyes closed during the functional imaging, and that they would have a 50-50 chance of receiving a pulsed magnetic field exposure that may have analgesic effects. Single-shot echo-planar BOLD images were acquired (17, 5 mm-thick oblique slices, 64 x 64 resolution, 192 mm FOV, 3 s TR, 50 ms TE). Slices were primarily transversal, inclined when viewed sagittally so that the frontal sinuses were not included in the imaging volume.

fMRI images were acquired on a Siemens Avanto 1.5 T MRI while the thermal pain cycled on and off, 10 times for each round of functional imaging. Immediately after each round the subjects were asked to rate their subjective pain verbally over the intercom. The subjects then had a 45 minute "rest"

period within the MRI system during which time they were not allowed to move and were exposed to the PEMF, or a sham condition. Subjects' heads were gently restrained using the adjustable foam pads included with the Siemens Avanto head coil. The functional imaging and pain protocol was then repeated to obtain "post-exposure" data, following which T1-weighted anatomical images were obtained (3D MPRAGE sequence, 1 mm isovoxel resolution, 192 slices, 256x256 mm FOV).

Subjects were asked at the end of the study if they fell asleep during the 45-minute exposure period. Subjects were also considered to have fallen asleep if the experimenter heard snoring, or if the subjects were non-responsive when asked if they were ready for the second round of imaging. There was no objective measure of awake/sleep status.

The pulsed magnetic field exposure was done within the MRI system by programming the Z-gradient coils (the gradient along the bore of the magnet). The peak gradient strength was 2 mT/m, and the patient table was offset 10 cm cranially from the isocentre so that the field at the brow level was set to be 200 μT, the same field strength used in whole-body exposures (non-MRI) within our lab in the past with Helmholtz coils (Shupak *et al.*, 2004).

Functional image processing was done with Brain Voyager (Brain Innovation B.V., the Netherlands) v1.9.9. Individual datasets were preprocessed with temporal filtering (with a high pass filter that had a cut-off frequency of 3 cycles/scan), 3D motion correction, spatial smoothing

(Gaussian 8 mm FWHM) and then spatially normalized to Talairach space to be combined for a General Linear Model (GLM) group analysis. For the sake of analysis, the "pain" condition was defined to be the final 12 s of the plateau plus the 3 s ramp back to baseline; the "rest" condition was taken as the final 21 s of the baseline period [the remainder was discarded due to evidence from Owen *et al.* (2008), that blood flow from pain may last longer than the hemodynamic models predict]. Default 2-gamma hemodynamic response curves were used (Friston *et al.*, 1998). An average of all Talairach anatomical images was created to display the results of the GLM analysis to better demonstrate the actual neuroanatomical variability for our subject pool. For the pre-post contrast images, a Bonferroni correction was applied to a corrected $p<0.05$. All images are presented in the radiological convention (figure left is subject right).

Based on the initial results seen from the separate-group analysis within Brain Voyager and on the *a priori* knowledge of brain regions associated with pain processing and the results of previous work (Robertson *et al.*, 2010a), 3 cm^3 cubic regions of interest were chosen and the beta weights exported for analysis in SPSS to explore potential interactions (Repeated Measures General Linear Model with pre-/post-exposure as a within-subjects measure and condition as a between-subjects measure). An alpha level of $p<0.05$ was selected for statistical significance, with no

corrections made for testing multiple ROIs (4 total: anterior, dorsal-medial, posterior cingulate; right insula).

All procedures were approved by the University of Western Ontario Health Sciences Research Ethics Review Board (protocol #10059).

4.3 Results

62 subjects were recruited that passed the initial exclusion criteria; 1 subject was excluded that had an outlier decrease in their reported pain, and 2 subjects that did not report at least 6 out of 10 for their initial pain rating, for 59 subjects providing at least subjective data. For the fMRI analysis, a further 2 subjects were excluded due to data loss, 1 because they did not close their eyes as instructed, 15 subjects that fell asleep during the 45 minute exposure period, and 8 due to excessive head motion. 33 subjects were included in the final analysis (15 sham, 18 PEMF exposed) that remained awake, that were well balanced between males (7 sham, 8 PEMF) and females (8 sham, 10 PEMF).

Within the cingulate, three regions of interest were chosen: anterior, dorsal-medial, and posterior cingulate/precuneus area. A region of interest in the right insula was also selected. These 4 regions of interest were selected *a priori* based on the results of our previous study (Robertson *et al.*, 2010a). Two regions within the hippocampus/caudate nucleus were also planned based on the results of that study, however there was little pain-related

activation in that area prior to exposure in either group, so those regions were not included for the mixed model analysis.

A significant time (pre-post) by condition (sham, PEMF exposed) interaction was seen within the right insula ($F_{1,31} = 5.77$, $p<0.05$, partial $eta^2 = 0.16$, power = 0.64), and also a main effect of time ($F_{1,31} = 18.9$, $p<0.01$, partial $eta^2 = 0.38$, power = 0.99). See Table 4.1 for a summary of the statistical tests. A main effect of time was also seen in the anterior and dorsal-medial cingulate, along with a tendency towards significance for the time-by-condition interaction (anterior cingulate: main effect $F_{1,31} = 13.2$, $p<0.01$, partial $eta^2 = 0.30$, power = 0.94; interaction $F_{1,31} = 3.75$, $0.1>p>0.05$, partial $eta^2 = 0.11$, power = 0.47; dorsal-medial cingulate: main effect $F_{1,31} = 13.9$, $p<0.01$, partial $eta^2 = 0.31$, power = 0.95; interaction $F_{1,31} = 3.67$, $0.1>p>0.05$, partial $eta^2 = 0.10$, power = 0.43). There was no significant main effect of time or interaction with exposure condition for the posterior cingulate (main effect $F_{1,31} = 0.02$, $p>0.5$, partial $eta^2 = 0.001$, power = 0.05; interaction $F_{1,31} = 0.04$, $p>0.5$, partial $eta^2 = 0.001$, power = 0.05).

Within these three regions of interest, a significant difference exists in the pre-exposure beta weights (AC: $F_{1,31} = 9.70$, $p<0.01$; DC: $F_{1,31} = 10.4$, $p<0.01$; RI: $F_{1,31} = 19.0$, $p<0.01$), indicating that there may be an issue of accidental selection bias driving the interactions observed. This group difference in pre-exposure values was not present in the subjective pain ratings ($F_{1,31} = 1.00$, $p>0.3$).

There was a significant effect of sleep on the difference in subjective pain ratings ($F_{1,56} = 13.50$, $p<0.01$, partial $eta^2 = 0.19$, power = 0.95). Excluding those who fell asleep (21 sham, 24 PEMF exposed that remained awake) a significant main effect of time was observed ($F_{1,43} = 27.23$, $p<0.01$, partial $eta^2 = 0.39$, power = 1.0), but no interaction of the magnetic field with time for the subjective pain ratings in awake subjects ($F_{1,43} = 0.422$, $p>0.1$, partial $eta^2 = 0.01$, power = .10). There was a significant interaction with gender (time*condition*gender $F_{1,41} = 5.20$, $p < 0.05$, partial $eta^2 = 0.11$, power = 0.60), where females had a significant decrease in subjective pain ratings after exposure (females alone $N_{sham} = 10$, $N_{PEMF} = 13$, $F_{1,21} = 4.94$, $p<0.05$, partial $eta^2 = 0.19$, power = 0.56). A power analysis indicates that, if the observed excess 0.159/10 decrease in pain scores in the PEMF exposed group is valid for the general population, with the observed standard deviations, then 286 subjects would need to be recruited to detect that difference for an alpha level of 0.05 and a power of 0.5. See Table 4.1 for a summary of statistical tests.

A chi-squared test on subjects' believed condition confirmed that blinding was maintained (67.9% of sham believed they were in sham, 58.1% of PEMF exposed believed they were in sham, Chi-squared on exposed frequencies = 1.38, $p>0.1$).

Test	**p**	**F**	**partial eta²**	**power**
Time x condition interaction (RI)	$p<0.05$	$F_{1,31} = 5.77$	0.16	0.64
Main effect of time (RI)	$p<0.01$	$F_{1,31} = 18.9$	0.38	0.99
Time x condition interaction (AC)	$0.1>p>0.05$	$F_{1,31} = 3.75$	0.11	0.47
Main effect of time (AC)	$p<0.01$	$F_{1,31} = 13.2$	0.30	0.94
Time x condition interaction (DC)	$0.1>p>0.05$	$F_{1,31} = 3.67$	0.31	0.95
Main effect of time (DC)	$p<0.01$	$F_{1,31} = 13.9$	0.10	0.43
Time x condition interaction (PC)	$p>0.5$	$F_{1,31} = 0.04$	0.001	0.05
Main effect of time (PC)	$p>0.5$	$F_{1,31} = 0.02$	0.001	0.05
ANOVA of pre-exposure beta weights	AC: $p<0.01$ DC: $p<0.01$ RI: $p<0.01$	AC: $F_{1,31} = 9.70$ DC: $F_{1,31} = 10.4$ RI: $F_{1,31} = 19.0$		
ANOVA of pre-exposure subjective ratings	$p>0.3$	$F_{1,31} = 1.00$		
Main effect of sleep on subjective ratings	$p<0.01$	$F_{1,56} = 13.50$	0.19	0.95
Time x condition interaction on subjective ratings (awake subjects)	$p>0.1$	$F_{1,43} = 0.422$	0.01	0.10
Main effect of time (awake subjects)	$p<0.01$	$F_{1,43} = 27.23$	0.39	1.0

Table 4.1: Summary of statistical tests.

RI = right insula; AC = anterior cingulate; DC = dorsal-medial cingulate; PC = posterior cingulate.

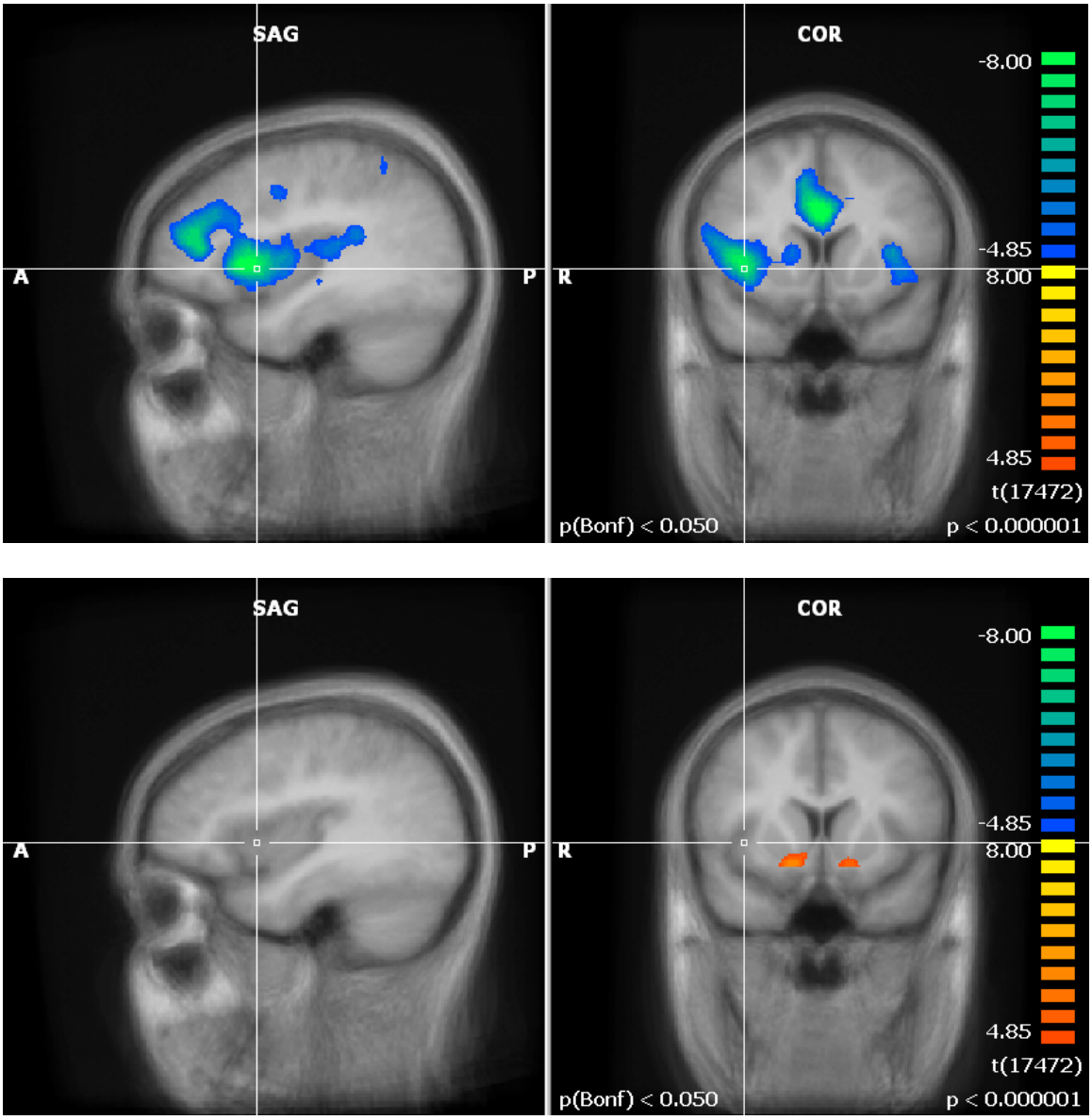

Figure 4.1: Right insula.

The difference in activation from the painful stimulus from post-exposure to pre-exposure for the PEMF exposed group (top) and the sham group (bottom), focused on the right insula. Blue/green colours indicate less activation after exposure than before.

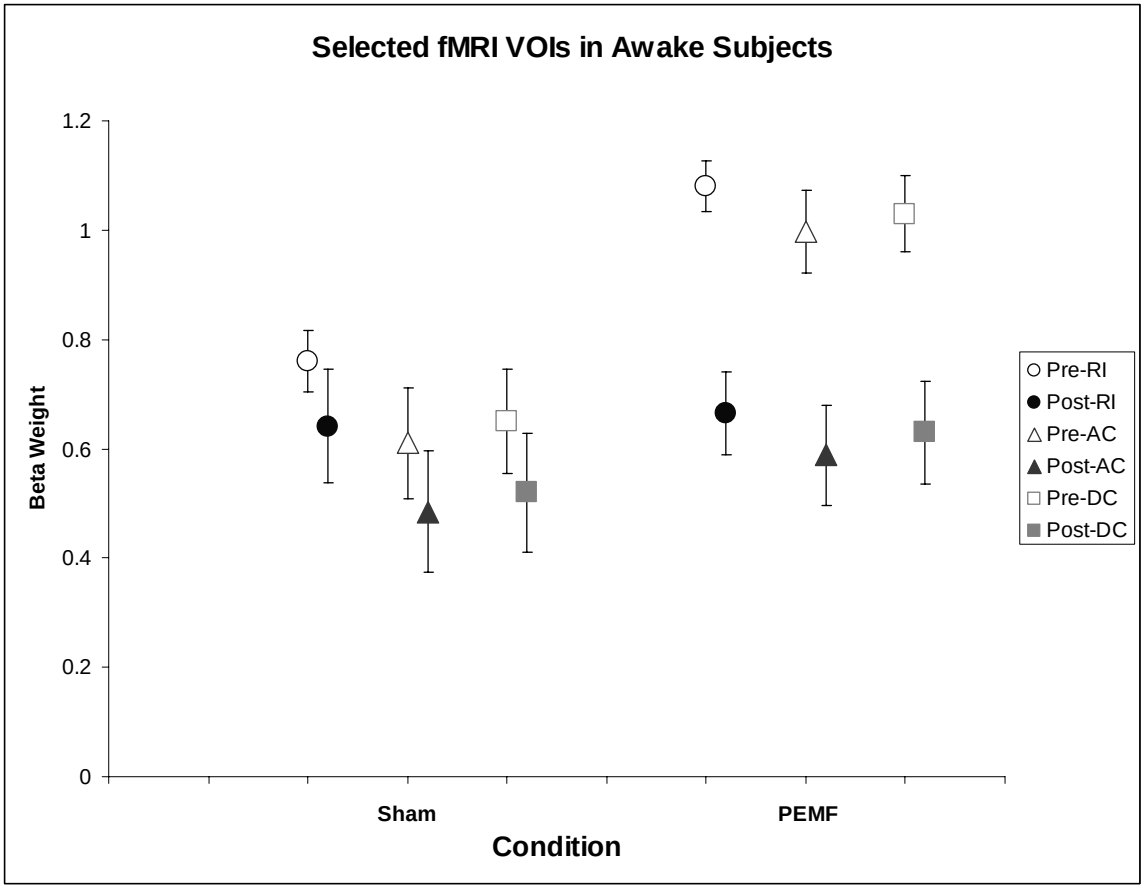

Figure 4.2: fMRI Activation in VOIs.

