RESEARCH AND COMMUNICATION PROJECT SUMMARIES

September 1994 – December 1998

Supported by

EMF RAPID

Electric and Magnetic Fields Research and Public Information Dissemination Program

(PL 102-486 Section 2118)

AND

NIEHS

National Institute of Environmental Health Sciences
National Institutes of Health
(PHS Act Section 301)

EMF RAPID

Electric and Magnetic Fields Research
and Public Information Dissemination Program

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PL102-486 Section 2118 & PHS Act Section 301

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NIEHS

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Table of Contents

Letter from Dr. Anne Sassaman, Director, Division of Extramural Research and Training, NIEHS v

EMF Research Program and Accomplishments vii

Project Listing by Funding Source and Mechanism xiii

Project Summaries

Investigator	Research Institution	Grant #	Page #
Aaron, Roy K, M.D.	Brown University	R01-ES07402	3
Anderson, Larry, Ph.D.	Battelle Pacific Northwest Laboratory	N01-ES65397	7
Astumian, Dean R. Ph.D.	The University of Chicago	R03-ES08913	11
Astumian, Dean R, Ph.D.	The University of Chicago	R29-ES06620	13
Balcer-Kubiczek, Elizabeth K, Ph.D.	University of Maryland	R01-ES07120	17
Balcer-Kubiczek, Elizabeth K, Ph.D.	University of Maryland	R03-ES08898	23
Barrett, J. Carl, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES23019	27
Bilski, Piotr, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES50138	29
Binninger, David M, Ph.D.	Florida Atlantic University	R01-ES07181	33
Blank, Martin, Ph.D.	Columbia University	R03-ES08855	37
Byus, Craig V, Ph.D.	University of California	R01-ES06128	41
Chignell, Colin F, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES50136	55
Costa, Lucio G, Ph.D.	University of Washington	R03-ES08851	57
Craviso, Gale, Ph.D.	University of Nevada	R03-ES08903	59
Dibirdik, Ilker, Ph.D.	University of Minnesota	R01-ES07175	63
Gandhi, Om, Sc.D, M.S.E.	University of Utah	R01-ES06292	67
Glickman, Barry W, Ph.D.	University of Victoria	R03-ES08894	71
Goldberg, Robert, Ph.D.	Information Ventures, Philadelphia, PA	PR702871	75
Graham, Charles, Ph.D.	Midwest Research Institute	R01-ES07053	81
Griffin, Guy David, Ph.D.	Oak Ridge National Laboratory	R03-ES08864	85
Grissom, Charles B, Ph.D.	University of Utah	R01-ES05728	89
Grissom, Charles B, Ph.D.	University of Utah	R03-ES08896	93
Goodman, Reba, Ph.D.	Columbia University	R03-ES08867	97
Hahn, Theodore J, M.D.	VA Medical Center	R03-ES08895	101

Hardisty, Jerry, DVM	Experimental Pathology Laboratories Inc., Research Triangle Park, NC	N01-ES35369	103
Harry, Jean, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES21164	105
Hei, Tom K, Ph.D.	Columbia University	R03-ES08926	107
Henderson, Ann S, Ph.D.	Hunter College-CUNY	R01-ES05735	109
Hong, Jau-Shyong, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES90083	113
Hunter, David J, Sc.D., M.P.H.	Brigham & Women's Hospital	Y01-CA62984	119
Hui, Sek Wen, Ph.D.	Roswell Park Cancer Institute	R01-ES07091	123
Kripke, Daniel F, Ph.D.	University of California, San Diego	R03-ES08930	127
Lai, Henry C, Ph.D.	University of Washington	R03-ES08865	129
Lai, Henry C, Ph.D.	University of Washington	R01-ES06290	133
Leske, M. Christina, Md.	State University of New York, Buffalo	Y01-CA62991	137
Liburdy, Robert P, Ph.D.	Lawrence Berkeley National Laboratory	R01-ES06401	141
Lieberman, Howard B, Ph.D.	Columbia University	R03-ES08931	147
Litovitz, Theodore A, Ph.D.	Catholic University Of America	R01-ES06872	155
Loberg, Lise I, Ph.D.	Illinois Institute of Technology Research Institute	R03-ES08921	159
Lotz, W. Gregory, Ph.D.	National Institute for Occupational Safety & Health	Y01-ES0032	163
Luben, Richard A, Ph.D.	University of California	R01-ES06410	169
Mandeville, Rosemonde, Ph.D.	University of Quebec, Laval	R01-ES07049	175
Marino, Andrew A, Ph.D.	Louisiana State University Medical Center, Shreveport	R01-ES05928	179
McCormick, David L, Ph.D.	Illinois Institute of Technology Research Institute	N01-ES25351	187
McCormick, David L, Ph.D.	Illinois Institute of Technology Research Institute	R01-ES07093	193
McCormick, David L, Ph.D.	Illinois Institute of Technology Research Institute	R03-ES08854	197
McDonald, Lee James, Ph.D.	Illinois Institute of Technology Research Institute	R03-ES08911	201
McLeod, P Kenneth J, Ph.D.	State University of New York	R01-ES06287	205
McMillian, Michael, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES90067	209
Mehta, Shashikant, Ph.D.	Brown University	R29-ES05970	211
Miller, Richard C, Ph.D.	Columbia University	R01-ES07124	213

Miller, Steven C, Ph.D.	SRI International	R01-ES07127	217
Murphy, Elizabeth, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES50156	221
Nuccitelli, Richard L, Ph.D.	University of California, Davis	R01-ES07133	223
Owen, Russell D, Ph.D.	Food and Drug Administration	Y01-1ES40272	229
Pennypacker, Keith, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES90066	233
Peters, John M, M.D.	University of Southern California	R01-ES06912	235
Portier, Christopher, Ph.D.	National Institute of Environmental Health Sciences	Z01-ES90602	239
Putney, James, Ph.D	National Institute of Environmental Health Sciences	Z01-ES90087	241
Reif, John S, Ph.D.	Department of Environmental Health, Colorado State	R01-ES08117	243
Reiter, Russel J, Ph.D.	University of Texas Health Science Center, San Antonio	R01-ES07132	245
Reiter, Russel J, Ph.D.	University of Texas, Health Science Center, San Antonio	R03-ES08890	249
Rinehart, Clifford A, Ph.D.	University of North Carolina at Chapel Hill	R01-ES07145	251
Ryan, Bernadette M, Ph.D.	Illinois Institute of Technology Research Institute	R03-ES08873	257
Saffer Jeffrey D, Ph.D.	Battelle Pacific Northwest Institute	R01-ES07122	261
Sheppard, Asher R, Ph.D.	Loma Linda University	R03-ES08920	265
Sisken, Jesse E, Ph.D.	University of Kentucky	R03-ES08878	269
Travlos, Gregory, DVM	National Institute of Environmental Health Sciences	Z01-ES21195	273
Williams, Jerry R, Ph.D.	Johns Hopkins University	R01-ES07076	281
Woloschak, Gayle E, Ph.D.	Argonne National Laboratory	R01-ES07141	285
Wrensch, Margaret, Ph.D, M.P.H.	University of California, San Francisco	R01-ES06285	287
Wu, Tai Tsun, Ph.D.	Harvard University	R01-ES-08051	293
Yellon, Steven M, Ph. D.	Loma Linda University, School Of Medicine	R01-ES-06137	297
Yost, Michael G, Ph.D.	University of Washington	R03-ES08877	305

NEMFAC Membership **319**

Interagency Committee Membership **319**

Budget **321**

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January 30, 1999

Dear Colleague:

I am pleased to provide this report on the Electric and Magnetic Fields (EMF) research, communication and evaluation activities that have been conducted by the National Institute of Environmental Health Sciences (NIEHS) over the past six years. This program was initiated by the NIEHS as a result of a mandate in Public Law 102 - 486 (Section 2118) which established the EMF Research and Public Information Dissemination (RAPID) Program to determine the health effects, if any, of EMF exposure. The EMF-RAPID Program involved both the NIEHS which led the biological research efforts and the U.S. Department of Energy which was responsible for administrative oversight and engineering research. This law as it related to the NIEHS directed the establishment of a comprehensive program to:

1. Determine whether or not exposure to EMF produced by the generation, transmission, and use of electric energy affects human health; and
2. Provide for dissemination of scientifically valid information on possible human health effects of EMFs.

As a result of this mandate and an existing concern of the NIEHS about possible health effects of EMF exposure, Dr. Kenneth Olden, Director of the NIEHS, directed that a comprehensive program be established that used not only the resources available through this law, but also other resources of the NIEHS to address this issue. As a result of these commitments, the NIEHS provided support for research, communication and evaluation projects in excess of $44 million. This funding was obtained through Section 2118, $30 million; NIEHS Research Program (PHS Act Section 301), $5.6 million; and the National Toxicology Program, $8.4 million.

I wish to acknowledge the many NIEHS staff who were involved in this program. Their efforts in implementing a complex and difficult program were critical to the EMF-RAPID Program's success.

I am confident that the products of this NIEHS-led research and communication program meet the high expectations of the Congress and the public.

Sincerely yours,

Anne Sassaman

Anne Sassaman, Ph.D.
Director, Division of Extramural
Research and Training

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EMF RESEARCH, COMMUNICATION AND EVALUATION PROJECT SUMMARIES

OVERVIEW

Widespread exposure to EMF is associated with the generation, transport and use of electricity. These low intensity, extremely low frequency fields were generally thought to be safe until a 1979 report suggested a possible association of increased childhood leukemia in homes predicted to have high magnetic fields. After numerous studies and more than 10 years of research, the question of possible health hazards from exposure to these fields had not been resolved. The Energy Policy Act (Public Law 102-486, Section 2118), which was signed into law in October 1992, authorized a five-year Electric and Magnetic Field (EMF) Research and Public Information Dissemination Program (RAPID) that was to be administered by the U. S. Department of Energy (DOE). The provisions of this Act were extended for one year in 1997, and it ended on December 31, 1998.

The NIEHS is the principal component within the National Institutes of Health (NIH) for support of basic research on environmental factors that contribute to human health problems and disease. Major emphasis by NIEHS is placed upon research examining those physical and chemical substances, including EMF, to which humans are exposed in their general environment as a result of human activities such as modern technologies and commercial processes. In this Act, NIEHS was designated as the lead agency for coordinating and conducting health effects studies and communicating the results to policy makers and the general public on the possible adverse effects, if any, of exposure to EMF from the generation, transport and use of electricity. In addition, in Section 2118 of the Act, the NIEHS was required to competitively solicit and select proposals to conduct research, communication and evaluation activities under the Program. At the end of five years, NIEHS was to report to Congress about whether power-line frequency EMF exposures pose a risk for human health. The NIEHS Director's Report to Congress is currently in the draft stage and will be submitted shortly.

Public Law 102-486 also called for a communication and outreach program. This was accomplished through a number of workshops, meetings, pamphlets, a web site and other venues.

This report is a compilation of the activities supported by the NIEHS in response to this mandate including projects funded by this Act (Section 2118) and by the NIEHS research appropriation (PHS Act, Section 301). Since funds were provided under Section 2118 and PHS Act, a project listing by funding source is given in addition to alphabetical and project number listings. All extramural projects supported by this program were competitively solicited, and all the research projects were rigorously peer-reviewed using the usual NIH process. Each research project is described and the finding(s) summarized.

Based on the broad research mandate under PL 102-486 Section 2118, a comprehensive research program was established that utilized both investigator-initiated and program-directed basic and applied research on the possible health effects of ELF-EMF. Because one goal of the program was to determine if a robust effect of EMFs could be identified, some projects were focused on identification and replication of EMF biological effects rather than on the possible mechanisms of EMF interactions. This program report summarizes the research supported under the EMF-RAPID Program, EMF research supported by NIEHS and the animal studies conducted by NIEHS as part of the National Toxicology Program. The intent of this report is to document the research, communication and evaluation activities of the program and to summarize the research findings to date. However, since research results often take several years to find their way into peer-reviewed literature, the effects of the EMF-RAPID Program will continue to impact this field for years to come.

An Interagency Committee was established by the Energy Policy Act to facilitate interagency coordination of EMF research and communication activities for the Federal agencies. An Advisory Committee (National Electric and Magnetic Fields Advisory Committee, NEMFAC), which included public advocates, union representatives, electric equipment manufacturers, experts in possible health effects of electric and magnetic fields, and experts in the

measurement and assessment of these fields, was established to advise the Interagency Committee. NEMFAC provided advice to the NIEHS on all activities conducted under this mandate.

RESEARCH GOALS AND SCOPE OF PROGRAM

At the initiation of the EMF-RAPID Program, several major epidemiological studies were in progress. These studies were to address the issue of childhood leukemia from residential exposure, adult leukemia and breast cancer from occupational and residential exposure, and brain cancer from occupational exposures. The NEMFAC's advice to NIEHS was that given the time constraints of the program and the ongoing epidemiology studies, the research focus should be on animal and cellular studies.

In addition to the Section 2118 funds, NIEHS through its own research program was funding some EMF research including collaborative epidemiology studies with the National Cancer Institute. At the same time, at the request of the DOE and prior to the EMF-RAPID Program, the NIEHS through the National Toxicology Program initiated toxicity and carcinogenicity studies of magnetic fields in rodents. Thus, EMF research begun under the EMF-RAPID Program was designed to accelerate and supplement the ongoing research programs at NIEHS and elsewhere.

A significant focus for the EMF-RAPID research program was to determine whether data existed or could be found to support the epidemiological associations between disease and magnetic field exposure. Several reports had suggested that magnetic field exposure caused cellular alterations. These data provided plausible mechanistic support for the epidemiological associations. Since these cellular changes had not been observed in other laboratories, a significant focus of the grants and contracts was on biological processes reported to be altered by EMF exposure. In addition to these projects, a collaborative effort between the DOE and NIEHS established four regional EMF exposure facilities that augmented and complimented the research supported by the grants and contracts. Two facilities were located in DOE laboratories (Pacific Northwest Laboratories, Richland, WA and Oakridge National Laboratories, Oakridge TN) while NIEHS oversaw EMF exposure facilities at the Food and Drug Administration (FDA, Rockville, MD) and at the National Institute for Occupational Safety and Health (NIOSH, Cincinnati, OH). The NIEHS used the regional exposure facilities to ensure standardized *in vitro* exposure units would be available to outside investigators for the conduct of pilot or hypothesis generating studies. Such facilities were considered critical because of the cost involved in construction of exposure facilities, and the need to have well characterized and complete information on the EMF exposures both *in vivo* and *in vitro*.

The advantages of these facilities were that they could recreate the complex magnetic exposures reported in the literature and that all exposures could be done using a blinded study protocol that has been demonstrated to minimize the chance for study bias. The focus of these regional exposure facilities was on the replication of the most important cellular effects of magnetic field exposures reported in the literature.

The EMF research projects were supported by a variety of mechanisms. The majority of the research funding (85%) was traditional NIH extramural grants and contracts. These projects were solicited by Requests for Applications/Proposals (RFA/Ps) in 1993 and 1996. The first grant awards were made in October 1994 within weeks of the first transfer of EMF-RAPID Funds to the NIEHS. These were standard three to four-year grant awards. In January 1997, a second set of grant awards for one to two-year research projects was funded with the focus on addressing questions that remained unanswered. The majority of the extramural research grants were to investigators exploring various mechanisms of cancer since this was the predominant concern raised by epidemiology studies. A smaller number of grants addressed the effect of magnetic fields on reproduction and development and on neurophysiology, especially the effect of magnetic fields on nocturnal melatonin levels. In addition, several animal studies were conducted using competitive contracting mechanisms. Interagency agreements with FDA and NIOSH were used to support the regional EMF exposure facilities and to address seminal EMF biological effects. Intramural NIEHS investigators competed for small intramural research awards.

These intramural research projects were designed to provide fundamental information on the biological processes that were the subject of the EMF studies.

In addition, in anticipation of the EMF-RAPID Program, the NIEHS initiated a number of activities to stimulate interest. First, an EMF workshop was convened in 1990 by the NIEHS' Division of Extramural Research and Training to define the state of the scientific knowledge and identify critical research areas, and in follow-up the NIEHS released a Program Announcement for EMF research. Also, the National Toxicology Program (NTP) released a Request for Proposals (RFP) in 1991 to evaluate the potential biological effects of EMF in a NTP two-year bioassay. As a result of these activities, the NIEHS was able to develop a comprehensive program that addressed the critical issues surrounding the EMF question using funds provided by Section 2118 (PL 102-486) and PHS Act appropriations.

Although several agencies had developed National EMF research plans, the National EMF Strategic Plan prepared by the DOE was used as the framework for the NIEHS EMF research program. This plan was the result of a collaborative process with input from Federal agencies, research and communications experts, the public and industry, and was developed specifically in anticipation of the legislation in PL 102-486.

The focus of the health effects research conducted under this program was in six major areas (five research and one communication):

Major Area	Principal Funding Source
a) epidemiological research	PHS Act
b) neurophysiological and behavioral research	Section 2118 and PHS Act
c) *in vivo* research	Section 2118 and PHS Act
d) cellular and genetic research	Section 2118 and PHS Act
e) exposure systems and methods	Section 2118
f) communication and information dissemination	Section 2118

For each of these areas brief summaries of the activities are provided below. Details of individual projects are given elsewhere in this document, and in the peer-reviewed literature.

1. **Epidemiological Research:**

The EMF RAPID Program did not focus on epidemiology studies because of the number and quality of ongoing studies. However, reports from collaborative studies with the National Cancer Institute supported by the NIEHS under the PHS Act show that neither occupational exposure or electric blanket use is associated with breast cancer in women. In addition, no links between EMF exposure and brain cancer were observed. In 1999 the results from these studies are expected to be published in the peer-reviewed literature. In addition to work directly supported by this program, major peer-reviewed epidemiology studies are being completed and reported.

2. **Neurophysiological and Behavioral Research**:

Research projects were solicited in six major areas: neuro-behavioral teratology, cardiovascular physiology, electrophysiology of the central nervous system, sleep and circadian rhythms, learning and performance, and field detection/sensitivity. In each of these areas, specific procedures were included for EMF field measurements and instrumentation protocols. Explicit efforts were made to replicate key published/reported research findings. In particular, small studies in several laboratories demonstrated effects of EMF exposure on melatonin levels. However, when these same investigators attempted to replicate the data in more comprehensive studies, no effects on melatonin were observed.

3. ***In Vivo* Research:**

Although the interaction of humans with EMF fields is of prime importance and concern, many areas of biological investigation are more efficiently conducted using other animal species. Fundamental research areas included

under this program included cancer research, reproduction, development, and endocrine and physiologic parameters that could be measured non-invasively. None of the *in vivo* studies supported by this program showed effects of EMF exposure on brain tissue or fetus development. In addition, effects on the endocrine system were not demonstrated.

The NIEHS also conducted a comprehensive NTP study that examined the effect of magnetic fields on cancer incidence, reproduction, development and other parameters typically assessed in a NTP two-year bioassay in rodents. No physiological effects on reproduction were seen after six months of continuous exposure. In addition, magnetic fields did not affect the developing rat fetus. Because melatonin changes were one of the seminal effects reported after EMF exposure, this parameter was measured in both rats and mice in an eight-week toxicity study. There was no demonstrated effect on clinical chemistry parameters, hematology or melatonin levels. In a two-year study involving 2,000 rats and mice, there were subtle increases in tumor rates in two exposure groups in male rats that were judged to be of equivocal significance. There were no exposure related increases in tumors in female rats or male and female mice. In a series of three studies, the EMF-RAPID Program evaluated the potential for magnetic field exposure to promote chemically-induced mammary cancer in the rat. In all three studies, there was no increase in the number or size of mammary gland tumors, and in several exposure groups, there were significant decreases in the number of mammary gland tumors.

4. **Cellular and Genetic Research:**

The basis for potential health effects, which might be caused by exposure to electric and magnetic fields, must lie in relevant biological responses. The understanding of any genetic, reproductive, endocrine, neurophysiological or other effect will be found ultimately at the cellular and molecular levels. Studies at the cellular level focused on the causal events relating field interaction to biological effects. Each study had as a critical component careful characterization and documentation of the field exposure that would allow other laboratories to replicate the study design. At the inception of the EMF-RAPID Program, alteration of *c-myc* messenger RNA expression in human leukemia cells seemed to offer evidence that magnetic fields, while not directly affecting DNA, might alter expression of genes leading to cancer. Because these data were fundamental to the hypothesis that EMF exposure affected cancer frequency in children, the NIEHS supported a wide range of EMF studies focused on genes and gene products. Although a few of the investigators reported effects for some parameters, the majority of the investigators reported no effects of EMF exposure on the genes, *c-myc* and *c-fos*. Small changes were reported in *c-jun* expression and other parameters in the c-*jun* pathway; however, one other investigator supported by the EMF RAPID program was unable to confirm this finding. In addition, effects on ornithine decarboxylase (ODC) and calcium flux changes, which have also been advanced as possible cellular processes affected by EMF exposure, could not be reproduced. Thus, no consistent effects that might be related to adverse health effects were seen at the cellular level.

5. **Exposure Systems and Methods:**

Significant advances in the EMF exposure systems used for *in vivo* and *in vitro* exposures were achieved under the EMF-RAPID Program that markedly improved the quality of the research conducted by investigators. First, there was a marked improvement in the quality of the EMF exposure systems. The DOE provided engineering and physics support to investigators. There were site visits to all the major investigators supported under this program; the National Institute of Standards and Technology (NIST) also collaborated in this effort. These quality assurance reviews identified several areas where subtle and in some cases, major deficiencies existed. This resulted in better exposure, better documentation of the exposure systems and a complete reporting of an exposure system's engineering and performance characteristics. The biological aspects of these studies were also improved because of a focus on using the NIH peer review system for supporting research. In particular, the NIH peer review system for projects requires a focus on hypothesis-driven research rather than phenomenology. The EMF-RAPID Program also attracted new and talented researchers to this area. Finally, through the regional EMF exposure units, there was a significant focus on replicating reported findings and conducting the studies in a completely blinded fashion to minimize potential study bias. In some experiments, the EMF exposure units were programmed (without

knowledge of the investigators), so that both exposure units were without magnetic fields (sham/sham experiments). These experimental conditions are important for inclusion in a study as internal controls. The comparison of experimental results between these sham/sham exposures often provided an indication of the amount of biological variation that was found in a study. It is interesting to note that all five of the cellular effects (ODC, gene expression, calcium flux, melatonin and growth of JB6 cells in agar) which were in the published literature at the initiation of the EMF-RAPID Program, were not reproduced when replication studies were conducted under carefully controlled conditions. It is worth noting that in some cases, the largest difference between groups occurred with sham/sham exposures thus suggesting that study design (e.g. sham/sham, blinding etc.) and experimental methodology (well characterized exposure systems) are extremely important for interpretation of EMF study results. This led several scientists to conclude that a study must include sham/sham exposures and be blinded before any effect is reported in the literature or at a scientific meeting

Communication Activities and Evaluation Process:

Communication and Outreach Activities

In addition to the research areas identified above, Public Law 102-486 also called for a communication and outreach program to make information about EMF available to the public and research community. To accomplish this mission, the NIEHS engaged in a variety of communication approaches to ensure wide dissemination of information. In collaboration with DOE, a question and answer booklet on EMF was developed and more than 100,000 copies were distributed including 10,000 in a Spanish version. A booklet, EMF in the Workplace, was also published that was developed with NIOSH and DOE and provides basic information in lay terms about EMF exposures in the workplace.

The NIEHS took the lead in developing a webpage for the EMF-RAPID Program that began operation on October 1, 1996 (www.niehs.nih.gov/emfrapid/home.htm). All of the EMF-RAPID Program's documents are available online. There are also links to other useful sites relating to EMF. This site received an average of 500 visits per day from approximately 21 countries.

The EMF-RAPID Program made available an EMF public information line where interested parties could call with questions about ELF-EMF and request information. The information line was open 10 hours a day for five days a week and received approximately 380 calls per month. Callers were provided copies of the EMF public information documents, and the operators were trained to give accurate responses to standard questions.

Health Effects Evaluation Process

As part of its mandate under the 1992 Energy Policy Act, the NIEHS is to report to the Congress about whether exposure to extremely low frequency (ELF) EMF has adverse effects on human health. The process the NIEHS used to evaluate any potential health hazards of these exposures was designed to be open, objective and scholarly, and contained a three-tier plan for collection and evaluation of the scientific information on ELF-EMF:

- A series of three science review symposia focused on 1) mechanistic research, 2) epidemiological research, and 3) laboratory research (animal and human). These workshops were aimed at including a broad spectrum of the research community in the evaluation of EMF health hazards, identifying key research findings and providing opinion on the quality of this research. Over 100 individuals registered for each meeting and included representatives from the public, stakeholders, regulatory agencies, advisory committees (NEMFAC and Interagency Committee) as well as scientists from varied disciplines both within and outside the field of EMF research.
- A working group meeting where a scientific panel reviewed historical and novel evidence on EMF and determined the strength of the evidence for human health and biological effects. Conclusions from the working group suggested areas of continuing concern such as childhood leukemia and adult leukemia.

- A period of public review for comment on EMF reports from the symposia and the working group prior to their use by the NIEHS in preparation of its report. During this period, the NIEHS hosted four public meetings (Tucson, AZ; Washington, DC; San Francisco, CA; and Chicago, IL) where individuals and groups could voice their comments. In addition, 178 written comments were also received during the comment period.

Access to these reports is available from the National Technical Information Service (703) 605-6000 or from the NIEHS web site (www.niehs.nih.gov/emfrapid/home.htm).

The following are the four documents that resulted from these meetings:

1. Portier C, Wolfe M, eds. EMF Science Review Symposium Breakout Group Reports for Theoretical Mechanisms and *In Vitro* Research Findings. Research Triangle Park, NC: National Institute of Environmental Health Sciences, 1997.
2. Portier C, Wolfe M, eds. EMF Science Review Symposium Breakout Group Reports for Epidemiological Research Findings. Research Triangle Park, NC: National Institute of Environmental Health Sciences, 1998.
3. Portier C, Wolfe M, eds. EMF Science Review Symposium Breakout Group Reports for Clinical and *In Vivo* Laboratory Findings. Research Triangle Park, NC: National Institute of Environmental Health Sciences, 1998.
4. Portier C, Wolfe M, eds. Assessment of Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields - NIEHS Working Group Report. Research Triangle Park, NC: National Institute of Environmental Health Sciences, 1998

Summary

This program was a collaborative effort between the DOE, NIEHS and other Federal agencies to develop a comprehensive data base for assessing the potential hazard of EMF exposure. The positive and productive interaction of multidisciplinary research teams in this program has served to re-enforce the importance of having physicists and engineers work together with biologists when evaluating physical agents. As the results of these studies are published in the peer-reviewed literature, the level of understanding about the interaction of biological systems with EMF of any intensity will be substantially enhanced. While this program answered some questions, it also raised other questions that may be addressed by further hypothesis-based, peer-reviewed research.

Electric and Magnetic Fields Research Projects by Funding Mechanism

A. Projects Funded Under PUBLIC LAW 102 - 486 (SECTION 2118), RAPID Program

1. Research Project Grants, R01s

Name	Project #	Institution	Project Title
Aaron	R01-ES07402	Brown University	EMF and Early Bone Development
Astumian	R29-ES06620	The University of Chicago	Study of the Electric Field Sensitivity of Yeast H+ ATPase
Balcer-Kubiczek	R01-ES07120	University of Maryland	Gene Expression Following 60-Hz Magnetic Field Exposure
Binninger	R01-ES07181	Florida Atlantic University	Effects of 60 Hz EMF on Transcription in Yeast
Byus	R01-ES06128	University of California Riverside, CA	Magnetic Fields Effects on Promotion of the Cancer
Graham	R01-ES07053	Midwest Research Institute	EMF Effects on Melatonin Hormones Immunity
Hei	R01-ES07091	Columbia University Institute	Effects and Mechanisms of EMF Signal Transduction
Lai	R01-ES6290	University of Washington	Effects of 60 Hz Magnetic Field on the Cholinergic System
Liburdy	R01-ES06401	Lawrence Berkeley National Laboratory	Magnetic Field Combinations and Lymphocyte Calcium Signaling
Litovitz	R01-ES06872	Catholic University of America	Studies of the Mechanism of EMF Induced Bioeffects
Luben	R01-ES06410	University of California Riverside, CA	Electromagnetic Effects on Membrane Receptor Function
Mandeville	R01-ES07049	University of Quebec	*In Vivo* Effects of 60 Magnetic Fields on Brain Tumor Promotion
Marino	R01-ES05928	Louisiana State University Medical Center, Shreveport, LA	Effect of 60-Hz Magnetic Fields On Lymphoid Phenotype
McCormick	R01-ES07093	Illinois Institute of Technology Research Institute	Mechanism of EMF Action in Human Breast Epithelial Cells

McLeod	R01-ES06287	State University of New York, Buffalo, NY	Perturbation of Cell Processes by ELF Electric Fields
Miller, R.	R01-ES07124	Columbia University	*In Vitro* Carcinogenesis by ELF Magnetic Fields
Miller, S.	R01-ES07127	SRI International	EMF Induced Signal Transduction and Gene Expression
Nuccitelli	R01-ES07133	University of California, Davis, CA	Effects of 60 Hz EM Fields on Human Keratinocytes
Reiter	R01-ES07132	University of Texas, Health Science Center, San Antonio, TX	60 HZ Magnetic Field Effects on *In Vivo* Pineal Melatonin
Rinehart	R01-ES07145	University of North Carolina, Chapel Hill, NC	Evaluation of Transforming Potential of EMF
Saffer	R01-ES07122	Battelle Pacific Northwest Laboratory	Cellular Effects of Weak Fields
Dibirdik	R01-ES07175	University of Minnesota	Protein Tyrosine Kinases and Electromagnetic Fields
Williams	R01-ES07076	Johns Hopkins University	Cellular Effects of Low Frequency Electromagnetic Fields
Woloschak	R01-ES07141	Argonne National Lab.	Changes in Gene Expression Accompanying Exposure to EMF
Yellon	R01-ES06137	Loma Lindi University	60 Hz Magnetic Field Clocks and Reproductive Development

1. Research Project Grants, R03s

Astumian	R03-ES08913	University of Chicago	Nonlinear Mechanisms for EMF Effects on Cells
Balcer-Kubiczek	R03-ES08898	University of Maryland Baltimore, Maryland	Molecular Effects of ELF Fields with Harmonic Content
Blank	R03-ES08855	Columbia University	Cytochrome Oxidase in Electric and Magnetic Fields
Costa	R03-ES08851	University of Washington	Exposure to EMF and Astrocytomas: *In vitro* Studies
Craviso	R03-ES08903	University of Nevada	Effects of EMFs on Calcium Levels in Excitable Cells
Glickman	R03-ES08894	University of Victoria	The Potential Mutagenicity and Co-Mutagenicity of Electrical Magnetic Fields

Goodman	R03-ES08867	Columbia University	EM Fields and Co-Promotion of Cancer
Griffin	R03-ES08864	Oak Ridge National Laboratory	Evaluation of Reported EMF Effects on Cell Communication
Grissom	R03-ES08896	University of Utah	Magnetic Field Dependence of Human Enzymes at Weak Fields
Hahn	R03-ES08895	VA Medical Center West Los Angeles	Brain Tumors in Mice Exposed to 60 Hz Magnetic Fields
Hei	R03-ES08926	Columbia University	EMF Environmental Carcinogens, and Co-Mutagenicity
Kripke	R03-ES08930	University of California, San Diego	EMF Effects on Melatonin Among Aging Volunteers
Lai	R03-ES08865	University of Washington	60 Hz Magnetic Field Induced DNA Damage
Lieberman	R03-ES08931	Columbia University	ELF Magnetic Fields, Gene Expression and the Cell Cycle
Loberg	R03-ES08921	Illinois Institute of Technology Research	EMF Effects on Cell Regulation in A-T Cells
McCormick	R03-ES08854	Illinois Institute of Technology Research	*In vivo* Responses to Complex EMF Exposure Metrics
McDonald	R03-ES08911	Illinois Institute of Technology Research	60 Hz Magnetic Fields and Cellular Stress Response
Reiter	R03-ES08890	University of Texas Health Science Center, San Antonio	Magnetic Field and *In vivo* DNA Damage
Ryan	R03-ES08873	Illinois Institute of Technology Research	Developmental Toxicology of EMF Exposure in Rats
Sheppard	R03-ES08920	Loma Linda University	Attributable Fraction Estimates for EMF Exposures
Sisken	R03-ES08878	University of Kentucky	Effects of 60 Hz EMF on Capacitative Calcium Entry
Yost	R03-ES08877	University of Washington	Extended Analysis of EMF Metrics and Melatonin Levels in Utility Workers

1. Research Project Contracts, N01s and Interageny Agreements Y01s

Anderson	N01-ES65397	Battelle Pacific Northwest Laboratory	National Toxicology Program (NTP) Studies to Evaluate the Potential of 50 or 60 Hz Magnetic Fields to Promote Breast Cancer in DMBA-treated Female Sprague Dawley Rats
Hardisty	N01-ES35369	Experimental Pathology Laboratories Inc., Research Triangle Park, NC	Pathology Support for EMF Animal Studies
Goldberg	PR-702871	Information Ventures, Inc. Philadelphia, PA.	Meta Analysis of Childhood Cancer Epidemiology
Lotz	Y01-ES0032	National Institute for Occupational Safety and Health	Effects of ELF Magnetic Field Exposure on Intracellular Calcium and Cell Proliferation *in vitro*
McCormick	N01-ES525351	Illinois Institute of Technology Research	Study to evaluate ODC in Mice
Owen	Y01-1ES40272	Federal Drug Administration	Power Line Frequency Magnetic Field Effects on Gene Expression and Enzyme Activity

1. Intramural Projects, Z01s

Barrett	Z01-ES-23019	National Institute of Environmental Health Sciences	Effects of Melatonin and EMF in Carcinogenesis
Bilski	Z01-ES-50138	National Institute of Environmental Health Sciences	The Influence of Electromagnetic Fields on the Reactions of Biological Transient Species
Chignell	Z01-ES-50136	National Institute of Environmental Health Sciences	The Role of Melatonin in the Biophysics Biological Effects of Magnetic Fields
Harry	Z01-ES-21164	National Institute of Environmental Health Sciences	Environmentally Enduced Alterations Neuron and Glia Dev. & Maturation
Hong	Z01-ES-90083	National Institute of Environmental Health Sciences	The Effect of 60 Hz EMF on Neurosciences, Tyrosine Phosphorylation
McMillian	Z01-ES-90067	National Institute of	EMF Effects on Brain Cell Cultures

		Environmental Health Sciences	Neurosciences
Murphy	Z01-ES-50156	National Institute of Environmental Health Sciences	Signaling Pathways Enhanced in the Oncostatic Properties of Melatonin
Pennypacker	Z01-ES-90066	National Institute of Environmental Health Sciences	Effects of EMF on Proto-Oncogene DNA Binding Activity During Brain Development
Portier	Z01-ES-90602	National Institute of Environmental Health Sciences	Hazard Identification, Estimates and Quantitative & Computational Biology Prediction of Risks from Exposure to EMF
Putney	Z01-ES-90087	National Institute of Environmental Health Sciences	Capacitative Calcium Entry
Travlos	Z01-ES-21195	National Institute of Environmental Health Sciences	The Effect of Light on Melatonin Levels in the Rat

1. Administration, Communication, Evaluation Activities,

Boorman		National Institute of Environmental Health Sciences	Communication & Other Activities
Boorman		National Institute of Environmental Health Sciences	Program Administration
Portier		National Institute of Environmental Health Sciences	Evaluation Program

B. Projects Funded Under PHS Act. Section 301, NIH/NIEHS Research Programs

1. Research Project Grants, R01s

Name	Project #	Institution	Project Title
Gandhi	R01-ES06292	University of Utah	Safety of Time Varying Magnetic Fields in MR Imaging
Grissom	R03-ES05728	University of Utah	Magnetic Spin Effects in Radical Enzymatic Reactions
Henderson	R01-ES05735	Hunter College-CUNY	Does Exposure to EM Fields Cause Cancer?
Hunter	U01-CA62984	Brigham & Womens's Hospital	Environmental Risk Factors and Breast Cancer in the NHS
Leske	U01-CA62991	University Medical Center at Stony Brook	EMF and Breast Cancer on Long Island
Liburdy	R01-ES06401	Lawrence Berkeley National Laboratory	Magnetic Field Combinations and Lymphocyte Calcium Signaling
Mehta	R29-ES05970	Brown University	Human B-cell Biology in Electric and Magnetic Environments
Peters	R01-ES06912	University of Southern CA	Magnetic Fields and Breast Cancer Risk
Reif	R01-ES08117	Colorado State University	Melatonin and Occupational Magnetic Field Exposure
Weaver	R01-ES06010	Massachusetts Institute of Technology	Bioelectromagnetic Mechanisms and Thresholds
Wrensch	R01-ES06285	University of California	Electromagnetic Fields and Adult Malignant Glioma
Wu	R01-ES-08051	Harvard University	EM Fields Induced in the Human Body: A New Approach

1. Research Project Contracts, N01s

McCormick	N01-ES25351	Illinois Institute of Technology Research	Studies to Evaluate the Toxicity and Potential Carcinogenicity of 60 Hz Magnetic Fields in Laboratory Animals

Research Projects

TITLE: **EMF and Early Bone Development**

Principal Investigator: Roy K. *Aaron*, M.D.
Orthopedic Department,
Brown University School of Medicine

Health Relevance: Cancer

Research Area: Studies involving whole animals; Cellular Processes

R01-ES07402 **Start Date:** 9/25/95 **End Date:** 8/31/98

Abstract

Power frequency (60 Hz) EMF have been implicated as affecting a number of biological processes involving cell replication and differentiation, including teratogenesis and neoplasia. The influence of these fields upon cell cycle activity has been associated with a number of membrane-mediated signaling mechanisms but the effects on phenotypic or genotypic expression have not been explained. This study examines the hypothesis that exposure to 60 Hz magnetic fields may produce alterations in endochondral ossification, the physiological process central to skeletal embryogenesis and longitudinal growth. In particular, we examine 60 Hz field effects on proliferation and differentiation and the genomic regulation of these key events in development.

This study utilizes an experimental model of endochondral ossification which has been well characterized in morphological, biochemical, and molecular terms and has been used to examine proliferation and differentiation in the context of development. This model is used to explore dosimetric considerations of 60 Hz exposure and to determine if there are developmental stages of particular sensitivity to these fields. Based on the results of previous studies, our studies are expected to reveal changes in cell differentiation and extracellular matrix synthesis in response to field stimulation.

The studies reported here describe the dose-dependent ability of power frequency fields to accelerate cell differentiation *in vivo* in an important developmental process, endochondral ossification. The acceleration of chondrogenic differentiation is associated with increased expression of $TGF\beta_1$ mRNA and protein. $TGF\beta_1$ is an important multifunctional signaling cytokine which regulates proliferation and differentiation in many tissues, notably cartilage and bone. The data suggest that the stimulation of $TGF\beta_1$ may be a mechanism through which EMF affects complex tissue behavior such as cell differentiation and through which the effects of EMF may be amplified.

Experimental Design and Exposure Conditions

The first aim of the study is to determine the response of the biologic system to varying field intensity levels and exposure durations. The exposure parameters providing the greatest biological response will then be used to identify the developmental stage most sensitive to ELF stimulation. On the basis of previous studies, this will most likely be the mesenchymal or progenitor cell pool (days 1-3 of development).

The second aim will describe the sequence of gene expression involved in the differentiation of chondrocytes from a mesenchymal progenitor cell pool in order to gain insight into the mechanism of activity of ELF field stimulation. Specific proliferation - and differentiation - associated genes will be identified which are expressed during chondrogenic differentiation. The genes studied will be those associated with growth (*c-fos, c-jun, c-myc*) and phenotypic expression (proteoglycan core protein, types II and X collagen, TGFβ, alkaline phosphatase).

The 60 Hz exposure device was designed and built according to specifications of the National Institute of Environmental Health Sciences (System LES-106, Electric Research and Management, State College, PA.) The estimated induced field at 0.1 mT in the rat is 3 mV/m (dB/dT = 3.77×10^{-2}T/s RMS). These exposure levels can be found in ambient home or work environments. The exposure equipment consists two quadruple coil sets containing a total of 16 coils. Each quadruple has 8 coil sets arranged in a side by side configuration utilizing two 4 coil Merritt arrangements (units "A" and "B"). Each coil in the 16 coil set has two windings in a bifilar, twisted wire construction to allow the application of AC current producing a magnetic field of 0.01-0.1 mT at 60 Hz when connected "aiding" and producing essentially no field when connected "opposing". This allows for active and sham experimental conditions.

AC field uniformity in the animal space is within $\pm$ 5% and vibration is less than 0.2 m/s^2 at 120 Hz. When energized at 0.1 mT and 60 Hz, stray electric fields in the animal exposure spaces were below 30 V/m and the acoustic noise in the 125 Hz octave band was less than 1 dB above normal background noise of 55 dB. Maximal heat dissipation of the coils was 55 W. The identical sham and exposure units were placed with their centers 12 feet apart in the opposite corners of the room. The orientation of the coils and the axis of the AC field were parallel to the direction of the horizontal geomagnetic field in the room. The horizontal geomagnetic field was 0.157 G at unit "A" and 0.181 G at unit "B". The vertical geomagnetic was 0.399 G at unit "A" and 0.359 G at unit "B".

Quality Assurance Measures

Animal exposure is performed double-blind to eliminate investigator bias. All biochemical tests are run with appropriate controls in duplicate.

Digital equipment controlled the exposure parameters from a PC keyboard and allows a gradual ramping up of the magnetic field and gradual turn off to avoid switching transients. An American Power Conversion line conditioner is placed ahead of a Variac to limit excessive 60 Hz line voltage variations.

Results

Significant increases in chondrogenesis were observed at 0.07 mT and 0.1 mT with the peak response occurring at 0.1 mT. The chondrogenic response to the 0.1 mT field was significantly greater than the response to 0.07 mT ($p=0.03$). A dose response in terms of exposure duration was observed as an increase in chondrogenesis at 7-9 hours of daily exposure. At higher and lower exposure levels the increase in chondrogenic differentiation was not observed indicating an exposure window of biological response. This field exhibited maximum stimulation of chondrogenic differentiation at 0.1mT and 7 hours of daily exposure. Maximal chondrogenesis was increased 33% over sham-treated controls at optimal exposure conditions.

Chondrogenic differentiation was investigated in more detail at optimal exposure parameters (0.1 mT, 7 h/d). Stimulation of chondrogenic differentiation was confirmed by increased expression of mRNA for the extracellular matrix proteins, PG and type II collagen (Table 1). Quantitative mRNA levels for PG were increased 57% in actively stimulated compared to sham stimulated ossicles. mRNA for type II collagen was correspondingly increased 56%. An increase in extracellular matrix deposition of PG and type II collagen was demonstrated by increased immunofluorescence of the matrix and by an increase in the immunopositive cells. $TGF\beta_1$ production in response to EMF was investigated by measuring changes in mRNA and active protein levels and by quantitating immunopositive cells (Table 2). mRNA levels were increased 68% in experimental compared to control ossicles. The active protein deposited in the extracellular matrix was increased 25%, and the number of immunopositive cells was increased 119%, in actively stimulated ossicles compared to sham stimulated controls.

Table 1: Extracellular Matrix Protein mRNA at 0.1 mT and 7 hours/day.
(O.D. Normalized to 18 S Protein)

	Sham Exposed	Active Exposed	p
Proteoglycan	95.9 ± 10.8	150.2 ± 17.9	0.03
Type II Collagen	24.5 + 2.2	38.2 + 5.2	0.04

Table 2: TGF?1 mRNA and Protein at 0.1 mT and 7 hours/day.

	Sham Exposed	Active Exposed	p
mRNA (O.D.)	189.7 ± 36.6	318.4 ± 37.6	0.03
Active Protein (pg/mg)	66.8 ± 2.8	83.3 ± 4.4	0.005
Immunopositive Cells (n)	227.7 ± 55.8	499.0 ± 6.0	0.005

Discussion and Contribution to Understanding Biological Effects of EMF

Power frequency fields in this study were in the general range of those comprising environment, home and work exposure. Field strengths varied from 0.01-0.1 mT. Field strengths commonly experienced in some work places and under transmission and distribution lines have been reported to be in the same range. Previous *in vivo* studies with sine waves of varying frequency and amplitude have demonstrated a biologically effective window at 15-150 Hz, inducing an estimated peak electric field in the tissue of 1 mV/m. In that model of bone resumption, a greater biological effect was seen at 15 and 75 Hz. In our study of 60 Hz fields, the calculated induced field in the tissue was 3 mV/m.

The maximum chondrogenic response within the range of amplitudes studied was to the 0.1 mT field. Data presented here expresses dosimetry also as a function of exposure duration. A chondrogenic response was observed at an exposure window of 7-9 h/d. Since the studies were designed to demonstrate biological activity at environmental exposure levels, these data do not necessarily define optimum dosing conditions for a 60 Hz sine wave from the therapeutic point of view.

A considerable amount of work with power frequency fields has assumed the perspective of exposure hazard and has centered on cancer promotion, teratology or reproductive loss, and physiological changes especially behavioral and cardiovascular. By and large, although these fields clearly can elicit responses from various biological systems, *in vitro, in vivo* and clinical studies have not demonstrated clear-cut exposure hazards to 60 Hz fields.

Another line of investigation with EMF in the ELF range has dealt with promotion of connective tissue repair. These studies have generally shown robust results in terms of a stimulation of physiologic repair and remodeling in bone, cartilage, and soft tissues. The cell biology underlying these physiologic observations consists largely of enhanced differentiation of progenitor cells and an increase in extracellular matrix synthesis. While enhanced proliferation of some cells can be shown *in vitro*, in more complex *in vivo* systems culminating in repair, differentiation to a mature phenotype and enhanced extracellular matrix synthesis has been the underlying mechanism in successful reconstitution of connective tissues. Since there is a general inverse relationship between proliferation and differentiation, these effects of EMF have largely been considered maturational.

Our data with power frequency fields confirms a large body of work, including our own, with therapeutic PEMF. It confirms that 60 Hz, 0.1 mT fields have significant biological activity characterized by enhanced extracellular matrix synthesis - a function of phenotypically mature, or differentiated, cells.

This study demonstrates biological activity of 60 Hz EMF on an important developmental pathway having broad applications to embryogenesis, growth, and repair. The specificity of EMF effects on various tissues is clearly determined by the specific tissues stimulated and by the predominant cell cycle activity of the cell population resident in the tissue. The results presented in this study are consistent with previous studies on accelerated repair and our current understanding is that EMF has important biological activity in connective tissues which is, by and large, pro-maturational and pro-differentiative. While some proliferation in early repair might be seen, EMF enhances differentiation and differentiative functions such as extracellular matrix protein synthesis.

These observations indicate a fundamental regulatory effect of 60 Hz EMF on basic cell cycle activities. Our data permits the hypothesis of an intermediary mechanism of EMF activity namely, increased synthesis of $TGF\beta_1$. $TGF\beta_1$ is a multifunctional growth factor, part of a large gene family with significant homologies to the bone morphogenic proteins (BMP) and to primitive genes such as decapentaplegic (dpp) complex in Drosophila, and Vg-1 of Xenopus. Other laboratories have shown increases in growth factors of the BMP and IGF-2 families by EMF and have suggested that stimulation of growth factors may be a method of biological amplification of EMF effects. Members of the $TGF\beta_1$/BMP gene family, particularly $TGF\beta_1$, have profound effects on a number of cell and tissue types. *In vitro* and *in vivo* studies have suggested a regulatory role for $TGF\beta_1$ in osteogenesis and chondrogenesis. In addition, $TGF\beta_1$ regulates soft tissue healing by acting as a chemoattractant for fibroblasts and as a stimulator of type I collagen synthesis. $TGF\beta_1$ is released by degranulating platelets and is present in inflammatory cells. Other studies have shown mitogenic effects and tumor promotion.

These studies suggest that $TGF\beta_1$ is an important signaling cytokine in the regulation of cell differentiation and proliferation and, therefore, is important in developmental processes (including embryogenesis, growth and repair) and in oncogenesis. Our data demonstrate that transcription of mRNA for $TGF\beta_1$ as well as the accumulation of active $TGF\beta_1$ protein are upregulated by 60 Hz fields coincident with accelerated chondrogenic differentiation, suggesting these EMF effects may be mediated by $TGF\beta_1$. Since $TGF\beta_1$ has multiple regulatory roles in many tissues, the ability of EMF to regulate $TGF\beta_1$ suggests that $TGF\beta_1$ may be a general effector of EMF activity and may account for the wide variety of effects observed with EMF stimulation.

Publications From This Grant

1. Ciombor D McK and Aaron RK. "EMF stimulates cartilage differentiation in endochondral ossification coincident with an increase in TGFβ expression." In: Electricity and Magnetism in Biology and Medicine. Ed. F. Bersani. Plenum Press. In press, 1998.
2. Aaron RK, Ciombor D McK, Keeping H, Wang ,S Capuano A and Polk C "Power frequency fields promote cell differentiation coincident with an increase in TGFβ expression." Bioelectromagnetics, Submitted, 1998.

Other EMF Publications

1. Aaron RK and Ciombor D. McK. "Acceleration of experimental endochondral ossification by biophysical stimulation of the progenitor cell pool." J. Ortho. Res. 14:582;1996.
2. Polk C, Aaron RK, and Ciombor D McK. "Therapeutic applications of low frequency electric and magnetic fields in the US." In: Treatment with Electromagnetic Fields, Physical Foundations - Biological Effects - Medical Applications. Ed: J.P. Stossel. Schattauer, Stuttgart, Germany, 1997.
3. Aaron RK, Ciombor D McK and Polk C. "Stimulation of chondrogenic differentiation in endochondral bone formation - Implications in skeletal morphogenesis." In: Skeletal Morphogenesis. Ed. M. Ehrlich. AAOS, Chicago, IL , 1997.
4. Ciombor D McK, Aaron RK, Lester G and Caterson B. "Chondrogenic differentiation in endochondral ossification and its stimulation by electrical signals." Submitted, 1998.

TITLE: **National Toxicology Program (NTP) Studies to Evaluate the Potential of 50 or 60 Hz Magnetic Fields to Promote Breast Cancer in DMBA-treated Female Sprague Dawley Rats**

Principal Investigator: Larry *Anderson, Ph.D.*
Battelle Pacific Northwest Laboratory
Richland, Washington

Health Relevance: Cancer

Research Area: Studies involving whole animals; tumor promotion

N01-ES65397 **Start Date:** 3/1/96 **End Date:** 10/31/97

Abstract

The studies are designed to determine whether 50 or 60 Hz sinusoidal or non sinusoidal magnetic fields have co-promotional activity in well characterized models of breast cancer carcinogenicity, such as the dimethylbenzanthrocene (DMBA) treated female Sprague Dawley rats. The major efforts will be to validate the positive effects of magnetic field exposure in this model as reported by Löscher *et al* (2, 7-10). Löscher (9) also reported that magnetic field exposures significantly reduced nocturnal melatonin levels in these rats so this measurement is also included in this study. Others have also suggested that magnetic field exposure can alter melatonin levels in rats (5, 6). The role of exposure to magnetic fields associated with the use of electric power has been postulated to have the potential to increase breast cancer in humans (12). The 26 week study may offer a greater opportunity for finding a weak promotional effect of magnetic field exposures on DMBA-initiated breast cancer in the rat.

Electric and magnetic fields (EMF) are associated with the production, transmission, and use of electricity; thus, the potential for human exposure is high. These electric and magnetic fields are predominantly of low frequency (60 Hz in the United States and 50 Hz in Europe) and generally of low intensity. Because some epidemiology studies and initiation/promotion studies in rats have suggested a potential for increased breast cancer rates with increasing magnetic field exposure, the ability of 50- and 60-Hz magnetic fields to promote mammary gland tumors initiated by the administration of 7,12-dimethylbenz(a)anthracene (DMBA) was examined in female Sprague-Dawley rats in 13- and 26-week whole-body exposure studies. Additional animals were evaluated for changes in pineal gland and serum melatonin concentrations.

First 13-Week Study

Groups of 100 female rats were administered 20 mg DMBA (four weekly gavage doses of 5 mg in sesame oil) or administered 20 mg DMBA and exposed to 1 G 50-Hz, 5 G 50-Hz, or 1 G 60-Hz magnetic fields for 18.5 hours per day, 7 days per week, for 13 weeks. A group of 100 vehicle control rats was administered only sesame oil on the same schedule. Additional groups of 10 rats receiving similar treatment were evaluated for pineal gland and serum melatonin concentrations at 4, 8, or 12 weeks.

All vehicle control rats survived to the end of the study. Of the animals administered 20 mg DMBA, six rats in the DMBA control group, 13 in the DMBA/1 G 50-Hz group, eight in the DMBA/5 G 50-Hz group, and five in the DMBA/1 G 60-Hz group died or were removed from the study prior to the final necropsy. Final mean body weights and body weight gains of the DMBA/1 G 50-Hz and DMBA/1 G 60-Hz groups and the mean body weight gain of the DMBA/5 G 50-Hz group were slightly greater than those of the DMBA control group. Clinical findings including torso masses and ulcers were attributed to DMBA administration.

The numbers of palpable mammary gland tumors, tumor sizes, and total tumor areas in DMBA/magnetic field groups were similar to those in the DMBA control group. Relative to the DMBA control group, exposure to magnetic fields did not significantly affect overall incidences of mammary gland neoplasms or non-neoplastic lesions in the DMBA/magnetic field groups.

Second 13-Week Study

Groups of 100 female rats were administered 8 mg DMBA (four weekly gavage doses of 2 mg in sesame oil) or administered 8 mg DMBA and exposed to 1 G 50-Hz or 5 G 50-Hz magnetic fields for 18.5 hours per day, 7 days per week, for 13 weeks. Additional groups of 10 rats receiving similar treatment were evaluated for pineal gland and serum melatonin concentrations at 4, 8, or 12 weeks.

Except for one rat in the DMBA/5 G 50-Hz group, all rats survived until the end of the study. Final mean body weights of DMBA/magnetic field groups were similar to those of the DMBA control group. Clinical findings including torso masses and ulcers were attributed to DMBA administration.

The numbers of palpable mammary gland tumors, tumor sizes, and total tumor areas in DMBA/magnetic field groups were similar to those in the DMBA control group. Relative to the DMBA control group, exposure to magnetic fields did not significantly affect overall incidences of mammary gland neoplasms or nonneoplastic lesions in the DMBA/magnetic field groups.

26-Week Study

Groups of 100 female rats were administered 10 mg DMBA (in sesame oil) by gavage or administered 10 mg DMBA followed by exposure to 1 G 50-Hz, 5 G 50-Hz, or 1 G 60-Hz magnetic fields for 18.5 hours per day, 7 days per week, for 26 weeks. Another group of 100 vehicle control rats were administered only sesame oil. Additional groups of 10 rats receiving similar treatment were evaluated for pineal gland and serum melatonin concentrations at 4, 8, or 12 weeks.

All rats in the vehicle control group survived until the end of the study. Twelve rats in the DMBA control group, 15 in the DMBA/1 G 50-Hz group, nine in the DMBA/5 G 50-Hz group, and six in the DMBA/1 G 60-Hz group died or were removed during the study. The final mean body weights and body weight gains of the DMBA/1 G 50-Hz and DMBA/5 G 50-Hz groups were significantly greater than those of the DMBA control group. Clinical findings including torso masses, abscesses, and ulcers were attributed to DMBA administration. The pineal gland melatonin concentrations of DMBA/5 G 50-Hz and DMBA/1 G 60-Hz rats were significantly greater than that of the DMBA controls at week 12; however, these data were highly variable between individual animals within each group.

The numbers of palpable mammary gland tumors, tumor sizes, and total tumor areas in DMBA/magnetic field groups were similar to those in the DMBA controls. The incidences of mammary gland carcinoma (including multiple) in the DMBA/1 G 60-Hz group were significantly decreased relative to the DMBA control group.

Results

In an initiation/promotion study in which female Sprague-Dawley rats were initiated by four weekly doses of 5 mg DMBA per rat beginning at 50 days of age and exposed to 50-Hz magnetic fields at 1 or 5 G field intensities or to 1 G 60-Hz magnetic fields for 13 weeks, there was no evidence that magnetic fields promoted the development of mammary gland neoplasms. The prevalence and multiplicity of mammary gland carcinomas in all DMBA groups limited the ability of this assay to detect a promoting effect of magnetic fields.

In an initiation/promotion study in which female Sprague-Dawley rats were initiated by four weekly doses of 2 mg DMBA per rat beginning at 50 days of age and exposed to 50-Hz magnetic fields at 1 or 5 G field intensities for 13 weeks, there was no evidence that magnetic fields promoted the development of mammary gland neoplasms.

In an initiation/promotion study in which female Sprague-Dawley rats were initiated by a single 10 mg DMBA dose at 50 days of age and then exposed to 50-Hz magnetic fields at 1 or 5 G field intensities or to 1 G 60-Hz magnetic fields for 26 weeks, there was no evidence that magnetic fields promoted the development of mammary gland neoplasms.

Summary of the 13- and 26-Week 7,12-Dimethylbenz(a)anthracene (DMBA) Initiation/ Magnetic Field Promotion Studies in Female Sprague-Dawley Rats

	First 13-Week Study	Second 13-Week Study	26-Week Study
Doses	20 mg DMBA control 20 mg DMBA/1 G 50 Hz 20 mg DMBA/5 G 50 Hz 20 mg DMBA/1 G 60 Hz	8 mg DMBA control 8 mg DMBA/1 G 50 Hz 8 mg DMBA/5 G 50 Hz	10 mg DMBA control 10 mg DMBA/1 G 50 Hz 10 mg DMBA/5 G 50 Hz 10 mg DMBA/1 G 60 Hz
Body weights	DMBA/1 G 50-Hz and DMBA/1 G 60-Hz groups greater than the DMBA control group	DMBA/magnetic field exposed groups similar to the DMBA control group	DMBA/1 G 50-Hz and DMBA/5 G 50-Hz groups greater than the DMBA control group
Survival rates	94/100, 87/100, 92/100, 95/100	100/100, 100/100, 99/100	88/100, 85/100, 91/100, 94/100
Mammary gland carcinoma (%)	92/100, 86/100, 96/100, 96/100	43/100, 48/100, 38/100	96/100, 90/100, 95/100, 85/100
Mammary gland fibroadenoma (%)	3/100, 2/100, 1/100, 1/100	None	71/100, 76/100, 73/100, 68/100
Evidence of promotional ability	No evidence	No evidence	No evidence

Discussion and Contribution to Understanding Biological Effects of EMF

The studies were designed to determine whether 50 or 60 Hz sinusoidal or non sinusoidal magnetic fields have co-promotional activity in well characterized models of breast cancer carinogenicity, such as the dimethylbenzanthrocene (DMBA) treated female Sprage Dawley rats. The major effort was to confirm the positive effects of magnetic field exposure in this model as reported by Löscher *et al* (Baum, *et al*, 1995, Löscher. *et al*, 1990 and 1994). Löscher (1994) also reported that magnetic field exposures significantly reduced nocturnal melatonin level in these rats so this measurement is also included in levels in rats (Lerchl, *et al*, 1990). These three studies failed to demonstrate a promotional effect of magnetic field exposure or mammory gland carcinomas in Sprague Dawley rats.

Publications From This Grant

1. National Toxicology Program. NTP technical report (studies of magnetic field promotion (DMBA initiation) in Sprague Dawley rats (gavage / wholebody exposure studies) NTP TR 489, NIH Publication No. 98-3979 U.S. Dept of Health and Human Services. Public Health Service, (1998).

TITLE: **Nonlinear Mechanisms for EMF Effects on Cells**

Principal Investigator: Dean R. *Astumian, Ph.D.*
The University of Chicago
Chicago, Illinois

Health Relevance: Biophysics of Cell Field Interaction

Research Area: Cellular Function, Biophysics/Field Interactions, Cellular Processes

RO3-ES08913 **Start Date:** 1/10/97 **End Date:** 1/09/99

Abstract

Our overall goal is (A) to further develop the formal analogy between occurrence of a biological effect and measurement of an EMF (electromagnetic field) exposure by the biological system, and (B) to then investigate several classes of biophysical mechanism. This general approach will allow us to appropriately develop theories of biophysical mechanism that deal with biological relevant EMF exposure conditions. As part of this general aim we will:

- Focus on the change in the number of molecules, (n)EMF, in a biochemical pathway that arises because of an EMF exposure. Here EMF is analogous to a measurement "signal" ("S").
- Develop theories of biophysical mechanism that explicitly describe the dependence of (n)EMF on exposure parameters and on properties of the biological system.
- Identify and describe other influences that result in competing changes for the same type of molecular in that pathway. To be regarded as causing a biological effect, the EMF induced change must at least be comparable to shot noise. (n)EMF, which is a fundamental random fluctuation in molecular (or ionic) number. Other competing changes may arise from mechanical influences, normal physiological variations of biochemical levels, or other sources.
- Estimate threshold EMF exposure conditions based on field strengths, exposure time and properties of the biological system. This will involve a generalized signal-to-noise ratio criterion that includes competing molecular changes due to noise ("N"), interference ("I"), background ("B") and modulating influences ("M"), usually spatial and temporal temperature variations. Further development will yield a general, biologically relevant framework within which specific candidate biophysical mechanics can be investigated. Motivation is to find "Causal Agent" of EMF exposures. This general aim is highly relevant to bioelectromagnetics research and to current and future efforts to regulate and mitigate EMF. Throughout we will attempt to construct the most sensitive possible theoretical models, to explain biological effects at very weak fields.

Experimental Design and Exposure Conditions

None.

Quality Assurance Measures

The work is theoretical. We will check our math, and make sure our numerical computations converge.

Results

We have shown that Brownian motors and pumps (such as kinesin and RNA polymerase motors and NaK ATPase ion pumps) modulated by a non-equilibrium chemical reaction such as ATP hydrolysis can be far more sensitive to an applied external force than a system at equilibrium. We have also demonstrated that a far from equilibrium process can drive the appearance of spontaneous coherence and self-organized oscillations.

Discussion and Contribution to Understanding Biological Effects of EMF

The theoretical mechanisms investigated in this project have shown the types of biological systems (Brownian motors, NaK ATPase ion pumps) that are likely to be most sensitive to applied AC electric or magnetic fields. However, none of these systems are responsive at environmental EMF levels.

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2. Tarlie M, and Astumian RD. "Optimal modulation of a brownian ratchet." Proc. Natl. Acad. Sci. 95: 2039-2043, 1998.
3. Bier M and Astumian RD. "What is Adiabaticity- Suggestions from a fluctuating Lineal Potential." Physics Letters A. 247: 385-390, 1998.
4. Derenyi I and Astumian RD. "Spontaneous coherence and oscillations by a membrane transporter in an RLC circuit." Phys. Rev. Letts, 80: 4602-4605, 1998.
5. Adair R, Astumian RD and Weaver J. "On the detection of weak electric fields by sharks, rays and skates." Chaos, 8: 576—587, 1998.
6. Astumian RD and Derenyi I. "Fluctuation driven transport and models of molecular motors and pumps." European Biophysical Journal, 27: 474-489, 1998.
7. Astumian RD and Moss F. " Overview the constructive role of noise in fluctuation driven transport and stochastic resonance." Chaos, 8: 533-538, Sept. 1998
8. Weaver JC, Vaughn T, Adair RK and Astumian RD. "Theoretical limits on the thresholds for the response of long cells to weak, extremely low frequency fields due to ionic and molecular flux rectification." Biophysial J., 75: 2251-2254, 1998.

Other EMF Publications

1. Astumian RD, Weaver JC and Adair RK. "Rectification and Stochastic Resonance for signal averaging of weak electric fields in biological systems." Proc. Natl. Acad. Sci. USA 92: 3740-3743, 1995.
2. Holian O, Astumian RD, Lee RC, Reyes BM, Attar BM and Walter RJ. "Protein Kinase C activity is altered in HL60 cells exposed to 60 Hz AC electric fields." Bioelectromagnetics, 17:504-509, 1996.

TITLE: **Study of the Electric Field Sensitivity of Yeast H+ ATPase**

Principal Investigator: Dean R. *Astumian*, Ph.D.
The University of Chicago
Chicago, Illinois

Health Relevance: Biophysics of Cell Field Interactions

Research Area: Cellular Function, Biophysics Cell/Field Interactions, Cellular Processes

R29-ES06620 **Start Date:** 09/28/94 **End Date:** 08/31/99

Abstract

The focus of this research project is to study ion transport proteins in order to determine possible mechanisms of interaction between electric fields and biological systems. Many experiments have focused on what we refer to as "downstream" effects, effects that may be caused by electric field exposure but require several intermediate processes before a biological response is seen. Thus, any condition of the cell that influences any of the intermediary states can change the overall response to a field in sometimes difficult to understand ways. Our approach is to study a reaction where the process that is the primary site of interaction can be directly assayed and studied as a function of field parameters such as frequency and amplitude. While ultimately, we are interested in the effects of 60 Hz fields at amplitudes below a few mV/cm, it is essential to investigate the mechanism of interaction over a wide range of frequencies and amplitudes to establish a baseline understanding of how electric fields and cells may interact.

Our specific hypothesis is that the proton ATPase that we are studying will have similar field dependence to the related protein Sodium-Potassium ATPase (NaKATPase) for which both frequency and amplitude optima have been observed. The proton ATPase is a simpler system, and so a more exhaustive study than is possible with NaKATPase can be accomplished. Specifically, we want to understand the biophysics behind the frequency and amplitude peaks. Many theories have been advanced which can only be tested be studying the behavior under many different conditions and comparing the results between these experiments. Ultimately, we anticipate that our research will provide insight that will help us to predict what proteins are likely to be very sensitive to electric fields, and under what conditions.

Experimental Design and Exposure Conditions

Our experimental design employs an electric field applied to a suspension of yeast proteoliposomes that contain the proton ATPase using plane parallel electrodes. As a precaution against artifacts resulting from electrolysis products, we separate the electrode from the sample solution by using a salt bridge, although preliminary experiments suggest that this cumbersome precaution is not necessary and we may dispense with the salt bridge, particularly for the very low field experiments. When the proteoliposome are made by breaking open whole yeast cells, most of the vesicles turn inside out. In this topology, the ATPase site faces outside, and the ATP mediated proton pumping is directed from out to in. This makes the assay quite convenient, since the level of ATP can be changed from the outside, and the proton uptake into the vesicle can be followed by acridine orange (a weak base) localization into the vesicle as an optical signal. All of these facts combine to make it possible to carry out experiments at many different field strengths and amplitudes ranging from .01 mV/cm to 1 V/cm, and 1 Hz to 100,000 Hz. The measured quantities are the steady state rates of proton uptake and ATP hydrolysis so transients are not expected to play a role.

In order to get a better handle on the experimental conditions optimal for field stimulated transport, we have renewed a collaboration with Prof. Tian Tsong at the University of Minnesota. In this work, we are investigating whether added noise can enhance the effect of an applied ac field by a mechanism known as stochastic resonance. In these experiments (carried out on NaK ATPase, for which the field dependence is best known)

we showed that the addition of a small amplitude external white noise electronically could actually increase the response to an applied ac field. However, as the noise increased, the effect due to the applied ac field went down, and was ultimately abolished or masked altogether.

Quality Assurance Measures

Many of the types of problems that plague very week field experiments using inductive coupling, such as stray fields, etc. are not relevant for the type of experiments we are performing. A particular advantage is the experiments will always include zero field (no exposure) which serves as a negative control, and high field (1 V/cm) where an effect is always observed, serving as a positive control. All biochemical tests will be run with appropriate controls in duplicate.

Results

To date, none of our work supports the idea of a weak field causing any detectable effect on the yeast proton ATPase. The biological variability was very high in all cases, dramatically decreasing our confidence in those cases where a small effect was apparently observed (at larger amplitudes). Cooling the yeast (unlike the case with erythrocytes) increased the variability from sample to sample.

The work on stochastic resonance and NaK ATPase is much more promising. The preliminary results show a definite effect of added noise. If the ac field is at the optimum voltage and frequency, adding noise always decreases the response. However, at non-optimal amplitudes and frequencies, adding noise in many cases increased the systems response, although typically by a factor of two, and not by several orders of magnitude as claimed in several theoretical works.

Discussion and Contribution to Understanding Biological Effects of EMF

We have not been able to demonstrate any reproducible effect of an applied AC electric field on the yeast proton ATPase. The system shows a tremendous amount of variability from day to day, and even when normalized, the control samples are indistinguishable from the samples exposed to the field. This highlights the necessity of very many and very rigorous controls for all experiments on the effects of weak electric fields on biological systems.

The results on the NaK ATPase illustrate the possible constructive role of noise in allowing detection of weak signals, although not to the tremendously large extent suggested by some theories.

Our work, while not directly showing very weak field effects, does provide guidelines for understanding theoretical basis for interactions between endogenous noise and external signals.

Publications From This Grant

1. Astumian RD and Bier M. "Fluctuation driven ratchets - molecular motors." Phys. Rev. Letters 72:1766-1769, 1994.
2. Astumian RD. "Electroconformational coupling of membrane proteins." Annals. N.Y. Acad. Sci. 720: 136-141, 1994.
3. Astumian RD, Weaver JC and Adair RK. "Rectification and stochastic resonance for signal averaging of weak electric fields in biological systems." Proc. Natl. Acad. Sci. USA 92: 3740-3743, 1995.
4. Bier M and Astumian RD. "Biased brownian motion as the operating principle for microscopic engines." Bioelec. Bioenerg., 39:67-75, 1996.

5. Astumian RD and Brier M. “Mechanochemical coupling of molecular motors to ATP hydrolysis.” Biophys. Journal. 70:637-653, 1996.

6. Bier M and Astumian RD. “Biasing brownian motion in opposite directions in a 3-State fluctuating potential: an application for the separation of small particles.” Phys. Rev. Letters 76:4277-4280, 1996.

7. Astumian RD. “Adiabatic theory for fluctuation induced motion on a periodic potential.” J. Phys. Chem. 100: 19075-19081, 1996.

8. Holian O, Astumian RD, Lee RC, Reyes HM, Attar BM and Walter RJ “Protein Kinase C activity is altered in HL60 cells exposed to 60 Hz AC electric fields.” Bioelectromagnetics, 17:504-509, 1996.

9. Astumian RD. “Thermodynamics and kinetics of brownian motors.” Science, 276: 917-922, 1997.

10. Astumian RD, Adair RK and Weaver JC. “Stochastic resonance at the single cell level.” Nature, 388: 632-633, 1997.

11. Derenyi I and Astumian RD. “Spontaneous coherence and oscillations by a membrane transporter in an RLC circuit.” Submitted, Phys. Rev. Letts. 1997.

Other EMF Publications

None.

TITLE: **Gene Expression Following 60 Hz Magnetic Field Exposure**

Principal Investigator: Elizabeth K. *Balcer-Kubiczek*, Ph.D.
University of Maryland at Baltimore
Baltimore, Maryland

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Cycle Control; Signal Transduction

R01-ES07120 **Start Date:** 09/28/94 **End Date:** 08/31/98

Abstract

Our objective has been to test the hypothesis that 60 Hz magnetic fields (MF) have a direct effect on gene transcription. Accordingly, our project had two aims: (1) to compare messenger RNA (mRNA) levels of selected candidate genes in MF/exposed versus control cells using several patterns of exposure and post-exposure times; (2) to identify other known and novel differentially expressed genes after several exposure conditions studied in Aim 1.

Our Aim 1 studies included genes, cells, 60/Hz magnetic field exposure parameters previously investigated and published by others. All these studies also included non-MF exposures to physical or chemical agents known to affect transcription of our selected candidate genes. Only positive-control non-EMF treatments, including X-irradiation (doses up to 20 Gy), heat (45.5^{o}C for 9 min) or 12-O-tetra-decanoylphorbol-13-acetate (TPA; 50 ng/ml for 3 h) significantly and reproducibly altered mRNA levels of several genes, including *c-fos, c-myc, bcl-2, egr-1, gadd45, mdm2,* PCNA, and *Waf1/Cip1*.

In Aim 2 studies, we screened a panel of 1920 randomly selected cDNAs in order to discover other genes that were differentially expressed in HL60 cells exposed to 60Hz magnetic fields (2 mT) or X rays (5 Gy) versus unexposed cells. Identification of these full-length clones was accomplished using our unbiased two gel cDNA library screening method (BIGEL). Eighteen differentially expressed cDNAs were recovered from the panel of 1920 clones in X-irradiated versus control HL60 cells. Differential expression in experimental versus control cells was independently confirmed by Northern blotting of paired total RNA samples hybridized to each of the 18 clonespecific cDNA probes. DNA sequencing revealed that 17 of the 18 cDNA clones produced database matches with genes related to glucose /protein /cell growth, energy metabolism, or oxidative stress apoptosis (such as *c-myc*, ras-like protein mRNA, peptide elongation factor-1 , cytochrome c oxidase, and triosephosphate isomerase). In contrast, comparative analysis of the same 1920 sequences revealed no differences in expression levels in magnetic field versus sham-exposed cells. Thus, BIGEL is a highly sensitive approach to identifying differentially expressed genes from a very large gene pool.

It has shown that differential gene expression was induced in a small proportion (less than 1%) of this pool by ionizing radiation, but not by 60 Hz magnetic at 2 mT. Laboratory reports on gene expression following magnetic field exposure have been controversial, although the weight of evidence suggests no effect of magnetic fields. We searched for changes in gene expression, including possible delayed expression, using a higher flux density and longer exposure duration than investigated by others in studies that yielded negative results. Our studies do not support a hypothesis that gene transcription is affected by exposure to MF and, therefore, that there are biological consequences at a cell level of exposure to magnetic fields.

Experimental Design and Exposure Conditions

Because of the claim that previous contradictory results may have been associated with the p53 status of the investigated cell lines, the cell lines chosen for investigation exhibited different p53 status; for details, see ref. [2,3]. The majority of this work used human leukemia HL60 cells, which are null for p53. Other investigated cell lines were established from the following human tumor tissues: breast (MCF7 WT cells; p53+/+), brain (A172 cells; p53/), and cervix (HeLa cells; clone HeLa S3; p53+/). All cell lines were cultured and exposed to treatment in the appropriate medium supplemented with 10 % fetal bovine serum. Cell lines and post exposure expression times were examined together in large experiments which were repeated 2 to 5 times.

60 Hz magnetic field exposure system: The exposure system has been described [1]. Briefly, cells were exposed in tissue culture flasks filled with culture media and placed in the central planes of long solenoids. The growing cells formed a monolayer parallel to the field lines. A sham-control solenoid was bifilar-wound and energized at the same level as the exposure solenoids. Consequently, control cells experienced zero magnetic field, but were subject to the same level of possible disturbances, including local acoustic noise and convective heating in field exposed cells. To eliminate the geomagnetic and other background DC/AC fields inside the solenoids, they were enclosed in individual magnetic shield cylinders. Strict temperature control was provided by partial immersion of the solenoids and flasks in a circulating temperature controlled water bath.

Magnetic field measurements: Measurements of the fields within the exposure coils were performed as previously described [1]. Mean, minimum, and maximum field levels were logged, and indicated stability to ± 3 % over 24 h; occasional transient power losses were noted in a few runs; no distortion of the 60 Hz magnetic field wave form was evident in oscilloscope displays; measurements of the power spectrum of exposure and background fields revealed a third harmonic component 20 to 40 dB down from the 60 Hz fundamental, according to spot measurements. Exposure flasks were manually introduced into energized solenoids, and removed prior to turning them off in order to minimize transient fields. Possible accessory field exposure was minimized by the use of the magnetic shields during experimental treatments, the use of a double-walled container fabricated from 0.13cm A mμmetal to carry cells to and from the MF exposure facility, and the fact that cells were maintained in incubators with MF levels in their chambers not exceeding a range between 0.1 to 1 μT.

Quality Assurance Measures

During handling and maintenance, cells are shielded from external fields. Flasks are transported between the MF exposure facility and our molecular biology laboratory (a 125m distance) in a custom-made mμ-metal container. Field levels in hood areas have been measured to be less than 0.1 μT. Cell cultures are grown in commercial CO_2-incubators. AC/DC fields inside the incubators' chambers were mapped; we use the incubators and/or incubator shelves with the lowest fields (less than 1 μT). Fields mapped in other work spaces and areas are less than 0.2 μT. Our 60 Hz magnetic field exposure facility is consistent with the characterizations suggested by the NIEHS RAPID Program. Temperature and exposure parameters are monitored and recorded during experiments. Cells are exposed in a stringently controlled environment and assays are performed under blind conditions. RNA samples are coded by individuals not involved in performing molecular assays. In addition, in some of the AIM 1 experiments, the coded RNA samples obtained are divided between two teams and analyzed independently in two laboratories [in the Department of Radiation Oncology (EKBK) or Medicine (SJM)]. The autoradiographic data are analyzed statistically to avoid subjective interpretation. Positive (nonMFs) controls are included.

Results

Note: Results shown are only from experiments that included all MF and non-MF groups, as listed under an individual study description.

Experimental Series # 1:
Cells: HL60
MF exposure parameters: 0.0057 or 0.57 mT for 10 to 40 min
Sampling time (T) after MF: immediate (T = 0 h)
Non MF exposures: Heat, 45.5^{o}C for 9 min (T = 0, 1 or 4 h);
500 ng ml1 TPA for 3 h (T = 0 h)
Transcripts studied: *c-myc, c-fos, actin*, GAPDH mRNA, 28S/18S ribosomal mRNA
Method: RNA/DNA hybridization (Northern blot)
MF effect: Not Significant

Experimental Series # 2:
Cells: MCF7 WT, A172, and HeLa S3
MF exposure parameters: 2 mT for 24 h
Sampling time (T) after MF: T = 0, 3, 24 or 48 h
Non-MF exposures: 500 ng ml1 TPA for 3 h (T = 0 h)
Transcripts studied: *c-myc, c-fos, actin*, GAPDH mRNA, *Csa19*
Method: RNA/DNA hybridization (Northern blot)
MF effect: Not Significant

Experimental Series # 3:
Cells: HL60
MF exposure parameters: 2 mT for 3 h
Sampling time (T) after MF: T = 0 h
Non-MF exposures: 5 Gy of X rays (T = 3 h)
Method: BIGEL (1920 randomly-selected cDNAs from HL60 cell cDNA library)
MF effect: No differentially- expressed cDNAs detected in mRNA samples from MF-exposed versus control cells (0/1920 cDNAs).

Experimental Series # 4:
Cells: HL60
MF exposure parameters: 2 mT for 3 h
Sampling time (T) after MF: T = 0 h
Non-MF exposures: 5 Gy of X rays (T = 3 h)
Transcripts studied: *egr-1, c-fos, bc12*, translocated t(8;14) *c-myc*, cytochrome c oxidase subunit II mRNA, TI227H, ribosomal protein L12 mRNA, EST 01598, triosephosphate isomerase mRNA, CpG island DNA (clone 133f10), mRNA for myoblast KIAA021, EST 85056, acidic phosphoprotein PO mRNA, novel mRNA elongation factor 1 mRNA, neuroleukin, nineteen-KD inactivating protein (bc12-like), RAN/TC4, GATA3. NF45 and Cu 2U-dependent super-oxide dismutase. Note: except for *egr1, bcl2* and *c-fos*, all the other transcripts were isolated by BIGEL using materials from X-irradiated versus control HL60 cells.
Methods: RNA/DNA hybridization (Northern blot)
MF effect: Not Significant

Our results do not support the previous conclusions by others: we did not see MF-inducible gene expression for field intensities and by others exposure durations investigated previously. In contrast, we found that the variation of *c-myc, beta actin* and *c-fos* in MF exposed or control HL60 cells was nonspecific, i.e., significantly shared with the GAPDH transcript, 18S rRNA and with the total RNA (represented by 28S rRNA). In all the cases, the fluctuations of mRNA in exposed cells was similar to those in sham-exposed cells, i.e. ± 2.5 standard deviations. Such variations are expected for *c-myc* and *actin*, whose basal levels are high in HL60 cells. The *c-fos* transcripts are

not elevated by MF exposure, in contrast to our earlier preliminary report. Similar MF-exposures also did not induce the ODC transcription; this result confirms the previous observations published in the literature on MF effects.

Our lack of confirmation of MF effects on *c-myc*, *c-fos*, *ODC* and *beta actin* certainly does not preclude the possibility of observing positive molecular responses for other genes and/or MF exposure conditions.

Discussion and Contribution to Understanding Biological Effects of EMF

Referring first to our data from Aim 1 experiments that used a candidate-gene approach, we observed several instances of expression changes in MF-exposed versus control cells ranging up to approximately 50 % without evidence of statistical significance. In contrast, our positive control data exemplify much larger expression ratios up to 1000-fold. In addition, the negative results with MFs offer some degree of generality, at least with respect to tissue origin of cells, cell geometry at time of MF exposure (monolayer versus suspension cultures), p53 status, and post-exposure kinetics of gene expression. It has recently been suggested that a possible mechanism of MF-induced carcinogenesis could be the stabilization of p53 protein via MF-induced heat shock protein interactions with p53. Stable, long-lived p53 protein is a marker for more than 60 % of human tumors. Our negative results for several genes, including *c-myc* and *c-fos*, were obtained for cells containing null, mutated, and wild type p53, arguing against the above mentioned involvement of p53 in MF effects.

Using human HL60 cells as a model system, our Aim 2 studies compared the early effects of X rays or magnetic fields on the expression of 1920 genes selected at random from an HL60 cell cDNA library. We found that, compared to matched controls, X-irradiation altered expression in 18 of the 1920 transcripts, whereas MF exposure had no effect on the same group of 1920 transcripts. Assuming a total of 15,000 different genes actively expressed and represented in our cDNA library, we extrapolate to a 95% upper confidence limit of 187 for the number of early radiation-responsive genes in the entire cDNA library, according to the hypergeometric distribution appropriate to the present case of sampling without replacement. Similar analyses of our negative MF data suggest a 95% upper confidence limit of 20 MF responsive genes in the entire cDNA library. It is possible that our expression analysis by BIGEL failed to detect transcripts altered by MFs. However, in support of our MF findings, no differentially expressed transcripts were identified by differential display in HL60 cells after a 5.7 µT, 20 min MF exposure, relative to control cells. Thus, the MF results reported here and previously are consistent with a transcriptome-wide null result, and with the fact that there is no proven mechanistic support for an effect of MF exposure on gene expression.

Of 18 differentially expressed cDNAs in X-irradiated HL60 cells, some genes are tissue and disease-specific. We found that HL60 cells exhibit two common markers of human lymphoid malignancies: null p53 and constitutively over-expressed *c-myc* activated via chromosomal translocation between chromosome 8 and 14. These findings may be important for interpreting positive/negative MF results with HL60 cells. The identification of new radiation responsive transcripts provides some insight into the pathogenesis of biochemical and morphological changes induced by X-rays, but not by MFs, in HL60 cells. The protein products encoded by five identified genes, COII, TPI, L12, P0, and EF1, are important components of the energy metabolism or carbohydrate or protein metabolic pathways. Down-regulation of these genes in X-irradiated versus control cells signifies that early metabolic changes occur prior to overt cellular consequences, including cell cycle perturbation and death. Our study has also identified genes associated with mitochondria, ribosomes and the plasma membrane, changes in the expression of which may indicate pre-apoptotic changes in X-irradiated HL60 cells. Interestingly, the X-ray-induced reduction in the mitochondrial mRNA for COII appears to be a result of injury to both the mitochondrial inner membrane and mitochondrial. Of note, ribosomal EF1 functions not only in translation but also in regulation of parts of the cytoskeleton involved in apoptosis. Neither of these transcripts was found to be affected by MFs. The TC4 gene is a novel member of the ras-like gene family that includes the rho group. As both ras and rho gene products control signaling pathways, our negative findings with MFs have important implications for mechanistic models of MF action on cells.

Publications From This Grant

1. Balcer-Kubiczek EK, Zhang XF, Harrison GH, McCready WA, Shi Z-M, Han L-H, Abraham JM, Ampey LL, Meltzer SJ, Jacobs MC and Davis CC. "Rodent cell transformation and immediate early gene expression following 60Hz magnetic field exposure." Envir. Health Persp. 104: 1188-1198, 1996.
2. Harrison GH, Balcer-Kubiczek EK, Shi Z-M, Zhang X-F, McCready WA and Davis CC. "Kinetics of gene expression following exposure to 60Hz, 2 mT magnetic fields in three human cell lines." Bioelectrochem. & Bioeng. 43: 16, 1997.
3. Balcer-Kubiczek EK, Meltzer SJ, Han L-H, Zhang X-F, Shi Z-M, Harrison GH, and Abraham JM. "Csa19, a radiation-responsive human gene, identified in an unbiased two-gel cDNA library screening method in human cancer cells." Oncogene 14: 3051-3057, 1997.
4. Balcer-Kubiczek EK, Zhang X-F, Han L-H, Harrison GH, Davis CC, Zhou X-J, Ioffe V, McCready WA, Abraham JM and Meltzer SJ. "BIGEL Analysis of gene expression in HL60 cells exposed to X-rays or 60-Hz magnetic fields." Rad. Res. 150: 663-672, 1998.

Other EMF Publications

None

TITLE: **Molecular Effects of ELF Fields with Harmonic Content**

Principal Investigator: Elizabeth K. Balcer-Kubiczek, Ph.D.

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Cycle Control, Signal Transduction

RO3-ES08898 **Start Date:** 01/10/97 **End Date:** 01/09/99

Abstract

This proposal has been prepared in response to the request for applications RFA ES-096-07 entitled "Biological Effects Power Frequency EMF". In particular, we respond to the need for research on "the potential biological effects for exposure conditions such as harmonics" and for research on genetic effects of such EMFs. We propose to investigate immediate early gene transcription induced by 60 Hz electromagnetic fields (EMF) with a higher harmonic content. The existing solenoid-based magnetic field exposure system will be powered by a square-wave electric input, instead of a sine-wave electric input. We will test the hypothesis that exposure to EMFs with higher frequency-components alter expression of immediate early genes and, therefore, that there is a risk for EMF-induced adverse health effects, including cancer. Broad frequency EMFs that include harmonics in addition to 60 Hz are common in work and other environments. The current experimental database does not include frequency spectra as a variable. Occupational or domestic exposure to such fields, if biologically active, may represent a long-term health risk to humans. We will use gene transcription as a model of an EMF-induced response. The specific aim of the proposal is to identify differentially expressed known and novel immediate early genes in human HL60 or MCF7 cells following 2 mT, 3 or 24 h exposure at post-exposure times of 0 and 3 h. We have developed and successfully tested a polymerase chain reaction-based technique (BIGEL) for the rapid complementary DNA (cDNA) screening and isolation of differentially expressed genes among randomly-selected genes from a cell-specific cDNA library in large enough quantities for sequencing.

Experimental Design and Exposure Conditions

1. Experimental Design

Experiments defined below are currently underway using HL60 cells as a model system. The following EMF conditions will be investigated in the proposed studies:

Exposure duration (h):	3, 24
Field level (mT):	2
Post-exposure lag (h):	0, 3

Although our results to date with sinusoidal 60-Hz magnetic fields have been negative, it is the proposal's hypothesis that 60-Hz square-wave exposure will induce positive responses. We will screen 10,000 genes per library over the period of the grant, 200 randomly-selected genes per membrane, regardless of possible ongoing negative results. This represents a significant proportion of the number of genes expressed in all but CNS tissues. As a backup strategy in case of ongoing negative results, we will investigate new candidate genes which are being identified in parallel funded studies (NASA NAGW-4392) using Northern blot analysis of RNA from EMF and sham-exposed cells.

We use a minimum of three field-and one sham-exposed 75-cm2 flasks each containing about $3x10^7$ cells for each of four experimental conditions listed above. Cells will be exposed in flasks filled with medium. RNA samples

extracted from the two groups will coded as described. Differential expression will be claimed only if three independent experiments produce reproduce similar results in Northern blot analysis.

2. Two-gel differential gene expression (BIGEL) assay

Cell-type specific cDNA libraries are used with BIGEL, a technique for panoramic survey of genes whose expression is altered by an agent of interest. The BIGEL protocol as well as its applications have recently been published (Oncogene 14, 3051-3057, 1997).

Confirmation of differentially expressed genes is be obtained not only by change in its expression level, but by reconfirmation of the length of the band and, most importantly, by Northern blot analysis of RNA isolated from control and test cells, and probed with 32P-labeled differentially-expressed cDNA. The gene identification step involves sequencing of the cDNA clone using Sequenase PCR Product sequencing kit. Homology searches of the GenBank, EMBL, are conducted using Genetics Computer Group software, accessible to us via the Internet.

Quality Assurance Measures

During handling and maintenance, cells are shielded from external fields. Flasks are transported between the MF exposure facility and our molecular biology laboratory (a 125-m distance) in a custom-made mμ-metal container.

Field levels in hood areas have been measured to be less than 0.1 μT. Cell cultures are grown in commercial CO_2-incubators. AC/DC fields inside the incubators' chambers were mapped; we use the incubators and/or incubator shelves with the lowest fields (less than 1 μT). Fields mapped in other work spaces and areas are less than 0.2 μT.

Our 60-Hz magnetic field exposure facility is consistent with the characterizations suggested by the NIEHS RAPID Program. Temperature and exposure parameters are monitored and recorded during experiments. Cells are exposed in a stringently controlled environment and assays are performed under blind conditions. RNA samples are coded by individuals not involved in performing molecular assays.

Two types of positive controls are included: (a) non-MF treatments; (b) genes whose expression is treatment/cell line specific. In the case of p53-null HL60 cells, positive-control genes include immediate early response genes, *c-fos* and *egr-1*.

Results

Exposure system modification for 60-Hz square-wave exposure

The exposure system has been constructed, as proposed. Our present 60-Hz exposure has a dual capacity for exposing mammalian cells in standard tissue culture flasks to either square-wave or sine-wave 60-Hz magnetic field, or to both simultaneously. The system modifications include the addition of a Hewlett Packard model 3311A function generator that drives up to three audio system amplifiers. Other key-components, including mμ-metal shielding, temperature control and data logging systems are as previously described (Envir. Health Perspect. 104, 1188-1198, 1996), and approved for the single-mode 60-Hz sine-wave magnetic field exposure system. Extensive measurements of generated fields when system is operated in the single square-wave mode, or in square-and sine-wave modes were performed (in collaboration with C.C Davis, the project engineer).

