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# DNA DAMAGE ASSESSMENT OF HUMAN LYMPHOCYTES AFTER WHOLE BODY EXPOSURE TO A SINUSOIDAL 60 Hz, 200 pT MAGNETIC FIELD

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# DNA DAMAGE ASSESSMENT OF HUMAN LYMPHOCYTES AFTER WHOLE BODY EXPOSURE TO A SINUSOIDAL 60 Hz, 200 μT MAGNETIC FIELD

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## ABSTRACT

*Introduction:* Due to electricity production, transmission and commercial and residential uses, humans are exposed to extremely low frequency electromagnetic fields (ELF-EMF). Based on results from human epidemiological reports, animal *in vivo* and cell *in vitro* studies on the chronic exposure to ELF-EMF, adverse health effects of ELF-EMF remain controversial. *Objective and Methods:* The aim of this pilot project was to examine and compare DNA damage and clastogenic effects detected by the alkaline comet assay and the cytokinesis-block micronucleus assay, respectively, in peripheral human blood lymphocytes collected from healthy adult volunteers before and after (whole-body) exposure to a 200 μT, 60 Hz ELF-EMF. *Results:* There was no significant difference between pre- and post-exposure samples ($p>0.05$). Moreover, magnetic field-exposed volunteers were not significantly different from sham-exposed subjects ($p>0.05$). *Conclusion:* This study found no evidence that an acute, whole-body exposure to the magnetic field could cause DNA damage in human lymphocytes.

**Keywords**

Extremely low frequency electromagnetic fields, cytogenetics, alkaline comet assay, cytokinesis-block micronucleus assay, human lymphocytes

## CO-AUTHOR STATEMENT

**Genevieve Albert:** Performed all manuscript writing, human exposure experiments, data collection, and cell irradiation. Performed most blood sample collections and data analysis.

**Pascale Bellier:** Assisted with experimental design and manuscript review

**Charles Cook:** Assisted with manuscript review (Appendix C).

**Leonora Marro:** Assisted with statistical design and analysis.

**James McNamee:** Assisted with experimental design, data analysis and manuscript review.

**Frank Prato:** Co-supervisor, assisted with experimental design and manuscript review.

**Alex Thomas:** Co-supervisor, assisted with experimental design and manuscript review.

**Vijayalaxmi:** Assisted with experimental design and manuscript review.

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| | |
|---|---|
| BN(C) | Binucleate (cell) |
| CA | Chromosomal aberrations |
| CBMN | Cytokinesis-block micronucleus assay |
| DBS | Deep brain stimulation |
| DSB | Double strand break(s) |
| ELF-EMF | Extremely low frequency electromagnetic field |
| Gy | Gray |
| MN | Micronuclei |
| MF | Magnetic field |
| SCGE | Single-cell electrophoresis gel |
| SCE | Sister chromatid exchange |
| SSB | Single strand break(s) |
| TMS | Transcranial magnetic stimulation |
| VNS | Vagal nerve stimulation |
| (μ, m)T | micro- milli- Tesla |

## GLOSSARY

| | |
|---|---|
| Acentric | Chromosomal fragment resulting from the lack of a centromere |
| Anaphase | The stage of mitosis in which the chromosomes move to opposite ends of the mitotic spindle |
| Clastogenic | Capable of causing breakage of chromosomes |
| Cytogenetic | Study of cellular components, particularly chromosomes |
| Diploid fibroblasts | A cell, with 2 sets of chromosomes, found within fibrous connective tissue and associated with the formation of collagen fibers |
| Genotoxic | Able to cause DNA damage |
| Mitogen | An agent that can induce mitosis in certain eukaryotic cells |
| Mutagen | A physical/biological/chemical agent that can induce or increase the frequency of mutation in an organism |
| Telophase | The final stage of mitosis during which the chromosomes of daughter cells are grouped in new nuclei |

## PREFACE

The interaction between humans and electric and magnetic fields has been studied in depth for over a century. There are two basic areas of research studying the effects of extremely low frequency electromagnetic fields (ELF-EMF) on biological systems. The first area of research studies ELF-EMF resulting in harmful health effects, such as cancer. The second area of research investigates how ELF-EMF can improve human quality of life, for example, by helping those suffering from neurological disorders.

Since 1979, there have been controversial studies indicating that weak magnetic fields can lead to DNA damage, resulting in higher incidences of cancer. Other studies have not been able to confirm these results. There was a need for a well-controlled and reproducible study on the possible cytogenetic effects of ELF-EMF. The current study investigated the effects of human whole-body exposure to ELF-EMF and DNA damage in lymphocytes.

Exposure to electrical and magnetic fields has been proposed as a possible means of providing therapeutic neuromodulation with the hopes of improving human quality of life. Deep brain, vagal and transcranial magnetic stimulation are current techniques used in human treatment. The review in Appendix C combines stimulation parameters developed in animal models and neuromodulation with the expectation of gaining a greater understanding of the mechanisms and neurobiological effects of these neuromodulation devices.

# CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

For over a century, the general public has enjoyed the benefits from the use of electricity. Due to the production of electricity, its transmission through power-lines, and commercial and residential uses, humans are now continually exposed to extremely low frequency electromagnetic fields (ELF-EMF). In 1979, scientists, epidemiologists and the public were alerted to the potential harmful health effects of chronic ELF-EMF exposure; children with a higher incidence of cancer resided in homes exposed to higher EMF than healthy control children (Wertheimer and Leeper, 1979). This controversial finding promoted three decades of epidemiological and scientific research into the effects of magnetic fields on biological systems. In 2002, after reviewing all data relating to ELF-EMF exposure, the International Agency for Research on Cancer (IARC) concluded that there was limited evidence of carcinogenicity in relation to childhood leukemia, and inadequate evidence that ELF-EMF is carcinogenic in human adults and experimental animals. Despite limited and inadequate evidence, ELF-EMF were classified in Group 2B, as "possible carcinogens to humans".

In 2005, Vijayalaxmi and Obe assessed the scientific literature on the genotoxic potential of ELF-EMF in biological systems. Among a total of 63 published reports during 1990-2003 (including *in vitro*, *in vivo*, animal and human studies), the conclusions from 29 investigations (46% of the investigations) did not identify an increase in cytogenetic damage, 14 studies (22%) indicated genotoxic potential, and 20 reports

(32%) were inconclusive. While most *in vitro* studies have demonstrated no evidence of 60 Hz ELF-EMF-induced genotoxicity (Miyakoshi *et al.*, 2000; Heredia-Rojas *et al.*, 2001; Cho and Chung, 2003), a small number of animal *in vivo* studies have demonstrated some evidence that 60 Hz ELF-EMF may induce DNA damage (Lai and Singh, 1997a; 1997b; Singh and Lai, 1998). Although a few human ELF-EMF whole-body exposure studies have been conducted, these studies have been limited to individuals exposed occupationally to EMF where precise exposure conditions were unknown (Ciccone *et al.*, 1993; Khalil *et al.*, 1993; Skyberg *et al.*, 1993, 2001; Valjus *et al.*, 1993). There have also been a number of epidemiological investigations attempting to clarify the association between MF and the incidence of human cancers (Alhom *et al.*, 2000; Auvinen *et al.*, 2000; Villeneuve *et al.*, 2000a, 2000b; Savitz *et al.*, 2000; Kheifets 1999). Alhom *et al.*, (2001) concluded that: "Overall, despite 20 years of extensive epidemiologic investigation of the relation of EMF to risk of chronic disease, there are still epidemiologic questions that need to be resolved".

Based on the recommendations for future research proposed by Vijayalaxmi and Obe (2005), the present investigation is a first-of-its-kind pilot study. It is a well-coordinated, collaborative study (Lawson Health Research Institute, the London Regional Cancer Program, and Health Canada) exploring the possible cytogenetic effects of whole body exposure to 60 Hz, 200 μT ELF-EMF in humans.

## 1.2 Electromagnetic radiation: ionizing and non-ionizing radiation

Non-ionizing (e.g. ELF-EMF) and ionizing radiation (e.g. gamma-irradiation) differ in frequency, wavelength and energy (Table 1).

### 1.2.1 Non-ionizing radiation (ELF-EMF)

Wherever electricity is generated, transmitted or used, ELF-EMFs are produced: these fields are found in our communities, homes and workplaces. Extremely low frequency (ELF) radiation belongs in the non-ionizing radiation portion of the electromagnetic spectrum among radiowaves (AM and FM), microwaves, and light waves including infrared and visible light. ELF-EMF consist of very low energy radiation in comparison to ionizing energy sources such as gamma-radiation, and do not have the required energy to directly break chemical bonds (Valberg et al., 1997). Electromagnetic fields (EMF) are composed of an electric and magnetic field components. The electric field is created by the presence of an electric charge and the magnetic field by the motion of electric charges. The magnetic field describes the magnitude and direction of the force exerted on a nearby current. The strength of the magnetic field is proportional to the current drawn from the source to which it is connected, and this strength, known as the magnetic flux density is measured in tesla (T) (Portier and Wolf (NIEHS), 1998). Within the home and community, humans are exposed to EMF that typically do not exceed 150 μT. Some magnetic fields up to 270 μT can be found around generating stations and substations (WHO, 1998).

| Source | Frequency (Hertz) | Wavelength (meters) | Energy (eV) |
|---|---|---|---|
| ELF (i.e. Power transmission) Non-ionizing | $3 - 3.0 \times 10^{2}$ | $10^{6}$-$10^{8}$ | $10^{-14}$-$10^{-13}$ |
| Gamma rays (i.e. $^{60}$Cobalt) Ionizing | $10^{18}$-$10^{20}$ | $10^{-12}$-$10^{-10}$ | $10^{4}$-$10^{6}$ |

**Table 1.1** Comparison between ELF-radiation and gamma-radiation with the following parameters: frequency, wavelength, and energy.

The MF is influenced by how quickly the current is alternating in the power-line source. The frequency of the cycles is measured in Hertz (1 Hz = 1 cycle per second). In North America, the alternating current flowing in the electric power system is 60 Hz; this frequency falls between 3-300 Hz and is categorized as extremely low frequency (ELF) in the electromagnetic spectrum (Poole & Ozonoff, 1996; Portier and Wolfe (NIEHS), 1998). Magnetic fields can easily penetrate buildings and biological tissues and are strongest close to the source and diminish exponentially with distance. These fields are very difficult to shield (Valberg *et al.*, 1997), although shielding can be achieved by using metal with high permeability (for example 1 mm thick Mu metal can attenuate ambient magnetic fields from 0-100 Hz by a factor of approximately 100, as described in Choleris *et al.*, 2001).

EMF exposure standards for the general public have been developed by the International Commission on Non-ionizing Radiation Protection (ICNIRP). For both 50 and 60 Hz, the ICNIRP exposure standards for the general public are 100 μT for continuous exposure and 1000 μT for short-term exposure (Jammet *et al.*, 1990). Health and safety standards for continuous ELF-EMF exposures have not been determined by the World Health Organization (WHO) nor by Health Canada. In fact, Health Canada

"does not consider guidelines necessary because the scientific evidence is not strong enough to conclude that typical exposures cause health problems" (Health Canada, 2004). WHO on the other hand stated the following "Limits of EMF exposure recommended in many countries are broadly similar to those of ICNIRP, which is a non-governmental organization (NGO) formally recognized by WHO..." (WHO, 1998).

In the present study, a 60 Hz magnetic field with an intensity of 200 μT was used. The 60 Hz MF is representative of the sinusoidal waveform that is used in electricity transmission and distribution in North America. The signal for the experiment was derived from the AC power lines used within St. Joseph's Health Care, in London, Ontario. This waveform is 60 Hz sinusoidal and it contained various random noise and distortions typical of the AC power grid (IEEE Standard, 1159-1995). In the magnetic field chamber, the ambient static field was ~50 μT and the ambient time-varying field strength at 60 Hz was ~0.08 μT.

A 200 μT magnetic field has been previously reported to have biological effects in humans. Shupak et al. (2004) observed an analgesic effect and Thomas *et al.*, (2001a, 2001b) observed that an ELF-EMF at 200 μT influences human postural sway. However, it should be noted that a special ELF-EMF computer generated pulse, the complex neuroelectromagnetic pulse (CNP), was required to elicit these responses.

### 1.2.2 Ionizing radiation

Sources of ionizing energy include ultraviolet, gamma, and x-rays. Unlike non-ionizing radiation, ionizing radiation releases large amounts of energy when it interacts

with biological tissue. This energy is strong enough to break strong chemical bonds such as those found in DNA. Gamma rays do not produce chemical and biological damage themselves, but when they are absorbed into tissues, they release their energy to produce fast-moving charged particles and free radicals that in turn are able to produce damage. The principal target for radiation-induced cell lethality is DNA damage (Hall and Giaccia, 2006). Damage to DNA is usually expressed as base damage, single-strand breaks (SSB), double-strand breaks (DSB), or crosslinks between the DNA double helix and proteins (Feinendegen *et al.*, 2007).

The average human is exposed to natural sources of radiation including cosmic rays, terrestrial radiation and radionuclides naturally present in the body (inhaled or ingested). From these naturally occurring sources, the general public is exposed annually to approximately 3.6 milli-Sievert (mSv) (Hall and Giaccia, 2006). The unit Sievert (Sv) is the product of absorbed dose (in gray) and radiation weighting factor (type and energy range). The annual limitation of radiation exposure for members of the public from human made sources is 1 mSv and for occupational workers 50 mSv (Health Canada, 2008; Hall and Giaccia, 2006). The medical exposures are excluded from these limitations because it is assumed that they confer personal benefit to the exposed person such as a chest x-ray (0.1 mSv), a spleen positron-emission tomogragraphy (PET) (3.7 mSv) or cranical computed tomography (CT) (50 mSv) (Hall and Giaccia, 2006).

In the present study, DNA damage induced by an exposure to 1.5 Gy gamma-radiation in human lymphocytes was used for the positive control. The unit Gray is used when defining absorbed dose, the energy absorbed per unit mass (joules per kilogram) (Hall and Giaccia, 2006). For most cells cultured *in vitro* and exposed to radiation, the

$D_0$ (the dose required to reduce cellular survival to e-1 = 0.37, 37% survival, on the linear portion of the dose survival curve) of survival curves falls in the range of 1 to 2 Gy (Hall and Giaccia, 2006).

The radiation for the current study was produced by the $^{60}$Cobalt source, an Eldorado 6 teletherapy unit located at London Regional Cancer Program (LRCP). $^{60}$Cobalt decays with a half-life of 5.26 years and produces two gamma rays, one at 1.17 MeV and another at 1.33 MeV.

### 1.3 Mutagenesis and cancer

Concerns about adverse health effects due to ELF-EMF exposure originated in 1979 when childhood leukemia was reported to be weakly associated to ELF-EMF exposure (Wertheimer and Leeper, 1979). A large body of high-quality epidemiological data exists for childhood leukemia and brain tumours, and for occupational exposure in relation to adult leukemia and brain tumours. Among all the outcomes evaluated in epidemiologic studies of EMF, pooled meta-analyses have shown that an average residential exposure above 0.4 μT may be associated with a small increased risk of childhood leukemia (Ahlbom *et al.*, 2000, 2001). Leukemia is the most common type of cancer reported in children and it arises when a B or T cell (lymphocytes, a part of the immune system) transform. These transformed cells then divide and generate more like-cells, which are highly malignant (Lightfoot, 2005).

To study if ELF-EMF may have the potential to cause cancer, it is important to understand the mechanisms of carcinogenesis in the human body after acute or chronic

exposure to an environmental (physical, chemical and or/biological) agent. Although the exact mechanisms for the development of human cancers are poorly understood, several *in vivo* and *in vitro* investigations have provided sufficient information to assess the potential of an environmental agent to contribute to carcinogenesis.

Carcinogenesis, the process by which normal cells are transformed into cancer cells, has basic principles outlined by Cohen and Ellwein (1991). These include, but are not limited to the following:

1. Genetic errors occur in normal cells that have the potential to become cancer
2. More than one genetic mistake must occur
3. Somatic mutations occur, resulting from DNA replication infidelity

It is possible for an environmental agent (biological/chemical or physical) to increase the risk of cancer development. There are two possible pathways in which an agent can induce carcinogenesis: the genotoxic pathway or the epigenetic pathway. In the genotoxic pathway, the agent induces direct DNA damage in the cell. After this damage has occurred, there are three possible outcomes for the cell: 1) the cell can undertake DNA repair 2) if the DNA damage cannot be repaired, the cell can go through organized cell death (apoptosis), and 3) if the DNA damage cannot be repaired or it is not repaired correctly, this will result in unrepaired/misrepaired DNA. An accumulation of unrepaired/misrepaired DNA can lead to the transformation of carcinogenesis. In the epigenetic pathway, the environmental agent does not cause genotoxic effects itself. Rather, the agent is able to increase the genotoxic potential of other agents (i.e. by

causing proliferation), resulting in DNA repair interference and allowing a cell with DNA damage to survive and undergo cell division (Cohen and Ellwein, 1991; Vijayalaxmi and Obe, 2005).

## 1.4 Cytogenetic and DNA damage assays

In recent years, classical cytogenetic methods such as the chromosomal aberration (CA) assay, cytokinesis-block micronucleus assay (CBMN), and sister chromatid exchange (SCE) assay have been used to assess chromosomal damage of instability of test compounds. Experimental techniques such as the alkaline comet assay have also been used to assess the potential genotoxicity of a large number of environmental agents. It has been observed that many environmental agents that are carcinogenic are also genotoxic/mutagenic agents (Ames *et al.*, 1973; Tennant and Zeiger, 1993).

### 1.4.1 Alkaline comet assay

The single-cell gel electrophoresis (SCGE) assay is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells and it was first pioneered by Ostling and Johanson (1984) and later modified by Singh *et al.*, (1988). This assay is applicable for detecting a wide range DNA damage. Relative to other genotoxicity tests, the advantages of the comet assay include its demonstrated sensitivity for detecting low levels of DNA damage, from 0.05 - 0.5 Gy exposure (McNamee *et al.*, 2000; Malyapa *et al.*, 1998; Vijayalaxmi *et al.*, 1992; Vijayalaxmi *et al.*, 1993).

Moreover, in comparison to the CBMN, chromosomal aberration and sister-chromatid exchanges assays, the alkaline comet assay requires a small number of cells (~10,000) per sample. It can be used on proliferating or non-proliferating cells, and there is a low cost associated with the assay (Dhawan *et al.*, 2008).

The assay can be described as follows. Briefly, individual cells are embedded in a thin agarose gel on a microscope slide. Cells are lysed in a solution consisting of high salts and detergents, and most cellular and nuclear proteins are removed and the compact DNA structure is disrupted (Collins *et al.*, 2008). The DNA is then unwound under alkaline conditions. Following the unwinding step, the negatively charged DNA is electrophoresed, and broken or damaged DNA fragments are able to migrate away from the nucleus towards the anode. The image obtained looks like a "comet" with a distinct head (comprised of intact DNA) and a tail (consisting of damaged or broken pieces of DNA) (Figure 1.1A). The evaluation of the fluorescently dyed DNA in the comet includes, but is not limited to the following parameters: comet length, comet tail length, comet tail ratio, and comet tail moment. The comet length is the entire length of the comet (μm) while the comet tail length is the distance from the leading edge of the comet head to the leading edge of the tail (μm). The comet tail ratio is calculated as the relative fluorescence intensity in the comet tail region to that in the entire comet. The comet tail moment is calculated as the tail ratio multiplied by the distance (μm) between the centers of gravity of the head and tail regions (Hellman *et al.*, 1995; McNamee *et al.*, 2002).

### 1.4.2 Cytokinesis-block micronucleus assay

Micronuclei (MN) are small DNA-containing extra-nuclear bodies. MN arise in dividing cells from acentric chromosome/chromatid fragments and/or whole chromosomes/chromatids that lag behind in anaphase and are not included in the daughter nuclei in telophase (Fenech 1993). Micronuclei containing chromosomal fragments may result from direct double-strand DNA breakage, conversion of single-strand breaks (SSB) into double-strand breaks (DBS) after cell replication, or inhibition of DNA synthesis (Mateuca *et al.*, 2006). The CBMN assay is considered as a standard assay for assessing chromosome instability and mitotic dysfunction (Mateuca *et al.*, 2006). Besides its capacity to detect micronuclei, the CBMN assay can also provide information of the proliferative capacity of cells. Scoring of micronuclei can be performed relatively easily because it is simpler, requires less training, and is less time consuming than other cytogenetic techniques, such as the chromosomal abberation (CA) assay (Norppa *et al.*, 2003) (Figure 1.1.B).

The CBMN assay consists of two culturing steps. The first involves the induction of cell division (of lymphocytes) using a T-cell mitogen (such as phytohemagglutinin). The second step involves culturing in the presence of cytochalasin-B, added 44 hours after the start of phytohemagglutinin-stimulation to stop dividing cells from undergoing cytokinesis. This process allows for mononucleated, binucleated, and polynucleated cells to be distinguished. Binucleated cells have completed one nuclear division during the *in vitro* culture and only binucleated cells are assessed for the presence of micronuclei (MN) (Fenech and Morley 1985, 1986).

There is variability in the baseline MN frequency within the human donor population due to influences such as age, gender, nutrition and lifestyle factors (e.g. smoking). For this reason, the CBMN assay has an average sensitivity limit of 0.2 - 0.3 Gy for ionizing radiation (Vral *et al.*, 1997; Touil *et al.*, 2002; He *et al.*, 2000; Streffer *et al.*, 1998; Thierens *et al.*, 1991; Huber *et al.*, 1983).

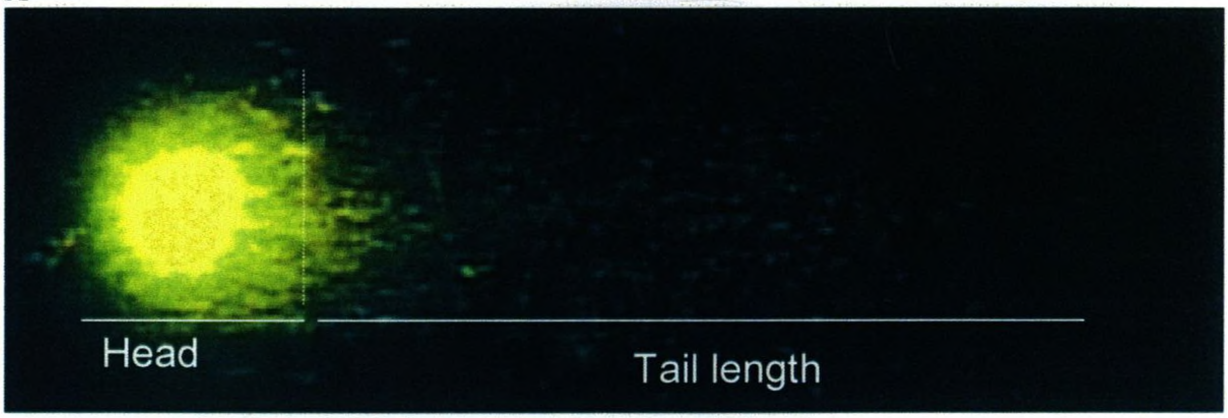
However, Fenech and Morley (1986) observed that it was possible to detect increases in MN induction following exposures as low as 0.05 Gy if pre-exposure controls were used.

### 1.4.3 Other cytogenetic assays

Two other cytogenetic assays commonly used in the study of potentially genotoxic agents are the chromosome aberration (CA) and sister exchange chromatid (SCE) assays. The CA assay is used to observe chromosomal aberrations in metaphase-arrested cells that have been fixed, spread on a microscope slide, and stained. Structural chromosomal aberrations result from direct DNA breakage, replication on a damaged DNA template, inhibition of DNA synthesis, and other mechanisms (Albertini *et al.*, 2000). Structural CAs have been detected at radiation doses of 0.5 Gy (Mateuca *et al.*, 2006).

The SCE assay measures SCEs that arise from the reciprocal exchange of DNA between two sister chromatids of a replicated chromosome. The frequency of SCEs in eukaryote cells is increased by exposure to genotoxic agents that induce DNA damage and are capable of interfering with DNA replication (Albertini *et al.*, 2000).

A




B

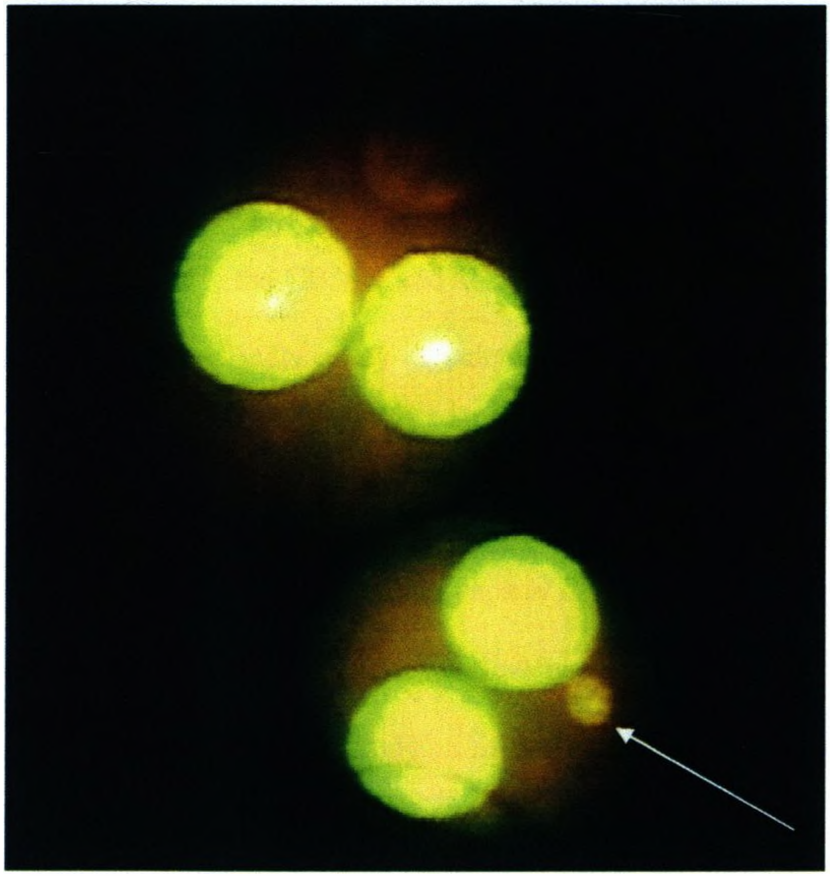


**Figure 1.1** DNA comet and binucleate cell. A: A DNA comet from a human lymphocyte. The nuclei acid was stained with SYBR Gold and visualized with the fluorescence microscope at 330x magnification. Intact DNA is in the head and fragmented DNA in the tail. B: Binucleated human lymphocyte containing one micronucleus (arrow). Acridine Orange stained cells were visualized with the fluorescence microscopy at 400x magnification. Nuclear material appears in yellow/green and cytoplasm.

SCE analysis has become accepted as a sensitive means of monitoring DNA damage in human populations. However, it has been observed that ionizing radiation is a poor inducer of SCE (Gundy *et al.*, 1984; Marin and Prescott, 1964; Gatti and Olivieri, 1973; Gibson and Prescott, 1972).

### 1.4.4 The best cytogenetic assays for DNA damage detection

Compared to other cytogenetic assays, there are several advantages to using the CBMN and alkaline comet assay for detection of DNA and clastogen damage. Firstly, in comparison with the CA and SCE assays, the scoring of MN and comets is simpler, requires less training, and is less time consuming (Mateuca *et al.*, 2006, Norppa *et al.*, 2003). Secondly, the CBMN and alkaline comet assay are sensitive to ionizing radiation exposure. The CBMN assay can detect a radiation dose of 0.2-0.3 (Vral *et al.*, 1997; Touil *et al.*, 2002; He *et al.*, 2000; Streffer *et al.*, 1998; Thierens *et al.*, 1991; Huber *et al.*, 1983) and the alkaline comet assay can detect DNA damage at doses of 0.05 to 5 Gy or more (McNamee *et al.*, 2000; Malyapa *et al.*, 1998; Vijayalaxmi *et al.*, 1992; 1993). For these reasons, the CBMN and alkaline comet assays are well chosen as the preferred genotoxicity assays in this study.