Activation with pain in selected volumes of interest for the fMRI analysis. Pre-exposure symbols are white, post-exposure are dark for the right insula (RI – circles), anterior cingulate (AC – triangles), and dorsal-medial cingulate (DC – squares). Sham exposure (N = 15) is on the left, PEMF exposure (N = 18) on the right.

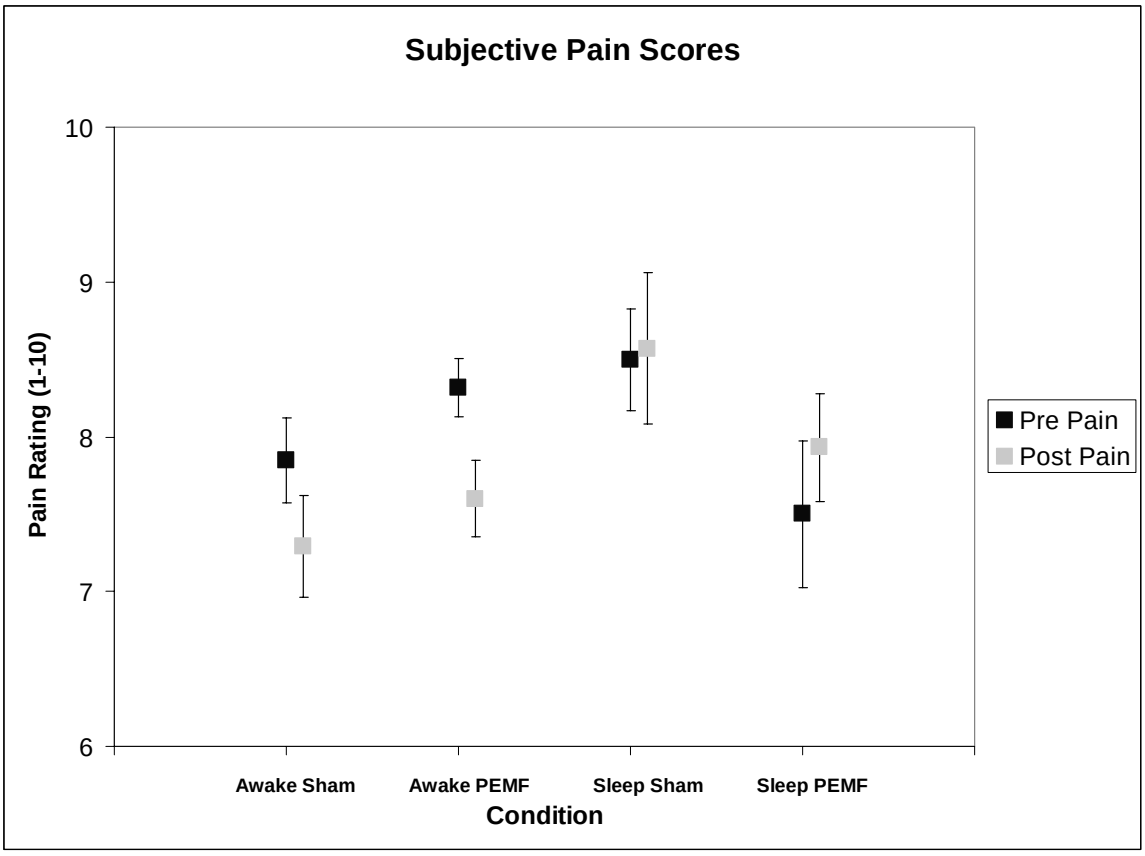

Figure 4.3: Subjective pain scores in awake vs asleep subjects.

A significant main effect of sleep is present in the difference between pre- (dark squares) and post- (light squares) exposure. Error bars represent SEM.

4.4 Discussion

Previously, we presented the results of a functional imaging study demonstrating decreases in pain processing in brain areas associated with the affective component of pain as a result of a pulsed electromagnetic field exposure (Robertson *et al.*, 2010a). However, that study used a 15-minute exposure, which was shorter than had been used in any human pain-related PEMF experiment in our lab previously. There was no change in subjective ratings with that short exposure, possibly due to exposure duration, number of subjects in the study, or other confounds of the MRI environment. The present study, using a 45-minute exposure sought to determine whether the duration of the exposure was the cause for not seeing subjective changes, or if perhaps the environment of the MR imaging itself could be a confound (Prato *et al.*, 1987, 1992).

Unfortunately, the long exposure period within the MRI lead to a number of subjects falling asleep, and there was a significant effect of sleep on subjective pain scores. As this was a serendipitous finding, there was no objective measure of whether or not a subject fell asleep (or for how long), it is possible that other undetected sleepers could be skewing the results of the subjects considered awake above. This serendipitous finding will be important for the design of future studies on nociception/pain and the use of PEMF that involve long periods of relaxation.

Changes in brain activity were seen within the same regions as the previous study, indicating that fMRI may continue to be a useful tool that is more sensitive to subtle changes in pain and affective processing than subjective measures.

4.5 Acknowledgements

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Chapter 5: The Effects of ELF Magnetic Field Exposure on Combined MRI-EEG Measures of a Painful Thermal Stimulus in Humans

By: John A. Robertson, Nicole Juen, Julien Modolo, Jodi E. Miller, Jean Théberge, Frank S. Prato, Alex W. Thomas

5.1 Introduction

Pulsed, extremely low-frequency (ELF, from DC to 300 Hz) magnetic fields (MF) have been shown to affect pain sensitivity in snails, rodents and humans (Del Seppia *et al.*, 2007; Prato *et al.*, 2000; Thomas *et al.*, 1997[a]). ELF MF can have different effects on nociception depending on a variety of parameters of the magnetic field (and light) exposure, increasing or decreasing sensitivity to a noxious stimulus. Inhibition of nociception was found to be stronger with a specific pulsed electromagnetic field (PEMF) (Thomas *et al.*, 1997[a]), leading to further studies with humans, in both normal controls and chronic pain patients (Shupak *et al.*, 2004, 2006).

To investigate the ways in which this pulsed magnetic field could affect pain neuroprocessing in normal volunteers, a functional magnetic resonance imaging (fMRI) study was undertaken (Robertson *et al*, 2010a). Significant effects of the pulsed magnetic field exposure were seen as changes between pre- and post-exposure in the amount of activation in pain-related brain

areas, using a 15-minute, 200 μT exposure paradigm. However, no significant changes in subjective pain ratings were observed in these studies, which was a departure from previous work with these PEMF. This may have been due to competing effects of the imaging fields increasing sensitivity, as there is evidence that the magnetic fields of the MRI environment may inhibit opioid-related analgesia (Prato *et al.*, 1987; Teskey *et al.*, 1988; Prato *et al.*, 1992; Laszlo and Gyires, 2009). We also hypothesized that the short exposure duration may have had a role to play, leading to a study with a 45-minute exposure period being performed, which similarly found significant interactions between the exposure and time for the fMRI signal in the right insula (Robertson *et al.*, 2011).

The 45-minute study (Robertson *et al.*, 2011) found that subjects falling asleep were a significant confounder on subjective ratings, and a good, objective measure of sleep status was not available. However, with the addition of an MRI-compatible electroencephalography (EEG) system to our site, we have a means to more objectively evaluate whether subjects fall asleep, and also to simultaneously evaluate changes in EEG measures of neural activity.

EEG offers some advantages over our BOLD-fMRI boxcar-epoch technique, namely that it does not require a task to evaluate (i.e., we can look at resting brain function), and that the temporal resolution is much higher. EEG has been an active area of interest for bioelectromagnetics researchers,

with the alpha frequency band representing a feature of particular interest (Cook *et al.*, 2002; 2006).

For the specific pulsed magnetic field investigated here, Cook *et al.* (2004) found that resting alpha activity was briefly increased over the occipital electrodes, relative to the sham difference, immediately following exposure, which then reversed to become a decrease several minutes following the end of the exposure. Using a method that permitted recordings during exposure (Cook *et al.*, 2009), a transient decrease in alpha activity was found during exposure.

Though animal work indicated that the specific PEMF used in our lab was more effective at inducing analgesia than a field with a different pulseform (Thomas *et al.*, 1997[a]), this has not as yet been confirmed in a study with human volunteers. Here we have selected a 60 Hz sinusoidal field as the non-specific MF exposure. This is a common non-specific magnetic field exposure, and also of interest due to its use in power transmission. Ghione *et al.* (2005) found that a 80 μT 50 Hz magnetic field exposure increased occipital alpha activity, and a 40 (but not 80) μT also decreased a pain threshold measurement.

The present study utilizes both functional MRI and EEG to investigate the effects of a specific pulsed magnetic field as well as a 60 Hz sinusoidal magnetic field in humans. Resting EEG, fMRI of a painful thermal stimulus, and subjective ratings of said pain were the observables of interest.

5.2 Methods

Right-handed healthy adult subjects aged 18-55 were recruited to participate in a functional magnetic resonance imaging (fMRI) study. Exclusion criteria included claustrophobia, nerve damage to the hand, analgesic use on the day of the study, alcohol use on the day of the study, caffeine or nicotine intake within 6 hours of the study, or the inability to lie still for an hour, as well as any other MRI exclusion criteria (e.g.: cardiac pacemakers). Subjects were blinded to their condition of sham vs pulsed magnetic field exposure. Experimenters were blinded to the condition until after it commenced, but had limited interaction with the subjects beyond that point, as the subjects were within the MRI system.

After informed consent, subjects were fitted with a Neuroscan MagLink 64-channel MRI-compatible EEG cap (Compumedics Neuroscan, Charlotte, NC, USA) with electrodes in the 10-20 configuration. A common reference electrode was located at the CPZ position, and a ground electrode at the FCZ position. Subjects' scalps were gently abraded with a wire brush, and the sites for skin electrodes (M1, M2, VEOG, and EKG) cleaned/abraded with NuPrep (Weaver and Company, CO, USA). The cap was then placed on the subjects' head, and each electrode filled with QuikGel (Compumedics Neuroscan, Charlotte, NC, USA). Gentle abrasion with a blunt syringe was used as needed to improve impedances to 15 kΩ or lower.

Subjects were given acute thermal pain with a Medoc Pathway (Medoc, Israel). A 1.6 x 1.6 cm Peltier thermode probe was attached to the hypothenar region of the right hand and heated under computer control (baseline of 31°C for 30 seconds, non-painful warmth of 40°C for 9 s, then painful heat stayed on for 24 seconds, with 3 second ramps in-between each plateau change; two, 0.5 second, 1.5°C dips in temperature were included in the painful heat plateau). Each subject underwent a test prior to the fMRI to determine their individual pain tolerance. The target temperature was adjusted individually to attain a subjective pain rating of at least 7/10 on a verbal analog scale (1-10). Subjects were asked to confirm that they could tolerate that level of pain without moving when in the scanner. Actual painful heat plateau temperatures varied between 47.5 and 50.0°C, depending on the subject.

After thermal pain pre-testing, subjects were placed in the MRI system, told to hold still and keep their eyes closed during the functional imaging, and that they would have a 50-50 chance of receiving a pulsed magnetic field exposure. Single-shot echo-planar BOLD images were acquired (30, 3.2 mm-thick oblique slices, 64 x 64 resolution, 205 mm FOV, 3 s TR, 30 ms TE). Slices were primarily transversal, inclined when viewed sagittally so that the frontal sinuses were not included in the imaging volume.

Subjects' heads were gently restrained using the adjustable foam pads included with the Siemens Verio 12-channel head coil, with a piece of 3M micropore tape (3M, MN, USA) placed across their forehead to reinforce the

need to hold still. Functional MRI images were acquired on a Siemens Verio 3.0 T MRI while the thermal pain cycled on and off, 10 times for each round of functional imaging. Immediately after each round of imaging the subjects were asked to rate their subjective pain verbally over the intercom. The subjects then had a 40 minute "rest" period within the MRI system during which time they were not allowed to move and were exposed to either 1) the PEMF, 2) 60 Hz MF (both at 200 μT peak), or 3) a sham condition for 30 minutes. A 5-minute rest recording before and after the exposure period rounded out the 40-minute rest period. At the beginning of the rest period, subjects were asked to close their eyes and relax without falling asleep, and a 10-minute EEG recording was initiated. At the 5-minute mark in this period the exposure condition was initiated. After the EEG recording, subjects were instructed to open their eyes, and respond to the intercom to ensure they were still awake. Halfway through the exposure condition, another 5 minute EEG recording was made, with the subjects again instructed to close their eyes, and then after the recording to open them and try to stay awake. Five minutes prior to the end of the exposure condition, another 10 minute eyes-closed EEG recording was made, covering the final 5 minutes of the exposure condition as well as the transition to the rest condition. The functional imaging and pain protocol was then repeated to obtain "post-exposure" data following which T1-weighted anatomical images were obtained (3D FLASH sequence, 1 mm isovoxel resolution, 176 slices, 256x232 mm FOV). Eyes-

closed EEG recordings were also made during each of the 12-minute functional MRI and thermal stimulus acquisitions. See figure 5.1 for a schematic of the experimental timeline.

As the introduced pulsed magnetic field can produce some acoustic noise within the MRI system, a simulation of the sound of the PEMF was created and played back via the speaker system of the MRI during the sham and 60 Hz conditions so that all 3 conditions would be as similar as possible. Due to the difficulty in using microphones within the MRI environment, the volume was balanced by ear until 3 experimenters (JAR, NJ, JM) agreed that the sound from the real pulsed magnetic field exposure and the speakers were indistinguishable.

The 60 Hz and pulsed magnetic field exposure was implemented within the MRI system by programming the Z-gradient coils (the gradient along the bore of the magnet). The peak gradient strength was 2 mT/m, and the patient table was offset 10 cm cranially from the isocentre so that the field at the brow level was set to be 200 μT, the same field strength used in whole-body exposures (non-MRI) within our lab in the past with Helmholtz coils (Shupak *et al.*, 2004; Cook *et al.*, 2004).

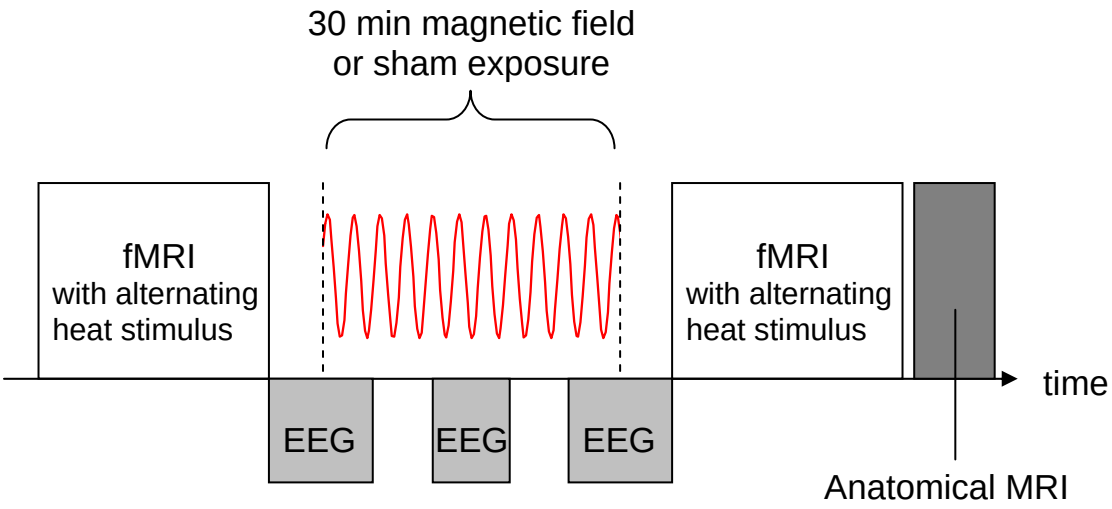

Figure 5.1: A schematic of the experimental timeline.

Functional MRI data was acquired as the painful thermal stimulus cycled on and off. Two blocks of fMRI were included, before and after the 30-minute magnetic field or sham exposure. Resting eyes-closed EEG recordings were also acquired from 5 minutes before the onset of the exposure condition, until 5 minutes after onset; for 5 minutes in the middle of the exposure period; and for 5 minutes before the cessation of the field, continuing until 5 minutes after offset. Anatomical images were collected at the end.

Subjects were asked at the end of the study if they fell asleep during the 40-minute exposure/rest period. In addition to self-reporting, subjects were considered to have fallen asleep if the experimenter heard snoring, or if the subjects were non-responsive when asked if they were ready for the second round of imaging or any of the EEG recordings during the exposure/rest period. Subjects were also asked if they believed they were exposed to a magnetic field, or a sham condition.