Search for MF-inducible genes in human HL60 cells

Experiments used actively-growing suspension cultures, final concentration 5×10^5 cells/ml. Panoramic gene analysis utilizing BIGEL requires custom preparation of multiple sets of duplicate membranes for Southern blotting, each membrane containing 192 different genes cDNAs selected at random from HL60 cell cDNA library; five membranes containing approximately 1000 cDNAs have been prepared to date. Complex cDNA probes for side-by-side Southern blotting are generated by reverse transcribing total mRNA isolated from experimental and control cells. To obtain enough material for probe preparation, 5×10^8 HL60 cells were exposed square-wave MF at 2 mT for 24 h. Cells were lysed for RNA extraction immediately following a 24-h exposure. One positive band corresponding to a 1.5 kb cDNA has been observed on primary screen membranes. We observed the relatively high signal in this band following hybridization with total cDNA probe from control cells and the absence of any

detectable signal following hybridization with total cDNA probe from MF-exposed cells. The gene exhibiting such a great difference in expression is likely to be biologically important. Our near-future plans include: (a) Isolation of the gene corresponding to this differentially-expressed band via second screening; (b) Confirmation of differential expression in exposed versus control cells via Northern blotting using differentially-expressed clone specific cDNA; (c) identification via sequencing and database searches; (d) examination for dose-dependence and kinetics.

Discussion and Contribution to Understanding Biological Effects of EMF

The identification and isolation of genes whose expression is markedly altered in cells of one type versus another has provided clues to understanding many biological processes, including neoplastic transformation. We have identified one differentially-expressed cDNA clone among 1000 cDNAs by comparing mRNAs exposed versus control cells; 60-Hz square-wave-exposure conditions were: 2 mT for 24 h, a 24-h post-exposure delay. This result, if confirmed by further analyses, may have a meaningful impact on assessment of the potential link between MF field with a higher harmonics content and biological effects.

Publications From This Grant

None.

Other EMF Publications

Peer-reviewed publications:

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2. Harrison GH, Balcer-Kubiczek EK, Shi Z-M, Zhang X-F, McCready WA and Davis CC. “Kinetics of gene expression following exposure to 60-Hz, 2 mT magnetic fields in three human cell lines.” Bioelectrochem. & Bioeng. 43, 1-6, 1997.
3. Balcer-Kubiczek EK, Meltzer SJ, Han L-H, Zhang X-F, Shi Z-M, Harrison GH, and Abraham JM. “Csa-19, a radiation-responsive human gene, identified in an unbiased two-gel cDNA library screening method in human cancer cells.” Oncogene 14, 3051-3057, 1997.
4. Balcer-Kubiczek EK, Zhang X-F, Han L-H, Harrison GH, Davis CC, Zhou X-J, Ioffe V, McCready WA, Abraham JM and Meltzer SJ. “BIGEL Analysis of gene expression in HL60 cells exposed to X-rays or 60-Hz magnetic fields.” Rad. Res. 150: 1-000, 1998.

TITLE: **Effects of Melatonin of EMF in Carcinogenesis**

Principal Investigator: J. Carl *Barrett*, Ph.D.
Laboratory of Molecular Carcinogenesis, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Cell Function; Cell Processes; Melatonin

Z01-ES23019 **Start Date**: 3/94 **End Date:** 9/98

Abstract

Evidence indicates that electromagnetic fields (EMF) reduce both the quantity of melatonin produced in the pineal gland and its effectiveness at the target site. Melatonin is an important indolic hormone in controlling biological rhythms and produced primarily at night in humans. Melatonin's high affinity membrane receptors are found exclusively in the brain and pituitary. However, recent evidence suggests that melatonin contains binding sites in many peripheral organs besides the brain. These binding sites are putative nuclear receptors for melatonin. Two nuclear receptors have been tentatively identified as RZRa and RZRb, which are members of the retinoic acid family of receptors. RZRb is found in the brain, while RZRa is generally found peripherally. Electromagnetic fields may reduce the quantity and effectiveness of melatonin in the body at these sites. We are studying how this may effect proliferation of normal and cancerous tissue.

Previous research indicated that melatonin can attenuate proliferation in estrogen responsive tissues. We are examining this hypothesis, and also how growth factor induced proliferation can be affected by melatonin. Melatonin reduced growth in MCF-7 (breast cancer, estrogen receptor positive [ER+]) and BG1 (ovarian cancer, ER+) cells at 10^9M concentrations with estradiol (10^8M) present. The concentration of melatonin which attenuated proliferation is considered physiological at night. However, treatment with estradiol reversed the melatonin attenuation of growth. Estradiol may elicit its proliferative capabilities through increased production or sensitivity to growth hormones. Therefore, we examined the effects of melatonin upon growth factor induced proliferation in MCF-7, BG1 and HeLa cells (cervical cancer ER). Epidermal growth factor (EGF) induced proliferation was attenuated at pharmacological concentrations (10^5M) in MCF-7 cells, but not in BG1 and HeLa cells. In contrast, insulin-like growth factor-1 (IGF1) induced proliferation was attenuated at physiological and pharmacological concentrations (10^9M to 10^5M) of melatonin in BG1 cells, but not MCF-7 cells. These experiments demonstrate that melatonin attenuates growth factor stimulated proliferation, and that melatonin has different responses towards growth factors in distinct cells lines. Use of Northern blots for pS2 and cell cycle experiments corroborated this work.

Experimental Design and Exposure Conditions

Cells were grown in 5% FBS, 1% Kanamycin and DMEM/F12 media. Twenty-four hours prior to the beginning of experimentation the media was changed to 10% DCC filtered FBS, 1% Kanamycin in DMEM/F12 to remove steroid hormones. Cells were then trypsinized and 20,000 cells/well were placed in 12-well plates. After 24 hours cells were treated with melatonin (10^{11}M, 10^9M, 10^7M and 10^5M) in 10% DCC filtered media for estrogen studies or in no serum media for growth factor studies. 0 melatonin controls contained 0.1% ethanol. Twenty-four hours following the melatonin addition, 10^8M estradiol, 10ng/mL EGF or 10ng/mL IGF1 was added. Five days later, cells were examined under a light microscope and assayed for proliferation colorimetrically (MTT) or counted on a Coulter counter.

Quality Assurance Measures

Dr. Martin Misakian (NIST) examined EMF fields in all incubators to make sure all exposures were done below 12m Gauss. Experiments were conducted in portions of the incubator with lower field levels. All experiments were done in the same cell incubator to keep EMF exposure constant for all melatonin treatments.

All studies are done with a positive control and negative control (growth factor, but no melatonin and no growth factor or melatonin present). Melatonin experiments are done without the lights on in the laminar flow hoods. Melatonin solutions are prepared fresh before each experiment.

Results

We have found that melatonin inhibits growth in estrogen responsive tissues. It also reduced EGF induced proliferation in MCF-7 cells at pharmacological concentrations and IGF1 induced proliferation in BG1 cells at both physiological and pharmacological concentrations. These results support the results of Dr. Blask, and add to them by demonstrating that IGF1 induced proliferation can be attenuated, and that the growth of ER+ ovarian cancer cells can also be attenuated.

These results suggest that some cancerous growth may be attenuated by melatonin treatment, and that reduction of melatonin levels or melatonin sensitivity by EMF may increase growth by reducing melatonin's antiproliferative capabilities. However, estradiol eliminates melatonin's effects, thus melatonin may not be physiologically relevant in ER+ tissues. These data will be used for a collaborative project among NIOSH, the Laboratory of Molecular Biophysics, NIEHS, and the Laboratory of Molecular Carcinogenesis, NIEHS to assess the effects of EMF on melatonin activity.

Discussion and Contribution to Understanding Biological Effects of EMF

This study does not determine direct EMF effects. However, it does examine the effects of melatonin on proliferation of cancer cells, and it has been hypothesized that EMF acts by perturbing melatonin concentrations.

Publications From This Grant

1. Baldwin WS and Barrett JC. “Melatonin: Receptor-medicated events that may affect breast and other steriod hormone-dependent cancers.” Mol. Carcinog., 21:149-155, 1998
2. Baldwin WS, Travlos G, Risinger JI, and Barrett JC. “Melatonin does not inhibit estradiol-stimulated proliferation in BG1 and MCF-7 cells.” Submitted Carcinognesis, In Press, 1998.
3. Baldwin WS and Barrett JC. “Melatonin Attenuates Hydrogen Peroxide Toxicity in MCF Cells Only at Pharmacological Concentrations.” Biochem & Research Comm. 250: 602-605 1998.
4. Baldwin WS, Curtis SW, Cauthen CA, Risinger JI, Korach KS and Barrett JC. “BG-1 ovarian cell line: and alternative mmodel for examining estrogen – dependent growth in vitro. “*In vitro* Cell. Dev. Biol. 34: 649-654, 1998.

Other EMF Publications

None

TITLE: **The Influence of Electromagnetic Fields on the Reactions of Biological Transient Species**

Principal Investigator: Piotr *Bilski*, Ph.D.
Laboratory of Molecular Biophysics, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Cellular Function, Biophysics of Cell Field Interaction, Free Radicals

Z01-ES50138 **Start Date:** 9/93 **End Date:** 9/95

Abstract

Prolongation of the life of free radicals has been postulated to be an effect of EMF exposure. Transient biological species which are involved in regulatory physiological and pathogenic processes are difficult to investigate directly because *in vivo* and *in vitro* their concentration is usually very low. However, such, or related species may be produced in high yield during laser pulse irradiation. An additional factor that may prolong the lifetime of transient species is heterogeneous environment. Many of the biological transients are neutral radicals and radical ions, which, due to their paramagnetic nature, interact with electromagnetic fields. Such an interaction is a foundation for EPR spectroscopy which has application for the investigation of the reactions of biological radicals. Radical ions may be monitored additionally as electrical current or electrical conductivity, which features a high sensitivity. We have set up appropriate equipment to measure both EPR and conductivity, and to spectrally monitor the transient species in the presence of EMF. We have also characterized model micellar systems to investigate the radicals and reactive oxygen species that are produced photochemically during photosensitization processes. To observe photoconductivity and EPR signals simultaneously in the same sample we have chosen the a system using trinitrobenzene (TNB) and tetrahydrofuran ether. Upon UV excitation, the radical anion of TNB is photoproduced, accounting for the transient electrical current that has been investigated in the absence of external magnetic field. We have described the photosensitization in micellar solution of Rose Bengal photosensitizer, and characterized singlet molecular oxygen, a known precursor of radical processes, in different environments. Singlet oxygen and other reactive species may, for example, affect melatonin, an important hormone whose physiological level has been reported to be sensitive to EMF. Our unique apparatus resources provide a solid scientific foundation to further investigate the influence of EMF on biological processes.

Experimental Design and Exposure Conditions

To investigate transient species produced in photochemical experiments, we had to characterize model systems and to prepare instrumentation. We have constructed and optimized our pulse and steady-state singlet oxygen spectrophotometers to directly detect extremely weak phosphorescence of singlet molecular oxygen that may be used a promoter of radical processes. We have also assembled a set up for laser exposures in the present of EM field with time-resolved detection. For EPR-photoconductivity measurements, we chose a direct current (DC) technique for precise measurements of small ohmic conductivities to extract rate constant for bulk relaxation and the ion mobilities in the absence and presence of EMF. At the same time, we characterized our model systems, which consisted of differently charged micelles to model biological heterogeneous environments.

Quality Assurance Measures

None

Results

A magnetic field affects the behavior of free radicals. A dominant process is an increase in the yield of radicals that escape from the triplet pair in the presence of EMF. This process can be easily observed in heterogeneous media like micelles or other molecular aggregates in which a higher level of molecular arrangement may be an important factor. The cellular subcompartments (membranes, DNA, proteins, mitochondria, etc.) might function as "reaction vessels" for radicals that can be influenced by an externally applied magnetic field in a similar way as radicals in the molecular aggregates of detergent we are testing. For modeling studies, we generated radical pairs in photochemical reactions *via* electron transfer or energy transfer processes. It is of interest to find appropriate heterogeneous systems for photochemical generation of radical in the presence of EMF. We have characterized the photosensitization in differently charged micelles, which is an elegant way to control energy transfer processes. We have also extended our studies on larger and higher organized premicellar aggregates. Some of these aggregates show a free exciton transition, which indicates a high degree of molecular organization, and is promising to control electron transfer reactions by irradiation of Rose Bengal photosensitizer. We have applied these systems to study the reactions involving nitric oxide during the oxidation of the *aci*-nitro moiety by singlet molecular oxygen, an important oxidant promoting radical processes. Singlet oxygen is formed in numerous photochemical processes and can be detected by its phosphorescence. To interpret the $^1O_2$ phosphorescence data correctly, we had to establish how singlet oxygen properties are affected by different environments. We have already measured the influence of polarity, proticity and polarizability in a number of solvents and solvent mixtures, and in heterogeneous systems. Free radicals may be neutral or charges species observable by its electrical current. We had tried to study this property by UV-irradiating the solution of trinitrobenzene in tetrahydrofurane to produce radical ions that are observed as electrical current. This process is affected by space charge separation depending on the radical ion concentration. Radical and ion radical pair recombination may be affected by the magnetic field. The new information we gather from the investigations in model photochemical systems are used to prepare and to interpret *in vitro* experiments, which are necessary for better understanding of molecular processes responsible for *in vivo* EMF effects.

Irradiation of TNB in THF efficiently produces radical ions that are observed as electrical current. Electrochemical reactions on the electrodes are negligible but they may increase in degassed solutions, and when the electrode surface is covered with colloidal platinum. There is a significant space charge separation, which depends on the radical ion concentration. It has been proven that the pulse relaxation of photoconductivity in ethereal solutions of TNB is satisfactorily described by an exponential decay because ions undergo pseudo first order recombination in solution, and the ions discharge on the electrodes. Slow bulk neutralization is of the first-kinetic order, which suggests a rearrangement of the charged forms prior to their neutralization. This component must involve radical and ion radical pair recombination, and may be affected by the magnetic field. This area of research will be continued.

Discussion and Contribution to Understanding Biological Effects of EMF

This research has contributed the understanding of the possible putative mechanisms for 60 Hz EMF on biological processes.

Publications From This Grant

1. Bilski P and Chignell CF. "Properties of differently charged micelles containing Rose Bengal: Application in photosensitization studies." Photochem. Photobiol. A: Chem. 77: 49-58, 1994.
2. Bilski P, Dabestani R and Chignell CF. "Photoprocesses of Eosine and Rose Bengal ion pairs with cationic surfactant in nonpolar solvents. Application in photosensitization studies." Photochem. Photobiol, A: Chem. 79, 121-130, 1994.

3. Bilski P, Reszka K and Chignell CF. “Oxidation of aci-nitromethane by singlet oxygen” J. Am. Chem. Soc. 116, 9883-9889, 1994.

4. Bilski P and Chignell CF. “Optimization of a pulse laser spectrometer for the measurement of the kinetics of singlet oxygen decay in solution.” J. Biochem. Biophys. Methods 33, 73-80, 1996.

5. Bilski P, Holt RN, and Chignell CF. “Properties of singlet molecular oxygen in binary solvent mixtures of different proticity and polarity.” Photochem. Photobiol., A: Chem. 109, 243-249, 1997.

6. Bilski P, Holt RN and Chignell CF. “Premicellar aggregates of Rose Bengal with cationic and zwitterionic surfactants.” Photochem. Photobiol., A: Chem. 10, 67-74, 1997.

Other EMF Publications

None.

TITLE: **Effects of 60 Hz EMF on Transcription in Yeast**

Principal Investigator: David M. *Binninger*, Ph.D.
Florida Atlantic University
Boca Raton, Florida

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Yeast

R01-ES07181 **Start Date:** 09/28/94 **End Date:** 08/31/97

Abstract

Numerous epidemiological studies have implicated exposure to power line (60 Hz) electromagnetic fields (EMF) as a possible contributor in the development of certain human cancers. Under laboratory conditions, a variety of cellular processes that have known roles in tumor progression have also been reported to be altered in response to EMF. Thus, available data suggest a plausible role for EMF in human carcinogenesis; however, we have only a rudimentary understanding of the mechanisms controlling the interactions of EMF with living cells.

This research project focuses on one of the reported biological effects, the effects of 60 Hz power line EMF on gene expression using *Saccharomyces cerevisiae* (commonly known as brewer's yeast) as the experimental model. This unicellular organism has been developed into a model for molecular biologists because it provides experimental approaches that are either more straight forward or not yet available for more complex types of cells. There is considerable evidence to show that fundamental cellular processes are conserved throughout all eukaryotic cell types. Thus, a clearer understanding of how EMF affect gene expression at the molecular and genetic level in yeast will provide insight into how this biological effect occurs in more complex cells, especially human cells. Findings from these studies will help to evaluate more critically whether 60 Hz EMF present a human health risk.

We have found that gene expression in yeast can be affected by both brief (20 min) (see section on replication experiments) and prolonged EMF exposure over a period of approximately 15 cell generations However, the effect on gene expression is highly gene specific. We have molecular clones for two yeast genes that are induced by EMF and one gene whose expression is diminished. These clones provide important probes to further investigate the effect of EMF on gene expression at the molecular and genetic levels. We are also using bioinformatic approaches to determine whether these or other EMF-responsive genes in yeast identify functional homologues of human genes that are known to be involved in some aspect of cancer or other disease processes. If so, it suggests the hypothesis that expression of some disease-associated genes in humans are affected by EMF.

Experimental Design and Exposure Conditions

<u>EMF Exposure Protocol:</u> A small culture of yeast cells (strain D7, ade2-40/ade2-119, trp5-12/trp5-27, ilvl-92/ilvl-92) was grown overnight with vigorous shaking at 30° C in yeast-peptone-dextrose (YPD) medium. The next day, the cell culture was diluted to a density of approximately 6,000 cells/ml. A 100 ml sample of diluted cells was transferred to each of two identical 500-ml Erlenmeyer flasks. Each flask was attached to a platform shaker located in a 30° C walk-in warm room. Each shaker was adjusted to 270 rpm using an electronic stroboscopic tachometer. The earth's static magnetic fields inside the room were measured at 42 microtesla with an angle of inclination of 68 N.

For the EMF-exposed cells, uniform 60 Hz AC magnetic fields were generated by a one meter square coil having 62 turns of 14 AWG magnet wire (constructed to IEEE standard 644.1987). A commercial 120V AC power source

was connected to an autotransformer to regulate the voltage supplied to the coil. The yeast cells were incubated continuously in the presence (or absence) of 20 microtesla 60 Hz AC magnetic fields. To avoid possible effects of transients, the coil was energized prior to placing the cells in the field and was turned off after the cells were removed.

Molecular Analysis of Effects on Gene Expression: At specified time points, cell samples were removed from each culture and chilled by pouring over crushed ice. The cells were then washed once with ice-cold water and stored at 70°C. Total cellular RNA was extracted from each cell sample. Depending on the biological endpoint being monitored, the purified RNA was used for *in vitro* protein synthesis, RNA (Northern) hybridization analysis, or mRNA differential display. These are well-developed techniques that are routinely used in molecular biology laboratories. Additional details are provided in the Section on Results and Discussion.

Quality Assurance Measures

Monitoring of experimental parameters included uniformity of the applied EMF, stray field exposure and temperature control. The strength of the EMF was monitored continuously with a battery-powered EMDEX-C recorder. To date, we have measured less than 5% variation in field strength during any 24 hour EMF-exposure period. Mμ- metal shielding was not used since the size of the warm room allowed the control and EMF-exposed samples to be separated by more than 2.5 meters. Detailed magnetic field mapping under actual experimental conditions showed that stray EMF exposure of the control cells was less than 0.2 microtesla.

Temperature control in the warm room was monitored by direct measurement of culture media temperatures. The average temperature difference between the control and EMF-exposed culture media was 0.2 C while the maximum difference was 0.4 C.

To further rule-out experimental artifacts that might be attributed to the EMF exposure, periodic sham/sham experiments were performed. To increase scientific rigor a “double blind” protocol in which the molecular biologist does not know which coil is activated is used.

Results

We have shown that expression of numerous yeast genes can be altered by continuous exposure to 20μT 60 Hz AC magnetic fields for 24 hours [Binninger and Ungvichian (1997)]. The largest fraction (44-67%) of genes evaluated were not affected. However, the abundance of 26-38% of the mRNA levels were increased at least 2-fold following exposure to EMF. We also consistently found a smaller proportion of mRNAs (7-18%) whose abundance declined in response to the EMF-exposure.

Using mRNA differential display, we have cloned three yeast genes (designated EMF-A, EMF-B and EMF-C) whose expression can to be affected in the presence of 20μT 60 Hz AC magnetic fields. Expression of both the EMF-A and EMF-B genes can be stimulated by the EMF-exposure whereas expression of the EMF-C gene is diminished. The complete DNA sequence of each gene was retrieved from the Yeast Genome Database (public domain). Molecular probes for all three genes were synthesized by PCR amplification using gene-specific oligonucleotide primers. These molecular clones are being used to develop a yeast genetic model to identify additional genes whose activity is affected by EMF with special interest in genes related to those involved in human carcinogenesis. Additionally, our model system offers the possibility of identifying genes related to the mechanism of EMF signal transduction (see next section for additional discussion).

Replication Experiments: With the enthusiastic cooperation of Dr. Reba Goodman (Columbia University, New York) we have replicated her published experiments demonstrating EMF induced changes in gene expression in yeast (Weisbrot *et al.*, Bioelectrochem. Bioenerget 31:167-177, 1993). To fully replicate her experiments, the EMF-exposure system used for the original experiments was shipped to our laboratory at FAU. Results from 11 independent experiments showed that the average mRNA level of the stress inducible SSA1 gene was 40% greater in cells exposed to 5.6 microtesla (rms) 60 Hz AC magnetic fields for 20 min. The 95% confidence interval was 18%. This modest increase in SSA1 mRNA abundance in the EMF-exposed cells is consistent with the published report of a 70% increase. The abundance of the 18S rRNA transcript was used as an internal control. Its level was unaffected with the expected average Exposed/Control (E/C) ratio of 1.0 with a 95% confidence limit of 0.06.

Discussion and Contribution to Understanding Biological Effects of EMF

The focus of the EMF RAPID program is to understand how biological systems interact with EMF and especially whether those effects might be harmful to humans. Numerous lines of evidence show that EMF can affect known biochemical and signal transduction pathways which can contribute to human cancer development. However, the mechanism by which EMF initiates these biological responses is completely unknown. Fundamental principles of molecular biology predict that EMF must directly interact with one or more cellular molecules, probably proteins. The biochemical and/or biophysical properties of the target molecule(s) must be altered in some manner to initiate the cascade of events that ultimately leads to the observed biological endpoints. The powerful genetic techniques available for yeast offer the opportunity to identify the molecular components and corresponding genes in this putative signal transduction pathway.

As a possible health risk, it is especially important to identify cellular processes related to known mechanisms of carcinogenesis that can be affected by EMF. An unexpected outcome of cloning human genes has been the discovery that many cancer-related genes are very similar in all eucaryotes--from yeast to humans. Thus, yeast is proving to be a valuable experimental model in many areas of human cancer research. By exploiting the genetic and recombinant DNA technology for yeast, we will gain insight into the mechanism of cellular interactions with EMF. Additionally, we are utilizing the rapidly developing field of computational genomics (bioinformatics) to extend our findings with yeast to human cells to explore how EMF might contribute to human cancer.

Publications From This Grant

1. Binninger DM and Ungvichian V. "Effects of 60 Hz AC magnetic fields on gene expression following exposure over multiple cell generations using *Saccharomyces cerevisiae*." Bioelectrochemistry and Bioenergetics 43 (1): 83-89, 1997.
2. Binninger DM, Busch A and Ungvichian V. "Molecular Cloning and Characterization of Yeast Genes Whose Expression Are Altered by 60 Hz AC Magnetic Fields." submitted, 1998.
3. Binninger DM and Ungvichian V. "Effects of 60 Hz EMF on Gene Expression In Yeast: A Replication Study." To be submitted, 1998.

Other EMF Publications

None.

TITLE: **Cytochrome Oxidase in Electric and Magnetic Fields**

Principal Investigator: Martin *Blank*, Ph.D.
Columbia University
New York, New York

Health Relevance: Cellular Biophysics

Research Area: Cellular Function; Cellular Processes; Enzyme Pathways

RO3-ES08855 **Start Date:** 02/01/97 **End Date:** 01/31/99

Abstract

Electromagnetic (EM) fields cause many changes in cells, but no known mechanisms link cellular changes to health risks. A possible link to disease is the "stress" response in cells, a known reaction to harmful stimuli that is activated by EM fields. However, it is not known how EM fields initiate this response. Information about physical transduction mechanisms is needed to understand the link to disease, and to define relevant exposure parameters in a hazard assessment model.

Studies of mechanism that have identified thresholds and molecular processes in physical transduction, have been based on Na,K-ATPase function in separate electric and magnetic fields. The dependence of both field effects on enzyme activity indicates that the initial reactions appear to be related to charge movements during enzyme function. Differences between the field effects can be explained by the different degrees of penetration of the individual fields into cells. To continue this research with greater control of charge movements, the proposed mechanism will be tested with cytochrome oxidase, a well defined electron transport enzyme in the mitochondrial oxidation chain. Techniques for assaying and controlling electron flow in cytochrome oxidase activity, as well as the instrumentation to control exposure to electric and magnetic fields, will be used to study the role of charge movements during signal transduction and to define thresholds.

The long term objective is to characterize the mechanism of EM field signal transduction by cells and to determine environmentally relevant exposure parameters. Knowledge of the mechanism will clarify the relation between EM field exposure and disease related changes in cells. It will then be possible to define relevant exposure parameters in a hazard assessment model. The specific aims of this study are to determine:

a) The relation between changes in cytochrome oxidase function in electric and magnetic fields, and the rate of electron transport.

b) The electric and magnetic field thresholds for changes in cytochrome oxidase function.

Experimental Design and Exposure Conditions

Experimental Design: Cytochrome oxidase was prepared from livers of Sprague-Dawley rats. Protein concentrations were in the range of 4 mg protein/ml, and enzyme activities of different preparations were in the range of 10^{15}-10^{16} electrons/mg protein/min. Enzyme activity was determined by a standard method (Smith), where the rate of oxidation of reduced cytochrome C is measured spectrophotometrically by the decrease in optical density at 550mμ (the peak in the α band of the absorption spectrum of cytochrome C), as the reduced cytochrome is oxidized. Addition of $K_3Fe(CN)_6$ solution at the end of an experiment was used to determine the optical density of completely oxidized cytochrome C. Sham controls were run with no applied field.

When studying the effects of magnetic fields, the data points were the average of at least four (generally 5-8) determinations and four (generally 5-8) controls, and the standard errors were around 5%. The rate data were reported as in past studies with the Na,K-ATPase, as the ratio M/C, the value in Magnetic field / Control activity. Experiments, using cytochrome C preparations that initially contain only the reduced form, can also be analyzed in terms of fundamental rate constants when excess reducing agent used in the preparation is removed by dialysis. When this is done, concentrations of oxidized and reduced cytochrome C at any time were determined from OD_{550} readings. The results of the two types of analysis, the steady state and the nonsteady state rapidly equilibrating reaction, lead to comparable results.

Magnetic Field Exposure Conditions: We have been using a unique exposure system for studying the effects of separately controlled electric or magnetic fields, as well as the phase angle between them when they act simultaneously. The instrument was designed and constructed by Electric Research & Management, Pittsburgh, with the following specifications: frequency range: 1 - 3000Hz, magnetic field: 0 - 1 mT, electric field: 0.2 - 200 mV/cm, phase between electric and magnetic fields: 0 - 359 degrees. In this study, we have used 60 Hz 10μT magnetic fields, which are uniform within 3% in the 5cm cube volume used for exposure. This volume was kept at constant temperature with circulated water from a thermostat. Fred Dietrich (Electric Research and Management, Inc) and Martin Misakian (NIST) checked the equipment and found that it was performing within specs, and that stray fields were below 0.1μT.

Quality Assurance Measures

As in our previous studies with the Na,K-ATPase, we have measured the effects of magnetic fields on the activity of cytochrome oxidase by comparing the magnetic field exposed (M) to the control (C) and calculating the ratio, M/C. In this work, we have used normal experimental procedures involving replicate runs, as well as statistical analysis of results to obtain averages and standard errors. Cytochrome oxidase enables us to also measure the effect of magnetic fields by determining the reaction rate constant, the physical property normally used to describe reaction rates. By comparing the effects on the rate constant with the results using the previous measure, we have validated the older measure in terms of a physical property.

Results

In our earlier studies with the enzyme, Na,K-ATPase, we developed the mobile charge interaction (MCI) hypothesis to account for effects of electromagnetic fields on charge movements during enzyme activity. In this project, we are testing the MCI mechanism using cytochrome oxidase, a well defined electron transport enzyme in the mitochondrial oxidation chain that oxidizes cytochrome C and becomes reduced when combining with oxygen. Changes in the oxidation state of cytochrome C can be varied by controlling the concentrations of oxidized and reduced forms of cytochrome C. Our goals the first year were to study the effect of charge flow rate, and to measure thresholds.

Cytochrome C solutions were reduced by adding varying amounts of ascorbate to change the balance between reduced and oxidized species present initially, (and therefor, the effective rate of oxidation). We studied the effects of magnetic fields as a function of the net reaction rate, and the results, reported as M/C, the rate in Magnetic field divided by the Control rate, support the MCI model. The effects of magnetic fields on this enzyme reaction are similar to effects on the Na,KATPase reaction in that:

60 Hz magnetic fields accelerate the reaction (M/C>1), apparently in competition with normal electron transport by the enzyme. When the basal rate is very fast, about $5x10^{16}$ electrons/mg protein/min, there is no increase due to the field. When the basal rate is slowed down to about one third of that value, M/C increases to about 1.3-1.4. M/C data at low field strengths suggest that the threshold is below1.0 μT.

We have also done experiments removing excess ascorbate from cytochrome C preparations by dialysis, so that all of the cytochrome C is initially in the reduced form. Under these conditions, the rate data yield reaction rate constants, fundamental chemical reaction properties. These experiments show similar effects of magnetic fields:

60 Hz magnetic fields increase the oxidation rate constant by 20-30% at field strengths below 3 μT and by a factor of about 2 between 6-10 μT.

Data at low field strengths suggest that the threshold is below 0.5μT, in the same range as thresholds determined by the M/C extrapolation, as well as earlier results for Na,K-ATPase function (0.3μT) and stimulation of transcription (<0.8μT). Magnetic field stimulation of transcription could occur by an MCI mechanism, where the field interacts with electrons moving along stacked base pairs in DNA.

At 10μT, the rate constant varies with frequency in the range 10-2500 Hz, with a maximum on the range 500-1000 Hz.

Discussion and Contribution to Understanding Biological Effects of EMF

We have demonstrated large effects of 60 Hz magnetic fields on the cytochrome oxidase reaction rate, i.e., up to a doubling of the initial rate (at low rates of oxidation), and a threshold field strength in the range of environmental fields. This experimental evidence provides a rationale for epidemiology studies indicating health effects due to environmental level magnetic fields.

The results support the MCI mechanism of interaction, where magnetic fields interact with moving charges in enzymes as they would in any conductor. The magnetic field appears to compete with the intrinsic mechanism of charge flow, and is overwhelmed at very high intrinsic flow rates. This may account for the therapeutic efficacy of magnetic fields in bone fractures, where function is reduced, while there does not appear to be an effect on normal bone.

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2. Blank M and Soo L "Magnetic fields accelerate electron transport rate constants in cytochrome oxidase reaction." Bioelectrochem. Bioenerg., 45:00-00, 1998.
3. Blank M and Soo L "Frequency dependence of cytochrome oxidate rate constants." Bioelectrochem. Bioenerg In press 1998.

Other EMF Publications

1. Lin H, Head M, Blank M, Han L, Jin M and Goodman R. "*C-Myc*-Mediated Transactivation of HSP70 Expression Following Exposure to Magnetic Fields." J. Cellular Biochem., 69:181-188, 1998.
2. Goodman R and Blank M. "Magnetic field stress induces expression of hsp70." Cell Stress and Chaperones, 3: 00-00, 1998.
3. Lin H, Opler M, Head M, Blank M and Goodman R. "Electronmagnetic field exposure induces rapid, transitory heat shock factor activation in human cells." J. Cellular Biochem. 66: 482-488, 1997.
4. Jin M, Lin H, Opler M, Maurer S, Blank M and Goodman R. "Biological and technical variables in *c-myc* expression in HL60 cells exposed to 60 Hz electromagetic fields." Bioelectrochem. Bioenerg. 44:111-120, 1997.

TITLE: **Magnetic Fields Effects on the Promotion of Cancer**

Principal Investigator: Craig V. *Byus*, Ph.D.
University of California
Riverside, California

Health Relevance: Cancer

Research Area: Toxicology in Whole Animals, Tumor Initiation/Promotion, Dermal-mice

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Abstract

The potential relationship between exposure of humans to low-frequency electric and magnetic fields and an increased risk for cancer continues to be highly debated. While this extremely complex topic is beyond the space available for appropriate review and exceeds in numerous areas our own area of expertise, there are several aspects of this debate that warrant some general comment. At least two large studies have recently been published which present a meta-analysis of 11-13 of the major studies relating residential proximity to electricity transmission and distribution equipment, and the risk of childhood leukemia, lymphoma, and nervous system tumors and all cancers. Of the various methods of measurement of EMF (wiring configuration codes, distance to power distribution sites, spot measurements of magnetic fields, calculated indices based on distance to power distribution equipment and historic load data) the wire-code measurement technique continued to have a positive association with a significantly increased risk for all three cancer types. Spot measurements consistently showed non-significant odds ratios (OR), and distance measurements in the calculated indices produced risk estimates which were significant only for leukemia. Also, some of the recent occupational studies indicated increased cancer risks for higher exposures to the magnetic field or the electric field, or combined exposure to both fields. The results for the electric field exposure and the combined field exposure in the Miller *et al*. 1996, study make the use of the organ and tissue induced fields and currents as a dosimetric measure more viable and better justified than ever before.

Regarding the potential association for EMF exposure to human cancer on the basis of epidemiology, Savitz we believe, states it best when he says that ". . . studies have tended to confirm that association, although the evidence falls short of demonstrating a cause association between magnetic fields and cancer." Recently the National Academy of Sciences report , while stating that "the current body of evidence does not show that exposure to these fields presents a human-health hazard", indicated that its conclusions did not consider the occupational studies. Furthermore, only a few areas were recommended for future research; among them animal studies involving cocarcinogenesis and mammary tumor development, and engineering studies of induced fields and currents and tissue heterogenuities.

The initiation-promotion model of epidermal carcinogenesis remains one of the most appropriate animal correlates of human cancer(s) of epithelial cell origin. Many important concepts relevant to the etiology and prevention of epithelial cell cancer were observed initially in this mouse model. The multistage process of carcinogenesis including initiation, promotion, co-promotion, progression and regression, can be clearly defined in this system and studied in a very quantifiable manner. We continue to believe that the epidermal mouse model is particularly suited for study of potential magnetic field effects even though none of the epidemiology studies suggested that EMF is related to skin cancer. Magnetic fields of low intensity do not appear to have effects of large amplitude upon biochemical processes. The long-term effects of magnetic fields upon organisms, if they exist at all, must be teased carefully from measurements made upon true sham-exposed controls. However, this does not mean

necessarily that the potential risk of EMF exposure is insignificant for the human population. In this regard, the two-stage model of epidermal carcinogenesis is appropriate for EMF studies since: spontaneous papilloma incidence is nearly nonexistent; the tumors can be observed and scored over many months without resorting to animal sacrifice; potential effects upon tumor incidence, latency, and multiplicity can be determined independently and in a highly quantitative manner; molecular markers of tumor progression have been clearly established and dosimetry considerations are simpler due to the surface location of the tumors. Furthermore, in no other animal model can the process of co-promotion or carcinogenesis be so carefully characterized (see below). (There are also a number of disadvantages to this model. Chief among them is the heterogeneous nature of the tissue involved.)

Five laboratories, including our own, have now completed a series of investigations designed to assess the ability of 50-60 Hz magnetic fields to serve both as promotion and co-promotion stimulus in the mouse initiation-promotion model of epidermal carcinogenesis. While these studies were all of high caliber in regards to exposure parameters and experimental design, no clear-cut or unequivocal conclusion can be drawn concerning the potential of EMF to alter the carcinogenesis process. In an attempt to aid in the review of this proposal the salient features of all of these studies are summarized in Table 1. A subset of the investigations dealt with the ability of magnetic field exposure to serve as a promoting agent. As such, animals were treated with an initiator (DMBA or UV) and exposed to magnetic fields for varying lengths of time. In the co-promotion protocols, the animals were initiated with DMBA, promoted with various doses of TPA, and co-promoted with magnetic fields. In our opinion, a careful analysis of the data *in toto* supports the single animal co-promotion protocol described in the Proposed Studies.

It is apparent from Table 1, that among the promotion studies only in the study of K2 mice was a significant magnetic-field promotional effect observed. This strain of mice is ODC-transgenic and has constitutively elevated ODC levels in virtually all tissues. This perhaps may explain the different response to the magnetic field of this strain as compared with the other two.

The co-promotional studies present a mixed picture. One observation is apparent, that TPA doses producing about 90% incidence or tumor multiplicities >1-2/animal at 40+ weeks preclude any additional effect (or its observation) by the magnetic field. TPA doses of 0.5 μg and above also preclude any field effect upon co-promotion (McLean *et al*., 1991, Byus *et al*., 1997, and the EPRI study). The low TPA data are suggestive, but not conclusive. Firstly they indicate a possible co-promotional effect after about 35-40 weeks, when the tumor incidence in control animals is at about 20%. (This is supported by the statistically significant result of Protocol 1 in the Byus *et.al* study). and by a relatively large but not statistically significant effect in the study by Stuchly *et al*. 1992. Secondly, another sub-set of data indicate that fields may accelerate tumor development early without affecting the final outcome (Statistically significant results in Stuchly *et al* 1992 and Byus *et. al*., 1997). Thirdly, there is one study indicating a field effect on the conversion of papillomas to carcinomas (McLean *et al*., 1995).

On the basis of all the studies completed to date we trust that at least one additional animal tumor experiment needs to be performed to tie up a number of loose ends in relation to defining the potential ability of magnetic field to serve as a co-promoter in this model. In order to observe a co-promotion effect of magnetic fields the data indicate that the dose of TPA employed must elicit a low, near threshold tumor response (tumor incidence and multiplicity). The factors which need to be addressed are identification of the appropriate (near threshold) low dose of TPA (0.1-0.2 μg, 2x/wk) for observing a magnetic field co-promotional effect, evaluation of the potential role the overall tumor burden in measuring this field effect, and investigation into the possibility that there exists a subpopulation of cells/tumors appearing either early or late with altered growth rates which are sensitive to magnetic field co-promotion (see Proposed Studies).

The second area of the proposed investigation relates to ODC activity and polyamine metabolism. The principal investigator began investigations into polyamine metabolism as a Ph.D. student in biochemistry, and has continued to investigate the role of ornithine decarboxylase (ODC) and polyamine metabolism in a variety of growth processes including development and during carcinogenesis throughout his career. The continued investigation of the potential role of ODC and polyamine metabolism following exposure of cells and tissues to low-energy magnetic fields, remains a very important area of research for two important reasons: such information supports the suggestion that magnetic fields can indeed effect intracellular molecular processes and provides a potential mechanism through which magnetic fields may alter cancer etiology.

A significant criticism of EMF research in relation to potential health effects has been directed at the fact that presumably there can be no adverse health effects of EMF because EMF can not cause any effects in cells or tissues above those caused by thermal noise. In this regard, the most reproducible effect of low-intensity, low-energy EMF exposure has been an elevation in ODC activity the controlling enzyme in polyamine metabolism. Four separate laboratories have now reported alterations in ODC activity in cultured cells following exposure to a variety of electromagnetic fields including amplitude-modulated RF, 60 Hz electric and 50/60 Hz magnetic fields. Most of these studies have involved transient exposure to athermal magnetic fields on the order of 0.1 to 1.0 mT for time periods ranging from 1-24 hours, have used various cultured cells and have observed changes in ODC on the order of 50% to three- to four-fold greater than sham-exposed values. (Litovitz *et al*. 1991-1994).

Table 1 Magnetic Field Promotion/Co-promotion Studies in the Mouse Epidermis Promotion Protocols

Mouse Strain	Initiator/Promoter	EMF Exposure	Results (*NOE-no observed effect)	Tumor Incidence/ Multiplicity (tumors/animal)
NMRI/HAN (30 mice/gp) Rannug *et al.*, 1993	DMBA	0.05 and 0.5 mT (50 Hz) 18-21 h/d	NOE* at 103 wks	30-40%/0.8-1.0
Sencar (40 mice/gp) Rannug *et al.*, 1994	DMBA	0.05 and 0.5 mT (50 Hz, continuous or intermittent -15s on/off) 18-21 h/d	Suggestive weak promoting effect of intermittent MF exposure; dose response (p=0.045) for intermittent MF at 104 wks NOE for continuous MF	10-15%/0.1-0.4
Sencar (32 mice/gp) Stuchly *et al.*, 1991 McLean *et al*., 1991	DMBA	2 mT (60 Hz) 6 h/d, 5 d/wk	NOE at 23 wks	0%/0 (no tumors observed)
K2 (ODC transgenic) (44 mice/gp) **Juutilainen *et al.*, 1996	UV	0.1 mT (50 Hz) 20 h/d, 7 d/wk	2x increase in multiplicity and incidence at 46 wks	30(60%/0.5(0.9

Co-Promotion Protocols

Sencar (32 mice/gp) McLean *et al.*, 1991	DMBA/TPA 1 (g/1x/wk	2 mT (60 Hz) 6h/d, 5d/wk	NOE at 21 wks	95%/10.0
Sencar (48 mice/gp) Stuchly *et al.*, 1992	DMBA/TPA 0.3 (g, 1x/wk	2 mT (60 Hz) 6h/d, 5 d/wk	3 fold increase in tumor incidence and multiplicity at 18 wks and earlier (p < 0.05) Not statistically significant at 23 wks	8(24%/0.65(1.9 20(30%/1(2
Sencar (48 mice/gp) McLean *et al.*, 1995	DMBA/TPA 0.3 (g, 1x/wk (for 23 wks only)	2 mT (60 Hz) 6h/d, 5d/wk for 52 weeks	Significant (p<0.03) increase in the fraction of mice in the field-exposed group with squamous cell carcinomas (SCC) compared to sham controls at 52 wks	1 SCC (8 SCC
Sencar (56 mice/gp) **EPRI study	DMBA/TPA 0.5, 1.0 and 2.0 (g, 2x/wk	2 mT (60 Hz) 6h/d, 5d/wk	NOE at 23 wks at any TPA dose	(0.5 (g) 50%/2 (1.0 (g) 90%/7 (2.0 (g) 100%/11.0

Sencar (60 mice/gp) Protocol 1: Byus *et al.*, 1997a	DMBA/TPA 0.25 (g and 0.5 (g, 2x/wk	2 mT (60 Hz) 7h/d, 7d/wk	Significant ($p < 0.04$) increase in tumor incidence (73%) and multiplicity (39%) with 0.25 (g TPA at 43 wks (late effect) NOE with 0.5 (g TPA at 33 wks	20(52%/1.3(1.8 80-90%/4
Sencar (60 mice/gp)	DMBA/TPA 0.25 (g, 2x/wk	0.2 mT (continuous)	20-25% increase in tumor incidence and multiplicity at 43 wks and earlier beginning at 10 wks	98%/5.0
Protocol 2:		2 mT (continuous)	Increased multiplicity early only (10-25 wks) NOE at 43 wks	90%/4.0
Byus *et al.*, 1997b		2 mT (intermittent)	Increased multiplicity early only (10-20 wks) NOE at 43 wks	90%/3.5-4.0

Refined by the mechanism by which EMF exposure leads to an increase in ODC activity in cultured cells by determining that the low frequency (60 Hz) magnetic field must be coherent for some minimal length of time (on the order of 10 seconds) in order to effect the alteration of this enzyme.

Of further significance at least four separate laboratories have now measured alterations in ODC activity in animal tissues brought about by exposure to 50-60 Hz magnetic fields *in vivo*. Loscher has recently reported that exposure of rats for a period of six weeks to a 50 Hz magnetic field with a flux density of 50 μT increased ODC activity (2-3 x) in the mammary tissue and the spleen (but not in the liver, small intestine, bone marrow and ear skin). This study was done in conjunction with further investigations into the reported ability of magnetic fields to increase the incidence of mammary carcinogenesis following treatment with DMBA. Litovitz has shown that a 60 Hz sinusoidal magnetic field at flux density of 4 μT was capable of significantly enhancing the ODC activity observed during the first peak in the timecourse at 15 hours of gastrulation in chicken embryos.

Most importantly Mandeville *et al.* 1996 in conjunction with a large primary brain tumor experiment in rats, performed a landmark experiment involving ODC activity. These investigators have reported that ODC activity in the brain, liver, intestine, spinal cord and kidneys all show an enhanced activity of ODC after 5, 15, and 32 weeks of exposure when compared to sham-exposed animals. Moreover, these investigators reported a linear dose-response for the increase in ODC activity in relation to magnetic field flux density in several of the measured organs. They concluded that their results clearly demonstrated an increase in ODC activity in organs of animals exposed to EMF. Interestingly, neither our own work, nor the large study supported by EPRI measured any significant differences in epidermal ODC activity before or after TPA following magnetic field exposure. Juutalainen *et al.* 1995, also observed no change in ODC however, but reported a 2-3 fold increase in epidermal putrescine and spermidine following a 24 hour exposure to a magnetic field intensity of 100 μT in conjunction with their tumor experiment using the ODC transgenic mouse (K2).

In summary, the increase in ODC activity either in cell culture or whole animals or embryos following exposure to low-intensity magnetic fields, is the most robust observation made to date, with at least seven separate laboratories worldwide reporting this effect and using a variety of exposure systems. Whether these effects are related to an alteration in cancer etiology is a separate question to be considered.

Mechanistically, polyamines have been shown to play an essential role in cell growth and differentiation. In addition to known genetic lesions in protooncogene and tumor suppressor genes, recent findings in both mouse and human tumors suggest that aberrant regulation of ODC can play an important role in tumor development. Over-expression of ODC either dramatically increased the transforming activity of a mutated ras gene in R6 fibroblast or resulted in the transformation of immortalized NIH3T3 cells. Since most human tumors originate in epithelial tissues, it is important to understand the mechanism in neoplastic development in an epithelial system. For this reason, I believe the manuscript from the laboratory of Dr. Susan Gilmore and associates entitled "The Role of

Ornithine Decarboxylase in Epidermal Tumorigenesis" is particularly relevant to this grant and to the study of polyamines and cancer in general. Using stable cDNA constructs of ODC to over-express ODC activity in a variety of particular cell lines, this investigator showed conclusively that ODC over-expression by itself was not sufficient to induce tumors in normal epithelial cells but that increased expression of ODC enhanced tumor development in initiated, pre-malignant epidermal cells. More importantly, this enhanced tumor development was not dependent upon ODC itself, but was mediated by an increase in intracellular putrescine (not spermidine or spermine) and exported putrescine. These investigators provided the first direct evidence that elevated ODC and putrescine levels (intracellular and extracellular) could contribute to tumor development in an epithelial cell type. Also important, the growth rate was not increased in several epidermal cells that they tested which had over-expression of ODC and elevated polyamine levels. They further suggested that the elevated polyamine content in these epidermal cells might have contributed to defects in the cells' extracellular matrix, resulting in detachment of cells or induced programmed cell death (apoptosis). They suggested that the electrostatic interactions of putrescine and polyamines with the anionic components of membranes could alter a number of specific membrane functions. These include known polyamine sensitive effects such as transduction of extracellular messages by enhancing calcium influx across the membrane, stimulating G-proteins and enhancing phosphorylation of cellular proteins. They also suggested that since polyamines and putrescine have been shown to be putative angiogenic factors, that the putrescine excreted by the ODC-infected cells could be causing a growth of tumors by polyamine-induced angiogenesis, since the tumors from these cells were all very highly vascularized.

Experimental Design and Exposure Conditions

1. Dates and Personnel

This grant began on November 1, 1992 and funding concluded in October 1996. The Study Section recommended budget for this original proposal was cut significantly prior to funding. The budget for year 4 also reflected more than a 50% reduction in comparison to years 1-3. We were overly optimistic in the original proposal concerning the time required for design, construction, set-up and standardization of the *in vivo* and *in vitro* exposure system which was not commercially available in its current design and configuration. However, we have completed most of the original specific aims. We have changed some of the aims as data became available and have pursued more promising avenues consistent with the original hypothesis where appropriate. **The papers and manuscripts supported by the grant are included at the beginning of the References section**. The continued collaboration and interactions between our two laboratories has proven to be productive and rewarding. Even though the laboratories are separated by more than 2,000 miles, we have maintained close contact through e-mail, telephone, extended visits and mutually attended meetings. We believe this interaction between an engineer expert in EMF-coupling to biological systems and a biochemist investigator concerned with cellular markers and animal models of carcinogenesis is a major strength of this proposal.

2. Animal and Cell Exposure Systems

State-of-the-art exposure systems for 60 Hz magnetic fields were designed, built and tested before their use in the experiments. This was a major accomplishment of the grant. Numerous new solutions were implemented, using extensive computer optimization procedures. Such analysis was performed not only to meet the technical requirements, but to do it efficiently and within a limited space afforded both in the animal facility for the *in vivo* systems, and in a single incubator for the *in-vitro* systems.

All the systems were based on Merritt coils. Merritt's coil system is characterized by a good access of light and air to the cages through the space between the coils. To analyze the magnetic fields generated by currents in thin wires in the absence of ferromagnetic materials the Biot-Savart law was used. Based on this algorithm a field modeling computer program was written.

Our animal magnetic field exposure facility is one of the few (if not the only one) where truly sham-exposed (energized out-of-phase coils) animals are in the same animal room with the magnetic field exposed animals. In most other animal exposure scenarios the "sham" group must be located a large distance from the "exposed" animals usually in another room or facility. The *in vitro* system similarly is one of the few (only one?) that can expose cells in culture to true sham-exposure (energized coils out-of-phase) and 3 different magnetic field intensities in the same incubator with the identical CO_2 supply.

3. Papilloma Co-promoting Activity of 60 Hz Magnetic Fields in the Multi-Stage-Model of Skin Carcinogenesis in the Sencar Mouse

Animal Data: We have now completed and analyzed the data from two animal protocols assessing the ability of magnetic fields to serve as co-promotion stimulus in a Sencar mouse model. Each protocol required approximately 1.2 years to perform (this includes time to obtain the age- and weight-matched animals, quarantine the animals, shave them, treat with the carcinogen DMBA, wait up to 2 weeks, promote with TPA for up to 43 weeks, harvest and analyze the tumors, and analyze the data). Only a summary of the data and its significance will be included here and in Table 1.
It has always been our opinion (confirmed by recent findings) that the maximal likelihood of observing a co-promotional effect of magnetic fields (if one exists) would occur at the low doses of TPA which yield an overall low tumor response. The rationale for this opinion has both statistical and mechanistic components. The statistical power to measure an increased tumor response in the sham-exposed groups is the greatest when tumor incidence and total tumor numbers are low. This is an extremely important consideration in any animal cancer experiments employing small numbers of animals designed to show an increase in tumor response following exposure to some agent. Maximal statistical power is also particularly relevant to support an observation of no effect. Mechanistically, our hypothesis has been that magnetic field effects upon cancer etiology (if they exist) are not of high magnitude and are not easily observable using conventional assays due to a limited/plateauing dose response. Chemical effects in animals, on the other hand, can be observed by increasing dosage up to 5-6 orders of magnitude above environmentally relevant levels. Furthermore, in the co-promotion assay, even TPA doses well below those eliciting the maximal tumor promotion response (1-2.0 (g; 2x/wk) might mask a potential magnetic field response. (We realized that low doses of TPA such as 0.25 μg produce a variable tumor response in the different animal populations used for replicate protocols. But, we failed to realize the degree of this variability)

In order to assess the co-promoting ability of magnetic fields in the mouse it was crucial to establish the dose-dependent ability of TPA or phorbol-ester tumor promoter alone on tumor incidence and multiplicity in the Sencar mouse. During the initial year of the grant while the magnetic field exposure apparatus was being designed and constructed, a dose-response relationship for the promotion of papilloma formation by TPA following initiation with 10 nm of DMBA was established. Groups of Sencar weight matched animals were initiated with DMBA and subsequently treated twice weekly with 0.25, 0.5, 1.0 and 2.0 μg of TPA dissolved in acetone. An additional group was treated with acetone alone. The results obtained are typical for this model for all parameters monitored, namely: percentage of animals with tumors, tumor multiplicity (tumors/animal) and latency.

Magnetic field co-promotion Protocol 1: Two suboptimal promoting levels of TPA (0.25 and 0.5 μg, 2 /wk) were chosen to test magnetic field co-promotion. Groups of 56-60 each were either sham-exposed (< 0.1 (T) or exposed to 2 mT, 60 Hz magnetic fields 7 h/d, 7 d/wk. The two groups of animals receiving 0.5 μg of TPA 2 x/wk were treated for a period of 33 weeks and those receiving 0.25 μg TPA 2x/wk for 43 weeks.

No statistically significant effects were observed in our animals treated with 0.5 μg TPA for 33 weeks. The same lack of co-promotion effects was also reported for the 0.5 μg TPA dose following 23 weeks in the EPRI study.

In the animals treated with the 0.25 μg TPA 2x/wk, and exposed to the 2 mT, 60 Hz magnetic field, a 73% increase in tumor incidence relative to the sham-exposed controls was observed. Average tumor incidence in the sham-exposed animals at 43 weeks of exposure was 30% and in the field-exposed animals, was 52%. A log-rank comparison of tumor incidence curves and both one-sided and two-sided chi square analysis at individual points throughout the tumor protocol supported a reasonably high degree of statistical significance for this difference. In terms of tumor multiplicity (average number of tumors per mouse) there was also a statistically significant difference between the field- and sham-exposed animals at 43 weeks of exposure. Sham animals had 1.3 tumors per animals, whereas field-exposed animals had 1.8 tumors per animal. In terms of total tumors: there was also a 39% increase in total tumors in the animals exposed to the 2 mT magnetic field. (The analysis of total tumors is another way of representing the data on tumorigenicity and can be used to compare groups as well, provided each group contains the same number of animals.) It was also apparent that the average numbers of tumors per mouse and total tumors were virtually identical in the sham field-exposed animals throughout a 30-week period of promotion/co-promotion.

Byus has communicated extensively with a number of experts in the field of epidermal carcinogenesis, and obtained several suggestions to explain the late effect of the magnetic field co-promotion. A population of tumors which are distinct from the majority and appeared to arise late in the protocol following 30 weeks of treatment with TPA and EMF. There are considerable data in the literature characterizing various populations of papillomas of varying growth rates, dependent upon TPA containing different tumor markers, and likelihood to develop into carcinomas. It is conceivable that the papillomas arising after 30 weeks of magnetic field exposure in these animals with a low percent tumor incidence and tumors represent a distinct EMF-sensitive population. The subset of tumors may further possess a unique distribution of tumor markers compared to those tumors arising earlier or in the sham-exposed animals. It is also conceivable that the observed increase in tumors, while statistically significant, is an artifact of animal variability and low numbers of tumors.

Co-promotion animal protocol 2: A determination of the biological parameters of the "dose" of magnetic field exposure is of extreme importance in understanding the potential risk of magnetic field exposure to the human population. Relevant parameters of dose may be: magnetic field intensity or flux density, total time of exposure, intermittent vs. continuous exposure, and the associated transient magnetic fields and their accompanying electric fields if present. In keeping with the Specific Aims in the original proposal, we attempted to address the aspect of relevant magnetic field intensity or flux density. Four groups of 60 Sencar mice each were treated with 10 nM DMBA as described previously and then were treated with 0.25μg TPA twice weekly begun 11 days after initiation with DMBA. The four groups were additionally exposed to continuous or intermittent 60 Hz magnetic fields in the following manner: (1) sham-exposed control, < 0.1 (g μ2). animals exposed to 2 mT continuous, 7 h/day, 7 d/wk (as a replication of the previous exposure); (3). animals exposed to 0.2 mT continuous 7 h/d, 7 d/wk continuous; and (4). animals exposed to 2 mT intermittent, 30 min on, 30 min off for 7 h/d, 7 d/wk.. Transients were intentionally eliminated through the design of the on/off switching.

As indicated earlier (Table 1) there was an unexpected problem with this experiment, as this batch of animals had a profoundly different dose-response from the previous two i.e., higher tumor incidence and multiplicities at 43 weeks. Several possible reasons for the difference, such as DMBA and TPA purity/specific lot, were thoroughly examined and ruled out. This response limited our ability statistically and perhaps mechanistically to obtain the expected significant data. However, the animals exposed to the 0.2 mT magnetic field continuously for 7h/d exhibited a slight increase in tumor incidence at 43 weeks. The log-rank test analyzed as survival curve data throughout the 43 week protocol had a chi square of 0.06, just outside of the range of being significant. However, analysis with the Fisher's exact test, both one-sided and two-sided, as well as, chi square analysis at 25, 35, and 43 weeks did show a significant difference in tumor incidence between the sham-exposed and the 0.2 mT continuous exposed animals, on the order of 20%. In terms of the total numbers of tumors observed throughout the 43-week period as well as the tumor multiplicity or average number of tumors/papillomas per animal, there was statistically significant 20-25% increase in the animals exposed to the 0.2 mT magnetic field in comparison to the sham-exposed animals at the conclusion of the experiment at 43 weeks and a number of points earlier in the treatment (19). Interestingly, in this protocol, the difference between the 0.2 mT and sham-exposed animals in terms of tumor number was observed early in the tumor promotion protocol rather than later as seen in our previous experiment. In this regard, a significant difference in both tumor multiplicity and total tumors occurred between 10-15 weeks of treatment and increased slightly throughout a 43-week period of promotion/co-promotion.

The animals exposed to the 2 mT continuous magnetic field (promoted with 0.25 μg of TPA), did not show any significant difference in total tumors, tumor incidence, or tumor multiplicity, at 43 weeks, in contrast to what was observed in our previous protocol. However, there was an increase in total tumors and average number of tumors relative to sham-exposed controls between 10 and 22 weeks that did achieve some degree of statistical significance. This was similar to the observation made by Stuchly *et al*. 1992 of an early 2 mT co-promotion effect (Table 1). After 25 weeks the rate of increase of tumors in the 2 mT continuous exposed animals decreased and paralleled nearly exactly that in the sham-exposed control.

In the group of mice exposed to 2 mT intermittent, there also was an early increase in total tumors. However, by the conclusion of the experiment at 43 weeks, there was no statistically significant difference between the tumor incidence or tumor number between the sham-exposed and EMF-treated groups.

In terms of tumor weights at the conclusion of the experiment, the tumors from the 0.2 mT continuously exposed groups were 40% heavier than those of the sham-exposed control. The tumors from the animals exposed to the 2

mT continuous field had also a statistically significant increase in mean size relative to the sham-exposed animals. This effect on tumor weights brought about by magnetic field exposure also supports the suggestion of possibly different tumor papilloma populations with different growth rates. However, it is also possible that there could be a magnetic field effect upon tumor size which is independent of the tumor number. There was no statistical difference between the weights of tumors in the sham-exposed animals and those exposed to the 2 mT intermittent field.

There were several important differences and similarities between the two tumor protocols performed in this grant period. A major difference between the response to 0.25µg TPA 2x/wk in protocols 1 and 2 was that in protocol 2, the tumor incidence, tumor multiplicity and total tumors was significantly greater in all animals as compared to protocol 1, even though they were both promoted with 0.25 µg of TPA twice weekly. The overall tumor incidence was 85-98% in protocol 2, whereas in protocol 1, the incidence was closer to 30%. In terms of tumor multiplicity, in protocol 2, 0.25µg TPA resulted in approximately 4 tumors per animals, whereas only 1.4 tumors per animal was achieved by the same tumor promoter dose in protocol 1. A review of the literature in terms of tumor burden in response to varying doses of TPA promotion reveal that there is considerable variability among the tumor response seen in animals exposed to suboptimal levels of TPA. Discussions with a number of experts in the field of epidermal carcinogenesis, including Dr. John DiGiovanni at the M.D. Anderson Cancer Center, suggest that this variability among tumor incidence and tumor multiplicity at the low levels of TPA is to be expected and not at all indicative of any defect or problem in the way in which the experiments were performed. The tumors in protocol 2 from the 0.25 µg TPA animals were also approximately twice as large by weight at 43 weeks as those seen in protocol 1, which is in keeping with the data showing increased incidence and multiplicity.

The other major difference between protocol 1 and 2 was that the field-mediated increase in total tumor number and multiplicity as well as some effect on incidence was observed early during co-promotion rather than later as had been observed in protocol 1. In the first protocol which, had low tumor incidence and low tumor number, the field effect was observed to occur later in co-promotion, while in protocol 2, the field-mediated stimulation in these parameters was seen earlier rather than later. This can be seen as the deviation in these tumor curves between weeks 10 and 20 among all magnetic field exposed groups of animals. The Stuchly *et al.* 1992 co-promotion protocol also showed an increase early in co-promotion by a continuous 2 mT magnetic field. However, these investigators did not promote the animals beyond 23 weeks (and they used 0.3µg TPA 1x/wk). The data indicates that the overall tumor burden and/or the dose of TPA employed is of paramount importance in observing a co-promotion effect if one really exists.

<u>Analysis of Ornithine Decarboxylase and Polyamines:</u> At the conclusion of the tumor protocol 1, the animals treated with 0.25µg TPA for 43 weeks were sacrificed and all the tumors removed and weighed as described. The tumors were then assayed for the activity of ODC and a representative subset was submitted to polyamine analysis. Both the papillomas, as well as, the epidermis in those animals which did not have tumors were analyzed for polyamines and ODC. In the papillomas the levels of putrescine, cadaverine, spermidine and spermine were comparable to what has been reported by others. The presence of cadaverine may be indicative of bacterial contamination of the tumors. However, cadaverine has been reported by this laboratory to be a "naturally-occurring" polyamine in mammalian cells. There was no statistical difference between any of the levels of polyamines from the field- or sham-exposed tumors. Papilloma ODC activity was measured five days after the last 0.25µg TPA treatment, and this constitutive level of ODC in the sham- and exposed-animals also exhibited no statistically significant difference.

The level of ODC and polyamines in the epidermis of those animals which did not have tumors following 43 weeks of treatment were also analyzed for ODC activity and polyamine levels. It was important to assess if these parameters were altered in the epidermal as opposed to the tumor tissue of animals chronically exposed to magnetic fields and treated twice weekly with TPA. The epidermal polyamine levels were somewhat different than those observed in the papillomas. Most evident was a decreased level of putrescine and an increase in spermine as has been reported by others. None of the polyamine levels were significantly altered in the epidermis of the sham- versus exposed-animals. The activity of ODC in the epidermis at the constitutive level five days post 0.25µg TPA was below the delectability of our assay and showed no difference between sham- and field-exposed. However, an additional group of animals was treated with 1.5µg of TPA and ODC assayed after five hours (the time of maximal ODC activity following this dose of TPA). The activity of ODC in the epidermis five hours post TPA increased

markedly and yet showed no difference between the sham- and exposed-animals. In the EPRI study, epidermal ODC activity was measured 6 h after 1, 2, or 5 weeks of TPA promotion (2µg, 1 (µg, or 0.5µg, 2x/week) and there was no significant difference between ambient and field-exposed groups (polyamine levels were not determined).

This analysis for EMF-mediated alterations in ODC activity and polyamine levels in the mouse epidermis *in vivo* was extensive, yet no effect was observed. This could be because the analysis wasn't extensive enough. Juutilainen reported no magnetic field-induced changes in epidermal ODC in the K2 transgenic line following only 24 h of exposure, but did observe a 2-fold increase in putrescine. Perhaps there are transient effects upon polyamine metabolism in specific stem cell populations in this heterogeneous tissue which are not observable when the entire epidermis is assayed. It is also conceivable that no effect exists in this tissue. This lack of an observable magnetic field response in ODC/polyamines (confirmed in part by the EPRI study in the epidermis served as the basis for our proposal to refocus our efforts in relation to this magnetic field marker to other tissues (see Proposed Studies).

4. Evaluation of Induced Electric Currents and Fields

In keeping with the original aims, induced electric currents in models of man, rat and mouse from 60 Hz magnetic fields were computed. All models had realistic shapes. The model of man was analyzed for two cases; with a homogeneous average tissue conductivity and a heterogeneous model. The heterogeneous model was based on the cross-sectional anatomical diagrams of a man model 177 cm tall and weighing 70 kg. The impedance method was used. After discretization, this model consisted of 37,000 cubical cells of an equal size of 1.31 cm. An electrical conductivity value was assigned to each cell, according to the tissue type it belonged to. For the homogeneous model, a conductivity of 0.07 S/m was assumed. The models of rat and mouse were homogeneous and did not include their tails. The body contours were obtained from their magnetic resonant images (MRI). These homogeneous models had realistic shapes. The rat and mouse models were discretized into 112 000 and 29,600 cubical cells of 1.5 mm and 1.0 mm, respectively. Their electrical conductivity was assumed equal to the man average conductivity, i.e., 0.07 S/m.

The results for various models and species, as well as different orientations of the magnetic field were compared. The data presented were useful as the first step in dosimetry for 60 Hz magnetic fields, and for inter-species scaling of biological interactions related to the tissue induced electric currents. A comparison of the induced electric fields and currents in homogeneous models of three species is given in Table 1.

<u>Table</u> 2. Comparison of the current densities induced by a 60 Hz magnetic field of 1 mT in homogeneous models (60).

	Current Density (((/m2)	
Model	Average	Maximum
Human	1.3	10.9
Rat	0.31	1.27
Mouse	0.12	0.41

The results obtained provided a useful insight into interspecies scaling and its limitations. The main difficulty in scaling was due to the different spatial distribution of the induced current. The maximum current densities were typically about an order of magnitude greater than average. Furthermore, in these calculations the models quality was limited, and the human body was not divided into sufficiently small sub-volumes. Detailed modeling with a high quality model of the human body and more efficient computational technique was performed under another grant The results of these computations clearly indicate that the differences are even greater when more detailed tissue anatomy and morphology are considered. Our recent more accurate modeling with resolution of 3.6 mm and an anatomically correct model of the human body and heterogeneous correct conductivity values gave the whole body average and the maximum current density of 3.4mA/m^2 and 77mA/m^2, respectively. The maximum current density was not in the skin, but CSF, and other tissues with high current densities were in blood, brain and prostate. Noting that the simplified modeling gives estimates that are lower, and does not provide any information about organs and tissues with higher levels, the importance of high resolution modeling of anatomically correct representation of mammalian bodies becomes evident. The recent accurate computations were carefully verified.

Our calculations confirm that the induced currents are weak compared to the endogenous currents for most environmental exposures, but their spatial distributions in the body are certainly very different. Typical endogenous currents are of the order of 1 mA/m^2. To induce that current density, the external field of the magnetic flux density about 13 mT is required if the maximum current density of 77mA/m2 is considered . For mice 2 mT exposure field induces maximum current densities of about 0.8 mA/m^2 according to Table 2. This reference data, when improved could be used more reliably for the human exposure scaling.

At the onset of this project, it was anticipated that human and animal anatomical models of the satisfactory resolution could be obtained from other researchers. This, however, turned out not to be feasible. Therefore, we had to develop image segmentation algorithms in our laboratory, thus expanding resources on an additional task and delaying the achievement of all the aspects of the planned specific aims. Furthermore, the impedance method suffers from certain limitations as it tends to converge to a wrong solution when air cavities of certain shapes are present. Our previous human model with a resolution of 1.3 cm did not include air cavities (anatomically incorrect). To be able to analyze the model with air cavities, we have developed computer software based on the scalar-vector potential formulation of the problem and the finite difference method numerical solution. Our segmentation software can and is planned be used to obtain a heterogeneous models of a mouse and a rat used in the proposed study.

The currently obtained detailed dosimetry for the human body will assist in scaling of the data from the already completed and the proposed animal experiments. The electric fields produced by the magnetic induction can in turn be used for modeling of membrane potential in cells connected by gap junctions in tissues that have such structures.

5. Effect of Magnetic Fields on ODC and Putrescine Export

An increase in ODC activity in cell lines and tissues exposed to low-energy EMF has been observed by a number of investigators. Although highly disputed, data in the literature suggested that 60 Hz magnetic fields were capable of altering gene expression. Given the well-characterized regulation of ODC activity brought about by specific gene expression by hormones and growth factors, we investigated extensively the possibility that magnetic field-mediated increases in ODC activity was brought about by elevated expression of ODC mRNA. Following an extensive series of experimentation we concluded that low-frequency modulated RF fields and pulsed magnetic field exposure which resulted in increases in ODC activity did not alter the steady-state level of the mRNA coding for ODC using Northern analysis. We subsequently investigated other potential mechanisms (other than transcription) by which EMF exposure might lead to altered ODC activity.

Considerable effort has gone into the understanding of the regulation of ODC at the transcriptional, post-transcriptional, and translational level. Currently, it is believed that while ODC can be regulated at the transcriptional level, the bulk of the regulation of the enzyme activity occurs post-translationally via ODC turnover. The regulation of ODC turnover is mediated by the putrescine and spermidine through another protein called the ODC-antizyme. The antizyme binds to ODC and causes its rapid degradation. The synthesis of the antizyme in the cell is controlled by putrescine and spermidine by a novel mechanism involving translational frameshifting. Thus, the intracellular concentration of putrescine and spermidine becomes feedback regulators of their own biosynthesis through antizyme-mediated ODC degradation. The $t_{1/2}$ of ODC activity and protein can be as short as 15 min when antizyme is bound to ODC.

During the previous grant period, we have studied and characterized what we believe is the major mechanism by which cells control or regulate rapidly the amount of putrescine and thus ODC which is present inside the cell. We have determined that cells in culture and primary cells in animals export or transport large amounts of putrescine from inside the cell to outside the cell. The data indicate that significantly more putrescine is exported by cells than is used for the intracellular biosynthesis of the polyamines. The process of putrescine efflux from the cell appears to be a highly regulated process stimulated by a number of factors known to increase polyamine biosynthesis, including the intracellular levels of putrescine itself. This putrescine and cadaverine export process is protein-mediated and can occur uphill against a concentration gradient. The putrescine exporter does not appear to be energy-dependent, and also appears to lack sodium and calcium dependence. There is a high degree of substrate selectivity on the order of cadavering > putrescine >> spermidine > spermine. The putrescine export capacity of the polyamine transporter appears to be quit large, since low levels of intracellular putrescine can be maintained even in the presence of 10-fold or greater external concentration gradients. We have studied this

phenomenon of putrescine export and published a series of manuscripts supported in part by this grant describing this process in various cell types.

We determined further that this process of putrescine/cadaverine export was sensitive to magnetic fields and could be a major mechanism by which magnetic fields lead to an increase in ODC activity. However, we have made considerably more progress characterizing putrescine export in general than specifically the effect of EMF magnetic fields on this process. Given the critique of this aspect of our work, we are not proposing to continue it here (see below).

Our working hypothesis is that exposure of cells to magnetic fields might directly or indirectly accelerate the export of putrescine from the cell. As the level of putrescine intracellularly decreases in response to an increased level of export, the cell would respond by a transient increase in the activity of ODC. In addition, it should be mentioned that at least one other laboratory, that of Dr. C. Cain at Loma Linda VA, has also measured increased amounts of putrescine exported from C3H/10/$T_{1/2}$ fibroblasts following transient exposure to 1.0 mT 60 Hz magnetic fields. We intend to continue to actively investigate magnetic-field induced alterations in this putrescine export process with our *in vitro* exposure apparatus, using a small amount of funds available from other sources. We have made considerable progress in cloning this transport system and hope to be able to characterize its importance during apoptosis, cell growth and potential interaction with magnetic fields.

6. Evaluation of Induced Currents on Subcellular Level (Micro-dosimetry)

One of the key questions related to interactions of low-frequency magnetic fields with biological systems is which parameters of the exposure field are responsible for observed effects. Knowledge of the induced electric field and current in various experimental in vitro systems is important for this purpose. In our initial study, the 3D impedance method was used to model spatial patterns of induced electric fields and currents in two preparations of cells. A cell monolayer with a random distribution of cells and a confluent monolayer of cells with gap junctions were considered. The cell membranes have the conductivity of the order of 10^{-5} to 10^{-7} S/m. The random model indicated that for higher cell densities the pattern of the induced current flow had a limited dependence on the size and shape of the container in which the cells were placed. It depended mostly on the actual cell placement. These results clearly indicate that cell density, if sufficiently high, changes the induced fields and currents and through this mechanism may contribute to the observed biological response.

A small cluster of cells connected by gap junctions was also analyzed. Gap junctions predictably increased both the average and maximum current densities as compared with those in a single cell in the same magnetic field. For a gap junction conductivity of 0.5 S/m, the same as assumed for the cytoplasm, the average current density increased with an increase in the surface area of the gaps. The average, however, still remained lower than the average for the same ring of cells entirely open. Our results also emphasize a significant error that is frequently due to simplified computations, in which the current density is computed directly from a radius of the ring (directly from Faraday's law). More detailed description and results are given in our publication.

The difficulties with the rectangular meshes and the impedance method provided the motivation for our exploration of the finite element method (FEM) to model induced currents on the cellular level. We applied the FEM method to analyze two models for cells in culture exposed to a uniform low frequency (60 Hz) magnetic field. The results obtained in this investigation were in general agreement with the results of our earlier modeling that used cubic cells and the impedance method. But there are some differences. Higher than previously anticipated current densities in gap junctions are obtained and are believed to reflect the reality.

What is the significance of the data we have obtained for possible interactions of magnetic fields with biological cells? The values of induced current densities and corresponding electric fields for 60 Hz at 1 mT and for very small cell clusters are very low. However, they do increase with the cluster size, if not exactly directly proportionally to the cluster equivalent radius, pretty close to that. Furthermore, it can be shown, that the quasi-static approximation is valid up to about 100 kHz for a conductive object of the human size and electrical properties. That means that for e.g. transient magnetic fields of 100 mT at 60 kHz, the current densities are 10^5 greater.

Our investigations thus far have not included membrane potential changes in cells connected by gap junctions due to the electric field in the extra-cellular space or solution (for *in-vitro* preparations). It is well known, that the greater the cell dimensions, the higher the membrane potential induced by the electric field at low frequencies for

cells in conductive medium. Evaluation of membrane potential change in cells connected by gap junctions forming both "chains" and clusters of various dimensions is a natural extension of our modeling of clusters with gap junctions in the magnetic field.

Recently, we have commenced an analysis of chains and clusters of various sizes and shapes in the electric field induced in tissue by a magnetic field or an electric field. A copy of the paper submitted for publication is enclosed as an Appendix. The finite element method (FEM) was used under the static field approximation, i.e. only conduction currents were taken into account and the displacement currents were neglected. Effects of gap conductivity and size were investigated in addition to consideration of various geometries of cells connected by gap junctions. The main findings can be summarized as follows:

- For gap-connected cell chains shorter than the chain characteristic length defined in the leaky cable model, a prolate spheroid or ellipsoid model is applicable with errors of the order of 5%, while the leaky cable model is much less accurate. For chain lengths above about 4 characteristic lengths (and provided that the length of the chain is much greater than the diameter), the leaky cable model proposed earlier by Cooper is accurate within 5%;
- The leaky cable model does not provide accurate results for assemblies of cells that are connected by gap junctions in more than one direction, e.g. in the case of organs such as the heart, or tissues such as the skin;
- Neither this static analysis nor the leaky cable model can take into consideration the frequency behavior of membranes that includes their capacitance (and therefore the displacement current. For longer chains and bigger cell assemblies the current due to the membrane capacitance may significantly reduce the membrane potential.

The initial conclusion of that investigation is that common environmental levels of 60 Hz of less than 1 mT are not capable of inducing 1 mV across cell membrane of gap-connected cells even in large human organs such as the heart. However, the induced membrane potential for fields used in the gap-connected skin cells of mice used in this experiment such evaluation has not been made, mostly due to the lack of reliable data on the organ and tissue induced electric fields. Similarly, the frequency behavior of the induced membrane potential has not been evaluated, yet.

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2. Byus CV, Kartun K, Pieper SE and Adey WR. "Increased ornithine decarboxylase activity in cultured cells exposed to low-energy modulated microwave fields and phorbol ether tumor promoters." Cancer Res. 48:4222-4226, 1988.

TITLE: **The Role of Melatonin in the Biological Effects of Magnetic Fields**

Principal Investigator: Colin F. *Chignell*, Ph.D.
Laboratory of Pharmacology and Chemistry, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Cellular Function; Biophysics of Cell/Field Interactions; Melatonin and Free Radicals

Z01-ES50136 **Start Date:** 9/93 **End Date:** 9/95

Abstract

Melatonin (N-acetyl-5-methoxytryptamine, MLT), an indole hormone synthesized primarily in the pineal gland and the retina, controls several essential physiological functions associated with circadian and seasonal rhythms. It is involved in immune responses, aging processes and age-related diseases. Because all these process are associated with oxidative damage of vital cell components, it has been postulated that melatonin may function as a natural antioxidant and free radical scavenger. It has further been suggested that EMF induced decrease in nocturnal levels of melatonin may be responsible for the weak association between environmental EMF exposure and cancer.

Experimental Design and Exposure Conditions

Singlet oxygen was photogenerated by a single laser pulse excitation (exc = 335 nm) of perinaphthalene in acetonitrile. The interaction between singlet oxygen and quenchers (melatonin, 6-hydroxymelatonin, etc.,) was measured by monitoring singlet oxygen radiative lifetime at 1270 nm at various quencher concentrations. Reaction of melatonin with free radicals was measured using the EPR/spin trapping technique.

Quality Assurance Measures

Rate constants for reactions with singlet oxygen were calculated from a plot relating singlet oxygen lifetime to quencher concentration. The data for the radicals was obtained by competition with the spin trap, 5,5-dimethyl-11-pyrroline-N-oxide using known literature values for the rate constants.

Results

We have found that all the indoles including MLT react with •OH at near diffusion controlled rates, k_r(indole/•OH)~10^{10} $M^{-1}s^{-1}$, for MLT k^1,=2.6x10^{10} $M^{-1}s^{-1}$]. The interaction of the indoles with •CH_3 radicals is less efficient and occurs with a rate constant of ~10^7 $M^{-1}s^{-1}$. In contrast scavenging of •OCH_3 is very fast process. (k~10^9 $M^{-1}s^{-1}$). We found no evidence for a reaction between MLT and superoxide. The $^1O_2$ quenching efficacy depends on the position and the nature of the substituent (s) of the indole nucleus and also on the reaction environment. In CH_3CN and CD_3OD the $^1O_2$ quenching occurs with the rate ~10^7-10^8 $M^{-1}s^{-1}$, while in D_2O the quenching is more than ten fold faster. The melatonin precursor serotonin generated hydroxyl radicals in the presence of ferric iron and H_2O_2, and the melatonin metabolic

6-hydroxymelatonin generated copious amounts of •OH radicals even in the absence of exogeneously added H_2O_2.

Given the reported low toxicity of MLT, its ready accumulation in all types of cells due to its high lipophilicity and distribution throughout the entire body *via* the blood stream, melatonin may be considered as a potential antioxidant when taken in pharmacological doses. However, the physiological concentrations of melatonin (100 pg/ml in the plasma) are probably too low for the hormone to act as a physiological antioxidant. Thus it seems highly improbable that the biological effects of EMF can be exerted via a modulation of melatonin levels.

Discussion and Contribution to Understanding Biological Effects of EMF

Constant exposure to electromagnetic fields (EMF) may constitute a risk to human health. Recent hypotheses link the slightly higher cancer incidence in the population exposed to EMF to decreased nocturnal production of the pineal hormone melatonin. Carcinogenesis is a free radical process which could potentially be inhibited by melatonin acting as a natural free radical antioxidant. Our studies indicate that although melatonin is a good scavenger for oxygen-centered free radicals and singlet oxygen the serum concentrations of this hormone are too low (pg/100 ml) for it to act as a physiological antioxidant.

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Other EMF Publications

None

TITLE: **Exposure to EMF and Astrocytomas: *In Vitro* Studies**

Principal Investigator: Lucio G. *Costa*, Ph.D.
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Health Relevance: Cancer

Research Area: Cellular Function; Cellular Proliferation; Signal Transduction

RO3-ES08851 **Start Date:** 01/10/97 **End Date:** 12/31/98

Abstract

Epidemiological studies suggest that exposure to electromagnetic fields (EMF) is associated with an increased incidence of certain cancers, most notably brain tumors, and in particular astrocytomas. No studies have, however, investigated the biological processes that may account, at the cellular level, for such epidemiological finds. EMF do not appear to have cancer-initiating capabilities, but may rather act as tumor promoters. Alterations in calcium homeostasis, protein kinase activity and gene expression have been reported as a result of EMF exposure and may be involved in their ability to increase cellular proliferation. This proposal will focus on the ability of 60 Hz EMF exposure to increase the basal and mitogen-stimulated proliferation of human astrocytoma cells, and on the role of protein kinase C (PKC) in this process. Proliferation of astrocytoma cells exposed to different levels and for different durations to EMF will be assessed by ^{3}H thymidine incorporation and by flow cytometry. The ability of EMF to potentiate the effect of a number of mitogens will be tested. To determine whether astrocytoma cells are uniquely sensitive to EMF, nontransformed astrocytes and other tumor cell lines will also be tested under similar protocols. As PKC plays a primary role in proliferation of astrocytoma cells, its involvement in the proliferative action of EMF and its augmentation of mitogens' effects will be investigated by the use of specific inhibitors and by direct measurements of PKC activation. These studies will address the possible biological basis for health effect, brain tumors, most often associated with EMF exposure, and will provide insights on a potential cellular and molecular mechanism. As such, these results will be relevant to further assess and evaluate the risk of brain tumors in humans exposed to EMF.

Experimental Design and Exposure Conditions

The human astrocytoma 1321N2 cell line is being used in this study. Proliferation is measured by ^{3}H-thymidine incorporation, flow cytometry or direct cell counting. Mitogens are added to the cells for a period of 24-48 hours. For proliferation experiments, cells are usually seeded in 24-well plates. Cells are exposed to EMF (0.61-.2G) in a shielded incubator coil system based on a 4 coil Merritt design similar to that of Dr. Robert Liburdy, built by Dr. Michael Yost. A double incubator houses the duplicate shielded coil systems.

Quality Assurance Measures

Magnetic field levels inside both incubators are monitored with EmdexLite dosimeters. Temperature in the cell culture medium is also monitored. Positive controls for cell proliferation are run in each experiment.

Results

Exposure of human astrocytoma cells to EMF (1.2G) caused an increase in cell proliferation, measured by both ^{3}H-thymidine incorporation into DNA and by flow cytometry. EMF also augmented the mitogenic effect of the phorbel ester TPA and the cholinergic agonist carbachol by 80%. Experiments utilizing plates of different radii indicated that the effect of EMF was due to magnetic rather than electric fields. The protein kinase C inhibitor GF109203X, and down-regulation of PKC, inhibited the effect of EMF on basal and agonist-stimulated proliferation, suggesting a role for this signal transduction pathway in Em's mitogenic action.

Discussion and Contribution to Understanding Biological Effects of EMF

Epidemiological studies suggest that there is a pervasive association between brain tumors, most notably astrocytomas, and exposure to EMF. These studies were aimed at addressing an issue which is directly relevant to the development of a mechanistically based understanding of the relationship between EMF exposure and brain tumors, as they are testing the hypothesis that EMF is a mitogen for human astrocytoma cells. At very high EMF exposure levels a mitogenic effect was observed, but these data do not provide support for the hypothesis that EMF at levels seen in the environment has significant biological effects.

Publications From This Grant

1. Wei M, Guizzetti N, Yost N and Costa LG. "The effect of 60 Hz electric and magnetic fields on the proliferation of human astrocytoma cells." Submitted, 1998.

Other EMF Publications

None

TITLE: **Effects of EMFs on Calcium Levels in Excitable Cells**

Principal Investigator: Gale Louise *Craviso*, Ph.D.
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Reno, Nevada

Health Relevance: Neurobehavioral

Research Area: Cellular function; Cellular Processes; Calcium Flux

R03-ES08903 **Start Date:** 1/10/97 **End Date:** 1/09/99

Abstract

Epidemiological studies have suggested that exposure to 60 Hz electromagnetic fields (EMFs) may have adverse effects on the nervous system. However, there is currently little information regarding how EMFs and neural cells may interact. The goal of our research is to provide data that will begin to bridge this gap. Results of preliminary studies show that isolated adrenal medullary chromaffin cells, a well-characterized cell culture model of neural-type cells, undergo an increase in intracellular calcium in response to a magnetic flux density of around 1 mT. In these studies, intracellular calcium level was monitored by fluorescence video imaging of the cells during EMF exposure. The overall aim of the proposed research is to continue to use fluorescence imaging of intracellular calcium in chromaffin cells to define the EMF-induced calcium response as a function of several EMF parameters. The specific field exposure parameters to be studied are: 1) the range of magnetic flux densities that elicit responses: 2) the frequencies of EMF that cause changes in calcium: 3) the relationship between the duration of the EMF exposure and calcium responses; 4) the effect of single versus multiple EMF exposures on calcium responses; and, 5) the effect of transients on calcium responses. Data derived from these studies will be important for helping to evaluate more critically whether environmental EMFs present a human health risk since EMF exposures in school, residential, occupational and transportation environments represent a complex mixture of field intensities, exposure durations, frequencies and transients.

Experimental Design and Exposure Conditions

Overview: The primary technique to be used in this study is fluorescence video imaging of $[Ca^{2+}]i$. Through the use of microscopy and the fluorescent calcium binding dye fluo-3, $[Ca^{2+}]i$ is monitored in several cells at a time during magnetic field exposure. The advantages of this approach include not only the ability to measure $[Ca^{2+}]i$ changes directly during EMF exposure in individual cells but also the ability to document dynamic aspects of the changes in $[Ca^{2+}]i$ such as calcium oscillations and calcium transients. Primary cultures of bovine adrenal medullary chromaffin cells, which are a well-characterized, biologically-relevant *in vitro* model of neural-type cells are the cell model employed in this study. The time between placement of cells into culture and initiation of EMF exposure will be from one to 8 days. Cells are plated at low density (1.5×10^5 cells/ml) in round, collagen-coated or poly-D-lysine-coated plastic petri dishes that are 35 mm in diameter. Attachment of cells to the dishes is carried out one day before initiation of the experiment. Cells are transported from the Principal Investigator's tissue culture facility to the EMF-imaging facility in a custom-made mμ-metal box.

Procedure for Loading Chromaffin Cells With Fluo-3

1. Culture dish removed from cell culture incubator and carried over to the lab bench where the medium is aspirated
2. Medium replaced with 1.25 ml of a balanced salt solution containing fluo-3AM (6 μM)
3. Cells returned to incubator where they are kept for 45 minutes to load dye
4. Cells removed from incubator and carried over to the lab bench where the fluo-3 solution is aspirated
5. Two ml of dye-free balanced salt solution added to cells
6. Culture dish placed on microscope stage (the microscope is enclosed in a rectangular mμ-metal enclosure) and viewed with a 10X microscope objective. Cells are illuminated at 490 nm by passing light generated by a mercury arc lamp through an interference filter and dichroic mirror. Fluorescent emissions >520 nm (reflected by the dichroic mirror) are collected by an intensified video camera. Video sequences are stored on a video cassette recorder (VCR) and digitized by a frame grabber. Computerized analysis of sequences on a frame-by-frame basis is performed by computing the average fluorescence of each cell.

Note: Steps 1, 2 4 and 5 are carried out in mμ metal shielding.

EMF Exposure system: For EMF exposure of cells, a magnetic field is generated by a pair of 16 cm diameter Helmholtz coils (distance between the coils is 8 cm) mounted on a non-metallic framework that is resistant to warping. Each coil has 25 turns of twisted enameled copper bifilar wire (16 gauge), using an optimal pitch of 0.3 - 0.5 twists/cm such that the direction of the currents through the two strands of bifilar wire is made either parallel for field exposure or antiparalled for skin exposure.

Quality Assurance Measures

EMF exposure system: We have put a great deal of effort into obviating the problem of perturbations in the applied magnetic field due to metallic components of the microscope objective and microscope stage by: a) fabricating a high-resolution objective entirely of plastic, and b) completely eliminating all metallic materials on the microscope stage in the vicinity where the cells are placed during imaging. In addition, our exposure system was inspected by W/L Associates, Ltd., on September 16, 1997. We have received their report and address all of the points that were raised regarding various field measurements.

Accessory non-EMF exposure: a) Temperature is continuously monitored by a thermistor probe attached to the edge of the inside of the culture dish and is kept constant at 36.5^{o} C by perfusing the cells with a balanced salt solution that is warmed by passing through a glass heating coil. At the magnetic field intensities employed thus far, no deviation in temperature from 36.5^{o} C has been observed. b) To minimize any vibrations that may be transferred to the cells from the coils, the framework holding the coils is isolated from the microscope stage. Vibrations are further minimized by resting the framework that holds the coils on foam rubber.

Other: Magnetic flux density is measured before and after each exposure. During experiments, magnetic field intensity is continuously monitored by continuously measuring current through the Helmholtz coils. All experiments are carried out with a positive control that consists of addition of a depolarizing agent to the cells several minutes after the exposure (sham or field) is terminated. Moreover, studies are conducted under double-blind conditions; the engineer sets the DPDT switch that controls the direction of the current in the two strands of bifilar wire and the individual conducting the imaging experiments does know whether cells are exposed or under sham conditions. Only after the $[Ca^{2+}]i$ responses are measured are is sham/exposed code broken. Our experimental protocols were reviewed by W/L Associates, Ltd., during their site visit.

Results

Studies are in progress in which we are determining: (1) what 60 Hz EMF intensities cause a change in $[Ca^{2+}]i$ and (2) what frequencies of EMFs cause a change in $[Ca^{2+}]i$. The data are currently being evaluated and no definitive results are available at this time.

Discussion and Contribution to Understanding Biological Effects of EMF

These studies are directed toward an ultimate goal of deriving a basic understanding of the mechanisms by which EMFs can interact with excitable cells, such as those of the nervous system, and alter their function. By defining the EMF-induced $[Ca^{2+}]i$ response in chromaffin cells as a function of several EMF parameters such as field intensity, duration of exposure, number of exposures, frequency and transients, we expect to provide a general basis for extrapolation of *in vitro* exposure effects to *in vivo* exposure situations. In this regard, some of the difficulties in establishing a positive link between exposure to magnetic fields and adverse neurological effects may well be due to the wide range of environmental exposure conditions where field strength, duration and other parameters continuously vary.