## 1.5 Literature review: ELF-EMF and DNA damage

In 2005, Vijayalaxmi and Obe published the review "Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency

electromagnetic radiation: a review and future research recommendations". Vijayalaxmi and Obe (2005) reviewed investigations between the years 1990-2003 that involved the genotoxic potential of exposure to ELF-EMF in animals, cultured rodent and human cells, and freshly collected human blood lymphocytes. In those studies observing genotoxic effects (not epigenetic) of ELF-EMF, there was an increase in DNA damage observed in 13 studies, no increase in DNA damage observed in 19 studies, and inconclusive effects in 8 studies. Of these studies, 5 reported no DNA damage from 60 Hz MF, while 3 observed increased DNA damage (Table 1.2). In the time period since (2004-present), 8 studies have evaluated the genotoxic effects of 50/60 Hz ELF-EMF. Three studies observed an increase in DNA damage, while 5 studies did not observe any increase in DNA damage (Table 1.3). As such, the issue of whether 60 Hz MF can cause DNA damage remains inconclusive.

### 1.5.1 1988-2003 studies regarding 60 Hz ELF-EMF

Due to limited human whole body ELF-EMF exposure studies, much of the health and safety exposure limits and recommendations have been based on animal and cellular studies. Studies performed by Lai and Singh (1997a, 1997b) and Singh and Lai (1998) have observed that rats exposed to a 60 Hz field at intensities of 0.1, 0.25 and 0.5 mT for 2 hours showed an increase in DNA damage and DNA crosslinks in brain cells of exposed animals. Similar effects were not observed in human lymphocytes or hamster ovary cells after *in vitro* exposure, nor in mouse brain cells after *in vivo ELF-EMF* exposure (Cho *et al.*, 2003; McNamee *et al.*, 2002; Skyberg *et al.*, 2001; Heredia-Rojas *et*

*al.*, 2001; Reese *et al.*, 1988). Human lymphocytes exposed to a 60 Hz field ranging between 0.8-2.0 mT for 24-72 hours did not show any evidence of increase in MN, SCE or DNA damage (Cho *et al.*, 2003; Skyberg *et al.*, 2001; Heredia-Rojas *et al.*, 2001). Brain cells of mice and hamster ovary cells exposed to the 60 Hz field at strengths between 0.1-2mT for 1-2 hours also did not show any evidence of increased DNA damage (McNamee *et al.*, 2002; Reese *et al.*, 1988).

### 1.5.2 2004-present studies regarding 50/60 Hz ELF-EMF

Three studies observed an increase in DNA damage after electromagnetic field exposure. Lai and Singh (2004) exposed rats to a whole body exposure of 60 Hz sinusoidal, 0.01 mT magnetic field for 24-48 hours and then studied DNA damage in rat brain cells using the comet assay. They observed that an exposure to 60 Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA damage. Moreover, by prolonging the exposure to 48 hours, the increase was larger. Winker *et al.*, (2005) and Wahab *et al.*, (2007) exposed human cells to a 50 Hz, 1 mT magnetic field, human diploid fibroblasts with a 5 min on and 5 min off for 2-24 hours and peripheral blood lymphocytes for 72 hours respectively. Cytotoxic damage was observed using the CBMN, CA (Winker et al., 2005) and SCE (Wahab *et al.*, 2007) assays. Winker *et al.*, (2005) observed that an intermittent exposure (5 mins on, 10 mins off) to a 50 Hz magnetic field caused a time-dependent increase in MN in cultured fibroblasts and that chromosomal aberrations were increased up to 10-fold above basal levels.

Summary Table 1.2

| Paper | Frequency (Hertz) | Field strength (milli-Tesla) | Exposure (hours) | Cells | Cytogenetic Assay | Effect | Proposed mechanism |
|---|---|---|---|---|---|---|---|
| Cho *et al.*, 2003 | 60 Hz | 0.8 mT | 24 hr | Human lymphocytes | MN and SCE | None | None |
| McNamee *et al.*, 2002 | 60 Hz | 1 mT | 2 hr | Whole body 10-day-old mice, brain cells | Comet assay | None | None |
| Skyberg *et al.*, 2001 | 60 Hz | 1.9 mT | unknown | Human whole body, lymphocytes | CA | None | None |
| Heredia-Rojas *et al.*, 2001 | 60 Hz | 1, 1.5, 2 mT | 72 hr | Human lymphocytes | SCE | None | None |
| Singh and Lai, 1998 | 60 Hz | 0.5 mT | 2 hr | Whole body rat, brain cells | Comet assay | Increase in DNA damage | DNA-protein and DNA-DNA crosslinks |
| Lai and Singh, 1997a | 60 Hz | 0.1, 0.25, 0.5 mT | 2 hr | Whole body rat, brain cells | Comet assay | Increase in DNA damage | Affect enzymatic processes in DNA repair |

| Paper | Frequency (Hertz) | Field strength (milli-Tesla) | Exposure (hours) | Cells | Cytogenetic Assay | Effect | Proposed mechanism |
|---|---|---|---|---|---|---|---|
| Lai and Singh, 1997b | 60 Hz | 0.5 mT | 2 hr | Whole body rat, brain cells | Comet assay | Increase in DNA damage | Effects of magnetic fields are mediated by free radicals |
| Reese *et al.*, 1988 | 60 Hz | 0.1, 2 mT | 1 hr | Chinese hamster ovary cells | Alkaline elution | None | None |

**Table 1.2** 60 Hz ELF-EMF from 1988-2003. MN: micronuclei; SCE: sister chromatid exchange; CA: chromosomal aberration. See text for details.

Summary Table 1.3

| Paper | Frequency (Hertz) | Field strength (milli-Tesla) | Exposure | Cells | Cytogenetic Assay | Effect | Proposed mechanism |
|---|---|---|---|---|---|---|---|
| Wahab et al., (2007) | 50 Hz | 1 mT | 72 hr | Human peripheral blood lymphocytes | SCE | Increase of SCE | Incidence of SCE caused by DNA crosslinks |
| McNamee et al., (2005) | 60 Hz | 0, 0.1, 1,2 mT | 2 hr | Whole body mouse, brain cells | Comet assay | None | None |
| Winker et al., (2005) | 50 Hz | 1 mT | 5 min field-on/10 min field-off, 2-24 hr | Human diploid fibroblasts | MN and CA | Increase in MN and chromo-somal aberrations | 1) Slow induction of DNA-repair processes 2) Initiate transcription, cause chain separation at specific DNA sequences |
| Luceri et al., (2005) | 50 Hz | 1, 10, 100 micro T | 18 hr | Human peripheral blood lymphocytes | Comet assay | None | None |

Summary Table 1.3

| Paper | Frequency (Hertz) | Field strength (milli-Tesla) | Exposure | Cells | Cytogenetic Assay | Effect | Proposed mechanism |
|---|---|---|---|---|---|---|---|
| Scarfi et al., (2005) | 50 Hz | 1 mT | 5min field-on/10 min field-off, 15,24 hr | Human diploid fibroblasts | MN assay, comet assay | None | None |
| Stronati et al., (2004) | 50 Hz | 1 mT | 2 hr | Human peripheral blood lymphocytes | SCE, CA, MN, comet assay | None | None |
| Testa et al., (2004) | 50 Hz | 1 mT | 48 hr | Human peripheral blood cells | Comet assay, SCE, CA, MN | None | None |
| Lai and Singh (2004) | 60 Hz | 0.01 mT | 24 or 48 hr | Whole body rat, brain cells | Comet assay | Increase in DNA damage | Free radicals |

**Table 1.3** 50/60 Hz summary table 2004-present. MN: micronuclei; SCE: sister chromatid exchange; CA: chromosomal abberation. See text for details.

The square continuous waveform used by Wahab *et al.*, (2007) increased the number of SCE in dividing human peripheral blood lymphocytes.

Between the years of 2004-2005, five studies found no increase in DNA damage. Stronati *et al.*, (2004), Testa *et al.*, (2004), and Scarfi *et al.*, (2005) exposed human cells to 50 Hz, 1 mT magnetic field. After a 2 hour exposure (Stronati *et al.*, 2004) and a 48 hour exposure (Testa *et al.*, (2004) found no increase in SCE, CA, MN or DNA damage in the comet assay in human peripheral lymphocytes. Moreover, an intermittent field exposure (5 minutes on, 5 minutes off) with a maximum of 24 hours did not show any effect in human diploid fibroblasts after using the comet and CBMN assays (Scarfi *et al.*, 2005). Luceri *et al.*, (2005) exposed human peripheral blood lymphocytes to a 50 Hz frequency at field strengths of 1, 10, and 100 μT. They observed no increase in DNA damage using the alkaline comet assay. In 2005, McNamee et al. exposed mice and immature mice to a 60 Hz, 0.1, 1, or 2 mT magnetic fields for 2 hours. Brain cells were observed for DNA damage by the comet assay at 0, 2 and 4 hours after magnetic field exposure. McNamee *et al.*, (2005) observed no significant increase in DNA damage in brain cells from any animal models at any time or exposure level after exposure.

### 1.5.3 Hypotheses for ELF-EMF DNA damage

As non-ionizing energy, 60 Hz ELF-EMF does not have sufficient energy to break the chemical bonds that make up DNA (Valberg et al., 1997). For this reason, the previously mentioned studies that have observed DNA damage after ELF-EMF exposure in human, animal, or cells have suggested the following hypotheses.

It has been proposed that the induction of DNA-repair processes is slowed when cells are exposed intermittently to ELF-EMF (Winker *et al.*, 2005; Ivancsits *et al.*, 2002). Ivancsits et al. (2002) observed that at an intermittence exposure of 5 minutes on and 10 minutes off to ELF-EMF produced the highest amount of damage whereas longer periods of rest (over 15 minutes) had no significant effect compared to controls.

It was hypothesized that extended off-times allowed the cell to remove ELF-induced damage by DNA repair mechanisms. Moreover, Lai and Singh (1997b) hypothesize that a 60 Hz magnetic field could affect the enzymatic processes of DNA repair by affecting the activity of poly-ADP-ribose polymerization, an enzymatic activity involved in DNA repair (Phillips *et al.*, 2005); thus, leading to an accumulation of DNA damage.

It has also been suggested that EMF can initiate transcription and then cause chain separation at specific DNA sequences (Winker *et al.*, 2005; Ivancsits *et al.*, 2002). ELF-EMF exposure may initiate transcription by interacting with moving electrons in DNA based on repulsive (Lorentz) forces, which may then cause chain separation at specific DNA sequences (nCTCTn) (Blank and Goodman, 2000; Winker *et al.*, 2005; Ivancsits *et al.*, 2002). Wahab *et al.*, (2007) and Lai and Singh (1998) propose that ELF-EMF can induce DNA to DNA or DNA to protein crosslinks. Wahab *et al.*, (2007) observed that after an ELF-EMF exposure, there was an induction of SCE in human lymphocytes, and Lai and Singh (1998) observed an increase of DNA crosslinks in rat brain cells after an exposure to a 60 Hz MF. Wahab *et al.*, (2007) compared the cross-linking ability of ELF-EMF to a known crosslink inducing agent, mitomycin C, and observed that the pattern of effects was similar; thus, ELF-EMF damage could result in DNA crosslinks.

Finally, it has been suggested that the effects of MF are mediated by free radicals (Lai and Singh 2004). Data from their experiments suggest that magnetic-field-induced DNA strand breaks are caused by an iron-mediated free radical process, probably via the Fenton reaction, which converts hydrogen peroxide to the more potent and toxic hydroxy radical.

Since magnetic fields at the power-line frequencies do not have sufficient energy to break chemical bonds that would result in DNA damage in cells, scientists have come up with other hypotheses. In 1997, Valberg *et al.*, published "Can low-level 50/60 Hz electric and magnetic fields cause biological effects?". In this review, Valberg *et al.*, (1997) rejects the theories that MFs can cause biological effects due to forces on ions and charged cell proteins or by the modulation of free radicals.

EMF-accelerated ions have an eV/bond energy of $10^{-8}$ whereas a hydrogen bond, single covalent bond, double covalent bond and triple covalent bond have eV/bond energies of 0.1, 3.6, 5.1, and 9.5, respectively. Therefore, these EMF-accelerated ions do not have the necessary energies to modify any bonds. Moreover, the biologically generated forces between cell proteins and structures appear to be far greater than any force generated by EMFs. A cell membrane field of approximately 14 V/m is produced by EMFs whereas a naturally occurring membrane field of $10^{7}$ V/m exists in the cell (Valberg *et al.*, 1997). Finally, the effects of even very small magnetic fields on chemical reactions involving free radicals are well established and reproducible in theory and in *in vitro* studies. However, confirming that ELF-EMFs have an effect in biological and biochemical systems remains unresolved (Brocklehurst and McLauchlan, 1996).

At present, there is no consistent or independently reproducible evidence that ELF-EMF induce DNA damage and no mechanisms of interaction is supported by empirical evidence.

### 1.5.4 Current thesis hypothesis

The current project is a first-of-its-kind collaborative pilot investigation of the extent of primary DNA and clastogenetic damage in peripheral blood lymphocytes of healthy human volunteers exposed for 4 hours to an acute whole-body 60 Hz, 200 μT ELF-EMF. If magnetic fields can result in adverse health effects, it is hypothesized that a 60 Hz ELF-EMF exposure will increase DNA damage and MN induction in human lymphocytes.

## 1.6 References

Ahlbom A, Day N, Feychting M, Roman E, Skinner J, Dockerty J, Linet M, McBride M, Michaelis J, Olsen JH, Tynes T, Verkasalo PK. 2000. A pooled analysis of magnetic fields and childhood leukemia. Br J Cancer 83: 692-698.

Ahlbom IC, Cardis E, Green A, Linet M, Savitz D, Swerdlow A, ICNIRP (International Commission for Non-Ionizing Radiation Protection) Standing Committee on Epidemiology). 2001. Review of the epidemiologic literature on EMF and Health. Environ Health Perspect 109: 911-933.

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Aitio A. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res: 111-172.

Ames BN. 1973. Carcinogens are mutagens: their detection and classification. Environ Health Perspect 6: 115-118.

Auvinen A, Linet MS, Hatch EE, Kleinerman RA, Robison LL, Kaune WT, Misakian M, Niwa S, Wacholder S, Tarone RE. 2000. Extremely low-frequency magnetic fields and childhood acute lymphoblastic leukemia: an exploratory analysis of alternative exposure metrics. Am J Epidemiol 152: 20-31.

Blank M, Goodman R. 2000. Stimulation of the stress response by low frequency electromagnetic fields: possibility of direct interaction with DNA, IEEE Trans Plasma Sci 28: 168-172.

Cho YH, Chung HW. 2003. The effect of extremely low frequency electromagnetic fields (ELF-EMF) on the frequency of micronuclei and sister chromatid exchange in human lymphocytes induced by benzo(a)pyrene. Toxicol Lett 143: 37-44.

Choleris E, Del Seppia C, Thomas AW, Luschi P, Chione S, Moran GR, Prato FS. 2001. Shielding, but not zeroing of the ambient magnetic field reduces stress-induced analgesia in mice. Proc R Soc Lond B 269: 193-201.

Ciccone G, Mirabelli D, Leis A, Gavarotti P, Rege-Cambrin G, Davico L, Vineis P. 1993. Myeloid leukemias and myelodisplastic syndromes: Chemical exposure, histologic subtype and cytogenetics in a case-control study. Cancer Genet Cytogenet 68: 135-139.

Cohen SM, Ellwein LB. 1991. Genetic errors, cell proliferation, and carcinogenesis. Cancer Res 51: 6493-6505.

Collins AR, Oscoz AA, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, Smith CC, Stetina R. 2008. The comet assay: topical issues. Mutagenesis 2008 Feb 17 [Epub ahead of print].

Dhawan A, Bajpayee M, Parmar D. 2008. Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol Toxicol 2008 Apr 22 [Epub ahead of print].

Feinendegen LE, Pollycove M, Neumann RD. 2007. Whole-body response to low-level radiation exposure: New concepts in mammalian radiobiology. Exp Hematol 35: 37-46.

Fenech M and Morley AA. 1985. Measurement of micronuclei in lymphocytes. Mut Res 147: 29-36.

Fenech M and Morley AA. 1986. Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low does X-irradiation. Mut Res 161: 193-198.

Fenech M. 1993. The cytokinesis-block miconucleus technique: a detailed description of the method and its application to genotoxicity studies in human population. Mut Res 285: 35-44.

Gatti M and Olivieri G. 1973. The effect of X-rays on labeling pattern of $M_1$ and $M_2$ chromosomes in Chinese hamster cells. Mutat Res 178: 101-112.

Gibson DA and Prescott DM. 1972. Induction of sister chromatid exchanges in chromosome exchanges in chromosome of rat kangaroo cells bby tritium incorporated into DNA. Exp Cell Res 74: 397-402.

Gundy S, Varga L, Bender MA. 1984. Sister chromatid exchange frequency in human lymphocytes exposed to ionizing radiation in vivo and in vitro. Radiat Res 100: 47-54.

Hall EJ and Giaccia AJ. 2006. Radiobiology for the radiologist, 6th ed. Lippincott Williams & Wilkins, Philadelphia. 546 pages.

He JL, Chen WL, Jin LF, Jin HY. 2000. Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxic effects of X-ray radiation. Mutat Res 469: 223-231.

Health Canada. 2004. Electric and magnetic fields at extremely low frequencies. It's your health. http://www.hc-sc.gc.ca/iyh-vsv/environ/magnet_e.html Accessed May 21, 2008.

Hellman B, Vaghef H, Boström B. 1995. The concepts of tail moment and tail inertia in the single cell gel electrophoresis assay. Mutat Res 336: 123-131.

Heredia-Rojas JA, Rodriguez-De La Fuente OA, Velazco-Campos RM, Leal-Garza CH, Rodriguez-Flores LE, Fuente-Cortez B. 2001. Cytological effects of 60 Hz magnetic fields on human lymphocytes in vitro: sister-chromatid exchanges, cell kinetics and mitotic rate. Bioelectromagnetics 14: 545-551.

Huber R, Streng S, Bauchinger M. 1983. The suitability of the human lymphocyte micronucleus assay system for biological dosimetry. Mutat Res 111: 185-193.

IARC (International Agency for Research on Cancer). 2002. Non-ionizing radiation. Part I. Static and Extremely Low-frequency Electric and Magnetic Fields. IARC Monograph Evaluation of Carcinogenic Risk to Humans 80: 1-395.

IEEE 1159-1995. IEEE Recommended Practice for Monitoring Electric Power Quality Institute of Electrical and Electronics Engineers. 01-May-1995. 70 pages.

Ivancsits S, Diem E, Pilger A, Rüdiger HW, Jahn O. 2002. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res 519: 1-13.

Jammet HP, Bernhardt JH, Bosnjakovic BFM, Czerski P, Grandolfo M, harder D, Knave B, Marshall J, Repacholi MH, Sliney DH, Stolwijk DH. 1990. Interim guidelines on limits of exposure to 50/60 Hz electric and magnetic fields. Health Phy 58: 113-122.

Khalil AM, Quasem W, Amoura F. 1993. Cytogenetic changes in human lymphocytes from workers occupationally exposed to high-voltage electromagnetic fields. Electro Magnetobiol 12: 17-26.

Kheifets LI, Gilbert ES, Sussman SS, Guénel P, Sahl JD, Savitz DA, Thériault G. 1999. Comparative analyses of the studies of magnetic fields and cancer in electric utility workers: studies from France, Canada, and the United States. Occup Environ Med 56: 567-574.

Lai H, Singh NP. 1997a. Melatonin and N-tert-butyl-a-phenyl-nitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J. Pineal Res 22: 152-162.

Lai H, Singh NP. 1997b. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics 18: 156-165.

Lai H, Singh NP. 2004. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect 112: 687-694.

Lightfoot T. 2005. Aetiology of childhood leukemia. Bioelectromagnetics (suppl 7): S5-S11.

Luceri C, De Filippo C, Giovannelli L, Blangiardo M, Cavalieri D, Aglietti F, Pampaloni M, Andreuccetti D, Pieri L, Bambi F, Biggeri A, Dolara P. 2005. Extremely low-frequency electromagnetic fields do not affect DNA damage and gene expression profiles of yeast and human lymphocytes. Radiat Res 164: 277-285.

Malyapa RS, Bi C, Ahern EW, Roti Roti JL. 1998. Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation. Radiat Res 149: 396-400.

Marin G and Prescott DM. 1964. The frequency of sister chromatid exchanges following exposures to varying doses of $^{3}$H-thymidine or X-rays. J Cell Biol 21: 159-167.

Mateuca R, Lombaert N, Aka PV, Decordier I, Kirsch-Volders M. 2006. Chromosomal changes: induction, detection methods and applicability in human biomonitoring. Biochimie 88: 1515-1531.

McNamee JP, McLean JR, Ferrarotto CL, Bellier PV. 2000. Comet assay: rapid processing of multiple samples. Mutat Res 466: 63-69.

McNamee JP, Bellier PV, McLean JR, Marro L, Gajda GB, Thansandote A. 2002. DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field. Mutat Res 513: 121-133.

McNamee JP, Bellier PV, Chauhan V, Gajda GB, Lemay E, Thansandote A. 2005. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. Radiat Res 164: 791-797.

Miyakoshi J, Yoshida M, Shibuya K, Hiraoka M. 2000. Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks. J. Radiat Res 41: 292-302.

Norppa H, Falck GC. 2003. What do human micronuclei contain? Mutagenesis 18: 221-223.

Ostling O, Johanson KJ. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun 123: 291-298.

Phillips JL, Campell-Beachler M, Ishida-Jones T, Haggren W, Adey WR. 1995. Exposure of PC12 cells to a 1 G sinusoidal magnetic field at 60 Hz: Effect on the activity of the DNA repair enzyme poly(ADP-ribose) polymerase. Paper presented at the Annual Review of Research on Biological Effects of Electric and Magnetic Fields from the Generation, Delivery and Use of Electricity Palm Springs, CA, November 12–16, 1995.

Poole C and Ozonoff D. 1996. Magnetic fields and childhood cancers: an investigation of dose response analyses. IEEE Eng Med Biol 15: 41-49.

Portier CJ and Wolfe MS, Eds. 1998. Assessment of health effects from exposure to power-line frequency electric and magnetic fields. Working group report, NIEHS, 523 pps.

Reese JA, Jostes RF, Frazier ME. 1988. Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks. Bioelectromagnetics 9: 237-247.

Savitz DA, Cai J, van Wijngaarden E, Loomis D, Mihlan G, Dufort V, Kleckner RC, Nylander-French L, Kromhout H, Zhou H. 2000. Case-cohort analysis of brain cancer and leukemia in electric utility workers using a refined magnetic field job-exposure matrix. Am J Ind Med 38: 417-425.

Scarfi MR, Sannino A, Perrotta A, Sarti M, Mesirca P, Bersani F. 2005. Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study. Radiat Res 164: 270-276.

Shupak NM, Prato FS, Thomas AW. 2004. Human exposure to a specific pulsed magnetic field: effects on thermal sensory and pain thresholds. Neurosci Lett 363: 157-162.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175: 184-191.

Singh N, Lai H. 1998. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutat Res 400: 313-320.

Skyberg K, Hansteen IL, Vistnes AI. 2001. Chromosomal aberrations in lymphocytes of employees in transformer and generator production exposed to electromagnetic fields and mineral oil. Bioelectromagnetics 22: 150-160.

Skyberg K, Hansteen IL, Vistnes AI. 1993. Chromosome aberrations in lymphocytes of high-voltage laboratory cable splicers exposed to electromagnetic fields. Scand J Work Env Health 19: 29-34.

Streffer C, Müller WU, Kryscio A, Böcker W. 1998. Micronuclei-biological indicator for retrospective dosimetry after exposure to ionizing radiation. Mutat Res 404: 101-105.

Stronati L, Testa A, Villani P, Marino C, Lovisolo GA, Conti D, Russo F, Fresegna AM, Cordelli E. 2004. Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analysis. Bioelectromagnetics 25: 41-48.

Tennant RW, Zeiger E. 1993. Genetic toxicology: current status of methods of carcinogen identification. Environ Health Perspect 100: 307-15. Review

Testa A, Cordelli E, Stronati L, Mariono C, Lovisolo GA, Fresegna AM, Conti D, Vallani P. 2004. Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood lymphocytes using different cytogenetic assays. Bioelectromagnetics 25: 613-619.

Thierens H, Vral A, de Ridder L. 1991. Biological dosimetry using the micronucleus assay for lymphocytes: interindividual differences in dose response. Health Phys 61: 623-630.

Thomas AW, Drost DJ, Prato FS. 2001a. Human subjects exposed to a specific pulsed (200 microT) magnetic field: effects on normal standing balance. Neurosci Lett 297: 121-124.

Thomas AW, White KP, Drost DJ, Cook CM, Prato FS. 2001b. A comparison of rheumatoid arthritis and fibromyalgia patients and healthy controls exposed to a pulsed (200 microT) magnetic field: effects on normal standing balance. Neurosci Lett 309: 17-20.

Touil N, Aka PV, Buchet JP, Thierens H, Kirsch-Volders M. 2002. Assessment of genotoxic effects related to chronic low level exposure to ionizing radiation using biomarkers for DNA damage and repair. Mutagenesis 17: 223-232.

Valberg PA, Kavet R, Rafferty CN. 1997. Can low-level 50/60-Hz electric and magnetic fields cause biological effects. Radiat Res 148: 2-21.

Valjus J, Norppa H, Jarventaus H, Sorsa M, Nykyri E, Salamaa S, Jarvinen P, Kajander J. 1993. Analysis of chromosomal aberrations, sister chromatid exchanges and micronuclei among power linesmen with long-term exposure to 50-Hz electromagnetic fields. Radiat Env Biophy 32: 325-336.

Vijayalaxmi, Tice RR, Struss GHS. 1992. Assessment of radiation-induced DNA damage in human blood lymphocytes using the single-cell gel electrophoresis technique. Mut Res 271: 243-252.

Vijayalaxmi, Struss GHS, Tice RR. 1993. An analysis of γ-ray-induced DNA damage in human blood leukocytes, lymphocytes and granulocytes. Mut Res 292: 123-128.

Vijayalaxmi and Obe G. 2005. Controversial cytogenetic observations in mammalian cells exposed to extremely low frequency electromagnetic radiation. A review and future research. Bioelectromagnetics 26: 412-430.

Villeneuve PJ, Agnew DA, Miller AB, Corey PN, Purdham JT. 2000a. Leukemia in electric utility workers: the evaluation of alternative indices of exposure to 60 Hz electricand magnetic fields. Am J Ind Med 37: 607-617.

Villeneuve PJ, Agnew DA, Miller AB, Corey PN. 2000b. Non-Hodgkin's lymphoma among electric utility workers in Ontario: the evaluation of alternate indices of exposure to 60 Hz electric and magnetic fields. Occup Environ Med 57: 249-257.

Vral A, Thierens H, De Ridder L. 1997. In vitro micronucleus-centromere assay to detect radiation-damage induced by low doses in human lymphocytes. Int J Radiat Biol 71: 61-68.

Wahab MA, Podd JV, Rapley BI, Rowland RE. 2007. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 550 Hz magnetic fields. Bioelectromagnetics 28: 281-288.

Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. 1979. Am J Epidemiol 109: 273-284.

Winker, R, Ivancsits S, Pilger A, Adlkofer F, Rudiger HW. 2005. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. Mutat Res 585: 43-49.

World Health Organization (WHO). 1998. Electromagnetic fields and public health: extremely low frequency (ELF). Fact sheet No. 205. http://www.who.int/mediacentre/factsheets/fs205/en/ Accessed April 15th 2008

# CHAPTER 2: MAGNETIC FIELDS AND DNA DAMAGE

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**Assessment of genetic damage in peripheral blood lymphocytes of human volunteers exposed (whole-body) to a 200 μT, 60 Hz magnetic field**

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## 2.1 Introduction

The transmission, distribution and use of electricity for a variety of commercial and residential applications has resulted in chronic exposure of much of the world's population to an ever-increasing level of extremely low frequency electric and magnetic fields (ELF-EMF). Despite numerous human epidemiological and laboratory studies, the association between chronic exposure to ELF-EMF and the occurrence of long-term adverse human health effects remains controversial. One area of intense research involves investigating the potential of ELF-EMF to cause genotoxic damage in mammalian somatic cells (Vijayalaxmi and Obe, 2005).