Functional image processing was done with Brain Voyager v2.1.2 (Brain Innovation B.V., the Netherlands). 3D motion correction was performed by the Siemens Verio system using the retrospective method bundled with the BOLD sequence. Individual datasets were preprocessed with temporal filtering (with a Fourier General Linear Model high-pass filter that had a cut-off frequency of 2 cycles per 12-minute fMRI session), and mean intensity adjustments to remove random scanner artifacts. Individual data was then spatially normalized to Talairach space and spatially smoothed (Gaussian 6 mm FWHM) to be combined for a General Linear Model (GLM) group analysis. For the sake of analysis, the "pain" condition was defined to be the final 12 s of the plateau plus the 3 s ramp back to baseline; the "rest" condition was taken as the final 21 s of the baseline period [the remainder was discarded due to evidence from Owen et al., 2008, that blood flow from pain may last longer than the hemodynamic models predict. Default 2-gamma hemodynamic response curves were used (Friston *et al*., 1998). An

average of all Talairach anatomical images was created to display the results of the GLM analysis to better demonstrate the actual neuroanatomical variability for our subject pool. For the post-pre contrast images, a Bonferroni correction was applied to a corrected $p<0.05$. All images are presented in the radiological convention (figure left is subject right).

Based on the initial results seen from the separate-group analysis within Brain Voyager and on the *a priori* knowledge of brain regions associated with pain processing and the results of previous work (Robertson *et al.*, 2010a,b), 3 cm^3 cubic regions of interest were chosen and the beta weights exported for analysis in PASW/SPSS statistics (version 18, IBM Corp., Armonk, New York, USA) to explore potential interactions. An alpha level of $p<0.05$ was selected for statistical significance, with no corrections made for testing multiple ROIs (2 total: anterior cingulate; right insula).

EEG preprocessing was performed in Neuroscan Maglink RT Edit 4.5 (Compumedics Neuroscan, Charlotte, NC). EEG data from the 5 minutes prior to and immediately following the exposure period was bandpass filtered (1-50 Hz) and EKG reduction performed to compensate for the ballistocardiogram and EKG artifact. Then approximately a 1-minute artifact-free selection just before the field onset and another 1-minute selection just after the field offset (maximum of 2.5 minutes from the exposure transition) was exported to MATlab. A fast Fourier transform was performed on rolling 2.048-second intervals of this selection (which were

Hamming windowed), and the absolute value of the resulting frequency spectrum from each interval was averaged to give the final average spectrum for each subject. The components of this spectrum were further averaged in separate frequency bins for alpha (8.3-12.2 Hz), beta (12.7-30.3 Hz), gamma (30.8-49.8 Hz), delta (1-3.9 Hz), and theta (4.4-7.8 Hz) EEG bands. The change in power over time within each frequency band was then analyzed across subjects within PASW/SPSS statistics, with the *a priori* hypothesis to focus on changes in the alpha band across the occipital region (average of O1, OZ, O2) (Cook *et al*., 2004).

All procedures were approved by the University of Western Ontario Health Sciences Research Ethics Review Board (protocol #16115).

5.3 Results

A total of 67 subjects were recruited to participate in the study. Two subjects withdrew prior to the end of the study, and a further four subjects were removed from the analysis due to the Medoc probe falling off their hand, falling asleep, medication use, or feelings of claustrophobia (one each). Thus 61 subjects have been included with at least subjective data (Sham N = 20, 8 female; PEMF N = 23, 15 female; 60 Hz N = 18, 11 female). For the functional MRI artifact-free data from 43 subjects was included in the analysis (Sham N = 16, 7 female; PEMF N = 15, 8 female; 60 Hz N = 12, 7 female). For the EEG analysis, artifact-free data from 42 subjects were included – a different subset of subjects than the fMRI data, as some subjects had good EEG data

but not fMRI data, and vice-versa – 10 of the subjects excluded in the EEG analysis were included in the fMRI analysis (Sham N = 14, PEMF N = 17, 60 Hz N = 11).

5.3.1 Subjective data

There was a significant main effect of time in the subjective pain ratings, with subjects reporting less pain after the exposure session ($p < 0.01$, $F_{1,58} = 26.27$, partial $eta^2 = 0.312$, power = 0.99), but no significant time by condition interaction ($p > 0.1$, $F_{2,58} = 0.976$, partial $eta^2 = 0.033$, power = 0.21). See figure 5.2.

When asked whether they believed they were exposed to a magnetic field or not, 5/20 subjects in the sham group believed they were in the sham exposure, 15/23 of the PEMF group believed they were in the sham group, and 6/18 of the 60 Hz group believed they were in the sham group. A Chi-square test indicates that only the responses of the sham group were significantly different than a predicted 50% response ($\chi^2_{1d.f.} = 5.0$, $p < 0.05$). However, as the total of all subjects in all groups was not significantly different than 50% (26/61 subjects across all groups believed they were in the sham condition), and since the guesses of the subjects in the sham group were wrong, we do not believe that the blinding of the experiment was compromised. The possibility that the simulated sound introduced for the sham and 60 Hz conditions did make the environment for those conditions different from the PEMF exposure cannot be ruled out though (e.g., if the

simulated sound was more noticeable than the actual acoustic noise of the gradients).

5.3.2 fMRI data

Within the anterior cingulate region of interest, there was a significant time by condition interaction ($p < 0.05$, $F_{2,40} = 3.57$, partial $eta^2 = 0.151$, power = 0.63), as well as a main effect of time ($p < 0.01$, $F_{1,40} = 15.22$, partial $eta^2 = 0.276$, power = 0.97). See figures 5.3 and 5.4. For the right insula, there was a significant main effect of time ($p < 0.05$, $F_{2,40} = 4.39$, partial $eta^2 = 0.099$, power = 0.53), but no interaction with condition ($p > 0.1$, $F_{2,40} = 1.27$, partial $eta^2 = 0.060$, power = 0.26). See figure 5.5.

5.3.3 EEG data

No significant interaction was observed between pre- and post-exposure EEG alpha activity over the occipital cortex (average of O1, OZ, and O2 electrodes) and magnetic field exposure condition ($p > 0.1$, $F_{2,39} = 1.21$, partial $eta^2 = 0.058$, power = 0.25). See figure 5.6. Although not part of our *a priori* hypothesis, the other frequency bands were also examined over this region, and similarly no significant interaction was observed.

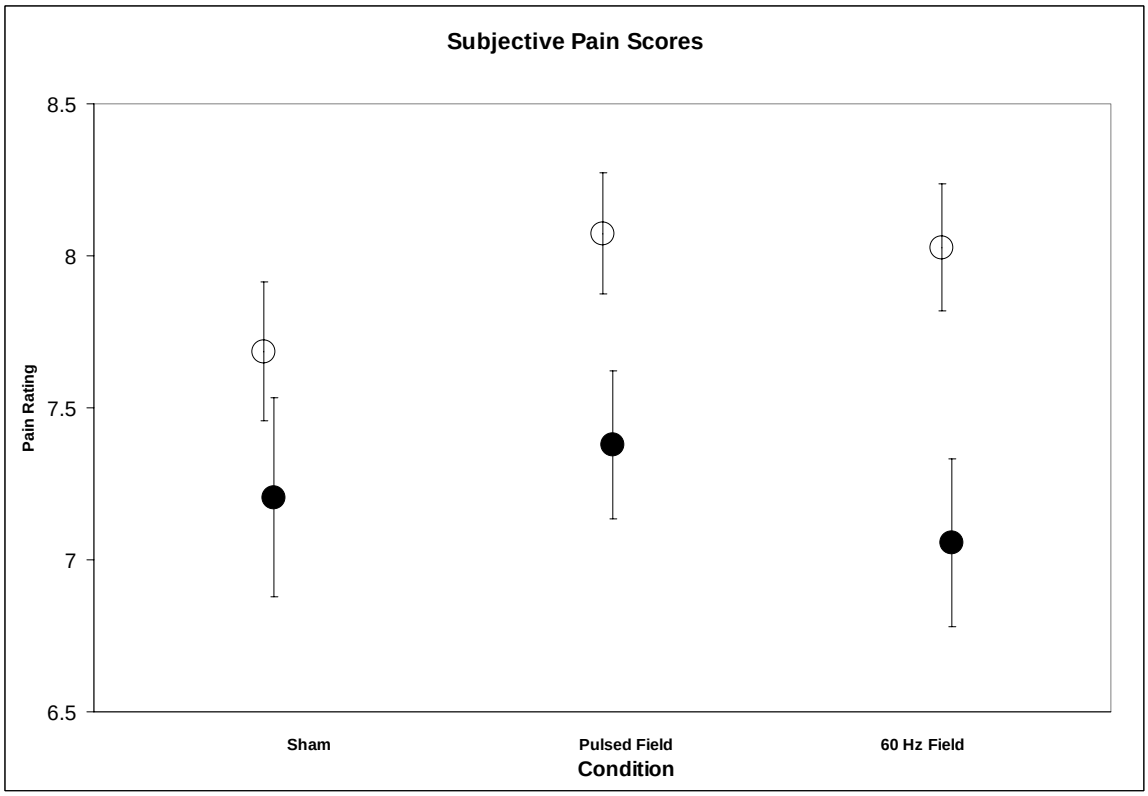

Figure 5.2: Subjective pain score data.

Subjective pain score data for sham (N=20), pulsed field (N=23), and 60 Hz (N=18) exposed groups. Pre-exposure scores are in the open circles, post-exposure scores in the closed circles. Error bars are +/- SEM.

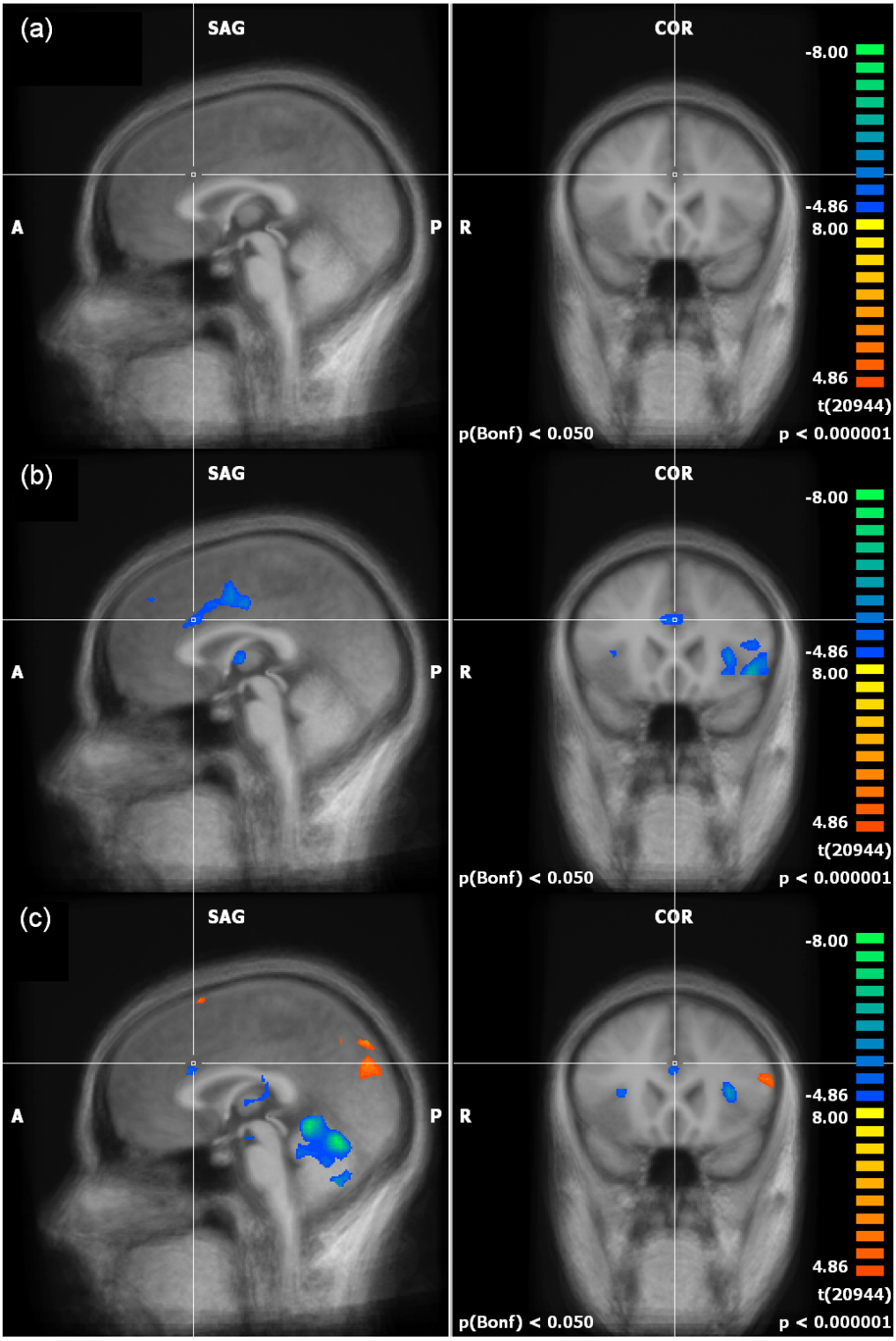

Figure 5.3: Anterior cingulate ROI.

Change in activation from post-exposure to pre-exposure within the anterior cingulate in (a) sham, (b) PEMF, (c) 60 Hz. Blue/green colours indicate less activation after exposure than before.

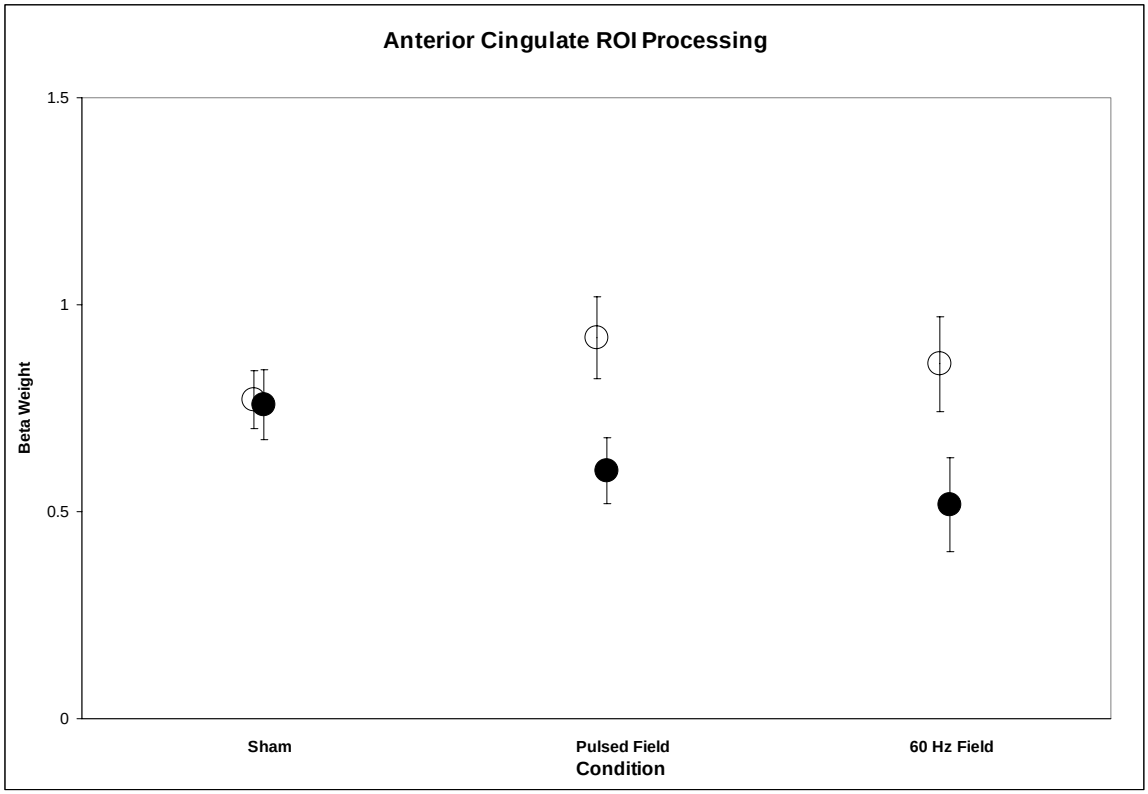

Figure 5.4: Anterior cingulate volume of interest activations.

Activation with pain in the anterior cingulate volume of interest for the fMRI analysis. Pre-exposure symbols are white, post-exposure are black. Sham exposure (N = 16) is on the left, PEMF exposure (N = 15) in the middle, and 60 Hz exposure (N = 12) on the right. Error bars are +/- SEM.