Publications From This Grant

1. Publicover NG, Marsh CG, Vincze CA, Craviso GL and Chatterjee I. "Effects of microscope objectives on magnetic fields." Re-submitted to Bioelectromagnetics , 11/98.

Other EMF Publications

1. Livingston GK, Witt KL, Gandhi OP, Chatteyee I and Roti Roti J. "Representative Integriety of mammalian cells exposed to power frequency electromagnetic fields." Envir. Molec. Mutagenesis, 17:49-58, 1991.

TITLE: **Protein Tyrosine Kinases and Electromagnetic Fields**

Principal Investigator: Ilker *Dibirdik*, Ph.D. (formerly Uckun, FM)
University of Minnesota
Minneapolis, Minnesota

Health Relevance: Cancer

Research Area: Cell Proliferation, Cellular Processes

R01-ES07175 **Start Date:** 9/25/95 **End Date:** 8/31/98

Abstract

The focus of this research grant is to further the understanding of how exposure to electromagnetic fields may affect the survival machinery in immature cells of the immune system. Specifically, we will examine if the tyrosine kinases, enzymes known to play a pivotal role in development and growth of immature cells as well as progression of leukemias and lymphomas, might be stimulated by electromagnetic field exposure. Preliminary studies conducted in our laboratory provided experimental evidence that two classes of tyrosine kinases are stimulated by EMF exposure. The goal of the proposed research is the elucidation of the molecular mechanism by which low energy electromagnetic fields initiate a cascade of cytoplasmic and nuclear signaling events in human lymphohematopoietic cells. Our research plan involves (a) immune complex kinase assays to examine the effects of EMF on the enzymatic activity of specific Src-family and non-Src family protein tyrosine kinases (PTK) in normal and leukemic B-cell precursors corresponding to discrete developmental stages of human B-cell ontogeny, (b) detailed biochemical and biologic studies of CD19 receptor function in B-cell precursors following EMF exposure, (c) use of CD19 receptor directed tyrosine kinase inhibitors to elucidate the role of CD19-receptor associated tyrosine kinases in the pleiotropic effects of EMF including activation of protein kinase C and *c-jun* protooncogene, (d) use of two distinct model systems (viz., K562-CD19 and UPN1lyn models) to examine the importance of CD19 receptor and LYN kinase in EMF induced biochemical signals, (e) correlative studies to explore the relationship between EMF responses of leukemic cells and various biomarkers which are routinely measured as well as (f) statistical analyses to examine the relationship between various biomarkers, clinical/laboratory data and residential EMF measurements. From each newly diagnosed acute lymphoblastic leukemia case, we routinely obtain several hundred million leukemic cells. Therefore, more than sufficient cells will be available for the proposed experiments which will require 50-100 million cells. In addition, our data will be interfaced with the residential EMF data generated in the CCG-E15 epidemiology study. We therefore believe that our proposed research will significantly contribute to the current knowledge of the biologic effects of EMF and their possible molecular mechanisms.

Experimental Design and Exposure Conditions

Cells in suspension will be exposed to 60 Hz sinusoidally oscillating EMF between 10 milligauss and 10 gauss (initial experiments will be done at 1 gauss). We will use the Merritt four-coil array system (ratio of windings 26:11:11:26), with further modifications as suggested by Kirschvink (M/K coil system). In this system the antiparallel vs parallel (control vs experimental) current flow can be selected by a pari of independently operating double-pole, double-throw switches. This allows for double-blind experiments in which the two switches (concealed in separate boxes) are set by different individuals, so that neither the investigator nor the laboratory personnel know which coil is experimental and which is control until the

data are processed and the switch positions are decoded. Alternatively, a computer randomization algorithm with computer-controlled relays can be used to eliminate any investigator participation in choosing the coil activation direction. We will use two identical incubators and two identical M/K coil systems. This will allow control and experimental exposures to be carried out under identical conditions simultaneously. Both incubators will be fitted with magnetoabsorptive steel (mμ metal) shields placed so as to shield the exposure system from fields produced by the warming and temperature circuitry in the top of the incubator. One set of coils will be energized with the current running in parallel (i.e. producing magnetic field) and the other with the current flowing antiparallel (i.e. zero induced magnetic field), using double blind randomization as described above for all experiments. These coils will be energized by a power amplifier (Kepco BOP 20-10M) driven by a function generator (Beckman) producing 60 Hz sine wave AC current in the coils. The AC magnetic field intensity within the exposure volume will be calibrated using a Hall-effect transverse probe (FG Bell) and a gaussmeter (Bell model 610). Waveforms will be monitored continuously using a digital oscilloscope with leads for both the electrical power supply and the gauss meter. Field strength will be recorded with a strip chart recorder attached to the output of the gauss meter. Static geomagnetic fields and their orientation will be recorded daily before and after each experiment. If findings by us or others indicate it is desirable, the geomagnetic field can be canceled or modified using properly oriented DC "bucking" coils; we do not intend at this time to investigate the geomagnetic field as high priority experimental variable but this can be incorporated in the experiments easily if future findings by others confirm its importance.

Quality Assurance Measures

Duplicate cell cultures will be placed in duplicate exposure devices in the duplicate incubators. All experiments will be double-blinded by having the principal investigator set one pair of DPDT switches which will not be visible to the graduate research assistant doing the experimental manipulation, after which the technician will set a second set of switches, whose settings will be known only to that individual. The wiring of the dual pairs of switches will be set up in such a way that one device will be activated and the other will not, but neither the investigator nor the technician will know which is which until their separate records are checked after the data are calculated. Fields will be calibrated in both exposure devices before the double blinding procedure is carried out, such that the field is 1.0 gauss + 1 % in the experimental device for the initial series of experiments. Immediately at the end of exposure, analysis of experimental variables will be carried out as described below. [NB: Although preliminary data have been obtained at 1 gauss and the basic plan is to continue using this field strength for most experiments, we will also determine whether the effects noted can be observed at lower field strengths. If we find that a lower field strength (e.g.100 mG or less) still allows observation of the key phenomena, then we will do some series of experiments at the lower levels since these are closer to the levels which may be found in households potentially at risk from EMF biohazards (if such hazards exist).

Results

B-lineage acute lymphoblastic leukemia (ALL) is a heterogeneous groups of diseases that are thought to originate from putative developmental lesions in normal B-lymphocyte precursor clones during early phases of ontogeny, which allegedly lead to maturational arrest at discrete stages of lymphopoiesis. A point of experimental focus in current EMF research is the investigation of EMF effects on signal transduction pathways. It has been noted that EMF exposure activates a proteinkinase C (PKC)-linked signaling cascade. However, EMF induced signaling events proximal to PKC activation have not been evaluated. Protein tyrosine kinases (PTK) participate and play pivotal and myriad roles in initiation of signal cascades that affect proliferation and survival of human B-lineage lymphoid cells. Recent studies indicate that stimulation of protein kinases in B-lymphocyte precursors is a requisite step in the generation of the pleiotropic effects of ionizing radiation, including the activation of PKC and PKC-dependent serine kinases, nuclear factor kappa B (NKfB), as well as c-jun protooncogene expression. The purpose of this study was to examine the role of *PTK* in EMF-induced activation of the PKC pathway in B-lineage lymphoid cells. Our studies provide direct evidence that exposure of B-lineage lymphoid cells to low

energy electromagnetic fields (EMF) stimulates the protein tyrosine kinases LYN and SYK, results in tyrosine phosphorylation of multiple electrophoretically distinct substrates, and leads to downstream activation of protein kinase C (PKC). EMF exposure enhances protein tyrosine phosphorylation in SYK deficient but not in LYN deficient B-lineage lymphoid cells and stimulates LYN kinase activity in wild-type as well as SYK-deficient B-lineage lymphoid cells. These results indicate that activation of LYN kinase is sufficient and mandatory for EMF induced tyrosine phosphorylation in B-lineage lymphoid cells. The PKC activity increases later than the LYN activity and pretreatment with the PTK inhibitors genistein or herbimycin A abrogates the EMF-induced PKC signal. Thus, stimulation of LYN is a proximal and mandatory step in EMF-induced activation of PKC in B-lineage lymphoid cells. Our observations prompt the hypothesis that a delicate growth regulatory balance might be altered in B-lineage lymphoid cells by EMF-induced activation of LYN.

Discussion and Contribution to Understanding Biological Effects of EMF

Our studies have examined if the tyrosine kinases, enzymes known to play a pivotal role in development and growth of immature cells as well as progression of leukemias and lymphomas, might be stimulated by electromagnetic field exposure. In the exposure conditions in these studies, a effect an Tyrosine kinases was observed. Details are cited in the publications from these studies. These results provided a possible molecular mechanism for the biological effects.

Publications From This Grant

1. Dibirdik I, Kristupaitis D, Kurosaki T, Tuel-Ahlgren L, Chu A, Pon D, Tuong D, Luben RA and Uckun FM. "Simulation of Src family protein tyrosine kinases as a proximal and mandatory step for SYK kinase-dependent phospholipase C gamma2 activation in lymphoma B-cells exposed to low energy electromagnetic fields." J. Biol. Chem. 273: 4035-4039, 1998.
2. Krisupailtis D, Dibirdik I, Vassilev A, Mahajan S, Kurosaki T, Chu A, Tuel-Ahigren L, Tuong D, Pond D, Luben RA and Uckun FM. "Electromagnetic field stimulation of Bruton's tyrosine kinase, J. Biol. Chem., 273 (20): 12397-12401, 1998.

Other EMF Publications

None.

TITLE: **Safety of Time Varying Magnetic Fields in MR Imaging.**

Principal Investigator: Om *Gandhi*, Sc.D., M.S.E.
University of Utah
Salt Lake City, Utah

Health Relevance: Cellular Biophysics

Research Area: Biophysics of Cell Field Interaction

R01-ES06292 **Start Date:** 09/01/93 **End Date:** 08/31/96

Abstract

Over the past five to ten years, magnetic resonance imaging (MRI) has become an important tool for medical diagnostic applications. In the early days of MRI, most people thought that MRI systems posed no hazards to patients or operating personnel because no X-rays were involved in the imaging process and the magnetic field strengths used were not considered harmful. The frequencies were low enough that the radio frequency (RF) magnetic fields did not induce appreciable internal current densities, and negligible RF heating was expected. Also fairly low rates of switching of magnetic fields were used which did not result in perceptible induced currents in comments due to switched gradient magnetic fields. Newer techniques are leading to the use of higher static magnetic fields; more rapidly switched gradient fields and higher RF magnetic fields. Although use of the increasingly stronger electromagnetic fields is causing concern about patient safety, to date, only simplified homogeneous spherical, cylindrical and disc models have been used to obtain induced current density distributions and rates of energy absorption (specific absorption ratesor SARs). Since this knowledge is vitally important, particularly for the critical regions of the body, we will adapt the anatomically based modeling techniques that we have successfully used for electromagnetic dosimetry.

We propose to use the previously tested highly efficient impedance method and its new generalization for SARs for RF magnetic fields, or realistic polarizations and variations typical of present and planned MRI systems. We also plan to use the newly developed modified finite-difference time-domain method, which has been used successfully for a wide variety of RF electromagnetic exposure conditions. For the various calculations we will use the new high-resolution model of a human volunteer based on MRI scans with resolutions of 1.875-3 mm) and its scaled versions where different scaling factors x, y, and z on the order of 0.6-1.0 will be taken to obtain models of different heights and weights representative of human adults and children. This latter step is needed since induced current densities and SARs are strongly dependent on the size and shape of the body. The current densities and SARs thus calculated will be compared with the safety guidelines suggested by USFDA and NRPB (U.K.).

Experimental Design and Exposure Conditions

Calculation of SARs for Body Coil: For SAR calculations we have used a realistic geometry of a 16-rung shielded bird cage body coil that is typically used in the MR imagers of today. Diameter and length of the bird cage are 56 cm each, while the diameter of the shield is 65.5 cm. To obtain circular polarization of the RF magnetic fields, each of the rungs is assumed to be fed with equal magnitude currents that are 22.5^0 out of phase relative to the previous rung. We have used the finite-difference time-domain method to calculate the induced electric and magnetic fields and SARs first in symmetically – placed homogeneous cylinders to verify the accuracy of the computer code, and subsequently in a 6mm resolution, 30-tissue,

anatomically-based model of the human body. The SAR calculations have been done at the presently used RF frequency of 64MHz (used in 1.5T mr imagers) as well as for higher magnetic field imagers that may use 128MHz and 170 MHz for ratio frequencies. (up to 4T machines).

Calculations of SARs for the Presently Used Head Coil: For these calculations we have taken the geometry and the parameters of the present-day commonly used head coil. Head coils are also made of a bird cage of 16-current-carrying rungs for which a progressive phase shift of 22.5^0 in the currents of the adjacent conductors is used to obtain circularly polarized RF magnetic fields. Diameter and length of the bird cage are 28.5 cm and 39.3 cm, respectively. As for the body coil the SAR's have been calculated for RF magnetic fields at 64, 128 and 170 MHz. (up to 4T machines).

Calculations of SARs for Proposed High-Frequency Head Coils: Tuned resonant-type higher-frequency head coils have recently been proposed and are being developed for improved NMR imaging with a higher signal-to-noise ratio and spatial resolution. We have used physical dimensions typical of such coils for frequencies up to the 400 MHz that are planned for those coils.

Currents Induced by Switched Gradient Magnetic Fields: We have used the impedance method to calculate the currents induced in a 0.655-cm (nominal 1/4 ") resolution, anatomically based model of the human body for switched gradient magnetic fields of a Maxwell pair of single-turn coils, each of diameter 0.654 m that are placed at an axial distance of 0.5664 m from each other. This is one of the typical geometries used for z-gradient coils for the present-day 1.5T MR imagers. These calculations have allowed us to determine the maximum values of dB/dT (switched magnetic fields) that may be used without the possibility of tingling/prickling sensations in the subject's body.

Quality Assurance Measures

Our objectives, as stated in the proposal were to: 1. Extend the impedance method to higher frequencies by including inductances, and then to validate this formulation by comparing impedance-method solutions with known analytical solutions for configurations such as solid and layered spheres. 2. Evaluate the relative merits of the generalized impedance method compared to the modified FDTD method for calculating SARs produced by RF magnetic fields in MRI systems. 3. Calculate the SARs using both methods and compare results to establish the validity of the methods. Then calculate the SARs produced by MRI systems in our new high-resolution anatomically based models of the human body.

Results

Calculation of SARs for Body Coil: The SAR calculations have been done for the presently used radio frequency of 64 MHZ for 1.5T MR imagers and for higher frequencies up to 170 MHZ that may be used in the future. This has allowed us to obtain the peak SARs for any 1 kg of tissue for which upper limits have been suggested in the NRPB safety guidelines.

Calculations of SARs for the Presently Used Head Coil: Using the finite-difference time-domain method, we have calculated the magnetic fields in the center of the head coil when it is empty or filled with 2/3-muscle-equivalent cylinder of diameter 21 cm. A cell size of 0.6 cm is used for the calculations. A 0.6 cm resolution model of the human head, neck, and upper torso is used to calculate the SAR distributions not only at the presently used radio frequency of 64 MHZ for 1.5 T MR imagers, but also at higher frequencies up to 170 MHZ that may be used in the future. For all of these calculations we have assumed an outer shield of diameter 65.5 cm.

Calculations of SARs for Proposed High-Frequency Head Coils: We have used physical dimensions typical of such coils and have calculated the SAR distributions of the 0.6 cm resolution anatomically based model of the head, neck and upper torso for frequencies up to 400 MHz that are planned for these coils. Because of the resonant nature of the coil, a half cosine-wave-type current variation is assumed along the length of the rungs. Also, a 22.5^0 progressive shift between adjacent rungs is assumed to obtain circularly polarized RF magnetic fields.

Currents induced by Switched Gradient Magnetic Fields. Using the impedance method we have calculated the induced current density distributions in the 0.6-cm-resolution model of the human body for which anisotropic conductivities have been taken for heart and skeletal muscle. In order to increase the resolution of the model to dimensions on the order of 2-3 mm to properly model the fat or bone to wet tissue interfaces, it is important to truncate the model to regions of high current densities which correspond to the regions of the body where high values of dB/dt are created. We have verified that because the induced currents are primarily circulatory in nature, it is possible to truncate the models to focus on the high-current density regions. This approach results in considerable savings in computer memory and computation times. The highest induced current densities have thus been calculated for the various pulse sequences that are typically used in present-day imagers.

Discussion and Contribution to Understanding Biological Effects of EMF

This study provides important mathematical high resolution modeling for estimating tissue absorption of electromagnetic energy for many frequencies. This computer model will help researchers better estimate the absorption of electromagnetic energy with field exposures. With this knowledge fundamental questions regarding EMF exposure in biological samples can be better addressed. By applying these high resolution molding techniques to inverse problems we may in the future be able to use the externally measured MCG and MEG data to determine the irregularities in the electrophysiology of heart and brain respectively.

Publications From This Grant

1. Furse CM, Yu QS and Gandhi OP. “Validation of the finite-difference time-domain method for near-field bioelectromagnetic simulations.” Microwave and Optical Technology Letters 16: 341-345, 1997.
2. Gandhi OP and Furse CM. “Currents induced in the human body for exposure to ultra wideband electromagnetic pulses.” IEEE Transactions on Electromagnetic Compatibility 39: 174-180, 1997.
3. Lazzi G and Gandhi OP. “Inversion theory as applied to the optimisation of conductivity profile for PML absorbing boundary condition for FDTD code.” Electronics Letters 33: 6-14, 1997.
4. Gandhi OP, Wu D, Chen JY and Conover DL. “Induced current and SAR distributions for a worker model exposed to an RF dielectric heater under simulated workplace conditions.” Health Physics 72: 236-242, 1997.
5. Gandhi OP, Lazzi G and Furse CM. “Electromagnetic absorption in the human head and neck for mobile telephones at 835 and 1900 MHz.” IEEE Transactions On Microwave Theory and Techniques 44: 1884-1897, 1996.
6. Furse CM and Gandhi OP. “A memory efficient method of calculating specific absorption rate in CW FDTD simulations.” IEEE Transactions On Biomedical Engineering 43: 558-560, 1996.
7. Gandhi OP. “d'Arsonval medal: Address - Some bioelectromagnetics research at the University of Utah: Acceptance speech on the occasion of receiving the d'Arsonval medal.” Bioelectromagnetics 17: 3-9 , 1996.
8. Gandhi OP and Chen JY. “Electromagnetic absorption in the human head from experimental 6-GHz handheld transceivers.” IEEE Transactions on Electromagnetic Compatibilty .37: 6-8, 1995.

Other EMF Publications

None.

TITLE: **The Potential Mutagenicity and Co-Mutagenicity of Electrical Magnetic Fields**

Principal Investigator: Barry W. *Glickman*, Ph.D.
School of Medicine, University of Victoria, Victoria, British Columbia, Canada

Health Relevance: Cancer

Research Area: Mutagenicity

R03-ES08894 **Start Date:** 3/20/97 **End Date:** 2/28/98

Abstract:

This proposal focuses on the potential contribution of electromagnetic fields (EMF) to mutagenesis in conjunction with free-radical producing mutagens. These experiments are motivated by the recent chemical studies demonstrating the stabilization of free radicals by EMF. We propose to examine the potential mutagenic enhancing effects of EMF using the *lacI* transgenic rat embryo fibroblast cell line, R2LIZ. *LacI* mutant frequencies will be determined from untreated cells and cells exposed to the mutagens adriamycin, nickel, or ethylnitrosourea (ENU) in the presence or absence of a 60 Hz magnetic field. In addition, mutational spectra for each exposure group will be ascertained by automated DNA sequencing of the *lacI* mutational target, to detect any sequence changes induced by the field and the concurrent mutagen exposures.

We have also exposed Big Blue mice to 3 mT 60Hz fields for extended periods and will now examine the influence of EMF on mutation frequency *in vivo* in these animals.

Experimental Design and Exposure Conditions

The mutagens were chosen for their known mutagenic ability and suspected mechanisms of mutagenesis. Adriamycin and nickel (in the form of NiCl and $NiSO_4$) are test chemicals in that they have the potential of damaging DNA via the formation of free radicals (ultimately superoxides and hydroxyl radicals). As an alkylating agent ENU is not thought to incur mutations through any radical mechanism, and so was chosen as the control chemical. Since the magnitude of any MF mutagenic enhancement is unknown, several chemical concentrations were tested in order to optimize the sensitivity of the experiments.

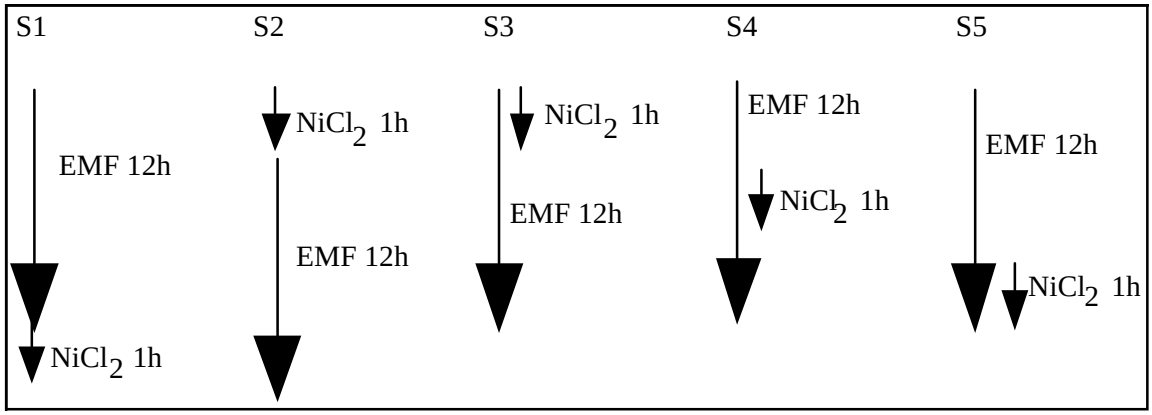

Figure 1

In terms of chemical-field co-exposure, five protocols were used. The protocols were chosen in order to detect any possible influence of the 2 mT magnetic field on the mutagenicity of the chemical ($NiCl_2$ in Figure 1). For example, S1 tests the possibility that the magnetic field alters the cellular potential for free radical induced DNA damage. In S2 the cells were pre-exposed for 1 hour, washed, and then put into the MF exposure apparatus after the design of Obe and Rosenthal (1989). Here the cells are "co-exposed" only in terms of the field and the chemical which has remained in the cell after the washing procedure. S3, S4 and S5 are all concurrent exposures in that the chemical treatment coincides with MF exposure, where after the cells were washed and put back into the field. All protocols subsequently allow 120 hours of growth to confluency within the MF, allowing time for the fixation of mutation. By comparing the results obtained from these protocols, one may discern between an MF enhancement of mutagenesis by a mechanism such as radical stabilization (which should be revealed in S2, S3, S4 and S5) and the confounding possibility of a MF enhancement of cell-membrane traversal of the mutagens (which would be indicated by enhancements in S3, S4 and S5 but not S2).

Quality Assurance Measures

Results will be prepared for publication and peer review.

Results

Transgenic Cell Line Study: Figure 2 shows the survival data for the exposure protocols. In all exposure protocols, nickel exposure led to a significantly lower survival than the control. Application of the magnetic field, however, did not significantly reduce the survival relative the nickel only exposure group. It is evident from Figure 2 that the 2 mT magnetic field does not enhance the toxicity of the nickel compound in the R2 cells. Neither does the magnetic field in the absence of the nickel compound inhibit survivorship.

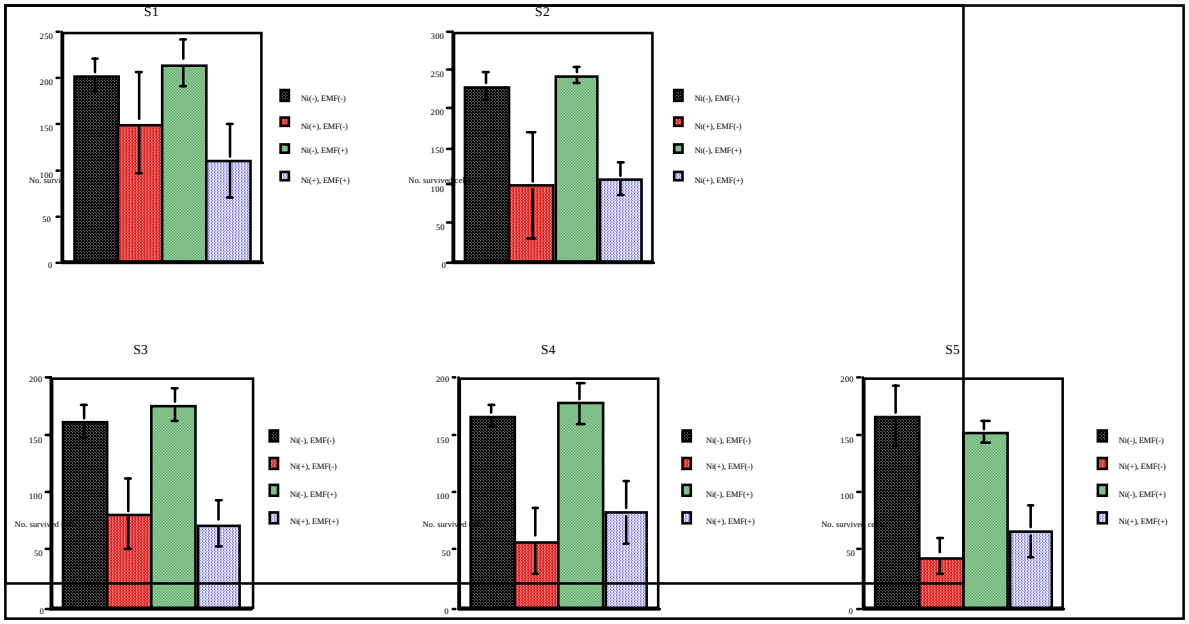

Figure 2

To date we have carried out the exposures for the purposes of mutant frequency determination for S1, S2, S3, S4 and S5. DNA from these exposure groups has been isolated and stored at 4°C.

Animal Studies: Twenty four Big Blue mice have been treated four months as follows in groups of six animals each: controls; EMF exposed 3 mT for 8 hours per day, 5 days for weeks for 12 weeks; dimethylbenzanthacene (DMBA) topically treated animals; and, DMBA and EMF treated animals. The purpose of the last two groups is to test the free radical hypothesis *in vivo*. All animal treatments have been completed. The analysis of the DNA for mutation has been initiated.

Discussion and Contributing to Understanding Biological Effects of EMF

As a necessary first step to mutant frequency comparison between magnetic field exposed and non-exposed cell populations, we have completed a thorough study of the possible effect of a 2mT magnetic field on cell survival. The results indicate that the magnetic field does not enhance the toxicity of $NiCl_2$. Since oxidative damage is not limited to the nucleic acids, but also damages lipids, proteins and other cellular functions, this result could be interpreted as a preliminary indication that a radical stabilization effect, if induced at all by the magnetic field, would be very weak in this system. However, the potential impact of the magnetic field on oxidative mutagenesis in this system will only be known from the determination of mutant frequency in the various exposure groups. This work is in progress.

Publications From This Grant

1. Suri A. "A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor co-mutagenic in the presence of menadione and MNU in a transgenic rat cell line." Mutation Research, 372, 23-31, 1996.
2. Suri A, de Boer J, Kusser W and Glickman B. "A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor co-mutagenic in the presence of trenimon in a transgenic rat cell line." Mutation Research, 372: 23-31 1998.

Other EMF Publications

None

TITLE: **Meta-Analysis of Childhood Cancer Epidemiology**

Principal Investigator: Robert *Goldberg*, Ph.D.
Information Ventures, Inc.
Philadelphia, PA

Health Relevance: Cancer

Research Area: Studies Involving People: Epidemiological Research

PR-702871 **Start Date:** 9/10/97 **End Date:** 5/19/98

Abstract

Epidemiology studies published for the relationship between EMF exposure and childhood cancers vary considerably for such criteria as the exposure assessment strategies used, the cut-off points for exposures, and the number of exposed cases and controls; therefore, simple comparisons of reported results are not possible. This project is to conduct a meta-analysis on the association between exposures to electric and magnetic fields (EMF) and childhood cancer including the examination of dose-response relationships. A major goal of this meta-analysis is the determination of the strength of association between exposure measures (dose) and response for the stronger findings of potential EMF carcinogenicity (e.g., childhood leukemia, central nervous system tumors). This meta-analysis extracted from the available peer-reviewed literature of epidemiological studies on this topic all available data in as detailed a form as possible and combined this information into the appropriate statistical analysis.

Experimental Design and Exposure Conditions

(A) Study Analysis

Statistics that incorporate the individual effect sizes use either of two statistical models: (1) *fixed effects,* and (2) *random effects.* Typically, data are combined into an average, or weighted, effect measure (e.g, odds ratio, regression coefficient) and an estimated standard error for that coefficient. These weights are of two types. One set of weights gives more emphasis to studies that have lower stochastic error in estimating the coefficient, often using the inverse of the variance of the coefficient. This is the fixed effect model. Alternately, it may be assumed that the true risk may vary between studies because of components of variation. The first is the within study variance, and the second is the random study effect incorporating the estimated variance in the true coefficient between studies. Model choice may be based on results of the chi-squared (or Q-test) for heterogeneity which assesses constancy of treatment effects. Once heterogeneity is detected, identifying its sources can be one of the most informative aspects of meta-analysis although some simply report the random effects result.

(B) Influence Analysis

Influence analysis was conducted by recalculating of summary indices or a set of studies leaving out one study at a time, and doing so for each study. It indicates the importance of each individual study in the combined summary statistic to determine whether any of the studies has a dominant effect.

(C) Publication Bias

Publication bias is the differential publication of studies based on their results. If present, one expects that studies with positive results are more likely to be published than ones with null results. One can combine z-scores of individual published studies to assess the sensitivity of the results to publication bias and

determine the number of additional null (unpublished) studies needed to reduce a statistically significant combined effect to non-significance. This number is the so-called Fail Safe N.

Using the combined effect measure, one can assess how large a study would be required to balance the average of reported results if they were due to random fluctuations. We can determine the size of a single study that would be needed to give a null summary statistic (i.e., An odds ratio of 1.0). If that hypothetical study had equal numbers of cases and controls, had an exposure prevalence equal to that observed in reported studies, and had an odds ratio equal to the reciprocal of the reported average. Unlike the Fail Safe N, this calculation uses the size of the effect measure, weights each study result by the inverse of its variance, hypothesizes a study with a protective rather than null effect and seeks a null rather than non-significant combined effect.

Results

The Meta-analysis was conducted by calculated fixed and random effects odds ratios for dichotomous cutpoints, calculated the heterogeneity of the studies, conducted an influence analyses on all of these, and conducted a publication bias assessment. Results are provided for the meta-analysis of studies on leukemia. The first row contains the summary results for all studies together. The first row lists the number of observed and expected exposure cases summed over all studies, the probability of rejecting the null hypothesis for all five studies combined, the fixed effects odds ratio and its 95% confidence interval, the probability that the studies are heterogeneous, and the random effects odds ratio and its 95% confidence interval. This row provides the traditional meta-analysis results.

The table provides a summary of all analyses. Random effects (meta-analyses) results for all the analyses are greater than 1.0, with several statistically significant. The size of a study needed to balance the observed results is extremely large. Types of exposure methodologies (study, column 1) for which there is a insufficient number of studies to conduct a meta-analysis have N/A in the OR random effect column.

Meta-Analysis Summary

Leukemia

Study	Number of Studies included	Exposed Cases	Expected Exposed Cases	Combined p's	OR ? effects	P1 (Q?)	OR random effects	Fail-Safe N	Sample Size Needed
Calculated Fields	5	20	14.23	0.07	1.63 (0.99-2.69)	0.42	1.63 (0.99-2.69)	-	3268
Measured Fields	6	125	104.22	0.05	1.25 (0.95-1.63)	0.10	1.26 (0.80-1.98)	-	2454
Calculated and Measured Fields	10	141	113.34	0.01	1.36 (1.07-1.73	0.16	1.42 (1.00-2.00)	7.19	6153
Wire Codes	5	336	251.93	<0.01	1.32 (1.09-1.61	0.03	1.39(1.00-1.95)	1254	1898
Proximity to Transmission Lines	3	26	24.58	0.07	1.19 (0.74-1.91)	0.04	1.30 (0.54-3.10)	-	959
Proximity to Distribution Lines	2	92	76.48	0.09	1.30 (0.82-2.04)		N/A	-	299
Proximity to Substations	2	10	10.64	0.20	0.81 (0.31-2.10)	0.06	N/A	-	323
Proximity to Transformers	2	25	19.76	0.27	1.29 (0.67-2.49)		N/A	-	338
Proximity to Electrical Facilities	11	375	284.58	<0.01	1.32 (1.10-1.57)	0.06	1.37 (1.05-1.79)	30.29	3433

Brain and Central Nervous System Tumors

Study	Number of Studies included	Exposed Cases	Expected Exposed Cases	Combined p's	OR ? effects	P1 (Q?)	OR random effects	Fail-Safe N	Sample Size Needed
Calculated Fields	4	13	12.64	0.17	1.37 (0.61-2.31)	0.22	1.22 (0.61-2.41)	-	2699
Measured Fields	4	36	23.50	0.10	1.39 (0.62-2.36)	0.99	1.39 (0.82-2.36)	-	612
Calculated and Measured Fields	7	44	31.55	0.06	1.43 (0.96-2.13)	0.31	1.40 (0.89-2.20)	-	3414
Wire Codes	4	193	211.71	0.03	1.02 (0.80-1.32)	0.01	1.24 (0.72-2.15)	-	1106
Proximity to Electrical Facilities	6	208	29.33	0.09	1.00 (0.79-1.25)	0.03	1.11 (0.74-1.67)	-	1790

Discussion

The goal of this project is to conduct a meta-analysis of studies investigating the possible association between exposure to magnetic fields and the incidence (or mortality) of childhood cancer. While several meta-analyses have already been conducted. This report extends these meta-analyses by providing:

1. Analyses including several more recent studies:
2. A more careful and detailed review of the exposure metrics motivating and justifying particular analyses
3. Estimates for fixed effects, random effects, heterogeneity, fail-safe N, and sample size needed, for a variety of exposure classifications:
4. Analyses stratified by various study characteristics to provide preliminary evaluation of possible confounding:
5. Analyses for publication bias, including heterogeneity assessment.

To assess the consistency and comparability of the studies, we reviewed the exposure metrics used in these studies, Comparability was assessed in terms of what the metric assessed (e.g., proximity to lines, observed field), characteristics of the source (e.g., transmission lines, distribution lines and the context in which the exposure was evaluated (e.g., country or region of the country in which wire codes were assessed). From this, we derived four main groupings of exposures: calculated fields, measured fields, wire codes, and proximity to electrical facilities. The latter was further subdivided by source. While several other differences among the exposure metrics were noted (e.g., spot versus 24-hour measurements, peak versus mean exposure), there was not sufficient variation in study design to enable us to form more fine-scaled groupings.

In reviewing the results of our analyses, there are a few interesting observations. First, the homogeneity and trend analysis revealed that most of the studies are homogeneous across exposure categories (within and exposure metric) and do not show statistically significant trends. Exceptions for leukemia are Wertheimer and Leeper (wire codes), Tomenius (spot measures), Feychting (calculated fields), Michaelis (24-hour measures (and for brain tumors are Wertheimer and Leeper (wire codes), Tomenius (spot measures), Savitz (wire codes), Tynes (calculated fields) and Gurney (wire codes). Interpreting this result is complicated by the lack of a clear authoritative exposure metric: each of the exposure categories is based on a physical attribute of the electromagnetic field exposure which cannot be reliably linked with a biological effect and therefore has to be considered a surrogate for exposure rather than a “true” exposure metric. It is possible that there is better reliability for certain metrics in certain geographic areas or with certain subtle variations in the assessment methods, but given the lack of understanding about the biological basis for an effect of electromagnetic field exposure (if any), any adjustment on this basis is purely speculative, In addition, the methods used to assess heterogeneity and trend have low statistical power making these relatively inconsistent results difficult to interpret.

Results from the meta-analyses in which the exposure data are dichotomized (see table) generally are positive and several are statistically significant. In most cases, none of the individual studies are particularly (or disproportionately) influential throughout the body of analyses.

Our analyses also provide little evidence for publication bias. The number of unpublished studies needed to balance those published (the Fail-Safe N) generally were greater then the number published. However, for studies in which the results were not statistically significant, this index is not meaningful. The funnel graph for measured and calculated fields does not show a deficit of studies although that for proximity to electrical facilities shows a deficit of estimates near and below the null value.

Discussion and Contribution to Understanding Biological Effects of EMF

In reaching final conclusions about the interpretation of these studies, one must consider four factors: the number of studies in an analysis, the heterogeneity, the effect size and the sensitive (robustness).

Overall, we see largely positive results with small effect sizes. The results are robust but there is considerable heterogeneity. If one chooses to use the summary estimates for interpretation, they suggest perhaps as much as a 15 to 25 % increase in the childhood leukemia rate and increase no greater than about 10% in the childhood brain

cancer rate for a 0.1 μT increase in magnetic field exposure. The childhood brain cancer data are more limited, less consistent and less compelling than the childhood leukemia data.

Publications From This Grant

1. Wartenberg D, Dietrich F, Goldberg R, Poole C and Savitz D. "A meta-analysis of studies of childhood cancer and residential exposure to magnetic fields." In preparation, 1998.

TITLE: **EMF Effects on Melatonin Hormones and Immunity**

Principal Investigator: Charles *Graham*, Ph.D.
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Health Relevance: Cancer

Research Area: Studies Involving People; Melatonin Production; Immunological Effects

R01-ES07053 **Start Date:** 9/28/94 **End Date:** 8/31/98

Abstract

Research is needed to identify a possible biological mechanism to account for the statistical link found in some epidemiological studies between exposure to magnetic fields and increased cancer risk. Melatonin (MLT), a hormone secreted primarily at night by the pineal gland, has been suggested as a candidate for this link. MLT stimulates the immune system, regulates reproductive hormones, and is reported to be part of the body's natural defenses against cancer. Studies with rodents suggest that nocturnal MLT levels may be suppressed by EMF exposure. Previously, we performed the first double-blind, laboratory-based study of magnetic field effects on nocturnal MLT levels in human volunteers (Graham, *et al*, 1996). Intermittent exposure to a 60 Hz, circularly-polarized magnetic field at 200 mG suppressed nocturnal melatonin levels in men with pre-existing low levels of MLT (low secreters). Low secreters also showed greater suppression of MLT when exposed to bright light at night. This suggested: some individuals may be more sensitive than others to exposure effects; similar mechanisms may be involved in field and light-induced suppression of MLT; and peak MLT level may index individual sensitivity to exposure. While these results support the melatonin hypothesis, they leave many questions unanswered. A key issue is whether exposure effects on MLT in humans are associated with further "downstream" alterations in immune system activity and/or regulation of the reproductive hormones. The purpose of this grant is to perform a series of double-blind exposure studies: (1) to replicate our initial human study, and further define effective exposure characteristics; (2) to determine whether similar effects happen in women; (3) to test the hypothesis that field-related suppression of MLT is associated with alterations in relevant reproductive hormones; and (4) to test the hypothesis that field-related suppression of MLT is associated with changes in specific immune system components. The studies will take account of individual differences in pre-exposure MLT level, and women volunteers will be tested in both the luteal and follicular phases of the menstrual cycle. Outcome measures include: MLT and its major urinary metabolite (aMT6s), the reproductive hormones testosterone, estradiol, and prolactin, selected cytokines (IL2, IFN), and measures of immune system activity and function (T, B and NK-cell counts and function). Information of the type to be gained in these studies is needed to aid in risk assessment, and, if no meaningful effects are found, to alleviate unwarranted public concern.

Experimental Design and Exposure Conditions

Study 1: This initial double-blind study was designed to replicate our previous findings and to evaluate additional exposure conditions and measures. After pre-screening for peak MLT level and light sensitivity, 80 men were randomly assigned to four independent groups. Each man slept overnight (2300-0700 hrs) in our laboratory exposure facilities under one of the following conditions: (1) no exposure sham control; (2) continuous magnetic field exposure; (3) intermittent exposure to a magnetic field with minimal harmonic content; or (4) intermittent exposure to a magnetic field with high harmonic content. Testing was performed with field strength set at 200 mG in all exposure conditions, and the previous pattern of intermittent exposure was used. Serial blood samples were

collected via in-dwelling catheter over the test night and urine samples were collected before and after exposure. Outcome measures included: MLT, testosterone, an immune panel (T-cells CD4, CD8, CD25; B cells, NK cells, monocytes, and CBC with differential), and urinary measures of MLT and its metabolite.

Study 2: This double-blind study was performed to determine if the effects seen in men also occur in women. Twenty-two healthy young women, with regular and predictable menstrual cycles were evaluated in two all night test sessions spaced one month apart (sessions occurred between days 38 of the menstrual cycle). The women were sham exposed in one session, and exposed to the intermittent 200 mG field in the other session. Test order was counterbalanced across subjects. Serial blood samples and urine samples were collected. Outcome measures included: MLT, estradiol, prolactin, cortisol, selected immune measures (CD3, CD4, CD8, CD56), and urinary MLT and aMT6s.

Study 3: This ongoing study will evaluate the reliability of the Study 1 results, and also provide new information about the effects of multiple nights of exposure. A total of 50 healthy young men are being evaluated. Subjects, assigned at random to sham control and intermittent 200 mG exposure test groups, sleep overnight in the lab for up to four consecutive nights. Outcome measures include: MLT, testosterone, an immune panel, and morning urinary measures of MLT and its metabolite.

Study 4: The ongoing study will evaluate the reliability of the results found with women in Study 2. It is designed to reduce the variability found earlier in regard to estradiol. The 24 young women in study, are being evaluated over two consecutive all night test sessions that occur between days 38 of each individual's menstrual cycle. The women are sham exposed in one session. In the other session, they are exposed to the previous intermittent field condition. Test order is counter-balanced across subjects. Outcome measures include: MLT, estradiol, prolactin, selected immune measures, and urinary MLT and aMT6s.

Pilot work is underway for a final study (study 5) to be completed by the end of the year. To date exposure has not altered nocturnal MLT levels. This has not allowed us to test the basic underlying assumption of the melatonin hypothesis: namely, that reduced nocturnal MLT results in increased levels of estradiol and altered immune system. This study directly addressed this issue. Women (n=24) will be tested in groups of four over a continuous 48-hr test session. Half will be in the luteal and half in the follicular phase of the menstrual cycle. Each women will serve as her own control. One night they will sleep in the dark from 11pm to 7 am. Another night, they will be exposed to bright light for 4 hrs. This exposure is sufficient to reduce plasma MLT AUC by approximately 60% and to markedly shift the time of peak MLT concentration. Serial blood samples and 24 hr urines will be obtained for assay of MLT, estrodiol, progestrone, Immune Panel(CD3, CD4, CD8, CD56), Production Of Cytokines (1L1, 2 and 6)By activated lymphocytes, and urinary mT6s.

No human subjects problems have been encountered during performance of the above studies, and the exposure facility has functioned as expected. Project studies are performed in the unique exposure test facility at Midwest Research Institute. This is the only facility in the United States specifically designed for exposure research with human volunteers. Subjects are exposed to uniform (<5%) circularly-polarized, 60 Hz magnetic fields. The magnetic field along each axis is generated by two concentric systems, each consisting of four square coils. Each axis is independently energized. The horizontal field current is shifted from the vertical field current by a phase angle of 90 degrees. Intermittent exposure conditions are created by presenting the magnetic field in alternating 1hr field-on and field-off periods. The field is further switched on and off every 15 sec throughout each of the 1hr field on periods. Switching is accomplished by a zero-crossing technique designed to produce field changes free of transients. During all test sessions, subjects sleep on a cot with their bodies oriented in line with the horizontal component of the magnetic field (north to south). Facility exposure characteristics have been verified by the Department of Energy in support of the RAPID program.

Quality Assurance Measures

In each study, project staff follow a systematic protocol using test instruments with NIST traceability to verify and calibrate exposure characteristics. In each test session, a continuous computer log is maintained to verify that the volunteer is exposed as planned. Chart recordings are maintained of ambient temperature and relative humidity in the facility throughout each session. All studies include a no-exposure sham control condition. All studies are conducted under verified double-blind test conditions to control for the effects of expectations on the part of both

the subjects and the experimenters. Biological assays are performed blind. Data handling operations and decisions are made prior to breaking the double-blind code. Laboratory lighting conditions are verified using a photometer with NIST traceable calibration.

Results

Study 1: No significant field effects on MLT, and no evidence of enhanced sensitivity in low secreters, was found. Compared to sham control, however, field exposure significantly ($p < 0.05$): (1) reduced the typical nocturnal rise in testosterone for low MLT secreters, but not for high MLT secreters; (2) reduced circulating levels of leukocytes and lymphocytes; (3) increased the number of T helper cells; (4) altered the CD4:CD8 ratio; and (5) was associated with a greater decline in circulating NK cells over the night.

Study 2: No field effects on MLT, and no evidence for enhanced sensitivity in low secreters, was found. The range of MLT values (15 - 238 pg/ml) was much greater in woman than men. The number of white blood cells in circulation in the morning was increased when peak nocturnal concentrations of MLT were delayed. This relationship was observed in women both the sham and exposed sessions, and with men in the previous study. Alterations in the CD4/CD8 ratio occurred during exposure, and this effect was primarily attributable to changes in T helper cells. No field effects on estradiol were found when all the women were included in the analysis. Five women, however, had marked differences in estradiol levels at the start of their two test sessions. A significant exposure effect was found when these women were deleted. The data were highly variable, however, and no conclusions could be made regarding exposure effects on estradiol.

Studies 3, 4 and 5: We expect to complete these studies in the near future. No results are available at this time. Additional research was performed to examine the extent to which aMT6s, the major urinary metabolite of MLT, accurately reflects integrated or peak nocturnal blood values of MLT. The urinary assay could be a powerful tool for epidemiological studies. Two factors currently limit widespread application of this technique. It is not known if the relationship between MLT and aMT6s is altered as a person grows older, or if it is similar for women and men. To address these issues, we collected and compared relevant blood and urinary measures. In a sample of men almost four times larger than any previously reported, morning aMT6s levels corrected for creatinine were highly correlated with total nocturnal plasma MLT($r = .78$, $p < .0001$), and with peak nocturnal MLT levels ($r = .72$, $p < .0001$). High versus low nocturnal MLT secretion patterns were well differentiated by morning aMT6s levels alone ($p < .0001$). We also assessed similar data in a sample of 29 women age 40 to 70 yrs, in a study sponsored by the Electric Power Research Institute. Corrected morning aMT6s levels correlated .89 ($p < .0005$) with total nocturnal plasma MLT, and .90 ($p < .0005$) with peak nocturnal MLT levels. Urinary MLT in the morning sample also was a good predictor of nocturnal blood levels of MLT, correlating .84 ($p < .0005$) with total plasma MLT, and .83 ($p < .0005$) with peak value. High versus low nocturnal MLT secretion patterns were well differentiated in the women by morning aMT6s levels alone ($p < .0001$). Nine of the 29 women were on hormone replacement therapy; no significant differences in plasma MLT, urinary MLT, or aMT6s were found between the groups. These results provide the type of laboratory based data needed to estimate the value of including morning melatonin metabolite levels in epidemiological studies.

Discussion and Contribution to Understanding Biological Effects of EMF

Laboratory-based studies aimed at elucidating possible mechanisms by which cancer risk could be increased by EMF exposure are of the highest priority. This project makes an important contribution to this issue by testing research questions derived from one of the most widely known hypotheses concerning the connection between magnetic field exposure and cancer risk. Both MLT-related changes in hormones, and the effects of exposure on immune function are addressed. The existing research base is extended by testing both men and women, by further defining effective field characteristics, and by including replicate studies of any effects observed. Information of the type to be obtained in this project is needed to aid in risk assessment, and, if no meaningful effects are found, to alleviate unwarranted public concern.

Publications From This Grant

1. Graham C and Gibertini M. "Human exposure to magnetic fields: Effects on melatonin, hormones and immunity." In Stevens, *et al* (eds.) The Melatonin Hypothesis: Breast cancer and the use of electric power Battelle Press, 1997.
2. Graham C, Cook MR, Kavet R, Sastre A and Smith K. "Prediction of nocturnal plasma melatonin from morning urinary measures." Journal of Pineal Research 24: 230-238, 1998.
3. Gibertini M, Graham C and Cook MR. "Self report of circadian type reflects the phase of the melatonin rhythm." Biological Psychology In Press, 1998.
4. Graham C and Cook MR. "Human sleep in 60 HZ magnetic fields." Bioelectromagnetics In press, 1998.
5. Doynov P, Cohen HD, Cook MR and Graham C. "Test facility for human exposure to AC and DC magnetic fields." Bioelectromagnetics In press, 1998.
6. Graham C, Cook MR, Sastra TA, Riffle D and Gerkovich MM. "Multiple night exposure to 60 Hz magnetic fields effects on melatonin and its metabolite" J. Pineal Res. Submitted, 1998.

Other EMF Publications

1. Graham C, Cook MR, Riffle DW, Gerkovich M and Cohen HD. "Nocturnal melatonin levels in human volunteers exposed to intermittent 60Hz magnetic fields." Bioelectromagnetics 17: 263-273, 1996.
2. Graham C, Cook MR, Riffle DW, Gerkovich M and Cohen HD. "Human Melatonin During Continuous Magnetic Field Exposure." Bioelectromagnetics 18(2):166-171,1997.
3. Sastre A, Cook MR and Graham CR. " Nocturnal exposure to intermittent 60Hz magnetic fields alters human cardiac rhythm." Bioelectromagnetics 19: 98-106, 1998.

TITLE: **Evaluation of Reported EMF Effects on Cell Communication**

Principal Investigator: Guy David *Griffin*, Ph.D.
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Health Relevance: Cancer

Research Area: Cellular Function; Cell Signaling; Cell Communication

RO3-ES08864 **Start Date:** 4/20/97 **End Date:** 3/31/99

Abstract

The major objective is to replicate studies reported by Benane *et al.* (1996) in which Clone 9 cells, treated with concentrations of chloral hydrate which partially inhibit gap junctional intercellular communication (GJIC), were exposed to power frequency, i.e., extremely-low-frequency (ELF), electromagnetic fields (EMFs). A further reduction in GJIC produced by the ELF-EMF was reported and found to be consistent with the Ion Parametric Resonance (IPR) model, proposed by Blackman *et al*. (1993), which predicts distinct magnetic field interactions with biological systems. Equipment and an expertise in all relevant experimental techniques is already in place to perform these replication studies at Oak Ridge National Laboratory (ORNL).

Chloral hydrate (CH) dose–response studies have been done, and a concentration of 1.5mM CH has been chosen to use in exposure experiments, Fourteen magnetic field exposure experiments have been carried out, plus 6 other experiments that were sham/sham (i.e. no magnetic field). Replicate dishes (triplicate) were exposed in two identical exposure chambers and all experiments were blinded. Sham exposures showed there was no difference between the two exposure chambers. In the 14 exposure experiments, mean GJIC for all exposed dishes was 10.5 $\pm$ 1.9 (std.dev.), and mean GJIC for non-exposed, dishes was 10.8 $\pm$2.1. No consistent effect of magnetic field exposure was observed, whether actual GJIC data was compared, or whether GJIC among different experiments was normalized as a percentage of control (i.e. not treated with CH) dish GJIC. No GJIC effects from magnetic field exposure were observed.

If indeed it is found that CGIC is affected by ELF-EMFs, then this study will provide important data which can be used to begin to mechanistically explain the role of these fields in tumorigenesis.

Experimental Design and Exposure Conditions

The initial experimental design was to closely replicate the experiments in Dr. Blackman's laboratory, in which cell-cell communication in a rat liver cell line was found to be inhibited by an ELF magnetic field in accordance with predictions of the IPR Model. Clone 9 cells were grown in F-12 nutrient medium supplemented with 10% fetal bovine serum and antibiotics. Cells were passaged into 35mm tissue culture dishes and grown to confluency prior to scrape loading experiments. When the cells reach confluency in the dishes, some dishes were treated with chloral hydrate. The concentration of chloral hydrate used must be determined empirically by performing some dose-response studies. Certain chloral hydrate treated dishes and untreated dishes will be exposed to an ELF field. The field exposure conditions used by Blackman were a 45 Hz AC field over a flux density range of 79-499 mGrms with a 366 mG parallel, <2 mG perpendicular DC field over a time period of 30 minutes. Instead of a range of exposure field strengths to test the IPR model (validation of the IPR model is not the purpose of this replication effort), only one exposure condition will be used: 238 mG rms AC, 45Hz with a parallel 366mG DC field for 30 minutes. Blackman has found that ELF field exposure produces an additional inhibition of cell communication beyond that produced by the chemical.

Certain preliminary experiments to characterize the system need to be carried out, such as determining the optimal initial cell seeding density in the 35mm dishes for subsequent maximal cell-cell communication, and determining the dose of chloral hydrate which produces a reasonable inhibition of cell communication without excessive cytotoxic effects. Following establishment of these experimental parameters, dishes will be exposed to ELF fields in the exposure system described below.

The exposure system used very closely mimic the exposure setup used in Blackman's laboratory. Two sets of double wound Helmholtz coils have been prepared for these experiments by Fred Detrich of ERM Co. The double wound configuration allows one of the coils to serve as a control (i.e. no magnetic field) system for the other coil. Current will flow antiparallel in the control coil, thus exposing the cultures to identical heating without producing a magnetic field exposure in this system. Each of the Helmholtz coils is completely enclosed in a mμ metal box, which has appropriate venting to allow circulation of incubator air within the box. The mμ metal boxes serve to shield the cultures within from stray magnetic fields from the incubator itself or the exterior room. The Helmholtz coils are energized by a signal generator (Wavetech model 19 function generator) to produce an AC magnetic field. The strength and frequency of this field can be adjusted as desired. In addition, a DC current supply (Kepco) is used to generate a DC field in the Helmholtz coils per Blackman's conditions. Both Helmholtz coils/mμ metal boxes are located in separate chambers of a standard water-jacketed, 5% CO^2 cell culture incubator (Cellstar model, Queue Systems Inc). There is room to expose four 60 mm dishes or six 35mm dishes in each Helmholtz coil. An additional feature of this exposure system is the ability to completely double blind any experiments as desired. A key-lock control box provides three options: either one of the Helmholtz coils may be activated to produce a field, or both coils may be de-energized to provide a sham/sham exposure. The person doing the experiment simply places the dishes in the exposure chambers, leaves the room, and another individual sets the control box to produce an exposure (or sham/sham) which is unknown to the individual doing the experiment. This is a highly important step to insure a non-biased approach to evaluating the cell communication in individual dishes. If reasonable replication of Blackman's reported effects can be observed in the Helmholtz coils, some exposures may be done using the regional ELF exposure facility at ORNL.

Quality Assurance Measures

There are several separate areas of quality assurance regarding these experiments. First, Clone 9 cells were obtained from ATCC(as specified by Blackman) and checked routinely for presence of mycoplasma contamination. Medium and sera were purchased from the same source used by Blackman (Life Technologies; Gibco BRL). Multiple stocks of low passage cells have been frozen and stored in liquid N_2 so that new cultures may be initiated at regular intervals. Cell cultures were discarded when the passage number reached 30. Second, there are quality assurance checks in regard to the exposure system. A Bartington Flux Gate Magnetometer (Model Mg-0_3MC) has been used to measure the AC and DC fields in the exposure system and to map the uniformity of the magnetic field within the Helmholtz coils where the dishes will be exposed. Measurements using a flux gate magnetometer have demonstrated that the field is uniform ($\pm$ 5% variation) over the region where the dishes are placed. Thermister probes (Omega) are used to monitor incubator temperatures in the Helmholtz coils on a continuous basis. Temperatures within the two Helmholtz coil systems have been adjusted to remain within $<0.2\ ^0$ C of each other.

A Fyrite indicator is used to measure CO_2 concentration within the incubator chambers at the beginning and termination of each exposure. A further quality assurance safeguard against experimental bias is the lock box which enables the experiment to be blinded with regard to the experimentalist. Third, there are quality assurance concerns regarding the overall experimental design for exposure experiments. These relate to how many replications need to be done for valid statistical analysis, how many dishes should be exposed per experiment, how many sham/sham exposures need to be done, etc. in which the various experiments should be performed. Two visits to Dr. Blackman’s laboratory have been made, and all experiments are being carried out in accordance with his overall experimental design in regard to exposures.

Results

Characterization of the GJIC responses of the Clone 9 cells, and the effects of different doses of chloral hydrate on cell-cell communication have been the main foci of experimental work. Characterization of GJIC as a function of

initial seeding density and the effect of serum concentration have been studied. Using either 5 or 10% fetal bovine serum supplemented medium made little difference in the extent of cell communication in confluent Clone 9 cell cultures, nor could overall differences in growth be distinguished. Initial cell seeding density was examined by preparing replicate cultures at an initial seeding innoculum of 1, 2.5, and 5 x 10^5 cells per dish, and growing the cells to confluence. The cultures reached confluence at different times (3 - 5 days after seeding) and cell communication was assessed on the day when confluence was just attained. We found that an initial seeding of 1 x 10^5 cells produced the most extensive GJIC. Usually the dye could be detected in 13 to 14 cell layers away from the scrape line, and this was consistent along the length of the line. Experiments were also carried out to examine the influence of time of incubation with the loading dye (lucifer yellow) on the subsequent analysis of GJIC. Times of 2,5,10, and 15 minutes were evaluated. Incubations of 5 minutes produced more extensive communication than 2 minutes, but no significant difference between 5, 10 or 15 minutes could be observed. An incubation time of 5 minutes has been chosen as standard. We have also evaluated the inhibitory effect of chloral hydrate on cell communication in the Clone 9 cells. Doses of chloral hydrate over the range of 1-5 mM (final concentration) have been used to establish a dose-response relationship regarding the effect of chloral hydrate concentration on GJIC. Good reproducibility with respect to GJIC inhibition in different dose-response studies. A concentration of 1.5mM chloral hydrate was chosen for exposure experiments. This concentration produced GJIC which was 60-70% of control (not treated with chloral hydrate) GJIC.

Discussion and Contribution to Understanding Biological Effects of EMF

The results from this project in contrast to those reported by Blackman, demonstrated no effects of EMF on the parameters studies. Thus, for the biological systems and EMF exposures used in these studies, a biologically relevant and robust effect was not observed. These data do not support the hypothesis EMF exposure can inhibit cell-cell communication.

Publications From This Grant

1. Griffin GD, Dowray V, Miller EJ, Williams W and Gailey, PC. “Effects of magnetic field exposure on gap junctional communication in clone 9 cells treated with chloral nydrate: a replication study.” Bioelectromagnetics, Submitted, 1998.

Other EMF Publications

None

TITLE: **Magnetic Spin Effects in Radical Enzymatic Reactions**

Principal Investigator: Charles B. *Grissom*, Ph.D.
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Health Relevance: Biophysics of Cellular Function

Research Area: Cellular Function; Biophysics Cell/Field; Cell signaling

R01-ES05728 **Start Date:** 4/15/93 **End Date:** 3/31/97

Abstract

The objective of this project was to study the influence of static (DC) magnetic fields on enzymatic reactions with radical (unpaired electron) intermediates in order to understand how environmental magnetic fields might influence biological processes through changes in radical pair recombination. A secondary goal of this work was to develop new tools to study the catalytic mechanism of enzymes with radical intermediates.

Only biochemical reactions that involve more than one unpaired electron are affected by a magnetic field. Most enzymes do not involve radical intermediates and should be unaffected by a change in magnetic field. However, more than 50 enzymes are believed to generate free radical intermediates during catalysis and may be subject to the influence of external magnetic fields.

The Grissom research group has shown that the activity of the B12-dependent enzyme ethanolamine ammonia lyase changes with magnetic field. This is the first magnetic field effect in an enzyme-catalyzed reaction with known radical pair intermediates.

Experimental Design and Exposure Conditions

The effect of an external magnetic field on enzymatic reaction rates can be determined in the same way classical enzyme kinetic parameters are determined. Enzymes with chromogenic substrates or products are followed spectrophotometrically. If no convenient chromophore is available for direct measurement of enzyme rates, then a fixed-time assay with HPLC or radionuclide detection of products or residual substrate is used. We have two electromagnet systems relevant to this work. (System 1): A single-beam UV/Vis spectrophotometer with an electromagnet for thermostatted steady-state kinetic measurements in the range 0-2500 gauss. (System 2): A rapid-scanning stopped-flow spectrophotometer with an electromagnet for rapid kinetic measurements also in the range of 0-6500 gauss. This instrument has some very desirable characteristics for our work with co-balamins and heme enzymes; it acquires an absorbence spectrum at a rate of 1,000 Hz with photomultiplier sensitivity. This is accomplished with a scanning wheel and double monochromator design that is unique to the OLIS, Inc. instrument. This system is equipped with an electromagnet and gaussmeter.

Quality Assurance Measures

The spectrophotometers used in these experiments are shielded from the electromagnet, and we have tested these systems for adventitious magnetic field effects by looking for the influence of the magnetic field on enzymes without radical pair intermediates. No magnetic field effect was observed on these "control" enzymes.

Furthermore, our magnetic field effect results with B12 ethanolamine ammonia lyase have been repeated and verified by Professor Ruma Banerjee in the Department of Biological Chemistry at the University of Nebraska. A

manuscript detailing this replication has been published [Taoka S, Padmakumar P, Grissom C.B, Banerjee R "Variable magnetic field effects on coenzyme B12 Dependent enzymes and validation of the magnetic field dependence of ethanolamine ammonia lyase." Bioelectromagnetics 18, 506-513, 1997]. Additional theoretical support for the effect of magnetic fields on enzymatic reactions, specifically ethanolamine ammonia lyase, has been provided by Walleczek and Eichwald .

Results

Magnetic field effects on radical pair recombination rates tend to exhibit "window" effects, such that higher fields do not always produce correspondingly larger effects. Because the fields are static, a simple laboratory electromagnet is adequate to achieve stable magnetic fields up to 10,000 gauss. The effect of a magnetic field on an enzymatic reaction can be observed by a change in the steady-state kinetic parameters, as well as by a change in the stopped-flow kinetic parameters (two different measurement techniques and two different instruments). The same magnetic field effect can be seen on the rate of photolysis of the vitamin B12 cofactor (without enzyme).

The kinetic parameter Vmax/Km (where Km is the Miachelis constant) for the coenzyme B12 dependent enzyme ethanolamine ammonia lyase is decreased 25 percent by a static magnetic field near 1000 gauss with unlabeled ethanolamine, and decreased 60 percent near 1500 gauss with perdeuterated ethanolamine. This effect is caused by a magnetic field induced change in the rate of intersystem crossing between the singlet and triplet electron spin states in the cob(II)alamin:5'deoxyadenosyl radical spin-correlated radical pair. This result has been independently verified and reproduced by another laboratory. Furthermore, the magnetic field dependence of ethanolamine ammonia lyase can be simulated by existing models of magnetic field dependent recombination in tightly held radical pairs.

The rate of another B12 enzyme, methylmalonyl CoA mutase (from humans), is not altered by a magnetic field, in spite of many similarities between ethanolamine ammonia lyase and methylmalonyl CoA mutase.

Discussion and Contribution to Understanding Biological Effects of EMF

The theory that describes magnetic field effects in chemical reactions had not been combined with the common biochemical formalisms of enzyme kinetics to describe the conditions under which an enzyme might be magnetic field dependent. Experimental data from our laboratory and other research groups now confirm the hypothesis that enzymatic reactions with radical pair intermediates should be susceptible to the effects of a DC magnetic field.

The fields at which these effects have been demonstrated are 10 gauss and above and care should be exercised when extrapolating to lower magnetic field strengths that are relevant to environmental exposures.

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Other EMF Publications

None.

TITLE: **Magnetic Field Dependence of Human Enzymes at Weak Fields**

Principal Investigator: Charles B. *Grissom*, Ph.D.
University of Utah,
Salt Lake City, Utah

Health Relevance: Biophysics of Cellular Function

Research Area: Cellular Function; Biophysics Cell/Field; Cell signaling

R03-ES08896 **Start Date:** 1/15/97 **End Date:** 12/31/98

Abstract

The objective of this work is to study the influence of weak DC magnetic fields (0-10 Gauss) on several enzymatic reactions that are important in human biochemistry. The heme enzymes nitric oxide synthase, cytochrome P450, and cytochrome C peroxidase, as well as the B12 dependent enzyme methionine synthase, are reasonable candidates for the site of interaction of 0-10 Gauss magnetic fields with biological systems. A magnetic field induced change in the level of nitric oxide, (as produced by nitric oxide synthase), could alter intracellular calcium levels and thereby change calcium efflux rates. This would provide the first causal link between molecular magnetic field effects and a cellular or physiological endpoint of magnetic field exposure.

In recent work, we have shown that the redox cycle of the heme-containing enzyme, horseradish peroxidase, is altered by a magnetic field as weak as 10 Gauss. The biphasic magnetic field dependence coincides exactly with predictions of the semi-classical model of magnetic field dependent radical pair recombination.

This work may be an important contribution towards a complete understanding of the possible health effects of environmental magnetic field exposure. In all cases, the postulated mechanism of interaction is through changes in radical pair recombination, where one radical species is a metal ion (iron in heme, or cobalt in B12).

Experimental Design and Exposure Conditions

The effect of an external magnetic field on enzymatic reaction rates can be determined in the same way classical enzyme kinetic parameters are determined. Enzymes with chromogenic substrates or products can be followed spectrophotometrically. If no convenient chromophore is available for direct measurement of enzyme rates, then a fixed-time assay with HPLC or radionuclide detection of products or residual substrate can be used. We have two electromagnet systems relevant to this work.

System 1): A single beam UV/Vis spectrophotometer with an electromagnet for thermo-statted steady-state kinetic measurements in the range 0-2500 Gauss.

System 2): A rapid-scanning stopped-flow spectrophotometer with an electromagnet for rapid kinetic measurements also in the range of 0-6500 Gauss. This instrument has some very desirable characteristics for our work with co-balamins and heme enzymes: it acquires an absorbance spectrum at a rate of 1,000 Hz with photomultiplier sensitivity. This is accomplished with a scanning wheel and double monochromator design that is unique to the OLIS, Inc. instrument. This system is equipped with an electromagnet and gaussmeter.

Quality Assurance Measures

The spectrophotometers used in these experiments are shielded from the electromagnet, and we have tested these systems for adventitious magnetic field effects by looking for the influence of the magnetic field on enzymes without radical pair intermediates. No magnetic field effect was observed on these "control" enzymes.

Results

For the first time, kinetic magnetic field effects have been used to study the redox cycling of the paramagnetic intermediates in a heme-containing enzyme. In the horseradish peroxidase (HRP) reaction, the rate of conversion of compound I (HRPI) to compound II (HRPII) decreases by 15% at 750 Gauss, and the rate of conversion of compound II to native HRP decreases by 35% at 750 Gauss. This is consistent with the reversible formation of a triplet radical pair (rp) in the single electron transfer from diamagnetic substrate to high-spin heme.

Stopped-flow kinetic traces were collected in a random order of magnetic field strengths, but always with a corresponding 0 magnetic field control trace that was collected immediately before or after the non-zero magnetic field trace. This procedure allows the precise comparison of kinetic traces as relative rates. Figure 1 shows the magnetic field dependence of the relative rate constants, k1 and k2 (Scheme I). Both k1 and k2 are net rate constants that include any possible reversal of these steps. Typical values of k1 and k2 at 0 magnetic field are about 6 and 20 s1, respectively. The standard error reported for each data point reflects an average value obtained from multiple measurements at each magnetic field, but collected in a random order.

At 10 Gauss, a slight increase in k1 and k2 is observed, followed by a substantial decrease in k1 and k2. At higher magnetic field strength, k1 and k2 return to the values observed at 0 Gauss. The same magnetic field dependence of k1 and k2 was observed in two replications of the data set reported herein.

Multiple paramagnetic species enter into the HRP catalytic cycle, including the porphyrin radical cation, native HRP [Fe(III)], HRPI / HRPII [Fe(IV)], and the two substrate radicals. A combination of any two can constitute a paramagnetic (radical) pair that can undergo spin-selective processes, wherein at least one reaction pathway is spin-forbidden.

Identification of a unique magneto-sensitive step is not yet possible, but the most likely candidate occurs in the electron transfer complex formed between HRPII and substrate. The transfer of one electron from substrate to HRPII regenerates native HRP and, according to the rules of total electron spin retention, should produce the triplet RP highspin HRPII, Fe(IV), S=1) that partitions between formation of native HRP and recombination to regenerate HRPII. In this unusual case, the singlet RP is unreactive towards RP recombination because it would lead to formation of low-spin Fe(IV) and loss of conservation of total angular momentum. The sign of the calculated magnetic field dependence of RP recombination agrees with a scheme in which the triplet RP is formed initially, and only the triplet RP can undergo recombination.

This observation has profound implications for the role of electron spin in all reactions that involve high-spin metal ions. When a diamagnetic substrate donates an electron to a high-spin paramagnetic acceptor, total electron spin will be conserved and reverse electron transfer will be limited to the triplet spin state. Intersystem crossing to the singlet spin state creates a species that is unreactive towards reverse electron transfer.

Discussion and Contribution to Understanding Biological Effects of EMF

Our work is relevant to the broader question of whether biologically significant magnetic field effects exist. A better understanding of magnetic field effects in enzymatic systems will benefit the biological EMF community as it collectively seeks to answer the more difficult question of whether these effects are significant to human health. Our laboratory will continue to relate our work to this important health, social, and political issue. We will continue to publish significant "negative" findings of magnetic field effects in enzymatic reactions, as well as our "positive" results. Our laboratory will continue to serve as a resource to those scientists in the biological EMF community who are interested in magnetic field effects on biological radical pair reactions.

In a very exciting development, we have shown that the heme enzyme horseradish peroxidase exhibits a magnetic field dependence in the range 0-1000 Gauss, with a prominent low-field feature at 10 Gauss. This opens the door

to our future study of magnetic field effects at environmentally relevant fields of 0-10 Gauss, where our calculations predict other heme enzymes, such as cytochrome P450, cytochrome C peroxidase, and nitric oxide synthase, may also be magnetic field dependent. If the activity of cytochrome P450 is altered by a magnetic field, this may be a possible mechanism for tumor promotion by magnetic fields.

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Other EMF Publications

None.

TITLE: **EM Fields and Co-Promotion of Cancer**

Principal Investigator: Reba *Goodman*, Ph.D.
Columbia University

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Tumor Promotion

RO3-ES08867 **Start Date:** 01/10/97 **End Date:** 01/09/99

Abstract

The linking of cancer to environmental electromagnetic fields (EMFs) is supported by persistent, though by no means conclusive, evidence. This is borne out by epidemiological studies, where exposure assessment is usually based on time-weighted averages. This classification ignores the realities of human exposures, which are intermittent and transient. Recent animal and cell studies point to intermittency in EM field exposure as increasing bioresponse levels, particularly in conjunction with a chemical carcinogen, suggesting its role in promotion/progression.

We propose to investigate EM fields as a possible co-promoter under conditions of intermittent exposures during the suppression and induction of neoplastic transformation. The INITC3H/10T1/2 mouse fibroblast cells provide an ideal *in vitro* model for such a study. They have been carcinogenically transformed by methylcholanthrene, but the expression of the neoplastic phenotype is suppressed indefinitely by the presence of retinoic acetate (RAC) in the culture medium. Upon withdrawal of RAC, neoplastic expression is observable in two to three weeks (manifested phenotypically as the loss of contact inhibition). This system, with documented relevance to the cancer process, allows us to test our hypothesis that a more pronounced response in gene expression occurs in cells exposed intermittently to EMF than in cells exposed continuously.

We will evaluate the effects of exposure intermittency during suppression and induction of neoplastic transformation at both the phenotypic level and the level of gene expression in INITC3H/10T1/2 cells. Specifically, changes in the expression of the tumor suppressor p53 and the oncogene *c-myc* at the transcriptional level and their upstream molecular sequelae, will be determined under both continuous and intermittent EMF exposures conditions. The transcription factor requirements for expression of *c- myc* and p53 during induction and suppression of neoplastic expression will be determined by using a combination of reporter and electrophoretic mobility shift assays (including competition assays and supershift assays). These data will determine the dose response metrics in the cellular response to intermittent exposures, indicate whether EM field co-promotion occurs through the same documented pathways as do chemical tumor promoters, and contribute to a better understanding of the risk associated with different environmental signals.

Experimental Design and Exposure Conditions

A. Phase I. Suppression of neoplastic state

- Establish base-line response (including thresholds) for messenger RNA levels and rates of transcription p53 and *c-myc* in INITC3H10T1/2 plus RAC for continuous exposure.
- exposure times: 4-60 minutes; 1-7 hours
- field strengths:800uT to 0.8uT

- Establish base-line response (including thresholds) for messenger RNA levels and rates of transcription p53 and *c-myc* in INITC3H10T1/2 plus RAC for intermittent exposure.

- exposure 20 mins to 800uT, 80uT, 8uT or 0.8uT then -take samples for RNA at 40 and 60 mins; 2, 3, 5 hours.
- Re-stimulation with different field strength at time points during decrease in p53/myc transcript levels -changes in p53 and /or *c-myc* are determined, then field-responsive regions of the promoters for p53 and myc will be sought using specific constructs containing deletion mutations or site specific mutagenesis with a CAT expression vector.
- EMSAs will identify regions within p53 and *c-myc* promoters that bind potential transcription factors. Oligonucleotide competition studies will identify these factors. In some cases binding sites for transcription factors are deleted and the effect of the deletions on promoter activity determined.
- EM field-effects on sequence-specific DNA binding proteins/ transcription factors AP1, AP2, AP3, OCT1, CTF/NF1, GRE, CREB, SP1, and NF-kappaB will be determined. Specificity of the DNA-binding protein for the putative binding site is established by competition experiments using natural or synthetic DNAs or oligonucleotides corresponding to the binding sites of the same and other DNA binding proteins.
- The sequence of the EM field-sensitive regions will be identified and compared with each other to determine whether similar or identical DNA sequences are EM field targets.
- phase I will take 6 months.

B. Phase II. Full transformation (determined by phenotypic changes in cell morphology indicating loss of contact inhibited state) Establish base-line response (and thresholds) for messenger RNA levels and rates of transcription p53 and *c-myc* in INITC3H10T1/2 minus RAC for continuous exposure.

- Exposure times: 4-60 minutes; 1-7 hours
- field strengths:800uT to 0.8uT
- establish base-line response (and thresholds) for messenger RNA levels and rates of transcription p53 and *c-myc* in INITC3H10T1/2 minus RAC for intermittent exposure.
- exposures 20 mins to 800uT, 80uT, 8uT or 0.8uT then take samples at 40 and 60 mins; 2, 3, 5 hours.
- Re-stimulation with different field strength at time points during decrease in p53/myc transcript levels
- If change in p53 and/or myc are determined at this point during suppression of transformation, we will proceed as in Phase I.
- Phase II will take 6 months

Having established baseline data, and (hopefully) thresholds for effects, we are now ready to examine the effect of intermittent exposures on the transition from suppression to full transformation.

C. Phase III. Effect of short intermittent exposures during induction of transformation: days 2-21+ (i.e., period from initial removal of RAC to total transformation as determined by photomicroscopic examination of phenotypic transformation from contact-inhibited to tumorigenic state) Experiments will be performed on days 1, 7, 14, and 21 after removal of RAC.

- Messenger RNA levels and rates of transcription p53 and *c-myc* in INITC3H10T1/2 minus RAC for intermittent exposure.
- Thresholds are expected to be defined by Phases I and II. Therefore, the Phase III protocol will use field strengths within threshold limits for stimulation and re-stimulation. First exposure will be 20 minutes, initially.
- Exposure 20 mins to 800uT, 80uT, 8uT or 0.8uT then take samples for RNA at 40 and 60 mins; 2, 3, 5 hours.
- Re-stimulation with different field strength at time points during decrease in p53/myc transcript levels .
- If changes in p53 and/or myc are determined during suppression of transformation, we will proceed as in (1) through (4) above in Phases I and II.
- Phase III will take 12 months.

Quality Assurance Measures

Sham/sham controls: The adequacy of controls is vital and sham/sham experiments are included routinely. The coils are double wound so that, depending on the direction of current flow, either an exposure field or sham field can be created. When the two windings are attached so that the current flows in opposite directions in each winding, then the field produced by each winding will cancel the field produced by the other winding. The net result of this cancellation is that no field is produced, yet the same current is flowing through the coils, producing identical heating.

Statistical analyses: In every experiment a sufficient number of separate exposures will be performed to assure statistical significance. Data are entered in QuatroPro for analysis. The results are examined with a two-tailed t-test to test the hypothesis that the ratio of the exposed samples over the control samples is equal to unity. Statistical significance using the multifactor analysis of variance program (INSTAT) is also used.

Data management: Each experiment is repeated a minimum of three times (i.e., three separate experiments). Each sample (from each experiment) is tested three times by different technical personnel. Samples are coded. The technical staff do not know which sample is experimental and which is control. The code is not broken until data have been analyzed. There is an exposure blinding system utilizing dual switches.

Results

Results (Feb. 1, 1997 to December 1, 1997):
INIT/C3H/10T1/2 cells. Exposed to 8uT, 60Hz.Western blot analyses of hsp70.

- Intermittent magnetic field stimulation during suppression (plus retinoic acetate; + RAC) and induction of neoplastic transformation (withdrawal of retinoic acetate; -RAC) on levels of stress response hsp70
- Base-line response/ thresholds for stress response protein hsp70kDa levels and rates of synthesis plus RAC for continuous exposure.
- Base-line response/ thresholds for stress response protein hsp70kDa plus RAC for intermittent exposure during suppression of neoplastic state.
- Base-line response/ thresholds) for stress response protein hsp70kDa minus RAC for continuous exposure during full transformation to neoplasia.
- Base-line response (and thresholds) for hsp70kDa levels and rates of synthesis minus RAC for intermittent exposure during full transformation to neoplasia.
- Activation of the transcription factor AP-1 (a fos/jun protein) is enhanced during transformation and this enhanced AP-1 activation is increased by magnetic field stimulation.

We have established:

- Dose-response and time-response parameters to define the effect of intermittency (as compared with continuous exposure) on the suppression and induction of neoplastic transformation.
- The base-line response and thresholds for stress response protein hsp70kDa levels and rates of synthesis in INITC3H10T1/2 plus RAC for continuous exposure during suppression of neoplastic state and during full transformation to neoplastic state.
- The base-line response and thresholds for stress response protein hsp70kDa plus RAC for intermittent exposure during suppression of neoplastic state and during full transformation.
- Activation of the transcription factor AP-1 (a fos/jun protein) is enhanced during transformation and this enhanced AP-1 activation is increased by magnetic field stimulation.

We are investigating whether magnetic fields are a possible co-promoter in the development of neoplasia using INITC3H/10T1/2 mouse fibroblast cells. The exposure conditions for these studies involve short intermittent exposures during the suppression and induction of neoplastic transformation. These cells provide an ideal *in vitro* model for such a study; they have been carcinogenically transformed by methylcholanthrene, but the expression of the neoplastic phenotype is suppressed indefinitely by the presence of retinoic acetate (RAC) in the culture

medium. Upon withdrawal of RAC, neoplastic expression is observable in 8-10 days (manifested phenotypically as the loss of contact inhibition).

Although we have only been engaged in this project for ten months, our data already show that cells undergoing suppression or induction of neoplastic transformation display a more pronounced response in gene expression to intermittent magnetic field exposures than to continuous exposure. A significant feature of our data is the demonstration that exposure to magnetic fields exacerbates the stress response as cells go from the "normal" to "neoplastic" cellular condition and that the transcription factor AP-1 is activated in those cells exposed to magnetic field during the transformation process. The linking of cancer to environmental electromagnetic fields is supported by persistent, though by no means conclusive, evidence. This is borne out by epidemiological studies, whose greatest challenge is the classification of these exposures. Exposure assessment in such studies are, mostly, based on time-weighted averages. This classification, however, ignores the realities of human exposures, which are intermittent and transient.

Publications From This Grant

None.

Other EMF Publications

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10. Goodman R and Blank M. "Non-thermal low-energy agent that induces stress response proteins: magnetic fields." Cell Stress & Chaperones, In press. 1998.

TITLE: **Brain Tumors in Mice Exposed to 60 Hz Magnetic Fields**

Principal Investigator: Theodore J. *Hahn, M.D.*
VA Medical Center
Los Angeles, California

Health Relevance: Cancer

Research Area: Studies Involving Whole Animals; Neoplastic Transformation; Tumor/Promotion

RO3-ES08895 **Start Date:** 1/18/97 **End Date:** 12/31/98

Abstract

Recently published data suggest that electric/or magnetic field (EMF) exposure may be associated with an increased incidence of neoplasia, particularly lymphoma and brain cancer. However, to date most of the evidence linking EMF exposure to increased brain cancer incidence has been derived from epidemiological studies, which are limited by the presence of multiple unidentified confounding factors. This study tests the hypothesis that continuous exposure to MF can induce or promote tumor incidence in mice, either independent of, or in association with, fractionated ionizing radiation exposure. Brain tissue will be utilized from a large-scale lifetime study of the effects of continuous exposure of C57BL/6 female mice to 14.1 Gauss (G) circularly polarized 60 Hz MF having 10 G horizontal and vertical components. In addition, mice will receive four levels of total ionizing radiation dose; 0 Gray (Gy), 3.5 Gy, 4.75 Gy, 6.0 Gy. The hemotoxylin and eosin stained brain tissue sections will be evaluated at three levels; optic chiasma, mammilary body, and pons. The time-dependent incidence of gliosos, astrocytoma, oligodendroglioma, mixed glioma, anaplastic glioma, meningioma, ependymoma, choroid plexus tumor and lipoma will be evaluated in mice which received continuous exposure to MF versus unexposed animals. Results will determine whether there is a significant effect of MF on the promotion or induction of brain tumors in mice.

Experimental Design and Exposure Conditions

The experimental design consists of the 8 cells in the 2x4 contingency table shown below. This design is the basis for the statistical analysis for the effect of MF on radiation induced and spontaneous brain cancer.

		Ionizing Radiation (R) Cobalt-60			
		0	**350**	**475**	**600**
Magnetic	0	190*	190	190	190
Field (G)	10	380	380	380	380

***No. of mice in treatment group**

Brain slides are also being read from an additional group of 380 mice which served as room controls, and received neither ionizing radiation or MF exposure. The hematoxylin and eosin stained brain tissue from each animal will be evaluated at 3 NTP specified levels: 1) at the optic chiasma, 2) at the caudal border of the mammilary body, 3) 2 mm caudal to the transverse fibers of the pons. Diagnosis and classification of toxic changes and tumor type will be made according to the standardized system of Morphologic and Diagnostic Criteria for Toxicologic Pathology which summarizes the diagnostic criteria for brain tumors in rodents. The following primary brain proliferative lesions will be documented: gliosis, astrocytoma [benign (B) or malignant (M)], oligodendroglioma (B or M), mixed glioma (B or M), anaplastic glioma (M), meningioma (B), meningiosarcoma (M), lipoma (B), ependymoma

(B or M), choroid plexus tumor (B or M). The following secondary brain proliferative lesions will be documented: carcinoma, not otherwise specified (NOS), adenocarcinoma, NOS, sarcoma, NOS.

Mice were exposed to 14.1 G (10 G horizontal and vertical components) MF for 18 hours/day beginning at 4 weeks of age, and continuing until death or terminal sacrifice at 29 months of age. Because they produce more uniform and average higher induced currents in experimental animals, circularly polarized MF were selected. Ten G is the approximate maximum practical exposure level achievable under current engineering designs for exposure systems. It is well below the acute toxic level for rodents. In addition rodents require a ten-to-twenty-fold greater MF exposure than that required by humans to induce a similar current density within the body. Each exposure module consisted of a specially built non-magnetic cage support structure surrounded by MF generating coils, which generated both vertical and horizontal fields. The uniformity and magnitude of MF within the energized modules were within 11% of the set value of 10 G for both the horizontal and vertical components. Harmonic distortion was <2%. Special switching circuitry eliminated transients during the "turning on" and "turning off" procedures.

Quality Assurance Measures

Quality Assurance activity associated with study thus far have included:
Review and approval of protocol
Review and approval of Standard Operating Procedures
Review and approval of forms to be used for recording data
Inspection/review of slide receipt.

Results

No increase in Brain Tumors or pre-neoplastic lesions was found in exposed mice compared to controls

Discussion and Contribution to Understanding Biological Effects of EMF

The results from this study will help suggest that MF do not promote or induce brain tumors in mice. The results of the study are expected to be published in 1999.

Publications From This Grant

Manuscript in preparation

Other EMF Publications

None.

TITLE: **Pathology Support for EMF Animal Studies**

Principal Investigator: Jerry *Hardisty*, DVM
Experimental Pathology Laboratory,
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Animal Studies; Brain Cancer; Leukemia

NO1-ES35369 **Start Date:** 1/1/95 **End Date:** 12/31/98

Abstract

The major objective was to provide quality assurance pathology review for major EMF animal studies including a review of the pathology specimens and a review of the pathology diagnoses to assure that the pathology criteria meet international standards.

The pathology review was conducted on four studies. These included the standard bioassay of up to 20 G fields in female F344 rats conducted at the University of Quebec, an initiation/promotion study of brain cancer in F344 rats conducted at the University of Quebec, an Electric Power Research Institute study of leukemia in C57 Black mice conducted at the University of California at Los Angeles (UCLA) and a brain cancer study in C57 Black mice conducted at UCLA. The review involved a quality assurance (QA) evaluation of the pathology specimens to assure that there was documentation between the histological slides, the paraffin blocks and the animal records. A QA pathologist then reviewed all of the neoplasms diagnosed in the study and 20% or more of the tissues for which there was no tumor diagnoses. When discrepancies were found, these were reviewed together with the study pathologist. At the end of this procedure, a Pathology Working Group (PWG) consisting of 6 to 10 experts was convened to review representative cases and cases where differences of opinion existed between study and QA pathologist. This was done in a blinded fashion with participation of both study and QA pathologists. When the majority PWG opinion differed from the study pathologist, the diagnosis was changed to reflect this opinion. Thus, the final study diagnoses reflect the opinions of the PWG, the study and QA pathologists. In all cases, only minor data discrepancies were found and resolved. Similarly there were only minor changes in the diagnostic terminology but now all four studies are consistent with international standards for the rodent pathology terminology and criteria.

Experimental Study Design and Exposure Conditions

In the study of Mandeville at the University of Quebec, groups of 50 female Fischer 344/N rats were exposed to 20 mG, 200 mG, 2 G, or 20 G magnetic fields. The pathology review focused on breast cancer, leukemia and brain cancer since the study pathologist did not find increased cancer rates at any site. The pathology review confirmed this finding and this was supported by the PWG review. This study has now been published (Mandeville *et al*, 1997).

In a second study at the University of Quebec, groups of pregnant female rats were exposed to Ethyl Nitrosourea (ENU) at day 18 of gestation or saline. Groups of 50 female offspring from these groups were subsequently exposed to 20 mG, 200 mG, 2 G, or 20 G magnetic fields. The pathology review focused on brain cancer since ENU increases cancer at this site. The study pathology pathologist found that magnetic field exposure did not alter the time of onset or incidence of brain cancer following ENU. The pathology review confirmed this finding and this was supported by the PWG review. This study has now been submitted for publication (Mandeville *et al*, 1999).

In a study of possible lymphoma promotion by magnetic fields, C57 Black mice were exposed to 0, 350, 475 or 600 R of ionizing radiation followed by subsequent exposure to 14.1 G circularly polarized magnetic fields or to sham exposure. There were 190 mice at each level of radiation that were sham exposed and 380 mice at each level of radiation that were subsequently exposed to the 14.1 G magnetic fields. The mice were allowed to live until the appearance of lymphoma with the remaining mice killed at 30 months. The study pathologist used the Pattengale classification to separate the mouse lymphomas into the various subtypes including the B and T cell lymphomas. The QA pathologists reviewed all the cases of lymphoma (more than 1500 mice) plus in the remaining mice, lymphoid organs were examined including thymus, spleen. lymph nodes. The liver was also examined since this is an early site for lymphoma involvement. The study pathologist had done a remarkable job of this complex classification and there were few diagnostic discrepancies. A PWG with expertise in mouse lymphomas was convened to review representative cases for each lymphoma type and to review diagnostic differences. The final study diagnoses represent the opinions of the study pathologist, the QA pathologist and the PWG. This study has now been submitted for publication (Babbitt *et al*, 1999).

In the study of possible lymphoma promotion by magnetic fields, the brains were also examined from the C57 Black mice that were exposed to 0, 350, 475 or 600 R of ionizing radiation followed by subsequent exposure to 14.1 G circularly polarized magnetic fields or to sham exposure. The study pathologist reviewed the brain looking for glial tumors and evidence of glial proliferative lesions. The QA pathologist reviewed all of the brains with glial tumors, with glial proliferations and 20% of the brains for which no glial proliferative diagnoses was made. There were few diagnostic discrepancies. A PWG with expertise in mouse brain tumors was convened to review all cases of brain tumors and glial proliferations. Very few brain tumors were found and there was no association with magnetic field exposure. The final study diagnoses represent the opinions of the study pathologist, the QA pathologist and the PWG. This study is now being prepared for publication (Babbitt and Kharazi, 1999).

Results

This pathology review assured that 4 major animal studies meet international standards and should provide greater acceptance of the results. In all four studies, there was no alteration in the pattern, incidence or onset of cancer with magnetic field exposure.

Discussion and Contribution to Understanding Biological Effects of EMF

The results from the studies subjected to pathology review demonstrated no effects of EMF on cancer in standard and initiation/promotion animal studies. These data do not support the hypothesis EMF exposure promotes the cancer process.