While most *in vitro* studies have demonstrated no evidence of 60 Hz ELF-EMF-induced genotoxicity (Miyakoshi *et al.*, 2000; Heredia-Rojas *et al.*, 2001; Cho and Chung, 2003), a small number of animal studies have demonstrated that 60 Hz ELF-EMF may induce DNA damage in the rodent brain (Lai and Singh, 1997a; 1997b; Singh and

Lai, 1998). Although a few human ELF-EMF whole-body exposure studies have been conducted, these studies have been limited to individuals exposed occupationally to ELF-EMF where precise exposure conditions were unknown (Ciccone *et al.*, 1993; Khalil *et al.*, 1993; Skyberg *et al.*, 1993, 2001; Valjus *et al.*, 1993). Thus, whole body human ELF-EMF exposure studies are important for providing human health and safety information.

The current study was undertaken to investigate whether an acute, whole-body exposure of 200 μT, 60 Hz magnetic field was capable of inducing detectable levels of either primary DNA damage or clastogenic damage (chromosomal breakage or disruption) in peripheral human blood lymphocytes from healthy adult volunteers. Unlike previous human studies, limited to occupational exposures, the current study investigated human whole-body exposure in a *controlled* laboratory setting. While the magnetic flux density tested in this study is relatively high compared to daily exposures encountered by the general public, such fields are encountered in some occupational settings and have been reported to elicit genotoxic effects in some rodent studies (Lai and Singh, 1997a; 1997b; Singh and Lai 1998).

## 2.2 Materials and Methods

### 2.2.1 Donors

A total of 30 (15 males and 15 females) non-smoking, non-pregnant human volunteers (aged 20-45) were recruited over a 10 month period, from a university population through poster advertisement in accordance with a protocol approved by the

Heath Sciences Research and Ethics Board (HSREB) at The University of Western Ontario. Consent forms and questionnaires were obtained from each donor to capture information about age, gender and general health and well-being. Subjects were blinded and randomly assigned into one of two groups: 1) 4 h exposure to a 200 μT, 60 Hz magnetic field or, 2) 4 h exposure to sham conditions. All subjects were exposed to sham (no field) or magnetic field conditions in the human exposure chamber located at the Lawson Health Research Institute at St. Joseph's Health Care in London, Ontario. Blood samples were collected from each subject both immediately prior to and immediately following the 4 h exposure period by venipuncture of the cubital or cephalic vein into 6 mL sodium heparin tubes (BD Vacutainer®). For each subject, an aliquot of the pre-exposure blood was used as a negative-control, while a second aliquot was exposed to 1.5 Gy $^{60}$Co γ–irradiation for use as a positive-control. Blood was collected from an additional 3 male and 3 female subjects for generating γ-radiation ($Co^{60}$ γ-irradiation, dose rate ~3 Gy/min) dose-response curves for the alkaline comet assay and cytokinesis-block micronucleus (CBMN) assays. Whole blood samples were diluted 1:9 with pre-warmed (37°C) culture medium [RPMI 1640 containing 10% Fetal Bovine Serum and 2 mM l-glutamine (Invitrogen), 1% penicillin/streptomycin (Sigma)]. From each sample, 50 μL aliquots were removed for the alkaline comet assay and cell viability assessment (Strauss *et al.*, 1991). The remaining blood was used for the CBMN assay.

### 2.2.2 Exposure system and condition

The human magnetic field exposure chamber has been previously described (Thomas *et al.* (2001a, 2001b), Shupak *et al.* 2004). Briefly, it consisted of three orthogonal square coil pairs: (i) 2 m square x 1 m separation, east-west horizontal axis, (ii) 1.75 m x 0.875 m separation, north-south horizontal axis, and (iii) 1.5 m x 0.75 m separation, vertical axis. In this study, only the vertical axis coil pair was powered. The 60 Hz sinusoidal waveform signal was derived from the AC power lines regularly used for electricity transmission and distribution in North America. The waveform signal contained various random noise and distortions typical of the AC power grid (IEEE Standard, 1159-1995). The signal was obtained from a step down transformer connected to the hydro mains and buffered by a low frequency operation amplifier (op-amp) with adjustable gain, to drive the input of a Techron (model 7780E6) power amplifier. The output of this amplifier drove the Helmholtz like coils to obtain a flux density of 200 μT within the human exposure chamber. The ambient field in the exposure system was 0.08 μT.

During the 4 h magnetic field or sham exposure period, each volunteer was seated in a padded reclining chair and entertainment was provided on a 24" LCD TV monitor with audio. The controls were located away from the exposure chamber and lighting was provided by 12 offset overhead dimmable fluorescent track lights. The exposure was continuously monitored using a Bartington 3D Fluxgate magnetometer probe and rack-mounted signal-conditioning unit (Bartington Instrumental Ltd.,Oxford, England) via a digital to analog converter.

### 2.2.3 Cell viability data

Cell viability was assessed for each of the treatment groups (negative-control, positive-control, and EMF/sham post-exposure) for each donor using the dual-stain viability assay (Strauss, 1991). No differences in viability were identified among the treatment groups for any of the donors, as cell viability remained >96% for all samples (please see data in Appendix B).

### 2.2.4 Alkaline Comet Assay

Due to the ability of visible light to induce damage in naked DNA, all steps of the comet assay were performed in subdued light. A 50 µL aliquot taken from the cell culture was gently mixed with 500 µL of pre-warmed (37°C) 1% Low Melting Point (LMP) agarose (Fisher Scientific) prepared in $Ca^{+}/Mg^{2+}$ -free PBS. A 75 µL aliquot of the cell suspension was then cast onto each well of a 2-well Trevigen Cometslide™ (Cedarlane Laboratories, Burlington, ON) and allowed to solidify for 10 minutes. The remainder of the alkaline comet assay was conducted as previously described (McNamee *et al.*, 2005). Briefly, the slides were placed into 80 mL of cold lysis buffer (2.5M NaCl, 100mM tetra-sodium EDTA, 10mM Tris base, 1% N-lauryl sarcosine, pH 10.0, supplemented immediately prior to use with 1% v/v Triton-X100) and maintained at 4°C in the dark overnight. The next day, the slides were rinsed with distilled water, followed by a 30-min equilibration in 80 mL alkaline unwinding/electrophoresis buffer (0.3M NaOH, 10 mM tetra-sodium EDTA, 0.1 w/v 8-hydroxyquinoline, and 2% (v/v) DMSO;

pH 13.1). The slides were then electrophoresed in a submarine gel electrophoresis unit (Wide Mini Sub Cell, Bio-Rad, Mississauga, Ontario, Canada) containing 520 mL fresh alkaline unwinding/electrophoresis buffer at 1 V/cm for 20 min. After electrophoresis, the slides were transferred to neutralizing buffer (80 mL of 1 M ammonium acetate solution; pH 7.0) for 30 min. Finally, the gels were submersed in 100% ethanol for 2 hours and then allowed to air-dry overnight.

For analysis, slides were stained with a 1/10000x dilution of SYBR Gold solution (Molecular Probes, Invitrogen, Burlington, Ontario, Canada) for 10 min and then rinsed with distilled water. From each culture, a total of 50 cells were analyzed to determine the extent of DNA damage. The comet head and tail regions were defined manually at x330 magnification on an Olympus BX-60 fluorescence microscope using a 'NB' filter cube, a programmable Hitachi KP-D581 digital camera and the Alkomet v3.1 image analysis system (McNamee *et al.*, 2000). Each comet was analyzed to capture the following DNA damage parameters: Tail Ratio (TR, % DNA in tail), calculated as the fluorescence intensity in the comet tail region relative to that in the entire comet; Tail Moment (TM), calculated as the TR multiplied by the distance (μm) between the centers of gravity of the head and tail regions; and, comet Tail Length (TL), calculated as the distance from the leading edge of the comet head to the leading edge of the tail (Hellman *et al.*, 1995; McNamee *et al.*, 2002).

### 2.2.5 Cytokinesis-blocked micronucleus (CBMN) assay

For each sample, a 10 mL aliquot of the blood culture was mitogen-stimulated with 1% phytohemagglutinin (PHA) (Invitrogen, Burlington, Ontario, Canada), then transferred to Corning 25 $cm^2$ vented rectangular cell culture flasks and incubated (37°C, 95% air/5% $CO_2$) for 44 hours in a Mu-metal enclosure that attenuated magnetic fields originating from the incubator in the 1-100 Hz range by a factor of up to 100 (Choleris *et al.*, 2001). Cytochalaisin-B (Sigma, Oakville, Ontario, Canada) was then added at a final concentration of 4 μg/mL and the lymphocytes were cultured for an additional 28 hours. The cell suspension was then transferred into 15 mL conical tubes and pelleted by centrifugation at 200 x g for 8 minutes. The supernatant was removed and the pellet was re-suspended in 10 mL of a hypotonic (75 mM KCl) solution for 5 minutes at room temperature. This step was followed by the addition of 2 mL of a 5:1 methanol (Sigma, Oakville, Ontario, Canada):glacial acetic acid (Fisher Scientific, Ottawa, Ontario, Canada) fixative and incubated for 10 minutes. The cells were again pelleted at 200 x g for 8 minutes and the supernatant was removed and the cells were re-suspended in 10 mL of fixative and allowed to stand for 10 min. The process of adding 10 mL of the fixing agent and pelleting the cells was repeated a total of 3 times to remove cellular debris. Finally, cells were re-suspended in fixative with the addition of 250 μL of 37% (v/v) formaldehyde, pelleted at 200 x g for 8 minutes and then re-suspended in 100 μL fixative to achieve the desired cell concentration for slide preparation. The cell suspension was dropped onto ice-cold slides, washed with fixative and then dried immediately over a 60°C steam bath. Slides were allowed to dry overnight on a warming plate (37°C), and

then they were stained with 10 μg/mL Acridine Orange (Sigma, Oakville, Ontario, Canada) on the day of analysis. The slides were visualized under fluorescence using a 40X objective (UPlanSApo, Olympus) and a 'NB' filter cube on a Olympus BX-60 microscope.

Micronuclei (MN) were scored using similar criteria described by Fenech *et al.*, (2003). Briefly, for each sample, a total of 500 consecutive cells were examined to determine the frequency of binucleate (BN) cells, and a total of 1000 BN cells were analyzed to record the incidence of cells with one MN (BN1MN), two MN (BN2MN) or three and more MN (BN≥3MN). The slides were blinded prior to scoring and the results were not decoded until all samples were analyzed. Two independent scorers evaluated 500 BNC for each sample in a blinded fashion. Since there were no statistical differences in scoring between individuals, the data from the two scorers were combined to achieve a count for the number of MN/1000 BN cells (BNC).

### 2.2.6 Statistical analysis

Statistical Analysis System (SAS 2006) Version 9.1 for Windows was used for all analyses. The data from the comets were analyzed using a randomized complete block design (RCBD) ANOVA with volunteers being treated as the block variable. An RCBD ANOVA was conducted on the increasing gamma-radiation dose-response data for each of the variables tested. If the ANOVA was significant ($p<0.05$), then Dunnett's multiple comparison test was performed to compare each radiation dose (0.1, 0.3, 0.5, 1.0, 1.5 Gy) to the control (0.0 Gy). A second RCBD ANOVA was performed on the magnetic field-

exposed and sham-exposed samples for the negative-control (pre-exposure), positive-control (γ-irradiation) and post-exposure data. If the ANOVA was significant, then Tukey's multiple comparison test was applied. This comparison was to ensure that the observations made in negative-control (pre-exposure) samples from magnetic-field exposed and sham-exposed individuals were not significantly different from each other but significantly differed from their positive controls exposed to 1.5 Gy γ-radiation. Assumptions for the above tests included normality and constant variance across treatment groups. These assumptions were tested using the Anderson-Darling test for normality and Bartlett's likelihood ratio test for homogeneity of variances. When the assumptions were not validated then the tests were considered invalid. If the assumptions were not satisfied then the natural log transformation was applied to the variables in question and the assumptions were retested.

The data obtained from CBMN analysis were analyzed using a Poisson regression model with volunteers being treated as the block variable in the randomized complete block design. A Poisson regression model was used on the data obtained from increasing γ-radiation dose-response. If the effect of γ-radiation dose-response was significant, then the differences between all γ-radiation dose levels and pre-exposure samples were tested using a Bonferroni corrected α-level, to ensure that the overall type I error from multiple comparisons was less than or equal to 0.05. A second Poisson regression was fit to the sham/magnetic field (post-exposure) group and both the negative-control (pre-exposure) and positive-control (γ-irradiation) groups. This comparison was to ensure that the results in negative- and post-exposure samples were significantly different from the positive control samples exposed to 1.5 Gy γ-radiation. Lack of fit tests for the Poisson

regression models were also conducted using Pearson Chi-square Deviance test. If under-/over- dispersion was present in the data then the model was adjusted by using the Pearson chi square scalar analysis.

To compare the magnetic field-exposed and sham-exposed groups, it was first necessary to correct the data by the baseline levels for each of the variables (TR, TM, TL, and MN). This was achieved by the subtraction of the negative-control (pre-exposure) baseline level of each volunteer from their corresponding post-exposure level, and then dividing by the pre-exposure value. The magnetic field-exposed and sham-exposed groups were then compared using a one-way ANOVA. Assumptions of normality and equal variance were verified using Anderson Darling test and Bartlett's test for homogeneity of variance.

## 2.3 Results

### 2.3.1 γ-irradiation dose-response

The alkaline comet assay and CBMN were first standardized using the blood samples exposed to increasing doses of γ-radiation. The data presented in Figures 2.1a-c indicate the typical radiation dose-dependent increase in TL, TR and TM, respectively. The overall F test for treatment level from the RCBD ANOVA was significant for TL ($F_{(5,25)}$=27.32, $p<0.0001$), TR ($F_{(5,25)}$=18.45, $p<0.0001$), and TM ($F_{(5,25)}$=28.61, $p<0.0001$). All of these indices were significantly elevated when the cells were exposed to 0.5 Gy (TL ($p<0.0026$), TR ($p<0.0055$) and TM (0.0001)).

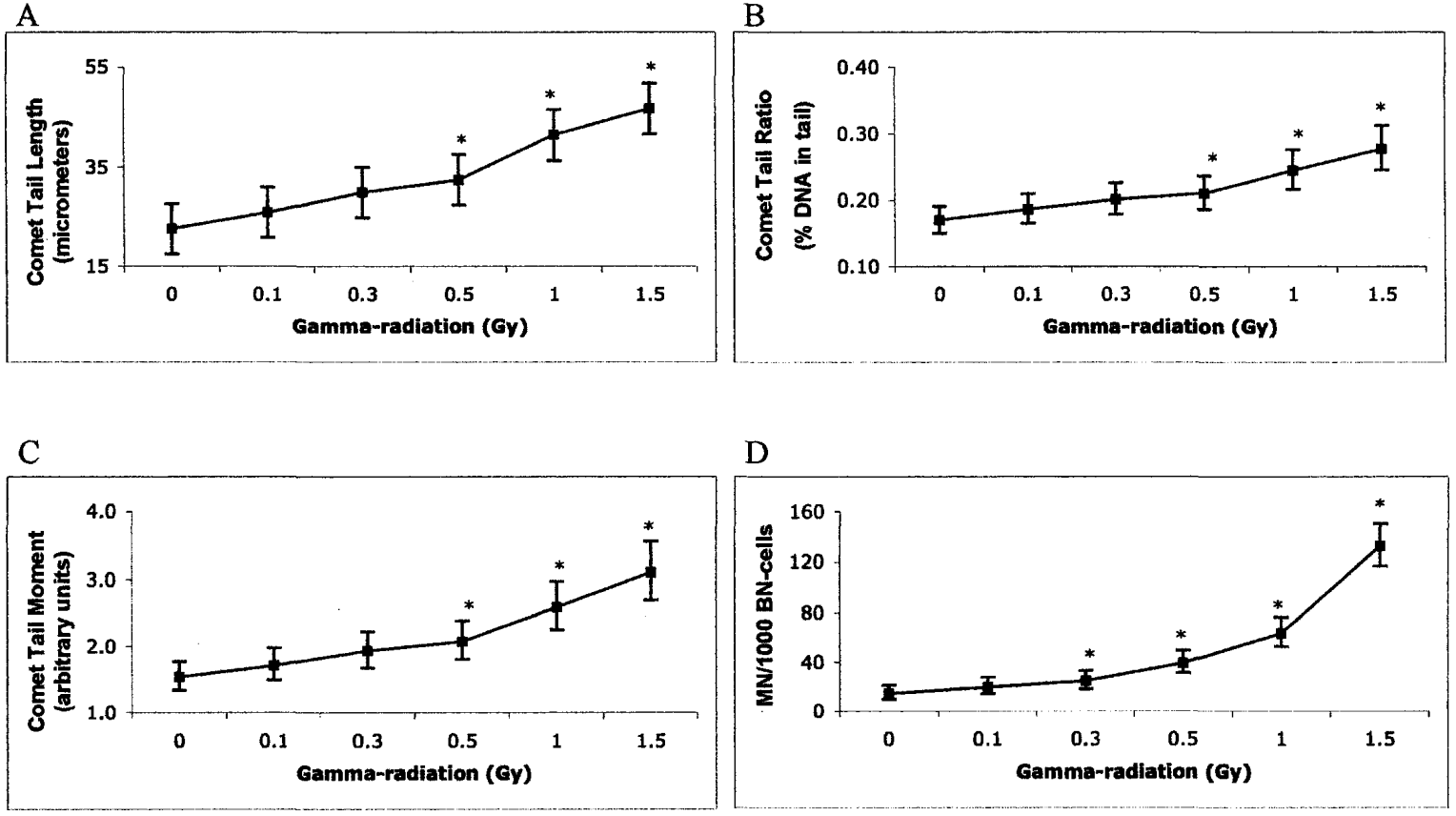


**Figure 2.1** DNA damage evaluated in the peripheral blood lymphocytes of human volunteers exposed *in vitro* to increasing doses of gamma-radiation. This experiment was conducted to standardize the assays used to determine the extent of DNA damage and the incidence of MN. A: Comet tail length (μm). B: Comet tail ratio (% DNA in Tail). C: Comet tail moment (arbitrary units). D: Incidence of MN in 1000 BN-cells. Each data point represents the mean (± 95% CI) from six subjects (three males and three females).
*: Significant ($p<0.05$) increase compared with 0 Gy.

For the CBMN assay, the overall F test from the Poisson regression model for treatment levels was significant ($F_{(5,25)}$ =112.47, $p<0.0001$). The Poisson regression models were corrected for over-dispersion by the Pearson chi-square scalar. The MN data presented in Figure 2.1d show the typical positive correlation with increasing γ-radiation dose. A marginally significant increase in the frequency of MN was observed when the cells were exposed at a dose of 0.3 Gy ($p<0.025$).

### 2.3.2 Magnetic field and sham exposures

All data obtained from the alkaline comet assay (TL, TR and TM) and CBMN (Proliferative index (PI), % BN-cells and total MN/1000 BN-cells) in individual volunteers as well as the mean from all subjects are presented in Tables 2.1 to 2.4. For both genotoxicity end-points, in each volunteer as well as the mean from all volunteers in magnetic field and sham-exposed groups: (i) the extent of genetic damage in the lymphocytes in negative-control (pre-exposure) samples was not significantly different from those obtained in post-exposure samples ($p>0.05$), (ii) the indices recorded in magnetic field-exposed volunteers were not significantly different from those in sham-exposed subjects ($p>0.05$), (iii) there was no significant difference when the data were analyzed for males and females separately, and (iv) in contrast, the response of the cells in both negative-control (pre-exposure) and post-sham/magnetic field exposure samples differed significantly from the positive control cells exposed *in vitro* to γ-radiation ($p<0.0001$).

| Volunteers | Tail Length (TL) | | | Tail Moment (TM) | | | Tail Ratio (TR) | | |
|---|---|---|---|---|---|---|---|---|---|
| ELF-EMF-exposed: | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls |
| | | | | | | | | | |
| 1 - Male | 21.5 | 22.9 | 50.3 | 1.5 | 1.4 | 3.0 | 0.2 | 0.2 | 0.3 |
| 2 - Male | 20.2 | 15.8 | 56.2 | 1.6 | 1.3 | 3.3 | 0.2 | 0.2 | 0.3 |
| 3 - Male | 21.7 | 16.6 | 45.8 | 1.8 | 1.4 | 2.9 | 0.2 | 0.2 | 0.3 |
| 4 - Male | 19.1 | 18.3 | 44.2 | 1.6 | 1.7 | 3.2 | 0.2 | 0.2 | 0.3 |
| 5 - Male | 23.3 | 31.7 | 59.5 | 1.6 | 1.6 | 3.3 | 0.2 | 0.2 | 0.3 |
| 6 - Male | 26.1 | 26.6 | 65.0 | 1.7 | 1.8 | 4.6 | 0.2 | 0.2 | 0.3 |
| 7 - Male | 26.6 | 31.2 | 66.6 | 1.5 | 1.7 | 3.6 | 0.2 | 0.2 | 0.3 |
| 8 - Male | 36.2 | 33.1 | 58.9 | 2.1 | 2.0 | 3.6 | 0.2 | 0.2 | 0.3 |
| 9 - Male | 40.2 | 44.4 | 64.4 | 2.4 | 2.4 | 3.4 | 0.2 | 0.2 | 0.3 |
| 10 - Male | 25.1 | 20.5 | 67.4 | 1.4 | 1.2 | 4.3 | 0.2 | 0.2 | 0.4 |
| 11 - Female | 18.1 | 29.7 | 49.0 | 1.4 | 1.8 | 3.3 | 0.2 | 0.2 | 0.3 |
| 12 - Female | 39.1 | 30.0 | 63.8 | 2.3 | 1.7 | 3.8 | 0.2 | 0.2 | 0.3 |
| 13 - Female | 26.6 | 31.8 | 41.1 | 1.3 | 1.3 | 3.0 | 0.1 | 0.1 | 0.3 |
| 14 - Female | 23.5 | 16.2 | 50.3 | 1.3 | 1.1 | 2.9 | 0.1 | 0.1 | 0.3 |
| 15 - Female | 21.7 | 22.5 | 54.8 | 1.3 | 1.4 | 3.3 | 0.1 | 0.2 | 0.3 |
| 16 - Female | 26.4 | 41.3 | 60.6 | 1.6 | 2.0 | 3.6 | 0.2 | 0.2 | 0.3 |
| 17 - Female | 28.6 | 41.6 | 63.2 | 1.7 | 1.7 | 3.7 | 0.2 | 0.2 | 0.3 |
| 18 - Female | 38.6 | 45.5 | 71.6 | 2.2 | 2.3 | 4.5 | 0.2 | 0.2 | 0.3 |
| 19 - Female | 31.6 | 26.9 | 54.3 | 1.7 | 1.5 | 3.2 | 0.2 | 0.2 | 0.3 |
| 20 - Female | 44.4 | 33.1 | 64.1 | 2.7 | 2.1 | 4.4 | 0.2 | 0.2 | 0.3 |
| Mean | 27.9 | 29.0 | 57.6 | 1.7 | 1.7 | 3.5 | 0.2 | 0.2 | 0.3 |
| 95 % CI | 24.8,31.0 | 25.9,32.1 | 54.5,60.6 | 1.6,1.9 | 1.5,1.8 | 3.4,3.7 | 0.2,0.2 | 0.2,0.2 | 0.3,0.3 |

**Table 2.1** Evaluation of comets from the alkaline comet assay measured as comet TL, TR and TM in the peripheral blood lymphocytes of healthy human volunteers ELF-EMF-exposed (whole-body) to 60 Hz ELF-EMF at a flux density of 200 mT for 4 hours.

| Volunteers | Tail Length (TL) | | | Tail Moment (TM) | | | Tail Ratio (TR) | | |
|---|---|---|---|---|---|---|---|---|---|
| Sham-exposed: | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive Controls |
| 1 – Male | 9.0 | 9.4 | 42.2 | 0.9 | 1.0 | 2.6 | 0.1 | 0.1 | 0.2 |
| 2 – Male | 23.7 | 27.7 | 58.8 | 1.2 | 1.4 | 3.4 | 0.1 | 0.1 | 0.3 |
| 3 – Male | 31.0 | 28.3 | 70.6 | 1.9 | 1.5 | 4.2 | 0.2 | 0.2 | 0.3 |
| 4 – Male | 31.4 | 34.5 | 57.1 | 2.0 | 2.1 | 3.9 | 0.2 | 0.2 | 0.3 |
| 5 – Male | 27.3 | 19.1 | 55.7 | 1.9 | 1.5 | 2.9 | 0.2 | 0.2 | 0.3 |
| 6 – Female | 17.6 | 21.3 | 51.8 | 1.4 | 1.2 | 2.7 | 0.2 | 0.1 | 0.2 |
| 7 – Female | 35.8 | 19.8 | 62.6 | 1.9 | 1.3 | 3.2 | 0.2 | 0.2 | 0.3 |
| 8 – Female | 27.4 | 27.7 | 62.7 | 1.7 | 1.5 | 3.5 | 0.2 | 0.2 | 0.3 |
| 9 – Female | 29.8 | 42.3 | 73.4 | 1.6 | 2.3 | 4.1 | 0.2 | 0.2 | 0.3 |
| 10 – Female | 33.1 | 32.1 | 69.0 | 1.6 | 1.5 | 4.1 | 0.2 | 0.2 | 0.3 |
| Mean | 26.6 | 26.2 | 60.4 | 1.6 | 1.5 | 3.4 | 0.2 | 0.2 | 0.3 |
| 95 % CI | 22.8,30.4 | 22.4,30.3 | 56.6,64.2 | 1.3,1.9 | 1.3,1.8 | 3.2,3.7 | 0.1,0.2 | 0.1,0.2 | 0.3,0.3 |

**Table 2.2** Evaluation of comets from the alkaline comet assay measured as comet TL, TR and TM in the peripheral blood lymphocytes of healthy human volunteers sham-exposed (whole-body) to 60 Hz ELF-EMF at a flux density of 200 mT for 4 hours.

| Volunteers | Proliferation Index | | | % BN-cells | | | MN/1000 BN-cells | | |
|---|---|---|---|---|---|---|---|---|---|
| ELF-EMF-exposed: | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls |
| 1 - Male | 2.5 | 2.5 | 2.4 | 47 | 42 | 46 | 10 | 12 | 136 |
| 2 - Male | 2.5 | 2.6 | 2.4 | 47 | 33 | 40 | 11 | 10 | 100 |
| 3 - Male | 2.1 | 2.4 | 2.0 | 41 | 42 | 37 | 23 | 10 | 149 |
| 4 - Male | 2.5 | 2.5 | 2.4 | 39 | 38 | 40 | 10 | 14 | 116 |
| 5 - Male | 2.3 | 2.1 | 1.9 | 51 | 51 | 49 | 8 | 8 | 155 |
| 6 - Male | 2.1 | 2.1 | 1.9 | 51 | 41 | 41 | 9 | 20 | 170 |
| 7 - Male | 2.4 | 2.5 | 2.0 | 40 | 39 | 38 | 7 | 5 | 106 |
| 8 - Male | 2.3 | 2.4 | 2.2 | 42 | 43 | 44 | 12 | 13 | 132 |
| 9 - Male | 2.3 | 2.1 | 2.1 | 44 | 40 | 38 | 12 | 15 | 122 |
| 10 - Male | 2.1 | 2.0 | 2.4 | 49 | 42 | 48 | 9 | 14 | 102 |
| 11 - Female | 1.7 | 2.5 | 2.3 | 34 | 43 | 48 | 40 | 8 | 148 |
| 12 - Female | 2.0 | 2.3 | 2.0 | 39 | 41 | 40 | 11 | 14 | 144 |
| 13 - Female | 2.3 | 2.4 | 2.1 | 39 | 42 | 42 | 9 | 14 | 115 |
| 14 - Female | 2.1 | 2.2 | 1.8 | 41 | 43 | 43 | 21 | 34 | 162 |
| 15 - Female | 1.8 | 2.0 | 1.7 | 44 | 39 | 36 | 19 | 12 | 150 |
| 16 - Female | 2.5 | 2.4 | 2.2 | 43 | 42 | 46 | 13 | 12 | 158 |
| 17 - Female | 2.3 | 2.3 | 2.0 | 47 | 39 | 39 | 20 | 15 | 144 |
| 18 - Female | 2.3 | 2.3 | 2.3 | 50 | 46 | 45 | 15 | 8 | 123 |
| 19 - Female | 2.5 | 2.5 | 2.2 | 45 | 48 | 49 | 11 | 17 | 124 |
| 20 - Female | 2.2 | 2.2 | 2.1 | 48 | 45 | 44 | 12 | 4 | 139 |
| Mean | 2.2 | 2.3 | 2.1 | 44 | 42 | 43 | 14 | 13 | 135 |
| 95 % CI | 2.1,2.3 | 2.2,2.4 | 2.0,2.2 | 42,46 | 40,44 | 41,45 | 11,17 | 10,16 | 123,143 |

**Table 2.3** The proliferation index, % binucleate cells (BN-cells) and total micronuclei (MN) in the peripheral blood lymphocytes of healthy human volunteers ELF-EMF-exposed (whole-body) to 60 Hz ELF-EMF at a flux density of 200 μT continuously for 4 hours.

| Volunteers | Proliferation Index | | | % BN-cells | | | MN/1000 BN-cells | | |
|---|---|---|---|---|---|---|---|---|---|
| Sham-exposed: | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls | Pre-exposure | Post-exposure | Positive controls |
| 1 - Male | 2.3 | 2.4 | 2.2 | 45 | 39 | 44 | 11 | 16 | 154 |
| 2 - Male | 2.4 | 2.5 | 2.3 | 42 | 41 | 42 | 6 | 4 | 123 |
| 3 - Male | 2.1 | 2.3 | 2.3 | 39 | 43 | 41 | 19 | 11 | 168 |
| 4 - Male | 2.3 | 2.2 | 2.2 | 46 | 43 | 45 | 10 | 11 | 104 |
| 5 - Male | 2.4 | 2.1 | 1.9 | 43 | 38 | 41 | 17 | 17 | 165 |
| 6 - Female | 2.6 | 2.4 | 2.2 | 41 | 42 | 40 | 7 | 11 | 146 |
| 7 - Female | 2.4 | 2.3 | 2.0 | 49 | 42 | 46 | 5 | 5 | 137 |
| 8 - Female | 2.0 | 2.2 | 1.9 | 51 | 46 | 46 | 11 | 6 | 159 |
| 9 - Female | 2.3 | 2.6 | 2.2 | 47 | 44 | 44 | 10 | 8 | 136 |
| 10 - Female | 2.0 | 2.2 | 1.9 | 46 | 45 | 46 | 8 | 7 | 119 |
| Mean | 2.3 | 2.3 | 2.1 | 45 | 42 | 43 | 10 | 9 | 139 |
| 95 % CI | 2.3,2.3 | 2.3,2.3 | 2.1,2.1 | 43,47 | 40,44 | 41,45 | 8,13 | 7,13 | 129,150 |

**Table 2.4** The proliferation index, % binucleate cells (BN-cells) and total micronuclei (MN) in the peripheral blood lymphocytes of healthy human volunteers sham-exposed (whole-body) to 60 Hz ELF-EMF at a flux density of 200 μT continuously for 4 hours.