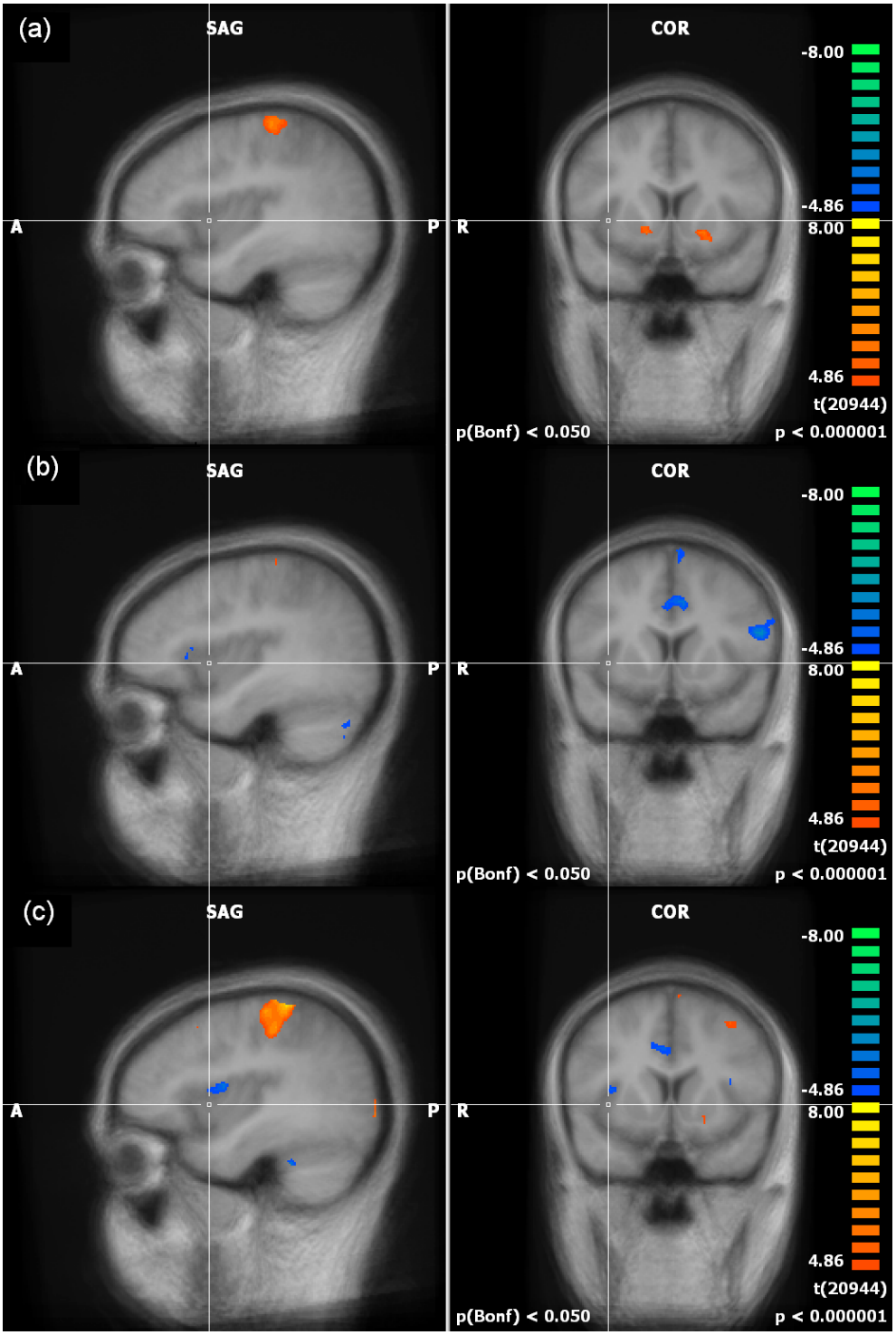

Figure 5.5: Right Insula ROI.

Change in activation from post-exposure to pre-exposure within the anterior cingulate in (a) sham, (b) PEMF, (c) 60 Hz. Blue/green colours indicate less activation after exposure than before.

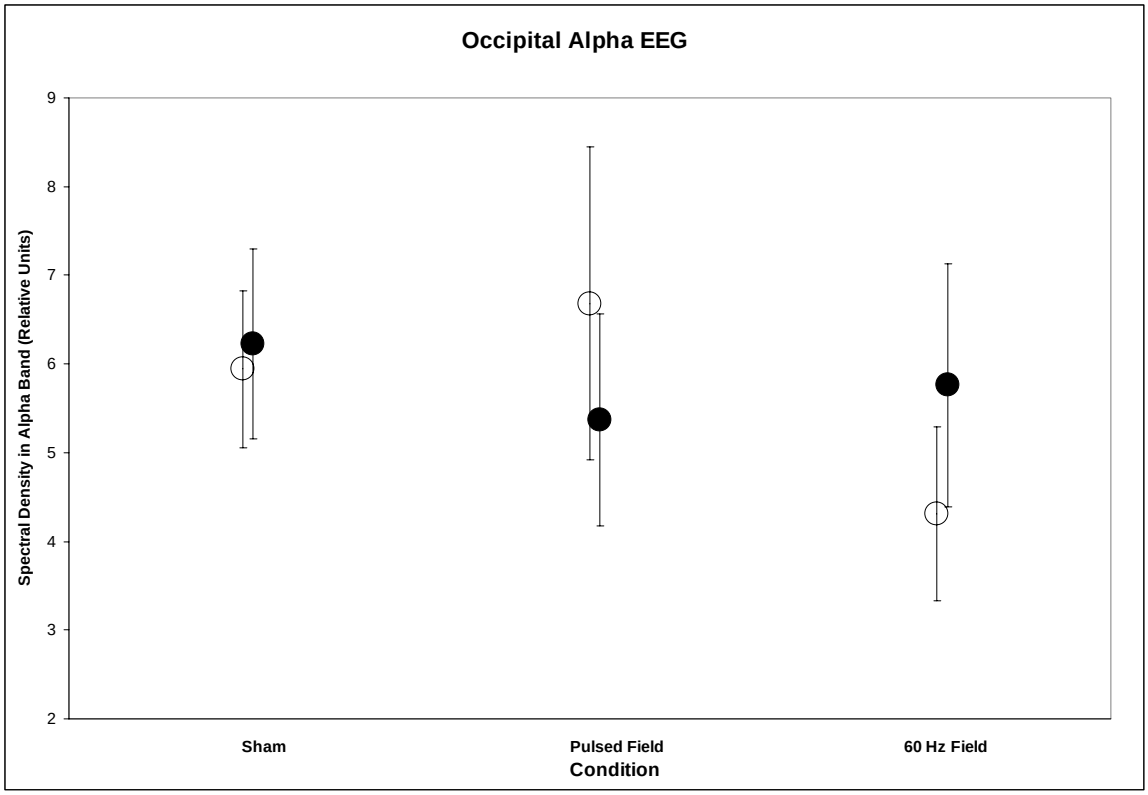

Figure 5.6: EEG Alpha activity.

EEG Alpha activity over the occipital electrodes (average of O1, OZ, O2). Pre-exposure symbols are white, post-exposure are black. Sham exposure (N = 14) is on the left, PEMF exposure (N = 17) in the middle, and 60 Hz exposure (N = 11) on the right. Error bars are +/- SEM.

5.4 Discussion

The results presented suggest that the same type of changes in functional processing (decreased activation in the insula and anterior cingulate) with pulsed magnetic field exposure have been repeated consistently across three studies (Robertson *et al.*, 2010a, 2011), though the decreases seen in the right insula were not significant in the present study. Non-significant decreases in subjective pain ratings were observed alongside these functional activation interactions, which is also consistent with the previous studies.

The potential influence of the imaging magnetic fields inherent to the MRI cannot be ignored, although at the same time they are difficult to control for. The analgesia associated with the pulsed magnetic field is likely opioid-related, as it can be reversed by opioid antagonists (Thomas *et al.*, 1997[a], 1997[b]). The evidence indicates that the time-varying magnetic fields of the MRI environment may inhibit opioid-related analgesia in snails and mice (Prato *et al.*, 1987; Teskey *et al.*, 1988; *Prato et al.*, 1992), however, the 3 T static field may have an analgesic effect of its own in mice (Laszlo and Gyires, 2009). By placing the anatomical scans at the end of the study, we reduced as much as possible the potential confounds from the extra exposures, as the study measurements were complete prior to the anatomical acquisitions.

The addition of EEG allowed us to examine changes in resting brain activity without the need for a task, and the sets of subtractions that take

place with fMRI. We did not observe any significant interaction of the magnetic field exposure with time in the alpha band, though the direction of the non-significant changes seen was interesting. For the pulsed magnetic field exposed group, a slight decrease in occipital alpha raises the question of whether time since off-set or on-set is important: Cook *et al* (2004), using the same pulseform, found a significant increase in alpha relative to sham for the first minute following a short exposure, which then reversed after 7 minutes. If the time since the pulsed field onset is important, then the later decrease may be the appropriate comparator for this study, indicating that our non-significant results are consistent with earlier work: here, a 30 minute exposure, in Cook *et al.* (2004), 7 minutes following the offset of a 15 minute exposure, or 22 minutes since the field onset. If instead the time since the end of the exposure is important, then our results appear to indicate a change in the opposite direction. For the 60 Hz field, the opposite was seen here, with a slight, non-significant increase in occipital alpha power; with 50 Hz Ghione *et al.* (2005) also found an increase in occipital alpha activity. Thus it appears that whatever effects magnetic field exposure may have on resting EEG, they are not necessarily related to changes in pain processing, as both fields had similar effects in that regard.

5.5 Acknowledgements

The authors would like to thank Dr. Dwight Moulin, Dr. Alexandre Legros, Dr. Keith St. Lawrence, Dr. Tim DeVito, Dr. Ronnie Abi-Raad, Mr.

John Butler, Mr. John Patrick, and Mr. Lynn Keenliside for their assistance with this project. This project was funded by a scholarship from the Canadian Natural Sciences and Engineering Research Council (J. Robertson), support from Baylis Medical Inc., and grants from the Canadian Institutes of Health Research and Canadian Foundation for Innovation (F. Prato *et al*.).

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General Conclusion

Here we have demonstrated that a specific pulsed magnetic field with analgesic effects does affect the BOLD activation measures of an acute thermal pain stimulus as measured with functional magnetic resonance imaging. Moreover, the effects were seen specifically in the affective processing of pain, which aligns well with the previous studies that found an effect on pain thresholds but not sensory thresholds, that is, that the pulsed magnetic field in question had analgesic but not anesthetic effects.

Whether magnetic fields can affect human behaviour is a controversial question and an active area of research. Many studies in bioelectromagnetics focus on determining the potential harmful effects of fields encountered in everyday life. This research shows that a pulsed magnetic field can have an influence on pain processing, demonstrating both that magnetic field exposure can have an effect, and that these biological interactions are not necessarily deleterious. Since the specific pulsed magnetic field employed here is a different pattern and a higher field strength than the typical daily ELF MF exposure for the public, it is inappropriate to extrapolate these results to potential effects of ambient exposures.

Utilizing the gradient hardware of an MRI system to introduce a specific pulsed magnetic field for research purposes is a novel technique. This advancement is now also being used by other researchers such as Dr. Alexandre Legros to investigate the effects of 60 Hz magnetic fields on finger

tapping and mental rotation manipulation tasks. The potential confounding effects of the other MRI fields are an ever-present concern, however, their presence in both the sham and exposed conditions should limit the extent of the confound. The fMRI activation changes observed did agree well with what we expected based on the previous non-MRI studies, with the exception of the 60 Hz sinusoidal exposure in Chapter 5, which we did not hypothesize would have similar effects to the specific pulsed magnetic field.

A potential uncontrolled variable, brief sleep, has also been identified in the course of these studies. The study described in Chapter 5 included mechanisms to try to keep subjects awake, or to objectively qualify sleep status with an EEG, and future studies should also utilize these methods. Future work would include more purposefully investigating the relationship between magnetic field exposure, sleep, and analgesia.

Other avenues for future investigation include the use of these novel MRI techniques to further investigate aspects of pulsed magnetic field effects. For example, early work with snails indicated that alternative pulsed magnetic field waveforms did not have anti-nociceptive effects. This could be verified in humans, and extended to also investigate the effects of other pulseforms, such as MR imaging sequences. With noxious heat and cognitive tasks, functional imaging could be used to determine the pattern-dependence of these magnetic field effects on brain function.

Light-dependence has also been found in previous studies, and is a commonly reported feature of a wide range bioelectromagnetics effects, from animal navigation to nociception or even human standing balance. The effects of light levels on the magnetic field exposure-related change in pain processing is ripe for investigation. Indeed, it is possible that the previously reported effect of subjects falling asleep is a manifestation of a light dependency (i.e.: with eyes closed), though the sleep state itself is likely more salient.

Finally, it would be interesting to investigate what effects magnetic field exposure has on chronic pain neuroprocessing. For that application, which requires examining baseline changes without an altering stimulus, our fMRI-BOLD technique would not be appropriate. Other functional imaging techniques such as arterial spin labeling or positron emission tomography can instead be introduced to permit the investigation of magnetic field effects on chronic pain in future studies.

Bioelectromagnetics is an exciting field with many potential beneficial medical applications, and even more open questions about the potential effects and mechanisms of various fields. The addition of functional imaging tools opens even more avenues of research, and allows for a set of more objective study observables.

Appendix A: Ethics Approval

This project involved the use of human volunteers, with approval from the University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB). The research was conducted under two approved protocols:

- Protocol # 10059, "Using functional MRI to assess the effects of pulsed low-frequency magnetic fields on human perception of pain" for the work reported in Chapters [2-4]. See figure A.1.
- Prototcol #16115, "Using the combined technology of functional MRI and EEG to assess the effects of pulsed, low-frequency magnetic fields on human perception of pain" for the work reported in Chapter [5]. See figure A.2.

Office of Research Ethics

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Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. A.W. Thomas

Review Number: 10059 **Revision Number:** 6

Protocol Title: Using functional MRI to assess the effects of pulsed low-frequency magnetic fields on human perception of pain

Department and Institution: Medical Biophysics, Lawson Research Institute

Sponsor:

Ethics Approval Date: July 24, 2006 **Expiry Date:** December 31, 2008

Documents Reviewed and Approved: Addition of a co-investigator

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted full board approval to the above named research study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;

b) all adverse and unexpected experiences or events that are both serious and unexpected;

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Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

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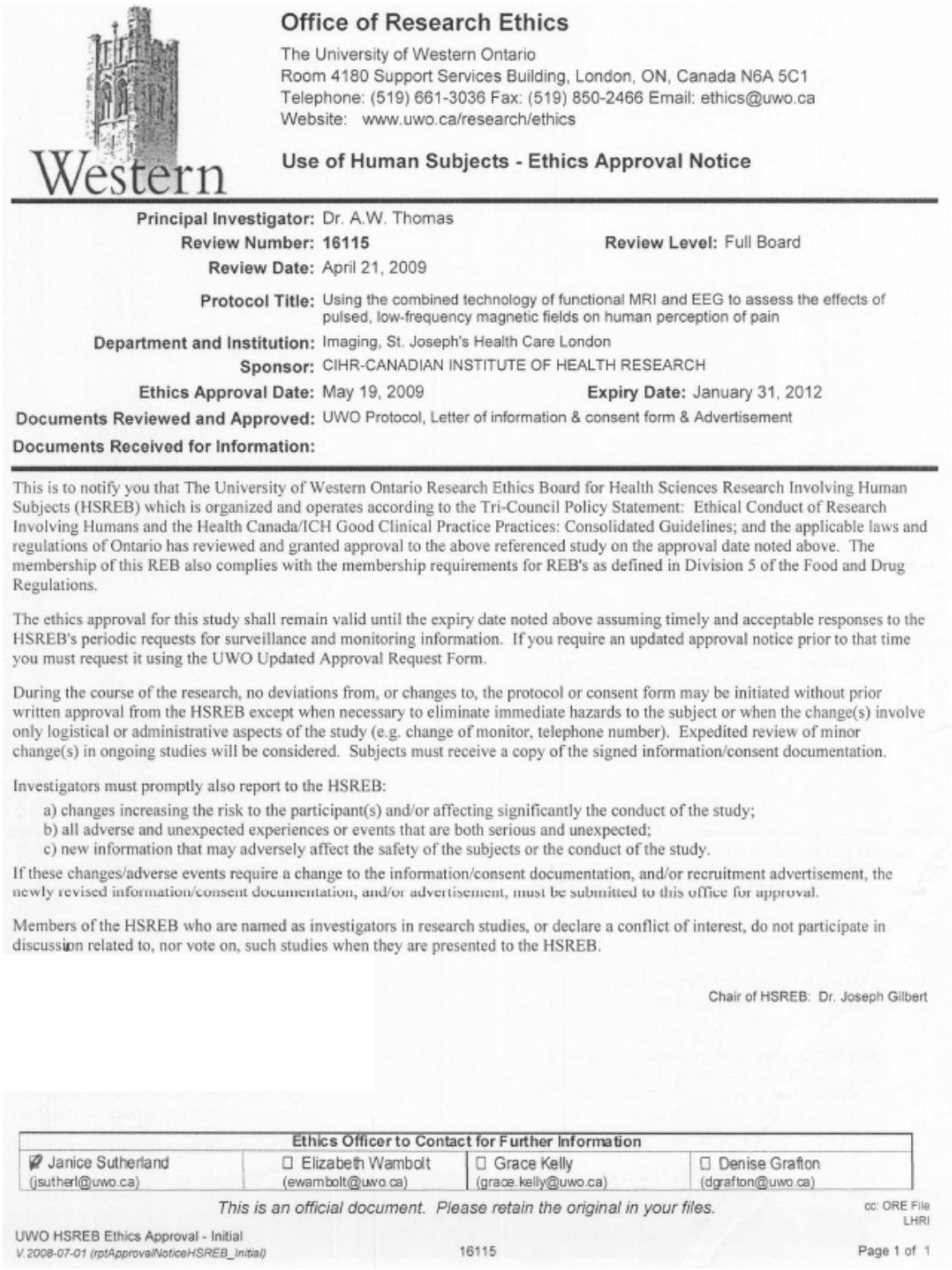
Figure A.1: HSREB approval for protocol 10059.