Publications From Studies Reviewed by the Pathology Contract

1. Mandeville R, Franco E, Sidrac-Ghali S, Paris-Nadon L, Rocheleau N, Mercier G, Desy M, and Gaboury L Evaluation of the potential carcinogenicity of 60 Hz linear sinusoidal continuous-wave magnetic fields in Fischer F344 rats. FASEB Journal 11, 1127-1136, 1997.
2. Mandeville R, Franco E, Sidrac-Ghali S, Paris-Nadon L, Rocheleau N, Mercier G, Desy M, Devaux C, and Gaboury L "Evaluation of the potential promoting effect of 60 Hz magnetic fields on N-ethyl-N-nitrosourea induced neurogenic tumors in female Fischer F344 rats. Submitted 1999.
3. Babbitt JT, Kharazi AI, Taylor JMG, Rafferty CN and Hahn TJM, Leukemia/Lymphoma in mice exposed to 60-Hz magnetic fields. Results of a chronic exposure study. Submitted, 1999.
4. Babbitt JT, Kharazi AI, Taylor JMG, Rafferty CN and Hahn TJM, Glial tumors in mice exposed to 60-Hz magnetic fields. Results of a chronic exposure study. In preparation, 1999.

Other EMF Publications

None

TITLE: **Alterations in GAP-43 mRNA as a Marker for Developmental Changes in the Cortical Hemispheres and Cerebellum Following Perinatal Exposure to Environmental Agents (EMF)**

Principal Investigator: *Jean Harry*, Ph.D.
Laboratory of Toxicology, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Neurobehavioral Dysfunction

Research Area: Whole Animals Toxicology, Reproductive Teratology, Neural Development

Z01-ES21164 **Start Date:** 9/96 **End Date:** 9/97

Abstract

This developmental period is highly sensitive to environmental insults. This project addresses the possible effects of electromagnetic fields (EMF) during the neonatal period. Studies in our laboratory have demonstrated that exposure to a developmental neurotoxicant can result in a shift in the developmental pattern for mRNAs associated with the outgrowth of the neuronal axon. During development, the axon of the neuron elongates to form connections (synapses) with various target sites. This process is guided by the axonal growth cone at the leading edge of the axon and growth associated protein-43 (GAP-43) is enriched at the tip of the growing axon at the cytosolic surface of the growth cone membrane. Alterations in this protein during critical periods of development could result in altered axonal elongation and subsequent connectivity of the neural network. Previous experiments suggested that exposure to EMFs during gestation and early development altered the levels of this mRNA at one particular time in rat brain development, postnatal day 3. This pilot study suggested that the developmental pattern for GAP-43 mRNA in the cerebellum of the brain may be altered in EMF exposed animals. The subsequent study has attempted to replicate the initial findings of the pilot study and to extend the time and regions of the brain for examination. Groups of rats were exposed gestationally and during early postnatal development to various types of EMFs, the study is still ongoing and the sample code has not been revealed. As to date, we have examined the levels of mRNA for GAP-43 in the cerebral hemisphere of rats from each exposure group and no changes in the levels or pattern have been observed. Samples are currently being analyzed from the cerebellum, a postnatally developing site that previously showed an increase in GAP-43 mRNA levels. In addition, the mRNA levels for various brain associated growth factors are being analyzed from the same samples. The previous pilot study was limited in number of animals, exposure conditions, sample collection, and age of examination. This replication study included multiple time points and additional growth associated endpoints in order to determine the reproductive validity of the data from the previous study and to determine the biological relevance of the findings.

Experimental Design and Exposure Conditions

Pregnant rats were exposed to EMF for 18.5 hours per day or intermittently (1hr on 1hr off for 18.5 hours) during their entire pregnancies and during the postnatal period using the continuous breeding protocol at IIT Research Institute. The exposure conditions were control, 20 mG, 2G, and 10G continuous exposure, 10G intermittent exposure. Brain tissue was collected from postnatal day 1, 3, 6, and 9, coded, and shipped on dry ice to NIEHS.

RNA was isolated from the cerebrum and the cerebellum at each time point, subjected to Northern blot analysis and probed with radio-labeled cDNA for GAP-43 and Actin. The amount of radioactivity in each band

representing mRNA for GAP-43 or Actin was determined. Data was expressed as a ratio of GAP-43 to Actin mRNA. Total RNA samples from each region and each time was analyzed by Rnase protection assay for various brain associated growth factors.

Quality Assurance Measures

The identity of the exposure conditions has remained unknown throughout these experiments. For each time point, 6 samples per exposure condition were isolated together, run on the same gel, and hybridized together to minimize variability. Exposure QA measures are associated with the ITI Research Institute.

Results

The data collection is currently in progress therefore the code of the samples has not been broken with regard to exposure condition. However, the data on mRNA levels for GAP-43 in the cerebrum of male rats is showing no differences between the 5 exposure groups across the developmental ages. Initial examination of growth factor mRNA levels also shows no differences between any of the 5 exposure groups with regard to the developmental of the cerebrum. Data is being analyzed for the cerebrum of the females and for the cerebellum of both males and females with regard to GAP-43 and the experiments are underway to examine the brain associated growth factors mRNA levels in the cerebellum.

Discussion and Contribution to Understanding Biological Effects of EMF

To replicate previous findings of an elevation in GAP-43 mRNA at one age in the cerebellum and to determine a shift in the profile of alteration during development and to validate the biological significance by examining other developmentally associated events.

Publications From This Grant

Manuscript being reviewed prior to submission

Other EMF Publications

None.

TITLE: **EMF Environmental Carcinogens and Co-Mutagenicity**

Principal Investigator: Tom K. *Hei*, Ph.D.
Columbia University
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Health Relevance: Cancer

Research Area: Cellular Function, Gene Expression, Tumor/promotion

RO3-ES08926 **Start Date:** 01/10/97 **End Date:** 01/09/99

Abstract

Accurate risk assessment for human exposure to ELF-EM has been hampered by marginal epidemiological data and the lack of corroborating experimental data. Studies designed to identify a link between cancer induction and exposure to MF have resulted in a large body of conflicting results over the years. There is recent evidence to suggest that ELF-MF may function as a tumor promoter, and as a co-mutagen with radiation and chemical carcinogens in *in vitro* studies. Preliminary studies by the applicant have shown a 2-3 fold enhancement in the mutagenicity of the direct alkylating agent, MNNG, by magnetic fields using a well-defined, highly sensitive mutation assay system. The present project proposes to extend these findings by examining, in double blind designed studies, the combined mutagenic effects of ELF-MF with either gamma-rays or benzo(a)pyrene at both the cellular and molecular levels using a well-characterized ELF-EM exposure device. Specifically, the quantitative mutagenic data will be used in assessing the mode of interaction between ELF-MF and the other two known human carcinogens. The types of mutants generated will be used to ascertain qualitatively the presence or absence of specific signature changes at the gene level. The MC-2XC concentric coil *in vitro* exposure system that meets all the current system design requirements of the National Institute of Environmental Health Sciences (NIEHS) will be used. Mutations will be scored at both the SI and HGPRT loci using the human-hamster hybrid AL cells. By using specific DNA probes of other genes that have been regionally mapped to various sites on chromosome 11, the molecular basis for mutations in the AL cells will also be examined. Because of the demonstrated sensitivity of the AL mutation system, it would be possible to examine the mutagenic/co-mutagenic effects of ELF-MF in conjunction with low doses of radiation or chemicals that would be impractical to detect in human epidemiological surveys, or indeed in most experimental animal systems.

Experimental Design and Exposure Conditions

The MC-2XC concentric coil *in vitro* exposure system designed and manufactured by Columbia Magnetic Incorporation were used in the study. This exposure device circumvents a number of problems commonly found in other EMF exposure systems such as uniformity of field stray field containment and the inclusion of sham exposure area. Briefly the MC-2XC system powered at 50-60 Hz by use of a variable transformer utilizes two Merritt 4 coil sets arranged in a concentric configuration to limit stray fields. Exposure chambers are connected to a commercial incubator that is held remote so that the required cell condition is maintained without interfering with the magnetic fields. One chamber is designed for sham exposures and therefore the system contains an active compensation device to cancel weak magnetic fields while remote exposure chambers are 0.1 degrees Celsius with magnetic field uniformity better than 5%. The dosimetry of the ELF-MF was determined using (1) a Magnetic Sciences International gaussmeter with a range of .1mG to 200G and an accuracy of 2% of reading and (2) a Walker Scientific Hall effect gaussmeter. This is a general purpose instrument capable of both AC and DC measurement and has a range over three scales of 100mG to 19.99kG. These instrument were also used to map the exposure to

ambient stray fields to which cells may be exposed during preparation and enroute from and to the exposure facility.

Cell Culture and Mutagenesis Assay: The dose response survival and mutation induction by graded doses of gamma-rays either alone or in combination with a magnetic field were examined using the human-hamster hybrid cells. The hybrid cells which contain a standard set of CHO-K1 chromosomes and a single copy of human chromosomes 11, express several cell-surface markers that render the cells sensitive to killing by different specific monoclonal and polyclonial antibodies in the presence of complement. Cells are maintained in Ham F-12 medium supplemented with 8% heat-inactivated fetal bovine serum, and 2X normal glycine. Exponentially growing cells planted in 60mm diameter tissue culture plastic dishes were irradiated with either a 1.5 or 3.0 Gy dose of rays from a Cesiun irradiator at an absorbed dose rate of 1.8 Gy/min. After irradiation cultures were exposed to a magnetic field for periods ranging from 24 hours to 7 days. After treatment cultures were trypsinized off the dishes and subcultured for both survival and magnetic assays.

Quality Assurance Measures

Magnetic fields are monitored routinely in the irradiation chamber utilizing a magnetometer probe manufactured by Walker Scientific, Worcester, MA. The probe has a rms of SYMBOL 177 \f "Symbol" 0.3% for the mean gauss value and a resolution of SYMBOL 177 \f "Symbol" 1% for the peak magnetic field. Incubators have been surveyed for background gradients of AC magnetic fields (usually ranging from a few milligauss to 1 gauss). The laboratory housing the Quad4x exposure system as well as the passage way to the tissue preparation laboratory have been surveyed and found to have an acceptably low stray magnetic field flux. In addition, the tissue preparation laboratory has been surveyed and found to have negligible stray fields.

When performing sham exposures, the toggle switch is set to "sham" causing the energized twisted bifilary wires in series opposing coils to nullify the induction of magnetic fields. Equal currents flow in both exposed and sham coils to provide identical exposure conditions (but no magnetic field) so that double-blinding can occur in order to minimize experimenter bias and sensory queues to the animals. The physicist involved with this study sets the irradiator to either energize or produce a null field so the biologist and laboratory technician do not know if the embryos are being exposed to magnetic fields or treated to a sham irradiation.

Results

Exposure of A cells to a magnetic field alone for a period up to 7 days was non-cytotoxic and non-mutagenic. While cells irradiated with graded doses of rays alone demonstrated a survival level and frequencies of mutation consistent with previously obtained data, concurrent treatment with magnetic fields did not enhance the cytoxicity nor mutagenicity of irradiation. An analysis of the spectrum of mutants generated from the various treatments can provide useful information on the molecular mechanisms involved in mutagenesis.

Discussion and Contribution to Understanding Biological Effects of EMF

Accurate risk assessment for human exposure to magnetic field has been hampered by marginal epidemiology data and the lack of combining experimental data. Studies designed to identify a link between cancer induction and exposure to magnetic fields have resulted in a large body of conflicting results over the years.

Publications From This Grant

1. Ansari R and Hei TK. “Mutagenicity of environmental EMF with other genotoxic agents in mammalian cells.” Submitted, 1998.

Other EMF Publications

None.

TITLE: **Does Exposure to EM Fields Cause Cancer?**

Principal Investigator: Ann S. *Henderson*, Ph.D.
Hunter College-CUNY,
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Health Relevance: Cancer

Research Area: Cellular function; Gene expression; Signal transduction

R01-ES05735 **Start Date:** 5/21/91 **End Date:** 4/30/97

Abstract

This research is designed to determine if a relationship exists between exposure to electric and magnetic fields (EMF) at 60 Hz and cancer. It was previously proposed that some form of transduction process at the cell surface/membrane is altered by EMF exposure. We hypothesize that this change is reflected in regulation of transcription at the DNA level. The proposed mechanism is of particular importance in light of the effects ascribed to EMF exposure, since a cascade of events could result from activation of a particular signal transduction pathway. EMF exposure causes changes at the cell surface that could affect receptor binding or activation (Luben and others). Other evidence (Adey, Liburdy, Blackman and others) for membrane involvement comes from studies of changes in Ca^{+2} flux in cells exposed to EMF. Thus, the existing data is in agreement with proposed mechanisms that EMF effects are initiated at the cell surface, leading to signal transduction events that could ultimately affect transcription.

Our goal is to obtain specific, measurable experimental endpoints that will allow us to determine whether EMF exposure is an initiating factor in neoplastic transformation and/or if exposure can mimic characteristics of the "second or other step" counterpart in the multiple independent events leading to neoplastic disease. The specific question is whether characteristics of neoplastic transformation and/or immortalization occur in cells exposed to extremely low frequency (elf) EMF at 60 Hz under environmentally significant conditions.

The specific goals of this research project are:

- Aim 1. To determine in cultured human cells exposed to defined EMF: (a) if the expression of cellular proto-oncogenes is altered; (b) if changes in transcription are present in all cell types; i.e., those derived from both neoplastic and "normal" cells, the time course of the response and if it consistent with that observed for known promoting factors, such as TPA.
- Aim 2. To investigate the role of signal transduction in cell response to EMF; can a relation with signal transduction (and the cell membrane) be made as predicted on the basis of calcium flux studies?

Experimental Design and Exposure Conditions

Cultured human HL60 cells at a density of 1 x 10^6 cells/ml are exposed to a 60 Hz EMF at 60, 600 or 6000 mG (rms) for 20 minutes. Biochemical and analytical procedures follow Interagency Agreement YOI-ES-40272 for protocols for extraction of RNA, dot blots and northern hybridizations. Tests of RNA integrity following extractions is also described in this document. Dot blots, northern hybridizations, and RT-PCR are used for measurement of transcript levels in each experimental set.

The cultured cells are exposed to EMF using Helmholtz coils, as employed in earlier experiments. The coils were made using 164 turn rectangular windings of gauge 19 magnet wire measuring approximately 13 cm x 14 cm with 8 cm spacing. The coils are 1 cm in diameter wound around an approximate square form. They are positioned with

their central axis horizontal; samples are placed on a Plexiglas stand in the horizontal plane in an area shown to have a uniform magnetic field and maximum field strength. Exposures take place in a mμ metal can. We are currently using a Double Blind Helmholtz Coil Exposure System designed by Electric Research and Management, Inc, (ERM). All features of our previous system were incorporated into the new design, such as coil dimensions, wire size, number of turns, coil form dimensions, coil form material, shielding enclosure dimensions and shielding enclosure material. This provides two functional units for simultaneous sham exposures. There are dual switches for selecting module controls; one set is inside a locked cabinet.

Single continuous exposures ranging from 2 minutes to 1 hour are carried out at a magnetic field intensities of 60 or 600 mG at 60 Hz with usual conditions equaling 20 minutes exposure with no post-exposure time. Harmonic content is sinusoidal; polarization is linear. The DC magnetic field is 82 mG 12.7^{o} [100 vertical; 0 horizontal]. The X component of DC fields is parallel to the AC field. The cells are shielded in a mμ metal container during exposure. The horizontal DC magnetic field in the container is less than 20 x 10^{7} tesla (measurement by M. Misakian from NIST). Fields are turned on 30 minutes prior to exposure; and the cells removed rapidly before turning off the field. Square 5 x 5 cm (T25) flasks are used for exposures. The bottom of the flask is 2 cm below the axis level. The height from flask bottom to top surface of liquid is about 1.1 cm; the height of the liquid is 0.6 cm. The cells are in suspension culture, but rest on the bottom of the flasks; the concentration is about 1 x 10^{6} cells / ml. The cells are maintained in T75 flasks. A magnetic field survey in all portions of the laboratory is routinely done. The area around the exposure apparatus and preparation bench are monitored before each experiment (control and experimental samples remain in the same flasks and are treated identically until exposure). The field inside the mμ metal container is 0.8 mG. Ambient static magnetic fields inside the mμ metal can at its normal position are: Horizontal (eastwest): 3.2 mG; Horizontal (northsouth): 4.7 mG; Vertical: ~ 10.5 mG; Total: 1112.9 mG (measurements by F. Dietrich, from ERM). No vibration or warming of coils is detectable.

Quality Assurance Measures

Control and experimental cells are taken from the same flask about one hour prior to exposures. The cells are exposed in a mμ metal container; non-exposed controls are held in a mμ metal container within a separate compartment of the same incubator; sham-exposed cells are placed under identical conditions as exposed cells. There is no apparent difference in results from "sham" exposed cells and cells held in a separate incubator compartment with no exposure. Each experiment series is repeated a minimum of 3 times; most are repeated more than 5 times. Three different assays are currently done for each transcript level measured: "dot blots", northern hybridizations and RT-PCR. For hybridization experiments done since 1993, the internal control is 2-microglobulin (2M). In Northern blots, 2M is hybridized coordinately with the transcript of interest and the results standardized relative to the amount of 2M; in RT-PCR, multiplexing is used (with both primers).

Experiments are performed in a small laboratory which is removed from possible sources of extraneous signals (elevator, NMR, X-ray diffraction, heavy equipment, etc.). The laboratory and equipment are monitored routinely by outside consultants. A map has been made of all possible sources of extraneous fields in the area of the exposure apparatus. The field inside the incubator used for exposures has been relatively constant at about 2 mG over a 5 year period of monitoring. A water-jacketed incubator is used.

A research associate is in charge of maintaining quality control for biological experiments, i.e., that appropriate procedures are followed, that coding takes place, reagents are standardized, all buffers sterile, etc. The laboratory is monitored before each experiment using a METEX digital multimeter. Temperature is monitored using a Physitemp thermocouple probe that is sensitive to 0.1^{o}C.

Results

RNA transcripts (steady state levels) in HL60 cells are increased in the presence of 60 Hz G) continuous wave sinusoidal fields. These experiments used hybridization methods for detection. In more recent experiments, we used quantitative RT-PCR (multiplexing with 2 microglobulin primers) to confirm increases in steady state levels of transcripts for c-*myc* and c-*fos* in HL60 cells exposed to 60 Hz EMF (60 mG) for 20 minutes. The response to EMF is rapid. Four minutes is the earliest time point after exposure that an increase in *c-myc* or *c-fos* transcripts can be measured. At the field strengths used in most of our experiments (60 or 600 mG), the increase in *c-myc*

transcripts peaks at 20 minutes. Following 20 minute exposures, removal of cells at various time points shows that the effect is diminished with time. There may be overlapping pathways between heat shock and exposure to EMF. Although hsp70 mRNA is increased (as possibly other heat shock proteins on the basis of 2D gel electrophoresis) in the presence of EMF, the reaction is not heat shock *per se*.

The cell membrane is important in the response to EMF. Treatment of cells with colchicine disturbs microtubules and decreases plasma membrane fluctuations. Cytochalasin treatment influences microfilament structure and increases local membrane fluctuations. *C-fos* transcript levels are decreased in the presence of colchicine (+EMF), but enhanced in cytochalasin B (+EMF). We conclude that there is a correlation between the amplitude of plasma membrane fluctuations and the response of the cell to EMF.

Reduction or absence of extracellular calcium negates the effect of EMF on transcript levels. The effect of EMF on steady state levels of *c-fos* and *c-myc* is suppressed when HL60 cells are placed under conditions of calcium reduction or the absence of calcium (EGTA).

Calcium channel blockers negate the effect of EMF. Calcium flux through transmembrane calcium channels was blocked 10 minutes prior to EMF exposure by the use of dependent calcium channel blocker, Verapamil (100 M).The steady state level of *c-myc* mRNA was increased after EMF exposure, but the effect was blocked by adding Verapamil 10 minutes before exposure. B_2-microglobulin gene expression was used as an internal control.

CAT expression is increased in HeLa B2- cells exposed to EMF following transfection of portions of the *c-myc* upstream control elements linked to a CAT reporter gene . *C-myc* upstream regulatory regions were transfected into both mouse (with integration) and human (transient) cells as CAT constructs. The presence of the 2-3 kb domain upstream of the human gene results in increased expression of CAT following 20 minute exposure of HeLa cells to 60 Hz EMF (60 mG). The transfection of a similar region from mouse *c-myc* regulatory DNA showed increased expression in mouse myeloma cells (PX3) [the maximum increase was at a B field of 600 mG]. Specific portions of the promoter region were deleted from the human promoter construct and introduced into cells to determine the responsive region to EMF. The region is less than 800 bp and is located between 353 and 1257 relative to the P1 promoter. Experiments in progress are designed to identify a more precise region.

Various portions of upstream regulatory regions of human c-*fos* (0 to 700 bp) were linked to CAT constructs as a reporter gene. Maximum expression (~+25%) is seen within 20 minutes of exposure using the complete construct, but the activity drops rapidly. Deletion analysis of the upstream regulatory DNA narrowed the responsive region to be the AP1 site. Fos protein is phosphorylated at serine residues. Other studies have shown that phosphorylation causes the protein, in conjugation with Jun, to down regulate its own promoter. Preliminary results, using immunoprecipitation, show that phosphorylation of the *c-fos* protein is increased in cells exposed to EM fields for 20 minutes. Protein was measured one hour after exposure, the point at which the protein was shown to peak. The increase in phosphorylation was about 30%. These studies suggest that EMF can enhance and down regulate the *c-fos* gene in a manner which is analogous to other growth factors.

Discussion and Contribution to Understanding Biological Effects of EMF

The possibility of a negative health effect from EMF exposure was initially based on epidemiological studies that linked EMF to increased cancer incidence. The presence of extenuating factors, however, often makes such studies difficult to interpret, and the evidence for EMF association with cancer has been inconsistent. The conclusion is that other avenues must be sought to confirm or negate the proposed correlation between EMF exposure and cancer or any other outcome. It is highly unlikely that EMF exposure could initiate tumor development. This suggests that, if EMF exposure plays a role in tumorigenesis, it must promote a process that is already initiated. One feasible means of promotion is via growth related pathways, the approach taken in the present studies. Research supports the hypothesis that EMF exposure can affect cell division and there is persuasive experimental evidence that EMF exposure can be related to growth. Thus, a feasible working hypothesis is that some form of transduction process at the cell surface is altered by EMFs, and that this change is reflected in cell regulation. If this is the case, the normal counterparts of oncogenes could be subject to disregulation in the presence of EMF. If this is true, proof will lie in the time frame of expression of interrelated transcripts, particularly those in the "early response" category. The presence of increased transcript levels in cells exposed to EMFs has been reported by this and other laboratories. We have observed a low, but significant, increase in steady state transcript levels of both *c-fos* and *c-*

myc following 60 Hertz (60 mG) EMF exposures within the expected time frames. The most compelling evidence for the validity of increased steady state transcripts of oncogenes is that the effect can be abolished by inhibition of transduction pathways proposed as important in field-cellular interactions. Our experiments have shown this to be the case.

Publications From This Grant

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2. Goodman R, Chizmadzhev Y and Henderson A. "Electromagnetic fields and cells." J. Cellular Biochemistry 52: 16, 1993.
3. Broude N, Karabakhtsian R, Shalts N, Goodman R, and Henderson A. "Correlation between the amplitude of plasma fluctuations and the response of cells to electric and magnetic (EM) fields." Bioelect. Bioenerget. 31: 19-23, 1994.
4. Lin H, Goodman R, and Henderson A. "Specific region of the *c-myc* promoter is responsive to electric and magnetic fields." J. Cell. Biochemistry 55:18, 1994.
5. Goodman R, Blank M, Lin H, Khorkova O, Soo L, Weisbrot L and Henderson A. "Increased levels of hsp70 transcripts induced when cells are exposed to low frequency electromagnetic fields." Bioelectrochem. Bioenerget.33: 115-120, 1994.
6. Gold S, Goodman R and Henderson A. "Exposure of human cells to electric and magnetic fields results in increased levels of Tantigen mRNA and protein." Bioelectromagnetics 15: 329-336, 1994.
7. Karabakhtsian R, Broud N, Shalts N, Kochlatyi S, Goodman R and Henderson A. "Calcium is necessary in the cell response to EM fields." FEBS LETT. 349: 16, 1994.
8. Rao S and Henderson A. "Regulation of *c-fos* is affected by electromagnetic fields." J. Cell Biochem. 63: 358-365, 1996.
9. Kochlaty S, Shalts N and Henderson A. "Short exposures to 60 Hz electromagnetic fields alters steady state transcript levels in HL60 cells."Resubmitted to Rad. Res., 1998.
10. Tao Q, Mii A and Henderson A. "Can EMF exposure emulate a tumor promoter? Designing endpoints." Electricity and Magnetism in Biology and Medicine, Bersani, F, Editor. Plenum Press, New York, in press, 1998.
11. Tao Q and Henderson A. "EMF induces differentiation in HL-60 cells." J.Cell. Biochem., in press, 1998.

Other EMF Publications

None.

TITLE: **The Effect of 60 Hz EMF on Tyrosine Phosphorylation**

Principal Investigator: Jau-Shyong *Hong*, Ph.D.
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Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Cellular Function, Cellular Processes, Signal Transduction

Z01-ES90083 **Start Date:** 10/93 **End Date:** 09/97

Abstract

Biological and epidemiological studies have suggested that low-frequency electromagnetic fields (EMF) may adversely affect human health. However, the mode of action of EMF on cells is largely unknown. On the other hand, DC electric fields have long been known to be an effective stimulus in cell activation. For example, DC fields cause polarized cell movement, neurite outgrowth and clustering of integral membrane proteins. We found recently that some of its effect may be mediated by the activation of receptor tyrosine kinases (see publication). These kinases are normally activated by binding of specific ligands such as peptide growth factors. It now appears that DC electric fields can activate these kinases directly in the absence of ligands. We are now interested in applying this new concept to study the effect of EMF in cell activation. As a first step, we plan to examine the effect of 60 Hz EMF on cultured human epidermoid carcinoma A431 cells. These cells have an abundance of receptors for epidermal growth factor (EGF) on their surface. The EGF receptor is a prototype of the receptor for tyrosine kinase, which is normally activated by EGF. Upon ligand binding, these receptors become dimerized and tyrosine becomes phosphorylated. By probing the Western blot of the lysate of A431 cells with an antibody to phosphotyrosine, we are able to demonstrate the tyrosine phosphorylation of the EGF receptor. Control cultures not treated with ligand showed a very low level of tyrosine phosphorylation. We are now in a position to test whether EMF can mimic the ligand causing tyrosine phosphorylation of EGF receptors in A431 cells. Since tyrosine phosphorylation of receptor tyrosine kinases is an integral step involved in stimulating cell proliferation and transformation, these studies may shed light on the molecular mechanisms of EMF-mediated cellular responses.

Experimental Design and Exposure Conditions

A431 cells are grown in tissue culture flasks. Before exposure, the cells are serum-starved to avoid the activation of receptor tyrosine kinases by factors in the serum. The cultures are exposed to EMF in a modified tissue culture incubator equipped with a double-turn Helmholtz coil which is 44.2 cm in diameter and is wound with 0.82 mm wire. The two coils are connected in series and are placed with their planes perpendicular to the ground. The culture flasks are placed with their bottom parallel to the axis of the coil. The AC current is delivered by a Wavetek function generator. After EMF exposure for a duration ranging from 0.5 to 8 hours, the cultures are processed for Western blots to probe the tyrosine phosphorylation of the EGF receptor.

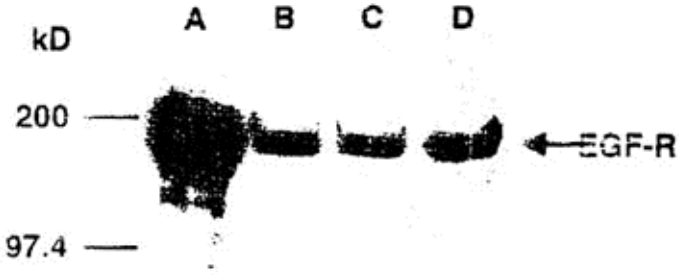
Quality Assurance Measures

Two identical incubator setups are available for this study. Thus, a test and a control exposure can be conducted in parallel. These setups were assembled and calibrated by the US EPA. Since cells are placed in enclosed, grounded incubators, stray fields are avoided.

Results

Accomplishments: We have employed cultured human epidermoid carcinoma A431 cells as a model to examine the effects of 60 Hz EMFs on receptor tyrosine kinases. These cells have an abundance of receptors for epidermal growth factor (EGF) on their surface. Upon ligand binding, these receptors dimerize and autophosphorylate tyrosines. By probing Western blots of the lysates of A431 cells with an antibody to phosphotyrosine, we were able to demonstrate the tyrosine phosphorylation of the EGF receptor with an apparent molecular weight around 170 kDa (Figure 1). Two series of experiments were conducted to examine the effects of 60 Hz EMFs on EGF receptor tyrosine phosphorylation in A431 cells. Initially, cells were exposed to a field strength of 1 gauss (using the EMF generator in Dr. C. Rinehart•s laboratory at UNC) for 30, 60, 90, and 120 minutes with corresponding controls which were placed in the incubator with no field strength. Results indicated that the phosphorylation patterns were the same between the EMF exposed and the control groups. This experiment was repeated twice with similar results (Figure 3 & 4). The second series of experiments was to extend the EMFs exposure to longer periods of 2, 4, 6, and 8 hours. Results from two independent experiments showed no effect of EMFs exposure (Figures 5 & 6). To further confirm these negative results, we have conducted the same exposure using the setup in Dr. C. Blackman•s laboratory at EPA, and the results were similar to those obtained at UNC (Figures 7 & 8). Thus, we conclude that unlike DC electrical fields, EMFs fail to affect receptor tyrosine kinases in A431 cells. Currently, we are studying the effects of EMFs exposure on the phosphorylation of NGF receptors in PC12 cells since it was reported that EMFs exposure prevents NGF-induced neurite outgrowth in this cell line.

Exposure in Dr. Bill Koch's setup (Dept. of Cell Biology and Anatomy, (UNC-CH) at a strength of 10 mGauss for 8 hours.



A A431 phosphorylated membrane
B EMF-exposed
C EMF control
D A431 control

Figure 2.

Exposure in Dr. Cliff Rinehart's Setup (Lineberger Cancer Center, UNC-CH) at a field strength of 1 Gauss.

Exposure in Dr. Cliff Rinehart's setup (Lineberger Cancer Center, (UNC-CH) at a field strength of 1 Gauss.

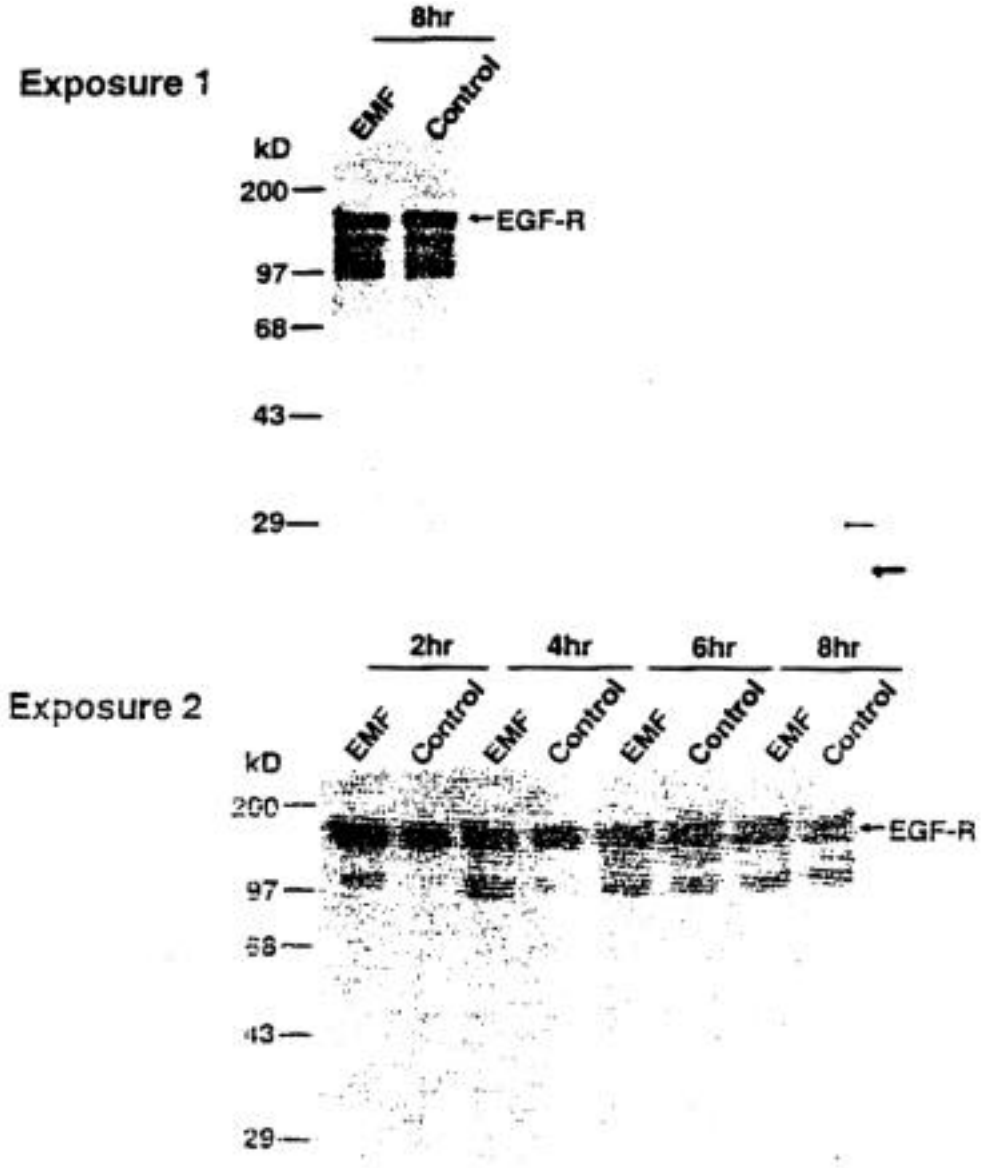
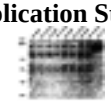


Figure 5.

Exposure in Dr. Cliff Reiehart's Setup (Lineberger Cancer Center, UNC-CH,) at a Field Strenght of 1 Gauss.

(Replication Study)



Exposure in Dr. Carl Blackman's Setup (EPA) at a field strength of 0.3 Gauss.

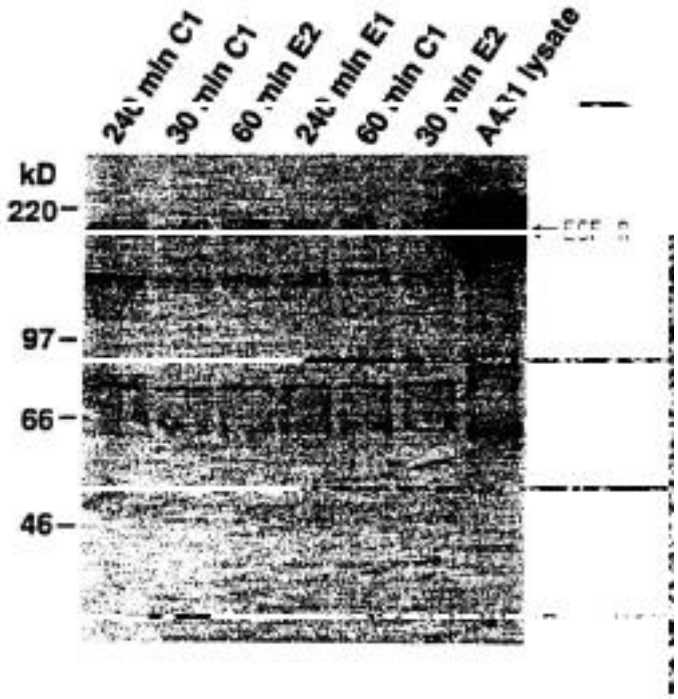


Figure 7.

Discussion and Contribution to Understanding Biological Effects of EMF

Biological and epidemiological studies have suggested that low frequency EMFs may adversely affect human health. However, the mode of action of EMFs at the cellular level is largely unknown; particularly, the potential effects on signal transduction mechanisms on the cell surface receptors have not been reported. This project was the first attempt to elucidate whether EMFs may affect certain cellular functions by acting on EGF receptor phosphorylation. The results obtained from experiments conducted in two different setups (UNC, Chapel Hill and EPA) showed consistent lack of effect of EMF at different exposure intensities and time periods on the tyrosine phosphorylation of A431 cells. Since tyrosine phosphorylation of certain growth receptors is a crucial step for carcinogenesis, the lack of effect of EMF on EGF receptor phosphorylation do not support the hypothesis that EMF cause cancer through this signal transduction step. Our results are consistent with the recent reports which generally show lack of effects of EMF on carcinogenesis.

Publications From This Grant

None.

Other EMF Publications

1. Peng HB, Baker LP, and Dai Z. "A role of tyrosine phosphorylation in the formation of acetylcholine receptor clusters induced by electric fields in cultured *Xenopus* muscle cells". J. Cell Biol. 120: 197-204, 1993.

Other EMF Publications

1. Peng HB, Baker LP, and Dai Z. "A role of tyrosine phosphorylation in the formation of acetylcholine receptor clusters induced by electric fields in cultured *Xenopus* muscle cells". J. Cell Biol. 120: 197-204, 1993.

TITLE: **Environmental Risk Factors and Breast Cancer in the NHS**

Principal Investigator: David J. *Hunter*, Sc.D., M.P.H.
Channing Laboratory, Brigham and Women's Hospital
Boston, Massachusetts

Health Relevance: Cancer

Research Area: Studies with People; Epidemiology Research; Dietary Factors

U01-CA62984 **Start Date:** 9/30/93 **End Date:** 9/29/98

Abstract

Research into potential environmental causes of breast cancer has been stimulated by reports of an increased incidence of the disease over time both in developed and developing countries. To date it has been extremely difficult to separate these temporal trends from issues related to better diagnostic techniques or more judicious, or at least more frequent, use of screening techniques. More recently concern has been expressed in regard to specific environmental agents because of regional differences in breast cancer incidence and mortality rates.

This is an epidemiological project designed to address a number of hypotheses related to environmental risks for breast cancer:

Specific Aim. Are DDE (bis[4dichlorophenyl]1,1dichloroethane)/PCB (polychlorinated biphenyl compounds) levels related to breast cancer in a nested case-control study?
Specific Aim. Are regional differences in breast cancer rates explained by known breast cancer risk factors, or variations in diet or alcohol consumption?
Specific Aim. Does regular overnight use of an electric blanket increase risk of breast cancer?
Specific Aim. Are levels of 2,5-hydroxy vitamin D related to breast cancer in a nested case-control study?

Experimental Design and Exposure Conditions

This study takes advantage of both the available data in an ongoing well-characterized prospective cohort of middle-aged women, as well as biological indicators of exposure to assess environmental risk factors for breast cancer in the Nurses' Health Study. The Nurses' Health Study was begun in 1976 with the establishment of a cohort of 120,700 women aged 30-55 and was principally designed to assess risk factors for breast cancer. Cohort members initially resided in 11 geographically dispersed states representing the Northeast (Massachusetts, Connecticut, New York, New Jersey, Pennsylvania), Midwest (Ohio, Michigan), the West (California) and the South (Florida, Maryland, Texas); although the majority of participants still reside in the original 11 states, members of the cohort currently live in all 50 states. These women report on exposures and disease outcomes every two years and followup in the cohort has been maintained at a level of better than 95% through 1990.

To accomplish the specific aims we have utilized prospective data from the Nurses cohort, and perform nested case-control studies based in the cohort of 33,000 women from whom we obtained a blood sample in 1989-90. We analyzed levels of DDE and PCBs among 240 women who developed breast cancer after giving us a blood sample and before June 1 1992, and compared these levels among 240 controls matched on year of birth and month of blood return. From June 1 1992 through June 1 1994 an additional 144 cases of breast cancer were diagnosed among the women who provided blood samples. Analyses of those cases and their matched controls are nearing completion. Similarly, we are analyzing these specimens for 2,5-hydroxy vitamin D, the major circulating metabolite of vitamin D.

In 1992 we inquired about electric blanket use. Use of electric blankets is an important source of exposure to EMF and it has been implicated in breast cancer etiology in a previous case-control study. We will study this exposure prospectively. In two years of follow-up, 464 cases of breast cancer occurred among the 78,564 women who answered the question about electric blanket use and were cancer-free in 1992.

We studied regional variations in the incidence and mortality of breast cancer prospectively from the start of the study in 1976. We examined the prevalence of specific risk factors, including screening behavior, in each region, and controlled for these risk factors to assess the extent to which they explain regional variations in incidence and mortality.

Quality Assurance Measures

An issue in any cohort study is the generalizability of the results. We have compared the age-adjusted breast cancer incidence in the Nurses' Health Study with that reported from the NCI Surveillance, Epidemiology and End Results (SEER) Program, which is based on population registries for cancer incidence that cover approximately 10% of the US population. For the period 1976 to 1990, the overall incidence rates observed in the Nurses' Health Study was 8% higher than the expected incidence rate calculated using the SEER program incidence rates for white women (Laden *et al*, 1997b). This small difference suggests (but does not prove) that results from the NHS are likely to be generalizable. Furthermore, from comparisons with 1990 Census data, we found that, with the exception of an under-ascertainment of women in large urban counties in the Northeast and in small counties in the South, the county-specific geographic distribution of participants in the NHS is remarkably representative of the white population of the original 11 states (Laden *et al*, 1997b)

DDE and PCB determinations: In year one, Mary Wolff of the Mt. Sinai Hospital, New York City, developed methods to analyze organochlorine pesticide residues in 0.5 ml samples of plasma rather than 1 ml samples. We then tested these procedures in a blinded quality control test. We submitted 25 blinded split specimens from 12 individuals under code to her laboratory for analysis. The results indicated excellent assay precision. For DDE the within-to-between variance ratio was 0.04, for total PCBs this was 0.02. The Spearman correlation between samples was 0.96 for DDE, 0.98 for PCBs. The mean coefficient of variation between specimens (s.d./mean) percent was 6.3 for DDE, 7.5 for PCB. For DDE the concentration was not significantly correlated with the CV percent ($r = 0.07$, $p = 0.74$). For PCB the mean was inversely correlated with CV percent ($r = 0.54$, $p = 0.01$), implied that the assay was less precise at lower PCB concentrations. Levels of DDE and PCBs were not significantly correlated ($r = 0.10$, $p = 0.65$) (Laden *et al*, 1997a).

Results

The relationship of DDE/PCB levels to breast cancer: Data on DDE were available on 236 breast cancer case-control pairs, and data on PCBs were available for 230 pairs. Our data do not support the hypothesis that exposure to DDT and PCBs increases the risk of breast cancer. The median level of DDE was lower among case patients than among controls (4.71 vs. 5.35 ppb, p=0.14), as was the median level of PCBs (4.49 vs. 4.68 ppb, p=0.72). The multivariate relative risk of breast cancer for women in the highest quintile of exposure as compared with women in the lowest quintile was 0.72 for DDE (95%CI=0.321.40) and 0.66 for PCBs (95%CI=0.321.37). Exposure to high levels of both DDE and PCBs was associated with a non-significantly lower risk of breast cancer (relative risk for women in the highest quintiles of both DDE and PCBs as compared with women in the lowest, 0.43 (95%CI=0.131.44) (Hunter *et al*, 1997)

Regional differences in breast cancer rates and known breast cancer risk factor: We identified 2603 incident cases of invasive breast cancer through 1992 (1,794,565 person years of follow-up). We calculated relative risks adjusted for age and established risk factors comparing California, the Northeast, and the Midwest with the South. For pre-menopausal women, there was little evidence of regional variation in breast cancer incidence rates, either in age adjusted or in multi-variate adjusted analyses. For postmenopausal women in California, age-adjusted risk was modestly elevated (RR=1.24; 95%CI=1.051.47); after adjusting for age and for established risk factors, the excess rate in California was attenuated by 25% (RR=1.18; 95%CI=1.001.40). No excess of breast cancer incidence was observed for postmenopausal women in either the Northeast or the Midwest. In conclusion, we observed little regional variation in age-adjusted breast cancer incidence, with the exception of a modest excess for

postmenopausal women in California. Adjustment for differences in the distribution of established risk factors explained some of the excess risk in California (Laden *et al*, 1997b).

Regular use of electric blankets and breast cancer: We have completed our substudy to analyze the reproducibility of the questions we asked prospectively on the Nurses' Health Study Questionnaire on electric blanket and waterbed use. We mailed a much more detailed instrument to 500 cohort members, inquiring about lifetime patterns of electric blanket and waterbed use. We received questionnaires back from 421 women. Comparison between the detailed instrument and the original questionnaire suggests that concordance is high for a history of never or very infrequent electric blanket use, as well as a history of long duration and frequent electric blanket use, suggesting that we can discriminate these two extremes of exposure. For example, comparing the supplemental questionnaire with the 1992 Nurses' Health Study Questionnaire information, and asking about electric blanket use between 1989 and 1991 categorized as 0 years or 3+ years, the Nurses' Health Study Questionnaire information had sensitivity of 0.94, and specificity of 0.95 compared with the supplemental questionnaire. Analyses of the relation of electric blanket use and breast cancer are currently underway.

Levels of 2,5-hydroxy vitamin D and breast cancer: We have continued to send specimens to Dr. Michael Holick, at Boston University, and vitamin D analyses are ongoing.

Discussion and Contribution to Understanding Biological Effects of EMF

We have studied whether electric blanket use (a major source of EMF exposure in women who use electric blankets) is related to breast cancer risk. Preliminary analyses do not suggest such a risk, although more detailed analyses are ongoing with further follow-up.

Publications From This Grant

None

Other EMF Publications

None.

TITLE: **Effects and Mechanisms of EMF Signal Transduction**

Principal Investigator: Sek Wen *Hui*, Ph.D.
Roswell Park Cancer Institute,
Buffalo, New York

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Proliferation

R01-ES07091 **Start Date:** 09/28/94 **End Date:** 08/31/98

Abstract

The purpose of this research grant is to investigate the effects of exposure to environmental level electromagnetic fields (EMF) on cellular signal transduction, and possible effects on a known model for carcinogenesis.

There are many conflicting reports that environmental EMF may cause cancer and other ailments, through mechanisms that are not understood at the present. There are also *in vitro* studies reporting conflicting results on environmental level EMF altering cellular functions, several of which may be related to carcinogenesis. The cellular functions may be better defined and controlled in a laboratory setting, and may shed light on the underlying mechanisms for reputed animal and human responses to environmental EMF. The goal of this project is to define a molecular mechanism for the reported effects of weak, low frequency electromagnetic field on cellular functions and if and how these effects are related to cancer etiology.

The proposed work will begin with an attempt to reproduce the magnetic field exposure effect on the expression of proto-oncogenes in lymphoblastoid cells [Phillips *et al*., Biochem. Biophys. Acta. 1132:140144, 1992]. After the effect is verified, so that a base line for parameter comparison is established, the hypothesis that electromagnetic field acts as a promoter rather than as an initiator will be tested using a two-stage carcinogenesis model system, i.e. the methyl-cholanthrene-initiated, retinoid suppressed C3H/10T1/2 fibroblasts cells.

We will examine the reputed carcinogenic effect of electromagnetic field signal amplification through the secondary messenger system, such as induced calcium signal, protein kinase C activation and other downstream functions of the cell. The outcome of this study will help to explain, and to define the significance limits of elusive cellular effects of weak electromagnetic field corresponding to environmental exposures.

Experimental Design and Exposure Conditions

The work will begin with an attempt to reproduce the 0.1 mT, 60 Hz magnetic field exposure effect on the expression of *c-fos* and *c-myc* proto-oncogenes in CEMCM^3 T-lymphoblastoid cells [Phillips ***et al***., Biochem. Biophys. Acta. 1132:140144, 1992], in order to bring the experimental conditions to the same baseline as certain published works. The magnetic field exposure is carried out in an exposure system duplicating the Phillips set up, with identical sham coils. After the effect is verified, further experiments employing 60 Hz electric fields of 2200 mV/m, (corresponding to about 3300 mA/m2 of current density in the culture medium), in addition to the magnetic field-induced current, will be performed. We then proceed to test the hypothesis that electromagnetic fields act as a promoter rather than as an initiator in carcinogenesis, using a two-stage carcinogenesis model system, i.e. the methylcholanthrene-initiated, retinoid suppressed C3H/10T1/2 fibroblasts. The timing and the extent of expression of the transformed phenotype, proto-oncogene expression, and proliferation of initiated cells will be determined. In order to identify the effective EMF signal and the amplification steps for the cellular responses, electromagnetic field-induced calcium signal, protein kinase C activation and other downstream functions will be measured in these

cells. The origin and mechanism of inducing calcium signal by electromagnetic field will be investigated, by using channel blockers, calmodulin inhibitors, and calcium pump modulators, and by matching the response frequency windows with hypothetical resonance amplification by intra, extra, and transmembrane circuits.

Quality Assurance Measures

The magnetic field exposure system is constructed by Electric Research and Management, Inc. (Pittsburgh, PA) as a duplication of the Phillips set up at Loma Linda, CA. It consists of two identical, double wound Helmholtz sets encased in acrylic frames, such that each set can be energized in reinforcing or bucking current directions. Sham exposure is done in the bucking current mode, so the differences in thermal and vibrational environment between the exposed and sham-exposed samples can be minimized. The fields are ramped on/off with cells within the center portion of the Helmholtz set where deviation of field strength is within 1% accuracy, thus switching transients are eliminated. The AC and DC field strengths are monitored with a Bartington 3axes magnetic field sensor. The earth magnetic field of 580 mG and 59° declination is identical in both incubators and is not shielded. Field exposed and sham exposed conditions were interchangeable in 2 identical incubators placed 3 m apart. The fan motors of the incubators have been replaced to minimize stray AC fields. The AC components of stray magnetic fields from the incubator heater and electronic control never exceed 3 mG within the sample volume. The control unit of the Helmholtz sets has a locked blind switch so that the experimenters do not know which is the sham set. The biochemical assays are also blind-coded. Exposures are always done in identical pairs with alternating incubators, so that slight differences between incubators are averaged. The exposure system has been checked by the DOE site visit team of Wisecup, Dietrich and Gailey for the EMF *RAPID* program on October 31, 1996.

Results

Replication Experiment: expression of proto-oncogenes as a result of exposure to 0.1 mT, 60 Hz magnetic field. The mandated duplication experiment to reproduced the results of Phillips *et al.*, on the oncogene expression in CEMCM3 cells, as a result of exposure to 0.1 mT, 60 Hz magnetic field, has been completed. Using a duplicating exposure system made by Electric Research and Management, Inc., we have performed up to 22 sets of repeating experiments on transcription rates, and up to 8 sets of repeating experiments on transcription levels, using the same cell line, cell density (10^6 cells/ml) during EMF exposure, culturing conditions, assaying methods as those described by Phillips *et al*. Realizing the variability from experiment to experiment, we took care to obtain data for all EMF exposure time points in the same experimental set, with the same plating of cells. Furthermore, the quantitation of proto-oncogene expression was normalized to the expression of a housekeeping gene G3PDH, a step not taken in the original Phillips *et al*. experiment. The results from the exposed cells were compared to those of the sham exposed cells. When the data from all duplicating sets of experiments were pooled, we found no significant difference in either the transcription rate or the transcription level between the EMF-exposed and sham-exposed cells at all exposure time points. The results disagreed with those reported by Phillips *et al*.

Proliferation rate of carcinogenically initiated INITC3H/10T1/2 cells in response to 60 Hz magnetic field. Our model for carcinogenisis is the methylcholanthrene transformed but retinyl-acetate (RAC) controlled INITC3H/10T1/2 cells, which are very sensitive to carcinogenic promotion after the withdrawal of RAC. Uncontrolled proliferation becomes apparent at 8 to 21 days after RAC withdrawal. Phorbol acetate treatment accelerates the progression. INIT cells were exposed to 60 Hz, 0.10.4 mT magnetic fields for various periods of time. The proliferation rate was measured by ^{3}H-thymidine incorporation. The results from EMF-exposed cells were compared to those of sham-exposed cells, as well as to those from unexposed cells. Both EMF-exposed and sham-exposed cells showed significant increase of proliferation rate from those of unexposed cells. There was a slightly higher rate in sham-exposed cells than EMF-exposed cells. The difference was time and dose-dependent beyond a threshold level, and was insensitive to exposure protocol. The same effect may be reproduced by raising the temperature of the incubators by a fraction of a degree. No difference in the expression of *c-myc* was detected, either by EMF exposure or by temperature modulation. Several other cells lines, including Jurkat cells, were found also to be proliferation-sensitive to such a slight temperature increase. Incidentally, EMF and sham-exposures also caused a slight increase in sample temperature by a fraction of a degree, due to the heating effect of the Helmholtz coils in the incubators. Therefore, the difference between EMF or sham-exposed and unexposed cells may be

attributed mainly to the temperature difference in the incubators produced by the exposure coils. Any field effects were likely to be masked by the temperature effect.

Intracellular calcium level in response to an external electric field. The intracellular calcium level in Jurkat cells was measured by fluorescence of Fura2 or Fluo3 dyes. Both spectrometric and microscopic measurements were made, and calibrated by ionomycin and manganese. We have examined the changes of calcium levels in CEMCM3, Wilson, L1210 and Jurkat lymphoid cells, as well as C3H/10T1/2 fibroblasts and CHO cells, as a result of an imposing 60 Hz electric field up to 100 mV/cm. The electric field was imposed by a pair of gel bridges. A slight decrease of fluorescence level was detected as a result of electric field imposition, but the decrease was determined to be a fluorescence artifact of Joule heating of the dye. No changes of intracellular calcium level was attributable to the imposing electric field. The natural intracellular calcium level oscillation was detected in a population of Jurkat cells. Its influence by the external electric field is still under investigation.

Discussion and Contribution to Understanding Biological Effects of EMF

The results of two major studies of our project, namely, the duplicating experiments of proto-oncogene expression in CEMCM3 T-lymphoblastoid cells upon exposures to 0.1 mT, 60 Hz magnetic field, and testing the carcinogenic promotion effect of 0.10.4 mT, 60 Hz magnetic field on INIT/10T1/2 cells, have been negative. The former study casts doubt on the reproducibility of a reported finding, after exhausting effort to duplicate the conditions. The detected "EMF effect" on the proliferation of INIT/10T1/2 cells in the latter study may be attributed to a small temperature increase due to the excitation coils used to produce the magnetic field, but not the field itself. Insofar as our studies, the biological effects of 0.1 mT, 60 Hz magnetic field, which we set out to measure, are not detected by the main stream biochemical methods we applied.

Our studies have contributed negatively to the general robustness tests of the reported EMF effects demanded by the RAPID program. Our finding of the extreme temperature sensitivity of some cell lines places a cautionary note in interpreting results from other studies of biological effects of EMF.

Publications From This Grant

1. Jahreis GP, Zhao YL, Johnson PG, and Hui SW. “Absence of 60 HZ 0.1 mT magnetic field induced changes in oncogene transcription rates or levels in $CEMCM_3$ cells.” Biochem Biophys. Acta, In press, 1998.

Other EMF Publications

1. Hui SW. "Electric Field Induced Calcium Flux and Changes in Cell Shape, Motility and Cytoskeleton". In: Biological Effects of Electric and Magnetic Fields (D.O. Carpenter, ed., Academic Press, vol. 2, pp. 83-101, 1994.

TITLE: **EMF Effects on Melatonin among Aging Volunteers**

Principal Investigator: *Daniel F. Kripke, Ph.D.*
University of California, San Diego
La Jolla, California

Health Relevance: Neurobehavioral

Research Area: Studies Involving Humans; Melatonin; Aging

RO3-ES08930 **Start Date:** 01/15/97 **End Date:** 12/30/98

Abstract

This is a small grant proposal in response to RFA ES06007, "Biological effects of power frequency EMF." It has been hypothesized that EMF (electric and magnetic fields) may suppress melatonin, and through this mechanism, EMF might mediate morbidity and mortality. The problem may be particularly severe for elderly people who have low melatonin. The hypothesis that melatonin prevents aging, e.g., aging related to free-radical damage and cancer, has recently received sensational news coverage. Millions of Americans fearful of melatonin deficiency may be buying products from health food stores labeled as melatonin. The investigators propose to critically test whether EMF in the bedroom influences daily melatonin excretion. This small grant will permit the investigators to collect home EMF recordings to combine with data being collected for other projects on melatonin excretion and bedtime light levels. The correlation of EMF resultant vector strength and nightly melatonin excretion will be determined, to establish if EMF could be a substantial regulator of melatonin production. Statistical controls for age, gender, light levels, and drug intake will be implemented. This study will establish whether EMF could be an important source of variance in melatonin excretion. In conjunction with ongoing studies, possible indirect EMF effects through melatonin on morbidity and mortality will be considered.

Experimental Design and Exposure Conditions

Subjects are being recruited and observed through AG12364 and HL55983, which support Actillume monitoring, sleep logs, mood ratings, and urine collections for 6-sulphatoxymelatonin. The sleep period (i.e., time-in-bed) is estimated to the minute from activity and illumination data, supplemented by sleep logs.

An Emdex Lite instrument is placed as close as possible to the subject's head during sleep, usually under the mattress, between the mattress and the box spring. The instrument is set to record each of 3 orthogonal EMF vectors (from which the triaxial resultant vector is computed), sampling and storing the data every 30 seconds. The instrument is left in place for approximately 7 days of monitoring, and then is returned to the laboratory for data recovery. The primary computed descriptor of EMF is the mean resultant vector (in milligauss) while the volunteer is in bed. Peak values (in bed) are also computed. EMF when the subject is not in bed is also computed, since among these elderly volunteers, the assumption that the volunteer is nearby in the house might be correct for the majority of the day. In addition, spot measurements of EMF at the actual top of the pillow are taken, with carefully determined spacial vectors, to complement the longerterm placements. For practical comfort, the meter is not placed on top of the pillow when the volunteer is sleeping.

We record 1-minute wrist activity and illumination data from the Actillume device. The data reflects the geometric mean of illumination at the wrist, the mean acceleration per minute, and the maximal 10 seconds of acceleration per minute. Light exposure is determined via an light sensor, calibrated monthly.

During two 24-hour intervals (at home), volunteers are asked to urinate approximately every 2 hours during wakefulness. Spontaneous nocturnal voidings are sampled, and most elderly volunteers awaken during mid-sleep to urinate. Most volunteers supply 10-15 samples per 24 hour. Volunteers measure volumes and freeze duplicate sample tubes from each sample in their home freezers.

Total daily excretion patterns of 6-sulphatoxymelatonin are estimated from the sum of each sample's concentration multiplied by volume. The primary dependent variable is the mean level of 6-sulphatoxymelatonin during the sleep period.

During the home recordings, subjects complete mood questionnaires. These questionnaires consist of the CES-D scale, a self-rating scale specifically designed for community epidemiological surveys, plus 8 items reflecting atypical Seasonal Affective Disorder symptoms derived from the SIGH-SAD-SR. We have previously found that these scales are correlated with low illumination.

Quality Assurance Measures

The laboratory's Emdex Lite meters are frequently arrayed in parallel in strong EMF, to determine that all meters maintain the same calibration sensitivity.

The major metabolite of melatonin, urinary 6-sulphatoxymelatonin, is assayed in duplicate by ELISA, using microwell kits supplied by DiagnosTech (formerly ELIAS, USA, Inc., Osceola, WI). Standards are assayed with each microwell plate. We have cross-validated the 6-SMT ELISA in our laboratory by correlating results (n>40) with two established RIA techniques (r=0.90-0.97). In our laboratory, the intra-assay coefficient of variation is 3.3% and the inter-assay CV is 13.7%, which is excellent for a metabolite in which the physiologic variation is several hundred percent. CV values for duplicate determinations of individual samples are generally <7%.

Results

These interim analyses are reported for the purpose of monitoring the study's progress, but do not form a basis for positive or negative conclusions.

Median EMF exposure during the sleep period was 0.48 milligauss. The range varied from 0.08 to 34 milligauss. The highest EMF value exceeded the group median by 8 standard deviations, and, therefore, might be regarded as a statistical outlier. All other EMF values were within 2 standard deviations of the median. Median 6-SMT excretion during the sleep period was 1058 ng hr-1; the range was 26-5185 ng hr1.

The Figure displays the association between EMF and 6-SMT excretion during the sleep period among all subjects, with log-transformation of the EMF variable. No association was found (Rs=0.03, p=0.77, n=73). Moreover, no association was found following removal of the single extreme EMF value (34 milligauss) (Rs=0.05, p=0.71, n=72).

Discussion and Contribution to Understanding Biological Effects of EMF

At this writing, the study has not yet finished the first of two years of data collection. No firm conclusions can be drawn from the data until the pre-planned number of subjects have been assessed and final statistical analyses are completed.

Publications From This Grant:

None

Other EMF Publications:

None

TITLE: **60 Hz Magnetic Field-induced DNA Damage**

Principal Investigator: Henry C. *Lai*, Ph.D.
University of Washington,
Seattle, Washington

Health Relevance: Cancer

Research Area: Cellular Function; DNA Damage; Free Radicals

RO3-ES08865 **Start Date:** 1/18/97 **End Date:** 12/31/98

Abstract

Recent research in our laboratory shows that rats acutely (2 hrs) exposed to a 60 Hz magnetic field (0.10.5 mT) have an increase in DNA single and double strand breaks in their brain cells. Experiments are proposed to further investigate the mechanism by which exposure to a 60 Hz magnetic field causes DNA damages in brain cells of the rat. Specifically, the role played by free radicals will be investigated, since our preliminary research also shows that treatment of rats before exposure with free radical scavengers (melatonin and a spin trap compound) can block the DNA damaging effect of magnetic fields. Free radicals have recently been implicated in the biological effects of extremely low frequency (ELF) magnetic fields. Experiments are designed to investigate the involvement of free radicals in magnetic field-induced DNA strand breaks in cells. This proposed research has the following specific aims:

- To further confirm the blocking effect of melatonin on magnetic field-induced DNA single and double strand breaks in brain cells of rats, a (drug) dose-response study will be carried out.
- To provide further support for the hypothesis that free radicals are involved in the effect of magnetic fields, animals will be treated with different spintrap compounds and the vitamin E analog trolox to investigate whether they can block the effects of magnetic fields on DNA strand breaks in rat brain cells. Spintrap compounds and trolox are efficient free radical scavengers.

This is an important new direction in ELF electromagnetic field bioeffect research because DNA damage is closely related to human health risk. Particularly, DNA damage in brain cells could affect neurological functions and also possibly lead to carcinogenesis and neurodegenerative diseases. It is hoped that data from this proposed research will help in identifying possible causal connections of exposure to power frequency magnetic fields and biological effects and also in the hazard assessment of power frequency electromagnetic fields.

Experimental Design and Exposure Conditions

Laboratory rats are used in this research project. Animals are exposed to 60 Hz magnetic fields in a Helmholtz coil-pair system. The Helmholtz coils are covered by aluminum plates to shield off electric fields so that animals are only exposed to magnetic fields. The field distribution and uniformity within the exposure coils have been calculated theoretically and measured with magnetic field meters. The exposure system and environment have been site-visited and validated by the DOE exposure system expert team. In the research, rats are exposed to the magnetic field at different intensities and durations. DNA strand breaks in brain cells are studied by the microgel electrophoresis assay.

Quality Assurance Measures

To ensure credibility of the research all the experiments carried out in this research project are run blind, i.e., the experimenters doing measurement and data collection do not know the treatment conditions of the animals being studied. Magnetic field exposure and data collection are done by different experimenters. Animals used as controls are exposed with the Helmholtz coils in the "bucking mode", i.e., the coils are activated with the electricity flowing in opposite directions so that the magnetic fields generated by the two coils cancel one another. This controls for the possible effects of vibration and heat generation, which can affect the responses of the animals.

Results

The following is a brief description of the results of experiments performed:

- Acute exposure to a 60 Hz magnetic field caused a dose dependent increase in DNA single and double strand breaks in brain cells of the rat.
- Increase in DNA strand breaks was observed at 0.01 mT after 24 hours of exposure. Exposure for 48 hrs. at the same intensity caused a further increase in breaks suggesting that the effect is cumulative over time.
- The effect of magnetic field on DNA strand breaks can be blocked by treating rats with free radical scavengers and iron chelator.
- Both DNA protein and DNA-DNA crosslinks were observed in brain cells of the rats after acute exposure to the magnetic field. An increase in apoptosis in brain cells was also observed.
- We have hypothesized based on the above findings that magnetic fields act through an iron dependent process (Fenton reaction) that causes an increase in free radical formation. Free radicals in turn cause DNA damage. Since cancer cells have a higher rate of iron metabolism compared to normal cells we have also found that cancer cells are more susceptible to magnetic fields than normal cells.

Discussion and Contribution to Understanding Biological Effects of EMF

Data from this research indicate that free radicals are involved in magnetic field-induced DNA strand breaks in brain cells. This finding could have an important implication on the health hazardous effects of magnetic field exposure. Involvement of free radicals in human diseases, such as cancer and atherosclerosis, have been suggested. Free radicals also play an important role in aging processes, which has been suggested to be a consequence of accumulated oxidative damage to body tissues. Relevant to our finding is that brain cells are known to be particularly susceptible to oxidative damage by free radicals. Involvement of free radicals in neurodegenerative diseases, such as Alzheimer, Huntington, and Parkinson, has been suggested. Our findings may be relevant to recent epidemiological studies reporting that occupational exposure to EMF increased the risk of development of Alzheimer's disease and amyotropic lateral sclerosis. (Davanipour *et al*. (1997) “Amyotropic lateral sclerosis and occupational exposure to electromagnetic fields”. Bioelectromagnetics 18: 28-35; Sobel *et al*. (1995) “Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease.” Am J Epidemiol 142:515-524).

On the other hand, recent research has suggested that free radicals can serve as stimulators of signal transduction in cells. Superoxides, hydrogen peroxide, hydoxyl radical, lipid hydroperoxides, nitric oxide can be signaling molecules under subtoxic condition. As a consequence of increase in free radicals, various cellular and physiological processes can be affected including: gene expression, transcription factor activation, Ca^{2+}signaling, protein phosphorylation, release of calcium from intracellular storage sites, cell growth, chemotaxis, and apoptosis. Effects of magnetic field exposure on free radical kinetics could affect these cellular functions.

Further experiments should be carried out to study the free radical hypothesis, for example, by studying other oxidative damages to macromolecules such as DNA oxidative base damage, protein oxidation, and lipid peroxidation.

Publications From This Grant

1. Lai HC and Singh NP. “Melatonin and N-tert-butyl-alpha-phenynitrone blocked 60 Hz magnetic field-induced DNA strand breaks in rat brain cells.” J. Pineal Res. 22:152-162, 1997.

2. Singh NP and Lai HC. “60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells.” Mutation Research, In press, 1998.

Other EMF Publications

1. Lai HC. “Spatial learning deficit in the rat after exposure to a 60 Hz magnetic field.” Bioelectromagnetics 17:494-496, 1996.
2. Lai HC and Singh NP. “Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells.” Bioelectromagnetics 18:156-165, 1997.
3. Lai HC, Carino MA, and Ushijima I. “Acute exposure to a 60 Hz magnetic field affects rat’s water maze performance.” Bioelectromagnetics. 19:117-120, 1998.

TITLE: **60-Hz Magnetic Field Effects on Cholinergic System**

Principal Investigator: Henry C. *Lai*, Ph.D.
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Seattle, Washington

Health Relevance: Neurobehavioral Dysfunction

Research Area: Neurobehavior, Cholinergic System

RO3-ES06290 **Start Date:** 1/10/97 **End Date:** 12/31/98

Abstract

We proposed experiments to study the effects of extremely low-frequency (ELF) magnetic field exposure on neurological functions and behavior in the rat. From the data of our preliminary experiments, we hypothesize that acute exposure to ELF magnetic fields activates endogenous opioids, which in turn causes a decrease in cholinergic activity in the brain. These changes in central cholinergic activity lead to a deficit in learning and memory functions. We intend to further investigate and characterize this chain of events through the following experiments: (1) A study to investigate the effects of acute exposure to magnetic fields of different combinations of intensity and duration, and of different frequencies on sodium-dependent high-affinity choline uptake (HACU), an index of cholinergic activity in different regions of the rat brain. Possible difference in responses between male and female rats to the magnetic field also will be investigated. (2) A study to investigate the effects of repeated magnetic field exposure on HACU and muscarinic cholinergic receptors in different regions of the brain. (3) A study to investigate the involvement of opioid receptor subtypes (mu, delta, and kappa) on the effect of magnetic fields on cholinergic systems; and (4) A study to investigate the effect of magnetic field exposure on performance in the radial-arm maze, a behavioral task involving spatial memory functions. Since it is well known that radial-arm maze performance depends on central cholinergic activity, behavioral deficits will be correlated with the changes in cholinergic activity in the brain. It is hoped that the data from these proposed studies will identify the exposure conditions that may trigger deleterious effects, reveal the neural mechanisms affected by ELF magnetic fields, and help in the risk assessment and setting of magnetic field-exposure guidelines in the public and occupational environments.

Methodology

Laboratory rats are used in this research project. Animals are exposed to 60-Hz magnetic fields in a Helmholtz coil-pair system. The Helmholtz coils are covered by aluminum plates to shield off the electric field so that animals are only exposed to magnetic fields. The field distribution and uniformity within the exposure coils have been calculated theoretically and measured with magnetic field meters. The exposure system and environment have been validated by the DOE exposure system expert team. In the research, rats are exposed to the magnetic field at different intensities and durations. Biochemical, pharmacological, and behavioral methods are used to investigated the effects of magnetic field exposure.

To ensure credibility of the research all the experiments carried out in this research project are run blind, i.e., the experimenters doing measurement and data collection do not know the treatment conditions of the animals being studied. Magnetic field exposure and data collection are done by different experimenters. As a control of the experiments, animals used as controls are exposed with the Helmholtz coils in the "bucking mode", i.e., the coils are activated with the electricity flowing in opposite directions so that the magnetic fields generated by the two coils cancel one another. This controls for the possible effects of vibration and heat generation, which can affect the responses of the animals.

Results

The following is a brief description of the experiments performed:

(a) Effect of different intensities of exposure to a 60-Hz magnetic field on cholinergic activities in the frontal cortex and hippocampus of the rat.

In this experiment, rats were exposed to a 60-Hz magnetic field of different flux densities (0.5, 1.0, 1.5, 2.0 mT) for 60 min in the Helmholtz coil pair exposure system. Controls for each flux density were rats exposed with coils activated in the "bucking" mode. Cholinergic activity as measured by high-affinity choline uptake was determined in the frontal cortex and hippocampus immediately after exposure. These two areas of the brain were studied because they contain high density of cholinergic innervations and are involved in learning and memory functions. Data from this series of experiments show that in both brain areas, a decrease in cholinergic activity was observed when the flux density was at 2.0 mT. No significant effect was observed at lower flux densities.

(b) Effects of different durations of exposure on cholinergic activities in the frontal cortex and hippocampus of the rat.

In this next series of experiments, rats were exposed to a 60-Hz magnetic field at 1.0 mT for 30, 45, 60, or 90 min in the Helmholtz coil system. Cholinergic activity in the frontal cortex and hippocampus was measured immediately after exposure. Controls were animals exposed in the "bucking" mode for the same period of time. Data show that after 90 min of exposure, a decrease in cholinergic activity was observed in both brain areas.

Data from these two series of experiments show that intensity and duration of magnetic field can interact. In the first experiment, significant response was not observed when the rats were exposed for 60 min at 1.0 mT, whereas an effect was observed after 60 min of exposure at 2.0 mT. In the second experiment, we found that when the exposure duration was prolonged to 90 min at 1.0 mT, a significant effect was observed. Thus, high intensity/short duration exposure can produce a similar effect as by lower intensity/longer duration exposure. This finding could have important implication in the determination of health risk in situations when humans are exposed to low-intensity magnetic field for prolonged period of time. To my knowledge, this is the first time such a relationship of interaction between intensify and duration of electromagnetic field exposure is reported in bioelectromagnetics research.

(c) Involvement of different subtypes of opioid receptors in magnetic-field-induced decrease in cholinergic activity in the rat brain.

In previous research, we have found that 60-Hz magnetic field-induced decrease in cholinergic activity in the frontal cortex and hippocampus of the rat was mediated by endogenous opioids in the brain because it can be blocked by pretreatment with the opiate antagonist naltrexone but not by the peripheral antagonist naloxone methiodide. In a follow-up study, the involvement of opioid receptor subtypes was investigated. Rats were pretreated by injection into the cerebroventricles of the mu-opioid receptor antagonist, β-funaltrexamine, or the delta-opioid receptor antagonist, naltrindole, before exposure to a 60-Hz magnetic field (2 mT, 1 hour). It was found that the effects of magnetic field on cholinergic activity in the frontal cortex and hippocampus were blocked by the drug treatments. This indicate that both mu and delta opioid receptors in the brain are involved in the magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat.

(d) Effects of repeated magnetic field exposure on cholinergic receptors in the brain of the rat.

An experiment was carried out to study the effect of repeated exposure to magnetic field on cholinergic receptors in the brain. As an adaptation process, neurotransmitter receptors usually change as a result of repeated increase or decrease in transmitter activity. In this experiment, rats were exposed 1 hr per day for 10 days to a 60-Hz magnetic field at a flux density of 2 mT. Receptors in the brain were assayed immediately after the last exposure. We found no significant difference in the concentration and affinity of cholinergic muscarinic receptors in the frontal cortex and hippocampus of the magnetic field-exposed rats compared with those of rats exposed similarly in the "bucking" mode.

(e) Investigation into the effect of acute 60-Hz magnetic field exposure on 'working' and 'reference' memory functions.

Since cholinergic systems in the brain are involved in learning and memory, we investigated whether magnetic field exposure affects these behavioral functions. We found that acute magnetic field exposure caused a deficit in memory functions in the rat. Exposed rats showed difficulty in memorizing locations of objects in their environment.

Two types of memory functions were studied: 'working' and 'reference' memory. 'Working' memory in the rat was studied with the radial arm maze, in which a rat was trained to obtain food reward from arms protruding from a center hub and to remember which arms it has taken food from and not to re-enter. Thus, the memory involved varies according to the responses of the animal in a particular training session. This is sometimes also referred to as 'short-term' memory. In addition, the effect of magnetic field exposure on 'reference' memory was studied using the Morris water-maze. In this behavioral model, rats are required to locate a submerged platform in a circular pool containing opaque water. It is assumed that a rat locates the platform by learning spatial cues in the environment to form a reference map.

In the first experiment, rats were trained in ten daily sessions to perform in a 12-arm radial maze. Exposure to a 60 Hz magnetic field (45 min, 0.75 mT) immediately before each training session significantly retarded the learning. Pretreatment with the cholinergic agonist physostigmine before magnetic field exposure reversed its effect on spatial learning. Data from this experiment indicate that magnetic field-induced spatial 'working' memory deficit is caused by the effect of the field on cholinergic systems.

Reference memory function was investigated using the Morris water maze. In the experiment, rats were trained in 6 sessions to swim and locate a submerged platform in a circular pool. Before each training session, two groups of rats were either exposed to the magnetic field (1 mT, 60 min) or 'sham' exposed. A group of unhandled animals was also included in this experiment. In a following session, the platform was removed and each rat was allow to swim in the pool for 1 min. The time spent in the quadrant of the pool where the platform had been located was score. This measures the ability of the rat to remember the location of the platform and is a measure of 'reference' memory. There was no significant difference in the rate of learning between the magnetic-field- and sham-exposed rats. However, there was a significant difference in the time spent in the previously platformed quadrant, with the magnetic field exposed rats spent significantly less time in it. The fact that during the probe trial magnetic field-exposed rats did not show a swim pattern indicative of searching for the 'missing' platform in the quadrant suggests that no 'place' learning had occurred in these animals. They probably located the platform during the learning trials using other learning strategies. Results from this experiment indicate that magnetic field exposure affects the formation of spatial 'reference' memory in the rat.

(f) Investigation into the effects of 60-Hz magnetic exposure and DNA strand breaks in brain cells of the rat.

We investigated the effects of magnetic field exposure on DNA in brain cells of the rat. We found that acute magnetic field exposure caused two types of DNA damage, single and double strand breaks. The effect is proportional to the intensity of the magnetic field (0.1-0.5 mT), i.e., a higher intensity of magnetic field causes a larger increase in single and double strand DNA breaks in rat brain cells. We have recently found that exposure to a 60-Hz magnetic field at 0.01 mT for 24 hrs could consistently cause an increase in both DNA single and double strand breaks in rat brain cells, and extending the exposure time to 48 hrs caused a further increase in both types of breaks. Thus, we again show that the duration of exposure has a significant contribution to the effect.

These data are important since DNA strand breaks can cause disruption in cell functions, cell death, and lead to cancer development. Further research into the effect of magnetic field exposure on DNA strand breaks has been supported by a RO-3 grant from the EMF RAPID program. Progress in that research is discussed in the report on grant RO3 ES-08865: "60 Hz magnetic field-induced DNA damage."

Conclusion

The main goal of this research project is to investigate the effects of exposure to power-line frequency magnetic fields on the central nervous system and its functions . Our research described above showed effects from molecular

and chemical changes in the brain to behavioral effects. These effects could have important implication in considering the possible health hazards of environmental ELF EMF exposure.

Cholinergic systems in the brain are involved in many important physiological and behavioral functions, including memory. Our research also implicates the effect of magnetic field exposure on the activity of another type of chemicals in the brain, the endogenous opioids, which are also involved in important brain functions, such as pain perception and coping with stress. We found that the magnetic field-induced increase in endogenous opioid activity in turn causes the decrease in cholinergic activity we observed in our experiments.

We have found that acute exposure to a 60-Hz magnetic field affected 'working' (short-term) spatial memory and 'reference' (long-term) memory in the rat. The finding that magnetic field exposure causes 'working memory' has been replicated by a laboratory in England in mice exposed to a 50-Hz magnetic field. These behavioral deficits could be related to the magnetic field-induced decreases in cholinergic activity in the brain, since central cholinergic systems are involved in spatial 'working' and 'reference' memory functions. Much work has to be done to further explore the effects of magnetic fields on spatial learning. Due to the relevance of spatial learning in rodents to human health conditions and the fact that much is known about the neural mechanisms of spatial learning, these experiments provide a powerful mean for the investigation of the effects of magnetic field exposure on functions of the central nervous system.

The finding that magnetic field exposure can lead to DNA damages in cells is also an important discovery. This effect has since been confirmed independently by two other laboratories in a human cancer cell line and normal human lymphocytes. An increase in DNA strand breaks in body cells could have an important implication on the possible health effect of exposure to ELF magnetic fields in the environment. According to the multi-step and clonal model of origin of cancer, tumorogenesis is a multi-stage process, mainly initiation followed by promotion, beginning with only one cell escaping from immune surveillance. Damage to cellular DNA or lack of its repair could be an initial event in carcinogenesis (clonal origin). Cumulative damage in DNA in cells also has been shown during aging. Several investigators contend that cumulated DNA damage in neurons and other post-mitotic cells is the primary factor in aging of an organism. Cumulative DNA damage in cells, particularly in neurons, has also been associated with Alzheimer's disease Huntington's disease, and Parkinson's disease. This may be relevant to recent epidemiological studies reporting that occupational exposure to electromagnetic fields could increase the risks of development of Alzheimer's disease and amyotropic lateral sclerosis. Thus, DNA strand breaks could lead to disruption of cell functions and carcinogenesis, and a relationship between an increase in DNA strand breaks and aging and neurodegenerative disorders has been suggested. It is imperative that further studies should be carried out to characterize and understand the effect of ELF magnetic field exposure on DNA damage in cells.

Publications From This Grant

None

Other EMF Publications

1. Lai H. “Spatial learning deficit in the rat after exposure to a 60 Hz magnetic field.” Bioelectromagnetics 17: 494-496, 1996.
2. Lai H and Singh NP. “Acute exposure to a 60-Hz magnetic field increases DNA strand breaks in rat brain cells.” Bioelectromagnetics 18: 156-165, 1997.
3. Lai H and Singh NP. “Melatonin and a spin-trap compound blocked 60-Hz magnetic field-induced DNA strand breaks in rat brain cells.” J. Pineal Res. 22: 152-162, 1997.
4. Lai H, Carino MA and Ushijima I. “Acute exposure to a 60 Hz magnetic field affects rats’ water maze performance.” Bioelectromagnetics. 19: 117-122, 1998.

TITLE: **EMF and Breast Cancer on Long Island Study**

Principal Investigator: M. Christina *Leske*, MD, MPH
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Health Relevance: Cancer

Research Area: Studies Involving People, Epidemiologic Research, Breast Cancer

U01-CA62991 **Start Date:** 9/30/93 **End Date:** 8/31/98

Abstract

Breast cancer incidence rates for Long Island women have been among the highest in New York State for many years. This has raised major community concerns about possible environmental causes that may add to breast cancer risk in this geographic area. Recent studies have suggested that electric and magnetic fields (EMF) may increase the risk of breast cancer. The hypothesis for the association is based on experimental evidence that light and extremely low frequency EMF affect melatonin production by the pineal gland, thus influencing mammary carcinogenesis in laboratory studies. Given the biologic plausibility of the hypothesized association between EMF and breast cancer, coupled with the unexplained high incidence rates of breast cancer among Long Island women, an investigation of EMF and breast cancer is warranted.

The *EMF and Breast Cancer on Long Island Study (EBCLIS)* is a case-control study with the major aim of evaluating the association between EMF exposures and breast cancer among Long Island women. The *EBCLIS* is being conducted by the Department of Preventive Medicine at the University Medical Center at Stony Brook. The study is linked to the Long Island Breast Cancer Study Project (LIBCSP), which includes several breast cancer studies conducted by a consortium of medical, academic, and research institutions. The broad objective of the LIBCSP is to explore the relationship between breast cancer on Long Island and specific environmental factors designated by a congressional mandate. One of the factors specified by this legislation is EMF exposure.

The centerpiece of the LIBCSP is a population-based, case-control study led by the Columbia University School of Public Health along with other institutions on Long Island and New York City, which was designed to evaluate associations with organochlorine pesticides and polycyclic aromatic hydrocarbons. To increase efficiency and avoid overlap in ascertainment and data collection on cases and controls, *EBCLIS* has been structured as a companion study to the Columbia-led study. Therefore, *EBCLIS* will be conducted by collecting EMF exposure data on a subset of the Columbia case-control study participants.

The specific aims are as follows:

- To evaluate the hypothesis of an association between EMF and breast cancer, magnetic field exposures of breast cancer cases will be compared to those of control women without the disease.
- To determine EMF exposures, participants are asked questions about EMF exposure and the following EMF measurements will be taken in the home: spot measurements, 24-hour measurements, and ground current test-load measurements. The electrical wiring configuration of each residence will be viewed from outside the home and coded using the Wertheimer-Leeper system.
- To enhance interpretation of exposure data, both cases and controls are women who have lived in the same Long Island residence (in Nassau or Suffolk counties) for at least 15 years.

Experimental Design and Exposure Conditions

Women who meet the study eligibility criteria (600 cases and 600 controls) are selected from the LIBCSP's Columbia case-control study. Cases will be recruited such that every woman eligible will be recruited until the total sample size is achieved. EMF data collection began January, 1997. We plan to periodically assess the age comparability of cases and controls throughout the study and making adjustments, if needed, to maintain age balance.

EMF measurements will be as follows:

- Questionnaire: All 3,200 LIBCSP participants are asked questions on occupational history, residential history, and appliance use. All 1,200 *EBCLIS* participants are asked questions about occupational EMF exposure; shift work; electric train travel; car use; light at night; and additional appliances not included in the LIBCSP questionnaire.
- Spot measurements of time-varying magnetic fields are recorded with an EMDEX II meter. Spot measurements are taken at the following locations: outside the front door of the residence; center of the bedroom; and center of a room (lived-in room) where the participant spent most of her time during the reference period.
- Ground current test-load measurements: EMDEX II meters are used to measure the change in magnetic fields due to ground current as a test-load cycles through three stages: off, low, and high. These measurements are taken concurrently with the spot measurements in the center of the bedroom and center of the lived-in room.
- 24-hour measurements of time-varying magnetic fields are taken with EMDEX II meters in the bedroom and in the lived-in room. The meters continually take EMF measurements every fifteen seconds over the 24-hour period.
- Wire coding: the electrical wiring configuration of each residence will be viewed from outside the home and coded using the Wertheimer-Leeper system. The wire coding of all *EBCLIS* eligible women will be done in batches, during the course of the data collection period.

Quality Assurance Measures

It is important to coordinate the timing of accrual of cases and controls, so that there is a steady accrual of each. If not, the cases and controls might vary by season. It is likely that magnetic field levels measured in many Long Island homes will exhibit a seasonal dependence, with the largest fields measured during hot days in the summer when demand for electrical power is highest. Thus, it is important to minimize seasonal differences between the times when cases and controls undergo their magnetic field measurements. To achieve this goal, cases and controls are measured at the same rate throughout the study.

Quality control measures are included into the study protocol for all data collection procedures. The wire code, EMDEX II measurements, and ground current data will be evaluated, as well as the interview data acquired in the study. The largest risks for decreasing the quality of the data are instrumental failures that go unrecognized and deviations from the experimental protocol. Consequently, daily checks of the functioning and gross calibration of the EMDEX II meters and ground current test-loads will be performed. Also rigorous evaluation of data collection to maintain quality and accuracy of the data will be performed.

Results

Data collection began January 1997 and will continue through the latter part of 1998. Results of the study are expected in early 2000.

Discussion and Contribution to Understanding Biological Effects of EMF

This project will help educate if a relationship exits between EMF and breast cancer, based upon the hypothesis that light and extremely low EMF effect melatonin production by the pineal gland.

Publications From This Grant

None.

Other EMF Publications

None.

TITLE: **Magnetic Field Combinations and Lymphocyte Calcium Signaling**

Principal Investigator: Robert P. *Liburdy*, Ph.D.
Lawrence Berkeley National Laboratory,
Berkeley, California

Health Relevance: Immunological Effects

Research Area: Cellular Function; Cell Proliferation; Calcium

R01-ES06401 **Start Date:** 6/01/94 **End Date:** 7/31/98

Abstract

In order for biological experiments to predict whether exposure to electromagnetic fields (EMF) causes cellular changes that may lead to the development of cancer and other adverse effects, reliable models and reproducible exposure conditions are a must. We propose to test the hypothesis that time-varying (AC) magnetic fields interact with biological systems in a fundamentally different way if a static (DC) magnetic field is present. Several theoretical models predict that the frequency, intensity, and orientation of the AC field, and the intensity and orientation of the DC field must all be rigorously controlled and varied in a specific manner in order to observe reproducible, ion-specific biological effects. Models discussed by Blackman/Blanchard, Ledvev, and Liboff will be examined; these are collectively referred to as the AC/DC model.

Our laboratory first reported that an AC/DC field combination conforming to this theory significantly decreased mitogen-activated calcium transport in rat lymphocytes, and that AC and DC fields alone had no effect [1.8 AC/DC intensity ratio, 231 mG DC, 16 Hz AC for $45Ca^{+2}$ Yost & Liburdy, 1992]. Resting cells did not respond to this AC/DC field combination indicating that calcium signaling is important.

Preliminary data show that $45Ca^{+2}$ influx is decreased or increased as the AC/DC field ratio is varied according to parametric theory. This is supported by the first real time fluorescence measurements of $[Ca^{+}2]i$ for Con-A activated rat thymocytes during AC/DC field exposures [1.8 AC/DC]; the plateau phase of $[Ca^{+2}]i$ decreases significantly after 100 seconds of mitogen activation. We also present the first real-time fluorescence measurements of $[Ca^{+2}]i$ and [pH]i during AC/DC field exposure in the presence of a second mitogen, TPA, and both are markedly diminished at the same field conditions [1.8 AC/DC] where an effect is predicted. The above data, obtained using different exposure systems, mitogens, and calcium assays, are consistent with Lednev theory, and strongly implicate calcium and the cell membrane as an interaction site. <u>The primary hypothesis</u> we will test, therefore, is that specific AC/DC field combinations increase or decrease calcium signaling in mitogen-activated thymocytes according to Lednev parametric theory. While monitoring $[Ca^{+2}]i$ and [pH]i, we will systematically vary the AC/DC field intensity ratio, conduct separate AC and DC field exposures, and vary the frequency of the AC field according to theory. Results will be correlated across three different exposure systems for cells exposed in a cuvette, a microtitre plate, or a microscope perfusion chamber. Each system can address different questions, and this is a major strength of the study. Mechanism studies will test calcium ion blocking and substitution, and mitogen binding.

<u>A secondary hypothesis</u> to be tested is that AC/DC field combinations which increase or decrease calcium influx may increase or decrease cell viability and proliferation, respectively. A significant increase in viable cells is seen during co-culture with Con-A, with PHA, or with a Ab directed against the T-cell receptor, in the presence of AC/DC fields that increase calcium influx [5.3 AC/DC intensity ratio, 231mG DC, 16 Hz AC]. This is consistent with calcium signaling in mitogen-activated thymocytes. Selected AC/DC fields ratios that alter calcium signaling will be tested for changes in cell viability and cell proliferation (DNA synthesis).

Experimental Design and Exposure Conditions

Model System: In these studies rat thymic lymphocytes or human T-cells will be used in *in vitro* experiments. Cells will be evaluated for changes in intracellular calcium using ratiometric fluorescent probes sensitive to calcium during activation of signal transduction. As discussed above a number of different agents will be used to activate the cells. Down-stream events following activation, such as DNA synthesis and cell proliferation will also be assessed. We have also evaluated a second model system for lymphocyte mitogen-activated signal transduction, the human T-lymphocyte cell line, Jurkat E6.1. This cell line is perhaps the best characterized lymphocyte model system available for signal transduction studies.

Exposure Systems: We have designed a unique cell culture exposure system involving the use of a gradient coil in a modified Merritt 4-coil configuration so as to establish a gradient time-varying (AC) magnetic field superimposed upon a uniform static magnetic field (DC). This will result in a continuously varying combination of AC/DC magnetic field intensity ratios. Two 96-well plates will be placed in this field so that cells in the wells will be exposed to a variety of AC/DC magnetic field ratios with 16 different ratios corresponding to 8-replicates at each ratio. This results in an excellent structure that lends itself to rigorous statistical analysis. This gradient coil will be operated inside of a mμ metal chamber which will be placed inside of a commercial cell culture incubator. The use of a mμ metal chamber is critical since it acts as a shield against both electric and magnetic fields that are generated during the operation of the commercial cell incubator. These endogenous magnetic fields can be considerable in intensity and the waveforms can vary from 60Hz sinusoidal to pulsed, intermittent bursts. We routinely map the magnetic fields before and after experiments, and we monitor temperature and CO_2 continuously and record values daily. We also plan to perform exposures of cells on a microscope stage and we have designed a special coil for these experiments. In our laboratory we also have a series of existing Helmholtz coil pairs that can be used to expose cuvettes or small volume test tubes to well-defined AC/DC magnetic field combinations. This includes a set of Helmholtz coils that can be operated to obtain real-time fluorescence measurements during exposure to AC/DC magnetic field combinations.