## 2.4 Discussion

Over the past 30 years, the potential of ELF-EMF to cause adverse health effects in humans has been intensely investigated, yet the issue of whether ELF-EMF can induce DNA damage in human and animal cells after *in vitro* exposure or in animals after *in vivo* exposure remains controversial (Vijayalaxmi and Obe, 2005). Studies performed by Lai and Singh (1997a, 1997b) and Singh and Lai (1998) observed that rats exposed to a 60 Hz magnetic field for 2 hours at intensities of 0.1, 0.25 and 0.5 mT showed an increase in DNA damage in brain cells. Moreover, Svedenstal *et al.*, (1999a, 1999b) observed that adult mice exposed to a 50 Hz magnetic field showed increased DNA damage in brain cells after 32 days magnetic field exposure at an intensity of 8 μT and at 14 days for mice exposed to an intensity of 0.5 mT. However, McNamee *et al.* (2002, 2005) found no evidence of DNA damage in the cerebellum of 10-day-old mice exposed for 2 hours at 1 mT 60 Hz magnetic field or any DNA damage in cerebellar or whole brain homogenates obtained from adult rats, mice and immature mice exposed to 60 Hz magnetic fields at 0.1, 1 or 2 mT for 2 hours. At present the reason for these discrepancies remains unresolved.

Despite numerous epidemiological studies, the possible association between MF exposure and the incidence of human cancers also remains controversial (Alhorn *et al.*, 2000; Auvinen *et al.*, 2000; Villeneuve *et al.*, 2000a, 2000b; Savitz *et al.*, 2000; Kheifets, 1999). This is largely due to two major issues that have plagued ELF-EMF epidemiological studies to date. The first is that it is not known which exposure metrics are the most relevant for conducting such studies since no biological mechanism of

interaction has been established. Secondly, since the mechanism of interaction is unidentified, it remains unknown to what extent possible confounding variables are being controlled for in such studies. Thus, the possible association between ELF-EMF exposure and childhood leukemia may be greater or less than what is currently estimated. However, despite these uncertainties, in 2002 the International Agency for Research on Cancer (IARC) classified ELF-EMF as "possibly carcinogenic to humans" (Group 2B). This decision was based upon a small but consistent association between ELF-EMF exposure and childhood leukemia.

Due to the uncertainties outline above, our laboratory initiated a "first-of-its-kind" investigation of human whole-body exposure to a well-controlled, measurable and reproducible magnetic field exposure followed by the observation of cytogenetic effects on peripheral human lymphocytes. Using this model, a variety of exposure conditions (electric fields, transients etc...) and metrics can be carefully controlled and sequentially tested in an effort towards identifying possible biological mechanisms of interaction in humans which may be relevant to human risk assessment from ELF-EMF. Systems that provide human whole-body exposure to ELF-EMF with well-controlled, measurable and reproducible conditions may provide unique insight into possible mechanisms of interaction between these fields and human tissue.

In the current study, we first investigated the sensitivity of the alkaline comet assay and the CBMN assay by exposing human lymphocytes to radiation ($^{60}$Co $\gamma$-irradiation) to generate dose-response curves. The sensitivity level of the CBMN assay in this study was established at 0.3 Gy, similar to that observed in comparable studies with relatively low sample numbers (He *et al.*, 2000; Streffer *et al.*, 1998; Thierens *et al.*,

1991; Huber *et al.*, 1983). However, the sensitivity limit of the alkaline comet assay for all endpoints tested in this study was 0.5 Gy, slightly higher than that reported in previously published studies (Singh *et al.*, 1994 ; McNamee *et al.*, 2000; Malyapa *et al.* 1998). Considerable donor to donor variability in background levels of DNA damage were observed in this study, which adversely affected the sensitivity limit analysis for the comet assay data. This was possibly due to the fact that sample collection was performed over a prolonged period of time (nearly 1 year) rather than during a short duration (weeks) in a laboratory setting.

After the dose-response curves were established, we examined the effect of a 4-h human whole body exposure to a 200 μT, 60 Hz magnetic field on the induction of DNA damage in lymphocytes. Primary DNA damage was assessed using the alkaline comet assay and three parameters (TR, TM, TL) were recorded for each comet. There was no evidence of increased DNA damage detected by any of these parameters. Moreover, when MF exposed lymphocytes were stimulated with PHA and processed using the CBMN assay, there was no indication of increased MN formation. The results obtained in the current pilot study provide no evidence to support the hypothesis that 200 μT, 60 Hz magnetic field induces DNA damage in lymphocytes.

Future studies are required to investigate the effect of sub-acute (several days to weeks) magnetic field exposures on cytogenetic endpoints in adults. Moreover, it would be informative to study the cytogenetic effects in human lymphocytes after a whole body exposure to a strong magnetic field, such as the static, gradient and radio frequencies used in magnetic resonance imaging (MRI) systems. Finally, the application of high-

throughput transcriptomics and/or proteomics approaches may also provide insight into possible mechanisms of interaction.

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**2.5 References**

Ahlbom A, Day N, Feychting M, Roman E, Skinner J, Dockerty J, Linet M, McBride M, Michaelis J, Olsen JH, Tynes T, Verkasalo PK. 2000. A pooled analysis of magnetic fields and childhood leukemia. Brit J Cancer 83: 692-698.

Ahlbom IC, Cardis E, Green A, Linet M, Savitz D, Swerdlow A, ICNIRP (International Commission for Non-Ionizing Radiation Protection) Standing Committee on Epidemiology). 2001. Review of the epidemiologic literature on EMF and Health. Environ Health Perspect 109: 911-933.

Auvinen A, Linet MS, Hatch EE, Kleinerman RA, Robison LL, Kaune WT, Misakian M, Niwa S, Wacholder S, Tarone RE. 2000. Extremely low-frequency magnetic fields and childhood acute lymphoblastic leukemia: an exploratory analysis of alternative exposure metrics. Am J Epidemiol 152: 20-31.

Cho YH, Chung HW. 2003. The effect of extremely low frequency electromagnetic fields (ELF-EMF) on the frequency of micronuclei and sister chromatid exchange in human lymphocytes induced by benzo(a)pyrene. Toxicol Lett 143: 37-44.

Choleris E, Del Seppia C, Thomas AW, Luschi P, Chione S, Moran GR, Prato FS. 2001. Shielding, but not zeroing of the ambient magnetic field reduces stress-induced analgesia in mice. Proceedings of the Royal Society of London: Biological Sciences 269: 193-201.

Ciccone G, Mirabelli D, Leis A, Gavarotti P, Rege-Cambrin G, Davico L, Vineis P. 1993. Myeloid leukemias and myelodisplastic syndromes: Chemical exposure, histologic subtype and cytogenetics in a case-control study. Cancer Genet Cytogenet 68: 135-139.

He JL, Chen WL, Jin LF, Jin HY. 2000. Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxic effects of X-ray radiation. Mutat Res 469: 223-31.

Hellman B, Vaghef H, Bostrom B. 1995. The concepts of tail moment and tail inertia in the single cell gel electrophoresis assay. Mutat Res 336: 123-131.

Heredia-Rojas JA, Rodriguez-De La Fuente OA, Velazco-Campos RM, Leal-Garza CH, Rodriguez-Flores LE, Fuente-Cortez B. 2001. Cytological effects of 60 Hz magnetic fields on human lymphocytes in vitro: sister-chromatid exchanges, cell kinetics and mitotic rate. Bioelectromagnetics 14: 545-551.

Huber R, Streng S, Bauchinger M. 1983. The suitability of the human lymphocyte micronucleus assay system for biological dosimetry. Mutat Res 111: 185-193.

IARC (International Agency for Research on Cancer). 2002. Non-ionizing radiation. Part I. Static and Extremely Low-frequency Electric and Magnetic Fields. IARC Monograph Evaluation of Carcinogenic Risk to Humans 80: 1-395.

IEEE 1159-1995. 1995. IEEE Recommended Practice for Monitoring Electric Power Quality Institute of Electrical and Electronics Engineers. 70 pages.

Khalil AM, Quasem W, Amoura F. 1993. Cytogenetic changes in human lymphocytes from workers occupationally exposed to high-voltage electromagnetic fields. Electromagn Biol Med 12: 17-26.

Kheifets LI, Gilbert ES, Sussman SS, Guénel P, Sahl JD, Savitz DA, Thériault G. 1999. Comparative analyses of the studies of magnetic fields and cancer in electric utility workers: studies from France, Canada, and the United States. J Occup Environ Med 56: 567-574.

Lai H, Singh NP. 1997a. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics 18: 156-165.

Lai H, Singh NP. 1997b. Melatonin and N-tert-butyl-a-phenyl-nitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J Pineal Res 22: 152-162.

Malyapa RS, Bi C, Ahern EW, Roti Roti JL. 1998. Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation. Radiat Res 149: 396-400.

McNamee JP, McLean JR, Ferrarotto CL, Bellier PV. 2000. Comet assay: rapid processing of multiple samples. Mutat Res 466: 63-69.

McNamee JP, Bellier PV, Gajda GB, Miller SM, Lemay EP, Lavallee BF, Marro L, Thansandote A. 2002. DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field. Radiat Res 158: 523-533.

McNamee JP, Bellier PV, Chauhan V, Gajda GB, Lemay E, Thansandote A. 2005. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. Radiat Res 164: 791-797.

Miyakoshi J, Yoshida M, Shibuya K, Hiraoka M. 2000. Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks. J Radiat Res (Tokyo) 41: 292-302.

SAS (Statistical Analysis System). 2006. Version 9.1 for Windows, SAS Institute. Cary, North Carolina.

Savitz DA, Cai J, van Wijngaarden E, Loomis D, Mihlan G, Dufort V, Kleckner RC, Nylander-French L, Kromhout H, Zhou H. 2000. Case-cohort analysis of brain cancer and leukemia in electric utility workers using a refined magnetic field job-exposure matrix. Am J Ind Med 38: 417-425.

Shupak NM, Prato FS, Thomas AW. 2004. Human exposure to a specific pulsed magnetic field: effects on thermal sensory and pain thresholds. Neurosci Lett 363: 157-162.

Singh NP, Lai H. 1998. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutat Res 400: 313-320.

Singh NP, Stephens RE, Schneider EL. 1994. Modifications of alkaline microgel electrophoresis for sensitive detection of DNA damage. International Int J Radiat Biol 66: 23-28.

Skyberg K, Hansteen IL, Vistnes AI. 2001. Chromosomal aberrations in lymphocytes of employees in transformer and generator production exposed to electromagnetic on choice of axis. J Clin Epidemiol 54: 1046-1055.

Skyberg K, Hansteen IL, Vistnes AI. 1993. Chromosome aberrations in lymphocytes of high-voltage laboratory cable splicers exposed to electromagnetic fields. Scand J Work Environ Health 19: 29-34.

Strauss, GH. 1991. Non-random cell killing in cryopreservation: Implications for performance of the battery of leukocyte tests (BLT), I. Toxic and immunotoxic effects. Mutat Res 252: 1-15.

Streffer C, Müller WU, Kryscio A, Böcker W. 1998. Micronuclei-biological indicator for retrospective dosimetry after exposure to ionizing radiation. Mutat Res 404: 101-105.

Svedenstal BM. Johanson KJ. Mattsson MO. Paulsson LE. 1999a. DNA damage, cell kinetics and ODC activities studied in CBA mice exposed to electromagnetic fields generated by transmission lines. In Vivo 13: 507-513.

Svedenstål BM, Johanson KJ, Mild KH. 1999b. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. In Vivo 13: 551-552.

Thierens H, Vral A, de Ridder L. 1991. Biological dosimetry using the micronucleus assay for lymphocytes: interindividual differences in dose response. Health Phys 61: 623-30.

Thomas AW, Drost DJ, Prato FS. 2001a. Magnetic field exposure and behavioral monitoring system. Bioelectromagnetics 22: 401-407.

Thomas AW, White KP, Drost DJ, Cook CM, Prato FS. 2001b. A comparison of rheumatoid arthritis and fibromyalgia patients and healthy controls exposed to a pulsed (200 microT) magnetic field: effects on normal standing balance. Neurosci Lett 309: 17-20.

Valjus J, Norppa H, Jarventaus H, Sorsa M, Nykyri E, Salamaa S, Jarvinen P, Kajander J. 1993. Analysis of chromosomal aberrations, sister chromatid exchanges and micronuclei among power linesmen with long-term exposure to 50-Hz electromagnetic fields. Radiat Environ Biophys 32: 325-336.

Villeneuve PJ, Agnew DA, Miller AB, Corey PN, Purdham JT. 2000a. Leukemia in electric utility workers: the evaluation of alternative indices of exposure to 60 Hz electricand magnetic fields. Am J Ind Med 37: 607-617.

Villeneuve PJ, Agnew DA, Miller AB, Corey PN. 2000b. Non-Hodgkin's lymphoma among electric utility workers in Ontario: the evaluation of alternate indices of exposure to 60 Hz electric and magnetic fields. J Occup Environ Med 57: 249-257.

Vijayalaxmi, Obe G. 2005. Controversial cytogenetic observations in mammalian cells exposed to extremely low frequency electromagnetic radiation. A review and future research. Bioelectromagnetics 26: 412-430.

## GENERAL CONCLUSIONS

The current study investigated whether ELF-EMF induced DNA or clastogenic damage in human lymphocytes after a whole-body exposure. The novelty of this study in comparison to other whole body exposure investigations was that volunteers were exposed to a well-controlled, measurable and reproducible magnetic field. With carefully controlled and established ELF-EMF metrics as well as the use of cytogenetic assays, it may be possible to identify the potential biological mechanisms of interaction between humans and ELF-EMF.

We examined the effect of a 4-hour human whole body exposure to a 200 μT, 60 Hz magnetic field on the induction of DNA damage in lymphocytes. Primary DNA damage was assessed using the alkaline comet assay. Three parameters for DNA damage detection (comet tail ratio (TR), tail length (TL) and tail moment(TM)) were recorded for each comet. There was no evidence of increased DNA damage detected by any of these parameters. When MF exposed lymphocytes were processed using the cytokinesis-block micronucleus assay, there was no indication of increased MN formation. The results obtained in the current study provide no evidence to support the hypothesis that a 200 μT, 60 Hz magnetic field induces DNA damage in lymphocytes.

Further investigation is needed to identify the effects of EMF exposure on the human body. Over the past two years, a laboratory setting has been established at the Lawson Health Research Institute, capable of investigating the possible genotoxic effects of human exposure to an ELF-EMF. Moreover, liaisons have been founded between the Lawson Health Research Institute in London, Ontario, the Regional London Cancer Program in London, Ontario, and Health Canada in Ottawa, Ontario. With an established

laboratory and a number of collaborators, the Lawson Health Research Institute is now an ideal site for ongoing EMF and genotoxicity investigations. It should be noted that investigations should not be limited to human ELF-EMF. The possible genotoxic effects of radio-frequency wireless communication devices, microwaves and strong EMF fields should also be considered.

In a collaborative initiative between the Lawson Health Research Institute and Dr. Vijayalaxmi from the Department of Radiation Oncology at the University of Texas Health Science Centre in San Antonio, Texas, we are currently investigating the possible genotoxic effects of exposing a rodent model to a prolonged and therapeutically relevant ELF-EMF. Mice will be exposed to the CNP (complex neuroelectromagnetic pulse), a low frequency (< 1000 Hz) ELF-EMF with a 1000 μT peak, for a period of 8 weeks. Blood will be drawn once every two weeks and bone marrow will be collected at the end of the 8-week exposure period. Both blood and bone marrow samples will be examined for micronuclei.

Future work in genotoxic and EMF research should include investigating the potential harmful effects of human exposure to a high strength magnetic field, such as those used in magnetic resonance imaging (MRI). Researchers at the Lawson Health Research Institute have access to a 1.5 Tesla (T) MRI. Human exposure in a MRI include a 1.5 T static magnetic field, a gradient frequency of ~ 1 kHz, and a radiofrequency (RF) of 64 MHz. A technique to the deliver the CNP magnetic field through the gradient coils of the MRI system has also been recently developed at the Lawson Health Institute. Possible human exposure conditions comprise of the following:

1. static magnetic field exposure only

2. static magnetic field exposure and the magnetic field gradient (normal or CNP)
3. static magnetic field and RF
4. static magnetic field, magnetic field gradient (normal or CNP) and RF.

It would be informative to obtain blood samples before and after a 1- or 2-hour human exposure to the aforementioned conditions to investigate the extent of genetic damage in peripheral blood lymphocytes of healthy human volunteers.

Finally, insight into the biological mechanism of ELF-EMF can be explored using genomic and proteonomic assays. Blood samples obtained before and after EMF exposure for the genotoxic assays can also be used in assays to analyze the identity, interactions and locations of proteins within a cell. Moreover, a more detailed analysis of genes from human volunteers can be observed changes after EMF exposure.

# APPENDIX B: Experimental details and additional data

## B.1 Positive control γ-irradiation

Samples, in conical tubes and gels on Cometslides™ , were irradiated at the London Health Sciences Centre, London, Ontario, using the cobalt 60 γ-irradiation source. The samples were placed 7.0 cm above the face plate. To determine the surface area of the tubes exposed to the irradiation, the following calculation was used:

$$1)\ D' = D'_{ref}\left[\frac{d_{ref} + d_{fp}}{d + d_{fp}}\right]^2$$

Where the distance from the face plate ($d_{ref}$) was 7 cm, the source to the face plate ($d_{fp}$) distance was 24 and sample distance (d) of 8.6 cm (7 cm distance from face plate + 1.6 cm for the conical tube width), the following equation was used to determine the percentage of the sample receiving radiation:

$$2)\ \frac{D'}{D'_{ref}} = \left[\frac{7cm + 24cm}{8.6cm + 24cm}\right]^2 = 90.4\%$$

The dose rate at 7.0 cm was interpolated from dose rate tables from 2006-2008. The $^{60}Co$ source decays with a half-life of 5.26 years. The table is based on a dose rate of 728.2 cGy/min measured on June 28$^{th}$, 2001. The values of the table are only valid at a distance of 7.0 cm above the faceplate.

The following equation was used to determine the dose rate at the desired distance.

3) $D'_{ref} = D'_1 + \frac{T - T_1}{T_2 - T_1}(D'_2 - D'_1)$

Values were obtained from the dose rates tables (at 7.0cm) for 2008.

An example:

$D'_1$ 294.2 cGy/min

$D'_2$ 293.4 cGy/min

$T_1$ May 13 2008 (Day 1)

$T_2$ May 20 2008 (Day 8)

T May 14 2008 (Day 2)

The dose rate for May 14$^{th}$ 2008 was determined to be 294.1 cGy/min.

Once the dose rate was determined, it was possible to calculate the irradiation time needed to radiate a sample at 1.5 Gy. The positive shutter correction time of 0.013 minutes must be accounted for the time needed for the source to slide into place.

Example:

$$\text{time} = \frac{1.5\ \text{Gy}}{2.941\ \text{Gy/min}^{-1}} + 0.0013\ \text{minutes} = 0.511\ \text{minutes} = 30.7\ \text{seconds}$$

# APPENDIX B: Experimental details and additional data

## B.2 Cell viability data

Cell viability summary:

| Samples | Total Viable cells | Non-viable cells | Viability (%) | Cell Concentration (cell/ml) |
|---|---|---|---|---|
| 19-24 | 191 | 0 | 100 | 3.2x10^6 |
| 25-30 | 250 | 3 | 99 | 4.2x10^6 |
| 31-32 | 211 | 7 | 97 | 3.6x10^6 |
| 33 | 235 | 0 | 100 | 3.9x10^6 |
| 34-36 | n/a | n/a | n/a | n/a |
| 37-38 | 203 | 1 | 100 | 3.4x10^6 |
| 39 | 206 | | 100 | 3.4x10^6 |
| 43-44 | 216 | | 100 | 3.6x10^6 |
| 45 | 236 | 2 | 99 | 4.0x10^6 |
| 46-47 | 150 | | 100 | 2.5x10^6 |
| 48 | 138 | | 100 | 2.3x10^6 |
| 55-56 | 168 | 1 | 99 | 2.8x10^6 |
| 57 | 237 | | 100 | 4.0x10^6 |
| 58-59 | 134 | | 100 | 2.2x10^6 |
| 60 | 210 | 1 | 100 | 3.5x10^6 |
| 61-62 | 143 | | 100 | 2.4x10^6 |
| 63-74 | 225 | | 100 | 3.8x10^6 |
| 75 | 270 | | 100 | 4.5x10^6 |
| 76-77 | 99 | | 100 | 1.7x10^6 |
| 78 | 194 | | 100 | 3.2x10^6 |
| 79-80 | 163 | | 100 | 2.7x10^6 |
| 81 | 160 | | 100 | 2.7x10^6 |
| 85-86 | 139 | 1 | 99 | 2.3x10^6 |
| 87 | 146 | | 100 | 2.4x10^6 |
| 88-89 | 163 | | 100 | 2.7x10^6 |
| 90 | 154 | | 100 | 2.6x10^6 |
| 91-92 | 191 | 2 | 99 | 3.2x10^6 |
| 93 | 223 | | 100 | 3.7x10^6 |
| 94-99 | 174 | 1 | 99 | 2.9x10^6 |
| 100-105 | 219 | | 100 | 3.7x10^6 |
| 106-107 | 214 | | 100 | 3.6x10^6 |
| 108 | 240 | | 100 | 4.0x10^6 |
| 109-110 | 137 | 5 | 96 | 2.4x10^6 |
| 111 | 148 | | 100 | 2.5x10^6 |
| 112-113 | 150 | | 100 | 2.5x10^6 |
| 114 | 119 | | 100 | 2.0x10^6 |

| Samples | Total Viable cells | Non-viable cells | Viability (%) | Cell Conc. (cell/ml) |
|---|---|---|---|---|
| 115-116 | 124 | 1 | 99 | 2.1x10^6 |
| 117 | 102 | | 100 | 1.7x10^6 |
| 121-122 | 170 | | 100 | 2.8x10^6 |
| 123 | 234 | 1 | 100 | 3.9x10^6 |
| 124-125 | 133 | | 100 | 2.2x10^6 |
| 126 | 164 | | 100 | 2.7x10^6 |
| 127-128 | 208 | | 100 | 3.5x10^6 |
| 129 | 242 | | 100 | 4.0x10^6 |
| 130-131 | 193 | 1 | 99 | 3.2x10^6 |
| 132 | 186 | | 100 | 3.1x10^6 |
| 133-134 | 187 | | 100 | 3.1x10^6 |
| 135 | 217 | | 100 | 3.6x10^6 |
| 136-137 | 251 | 1 | 100 | 4.2x10^6 |
| 138 | 212 | 1 | 100 | 3.6x10^6 |
| 139-140 | 240 | 1 | 100 | 4.0x10^6 |
| 141 | 206 | | 100 | 3.4x10^6 |
| 142-143 | 138 | | 100 | 2.3x10^6 |
| 144 | 164 | | 100 | 2.7x10^6 |
| 145-146 | 169 | | 100 | 2.8x10^6 |
| 147 | 190 | | 100 | 3.2x10^6 |
| 148-149 | 133 | | 100 | 2.2x10^6 |
| 150 | 156 | | 100 | 2.6x10^6 |
| 151-152 | 174 | | 100 | 2.9x10^6 |
| 153 | 194 | | 100 | 3.2x10^6 |
| 154-155 | 88 | | 100 | 1.5x10^6 |
| 156 | 115 | | 100 | 1.9x10^6 |
| 157-162 | 214 | | 100 | 3.6x10^6 |
| 172-178 | 170 | 1 | 99 | 2.9x10^6 |
| 178-179 | 124 | | 100 | 2.1x10^6 |
| 180 | 210 | | 100 | 3.5x10^6 |

**Table B.2** Human lymphocyte cell viability. Viable and non-viable cell counts obtained from six 1 $mm^2$ squares of a hemocytometer. Human lymphocyte cell concentrations and viability % were determined for each condition (pre-exposure, post-exposure, and positive control) for each subject.

## APPENDIX B: Experimental details and additional data

### B.3 CBMN scoring criteria

*Document provided by Dr. James McNamee from Health Canada*

The cytokinesis-blocked micronucleus (CBMN) assay is a measure of chromosome breakage and loss. It is important to specify the acceptance criteria for binucleate cells (BNC), and micronuclei (MN) within BNC, so that consistent scoring can be achieved between different scorers and laboratories. This will enable better precision of the assay and increase the reliability of the method for comparing DNA damage between samples.