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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. A.W. Thomas
Review Number: 16115 **Review Level:** Full Board
Review Date: April 21, 2009
Protocol Title: Using the combined technology of functional MRI and EEG to assess the effects of pulsed, low-frequency magnetic fields on human perception of pain
Department and Institution: Imaging, St. Joseph's Health Care London
Sponsor: CIHR-CANADIAN INSTITUTE OF HEALTH RESEARCH
Ethics Approval Date: May 19, 2009 **Expiry Date:** January 31, 2012
Documents Reviewed and Approved: UWO Protocol, Letter of information & consent form & Advertisement
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

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16115

Page 1 of 1

Figure A.2: HSREB approval for protocol 16115.

Appendix B: Suppl. info for Chapter 2

Note: a version of this chapter has been published as electronic supplementary information to an article published in the *Journal of the Royal Society: Interface*. With kind permission from the Royal Society, this supplementary information has been republished below. See Appendix F for more details on the copyright policy of Royal Society journals.

Low Frequency Pulsed Electromagnetic Field Exposure Can Alter Neuroprocessing in Humans: Supplementary Information

By: John A. Robertson, Jean Théberge, Julie Weller, Dick J. Drost, Frank S. Prato, and Alex W. Thomas.

B.1 Proposed mechanisms of action

There are a number of potential mechanisms explaining magnetic field effects on behaviour, and they are not mutually exclusive. Furthermore, various mechanisms may be light-dependent (Prato *et al*., 2009; Wiltschko and Wiltschko, 2006). Previous work in our lab with standing balance (Prato *et al*., 2001) also found a dependence on light conditions.

Magnetite-based effects depend on magnetic fields acting on particles of magnetic iron in the body (biological magnetite), which are coupled somehow

to biological sensors. The strong static field of the MRI would likely overwhelm any magnetite-based sensor from picking up the time-changing magnetic field (though like with scuba divers and pressure sensors, it's possible that the change in magnetic field can still be detected). It has not been conclusively shown that humans possess a magnetite-based magnetic sense, and if they do whether it is sensitive to changing fields or the static field direction only. Moreover, to produce a torque on biological magnetite time-varying fields should be applied perpendicular to the static field; in our MRI exposure the pulsed magnetic field was parallel to the main field, so is unlikely to have an effect (Adair, 1994).

Ion parametric resonance effects depend on combinations of static and time-varying fields to affect ions and ion channels in cells. The strong static field of the MRI does not preclude ion parametric resonance effects from acting, however it will almost certainly change the effect from that in previous experiments conducted in the Earth's static field. Previous work with land snails (Prato *et al.*, 2000) in our lab indicated that an ion parametric resonance mechanism may underlie changes in nociception with sinusoidal fields. For 1.5 T, the cyclotron frequency of most ions would be in the megahertz range, well above the frequency of our PEMF exposure. We do not believe that the analgesic effect of pulsed PEMFs act through an ion parametric resonance effect, but have not as of yet ruled that out.

The free radical mechanism allows magnetic fields to alter the recombination rates of free radicals in chemical reactions. While time-varying fields could potentially be detected biologically, it is the field strength that matters to the chemical reaction, so in an MRI the very strong static field would prevent the time-varying field from having an effect (i.e., it is unlikely that there is a window between 1.5000 T and 1.5002 T). For more on interaction mechanisms, see the NIEHS Working Group Report (1998, §4.8.3.5 for the radical pair mechanism).

This leaves us with induced currents. We believe that the PEMF studied here acts through an induced current mechanism. The electric fields induced by the time-changing field will not be altered by a static field (though it can be argued that the neurons' sensitivity to such might be altered), however these fields are admittedly weak and sub-threshold. Our hypothesis is that a stochastic resonance effect allows these weak sub-threshold induced currents to affect neural processing, by reinforcing a pattern of firing over time. In this case the pattern is important as it must be "biologically relevant" to build up in the brain.

B.2 Functional imaging

The functional imaging scan consisted of a single-shot echo-planar imaging acquisition, which produced gradients for the imaging that exceeded the strength of those being investigated in this study. There were 16 slices acquired within 2500 ms, with a 500 ms delay before the next volume

acquisition began (overall 3000 ms repetition time, TR). Each slice acquisition consisted of a "spoiler" gradient pulse, a slice select gradient with an RF pulse, an inversion RF pulse, and then the echo-planar readout. This portion accounted for the majority of the magnetic field exposure, with 67 ms of readout with strong gradient switching per slice.

The phase encode gradient sequence consisted of short blips of 0.05 ms, 3.5 mT/m peak amplitude, ramps of 0.02 ms (maximum gradient slew of 175 mT/m/ms). Since the slices were oblique, the phase encode direction was in the Y-Z plane (the gradient was a vector combination of the physical Y and Z gradients).

Readout: 0.1 ms ramps (full positive to full negative), plateau of 0.95 ms, 8 mT/m amplitude (maximum gradient slew of 160 mT/m/ms). For a position 5 cm from the centre (the maximum offset of the imaged volume, and the highest gradient field strength in the imaged volume), the change in magnetic field strength would be 8 T/s.

Note that because the distance from isocentre is different for the PEMF exposure and the fMRI imaging, the gradient slew rate for the PEMF exposure is another 2 times lower than the change in field strength alone would suggest (i.e.: 4 mT/m/ms). This is a large part of why the PEMF sequence does not produce much acoustic noise.

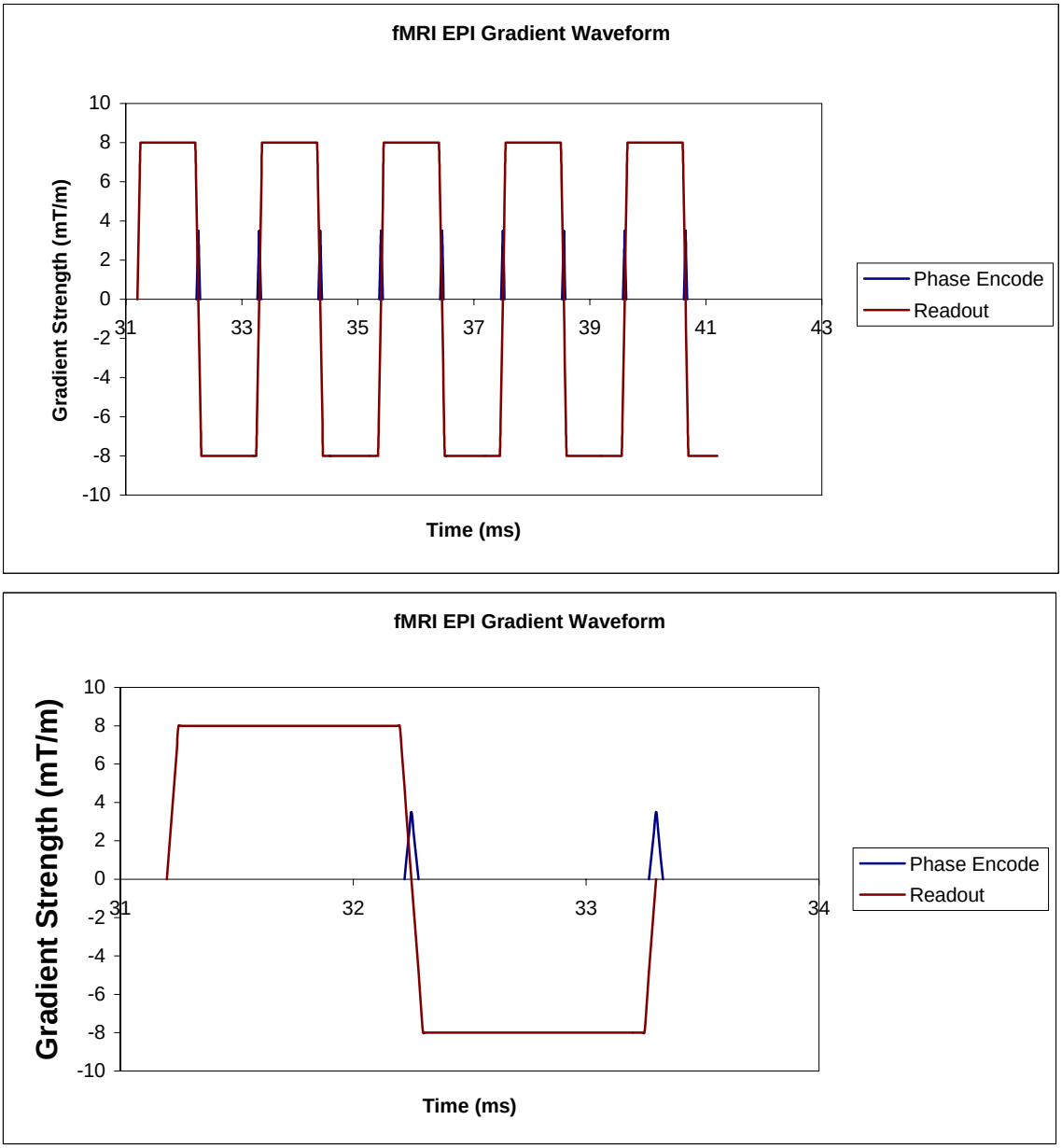

Figure B.1: EPI gradient waveform.

A portion of the fMRI EPI gradient waveform. There would be a total of 64 of these trapezoidal readout pulses (32 if one counts a cycle as including both the positive and negative lobes). A single cycle is shown in the bottom for additional detail.

B.3 Pulsed Field Exposure

As discussed in the main paper, the field strength of the PEMF tested was 200 μT at the brow of the head. This was achieved using a gradient magnetic field, so the peak field strength will vary linearly with position. The patient table of the MRI was moved 10 cm so as to offset isocentre (where the field strength of the gradient is zero), as demonstrated in Figure B.2. The table was moved for the sham condition as well, and moved back into the original position (with isocentre at the brow) prior to the second round of functional imaging.

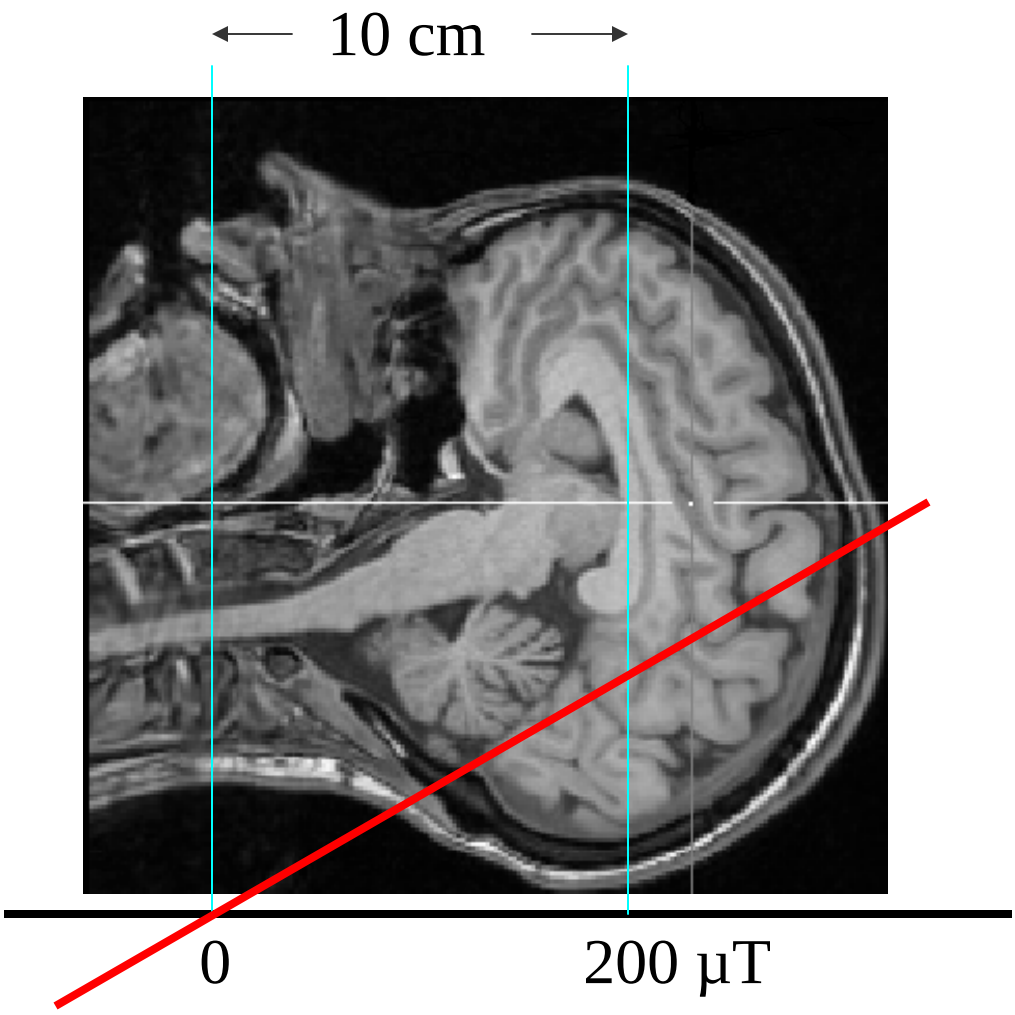

Figure B.2: Subject positioning.

The variation in field strength vs position displayed graphically. The isocentre of the MRI is at the 0 point. The linearly varying field strength is represented by the red line.

The electric field strength is a tricky number to come to. We have the magnetic field strength (200 uT at the brow of the head with a 2 mT/m gradient) and the pattern with which it varies (importantly, that the peak rate of change is 0.4 T/s at the level of the brow). However, the induced electric field depends on the area that magnetic flux crosses.

$\varepsilon = -(dB/dt)(A)$ [V]

For a circular area:

$\varepsilon = -(dB/dt)(\pi r^2)$ [V]

And the electric field is then:

$|E| = (dB/dt)(\pi r^2)/(2\pi r)$ [V/m]

$|E| = (dB/dt)(r)/(2)$ [V/m]

So for example, the electric field induced in a 1 cm radius of tissue with the PEMF would be 2 mV/m. This gives us an idea of the order-of-magnitude of the induced electric fields in this experiment, though realistic current loops may be larger than 1 cm radius (whole head) or smaller (fibre tracts or other structures within the brain).

B.3 Main Effects of Time

The region-of-interest analysis of the data focused on regions involved in the limbic and sensory system, locations we believed would highlight functional changes associated with pain. Furthermore, significant interactions between time (pre-post) and condition (PEMF exposed vs sham) were reported as these represented “the effect” of the PEMF exposure. There

were also some significant main effects of time, for example in the ipsilateral primary sensory area ($F_{1,29} = 7.184$, $p<0.05$, power = 0.736, partial eta-squared = 0.199), but not a significant interaction ($F_{1,29} = 1.091$, $p>0.3$). This main effect of time can be partly seen in Figure 2.3 of the paper where a large area of pre-post decrease can be seen particularly in the sham condition. While the volume of difference is smaller in the exposed condition, it is still present, but does not show in the slice highlighting the changes in the ipsilateral insula.

A significant main effect of time is not surprising in a study like this, where a task is repeated with a time delay; these can be due to adaptation or conversely sensitization, as well as learning effects, relaxation/anxiety from time spent in the magnet, and potentially bioeffects of the imaging and static magnetic fields (which both groups are exposed to).