In this reporting period we have also designed and characterized a unique and specialized *in vitro* magnetic field exposure system. This system is a computer-controlled, dual-channel spectrophotofluorimeter that has the following capabilities. This system can monitor real-time fluorescence and absorbance from two independent samples in their respective thermo-reguated cuvette holders located in physically separated modules. A fiber-optic quartz light pipe delivers source light from a computer-controlled dual monochromator and this light is split equally via a bifurcated light pipe attached to the two identical modules. Each module houses identical, matched photometers for collecting fluorescence and/or absorbance signals from the samples. There are excitation and emission monochromators. The two identical sample modules have also been fitted with two square Helmholtz coils for generating a uniform magnetic field.

We are not aware of a similar cuvette-based magnetic field exposure system with the above capabilities. This system will enable computer-controlled operation of experiments to monitor in real-time changes in fluorescence for intracellular calcium measurements, and it will enable the simultaneous monitoring of absorbance changes from the same sample. The latter can be used, for example, to follow changes in UV-VIS signals from dyes that report on redox activity in cells. Such cuvette-based data will represent an important set of endpoints for comparison and support of the calcium measurements we will conduct using a fluorescence plate reader for cell maintained in cell culture and exposed to magnetic fields over relatively long exposure periods.

Exposure Regime: AC and DC magnetic field intensities and frequency will be varied according to AC/DC field interaction models mentioned above. In general the AC/DC field intensity will be varied over several fold increases in magnitude to follow the argument of the appropriate Bessel function through multiple zero crossings of the curve. AC/DC field ratios will be varied, for example, from 1.75 -21.75 corresponding to six zero crossings of the Bessel function curve. An additional factor is specific tuning for the calcium ion. Initial exposures will start using 16 Hz AC field with a 208mG DC field tuned for Ca-40. AC and DC field orientation will be parallel. AC field intensities of rms vs. peak will be compared in these studies. Short-term exposures will be carried out for times up to 60 minutes in the calcium transport studies. Longer exposures of up to several days will be carried out for studies involving DNA synthesis and cell proliferation. Continuous sinusoidal waveforms will be used for AC magnetic fields.

Quality Assurance Measures

Exposure System: We use mμ metal shielding chambers to eliminate stray fields as a possible confounding factor in our *in vitro* exposures. We monitor temperature during exposures and in the cell culture exposures we monitor temperature and CO_2 daily. We use a Multiwave II to verify the shape of the sinusoidal waveform and the frequency composition. We have calibrated AC and DC monitoring equipment from several manufacturers which we cross calibrate against each other: Multiwave II with three-axis Bartington probes(1G and 5G); Leeper Monitor Industries AC probe; Bartington single-axis fluxgate probes (axial and transverse); F.W. Bell Hall Effect AC and DC magnetic field probes. In addition, we have cross calibrated several probes at Battelle Pacific Northwest Laboratories in the laboratory of Dr. Doug Miller.

Blinding: We have blinding protocols we can implement at three levels in the follow manner. First the experimenter is not aware of the treatment groups for activation of the lymphocytes (chemical blinding). Second the experimenter does not know which magnetic fields are generated in the experiment (magnetic field blinding). Third, the data can be analyzed and tested for statistical significance in a blinded manner and then the codes are broken (data analysis blinding).

Experimental Design: We will design experiments so that, wherever possible, paired treatment groups will be comprised of a) sham-sham treatment; b) sham-exposure treatment; and c) exposure-sham treatment. This will rule out any confounding effects due to un-energized exposure treatment.

Positive Controls. We will use various agents to trigger signal transduction in these lymphocyte studies. These responses will constitute the baseline defining the appropriate response of the lymphocytes to activating agents. We will use Con-A, PHA, or antibody directed against the T-cell receptor. Cells that fail to demonstrate a standard response will not be used in magnetic field experiments.

Results

For the first phase of this grant period there were three objectives identified in Table 1 (Timetable for Specific Aims). These were to build and test a Merritt coil. There is a Plexiglas platform that can be placed inside of the coil system to hold the microtitre plates in the central exposure volume. Considerable engineering effort was spent to design the coil exposure system so that a) the position of the four coils are adjustable on Plexiglas rods, b) the coil frame can be rotated 90 degrees if desired to orient the magnetic fields in a vertical position relative to the plane of the platform for the microtitre plates, and c) the platform for positioning the microtitre plates is adjustable in height and can accommodate two sets of adjacent microtitre plates using a double-decker design. The latter is important since we originally designed the exposure system to accommodate one set of adjacent plates. This improvement doubles the possible data for this exposure system.

Dr. Yost is responsible for fabricating a miniature AC magnetic field probe for use in the wells of the 96-well microtitre plates. This probe uses an inductive type pick-up modified from a commercially available core with >1000 turns. Induced voltage from the pick-up passes into a band-limited amplifier and integrator; this can be sent to a spectrum analyzer or RMS AC voltmeter for display. Test results for the miniature probe indicate high sensitivity (1mV/0.1uT) and nearly flat response (1.5%) over the ELF frequency range from 10 to 1000 Hz. The predicted field uniformity across the adjacent microtitre plates is depicted in a figure attached to the abstract, mentioned above. Dr. Yost has delivered this probe to LBL recently for use in the next reporting period.

A second, cuvette-based magnetic field exposure system has been set-up and is operational. This system is discussed above in the section on Experimental Design and Exposure Conditions. The system is unique to our knowledge in that it is a dual-beam, bifurcated fiberoptic-based system with both excitation and emission monochromators with the capability of obtaining fluorescence emission and excitation spectra, and of simultaneously obtaining absorbance spectra. The system is temperature-controlled, and there are identical Helmholtz-type exposure coils for use in generating AC and DC magnetic fields. We have used this system to carry out some of our cellular studies discussed below.

Cellular Studies

Intracellular Calcium Studies. We have conducted a series of studies using Jurkat E6.1 in which intracellular calcium levels have been monitored during exposure to a combination of 1G, 60 Hz magnetic fields and 0.4G static magnetic fields. These studies lymphocytes were either activated, or not, with antibody to the CD3 cell surface receptor to trigger calcium signaling. This response of Jurkat cells is well described in the literature, and we obtain reproducible increases in intracellular calcium over time, in a typical dose-response manner using anti CD3 antibody. This increase in calcium was elicited using anti CD3 antibody from two separate commercial sources (Becton-Dickenson, and Accurate Antibody). We have extended these studies by repeating these calcium measurements in the presence of a combination of 1G, 60 Hz magnetic fields and 0.4G static magnetic fields. In these tests we initially used anti CD3 antibody from Becton-Dickenson. We observe that over a series of multiple experiments an increase in intracellular calcium is detected when cells are activated in the presence of the magnetic fields. Such increases are not observed when cells are resting (not activated with antibody). This data suggests that this magnetic field combination can act to enhance or increase calcium signaling in response to anti CD3 antibody.

Scatchard Studies. The above studies were subsequently followed by experiments in which we tried to identify a possible biological event in the signal transduction pathway responsible for changes in calcium signaling during magnetic field exposure. To do this we conducted Scatchard studies to measure two important endpoints: the dissociation constant for antibody-receptor binding, and b) the number of antibody-receptor complexes on the cell surface. Antibody-receptor binding is the first step in the signal transduction pathway and it takes place at the surface of the cell membrane.

In these studies we employed anti CD3 antibody from both commercial sources mentioned above so that we could a) determine if changes in receptor-binding could be observed using two different antibodies to the CD3 receptor, and b) to verify that any changes we observed were or were not generalizeable to more than one receptor-binding antibody. We conducted a series of studies, at the magnetic field combination mentioned above, using Becton-Dickenson antibody. These exposures were brief for 10-15 minutes, at 37° C. Receptor binding was assessed using fluorescent-antibody. We observed over a series of experiments that the number of antibody-receptor complexes on the cell surface was increased by the magnetic field exposure. This increase in receptor binding was on the order of 25-30%, and this change represents a biologically significant alteration. Such an increase in receptor-binding offers a plausible explanation for the increase in calcium we observe. This is an important biological point, since an increase in receptor binding to the CD3 receptor would act to open more calcium channels and result in an increase in intracellular calcium, which is what we observe.

To confirm or refute these findings we conducted studies using a second antiCD3 antibody obtained from Accurate Antibody, Inc. In a series of experiments we observed that using this antibody to the CD3 receptor there was an increase in antibody-receptor binding in the presence of the magnetic fields. The absolute value of change was not identical to that observed for the Becton-Dickensen antibody, but the direction of the change (an increase) was consistent for both antibodies. This is perhaps to be expected since these two different antibodies are selective for different specialized structures on the CD3 molecule; thus, they bind to different sites. This difference in binding may be responsible for differences in the magnitude of the increase of receptor binding observed for both of the antibodies.

Dose-Response Studies. It is critical for a dose-response relationship to be established to support the observations of magnetic field bioeffects. Recently we have conducted magnetic field dose-response studies to extend our Scatchard data to higher field intensities. In these experiments the AC component of the magnetic field was increase from 1G to 10 G or 100 G. In general, across these studies we have observed that as AC field intensity is increased Scatchard data indicates that receptor-binding is enhanced. This increase does not appear to be linear with field strength, but it appears to be monotonically increasing, and there does not appear to be evidence for a "plateau" or saturation" for receptor-binding.

Replication Studies. It is critical for important bioeffects to be reliably tested by independent investigators. We contacted Dr. Walter Balcavage of the Indiana University Medical School, an experienced scientist in Scatchard and receptor binding research, and suggested a series of replication studies. Dr. Balcavage agreed to undertake these replication studies as an independent scientist; he has not previously interacted with our laboratory. We provided Dr. Balcavage with Jurkat cells from laboratory, as well as antibody, and an exposure coil system. We

also provided technical support to Dr. Balcavage's laboratory in the form of laboratory training in our research protocols employed in our studies.

Dr. Balcavage conducted a series of studies in which he repeated our original Scatchard experiments, using our original protocols, the same identical exposure coils, and the same cells and antibody. He conducted twelve blinded experiments and he observed that EMF resulted in an observed increase in receptor-binding in Jurkat lymphocytes. This is consistent with our previous observations, and Dr. Balcavage presented these findings at an international meeting (BEMS, 1996) as a successful replication study.

Discussion and Contribution to Understanding Biological Effects of EMF

The biological significance of the studies we are conducting relate to several important aspects of enhancing immunology function. In addition, if the observations we have made in our *in vitro* studies can be translated to the *in vivo* setting, the potential exists for the positive application of magnetic fields in medicine to enhance immune function.

The idea of magnetic fields enhancing signal transduction in the lymphocyte follows directly from our findings and the potential for positive applications in medicine is a plausible hypothesis based on our laboratory observations. In our studies we have observed that calcium influx and antibody binding to the CD3 receptor at the cell surface are enhanced in the presence of magnetic fields. The changes in intracellular calcium we observe are within normal limits of change of intracellular calcium when lymphocytes are activated; thus, magnetic fields increase intracellular calcium so that signal transduction is enhanced, but not to an extent that results in untoward consequences that effect, for example, cell viability. The parameters of intracellular calcium and receptor-ligand binding which we have investigated are important markers for signal transduction and both occur early in the signal transduction cascade, with receptor-ligand binding representing the first event that triggers this cascade. The observation that both parameters are enhanced is consistent since an increase in receptor-ligand binding to the CD3 receptor should result in a greater number of calcium ion channels opening, and, thus, an increase in calcium ion influx into the lymphocyte. The dose response relationship we have observed for this effect, and the independent replication, discussed below, of our original observation of magnetic fields enhancing antibody binding to the CD3 receptor further supports the credibility of these magnetic field induced changes.

Magnetic fields act synergistically with the activating agent anti CD3 antibody in our studies and enhance signal transduction in lymphocytes. A practical result of this synergistic interaction is that less antibody is required to achieve the same level of lymphocyte activation in the presence of the magnetic field. If this EMF effect is observed in the *in vivo* setting, the individual's lymphocytes and immune system could be "boosted." For individuals who are immunosuppressed, such as patients on drug regimes that have side effects which are immunosuppressive, or patients with disorders of the immune system involving an impairment of lymphocyte activation, or older patients who experience immune system impairment, the use of magnetic fields holds the potential to "boost" immune responsiveness.

Publications From This Grant

1. Roy S, Noda Y, Eckert V, Traber MG, Mori A, Liburdy RP and Packer L. "The Phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils is increased by a 0.1 mT (60 Hz) magnetic field." FEBS Letters, 376:164-5, 1995.

Other EMF Publications

1. Harland JD and Liburdy RP. “Environmental magnetic fields inhibit the antiproliferative action of tamoxifen and melatonin in a human breast cancer cell line.” Bioelectromagnetics 18:555-562, 1997.
2. Loscher W and Liburdy RP. “Animal and cellular studies on carcinogenic effects of low frequency (50/60-Hz) magnetic fields.” Mut. Res. 410:185-220, 1998.

3. Harland JD, Engstrom S and Liburdy RP. "Evidence for a slow timescale of interaction for magnetic fields inhibiting tamoxifen's antiproliferative action in human breast cancer cells." Cell Biochem. Biophys., in press, 1998.

4. Harland JD and Liburdy RP. "Cytostatic drugs act less effectively to inhibit breast cancer cell proliferation during co-treatment with environmental magnetic fields." Submitted, 1998.

TITLE: **ELF Magnetic Fields, Gene Expression and the Cell Cycle**

Principal Investigator: Howard B. *Lieberman*, Ph.D.
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New York, New York

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Signal Transduction

RO3-ES08931 **Start Date:** 01/15/97 **End Date:** 12/31/98

Abstract

Epidemiological studies suggest that extremely low frequency (ELF) magnetic fields (MF), which are generated from power transmission lines and other common electrical devices, can cause cancer. However, little laboratory data are available to either support or refute these findings, or define the critical biological targets or molecular response of cells to these fields. This proposal focuses on the identification of changes in protein synthesis profiles induced by ELF-MF exposure and the molecular mechanisms in control. The fission yeast *Schizosaccharomyces pombe* is used as a model system because it can serve as a sensitive tool to detect biological changes induced by ELF-MF and to dissect the genetic basis for any alterations observed. Our initial studies were carried out using the exposure device of Goodman and Henderson. Using 2D gel electrophoresis, we found that synthesis of three proteins (of approximately 500 proteins detected by this procedure) was transiently turned off in wildtype *S. pombe* cells after exposure to a 60 Hz 80μT MF for 20 min. The fact that only three proteins were affected by this treatment demonstrates the high specificity of the molecular response. Furthermore, following the same treatment, yeast cells containing the checkpoint control gene mutation ras 9::ura 4 also transiently cease synthesis of two of these proteins, but continue to make the third, indicating that regulation of this latter protein is rad 9 dependent and linked to cell cycle control. No new proteins were detected after ELF-MF treatment. These observations form the foundation for the current proposal. Studies in this application will be performed with a Magnacell MC2xc ELF-MF system, which is currently used by the NIOSH and the EPA for investigation and meets all requirements set forth by the NIEHS to generate true MF of defined intensities while minimizing the production of electric fields. The specific goals of this proposal are to identify the three regulated yeast proteins, and isolate and characterize the genes encoding these proteins. Regulation in other *S. pombe* check point control mutants, containing rad11, rad3136 or rad17W, will be examined to test whether the rad 9 dependent control is, in general, a checkpoint-related effect. The ability of ELF-MF to induce cell cycle delay in *S. pombe* cells will also be examined. Synthesis of these proteins in heat-shocked and gamma-irradiated cells will be examined to test whether they are part of pathways that generally respond to insults. Studies will also be conducted to identify the gene regulatory sites. Together, the results of this study should identify proteins regulated by ELF-MF and reveal the nature of the molecular mechanisms in control.

Experimental Design and Exposure Conditions

Exposure Conditions: For this proposal, we will use the MC2xc concentric coil *in vitro* system designed and manufactured by Columbia Magnetics, Inc. (Kennewick, WA) to generate ELF-MF. This equipment circumvents several problems commonly encountered by investigators studying ELF-MF i.e., exposure field uniformity, stray field containment, and suitable sham exposures. The MC2xc, powered at 50 or 60 Hz by a variable transformer, utilizes two Merritt 4-coil sets arranged in a concentric configuration to limit stray fields. Exposure chambers are connected to a commercial incubator (Forma model 3026), held remote so that the required culture atmosphere is obtained, but incubator MF do not interfere with exposure or sham field information. One chamber is used for sham exposures and thus the system contains an active compensation device to cancel weak ambient MF while

maintaining equal current in sham and exposure coils. Coil MF are calculated and controlled by computer (486/33MHz/8MB/230MV) which records temperature and MF. The system can be operated to facilitate blind experiments as to the field status of the chambers during experiments. The temperature difference in remote exposure chambers is 0.1 C with the MF uniformity better than 5%. In opposed current flow mode, field cancellation results in <1 volt meter. The system is housed in a fully equipped biology lab, and is located such that incidental MF from freezers, magnetic stirrers, current carrying transformers in walls, etc., are minimized.

Preliminary data was obtained using the Helmholtz coil-based equipment of Dr. Reba Goodman Columbia University. Our laboratory received the MC2xc machine. The new machine will be used for future experiments. We are aware of guidelines issued for quality control and these will be implemented.

Magnetic Field Exposure Parameters:

- MF strength: 10 T to 80 T
- Duration of each ELF-MF exposure: 20 min to 4 hrs
- Number of separate exposure periods: single exposure
- Field oscillation frequency: 60 Hz
- Polarization: linear
- Relative orientation of AC and DC MF: MF will be in the vertical plane. When oriented on the earth's geomagnetic axis, the earth's DC field is virtually eliminated
- Intermittency: continuous exposure
- Turn-on and turn-off transients: Fields will be activated before cells are introduced into the MC2xc. After cell removal, fields will be turned off
- Field spatial homogeneity: < 5%
- Field temporal homogeneity: no variations
- MF geometry relative to cells in device: vertical plane
- Accessory non-ELF-MF exposure: Neither heat nor vibration is detectable at 2.0 amps (approx. 1 mT). Exposure chambers are mechanically isolated from coils. The temperature difference in remote exposure chambers is 0.1^0C while incubator temperature stability is 0.4 C. Yeast cells, growing logarithmically in 15 ml of appropriate media, will be placed into T25 flasks, as described below, and inserted into incubators, which will not be CO_2-controlled (not necessary for *S. pombe*) and will be held at 30^0 C. One chamber is used for sham exposure and therefore the system contains an active compensation device to cancel weak MF while maintaining equal current in the sham and exposure coils. Coils are electrically shielded and doublewound using a twisted fibillary winding technology that provides the best possible cancellation in the counterphase mode. In opposed current flow mode, field cancellation results in <1 mG residual field at 1 amp and electric fields in exposure chambers <1 volt meter. The volume of media will be such that effects of CO_2/O_2 tension are negligible
- Incidental ELF-MF exposure: Incubators housing sham and treated cells will have internal shields to minimize incubator-induced fields. The MC2xc will be located such that incidental MF from freezers, magnetic stirrers, current carrying transformers in walls, etc., are minimized.
- Monitoring MF: Routine manual assessment of MF in incubators which house cells prior to and following exposure will be determined by devices described below.

Magnetic Field Dosimetry: MF at the site of exposure, and ambient stray fields encountered by cells during preparation and transit to and from the exposure lab, will be measured by:

- A Magnetic Sciences International gaussmeter (MSI25). This is a passive coil instrument with a range of 0.1 mG to 200 G, and an accuracy of 2% for readings. This device is factory calibrated and useful for quick checks of 60 Hz ELF. Because of its high sensitivity to very small fields, it is also useful to check the zero field sham region. It responds accurately to higher harmonics (up to 180 Hz). At higher frequencies, it has a linear response up to 4 kHz. It cannot be used for DC fields or for precision measurements.
- A Walker Scientific Hall effect gaussmeter (Model MG5DP). This general purpose instrument is capable of AC and DC measurement, with a true RMS reading for AC fields and a peak detection circuit for recording peak values. It has a range over three scales of 100 mG to 19.99 kG. Accuracy for RMS readings is frequency dependent, +\- 1% at 10 Hz-20 Hz for the 100 G scale, and +/- 0.3% for higher scales. DC accuracy is +/-

0.05%. The Hall effect probe has an active dimension of about 1 mm x 1 mm, and thus a spatial resolution more than adequate for mapping MF over the MC2xc. This will be the primary instrument for MF dosimetry and monitoring field uniformity.

Induced Electric Fields and Current Measurements: Exposure to an externally applied varying MF will induce an electric field and associated current in culture medium. Consequently, during *in vitro* studies with AC MF, cells will be exposed to at least three parameters: the MF, the induced electric field and an electrical current. These induced parameters will depend upon the exposure geometry and the culture medium conductivity. For *in vitro* experiments, the magnitude and direction of the induced fields and associated currents depend upon flask geometry and direction of AC MF relative to the flask. We are able to make direct measurements of currents and fields by use of a Keithley Model 237 Source/Measure Unit. This is a high precision and accuracy device that can either source voltage and measure current or source current and measure voltage. Its accuracy in current or voltage measuring modes is +/- 0.03%, except for currents of 0.1 to 10pA, at which it is 0.3%. It has a range of 1100.0 V in four ranges for voltage and 10 fA to 1 A for current. The induced field can be calculated easily if the MF is known, and the resulting induced current can be measured easily. Alternatively, it is possible to measure conductivity of the cell medium directly, and to calculate induced currents in the medium with the application of an AC MF. All that is required is a flask with electrodes implanted at various locations to serve as probes for measurement. This allows monitoring a parameter that may be of significance for expression of a given biological effect, or in any case verifying whether such parameters as medium conductivity or flask geometry are important.

Special Cell Culture Considerations During ELF-MF Exposure: Because of induced electric fields and attendant currents that depend upon flask geometry and relative orientation of the MF vector, it is necessary to note conditions under which cells are grown and exposed for data interpretation. The following relevant parameters will be recorded and monitored

- The MC2xc produces ELF-MF with the field vector oriented perpendicularly. Flasks will be horizontal
- It is expected that T25 flasks will be used for *in vitro* studies. Induced fields and currents will be determined, as was reported previously (Weisbrot *et al*., 1993)
- Flasks will be sterilized by gamma-rays
- CO_2/O_2 tension: The depth of the culture medium will be 25 mm, to maintain active growth
- Buffering and pH: Yeast growth medium is well-buffered to maintain physiological pH
- Temperature: The Forma incubator will maintain temperature at 30^0 C, +/-0.20 C
- Humidity: The incubator can maintain humidity at 95%. However, this is not crucial for yeast
- Initial cell density and growth phase: At 1 x 10^7 *S. pombe* cells/ml, there will be enough fresh medium and space to support growth during experiments
- Incubators will be surveyed for background ELFMF, and modified if necessary
- Incidental MF exposures will be monitored and recorded. The laboratory housing the MC2xc will be surveyed for background during experimentation. Passage ways from the exposure room to the cell manipulation lab will be surveyed during the working day to assess background field strength. The lab housing cells will be monitored twice daily to determine field strength imposed during the entire incubation period.

Quality Assurance Measures

To insure appropriate MF treatments and machine use, careful measurements are to be made by our in-house physicists/dosimetrists, Dr. Paul Kliauga and Dusan Srdoc, under the guidance of three external advisory committee members, Drs. Kenneth McLeod (Stony Brook University, NY), Fred Dietrich (Electric Research & Management, Inc., Pittsburgh, PA), and Tom Tenforde (Pacific Northwest, WA). The committee will meet once a year in New York to review progress and examine the MC2xc to ensure proper use. Dr. McLeod has already visited the lab, taken MF measurements, and has examined and approved our exposure system. As an added quality control, Dr. Kliauga or Dr. Srdoc will place all samples in the machine for simultaneous sham and MF treatment. Samples will be coded so the physicists will not know what the samples are, and they will not tell us which cells were sham or MF treated until after protein isolation and analyses or other appropriate types of processing and scoring have occurred.

Results

The preliminary data described in the original application resulted from experiments performed with the Helmholtz coil based system first constructed and used by Dr. Reba Goodman, who is currently at Columbia University. At the time, samples were exposed to a 60 Hz 80 T MF for 20 minutes. Since that time, our Department has obtained a NIEHS approved Magnacell MC2xc elf-MF system to perform the experiments outlined in the grant. Our initial studies with this system have failed to reproduce the changes in protein synthesis observed previously with the Helmholtz coil machine. The same ELF-MF exposure conditions were presumably applied to *S. pombe* samples, but no changes in protein synthesis were detected relative to sham-treated controls when the new system was used. The results indicate that there is a fundamental, but as of yet unidentified, difference between the Helmholtz coil system and the Magnacell MC2xc machine, which are both used routinely by other investigators for MF-related experiments. The reason for the machine-dependent differences in protein synthesis observed is being pursued.

Discussion and Contribution to Understanding Biological Effects of EMF

Numerous epidemiological studies suggest a correlation between exposure to extremely low frequency (ELF; up to approximately 100 Hz) magnetic fields (MF), primarily generated by power transmission lines and common electrical devices, and the induction of cancer in humans (Wertheimer and Leeper, 1979; Savitz and Calle, 1987; Stevens, 1987; Brown and Chattopadhyay, 1988; Bates, 1991; Goldberg and Creasey, 1991; Stevens *et al*., 1992). However, at best, these studies only suggest some relationship between ELF-MF and cancer, but do not conclusively prove cause and effect. In fact, to date, few reports describe laboratory studies that reproduce ELF-MF-induced cancer in animals or morphological transformation of cells *in vitro* (Hintenlang, 1993; Balcer-Kubiczek and Harrison, 1991). ELF-MF do not possess enough energy to permanently disrupt chemical bonds in DNA (Cohen *et al*., 1986; Reese, Jostes and Frazier, 1988; reviewed in Murphy *et al*., 1993). Therefore, any biological changes caused by these MF must be mediated through mechanisms that most likely do not involve DNA damage. Nevertheless, despite the limited laboratory studies linking deleterious biological effects with ELF-MF, the prevalence of ELF-MF generating devices in the environment, and the existence of epidemiological data suggesting that MF can induce cancer, are cause for concern.

Several studies indicate that ELF-MF can modify cell cycle kinetics. Khalil and Quassem (1991) demonstrated that short 50 Hz MF exposures can alter the mitotic index of human lymphocytes. Goodman, Greenebaum and Marron (1979) described studies with the slime mold, *Physarum polycephalum* that indicated ELF-MF can alter cell cycle kinetics in this organism. Furthermore, we detected different protein synthesis patterns in wildtype *S. pombe* as compared to rad9::ura4 checkpoint control mutant cells treated with ELF-MF. These studies suggest that ELF-MF can influence the cell cycle. The molecular mechanisms mediating the altered regulation, or its significance to cancer induction, are unknown. In addition, since changes in cellular membranes caused by ELF-MF have been detected (Adey and Sheppard, 1987), they may also be of importance in relation to cancer or other harmful effects caused by MF. Interestingly, cdc2, a protein important for cell cycle regulation, can phosphorylate lamin B, a major component of nuclear membranes (Dessev *et al*., 1991; Loscher *at al*., 1991).

Alterations in gene expression have been detected in cells exposed to DNA damaging agents, as well as heatshock (Hollander and Fornace, 1989; Woloschak and Chang-Liu, 1992). These studies have been useful for understanding the biological response of cells to treatment by these agents. Several studies indicate that MF can modify gene expression. Goodman and coworkers (1992) found that transcription, in general, can be increased in salivary glands of the dipteran, *Sciara coprophila* after exposure to ELF-MF, and alterations in protein synthesis patterns are also observed (Goodman and Henderson, 1988). There is some overlap between proteins synthesized in response to heatshock versus ELF-MF, but only the former causes a significant decrease in total cellular protein synthesis (Goodman and Henderson, 1988). In addition, transcription of beta-actin, histone H2B and *myc* RNA are also altered in human HL60 cells after MF treatment (Goodman *et al*., 1989). Peak increases in transcription occurred in a narrow window of exposure parameters (Wei, Goodman and Henderson, 1990). Analysis of *c-myc*/CAT gene fusions led Lin, Goodman and Henderson (1994) to identify a 904 basepair DNA region upstream of the *c-myc* gene that responds to ELF-MF exposure.

The use of mammalian cells to study cellular processes affected by ELF-MF in the lab pose several problems. However, use of the fission yeast *Schizosaccharomyces pombe* affords many advantages (Zhao and Lieberman, 1995). Yeast can be manipulated much more readily. They are less expensive to grow, and have a shorter doubling time (2 to 4 hours versus greater than 12 hours) and a higher plating efficiency (approx. 100%). Yeast genetics are comparatively well understood. Haploid cells can be diploids, and sporulation can be induced, resulting in the formation of haploid progeny. Haploid and diploid cells can be isolated and analyzed. Recombinant DNA methodologies are also more advanced in yeast systems. Many vectors are available for gene cloning and expression. Furthermore, homologous recombination systems are very active in yeast, permitting transplacement, whereby genes manipulated *in vitro* can efficiently replace those located on a chromosome.

Similarities in several aspects of yeast and mammalian cellular processes suggest that studies with *S. pombe* can provide insight into mammalian systems. The cloning of human cell-cycle-related genes (*cdc-2, cdc-25 and wee-1*) following functional complementation of *S. pombe* mutants (Lee and Nurse, 1987; Okazaki *et al*., 1990; Igarashi *et al*., 1991), cell division by binary fission (Gutz *et al*., 1974), the ability of *S. pombe* chromosomes to persist and replicate autonomously in mouse cells (Allshire *et al*., 1989), the ability to use SV40 promoter sequences (Jones *et al*., 1988), the prevalence of genes with introns (Mao *et al*., 1982; Hindley and Phear, 1984) and the appropriate processing of mammalian introns (Kaufer, Simanis and Nurse, 1985) exemplify the similarities between comparable cellular and molecular processes and structures in *S. pombe* and mammalian systems. Thus, the study of ELF-MF effects in *S. pombe* could provide important information not attainable by research focused solely on mammalian cells.

In terms of DNA repair, approx. 30 independent repair-related rad loci have been identified in *S. pombe* through genetic analyses (Schƒpbach, 1971; Nasim and Smith, 1975; Schmidt *et al*., 1989; AlKhodairy and Carr, 1992; AlKhodairy *et al.*, 1994; Ford *et al*., 1994; Tavassoli *et al*., 1995; we identified rad23, Lieberman *et al*., 1989). Alterations in rad1, rad3, rad9 or rad17 (Schƒpbach, 1971), four of these loci, cause extreme radio-sensitivity, indicating that the encoded proteins must play key roles in promoting cell survival following exposure to DNA damaging agents. Mutations within these genes also eliminate the ability of cells to delay cycling transiently in G2 after irradiation (i.e., G2/M checkpoint control; Hartwell and Weinert, 1989), a process in wildtype cells thought to provide extra time for repair of damaged DNA before entry into mitosis. Therefore, the inability to delay cycling is at least partly responsible for the radiosensitivity of these mutants. As such, rad1, rad3, rad9, and rad17 provide a link between radiation exposure and cell cycle control (AlKhodairy and Carr, 1992; Rowley, Subramani and Young, 1992). Interestingly, we found that ELF-MF turns off translation of three proteins in wildtype *S. pombe*, but rad9::ura4 cells continue to synthesize one of these even after exposure, suggesting a relationship between checkpoint control and ELF-MF.

This proposal focuses on three yeast proteins whose syntheses are regulated in response to ELF-MF. The goal of this grant is to identify these proteins, and clone and characterize the corresponding genes. The structure of the genes will be determined and the region of ELF-MF responsiveness will be defined. Since a link between a checkpoint control gene, rad9, and ELF-MF has been established, and the literature contains several examples of the ability of MF to alter cell cycle progression, this relationship will be examined further, both genetically using an array of *S. pombe* checkpoint control mutants and also by testing whether ELF-MF can induce a transient delay in cell cycle progression. This is especially important since we now have a human homologue of *S. pombe* rad9 (Lieberman *et al*., 1996), as well as a mouse version (H. Hang, H.B. Lieberman *et al*., manuscript in preparation), indicating that what we learn about mechanisms of cellular responsiveness to ELF-MF in yeast will have relevance to humans.

Publications From This Grant

None.

Other EMF Publications

None.

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TITLE: **Studies of the Mechanism of EMF Induced Bioeffects**

Principal Investigator: Theodore A. *Litovitz*, Ph.D.
Catholic University of America,
Washington, DC

Health Relevance: **C**ellular Function

Research Area: Cellular Processes; Ornithine Decarboxylase; Biophysics of Cell/Field Interactions

R01-ES06872 **Start Date:** 09/25/95 **End Date:** 08/31/98

Abstract

The existence of effects induced in biological systems by exposure to weak, extremely low frequency (ELF) electromagnetic fields (EMF) is gaining acceptance with some members of the scientific community. This fact, coupled with epidemiological data which suggest the possible involvement of such weak EMF in the incidence of leukemias and other cancers, has raised interest in the interactions of fields with living organisms. The cell-field interaction mechanism, however, is not understood. The research described in this proposal focuses on the investigation and characterization of the mechanisms by which cells detect, and respond, to weak, externally-impressed fields, while discriminating against intense, endogenous thermal noise fields. Most of the proposed research will be conducted using mammalian cultured cells as the target of EMF exposure, primarily applied as 60 Hz sinusoidal magnetic fields. The specific activity of the enzyme ornithine decarboxylase will serve as the biological endpoint for many of these studies. Another end point will a study of the effect of EMF induced stress proteins. The work will consist of a series of experiments directed toward four major goals:

- Preliminary studies have established that cellular detection of EMF involves at least two important time scales: a rather short "sensing" interval, and a somewhat longer "memory" time. The temporal detection parameters will be thoroughly characterized, and the resultant data used to describe the cell-field interaction mechanism.
- Experiments will examine the dose-response characteristics that determine cellular response to applied EMF. This will include an examination of amplitude thresholds for bio-response characteristics at various field frequencies, and an examination of what constitutes a "minimum effective dose" of field exposure.
- Applied ELF noise fields with electric field amplitudes 100-1000 times smaller than those calculated for thermal noise fields have been shown to reduce, or to eliminate, the typical cellular response to applied 60 Hz magnetic fields. A series of EMF exposures will be conducted to explore the crucial differences, in terms of such inhibitory effects, between such externally impressed noise fields and the endogenous thermal noise fields.
- Studies at Columbia University have shown that stress proteins are induced by weak magnetic fields. To explore the implications of this finding we will determine the survival rate of chick embryos exposed first to weak EMFs and then subjected to anoxic conditions. Our goal is determine if the induction of stress proteins by EMFs confers protection against schema/reperfusion.

Experimental Design and Exposure Conditions

Investigation of Temporal Sensing as a Feature of EM field detection: The heart of the proposed temporal sensing research is the execution of a set of experiments designed to explore thoroughly the kinetics of the sensing

(averaging) and memory processes. We shall investigate the dependence of these processes on the time variations in the field parameters (e.g. amplitude, frequency, and waveform) of the stimulating field.

Characterization of the Inhibitory Effects of superimposed EM noise fields: Our preliminary data have demonstrated that, under certain conditions, super-imposing an incoherent (noise) EM field on a coherent EM field can inhibit the induction of bioeffects. We propose to explore extensively the parameters of the noise field which are crucial for inhibition effects. For example, we shall consider such things as band width of noise, necessity for colinearity between coherent and incoherent fields, and time duration of noise. It is our hope to use this information to understand better how the cell discriminates against the local thermal noise fields and yet can be affected by an externally applied noise field which is 1,000 times smaller.

Determination of the Dose Response relationship between the EMF and the bioeffect: Our preliminary data (and those of others in the literature) suggest that a rather sharp, dose-response amplitude threshold exists for induction of bioeffects by an EMF. Our first studies in this area will have as their goal the confirmation and characterization of these thresholds. In addition we propose to utilize the sharpness of the thresholds in a study of the frequency dependence of the threshold amplitude in order to explore the importance of the dielectric constant (and therefore of counter-ions) in the field-cell interaction mechanism.

Determination of the effect of a "preconditioning" EMF exposure on the survival rate of chick embryos after simulated schema/reperfusion. Our preliminary data suggest that exposure to weak EM fields can inhibit the damage to anoxia in chick embryos. This will be done by placing the embryos in an argon atmosphere after exposure to a weak magnetic 60 Hz magnetic field. Our goal is to study this effect in greater detail to determine the feasibility of using weak EMFs to limit the damage due to heart attacks.

Very uniform magnetic fields can be generated over large regions using paired coils arranged in the Helmholtz configuration. This arrangement uses two circular coils of equal radius, positioned parallel to each other at a distance equal to the radius. The coils are connected in series, with the current traveling in the same angular direction in both coils. The resulting magnetic field is substantially uniform within a portion of the volume between the coils. The regions of a Helmholtz coil within which the magnetic field varies by not more than 5% is roughly equal to 12% of the volume between the coils. This corresponds to a cylinder concentric with the axis of the coils, and with a diameter equal to 35% of the diameter of the coils.

Cell monolayers will be exposed in 75 cm^2 tissue culture flasks containing 15 ml of nutrient medium. Three flasks will be stacked, one atop the other, and positioned within the Helmholtz coils such that the cell monolayers lie within a 2.5 radius concentric with the axis of the coils. A rectangular sample pattern will insure that the samples are correctly positioned within the Helmholtz coils. Each coil will be wound on a 15 inch aluminum form which will be electrically grounded. These coil dimensions insure that the magnetic field in the sample exposure region is within 5% of the axial field.

Quality Assurance Measures

To provide proper sham exposure conditions with the same apparatus and current flow, the coils were constructed using paired conductors placed adjacent to each other. Each coil consists of N turns of the paired conductors connected in series, such that electric current flows in the same direction through 2N loops, or in opposite directions through each wire of the paired conductors. In the first case the induced magnetic fields from each loop reinforce each other, and in the latter they cancel each other. The use of paired conductors insures optimum field cancellation. This arrangement enables experiments to be conducted under identical conditions for sham and exposed samples, except for the magnetic field. Each set of coils is housed in a separate incubator which has been calibrated to provide the same temperature as the two incubators that house the other sets of coils.

For the bulk of the proposed work we will use the activity of ornithine decarboxylase (ODC) to assess response to EM fields in the murine fibroblast cell line L929. Significant results obtained with L929 will be confirmed by limited replicate experiments using at least one other cell type. Such confirmation will be done using the Daudi human lymphoma line. When appropriate, we will also confirm the relevance of findings in cultured cells for an *in vivo*, multicellular system by investigating EMF-induced changes in ODC activity in chick embryos. ODC analyses will be run in triplicate.

Results

During the past year our research has had several remarkable new results and we have pushed ahead in several areas in order to exploit our discoveries. This results include the following,

- The discovery that weak ELF-EM fields can be used to protect against damage due to heart attacks (schema/reperfusion). This work was performed on chick embryos and was based upon the experimental results of Goodman and Blank at Columbia University. which suggested that weak EM fields could induce stress proteins. The results to-date indicate that a doubling in the survival rate can be achieved by use of short time (20 min) exposures to ELF-EM fields.
- The discovery that weak EM fields (a non-ionizing radiation) can be used to protect against damage due to ionizing radiation (e.g. UV light). We have found that exposure to weak ELF-EM fields for a period of 20 minutes induces protection against UV radiation which lasts for at least several hours. This result is consistent with the concepts of radio-adaptation. It is also consistent with the idea that stress proteins are involved in radio-protection.
- The discovery that 60 Hz mechanical vibrations can cause similar biological effects (specifically protection from anoxia) as 60 Hz EMFs. It appears that mechanical vibrations induce stress proteins in the same manner as does EM fields. Even the concepts of temporal sensing which we have developed for EM field induced bioeffects are found to apply here.
- The discovery that ELF-EM noise fields can inhibit the bioeffects induced by mechanical vibrations in a manner similar to the inhibition of coherent EMF induced bioeffects by EM noise. This suggests that mechanical vibration and EM fields utilize the same signal transduction pathways.
- The completion of a theory (based upon displacement of counter ions in the double layer) which explains how weak ELF-EM fields can induce biological effects. This theory predicts that weak electric fields are amplified near the surface of a cell in the region of the receptors. By comparison with the enhancement in the dielectric constant at low frequencies the data suggest an amplification in the field by a factor of over 100.
- The execution of a series of experiments which demonstrate that the theory correctly predicts the frequency dependence of the threshold electric field for induction of stress proteins.
- The study of the dose response characteristics of 3 different EM induced effects. a) EM induced enhancement in ODC activity, b) EM induced protection against anoxia/reoxygenation, and c) EM induced abnormalities in chick embryos (Juutilainen *et al*). We have demonstrated that the typical dose response, in these EM induced effects is sigmoidal with a typical threshold value in the range from I to 4 μT. This demonstrates that the EM dose response is typical of that of found for other physical and chemical stimuli.
- We have discovered that when an EM field is used to stimulate a stress response it can under chronic exposure conditions down regulate the response and cause the biological system to be more vulnerable to anoxia and other stresses.

Discussion and Contribution to Understanding Biological Effects of EMF

The sum total of our efforts prove that weak EM fields act like many other physical and chemical stimuli in biology. They induce bioeffects which can be adverse or beneficial for human health depending upon exposure conditions. The beneficial effects discovered above (protection against heart attacks) have the potential to save over 100,000 lives per year. The value of this one discovery could more than justify an EMF research budget 100 times that of the Rapid Program.

Publications From This Grant

1. Penafiel M, Litovitz TA, Krause D and Mullins JM. "The role of modulation in microwave induced bioeffects. " Bioelectromagnetics 18:132-41, 1997

2. Litovitz TA, Penafiel M, Krause D, Zhang D and Mullins JM. "The role of temporal sensing in bioelectromagnetic effects," Bioelectromagnetics 18:388-395, 1997.

3. Farrell JM, Barber M, Krause D and Litovitz TA. "The superposition of a temporally incoherent magnetic field inhibits 60 Hz-induced changes in the odc activity of developing chick embryos." Bioelectromagnetics 19:53-56, 1998.

4. DiCarlo AL, Farrell JM and Litovitz TA. "Myocardial Protection Conferred by Electromagnetic Fields." Circulation, In press, 1999.

5. DiCarlo AL, Farrell JM, and Litovitz TA. "A Model and Biomarker for Electromagnetic Field Effects: Cardiac Protection is Conferred by Exposure to to 60 Hz Magnetic Fields." Bioelectromagnetics, in press. 1998.

6. DiCarlo AL and Litovitz TA. "Is genetics the unrecognized confoudning factor in bioelectromagmetics flock dependence of field induced anoxia protection in chick embryos." Chemistry and Bioenerg., In press, 1998.

Other EMF Publications

1. Litovitz TA, Penafiel LM, Farrel JM, Krause D, Meister R and Mullins JM. "Bioeffects induced by exposure to microwaves are mitigated by ELF noise." Bioelectromagnetics 18:422-430. 1997.

2. Farrell JM, Litovitz TL, Penafiel LM, Montrose C J, Doinov P, Barber M, Brown KM and Litovitz T. "The Effect of pulsed and sinusoidal magnetic fields on the morphology of developing chick embryos." Bioelectromagnetics 18:431-438, 1997.

3. Farrell JM, Barber M, Krause D, Litovitz TA. " Effects of low frequency electromagnetic fields on the activity of ornithine decarboxylase in developing chicken embryos." Bioelectrochemistry and Bioenergetics 43:91-96, 1997.

Pertinent U.S. Patents (inventor or co-inventor):

1. Protection of Living Systems from the Effects of Schema, Anoxia and Hypoxia by use of Electromagnetic Fields (1997, pending).

TITLE: **EMF Effects on Cell Regulation in A-T Cells**

Principal Investigator: Lise I. *Loberg*, Ph.D.
IITRI Research Institute,
Chicago, Illinois

Health Relevance: Cancer

Research Area: Cellular Function; Cell Proliferation; Cellular Processes

RO3-ES08921 **Start Date:** 1/10/97 **End Date:** 12/31/98

Abstract

EMF is an ubiquitous environmental exposure which may play a role in the induction of cancer. The actions of EMF in biological systems may be enhanced when exposure occurs in combination with genotoxic insults. As such, a plausible mechanism of action of EMF may be modulation of cellular responses to agents which damage DNA. The hypothesis of the proposed program is that EMF exposure alters the molecular regulatory pathways which are normally activated in cells exposed to DNA damaging agents. The function of these pathways in normal cells is to induce cell cycle arrest, presumably to allow for repair of damage before DNA replication or mitosis takes place. A corollary hypothesis is that the effect of EMF exposure will be pronounced in cells cultured from patients with the genetic disease ataxia telangiectasia (AT). AT cells have defects in DNA damage-induced cell cycle arrest and are sensitive to cell killing and chromosome damage after exposure to other components of the electromagnetic spectrum (i.e. ionizing and ultraviolet radiation). In these experiments, AT and normal cells are exposed to mitomycin C, a DNA damaging agent and apoptosis inducer followed by immediate exposure to EMF. The molecular mechanism of EMF is investigated by measuring components of signal transduction pathways known to be involved in the cellular response to DNA damage. G1/S arrest is investigated by measuring p53 and related protein expression; DNA synthesis arrest is investigated by measuring expression of cyclin A; G2/M arrest is investigated by measuring cyclin B1. To elucidate whether EMF effects are transduced via cellular membranes, the MAP kinase cascade is investigated by measuring transcription of *c-jun* and activation of the signal transducing kinases ERK1 and ERK2. Survival of AT and normal cells following mitomycin C /EMF exposure is to be measured, to determine whether EMF effects on cells impact on measurable biologic outcomes. These experiments are designed to investigate EMF effects on proliferation and survival of cells with prior exposure to a genotoxic agent. In addition, the mechanism of EMF action is investigated by measuring various components of cellular regulatory pathways which lead to inhibition of cell cycle progression. These experiments will also determine whether cells cultured from AT patients have unusual sensitivity to EMF effects.

Experimental Design and Exposure Conditions

- Cell culture: Four lymphblastoid cultures, two AT and two normal, are maintained under standard growth conditions. Prior to experimentation, cells are maintained under low ambient EMF.
- DNA Damage: Mitomycin C induces base damage in DNA leading to inductor of p53 protein. Cell cultures are treated with 10μg/ml mitomycin C and immediately placed into the exposure chambers. Samples are removed at 4, 12 and 24 hours, and are prepared for analysis of protein expression.

- EMF Exposure: To determine whether EMF alone affects growth survival or protein expression in A-T compared to normal lymphoblasis cell cultures are exposed to EMF for 0, 3, 6 or 24 hours.

Exposure Conditions:

- Magnetic Field Polarization, Frequency, and Harmonic Content
- Linearly polarized, pure 60 Hz fields; < 3% total harmonic distortion.
- Magnetic Field Intensity Range 10 mG to 10 G.
- Relative Orientation of AC and DC Magnetic Fields AC fields vertical (parallel to primary component of DC field).
- Associated Electric Fields < 10 Volts/meter.
- Field Spatial Homogeneity Uniform to + 5% throughout exposure volume.
- Field Temporal Homogeneity No variations expected (experimental fields are derived from function generator, independent of line voltage). Field temporal homogeneity is determined periodically throughout research program.
- Field Monitoring Continuous throughout program (during both exposures and field-off conditions; monitoring includes both field strength and waveform).
- Geometry of Cell Culture System: Magnetic field exposure will be vertical (perpendicular to monolayer cultures of human mammary epithelial cells).
- Environmental Conditions: Temperature in all exposure chambers will be maintained at 37 + 0.2 ^{o}C, in an environment held at 5.0 + 0.5% CO_2. Environmental conditions in all EMF exposure chambers are controlled by a single incubator located remotely from the exposure chambers
- Stray Fields Laboratory site fully mapped for ambient 60 Hz and geomagnetic (dc) fields. Temporal characteristics of ambient magnetic fields have been determined; ambient magnetic fields are < 1 mG. No DC field cancellation. All incubators are Fraday-shielded.
- Sham Exposure Conditions (including crosstalk) 60 Hz fields in sham control module + fields generated by energized sham).
- Blinding All studies conducted using fully blinded design.
- To determine whether EMF affects mitomycin C-induced expresion of P53 protein cell cultures are exposed to both mitomycin C and EMF for 24 hours

- Signal Transduction: Cyclin A, cyclin B1, p53, p21, and GADD45 protein levels are measured via standard Western immunoblotting procedures. ERK1 and ERK2 kinase activity are measured via immunoprecipitation of the proteins, followed by phosphorylation of an appropriate substrate peptide. *C-jun* transcription is measured by Northern blotting.

- Cell Survival: Both short term and long term assays are used to measure the effects of EMF on survival of AT compared to normal cells. Trypan blue exclusion is the short term measure of cell killing and growth. Colony formation assays measure long term survival.

Quality Assurance Measures

- Ambient 60 Hz and Static Magnetic Fields Fully mapped by IITRI engineers prior to installation of exposure system and incubators. No DC field cancellation.
- Magnetic Field Monitoring Field strength and waveform monitored continuously throughout program (both during exposure periods and periods of module shutdown). Exposure system performance has been validated by a DOE site visit team.
- Study Blinding-All studies conducted using fully blinded design. Selection of sham/exposure, exposure/sham, or sham/sham design is made by computer; cell and molecular biology staff are unaware of exposure regimen until exposures are uncoded after completion of endpoint analyses.
- Positive Controls-Agents with known activity in modulating proteins of interest (e.g. mitomycin C induces p53) are included in the design of all studies. Loading controls are included in all studies to ensure no systematic bias in study design or performance.

Results

We have established four lymphoblastoid cell lines in the laboratory. Two cell lines were derived from ataxia telangiectasia (AT) patients, and two were derived from non-AT (normal) individuals. Growth curves for each cell line have been determined.

- 24-hour exposure to 10 G EMF does not produce changes in either short term growth or long term survival in A-T or normal lymphoblast cells.
- We have used the DNA-damaging agent mitomycin C to test EMF effects on DNA damage induced signaling p53 protein expression increases with time after exposure to mitomycin C. EMF at 10 G does not affect mitomycin C-induced p53 expression in either normal or A-T cells.
- No changes in p53 or p21 protein expression were detected in cells (either A-T or normal) after exposure to 10 G EMF for the above durations. An increase in p21 protein expression was measured in the two A-T cells lines following 24 hr exposure to 100mG. A decrease in p21 protein expression was measured in the two normal cell lines following exposure to 100mG. No effects were seen on p53 protein expression, or on p21 expression at the other exposure durations tested. It is important to note that while three separate cell cultures for each cell line were exposed to 100mG, this has not yet been repeated with an independent exposure. These results are being followed up.

Discussion and Contribution to Understanding Biological Effects of EMF

A critical consideration in evaluating the possible health effects of human exposure to power frequency magnetic fields is that such exposures may induce biological responses in sensitive sub-populations that are not seen in the general population. Such a mechanism could at least partially explain the overall lack of consistency of the results of epidemiology studies of EMF effects on cancer and other diseases.

As a result of a genetic defect, AT cells demonstrate aberrant responses to a variety of chemical and physical agents, including ionizing radiation, ultraviolet radiation and reactive oxygen species. Specifically, AT cells lack DNA damage-induced arrest of cell cycle progression and DNA replication seen in normal cells. This project investigates the influence of magnetic field exposure on DNA damage-induced changes in signal transduction, cell cycle progression and DNA replication. Comparison of the responses of AT and normal cells evaluates the involvement of the ATM gene product in the regulation of such changes. Comparative studies such as these may identify biochemical or molecular targets for EMF action, and will generate data relevant to possible effects on a genetically susceptible subpopulation.

Publications From This Grant

None

Other EMF Publications

1. Suri RB, Loberg LI, Stark BC and McCormick DL. “Dehydroepiandrosterone suppresses tumor promoter-induced but not basal *c-myc* expression in HBL100 human breast epithelial cells.” Proc. Amer. Assoc. Cancer Res., in press, 1998.

TITLE: **NIOSH Summary Report on EMF Research Conducted Under Interagency Agreement**

Principal Investigator: W. Gregory *Lotz*, Ph.D.
National Institute for Occupational Safety and Health (NIOSH), Cincinnati, Ohio

Health Relevance: Cancer

Research Area: Cellular Function; Calcium; Cellular Processes

Y1-ES0032 **Start Date:** 1994 **End Date:** 1998

Abstract

This research is part of the interagency effort to evaluate the potential health effects of extremely low frequency (ELF) electric and magnetic field (EMF) exposure. The principal objectives of this agreement are: (1) to establish and maintain a regional magnetic field exposure facility which will be used for *in vitro* research on EMF effects; and (2) to conduct directed research intended to replicate important findings on the cellular responses to EMF, concentrating on studies relating to cell signaling. Specifically, NIOSH has undertaken studies of two significant findings reported in the EMF literature; magnetic field effects on intracellular calcium and related cell signaling functions, and magnetic field effects on cellular proliferation and transformation.

In the area of intracellular calcium effects, NIOSH has, in consultation with the agencies collaborating on the regional exposure systems, conducted studies to replicate the experiments of E. Lindström *et al*, (J. Cell. Physiol. 156:395-398, 1993; Bioelectromagnetics 16:41-47, 1995; FEBS Lett. 359:151-154, 1995; FEBS Lett. 370:118-122, 1995) which have reported that transient changes in intracellular free calcium concentration were induced in the human leukemic Jurkat cell line of lymphocytes by exposure to a weak magnetic field at 50 Hz and other ELF.

The Lindström *et al* reports have the important feature of studying intracellular calcium changes directly during magnetic field exposure by using the fluorescent calcium-binding dye fura-2. The advantages of this approach for the study of intracellular calcium include continuous monitoring of intracellular free calcium levels, the ability to document dynamic changes (i.e., oscillations and transients), and the ability to measure changes in individual cells through the use of microscopy. A number of other investigators have reported in recent meetings on their efforts to replicate the findings of Lindström *et al*. The results of these studies have been mixed in confirming such changes in intracellular calcium, but none of these other studies have been published as full papers.

In a separate set of experiments, NIOSH is studying the effect of 60 Hz magnetic fields on the proliferation of the mouse epidermal cell line, JB6, in an effort to confirm the findings of R.W. West *et al*. (Bioelectrochem. Bioenerg. 34:39-43, 1994; Bioelectrochem. Bioenerg. 39:175-179, 1996), that exposure to ELF magnetic fields significantly increases the colony-forming efficiency in soft agar, an event associated with neoplastic transformation in these cells. The tumor-promoter-responsive JB6 cell line has been used in a number of EMF studies, including those looking at cell signaling processes and gene expression. The work of West *et al* is significant in reporting enhancement of cell growth during exposure to relatively low magnetic field strength *in vitro*, suggesting that changes in cell function occurring under magnetic field exposure may actually result in a change in cell proliferation. Changes in cell proliferation and/or transformation may be more indicative of changes that would have probable impact for potential health effects of magnetic field exposure of an intact organism.

In both studies, NIOSH work is being conducted in close consultation with the investigators whose original work is the source of the published reports of interest. This consultation includes face to face visits as well as continuing correspondence. Collaboration on these studies with the original investigators has been essential to this effort.

Experimental Design and Exposure Conditions

Intracellular Calcium: Cultured cells of the Jurkat line are placed on cover glasses coated with poly-L-lysine and incubated with the fluorescent dye, fura-2, following the procedures of Lindström *et al*. The cells are maintained in an open perfusion chamber on the stage of the microscope at 37 ^{o}C. Intracellular calcium activity in single cells is determined prior to, during, and after magnetic field exposure with a fluorescence microscopy imaging system in real time. As a positive control, intracellular calcium is also analyzed in the same cells stimulated with an anti-CD3 monoclonal antibody.

The exposure system for this study consists of a specially designed and fabricated coil placed on the stage of the microscope, and in which the open perfusion chamber resides. For each experiment, a baseline was obtained in the first 8 minutes followed by 50 Hz, 1.5 G magnetic field (MF), sham (SF) or no field (NF) conditions from 8 to 16 minutes. From 16 to 24 minutes, cells were exposed to 0.5 µg/ml anti-CD3.

A calcium transient was defined as a 3-fold increase of F340/F380 from baseline. The occurrence of a $[Ca^{2+}]_i$ transient for a cell in one of the three time periods-baseline, exposures, or positive control was designated by a "Y" or a "N." Cells responding to positive control are considered to possess the capacity to respond to magnetic fields, and were categorized together to give:

- **NNY, NYY, YNY, YYY.** Only cells with a stable $[Ca^{2+}]_i$ baseline in the first 8 minutes were considered, giving the following subpopulation of cells:
- **NNY, NYY** (Qualified Cells). The incidence of $[Ca^{2+}]_i$ transients during the exposure period was calculated as:
- **NYY/(NYY + NNY)** (Responding Cells/Qualified Cells).

Cell Proliferation: JB6 cells were taken from culture and suspended in a soft agar preparation in 60 mm dishes according to the procedure of West *et al*. Dishes were then exposed to the magnetic field or maintained under sham exposure conditions for 10 to 14 days before being scored for colony growth using an inverted microscope with a calibrated eyepiece reticule. Colony counting procedures were those of West *et al*. Assessment of colony growth was conducted in a blinded manner.

The exposure system for the JB6 cells was the DOE-supplied regional exposure system consisting of two identical chambers contained within a system of stacked Merritt coils providing an applied vertical magnetic field. Two additional pairs of Helmholtz coils provide field control in the other two axes orthogonal to the Merritt coils. The system is capable of providing or canceling specific static magnetic field conditions in addition to the applied AC magnetic field, but these features were not used for the JB6 cell exposures. Both exposure chambers were supplied with environmentally controlled air (temperature, humidity, CO_2) from a common incubator. The entire system was under computer control, and was operated so that which chamber was exposed and which was sham-exposed to the magnetic field was not known to the investigator until the code was broken after the scoring of the colonies had been completed. All exposures were to vertical, 60 Hz sinusoidal magnetic fields. Field strengths were 1.0 G and 10 G. Exposure duration was either 10 or 14 days. The experimental design is shown in Table 1.

Table 1: Experimental Design for JB6 Cell Proliferation Studies

TPA Concentration (ng/ml)	Field A	Field B
0.00	On/Off/Sham	On/Off/Sham
0.01	On/Off/Sham	On/Off/Sham
0.05	On/Off/Sham	On/Off/Sham
0.10	On/Off/Sham	On/Off/Sham
1.00	On/Off/Sham	On/Off/Sham

Quality Assurance Measures:

In coordination with the Steering Committee for the regional EMF exposure facilities, NIOSH worked with the Department of Energy and the National Institute for Standards and Technology (NIST) to assure quality and accuracy of exposure parameters and systems. These procedures included on-site testing and measurement by Dr.

Martin Misakian of NIST for confirmation of agreement between measured and calculated fields generated by the exposure system within the coils, assessment of geomagnetic static fields, and background magnetic field measurements in the laboratory around and within the coils. This includes an assessment of the frequency characteristics of the magnetic field, including the fundamental and harmonics, and survey for possible sources of magnetic fields not generated by the exposure system itself. These same procedures were followed for each exposure system (the Regional exposure system and the microscope stage exposure system) used for the two validation studies.

For quality assurance in the biological endpoints, the basic approach has been to rigorously establish the normal variability characteristics of the experimental model in our laboratory, including sham-sham runs of the protocol, then to evaluate the response of that model to an accepted positive control known to perturb the system, and finally to compare the response of the model to magnetic field exposure to the changes observed with a positive control. Experiments were conducted blind, so that the researcher scoring the biological endpoint of interest did not know the exposure status of the cells being scored. Only after the data had been compiled was the exposure code broken.

Results

Intracellular Calcium: In a completely blinded study, no significant differences were found in the incidence of $[Ca^{2+}]_i$ transients during exposure to a 50 Hz 1.5 G magnetic field.

The data, shown in Table 2, demonstrate that cells responsive to MF, if they exist, constitute a relatively small fraction of the total population.

Table 2. Effect of a 50 Hz 1.5 G Magnetic Field on [Ca2+]i Transients Incidence of [Ca2+]i transients in the exposure period among qualified cells1

Experiment[2]	Obj.[3]	Exposure Condition[4]	Total Cells[5]	NYY[6]	NYY+NNY[6]	NYY/(NYY + NNY)[7]
1 (N=8)	40X	NF	330	27	167	0.162 ± 0.097
		SF	333	29	174	0.179 ± 0.116
		MF	319	34	177	0.194 ± 0.100
2 (N=7)	40X	NF	363	25	232	0.119 ± 0.114
		SF	327	20	200	0.110 ± 0.080
		MF	324	25	212	0.132 ± 0.083
3 (N=5)	20X	NF	720	15	494	0.031 ± 0.013
		SF	795	10	576	0.017 ± 0.019
		MF	791	12	558	0.022 ± 0.007

1. Qualified cells include responses NYY and NNY.
2. The number of replicates are in parentheses.
3. Microscope objective magnification.
4. NF, no field; SF, sham field; MF, magnetic field (50 Hz, 1.5 G).
5. This number includes all possible responses.
6. Total number of qualified cells.
7. Mean ± SD for individual replicate values.

Cell Proliferation: Transformation frequency of JB6 cells displayed a dose-dependent response to increasing concentrations of TPA (Table 3). Co-exposure of cells to both TPA and the 1 G or 10 G, 60 Hz field did not result in any differences in transformation frequency for any TPA concentrations tested (0 to 1 ng/ml; Table 4 [1 G MF] and Table 5 [10 G MF]). In tests comparing cells obtained from ATCC or Dr. West and the same lot of serum, cells obtained from Dr. West produced significantly greater numbers of colonies without TPA treatment or the presence of MF.

In tests to determine reasons for the discrepancy between our results and those reported by West *et al*, factors such as serum lot, cell-plating technique, the effect of increasing or decreasing the time the cells are held at 42°C prior to plating and other experimenter-dependent factors were examined. Of the factors above, serum lot and cell

plating technique, particularly order of plating had the greatest effect on colony formation. The two serum lots examined were obtained from the same supplier (JRH Biochemicals). Initial growth tests determined that the two lots (JRH 3M and JRH 4M) were capable of supporting the JB6 cell line. JRH-3M was provided by Dr.West as a lot which gave consistent positive results in his laboratory. Samples of JRH 4M were provided to Dr. West and failed to show a positive effect of MF exposure on JB6 cell-proliferation in his laboratory. It was observed in our laboratories that JRH 3M caused a noticeable aggregation of cells despite repeated titration. Because the aggregated cells tended to settle to the bottom of the pipette during the plating procedure, and based on Dr. West's technique of plating 6 plates from a single 10 ml pipette, in our normal replication studies every effort was made to ensure that each treatment group had at least one dish from the first 4.5 ml of suspended cells. In tests of the effect of plating order on cell colony formation, significant differences were observed between JRH serum lots, 3M and 4M. As seen in Table 6, with JRH 3M serum there was a significant difference between colony counts of plates plated with the initial 4.5 ml of cell suspension versus the second 4.5 ml. This was not observed with JRH serum lot 4M.

Table 3: Anchorage-independent growth of JB6 cells treated with TPA and Sham/Sham Exposure to MF Values are number of colonies per dish (mean ± s.d.).

TPA Treatment (ng/ml)	Number of Colonies Chamber A	Number of Colonies Chamber B
0.00	6.00 ± 2.64	13.00 ± 1.15
0.01	17.5 ± 1.44	26.33 ± 5.84
0.03	45.83 ± 2.17	43.33 ± 2.85
0.10	285.33 ± 12.13	275.00 ± 13.87
1.00	359.00 ± 20.79	340.00 ± 11.72

Table 4: Anchorage-independent growth of JB6 cells in a 1.0 G MF. Values are number of colonies per dish (mean ± s.d.). GR = growth ratio (MF colonies/Control colonies)

Control (7 days)	MF (7 days)	GR	Control (14 days)	MF (14 days)	GR
16.0 ± 1.0	14.3 ± 3.1	0.89	49.0 ± 7.2	40.0 ± 5.6	0.82
16.3 ± 3.5	16.7 ± 3.7	1.02	6.3 ± 0.8	7.3 ± 1.45	1.16
9.7 ± 1.3	13.6 ± 3.7	1.40	12.4 ± 0.7*	11.8 ± 4.5	0.95
19.3 ± 2.7	19.2 ± 4.0	0.99	16.7 ± 1.7*	17.8 ± 3.2	1.06

* Experiments halted at day 10 instead of day 14.

Table 5: Anchorage-independent growth of JB6 cells (No-TPA) exposed to a 10.0 G MF. Values are number of colonies per dish (mean ± s.d.). GR = growth ratio (MF colonies/Control colonies)

Control (14 days)	MF (14 days)	GR
22.70 ± 1.76	12.67 ± 2.40	0.56
13.33 ± 1.85	14.33 ± 1.45	1.08
16.00 ± 1.00	18.67 ± 1.33	1.17
17.67 ± 4.37	13.00 ± 4.00	0.73
20.00 ± 2.00	23.67 ± 1.85	1.18
34.00 ± 4.02	45.73 ± 7.89	1.34

Table 6: Effect of serum lot (JRH 3M or JRH 4M) and plating order on anchorage-independent growth of JB6 cells treated with TPA (0.01 ng/ml). Values are number of colonies per dish (mean ± s.d.).

Plating Order	Serum	Number of Colonies
First 4.5 ml	JRH 3M	43.50 ±3.44
Second 4.5 ml	JRH 3M	23.00 ± 2.97
First 4.5 ml	JRH 4M	18.00 ± 5.71
Second 4.5 ml	JRH 4M	± 5.00

In Summary:

The data obtained using the NIOSH regional exposure facility have indicated that exposure of JB6 cells to a 1 or 10 Gauss, 60 Hz field does not alter anchorage-independent proliferation. MF exposure does not increase the effect of TPA on anchorage- independent proliferation. These results are in contrast to those published by West *et al*.

The influence of serum lot as a contributing factor in the differences in the data reported here and those reported by West *et al*. (1994, 1996) cannot be discounted. In experiments with JRH serum lot 3M (a serum lot previously reported to yield an increase in JB6 anchorage independent growth) order of cell plating was seen to produce apparent increases in cell proliferation similar in magnitude to those reported by the previous researchers (West *et al*., 1994; 1996).

Discussion and Contribution to Understanding Biological Effects of EMF

Intracellular Calcium: The NIOSH data acquired thus far do not agree with the reports of magnetic field-stimulated changes in intracellular calcium levels. Two hypotheses are being considered that may explain the differences between our results and those of Lindström *et al*. One deals with the possible potentiation of a response related to the UV exposure, as described in two recent publications. The other hypothesis is that a small sub-population of the Jurkat cells is responsive to magnetic field exposure, but that the result of that response is masked by the statistical average of the larger number of cells we analyzed in a group. If this is true, then Lindström would observe those sensitive cells because of her pre-selection of individual cells for study. The finding that a small, but identifiable proportion of cells was responsive to magnetic field exposure would be very significant, revealing an *in vitro* effect on intracellular calcium that would be amenable to further study. However, if we are unable to obtain data supporting this "sensitive subpopulation of cells" hypothesis, then the significance of the reports by the Lindström group would be diminished. If it turns out that ultraviolet radiation potentiates the response to magnetic fields, this may explain an effect of combined exposure that may also provide valuable elucidation of reported EMF effects.

Cell Proliferation: In this case the NIOSH data again do not agree with the original reports by West *et al*. The data obtained using the NIOSH regional exposure facility indicate that exposure of JB6 cells to a 1 or 10 G, 60 Hz MF does not increase the anchorage-independent proliferation of promotion sensitive JB6 cells. In addition, the data support the conclusion that exposure to 1 or 10 G MF does not enhance the effect of TPA on cellular transformation frequency.

Extensive efforts have been made to decrease intra-assay variability while duplicating the experimental design of West *et al* (1994); cells, serum and other cell culture supplies were either from the same commercial source or actually supplied by Dr. West. The methods used in the present study were identical to those of West *et al.* (1994;1996) except for the use of the DOE regional facility for cell exposures. Despite these efforts, our findings were different than those previously reported (West *et al.*, 1994; West *et al.*, 1996).

The possibility that serum lot contributed to differences between the data reported here and those reported by West *et al*. (1994, 1996) can not be discounted. As indicated in Table 6 plating order with JRH serum lot 3M (a serum lot previously reported to yield an increase in JB6 anchorage independent growth) was seen to produce apparent increases in cell proliferation similar in magnitude to those reported by previous researchers (West *et al*., 1994;1996), Because our experimental design always included three groups (EMF-exposed, sham and control) of 5 TPA concentrations and exposure assignment of these was random, the likelihood of plating order contributing

to erroneous interpretation was lessened. In previous studies (West *et al.*, 1994: 1996) only 2 groups of cells were included with no internal control (no TPA). While not discounting the effect of MF exposure, if only 2 groups of cells are plated (as in the previous publications), the differences that could result from plating order may be inadvertently attributed to EMF exposure.

In addition to the close cooperation NIOSH has had with Dr. West at the NCTR, NIOSH was in continuous consultation with the laboratory of Dr. Jeffery Saffer at Battelle Pacific Northwest Laboratories. The NIOSH JB6-replication effort closely mirrors that of Dr. Saffer in that both always included the positive control, TPA in each assay. In concordance with the NIOSH effort to date, Dr. Saffer has reported (Saffer *et al*., 1997) their inability to reproduce the findings of West *et al* (1994;1996).

The fact that neither Saffer *et al*., nor NIOSH has been able to confirm the West *et al*. findings on JB6 proliferation greatly diminishes the significance of this report. The possibility that serum lot, and the absence of positive controls in the work by West *et al*. may explain the difference in findings among these laboratories also suggests that the magnetic field exposures tested do not affect JB6 proliferation.

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2. Snawder JE, Edwards RM and Conover DL. "Effect of magnetic field exposure on anchorage-independent growth of a promoter sensitive mouse epidermal cell line (JB6)." Environ Health Persp., In press, 1998.
3. Bowman JD, Sivaganesan S, Elmore RT, Smith RJ and Stayner L. "Meta-Analysis of the exposure-response relationships for occupational magnetic fields with brain cancer and leukemia." In preparation, 1998.

Other EMF Publications

1. Kim YV, Conover DL, Lotz WG and Cleary SF. "Electric field-induced changes in agonist-stimulated calcium fluxes on human HL-60 leukemia cells". Bioelectromagnetics, in press, 1998.
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3. Bowman JD, Thomas DC, Jiang L, Jiang F, Feng J and Peters JM. "Residential magnetic fields predicted from wiring configurations: I. Exposure model." Biolelectromagnetics, in press, 1998.
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TITLE: **Electromagnetic Effects on Membrane Receptor Function**

Principal Investigator: Richard A. *Luben*, Ph.D.
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Health Relevance: Cancer

Research Area: Cellular Function, Gene Expression, Signal Transduction

R01-ES06410 **Start Date:** 08/01/94 **End Date:** 07/31/98

Abstract

There is at present no accepted molecular mechanism to explain how disease processes *in vivo* could be caused by exposure to low levels of non-ionizing, non-thermogenic electromagnetic fields (EMF). Based on our previous work with bone cells and other cell types, we have hypothesized that EMF interacts with cells primarily at the level of membrane signal transduction mechanisms. We have shown that a class of cell membrane receptors known as G protein linked receptors, which serve as receptors for hormones, neurotransmitters and other cell regulators, are modulated by exposure to EMF as low as 1 gauss. In experiments we have demonstrated that bone cells exposed to a 1 gauss, 60 Hz sinusoidally varying magnetic field exhibit a transient activation, followed by a prolonged down-regulation, of protein kinase C (PKC), an enzyme which participates in the function of G protein linked receptors. Other experiments indicate that the same field desensitizes parathyroid hormone (PTH) and adrenergic receptors in bone cells, effects which could be secondary to activation or down-regulation of PKC. PKC is also the site of action of tumor promoting agents such as phorbol esters. We hypothesize that EMF actions on PKC and associated signal transduction pathways could allow EMF to regulate cell functions and/or act as a tumor promoter, either directly or in synergy with other agents in the environment of the cell. This project aims to study the biochemical mechanisms of effects of EMF on PKC and related components of signal transduction pathways. We exposed normal and neoplastic bone cells, lymphoid cells, and other relevant cell types in culture to 60 Hz sinusoidally varying magnetic fields between 10 milligauss and 10 gauss (initial experiments will be done at 1 gauss until we obtain more information on the mechanisms of the effects we have already observed). We determined the activity of G protein linked membrane receptors in the cells by measuring their responses to appropriate hormones in terms of PKC activation, translocation and down regulation, inositol phospholipid turnover, calcium ion transport and adenyl cyclase activation. We studied in detail the regulation of PKC in terms of EMF effects on sensitivity to activating agents (including tumor promoters), inhibitors, isoenzyme profiles, degradation kinetics, and modulation by phospholipase C. We determined whether the observed effects of EMF on PKC are reflected by changes in other pathways and/or expression of genes which are normally regulated by PKC. We hope by these studies to establish a mechanistic framework within which the possible effects of EMF on health can be understood at the molecular level.

Experimental Design and Exposure Conditions

Exposures were done in identical tissue culture incubators fitted with identical modified Merritt coil apparatus designed by Dr. Maria Stuchly. These coils are wound in a bifilar manner to allow current-on, zero-field sham exposures, and incorporate compensating Helmholtz coils outside the Merritt coil to cancel field spillover. Identical samples will be treated using double-blind conditions with computer-randomized double DODT switches to conceal the identity of sham and exposed samples until after assays are completed.

Magnetic field intensity: Initial experiments: 1G, 60 Hz. Experiments on relevant effects will be repeated at 100 mG, then if appropriate at 10 mG.

- Duration of exposures: Single exposures for 15 sec @ 24 hr, followed by field off for 24 hr to observed activities during recovery.
- Number of separate exposure periods: single exposures.
- Circadian time of exposure: not applicable (cultured cells will be used for all experiments).
- Field oscillation frequency: 60 Hz.
- Harmonic content: to be determined by Dr. Stuchly; design parameters <0.1% content of harmonics.
- Polarization: linear.
- Static magnetic field: strength and orientation will be measured for experiments but not corrected.
- Relative orientation of AC and DC fields: AC field vertical, DC field contributed by Earth's field orientation (approx 45E vertical and 15E horizontal).
- Associated electric fields: to be determined by Dr. Stuchly; approx. 0.1 mV/m for 1 G magnetic field.
- Intermittency: continuous exposure.
- Turn-on and turn-off transients: Fields will be turned on before introducing cells to the coils; cells will be removed before the fields are turned off.
- Spatial homogeneity: <0.2% within the central 20x20x20 cm volume of the coil where all exposures will be done.
- Temporal homogeneity: none expected (will be monitored by recording).
- Geometry of the cell culture system: cells will be exposed in 30 mm dishes or T75 flasks. Field will be perpendicular to cell layers.
- Geometry and movement of exposed animals: N/A
- Accessory non-EMF exposure: vibrations will be measured in exposed and sham coils. Temperature 37° (0.2E, CO_2 5% (0.05% in both incubators (monitored).
- Incidental EMF exposure: Cells will be handled to minimize fields during handling and culture outside the exposure incubators. Field conditions in all incubators and handling areas will be mapped, monitored and reported.

Quality Assurance Measures

We used two identical incubators and two identical coil systems. This will allow control and experimental exposures to be carried out under identical conditions simultaneously. One set of coils will be energized with the current running in parallel (i.e. producing a magnetic field) and the other with the current flowing antiparallel (i.e. zero induced magnetic field), using double-blind randomization as described above for all experiments. These coils will be energized by a power amplifier (Kepco BOP 20-10M) driven by a function generator (H-P) producing 60 Hz sine wave AC current in the coils. Waveforms were monitored continuously using a digital oscilloscope with leads for both the electrical power supply and the gauss meter. Field strength were recorded with a strip chart recorder attached to the output of the gauss meter. Static geomagnetic fields and their orientation will be recorded daily before and after each experiment. If findings by us or others indicate it is desirable, the geomagnetic field can be canceled or modified using properly oriented DC "bucking" coils; we do not intend at this time to investigate the geomagnetic field as a high priority experimental variable but this can be incorporated in the experiments easily if future findings by others confirm its importance.

All experiments were double-blinded by having the principal investigator set one pair of DODT switches which will not be visible to the technician doing the experimental manipulation, after which the technician will set a second set of switches, whose settings will be known only to that individual. The wiring of the dual pairs of switches were set up in such a way that one device will be activated and the other will not, but neither the investigator nor the technician will know which is which until their separate records are checked after the data are calculated. Fields were calibrated in both exposure devices before the double blinding procedure is carried out, such that the field is 1.0 G +1% in the experimental device for the initial series of experiments. Immediately at the end of exposure, analysis of experimental variables were carried out without breaking the exposure codes until calculations are completed.

Results

Specific Aim 1. We have established our new exposure facilities using equipment purchased in the first year of the grant. The new coils were fabricated for us by the Loma Linda engineering facilities and have been inspected by both our local engineers and a DOE-RAPID site visit team. The facility is operating as expected with excellent uniformity and reproducibility. We were able to purchase three identical incubators so that the electromagnetic environment of the cells is the same during propagation and passage (incubator 1) as it is in either sham or exposed experimental systems (incubators 2 and 3). We have studied time course and dose response of PKC in both cell membrane and cytosolic fractions, using EMF exposure at 1.0 gauss rms, and other treatments known to stimulate PKC activity such as parathyroid hormone (PTH) and the tumor promoting phorbol ester (TPA). We attempted to avoid use of the commercial kits for PKC activity by preparing all our own reagents, but this proved to be not as reproducible as using the kits and ultimately no less expensive. Nevertheless, the primary findings of EMF stimulation of PKC activity, followed by down-regulation, have been replicated in bone cells.

We have also studied the effects of EMF on PKC activity in human leukemia cells, in collaboration with Dr. Fatih Uckun of the University of Minnesota. These results is interesting, since the activity of PKC is known to be related to the survival of leukemic B cell clones by way of the NK-6B transcriptional regulation pathway. This collaborative investigation is interesting, since B-cell leukemias are the primary leukemias of childhood and these are the diseases which have been most consistently linked to environmental EMF in epidemiological studies. We are continuing to pursue the investigations both in bone cells and in human B leukemia cells. Other leukemias such as HL-60 have been investigated in preliminary experiments during the past year, but we found no consistent changes in PKC activity and we have thus not pursued these any further. If time is available, we may examine the lack of response of HL-60 as a negative finding in the future.

Also in specific aim 1, we have developed our Western blotting assay for PKC isozymes in the bone cells and human B cell leukemias. Preliminary evidence using this assay indicates that the beta isozyme of PKC is primarily affected by EMF exposure both in bone cells (down-regulation) and in leukemia cells (induction). These results are very interesting in view of the differences in responsiveness of the two different cell types to EMF both *in vivo* and *in vitro*. Two issues are important here: a) the possibility that only the beta isozyme is affected (alpha and gamma do not appear to change, although this needs to be confirmed); and b) the fact that EMF down-regulates this isozyme in bone (where proliferation is inhibited and differentiated functions are stimulated) but up-regulates it in B cells (where proliferation is stimulated and differentiation is inhibited). These findings are consistent with previous general observations both in cell lines and *in vivo*, and suggest possible mechanisms involving PKC-in regulating these effects.

Increased bone growth and increased bone-forming activities of bone cells (osteoblasts) have been observed in response to treatment with pulsed or continuously varying electromagnetic fields (EMF) as low as 10 mT. In the past year we have examined in more detail the hormone and growth factor related mechanisms influenced in bone cells by EMF exposure. As previously reported, we found that PKC activity was increased in the membrane fraction of the cells by treatment with EMF alone. This increase in activity was accompanied by the translocation of PKC isoenzyme alpha (PKCa) from the soluble to the particulate (membrane) fraction of the cells. The PKC beta isoform was not directly influenced by EMF exposure and its abundance in the cell was not changed. Despite the translocation and activation of PKC, phospholipase C (PLC) activity was not activated by EMF treatment. This suggests that EMF may increase the abundance of di-acylglycerol (DAG), the activator of PKC, in the membrane by a non-PLC-dependent process, such as increasing phosphotidylinositol kinase (PIK) activity. We are currently examining this hypothesis in greater detail. We also showed that treatment of bone cells with EMF increased both protein tyrosine kinase (PTK) activity as we had previously reported for human leukemia cells. In other studies, EMF was found to have little effect on the vitamin D activation of PKC in bone cells, indicating that intracellular hormone receptors fail to respond to EMF in the same manner as do membrane receptors.

The current series of findings demonstrate that our previous observations of direct effects of EMF are repeatable, and that bone cells respond in a highly complex, specific and coordinated manner to EMF exposures. It seems logical to hypothesize that other cell types may exhibit not only normal but also pathological responses to EMF which are mediated by membrane signal transduction pathways. In an attempt to test this hypothesis, we have extended our studies to new receptor-mediated model systems in the past year. The phosphorylation of the T-cell receptor zeta subunit in human Jurkat T-cells was found to be inhibited by exposure of cells to EMF, suggesting a

desensitization of the growth-regulatory receptor similar to that seen in both bone cells and human B-cells. We also have examined apoptotic events in normal and transformed human cell line, showing that transformed cells responded to EMF with increased apoptotic events including micronucleus formation, while non-transformed cells did not. Finally, in a system which may be dependent on the activity of the estrogen receptor, we have replicated Liburdy's findings that EMF caused an increased growth rate in human breast cancer cells treated with melatonin. A general picture has begun to emerge in our research of EMF producing failures in growth-regulatory receptor-mediated pathways in transformed cells. This would be consistent with a possible role of EMF as a tumor promoter or co-promoter. We are focusing the current year's work on extending these preliminary findings.

Specific Aim 2. We have instituted a collaboration with Dr. Robert Stagg of the Loma Linda VA hospital to measure intracellular calcium ion levels using digital microscopy. This work has just begun due to delay in getting the exposure coils and other equipment set up, but we expect significant results in the coming year. With respect to PKC and phosphoinositide turnover, a new graduate student in the lab has begun to learn the required techniques and assays to carry out these experiments.

Specific Aim 3. Several lines of experimentation have been instituted on this aim. We have done correlative studies on the relationship between PKC activity levels and cAMP levels (and PKA activity levels) in bone cells. The results suggest that hormonally mediated cAMP/PKA pathways are inhibited in bone cells by EMF exposure. This inhibition is maximal in the first 2 hours after the shutoff of the field, and is correlated with increased levels of PKC during this time period. These findings, which confirm previous preliminary studies, suggest that intermittent exposure to the field (i.e. an exposure long enough to produce partial down-regulation of PKC followed by turning off the field long enough to allow rebound of the PKC activity) may be an important factor in producing at least some of the important metabolic effects in bone cells. We have only begun to study leukemia cells in this paradigm and we do not yet have any results to report.

Also in specific aim 3, we have studied protein tyrosine kinases (PTKs) of the src family in human leukemia cells. These studies have shown that EMF exposures stimulate the lyn and syk PTKs in NALM-6 pre-leukemia cells. These PTKs are known to be involved in the signal transduction pathways regulating cell proliferation, differentiation and apoptosis in leukemia cell clones, and their activation is important in activating PLC and PKC required for activation of nuclear transcriptional processes. We are continuing these studies and have begun to examine whether other PTKs are regulated by EMF in bone cells. For example, the src PTK is known to be crucial in bone cell differentiation (also using PKC-mediated nuclear transcription pathways) and thus is a possible candidate in the effects of EMF on bone cells.

Significance: The studies carried out in the first year have confirmed our preliminary observations that the enzyme designated PKC, and cellular pathways related to PKC, are influenced by relatively low energy electromagnetic fields. The enzyme PKC is known to be an important regulator of cell proliferation (an increase in cell numbers, which is required for normal growth, but can also be involved in abnormal cell multiplication such as in cancer). PKC is also involved in the process of regulating cell differentiation (the appearance of specialized functions in cells, such as in cases where bone healing is promoted by EMF). Thus these findings suggest a single enzyme, PKC, as possible means by which EMF may have either beneficial effects on cells (e.g. bone healing), or unhealthy effects in other cells (e.g. possibly aiding the survival of some leukemia cells). We have also shown that EMF can stimulate enzymes in human leukemia cells which are associated with transcription of genes in the nucleus (pathways also involving PKC). We plan to continue to study these effects in hope of learning more about whether these enzymes are actually involved in either positive or negative changes in cells in the living organism.

Discussion and Contribution to Understanding Biological Effects of EMF

The research conducted under this grant has investigated potential mechanisms of cellular responses to low-frequency electromagnetic fields. We have focused on signal transduction mechanisms and effector pathways which are linked to the control of cell proliferation, as a way of assessing whether there are plausible linkages between environmental EMF exposure and cancer. Our findings indicate that for 60 Hz field strengths of 0.1 mT and above, there are changes in the activation of protein kinase C (PKC), protein tyrosine kinases (PTKs), and apoptosis in several cell types. In particular, the changes observed in human leukemia cells are in pathways that would favor increased clonal survival and possible leukemogenesis. However, at field strengths lower than 0.1 mT

we have been unable to demonstrate any reproducible effects on these pathways. These studies suggest that, if there is any mechanistic connection between environmental EMF exposure and leukemia, the mechanisms involved are likely to involve short exposures to high field strengths rather than chronic exposure to constant background fields of 1 microtesla or less.

In another set of studies, we were able to replicate the findings of Liburdy, *et al*., demonstrating that exposure to a field of 0.12 microtesla produced a significant blockage of the anti-proliferative effects of melatonin and tamoxifen on human MCF-7 breast cancer cells. The effects of EMF were found to be extremely dependent upon culture conditions, serum, and source of cells. In addition, these effects were only transiently expressed during the log phase of proliferation of the cells, and did not change the final density of cells achieved in the cultures. Our preliminary experiments suggest that the ability to detect these effects of EMF is dependent upon the level of the beta isoform of the estrogen receptor being expressed in the cells under analysis. We cannot conclude at this point that these results demonstrate a tumorigenic mechanism which might operate *in vivo*, but the data do suggest that closer investigation is warranted of the potential connections among EMF, breast cancer cells, and the estrogen receptor (especially the beta isoform).

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TITLE: ***In Vivo* Effects of 60 Hz Magnetic Fields on Brain Tumour Promotion**

Principal Investigator: Rosemonde *Mandeville* MB, ChB, PhD
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Health Relevance: Cancer

Research Area: Toxicological studies using whole animals, Short term studies in whole animals, Tumour promotion

R01-ES07049 **Start Date:** 9/28/94 **End Date:** 8/31/97

Abstract

Several epidemiological studies published in the scientific literature implicate exposure to electric and magnetic fields in the development of cancer of the breast, leukemia, malignant glioma, and other diseases. Some studies have suggested that magnetic fields (MF) act as tumor promoters. The objective of this research program is to determine whether prolonged exposure to 60 Hz linear, sinusoidal, continuos-wave MF exerts tumor promoting effects on neurogenic tumors in Fischer rats. Tumors will be initiated by the transplacental exposure to a sub-threshold dose of a chemical carcinogen N-Ethyl-N-nitrosourea (ENU).

To achieve this objective two studies are planned to take place between 1995 and 1997:

- TPA (12-O-tetradecanoyl-phorbol-13-acetate) Promotion Study; The is first study will both validate the applicability of the two-stage theory of carcinogenesis to neurogenic tumors in Fischer rats and quantitate the promoting effect of TPA on macrotumours, microtumours and early proliferative lesions. We are also planning to identify stable and reproducible changes that play an important role in ENU-tumor promotion at the biochemical (ornithine decarboxylase activity) and molecular (dominant oncogene and tumor suppressor gene expression) levels. This quantitative study is crucial to establish a laboratory baseline for the evaluation of brain-tumor promotion in Fischer rats so as to allow the estimation of the bio-equivalence of MF-promoting effect.
- Magnetic Field Promotion Study; In the MF promotion study, in addition to establishing whether 60 Hz fields could promote neurogenic tumors in the Fischer rats, this study will quantitate the promoting effect of MF on macrotumours, microtumours and early proliferative lesions.

The present research program will provide direct answers to several important questions concerning the possible tumor promoting effect of 60 Hz linear, sinusoidal continuous-wave MF

- Can chronic exposure to MF promote the development of neurogenic tumors initiated by a chemical carcinogen (ENU) in Fischer rats?
- In the case of tumor promotion, is there a dose-response effect (2, 20, 200 and 2000 µT)?
- What is the bio-equivalence to a classical tumor promoter, TPA?
- Can we identify stable and reproducible changes that play an important role in tumor promotion?
- Accordingly, the aims for our efforts was to define the robustness of MF promoting effect and determine the biological significance of such an effect.

Experimental Design and Exposure Conditions

The graded TPA Brain Tumor promotion Study.
Seven groups of female F344 rats (Taconic Farms) were used in this study. Pregnant females were given a sub-threshold dose of ENU (5 mg/kg) on the 18th day of gestation. TPA was dissolved in acetone and diluted with saline. Fifty offspring from each group were treated with TPA i.p. on days 19, 20 and 21 in the prenatal period and twice per week in the postnatal period starting on the second week and for the duration of the experiment. Animals were sacrificed at 60 weeks of age. Animals were assigned at random to treatment and control groups. Seven different concentrations of TPA were used: 0.01, 0.05, 0.1, 1,5, and 10 mg/kg.

The Magnetic Fields Brain Tumor Promotion Study.

Eight groups of 50 female F1 rats were used in this study. This is based on two considerations: First, sample size calculation determining that 50 animals per dose group will provide 90% power to detect an increase in cancer incidence of 25%-30%. Second, we have elected to use only females in this study mainly because data generated from our preliminary study (ENU#90) have shown no statistical significant difference in ENU-induced tumors between gender. Moreover, because of the big difference in size between males and females at 14 months of age, only 1 male could be housed in our custom made cages.

Pregnant females were listed in numerical order based on Day 1 of gestation and assigned a random number corresponding to ENU (treatment) or control and field intensity (color coded). This was a computer-assisted randomization program developed specifically for these MF studies in our Institute. In the selection of the 50 females we used the largest possible number of litter. Ideally we took only two females from each litter.