This section describes the criteria that we would like our four core labs to follow for emergency biodosimetry purposes. The criteria are very similar, but not exactly the same as, the criteria described by Fenech *et al.*, (Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., and Zeiger, E. HUMN project: detailed description of the scoring criteria for cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mut. Res.* 534 65-75 (2003)). This paper provides some useful pictures of Giemsa-stained CBMN cells that can be used for reference when learning the technique. However, we find Acridine Orangestained cells far easier to score in comparison to Giemsa-stained cells as Acridine Orange-stained cells will stain the nucleus green/yellow and the cytoplasm red.

CBMN scoring can be divided into three steps:

1. Determine if a cell may be considered for scoring (e.g. Is the cell an intact binucleate cell (BNC)?).
2. Determine if the BNC contains a MN (or more than one MN). MN are morphologically identical, but smaller than, the main nuclei.
3. After the desired number of BNC have been scored, determine the frequency of mono-, bi-, tri- and quadra-nucleated cells to allow the Proliferative Index and Binucleate Frequency to be calculated.

### B.3.1 Determining eligible binucleated cells

Cells that have undergone one nuclear (but not cytoplasmic) division, after whole blood culture and subsequent cytochalasin B block, will be binucleated. These BNC are the cells of interest for MN scoring. A BNC is considered eligible for scoring if it has a relatively intact cytoplasmic boundary with two clearly defined nuclei. A small degree of tearing in the cytoplasm is tolerated (an artifact of slide preparation), provided that the scorer is confident that any MN that might have been present within the torn area would still be associated with the cell in question. Any cells that have the look of having committed toward apoptosis should not be scored as an eligible BN cell for the CBMN assay.

Specifically, the following criteria must be met for a BNC to be enumerated:

1. The cytoplasmic boundary of the cell should be intact and clearly distinguishable from adjacent cells.
2. The cell is binucleated.
3. The nuclei are round or oval-shaped, however one 'dent' in each nuclei (kidney-shaped) is tolerated.
4. The two nuclei must have intact nuclear membranes.
5. Both nuclei must be situated within the same cytoplasmic boundary.
6. The two nuclei should be of approximately the same size, staining pattern and staining intensity.
7. The two nuclei may be attached by a fine nucleoplasmic bridge which is no wider than one-fourth of the
8. largest nuclear diameter (see below).
9. The two nuclei may touch, but ideally they should not overlap. If the nuclei do overlap, they may still
10. be scored as a BNC as a long as at least one nuclear membrane is discernable in the overlap area.
11. The binucleated cell in question should not appear to have committed toward apoptosis.

B.3.2 Micronucleus (MN) scoring criteria

MN are scored only within eligible BNC. Typically, 1000 BNC are scored per sample for dose-response curves or experimental studies. However, a lower number of BNC may be suitable for triage situations and experiments planned over the next 3 months will evaluate this possibility.

Specifically, the following criteria must be met for a MN to be enumerated:

1. The MN diameter should range from 1/2.5 to 1/16th of the diameter of the main nuclei of the BNC.
2. MN are round or oval in shape.
3. MN are non-refractile and therefore can be distinguished from artefacts such as staining debris.
4. The MN membrane is not linked or connected to the main nuclei (these are classified as nuclear buds).
5. MN may touch the main nuclei, but the MN boundary must be distinguishable from the nuclear boundary.
6. MN should have approximately the same staining intensity as the main nuclei, however, in our experience we have noted that micronuclei can sometimes stain with less intensity than the main nuclei. These MN are currently scored both at HC and AECL.
7. More than one MN may be present within a BNC. BNC bearing one, two and three MN should be enumerated separately (on separate keys of the tally) as this

allows greater flexibility in presenting the scoring data. BNC bearing more than 3 MN are not included in either the MN tally or the BNC tally, as there is a risk that these cells may actually be undergoing apoptosis.

8. The approximate size of a typical BNC containing the maximum and minimum size of scorable MN (1/2.5 diameter MN to nucleus, 1/16 diameter MN to nucleus)

Occasionally, nucleoplasmic bridges (NPB) may be observed in binucleated cells. They are thought to originate from rearranged chromosomes with more than one centromere (e.g.: dicentric chromosomes). A separate tally of BNC containing NPB may be kept for reporting purposes. BNC bearing NPB may still be considered for scoring provided the following criteria are met:

1. The NPB is a continuous link between the two nuclei
2. The width of the bridge does not exceed one-fourth of the diameter of the nuclei
3. The bridge has the same staining characteristics as the main nuclei
4. More than one bridge may be observed within the BNC, however the one-fourth rule will apply for the sum of the bridges.
5. A BNC with an NPB may contain a MN, and can be scored as such, as long as the MN meets the criteria described above.

### B.3.3 Proliferative Index (PI) and Binucleate Frequency (BNF)

It is useful to score slides not only for the presence of BNC, but also for the ratio of BNC cells to mono-, tri- and quadra-nucleated cells. This can give an indication of the health of the culture, the appropriateness of the culture conditions and whether cytochalasin B was added at the optimum time. While BNC frequency is quoted most often, but we have found that calculating the Proliferative Index (PI) is a more descriptive indicator. At Health Canada, typically a PI of 1.8 to 2.2 is achieved. BNF and PI can be determined during a separate set of scoring (after CBMN scoring) of approximately 500 cells. Choose a position near the middle of a slide to scan. Every lymphocyte is scored, whether mono-, bi-, tri-, or quadranucleated (with the exception of apoptotic cells), provided that the nuclei are clearly contained within a cytoplasmic boundary. All lymphocytes (whether they would be appropriate for CBMN scoring or not) are tallied (i.e.: torn cells are still counted). Neutrophils and cell debris are ignored.

BNC Frequency (or %BNC) is calculated by the following formula:

%BNC = (# BNC/Total Lymphocytes)*100%

Proliferative Index (or PI) is calculated by the following formula:

$$PI = \frac{((1*\#mononucleated)+(2*\#binucleated)+(3*\#trinucleated)+(4*\#quadranucleated))}{\text{Total \# of Lymphocytes evaluated}}$$

# APPENDIX C: LITERATURE REVIEW: STIMULATION PARAMETERS

This appendix has been submitted to the peer-reviewed journal *Neuroscience & Biobehavioral Reviews* on April 10th 2008.

## Deep brain stimulation, vagal nerve stimulation and transcranial stimulation: An overview of stimulation parameters and neurotransmitter release

G.C. Albert, C.M. Cook, F.S. Prato, and A.W. Thomas

### C.1 Introduction

Neurological disorders (NDs) encompass a vast spectrum of medical problems that span development, from neonates to the geriatrics. It is estimated that approximately 1 in 4 adults suffer from a diagnosable disorder, which has a significant impact on the lives of the individuals suffering from them, their families and society as a whole (Kessler *et al.*, 2005). Progressive NDs such as Parkinson's Disease (PD), can lead to a dependence on health care systems. This is a grave economical concern because as the worldwide population ages, many progressive NDs are on the rise (Huse *et al.*, 2005; Bertolote *et al.*, 2006).

Neuroscientists consistently strive towards discovering better diagnoses and treatments for NDs. Considering the large variations in type, treatment is typically largely focused on pharmacological interventions. However, even targeted pharmacological agents have failed to provide complete relief of symptoms and their prolonged use are often limited by complications and side effects. Considering these

points, there has been a renewed interest in assessing the effectiveness of alternative treatment modalities such as brain stimulation for the treatment of certain NDs.

There is a long history of using electrical and magnetic stimulation methods to manipulate central nervous system activity (Andrews, 2003; Gildenberg, 2005). With the success of pharmaceutical treatments, brain stimulation techniques remained relatively coarse and non-directed, such as electroconvulsive therapy (ECT). However, research over the past decade has seen significant advances in various invasive and non-invasive stimulation therapies that provide increased relief of symptoms with fewer serious side effects, such as Transcranial Magnetic Stimulation (TMS), Deep Brain Stimulation (DBS) and Vagal Nerve Stimulation (VNS). Interestingly, scientific and clinical applications have largely preceded the development of extensive animal models, presenting a challenge for researchers. Without adequate animal models, little information exists on how these treatments alleviate symptoms in humans nor are there existing mechanisms to explain the biological effects of the devices. Some research exists examining the effects of electrical stimulation on animal models through voltammetry and microdialysis. However, these studies currently have little or no relationship to devices used with humans in a clinical setting. While animal models have not had a practical effect on the development of treatments within humans, animal research has helped us to map the function areas of the brain. These studies have determined which sites of the brain to stimulate to induce a predictable response, and which electrical parameters will have a reliable and reproducible effect on neurotransmitter release. Specific stimulation parameters in human subjects have not been determined; hence, our understanding is somewhat deficient as treatments are not

currently as focused and consistent as they potentially could be. This review combines the stimulation parameters developed in animal models and the neuromodulation techniques used in human treatment. Combining these two bodies of information will greatly improve our understanding of the mechanisms and neurobiological effects of the devices, which in turn may significantly advance the effective treatment of many neurological disorders.

### C.2 Monitoring neurotransmitter release

To understand brain neuromodulation, it is essential to have technologies that can detect, identify, and quantify neurotransmitter release. Two popular methods are cyclic voltammetry and microdialysis.

Fast-scan cyclic voltammetry is used to investigate neurotransmission *in vivo* and *in vitro*. It is also a valuable tool to evaluate animal models of disease involving neurotransmitter imbalance such as dopamine release in PD. Cyclic voltammetry can measure neurotransmitter release on a subsecond time-scale, making it possible to monitor the neurotransmitter release as it occurs. It also has the benefit of being able to measure the increases and decreases in neurotransmitter release. A micro-meter probe is used in this procedure, keeping tissue damage to a minimum (Robinson *et al.*, 2003). Cyclic voltammetry works by placing a carbon-fiber microelectrode in the area of the brain being monitored for neurotransmitter release. Neurotransmitter release is then induced by electrical or pharmacological stimulation, causing excitation of the neuronal compartment. Microelectrodes can detect neurotransmitters because the potential of the

electrode is linearly scanned, and the neurotransmitters adjacent to the electrode will be oxidized or reduced. The current is measured, which provides information that identifies the released chemical. The current output data, which has a large background current, is known as a cyclic voltammogram. The voltammogram has a background in the output, which prevents clear readout; however, the background can be subtracted to reveal changes in the current. The resulting 'background subtracted' cyclic voltamogram indicates that the change in current is attributable to the oxidation/reduction of a specific neurotransmitter (Robinson *et al.*, 2003). During stimulation, the magnitude of the current increases as a result of neurotransmitter release and then decreases as a result of neurotransmitter uptake. The change in the current output signal within the cyclic voltammogram indicates a fluctuation in neurotransmitter release. The neurotransmitter concentration can be determined by an *in vitro* calibration that is collected from the current output data (Robinson *et al.*, 2003).

The basics of microdialysis involve the implantation of a probe/catheter used for tissue implantation and a perfusion pump, which circulates fluid from a reservoir to the probe and then back into a collection tube. The probe is small in diameter and the tip has a semi-permeable membrane. The probe contains a solution that is isotonic to the cerebral environment which allows for mass transport of molecules across the probe membrane, driven by simple diffusion along the concentration gradient. Therefore, neurotransmitters will diffuse into the fluid of the probe. At a constant rate, the concentration of a particular neurotransmitter in the probe's fluid will be proportional to the concentration of neurotransmitter in the interstitial fluid in the area being tested. This ratio is referred to as the relative recovery. This technique enables frequent sampling of

chemical changes in the interstitial fluid with maximal time resolution (Hillered *et al.*, 2005).

It should be noted that *in vivo* voltammetry offers significant improvements over microdialysis in both spatial and temporal resolution. With regard to spatial resolution, the sampling area of the typical microdialysis probe is difficult to quantify precisely. In addition to the difficulties associated with quantifying the sampling area, most commercial microdialysis probes are relatively large, making attempts to study small regions of the brain difficult. The large size of the microdialysis probe makes it difficult to functionally differentiate neighbouring areas within the extended sampling area. At the level of temporal resolution, microdialysis is limited by the analytical method High Performance Liquid Chromatography (HPLC), where samples need to be collected within minutes instead of seconds (Bruno *et al.*, 2006). Finally, Westerink & Justice Jr., (1991) concluded that for short-term and well-defined stimuli such as electrical stimulation, voltammetry is most useful.

### C.2.1 Electrical stimulation

Electrical stimulation is hypothesized to alleviate the symptoms of NDs by controlling the release of specific neurotransmitters. A neurostimulator consists of a battery power supply, a pair of electrodes in contact with tissue and extension wires to connect the electrodes to the battery. Extracellular stimulation uses the electrodes to generate voltage/current fields (Rise, 2000). The applied stimulus must depolarize the nerve membrane sufficiently to enable the generation of an action potential along the

nerve track (axon). Important considerations for the excitation process include knowing the orientation of nerve cells relative to the electric field. The orientation of the nerve cells must be determined because the voltage along the axis of an axon determines the efficacy of neural activation. Furthermore, stimulation applied parallel to a nerve fiber is more effective than stimulation applied perpendicular to the fiber, and stimulation with a uniform electric field is ineffective (Testerman *et al.*, 2006).

Careful selection of stimuli can be applied to achieve a desired outcome, since the choice of stimuli will affect a physiological response (Grill *et al.*, 1995). Different subunits of a neuron must be considered, such as the dendrites, soma, axon hillocks, nodes, internodes and unmyelinated terminals, since they all have different electrical properties. Testerman *et al.*, (2006) and Rise (2000) highlight four general rules that apply to most applications of neurostimulation:

1) Nerve cells further away from the electrode are less likely to be stimulated.
2) Axons require lower amplitudes to be stimulated than nerve cell bodies.
3) A lower stimulation amplitude is more effective when stimulating larger axons versus smaller axons.
4) The branching process of axons allows for easier activation by stimulation in comparison to axons without branching.

In some instances, the purpose of electrical stimulation is not to increase the level of neurotransmitters but to cease their over-abundant release. In this case, it is better to either lesion the neuronal network or abolish the messaging system through electrical

stimulation; thus, creating an inhibitory-like effect (Benabid, 2003). This inhibition effect is possible through two mechanisms described by Benabid (2003). The first mechanism, known as jamming, involves an alteration of the firing pattern to a meaningless and inefficient signal. The second mechanism consists of the loss of electrogenic properties or synaptic inhibition in the neuron; thus, resulting in the suppression of firing. In addition to Benabid's observations, Rattay (1999) documented that it is possible to stop the propagation of the unwanted natural neural activities towards the axon. This is achieved by applying a negative stimuli from an electrode positioned in front of the dendrites, causing a strong hyperpolarization at the beginning of the axon.

### C.2.2 Electrodes

Electrodes play a very important role in neurostimulation. The type or positioning of the electrode can specify how effictively the neuron is stimulated. For example, the efficacy of stimulation decreases rapidly with distance (Rattay, 1990, 1999); the electrode will be most effective if placed close to the neuron of interest. Electrodes work by generating a voltage/current field distributed to the neuronal tissue. The current flow requires a negative cathode and a positive anode. The type of current applied can greatly affect a neuron, for example, excitation of the neurons with electrodes in the vicinity of the axon with negative currents is easier compared to positive stimuli (Rattay, 1999).

The most common electrode forms are monopolar and bipolar. The monopolar electrode is typically negative and situated near or within the tissue to be stimulated.

Bipolar electrodes use both positive and negative electrodes near or within the nervous tissue targeted for stimulation that have the same or similar surface areas (Rise, 2000).

### C.2.3 Stimulation waveform

The stimulation waveform is made up of standard parameters including amplitude, pulse width, and stimulation frequency. Generally, researchers use the terms voltage and current interchangeably to denote the amplitude of the waveform. If the tissue in question is the brain, and if we assume that brain conductivity is more or less constant, then there will be a linear relationship between the two and either can be used to describe the amplitude. However, there may be variations in the brain and specifically within the white matter, and the conductivity may vary depending on the orientation of the fiber tracts and the stimulation device. The current can be applied in a monophasic or a biphasic waveform. The pulse width is the duration of the stimulus pulse, or in other words, the time that the voltage is applied to the electrode. The duty cycle is defined as the percentage of time during which stimulation occurs, calculated as stimulation time divided by the sum of signal ON and OFF times multiplied by 100 (Labiner & Ahern, 2007).

Within certain ranges, the interaction of the stimulus amplitude and the pulse width are largely independent of the stimulus frequency, and are dominated by the properties of the tissue near the electrode (Rise, 2000). The stimulation frequency is the number of times the stimulus pulse is applied in a period of time. It is largely mediated through its influence on the electro-chemical activity within the overall neural network.

The frequency effects are much more dependent on the particular stimulation therapies, and changes in frequency are generally less effective than changes in amplitude or pulse width in producing clinically measurable effects (Testerman *et al.*, 2006).

## C.3 Literature review of animal studies

This compiled research, Table C.1, is limited only to those studies that focused on specific stimulation parameters, such as frequency, amplitude, pulse number, and stimulation train. A successful stimulating waveform must take into consideration the neurotransmitter being released, the area of the brain being stimulated and the region of the brain releasing the neurotransmitter.

### C.3.1 Frequency

A frequency waveform can elicit the release of a neurotransmitter and determine the quantity of neurotransmitter that is released. While neurotransmitter release is frequency dependent; there is a frequency plateau at which the neuron will keep firing but there will be no increase in the amount of neurotransmitter released (Wightman & Zimmerman, 1990; John *et al.*, 2006). The ability of the frequency to elicit a response is also determined by the gender of the tested species (Walker *et al.*, 2000) as well as anesthetized versus non-anesthetized animals (Garris *et al.*, 1997).

Peak releases of dopamine were observed between 37-75 Hz (15 pulses, 300 μA) by Lee *et al.*, (2006) when stimulating the subthalamic nucleus, and 60 Hz (10 s

stimulation train, 80 μA) for dopamine release in the nucleus accumbens and caudate nucleus (Kuhr, 1986). Dopamine release does not occur when the nucleus accumbens and caudate nucleus are stimulated at frequencies of <30 Hz or >240 Hz (Kuhr, 1986). A significant increase in dopamine release was observed by You *et al.*, (1998) in the nucleus accumbens septi at 25 Hz (train duration 0.5 sec; pulse width 0.1 msec; inter-stimulation interval, 2 sec and current intensity 500 μA) with only a marginally higher increase at higher frequencies. In addition to dopamine release, serotonin, glutamate and gamma-aminobutyric acid (GABA) release are also frequency dependent. Bunin et al., 1998a observed a large change in the maximal concentration of serotonin release when the frequency changed from 10 to 20 Hz (other variables not mentioned). It was also reported by John *et al.*, (2006) that serotonin frequency-independence occurs at >20 Hz (30 pulse stimulations at 350 μA). Glutamate on the other hand, has increased release over a range of 25 to 400 Hz, with a maximal release at >50 Hz in the nucleus accumbens septi (0.5 sec trains of 0.1 msec pulses with an intertrain interval of 2 sec; at 500 μA)(You et al., 2006). Windels *et al.*, (2003) noted that a frequency of 130 Hz (500 μA) provoked a maximum release of glutamate in both the Globus Pallidus (GPi) and Substantia Nigra reticulata (SNr), whereas 60 or 350 Hz (at 500 μA) induced a lesser effect, and 10 Hz had no effect. This group also observed that increasing frequency during electrical stimulation of the STN induced increases of GABA release within the SNr, while they remained stable in the GPi.

Summary Table C.1

| Author | Neuro-transmitter | Animal | Frequency (Hz) | Current (μA) | Pulse or stimulation train | Brain region stimulated | Brain region release | Neuro-transmitter release |
|---|---|---|---|---|---|---|---|---|
| Kuhr et al., 1984 | Dopamine | Anes-thetized rat | 60 | 60 | 10 s stim. | Medial forebrain bundle | Caudate nucleus | Maximum release |
| Kuhr et al., 1986 | Dopamine | Anes-thetized rat | 60 | 60 | 10s | Medial forebrain bundle | Caudate Nucleus | Best release |
| | | | 60 | 70 | 10s | Medial forebrain bundle | Nucleus accumbens | Best release |
| Garris & Wightman, 1994 | Dopamine | *in vivo* rat | 60 | 300 | n/a | Medial forebrain bundle, ventral tegmental area/substantia nigra region | Medial prefrontal cortex | Highest concentration release |
| | | | 50 | 300 | 120 stim pulses | Medial forebrain bundle, ventral tegmental area/substantia nigra region | Basal lateral amygdalloid nucleus | Highest concentration release |
| | | | 60 | 300 | n/a | Medial forebrain bundle, ventral tegmental area/substantia nigra region | Caudate-putamen | Highest concentration release |
| | | | 60 | 300 | n/a | Medial forebrain bundle, ventral tegmental area/substantia nigra region | Nucleus accumbens | Highest concentration release |
| Garris PA et al., 1997 | Dopamine | Anes-thetized and freely moving rat | 60 | (+/-) 125 | 1 s | Substantia nigra | Striatum | Maximum release |

Summary Table C.1

| Author | Neuro-transmitter | Animal | Frequency (Hz) | Current (uA) | Pulse or stimulation train | Brain region stimulated | Brain region release | Neuro-transmitter release |
|---|---|---|---|---|---|---|---|---|
| Rice et al., 1997 | Dopamine | *in vitro* guinea pig slices | 10 | 20 V pulses | Train of 100 µs delivered for 10 s | Substantia nigra pars compacta | Substantia nigra and ventral tegmental Area | Maximum release |
| Bunin et al., 1998 (a) | Serotonin | *in vitro* rat brain slices | 100 | 350 | 20 pulses 2 ms dur. | 100-200 um away from carbon electrode | Dorsal raphe | Maximum release |
| | | | 100 | 350 | 20 pulses 2 ms dur. | 100-200 um away from carbon electrode | Substantia nigra reticulata | Maximum release |
| Bunin & Wightman, 1998 | Serotonin | *in vitro* rat brain slices | 100 | 350 | 2 msec per phase and 20 pulses | on slice surface (not specified) | Substantia nigra reticualta and dorsal raphe nucleus | Maximum release |
| You et al., 1998 | Glutamate | Freely moving rat | 50 | 500 and 700 | n/a | Ipsilateral of the medical prefrontal cortex | Nucleus accumbens speti | Maximum release |
| | Dopamine | | 25 | 250, 500 and 700 | n/a | Ipsilateral of the medical prefrontal cortex | Nucleus accumbens speti | Maximum release |

Summary Table C.1

| Author | Neuro-transmitter | Animal | Frequency (Hz) | Current (uA) | Pulse or stimulation train | Brain region stimulated | Brain region release | Neuro-transmitter release |
|---|---|---|---|---|---|---|---|---|
| Juckel et al., 1999 | Serotonin | Anes-thetized and awake rat | 60 | 150 | 1 s trains of 5 ms presented every 5 s for 20 mins | Left medial prefrontal cortex | Ipsilateral ventral hippocampus | Increased release |
| | | | 60 | 150 | 1 s trains of 5 ms presented every 5 s for 20 mins | Left medial prefrontal cortex | Amygdala | Increased release |
| Walker et al., 2000 | Dopamine | Anes-thetized and rat brain slices (male and female) | 60 | 300 | 2 msec phase, 120 pulses | Medial forebrain bundle | Caudate nucleus | Maximum release in male rat |
| | | | 60 | 200 | 2 msec phase, 120 pulses | Medial forebrain bundle | Caudate nucleus | Maximum release in female rat |

| Author | Neuro-transmitter | Animal | Frequency (Hz) | Current (uA) | Pulse or stimulation train | Brain region stimulated | Brain region release | Neuro-transmitter release |
|---|---|---|---|---|---|---|---|---|
| Windels et al., 2003 | Glutamate | Anes-thetized rat | 130 | 500 | 60 µsec | Subthalamic nucleus | Globus pallidus | Maximum release |
| | GABA | | 60 | 500 | 60 µsec | | | Increased release |
| Hentall et al., 2005 | Serotonin | Anes-thetized rat | 100 | 150 | 0.5 ms for 20s train | Nucleus raphe magnus | Lumbar spinal chord | Best result |
| Greco et al., 2006 | Dopamine | Anes-thetized Syrian hamster | 60 | 300 | 2ms phases pulse train 2s | Medial forebrain bundle in the lateral hypothalamus of the right brain | Caudate-putamen and core of the nucleus accumbens | Best result |
| John et al., 2006 | Dopamine | *in vitro* mouse brain slices | 30 | 350 | 1 ms, 30 pulses | 100-200 µm away from carbon electrode | Ventral tegmental area | Peak release |
| | Serotonin | | 60 | 350 | 1ms, 60 pulses | 100-200 µm away from carbon electrode | Substantia nigra pars compacta | Peak release |
| | Serotonin | | 40 | 30 | 1ms, 40 pulses | 100-200 µm away from carbon electrode | Substantia nigra reticulata | Peak release |
| Lee et al., 2006 | Dopamine | *in vitro* rat brain slices | 50 | 300 | 15 or 1000 pulses | Subthalamic nucleus | Striatum | Stimulus-time-locked |

**Table C.1** Electrical stimulation parameters for selective neurotransmitter release in animal models

It has been observed that frequency can have different neurological effects on male rats versus female, as well as in freely moving rats versus anesthetized rats. For example, Walker *et al.*, (2000) observed that 60 Hz (at 500 μA) stimulation elicited a higher dopamine release in female rats versus male rats (Walker *et al.*, 2000). Garris et al., (1997) observed that the rate of dopamine release was increased significantly at frequencies 20 and 60 Hz (0.4 s train duration, 125 μA) in freely moving rats when compared to anesthetized rats.

### C.3.2 Amplitude

Neurotransmitter release is current dependent (Juckel *et al.*, 1999), however, it is reported that high levels of current stimulation on subsequent days can impair dopamine release (Garris *et al.*, 1997). This may be due to lesions that are generated at the site of stimulation, resulting from higher amplitudes. There is some suggestion that such lesions can be caused by the electrochemical generation of free radicals during large amplitude stimulations (Garris *et al.*, 1997).

Lee *et al.*, (2006) tested a range of current intensities between 25 and 1600 μA in an attempt to elicit dopamine release in the striatum in rat brain slices. Although there was increased within this range, the maximal increase occurred between 200-600 μA (15 pulses at 50 Hz) after subthalamic stimulation. You *et al.*, (1998) observed a significant dopamine release from the nucleus accumbens at 250, 500 and 700 μA (100 Hz, train duration 0.5 sec; pulse width, 0.1 msec; and interstimulation interval 2 sec). In addition, Kuhr *et al.*, (1986) revealed that 25 μA (10 s, 60 Hz) stimulation of the medial forebrain

bundle was needed to evoke a significant response in the caudate nucleus and the nucleus accumbens. This dopamine release increased linearly and reached a maximum at 60 μA. Higher stimulation currents (above 60 μA) elicited dopamine responses that were independent of amplitude. Serotonin and glutamate also displayed amplitude dependencies. Juckel *et al.*, (1999) observed that the release of serotonin is current-dependent and not lateralized. Serotonin release at a current of <50 μA (0.5ms, 50 Hz) (Hentall et al., 2006) into the lumbar spinal-cord and a current of < 15 μA (20 pulses, 100 Hz) (Bunin & Wightman, 1998b) into the substantia nigra reticulata and dorsal raphe nucleus were usually found to be ineffective. Increases in serotonin release were dependent from 15-300 μA (20 pulses, 100 Hz) and then a continued release of serotonin with no increasing response was observed to occur at a current >300 μA (Bunin & Wightman, 1998b). With a stimulation of 350 μA (20 pulses, 100 Hz), the maximum concentration of serotonin released in the Dorsal Raphe (DR) was approximately twice that of the Substantia Nigra compacta (SNc) (Bunin et al., 1998a). Compared to the amplitudes needed by dopamine and serotonin, glutamate release was significant at quite high amplitudes of 500 and 700 μA (0.5 sec trains of 0.1 msec pulses with an intertrain interval of 2 sec, 100 Hz) (You *et al.*, 1998).