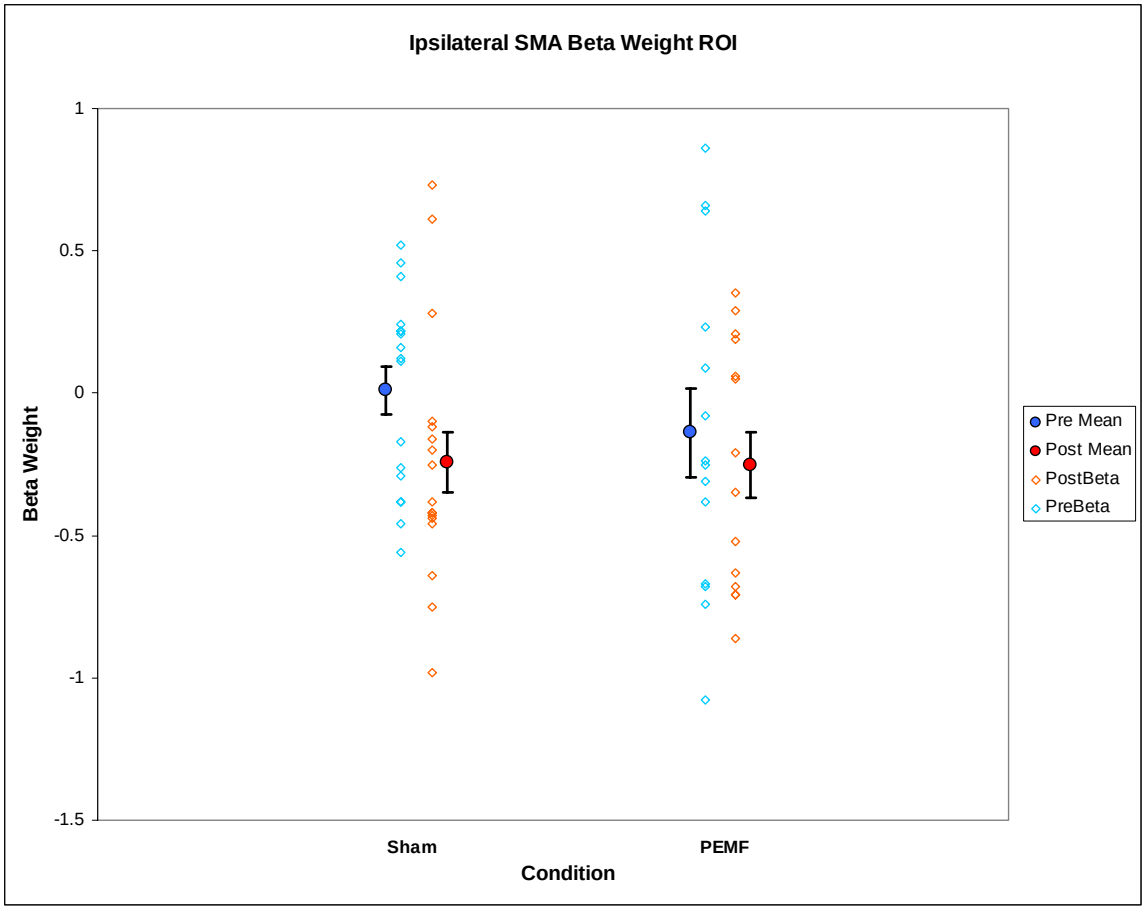

Figure B.3: Beta weight data for sensory-motor area.

The main effect of time for the ipsilateral sensory-motor area is illustrated in the plot of the beta weights. The beta weights for both groups decreased over time, there was no significant interaction with the magnetic field exposure condition for this area.

B.4 References

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Appendix C: Introducing a PEMF into an MRI System, a Methodological Discussion

By: John A. Robertson, Jean Théberge, Frank S. Prato, Alex W. Thomas

C.1 Introduction

Bioelectromagnetics is the study of how magnetic fields can affect biological systems. Recent research in the field has suggested that time-varying magnetic fields can affect human and animal behaviour. One of the most reproducible experimental paradigms is the interaction with opioid-related behaviour (Del Seppia *et al.*, 2007). There is evidence that at least some of these effects are due to changes within the brain rather than peripherally, as head only exposures have been utilized (Shupak *et al.*, 2006)

One modern technique for objective investigation of functional brain changes is functional MRI (fMRI). Standard fMRI provides an objective measure mainly dependent on changes in cerebral blood oxygenation levels which can help tease out the effects of certain magnetic fields on neuroprocessing.

Diagnostic purposes are also possible if differential effects of applied magnetic fields can be characterized. For example, Thomas *et al.* (2001, 2002) found that a pulsed magnetic field differentially affected the standing balance of rheumatoid arthritis patients, fibromyalgia patients, and healthy controls.

A speculative future application of this technique may be to search for a differential MRI-measureable response or "magneto-contrast" (using fMRI/BOLD, fMRI/ASL, or MR-spectroscopic) following a specific pulsed magnetic field exposure.

These potential future diagnostic tools and investigational methods will first, of course, require the ability to deliver specific magnetic field pulseforms within the MRI system's environment.

Here we show that there are many methods to bring arbitrary time-varying magnetic fields into the MRI environment for exposure purposes:

1. Specialized coils can be created to deliver the applied field, similar to gradient inserts currently used for certain imaging applications at present. This is indeed the method currently used when transcranial magnetic stimulation (TMS) is delivered inside the MRI. Dedicated hardware allows the investigator to create a coil to best meet the particular application, such as spatial uniformity or reduced acoustic noise and vibration to better maintain subject blinding. This specialized hardware however can be expensive to manufacture, and difficult to implement amongst the other MRI hardware (e.g., simply physically fitting another set of coils within the bore while still leaving room for the subject and a RF headcoil).

2. As can be seen in Figure C.1, the MRI system itself is composed of a series of nested electromagnets that can be commandeered to deliver arbitrary low frequency magnetic fields for exposure purposes.

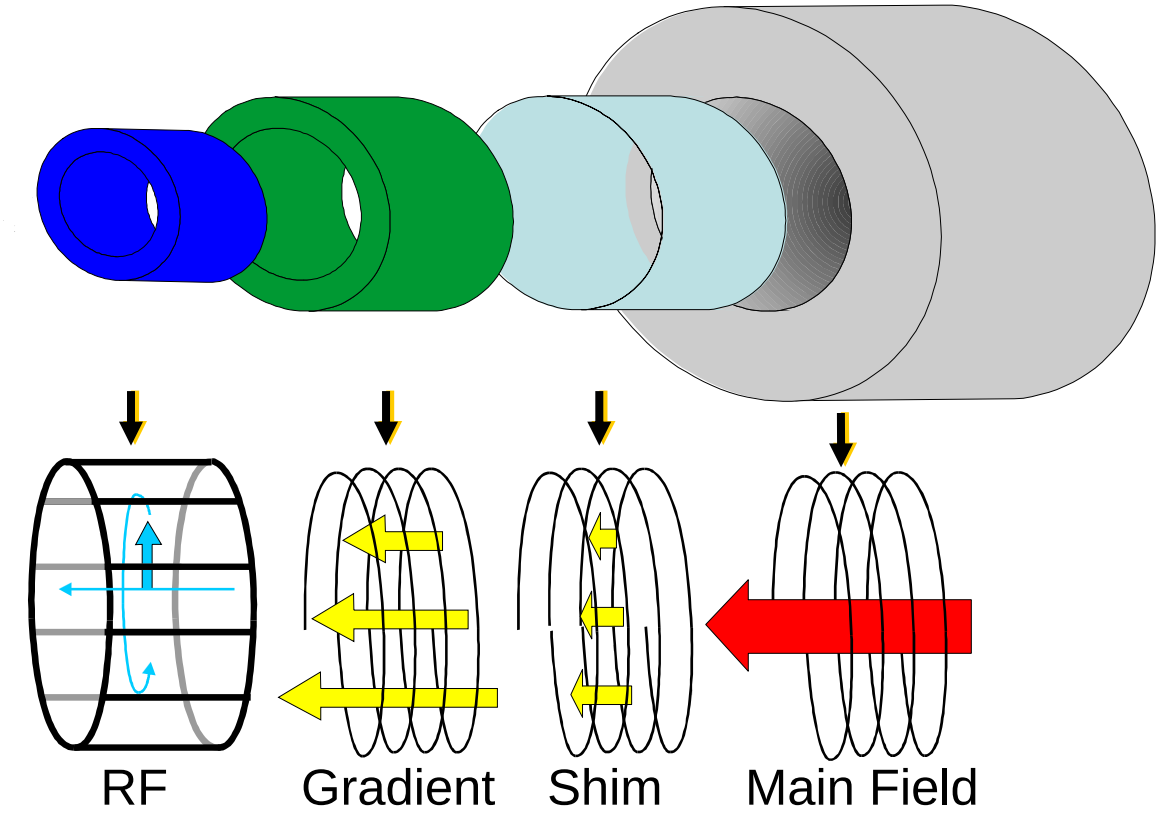

Figure C.1: An “exploded view” of a typical MRI system.

An “exploded view” of a typical MRI system showing that it is composed of nested electromagnets that produce the main static field, a set of shim coils to improve the homogeneity of the static field, the gradient coils to produce spatially-varying fields for the imaging, and a radiofrequency (RF) transmit coil.

2a. The main magnet winding, providing the static field ("B_0") is designed to produce a strong, uniform magnetic field. However, many modern magnets are closed-loop superconductors, and it is dangerous to attempt to tap into the high circulating current to introduce a time-varying component. Similarly, the power supply of resistive magnets is not generally designed to accept a time-varying input (with a strong DC offset of the MRI's main field).

2b. The shim coils, in particular the B_0 coil, where available, which can produce a uniform field near the centre of the bore, are physically suitable for producing low-strength time-varying magnetic fields. However, getting access to them on the software level is a challenge as many MRI systems were not designed with the notion of using these coils in such a way. Physical access to the shim coils (or their amplifiers) for hardware generation of arbitrary waveforms may be possible, however, we did not attempt this.

2c. Utilizing the gradient coils presents a set of compromises. They produce a non-uniform spatial field (a linear gradient, with a null point at the isocentre of the MRI, see figure C.2), and have not been designed with blinded studies in mind in terms of acoustics. However, they can produce powerful time-varying magnetic fields, and are easily accessible from the software level via the normal method of creating imaging sequences with arbitrary gradient waveform shapes. Moreover, the field strengths are well known and controlled for as part of the imaging system, leading to a high

confidence in delivered dose. This is the implementation we have utilized, and will be further discussed in the results.

2d. The radiofrequency transmit coils can also be used to deliver time-varying magnetic fields, especially if there is an interest in delivering a field at the frequency the coil is designed for (that is, the Larmor frequency of the nuclei that MRI is designed to examine). Since our research group is focused on low frequency magnetic field interactions, we have not examined the full capabilities of radiofrequency delivery. However, MRI systems are designed to provide fine control over the amplitude and frequency modulation of radiofrequency pulses.

C.2 Methods

We have utilized the gradient coils, specifically the Z-gradient (the gradient along the axis of the MRI), for the delivery of specific low frequency magnetic fields within the MRI system.

C.2.1 Programming

An arbitrary magnetic field waveform was produced by the gradient system by programming an "imaging sequence" with no RF pulses that produces no image, but simply consists of the desired waveform. The Siemens IDEA programming language was utilized. Specific challenges to the implementation include having to create a call to multiple waveform objects containing the point-by-point data on the arbitrary waveform when said

waveform is much longer than a typical waveform used in imaging. For example, the specific pulsed magnetic field investigated in Robertson *et al.* (2010) is over 5 seconds long, and it is sampled every 10 μs by the scanner, necessitating the use of multiple gradient waveform objects (of 8192 points each).

C.2.2 Integration with imaging

For the investigations in our lab, it is assumed that a prolonged exposure to the selected low frequency pulsed magnetic field would be required to observe any potential effect on neuroprocessing, so the arbitrary magnetic field was not interleaved with any imaging sequence. This also allowed us to move the patient table within the MRI to offset the isocentre differently for the exposure as compared to the table position for imaging. However, it is possible to create an imaging sequence that combines an active exposure magnetic field waveform between the repetitions ("T_R") of the imaging, at the expense of imaging data. For example, it is possible to deliver a specific investigational magnetic field for a few seconds at a time between the volume acquisitions of an fMRI sequence, although there will be no functional data collected for that time period, which may have repercussions for the quality of the fMRI data analysis. However, the use of the gradient system to deliver the exposure precludes *simultaneous* imaging and exposure.

C.2.3 Measurement

The gradients do not produce a magnetic field that is perfectly linear. Within the MRI system, these non-linearities are accounted for by software routines to compensate for geometric distortions in the images. We wished to know the actual magnetic field exposure, so we created a shielded 3-axis pick-up coil attached to a custom LabVIEW data acquisition system that measured the induced current of the time-changing magnetic fields, that is, the derivative of the time-changing fields. These were converted by our LabVIEW software back to a magnetic field value.

Acoustic transients are produced by magnetic field pulses of the gradient coils, and these can potentially confound any studies which require blinding between magnetic field exposure conditions. Acoustic measurements are particularly difficult within the MRI environment, so relative measurements were made by channeling the sound to a safe distance from the system with an acrylic tube, and then utilizing a Radioshack sound level meter (model 33 2055) and with a piezoelectric microphone (Bruel & Kjaer type 2801, Denmark). Subjective reports were also obtained from several volunteers.

C.3 Results

Our program did not attempt to circumvent the basic checks of the Siemens imaging system, one of which was a check of the resonant frequency of water before beginning the “imaging” sequence. In order for this check to pass and allow the sequence to proceed, there must be some signal (i.e.: water

or a subject) at isocentre. Because of this, we chose to offset the table cranially, so that isocentre was moved to close to the subject's mouth. It would be possible to offset the table in the other direction, so that isocentre was outside the subject's body, above the head. This would change the gradient of exposure, but would ensure that the entire brain received *some* dose. For this implementation, either the initial check must be disabled, or a bottle of water must be placed in the location where isocentre will be located.

Using our pick-up coil, we verified that within 10 cm of isocentre, the gradient fields were linear to within the sensitivity limits of our equipment (<1% deviation from linearity). At 20 cm however, the field strength was approx. 7% below the predicted field, as can be seen in figure C.3.

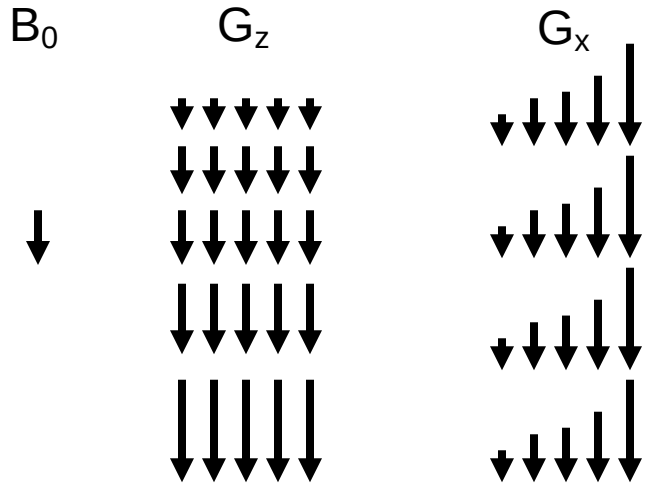

Figure C.2: Gradient field strengths.

A representation of the magnetic field strengths with gradients in the Z (G_z) or X (G_x) direction active. In all cases the field continues to point along the main axis of the MRI (B_0). Not to scale.

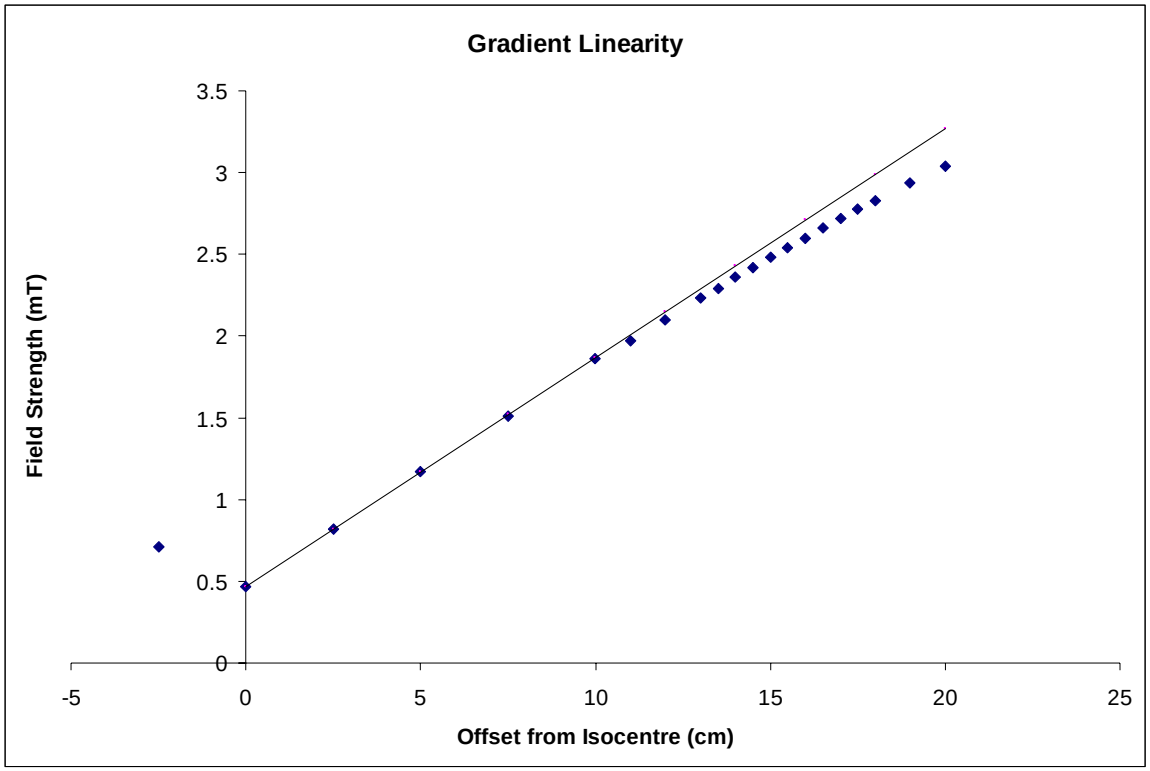

Figure C.3: Measured Z-gradient field strength and non-linearity.