Fields specifications. Animals were exposed to 60 Hz linear, sinusoidal, continuous-wave MF of different intensities ranging from <0.02 µT (Sham exposure) to 2, 20, 200, 2000 µT. Exposure was performed 20 hours per day (10 hours in the dark and 10 hours in the light). Exposure started in the prenatal period (18th day of gestation) and continued during weaning and active growth until advanced adulthood (60 weeks of age). Animals were assigned at random to treatment and control groups. This study was performed under stringently controlled environmental conditions with a constant light cycle (12/12 hours dark/light cycle).
Environmental Monitoring. Environmental monitoring tests were conducted in both holding and exposure rooms, these tests included (1) temperature; (2) humidity; (3) air flow; (4) photo period; (5) noise; (6) room access; and (7) AC fields. We also monitored ammonia and CO_2 levels after housing animals in units for a period of seven days.

Quality Assurance Measures

Animal Facility. Our animal facility has been approved by the Office for Protection and Research Risks (OPRR) and the assigned assurance number is #A5271-01 and became effective on 10/5/94. The approval period is five years, covers all Public Health Services (PHS) supported activities involving live vertebrate animals and will expire on 9/30/99.
Blinding and field rotation. In order to limit the effect of any potential environmental confounder in this study, we have elected to develop a rotation strategy that is completely randomized. This rotation strategy is based on the color code light coding provided in the exposure room to designate the exposure level assigned to each of the five groups of modules. The lights are red, green, yellow, blue, and white. The position of the module inside the room was changed. In this rotation, care was taken so as not to have sham and 2 T fields besides 2000 µT.

Results

The present study on the effect of magnetic field on brain tumor promotion started earlier than originally planned in order to shorten our study period and to allow us to complete the study and the final report before June 1998. Animals were received on the of June 21, 1995.

The experiment itself started on August 19, 1995 and the animal sacrifice was made in November 1996. The histopathology and results of this study were reported in the 3rd EMF *Rapid* Symposium in Phoenix Arizona, April 5-8, 1998.

Discussion and Contribution to Understanding Biological Effects of EMF

The findings from this ENU/MF's promotion study demonstrate that, under our defined experimental conditions, exposure to 60 Hz linear (single axis) sinusoidal continuous wave MF's had no effect on the survival of rats or on the number of animal bearing neurogenic tumors. These results suggest that MF's have no promoting effect on neurogenic tumors in the F344 rats exposed transplacentally to ENU.

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TITLE: **Effect of 60-Hz Magnetic Fields on Lymphoid Phenotype**

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Health Relevance: Cancer

Research Area: Toxicology Studies Using; Whole Animals; Short Term Studies; Immunosuppression

R01-ES05928 **Start Date:** 9/25/95 **End Date:** 8/31/98

Abstract

The ultimate aim of this research is to elucidate the biological and biophysical processes that subserve the empirical link between environmental electromagnetic fields (EMFs) and human disease. The basic hypothesis is that exposure to environmental EMFs can cause impaired immunosurveillance. The specific hypothesis being tested is that long-term exposure of mice to simulated environmental EMFs will cause changes in the immune system.

Comparisons were planned between the exposed and control groups to permit evaluation of 3 immune-system variables: (1) cellularity in the spleen, thymus, and bone marrow; (2) distribution of lymphocyte sub-populations in the spleen, thymus, and bone marrow; (3) functional activity of cytotoxic T lymphocytes and NK cells. The comparisons were based on changes in both the mean and variance, and were evaluated using the L test.

Homogeneous magnetic fields (Å5%) were established using an octapole arrangement of the Merritt 4-coil system. The mice were housed in a totally non-metallic environment, and were maintained in cages that had no direct mechanical path to the field coils, thereby minimizing the potential effect of vibration. Each octapole was operated in series resonance to eliminate the possible confounding effects of line-frequency harmonics. Fourier analysis of the coil current established that the strongest harmonics were more than 50 dB below the fundamental. The exposure and control units were operated at different ends of a dedicated room in an approved animal-care facility. The fringing fields of the exposure units were undetectable at the location of the control units. The ambient 60-Hz field at the control locations averaged less than 0.4 mG, and was never higher than 0.7 mG. Current, magnetic field, and temperature were continually recorded every 2 minutes to provide a historical record. The room was continuously maintained under temperature and humidity control. The room air was replaced 15 times per hour with fresh air.

Cell sub-populations were identified on the basis of antibody staining and flow cytometry as follows: Spleen, pre-B (CD45), immature B (IgM+/IgD-), mature B (IgM+/IgD+), T (CD90+/CD3+), NK (NK1.1); bone marrow, pre-B, immature B, and mature B; thymus, T, CD4+/CD8-, CD4-/CD8+, and CD4+/CD8+. *In vitro* allogeneic stimulation was used to induce formation of cytotoxic T lymphocytes (CTL) from spleen cells, and their proliferative response and cytotoxic activity were determined. Spleen cells treated *in vitro* with IL-2 for 18 hours were used to assess NK-cell cytotoxic activity. Serum corticosterone was measured by radioimmunoassay.

Partial results for lymphoid phenotype in male mice exposed for 1-49 days, 5000 mG, showed that EMF exposure resulted in changes in the distribution of immature B cells in the spleen and bone marrow, and T cells in the spleen and thymus. Under the same exposure conditions, the field had no effect on the distribution of NK cells, the proliferation of CTL, or the lytic activity of CTL or NK cells. Serum corticosterone levels in male mice exposed to 5000 mG, 175 days were significantly different than control levels.

One of the threshold considerations regarding the health and safety issue raised by environmental electromagnetic fields involves the choice of the biological model used to explicate putative EMF/organism interactions. The cybernetic (non-linear) model was chosen in this study for several reasons. First, it is well suited to attempts to resolve the most fundamental issue regarding EMF bioeffects — the occurrence of transduction. Second, the cybernetic model can harmonize reports from different laboratories, thereby resolving the apparent controversies. Third, a living organism is expected on theoretical grounds to exhibit non-linear responses to low-energy stimuli such as environmental EMFs because other conditions besides the EMF are important in determining the response of the animal.

Although important issues are not yet resolved in this study, the results showed that EMFs caused changes in the immune system, possibly mediated by the neuroendocrine system. This conclusion suggests the existence of a specific biological pathway linking environmental EMF exposure and human disease. The biological model employed in this study seems capable of providing a framework for explaining the laboratory and epidemiological reports involving EMF-induced effects.

Experimental Design and Exposure Conditions

Experimental Hypothesis: The ultimate aim of this research is to elucidate the biological and biophysical processes that subserve the empirical link between environmental electromagnetic fields (EMFs) and human disease. The basic hypothesis is that exposure to environmental EMFs can cause impaired immunosurveillance. The specific hypothesis being tested is that long-term exposure of mice to simulated environmental EMFs will cause changes in the immune system.

Because the particular immune-system changes in laboratory animals that indicate immunosuppression (have functional consequences for the animal with regard to disease endpoints) cannot generally be identified unambiguously, any experimental evidence supporting the specific hypothesis will be interpreted as support for the basic hypothesis. Such evidence will also be taken as proof that EMF signal transduction occurred, thereby implying that both a cellular location and a biophysical process must exist to mediate transduction.

Statistical Hypothesis: The choice of the statistical hypothesis was guided by the choice of the biological model used to rationalize inferences of cause-and-effect relationships. We assumed that the response of each EMF-exposed animal in the study was partially determined by the applied EMF, and partially determined by other factors that were not controlled explicitly, and that could never be controlled (because of the complexity of the animal). Consequently, the experimental evidence sought was a consistent occurrence of change in the immune system caused by the EMF, not a consistent pattern of changes in particular dependent variables.

A "change in the immune system" was defined in terms of justifiable rejection of hypotheses of the type $(\mu_1,\sigma^2_1) = (\mu_2,\sigma^2_2)$, where $\mu 1$ and σ^2_1 denote the mean and variance in a parameter measured in the EMF-exposed group, and μ_2 and σ^2_2 are the corresponding quantities in the control group. A rejection was justifiable when the associated error rates were acceptable (see below).

Comparisons were planned between exposed and control groups to permit evaluation of 3 immune system variables: (1) cellularity in the spleen, thymus, and bone marrow; (2) distribution of lymphocyte sub-populations in the spleen, thymus, and bone marrow; (3) functional activity of cytotoxic T lymphocytes (CTL) and NK cells. The variables were assessed by measuring 3, 12, and 3 parameters, respectively. A procedure was developed to permit statistical testing of the chosen hypotheses based on the use of L, a test statistic that can be expressed as the sum of L1 and L2, the log-likelihood ratio statistics for the variance and mean, respectively (3). The distribution of L is approximately chi-square with 2 degrees of freedom. Thus $(\mu_1,\sigma^2_1) = (\mu_2,\sigma^2_2)$ can be rejected if L > c22,a where a is the chosen error rate.

Chronicity Criterion: We expected that changes in the immune system would be chronic in the sense that the changes would persist during exposure. Additionally, as mentioned, we expected that the consequences of prolonged EMF exposure would not necessarily be reflected in uniform and consistent changes in the parameters that initially manifested the consequences of EMF exposure. These expectations were reconciled by requiring that

a particular parameter be statistically significant at two (or more) consecutive recovery times before rejecting the null hypothesis for the parameter (an "effect"). Thus, two consecutive statistically significant pair-wise comparisons for a particular parameter were accepted as indicating an effect of EMF exposure.

Family-Wise Error Rate: Each experiment consists of the exposure of different groups of male or female mice to a magnetic field of given strength for 1, 5, 10, 21, 49, 105, and 175 days. Measurements of 3 + 12 + 3 = 18 immune-system parameters were planned at each recovery time, with the intention to declare an effect whenever two statistically significant pair-wise comparisons (successes) were found. It was therefore necessary to ensure that the family-wise error rate remained within acceptable limits. We chose to do this by adjusting the comparison-wise error rate, q. It can be shown that if $q < 0.0285$, then the family-wise experimental error is $P < 0.05$ for the case of 7 recovery times. Thus, in a given experiment, we planned to declare an effect when at least 2 consecutive successes were observed at $P < 0.0285$ in any parameter.

Replicates: Because of practical limitations regarding the number of mice that could be measured on a given day, 3 replicates of each combination of exposure conditions were planned (generally, N=5 in the exposed and in the control group in each replicate), with the intention of combining the data for a given set of conditions in one overall test. For 3 replicates, overall L (the sum of the L values from each replicate) is approximately chi-square with 6 degrees of freedom under the hypothesis of no treatment effect. Overall L1 and L2 are also approximately chi-square, each with 3 degrees of freedom.

Methods: Cell sub-populations were identified on the basis of antibody staining and flow cytometry as follows: spleen, pre-B (CD45), immature B (IgM+/IgD-), mature B (IgM+/IgD+), T (CD90+/CD3+), NK (NK1.1); bone marrow, pre-B, immature B, and mature B; thymus, T, CD4+/CD8-, CD4-/CD8+, and CD4+/CD8+. *In vitro* allogeneic stimulation was used to induce formation of cytotoxic T lymphocytes (CTL) from spleen cells, and their proliferative response and cytotoxic activity were determined. Spleen cells treated *in vitro* with IL-2 for 18 hours were used to assess NK-cell cytotoxic activity. Serum corticosterone was measured by radioimmunoassay.

Quality Assurance Measures

Homogeneous magnetic fields (Å5%) were established using an octapole arrangement of the Merritt 4-coil system. The mice were housed in a totally non-metallic environment, and were maintained in cages that had no direct mechanical path to the field coils, thereby minimizing the potential effect of vibration. Each octapole was operated in series resonance to eliminate the possible confounding effects of line-frequency harmonics. Fourier analysis of the coil current established that the strongest harmonics were more than 50 dB below the fundamental.

The exposure and control units were operated at different ends of a dedicated room in an approved animal-care facility. The fringing fields of the exposure units were undetectable at the location of the control units. The ambient 60-Hz field at the control locations averaged less than 0.4 mG, and was never higher than 0.7 mG. Current, magnetic field, and temperature were continually recorded every 2 minutes to provide a historical record. The room was continuously maintained under temperature and humidity control. The room air was replaced 15 times per hour with fresh air.

Results

Partial results for male mice exposed for 1-49 days, 5000 mG, are shown in **Figure 1.** EMF exposure resulted in changes in the distribution of each of the listed sub-populations. Under the same exposure conditions, the field had no effect on the distribution of NK cells, the proliferation of CTL, or the lytic activity of CTL or NK cells.

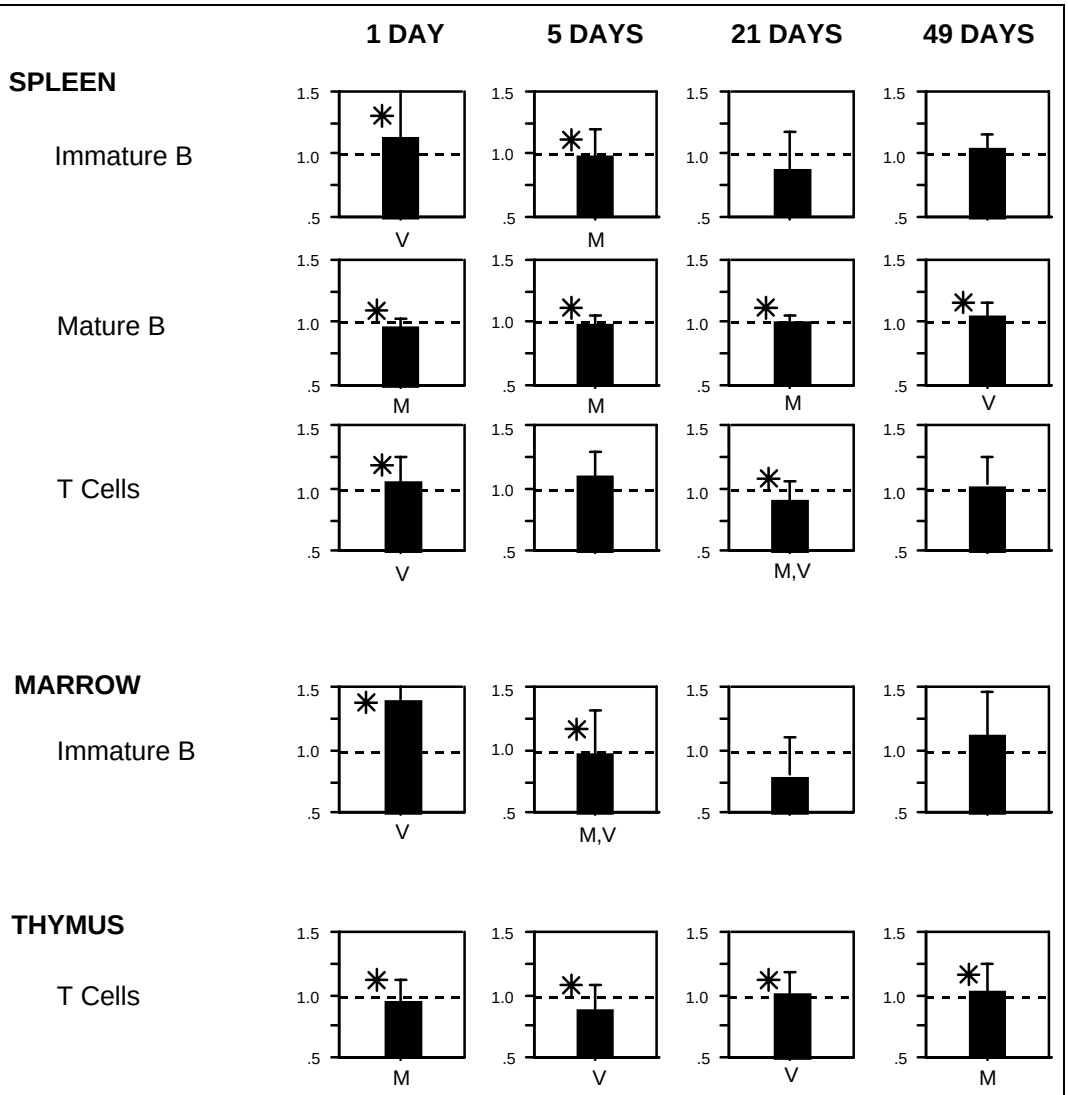

FIGURE 1. Effect of exposure of male mice to 5000 mG, 60 Hz for 1-49 days. At each time, the results of 3 replicates were combined (N=5 in the exposed and sham-exposed groups in each replicate). For purposes of graphical representation the data was normalized so that the mean of the control groups was 1.0. ✳ $P < 0.0285$, based on the L test. In the cases where the comparisons were significant, the basis of the significance is indicated (M, mean; V, variance).

The results of corticosterone determinations in the serum of mice exposed to 5000 mG, 175 days are shown in **Figure 2.**

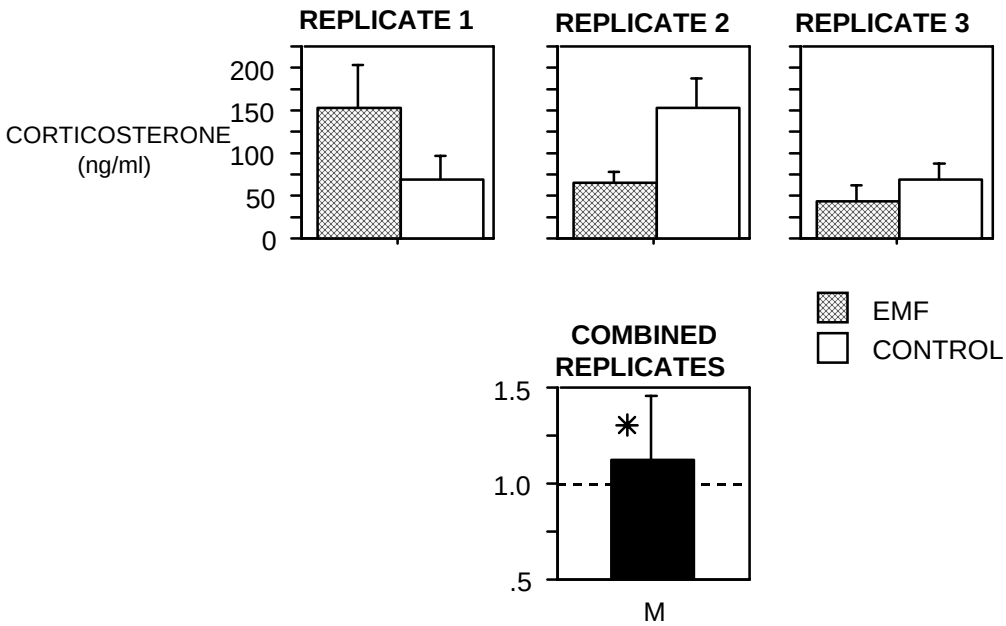

FIGURE 2. Effect of exposure of male mice to 5000 mG, 60 Hz, for 175 days. The results from each of 3 replicates (top) and the overall results (bottom) are shown. For display of the overall results, the data was normalized so that the mean of the control groups was 1.0. ✳P < 0.0285, based on the L test. M (mean) indicates the basis of the significance found in the L test.

The corresponding calculation for the L statistic (Table 1) clearly illustrates how test means can differ differently in each replicate and yet combine to evidence transduction.

REPLICATE NUMBER	EMF			CONTROL			L_1	L_2	L
	N	MEAN	SD	N	MEAN	SD			
1	5	151.80	111.28	5	68.40	64.66	1.406	2.330	3.737
2	5	66.80	26.49	4	153.50	69.44	3.612	6.090	9.701
3	4	42.75	37.11	4	69.75	34.50	0.021	1.388	1.409
							5.039	9.808	14.84 ✳

TABLE 1. Corticosterone levels in male mice exposed to 5000 mG, 175 days, in 3 replicates. ✳P < 0.0285.

To demonstrate the ability of the L test to discern differences between non-linear systems, the logistic equation was used to generate two non-linear data sets, each of which was sampled 15 times in 3 replicates (**Figure 3**).

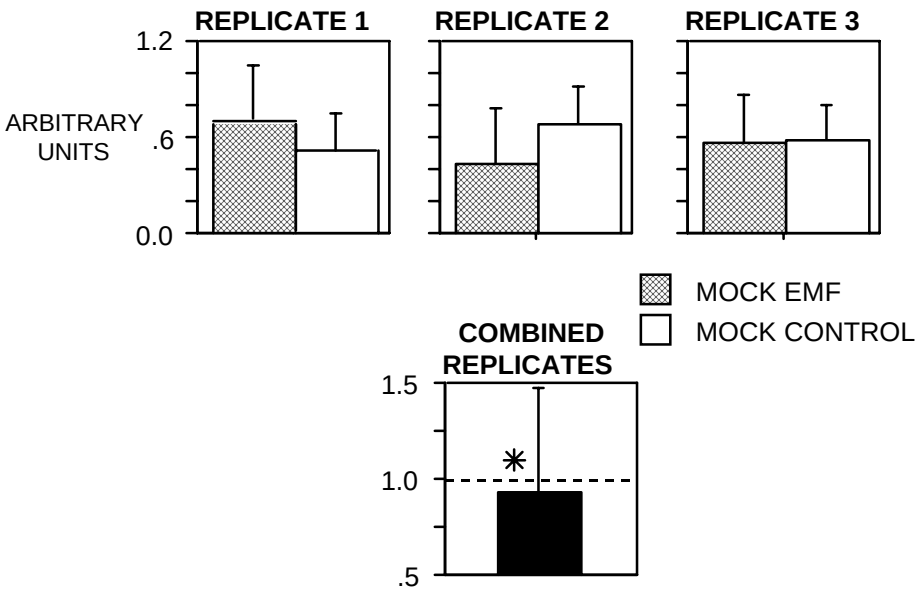

FIGURE 3. The results of random sampling from mock exposed and control groups generated by $x_k = 3.89x_{k-1}(1-x_{k-1})$ and $y_k = 3.99y_{k-1}(1-y_{k-1})$, respectively, with $x_0 = y_0 = 0.4$, k=1...100. Top, results from 3 replicates; bottom, overall results. For display of the overall results, the data was normalized so that the mean of the control groups was 1.0. * $P < 0.05$ based on the L test. M (mean) indicates the basis of the significance found using the L test.

The L test recognized the difference between the populations (Table 2) even though the groups in individual replicates differed differently (**Figure 3**).

REPLICATE NUMBER	EMF			CONTROL			L_1	L_2	L
	N	MEAN	SD	N	MEAN	SD			
1	15	0.698	0.350	15	0.524	0.251	1.609	2.348	3.957
2	15	0.438	0.339	15	0.685	0.217	2.949	5.191	8.140
3	15	0.569	0.295	15	0.577	0.214	1.494	0.006	1.500
							6.052	7.545	13.597 *

TABLE 2. Mock data obtained from random sampling of two non-linear systems. * $P < 0.05$. As a positive control, the procedure was repeated with all 6 samples drawn from the same data set but analyzed as indicated above. No differences were found, as expected ($L = 2.49$, $P > 0.05$).

Discussion and Contribution to Understanding Biological Effects of EMF

The health and safety issue raised by environmental electromagnetic fields is unsusceptible of resolution until several scientific, sociological, and procedural issues are resolved first. One of the threshold scientific issues involves the choice of the biological model used to explicate putative EMF/organism interactions. A physical model, by definition, is based on the assumption that the strength of the field principally determines the mean of the measured parameter, independent of the animal's genotype, history, and environment. The physical model is used, for example, to predict the toxic effects of chemicals.

A cybernetic model, in contrast, is defined as one based on the assumption that the instantaneous behavior of the organism is a result of the totality of the conditions present at the immediately preceding instant of time (27). Because the EMF is only one such condition, it (at most) only partially determines what can be observed. The biological variability ordinarily found in an ostensibly identical group of animals exemplifies application of the cybernetic model; although each measurement is determined and bounded, it is not predictable. The cybernetic model requires a reconceptualization of EMF/tissue interactions leading to acceptance of the idea that observables are (at most) only partially predictable, and need not fulfill a subjective requirement of consistency.

The cybernetic model was chosen in this study for several reasons. First, the seminal issue in all EMF bioeffects studies is whether or not EMF transduction occurred. An effect rationalized using the physical model is sufficient but not necessary to imply transduction because the physical model is derived from the cybernetic model in the limit. Thus, the absence of an effect based on use of the physical model would not imply that transduction did not occur. In contrast, in the cybernetic model, the absence of a change in the response of the organism to an EMF would reasonably imply that transduction did not occur. Thus the cybernetic model is singularly suited to attempts to resolve the most fundamental (and divisive) issue regarding EMF bioeffects — the occurrence of transduction.

Second, among the studies in which the physical model has been used, virtually every report of an EMF-induced bioeffect has been followed (or preceded) by a negative report involving a reasonably comparable biological system and the evaluation of a reasonably comparable experimental hypothesis. As a result, not a single EMF-induced bioeffect is presently accepted in the sense that general relativity, the theory of radioactive decay, or the law of gravity are accepted. On the other hand, in the case of one line of studies (those involving EMF-induced effects on growth rate) adoption of the cybernetic model led to a consistent answer to the question of whether the field actually caused biologic effects (7). The fact that the cybernetic model harmonized the implications of reports from different laboratories suggests that conflicts which flow from the use of the physical model are artifacts produced by the model, and that adoption of the cybernetic model would resolve the apparent controversies.

Finally, a living organism is perhaps the best possible example of a far-from-equilibrium system, and it is to be expected that responses to relatively low-energy stimuli will be non-linear in nature (because other conditions besides the EMF are important in determining the response of the animal). Indeed, it is probably the case that (with the exception of studies involving the measurement of parameters associated with immediate early stages of transduction) any EMF- induced biological effects that are not non-linear are either trivial or unrelated to the fundamental issue of health risks due to environmental electromagnetic fields. Thus, on theoretical grounds, only the cybernetic (non-linear) model is applicable to effects caused by environmental EMFs.

Although important issues are not yet resolved in this study, the results showed that EMFs can cause changes in the immune system, possibly mediated by the neuroendocrine system. This conclusion suggests the existence of a specific biological pathway linking environmental EMF exposure and human disease, and the model on which it was based seems capable of providing a framework for explaining the laboratory and epidemiological reports involving EMF-induced effects.

Publications From This Grant

1. Mailhes JB, Young D, Marino AA and London SN. “Electromagnetic fields enhance chemically-induced hyperploidy in mammalian oocytes.” Mutagenesis 12:347-351, 1997.

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1. Staczek J, Marino AA, Gilleland LB, Pizarro A and Gilleland HE Jr. "Low-frequency electromagnetic fields alter the replication cycle of MS2 bacteriophage." Current Microbiology, In Press, 1997.
2. Landry PS, Sadasivan KK, Marino AA and Albright, JA. "Electromagnetic fields can affect osteogenesis by increasing the rate of differentiation." Clin. Orthop. 338:262-270, 1997.
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TITLE: **Studies to Evaluate the Toxicity and Potential Carcinogenicity of 60 Hz Magnetic Fields in Laboratory Animals**

Principal Investigator: David L. *McCormick*, Ph.D.
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Health Relevance: Cancer

Research Area: Toxicology Studies Using Whole Animals; Lifetime/Generational Studies Cancer

N01-ES25351 **Start Date:** 8/10/92 **End Date:** 3/31/98

Abstract

The results of a number of epidemiological studies suggest that exposure to power frequency (60 Hz) magnetic fields may be associated with a variety of adverse health effects in humans. These effects include reproductive dysfunction, immune alterations, behavioral effects, and an elevated risk of cancer in several target organs. However, the *in vivo* experimental data base on which risk assessments for such effects can be developed is very limited. To address this data gap, a program to evaluate the toxicology and potential carcinogenicity of 60 Hz magnetic fields in experimental animals is in progress. This program is focused on studies using well-characterized animal model systems in which a significant toxicology/carcinogenicity data base has been published. All studies are being conducted in full compliance with Good Laboratory Practice (GLP) regulations, using a fully dedicated, 21,000 square foot magnetic field exposure facility that was constructed at IIT Research Institute specifically for the NIEHS/NTP electromagnetic field research program. Operation of the facility was validated independently by the National Institute of Standards and Technology prior to the initiation of the program, and is being revalidated annually throughout the project period.

In all studies, experimental groups include animals exposed continuously for 18.5 hours per day to linearly polarized sinusoidal 60 Hz magnetic field strengths of 0 G, 20 mG, 2 G, or 10 G, or intermittently (1 hr on/1 hr off) to 10 G fields. The program includes the following *in vivo* bioassays: a) 8-week toxicity studies in F344 rats and B6C3F1 mice, in which any effects of subchronic exposure to magnetic fields on overall animal health, specific organ function (monitored by clinical chemistry assays), and hematologic parameters are identified; b) a developmental toxicity (teratology) study in Sprague-Dawley rats, in which animals are exposed to magnetic fields *in utero* to assess possible effects on prenatal development; c) a multigeneration reproductive toxicity study in Sprague-Dawley rats using the Reproductive Assessment by Continuous Breeding (RACB) protocol, to identify any effects of magnetic field exposure on reproductive function; d) lymphoma promotion studies in PIM and p53 knockout transgenic mice, in which the influence of magnetic field exposure on lymphoma induction is assessed in animals that are genetically predisposed to the disease; e) immunotoxicology studies in mice, in which the influence of magnetic field exposure on lymphoid organ weight and cellularity, T-cell number and function, B-cell number and function, NK-cell function, composition of T-lymphocyte subsets, and host resistance to infection is determined; f) pineal function studies in F344 rats and B6C3F1 mice, in which the influence of magnetic fields on serum and pineal melatonin levels and pineal N-acetyltransferase activity is determined; and g) 2-year chronic toxicity/oncogenicity evaluations in F344 rats and B6C3F1 mice, in which the toxicity and potential carcinogenicity of magnetic fields are evaluated in animals exposed over the majority of their natural life span. Group sizes have been increased beyond those traditionally used in toxicity and carcinogenicity bioassays to increase statistical power, and to increase the likelihood of detecting small effects.

Experimental Design and Exposure Conditions

- Experimental Model Systems -- For most studies, F344 rats and B6C3F1 mice are being used. These model systems are used routinely by the National Toxicology Program for toxicology and carcinogenicity bioassays, and an extensive data base is available concerning in-life, clinical pathology, and histopathology parameters for these animal strains. Specialized endpoint studies (developmental and reproductive toxicity studies in Sprague-Dawley rats, lymphoma promotion studies in transgenic mice, specific immune function endpoint studies in several mouse strains) are being conducted in animal model systems for which an established data base is available.
- Experimental Endpoints -- The influence of exposure to 60 Hz magnetic fields on overall animal health (monitored by survival, body weight, clinical observations, clinical pathology, and histopathology evaluations), cancer incidence (monitored by clinical observation, gross pathology, and histopathology evaluations), reproductive function (monitored as number and frequency of successful matings, litter size and weight, and pup survival), developmental toxicity (monitored as fetal survival, weight, and incidence of birth defects), immune function (monitored by assessment of T-cell number and function, B-cell number and function, NK cell function, and host resistance to infectious challenge), and pineal function (monitored by quantitation of serum and pineal melatonin and pineal activity of N-acetyltransferase) is being determined in animals exposed to magnetic fields for periods of up to two years. Where available, appropriate positive control materials are included in experimental designs.
- Magnetic Field Polarization, Frequency, and Harmonic Content -- Linearly polarized, sinusoidal 60 Hz fields with < 3% total harmonic distortion. Fields are ramped on and off over 7 to 9 cycles to preclude the generation of magnetic field transients.
- Magnetic Field Intensity -- 0 G (sham control), 20 mG continuous, 2 G continuous, 10 G continuous, and 10 G intermittent (1 hr on, 1 hr off).
- Exposure Duration -- 18.5 hrs per day for up to 2 years.
- Relative Orientation of AC and DC Magnetic Fields -- AC fields horizontal (perpendicular to primary component of DC field).
- Associated Electric Fields -- < 100 Volts/meter.
- Field Spatial Homogeneity -- Uniform to + 10% throughout exposure volume.
- Field Temporal Homogeneity -- Measured continuously throughout program; temporal variability is < 5% over nearly 2 years of exposure system operation.
- Field Monitoring -- Continuous throughout program (during both exposures and field-off conditions); monitoring includes both field strength and waveform.
- Geometry of Animal Exposure -- Animals are allowed unrestricted mobility at all times during exposures.
- Environmental Conditions -- Environmental conditions in all animal exposure rooms are maintained in the ranges of 72 + 3°F, 50% + 15% relative humidity, and > 10 air changes per hour. Activation of magnetic field exposure modules has no effect on any environmental parameter. Environmental conditions are monitored continuously; sensors are alarmed to alert project personnel in the event of deviations from environmental set points.
- Other Environmental Cues -- Noise, vibration, and lighting in exposure rooms are monitored continuously. Noise levels vary by < 1 dB in the 125 Hz octave band when system is energized. Vibration is undetectable in animal cage racks. Lighting is controlled by automatic timer; light levels are identical in all exposure rooms.
- Stray Fields -- Laboratory site has been fully mapped for ambient 60 Hz and geomagnetic (dc) fields; temporal characteristics of ambient magnetic fields have been determined. Ambient magnetic fields in all animal exposure rooms are < 1 mG. No DC field cancellation is used. Ambient fields are monitored continuously in sham control room, and during field shutdown periods in all other rooms.
- Sham Exposure Conditions -- Ambient 60 Hz fields are monitored continuously in sham control room; ambient fields are uniformly < 1 mG.
- Crosstalk -- < 1 mG between rooms in worst-case room rotation scenario.

Quality Assurance Measures

Regulatory Compliance -- All studies conducted in full compliance with FDA Good Laboratory Practice (GLP) Regulations. The IITRI Quality Assurance Unit conducts a regular program of critical phase inspections and data

audits to ensure compliance with individual study protocols, IITRI Standard Operating Procedures (SOPs), and the NTP Statement of Work.

Ambient 60 Hz and Static Magnetic Fields -- Fully mapped by IITRI engineers prior to installation of exposure system and incubators. No DC field cancellation.

Magnetic Field Monitoring -- Field strength and waveform monitored continuously throughout program (both during exposure periods and periods of module shutdown). Exposure system performance inspected and validated by NIST prior to program initiation and annually throughout project period.

Control for Positional Effects -- A regular schedule of animal rotation (from top to bottom within a rack, from bay to bay within each exposure room, and between exposure rooms) is maintained to preclude the possibility that data may be confounded by positional effects within animal racks, animal rack bays, or animal exposure rooms.

Positive Controls -- Where available, appropriate positive control agents have been included in study designs.

Peer Review -- All histopathologic findings will be reviewed by a National Toxicology Program Pathology Working Group.

Results

TR-488

Toxicology and Carcinogenesis Studies of 60-HZ Magnetic Fields in F344/N Rats and B6C3F1 Mice (Whole-body Exposure Studies)

Abstract

Electric and magnetic fields (EMF) are associated with the production, transmission, and use of electricity; thus, the potential for human exposure is high. These electric and magnetic fields are predominantly of low frequency (60 Hz in the United States and 50 Hz in Europe) and generally of low intensity. Epidemiology studies have suggested a potential for increased breast cancer, brain cancer, and leukemia rates with increasing magnetic field exposure. Therefore, given the widespread exposure to low-intensity, 60-Hz magnetic fields in industrialized societies, standard toxicology studies and long-term carcinogenesis studies were conducted using traditional rodent models. Male and female F344/N rats and B6C3F1mice were exposed to 60-Hz magnetic fields by whole-body exposure for 2 years.

2-Year Study In Rats: Groups of 100 male and 100 female rats were exposed to 60-Hz magnetic fields at intensities of 0.02, 2, or 10 G for 18.5 hours per day, 7 days per week, for 106 weeks. Groups of 100 male and 100 female control rats were housed in the same exposure chambers without applied magnetic fields. Additional groups of 100 male and 100 female rats were intermittently exposed (1 hour on and 1 hour off) to a 10 G 60-Hz field 18.5 hours per day, 7 days per week, for 106 weeks.

Survival and Body Weights: Survival of exposed rats was similar to that of the control rats. Mean body weights of exposed groups of male and female rats were similar to those of the control groups throughout the study.

Pathology Findings: The incidences of thyroid gland C-cell adenoma and carcinoma in 0.02 G male rats and of adenoma and adenoma or carcinoma (combined) in 0.02 and 2 G males were significantly greater than in the control group. The incidence of mononuclear cell leukemia in males in the 10 G intermittent group was significantly less than in the control group.

2-Year Study In Mice: Groups of 100 male and 100 female mice were exposed to 60-Hz magnetic fields at intensities of 0.02, 2, or 10 G for 18.5 hours per day, 7 days per week, for 106 weeks. Groups of 100 male and 100 female control mice were housed in the same exposure chambers without applied magnetic fields. Additional groups of 100 male and 100 female mice were intermittently exposed (1 hour on and 1 hour off) to a 10 G 60-Hz field 18.5 hours per day, 7 days per week, for 106 weeks.

Survival and Body Weights: Survival of male mice exposed to 10 G was significantly less than that of control mice after 2 years; survival of all other exposed groups of mice was similar to that of control mice. Mean body weights of exposed groups of male and female mice were similar to those of the control groups throughout the study.

Pathology Findings: The incidences of alveolar/bronchiolar adenoma were significantly decreased in 0.02 and 2 G male mice and 2 G female mice relative to the control groups; the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were significantly less in males and females exposed to 2 G than in the control groups. In female mice, the incidence of malignant lymphoma in the 10 G intermittent group was significantly less than in the controls.

Results

Under the conditions of these 2-year whole-body exposure studies, there was equivocal evidence of carcinogenic activity of 60-Hz magnetic fields in male F344/N rats based on increased incidences of thyroid gland C-cell neoplasms in the 0.02 and 2G groups. There was no evidence of carcinogenic activity in female F344/N rats or male or female B6C3F1 mice exposed to 0.02, 2, or 10 G, or 10 G intermittent 60-Hz magnetic fields.

In exposed rats and mice there were no increased incidences of neoplasms at sites for which epidemiology studies have suggested association with magnetic fields (brain, mammary gland, leukemia).

Pathology Tables, Survival and Growth Curves from NTP 2-year Studies
Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 60-Hz Magnetic Fields

	Male F344/N Rats	**Female F344/N Rats**	**Male B6C3F1 Mice**	**FemaleB6C3F1 Mice**
Exposure field intensity	Control, 0.02, 2, or 10 G continuous, or 10 G intermittent	Control, 0.02, 2, or 10 G continuous, or 10 G intermittent	Control, 0.02, 2, or 10 G continuous, or 10 G intermittent	Control, 0.02, 2, or 10 G continuous, or 10 G intermittent
Body weights	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group
Survival rates	57/100, 46/100, 47/100, 48/100, 59/100	59/100, 68/100, 60/100, 61/100, 58/100	76/100, 72/100, 84/100, 62/100, 74/100	70/100, 74/100, 79/100, 74/100, 77/100
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	None	None	None
Uncertain findings	Thyroid Gland (C-cell):adenoma (15/99, 25/100, 26/100, 23/100, 18/100); carcinoma (1/99, 7/100, 4/100, 2/100, 5/100); adenoma or carcinoma (16/99, 31/100, 30/100, 25/100, 22/100)	None	None	None0
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	No evidence	No evidence

Eight-Week Toxicity Studies -- The results of 8-week toxicity studies were negative: sub-chronic exposure to magnetic fields had no biologically significant effect on any parameter measured in either sex of either F344 rats or B6C3F1 mice.

Developmental Toxicity Studies -- The conduct of gross external, visceral, cephalic, and skeletal evaluations on more than 3,000 Sprague-Dawley rat fetuses revealed no evidence of magnetic field teratogenicity. Fetal survival, fetal body weights, and maternal body weights in EMF-exposed groups did not differ from sham controls.

Reproductive Toxicity Studies -- The conduct of a multi-generation reproductive toxicity study in Sprague-Dawley rats using the Reproductive Assessment by Continuous Breeding (RACB) design revealed no adverse effect of magnetic field exposure on reproductive performance in either sex.

Lymphoma Promotion Studies -- Studies conducted in two strains of transgenic mice [PIM mice carrying the pim-1 oncogene and TSG-p53 mice, in which the tumor suppressor gene, p53, has been deleted] revealed no activity of 60 Hz magnetic field exposure as a promoter of hematopoietic neoplasia.

Pineal Function Studies -- Neither amplitude studies (in which pineal function is assessed at a single time point) in F344 rats or B6C3F1 mice nor time course studies (in which pineal function is characterized throughout the dark cycle) in B6C3F1 mice revealed any biologically significant effect of subchronic magnetic field exposure on serum or pineal melatonin content or pineal activity of N-acetyltransferase, the rate-limiting enzymatic step in melatonin biosynthesis.

Immunotoxicology Studies -- Subchronic exposure to magnetic fields had no effect on: a) lymphoid organ weight or cellularity in B6C3F1 mice; b) B-cell function in B6C3F1 mice measured by the antibody plaque-forming assay; c) T-cell function in BALB/c mice measured by delayed-type hypersensitivity responses; d) lymphocyte subsets in B6C3F1 mice, as enumerated via FACS analysis; or e) host resistance to infection with *Listeria monocytogenes*. Decreases in NK cell activity in B6C3F1 mice (assessed by YAC-1 cytolysis) have been observed in some studies. However, the influence of EMF on NK cell function is variable between experiments, and no clear pattern has emerged from a total of 4 replicates of the NK study design.

Two-Year Chronic Toxicity/Oncogenicity Studies -- Two year studies to evaluate the influence of chronic exposure to magnetic fields in F344 rats and B6C3F1 mice were recently completed. Group sizes of 100 animals per sex per group were used to increase the statistical power of the bioassays, and to increase the likelihood of detecting small effects. Chronic exposure to pure 60 Hz magnetic fields resulted in a statistically significant increase in mortality in male B6C3F1 mice, but not in female B6C3F1 mice or in either sex of F344 rats. Chronic exposure induced no gross toxicity, and had no effect on animal body weight. Peer review of histopathology evaluations (all gross lesions + approximately 50 tissues per animal for 1000 animals per study) is currently in progress.

Discussion and Contribution to Understanding Biological Effects of EMF

With the possible exception of a modest suppression of NK cell function in female mice, the results of studies that have been completed as part of the present program have not identified any biologically significant toxic effects of 60 Hz magnetic fields in either rats or mice. It is important to note, however, that peer review of histopathology data from the two-year chronic toxicity/oncogenicity studies in rats and mice will not be completed until early 1998. Because the program has been conducted using well-studied animal model systems and large group sizes, the lack of observed effects provides support for the view that environmental exposure to power frequency magnetic fields is not a significant risk factor for human disease.

The goals of the RAPID program include the development of experimental and epidemiological databases that can be used to support risk assessments of magnetic field exposure in humans. The present program will contribute directly to this program goal, in that an extensive series of biological evaluations of magnetic field action have been performed using model systems that have been used widely in the development of risk assessments for other agents.

The overall contribution of this program is the evaluation of the potential toxic and/or oncogenic activity of 60 Hz magnetic fields in well-studied animal model systems. Because no biologically significant effects have been seen to date, our data do not support the hypothesis that magnetic field exposure has significant deleterious effects on

animal health, reproduction, fetal development, immune function, or pineal function. Conclusions concerning the potential activity of 60 Hz magnetic fields in cancer induction must await completion of peer review of histopathology data from the two-year studies.

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Other EMF Publications

None

TITLE: **Mechanism of EMF Action in Human Breast Epithelial Cells**

Principal Investigator: David L. *McCormick*, Ph.D.
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Chicago, Illinois

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Proliferation

R01-ES07093 **Start Date:** 09/30/94 **End Date:** 08/31/98

Abstract

Increased exposure to power frequency magnetic fields (EMF) has been proposed as a causal factor in the rising incidence of breast cancer in the United States. However, plausible biological mechanisms for the putative tumor enhancing effects of EMF at the level of the breast epithelial cell are essentially lacking. The overall hypothesis being investigated in this program is that the effects of EMF on the breast are epigenetic in nature, and are effected through influences on the action of hormones and other regulators of breast epithelial cell differentiation and proliferation. Using primary cultures of normal human breast epithelial cells and the non-transformed human breast epithelial cell strain HBL-100 as an *in vitro* model systems, the program will: a) determine the influence of EMF + hormones on proliferation of human breast epithelial cells; and b) investigate alterations in expression of breast cancer-associated genes as a mechanism for EMF + hormone effects. Using an extensively validated *in vitro* EMF exposure system, initial studies focus on the effects of EMF alone on cell proliferation and the expression of known cancer-associated genes, including HER-2/neu, *c-myc*, p53, and cyclin E. Dose-response and time-response parameters will be determined to investigate the reversibility of induced effects, and the hypothesized existence of field strength "windows." After studies with EMF alone are completed, a series of addition and deletion experiments will be performed to study the interactions between EMF and hormones; these studies will be directed towards the identification of one or more endocrine mediators of EMF action in the breast. Should EMF be found to have no influence on the expression of the battery of breast cancer-associated genes heretofore evaluated, differential display studies will be performed with sham- and EMF-exposed cells in order to identify other genes in human breast epithelial cells whose expression is altered by EMF exposure. The results of these studies should identify one or more specific genes whose altered expression is associated with the proliferation of human breast epithelial cells, and will evaluate the role of endocrine status as a modifier of the biological activity of EMF.

Experimental Design and Exposure Conditions

- Experimental Model System -- Normal (non-transformed) human breast epithelial cells (primary cultures), and non-transformed HBL-100 human breast epithelial cells.
- Experimental Endpoints -- The influence of exposure to 60 Hz magnetic fields + mammotropic hormones on mammary epithelial cell proliferation and the expression of cancer-associated genes (at the mRNA and protein level) will be assessed. Cell proliferation/cytotoxicity of EMF will be quantitated by the MTT and/or XTT assays. The influence of EMF on gene expression will be evaluated by Northern and Western blot assays, by RNAse protection assays, and by differential display. Appropriate positive and negative control materials will be included in all assays.
- Magnetic Field Polarization, Frequency, and Harmonic Content -- Linearly polarized, pure 60 Hz fields; < 3% total harmonic distortion.
- Magnetic Field Intensity Range -- 10 mG to 10 G.

- Relative Orientation of AC and DC Magnetic Fields -- AC fields vertical (parallel to primary component of DC field).
- Associated Electric Fields -- < 10 Volts/meter.
- Field Spatial Homogeneity -- Uniform to + 5% throughout exposure volume.
- Field Temporal Homogeneity -- No variations expected (experimental fields are derived from function generator, independent of line voltage). Field temporal homogeneity is determined periodically throughout research program.
- Field Monitoring -- Continuous throughout program (during both exposures and field-off conditions; monitoring includes both field strength and waveform).
- Geometry of Cell Culture System -- Magnetic field exposure will be vertical (perpendicular to monolayer cultures of human mammary epithelial cells).
- Environmental Conditions -- Temperature in all exposure chambers will be maintained at 37 + 0.2 ^{o}C, in an environment held at 5.0 + 0.5% CO_2. Environmental conditions in all EMF exposure chambers are controlled by a single incubator located remotely from the exposure chambers.
- Stray Fields -- Laboratory site fully mapped for ambient 60 Hz and geomagnetic (dc) fields. Temporal characteristics of ambient magnetic fields have been determined; ambient magnetic fields are < 1 mG. No DC field cancellation. All incubators are Faraday-shielded.
- Sham Exposure Conditions (including cross-talk) -- 60 Hz fields in sham control incubator are < 1 mG (total of fields generated by cross-talk from energized experimental module + fields generated by energized sham).
- Blinding -- All studies conducted using fully blinded design.

Quality Assurance Measures

- Ambient 60 Hz and Static Magnetic Fields -- Fully mapped by IITRI engineers prior to installation of exposure system and incubators. No DC field cancellation.
- Magnetic Field Monitoring -- Field strength and waveform monitored continuously throughout program (both during exposure periods and periods of module shutdown). Exposure system performance has been validated by a DOE site visit team.
- Study Blinding -- All studies conducted using fully blinded design. Selection of sham/exposure, exposure/sham, or sham/sham design is made by computer; cell and molecular biology staff are unaware of exposure regimen until exposures are uncoded after completion of endpoint analyses.
- Positive Controls -- Agents with known activity in modulating expression of cancer-associated genes (tumor promoters, hormones, and chemopreventive agents) are included in the design of all studies. Housekeeping genes and loading controls are included in all studies to ensure no systematic bias in study design or performance.

Results

Initial work focused on replication of the studies of Goodman and Henderson, who reported that 60 Hz magnetic field exposure increases the expression of *c-myc* in HL-60 promyelocytic leukemia cells. The findings of Goodman and Henderson were not replicated: magnetic field exposure had no effect on either the basal expression of *c-myc* in HL-60 cells, or on the induction of *c-myc* expression by the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). Two sets of replication studies were conducted independently by two different molecular biologists in our laboratory; their results were comparable, and failed to confirm the findings of Goodman and Henderson.

After replication studies were completed, experimental work was focused on studies in two types of human breast epithelial cells. HBL-100 cells are estrogen receptor negative (ER-), and do not respond to the addition of exogenous estrogen; they serve as a model of hormone-independent breast cell growth. Primary serum-free cultures of normal (non-transformed) human mammary epithelial (HME) cells do increase their proliferation in response to low doses of estrogen or to the inclusion of phenol red in the medium; HME cells are used as a model of hormone-responsive breast epithelium.

Exposure to 60 Hz magnetic field strengths of 100 mG to 10 G for periods ranging from 20 minutes to 24 hours has no effect on hormone-independent cell proliferation or on the expression of cancer-associated genes (*c-myc*,

p53, or HER-2/neu) in either type of human breast epithelial cell. Furthermore, magnetic field exposure has no effect on the induction of *c-myc* expression by TPA or the induction of p53 expression by mitomycin C. Interaction experiments to study effects of magnetic fields + hormones in HME cells are in progress.

Because no alterations in the expression of known cancer-associated genes have been identified in either HME or HBL-100 cells, additional studies are in progress in which differential display technology is being used to identify other genes in HME cells whose expression is altered (by > 50%) by EMF exposure. These studies are being conducted in collaboration with Dr. Gayle Woloschak of Argonne National Laboratory. Initial experiments have identified four genes whose expression in HME cells and in HeLa cells may be up- or down-regulated by EMF. Preliminary sequence analyses have found no homology with known genes. This work is continuing; should a robust effect on any individual gene be identified, studies with that gene will be expanded to include considerations of dose-response and time-response parameters for alterations in its expression.

Discussion and Contribution to Understanding Biological Effects of EMF

No differences in the expression of a panel of known breast cancer-associated genes have been identified in HBL-100 or HME human breast epithelial cells exposed to 60 Hz magnetic fields. Similarly, 60 Hz magnetic fields do not alter the expression of these genes in cells exposed simultaneously to other modulators of gene expression. By contrast, however, four genes have been identified by differential display whose expression is up- or down-regulated in both HME cells and in HeLa cells exposed to 60 Hz magnetic fields. Although the function of these genes is not known, demonstration of their altered expression in cells exposed to magnetic fields suggests a novel magnetic field bioeffect.

This program contributes to RAPID program goals and to our understanding of the biological effects of magnetic fields by investigating molecular mechanisms of magnetic field action in a cell type that has been suggested as a target tissue for EMF action. Data obtained to date suggest that any influence of magnetic fields on human breast epithelial cells is unlikely to be mediated by effects on a currently identified oncogene or tumor-suppressor gene. However, several genes that may serve as targets for EMF action have been identified using differential display technology. The biological function of these genes remains to be elucidated.

Publications From This Grant

None

Other EMF Publications

None.

TITLE: ***In Vivo* Responses to Complex EMF Exposure Metrics**

Principal Investigator: David L. *McCormick*, Ph.D.
IIT Research Institute
Chicago, Illinois

Health Relevance: Neurobehavioral

Research Area: Studies Involving Whole Animals; Neurobehavioral; Melatonin

RO3-ES08854 **Start Date:** 1/10/97 **End Date:** 12/31/97

Abstract

Exposure to power frequency magnetic fields (EMF) has been proposed as a causal factor in the induction of a wide range of adverse health effects, including cancer. However, although the EMF epidemiology is suggestive, no conclusive data exist to support this hypothesis. To date, most *in vivo* experimental studies of EMF bioeffects have used an idealized metric in which animals are exposed continuously to pure, linearly polarized 50 or 60 Hz sine waves. A key limitation to the design of these studies, however, is that they use a highly idealized exposure metric that does not include several components of environmental EMF to which humans are routinely exposed. To address this critical data gap, we are investigating the biological activity of complex EMF metrics that include power frequency harmonics, transients, and time-varying magnetic fields, using pineal function in rats as a biomarker. Alterations in pineal enzyme activity and decreased serum melatonin have been implicated as potentially causal in carcinogenesis, reproductive dysfunction, and other adverse health effects; pineal function can be quantitated precisely, and is modulated by exposure to physical factors. As such, pineal function provides a sensitive *in vivo* biomarker with which to study EMF effects.

The goal of the program is to identify biologically active EMF metrics other than continuous 60 Hz sine waves, in order to support a more complete assessment of the health effects of EMF exposure. The program will investigate two hypotheses: (1) superimposition of power frequency harmonics or transients onto a pure 60 Hz magnetic field will induce biological responses that are not induced by exposure to pure 60 Hz magnetic fields alone, and (2) rapidly intermittent or time-varying exposure to EMF has biological activity that is not seen with continuous EMF exposure. To investigate these two hypotheses, timed-sacrifice studies will be performed to quantitate pineal function in F344 rats exposed to several complex EMF exposure metrics. Amplitude and/or time-shift effects will be identified through comparisons of serum melatonin, pineal melatonin, and pineal activity of N-acetyltransferase in EMF-exposed and control rats. EMF metrics to be evaluated will include pure continuous 60 Hz fields, intermittent 60 Hz fields, time-varying 60 Hz fields, pure continuous 180 Hz fields, 60 Hz fields onto which a 180 Hz harmonic has been superimposed, 60 Hz fields onto which 180 Hz, 300 Hz, and 420 Hz harmonics have been superimposed, and continuous and time-varying 60 Hz fields onto which a series of environmentally relevant transients have been superimposed. This approach will identify complex EMF metrics with biological activity; active EMF metrics may have activity as modifiers of human health.

Experimental Design and Exposure Conditions

- Experimental Model System -- F344 rats. This model has been used extensively in bioassays of magnetic field activity, including evaluations of pineal function.
- Experimental Endpoints -- The influence of exposure to magnetic fields on pineal function (serum and pineal melatonin levels and pineal activity of N-acetyltransferase) is being determined in animals exposed to

magnetic fields for four weeks. In addition, the influence of magnetic fields on the activity of ornithine decarboxylase (ODC) in tissues harvested from EMF-exposed rats is being determined.

- Magnetic Field Polarization, Frequency, and Harmonic Content -- Baseline metric: linearly polarized, sinusoidal 60 Hz fields with < 3% total harmonic distortion. Fields are ramped on and off over 7 to 9 cycles to preclude the generation of magnetic field transients. Harmonics: 180 Hz (3rd harmonic), or 180 Hz + 300 Hz + 420 Hz (3rd + 5th + 7th harmonics) superimposed on 60 Hz sine wave. Transients: 20 recorded transient events associated with transmission or use of electricity have been recorded and are superimposed randomly on the 60 Hz signal.
- Magnetic Field Intensity -- 0 G (sham control), or 2 G (total magnetic field exposure, all frequencies [60 Hz only, 60 Hz + harmonics, 60 Hz + transients]).
- Exposure Duration -- 18.5 hrs per day for four weeks.
- Relative Orientation of AC and DC Magnetic Fields -- AC fields horizontal (perpendicular to primary component of DC field).
- Associated Electric Fields -- < 100 Volts/meter.
- Field Spatial Homogeneity -- Uniform to + 10% throughout exposure volume.
- Field Temporal Homogeneity -- Measured continuously throughout program; temporal variability is < 5% over nearly 2 years of exposure system operation.
- Field Monitoring -- Continuous throughout program (during both exposures and field-off conditions); monitoring includes both field strength and waveform.
- Geometry of Animal Exposure -- Animals are allowed unrestricted mobility at all times during exposures.
- Environmental Conditions -- Environmental conditions in all animal exposure rooms are maintained in the ranges of 72 + 3 oF, 50% + 15% relative humidity, and > 10 air changes per hour. Activation of magnetic field exposure modules has no effect on any environmental parameter. Environmental conditions are monitored continuously; sensors are alarmed to alert project personnel in the event of deviations from environmental set points.
- Other Environmental Cues -- Noise, vibration, and lighting in exposure rooms are monitored continuously. Noise levels vary by < 1 dB in the 125 Hz octave band when system is energized. Vibration is undetectable in animal cage racks. Lighting is controlled by automatic timer; light levels are identical in all exposure rooms.
- Stray Fields -- Laboratory site has been fully mapped for ambient 60 Hz and geomagnetic (dc) fields; temporal characteristics of ambient magnetic fields have been determined. Ambient magnetic fields in all animal exposure rooms are < 1 mG. No DC field cancellation is used. Ambient fields are monitored continuously in sham control room, and during field shutdown periods in all other rooms.
- Sham Exposure Conditions -- Ambient 60 Hz fields are monitored continuously in sham control room; ambient fields are uniformly < 1 mG.
- Crosstalk -- < 1 mG between rooms in worst-case room rotation scenario.
- Quality Assurance Measures
- Regulatory Compliance -- All magnetic field exposures and pineal function evaluations are being conducted using procedures developed to be in full compliance with FDA Good Laboratory Practice (GLP) Regulations.
- Ambient 60 Hz and Static Magnetic Fields -- Fully mapped by IITRI engineers prior to installation of exposure system and incubators. No DC field cancellation.
- Magnetic Field Monitoring -- Field strength and waveform monitored continuously throughout program (both during exposure periods and periods of module shutdown).
- Control for Positional Effects -- A regular schedule of animal rotation (from top to bottom within a rack and from bay to bay within each exposure room is maintained to preclude the possibility that data may be confounded by positional effects within animal racks or animal rack bays.

Results

Studies are in progress; no final data are currently available.

Discussion and Contribution to Understanding Biological Effects of EMF

This program will contribute to overall RAPID program goals and to our understanding of the biological effects of EMF through the generation of data relevant to the possible biological activity of magnetic field metrics other than the pure continuous 50 or 60 Hz sine wave. Because environmental magnetic fields are time-varying, and contain harmonics and transients, evaluation of the possible activity of these "real-world" exposure metrics is critical to an overall evaluation of EMF health effects.

Publications From This Grant

None

Other EMF Publications

None.

TITLE: **60 Hz Magnetic Fields and Cellular Stress Response**

Principal Investigator: Lee James *McDonald*, Ph.D.
IIT Research Institute
Chicago, Illinois

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Cycle Control, Signal Transduction

R03-ES08911 **Start Date:** 1/10/97 **End Date:** 12/31/98

Abstract

Exposure to 60 Hz electromagnetic fields (EMF) may pose a human health hazard, based on epidemiological research indicating that it is a risk factor for the development of certain cancers. Currently, a goal is to design and perform experiments that will uncover the mechanisms by which EMF affects living systems at the cellular and biochemical levels of interaction. Cells respond to a variety of environmental changes and toxic insults such as elevated temperature, toxic chemical exposure, and several types of radiation, by a concerted mechanism of induction of protective proteins, known as the "stress" response. If EMF perturbs cells in any significant way, then this cell pathway is likely to be enacted. This proposal outlines a series of studies using a state-of-the-art *in vitro* EMF exposure facility to examine systematically the effects of 60 Hz EMF exposure on the stress response of cultured human cells. The potential induction of the cellular stress response by 60 Hz EMF will be addressed in a simple series of experiments, with, as integral to their design, specifications for the remote addition of stress-inducing reagents to cell cultures within the exposure field. Quantitative comparisons of sham-exposure versus EMF-exposure will be made in a blinded experimental design. Several endpoints will be followed in response to EMF-exposure alone and in combination with known stress-inducing treatments by oxidants or heavy metals. Immunological assays will be used to detect the induction of heat shock protein (HSP) expression for members of each of four major families of human HSP. In a separate set of experiments, a brief pretreatment with a low-level oxidant stress will be followed by a much higher toxic insult, and cell survival will be followed. The potential effects of 60 Hz EMF exposures on the stress response to either phase of these experiments will be determined. All together, these experiments will serve to thoroughly document the ability of 60 Hz EMF to induce a stress response in cultured human cells, and will aid in assessing the extent of risk to whole animals.

Experimental Design and Exposure Conditions

- Experimental Model System; Normal (non-transformed) human lymphoblastoid cells.
- Experimental Endpoints; The influence of exposure to 60 Hz magnetic fields + stress treatments on the expression of heatshock proteins (HSPs) will be assessed. Stress treatments include 20 minute exposure to 42 C, or treatment with approximately 10 M CdC/2. HSP expression will be evaluated by Western blot assays, using specific monoclonal antibodies. Cytotoxicity of stress exposures will be monitored, and appropriate positive and negative control materials will be included in all assays.
- Magnetic Field Polarization, Frequency, and Harmonic Content; Linearly polarized, pure 60 Hz fields; < 3% total harmonic distortion.
- Magnetic Field Intensity Range; 10 mG to 10 G.
- Relative Orientation of AC and DC Magnetic Fields; AC fields vertical (parallel to primary component of DC field).

- Associated Electric Fields; < 10 Volts/meter.
- Field Spatial Homogeneity; Uniform to + 5% throughout exposure volume.
- Field Temporal Homogeneity; No variations expected (experimental fields are derived from function generator, independent of line voltage). Field temporal homogeneity is determined periodically throughout research program.
- Field Monitoring; Continuous throughout program (during both exposures and field-off conditions; monitoring includes both field strength and waveform).
- Geometry of Cell Culture System; Magnetic field exposure will be vertical.
- Environmental Conditions; Temperature in all exposure chambers will be maintained at 37 + 0.2° C, in an environment held at 5.0 + 0.5% CO_2. Environmental conditions in all EMF exposure chambers are controlled by a single incubator located remotely from the exposure chambers.
- Stray Fields; Laboratory site fully mapped for ambient 60 Hz and geomagnetic (DC) fields. Temporal characteristics of ambient magnetic fields have been determined; ambient magnetic fields are < 1 mG. No DC field cancellation. All incubators are Faraday shielded.
- Sham Exposure Conditions (including crosstalk); 60 Hz fields in sham control incubator are < 1 mG (total of fields generated by crosstalk from energized experimental module + fields generated by energized sham).
- Blinding; All studies conducted using fully blinded design.

Quality Assurance Measures

- Ambient 60 Hz and Static Magnetic Fields; Fully mapped by IITRI engineers prior to installation of exposure system and incubators. No DC field cancellation.
- Magnetic Field Monitoring; Field strength and waveform monitored continuously throughout program (both during exposure periods and periods of module shutdown). Exposure system performance has been validated by a DOE site visit team.
- Study Blinding; All studies conducted using fully blinded design. Selection of sham/exposure, exposure/sham, or sham/sham design is made by computer; cell and molecular biology staff are unaware of exposure regimen until exposures are uncoded after completion of endpoint analyses.
- Positive Controls; Stress treatments known to induce expression of families of HSPs are included in the design of all studies. Constant gel loading with cell homogenate samples is controlled by matching the total protein. Commercially obtained HSP standards are included in all immunoblot analyses to ensure assay performance.

Results

Human cell cultures of several types have been established and investigated for potential stress responses to EMF; among the cell types being studied are human lymphoblastoid cells, normal human mammary epithelial cells and HBL100 cells, transformed human mammary epithelial cells. The latter two cell lines are under investigation because of a large body of work performed at IITRI with these cells in the *in vitro* 60 Hz EMF exposure facility. In addition, there are frozen stocks of cell culture homogenates from a thorough series of experiments in which these cells were exposed to EMF, at 0.1, 1, and 10 G for 1, 4, and 24 hours. We are in the process of analyzing these cell homogenates for the presence of protein markers of an induced stress response, with our antibody based assays.

Each of the cell types in use has been shown to mount a robust stress response after exposure to a standard stressing treatment, i.e. heat shock caused by incubation at 42° C for 20 minutes. In addition, the cells have been shown to respond to $CdCl_2$ exposure with a stress response, a finding that is being confirmed in a series of dose response design exposure experiments. There are several distinct families of "heat shock proteins" (HSPs) that may be induced in response to one or another stress exposure. In this program we proposed using specific, commercially available antibodies to measure the actual concentration of these proteins in cells receiving EMF or sham exposures. Antibodies were obtained that recognized HSP32, HSP60, HSP70, and HSP90. In the cell types being exposed to EMF and positive control stress treatments, the immunoassays for HSP60 and HSP70 have proved to be the most robust and reliable; these tests are being used for the majority of cell exposure experiments. The other assays, for HSP32 and HSP90, require further optimization prior to their addition to the battery of assays employed.

A number of EMF exposure experiments have been performed to date, mostly involving 24 hour exposure times at EMF field strengths from 0.1 to 10 G. After the exposure period, cells are collected and homogenized and the overall protein concentration assessed. Replicate samples of a constant protein content are subjected to SDS PAGE and Western blotting with the specific antibodies recognizing the HSPs. The strength of the signal on the blots is quantified by densitometric analysis of exposed X-ray films. Results of experiments and analyses performed up to the present time have not demonstrated statistically significant differences in the expression of HSPs in comparisons of sham exposed control cell cultures with EMF exposed cultures.

Discussion and Contribution to Understanding Biological Effects of EMF

Human cells of several types were exposed to 60 Hz EMF or sham-exposure control at different field strengths (0.1 to 10 G) for times up to 24 hours. In all studies to date, there has been no significant difference in the expression of heatshock proteins between EMF exposed and sham exposure cultures. Positive control treatments induced heat shock proteins in all experiments.

This program was designed to identify a potential mechanism by which powerline frequency (60 Hz) EMF could interact with cellular components, the so-called “stress” response. The stress response is a fundamental biochemical pathway that cells use to protect themselves when faced with potentially damaging conditions such as increased temperature, loss of essential nutrients, or the presence of toxic chemicals or oxidants. The protection afforded the cells by this response aids in protecting the body from insults both due to environmental factors and also to disease. If 60 Hz EMF is found to interact with the cellular stress response, it may have implications for disease processes from inflammation to cancer.

Publications From This Grant

None.

Other EMF Publications

None

TITLE: **Perturbation of Cell Processes by ELF Electric Fields**

Principal Investigator: Kenneth J. *McLeod*, Ph.D.
State University of New York
Stony Brook, New York

Health Relevance: Biophysics of Cellular Function

Research Area: Cellular Function; Cell Proliferation; Cell Cycle Kinetics

R01-ES06287 **Start Date:** 9/01/93 **End Date:** 8/31/97

Abstract

Exposure to electric and magnetic fields (EMFs) has been linked to various forms of cancer in numerous epidemiological studies. However, laboratory based studies have yet to determine a definitive biological mechanism which could explain these epidemiological findings. The principal objective of this work was to address the hypothesis that the response of cells *in vitro*, to extremely low frequency EMF exposure, is dependent on the physical relationship between the cells and the induced field. More specifically, we have proposed that the interaction of EMFs with living cells is through the electric forces developed at the surface of the cells, a hypothesis which predicts that cell size, shape, and surface charge density will critically affect field-cell interactions. Moreover, this hypothesis predicts that the characteristics of the induced electric field, including frequency, intensity, and duration will have specific effects on the responsiveness of exposed cells. To test this hypothesis, four specific aims were proposed: (1) to determine whether cells respond in a dose dependent manner to the magnitude of an induced electric field; (2) whether the biological response of a cell to an electric field is reflected in morphologic changes; (3) whether the biological response of a cell to induced electric field exposure is directly proportional to the mechanical perturbation imposed on the cell by electric forces; and, (4) whether the responsiveness of a cell line to electric field exposure can be predicted from its morphology. We observed that such direct forces may be capable of explaining some observed *in vitro* cellular effects of electric field exposure, but that there also appears to exist a second, and perhaps more relevant transduction mechanism related to cell substrate adhesion processes.

Experimental Design and Exposure Conditions

In these experiments, cells are exposed to electric fields through both magnetic induction and by direct coupling. Magnetic induction is accomplished through a solenoidal exposure apparatus. This exposure system ensures a uniform magnetic field exposure for all cells within an exposed sample and provides a rigorous sham exposure. The individual solenoid coils used for both control, sham and treatment exposures consist of 4 cm diameter, 25 cm long hollow cores with two windings. Because the coils are of identical construction, any coil can be used in a treatment, sham or control configuration. In the treatment configuration, the two windings on the solenoid coil are attached in series so as to produce aiding magnetic fields. In this configuration, the solenoids produce a 1.8 milliTesla rms magnetic flux density for a 10^6 milliamp rms current. The flux density varies less than 3% over the central 10 cm in the interior of the coil due to the use of additional windings on the ends of the solenoids, permitting up to six eightwell Nunc tissuetek plates (48 samples) to be simultaneously exposed to a uniform field. In the sham exposure configuration, the two windings of the solenoid coil are connected so as to produce opposing magnetic fields. In this configuration, the measured magnetic flux in the interior of the coil is less than 0.01 mT for a 10^6 milliampere current. A single current source (KrohnHite Model 75) is used to provide the current for both the sham and treatment solenoids so that any resistive heating in the two coils will be similar. Each coil presents a total resistance of 40 ohms, so that power dissipation in each coil is approximately 0.5 Watts. The

resulting power dissipation results in a temperature rise of the coils of 1.5 degrees C in both sham and exposure solenoids for a 30 Hz exposure condition. The control, sham, and exposure solenoids are placed on the same shelf of an incubator to ensure minimal variation in other environmental factors. The magnetically induced electric field and current density distributions within the individual square wells have been calculated with 20 micrometer resolution using a 2D impedance network solved via the method of successive over relaxation. The solution at the monolayer surface demonstrates a peak tangential (horizontal) electric field intensity of 6 microvolts/cm rms at the center of the well which decreases to zero at the edges of the well. Correspondingly, the normal (vertical) electric field is maximal (1 microvolt/cm) at the edge of the well, decaying to zero at the center of the well.

Pure electric field exposure is accomplished in a separate system where cells, growing on coverslips, are exposed in a long thin channel (2.5cm x 10 cm) through platinum electrodes coupled into the growth media through agar bridges. A voltage/current converter capable of sourcing up to 100 mA pp permits exposure of the cells to an induced field intensity of up to 1 V/m pp in media up to 4 mm deep. As electric field intensity is a function of media depth, the exact field intensity is monitored by an array Ag/AgCl electrodes mounted flush with the bottom of the exposure chamber.

Quality Assurance Measures

- Characterization of magnetic induction system
- Magnetic field intensity up to 2 mT rms
- Duration of EMF exposure 4 to 64 hours
- Field Frequencies 15 150 Hz
- Static magnetic field 507 milligauss at 85 degrees below horizontal
- Orientation of a.c. to d.c. magnetic field 85 degrees
- Associated electric field 8.4 microvolts/cm peak at 1.8 mT, 30 Hz
- Transient characteristics of exposure system: 74 mH, 40 ohms, implying 1.9 ms time constant
- Spatial homogeneity 3% over exposure range of solenoid
- NonEMF exposures Max temp rise in solenoids 1.5 degrees C rise in first 2 hours, sham and exposure coils similar within 0.1 degrees.
- Incidental exposures Fan in incubator produces ambient 60 Hz flux of 14.5 microTesla oriented horizontally, in line with solenoids.
- Characterization of electric field exposure system
- D.C. to 1 KHz
- Direct measurement of field during experiment with a resolution of 5 microvolts/cm.
- 0.5 mV/m to 1 V/m

Results

Significant results were obtained during the four years of this grant period in terms of advancing our understanding of the role of extracellular electric currents and their ability to influence cell function. We have been able to clearly demonstrate that in our EMF exposure system, using confluent cultures, cell response corresponds to the magnitude of the induced electric field, rather than to the applied magnetic field. Using a histologic staining procedure for alkaline phosphatase, a distinct spatial mapping of the cells demonstrates a pattern corresponding to the magnitude of the induced electric field.

This work has also provided a clear dose-response characterization, at least for bone cells *in vitro* (MC-3T3-E1, a non-transformed osteoblastic cell line). These studies suggest that the threshold for cellular responses to ELF electric fields may be as low as 1 microvolt/cm (100 mV/m). It is interesting to note that this field level is consistent with the field intensity shown to be capable of influencing bone adaptation *in vivo*.

We have shown that in non-confluent cultures of non-transformed cells, field exposure results in decreased doubling time (enhanced proliferation). This is in direct opposition to previous studies where we exposed transformed (non-anchorage dependent cells) to ELF electric fields, and a delay in growth resulted from field exposure. This would seem to suggest a strong influence of cell-substrate interaction in mediating ELF electric field effects.

A decrease in alkaline phosphatase activity is observed in field exposed MC-3T3 cells, consistent with a reduction in the typical transition time from late G1 to S phase. In addition a decreased fraction of cells in the G 2/M phase of the cycle is observed for field exposed cells. Again, this is a fundamentally different result than observed in transformed cells, where flow cytometric studies have confirmed that field exposure produces a delay in progression through the G2/M phase of the cell cycle.

We have been able to show that while induced surface charge and alterations in fixed surface charge both affect cell growth control, only induced surface charge perturbations (either a.c. or d.c.) result in cellular changes similar to those found in magnetic field exposed cells. These data support the contention that not only is it the electric field which is critical to ensure cellular responses, but it is the influence of the electric field with charges and/or dipoles at the outer surface of the cell, or cell substrate, which is critical in the transduction process.

We have undertaken a series of short duration (3 hour) pure electric field exposure experiments to address the potential role of direct interactions of ELF fields with cells. 60 Hz fields of 1 V/m applied for 3 hours results in a significant reorientation of the cells in the direction of the applied field, with the cells demonstrating a large projected area due to an increase in both the major and minor diameters of the cell. Similarly, 24 hour exposure of the same cells to a 1 mV/m magnetic field resulted in a reorientation of cells perpendicular to the magnetic field,(parallel to the induced electric field), with an increased area resulting from an increase in the major axis of these spindle shaped cells.

We have completed numerical modeling of the electromechanical interactions of AC fields with the cell. Two approaches have been taken, allowing us to compare the possible roles for a rectifying force (i.e. electric polarization force) and direct Coulombic forces which would need to be rectified by a secondary process within the cell to have an accumulated effect. Atomic force microscopy studies have been undertaken to test whether intrinsic rectification processes exist in the cell which could permit Coulombic forces to directly interact with the cell. These studies have demonstrated a long term effect of a small magnitude force (~ 1 pico Newton), sufficient to deform the cell by approximately 1 nanometer, wherein the cell actively responds through a change in cell height. These studies suggest a stochastic biasing of the "Brownian ratcheting" mechanism through which cytoskeletal polymerization occurs, thereby providing an intrinsic rectification mechanism which could help explain the influence of a.c. field effects on cells. The force required to see this phenomenon is consistent with that caused by the interaction of a 10 mV/m electric field with a cell supporting a typical surface charge of 0.1 Coulomb/m^2.

Finally, we have developed a transfected cell line wherein gap junctional communication has been substantially decreased. Exposure to low level, ELF fields shows these cells have a similar threshold level for response, suggesting that gap junctional coupling does not play a major role in the cellular sensitivity to extracellular electric currents in this intensity range.

Discussion and Contribution to Understanding Biological Effects of EMF

These studies demonstrate that the influence of time changing magnetic field exposure on biological systems is predominantly a result of the induced electric field. Non-thermal ELF electric field effects on cells *in vitro* have been observed in two distinct magnitude ranges. The lowest thresholds for which robust biological responses are observed are in the range of 0.1 millivolt/meter, with the effects appearing (in a non-transformed cell line) as a decrease in the cell cycle time, and an increase in cell size through attainment of a more spindle shaped form, with orientation parallel to the induced electric field. For higher intensities punt electric field (1 volt/meter) the effect of exposure are similar but occur faster.

We propose that the dominant effect at field exposures below 1mV/m is a secondary effect. That is, we believe the field alters the electrical double layer characteristics at the substrate surface, which influences cell behavior secondarily through alterations in cell adhesion characteristics (possibly mediated by changes in the adsorbed protein layer). At higher field intensities, the effect on cell behavior could be direct, involving Coulombic forces acting on the surface of the cell, with long term effects arising through stochastic biasing of ongoing cytoskeletal activities.

Recent modeling studies show that, at 60 Hz, a flux density of only 10 milligauss (1 microTesla is sufficient to induce an electric field intensity of 0.1mV/m in the human body. The implication of this research, therefore, is

that 60 Hz residential and commercial magnetic fields are sufficiently large to influence cell activity, if the cells in the body are in a state similar to those existing in these *in vitro* investigations.

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TITLE: **Receptor Regulation of Rat Enkephalin**

Principal Investigator: Michael *McMillian*, Ph.D.
Laboratory of Environmental Neurosciences, NIEHS
Research Triangle Park, NC

Health Relevance: Neurobehavioral Dysfunction

Research Area: Short term whole animal studies, Neurobehavior, Neuronal Development

Z01-ES90067 **Start Date:** 10/93 **End Date:** 9/95

Abstract

The central nervous system (CNS) is dependent on electrical signaling to form appropriate, complex connections and networks. The developing CNS is thus potentially among the most important targets for electromagnetic field (EMF) effects. Immune cells are reportedly among the most sensitive cells to EMF effects. Brain microglia, the resident immune cells of the CNS, arise from the same lineage as the monocytes of the peripheral immune system. Microglia can be stimulated to produce a number of cytokines, (including TNF alpha, IL-1,IL-6, and TGF beta) that have been shown to be important in certain aspects of brain development, as well as in inflammatory and neurotoxic responses to brain injury in adults. Additionally there are clinical reports of an increased incidence of rapidly growing astrocytomas after EMF exposure. We have developed mixed brain cell cultures as model systems to study mechanisms involved in neurotoxicity. Pronounced changes are seen in these cultures after exposure of neonatal rat pups to drugs, such as the NMDA receptor blocker MK801, and this model system should provide a useful screen for long-lived effects of EMF exposure on the developing brain.

The main purpose of this project was to examine the effects of EMF exposure in mixed brain cell cultures from neonatal pups. The cell culture studies yielded a number of differences between groups, but correlations with EMF dose-exposure were generally lacking. Basal [^{3}H]pdBu binding, which serves as an index for the number of neurons, was higher in cultures from EMF-exposed brains. This suggests that high EMF exposures did not kill neurons. EMF exposure increased the lipopoly saccharide (LPS)-induced activation of microglia. LPS-induced releases of IL-1 beta (primarily microglia-derived) and {Met5} enkephalin (primarily astrocyte-derived) were not significantly different between groups. In summary, this pilot study with few experimental animals showed that EMF exposure effects had minimal microglia responses which, in turn, may influence the neuro-immune functions during brain development. The goal of this research project is to evaluate the effects of electromagnetic field (EMF) exposure on neuronal development.

Experimental Design and Exposure Conditions

The Sprague-Dawley rats that were utilized in the study, "Studies to evaluate the toxic and carcinogenic potential of 60 Hz magnetic fields in laboratory animals for the National Toxicology Program" were studied in this proposal. In these experiments conducted in IIT Research Institute in Chicago, several pups were not used and were designated to be sacrificed. This study utilized these additional rats. The pregnant rats were exposed to continuous (18.5 hours/day) or intermittent (one hour on/one hour off during the exposure period) fields of EMF. Ten rat pups at postnatal day 1 were sacrificed from groups of rats exposed of EMF. Five groups of rat pups were studied: A): Control B): 20 mGauss continuous exposure C): 2 Gauss continuous exposure D): 10 Gauss intermittent exposure E): 10 Gauss continuous exposure mixed brain cell cultures were prepared according to the published method (McMillian *et al.*, 1992) and cells were incubated for 7-14 days before use. {^{3}H}pdBu binding which binds to

protein kinase C, was used as a marker for neurons. Immunocy to chemical studies using a specific marker (ox-42) were used for the activation of microglia. Levels of {Met5}-enkephalin and IL-1 beta were measured by radio immunoassay and ILISA, respectively.

Quality Assurance Measures

All exposure parameters were independently verified by the National Institute of Standards and Technology (NIST). All cell cultures were done on blinded samples. The code was broken only after all of the results were analyzed.

Results and Discussion

The cell culture studies yielded a number of significant differences between the five groups (A: sham, B: 20mG, C: 2G, D: 10G intermittent, E: 10G continuous), but correlations with EMF dose-exposure were generally lacking. Basal [^{3}H]pdBu binding, which binds to protein kinase C and can be used as an index for the number of neurons was higher in cultures from EMF-exposed brains. This suggests that EMF exposures did not kill neurons, but rather may have interfered with normal developmental loss of neurons. Group B plates showed the highest [^{3}H]pdBu binding in response to activation of protein kinase C by phorbol ester. Treatment with lipopoly saccharides (LPS), which activate microglia, decreased [^{3}H]pdBu binding in group C, D, and E, presumably due to the loss of neurons by microglia. This notion was consistent with the finding that microglia in these three groups were more activated than group A and B. Levels of {Met5}-enkephalin (predominantly reflecting astrocytes) were not altered in EMF-exposed groups, suggesting that astrocytes were not affected. Therefore, most of the changes observed in this study can be attributed to EMF effects on microglia responses. This notion supports the findings that immune cells are among the most sensitive cell to EMF because microglia arise from the same lineage as the monocytes of the peripheral immune system. Thus results obtained from this study suggest that EMF exposure during gestation may alter the neuro-immune function which may influence the brain development.

Discussion and Contribution to Understanding Biological Effects of EMF

None

Publications from this Grant

None

TITLE: **Human B-cell Biology in Electric and Magnetic Environment**

Principal Investigator: Shashikant *Mehta*, Ph.D.
Roger Williams Medical Center, Brown University
Providence, Rhode Island

Health Relevance: Cancer

Research Area: Cellular function, cell proliferation

R29-ES05970 **Start Date:** 09/30/96 **End Date:** 09/29/97

Abstract

T-cell derived cytokines such as IL-2 provide a signal for proliferation to activated human B-cells. Our earlier results demonstrated the modulation of IL-2 secretion by both PHA stimulated freshly prepared human T-cells and human leukemic T"Jurkat" cells. Since IL-2 induced growth of both activated T and B cells, our experimental objectives were to determine the growth of freshly prepared human peripheral blood T-cells in response to added PHA at a specified amplitude of alternating magnetic field (AMF) in the presence of varying amplitudes of the static magnetic field (SMF) strength. Various amplitudes of the SMF were used, because many reports from other laboratories indicate that AMF effects depend, among other factors, on the direction and amplitude of the SMF. In addition, we have also explored the response to AC/DC magnetic field combinations of the "Jurkat" leukemic T-cell line. It should be noted that Jurkat cells do not require IL-2 for their growth in contrast to either normal T or B cells. Our objectives were to (a) establish the existence or non-existence of a possible response (b) to relate the nature of any observable response to existing theoretical models and known biological processes.

Experimental Design and Exposure Conditions

Exposure System: Our exposure system was described in earlier progress reports and in reference 5. In all of our experiments culture plates were distributed equally (8 each) to two identical "satellite" chambers supplied with heated moist air and $C0_2$ from a central incubator. Both "satellites" were surrounded by identical coil systems that produced a SMF gradient so that plates located at different levels were exposed to different SMF levels between approximately 0 and 0.7 gauss. The “satellites" were also surrounded by a 4-coil Merritt system that was connected to a source of alternating current. Using this (bifilar) system in the active mode, a uniform AMF was produced in one of the "satellites".

Growth of Freshly Prepared Human T-cells: We have exposed fresh human peripheral blood cells from 19 healthy human donors to a 1 Gauss (G), 60 Hertz (Hz) AMF in the presence of parallel directed SMF of different magnitudes. Cells were stimulated with Phytohemaglutimin-M (PHA-M) and field exposed for 72 hours. [^{3}H]thymidine was added for the final 24 hours of incubation. Proliferation in the presence of AMF (exposed cultures) was compared with proliferation in the absence of AMF (controls) when exposed cultures and controls were subjected to approximately the same SMF. When samples from all 19 donors were considered, no effect on average proliferation R (exposed/control) was found that was statistically significant at the 0.05 level. Cells from 8 donors appeared to be sensitive to the AMF to the extent that R , evaluated at constant SMF, deviated by more than 25 per cent from unity. However cells from only 3 donors exhibited this sensitivity at an SMF value within the geomagnetic field range. Cells from 3 additional donors were exposed to 25 Hz AMF and from 3 others to 30 Hz AMF. The R values for these lower frequencies suggest a proliferative effect of AMF exposure of about 10 per cent at simultaneously present SMF levels below 0.54 G. When a proliferation ratio (R3) is evaluated to compare

exposure between large (0.55 to 0.82 G) and small (0.08 G) SMF in the absence of any AMF, using the control cultures from all 25 donors, a small (5%) depressive effect of the larger SMF was observed.

Response to AC/DC Magnetic Field Combinations of Jurkat Cells: Jurkat cells were grown routinely at a seeding density of 2.5 x 10^5 cells/ml. At either day 3 or 4, cells were washed, checked for viability, counted and replated in triplicate at 5.O x 10^5/ml near the center position of 96-well culture plates at a volume of 0.2 ml/well. Cultures were exposed to the SMF for 6 hours. Frequencies investigated thus far were 10 Hz, 20 Hz, 30 Hz, 40 Hz and 50 Hz. We are now working on 60 Hz exposure. The AMF at a 0.5 G level was always turned on for only the first 3 hours of incubation. ^{3}H-thymidine (6.7 Ci/mM, 1µCi/ml) to measure cell growth was added at the onset of each experiment. At the end of 6 hour incubation, cells were harvested and incorporated thymidine was assessed on a liquid scintillation counter. At least 4 experiments were performed at each frequency. Therefore each point on the graphs of the ratio R = (growth in the presence of AMF+SMF) / (growth in the presence of equal SMF in the absence of AMF) is based on at least 12 points of exposure data. That ratio was greater than one at some SMF levels at all investigated frequencies. However, R varied considerably with both SMF level and frequency and its average value (for 4 experiments at the same SMF/AMF combination) was as high as 1.4 at least at one frequency.

Results

Diverse biological effects of ELF low intensity magnetic field exposure have been reported which depend on, or are modulated by a simultaneously present static magnetic field. Examples are acceleration of early growth in some plant species, nociceptive response of land snails, Ca^{2+} flux in bone cells, Ca^{2+} release from neural tissue, control of neurite outgrowth and Ca signal transduction in mitogen stimulated rat thymocytes. In fresh human lymphocytes we have seen no effect of such field combinations, except in cells from some individuals, suggesting that a minority of human donors may be particularly field sensitive.

The results of experiments on Jurkat cells strongly suggest the existence of some SMFdependent "resonances". Thus far, however, attempts to quantitatively relate R values to specific theoretical models have not given unequivocal answers. Questions also remain as to which part of the cell cycle is sensitive to AMF/SMF exposure and whether effects on proliferation persist when AMF exposure is longer that 3 hours.

As with most *in vitro* experiments, extrapolation of results to human disease states is difficult. However, the experiments with Jurkat cells indicate that a 0.5 G AMF, in the presence of SMF, can accelerate the growth rate of at least one human cancer cell line.

Discussion and Contribution to Understanding Biological Effects of EMF

The analysis of our results with Jurkat cells at different frequencies does not show maximum or minimum response corresponding to a charge to mass (Q/m) ratio of any physiologically relevant ion. The data do suggest a response peak at a Q/m ratio for a charged phosphate group present during phosphorylation. To check for agreement (or disagreement) with proposed resonance theories we plan to run Jurkat cell growth experiments at constant SMF corresponding to Ca^{2+} resonance and phosphate groups

Publications From This Grant

None

Other EMF Publications

None.

TITLE: ***In Vitro* Carcinogenesis by ELF Magnetic Fields**

Principal Investigator: Richard C. *Miller*, Ph.D.
Columbia University,
New York, New York

Health Relevance: Cancer

Research Area: Gene Expression and Oncogenic Transformation From *In Vitro* and *In-Vivo* Exposure

R01-ES07124 **Start Date:** 9/30/94 **End Date:** 8/31/98

Abstract

Cancer incidence is increasing world wide. The dramatic increases of childhood cancers, mammary carcinomas, leukemias and neuroblastomas, observed primarily in industrialized countries, is cause for concern and has given rise to numerous epidemiological investigations which attempt to ascertain the etiologic agent(s) responsible. While the link between chemical induction and ionizing radiation induction of neoplasms has been well established, there is concern that the cancer rise may be linked to the increased proliferation of electrical transmission lines and electrical devices. Epidemiological studies have attempted to correlate exposure, particularly occupational exposures, to induced magnetic fields, principally those in the extremely low frequency (ELF) region (up to 100 Hz) with an increased risk of cancer induction. A large body of evidence suggests a causal link between ELF magnetic fields and cancer but, as yet, is not conclusive.

Experimental Design and Exposure Conditions

The *in vitro* magnetic field irradiator, Magnacell MC2X and the *in vivo* Quad-4x magnetic field irradiator meets all of the requirements specified by NIEHS for ELF magnetic field studies and is capable of neutralizing stray magnetic fields while producing a uniform magnetic field virtually free of heat, vibration or visual queues that could compromise experimental results. In addition, the irradiators are capable of producing identical exposure conditions when set in the 'sham' mode with the exception of ELF magnetic fields in order to provide double-blinded experiments.

The Quad-4x exposure system is comprised of 4 Merritt 4-twisted bifilary coil winding sets arranged in an octapole configuration. Magnetic fields are linearly polarized on a horizontal plane. Magnetic fields are generated by passing electric current through coils of wire threaded through plastic pipe arranged in rectangular patterns. Magnetic field strength is proportional to the current and the number of turns in each coil so that in this array the coils produce a magnetic field strength of 2.5 mT when the unit is operated at 5.5 amperes. Because of voltage drop due to coil resistance, calibration of the unit with a gaussmeter prior to each experiment must be performed to ensure precise exposures. A variable transformer and a jumper strip connector located in the electrical connector boxes on each module allow for selection of magnetic field strengths from ambient to 2.5 mT at the same input voltage. When performing sham exposures, the toggle switch is set to "sham" causing the energized twisted bifilary wires in series opposing coils to nullify the induction of magnetic fields. Equal currents flow in both exposed and sham coils.