In the rat model, the amplitude strength needed to obtain maximal neurotransmitter release depends on the animal's conscious state; whether the rat is chronically implanted with an electrode and conscious when the stimulation occurs, or the rat is anesthetized during stimulation. Garris *et al.*, (1997) did a comparison and observed that an amplitude of ±125 μA (1.0 s, 60 Hz) in chronically implanted rats during surgery was needed to achieve maximal dopamine release, while an amplitude of

± 300 μA (1.0 s, 60 Hz) was required in anesthetized rats. Current also has a different effect on male versus female animal test subjects. In the caudate nucleus, maximal dopamine concentrations (2 ms phases, 60 Hz) tended to plateau at lower currents, 200 μA, in female rats compared to that in male rats (300 μA) (Walker *et al.*, 2000).

### C.3.3 Pulse number and stimulation train

Fewer studies have examined the influence of varying pulse number and stimulation train on neurotransmitter release relative to those considering the influence of frequency and amplitude. In fact, a number of studies omit this information altogether preventing any definitive conclusions to be made from such limited observations.

John *et al.*, (2006) observed that peak-evoked neurotransmitter release is pulse number and frequency dependent in all midbrain regions studied and not dependent upon the absolute duration of stimulation. Moreover, Kuhr (1986), found that a 10s (60 Hz 100 μA) stimulation train showed a linear increase of dopamine release, whereas increasing this stimulation train to 30s did not further influence the outcome.

Hentall *et al.*, (2006) observed that a 20 s pulse train (50 Hz, 150 μA, 0.5 ms pulses) caused an increase in monoamine oxidation, compared to basal levels. Increasing the pulse width from 0.1 to 0.5 ms (50 Hz, 150 μA) caused a higher peak oxidation current, but a further increase from 0.5-1 ms produced little additional rise in peak oxidation current. Bunin *et al.*, (1998a) also observed that serotonin release increased with pulse widths of increasing duration (using 20 pulses at 100 Hz) with maximal serotonin increase occurring by augmenting the pulse number from 1-20 at 100 Hz, with

slight reduction in transmitter release per impulse later in the 20 s pulse train at all frequencies from 10-100Hz. Lee *et al.*, (2006) noted 15 pulses elicited stimulus-time locked increases in striatum dopamine release (50 Hz, 300 μA), whereas extended duration of stimulation to 1000 pulses evoked a brief increase in striatal dopamine efflux that was not stimulus-time-locked.

## C.4. Literature review of human studies

### C.4.1 Stimulation modalities

Irrespective of the mode of action, electrical or magnetic, these techniques involve altering neuronal activity. Deep Brain Stimulation (DBS), Vagal Nerve Stimulation (VNS) and Transcranial Magnetic Stimulation (TMS) all have the ability to elicit or inhibit neuronal firing, thus affecting the neurotransmitter concentration in different regions of the brain. Since neuropsychological and movement disorders have been extensively studied in animal, clinical and pharmaceutical studies, it has been possible to identify the neurotransmitter(s) involved in these disorders. This information provides a starting point when looking at stimulation modalities as treatment for neuropsychological and movement disorders. Please refer to Tables C.2a and C.2b.

C.2a

| Author | Psychological/Movement Disorder | Neurotransmitters involved |
|---|---|---|
| Merrill et al., 2006 | Alzheimer's | Acetylcholine, glutamate, norepinephrine, serotonin |
| Raike et al., 2005 | Dystonia | Dopamine |
| Ghijsen, 2007 | Epilepsy | GABA |
| Benito-Leon & Louis, 2006 | Essential Tremor | GABA, dopamine |
| Bohnen & Frey, 2007 | Parkinson's Disease | Dopamine |

C.2b

| Author | Psychological/Movement Disorder | Neurotransmitters involved |
|---|---|---|
| Bailer & Kaye, 2003 | Anorexia/Bulimia | Serotonin, dopamine, norepinephrine |
| George et al., 2002 | Anxiety | Norepinephrine |
| Nutt 2006; Velasco et al., 2005 | Depression | Dopamine, serotonin, noradrenaline |
| Shiah & Yatham 2000 | Mania | Serotonin |
| Dickel et al., 2007 | Obsessive Compulsive Disorder | Serotonin |
| Lewis & Lieberman, 2000; Carlsson et al., 2001 | Schizophrenia | Dopamine, noradrenaline, serotonin, acetylcholine, glutamate, GABA |
| Singer & Minzer, 2003 | Tourette's Syndrome | Dopamine, glutamate, GABA, serotonin |

**Table C.2** Neurotransmitter and ND pairings. A: Movement disorders paired with their respective neurotransmitters.

B: Neuropsychological disorders paired with their respective neurotransmitters.

### C.4.2 Deep Brain Stimulation (DBS)

DBS employs chronically implanted electrodes in the brain to electrically stimulate specific neuronal networks. In the 1960s, an early version of DBS that acted by lesioning neuronal networks was explored. By the early 1970s, DBS was used to therapeutically treat movement disorders and epilepsy (Perlmutter & Mink, 2006). Long-term chronic DBS treatment, using an implanted technology similar to a pacemaker, combined with chronically implanted deep brain electrodes, was finally developed and extensively studied by Benabid *et al.*, (1996). The components of the DBS equipment include an electrode(s) (depending on unilateral or bilateral stimulation) that is approximately 1.25 mm in diameter with four contacts, a pulse generator/battery and an extension connecting the electrode and the generator/battery (see Andrews, 2003 for a full description) In 1997, DBS treatment received Food and Drug Administration (FDA) approval in the United States (Andrews, 2003).

DBS is currently being explored as a treatment option for movement disorders and neuropsychiatric disorders including essential tremor, PD, Dystonia, Tourette Syndrome (TS), Obsessive-Compulsive Disorder (OBS) (Perlmutter & Mink, 2006) and Depression (Wichmann & Delong, 2006). The treatment of these movement and neuropsychiatric disorders involve the electrical stimulation of specific neuronal networks in the brain.

The benefit of DBS over other medical interventions is that it is a non-destructive and reversible treatment (Bittar, 2006). Since the effects are reversible, and the treatment is adaptable, DBS allows for future treatment options including neural grafts or stem cell

implantations (Benabid, 2003). When compared with pharmacological treatment, DBS has a lower risk of producing neurological deficits and can be used in conjunction with a drug regimen to obtain optimal results. A further advantage of DBS over other treatment options are stimulation that parameters can be easily adjusted to fit a patient's individual needs, providing maximum benefits and minimum side effects (Andrews, 2003). Furthermore, DBS should not exclude patients from future restorative therapies (Bittar, 2006). Concerning possible side effects of DBS, there is the small possibility of surgical complications, such as intracranial hemorrhage, infection, as well as stimulation-induced effects including delirium, mood changes (including depression and mania), and movement disorders (Holtzheimer & Nemeroff, 2006).

There is a great deal of speculation over what effects DBS have on the brain to function as a viable treatment. For patients suffering from movement disorders (PD, dystonia, essential tremor) DBS may replace tissue ablation in the basal ganglia and the thalamus. The permanent lesioning of neural tissue, ostensibly inhibiting neural activity has been a hypothesized mechanism behind with DBS mimicking this ablation, without causing permanent damage. Another hypothesis has been put forward that the therapeutic properties of high-frequency DBS, in both white and grey matter targets, are caused by an excitatory axonal response (Hardesty & Sackeim, 2006). Benabid (2003) has speculated that the mechanism of DBS is based on a combination of inhibition and excitation, which causes neuronal jamming and silencing, as well as axon stimulation.

McIntyre *et al.*, (2004) have been studying how DBS produces its therapeutic effects, and have been exploring the inhibition and excitation of neurons as a cause, citing

specific hypotheses in the literature (Table C.3) including depolarization blockade, synaptic inhibition and synaptic depression.

Garcia *et al.*, (2005) have suggested that the improvement of movement disorders after High Frequency Stimulation (HFS) DBS, a frequency between 120-180 Hz, is due to "parallel non-exclusive actions: silencing of ongoing activity and generation of an activity pattern in the gamma range". By switching off a disrupting activity, one that would cause the movement disorder, it is then possible to apply a new type of frequency that has beneficial effect. This has particular relevance in PD where there is abnormal synchronization and irregular discharges in the subthalamic nucleus that must be turned off to achieve therapeutic benefit (Garcia *et al.*, 2005).

### *C.4.2.1 DBS tissue targets*

Tissue targets are an important consideration when determining stimulation parameters. Common tissue targets in movement disorders have been the thalamus and ventralis intermediate nucleus (VIM), the globus pallidus (GPi) (Bittar, 2006) and the subthalamic nucleus (STN) (Perlmutter & Mink, 2006) respectively. Specific tissue targets have also been discovered for certain psychological disorders. The tissue targets for OBS are the internal capsule (Gabriels *et al.*, 2003; Abelson *et al.*, 2005; Cosyns *et al.*, 2003; Nuttin *et al.*, 1999, 2003; Tass, 2003; Abelson, 2005; Greenberg, 2006; Okun, 2007) and the ventral caudate nucleus (Aouizerate *et al.*, 2005). Targets being explored for depression are the subgenual cingulate (Mayberg, 2005) and the inferior thalamic peduncle (Jimenez *et al.*, 2005). For TS, sites that have been targeted include the

thalamus (Houeto *et al.*, 2005; Visser-Vandewalle *et al.*, 2003; Diederich *et al.*, 2005), the GPi (Diederich *et al.*, 2005; Houeto *et al.*, 2005) and the anterior limb of the internal capsule (Flaherty *et al.*, 2005).

In addition to classic NDs such as PD, there has been some interest in treating affective disorders. Major depression that is resistant to pharmacological intervention has been investigated using brain stimulation. A specific tissue target for depression that has been highlighted as being resistant to treatment is the subgenual anterior cingulate cortex (ACC) of the Brodmann area 25 (BA25) (Giacobbe & Kennedy, 2006). It is hypothesized that successful treatment of depression may rely upon some combination of deactivation of the ventral compartment and then reactivation of the dorsal compartment that would lead to an enhanced efficacy of the BA25 (Giacobbe & Kennedy, 2006). On the other hand, neuroimaging and neurocircuitry models have suggested that depression results from a dysfunction within a frontal cortical-subcortical-brainstem neural network (Holtzheimer & Nemeroff, 2006; Mayberg, 2003; Drevets & Raichle, 1992; Rauch, 2003).

### *C.4.2.2 DBS stimulation parameters*

The aim of DBS programming is to achieve optimal parameter settings to provide maximal symptom suppression, minimal side effects and maximal neurostimulator battery life (Volkmann *et al.*, 2002).

| Author | Theory | Description |
|---|---|---|
| Beurrier et al., 2001 | Depolarization blockade | Stimulation-induced alterations in the activation of voltage-gated currents that block neural output near the stimulating electrode. |
| Dostrovsky et al., 2003 | Synaptic inhibition | Indirect inhibition of neuronal output by means of activation of axon terminals that make synaptic connections with neurons near the stimulating electrode. |
| Montgomery & Baker 2002 | | Stimulation-induced disruption of pathologic network activity. |
| Urbano et al., 2002 | Synaptic depression | Synaptic transmission failure of the efferent output of stimulated neurons as a result of transmitter depletion. |

**C.3** Neuron inhibition and excitation theories

When using DBS for movement disorders, a stimulating parameter is applied as a waveform of adjustable duration, with a pulse width between 60-450 μsec, an amplitude with a voltage between 0-10.5 V, and a pulse frequency varying between 2-250 Hz (Hardesty & Sackeim, 2006). Andrews (2003), in a review of DBS, found more precise stimulation parameters for those suffering from movement disorders. He found that a pulse width between 60-90 μsec with an amplitude of 3.5 V, and a frequency rate of 130-185 Hz provided the best results. Volkmann *et al.*, (2002) found that a pulse width of approximately 65 μsec was ideal for thalamic stimulation and that a pulse width of 75 μsec was ideal for pallidal stimulation. In addition, frequencies above 50 Hz were necessary. Rizzone *et al.*, (2001) made a more detailed study of the variations within stimulation parameters in DBS treatment of the subthalamic nucleus in PD. This study looked at pulse width values of 60, 120, 210 and 410 μs and frequency values of 10, 50, 90, 170 Hz. They observed that increasing the frequency rate from 90 Hz to 130 and 170 Hz, at every pulse width value, progressively decreased the intensity of the stimulus necessary to reach a non-significant effect. Moreover, lengthening the pulse width from 60 μs to 210 or 450 μs progressively reduced the stimulus intensity necessary to obtain an effect.

Medtronic Inc. uses the following programming settings for the Activa Parkinson's Control Therapy: the typical final setting for amplitude is 2.5-3.5 V, the pulse width is between 60-120 μsec, and the rate (pulses per second) is 130-185 Hz. The polarity of the electrode depends upon the site of stimulation. STN patients use unipolar therapy, whereas GPi patients use unipolar or bipolar therapy (Medtronic Inc., Implant

Manual, 2006). The programming settings for the Activa Tremor Control Therapy are 1-3 V, 60-90 μsec, 130-185 Hz, with bipolar polarity.

DBS therapy is being explored as a treatment for psychiatric diseases; however, its therapeutic effects have not yet been established and the stimulation parameters are yet to be specified. Developments in the study of OCD, depression, and TS are underway. The effects of various stimulation parameters are described in Table C.4

### C.4.3 Vagal Nerve Stimulation (VNS)

Vagal nerve stimulation (VNS) became a beneficial form of therapy when it was initially developed as a treatment for epilepsy. FDA approval was granted in 1997 for the use of VNS as an anti-epileptic therapy and in 2005 for the treatment of resistant depression (Carpenter et al., 2006). The appeal of this therapy (which consists of using an electrode and a pulse generator/battery with a connecting extension or lead) is that the side effects are minimal. This is of particular interest for epileptic patients who must deal with the side effects of antiepileptic medications, such as sedation and allergic reaction (Gildenberg, 2005).

The electrode in contact with the vagus nerve is composed of three helical coils that encircle the nerve in the neck. The positive electrode comes in contact with the cephalad, the negative electrode with the immediate caudad and the anchoring coil at the further caudad. It is the left vagus that is primarily used since it has more afferent fibers (and less cardiac effect on stimulation) than the right vagus nerve (Andrews, 2003).

### *C.4.3.1 VNS Tissue Targets*

Despite clinical success with treating epilepsy and depression, the specific mechanism underlying VNS relief of neuropsychiatric symptoms is currently unknown. However, animal studies suggest that seizure relief post-VNS may be related to locus coeruleus activation (Krahl *et al.*, 1998; Labiner & Ahern 2007). Research has shown that the locus coeruleus is the major site in the brain in which norepinephrine-producing cells are localized and that these neurons have wide-ranging axons extending to the central tegmental tract in the brainstem, the thalamus, the hippocampus, amygdala and neocortex (Harden *et al.*, 2000; Van Brockstaele *et al.*, 1999). Dorr & Debonnel (2006) observed that VNS treatments induce large time-dependent increases in basal neuronal firing. These increases occur in the brainstem nuclei, releasing serotonin and norepinephrine into the dorsal raphe nucleus and locus coeruleus respectively. Follesa et al., (2007) have further clarified this potential mechanism, observing that acute VNS increased the norepinephrine concentration in the prefrontal cortex of the rat. It also increased neurotrotrophic factor and fibroblast growth factor in both the hippocampus and neocortex and decreased the abundance of nerve growth factor in the hippocampus.

Given its effect on the hippocampus, it has been suggested that VNS may also affect memory processes. Zuo *et al.*, (2007) suggest that neural activity in the vagus nerve, occurring as a result of changes in peripheral state, is an important mechanism by which emotional experiences and arousal can enhance the storage of memories of those experiences. Other areas of the brain that appear to be affected by VNS include the

limbic and paralimbic regions, areas of the brain that modulate mood in normal and abnormal populations (Phan *et al.*, 2004).

Harden et al., (2000) documented that VNS may have an effect on mood in epilepsy patients as measured by multiple mood and anxiety evaluations. It was hypothesized that stimulation of the noradrenergic system in the brain, leads to an increase in norepinephrine release, thus improving depression symptoms *and* having an antiseizure effect (Bunney & Davis, 1965; Krahl *et al.*, 1998). Alternatively, it is also possible that noradrenaline in the amygdala is increased by the direct projections of the noradrenergic neurons of the nucleus of the solitary tract (the A2 noradrenergic cell group), which project to the amygdala (Herbert and Saper, 1992) as well as the locus coeruleus.

In addition to VNS modulation of norepinephrine, effects have been found on other neurotransmitters such as serotonin, and GABA (Dorr & Debonnel, 2006; Groves & Brown, 2005; Harden *et al.*, 2000; Ben-Menachem *et al.*, 1995; Morossu *et al.*, 2003). As such, there is a suggestion that VNS can not only be an effective treatment for seizure but other neuropsychiatric disorders, which are associated with the aforementioned neurotransmitters. Modulation of the serotonergic system is certainly a possibility, as the main relay nuclei for the vagus nerve are the nuclei of the solitary tract, which in turn has a projection to the raphe nuclei. The cell bodies of the raphe nuclei are nearly the complete source of serotonin. There is evidence that the GABA receptor density in the hippocampus was significantly increased in responsive patients compared to the controls and non-responders (Marrosu *et al.*, 2003).

Summary Table C.4

| Author | Disorder | Polarity | Amplitude (Volts) | Frequency (Hz) | Pulse width (μsec) | Brain area stimulated | Effect |
|---|---|---|---|---|---|---|---|
| Nuttin et al., 1999 | OCD | Unipolar | 5 | 100 | 210 | Internal capsule | Reduction in OBS behavior |
| Nuttin et al., 2003 | OCD | Bipolar | 4-10.5 | 100 | 210 or 450 | Anterior limbs of the internal capsule | 3 of 6 patients responded with improvement |
| Ander-son & Ahmed, 2003 | OCD | Bipolar | 2 | 100 | 210 | Anterior capsule | All compulsions abated, no adverse effects |
| Tass et al., 2003 | OCD | Unipolar | 2 and 4 | 130 (square wave) | 90 | Ventral part of the anterior limb of the internal capsule and the nucleus accumbens and the rostro-caudal part of the bed nucleus of the striterminalis | Attenuated OCD symptoms in 2 of 3 patients |
| Abelson et al., 2005 | OCD | Unipolar and bipolar | 3.0-10.5 | 130 | 210 | Base of the internal capsule, at its junction with the nucleus accumbens | 3 patients showed improvement 1 patient had no significant result |

Summary Table C.4

| Author | Disorder | Polarity | Amplitude (Volts) | Frequency (Hz) | Pulse width (μsec) | Brain area stimulated | Effect |
|---|---|---|---|---|---|---|---|
| Greenberg et al., 2006 | OCD | Monopolar and bipolar | 2-6 | 130 | 90 and 210 | Anterior limb of the internal capsule just anterior to the rostral border of the anterior commissure in the coronal plane | Yale-Brown Obsessive Compulsive Scale scores decreased during DBS. |
| Okun et al., 2007 | OCD | Monopolar | 0,2,4,6,8 | 135 | 210 | Internal capsule and nucleus accumbens region (ventral regions) | Best results at lower voltage. Improves mood in the more ventral regions. |
| Mayberg et al., 2005 | Depression | Monopolar and Bipolar (unilateral/ Bilateral) | Progressively increased up to 9 | 130 | 30-250 60 | Subenual cingulated white matter (cg25WM), BA25 | Mean stimulation parameters at 6 months: 4V, 60 μsec, 130 Hz. Antidepressant response in 4/6 patients. |

| Author | Disorder | Polarity | Amplitude (Volts) | Frequency (Hz) | Pulse width (μsec) | Brain area stimulated | Effect |
|---|---|---|---|---|---|---|---|
| Diederich et al., 2005 | Tourette's syndrome | Bilateral and bipolar | 2 | N/A | 185 pulses per second 60 μsec in both channels to start, later 150 μsc in channel 1 and 120 μsc in channel 2 | Thalamic nuclei, globus pallidus, postero-ventrolateral | Decrease in mean tic frequency by 73% by Yale Global Tic Severity Scale scores |
| Acker-mans et al., 2006 | Tourette's syndrome | Patient 1: bilateral Patient 2: quadropolar | 6.4<br>3.1 | 130<br>170 | 120<br>210 | Patient 1: bilateral in the thalamus at the level of the nucleus ventro-oralis internus, the centromedian nucleus and the substantia periventricularis Patient 2: 4 electrodes implanted; 2 at the thalamic nuclei and 2 at the posteroventrolateral globus pallidus | In both patients, all major tic and compulsions disappeared. |

**C.4** DBS stimulation parameters for OCD, Depression and Tourette's syndrome.

In addition, VNS has also been shown to increase the levels of free GABA (Ben-Menachem *et al.*, 1995) and the serotonin metabolite 5-hydroxyindolacetic acid (Ben-Menachem *et al.*, 1995; Hammond *et al.*, 1992) in the cerebrospinal fluid.

### *C.4.3.2 VNS electrical stimulation parameters*

VNS has the potential to treat a variety of psychiatric disorders; however, the stimulation parameters used to treat these disorders have not yet been precisely determined. In the two FDA approved VNS treatments, both epileptic and depression VNS programming parameters have been established, although there are slight variations across studies. The typical values for VNS therapy in epilepsy, is a current between 1-2 mA, a rate between 20-30 Hz, a pulse width of 250-500 μsec, and a duty cycle of 10% (signal ON time of 30 seconds, and a signal OFF time at 5 minutes) (Andrews, 2003; Labiner and Ahern, 2007).

Few studies have significantly advanced other stimulation parameters, although some do exist for migraine and Alzheimer's (Table C.5). There are currently no publications on stimulation parameters for anxiety disorders or eating disorders in humans and VNS. However, there have been suggestions that VNS can be used as a treatment option in these areas. In an animal model, Sobocki et al., (2006) concluded that VNS affects body weight at the expense of body fat resources, without affecting metabolic rate. The stimulation parameters for this study were monopolar current impulses with amplitudes of 4 V, 0.5-ms duration, and frequency of 34 Hz supplied every 3 or 4 hours during a 24 hour period. In addition, Faris *et al.*, (2006) suggest that cyclic

increases in vagal activity drive the urge to binge-eat and vomit and thus, bulimia is a result of alternate vagal firing patterns. To date, no clinical studies have evaluated the effectiveness in alleviating eating disorders.

### C.4.4 Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive method of inducing current within the brain to alter neural activity (Maeda et al., 2003). Its use within both neuroscience and neuropsychiatry have grown exponentially over the last decade, with nearly 1500 published papers appearing in PubMed on "TMS" and "treatment". Clearly it is beyond the scope of this review to consider all of them.

Our aim is more modest, as we hope to consider the specific parameters associated with the treatment or alleviation of symptoms in various NDs. With its ability to quickly modulate brain function, it has been suggested that TMS can be a powerful therapeutic agent, particularly in movement and affective disorders. There has been success in alleviating symptoms in PD, depression, mania, OCD, TS and Schizophrenia; however, these are general observations and the neurobiological mechanism for these findings is unknown. By understanding the parameters associated with successful treatment, hopefully further light can be shed on the etiology of the various disorders.

| Author | Disorder | Current (mA) | Frequency (Hz) | Pulse width (μsec) | Duty cycle | Effect |
|---|---|---|---|---|---|---|
| Sadler et al., 2002 | Migraine<br><br>Migraine + Epilepsy | 0.25<br><br>1.75 | 30<br><br>20 | 500<br><br>250 | 'on' time 30s every 5 minutes<br>'on' time 7s, 'off' time 12s | Migraine attacks for case study (male), beneficial effect of VNS noted 8 weeks after implantation of stimulator |
| Hord et al., 2003 | Migraine | 1.0 | 30 | 500 | 30 s trains every 5 minutes | Antinociceptive effect was noticed 1 to 3 months after initiation of VNS in all 4 patients |
| Sjogren et al., 2002 | Mild to moderate Alzheimer's | 0.25 increased to 0.5 | 20 | 500 | 'on' 30s every 5 minutes | 3 and 6 months, treatment with VNS resulted in an improvement in cognitive functions, as measured by the change in total Alzheimer's Disease Assessment Scale Cognitive Subscale. |

**Table C.5** VNS Stimulation parameters for migraine, epilepsy and Alzheimer's disease.

### *C.4.4.1 TMS, neurotransmitters and mechanism*

The mechanism of TMS effects are not clear. Most basically, it achieves its effect by stimulating the cortex with a strong time-varying magnetic field (George *et al.*, 2004) with the aim of inducing electrical currents within the brain that can depolarize neurons or their axons. A coil of wire that can vary in terms of shape, size and orientation is placed above the scalp. According to Sekino & Ueno (2004), to obtain current distribution in larger and deeper areas, it is best to position a large, circular coil on the forehead. A strong electrical current is passed through the coil and the rapidly changing current creates a magnetic field (Rossini & Rossi, 2007). This magnetic field therein causes induced electrical currents intracranially within the cortex, achieved by using specific stimulation parameters such as the current amplitude, duration and direction (Andrews, 2003).

By repeating the stimulation rapidly for several seconds, "repetitive" transcranial magnetic stimulation (rTMS) is achieved. rTMS uses the motor threshold (MT), one of the most important parameters for stimulus application by its use in standardizing stimulus intensities between individuals and by defining application safety ranges (Hanajima *et al.*, 2007). The MT is the minimum intensity of stimulation required to induce a twitch of at least 50 μV in peak-to-peak amplitude in a muscle of interest (Maeda *et al.*, 2003). The resting MT (RMT), observed in relaxed muscle, is when there is a 50% probability of producing a response. The active motor threshold (AMT) in active muscle, in comparison to RMT is more difficult to define. AMT is the intensity at which motor evoked potentials (MEP) with an amplitude of around 200-300 μV can be

distinguished from the background activity in 50% of trials (Hanajima *et al.*, 2007; Rothwell *et al.*, 1999). For a detailed comparison of different methods for estimating MT in TMS applications, please refer to Hanajima *et al.*, (2007). Interestingly, Arai *et al.*, (2007) observed that the use of AMT or RMT and the use of monophasic and biphasic waveforms can have different effects on MEPs. After the use of 90% AMT, it was observed that MEPs were enhanced for a few minutes after both monophasic and biphasic rTMS. On the other hand, the enhancement of MEPs were larger and longer after monophasic rTMS at 90% RMT.

rTMS frequencies are generally differentiated into fast (>1 Hz) and slow (1 Hz or less) with specific effects associated with each (George *et al.*, 2004). Other parameters include the waveform of the magnetic field (biphasic or monophasic), the strength of the maximum magnetic field, the coil type, the stimulation intensity (percentage of the subject's MT), and the inter-train intervals (time between trains of rTMS).

To better understand how TMS exerts its biological effects, computational simulations have been developed to calculate hypothesized effects on arbitrary neuronal structures. A few different models have been proposed. In one model, Kamitani *et al.*, (2001) observed that a single magnetic pulse applied to a model of cortical neurons can induce a brief firing burst followed by a silent period of duration, which this group compared to TMS data. They suggested that calcium influx followed by the opening of calcium-dependent potassium channels was responsible for the bursting followed by a long hyper-polarization period. Miyawaki & Okada (2004) analyzed the response of a simple neuron network model of a sensory feature detector system to a TMS-like perturbation and observed that a TMS-like perturbation could *suppress* neuronal activity.

Another mechanism of action was proposed when TMS was used in an *in vitro* experiment. Ikeda *et al.*, (2004) observed at the cellular level that mRNA expression changes in monoamine transporter genes, which are targets for antidepressants and psychostimulants. They observed that chronic rTMS modulated the monoamine transporter genes, thus affecting the termination of monoaminergic synaptic transmission by rapid-take up of serotonin, dopamine, and norepinephrine.

Suggestions that TMS exerts effects through activation and inhibition have also been investigated in human studies. Shimamoto *et al.*, (2001) observed that rTMS is beneficial for PD symptoms and that it may act via inhibition of dopaminergic systems. Both Hallett (2000) and Gilbert *et al.*, (2005) have also noted a cortical inhibitory mechanism. When TMS was applied to the motor cortex, a MEP was produced. The MEP amplitude can be inhibited by activating inhibitory interneurons with a subthreshold stimulus given at 2-5 sec (Gilbert *et al.*, 2005) or at >5 seconds (Hallett 2000). Another example of inhibition was observed by Lang *et al.*, (2007) who found that continuous 1 Hz rTMS to the human motor cortex induces a transient decrease in corticospinal excitability. Intracortical inhibition is probably largely mediated by neurons containing GABA (Hallett 2000).