The RMS field strength of a time-varying (60 Hz sinusoidal) pattern applied with the Z-gradient of was measured as a function of position along the main axis of the MRI bore. A linear fit to the first 3 points is plotted for comparison, demonstrating that there is a non-linearity present at distances further from the isocentre.

Acoustic noise is difficult to measure within the MRI system. The sound of the functional imaging sequence was measured as having 5X the background sound pressure on the piezoelectric microphone, and an increase of 13 dB on the RadioShack sound level meter. Though the patterned sound of a pulsed 2 mT/m gradient could be heard by the experimenters, the increase in sound pressure could not be detected on either meter. For double-blinding purposes, we created a simulated sound of the magnetic field by creating a WAV sound file with MATlab (The MathWorks, Natick, MA) based on the timings of the specific gradient field. This simulated sound was then subjectively ("by ear") balanced to the level of the sound produced by the active pulsed magnetic field, both by volume and adjusting the bass/treble levels of the simulated sound. Even without this additional precaution, subjects wearing hearing protection were not able to determine their exposure condition in a 1.5 T MRI with a 2 mT/m pulsed gradient sequence (Robertson *et al.*, 2010).

The pulsed magnetic fields also create a minor amount of vibration of the MRI bore liner and possibly patient table, however we do not at present have the equipment to measure the mechanical vibrations.

C.4 Conclusion

We have successfully been able to create and deliver specific pulsed magnetic fields for bioelectromagnetics experiments by reprogramming the gradient coils of our MRI system using existing software tools. Other

implementation methods may be utilized, with each having different advantages. These techniques open a new avenue for bioelectromagnetics research and potential future treatment and diagnostic methodologies involving exposure to low frequency pulsed electromagnetic fields.

Practical experiments using these techniques will have to face the obvious confound that the MRI imaging involves various amplitudes and frequencies of magnetic field exposure, and that these may themselves affect behaviour and neuroprocessing (Prato *et al.*, 1987; Rohan *et al.*, 2004; de Vocht *et al.*, 2006) in ways that may add, oppose, or neutrally interact with the intended investigational magnetic field.

C.5 Acknowledgements

We would like to thank Mr. Lynn Keenliside, Mr. Michael Corbacio, Ms. Nicole Juen, Dr. Julien Modolo, Dr. Alexandre Legros, and Dr. Dick Drost of the Lawson Health Research Institute, and Dr. Tim DeVito of Siemens for their assistance with this project.

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Appendix D: Additional Experimental Details

D.1 Specific Pulsed Magnetic Field Waveform

The complex neuroelectromagnetic pulse ("CNP") waveform has been examined in a number of previous studies in snails, mice, and humans.

Along the way a number of hardware changes and improvements have been made, from the original 8-bit whole-body exposure systems for animals and humans, to the portable headsets used in chronic pain studies, to the exposures in the MRI for the experiments described here.

The original systems had a waveform that was sampled at 1 point every ms (i.e.: 1 kHz). The amplifiers and coils could not instantaneously change the field exposure, even though the set point was changed, so there was a slight modification of the waveform due to the hardware. It consisted of a series of small "steps" – the field would quickly (<0.5 ms) ramp to the next value, and then hold there until the next set point came in from the control computer. The maximum time-varying field for this system has been reported as "~0.7 T/s" (Prato *et al.*, 2001). This waveform has been measured and displayed in figure D.1.

When the portable head coil units and generators were invented, the new hardware sampled the waveform at a higher sampling rate. To fill in the missing points, the original waveform was linearly interpolated, leading to a slight change in the waveform and a decrease in the maximum time-varying field, to 0.4 T/s. This linearly interpolated pulsed field was also used on the

MRI for the first and second experiment at 1.5 T. See figure D.2 for this waveform.

To mimic more closely the original CNP, a pulseform sampled every 10 μs (100 kHz) was created, and the rate-of-change for every transition was set to 1 T/s. This also allowed us to test the effects of induced current (which would be 2.5 times higher than the previous experiment) without changing the general, lower-frequency waveform/shape, or the peak field strength. See figure D.3 for this waveform.

D.3 References

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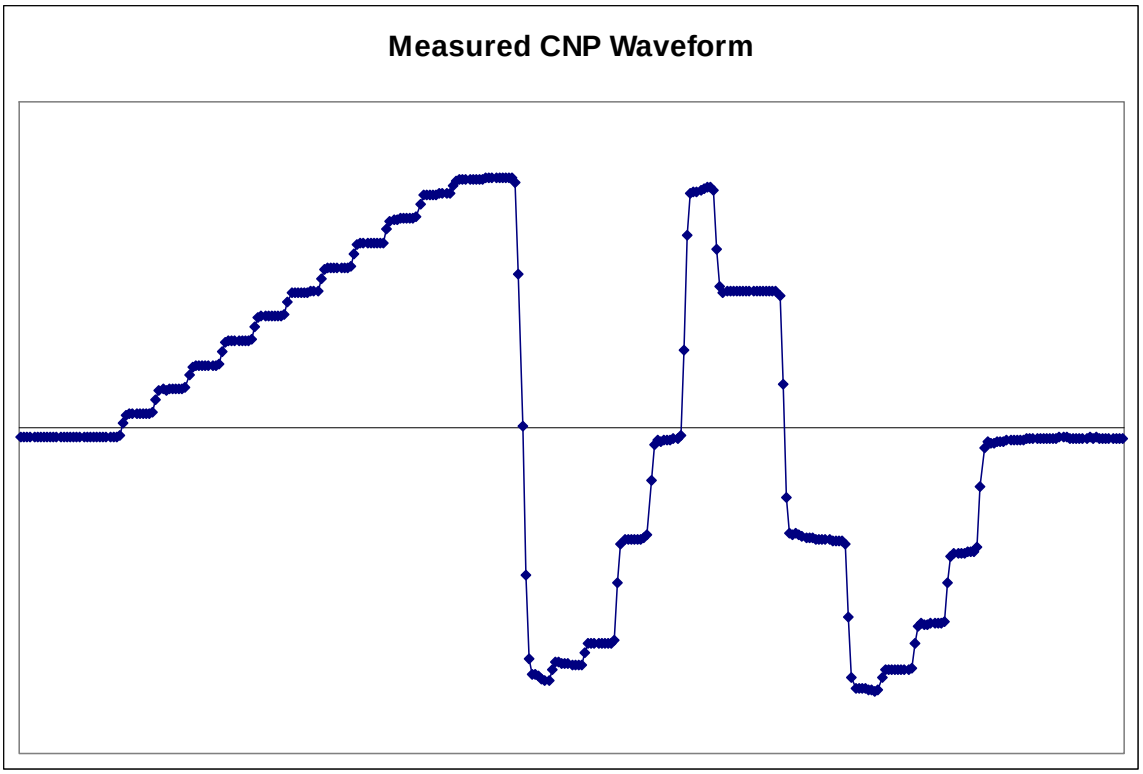

Figure D.1: Measured CNP Waveform.

CNP waveform measured from early human whole-body exposure equipment using the Bartington fluxgate magnetometer, 10 kHz sampling rate.

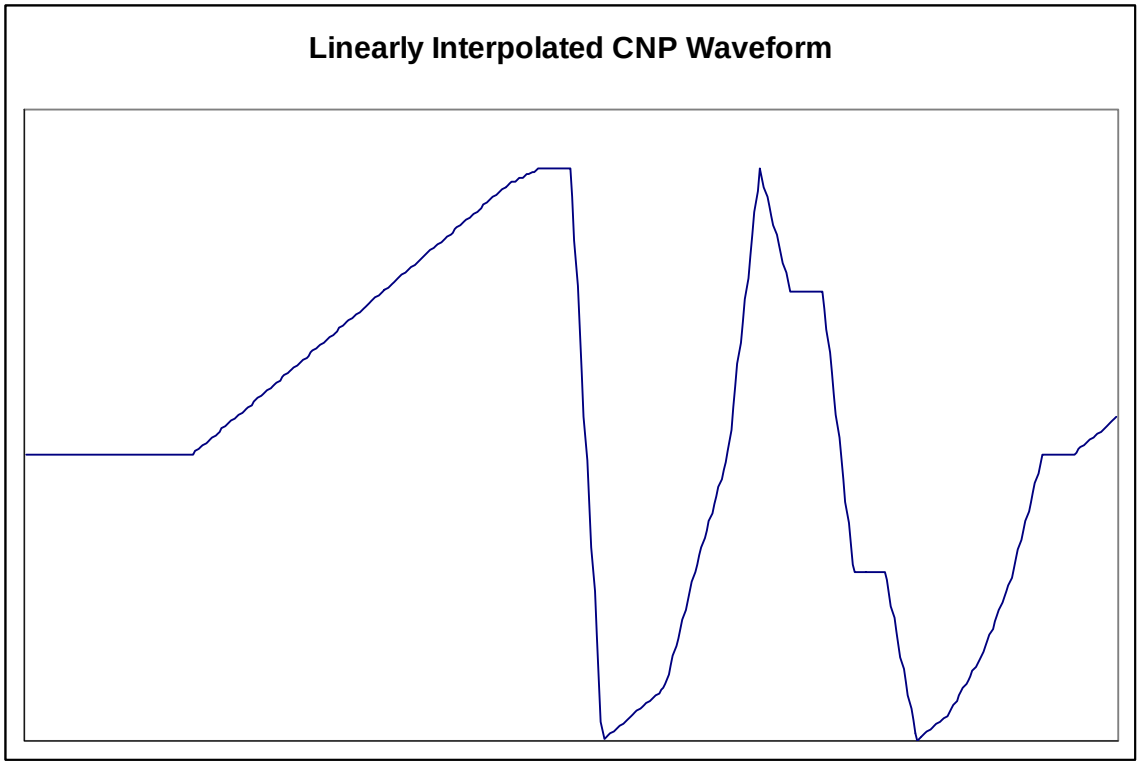

Figure D.2: Linearly interpolated CNP waveform (single pulse).

The waveform of the specific pulsed magnetic field with a linear interpolation of points, leading to a different rate-of-change (dB/dt) at different points in the waveform, with a maximum dB/dt of 0.4 T/s. This was the implementation used on the 1.5 T Seimens Avanto for the studies described in Chapters 2, 3, and 4.

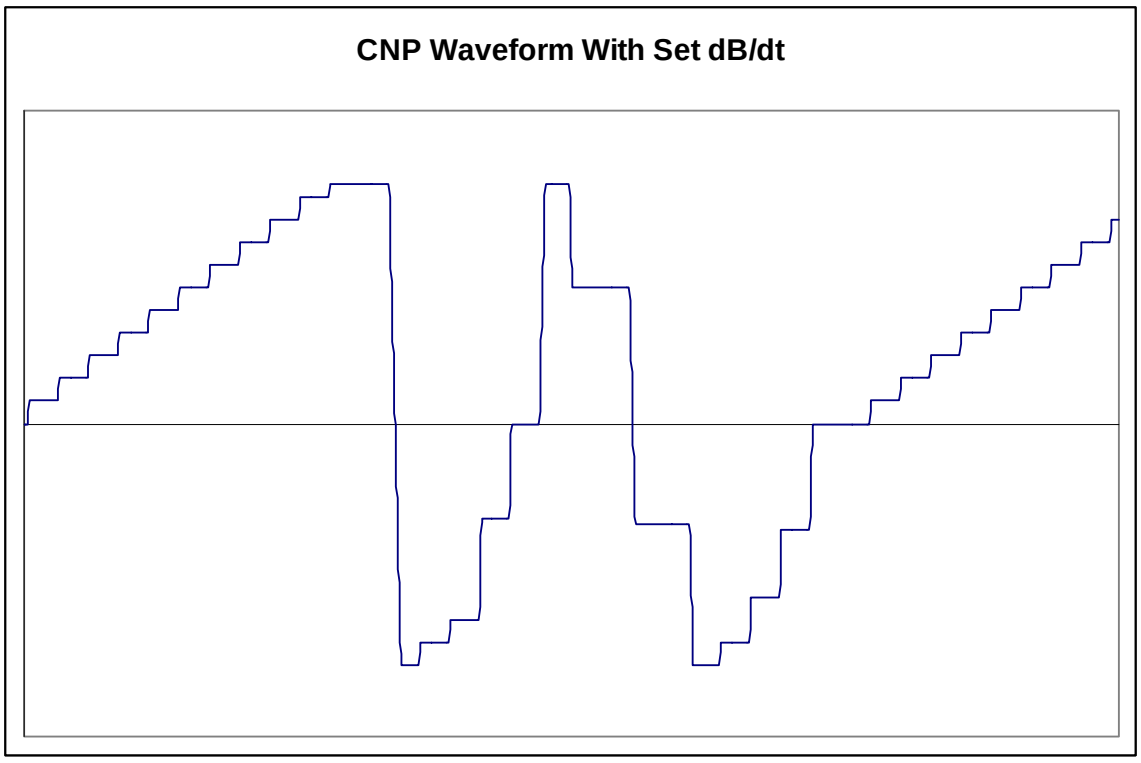

Figure D.3: CNP Waveform with 1 T/s rate of change.

The waveform with a set rate-of-change of 1 T/s, as used in the experiment on the 3T Verio MRI system (Chapter 5).

Appendix E: Evolution of Hybrid Functional Imaging in Bioelectromagnetics Research

Note: a version of this chapter has been published in the journal *The Environmentalist.*

Evolution of Hybrid Functional Imaging in Bioelectromagnetics Research

By: John A. Robertson, Alex W. Thomas, Julien Modolo, Jodi Miller, Nicole Juen, Alexandre Legros, Frank S. Prato

E.1 Introduction

Studies in animals and humans have demonstrated that there is the potential for pulsed electromagnetic fields to affect behaviour. One area of study in particular has been nociception, the reactions to noxious stimuli. Teskey *et al* (1988) found a reduction in analgesia from an opioid agonist

(fentanyl) after exposure to the time-varying gradient fields of a magnetic resonance imaging (MRI) system.

Working under the hypothesis that if one magnetic field could reduce analgesia, perhaps another could induce or augment analgesia, Thomas *et al* (1997) developed a specific pulsed magnetic field pattern for analgesic purposes known as the "complex neuroelectromagnetic pulse" (CNP). Studies in snails (Thomas *et al*, 1997) and mice (Shupak *et al*, 2004a) demonstrated that the CNP could produce analgesia after an acute exposure, and further that this effect could be ablated by pre-treatment with naloxone, an opioid antagonist.

This approach has translated to human studies on acute pain by Shupak *et al* (2004b), in healthy volunteers, and also to chronic pain conditions such as rheumatoid arthritis (Shupak *et al* 2006). Shupak *et al* (2004b), also observed that sensory thresholds to non-painful warmth (as tested by a Peltier thermode stimulator) were not affected by the CNP exposure. Thus the analgesic effect may be quite specific, and not due to some kind of general anesthesia, which is an important consideration for potential clinical use.

However, the effects in humans tend to be subtle, and difficult to detect. Moreover, the mechanism of action is not well known: are the fields affecting general systems within the brain, or only specific regions? What receptor systems are involved in the transduction mechanism? The evolution of hybrid (simultaneous modality) functional imaging techniques will be valuable in

getting answers to these questions, as well as others within the bioelectromagnetics research arena.

E.2 Functional Imaging Methods and History

Long before the invention of magnetic resonance imaging scanners, scientists and clinicians had a strong interest in examining the workings of the brain. That the brain did have anatomically distinct regions was an ongoing debate for some time, even after Brodmann created a detailed map of the various areas that possessed different histological staining characteristics (Brodmann, 1909).

The task then became identifying which regions of the brain were responsible for what types of behaviour and neuroprocessing. Invasive techniques utilizing stimulating electrodes were developed, allowing both the identification of seizure foci, as well as providing ways to create the first functional maps of the brain (Penfield and Jasper, 1954). More recently, transcranial magnetic stimulation (TMS) has been implemented as a non invasive way to stimulate specific brain regions which has also been used as a brain mapping tool (Wasserman *et al.* 1993).

Invasive surgery is always best left as a last resort, so neuropsychologists developed highly specific pencil & paper and psychophysical test regimes that are able to localize changes in neuroprocessing quite specifically (e.g., the Halstead-Reitan test battery: Reitan and Wolfson, 1993), giving informed speculation to neurosurgeons on

a good starting location for the location of a suspected lesion without having to take an exploratory look inside the skull. These test batteries, being non-invasive, also proved to be valuable research tools, and to this day help guide study design into the potential behavioural effects of many stimuli, including electromagnetic fields (Corbacio *et al*, in submission).