Magnetic Field Exposure Parameters For The Quad-4x Exposure Systems Of Animals Are As Follows:

- Magnetic field intensity: From ambient to 2.5 mT.
- Duration of each ELF magnetic field exposure: From 2 to 8 days
- Number of separate exposure periods: Single exposure.
- Circadian time of exposure: All animal experiments start at 12 noon and terminate 2, 4, 6 or 8 days later.
- Field oscillation frequency: 60 Hz.
- Polarization: Linear.
- Relative orientation of AC and DC magnetic fields: Magnetic field are in the horizontal plane. By use of bucking coils, earth's magnetic field can be virtually eliminated.
- Intermittency: Continuous exposure.
- Turn-on and turn-off transients: Fields are activated before animals are introduced to the exposure system. After cell removal from the irradiator, the irradiator is turned off.
- Field spatial homogeneity: Less than 5%.
- Field temporal homogeneity: No variations.
- Geometry and movement of exposed animals: Magnetic field and animals are in the horizontal plane. Both animal size and movement has been taken into account.
- Incidental ELF magnetic field exposure: The unit design minimizes natural background and stray fields to 0.1T. In addition, the location of the unit has been surveyed and found to have low ELF magnetic field strength in 4 of the 8-day periods measured (surveyed every 6 hr).
- Accessory non-ELF magnetic field exposure: There is no detectable vibration since the exposure chambers are isolated from the Merritt coils. In addition, the temperature rise in the chambers is less than 0.1° C. Also, coils are electrically shielded and double-wound (bifilar twisted) in order to cancel fields when set in the "sham" mode.

Quality Assurance Measures

Magnetic fields are monitored routinely in the irradiation chamber utilizing a magnetometer probe manufactured by Walker Scientific, Worcester, MA. The probe has an rms of 0.3% for the mean gauss value and a resolution of 1% for the peak magnetic field. Incubators have been surveyed for background gradients of ac magnetic fields (usually ranging from a few milligauss to 1 gauss). The laboratory housing the Quad-4x exposure system as well as the passage way to the tissue preparation laboratory have been surveyed and found to have an acceptably low stray magnetic field flux. In addition, the tissue preparation laboratory has been surveyed and found to have negligible stray fields.

When performing sham exposures, the toggle switch is set to "sham" causing the energized twisted bifilary wires in series opposing coils to nullify the induction of magnetic fields. Equal currents flow in both exposed and sham coils to provide identical exposure conditions (but no magnetic field) so that double-blinding can occur in order to minimize experimenter bias and sensory queues to the animals. The physicist involved with these studies sets the irradiator to either energize or produce a null field so the biologist and laboratory technician do not know if the embryos are being exposed to magnetic fields of treated to a sham irradiation.

Results

Gene Expression Studies: Experiments have been completed that were designed as "Replication" experiments. In these studies, mouse fibroblast C3H IOTI/2 cells and immortalized human breast cells (MCF-10) were examined for expression of *c-myc, c-fos* and p-53 after exposure to 60 Hz magnetic fields. Utilizing the Magnacell MC-2xc in vitro magnetic field irradiator, mouse cells were exposed for 10, 20, 40, 60 or 120 minutes to 5, 10, 50, 100 or 1000 µT magnetic fields of 60 Hz. Gene expression was determined by measuring m-RNA. As a positive control, either ionizing radiation (X rays) or the tumor promoter, 12-0-tetradecanoyl phorbol-13-acetate (TPA) was applied to cells examined simultaneously with cells exposed to 60 Hz magnetic fields. Under the conditions used, we were not able to observe magnetic field-induced gene expression in either cell line. Field strengths and exposure duration included conditions that were described as necessary to observe the "window effect" reported by Dr. Reba Goodman.

In Vitro Oncogenic Assay Studies: In order to examine the potential of 60 Hz magnetic fields to either induce or promote X-ray induced oncogenic transformation, C3H IOTI/2 cells were exposed *in vitro* to 100, 500 or 1000 µT of 60 Hz magnetic fields for 24 hr alone or were pretreated with 3 Gy of 250 kVp X rays immediately before exposure to magnetic fields. Based on the double-blinded studies, neither cell survival nor X-ray induced oncogenic transformation was significantly altered because of exposure to 60 Hz magnetic fields. In addition, C3H IOTI/2 cells were exposed to 200 and 1000 µT magnetic fields followed by treatment for 8 weeks with 0.1 µg/ml TPA. While TPA enhanced the frequency of X-ray induced oncogenic transformation, the addition of either 200 or 1000 µT magnetic fields for 24 hr did not significantly increase X-ray induced oncogenic transformation.

In vivo *oncogenic* assay *studies:* The Syrian hamster embryo model has been used extensively to study the process of oncogenic transformation. Embryos treated *in utero* were examined *in vitro* for morphologically altered colonies indicative of oncogenic transformation. Hamsters beginning on their fifteenth day of pregnancy (17.5-day gestation) were exposed for 2 days to 2.5 mT (2500 µT) of 60 Hz magnetic fields either before or after exposure to X rays. Embryos were surgically removed for growth *in vitro*. During the incubation period to observe oncogenic transformation, 0.1 µg/ml TPA was added to the cells to act as a promoter of oncogenesis. Cells exposed to the combination X rays, magnetic fields and TPA showed a significant rise in morphologic transformants compared to embryos exposed to X rays and TPA but no magnetic field.

Discussion and Contribution to Understanding Biological Effects of EMF

The C3H 10T1/2 cell *in vitro* transformation assay system and the *in utero* Syrian hamster embryo system have been used for over 30 years by investigators in this laboratory to identify chemical and physical carcinogens. Dose response relationships have been developed in order to extrapolate to low exposure dose effects for cancer risk estimation. The assay systems have proven themselves valuable as sensitive indicators of initiators of carcinogenesis as well as useful at identifying promoters of initiated cells. A wide range of field strengths and exposure times were applied to cells *in vitro* and embryos *in utero* to examine initiation, promotion and co-promotion with magnetic fields. Based on results of gene expression and *in vitro* oncogenic transformation studies, 60 Hz fields are neither mild nor potent carcinogens. This suggests that on balance, their contribution to the rising rate of cancer in industrialized societies is negligible. However, based on studies of late gestational age hamster embryos, x-ray initiated embryonic cells may be oncogenically promoted with power frequency magnetic fields and TPA. This suggests that for a subset of the general population, magnetic fields as a weak promoter.

Publications From This Grant

1. Miller RC, Richards M, Kliauga P, Martin SG and Hall EJ. “Carcinogenic potential of 60 Hz power frequency magnetic fields, I. Results from *in vitro* studies.” Submitted.
2. Miller RC, Gupta P, Kliauga P and Martin SG. “Carcinogenic potential of 60 Hz power frequency magnetic fields, I. Results from *in vivo* studies.” Submitted, 1997.

Other EMF Publications

None.

TITLE: **EMF Induced Signal Transduction and Gene Expression**

Principal Investigator: Steven C. *Miller*, Ph.D.
SRI International
Menlo Park, California

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Signal Transduction

R01-ES07127 **Start Date**: 9/28/94 **End Date:** 8/31/98

Abstract

Evidence from epidemiological studies has suggested a possible association between exposure to power frequency electric and magnetic fields (EMF) and increased incidence of cancers. Despite numerous studies on the biological effects of EMF, no clear consensus has been reached by the scientific community concerning the health effects of EMF. Well characterized EMF exposure systems and reproducible biological model systems are necessary to effectively investigate this problem.

Our aim is to evaluate the hypothesis that 60 Hz EMF may amplify the signal transduction pathway induced by the chemical tumor-promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Because the EMF exposure parameters and the fundamental principles by which the energy in EMF may be coupled to a biophysical process are unclear, our first goal was to use a sensitive model system that would allow both positive and negative controls to be included in the experimental design to systematically evaluate the effect of the EMF. This goal has been achieved by focusing our initial studies on the regulation of NF-kB or AP-1 dependent reporter gene expression in the human promonocytic cell line U937. Gene expression refers to the process of stimulating the production of messenger RNAs, which code for specific proteins. These unique proteins are coded for by specific genes, then take part in various cell activities, from growth and metabolism to cell division

Our model system measures gene expression using a model reporter gene with an assay system that detects the amount of reporter protein produced in cells. Thus, this system evaluates the ability of a 60 Hz EMF to modulate a TPA-induced signal transduction pathway by acting through the well-defined transcription factors:

NF-kB; the human immune deficiency virus type 1 long terminal repeat (HIV-1 LTR), which contains two copies of the kB sequence, was used as the biological sensor for the detection of signal transduction pathways acting on the HIV-1 LTR directed chloramphenicol acetyltransferase (CAT) reporter gene. In addition, another DNA construct containing four copies of the kB sequence (4XkBCAT) has been developed for use in this model system to demonstrate that effects are mediated through the NF-kB binding site.

AP-1; the CAT gene is placed downstream of a human metallothionein IIA minimal promoter and four AP-1 recognition sequences (4XAP-1CAT).

Experimental Design and Exposure Conditions

The reporter gene is introduced into U937 cells by electroporation, 24 h later, replicate cultures are treated with TPA to induce CAT expression. Replicate culture flasks are placed into a control, sham, or EMF exposure incubator and 24 h later harvested. Protein values are determined for cell extracts and the amount of CAT protein is determined by CAT ELISA. Because the CAT protein is relatively stable, measuring the amount of CAT protein measures the sum of gene expression from the time of electroporation, treatment with TPA, to the time of harvest. Because this methodology is very sensitive and reproducible it is particularly well suited for EMF studies.

Our experiments are performed in T-25 Corning flasks (25- cm^2) containing 5 ml of medium. Flasks are oriented in the magnetic field in the parallel configuration (the magnetic field is oriented parallel to the base of the flask). Culture flasks are placed into defined positions marked by a grid within the sham and exposure chambers which are temperature controlled (37 +/-0.1^{o}C) by circulating water through the jacket of the walls and doors of the chambers. A set of Helmholtz coils produce spatially uniform magnetic fields with a maximum field of about 13 gauss (1.3 mT). The field is generated from an unregulated commercial 60 Hz source using a variable autotransformer to control the field intensity. Experiments are done in replicates under blinded conditions to systematically evaluate the EMF exposure parameters. These studies demonstrated that the EMF exposure environment had no effect on NF-kB or AP-1 dependent signaling pathways in the U937 leukemia cell model (Miller *et al*., manuscript in preparation).

Experimental Design and Exposure Conditions for DT-40 Studies: In 1997 we initiated a focused replication effort using the DT-40 cell line and selected DT-40 mutants obtained from the Uckun laboratory. Cell lines, experimental protocols, and critical experimental details have been shared between the laboratories. However, a series of studies done under double-blinded conditions at SRI in the new exposure system (designed by ERM) show no effect of a 100 µT 60 Hz EMF on inositol 1,4,5-triphosphate (IP3) production (Miller *et al*., manuscript in preparation). The failure to replicate the results of the Uckun laboratory has focused our efforts on 1) biological differences and 2) exposure parameters.

Electric Research and Management (ERM) Design: The LES-002-1A-DC model exposure system was installed at SRI in November 1996. This state-of-the-art system is a computer controlled *in vitro* magnetic field exposure system capable of exposing cell cultures to linearly polarized vertical magnetic fields in the range of 0.1 µT to 1.0 mT from 10 to 80 Hz at 5-Hz intervals. In addition, the exposure system has direct current (DC) windings to provide magnetic fields up to 0.1 mT in a three-axis configuration using a Helmholtz-type arrangement. This feature provides the ability to generate an exposure environment (defined by the three-axis magnetic sensor) to the same particular combination of static (DC) and EMFs which are used in the exposure environments of other laboratories with reported EMF-induced bioeffects.

There are three chambers (A, B and S). Chambers A and B are capable of exposing cell cultures to magnetic fields, whereas the S chamber is not and is used for unexposed cultures. The coils surrounding chambers A and B are constructed in groups of eight per chamber in a four-coil Merritt arrangement with the outer coils bucking the inner coils to reduce the fields outside the assembly. The coils are wound in a bifilar arrangement to allow the coils to be energized in an opposing arrangement for SHAM conditions. The SHAM and stock fields are less than 0.1 µT above background with the active coils operating at 1.0 µT. Each chamber has a temperature monitor and a three-axis magnetic sensor. The environment is controlled by the common FORMA incubator with outputs to the computer to monitor temperatures, humidity, and CO_2 levels. The system cabinet houses a Techron Amplifier, a Wavetek Function Generator, and custom designed digital and analog circuitry.

Quality Assurance Measures

A site visit and project review coordinated by William Wisecup, Frederick Dietrich, and Paul Gailey of DOE was held on December 20, 1994 at SRI at the request of the Principal Investigator. The purpose of this site visit was to impartially investigate and document possible causes of repeatable results which seemed to reflect an effect related to position within the exposure system. Their review suggested a temperature gradient or a nonuniform field. A series of temperature and field measurements made by the site visit team with their equipment documented the presence of a temperature gradient that could account for the differences observed between exposed and non-exposed cells. This site visit greatly assisted the Principal Investigator in obtaining internal funds for designing a new exposure facility for these studies. The new system has two identical exposure units temperature controlled by water circulation to allow for the simultaneous incubation for control and EMF exposed cells. The original system is no longer in use.

DT-40 Replication Project: DT-40 and selected mutant lines are maintained in a mµ-metal shielded box placed within the top chamber of water-jacketed cell culture incubator (Queue Systems) with the door heater disabled. A detailed experimental protocol has been developed in collaboration with the Uckun laboratory for the initial studies evaluating the effect of a 100 µT 60 Hz EMF on IP3. This standard operating procedure (SOP) includes a positive

control agent in the study design. Cells are transported to the SRI EMF exposure facility in a mu metal shielded box. All exposures are done under blinded conditions in the LES-002-1A-DC model exposure system. A three-axis fluxgate (Bartington) sensor is used to record the AC and DC fields in the exposure incubators before and prior to EMF exposure. Magnetic field monitoring is done continuously during the exposure period.

To determine whether an experimentally defined exposure metric may be important in EMF exposure and bioeffects, the Bartington three-axis fluxgate magnetometer is used to measure the magnetic field characteristics that are then directly correlated with robust or no significant bioeffects.

Results

The DOE site visit revealed that a repeatable effect was related to position within the exposure system because of a temperature gradient. That our preliminary studies detected an artifact related to a temperature gradient in the exposure system attested to the sensitivity of the assay system. The modified exposure system demonstrated temperature control between control and EMF chambers at 37+/-0.1^{o} C. A blinded experimental protocol was used to assess possible field effects on TPA-induced signal transduction. A series of studies demonstrated no effect of 60 Hz EMF on NF-kB or AP-1 dependent pathways in U937 cells (Miller *et al.*, manuscript in preparation).

In the human B cell lymphoma cell line Daudi we found no effect of a 100 μT 60 Hz EMF on phosphotyrosine protein profiles. In contrast, samples treated with the positive control agent anti-IgM demonstrated robust changes in phosphotyrosine protein profiles compared to controls. Because our results were not consistent with the findings of Uckun *et al. (*Exposure of B-lineage lymphoid cells to low energy electromagnetic fields stimulates Lyn kinase; J. Biol. Chem. 270:27666-27670, 1995), in 1997 we initiated a focused replication effort using the DT-40 cell line and selected DT-40 mutants obtained from the Uckun laboratory. In the DT-40 model crosslinking of the BCR by anti-IgM antibodies activates a signaling cascade resulting in protein tyrosine kinase activation, tyrosine phosphorylation of substrate proteins, activation of phospholipase C (PLC-λ), inosito 1, 4, 5-triphosphate production, and the mobilization of intracellular Ca^{2+} from internal stores. Therefore, anti-IgM-induced signaling in the DT-40 model provides a well established biological framework for the assessment of a possible EMF-induced signaling mechanism.

A series of studies done under double-blind conditions at SRI in the new ERM exposure system show no effect of a 100 μT 60 Hz EMF on IP3 production. The failure to replicate the results of the Uckun laboratory has focused our efforts on 1) biological differences and 2) exposure parameters.

Discussion and Contribution to Understanding Biological Effects of EMF

Because our studies indicated no effect of EMFs in the U937 leukemia cell model, in 1997 we initiated a replication study focused on the results of Uckun *et al*. ("Exposure of B-lineage lymphoid cells to low energy electromagnetic fields stimulates lyn kinase:" J. Biol. Chem. 270:27666-27670, 1995). This significant publication demonstrated that even short exposure (1-30 min) to a 100 mT 60 Hz EMF resulted in an enhanced tyrosine phosphorylation profile of proteins, lyn and syk protein tyrosine kinase activation, followed by protein kinase C (PKC) stimulation. This seminal finding suggests an EMF dependent mechanism that has relevance to childhood leukemia. Replication of these findings would provide important new information to further contribute to the assessment of a possible EMF hazard to human health.

Because of our current understanding of the unique requirements for EMF replication studies, we are working in close collaboration with the Uckun laboratory. Because they have shown that the chicken lymphoma B cell line DT-40 is EMF responsive, this system is appropriate for further efforts to understand the role of protein tyrosine kinases in EMF-induced signal transduction. Moreover, because the DT-40 cell line expresses membrane-bound immunoglobulin (mIgM) it has been widely used to study B cell receptor (BCR)-induced signaling. Crosslinking of the BCR by anti-IgM antibodies activates a signaling cascade resulting in protein tyrosine kinase activation, tyrosine phosphorylation of substrate proteins, activation of phospholipase C (PLC-μ), inositol 1,4,5-triphosphate production, and the mobilization of intracellular Ca^{2+} from internal stores. Therefore, anti-IgM -induced signaling in the DT-40 model provides a well established biological framework for the assessment of a possible EMF-induced signaling mechanism.

Cell lines, experimental protocols, and critical experimental details have been shared. However, a series of studies done under blinded conditions in the ERM exposure system show no effect of a 100 μT 60 Hz EMF on IP3 production(Miller *et al.*, manuscript in preparation). The failure to replicate the results of the Uckun laboratory has focused our efforts on 1) biological differences and 2) exposure parameters. To determine whether an experimentally defined exposure metric may be important in EMF exposure and bioeffects, the Bartington three-axis fluxgate magnetometer is used to measure the magnetic field characteristics that are then directly correlated with robust or no significant bioeffects. Our current studies emphasize that to facilitate replication of published results the original investigator must define the physical and biological requirements responsible for a given response. Our studies have used leukemia cell model systems to evaluate the effect of 60 Hz EMFs on biological signaling cascades. In the human leukemia cell line U937 we found no effect on NF-kB or AP-1 dependent reporter gene expression (Miller *et al*., manuscript in preparation). In the human B cell lymphoma cell line Daudi we found no effect of a 100 mT 60 Hz EMF on phosphotyrosine protein profiles. Our results were not consistent with the findings reported by Uckun *et al*.(1995). Thus, in 1997 we initiated a focused replication effort using the DT-40 cell line and selected DT-40 mutants obtained from the Uckun laboratory. Our current studies in the DT-40 model system show no effect of a 100 mT 60 Hz EMF on IP3 production (Miller *et al.*, manuscript in preparation). Therefore, the further assessment of the possible human health hazards of EMF exposure depends, in part, upon establishing a causal connection between the existence of robust, replicable biological effects and the precise dosimetric parameters of EMF exposure upon which these effects depend.

Publications From This Grant

1. Miller SC and Furniss MJ. " Bruton's Tyrosine Kinase Activity and Inositol-1, 4,5-Trisphosphate Production are not altered in DT40 Lymphoma B Cells Exposed to Power line Frequency Magnetic Fields. J. Biol. Chem. 273, 32618-32626, 1998.
2. Miller SC, Haberer J, Venkatachalam U, and Furniss MJ. "NF-kB or AP-1 dependent reporter gene expression are not altered in human U937 cells exposed to power-line frequency magnetic fields." Radiation Research in press, 1998.

TITLE: **Signaling Pathways Involved in the Oncostatic Properties of Melatonin**

Principal Investigator: Elizabeth *Murphy*, Ph.D.
Laboratory of Molecular Biophysics, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Cell Function; Cell Processes; Cell Proliferation

Z01-ES50156 **Start Date:** 9/93 **End Date:** 9/95

Abstract

Epidemiological studies have suggested that exposure to electromagnetic fields (EMF) may cause an increased incidence of some forms of cancer; however, the mechanism responsible for this is unclear. The multistage process of neoplastic progression requires the uncoupling of proliferation and apoptosis in such a manner that the cancer cell acquires a proliferative advantage. In addition EMF has been associated with leukemia, a disease associated with aberrant inhibition of apoptosis. Furthermore, EMF is reported to alter Ca^{2+} homeostasis and alterations in Ca^{2+} homeostasis are reported to be involved in apoptosis. Our understanding of how EMF might alter apoptosis is hampered by our lack of understanding of the mechanisms regulating apoptosis. We are testing the hypothesis that EMF exposure alters Ca^{2+} homeostasis, and that altered Ca^{2+} homeostasis alters signaling pathways that lead to a proliferative advantage of the preneoplastic cell, and thus enhances neoplastic progression. To test this, we employed three lines of Syrian hamster embryo (SHE) cells that represent different stages of neoplastic progression. Earlier studies showed that, unlike normal diploid SHE cells whose growth arrests upon removal of serum, early preneoplastic cells, sup+I, undergo apoptosis at a high rate. Cells from a later stage of progression, sup-II, isolated from the same clone at a later passage, were resistant to apoptosis. We investigated Ca^{2+} homeostasis in these cells, and how it is altered by low serum, which induces apoptosis in the sup+I cells. We found differences in intracellular free Ca^{2+} levels, and also differences in endoplasmic reticulum Ca^{2+} compartmentalization, among the three cell lines, implying that Ca^{2+} regulation is modified during neoplastic conversion. We found a decrease in endoplasmic reticulum calcium in cells that will undergo apoptosis. This decrease in endoplasmic reticulum calcium is strongly associated with apoptosis; if we rescue cells from apoptosis we block the decrease in endoplasmic reticulum calcium. Furthermore this decrease in endoplasmic reticulum calcium appears to be due to altered plasma membrane calcium transport. Because EMF has been reported to alter membrane calcium transport this is a possible mechanism by which EMF could promote neoplasmic progression.

Experimental Design and Exposure Conditions

Syrian hamster embryo (SHE) cell lines and two immortalized cell lines derived from these cells were employed. The cell lines consist of normal diploid SHE cells, which growth arrest upon removal of serum, preneoplastic sup+I cells, which undergo apoptosis in low serum, and sup- cells which are resistant to apoptosis in low serum. We studied calcium homeostasis using fluorescent microscopy in cells loaded with the fluorescent calcium indicator, *fura-2*. Endoplasmic reticulum calcium was measured using thapsigargin releasable calcium.

Quality Assurance Measures

Not Applicable

Results

The goals of this study are to define changes in Ca^{2+} homeostasis associated with apoptosis. Fluorescent microscopy of fura-2 loaded cells was used to measure intracellular, free calcium concentrations (Cai) and endoplasmic reticulum (ER) Ca^{2+} content. The results indicate that the (Cai) level in logarithmically growing sup+I cells (~100 nM) was considerably lower than that observed in sup-II cells (~260 nM). Serum removal resulted in a reduction of (Cai) in the sup+I cells (~82 nM), whereas the (Cai) level in sup-II cells did not change. Endoplasmic reticulum (ER) calcium levels were determined by measuring thapsigargin-releasable Ca^{2+}. Reduced ER calcium was consistently observed in cells induced to undergo apoptosis. Specifically, thapsigargin-releasable Ca^{2+} was greatly reduced in sup+I cells (45 nM) as compared to sup-II cells (190 nM) after 4 h in low serum. When sup-II cells were placed under conditions that result in apoptosis (thapsigargin or okadaic acid), decreased ER calcium was also observed. To determine whether reduced ER calcium has a causative effect in apoptosis, ER calcium levels were exogenously increased in sup+I cells by raising extracellular Ca^{2+} to 3 mM; the ER calcium levels were maintained, and apoptosis was blocked. Studies were performed to assess the role of plasma membrane Ca^{2+} transport in the altered Ca^{2+} homeostasis and apoptosis. To measure whether Ca2+ entry was decreased in sup+I cells in low serum, Mn^{2+} uptake was used to monitor Ca2+ influx. The data show that in low serum the rate of thapsigargin-induced Mn^{2+} entry in sup+I cells was approximately 50% lower than that of sup-II cells. In further support of this hypothesis, thapsigargin-treated sup+I cells (low serum) showed decreased Ca^{2+} entry upon raising extracellular $Ca2^{2+}$ from 0 to 2 mM. These data show that preneoplastic sup+I cells, that show a high propensity to apoptosis under low serum conditions, have decreased Ca2+ entry across the plasma membrane. Therefore if EMF enhances Ca^{2+} across the plasma membrane it could interfere with apoptotic mechanisms and proliferative advantage by blocking cell death.

These data were used for a collaborative project between NIOSH, and the Laboratory of Molecular Carcinogenesis, NIEHS to assess the effect of EMF on calcium homeostasis and cell cycle regulation.

Discussion and Contribution to Understanding Biological Effects of EMF

This study shows that preneoplastic sup+I cells, which exhibit a high susceptibility to apoptotic death, have an altered Ca^{2+} homeostasis and that this altered Ca^{2+} homeostasis is causally involved in apoptosis. We further show that this altered Ca^{2+} homeostasis associated with apoptosis involves a reduced Ca^{2+} entry across the plasma membrane. Because EMF has been reported to alter membrane Ca^{2+} transport this is a possible mechanism by which EMF could promote neoplastic progression.

As discussed in the Report of the "EMF Science Review Symposium for Theoretical mechanisms and *in vitro* research findings.”" is noted that there is a tendency for EMF to alter Ca^{2+} in transformed cells. In addition, epidemiological studies have suggested that EMF may cause an increased incidence of some forms of cancer. As we have shown, preneoplastic cells have a propensity to undergo apoptosis. If EMF decreases the rate of apoptosis, it could have enhance promoter effect. Before we can investigate the mechanisms by which EMF might alter apoptosis, we first need to elucidate the mechanisms involved in apoptosis, particularly those involving alterations in Ca^{2+} homeostasis, since EMF is also reported to alter Ca^{2+} transport.

Publications From This Grant

1. Preston GA, Barrett JC, Biermann JA and Murphy E. “Effects of Alterations in Calcium Homeostasis on Apoptosis during Neoplastic Progression.” Cancer Research 57: 537-542, 1997.
2. Petranka J, Jayadev S, Baldwin W, Barrett, JC and Murphy E. “The oncostatic actions of melatonin: the role of the melatonin nuclear receptor.” J. of Pineal Res. Submitted, 1998.

Other EMF Publications

None.

TITLE: **Effects of 60 Hz EM Fields on Human Keratinocytes**

Principal Investigator: Richard L. *Nuccitelli*, Ph.D.
Section of Molecular & Cellular Biology,
University of California, Davis

Health Relevance: Cancer

Research Area: Cell Function; Cell Proliferation; Signal Transduction

R01-ES07133 **Start Date:** 09/28/94 **End Date:** 08/31/98

Abstract

This research project was designed to determine if 60 Hz electromagnetic fields (EMF) alter the differentiated cellular function of human skin cells (keratinocytes and fibroblasts) in culture. Since skin forms the outermost cellular layer on our bodies, it will be exposed to larger EMF from our environment than other cells in our body which are generally better shielded from such fields by the conductive fluid layer beneath our skin. We applied both electric and magnetic field strengths commonly found in our environment to skin fibroblasts and keratinocytes and looked for measurable changes in protein synthesis or differentiation-specific protein expression occur. Previous work from another lab has reported large increases in the rate of protein synthesis in response to 20 Hz EMF. We have recently completed our experiments attempting to replicate these results and were completely unsuccessful at both 20 and 60 Hz, 84 G fields. We could detect no significant difference in protein synthesis rates between exposed human fibroblasts and controls at 7, 14 or 21 days of exposure.

We next investigated the heat shock proteins, HSP27 and HSP70 in the lower field of 1 G. We could detect no change in phosphorylation, translocation or expression after exposure to this field strength for 5 min, 20 min, 2 h or 24 h on two different cell types. Since changes have been found in the laboratory of Reba Goodman, we are sending our technician to her lab for a week to conduct these experiments there. Up to this point we have been unable to detect any effects of 60 Hz electromagnetic fields on human keratinocytes.

Experimental Design and Exposure Conditions

We are using human keratinocytes and fibroblasts obtained from neonatal foreskins. Low passage stock cultures are stored in liquid nitrogen in one of our laboratories. For these studies, only cultures of low passage number (cumulative population doublings level of 30-34) have been used to exactly replicate the conditions of Rodemann, *et al*. (1989). In the first set of experiments the goal was to replicate Rodemann's observations that long term field exposure dramatically increases protein synthesis rates in human fibroblasts. The second aim was to determine if EMF exposure induces a sensitive stress indicator, heat shock protein, HSP27, to exhibit a change in phosphorylation, translocation or synthesis. These experiments were performed on keratinocytes.

The initial field condition used was 20 Hz, 80 G fields as used by Rodemann, *et al*. (1989). We used a similar solenoid coil exposure system that is cooled by circulating water and is housed in a 5% CO^2 water-jacketed incubator at 37 ^oC. We expanded this exposure protocol to include 60 Hz fields and lower field strengths spanning a range of 1 G to 80 G. For the lower field strengths, we used a Merritt coil system that is enclosed in a mμ metal box to shield all transients. A DC magnetic field was superimposed to mimic the earth's static field in the enclosure. For every experimental series we included EMF-exposed with sham-exposed as well as sham-sham controls, since two incubators were used in most experiments.

Quality Assurance Measures

The magnetic field strength is monitored daily using a Bell Model 620 Gauss meter with an axial probe. The temperature of the incubator is continuously monitored and recorded. For the rather large fields of 80 G, no mμ metal shielding was used around the solenoid coil, however, the controls are in a separate incubator inside a ventilated mμ metal box to screen out magnetic field transients in the incubator. For all of the 1 G experiments, a mμ metal box was used. Our coils were calibrated by a site visit team from the Department of Energy under the direction of Dr. Paul Gailey.

Results

Our initial studies were designed to replicate the study by Rodemann, *et al*. (1989) in which they reported that human skin fibroblasts increased their rate of protein synthesis in response to long-term exposure to 20 Hz, 8.4 mT (84 G) electromagnetic fields. They used vertically oriented solenoid coils in a 37°C, 5% CO^{2} incubator with stacks of tissue culture dishes along the center of the coils and exposed the cells to two six-hour exposures of 20 Hz, 8.4 mT electromagnetic fields per day for up to 21 days. They reported a very large increase in protein synthesis of up to 925% in 21 days.

Human skin fibroblast cultures were established and maintained essentially as described by Rodemann, *et al*. (1989). We used primary human fibroblasts derived from neonatal foreskins cultured in Dulbecco's modified Eagle's medium (DMEM, ICN Biomedicals Inc. Costa Mesa, CA) supplemented with 10% (v/v) bovine calf serum (Hyclone Laboratories Inc., Logan, UT) and antibiotics in an incubator maintained at 37°C, 5% CO^2 for up to 21 days with or without EMF exposure. Cells from 8-10 passages with a cumulative population doubling level of 35-40 were used. Cells were plated at a density of 2.0 x 10^4 per cm2 in 60 mm culture dishes (5.6 x 10^5/dish). Medium was changed twice a week. When the medium was renewed, the positions of the dishes within the solenoid coil were changed to randomize their locations within the coil over the duration of the experiment. Protein synthesis was assayed after 7, 14 and 21 days of incubation using triplicate plates of control and EMF-treated groups by measuring incorporation of [^{3}H] leucine into cellular proteins (Rodemann *et al*, 1987). Briefly, cells were labeled during the last 24 hours of exposure with 1.0: Ci/ml [^{3}H] leucine (New England Nuclear, Boston, MA) in 2.0 ml of DMEM. The radioactive labeling medium was aspirated, and the labeled monolayers of cells were rinsed 5 times with ice-cold Ca^{2+}- and Mg^{2+}- free phosphate buffered saline (PBS). The rinsed cells were treated with ice-cold 5% trichloroacetic acid (TCA) for 30 min. After the TCA-soluble fraction was removed, cells were rinsed 3 times with PBS, then extracted with 2.0 ml of 0.1 mM NaOH - 0.1% sodium dodecyl sulfate (SDS) solution for 30 min at room temperature.
The radioactivity in the sample extracts was quantified by liquid scintillation counting. The total amount of cellular proteins in the extracts were determined using the Bradford assay (Bio-Rad Laboratories, Richmond, CA). Our exposure system was an 8 inch diameter, water-jacketed, vertical solenoid coil of 392 turns, 2.3 S, 21.2 mH driven by a function generator producing a 20 or 60 Hz sine wave. Water was maintained at a constant temperature of 37°C and was circulated over the coil to remove any heat generated. We measured the field daily using a Bell model 9640 gaussmeter.

We have conducted 7 three-week-long experiments using a 20 Hz field and 2 experiments using a 60 Hz field. In none of these experiments have we observed a stimulation of protein synthesis. Instead, we find no significant difference from controls. We have also normalized on total cellular protein and this also indicates no significant difference from control groups. One very important difference in technique used here compared with that used by Rodemann is the amount of time that the cells were allowed to take up ^{3}H-leucine. We incubated the cells for 24 hr, but Rodemann only incubated for 6 hr. Our leucine uptake data indicate that at 6 hr the cells have not reached equilibrium with the label but are in the steepest region of the uptake curve. Thus, non-saturation of the precursor pool means he was measuring amino acid uptake differences rather than protein synthesis differences. These experiments were primarily carried out with a HaCaT keratinocyte cell line. We have repeated the experiments and confirmed the observations with normal keratinocytes derived from neonatal foreskin.

Our second set of studies have examined a very sensitive marker of cell stress in human keratinocytes, the heat shock protein, HSP 27. This protein exhibits three distinct changes in response to numerous stresses, including heat or low ATP levels: 1) there is an increase in its rate of production in the cell; 2) Some of the protein becomes phosphorylated and new bands can be observed on isoelectric focusing gels; 3) It is translocated into the nucleus. We have used both a cell line of human keratinocytes (HaCat cells) and a primary culture of human keratinocytes in this work.

In order to determine if the field exposure stimulated a transient response, we examined a range of exposure times, 5 min, 20 min, 2 hr and 24 hr. We also adopted a positive control to be sure that we could detect any HSP27 expression changes should they occur. The positive control was to expose a cell culture to 100 FM sodium arsenite ($NaAsO^2$) for 2 h. We could detect no change in HSP27 or actin levels of expression in HaCat cells in response to a 60 Hz, 1G field at these exposure times. This experiment was repeated using a primary culture of human keratinocytes with the same negative result (Figure 4, table 1). However, in both cases the positive control exhibited enhanced expression and phosphorylation of HSP27, indicating that our system was indeed capable of detecting any changes in protein synthesis that occurred.

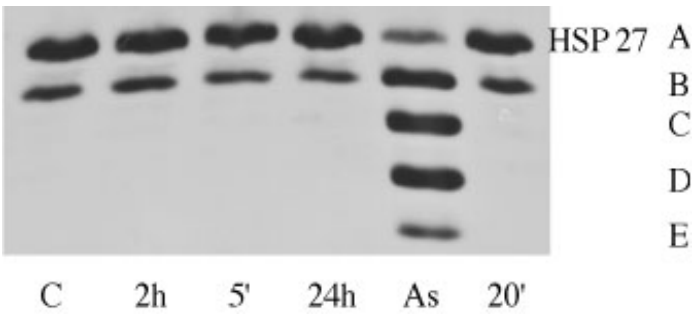

Figure 4. Isoelectric focusing gel, immunobloted using the ECL technique. One gauss field does not induce phosphorylation of HSP27 in normal human keratinocytes. Exposure times indicated below each lane. AC is control and was not exposed to EMF. AAs indicates a 2 h exposure to sodium arsenite as a positive control.

Next we examined protein synthesis levels of HSP27 following a 24 h exposure to a 60 Hz, 1 Gauss field. Controls included monitoring actin expression levels in all gels as well as exposure in a coil wound with parallel wires so that the same amount of current could be passed through the coil as in the normal test exposure system, but in an antiparallel manner so that the magnetic field produced by each winding would cancel out the other. All of the experiments were conducted in a blind manner in which the person running the gels was unaware of the content of each lane. The code was broken only after the data was analyzed. We could detect no significant difference in HSP 27 expression in response to this field exposure (Figure 5). The actin levels were also the same in each of these cultures. These data are plotted as relative densities and normalized densities (divided by the actin expression levels) in Figure 5.

We also examined the cells for translocation of HSP27 by using immunohistocytochemistry. While our positive controls exhibited excellent translocation into the nucleus, we were unable to detect any translocation of HSP27 into the nucleus of our exposed cells at any of the four exposure times used.

These experiments were primarily carried out with a HaCaT keratinocyte cell line. We have repeated the experiments and confirmed the observations with normal keratinocytes derived from neonatal foreskin. As observed in HaCaT keratinocytes, HSP27 in non-stressed normal keratinocytes appeared in a IEF blot as a major band of non-phosphorylated isoform A and a minor band of monophosphorylated isoform B. Sixty Hz, 1 G EMF exposure did not induce phosphorylation of HSP27 for either acute (5 - 20 min) or long term (up to 24 h) EMF exposure (Figure 4). In a positive control, cells were treated with 100 FM sodium arsenite (NaAsO2) for 2 h, and demonstrated the generation of three more acidic isoforms resulting from the progressive phosphorylation of HSP27. Note that EMF exposure did not affect the total amount of HSP27 isoforms, but the amount of HSP27 was enhanced significantly (over two-fold) in response to arsenite stimulation (Table 1). These observations suggest that EMF does not induce de novo synthesis of HSP27 in normal keratinocytes. This notion was further supported by quantitation of the bands on SDS-PAGE- immunoblots. After 24 h EMF exposure, the integrated intensity of HSP27 bands virtually remained unchanged compared to those of the cells not exposed to EMF (Figure 5). In positive controls, the total amount of HSP27 in arsenite-treated or heat-stressed samples increased about 50% (Figure 5).

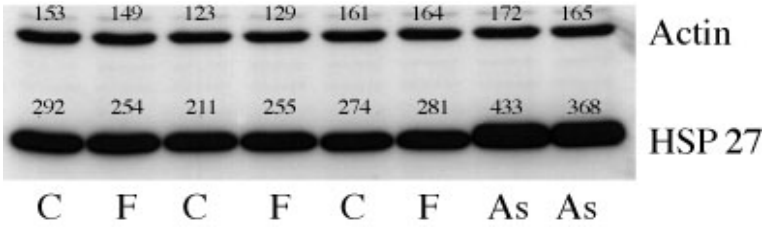

Figure 5. Western blot of SDS-PAGE of normal keratinocytes exposed to 1 G, 60 Hz EMF for 24 h. AC@ indicates control with no field exposure; AF@ indicates field exposure and As indicates the arsenite positive control. The numbers above each band indicated the integrated intensity of that band.

Goodman's group has observed that 80 FT, 60 Hz electromagnetic field induced a moderate (about 30-40%) increase in the expression of the 70 kD heat shock protein (HSP70) in HL60 line of leukemia cells. We have expanded our study to cellular effects of EMF on HSP70, trying to confirm their results. We grew HL60 cells, provided by Dr. Goodman, along with HaCaT keratinocytes, and exposed both of them to 80 FT EMF for 20 min, 2 h and 24 h. Our preliminary results show no significant changes in synthesis of HSP27 or HSP70 as a function of field exposure.

Discussion and Contribution to Understanding Biological Effects of EMF

Our results indicate no change in protein synthesis rates or changes in heat shock proteins by a wide range of field strengths from 1 G to 84 G, 60 Hz fields. We are unable to replicate the previous study of Rodemann, *et al*. (1989). Thus, we must conclude that these field strengths do not generate a detectable change in human skin cell protein synthesis in human skin cells exposed for as long as 3 weeks with two, six-hour exposures per day in vitro. In addition, we were unable to detect any change in HSP27 phosphorylation or translocation after up to 24 h in a 1 G, 60 Hz field, suggesting that this field strength is not influencing this common stress protein.

Publications From This Grant

1. Nishimura KY, Isseroff RR and Nuccitelli R. "Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds" J. Cell Sci., 109:199-207, 1996

Other EMF Publications

None.

TITLE: **Power Line Frequency Magnetic Field Effects on Gene Expression and Enzyme Activity**

Principal Investigator: Russell D. *Owen*, Ph.D.
Food and Drug Administration
Rockville, Maryland

Health Relevance: Cancer

Research Area: Gene Expression; Cellular Processes

Y01-ES40272 **Start Date:** 8/29/94 **End Date:** 9/30/97

Abstract

A number of epidemiological studies have suggested a small but statistically significant association between exposure to 60 Hz electromagnetic fields (EMF) and specific health problems. However, these studies have not been able to clearly link EMF and adverse health effects, and our current knowledge of the etiology of cancer is insufficient to explain the mechanisms for the carcinogenicity (or any other health effect) of EMF. In addition, the conclusions from such studies are often controversial and not definitive. Because the body of epidemiological studies are inconclusive, the scientific community has looked to laboratory studies for clarification of the biological effects of EMF. The results of some of these laboratory studies indicate that biochemical changes can occur at the cellular level when cells are exposed to EMF. Despite the number of studies on the biological effects of EMF, there remains considerable controversy concerning whether observed bioeffects would lead to health effects. Among the reasons for the controversy are reports of both positive and negative effects in different laboratories doing similar studies. Another reason is that many of the bioeffects reported in the peer-reviewed literature have not been replicated. Also, there are scientists who believe that power frequency fields contain too little energy to cause any biological effects. These scientists argue first, the energy of a 60 Hz electromagnetic wave is too weak to break chemical bonds, and second, natural electric fields in the body are greater than those that can be induced by common EMF exposure. Thus, there is not as yet a consensus on the biological effects of EMF on the human health consequences for any such bioeffects.

One of the highly cited and studied areas for EMF bioeffects is on the behavior of cells exposed to electric and/or magnetic fields. Studies have examined a wide range of endpoints. For example, *in vitro* studies report effects on cell membranes, RNA transcription, ornithine decarboxylase activity, calcium-ion efflux, and cellular response to hormones. These responses to EMF at the cellular level display a considerably complexity. There have been a number of studies initiated to replicate these earlier results that have not obtained similar results; therefore, there continues to be controversy. However, in most instances, investigators have not completely duplicated exposure conditions and assays, and therefore, the lack of response reported by other investigators may be due to differences in the protocols. Thus, there is a need for additional research, particularly careful replication studies, on the biological effects of EMF exposure, especially at power line frequency (60 Hz).

The main objective of this research project is to validate important findings of cellular responses of exposure to extremely low frequency (ELF) magnetic fields. Specifically, the cell environment (media, nutrients, temperature, humidity, etc.), as well as the specified field exposure conditions were duplicated as well as possible. Studies that supplement the rigorous replication experiments were performed. Two replication projects were initiated: (1) analysis of the expression of the *c-myc* proto-oncogene in promyelogenous leukemia (HL-60) cells, and (2) analysis of the activity of ornithine decarboxylase (ODC) in mouse L929 cells.

Experimental Design and Exposure Conditions

For all projects, cells were maintained in low field conditions using the protocols and culture conditions provided by the original investigators. Where practical, the same lots of serum and other reagents are used in the replication work as were used by the original investigators. Cells for each of the projects were obtained both from the ATCC and from the original investigators.

A number of exposure systems were used. An exposure system was designed and built using a pair of identical mμ metal enclosures to block out ambient fields (Davis *et al*, BEMS 17th Annual Meeting, 1995). This system has been used for each project. In addition, experiments were conducted in exposure systems provided by the original investigators and/or in the laboratories of the original investigators. A regional ELF-EMF exposure facility (described at the 1995 DOE Contractors Review) was used for some of the experiments. Control cells were sham exposed in the same incubator. Temperatures and magnetic fields were continuously monitored and recorded during the experiments.

Gene Expression in HL-60 Cells: Replication cell exposures were conducted at 60 Hz, 0.06 G for a 20 minute period of incubation. Gene expression relative to differing conditions of ELF-EMF was assayed as follows: Extraction of RNA from cells was performed by the method of Henderson and Goodman and analyzed by blot hybridization. Quantitation of radioactive hybridization data was achieved using a storage phosphor image analysis system.

ODC activity in mouse L929 cells: Replication cell exposures were performed using the conditions previously reported to give the maximal (two-fold) enhancement: 60 Hz, 0.1 G for four hours. Following exposure cells were lysed in buffer and assayed for ODC activity by incubation with ^{14}C-Ornithine with capture and quantitation of released $^{14}CO_2$.

Quality Assurance Measures

Work was reviewed by an Interagency EMF *In Vitro* Protocol Work Group (composed of representatives from NIEHS, NIOSH, FDA and DOE) at the discretion of the NIEHS. Reciprocal visits with the original investigators laboratories were made for each of the projects. Progress was presented at open scientific meetings. All work will be submitted for publication in the peer-reviewed scientific literature upon completion. All exposures are conducted in a blinded fashion, such that the respective identities of exposed and control samples remained unknown until all data were obtained.

The gene expression studies incorporates the following types of controls: Control baseline studies to determine the precision and accuracy of the assay methods. Positive control studies to provide a frame of reference for the magnitude of signals observed in the presence of ELF-EMF. Internal controls to assure that data are comparable both across different samples within an experiment as well as between separate experiments.

Results

Gene Expression: HL-60 human leukemia cells obtained from different sources were found to have different growth rates and morphologies. Sham-sham experiments showed that the assay methods were sufficiently sensitive and reproducible to detect effects of the magnitude that had been reported. Positive control studies with TPA showed the responsiveness of the cells. Replication experiments did not confirm an effect of magnetic field exposure on *c-myc* expression. Experiments included those addressing the impact of the following factors: source of cell line, magnitude of exposure, duration of exposure. Comparison of cDNA from exposed and control cell populations was performed to clone and identify genes that are differentially expressed in cells exposed to magnetic fields. Putative differentially expressed cDNAs cloned and sequenced in preparation for further analysis of the effect of ELF exposure on their transcripts.

Enzyme Activity: A series of sham-sham experiments showed that the assay methods were sufficiently sensitive and reproducible to detect effects of the magnitude that had been reported. Positive control studies with serum stimulation showed the responsiveness of the cells. Replication experiments did not reveal a statistically significant effect of magnetic field exposure on ODC activity. Experiments included those addressing the impact of the

following factors: source of cell line, magnitude of exposure, duration of exposure, exposure system used, reagent lots, temperature variation, static field component.

Discussion and Contribution to Understanding Biological Effects of EMF

No evidence was found to support the two major reported effects of EMF exposure on cells in culture that have been cited to explain the mechanism of an association between surrogates for magnetic field exposure and EMF health effects. These investigations are strengthened by the fact that comparable results were obtained using multiple exposure systems, as well as cells, materials, and methods obtained from the original investigators.

Publications From This Grant

1. Owen RD. " MYC mRNA abundance is unchanged in subcultures of HL6O cells exposed to power-lines frequency magnetic fields." Rad. Res. 150: 23-30, 1998.

Other EMF Publications

None

TITLE: **Regulation of AP-1 Transcription Factors and Opioid Peptides in Seizure Paradigms**

Principal Investigator: Keith *Pennypacker*, Ph.D.
Laboratory of Environmental Neurosciences, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Neuro-behavioral Dysfunction/brain development

Research Area: Toxicology studies using whole animals, Neuro-behavioral, Gene Expression

Z01-ES90067 **Start Date:** 10/93 **End Date:** 9/95

Abstract

There have been reports that exposure to magnetic fields might affect the nervous system causing neurobehavioral dysfunction. Previous studies from our laboratory showed that during early postnatal development, the brain contains high levels of AP-1 transcription factor DNA binding activity and *c-jun* transcription factor. The elevated content of these factors which regulate gene transcription appears to be correlated with cellular proliferation/differentiation in the brain. Thus, AP-1 DNA binding activity can serve as a sensitive index for early brain development. The purpose of this project is to examine the effects of EMF on the developing brain. Pregnant rats were exposed to different levels of EMF (0, 20mG, 2G and 10G) during the gestation period. Pups from different EMF exposed mothers were sacrificed at postnatal day 3 and the brain levels of AP-1DNA binding activity and c-jun immunoreactivity were determined. No change in *c-jun* binding activity was detected in the brains from rats exposed to EMF. The AP-1 binding activities were lower in EMF-exposed groups than the control group, although there was no clear dose-response relationship. This finding suggests that EMF affects the expression of certain genes during brain development by modulating the expression of AP-1 transcription factors.

Experimental Design and Exposure Conditions

The Sprague-Dawley rats that were utilized in the researched project, "Studies to the toxic and carcinogenic potential of 60 Hz magnetic fields in laboratory animals for the National Toxicology Program" were studied in this proposal. In these experiments conducted in IIT Research Institute in Chicago, several pups were not used and were designated to be sacrificed. This study utilized these additional rats. The pregnant rats were exposed to continuous (18.5 hours/day) or intermittent (one hour on/one hour off during the 18.5 hour exposure period) fields of EMF during the entire pregnancy and during the postnatal period. Ten rat pups at postnatal day 3 were sacrificed from groups of rats exposed of EMF. Five groups of rat pups were studied: A): Control B): 20 m Gauss continuous exposure C): 2 Gauss continuous exposure D): 10 Gauss intermittent exposure E): 10 Gauss continuous exposure brains were frozen in dry ice and brought back to NIEHS. Western blot analysis was used to determine levels of *c-jun*. Gel-shift mobility assay was used to determine the AP-1 DNA binding activity.

Quality Assurance Measures

The exposure fields were independently verified by the National Institute for Standards and Technology (NIST). All culture work was done on coded samples and the codes were broken only after all work had been completed.

Results and Discussion

Pregnant rats were exposed to different levels of EMF during the gestation period. Pups from different EMF exposed mothers were sacrificed at postnatal day 3 and the brain levels of AP-1 DNA binding activity and c-jun immunoreactivity were determined. No changes were revealed for *c-jun* immunoreactivity among the various groups. However, AP-1 DNA binding activity was affected in the brains from rats exposed to EMF. Except for the intermittently-exposed group (D), the AP-1 DNA binding activities of the other three treated groups were lower (15-25%) than that of the control group (A). Although the 2G-exposed group exhibited the least amount of AP-1 activity, there is no clear dose-response relationship among the different exposed groups. Earlier studies from our laboratory showed that AP-1 DNA binding activity is elevated and may be important in brain development. Therefore, the decrease in the AP-1 binding activity obtained from this study suggest that EMF affects neuronal development during the gestational period by influencing the expression of certain genes which are regulated by these transcriptional factors. The principal investigator left the Institute and this avenue of research was not continued.

Publications From This Grant

None

Other EMF Publications

None.

TITLE: **Magnetic Fields and Breast Cancer Risk**

Principal Investigator: John M. *Peters,* M.D.
University of Southern California
Los Angeles, California

Health Relevance: Cancer

Research Area: Studies Involving People; Epidemiological Research; Breast Cancer

R01-ES06912 **Start Date:** 9/8/94 **End Date:** 8/31/99

Abstract

In developing countries, increases in breast cancer incidence occur simultaneously with increases in industrialization. Increased exposure to magnetic fields has been offered as one possible explanation of this occurrence. Therefore, we are conducting a nested case-control study of the possible association between magnetic field and breast cancer risk in African-American and Latino women in Los Angeles County. The research questions being addressed are as follows:

Primary:

1. Is residential exposure to magnetic fields, as assessed by wiring configuration coding in homes occupied over the past 10 years before diagnosis, associated with the risk of breast cancer. Wiring configuration coding is a method that uses data on the types and distances to nearby outdoor electrical wiring to impute magnetic field levels in homes.
2. Do higher exposures to alternating current (AC) magnetic fields, as assessed by 7 days of measurements, increase a woman's risk of breast cancer.

Secondary

1. Do particular combinations of the alternating current (AC) magnetic field and the direct current (DC) magnetic field, increase the risk of breast cancer. The AC field results from our use of the 60 Hz electric power supplied by the utilities; the DC (or static) field results from the earth's magnetic field but is altered by the environment within residences. This hypothesis was prompted by observations of biologic effects at particular combinations of the AC and DC fields in several experimental systems.

Experimental Design and Exposure Conditions

The study population is composed of African-American and Latino women in Los Angeles County who responded to a mailed questionnaire on a variety of diet and lifestyle factors related to cancer risk. Women who did not report breast cancer on this questionnaire but who develop it during the 4.5 years of the study will be included. Controls will be randomly sampled from women who do not have breast cancer in the cohort. Magnetic field assessments are performed using wiring configuration coding of all homes occupied in Los Angeles County for at least 6 months during the 10 years prior to the reference date. The reference date for cases is the date of the diagnosis; for controls an equivalent date is generated.

Wiring configuration coding, in which data on the types and distances to nearby outdoor electrical wiring (i.e.: transmission and distribution lines) are used to impute magnetic field levels in all homes in Southern California occupied by subjects in the past 10 years. We are using a protocol developed for an NCI funded study of childhood leukemia which has been extensively tested. This is a modification of the Wertheimer Leeper wiring configuration

which has been associated with the risk of childhood cancer in a number of studies (Wertheimer and Leeper, 1979) but has not been well studied in relation to breast cancer. Wire coding involves drawing a map of the type of power lines running with 150 ft of the house and their distance from the house (250 ft. for transmission lines). The wire coding is being done in a blinded fashion and an actual wire code is assigned by a computer algorithm after specified data are abstracted from the map.

Direct recordings of alternating current (AC) magnetic fields in the home over 7 days using Emdex magnetic field meters (Enertech Consultants, Campbell CA). The meter takes measurement every 120 seconds. The Emdex lite meter is worn by the subject for the first day and then placed in a designated location (most occupied room other than the bedroom) for the remaining 6 days. After recording, we download the data to a laptop computer, check and then store the data for analysis.

DC (static) magnetic field measurements at several locations in the home using the Multiwave System II (ERM, State College PA). These are made along with corresponding spot measurements of the AC magnetic field at three locations in the bedroom (2 locations on the bed--where the subjects' head lies and where the chest lies), as well as in the bathroom, kitchen and living room. The use of Multiwave System II, which we were able to purchase thanks to supplementary funding from the ARMY enables these AC and DC spot measurements to be made simultaneously by the same instrument. This way we are certain of their relative orientation, which is important to testing the secondary hypothesis.

A questionnaire will be used to collect information on light at night (a potential confounder) and employment in occupations with potential exposure to EMF. Information on known risk factors for breast cancer will be obtained from the previously completed cohort study questionnaire.

Standard methods for the analysis of data from case-control studies will be used.

Quality Assurance Measures

Emdex meters and magnetometers are formally (a calibration unit for the Multiwaves) factory calibrated every 6 months but we calibrate them weekly at the office using equipment designed by our engineering consultant, William Kaune, and thus would identify any faulty machines (no problems to date). We will also send the Multiwaves to the factory for yearly calibration.

Wire coding is done blind to case or control status. Wire coders were trained by William Kaune and adhere to a standard protocol for drawing maps. The actual wire code is assigned by a computer algorithm to eliminate potential bias. Quality control for wire code entails having a random sample of wire maps redrawn by an experienced mapper, Bob Workley with Enertech Consultants. He did all wire coding for the large NCI funded study of childhood leukemia published this year. We then compare these maps to those of our study mappers and identify any discrepancies and discuss them while at the location. This will be repeated every 6 months. To date we have had excellent concordance.

Results

This is a 5 year epidemiological study. The first year was devoted to setting up all study protocols and pilot testing and then finalizing the protocols in the field. During the second year we altered the protocol to include the Multiwave System II meters for spot measurements based on the supplementary funding we received from the ARMY. Because of the long-term nature of this project, we are not able to present results at this time. We have completed interviews and home measurements for 191 cases and 174 controls. We have drawn wire maps for at least one residence on 216 cases and 230 controls. We have partial participation for an additional 18 cases and 34 controls. Participation rates are at the level we had predicted in the original submission which is substantially better than standard population based case-control studies in this ethnic mix. This is a benefit of the nested case-control study design.

Discussion and Contribution to Understanding Biological Effects of EMF

This research will help evaluate whether residential exposure to magnetic fields, as assessed by wiring configuration coding in homes occupied over the 10 years prior to diagnosis, are associated with the risk of breast cancer.

Publications From This Grant

None.

Other EMF Publications

None.

TITLE: **Development of a Mathematical Model of Melatonin Production, Distribution, and Elimination in Rats and Humans**

Principal Investigator: Christopher Portier, Ph.D.
Laboratory of Computational Biology & Risk Analysis, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Neuropharmacology

Research Area: Computational Biology; Pharmacokinetics; Melatonin

Z01-ES90602 **Start Date:** 9/95 **End Date:** 9/96

Abstract

There have been reports that exposure to electric and magnetic fields (EMF) lowers nocturnal melatonin levels. Our rationale is to separate the effect of lowered nocturnal melatonin levels and possible direct effects of magnetic fields from qualifiable sources of biological variability. We have demonstrated that a physiologically based pharmacokinetic (PBPK) model can describe the production, distribution, and elimination of melatonin in normally cycling Sprague-Dawley rats and in young (20-40 years old) male humans. Additionally, the model is capable of describing the absorption, distribution, and elimination of exogenously supplied melatonin in these species. This model will now be used as a tool to investigate individual human melatonin pharmacokinetics, as well as the effects of abnormal light cycles and EMF exposures on melatonin pharmacokinetics in rats and humans.

Experimental Design and Exposure Conditions

The subjects modeled are normally cycling Sprague-Dawley rats and young (20-40 years old) male humans. The models assume flow-limited distribution of melatonin to tissues and first order elimination by liver metabolism.

Quality Assurance Measures

All melatonin data, both endogenous plasma levels and plasma levels after administration of exogenous melatonin, were taken from peer-reviewed published literature. Model code in SCoP is available upon request. Executable models for IRIX 5.3 are also available upon request.

Results

A PBPK model has been developed to describe the production, disposition, and elimination of melatonin in rats and humans. The model reproduces both rat and human plasma melatonin levels from normal production and from exogenous administration. The distribution and elimination of melatonin are simulated on the basis that tissue distribution is flow-limited and that metabolism is not saturated at achieved concentrations, and that, in rats, metabolism is significantly faster than distribution. In the absence of data to the contrary, these assumptions allow a relatively simple PBPK model to simulate a relatively complex pharmacokinetic process.

Discussion and Contribution to Understanding Biological Effects of EMF

One of the basic questions underlying the proposed effects of EMF on melatonin is the biological relevance of a small (less than 30%) decrease in plasma melatonin. The model presented in this paper provides a quantitative

framework for assessing the impact of changes in melatonin production, elimination, and plasma levels on the concentration of melatonin in tissues of interest, including marrow, mammary gland, and brain.

Publications From This Grant

1. Blumenthal GM, Kohn MC and Portier CJ. "A mathematical model for pineal melatonin production in rats." Abstract, Toxicologist 31:737, 1997.
2. Blumenthal GM, Kohn MC and Portier CJ. "A mathematical model of production, distribution, and metabolism of melatonin in mammalian systems." Toxicol. Appl. Pharmacol 147:83-92, 1997.

Other EMF Publications

None.

TITLE: **Calcium Signaling in Cells of the Immune System**

Principal Investigator: James *Putney*, Ph.D
Laboratory of Cellular and Molecular Pharmacology, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Immune System Effects

Research Area: Cellular Function; Cellular Processes; Effect on Cell Calcium

Z01-ES90087 **Start Date:** 9/93 **End Date:** 9/95

Abstract

Of the cellular processes that might be affected by low frequency magnetic fields, the cellular transport of ions has been suggested as a target. Some recently published findings suggest that cellular calcium fluxes and calcium signaling are significantly affected by magnetic fields (Walleczek, J. , "Electromagnetic field effects on cells of the immune system: the role of calcium signaling." FASEB, J. 6: 3177-3185,1992). We are attempting to elucidate the subtle, subcellular changes that occur in cells in culture as a result of the action of hormones and growth factors, and also as a result of calcium distribution that may occur as a result of the position of magnetic field exposures, sensitive confocal imaging technology will be used. As model systems for these studies, we will utilize lymphocyte cell lines, S49 and Jurkat, both of which we have shown to have highly developed calcium signaling systems.

We have only recently brought our confocal microscope on-line; thus we have not yet applied this technology to studies of calcium signaling. However, we have been able to utilize other techniques to examine calcium signaling. As a result of this work, we have made some important strides in sorting out potential calcium mobilizing messengers in these model cell systems. For example, we have investigated the nature of the calcium mobilizing effects of a newly described material isolated from Jurkat cells, termed Calcium Influx Factor (CIF). Our findings cast doubt on the physiological role of this material in Jurkat cells and in other cell types. We have also investigated the possible action of cyclic GMP as a mediator of calcium signaling in non-excitable cells. Again our findings were largely negative. We are continuing to work on new ways that may reveal novel messengers or signaling systems that regulate cellular calcium homeostasis and which could be possible targets of disruption by magnetic field exposures.

Experimental Design and Exposure Conditions

At this stage there are no magnetic field exposures. Exposures will be initiated in collaboration with the regional facility at NIOSH where the exposure system is well described. The calcium culture studies are done following procedures that have been published in the literature.

Quality Assurance Measures

There are no magnetic field exposures at this time. The cell cultures follow standard good laboratory procedures.

Results

The studies have focused on messengers and growth factors that may affect calcium levels.

Specifically we have studied and documented the effects of cyclic GMP on calcium signaling in lymphocytes, as well as the effects of as yet uncharacterized materials present in extracts of stimulated lymphocytes. We have also initiated studies on the role of calcium signaling in apoptosis in lymphocytes.

These studies will be continued in collaboration with NIOSH.

Discussion and Contribution to Understanding Biological Effects of EMF

Reports in the literature describe effects of EMF on calcium signaling in lymphocytes. To understand how such effects could affect lymphocyte function and, perhaps, lead to transformation of lymphocytes, we have investigated how signaling pathways affect calcium, as well as how calcium signaling participates in the critical control processes of apoptosis.

Publications From This Grant

1. Bird St J and Putney JW. "Effect of inositol 1, 3, 4, 5-tetrakisphosphate on inositol trisphosphate-activated Ca2+ signaling in mouse lacrimal acinar cells." J. Biol. Chem. 271: 67-66-1996.
2. Bian X, Bird St. J and Putney, JW. "C-GMP is not required for capacitative Ca2+ entry in JURKAT T-lymphocytes." Cell Calcium 19: 351-354, 1996.
3. Louzao MC, Ribeiro CMP, Bird G St. J and Putney JW. "Cell type-specific modes of feedback regulation of capacitative calcium entry." J. Biol. Chem. 271: 14807-14813, 1996.
4. Lobaugh LA, Eisfelder B, Gibson K, Johnson GL and Putney JW. "Constitutive activation of a phosphoinositidase C-linked G-protein in murine fibroblasts decreases agonist-stimulated Ca2+ mobilization." Mol. Pharmacol. 50: 493-500, 1996.
5. Ribeiro CMP and Putney JW. "Differential effects of protein kinase C activation on calcium storage and capacitative calcium entry in NIH 3T3 cells." J. Biol. Chem. 271: 21522-21528, 1996.
6. Bian X, Hughes FM, Huang Y, Cidlowski JA and Putney JW. "Role of calcium in the induction of apoptosis by SERCA Ca2+-ATPase inhibitors and glucocorticoids in S49 lymphoma cells and in the suppression of apoptosis by the Bcl-2 oncogene." Am. J. Physiol. 272: C1241-C1249, 1997.
7. Bird G St J, Obie JF and Putney JW "Effect of cytoplasmic Ca2+ on (1,4,5) IP3 formation in vasopressin-activated hepatocytes." Cell Calcium 21: 253-256, 1997.
8. Ribeiro CMP, Reece J and Putney JW. "Role of the cytoskeleton in calcium signalling in NIH 3T3 cells. An intact cytoskeleton is required for agoinst-induced [Ca2+]i signalling, but not for capacitative calcium entry." J. Biol. Chem. 272: 26555-26561, 1997.

Other EMF Publications

None.

TITLE: **Melatonin and Occupational Magnetic Field Exposure**

Principal Investigator: John S. *Reif*
Department of Environmental Health, Colorado State
Fort Collins, Colorado

Health Relevance: Neurobehavioral

Research Area: Studies Involving People; Epidemiological Research; Melatonin

R01-ES08117 **Start Date:** 8/20/96 **End Date:** 7/31/98

Abstract

The objectives of this project are to determine whether reduced excretion of a melatonin metabolite observed in a pilot study of electric utility workers can be replicated (Study 1) and to assess whether melatonin is chronically suppressed in utility workers (Study 2). In Study 2, a subset of subjects from the pilot study will participate in repeated exposure assessment and melatonin assay over a one year period. Both studies are being performed by simultaneously measuring magnetic field exposures and melatonin production in each subject. Magnetic fields are measured on a 24hour basis using individual data-logging EMDEX II meters adapted to record light intensity concurrently. Melatonin production is evaluated by measuring urinary concentrations of its major metabolite, 6-hydroxymelatonin sulfate (6-OHMS). Field data collection for Study 1 (183 subjects) has been completed and data analyses are in progress. Field data collection for Study 2 (85 subjects) is in progress. A pilot study performed by our group indicated that under certain magnetic field exposure conditions electric utility workers had lower melatonin levels (1). This paper has been submitted to Mary Wolf for consideration by the NIEHS Working Group. Our results indicate that the temporal stability of 60 Hz magnetic field exposures in electric utility workers is a key factor associated with suppression of daytime and nocturnal melatonin secretion in humans and that exposure timing, duration and intensity also play important roles. The suppression of melatonin by 60 Hz magnetic fields may serve as an important model for understanding human biological responses to magnetic fields.

Experimental Design and Exposure Conditions

The objectives of this project are to determine whether reduced excretion of a melatonin metabolite observed in a pilot study of electric utility workers can be replicated (Study 1) and to assess whether melatonin is chronically suppressed in utility workers (Study 2). In Study 2, a subset of subjects from the pilot study will participate in repeated exposure assessment and melatonin assay over a one year period. Both studies are being performed by simultaneously measuring magnetic field exposures and melatonin production in each subject. Magnetic fields are measured on a 24-hour basis using individual data-logging EMDEX II meters adapted to record light intensity concurrently. Each participant wears the meter on their waist 24 hours per day for three work days, placing the meter next to their bed while they are asleep at night. The meter records magnetic field and ambient light exposures at 15 second intervals and subjects log the times they are at home, work, and asleep. Exposure metrics based on magnetic field intensity, cumulative exposure, intermittence, and temporal stability are calculated for each exposure period (work, home, sleep, other) by day. Melatonin production is evaluated by measuring urinary concentrations of its major metabolite, 6-hydroxymelatonin sulfate (6-OHMS), which is a valid marker of diurnal melatonin secretion in humans. Morning urine samples are collected daily for four days; a baseline sample is obtained prior to the beginning of the work week (Monday); participants then submit a sample on each morning for three consecutive work days (Tuesday, Wednesday, and Thursday). The entire volume of the first morning void is collected and pooled with any nighttime voids to provide a total overnight urine sample. A "spot" urine sample is also collected immediately following each work shift. Overnight 6-OHMS excretion, estimated as the product of

total overnight urine volume and the unadjusted 6-OHMS concentration, is calculated along with nocturnal and daytime 6-OHMS concentrations adjusted for creatinine (6-OHMS/cr).Quality Assurance Measures

A functional calibration test is performed on each EMDEX meter prior to use and at the end of each subject's measurement period. The meters are also calibrated bimonthly with a single copper coil magnetic field generating system using both the calculated magnetic fields obtained from the applied amperage and the measurements from a separate gauss meter. Light measurements are calibrated against a lux meter in a light exposure chamber and a calibration curve is then established for each meter. Data quality control criteria have been established and each EMDEX file is reviewed to ensure data integrity. Calibration logs and recordings of magnetic fields, light, and motion are inspected for each subject and day of testing. EMDEX data are excluded from the analysis if the meter is out of calibration, malfunctioning, or not worn.

Results

Field data collection for Study 1 has been completed and the data analyses are in progress. There are 22 females and 161 males (183 subjects total). The distribution of minority groups is: 91% White, 5% Hispanic, 2% Native American, 2% other. For Study 2, selection criteria have been established and subject recruitment and, field data collection are in progress.

Discussion and Contribution to Understanding Biological Effects of EMF

Virtually everyone is exposed to some degree to magnetic fields and electric utility workers are among the highest occupational exposures. Our study focuses on exposure levels that are typically encountered in the home and workplace, as well as elevated levels that are encountered by workers. Relevant exposure parameters that result in biological perturbations in humans must be defined before potential risks can be properly evaluated. The purpose of this project is to determine whether melatonin production in electric utility workers is suppressed by occupational or residential exposure to magnetic fields. A pilot study performed by our group indicated that under certain magnetic field exposure conditions electric utility workers had lower melatonin levels. Our results indicate that the temporal stability of the magnetic field exposure is a key factor associated with suppression of daytime and nocturnal melatonin secretion in humans and that exposure timing, duration and intensity also play important roles. If this study and others demonstrate a change in worker's production of melatonin, industry may need to consider limiting exposures for certain job categories in the future. The suppression of melatonin secretion by 60 Hz magnetic fields may serve as an important model for understanding human biological responses to magnetic fields.

Publications From This Grant

1. Burch JB, Reif JS, Yost MG, Keefe TJ and Pitrat CA. “Nocturnal excretion of a urinary melatonin metabolite in electric utility workers. ” Scand. J. Work, Environment & Health, 24:183-189 1998.
2. Burch JB, reif JS, Yost MG, Keefe TJ and Pitrat CA. “Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields.” Am. J. Epidemilogy, In Press, 1998.

Other EMF Publications

None

TITLE: **60 Hz Magnetic Field Effects on *In Vivo* Pineal Melatonin**

Principal Investigator: Russel J. *Reiter*, Ph.D.
University of Texas Health Science Center
San Antonio, Texas

Health Relevance: Cancer

Research Area: Toxicological studies using whole animals; Short term studies; Neurobiochemical effects

R01-ES07132 **Start Date:** 9/28/94 **End Date:** 8/31/98

Abstract

Over the last several years, research reports published in the biomedical literature have linked exposure to low level electric and magnetic fields with the development of cancers of differing etiologies. The purpose of this research project is to identify a biological mechanism which might explain these findings. The specific aims of the research are as follows:

- To test whether the Yellon exposure paradigm alters the ability of the pineal gland to produce melatonin at night in rats and hamsters. (The Yellon paradigm is the exposure of experimental animals to 60 Hz magnetic fields (MF) with a field strength of 1G for 15 min beginning two hours before lights off.)
- To determine the efficacy of acute nocturnal exposures to sinusoidal MF on the ability of the pineal gland to produce melatonin.
- To examine whether pineal glands isolated *in vivo* by means of superior cervical ganglionectomy respond to magnetic field exposure with altered melatonin levels.
- To test whether the retinas and the so-called biological clock, the suprachiasmatic nuclei (SCN), are involved in mediating the effects of MF on pineal melatonin synthesis and secretion.

Melatonin is the endpoint in these studies since it is a potent inhibitor of cancer cell growth and the initial damage to DNA. Melatonin's efficacy in these regards stems from its ability to act as a direct free radical scavenger and antioxidant. These findings relate to the reported increased incidence of cancer in some epidemiological reports related to magnetic field exposure.

The findings to date relative to melatonin levels in rats exposed to sinusoidal MF (1G, 15-60 min, 2 h before lights off during the day (aim 1, above) have been negative in terms of altering nighttime melatonin levels. These studies used 3 important parameters of pineal function, i.e., pineal N-acetyltransferase (NAT) activity, pineal melatonin, and plasma melatonin levels. All studies were conducted blind and assays used for the measurements worked well. Thus, we have concluded that late light period exposure is inconsequential in terms of the melatonin rhythm on the night following the exposure. Whereas there are obviously many other field parameters and conditions that could be tested, it is the current opinion that we should move to nighttime sinusoidal field exposures to determine their efficacy in terms of pineal melatonin production.

Quality Assurance Measures

The exposure equipment currently in use consists of two identical Merritt coil systems in which the investigators can place 4 large rodent cages. Both coils are connected to a computer system in another room so that during a specific exposure regimen, there is no need for anyone to be in the room with the animals. The computer is programmed to randomly select the true and sham exposure coils. Thus, during the exposure the people involved

do not know which coil is activated and which is bucked. The experiment supervisor, at the conclusion of an exposure, takes the hard copy print out and seals it until the data are tabulated. The individuals conducting the biochemical assays are unfamiliar with the exposure regimen and experimental design. Thus, at all steps the investigators are blind to the specific study being conducted. The instrumentation itself is checked on a regular basis by the consultant on the project, Mr. Fred Dietrich of Electric-Research and Management, Pittsburgh, PA. Biochemical assays are supervised by a Research Scientist, Dr. Dun-xian Tan. Stray fields in the sham coil are less than 2mG compared with the real exposure which are 0.5-1.5G. The coils, although not shielded, are separated by a distance of 3.5m. Room control animals, in addition to sham exposure animals, are used. Positive controls include animals acutely exposed to light at night. Light is a known suppressor of melatonin levels.

Results

A summary of studies in which Yellon type exposures (short-term daytime magnetic field exposures) were used is shown in the table below. In these studies the magnetic field exposure was initiated at 2 hours before darkness onset and continued for either 15 or 60 min. Animals were then killed prior to darkness onset (20:00 h) or during the night (at various times). None of the exposures altered pineal function in the light at 20:00h.

Exposure parameters	Results
1G, 15 min., 60 Hz, beginning 2h before darkness	No statistically significant changes in nocturnal increases in either pineal NAT, pineal melatonin or serum melatonin levels relative to sham controls at either 23:00 or 01:00 h (3 and 5 h after darkness onset, respectively).
1G, 15 min, 60 Hz, beginning 2 hours before darkness	No statistically significantly changes in nocturnal increases in either pineal NAT, pineal melatonin or serum melatonin levels relative to sham controls at 24:00 h (4 h after darkness onset). Positive controls (exposed to light) showed marked suppression of all parameters measured.
1.5G, 15 min., 60 Hz beginning 2 hours before darkness	No statistically significant changes in nocturnal increases in either pineal NAT, pineal melatonin or serum melatonin relative to sham controls at either 23:00 or 01:00 h (3 and 5 h after darkness onset).
1.0G, 15 min., 60 Hz beginning 2 hours before darkness	No statistically significant changes in nocturnal increase in either pineal NAT, pineal melatonin or serum melatonin relative to sham controls at either 22:00, 24:00 or 02:00 h (2, 4 and 6 hours after darkness onset).
1.0G, 60 min., 60 Hz beginning 2 hours before darkness	No statistically significant changes in nocturnal increases in either pineal NAT, pineal melatonin or serum melatonin levels relative to sham controls at 23:00 h. Positive controls (exposed to light) showed marked suppression of all parameters.
1.5G, 60 min., 60 Hz beginning 2 hours before darkness	No statistically significant changes in nocturnal increase in either pineal NAT, pineal melatonin or serum melatonin levels relative to sham controls at either 23:00 or 01:00 h (3 and 5 hours after darkness onset).
0.25G, 15 min., 60 Hz beginning 2 hours before darkness.	No statistically significant changes in pineal parameters relative to sham controls at 23:00 or 01:00 h.
1G, 15 min., 60 Hz intermittent (1min. on 1/min off), beginning 2 hours before darkness.	No statistically significant changes in pineal parameters relative to sham controls at 23:00 or 01:00 h.
0.5G, 15 min., 60 Hz beginning 2 hours before darkness	No statistically significant changes in nocturnal increases in either pineal NAT, pineal melatonin or serum melatonin relative to sham controls at 23:00 h

The following studies have been conducted in animals which were exposed to magnetic fields beginning at darkness onset and the effects on pineal melatonin synthesis were assessed.

Exposure Parameters	Results
1G, 3h, 60 Hz, beginning at darkness onset	No statistically significant change in nocturnal increases in either pineal NAT, pineal melatonin and serum melatonin relative to sham controls at 23:00 h (3 hours after darkness onset)
1G, 4 or 6 h, 60 Hz, beginning at	No statistically significant change in onset nocturnal increases in any pineal parameter

darkness onset	at either 24:00 02:00 h (4 and 6 hours after darkness onset)
1G, 4 or 6 h 60 Hz, intermittent (1 min on/1 min off) beginning at darkness onset	No statistically significant change in nocturnal increases in any pineal parameter at either 24:00 or 02:00 h (4 and 6 hours after darkness onset)

Because of the report by Selmaoui and Touitou, described above, a number of studies have been carried out using their exposure paradigm, i.e., 12 hours exposure beginning in the light and extending into the dark (total duration of 12 hours). While Selmaoui/Touitou used 50 Hz, we used 60 Hz fields.

Exposure Parameters	**Results**
1G, 12h, 60 Hz	No statistically significant changes in nocturnal increases in either pineal NAT, pineal melatonin and serum melatonin relative to sham controls at 24:00 h (4 hours after darkness onset)
1G, 12h, 60 Hz, intermittent (1 min on/1 min off)	No statistically significant change in nocturnal increases in any pineal parameter at 24:00 h (4 hours after darkness onset)
0.5G, 12h 60 Hz	No statistically significant change in nocturnal increases in any pineal parameter at 24:00 h (4 hours after darkness onset)
1G, 12h, 60 Hz	No statistically significant change in nocturnal increases in any pineal parameter at 24:00 h (4 hours after darkness onset)
1G, 12h, 60 Hz	No statistically significant change in nocturnal increases in any pineal parameter at 02:00 h (6 hours after darkness onset)

Addition studies using similar acute magnetic field exposure paradigns have usually yielded similar negative results.

Discussion and Contributions to Understanding Biological Effects of EMF

To date we have been unable to reliably demonstrate that the exposure of rats to either continuous or intermittent short term (up to 12 hours) 60 Hz magnetic fields alter pineal melatonin production or melatonin levels in the blood of albino rats. The number of magnetic field parameters that have been examined as to their potential effect in the synthesis of melatonin has been limited. Thus, field strengths have varied between 0.25-5.0G and the duration of exposure has been 15 min to 12 hours.

A depression of melatonin as a consequence of magnetic field exposure has been hypothesized to potentially account for the reported increased cancer risk in individuals exposed to magnetic fields. The present findings using acute magnetic field exposures in a field strength range that could be encountered in a residential or occupational setting does not support what is known as the melatonin hypothesis, ie., magnetic field exposure depresses melatonin levels which in turn causes the acceleration of tumor growth since melatonin is normally oncostatic. In usual settings humans would normally would be exposed to “high” magnetic fields for only a portion of each 24 hour period, ie., acutely exposed; thus, the currently used exposures would seem environmentally relevant. It is possible that repeated acute exposures e.g., each day for several weeks, may lead to a depression of melatonin levels. Also more complex magnetic fields e.g., those including transients, intermittency etc., could have effects on melatonin production or utilization that were not uncovered by the present studies.

If circulating melatonin levels are ever depressed as a consequence of magnetic field exposure, the depression may not be a result of a reduced production of melatonin in the pineal gland, but rather it could be a consequence of its increased utilization at the peripheral level.

Publications From This Grant

1. Rogers WR, Reiter RJ, Barlow-Walden LR, Smith AD and Orr JC. “Regularly-scheduled, daytime, slow-onset 60 Hz electric and magnetic field exposure does not depress serum melatonin concentration in non-human primates.” Bioelectromagnetics (Suppl. 3) 11:111-118, 1995.

2. Rogers WR, Reiter RJ and Orr JL. "The effects of exposure to 60 Hz electric and magnetic fields on melatonin in non-human primates might depend on specific aspects of field exposure." In: The Melatonin Hypothesis: Breast Cancer and Use of Electric Power, Wilson, BW, Anderson, L and Stevens, R. eds., Battelle Press, Columbus, pp. 429-470, 1997.

3. Rogers WR, Reiter RJ, Smith HD and Barlow-Walden L. "Rapid onset/offset variably scheduled 60 HZ electric and magnetic field exposure reduces nocturnal serum melatonin concentration in non-human primates. " Bioelectromagnetics Suppl 3: 119-122, 1995.

Other EMF Publications

None.

TITLE: **Magnetic Field and *In Vivo* DNA Damage**

Principal Investigator: Russel J. *Reiter*, Ph.D.
University of Texas Health Science Center
San Antonio, Texas

Health Relevance: Cancer

Research Area: Studies involving Whole Animals; DNA Damage; Free Radicals

R03-ES08890 **Start Date:** 01/10/97 **End Date:** 12/31/98

Abstract

The objective of these studies is to determine whether the exposure of experimental animals to 60 Hz magnetic fields alters the degree of damage of DNA which is a normal consequence of exposure to a carcinogen. Carcinogens, in many cases, damage DNA because they induce the formation in cells of highly toxic agents called free radicals; damage to DNA is a prelude to cancer. Since it has been shown that magnetic fields increase the mean intracellular concentrations of free radicals, we predict that the combined treatment of rats with the carcinogen safrole (to generate large numbers of free radicals) plus their exposure to a 60 Hz magnetic fields will lead to greater damage to DNA than that caused by the carcinogen only. To test these associations, carcinogen-treated rats will be exposed or sham-exposed to 60 Hz magnetic fields for 8 hours (to simulate duration of an occupational workday exposure). If magnetic field exposure increases DNA damage (measured in these studies using two different sensitive techniques) that results after carcinogen treatment, it could explain the means by which magnetic fields, although not directly carcinogenic, increase the incidence of cancer as reported in some epidemiological studies. Magnetic fields over a range of field strengths will be tested to identify possible dose-response relationships.

Quality Assurance Measures

The exposure equipment currently in use consists of two identical Merritt coil systems in which the investigators can place 4 large rodent cages. Both coils are connected to a computer system in another room so that during a specific exposure regimen, there is no need for anyone to be in the room with the animals. The computer is programmed to randomly select the true and sham exposure coils. Thus, during the exposure the people involved do not know which coil is activated and which is bucked. The experiment supervisor, at the conclusion of an exposure, takes the hard copy print out and seals it until the data are tabulated. The individuals conducting the biochemical assays are unfamiliar with the exposure regimen and experimental design. Thus, at all steps the investigators are blind to the specific study being conducted. The instrumentation itself is checked on a regular basis by the consultant on the project, Mr. Fred Dietrich of Electric-Research and Management, Pittsburgh, PA. Biochemical assays are supervised by a Research Scientist, Dr. Dun-xian Tan.

Stray fields in the sham coil are less than 2mG compared with the real exposure which are 0.5-1.0G. The coils, although not shielded, are separated by a distance of 3.5m. Room control animals, in addition to sham exposure animals, are used. Positive controls include animals acutely exposed to light at night. Light is a known suppressor of melatonin levels.

Results

In the studies performed to date, indirect measures of free radical damage to DNA, i.e., levels of 8-hydroxyguanosine (8-O HdG) and number of micronuclei, have been used to estimate the number of free radicals generated. When the carcinogen safrole, a free radical generating agent was given, hepatic levels of 8-0 HdG were increased after 8 hours. When safrole treated rats were additionally exposed to 1G sinusoid magnetic fields for the same 8 hours, 8-0 HdG levels were not further elevated. This suggests that magnetic field exposure did not increase free radical generation above that induced by the carcinogen. Similar results were obtained when the number of micronuclei were quantified. Like Lai and Singh, however, there was some indication that DNA damage was exaggerated by magnetic field exposure only (without carcinogen treatment). This increase has not proven statistically significant and we are further testing this possibility.

Discussion and Contribution to Understanding Biological Effects of EMF

There is some epidemiological evidence which suggests an increased cancer risk for individuals living or working in a higher than usual magnetic field environments. Several theories have been proposed to explain this alleged increased. The theory being tested here is that magnetic fields increase the half life of free radicals which then proceed to damage DNA. The purpose of the present experiments are to determine whether the reported increased cancer risk can be explained by free radical mechanisms.

Publications From This Grant

1. Reiter RJ. “Extremely low frequency fields and melatonin: animal studies.” In: Electromagnetic Fields Effects, Matthes, GT. Ed., 1997.

Other EMF Publications

None.

TITLE: **Evaluation of Transforming Potential of EMF**

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Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cellular Processes

R01-ES07145 **Start Date:** 9/28/94 **End Date:** 8/31/98

Abstract

Concerns about the carcinogenic potential of electric and magnetic fields (EMF) have been raised by a number of epidemiology studies. Carcinogenesis studies in rodents indicate that exposure to magnetic fields has promotional or copromotional effects on experimentally induced breast cancers suggesting that exposure to EMF may contribute to human cancers. Cancers of exocrine glands such as breast, endometrium and prostate arise from epithelial cells in a microenvironment that is dominated by stromal influences. These studies were designed to assess biological effects of exposure to 60 Hz EMF on human endometrial stromal fibroblasts (ESF) that may alter their interactions with epithelial cells, and thereby contribute to tumorigenesis. Short-term exposures of an hour or less had no effect on *c-myc* or *gro* mRNA levels, nor on progression through the cell cycle. However; repetitive, chronic exposures to 60 Hz EMF accelerated aging of the ESF by diminishing the number of cumulative population doublings the cell cultures experienced prior to replicative senescence. These results indicate that biological effects of exposure to magnetic fields may be insufficient to cause detectable changes in gene expression in short term studies. However, the effects of exposure may be cumulative, over time becoming sufficient to cause overt biological effects. Because aging of ESF disrupts their interactions with epithelial cells in ways that would facilitate tumorigenesis, EMF-induced aging of ESF may contribute to an increased incidence of malignancies.

A second series of experiments tested the effects of EMF on endometrial, ovarian and prostate cancer cells. Exposure to EMF accelerated the growth and other malignant-like behaviors of some of these cells. Endometrial and prostate cancer cells were more responsive to EMF than were ovarian cancer cells. In general, the less differentiated cells were more responsive to EMF than the better differentiated endometrial cancer cells. Because these cells are fully malignant, and less differentiated cells were more responsive than better differentiated cancer cells, EMF may alter tumor progression.

Experimental Design and Exposure Conditions

The exposure apparatus was constructed by EMF-RAD Consulting & Engineering Services, Ontario, Canada, and consists of dual counter-wound Hemholtz coils. Sham exposures were performed by energizing counter windings. All experiments were performed blind. Field strengths were measured by an internal Gauss meter, and confirmed by measurement with a hand-held field meter (Teslatronics). Stray fields are controlled by maintaining exposure within mμ metal boxes (Amμmeal). Cell cultures within CO_2 incubators were protected from stray and fan generated fields by culturing within m metal boxes. Ambient fields were measured at 1-2 mG in the exposure chambers and cell culturing chambers.

Sham and EMF exposures were conducted simultaneously on duplicate cell cultures. Exposures were conducted within mμ metal boxes in a separate CO_2 incubator. All experiments were performed blind. Short term exposures were for one h, chronic exposures were for 30 min, three exposures per week. All exposures were conducted at 60

Hz. Line voltage was held constant by a voltage stabilizer. Effects of short-term exposure to EMF on expression of the *c-myc* and *gro* genes. Conditionally immortalized human ESF (P2 cells) were serum starved for 48 hours to reduce *c-myc* levels. The cells were then stimulated with TPA (10 ng/ml) as a positive control for induction of *c-myc* expression, or exposed to 60 Hz EMF at various field strengths for 1 h. Cells were harvested at intervals of two and 4 hours after stimulation. Duplicate cell cultures at each field strength were exposed along with duplicate sham exposures.

Cell cycle effects: ESF or an immortalized variant (N5) were exposed to EMF (100 mG or 1G) for one hour. Cells were incubated with bromodeoxyuridine (10 μM) for 2 hours. ESF were fixed in ice-cold 70% ethanol and processed for flow cytometric analysis. Total was determined by fluorescence of propidium iodide, and newly synthesized DNA by incorporation of bromodeoxyuridine. For each sample 30,000 events were collected on a FACScan Analyzer (Becton-Dickenson) and analyzed using the Cicero analysis for Cyclops software (Cytomation, Fort Collins, CO).

Effect of EMF on aging of ESF: ESF were subcultured at a one to two split ratio upon reaching confluence. Each time a cell culture attained confluence was recorded as one cumulative population doubling. Replicative senescence was scored as the appearance of enlarged flattened cells and failure to attain confluence by three weeks after the previous serial passage. Experiments were performed blind. Cellular life span is measured as cumulative population doublings attained before the cell culture enters a nonproliferative, senescent state. End of proliferative life span (replicative senescence) was determined as failure to attain confluence by three weeks after passage at a one to two split ratio and the appearance of enlarged flattened cells. Independent replicate cell cultures were used for each treatment parameter. One set of cell cultures were treated with a mixture of growth factors (Mito+) that extends life span, and another set of cell cultures were treated with IL-1 (1.0 ng/ml) to reduce their replicative life span.

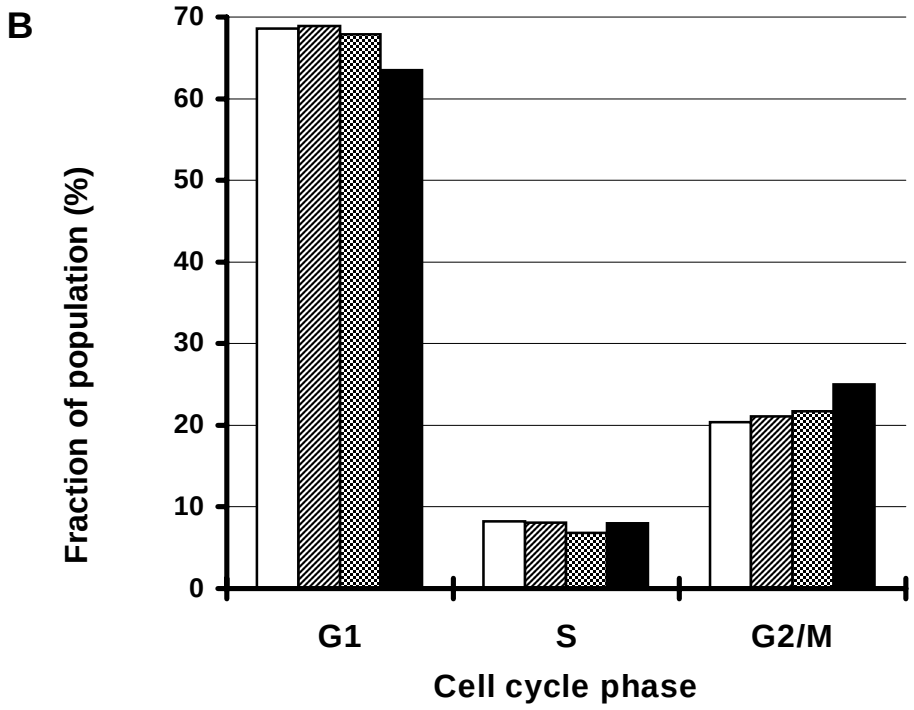

Figure Legend Effects of EMF on cell cycle of immortal ESF, (N5 cell line), panel A. Cell cycle distribution determined by flow cytometry. Sham exposed cell cultures, open bars; cell cultures exposed to EMF of 1 Gauss for 1 h, solid bars; cell cultures exposed to γ irradiation (800 rads), slashed bars; cell cultures exposed to taxol (2μM), cross hatched bars. Effects on normal ESF, panel B. Sham exposed cell cultures, open bars, cell cultures exposed to EMF, 100 mG for 1 h, slashed bars; cell cultures exposed to EMF at 1 Gauss, crossed hatched bars; cell cultures exposed to γ irradiation (300 rad), solid bars.