TMS has also been found to induce neurotransmitter release of dopamine and glutamate in an animal model (Zangen & Hyodo 2002). Dopamine and glutamate were increased in the nucleus accumbens of the rat when TMS was applied either over the frontal or caudal cortex, with acetylcholine concentrations being unaltered. An increase in glutamate concentrations after rTMS was also observed in human patients with unipolar major depression; state-dependent changes within the left dorsolateral prefrontal

cortex were involved in the glutamate system and it was determined that glutamate release can be reversed in a dose-dependent manner by rTMS (Luborzewski *et al.*, 2006). Michael et al., (2003) observed that changes in glutamate/glutamine levels are dependent on the pre-transcranial magnetic stimulation glutamate/glutamine concentrations. For example, when glutamine levels are minimal, there was a greater increase in glutamate/glutamine after short-or long-term stimulation. rTMS is also capable of modulating measures of motor cortex excitability which represent central inhibitory mechanisms related to the GABAnergic system (Maeda *et al.*, (2000). Various stimulation parameters and stimulation sites are being explored by rTMS for dopamine (Shimamoto *et al.*, 2001; Keck *et al.*, 2002; Zangen *et al.*, 2002; Strafella *et al.*, 2003; Pogarell *et al.*, 2005; Khedr *et al.*, 2007), glutamate (Zangen *et al.*, 2002; Michael *et al.*, 2003; Luborzewski *et al.*, 2007), glutamine (Michael *et al.*, 2003), serotonin (Sibon *et al.*, 2007), and GABA (Shimamoto *et al.*, 2001) release (please see Table C.6).

### *C.4.4.2 TMS Tissue targets*

A select group of brain areas are currently being explored as possible TMS stimulation sites for the treatment of movement and affective disorders. For PD, stimulation of the motor cortex (Mally and Stone, 1999a; Siebner *et al.*, 2000), primary motor area (Khedr *et al.*, 2007), left motor cortex (Lefaucheur *et al.*, 2004), dorsal premotor cortex (Buhmann *et al.*, 2004) or the bilateral frontal and occipital cortex (Ikeguchi *et al.*, 2002) have had beneficial effects upon Parkinsonian symptoms.

Summary Table C.6

| Author | Disorder/ neurotrans -mitter | Condition | Frequency and train | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Michael et al., 2003 | Glutamate and glutamine | Single session and 5 conse-cutive sessions | 20 Hz, 20 min/day 20 2s trains separated by 58s inter-train intervals | N/A | N/A | N/A | 80% MT | 800 | Left DPC | LDP cortex, right, DLPC, left cingulate cortex | rTMS may act via stimulation of glutama-tergic prefrontal neurons. |
| Lubor-zewski et al., 2007 | Unipolar major depression and glutamate | 10 days | 20 Hz, 50 trains of 2s | N/A | N/A | N/A | 100%RM T | 2000 | Left DPC | N/A | State-dependent changes, reversed in a dose-dependent manner by rTMS. |
| Zangen et al., 2002 | Dopamine and glutamate | non-repetitive 1 session | 2 | 90 | Mono-phasic | Electric field intensity of 500V/m | 98% of maximu m output | 200 | Frontal or caudal cortex | Nucleus accum-bens | Increase in dopamine and glutamate |

Summary Table C.6

| Author | Disorder/ neuro-transmitter | Condition | Frequency and train | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Shima-moto et al., 2001 | PD and dopamine | 1/week for 2 months | 0.2 Hz | N/A | N/A | 700 Volts | 100% RMT | 60 total, 30 per side of frontal area | Frontal areas | Lumbar cerebro-spinal fluid | PD symptoms alleviated |
| Keck et al., 2002 | Dopamine | 1 session | 20 trains at 20 hz for 2.5 s, intertrains 2.5 min | N/A | N/A | 120A/μs magnetic induction field max. 4T | 130% MT | 1000 | Left frontal cortex | Meso-limbic and meso-triatal system | Significant modulation of release patterns of dopamine in both systems |
| Stra-fella et al., 2003 | Dopamine | 3 rTMS blocks | 10 Hz, 15, 10-pulse trains of 1s duration, 10 mins apart | N/A | N/A | N/A | 90% RMT | N/A | Motor area 1 or the left occipital cortex | Ipsi-lateral putamen | Induced release of dopamine |

| Author | Disorder/ neuro-transmitter | Condition | Frequency and train | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Poga-rell et al., 2006 | Dopamine | Initial session, 5 sessions per week over 3 weeks with 1500 stimuli daily | 10 Hz, 30 trains of 10 s with 30s intertrain interval each | N/A | N/A | N/A | 100% RMT | 3000 | Left DPC | Striatum | Release of endogenous dopamine. |
| Khedr et al., 2007 | PD and dopamine | 6 sessions, once a day; 6 consecu-tive days | 25 | N/A | Biphasic | N/A | 100% RMT | 1000 for each hemi-sphere and extensor | Extensor digitorum brevis, right and left hemis-pheres | N/A | Increases in serum dopamine after six rTMS sessions. |

**Table C.6** TMS stimulation parameters and neurotransmitter release in humans. DPC: Dorsolateral prefrontal cortex; PD: Parkinson's Disease; MT: Motor Threshold, RMT: Resting Motor Threshold

For those suffering from depression, stimulation of the prefrontal cortex (Dragasevic *et al.*, 2002; Bortolomasi *et al.*, 2007), dorsolateral prefrontal cortex (Freighi *et al.*, 2004), left dorsolateral prefrontal cortex (George *et al.*, 2000; Avery *et al.*, 2006), right dorsolateral prefrontal cortex (Januel *et al.*, 2006), a combination of the right and then left dorsolateral prefrontal cotex (Fitzgerald *et al.*, 2006) have had antidepressant effects. A combination of left and right prefrontal cortex stimulation (Grisaru *et al.*, 1998; Garcia-toro *et al.*, 2006) has helped those suffering from depression as well as those suffering from mania. Obsessive-compulsive urges were relieved when the supplementary motor area (Mantovani *et al.*, 2006) or the left or right lateral prefrontal cortex (Greenberg *et al.*, 1997) were stimulated by TMS. To reduce hallucination frequency in those suffering from Schizophrenia, the left temporoparietal cortex was stimulated (Hoffman *et al.*, 1999, 2000, 2005; Chibbaro *et al.*, 2005). Moreover, stimulation of the left auditory cortex (D'Alfonso *et al.*, 2002) reduced auditory hallucinations. Other sites of the brain that should be explored by TMS in the treatment of Schizophrenia are the left anterior cingulate and the thalamus. According to a study performed by Theberge *et al.*, (2003) using the 4.0T proton $H^1$ magnetic resonance spectroscopy, those suffering with Schizophrenia had an imbalance in glutamate and glutamine in the anterior cingulate and glutamine in the thalamus.

### *C.4.4.3 TMS electrical stimulation parameters*

Studies focusing upon further understanding the stimulation parameters in TMS studies on animal models are numerous. Using microdialysis, Keck *et al.*, (2007) and

Kanno *et al.*, (2004) have observed dopamine release in the dorsal hippocampus and the dorsolateral striatum respectively in the rat after rTMS. Research by Kanno *et al.*, (2003) has observed serotonin release in the rat prefrontal cortex after rTMS. Stimulation parameters for dopamine release vary slightly between both studies with Keck et al., (2007) using a rTMS stimulation of 20 trains of 20 Hz, 2.5 seconds intervals and Kanno *et al.*, (2004) using a rTMS stimulation of 25 Hz for 1 second with 1 min intervals between trains with a total of 500 stimuli. Research led by Kanno et al., used the same stimulation parameters for dopamine release (2004) as for serotonin release (2003).

Finally, to understand rTMS as a therapeutic tool in a variety of psychiatric and neurological disorders, Post *et al.*, (1999) explored the mechanisms underlying rTMS and the potential therapeutic and possible adverse effects of its use. After 11 weeks of long-term rTMS treatment, there were no cognitive impairments or structural alterations in rat brains. In addition, this group suggests that rTMS could reduce detrimental effects of oxidative stressors such as amyloid beta and glutamate that can result in neuronal damage.

TMS has shown some success in alleviating symptoms with PD, depression, mania, and OCD and Schizophrenia. However, there are vast differences in stimulation parameters including frequency, intensity and number of stimuli pulses being used (please refer to Table C.7).

Frequency intensities explored for treatment range from 1 Hz up to 20 Hz. PD (Mally & Stone, 1999(a)(b); Siebner *et al.*, 2000; Khedr *et al.*, 2003; Buhmann *et al.*, 2004; Ikuguchi *et al.*, 2002, Lefaucheur *et al.*, 2004), depression (Dragasevic *et al.*, 2002; Fregni *et al.*, 2004; Bortolamasi *et al.*, 2007; Garcia-toro *et al.*, 2006; George *et al.*, 2000;

Fitzgerald *et al.*, 2006; Januel *et al.*, 2006; Avery *et al.*, 2006), OCD (Greenberg *et al.*, 1997; Mantovani *et al.*, 2006) and TS (Mantovani *et al.*, 2006) are using frequencies ranging between 1 and 20 Hz, mania (Grisaru *et al.*, 1998) is exploring 20 Hz, and Schizophrenia (Hoffman *et al.*, 1999, 2000, 2005; d'Alfonso *et al.*, 2002; Chibbaro *et al.*, 2005) at 1 Hz.

The motor excitability measures used for stimulation include MT, RMT and ATM. The use of RMT ranges from 80% to 110%. An RMT of 80% was used for PD (Lefaucheur *et al.*, 2004), depression (George *et al.*, 2000), and OCD (Greenberg *et al.*, 1997); an RMT of 90% for PD (Mally & Stone 1999; Siebner *et al.*, 2000), and depression (Januel *et al.*, 2006). Finally RMT at an intensity of 110% was used for depression (Garcia-toro *et al.*, 2006; Fitzgerald *et al.*, 2006; Avery *et al.*, 2006). The MT intensity used in depression were 120% (Khedr *et al.*, 2003), 110% (Fregni *et al.*, 2004) and 90% (Bortolomasi *et al.*, 2007). For Schizophrenia, only a MT of 80% (Hoffman 1999, 2000; D'Alfonso *et al.*, 2002) and 90% (Hoffman, 2005; Chibarro, 2005) were used. Mantovani *et al.*, 2006 used an MT at 100% for OCD and TS. For mania, a MT of 80% was used (Grisaru *et al.*, 1998). The only study that used ATM, at 80%, was Buhmann *et al.*, 2004 for PD.

Clearly, there is a significant variation in stimulation parameters. The number of pulses used per session and the number of sessions used in TMS can all lead to further variation in stimulation effects. Moreover, the risks and safety of rTMS (Wassermann, 1998; Chen *et al.*, 1997), and the basic principles and procedures for routine clinical applications of rTMS (Rossini *et al.*, 1994) must be taken into consideration when determining stimulation parameters.

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sibon et al., 2007 | Serotonin | 3 blocks of stimu-lation delivered 10 mins apart | 15 trains of 10 Hz | N/A | N/A | N/A | N/A | N/A | DPC | Limbic areas, left parahipp ocampus gyrus, right insula, right cingulate gyrus and cuneus | Modulation aspects of serotonin metabolism in limbic areas. |
| Mally & Stone, 1999a | PD | 10 days, 2 a day | 1Hz, 1 ms duration | N/A | N/A | N/A | 60% max output capacity of apparatus (max 2T) | 60 stimuli a day, 30 stimuli per session | Motor cortex | N/A | Significantl y improved symptoms, maintained for three months. |
| Mally & Stone, 1999b | PD | 7 days, 2 a day | 1 Hz | 100 | N/A | ~0.34T, 0.57T, or 0.80T | 90% RMT | 30 | Above vertex of the skull | N/A | Decrease in symptoms, Unified PD Rating Scale. |

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Siebner et al., 2000 | PD | Single session | 15 trains 5 Hz | N/A | Biphasic | N/A | 90% RTM | 2250 | Motor area 1 | N/A | Decrease in symptoms, Unified PD Rating Scale. |
| Khedr et al., 2007 | PD | 1 session a day, 10 days | 1000, 2 pulses at 5 Hz | N/A | N/A | N/A | 120% MT | 1000 and 500 pulses applied to each hemis-phere | Primary motor area | N/A | Lasting improve-ment. |
| Buh-mann et al., 2004 | PD | 1 session | 1 Hz | 300 | Biphasic | N/A | 80% ATM | 1200 | Dorsal pre-motor cortex | Ipsi-lateral Motor area 1 | Induced excitability changes in ipsilateral Motor area 1. |

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ike-guchi et al., 2002 | PD | 6 sessions 1 session, 10 mins a day | 0.2 Hz | N/A | N/A | N/A | 70% maximal output of device | 60 total, 30 per side of frontal area | Bilateral frontal cortex or occipital cortex | N/A | Improve-ment after frontal rTMS. |
| Lefau-cheur et al., 2004 | PD | 1 session | 0.5 Hz, 20 min stimu-lation | N/A | N/A | N/A | 80% RMT | 600 | Left motor cortex | N/A | Improved upper limb rigidity bilaterally and walking. |
| | | 1 session | 10 Hz, 20 trains of 10s (50s intertrain intervals) | N/A | N/A | N/A | 80% RMT | 2000 | Left motor cortex | N/A | Improved bradykin-esia and rigidigy of upper limb. |
| Draga-sevic et al., 2002 | DP in PD patients | 10 days | 0.5 Hz, 0.1 ms 5 series of 20 stimuli | N/A | N/A | N/A | 10% above MT | 100 | PC | N/A | Mild to moderate anti-depressant effects. |

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Borto-lomasi et al., 2007 | DP | 5 sessions for 1 week | 20 2s, 20 Hz | N/A | N/A | N/A | 90% MT | 800 stimuli per day | PC | N/A | Significant reduction of baseline depression. |
| Garcia-toro et al., 2006 | DP | 10 sessions | 1 and 20 | N/A | N/A | N/A | 110% RMT | 3000 | Left and right PC | N/A | Statistically significant changes, Hamilton Rating Scale for Depression. |
| George et al., 2000 | DP | 10 days for 2 weeks | 20 2-s, 20-Hz stimu-lations over 20 min | N/A | N/A | N/A | 80% RMT | 800 per session, 10 sessions per treatmen t phase | Left DPC | N/A | Anti-depressant effect. |

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fitz-gerald et al., 2006 | DP | 6 weeks, 5 days per week of high/low frequency Right side | 1 Hz, 3 trains of 140s, 30s interval between trains | N/A | N/A | N/A | 110% RMT | N/A | Right and then left DPC | N/A | Therapeutic response |
| Januel et al., 2006 | DP | 16 sessions | 1 Hz | N/A | N/A | N/A | 90% RMT | 120 | Right DPC | N/A | Free from medication for over a month. |
| Avery et al., 2006 | DP | 15 sessions | 10 Hz | N/A | N/A | N/A | 110% RMT | 1600 | Left DPC | N/A | Clinically significant anti-depressant. |
| Grisaru et al., 1998 | Mania | 10 days for 2 weeks | 20 Hz, 2s train, 20 trains a day, intertrain interval 1s | N/A | N/A | N/A | 80% MT mean left side 67%, mean right side 72% | N/A | Left PC, right PC | N/A | Suggests right prefrontal cortex stimulation beneficial, |

Summary Table C.7

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Green-berg et al., 1997 | OCD | N/A | 20 Hz for 2s per min for 20 min | N/A | N/A | N/A | 80% RMT | N/A | Left PC or right PC | N/A | Compulsive urges decreased. |
| Mantov ani et al., 2006 | OCD, TS | 10 daily sessions | 1 Hz | N/A | N/A | N/A | 100% MT | 1200 | Supple-mentary motor area | N/A | Statistically significant reductions, Yale-Brown Obsessive Compulsive Scale. |
| Hoff-man et al., 1999 | Schizo-phrenia | N/A | 1 Hz | N/A | N/A | N/A | 80% MT | 2400 | Left temporo -parietal cortex | N/A | Improve-ment in halluci-nation severity. |

| Author | Disorder/ neuro-transmitter | Condition | Train and Frequency | Pulse Width (μsec) | Wave-form | Magnetic field density | Intensity: motor threshold | # of stimuli or pulses per session | Stimu-lation site | Brain region affected | Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hoff-man et al., 2000 | Schizo-phrenia | 4 sessions, 4,8,12, and 16 mins | 1 Hz | N/A | N/A | N/A | 80% MT | 2400 | Left temporo -parietal cortex | N/A | Significant reduction in halluci-nations. |
| Hoff-man et al., 2005 | Schizo-phrenia | 9 sessions, 4, 8,12, and 16 mins from days 4 to 9 | 1 Hz | N/A | N/A | N/A | 90% MT | 7920 | Left temporo -parietal cortex | N/A | Halluci-nation frequency significantly decreased. |
| D'Al-fonso et al., 2002 | Schizo-phrenia | 2 weeks, 10 sessions, 20 mins each | 1 Hz | N/A | N/A | N/A | 80% MT | N/A | Left auditory cortex | N/A | Statistically significant improve-ment observed on halluci-nation scale. |
| Chib-baro et al., 2005 | Schizo-phrenia | 4 sessions, 15 mins each | 1 Hz | N/A | N/A | N/A | 90% MT | N/A | Left temporo -parietal cortex | N/A | Improve-ments in auditory halluci-nations. |

**Table C.7** Stimulation parameters for movement and neuropsychological disorder treatment using TMS. DLPC: Dorsolateral prefrontal cortex, PC: Prefrontal cortex; PD: Parkinson's Disease, OCD: Obsessive Compulsive Disorder, TS: Tourette's Syndrome, DP: Depression; MT: Motor Threshold, RMT: Resting Motor Threshold, ATM: Active Motor Threshold.

## C.5 Discussion

With a significant portion of our society affected by neurological disorders, the need for improved and more effective treatments are greater than ever before. Advancing current deep-brain stimulation (DBS), vagal-nerve stimulation (VNS), and transcranial magnetic stimulation (TMS) for clinical treatment may result in significant improvements in the quality of life for millions of people suffering from these diseases.

This review has also explored stimulation parameters currently used in DBS, VNS and TMS treatments for neurological disorders. While stimulation parameters for human devices are still being developed, all three stimulation techniques show great promise for treatment; however all three modalities still do not have clear mechanisms of action nor are the stimulation parameters yet defined for *specific* disorders. Through voltammetry and microdialysis in animal models, it has been possible to monitor the effects of electrical stimulation parameters resulting in the release of different concentrations of glutamate, GABA, serotonin and dopamine in different regions of the brain. The neurotransmitter release characteristics have been measured reliably and it has been observed that each neurotransmitter subtype and brain region may have their own optimal stimulation parameter. *If used in conjunction, the stimulation parameters and data obtained from both animal and human studies could result in a greater understanding of the biological effects of DBS, VNS, and TMS.* In addition to animal studies, studies that couple human stimulation devices with brain-imaging techniques may provide a powerful means to explore brain function in the *in vivo* human brain. For example, it is now possible to obtain brain-behaviour relations and to optimize the impact of TMS (Wagner

*et al.*, 2007) using various brain imaging techniques. It is also possible to observe the effect of TMS on local brain response (George and Belmaker, 2007). TMS has been paired with electroencephalography (EEG), positron emission tomography (PET), single-photon emission computed tomography (SPECT), near-infrared spectroscopy (NIRS), and functional magnetic resonance imaging (fMRI). Of all non-invasive imaging modalities, fMRI has provided the clearest insight into the regions of the brain affected during TMS (Wagner *et al.*, 2007) by showing where there have been increases/decreases in blood flow presumably coupled to increases/decreases in neuronal activity. However, magnetic resonance spectroscopy (MRS) has provided information on neurotransmitter concentrations in the brain and the combination of MRI and PET has provided information on the metabolic impact of brain stimulation with neurotransmitter receptor uptake (Wagner *et al.*, 2007). If TMS were paired with simultaneous PET and MRS, the metabolite distribution captured by MRS could be combined with the acquisition of PET images of metabolic activity or neuro-receptor densities (Raylman *et al.*, 2007). This could provide crucial information for those studying Schizophrenia because it would be possible to obtain simultaneous information on glutamate turnover by MRS and dopaminergic neurotransmission by PET measurements.

This review has provided an in-depth overview of electrical and magnetic stimulation parameters, and neurotransmitter release in animal and human models. The various animal and human research modalities that have been explored in this review have, until now, been studied in isolation. Our ultimate objective is to encourage further study that unites these modalities in tandem. Further research and collaboration between

those doing animal and human studies can result in more precise, measurable, and effective methods of treatment for human neurological disorder.

### C.6 Future research and speculation

One of the main enticements to produce this review was the current lack of a validated mechanism of action or even a reasonable theory of mechanism to propose for the effects of electrical or magnetic stimulation of the human brain. Here we see put together a selection of citations and potentially useful methods not normally associated with each other, and yet comparable on a number of levels. Both magnetic and electrical stimulation of neural tissue produce neuromodulatory effects through some as yet unknown mechanism, and voltammetry delineates a method of exciting neurotransmitter and neuromodulator release on demand locally and in remote regions of neural tissue. Each brain stimulation method outlines a range of excitatory parameters, often arbitrary and/or trial-and-error, to achieve an end goal (behavioral modification of some sort, or neurotransmitter release). Perhaps with the aid of voltammetry or similar assay, a more precise and predictive stimulation parameter or set of parameters could be applied with a better defined theory of action or proposed mechanism of action. We propose that these comparable paradigms be explored, using voltammetry or microdialysis and electrical and magnetic stimulation to better define the stimulation parameters required to produce optimal behavioral effects. We also speculate that these electrical and magnetic stimulation parameters will be found to produce a specific and selective neurotransmitter and neuromodulator release in regions of tissue remote to the stimulation site, and that

the parameters required for optimal stimulation will be initially tuned to the target tissue, time-variable, and far more complex than those currently being employed (see USPTO #6,234,953).

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## C.7 References

Abelson JL, Curtis GC, Sagher O, Albucher RC, Harrigan M, Taylor SF, Martis B, Giordani B. 2005. Deep brain stimulation for refractory obsessive-compulsive disorder. Biol Psychiatry 57: 510-516.

Ackermans L, Temel Y, Cath D, van der Linden C, Bruggeman R, Kleijer M, Nederveen P, Schruers K, Colle H, Tijssen MA, Visser-Vandewalle V, Dutch Flemish Tourette Surgery Study Group. 2006. Deep brain stimulation in Tourette's syndrome: two targets? Mov Disord 21: 709-713.

Anderson D, Ahmed A. 2003. Treatment of patients with intractable obsessive-compulsive disorder with anterior capsular stimulation. Case report. J Neurosurg 98: 1104-1108.

Andrews AJ. 2003. Neuromodulation I. techniques-deep brain stimulation, vagus nerve stimulation, and transcranial magnetic stimulation. Ann NY Acad Sci 993: 1-13.

Aouizerate B, Martin-Guehl C, Cuny E, Guehl D, Amieva H, Benazzouz A, Fabrigoule C, Allard M, Rougier A, Burbaud P, Tignol J, Bioulac B. 2005. Deep brain stimulation of the ventral striatum in the treatment of obsessive-compulsive disorder and major depression. Med Sci (Paris) 21: 811-813.

Arai N, Okabe S, Furubayashi T, Mochizuki H, Iwata NK, Hanajima R, Terao Y, Ugawa, Y. 2007. Differences in after-effects between monophasic and biphasic high-frequency rTMS of the human motor cortex. Clin Neurophys 118: 2227-2233.

Avery DH, Holtzheimer PE, Fawaz W, Russo J, Neumaier J, Dunner DL, Haynor DR, Claypoole KH, Waidik C, Roy-Byrne P. 2006. A controlled study of repetitive transcranial magnetic stimulation in medication-resistant major depression. Biol Psychiatry 59: 187-194.

Bailer UF, Kaye WH. 2003. A review of neuropeptide and neuroendocrine dysregulation in anorexia and bulimia nervosa. Curr. Drug Targets CNS Neurol Disord 2: 53-59. Review.

Benabid AL. 2003. Deep brain stimulation for Parkinson's Disease. Curr Opin Neurobiol 13: 696-706.

Benabid AL, Pollak P, Gao D, Hoffmann D, Limousin P, Gay E, Payen I, Benazzouz A. 1996. Chronic electrical stimulation of the ventralis intermedius nucleus of the thalamus as a treatment of movement disorders. J Neurosurg 84: 203-214.

Benito-Leon J, Louis ED. 2006. Essential tremor: emerging views of a common disorder. Nat Clin Pract Neurol 2: 666-678. Review.

Ben-Menachem E, Hamberger A, Hedner T, Hammond EJ, Uthman BM, Slater J, Treig T, Stefan H, Ramsay RE, Wernicke JF, Wilder BJ. 1995. Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. Epilepsy Res 20: 221-227.

Bertolote, JM, Dua, T, Janca, A, Muscetta, A, Saraceno, B, Saxena S. 2006. Chapter 4: conclusions and recommendations. In Neurological disorders: public health challenges. WHO, WHO press, Switzerland.

Beurrier C, Bioulac B, Audin J, Hammond C. 2001. High-frequency stimulation produces a transient blockage of voltage-gated currents in subthalamic neurons. J Neurophysiol 85: 1351-1356.

Bittar RG. 2006. Neuromodulation for movement disorders . J Clin Neurosci 13: 315-318.

Bohnen NI, Frey KA. 2007. Imaging of cholinergic and monoaminergic neurochemical changes in neurodegenerative disorders. Mol Imaging Biol 9: 243-257.

Bortolomasi M, Minelli A, Fuggetta G, Perini M, Comencini S, Fiaschi A, Manganotti P. 2007. Long-lasting effects of high frequency repetitive transcranial magnetic stimulation in major depressed patients. Psychiatry Res 150: 181-186.

Bruno JP, Gash C, Martin B, Zmarowshi A, Pomerleau F, Burmeister J, Huettl P, Gerhardt GA. 2006. Second-by-second measurement of acetylcholine release in prefrontal cortex. Eur J Neurosci 24: 2749-2757.

Buhmann C, Gorsler A, Baumer T, Hidding U, Demiralay C, Hinkelmann K, Weiller C, Siebner HR, Munchau A. 2004. Abnormal excitability of premotor-motor connections in de novo Parkinson's Disease. Brain 127: 2732-2746.

Bunin MA, Prioleau C, Mailman RB, Wightman RM. 1998a. Release and uptake rates of 5-hydroxytryptamine in the dorsal raphe and substantia nigra reticulata of the rat brain. J Neurochem 70: 1077-1087.

Bunin MA, Wightman RM. 1998b. Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: An investigation of extrasynaptic transmission. J Neurosci 18: 4854-4860.

Bunny WE Jr, Davis JM. 1965. Norepinephrine in depressive reactions. Arch Gen Psychiatry 13: 483-494. Review.

Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. 2001. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. Ann Rev Pharmacol Toxicol 41: 237-260.

Carpenter LL, Friehs GM, Tyrka AR, Rasmussen S, Price LH, Greenberg BD. 2006. Vagus nerve stimulation and deep brain stimulation for treatment resistant depression. Med Health RI 89: 137-141.

Chen R, Gerloff C, Classen J, Wassermann EM, Hallett M, Cohen LG. 1997. Safety of different inter-train intervals for repetitive transcranial magnetic stimulation and recommendations for safe ranges of stimulation parameters. Electroencephalogr Clin Neurophysiol 105: 415-421.

Chibbar G, Daniele M, Alagona G, Di Pasquale C, Cannavo M, Rapisarda V, Bella R, Pennisi G. 2005. Repetitive transcranial magnetic stimulation in schizophrenic patients reporting auditory hallucinations. Neurosci Lett 383: 54-57.

Cosyns P, Gabriels L, Nuttin B. 2003. Deep brain stimulation in treatment refractory obsessive-compulsive disorder. Verh K Acad Geneeskd Belg 65: 385-399.

d'Alfonso AA, Aleman A, Kessels RP, Schoten EA, Postma A, van Der Linden JA, Cahn W, Greene Y, de Hann, EH, Kahn RS. 2002. Transcranial magnetic stimulation of left auditory cortex in patients with schizophrenia: Effects on hallucinations and neurocognition. J Neuropsychiatry Clin Neurosci 14: 77-79.