Electroencephalography (EEG) is another valued tool in the clinic as well as the research institute. The electrical activity of the brain, and more precisely of large ensembles of pyramidal neurons with synchronous activity, can be detected on the surface of the scalp non-invasively using electrodes. Surface EEG has excellent temporal resolution, and still provides one of the most direct measures of electrical activity of the brain (short of invasive implanted electrodes). Though source localization methods are available for estimating the location of an electrically active region within the brain, the difficulty of the "inverse problem" – identifying spatially which area of the brain produced a given electrical recording in the EEG – has meant that EEG is often best paired with an additional method of interrogating the brain's function to determine location more precisely. However, it is possible to estimate EEG sources reasonably accurately by applying *a priori* assumptions and constraints to simplify the inverse problem, providing a spatial resolution on the order of cm^2 for the surface recordings and cm^3 for sources. To do this several specialized software packages are available on the

market from EEG system manufacturers, such as CURRY (Compumedics-Neuroscan, Charlotte, NC).

E.3 Functional Imaging Advantages and Uses

Two more recent additions to the repertoire of functional imaging are positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Both offer good spatial localization of activity in the brain, but have compromises in temporal resolution – a single fMRI volume takes on the order of a second to acquire, and several are needed to study activation changes, while PET can be slower again by an order of magnitude. Both also rely on measures of metabolic or hemodynamic activity rather than electrical activity directly.

PET involves the use of a radioactive tracer molecule. The canonical tracer being fluorodeoxyglucose (FDG), a glucose analog with a radioactive ^{18}F atom included. The FDG is taken up by cells with metabolic demand, just like glucose, but is not fully broken down, and remains sequestered in the cell. Radioactive decay of the ^{18}F is then detected by the PET scanner, and an image of metabolic demand is built up over time (Alavi *et al*, 1986). PET is a very useful tool for investigating chronic processes since the image is quite stable, and the data can be scaled to give absolute units of tracer uptake. It is not well suited to fast-changing paradigms as it takes some time to build up an image.

Other tracer molecules are being developed for PET, which will widen the scope of its applicability in bioelectromagnetics research, with the frontier of research promising labeled pharmaceuticals that would allow researchers to investigate the activity of specific receptor systems.

Functional Magnetic Resonance Imaging (fMRI) capitalizes on the fact that oxygenated and deoxygenated hemoglobin have different magnetic properties. As a region within the brain is activated, the metabolic demand increases, which triggers a compensatory increase in blood flow to the region. Because of a blood oxygenation overshoot, the region receives more oxygenated hemoglobin than during the resting state. The oxygenated blood has less of a magnetic moment than the deoxygenated blood, which causes less signal degradation from the surrounding protons (which supply the MRI signal). That ultimately leads to a slight signal difference that depends on the oxygenation in the blood, or Blood Oxygenation Level Dependent (BOLD) signal (Detre and Wang, 2002). This difference can be detected in an MRI image, but the magnitude of the BOLD change is fairly small. The technique is based on a subtraction between a rest and an activated state, and furthermore, the MRI signal suffers from a signal drift over longer periods of time. Thus, fMRI experiments require multiple repetitions of a stimulus in order to detect the functional changes, resulting in fMRI being best suited to investigating repeated, acute stimuli or behaviours over fairly short time

periods (typically, several seconds). Therefore, fMRI is a complementary imaging modality to PET.

Newer fMRI techniques aim to expand the toolset. Arterial Spin Labeling (ASL) can provide absolute measures of cerebral blood vs. the relative measures of BOLD/fMRI, which allows for the investigation of slower processes and chronic states (Detre and Wang, 2002). Another technique consists in studying functional connectivity, not using a simple subtraction of activity between an "on" and "off" state, but for correlations in the changes to the BOLD signal over time across brain regions, identifying which ones step up their metabolism in synchrony (Bluhm *et al*, 2007).

E.3.1 Hybrid Functional Imaging

Each of these imaging modalities represents a trade-off: fMRI and PET both have excellent spatial resolution, but are limited in their temporal resolution, and further are one step removed from directly measuring the brain's electrical activity, instead using hemodynamic or metabolic correlates. Also, EEG, EMG (electromyography) and ECG (electrocardiography) have excellent temporal resolution, but poor spatial resolution. Hybrid functional imaging combines the best attributes of each modality to give a better picture of what functional changes are taking place within the brain and body, with each modality helping to compensate for the shortcomings of others.

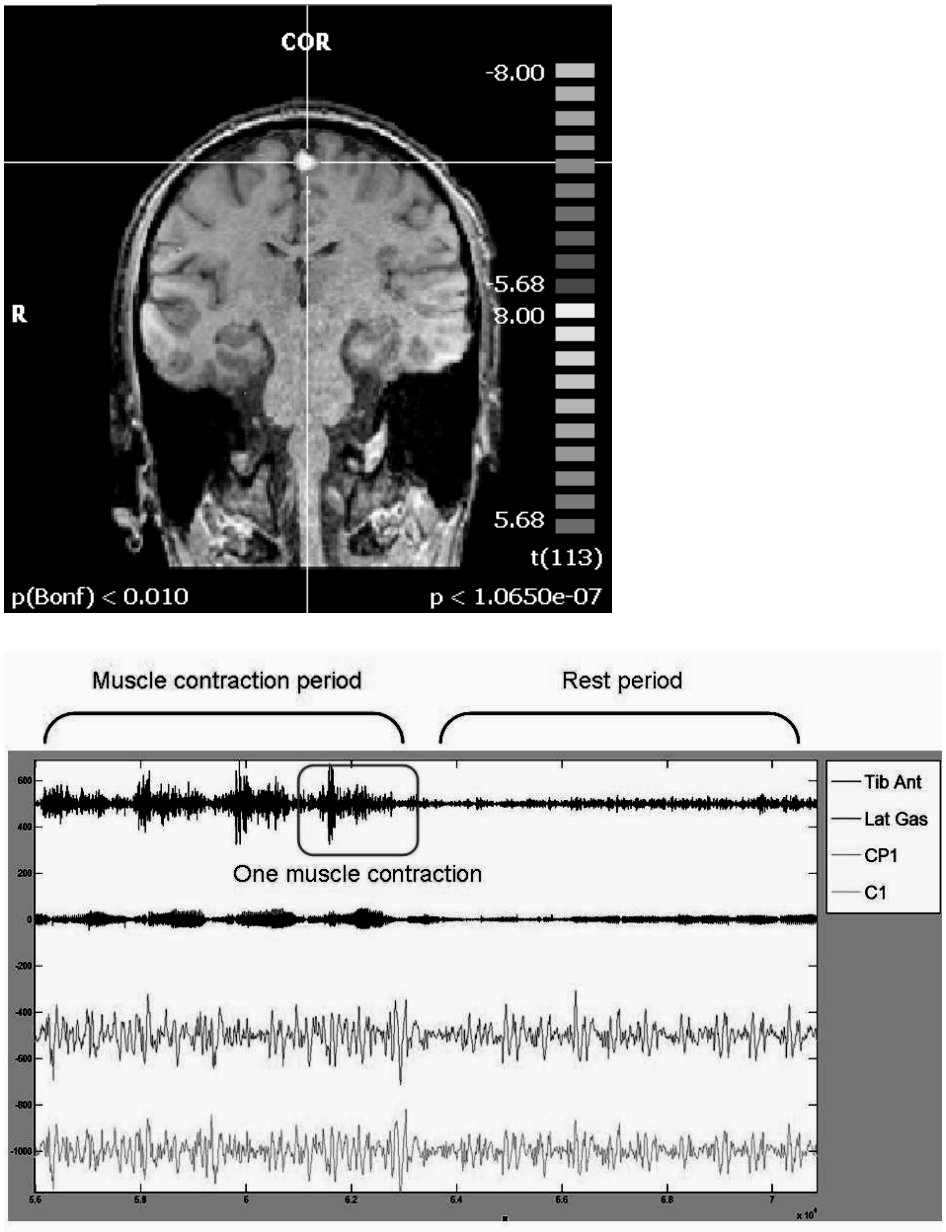

Figure E.1: Sample hybrid data.

Example of simultaneous acquisition of EEG, EMG, and fMRI data during an alternating muscular contraction with rest task in a single test subject. Top: fMRI-BOLD activation within the motor cortex represented by the bright region at the center of the crosshairs. Bottom: EMG (top two traces) and EEG (bottom two traces) recorded during one repetition of the muscle contraction and rest task. The artifacts imposed by the MRI imaging gradients have been suppressed in post-processing.

Combined fMRI-EEG systems are now leaving the bleeding edge of research possibilities, and are becoming commercially available. The strong, time-changing magnetic fields of the MRI system pose a challenge for recording EEG, but a usable EEG signal can be obtained with specialized hardware and software-based corrections. The combination of EMG, for recording the electrical activity of muscles, and clinically useful ECG for recording cardiac electrical activity may soon also be a commercial reality within the environment of an MRI scanner (van der Meer *et al*, 2010). See figure E.1 for an example of EEG-EMG-fMRI data.

PET has long been a target for hybrid imaging, often with X-ray Computed Tomography (CT) to provide anatomical images that localize the accompanying metabolic measurements of PET. Combining PET with the better soft-tissue contrast of MRI is also a goal, with the first hybrid systems presently entering service. This not only allows for the combination of MRI structural information with PET, but also the ability to acquire both fMRI and PET functional data together. Several MRI manufacturers are now producing hybrid MRI-PET systems.

E.4 Bringing Hybrid Functional Imaging to Bioelectromagnetics

High-quality, objective data on how the brain responds to magnetic field exposure is important in answering a number of questions in the field of bioelectromagnetics. EEG has already been used by several groups as one measure of functional changes (Cook *et al*, 2006; Croft *et al*, 2010). Due to

interference from the magnetic fields being investigated on the sensitive pickups of the EEG system, where the induced electric fields from the applied magnetic field exposure can dwarf the physiological electric fields of the brain, it has always been a challenge to design exposure and recording systems that provide usable EEG recordings. Fortunately for the bioelectromagnetics community, the integration of an EEG into an MRI system faces many of these same challenges due to the magnetic fields of the MRI system, and hybrid MRI-EEG systems can, to some extent, compensate for the confounding exposures in a bioelectromagnetics experiment (see Figure E.2). Interestingly, Robertson *et al* (2010) demonstrated that arbitrary magnetic fields could be generated by, and within, an MRI system using the existing MRI hardware. That capability allows for the use of MRI, and multimodality systems hybridized with MRI, to investigate the functional changes associated with magnetic field exposure.

Due to the limitations of the fMRI-BOLD technique, Robertson *et al* (2010) strictly examined the changes in the processing of a specific task (pain perception), but could not comment on whether the baseline functioning of the brain was altered by the magnetic field exposure. Bringing in complementary imaging modalities (fMRI-ASL, PET, EEG) will allow such questions to be answered. Indeed, one of the exciting aspects of the combination of PET and MRI will be to determine if the strong magnetic

fields and radiofrequency fields of the MR imaging processes themselves affect brain activity and metabolism.

E.4.1 The Next Evolution

This multimodality imaging capability will be important for acquiring a better understanding of how magnetic fields can affect certain behavioural end-points, such as pain processing, in healthy controls as well as patients with chronic conditions. As these techniques evolve, we may even be able to get answers within a single subject, which will be important for clinically-relevant individualized medicine.

The specific pulsed magnetic field known as the complex neuroelectromagnetic pulse (CNP) is an example of MF-based therapy that could benefit from functional imaging advances. It originally designed without the benefit of this technology, and it is quite likely that the CNP is generalized and not optimized as it currently exists. Adaptations of the specific pulse-form will likely be required to best treat individual patients for maximum efficacy. Real-time and individualized techniques are evolving, which will be particularly important for this field.

Opioid receptors or their analogues are present in virtually identical ways in all patients; many other pharmaceuticals are similarly basic enough to not show much of an individualized response. We cannot necessarily say the same of magnetic fields that are designed to alter neural processing in a specific way. Personalized treatment may be the way of the future, with the

first treatment session involving the fine-tuning of the magnetic field parameters to the individual's specific brain activity patterns in near-real-time, as measured by combined behavioural testing, EEG, fMRI, and PET.

The feedback required for tweaking, even with a fairly narrow parameter space, is simply not possible to provide with purely subjective measures of outcome. Thus the evolution of sensitive, near-real-time multimodality imaging techniques will advance the optimization of pulsed electromagnetic field therapies. Similarly, the specificity and breadth of objective measurements offered by hybrid and molecular functional imaging should enable the detection of potentially subtle, transient effects of weak magnetic fields in bioelectromagnetics research.

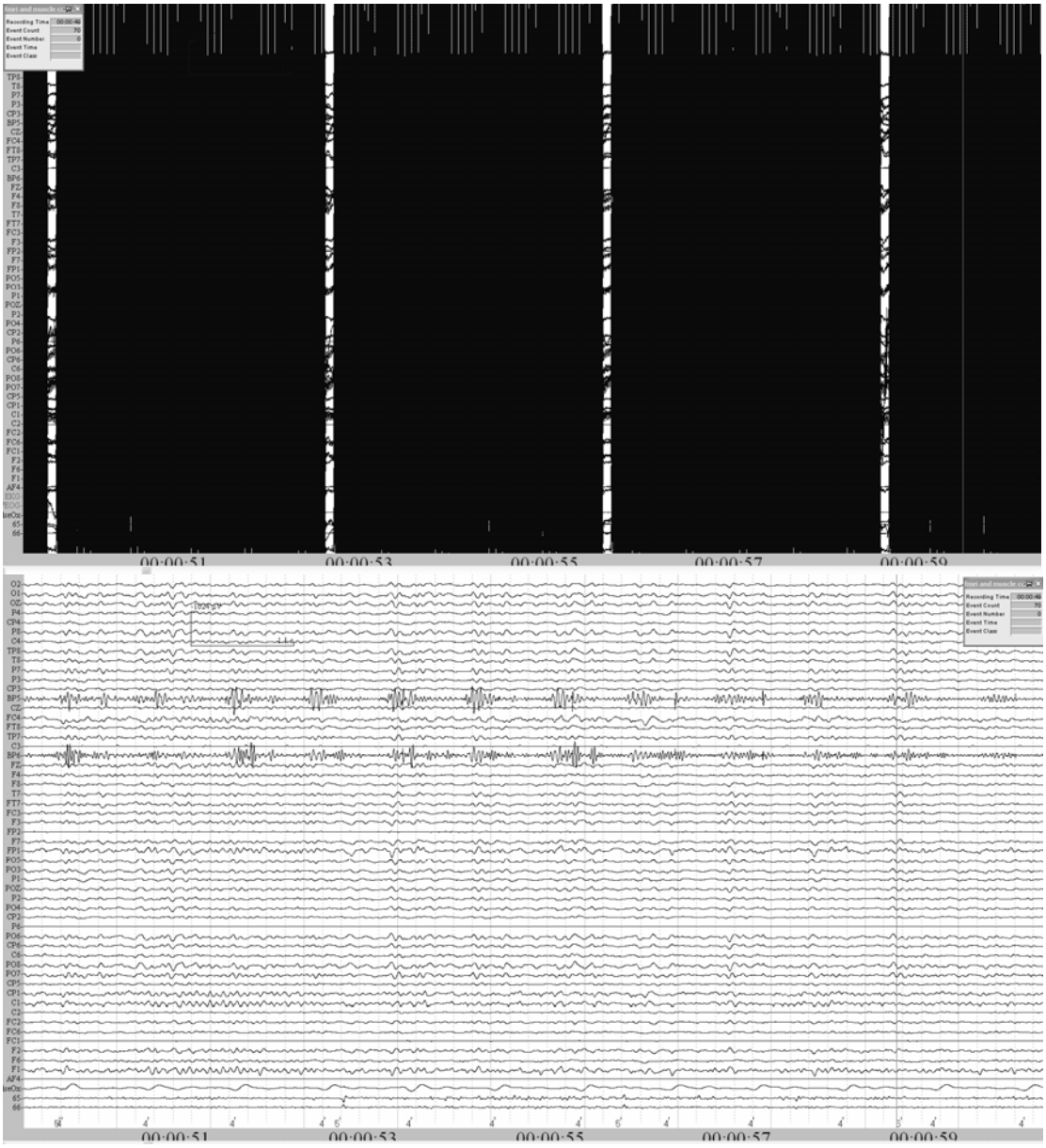

Figure E.2: EEG artifacts.

The artifacts in EEG signals from the induced fields due to the time-varying magnetic fields of the MRI are seen in the top as a near-saturation of the recording. The artifact can be suppressed, providing the physiological EEG information in the bottom.

E.5 Conclusion

Current research suggests that there is the potential for electromagnetic fields to affect biological systems, including human behaviour. However, the effects may be subtle and elusive, thus having a suite of sensitive and objective tools at their disposal is important for researchers. Hybrid multi-modality functional imaging provides data on the function and metabolism of a subject's brain, with different modalities contributing information across spatial and temporal scales. As the technology is developed and real-time techniques emerge, individualization of magnetic field pulses may become a reality.

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