Dickel DE, Veenstra-VanderWeele J, Bivens NC, Wu X, Fischer DJ, Van Etten-Lee M, Himle JA, Leventhal BL, Cook EH Jr, Hanna GL. 2007. Association studies of serotonin system candidate genes in early-onset obsessive-compulsive disorder. Biol. Psychiatry 61: 322-329.

Diederich NJ, Kalteis K, Stamenkovic M, Pieri V, Alesch F. 2005. Efficient internal pallidal stimulation in gilles de la tourette syndrome: A case report. Mov Disord 20: 1496-1499.

Dorr AE, Debonnel G. 2006. Effect of vagus nerve stimulation and noradrenergic transmission. J Pharmacol Exp Ther 318: 890-898.

Dostrovsky JO, Levy R, Wu JP, Hutchison WD, Tasker RR, Lozano AM. 2000. Microstimulation-induced inhibition of neuronal firing in human globus pallidus. J Neurophysiol 84: 570-574.

Dragasevic N, Potrebic A, Damjanovic A, Stefanova E, Kostic VS. 2002. Therapeutic efficacy of bilateral prefrontal slow repetitive transcranial magnetic stimulation in depressed patients with Parkinson's Disease: an open study. Mov Disord 17: 528-532.

Drevets WC, Raichle ME. 1992. Neuroanatomical circuits in depression: implications for treatment mechanisms. Psychopharmacol Bull 28: 261-274.

Faris PL, Eckert ED, Kim SW, Meller WH, Pardo JV, Goodale RL, Hartman BK. 2006. Evidence for a vagal pathophysiology for bulimia nervosa and the accompanying depressive symptoms. J Affect Disord 9: 79-90. Review.

Fitzgerald PB, Benitez J, de Castella A, Daskalakis ZJ, Brown TL, Kulkarni J. 2006. A randomized, controlled trial of sequential bilateral repetitive transcranial magnetic stimulation for treatment-resistant depression. Am J Psychiatry 163: 88-94.

Flaherty AW, Williams ZM, Amirnovin R, Kasper E, Rauch SL, Cosgrove GR, Eskandar EN. 2005. Deep brain stimulation of the anterior internal capsule for the treatment of Tourette syndrome: technical case report. Neurosurgery 57: E403.

Follesa P, Biggio F, Gorini G, Caria S, Talani G, Dazzi L, Puligheddu M, Marrosu F, Biggio G. 2007. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. Brain Res 1179: 28-34.

Fregni F, Santos CM, Myczkowski ML, Rigolino R, Gallucci-Neto J, Barbosa ER, Valente KD, Pascual-Leone A, Marcolin MA. 2004. Repetitive transcranial magnetic stimulation is as effective as fluoxetine in the treatment of depression in patients with Parkinson's Disease. J Neurol Neurosurg Psychiatry 75: 1171-1174.

Gabriels L, Cosyns P, Nuttin B, Demeulemeester H, Gybels J. 2003. Deep brain stimulation for treatment-refractory obsessive-compulsive disorder: psychopathological and neuropsychological outcome in three cases. Acta Psychiatr Scand 107: 275-282.

Garcia L, D'Alessandro G, Bioulac B, Hammond C. 2005. High-frequency stimulation in Parkinson's Disease: More or less? Trends Neurosci 28: 209-216.

Garcia-Toro M, Salva J, Daumal J, Andres J, Romera M, Lafau O, Echevarria M, Mestre M, Bosch C, Collado C, Ibarra O, Aguirre I. 2006. High (20-hz) and low (1-hz) frequency transcranial magnetic stimulation as adjuvant treatment in medication-resistant depression. Psychiatry Res 146: 53-57.

Garris PA, Wightman R.M. 1994. Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: An in vivo voltammetric study. J Neurosci 14: 442-450.

Garris PA, Christensen JRC, Rebec GV, Wightman MR. 1997. Real-time measurement of electrically evoked extracellular dopamine in the striatum of freely moving rats. J Neurochem 68: 152-161.

George MS, Nahas Z, Kozel FA, Li X, Yamanaka K, Mishory A, Hill S, Bohning DE. 2004. Repetitive Transcranial Magnetic Stimulation (rTMS) for Depression and Other Indications. In: Bioelectromagnetic Medicine. Rosch, P.J., Markov, M.S., Eds. Marcel Dekker, New York. Pp 293-312.

George MS, Nahas Z, Molloy M, Speer AM, Oliver NC, Li XB, Arana GW, Risch SC, Ballenger JC. 2000. A controlled trial of daily left prefrontal cortex TMS for treating depression. Biol Psychiatry 48: 962-970.

George MS, Nahas Z, Bohning DE, Kozel FA, Anderson B, Chae JH, Denslow S, Li X, Mu C. 2002. Vagus nerve stimulation therapy: a research update. Neurology 59: S56-S61.

George MS, Belmaker RH. 2007. Transcranial magnetic stimulation in clinical psychiatry. American Psychiatric Publishing, Inc., Washington, DC.

Ghijsen WEJM, Kuiderwijk M, Lopes da Silva FH. 2007. Electrically evoked GABA release in rat hippocampus CA1 region and its changes during kindling epileptogenesis. Brain Res 1135: 69-76.

Giacobbe P, Kennedy SH. 2006. Deep brain stimulation for treatment-resistant depression: a psychiatric perspective. Curr Psychiatry Rep 8: 437-444.

Gilbert DL, Sallee FR, Zhang J, Lipps TD, Wassermann EM. 2005. Transcranial magnetic stimulation-evoked cortical inhibition: a consistent marker of attention-deficit/hyperactivity disorder scores in tourette syndrome. Biol Psychiatry 57: 1597-1600.

Gildenberg PL. 2005. Evolution of neuromodulation. Stereotact Funct Neurosurg 83: 71-79.

Greco PG, Meisel RL, Heidenreich BA, Garris PA. 2006. Voltammetric measurement of electrically evoked dopamine levels in the striatum of the anesthetized Syrian hamster. J Neurosci Method 152: 55-64.

Greenberg BD, George MS, Martin JD, Benjamin J, Schlaepfer TE, Altemus M. Wassermann EM, Post RM, Murphy DL. 1997. Effect of prefrontal repetitive transcranial magnetic stimulation in obsessive-compulsive disorder: a preliminary study. Am J Psychiatry 154: 867-869.

Greenberg BD, Malone DA, Friehs GM, Rezai AR, Kubu CS, Malloy PF, Salloway SP, Okun MS, Goodman WK, Rasmussen SA. 2006. Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder. Neuropsychopharmacology 31: 2384-2393.

Grill WM, Mortimer JT. 1995. Stimulus waveform for selective neural stimulation. IEEE Eng Med Biol Mag. July/August: 375-385.

Grisaru N, Chudakov B, Yaroslavsky Y, Belmaker RH. 1998. Transcranial magnetic stimulation in mania: a controlled study. Am J Psychiatry 155: 1608-1610.

Groves DA, Brown VJ. 2005. Vagal nerve stimulation: A review of its applications and potential mechanisms that mediate its clinical effects. Neurosci Biobehav Rev 29: 493-500.

Hallett M. 2000. Transcranial magnetic stimulation and the human brain. Nature 406: 147-150.

Hammond EJ, Uthman BM, Wilder BJ, Ben-Menachem E, Hamberger AL, Hedner T, Ekman R. 1992. Neurochemical effects of vagus nerve stimulation in humans. Brain Res 583: 300-303.

Hanajima R, Wang R, Nakatani-Enomoto S, Hamada M, Terao Y, Furubayashi T, Okabe S, Inomata-Terada S, Yugeta A, Rothwell JC, Ugawa Y. 2007. Comparison of different methods for estimating motor threshold with transcranial magnetic stimulation. Clin Neurophysiol 118: 2120-2122.

Harden CL, Pulver MC, Ravdin LK, Nikolov B, Halper JP, Labar DR. 2000. A pilot study of mood in epilepsy patients treated with vagus nerve stimulation. Epilepsy Behav 1: 93-99.

Hardesty DE, Sackeim HA. 2006. Deep brain stimulation in movement and psychiatric disorders. Biol Psychiatry 61: 831-835.

Hentall ID, Pinzon A, Noga BR. 2006. Spatial and temporal patterns of serotonin release in the rat's lumbar spinal cord following electrical stimulation of the nucleus raphe magnus. Neuroscience 142: 893-903.

Herbert H, Saper CB. 1992. Organization of medullary adrenergic and noradrenergic projections to the periaqueductal gray matter in the rat. J Comp Neurol 315: 34-52.

Hillered L, Vespa PM, Hovda DA. 2005. Translational neurochemical research in acute human brain injury: The current status and potential future for cerebral microdialysis. J Neurotrauma 22: 3-41.

Hoffman RE, Boutros NN, Hu S, Berman RM, Krystal JH, Charney DS. 2000. Transcranial magnetic stimulation and auditory hallucinations in schizophrenia. Lancet 355: 1073-1075.

Hoffman RE, Boutros NN, Berman RM, Roessler E, Berger A, Krystal JH, Charney DS. 1999. Transcranial magnetic stimulation of left temporoparietal cortex in three patients reporting hallucinated "voices". Biol Psychiatry 46: 130-132.

Hoffman RE, Gueorguieva R, Hawkins KA, Varanko M, Boutros NN, Wu YT, Carroll K, Krystal JH. 2005. Temporoparietal transcranial magnetic stimulation for auditory hallucinations: Safety, efficacy and moderators in a fifty patient sample. Biol Psychiatry 58: 97-104.

Holtzheimer PE, Nemeroff CB. 2006. Advances in the treatment of depression. NeuroRx 3: 42-56.

Hord DE, Evans MS, Mueed S, Adamolekun B, Naritoku DK. 2003. The effect of vagus nerve stimulation on migraines. J Pain 4: 530-534.

Houeto JL, Karachi C, Mallet L, Pillon B, Yelnik J, Mesnage V, Welter ML, Navarro S, Pelissolo A, Damier P, Pidoux B, Dormont D, Cornu P, Agid Y. 2005. Tourette's syndrome and deep brain stimulation. J Neurol Neurosurg Psychiatr 76: 992-995.

Huse DM, Schulman K, Orsini L, Castelli-Haley J, Kennedy S, Lenhart G. 2005. Burden of illness in Parkinson's Disease. Mov Disord 20: 1449-1454.

Ikeda T, Kurosawa M, Uchikawa C, Kitayama S, Nukina N. 2004. Modulation of monoamine transporter expression and function by repetitive transcranial magnetic stimulation. Biochem Biophy. Res Commun 327: 218-224.

Januel D, Dumortier G, Verdon CM, Stamatiadis L, Saba G, Cabaret W, Benadhira R, Rocamora JF, Braha S, Kalalou K, Vicaut PE, Fermanian J. 2006. A double-blind sham controlled study of right prefrontal repetitive transcranial magnetic stimulation (rTMS): Therapeutic and cognitive effect in medication free unipolar depression during 4 weeks. Prog Neuropsychopharmacol Biol Psychiatry 30: 126-130.

Jiménez F, Velasco F, Salin-Pascual R, Hernández JA, Velasco M, Criales JL, Nicolini H. 2005. A patient with a resistant major depression disorder treated with deep brain stimulation in the inferior thalamic peduncle. Neurosurgery 57: 585-593.

John CE, Budygin EA, Mateo Y, Jones SR. 2006. Neurochemical characterization of the release and uptake of dopamine in ventral tegmental area and seotonin in substantia nigra of the mouse. J Neurochem 96: 267-282.

Juckel G, Mendlin A, Jacobs BL. 1999. Electrical stimulation of rat medial prefrontal cortex enhances forebrain serotonin output: Implications for electroconvulsive therpay and transcranial magnetic stimulation in depression. Neuropsychopharmacology 21: 391-398.

Kamitani Y, Bhalodia VM, Kubota Y, Shimojo S. 2001. A model of magnetic stimulation of neocortical neurons. Neurocomputing 38-40: 697-703.

Kanno M, Matsumoto M, Togashi H, Yoshioka M, Mano Y. 2003. Effects of acute repetitive transcranial magnetic stimulation on extracellular serotonin concentration in the rat prefrontal cortex. J Phramacol Sci 93: 451-457.

Kanno M, Matsumoto M, Togashi H, Yoshioka M, Mano Y. 2004. Effects of acute repetitive transcranial magnetic stimulation on dopamine release in the rat dorsolateral striatum. J Neurol Sci 217: 73-81.

Keck ME, Sillaber I, Ebner K, Welt T, Toschi N, Kaehler ST, Singewald N, Philippu A, Elbel GK, Wotjak CT, Holsboe, F, Landgraf R, Engelmann M. 2000. Acute transcranial magnetic stimulation of frontal brain regions selectively modulates the release of vasopressin, biogenic amines and amino acids in the rat brain. Eur J Neurosci 12: 3713-3720.

Keck ME, Welt T, Muller MB, Erhardt A, Ohl F, Toschi N, Holsboer F, Sillaber I. 2002. Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. Neuropharmacology 43: 101-109.

Kessler RC, Chiu WT, Demler O, Walters EE. 2005. Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). Arch Gen Psychiatry 62: 617-627.

Khedr EM, Rothwell JC, Shawky OA, Ahmed MA, Foly N, Hamdy A. 2007. Dopamine levels after repetitive transcranial magnetic stimulation of motor cortex in patients with Parkinson's Disease: Preliminary results. Mov Disord 22: 1046-1050.

Krahl SE, Clark KB, Smith DC, Browning RA. 1998. Locus coeruleus lesions suppress the seizure attenuating effects of vagus nerve stimulation. Epilepsia 39: 709-714.

Kuhr WG, Ewing AG, Caudill WL, Wightman RM. 1984. Monitoring the stimulated release of dopamine with in vivo voltammetry. I: Characterization of the response observed in the caudate nucleus of the rate. J Neurochochem 43: 560-569.

Kuhr WG, Bigelow JC, Wightman RM. 1986. In vivo comparison of the regulation of releasable dopamine in the caudate nucleus and the nucleus accumbens of the rat brain. J Neurosci 6: 974-982.

Labiner DM, Ahern GL. 2007. Vagus nerve stimulation therapy in depression and epilepsy: Therapeutic parameter settings. Acta Neurol Scand 115: 23-33.

Lang N, Speck S, Harms J, Rothkegel H, Paulus W, Sommer M. 2007. Dopaminergic potentiation of rTMS-induced motor cortex inhibition. Biol psychiatry 20: 687-702.

Lee KH, Chang SY, Kim U. 2004. Neurotransmitter release from high-frequency stimulation of the subthalamic nucleus. J Neurosurg 101: 511-517.

Lee KH, Blaha CD, Harris BT, Copper S, Hitti FL, Liether JC, Roberts DW, Kim U. 2006. Dopamine efflux in the rat striatum evoked by electrical stimualtion of the subthalamic nucleus: Potential mechanism of action in Parkinson's Disease. Eur J Neurosci 23: 1005-1014.

Lefaucheur JP, Drouot X, Von Raison F, Menard-Lefaucheur I, Cesaro P, Nguyen JP. 2004. Improvement of motor performance and modulation of cortical excitability by repetitive transcranial magnetic stimulation of the motor cortex in Parkinson's Disease. Clin Neurophysiol 115: 2530-2541.

Lewis DA, Lieberman JA. 2000. Catching up on schizophrenia: natural history and neurobiology. Neuron 28: 325-334.

Luborzewski A, Schubert F, Seifert F, Danker-Hopfe H, Brakemeier EL, Schlattmann P, Anghelescu I, Colla M, Bajbouj M. 2007. Metabolic alterations in the dorsolateral prefrontal cortex after treatment with high-frequency repetitive transcranial magnetic stimulation in patients with unipolar major depression. J Psychiatr Res 41: 606-15.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. 2000. Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. Clin Neurophysiol 111: 800-805.

Maeda F, Dubeau MC, Koski L, Lisan SH. 2003. Investigation of Mood Disorders by Transcranial Magnetic Stimulation. In: Brain imaging in affective disorders. Soares JC, Ed. Marcel Dekker, New York.

Mally J, Stone TW. 1999a. Improvement in Parkinsonian symptoms after repetitive transcranial magnetic stimulation. J Neurol Sci 162: 179-84.

Mally J, Stone TW. 1999b. Therapeutic and "dose-dependent" effect of repetitive microelectroshock induced by transcranial magnetic stimulation in Parkinson's Disease. J Neurosci Res 57: 935-40.

Mantovani A, Lisanby SH, Pieraccini F, Ulivelli M, Castrogiovanni P, Rossi S. 2005. Repetitive transcranial magnetic stimulation (rTMS) in the treatment of obsessive-compulsive disorder (OCD) and tourette's syndrome. Int J Neuropsychopharmacol 9: 95-100.

Marrosu F, Serra A, Maleci A, Puligheddu M, Biggio G, Piga M. 2003. Correlation between GABAa receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. Epilepsy Res 55: 59-70.

Mayberg HS. 2003. Positron emission tomography imaging in depression: a neural systems perspective. Neuroimaging Clin N Am 13: 805-813.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH. 2005. Deep brain stimulation for treatment-resistant depression. Neuron 45: 651-660.

McIntyre CC, Savasta M, Kerkerian-Le Goff L, Vitek JL. 2004. Uncovering the mechanism(s) of action of deep brain stimulation: Activation, inhibition, or both. Clin Neurophysiol 115: 1239-1248.

McIntyre CC, Savasta M, Walter BL. Vitek JL. 2004. How does deep brain stimulation work? Present understanding and future questions. J Clin Neurophysiol 21: 40-50.

Medtronic DBS™ Lead kit for deep brain stimulation. Implant manual. 2006. Medtronic Inc.
http://www.medtronic.com/physician/activa/downloadablefiles/197928_b_006.pdf. Accessed January 26, 2008.

Merrill CA, Jonsson MAG, Minthon L, Ejnell H, Silander HC, Blennow K, Karlsson M, Nordlund A, Rolstad S, Warkentin S, Ben-Menachem E, Sjogren MJC. 2006. Vagus nerve stimulation in patients with alzheimer's disease: Additional follow-up results of a pilot study through 1 year. J Clin Psychiatry 67: 1171-1178.

Michael N, Gosling M, Reutemann M, Kersting A, Heindel W, Arolt V, Pleiderer B. 2003. Metabolic changes after repetitive transcranial magnetic stimulation (rTMS) on the left prefrontal cortex: a sham-controlled proton magnetic resonance spectroscopy (1H MRS) study of the healthy brain. Eur J Neurosci 17: 2462-2468.

Miyawaki Y, Okada M. 2004. A network model of perceptual suppression induced by transcranial magnetic stimulation. Neural Comput 16: 309-331.

Montgomery EB, Baker KB. 2000. Mechanism of deep brain stimulation and future technical developments. Neurol. Res. 22: 259-266.

Nutt DJ. 2006. The role of dopamine and norepinephrine in depression and antidepressant treatment. J Clin Psychiatry 67: 3-8.

Nuttin B, Cosyns P, Demeulemeester H, Gybels J, Meyerson B. 1999. Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. Lancet 354: 1526.

Nuttin BJ, Gabriels LA, Cosyns PR, Meyerson BA, Andreewitch S, Sunaert SG, Maes AF, Dupont PJ, Gybels JM, Gielen F, Demeulemeester HG. 2003. Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. Neurosurgery 52: 1263-1272.

Okun MS, Mann G, Foote KD, Shapira NA, Bowers D, Springer U, Knight W, Martin P, Goodman WK. 2007. Deep brain stimulation in the internal capsule and nucleus accumbens region: Responses observed during active and sham programming. J Neurol Neurosurg Psychiat 78: 310-314.

Perlmutter JS, Mink JW. 2006. Deep brain stimulation. Annu Rev Neurosci 29: 229-257.

Phan KL, Wager TD, Taylor SF, Liberzon I. 2004. Functional neuroimaging of human emotions. CNS Spect 9: 258-66.

Pogarell O, Koch W, Popperl G, Tatsch K, Jakob F, Zwanzger P, Mulert C. Rupprecht R, Moller H, Hegerl U, Padberg F. 2006. Striatal dopamine release after prefrontal repetitive transcranial magnetic stimulation in major depression: Preliminary results of a dynamic [123I] IBZM SPECT study. J Psychiatr Res 40: 307-314.

Post A, Muller MB, Engelmann M, Keck ME. 1999. Repetitive transcranial magnetic stimulation in rats: evidence for a neuroprotective effect in vitro and in vivo. Eur J Neurosci 11: 3247-3254.

Raike RS, Jinnah HA, Hess EJ. 2005. Animal models of generalized dystonia. NeuroRx 2: 504-512.

Rattay F. 1990. Electrical nerve stimulation: theory, experiments and applications. Springer, New York.

Rattay F. 1999. The basic mechanism for the electrical stimulation of the nervous system. Neuroscience 89: 335-346.

Rauch SL. 2003. Neuroimaging and neurocircuitry models pertaining to the neurosurgical treatment of psychiatric disorders. Neurosurg Clin N Am 14: 213-223.

Raylan RR, Mayjewski S, Velan SS, Lemieux S, Kross B, Popov V, Smith MF, Weisenberger AG. 2007. Simultaneous acquisition of magnetic resonance spectroscopy (MRS) data and positron emission tomography (PET) images with a prototype MR-compatible, small animal PET imager. J Magn Reson 186: 305-310.

Rice ME, Cragg SJ, Greenfield SA. 1997. Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. J Neurophysiol 77: 853-862.

Rise MT. 2000. Instrumentation for neuromodulation. Arch Med Res 31: 237-247.

Rizzone M, Lanotte M, Bergamasco B, Tavella A, Torre E, Faccani G, Melcarne A, Lopiano L. 2001. Deep brain stimulation of the subthalamic nucleus in Parkinson's Disease: effects of variation in stimulation parameters. J Neurol Neurosurg Psychiatry 71: 215-219.

Robinson DL, Venton BJ, Heien MLAV, Wightman RM. 2003. Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. Clin Chem 49: 1763-1773.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CM, Maertens de Noordhout AL, Marsden CD, Murray NMF, Rothwell JC, Swash M, Tomberg C. 1994. Non-invasive electrical and magnetic stimulation of the brain , spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 91: 79-92.

Rossini PM, Rossi S. 2007. Transcranial magnetic stimulation. Neurology 68: 484-488.

Rothwell JC, Hallet M, Berardelli A, Eisen A, Rossini P, Paulus W. 1999. Magnetic stimulation: motor evoked potentials. The international federation of clinical neurophysiology. Electroencephalogr Clin Neurophysiol Suppl 52: 97-103.

Salder RM, Purdy RA, Rahey S. 2002. Vagal nerve stimulation aborts migraine in patients with intractable epilepsy. Cephalalgia 22: 482-484.

Sekino M, Ueno S. 2004. Numerical calculation of eddy currents in transcranial magnetic stimulation for psychiatric treatment. Neurol Clin Neurophysiol 2004: 88.

Shiah IS, Yatham LN. 2000. Serotonin in mani and the mechanism of action of mood stabilizers: a review of clinical studies. Bipolar Disord 2: 77-92.

Shimamoto H, Takasaki K, Shigemori M, Imaizumi T, Ayabe M, Shoji H. 2001. Therapeutic effect and mechanism of repetitive transcranial magnetic stimulation in Parkinson's Disease. J Neurol 248: III/48-III/52.

Sibon I, Strafella AP, Gravel P, Ko JH, Booij L, Soucy JP, Leyton M, Diksic M, Benkelfat C. 2007. Acute prefrontal cortex TMS in healthy volunteers: effects on brain 11C-alphaMtrp trapping. Neuroimage 34: 1658-1664.

Siebner HR, Peller M, Willoch F, Minoshima S, Boecker H, Auer C, Drzezga A, Conrad B, Bartenstein P. 2000. Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. Neurology 54: 956-963.

Singer HS, Minzer K. 2003. Neurobiology of tourette's syndrome: Concepts of neuroanatomic localization and neurochemical abnormalities. Brain Dev 25: S70-S84.

Sjogren MJC, Hellstrom PTO, Jonsson MAG, Runnerstam M, Silander HC, Ben-Menachem E. 2002. Cognition-enhancing effect of vagus nerve stimulation in patients with alzheimer's disease: A pilot study. J Clin Psychiatry 63: 972-980.

Sobocki J, Fourtanier G, Estany J, Otal P. 2006. Does vagal nerve stimulation affect body composition and metabolism? Experimental study of a new potential technique in bariatric surgery. Surgery 139: 209-216.

Strafella AP, Paus T, Fraraccio M, Dagher A. 2003. Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex. Brain 126: 2609-2615.

Tass PA, Klosterkotter J, Schneider F, Lenartz D, Koulousakis A, Sturm V. 2003. Obsessive-compulsive disorder: development of demand-controlled deep brain stimulation with methods from stochastic phase resetting. Neuropsychopharmacology 28: S27-S34. Review.

Testerman RL, Rise MT, Stypulkowski PH. 2006. Electrical stimulation as therpay for neurological disorders. IEEE Eng Med Biol Mag September/October: 74-78.

Theberge J, Bartha R., Drost DJ, Menon RS, Ashok M, Takhar J, Neufeld RW, Rogers J, Pavlosky W, Densmore M, Al-Semaan Y, Williamson PC. 2003. Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with Schizophrenia and healthy volunteers. Am J Psychiatry 159: 1944-1946.

Urbano FJ, Leznik E, Llinas RR. 2002. Cortical activation patterns evoked by afferent axons stimuli at different frequencies: An in vitro voltage-sensitive dye imaging study. Thalamus Rel Syst 1: 371-378.

Van Bockstaele EJ, Peoples J, Telegan P. 1999. Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway. J Comp Neurol 412: 410-428.

Velasco F, Velasco M, Jimenez F, Velasco A, Salin-Pascual R. 2005. Neurobiological background for performing surgical intervention in the inferior thalamic peduncle for treatment of major depression disorders. Neurosurgery 57: 439-448.

Visser-Vandewalle V, Temel Y, Boon P, Vreeling F, Colle H, Hoogland G, Groenewegen HJ, van der Linden, C. 2003. Chronic bilateral thalamic stimulation: a new therapeutic approach in intractable Tourette syndrome. Report of three cases. J Neurosurg 99: 11094-1100.

Volkmann J, Herzog J, Kopper F, Deuschl G. 2002. Introduction to the programming of deep brain stimulators. Mov Disord 17: S181-S187.

Wagner T, Valero-Cabre A, Pascual-Leone A. 2007. Noninvasive human brain stimulation. Annu Rev Biomed Eng 9: 527-565.

Walker QD, Rooney MB, Wightman RM, Kuhn CM. 2000. Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. Neuroscience 95: 1061-1070.

Wassermann EM. 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation. Electroencephalogr Clin Neurophysiol 108: 1-16.

Westerink BHC, Justice Jr., JB. 1991. Microdialysis compared with other in vivo release models. In Microdialysis in the neurosciences. Robinson TE, Justice Jr. JB, Eds. Amsterdam; New York: Elselvier. pp 23-42.

Wichmann T, Delong MR. 2006. Deep brain stimulation for neurologic and neuropsychiatric disorders. Neuron 52: 197-204.

Wightman MR, Zimmerman JB. 1990. Control of dopamine extracellular concentration in rat striutum by impulse flow and uptake. Brain Res Rev 15: 135-144.

Windels F, Bruet N, Poupard A, Feuerstein C, Bertrand A, Savasta M. 2003. Influence of the frequency parameter on extracellular glutamate and gamma-aminobutyric acid in substantia nigra and globus pallidus during electrical stimulation of subthalamic nucleus in rats. J Neurosci Res 72: 259-267.

You Z, Tzschentke TM, Brodin E, Wise RA. 1998. Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus accumbens: An in vivo microdialysis study in freely moving rats. J Neurosci 18: 6492-6500.

Zangen A, Hyodo K. 2002. Transcranial magnetic stimulation induces increases in extracellular levels of dopamine and glutamate in the nucleus accumbens. Neuroreport 13: 2401-2405.

Zuo Y, Smith DC, Jensen RA. 2007. Vagus nerve stimulation potentiates hippocampal LTP in Freely moving rats. Physiol Behav 90: 583-589.