Life span (as cumulative population doublings to replicative senescence).

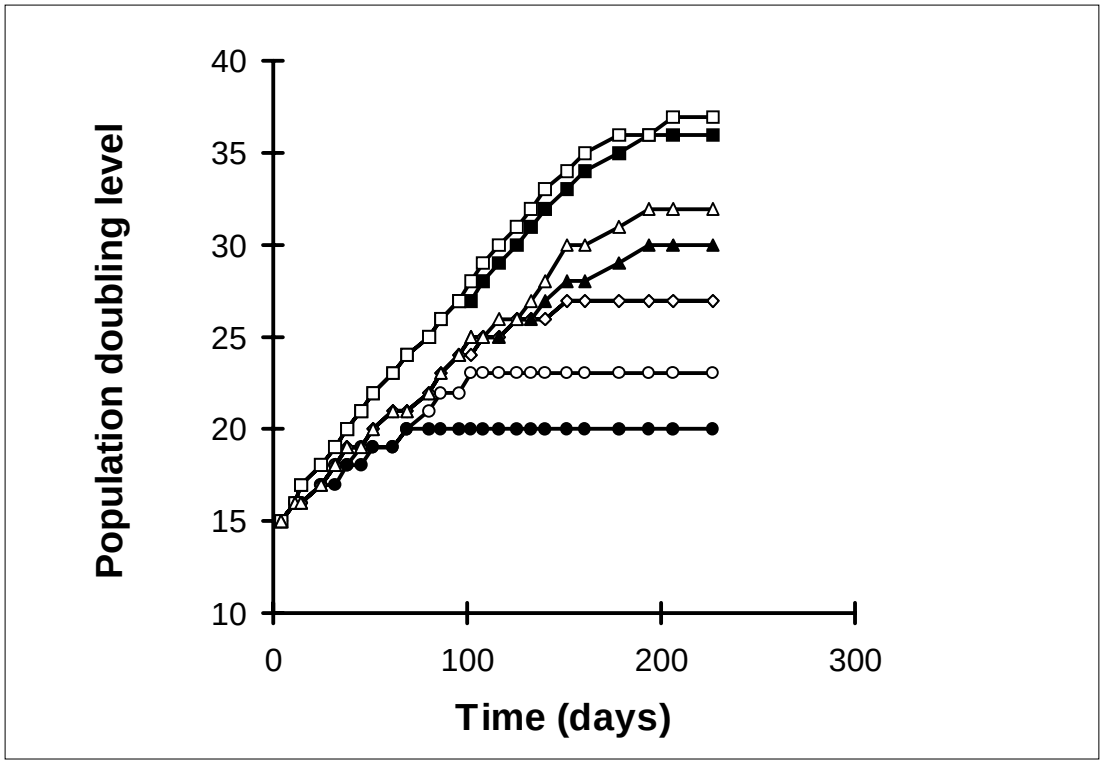

Figure Legend Effects of chronic exposure to EMF on growth rate and replicative life span of normal ESF. Mito+ treated-cultures, squares; sham-exposed cultures, triangles, 250 mG EMF, diamonds; IL-1β-treated cultures, circles.

Quality Assurance Measures

The exposure apparatus and other laboratory areas were inspected by a Department of Energy team. All experiments are done blind. The person evaluating the end point in question, be it lifespan, anchorage independent growth, or gene expression does not know the exposure conditions. In this way we will eliminate any subconscious bias. All experiments are performed in duplicate or in triplicate. Stray fields are measured with a portable gauss meter and were determined to be in the 1-2 mGauss range. Gene expression studies are assessed in triplicate. All studies include positive controls, and if possible negative controls. For example, in the studies of the effects of EMF on lifespan, Mito+TM (Collaborative Research) was used as the positive control, and interleukin-1 as the negative control.

Results

EMF effects of *c-myc* expression: We assessed the effects of short-term exposure to EMF on expression of the *c-myc* and gro genes. Conditionally immortalized human ESF (P2 cells) were serum starved for 48 hours to reduce *c-myc* levels. The cells were then stimulated with TPA (10 ng/ml) as a positive control for induction of *c-myc* expression, or exposed to 60 Hz EMF at various field strengths for 1 h. Cells were harvested at intervals of two and 4 hours after stimulation. Duplicate cell cultures at each field strength were exposed along with duplicate sham exposures. TPA but not EMF increased *c-myc* mRNA levels. *C-myc* mRNA levels in N5 cells were relatively invariant, regardless of treatment regimen

EMF effects of *gro* expression: The *gro* gene encodes the melanoma growth stimulatory activity protein. Because of the association of this gene with malignant transformation, we assessed the effect of EMF on *gro* levels in ESF. Treatment of ESF with IL-1 (1.0 ng/ml) or TPA (10 ng/ml) increases *gro* mRNA abundance in conditionally immortal ESF. Exposure to EMF at various field intensities for 1 hour had no effect on *gro* mRNA levels. This

experiment was repeated with another immortal endometrial stromal cell line, N5. Again, IL-1 and TPA increased *gro* mRNA levels. We did not detect any significant changes in the relative abundance of *gro* mRNA in response to exposure to EMF at field strengths ranging from 125 mGauss to 2.0 Gauss.

EMF effects on the cell cycle: Many regulatory factors in addition to *c-myc* influence cell proliferation by controlling the cell cycle. Because of this, the effect of exposure to EMF on the cell cycle of ESF was determined directly by assessing cell cycle kinetics in a cell strain and in a cell line. The ESF cell strain was exposed to ionizing radiation to serve as a positive control for inhibition of progress through the cell cycle. Because immortalized cells have often lost their checkpoint control, we treated the immortalized ESF with taxol to cause cell cycle arrest. Taxol stabilizes microtubules, preventing mitosis, and causing cells to arrest in G2/M. Immortalized ESF were exposed to EMF at 1 Gauss, 60 Hz, for 1 h or sham-exposed. Control cell cultures were exposed to either gamma-irradiation (800 rad) or to taxol to induce cell cycle arrest. Immortalized ESF treated with taxol and gamma-irradiated ESF accumulated in the G2/M portion of the cell cycle as compared to sham-exposed controls. EMF had no effect on the cell cycle. This experiment was repeated with normal ESF. Exposures were to field intensities of 100 mGauss or to 1 Gauss, 60 Hz, for 1 hour. There were no changes in the cell cycle as compared to sham-exposed cells after 7 hours or after 24 hours.

EMF effects on aging: These studies did not detect any short-term effects on gene expression or cell cycle distribution in normal or immortalized ESF. Reasoning that exposure to EMF may contribute to cancer by causing effects that are below the level of detection in short term assays, we proceeded to assess EMF effects on ESF that become manifest after repetitive exposures. This was done by determining the effects of exposure to EMF on the replicative life span of ESF. Cellular life span is measured as cumulative population doublings attained before the cell culture enters a nonproliferative, senescent state. End of proliferative life span (replicative senescence) was determined as failure to attain confluence by three weeks after passage at a one to two split ratio and the appearance of enlarged flattened cells. Independent replicate cell cultures were used for each treatment parameter. One set of cell cultures were treated with a mixture of growth factors (Mito+) that extends life span, and another set of cell cultures were treated with IL-1 (1.0 ng/ml) to reduce their replicative life span. Cells had a decreased life span when treated with IL-1, and an increased life span when treated with Mito+TM (Table 1). Exposure to fields of 50 or 125 mG had no effect upon life span when compared to sham-exposed cell cultures. However, exposure to EMF at 250 mG diminished replicative life span (Table 1). Proliferation rates were determined by plotting days in culture vs. cumulative population doublings. Analysis of the rates of proliferation show that there is no difference between EMF and sham-exposed cultures for the first 120 days of exposure. As with our studies of gene expression and cell cycle effects, these studies suggest that exposure to EMF at these field strengths has biological effects on human cells that may be below the limits of detection in short term studies, but may have effects which are cumulative, becoming manifest only after repeated exposures.

Table 1. Effect of EMF on Life span of Human Endometrial Stromal Fibroblasts

	sham (mG)			**EMF (mG)**			
IL-1β	50	125	250	50	125	250	Mito+™
20	29	31	30	31	25	27	36
23	29	33	32	33	33	27	37

EMF effects on tumor cells: EMF effects were assessed on a number of human endometrial, ovarian, and prostate cancer derived cell lines. Exposure to EMF increased cell number (by MTT metabolic activity assay) of 3/4 endometrial cancer cell lines, 1/6 ovarian cancer cell lines, and 2/2 prostate cancer cell lines. Significant EMF-induced increases in anchorage dependent proliferation were detected in 3/4 cell lines tested after 7 and 10 days of exposure to 60 Hz EMF at 2Gauss. The malignancy associated behavior of anchorage independent proliferation was increased in 2/3 cell lines tested. In general, the less differentiated the cell line, the more responsive it was to exposure to EMF.

Discussion and Contribution to Understanding Biological Effects of EMF

Most studies of risk assessment for exposure to EMF have concentrated on transient changes in gene expression induced by low field strengths. These have largely yielded negative results. In contrast, long term exposures to field intensities of 1 Gauss or more have identified consistent biological effects, including apparent tumor promotion. The studies with *c-myc* were designed to replicate the findings of Goodman and Henderson who reported that EMF increased expression of this gene. The studies with expression of *gro* /MGSA were designed to determine whether exposure to EMF caused changes in the expression of a gene that could regulate the proliferation and differentiation of epithelial cells. Short term exposure to EMF did not alter the relative abundance of the *c-myc* or *gro* mRNAs, suggesting that the effects of 60 Hz EMF if any at these field strengths are below the levels of detection. Cell proliferation could become dysregulated by other means. However, our studies of the effects of EMF on the cell cycle distribution indicates that short term exposures to 60 Hz EMF does not alter the cell cycle of ESF or of an immortalized variant. Similarly, in the long term aging study, there were no apparent EMF-induced alterations in proliferative rates of ESF for 120 days. After this time, a dose-dependent effect of accelerated aging was detected. These findings indicate that EMF effects on aging are slow and cumulative, requiring many cell divisions and months in culture to become evident. This effect was evident only after months of repetitive exposure to EMF, suggesting that some EMF effects may be weak, but cumulative. The mechanism whereby EMF accelerates aging is unclear at present. EMF induces oscillating Ca++ fluxes into the cell which in turn play a role in the maturation of cells. Increased influxes of Ca++ as a result of exposure to EMF could play a role in accelerated aging. Corresponding effects *in vivo* may require decades of human exposure before effects become detectable. This time scale is consistent with cancer and with other diseases associated with aging in humans. Cancer is an age-related disease, with increased age conferring a substantially increased risk for many cancers. Linkages of EMF to aging are also suggested by the report that exposure to EMF increases the risk of Alzheimer's disease, an aging-related neurologic syndrome.

Most tumors arise from epithelial cells, which must undergo a number of carcinogenic steps before becoming malignant. Tumor formation by the initiated or partially transformed cell is thought to require disruption of normal tissue homeostasis. Accelerated aging of ESF is one way in which EMF disrupts tissue homeostasis which depends heavily upon stromal/epithelial interactions in exocrine glands such as endometrium. As an example of this, we found that human ESF from adult donors inhibit malignancy associated behaviors such as anchorage independent proliferation. As ESF age, they lose this ability, creating conditions that would facilitate tumorigenesis. Accelerated aging of the microenvironment could contribute to the tumor-promotive effects of EMF that are evident in animal studies, and contribute to human cancers by facilitating tumorigenesis.

The studies of EMF effects on tumor cells provide several potentially important clues as the effects of EMF on human cancers. Steroid sex hormone responsive endometrial and prostate cancer cells were more responsive to the effects of exposure to EMF than were ovarian cancer cells, suggesting a cell lineage or cell type specificity to EMF effects. Within groupings of cancer cells from the same tissue, the less differentiated the cell, the more likely it was to be stimulated by exposure to EMF. This result suggests that EMF is more likely to act at the level of tumor progression than of tumor promotion or initiation.

Publications From This Grant

1. Rinehart CA and Torti VR. "Aging and Cancer; The Role of Stromal Interactions with Epithelial Cells." Molecular Carcinogenesis, 18: 187-192, 1997.
2. Rinehart CA, Parrish EA, Wanamaker R, Watson JM and Torti VR. "Effects of Short and Long Term Exposure to 60 Hz Magnetic Fields on Human Endometrial Stromal Fibroblasts." Submitted, 1998.
3. Watson JM, Parrish EA and Rinehart CA. "Potentiation of cancer cell growth *in vitro* by electromagnetic fields." In preparation.

TITLE: **Developmental Toxicity of EMF Exposure in Rats**

Principal Investigator: Bernadette M. *Ryan*, Ph.D.
IIT Research Institute
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Health Relevance: Reproduction

Research Area: Whole Animal Toxicology Studies; Developmental Toxicity

R03-ES08873 **Start Date:** 1/15/97 **End Date:** 12/31/97

Abstract

Considerable public concern has developed regarding possible adverse reproductive outcomes resulting from exposure to power frequency magnetic fields (EMF). Recently, the role of linearly polarized, transient-free, pure 60 Hz (the major component of power frequency EMF) was examined in a large scale mammalian study conducted for the National Toxicology Program (NTP) by our laboratory, and the results indicated a lack of adverse reproductive effects. Other investigators (Rommereim *et. al.*) have recently reported similar findings in a developmental toxicity study of 60 Hz magnetic fields. However, in order to define the potential health risk associated with EMF, it is crucial that other components such as harmonics and transients, in conjunction with 60 Hz magnetic fields be evaluated, especially since it is hypothesized that other components (harmonics and transients) or factors such as time-varying field intensities produce adverse health effects. Therefore, the aim of this project will be to evaluate the effects of EMF components (harmonics and transients) as well as time varying field intensities on reproductive outcome. Because the developing fetus consists of "sensitive" populations of cells undergoing numerous processes (e.g., proliferation, differentiation, migration, organogenesis and histogenesis), it can be used as a tool to examine the interactions of EMF with fetal cells to produce adverse developmental effects. Two studies will be conducted to accomplish these objectives; the first study will examine the potential of power frequency harmonics to adversely impact development of the fetus, while the second study will evaluate the effects of transients as well as time-varying field intensities on the same parameters. In addition to using established developmental toxicity endpoints to evaluate the effects of EMF, the studies will also attempt to assess injury to fetal brain cells by evaluating the levels of glial fibrillary acid protein (GFAP) from selected offspring. GFAP is the principal filament protein produced by astrocytes and its production is increased in response to CNS injury.

Experimental Design and Exposure Conditions

- Each exposures group will consist of approximately twenty sperm-positive Sprague-Dawley rats/group (Taconic Farms Inc.). Dams will be exposed from gestation day 6 through 19. Fetuses are collected by cesarean section and examined for reduced body weight, gross external abnormalities, visceral abnormalities and/or skeletal abnormalities. In addition to the standard developmental toxicity endpoints, GFAP will be evaluated in fetal brain tissue.
- Experimental endpoints - examination of the potential of harmonics or transients to alter fetal development .
- Exposure Duration: 18.5 hours/day during gestation days 6-19.
- Exposure groups: Harmonic Study-Control, 60 Hz Control, 180 Hz (3 rd harmonic), 60 Hz + 180 Hz superimposed; Transient Study-Control, 60 Hz Control, Transients, 60 Hz + superimposed transients (transients: 20 recorded transient events associated with transmission or use of electricity).
- Field intensities: 0 G-control or 2G-exposed
- Relative Orientation of AC and DC Magnetic Fields û AC fields horizontal (perpendicular to primary component of DC field).

- Associated Electric Fields: <100 Volts/meter.
- Field Spatial Homogeneity: uniform to ±10% throughout exposure volume.
- Field Temporal Homogeneity: measured continuously throughout program; temporal variability is <5%
- Field Monitoring: continuous throughout the program, when fields are energized or de-energized
- Environmental Conditions: Room temperature of the animal exposure rooms is maintained at 72± 30°F and 50%-15% relative humidity.
- Stray Fields: The laboratory site has been fully mapped for ambient 60 Hz and geomagnetic (dc) fields; temporal characteristics of ambient magnetic fields have been determined.
- Sham exposure conditions: Ambient 60 Hz fields are monitored continuously in sham control room and are less than 1 mG with no DC field.
- Cross-talk: less than 1mG between rooms.
- Other environmental cues: Noise, vibration and lighting in exposure rooms are monitored continuously. Noise levels < 1dB, vibration is undetectable in the animal cage racks and lighting is controlled by automatic timers; light levels are identical in all exposure rooms.

Quality Assurance Measures

- All standard teratology procedures are conducted under strict adherence to SOP 's (standard operating procedures).
- All data is reviewed on a daily basis.
- Exposure fields are monitored continuously.
- Observers are blind to exposure groups when performing the fetal evaluations.
- All fetal data is verified and/or reviewed by a second person.
- Data is collected with adherence to FDA-GLP 's (Good Laboratory Practice) Regulations.

Results

Exposure metrics (60Hz alone, harmonics – 180Hz alone, 60 Hz+180 Hz or transients superimposed on the 60 Hz signal) did not induce material or fetal toxicity. The magnetic field metrics that were studied had no biologically significant effects on litter size, litter weight of fetal development. Initial results from the glial fibillary acidic protein (GFAP) assay indicate a very low level, if any, of expression of GFAP. Additional assay refinement validation are being considered. A low incidence of the fetal malformations and variations were seen in all groups in the study including sham controls. With the exception of rib variants the incidence of grass external, visceral cephalic and skeletal anomalies were comparable in all groups; regardless of magnetic field exposure. The incidence of fetal anomalies in all exposed groups was comparable to that seen in either sham controls from this study or to in house historical control groups as well as historical control groups reported by MARTA/MTA; Published by Charles River.

Although the rib variants may appear to be an exposure related phenomenon direct causation to exposure is questionable since the incidence is within the range of in-house as well as published historical control ranges. In addition common rib variants such as the presence of a 14th rib are regarded as alternative normal patterns in development. Simarly variations such as wavy ribs may result from transitory development delays. Therefore, these data do not support the hypothesis that exposure to power frequency magnetic fields is a significant risk factor for the development fetus.

Discussion and Contribution to Understanding Biological Effects of EMF

The developing fetus represents a potential population of cells that could be uniquely sensitive to pertubation by EMF; it is a population of cells undergoing numerous processes (cell proliferation differentiation, migration) any of which could be disturbed and result is development toxicity. Overall this program assessed the potential of EMF to adversely impact cell process of the developing fetus using standard developmental toxicity endpoints. The studies did not show any adverse EMF related effects. These studies provide more comprehensive evaluation for the lack of potential hazard associated with human exposure to EMF and indicate that the developing fetus is not a sensitive population at risk from EMF exposure.

Publications From This Grant

None

Other EMF Publications

1. Ryan BM, Mallett E, Johnson TR, Gauger JR and McCormick DL. "Developmental Toxicity Study of 60 Hz (Power Frequency) Magnetic Fields in Rats." Teratology 54:73-83, 1996.
2. Ryan BM, Mallett E, Johnson TR, Gauger JR and McCormick DL. "Multi-generation reproductive toxicity assessment of 60 HZ magnetic fields using a continuous breeding protocol in rats." Teratology In Press, 1998.

TITLE: **Cellular Effects of Weak Electromagnetic Fields**

Principal Investigator: Jeffrey D. *Saffer*, Ph.D.
Battelle Memorial Institute; Pacific Northwest Laboratory
Richland, Washington

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Neoplastic Transformation

R01-ES07122 **Start Date:** 9/30/94 **End Date:** 8/31/98

Abstract

Extremely low frequency (ELF) electromagnetic fields (e.g., from power lines) have been implicated in carcinogenesis by epidemiological, animal, and *in vitro* studies. The long term goal of this research project is to discern a role of ELF field-induced changes in gene expression in enhanced transformation.

We have shown that ELF magnetic fields do not alter the expression of the MYC proto-oncogene in HL60 cells, a human leukemia line, as previously reported by others. To clarify further the limits of gene expression changes induced by magnetic fields in HL60 cells, differential display PCR, a method capable of examining thousands of genes, was used to determine whether any genes are field-responsive in those cells. No indication of gene expression changes was observed.

The failure to find an effect in HL60 cells does not shed light on the capability, or lack thereof, for ELF magnetic fields to contribute to neoplastic transformation since those cells are already transformed. The relevant processes may already be disrupted in those cells so that no further effect could be discerned. Therefore, recent work in this project focused on cells that are not fully cancerous. Specifically, since evidence suggests that magnetic fields are not capable of initiating cancer, but only in promoting transformation, we are using a cell line, JB6, that is initiated but not fully transformed.

In a soft agar assay, 60 Hz magnetic fields of 0.01, 0.1, 1.0, or 1.1 mT flux density did not induce anchorage-independent growth. In addition, these magnetic fields did not enhance tumor promoter-induced transformation showing no increase in the maximum number of transformed colonies and no shift in the dose-response curve. Thus, these data do not support the notion that environmental exposures to magnetic fields contribute to transformation.

Although no effect of magnetic fields was discerned in the JB6 cells, the experimental design used allowed discovery of novel processes involved in transformation. Specifically, p107, a retinoblastoma-related protein, is down-regulated during transformation and likely contributes to reestablishment of the cell cycle in cells grown under anchorage-independent conditions. In addition, a novel form of the transcription factor Sp1 was discovered.

Experimental Design and Exposure Conditions

The experimental approach consists of two parts. First, the possible effect of magnetic fields on gene expression in HL60 cells was examined using differential display PCR. This was to determine whether the lack of effect on MYC expression as observed in our preliminary work was consistent with the behavior of other genes in the cell. Second, the effect of magnetic fields on tumor promotion will be measured using the JB6 cell model system. A wide range of field conditions (0-1mT, 0-19 day exposures) have been used.

The magnetic field exposure system is a double-wound square 4-coil configuration (field variation within culture area < 0.5%) generating a vertical 60 Hz magnetic field with two identical chambers and coil sets that can be randomly assigned for exposure and sham. The static geomagnetic field measured with a gaussmeter with a Hall effect probe was 30.7 T, angle of inclination 30 degrees in one chamber and 32.8 T, angle of inclination 12 degrees in the other. The 60 Hz fields present at each culture dish location were measured before and after exposure using a power frequency field meter. For sham exposures, background fields were less than 0.1T.

HL60 cells (batch F-9085), a human promyelocytic leukemia line, were obtained from ATCC and grown in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin. JB6 cells (clone 41a) were grown in DMEM with 5% fetal bovine serum and penicillin/streptomycin. Both cell lines were immediately expanded in order to freeze 100 vials each to provide a uniform stock for subsequent experimentation.

For each experiment with HL60 cells, a new vial of frozen cells was thawed, expanded for 11 days and used at the same passage number. Cell growth was monitored daily throughout the expansion. Sixteen hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and resuspended in fresh medium at a concentration to give the appropriate density at time of exposure and plated in the outer ring of organ culture dishes. Dishes of cells were randomly picked and placed alternately into the two exposure chambers and allowed to equilibrate for 20 minutes prior to exposure. After exposure, dishes were removed from the chambers simultaneously and randomly coded by an investigator who did not know the field conditions in the chambers. Subsequent manipulations of cells were alternated between dishes from each chamber to avoid handling artifacts. Samples remained coded until all data analysis was completed. Similar handling is being used for JB6 cell experiments.

Steady-state RNA levels for specific genes were assessed using ribonuclease protection assays. Conditions were chosen to provide probe excess and a linear response. Differential display analysis was accomplished using RNAmap kits from GenHunter.

Transformation of JB6 cells was measured by standard soft agar growth assays. Anchorage-independent growth is measured in the presence or absence of various field conditions. The number and size distribution of resulting colonies and any possible shift in TPA dose-response is assessed in each assay.

Quality Assurance Measures

Cells are maintained at all times in a low AC field (< 3 T) environment except during a single centrifugation (1000 rpm) for HL60 cells. To measure the fields in the incubator, the laminar flow hood, and nearby areas an EMDEX II magnetic field data logger and a power frequency field meter were used.

As a positive control to define the resolution of the protection assay as well as to indicate that the cells respond normally to other agents, HL60 cells were treated with 3 nM 12-0-tetradecanoyl-phorbol-13 acetate (TPA), which induces a biphasic response in MYC mRNA levels. In contrast, beta-2 microglobulin was not responsive to TPA treatment. We observed an increase in MYC to 1.60 ± 0.37 (n=21) times the initial level within 30 min and a decrease to 0.29 ± 0.21 (n=21) after 90 min, consistent with previous observations.

For the differential display, each RNA sample was tested for genomic DNA contamination by performing a control reaction without reverse transcriptase. PCR products were analyzed on denaturing sequencing gels. Undried gels were exposed to film for 16-48 hours. Since ddPCR is characterized by some variability, PCR reactions were performed and analyzed in duplicate to differentiate between real differential expression and the random variation inherent in this system. In addition, any potential difference was assessed in an independent set of reactions.

For JB6 transformation assays, a series of assays were carried out to choose a suitable lot of serum, as defined by low background in the absence of TPA and a high transformation efficiency in the presence of the tumor promoter.

The biological effects of each magnetic field exposure condition were evaluated in a series of experiments involving all possible combinations of exposures: sham (left chamber) vs. exposed (right chamber), exposed (left) vs. sham (right), and sham vs. sham. The particular experimental set-up as well as the choice of exposed chamber is unknown to the investigator. Actual fields in both chambers are measured at the position of each culture dish.

Results

Analysis of gene expression changes induced by 60 Hz magnetic fields has been considered to support an association between magnetic field exposure and cancer risk. Several reports have indicated that these fields rapidly activate many genes in mammalian cells. However, previous studies in this area have not provided sufficient information to support the conclusions drawn. To clarify this controversial research, we have attempted to validate, under rigorously controlled conditions, key experiments on magnetic field-induction of gene expression. An extensive series of experiments, incorporating critical improvements in experimental design, most notably blind exposures and internal standards, was performed with human HL-60 and Daudi cells. Exposure conditions covered a range of flux densities (5.7 μT to 10 mT) and times (20 - 60 min). No alteration in MYC or beta-actin steady-state mRNA levels were observed. For example, ribonuclease protection assays of MYC expression relative to beta-2 microglobulin (an internal standard) failed to indicate an effect of a 5.7 μT, 60 Hz magnetic field in contrast to previously reported 2.6- to 4.5-fold increases. We attribute the difference in results to the lack of any controls or internal standards in the previously reported assays. The lack of an effect was not attributable to exposure geometry, timing of RNA preparation, or serum lot and concentration. To eliminate any remaining variables, exact replication was performed in one of the laboratories previously reporting gene expression effects; again no evidence for altered MYC expression was found. In addition, differential display PCR has indicated that ELF field-induced gene expression changes were not prevalent. Thus, the important connection between EMF and cancer drawn by previous reports is considerably weakened.

Since the above results were obtained with already transformed cells, they do not necessarily reflect the potential or lack thereof for magnetic fields to contribute to carcinogenesis. To address this directly, we carried out a series of experiments to determine whether magnetic fields can act as a tumor promoter. The data indicated that in well-controlled experiments power frequency magnetic fields do not induce transformation of JB6 cells nor do they influence tumor promoter-induced transformation. In a number of assays at a 0.01 and 1.0 mT flux density, the number of anchorage-independent colonies in the presence or absence of the field were remarkably similar, with the largest difference occurring in a sham-sham comparison. Similarly, there was no evidence in any experiment for an increase in colony numbers or a shift in the dose-response of JB6 cells concomitantly treated with TPA and the magnetic field. Magnetic field exposure also had no effect on EGF-induced transformation. Furthermore, the lack of a magnetic field effect was not due to the presence or absence of induced electric currents, since each experiment allowed discernment of a potential effect under both low and high induced currents.

In preparation for investigating the effect of magnetic field exposure on gene regulation, we carried out experiments to establish the nature of an unexpected small transcript for the transcription factor Sp1. The results led to the description of an alternatively spliced form of the Sp1 mRNA. It was further shown that this alternatively spliced message gives rise to a smaller protein, Sp1-S, containing only one of the two glutamine-rich domains of the full-length Sp1. Cotransfection studies demonstrated that Sp1-S is able to transactivate transcription from promoters containing multiple Sp1 binding sites, but its activity on promoters containing a single GC-box is greatly reduced compared to Sp1.

Finally, the cell cycle and transcription regulatory factor p107, a member of the retinoblastoma family, has been shown to be down-regulated during the time in which JB6 cells become irreversibly transformed. The loss of this tumor suppressor protein is consistent with the occurring transformation.

Discussion and Contribution to Understanding Biological Effects of EMF

The gene expression studies have received a great deal of attention since they refuted the long held belief that magnetic field exposure resulted in an increase in MYC expression in HL60 cells. The positive controls and internal standards provided clear support for the conclusions drawn and have served as an example of the rigor required for EMF experiments.

The recent results showing that magnetic field exposure also does not contribute to neoplastic transformation provided further evidence that typical environmental exposures to magnetic fields are unlikely to have adverse consequences with regard to cancer risk.

Publications From This Grant

1. Saffer J, Thurston S. "Cancer risk and electromagnetic fields." Nature 375:22-23, 1995.
2. Saffer J, Thurston S. " Short exposures to 60 Hz magnetic fields do not alter *MYC* expression in HL60 or Daudi cells." Radiat Res. 144:18-2, 1995.
3. Persengiev S, Saffer J, Kilpatrick D. "An alternatively spliced form of the transcription factor Sp1 containing only a single glutamine-rich transactivation domain." Proc Natl Acad Sci USA 92: 9107-9111, 1995.
4. Saffer JD, Phillips JL. "Evaluating the biological aspects of *in vitro* studies in bioelectromagnetics." Bioelectrochem Bioenergetics 40:1-7, 1996.
5. Saffer JD, Chen G, Colburn NH, Thurston SJ. "Power frequency magnetic fields do not contribute to transformation of JB6 cells." Carcinogenesis 18:1365-1370, 1997.

Other EMF Publications

None.

TITLE: **Attributable Fraction Estimates for EMF Exposures**

Principal Investigator: Asher R. *Sheppard*, Ph.D.
Loma Linda University
Loma Linda, California

Health Relevance: Exposure Metrics

Research Area: Biophysics/Field Interactions, Epidemiology, and Cancer/Leukemia

R03-ES08920 **Start Date:** 03/01/97 **End Date:** 02/28/99

Abstract

The proposed research will estimate the attributable fraction (AF) for childhood leukemia (CL) from exposures to power frequency magnetic fields (MFs) using novel methods for exposure assessment. CL is the most common childhood cancer and a significant factor in childhood morbidity and mortality, accounting for about 2400 deaths annually in the United States. There was a cumulative increase of 20% in the rate of incidence of CL over the period 1973-1991 (Landrigan, 1995). Data on exposure and risk-rather than summary risk estimates-will be taken from almost all available epidemiological studies on this topic. The resulting AF estimates and underlying epidemiological methods will contribute directly to development of hazard assessment models for the potential influence of EMFs on CL. In pilot work, epidemiological data from several studies on EMFs and CL have been acquired, put into a database, and verified to reproduce published risk estimates. The proposed advances in epidemiological research methodology and exposure assessment require these specific aims: 1) compilation of a larger database on CL and MF exposures from existing epidemiological studies; 2) creation of a MF-based exposure score for standardized exposure assessments; 3) estimation of the total exposed population; 4) incorporation of a dose-response model; 5) estimation of AF from the database (unlike meta-analyses which use summary data); 6) conduct sensitivity analyses to identify critical assumptions and needs for better data. Because major steps have already been taken towards these goals, it will be possible to complete this work within funding available from a RO3 grant. Follow-on work could apply the method to other diseases of children and adults potentially caused by EMF exposure.

Experimental Design and Exposure Conditions

None.

Quality Assurance Measures

Data incorporated into the database were obtained directly from authors of the original study and verified by reproducing results published by the study authors. Authors were consulted to resolve any discrepancies. A codebook was prepared to define the variables used in the database. Variable descriptions were obtained and clarified by direct contact with study authors. This database and related materials are regularly backed up to assure against loss.

Results

We have acquired data and permission to use them from 13 studies which obtained magnetic field estimates on a subject-by-subject basis and four more which have only aggregate exposure information provided in the publication. Overall, the available data include 3164 cases, 4896 controls, 35 subjects of a cohort study, and 144

subjects of an ecologic study. Of the case-control studies, the number of cases for which magnetic fields were measured by a meter, 1285, is nearly equal to the number, 1352, for which estimates were based on distance to the power line. Sometimes load data were obtained. Exposure data for another 791 cases are based entirely on surrogate measures. For control subjects, magnetic field estimates were obtained from field measurements, distance-based calculations, and surrogates for these numbers, respectively: 1612, 2614, 678.

Data have been transferred from native formats into a unified database in SAS format. The database has been verified by reproducing published information on odds ratios, etc.

We set this goal for exposure assessment: obtain from each study the magnetic field estimate which most nearly provided magnetic fields in the child's bedroom over the child's lifetime. For our first analysis we determined the measurement which most nearly met this goal for each study. Other magnetic field estimates were identified, evaluated, and prioritized for later use. Data from a recently published large National Cancer Institute study of childhood leukemia will be used for calibration of wire code-based exposures for those U.S.A. homes not represented by the Denver and EPRI studies.

Estimates of the attributable fraction will be made in the next period. The attached table indicates the studies incorporated in our analysis and the exposure assignment techniques employed for our preliminary analysis.

EXPOSURE ASSESSMENT

Study	**Cases / CTRLS**	**Preferred**	**Others Available**
Wertheimer & Leeper 1979	155/155	Group MF from 2 level wire code calibrated from Savitz data	2-level W/L wire code
Savitz *et al,* 1988	73/222	MF from spot measurement (low power) in child=s bedroom and MFs derived from 5-level W/L wire code	Other home locations, front door: high power spot measurements; 5-level W/L wire code
Fulton *et al.,* 1980	119/225	MF from wire code derived from primary data on distance, wire types	Distance to power line
Tomenius, 1986	243/212	MF at front door for diagnosis home	MF at front door of birth residence, Proximity (<150m) to transmission, substation
Coleman *et al.,* 1989	84/141	MF from power line – assign W/L wire code, use 1000 homes study to calibrate distance data: MF near substation using Bristol, Eng. Data	Proximity (within 100m) to substation
London *et al.,* 1991	169/149	24-h bedroom measurement (normal power)	Spot measurement: W/L wire code
Feychting and Ahbom 1993	38/152	MF calculated from load and distance for diagnosis date & averaged prior to diagnosis (homes & apartments)	Spot measurement, Calculated & estimated MF's fro 1, 5, 10 years prior to diagnosis
Verkasalo *et al.,* 1993	35 (exp. Info for 134,800)	MF calculated for annual average from map and load data	Calculated cumulative exposures
Olsen *et al.,* 1993	832/1658	MF calculated annual averages over life from maps & loads	Cumulative lifetime exposure

Michaelis *et al.*, in press	176/414	24/h average MF in child's bedroom	24-h median, nightime median in child=s bedroom; median mean home MF from spot meas; 24-h living room
Fajardo-Gutierrez, 1997	187-187	MF cal. From K/S wire code & 1000 homes	Distance from electrical facilities
Tynes & Haldorsen, 1997	148/579	MF annual averages from map & loads	Cumulative exposure
Myers *et al.*, 1990	< 180	Calculated MF	Distance to source
Lowenthal, 1991	49	Distance to power line	
Petridou *et al.*, 1994	136/187	Distance from poser line	Distance from substation
Lin *et al.*, 1994	67	To be determined	
Lin *et al.*, 1996	28	Spot MF measurement	Distance to line (D>100m or <100m)
Linet *et al.*, 1997*	624/615	Time-weighted average summary of measurements	24-h bedroom; spot meas. In rooms of home(s), at front door; wire codes

* Under consideration by NCI
No subject-specific data for studies shown with shaded background

Discussion and Contribution to Understanding Biological Effects of EMF

This research will yield estimates of the attributable fraction in the United States for childhood leukemia associated with magnetic field exposures. Our work may contribute to understanding whether magnetic field exposures are associated with excess risk of childhood leukemia and may indicate preferred exposure metrics.

Publications From This Grant

None

Other EMF Publications

None

TITLE: **Effects of 60 Hz EMF on Capacitative Calcium Entry**

Principal Investigator: Jesse E. *Sisken*, Ph.D.
University of Kentucky

Health Relevance: Cancer

Research Area: Cellular Function; Signal Transduction; Cellular Processes; Calcium Regulation

RO3-ES08878 **Start Date:** 1/10/97 **End Date:** 1/09/99

Abstract

Some epidemiological studies suggest that residential and occupational exposure to power frequency electromagnetic fields (PFEMF) may be a risk factor for the development of certain neoplasms. This association is often weak, however, and there is no generally accepted mechanism to explain how this might occur. The long-term goal of this work is to determine whether or not PFEMF (60 Hz) can affect those signal transduction mechanisms that regulate cellular proliferation and thus increase the likelihood of tumorigenesis. Calcium ions are major components of such regulatory pathways and there are suggestions in the literature that a particular calcium regulatory system, the "capacitative" calcium entry mechanism, might be sensitive to EMF. A plausible suggestion for why this might be so, especially in the case of stem cells responsible for maintaining renewal cell populations such as those of epithelial and hematopoetic tissues, is presented. If correct, this model could help explain how EMF might increase the risk of cancers and leukemias. The specific aim of this proposal is to test the first part of this hypothesis, i.e., to determine whether or not sinusoidal PFEMF can modulate the entry of Ca2+ through the capacitative entry system in cells.

Experimental Design and Exposure Conditions

Studies will be done with Swiss 3T3 and SV40-transformed Semian 3T3 cells, rat osteosarcoma cells (ROS 17/2.8) and normal human fibroblasts. The basic protocols will be to activate this system and determine whether or not 60 Hz EMF can modulate the rate at which Ca^{2+} flows through this system. Activation of this system will be accomplished by depleting the intracellular calcium store with mitogenic agents or the tumor promoter, thapsigargin. During the course of treatment, cells will be exposed to sinusoidal, 60 Hz EMF in the milliTesla range with induced electric fields in the 10 to 30 V/cm range using an air gap reactor system developed in this laboratory. One of the advantages of this system is that it allows for resolution between effects due to magnetic fields and those due to induced electric fields. Cytosolic free calcium levels will be monitored in real time by recording light output from the calcium specific, luminescent, photoprotein, aequorin.

Quality Assurance Measures

Our EMF exposure system was initially evaluated by Dr. Martin Misakian of the National Institute of Standards and Technology who found that it performed essentially as designed. More recently, Dr. Fred Dietrich performed some measurements of our system on Sept. 25, 1997 while here on an NIEHS-sponsored site visit. His measurements of the magnetic fields geneated at the site of the cells in our experimental system were in good accord with the original design, so that we are assured that our system performs as originally designed.

Results

We began this work with a series of experiments to first establish whether baseline cytosolic free calcium ion concentration ([Ca^{2+}]i) and agonist-induced calcium transients are affected by 60 Hz EMF in otherwise untreated, normal polarized cells. The work done to date has been on SV40-transformed Swiss 3T3 (SV3T3) cells. The general protocol utilized is to first record [Ca^{2+}]i levels for 400 seconds prior to turning on the fields (period A). Then, with fields turned on, record for another 400 seconds (period B), induce a calcium transient with bradykinin (peak 1) and record post peak baseline levels (period C). The fields are then turned off for 2 minutes while the medium is exchanged for fresh, bradykinin-free medium. They are turned on again and baselines are again recorded (period D), a second peak is induced with bradykinin (peak 2) and a post second peak baseline level (period E) is recorded. Overall, we record, in the same population of cells, a pre-EMF baseline level and, in the presence of EMF, 2 bradykinin-induced calcium transients and pre-peak and post-peak baseline levels surrounding each peak. Since the calcium transients induced by bradykinin are derived almost entirely from intracellular stores, by examining a second peak we learn whether or not the refilling of the intracellular calcium stores during EMF treatment following the first peak (which takes place from extracellular sources) is affected by the fields.

The experimental design is a 3 X 3 matrix. The studies are performed under 3 EMF field conditions (true sham, 3 Gauss and 20 Gauss) and at 3 different medium calcium concentrations (0.4 mM, 1.8 mM and 10 mM). The rationale behind this design is that if PFEMF can lead to a net entry of Ca^{2+} into cells, we should see stepwise increases in [Ca^{2+}]i as both the extracellular calcium concentration increases and with increasing magnetic flux densities. The greatest differences, therefore, should be between sham treated cells in 0.4 mM extracellular Ca^{2+} and 20 G treated cells in 10 mM extracellular Ca^{2+}. A total of 67 experiments has been performed with this methodology. Sample sizes for the individual treatments range from a minimum of 5 to a maximum of 14.

Table 1 (below) contains the data for the 5 baseline data periods listed above. As can be seen, the data show no obvious, stepwise or consistent changes as a result of exposure to EMF for any of the parameters measured. However, because the variances are high, we cannot totally rule out the possibility that a small effect is occurring that is not detectable due to the level of variation in the data. As expected, bradykinin-induced peaks, which we have previously shown to be insensitive to extracellular Ca^{2+} in SV3T3 cells (Newcomb, *et al*. Cell Calcium 14:539-549, 1993) are also unaffected by the PFEMF.

In a more recent series of experiments the same experimental protocol was utilized except that we reduced the BK concentration to 10nM and studied the effects of a 500 G EMF on cells in 10mM extracellular Ca^{2+}. Preliminary analyses indicate that, for the first time from this laboratory, we may be seeing increases in [Ca^{2+}] as a result of EMF treatment under these conditions. The effect is small, on the order on 10nM (i. e., about 10 percent) but statistically significant at the .01 level.

The experiments described above also served as controls for the second step in this work which is to examine the effects of PFEMF on the net entry of Ca^{2+} into cells whose capacitative entry system has been activated by the mumor promoter, thapsigargin (TG). In this work we have been using 2 different methodologies to activate the capacititative system. In the first, cells are treated with the tumor promoter, thapsigargin which irreversibly blocks the ATPasc responsible for pumping Ca^{2+} into the intracellular pool. This leads to an emptying of the pool and activation of the CRAC. This is done in normal, polarized cells and in cells exposed to high levels of K^+. In both cases, the culture media contains 10mM Ca^{2+} which is more that 5 times the levels found innormal media. In neither of the these types of experiments have we been able to observed any significant differences in the activity of this channel between cells exposed to 60 Hz, 500 G EMF and those exposed to true sham conditions.

Discussion and Contribution to Understanding Biological Effects of EMF

As described above, we have found statistically significant evidence for an effect of EMF on calcium regulation only in experiments in which cells in 10mM Ca^{2+} were exposed to 500 G EMF and sub-optimal concentrations of bradykinin. A more detailed study of this effect is in progress. Although the defect is small, insight into the mechanisms involved could provide a clearer basis for understanding the relationship between EMF and calcium regulation and might also enhance our understanding of how the capacitive calcium entry system is regulated. Overall, however, given the conditions required to obtain our results and the small magnitude of the effect

observed, it seems reasonable to doubt that the kinds of changes we observe are related to potential biological effects of EMF at the population level.

Publications From This Grant

None

Other EMF Publications

1. Sisken BF and Sisken JE. “Effects of electromagnetic field stimulation on cellular signal transduction mechanisms and analyses of the effects of low frequency electromagnetic fields on calcium "spiking" in ROS 17/2.8 cells.” Final report to Electric Power Research Institute, Palo Alto, California for contracts WO9103-01 and WO8021-14, TR-109489, 1997.
2. Mullins RD and Sisken JE. “An analsis of spontaneous calcium spiking behavior in ROS 17/2.8 Cells.” Bioelectromagnetics, Submitted, 1998.

TITLE: **The Effect of Altered Nocturnal Melatonin Levels on the Progression of Breast Cancer in Rats**

Principal Investigator: Gregory *Travlos*, DVM
Laboratory of Experimental Pathology, NIEHS
Research Triangle Park, North Carolina

Health Relevance: Cancer

Research Area: Toxicology Studies of Whole Animals; Tumor/promotion
Melatonin/Breast Cancer

Z01-ES21195 **Start Date:** 9/93 **End Date:** 9/96

Abstract

There have been reports that exposure to electric and magnetic fields (EMF) lowers nocturnal melatonin levels. Further it has been reported that exposure to magnetic fields will promote chemically-induced breast cancer in the rat. Our rationale is to separate the effect of lowered nocturnal melatonin levels from a possible direct effect of magnetic fields. We have demonstrated that one minute exposures of light will decrease nocturnal melatonin levels in the F344/N rat. Further we have demonstrated that multiple one minute exposures approximately two hours apart will allow a nocturnal rise in melatonin levels but will prevent peak levels. Since the reported effects of magnetic field exposure is a moderate decrease in peak melatonin levels, this model will allow us to test the effect of lowered melatonin levels in a rat breast cancer model. The rationale is that short-term light exposure at night or magnetic field exposure or both will cause a decrease in nocturnal melatonin levels and this may account for the reported association between exposures and increased breast cancer. Since light at night is a proven suppresser of melatonin levels, we will follow this course for investigating the health effects of decreased melatonin levels, thus avoiding the confounding factors that magnetic field exposure may produce. Yet our study will provide important information on with respect to possible adverse health effects associated with magnetic fields.

Experimental Design and Exposure Conditions

The model is MNU induced breast cancer in the F344/N rat. The melatonin in the blood and pineal gland is measured using HPLC techniques after extraction of the melatonin. Positive controls are run in each assay. The animals are coded and the assays run in a blind fashion. The experiments are run under GLP conditions.

Quality Assurance Measures

Assay standards, spiked serum samples and curves are run for each melatonin assay. The data will be reviewed by QA personnel.

Results

We previously reported that a light tight facility, in which the light cycle could be modulated, was constructed and that the light/dark cycle for female F344/N rat was switched from the standard nighttime to a 3 am to 3 pm dark cycle. Also, we demonstrated that we could reliably and reproducibly measure serum and pineal melatonin levels in the rat and that the normal nocturnal rise in melatonin would change to the ambient light/dark cycle by switching the dark cycle to 3 am-3 pm. Further we demonstrated that multiple (5 one-minute pulses of

incandescent light every two hours after the start of the dark phase) bursts of light decrease but not negate the nocturnal melatonin concentrations (Figure 1) and pineal SNAT activity (Figure 2).

We have demonstrated that the effect of the light-burst treatment caused significant decreases of serum melatonin concentration and pineal SNAT activity after 2-weeks of continued light burst exposures (Figures 3 and 4). The serum melatonin concentration decreases were not as severe as demonstrated in the 1-day exposure. Pineal melatonin concentration did not appear to be affected (Figure 5). Additionally there appeared to be a time period near the middle of the dark phase (approximately 9:00 for this light/dark cycle) during which the serum concentrations were more sensitive to the effects of light (Figure 3). Also, there appeared to be a developing phase shift advance in the melatonin cycle of the light-exposed animals (Figure 3). After 10-weeks of continued light burst exposures, the light exposed animals demonstrated a similar melatonin cycle as control animals but with what appeared to be somewhat decreased peak values (Figure 6). A more developed phase shift advance (with higher serum concentrations at 5:30 and 6:30) also occurred (Figure 6). There was still a significant decrease of pineal SNAT activity for the light-exposed animals (Figure 7) but pineal melatonin concentrations demonstrated an overall increase (Figure 8).

At necropsy in the 26-week MNU-induced mammary tumor model, there were no significant alterations in mass incidence (30/40 MNU control rats v. 29/40 MNU+light burst rats) or multiplicity (2.3 masses/tumor-bearing MNU control animal v. 1.9 masses/tumor-bearing MNU+light burst animal), and there may have been a propensity for larger masses in the MNU control rats (1.14 g total mass wght./tumor-bearing control animal v. 0.74 g total mass wght./tumor-bearing MNU+light bursts animal). We demonstrated that there were no apparent alterations of the serum or pineal melatonin concentrations or pineal SNAT activity cycles that would be attributed to MNU administration or prescence of tumor at 26 weeks post-MNU IP injection (Figures 9-11). Additionally, animals that continued to be exposed to light bursts for 26 weeks, had similar or higher serum melatonin concentrations compared to controls and their baseline values (during the end of the light phase at 2:30 and the beginning of the light phase at 15:30) remained elevated (Figure 9). Furthermore, an age cohort of rats that had been pinealectomized at 4 weeks of age had serum melatonin concentrations that were higher than expected (Figure 9). A very small sampling of pinealectomized rats at 2- and 10-weeks (n = 3/interim time point) demonstrated serum melatonin concentrations of <14 pg/mL regardless of the light phase (data not shown). Additionally, light bursts did not appear to affect pineal melatonin concentrations or pineal SNAT activities after 26-weeks of continued intermittent light exposures (Figures 10 and 11).

Discussion and Contribution to Understanding Biological Effects of EMF

Pineal melatonin production is governed by neuronal activity in the suprachiasmatic nuclei of the hypothalamus and requires intact sympathetic innervation of the pineal gland. Melatonin synthesis is synchronized to the prevailing daily light:dark cycle and exhibits a marked circadian rhythm with peak synthesis and secretion occurring at night. Melatonin has a major role mediating the effects of photoperiod on reproductive physiology and other endocrine organs. Additionally, it has been reported that melatonin has oncostatic, oncomodulatory, immunoregulatory, and antioxidant properties. Melatonin may have both direct and indirect beneficial effects on tumor development and maintaining a normal circadian rhythm of melatonin production may be beneficial in decreasing the risk of tumor development. Thus, if normally high nighttime melatonin concentrations are lowered by EMF or light, the oncostatic/antioxidant properties of this hormone may be altered thereby increasing a person's susceptibility to some cancers (for example, breast cancer).

The effect exposure to EMF has on the melatonin circadian rhythm appears to one of attenuation rather than abolition. Furthermore, there is evidence to indicate that the pineal gland is not directly sensitive to EMF but that it responds to the pulsed electric currents induced by rapid inversion of the magnetic field. For example, rats exposed to repeated instantaneous inversion of a magnetic field had a 50% reduction in circulating melatonin concentrations while rats exposed to manually inverted fields were unaffected. It was proposed that these electric currents produce a visual sensation in the retina which are interpreted as light and result in suppression of melatonin production. The eyes have often been considered the receptor of magnetic field changes. Since exposure to light at night is a proven suppresser of melatonin production, studies on the health effects of altered melatonin concentrations could be performed using intermittent light bursts, thus, avoiding the expense of building and validating an EMF exposure facility.

In our laboratory, a preliminary study indicated that using 5, one-minute, pulses of incandescent light administered every two hours during the dark phase of the light:dark cycle attenuated melatonin production without totally shutting down synthesis. The data suggest that this pulsed light paradigm may useful to evaluate the effects of a moderately suppressed melatonin rhythm, similar to what is caused by EMF, without having to construct, characterize, and validate a EMF exposure facility. Furthermore, the data suggest that intermittent light bursts suppress peak serum melatonin concentrations for 2 weeks and possibly 10 weeks. But that the magnitude of suppression decreases with time and at 26 weeks there appears to be no effect of light bursts on serum melatonin concentrations. Additionally, there appears to be a time period near the middle of the dark phase during which the serum melatonin concentrations are more sensitive to the effects of light exposure. These preliminary data suggest that intermittent light bursts have no effect on the development tumors in an MNU-induced tumor model in female F344/N rats. Furthermore, there is an indication that serum concentrations of melatonin may not be solely controlled by production by the pineal gland (for example, increased serum concentrations in pinealectomized animals) and/or release of pineal melatonin may not be simply related to diffusion (for example, increased pineal melatonin concentrations regardless of the pineal SNAT activity in the light exposed animals).

Figure 1. Serum melatonin concentration after five 1-minute light bursts during the dark phase

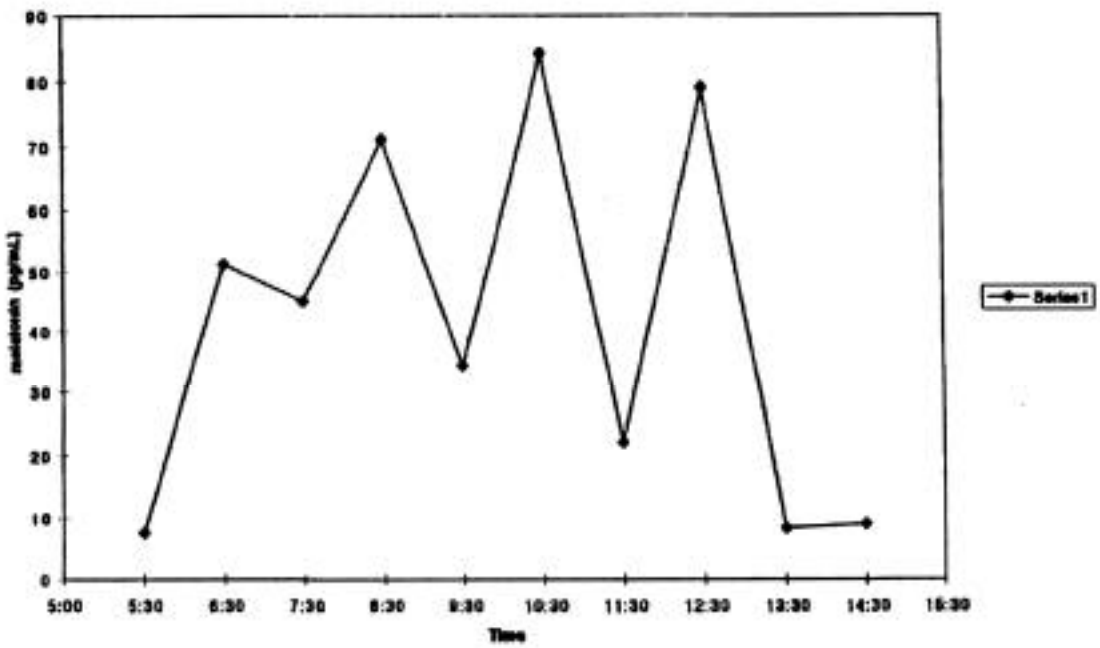


Figure 2. Pineal SNAT activity after five 1-minute light bursts during the dark phase

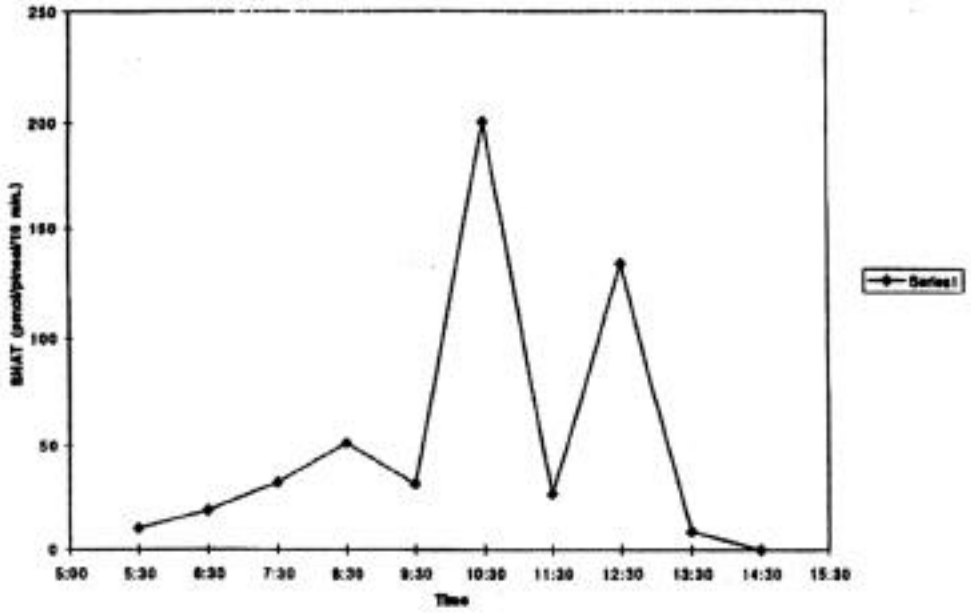


Figure 3. Serum melatonin concentration after two weeks of light bursts

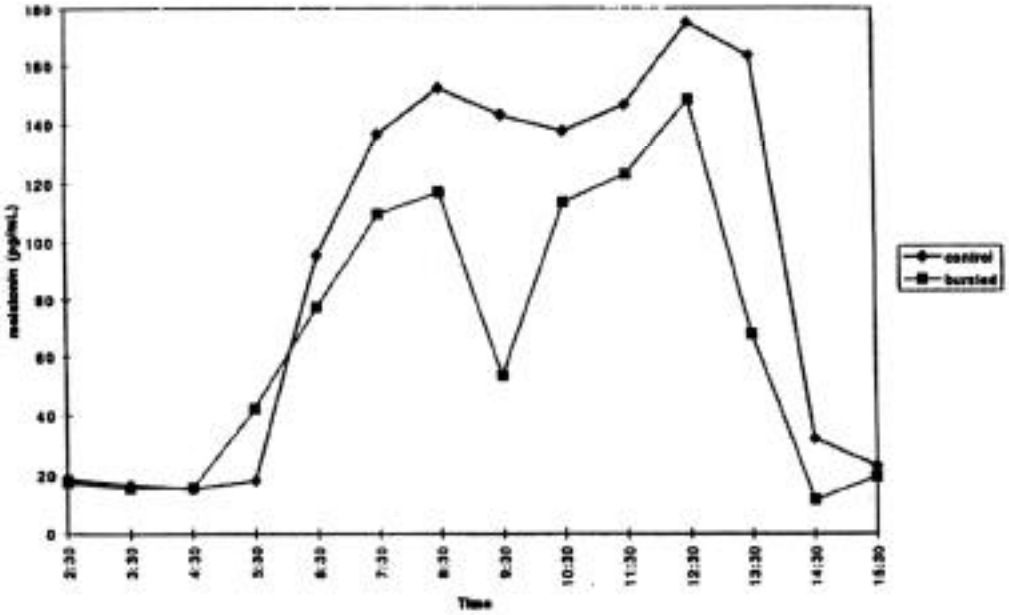


Figure 4. Pineal SNAT activity after two weeks of light bursts

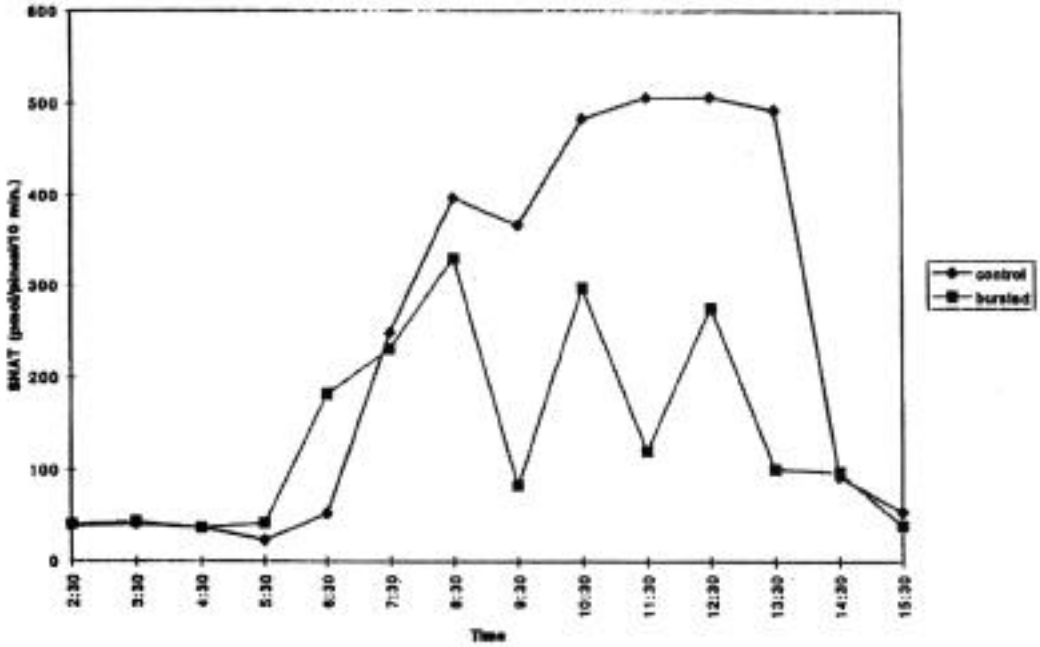


Figure 5. Pineal melatonin concentration after two weeks of light bursts

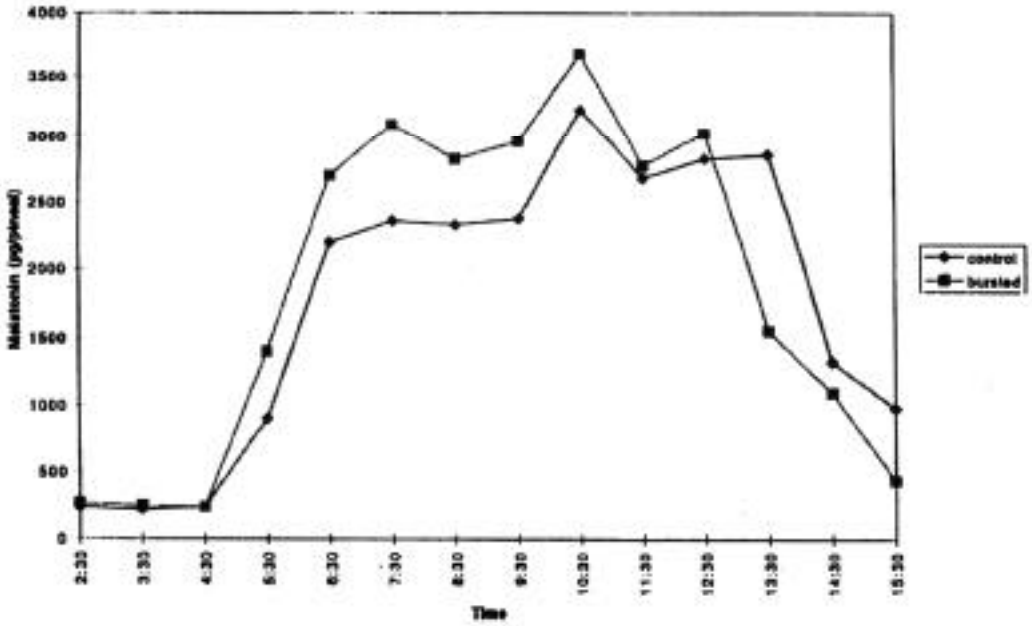


Figure 6. Serum melatonin concentration after ten weeks of light bursts

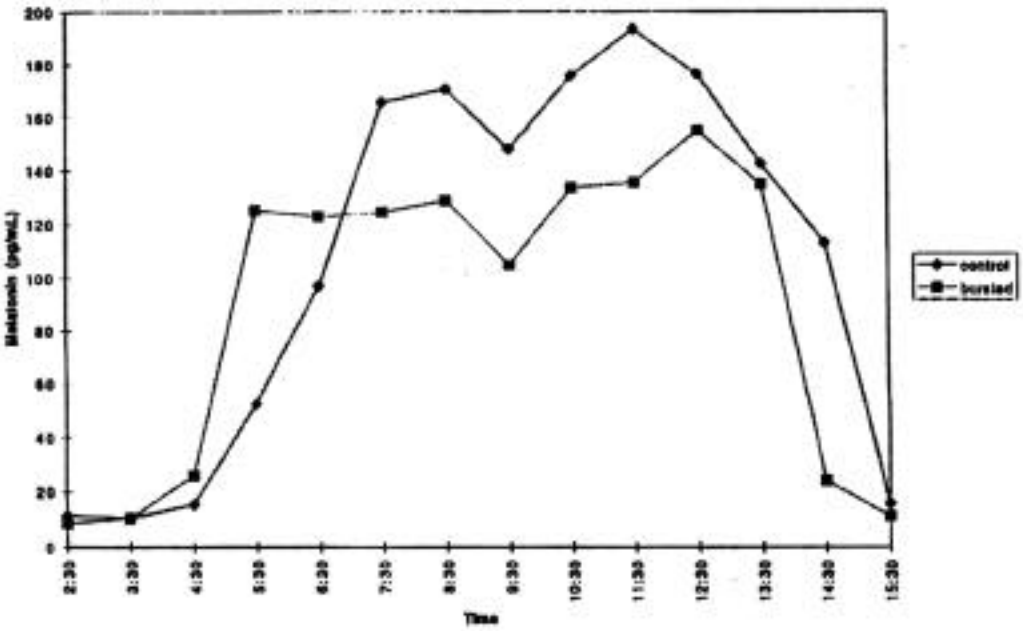


Figure 7. Pineal SNAT activity after ten weeks of light bursts

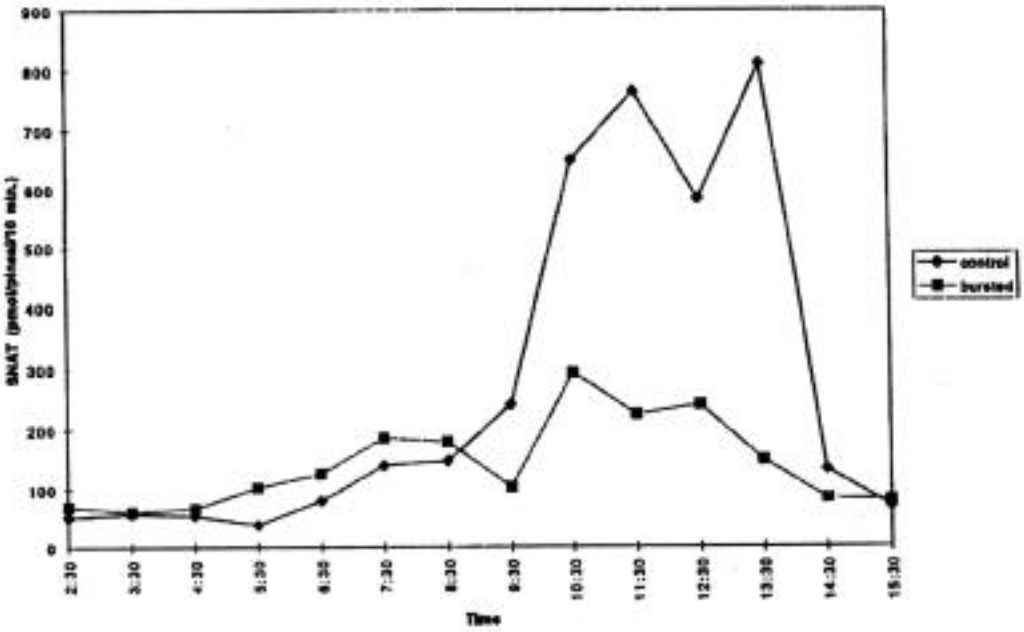


Figure 8. Pineal melatonin concentration after ten weeks of light bursts

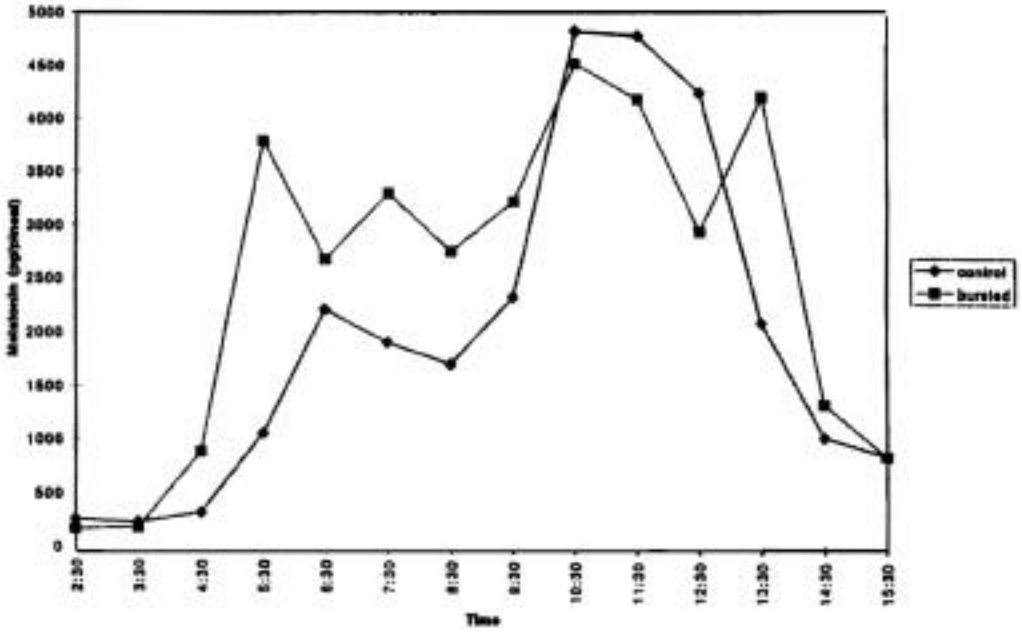


Figure 9. Serum melatonin concentration for the 28-Week MNU-induced mammary tumor study

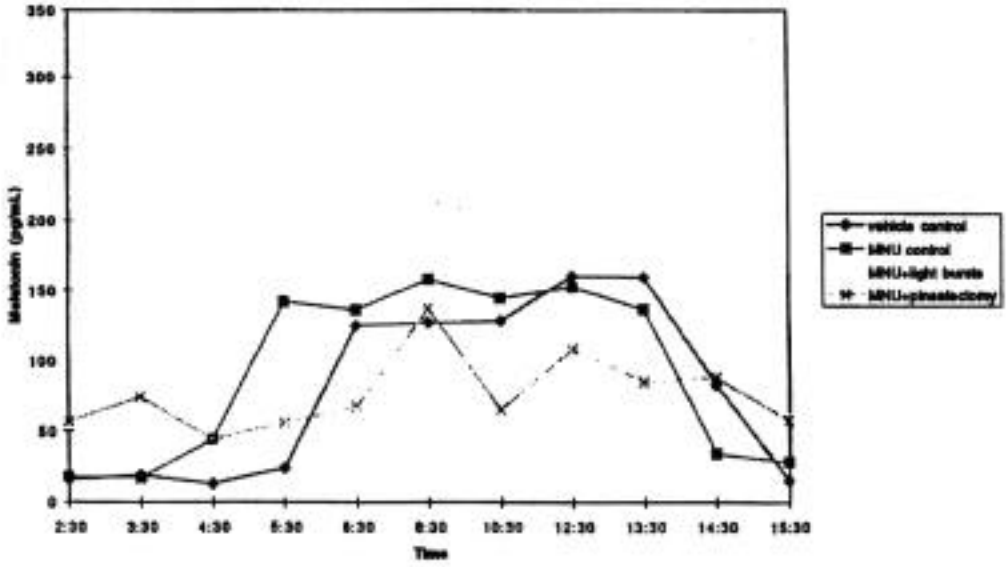


Figure 10. Pineal melatonin concentration for the 26-Week MNU-induced mammary tumor study

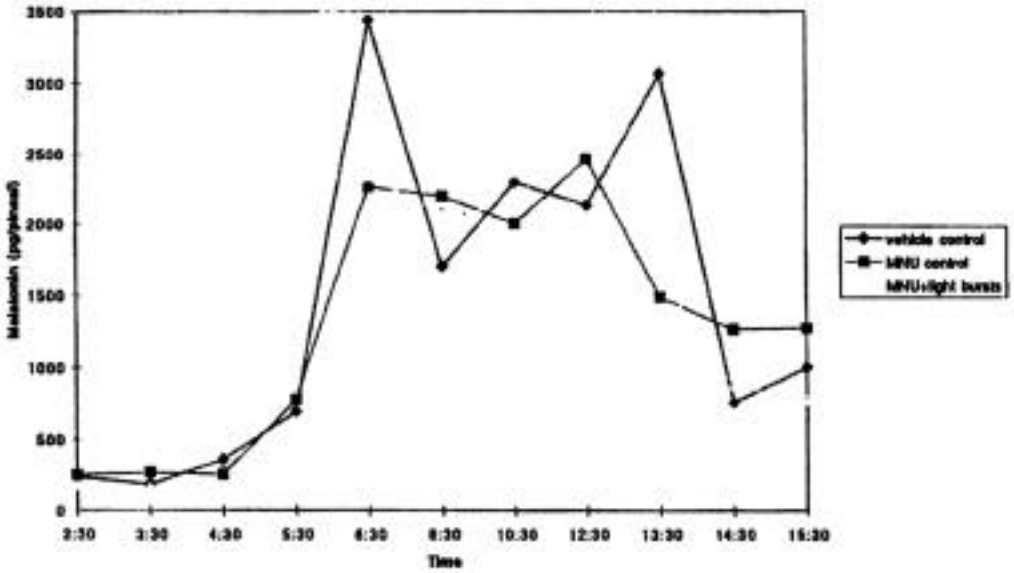
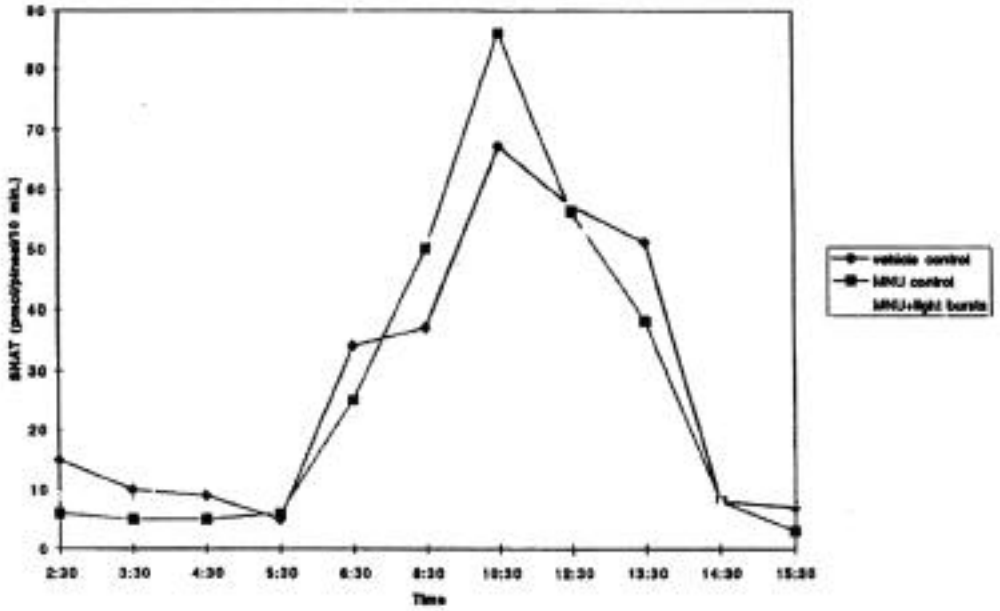


Figure 11. Pineal SNAT activity for the 26-Week MNU-induced tumor study



TITLE: **Cellular Effects of Low Frequency Electromagnetic Fields**

Principal Investigator: Jerry R. *Williams*
Johns Hopkins University

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Proliferation

R01-ES07076 **Start Date:** 9/28/94 **End Date:** 8/31/98

Abstract

There have been several claims made in the biomedical literature that exposure to low frequency electromagnetic fields (EMF) leads to an increase in cancer risk. The epidemiological data on this point is contradictory, and part of the skepticism which surrounds the issue derives from the absence of any well-established mechanism by which EMF could induce oncogenic changes. Evidence suggests that EMF fields do not lead to DNA damage or mutation. However, there are a number of studies which show that EMF lead to increased levels of a number of mRNAs, including *c-myc, c-fos, c-jun* and protein kinase C (PKC). All these genes are known to be implicated in malignant transformation. These findings need to be reproduced. Moreover a pathway has to be established showing the chain of events which lead from EMF exposure to gene expression.

We wish to examine the effect of EMF exposure on tumor cells with defined genetic abnormalities. We are particularly interested in small cell lung cancer lines, which have been extensively studied in this laboratory. These cells can spontaneously advance to a non-small cell phenotype. Transfection studies indicate that *c-myc* expression is a necessary component of this transition. In this system, EMF induction of *c-myc* may represent a mechanism of malignant progression.

We also wish to test the hypothesis that EMF may interact with environmental mutagens. We will address this question using cells deficient in mismatch repair, testing their response to ionizing radiation and alkylating agents in the presence or absence of EMF. These cells represent a hypermutation phenotype that is relevant to multistage carcinogenesis.

All results are negative. We found no EMF effect in these several systems.

Experimental Design and Exposure Conditions

The EMF exposure apparatus consists of two coil assemblies, arranged so that either can be activated. The experimenter does not know which cells have been exposed and which have been subjected to sham treatment.

The cell lines to be used are the human leukemic line HL60 and the human small cell lung cancer lines NCI H82, NCI H209 and NCI H249. EMF effects on *c-myc* expression have been noted in HL 60 cells. The lung cancer lines have been extensively studied in this laboratory with regard to c-myc regulation. Six human colon cancer lines will also be studied. Three of these are deficient in the mismatch repair pathway.

EMF effects have been noted in HL 60 cells at 5 T, 60 Hz after 20 minutes. These exposure conditions will be used first, though we plan to study the effect of field strength and frequency over the ranges 0.5 - 500 T and 15 - 120 Hz respectively. The exposure system can be ramped to eliminate transients; a comparison between cells exposed to ramped and unramped fields will be made.

Animal studies will be considered if the *in vitro* results warrant. The animal model has yet to be selected.

Quality Assurance Measures

Background fields in the exposure room have been monitored and found to be negligible in the area where the coils are situated. Stray fields were monitored by the installing engineer. The two exposure units do not interfere with each other. The experiments will be conducted double blind, in that the experimenter will not know which cells have been exposed to an EMF field and which are the sham-treated controls - nor will the individual who scores for biological response know treatment received by each group. Ionizing radiation will be used as a positive control for mutagenesis and cell cycle delay studies. Chemical treatments such as TPA and retinoic acid which are known to induce or repress the genes of interest will also be used as controls.

Results

Our results are completely negative. We have observed no reproducible patterns of gene expression for any cell line and for any EMF exposure.

Studies have been completed comparing the mutability of cells with and without mismatch repair capacity. The repair deficient cells have a much higher spontaneous rate of mutation at the hprt locus. They are also more prone to radiation-induced mutation. We see no effects of EMF in the cell system.

Similarly the small cell lung cancer (SCLC) cell model has been investigated for spontaneous and radiation-induced transition to a non small cell (NSCLC) phenotype. Low doses (up to 3 Gy) of ionizing radiation enhance the fraction of NSCLC recovered. However these NSCLC may be more radioresistant, raising the possibility that ionizing radiation is acting as a selective agent. The relative contributions of radiation induced mutations and selection in this system has yet to be determined.

Studies with EMF show no effect. HL60 cells do not appear to up-regulate *c-myc* mRNA in response to EMF. We compared the response of these cells to different field strengths and measured expressed levels as the ratio of c-myc expression in exposed versus control cells. To correct for differences in the amount of RNA loaded onto the gel, *c-myc* expression was expressed as a fraction of the housekeeping gene gapdh.

(*c-myc*/gapdh)Exposed/(*c-myc*/gapdh)Control

The null hypothesis was that this ratio should not differ from one. Of the five exposure conditions tested on these cells, none show any statistically significant effect on *c-myc* expression.

<u>TPA treatment of these cells resulted in a twofold induction of *c-myc* mRNA.</u>

The human Tcell leukemic cell line CEMCM3 has also been reported to up-regulate *c-myc* mRNA in response to EMF. However we have been unable to reproduce this result. Similar studies have been conducted with the human non-small cell lung cancer line, NCI-H82. We were unable to detect any change in *c-myc* expression using a field strength of 100uT and a 20 minute exposure.

We have investigated the possibility that EM fields in some way interfere with cellular responses to DNA damage. A human colorectal carcinoma cells expressing wild type p53 were exposed for 72 hours to a 60 Hz, 100 uT field, in the presence of tritiated water. Tritium was added to give a dose rate of 3cGy/hr. This treatment induced p53and p21 expression in the c ells. The presence or absence of EMF exposure did not affect these endpoints in any way.

Discussion and Contribution to Understanding Biological Effects of EMF

These studies will confirm or refute previous studies showing EMF induces cellular change in specific cell types. Positive results can lead to new research into possible mechanisms by which EMF may induce toxic cellular effects.

Publications From This Grant

1. Bean CP, Dietrich FM, Fox MS, Rigers WR, Stolwijk JJ and Williams JR. EMF research activities completed under the energy policy act of 1992, interim report,Committee to Review the Research Activities Completed Under the Energy Policy Act of 1992, National Academy Press, 1995.

2. Stevens CF, Savitz DA, Anderson LE, Driscoll DA, Gage FH, Garwin RL, Jelinski LW, Kelman BJ, Luben RA, Reiter RJ, Slovic P, Stolwijk J, Stuchley MA, Wartenberg D, Waugh JS and Williams JR. "Possible health effects of exposure to electric and magnetic fields, Committee on the Possible Effects of Electromagnetic Fields on Biologic Systems." National Academy Press, 1997.

Other EMF Publications

None.

TITLE: **Changes in Gene Expression Accompanying Exposure to EMF**

Principal Investigator: Gayle E. *Woloschak*, Ph.D.
Argonne National Laboratory
Argonne, Illinois

Health Relevance: Cancer

Research Area: Cellular Function; Gene Expression; Cell Cycle

R01-ES07141 **Start Date:** 9/28/94 **End Date:** 8/31/97

Abstract

Experiments were conducted (a) to determine whether EMF (60 Hz) affects gene expression and (b) if so, to identify the mechanism responsible. Using differential display approaches, we identified several genes that were modulated in response to EMF exposure (blind, 1G or 10G, 24 h) in HeLa cells. Investigators were blind to the exposed vs. control cells; treatments were 1G or 10G for 24 h. Northern blots confirmed the changes in expression observed using differential display. Two genes were induced and two genes were repressed following 1G EMF exposure. These changes in gene expression were no more than 2-1-fold in magnitude. The sequences were compared to GenBank, and while several matches were found, they were to ESTs or to known genes at low homology, suggesting that these genes are novel. A more detailed analysis of the 3 UTRs of several of these genes revealed the presence of possible consensus elements. In each case, oligonucleotides were synthesized and tested in an EMSA (electrophoretic mobility shift assay) for specific binding. While all of them bound proteins specifically, only four showed binding that was affected by EMF exposure. In each case, binding of proteins to the consensus element was increased more than five-fold using extracts from EMF-exposed cells (blind, 10G, 24 h) compared with extracts from sham-exposed cells. These elements are evolutionarily conserved, are found in 3 UTRs of EMF-affected genes, and show EMF- responsive protein binding patterns. They may be important in modulating EMF-induced gene expression or some other EMF-induced activity in the cell.

Experimental Design and Exposure Conditions

Exposures are done using continuous sinusoidal 60-Hz magnetic fields at intensities from 0-100 T (0-1000 mG) for lengths of time up to 72 h. We are currently using the human cell line HeLa, though we plan to test other cell lines as well (breast cell lines used by D. McCormick). RNA changes will be determined by differential display. In this technique, RNA is harvested and cDNA is synthesized using reverse transcriptase and then amplified. Identified bands are then tested by dot blots and Northern blots to ensure that levels of the transcript are changed in response to EMF. Later experiments will examine how these genes are regulated.

Quality Assurance Measures

We have set up several negative controls, such as monitoring expression of genes encoding ornithine decarboxylase and proliferating cell nuclear antigens. These genes are not affected by EMF exposure. We have also included some positive controls, particularly the use of ionizing radiation to induce known positive responses. All experiments are done blind using the EMF facilities at IIT Research Institute. Jim Gauger is the engineer for this facility. It includes two side-by-side incubators, one of which is exposed to EMF while the other is not. We also include 2 incubator controls (from 2 other incubators) in all experiments. There is much interest in the scientific community in establishing biological consequences of EMF exposure. Two major limitations to drawing firm conclusions on biologic consequences of EMF exposure have been quantitatively small changes in cellular

parameters following exposure and qualitatively poor reproducibility from experiment to experiment. These problems have especially plagued the gene expression area where only modest (if any) changes in gene expression have been reported following EMF exposure.

During the initial 3 years of this project we have examined approximately 10% of the genome of expressed sequences and have identified four transcripts which reproducibly change (1.5 2-fold) in expression following EMF exposure. Any of these changes alone would be considered of questionable importance since the changes are so small (no greater than two-fold). However, the fact that four genes all changed reproducibly way following EMF exposure suggests a possible biologic significance to the response. In addition, in at least two instances the changes occurred in cell lines of different origin (HeLa vs. breast cells). For that reason we are testing the hypothesis that genes modulated reproducibly following EMF exposure in cell lines of different type are part of the cellular response to EMF exposure.

Results

We have determined that very few genes are affected by EMF exposure. In fact, at early time points (within the first 6 h following exposure) we have not yet identified a reproducible change in expression either at the level of total transcription or when specific genes are studied. Recent preliminary studies have suggested that 24-72 h exposure times may lead to the modest induction of a few genes in response to EMF exposure. These were identified by the technique of differential display which resulted in a new band. A partial sequence of this band and a search through Genbank revealed identity with no known genes. Two genes were induced and two genes were repressed following 1G EMF exposure. These changes in gene expression were no more than 2 1-fold in magnitude. The sequences were compared to GenBank, and while several matches were found, they were to ESTs or to known genes at low homology, suggesting that these genes are novel. A more detailed analysis of the 3 UTRs of several of these genes revealed the presence of possible consensus elements. In each case, oligonucleotides were synthesized and tested in an EMSA (electrophoretic mobility shift assay) for specific binding. While all of them bound proteins specifically, only four showed binding that was affected by EMF exposure. In each case, binding of proteins to the consensus element was increased more than five-fold using extracts from EMF-exposed cells (blind, 10G, 24 h) compared with extracts from sham-exposed cells.

Discussion and Contribution to Understanding Biological Effects of EMF

None.

Publications From This Grant

1. Libertin CR, Panozzo J, Groh K, Chang-Liu C-M, Schreck S and Woloschak GE. "Effects of gamma rays, ultraviolet radiation, sunlight, microwaves, and electromagnetic fields on gene expression mediated by human immunodeficiency virus promoter." Radiation Research, 140:91-96, 1994.
2. Woloschak GE, Schreck S, Panozzo J, Chang-Liu C-M, and Libertin CR. "HIV expression is induced in dying cells." Biochim. Biophys. Acta, 1351:105 110, 1997.

Other EMF Publications

None.

TITLE: **Electromagnetic Fields and Adult Malignant Glioma**

Principal Investigator: Margaret *Wrensch*, Ph.D., M.P.H.
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San Francisco, California

Health Relevance: Cancer

Research Area: Studies Involving People; Epidemiological Research; Brain Cancer

R01-ES06285 **Start Date:** 12/1/92 **End Date:** 11/30/96

Abstract

Malignant glioma, the most common primary malignant brain tumor in adults, is debilitating and rapidly fatal. Very little is known about its causes; however, residential studies in children and occupational studies in adults have suggested that extremely-low-frequency electromagnetic field (EMF) exposures may be a factor. Studies of adult brain cancer have not yet undertaken an overall EMF exposure assessment incorporating residential, occupational, and other exposures. In a large, National Cancer Institute funded, population-based case-control study in the San Francisco Bay Area we examined a variety of potential risk factors for brain cancer including family and personal medical histories, occupation, smoking and alcohol use, and other characteristics. Cases (n=492; 82% of those eligible) were adults newly diagnosed with histologically confirmed glioma from 8/1/91-4/30/94. Age-, sex-, and race-frequency-matched controls (n=462; 63% of those eligible) were obtained through random-digit dialing. Structured interviews asked questions pertinent to EMF exposure including detailed occupational histories to enable classification of occupational EMF exposures with duration; history and frequency of use of specific appliances (or other electric devices such as ham radios); and all addresses where subjects lived 15 years prior to study entry. The residential EMF exposure assessment consisted of (1) indoor and outdoor home spot measures with EMDEX meters, (2) characterization of power lines, transformers, and substations near the residence, and (3) Wertheimer-Leeper type wire-codes for the residence. Current residences and all residences in the state of California of each subject for 7 years prior to study entry will be considered. A sample of all residences were reassessed, blindly. Epidemiological and industrial hygiene data are used to develop EMF exposure matrices based on external residential wiring and measured fields at or around the residence(s), occupation, and electric appliance use. Statistical analyses are underway to test hypotheses that cases were more likely than controls to have lived in a residence with high current vs. low current configuration wiring; that cases lived in residences with higher average EMF field strengths (or other metrics such as peaks, variance, or rate of change) than controls; and that cases had greater EMF exposure than controls based on each EMF exposure matrix and on a composite matrix.

Experimental Design and Exposure Conditions

<u>Residential exposure assessment protocol:</u>
Characterization of power lines:
Current or diagnosis residence and prior (7 year) residences:
Standardized drawings of all power lines within 150' of residence:
Categorization of up to 3 lines within 150' of residence:
Power lines were then coded according to the Wertheimer-Leeper (W-L) and the Kaune-Savitz (K-S) classification scheme.

<u>The Wertheimer-Leeper wire-codes are:</u>

VHCC: transmission lines, multiple 3-phase, or thick 3-phase 0-50';
thin 3-phase 0'-25';
OHCC: transmission lines, multiple 3-phase, or thick 3-phase 51' -129';
thin 3-phase 26' -64';
first span secondary 0-50'
OLCC: transmission lines, multiple 3-phase, or thick 3-phase 130' -150';
thin 3-phase 65'-129';
first span secondary 51' -129';
other above ground 0-129'
VLCC: thin 3-phase 130' -150';
first span secondary 130'-150';
other above ground 130' -150'
BURIED: Underground cable serving the residence
Substation: substation noted within 150' of residence

The Kaune-Savitz wire-codes are:

HIGH: Transmission line or three-phase primary within 65 feet of the residence
MEDIUM: Not in high category and transmission line or three-phase primary within 150 feet or open secondary within 85 feet of the residence
LOW: Not in high or medium category.

EMDEX spot-measurements: Six 10 second spot measurements were taken at each location and averaged. Measurements were taken with the meter at waist height, facing north. For index (current or diagnosis) residences, measurements were taken in the center of the kitchen, family room, and bedroom, at the front door, at the four outdoor corners of the residence, and under up to three power lines within 150' of the residence. For prior residences, measurements were taken at the front door and under up to three power lines within 150' of the residence.

Exposure parameters: For current residences, we measure RMS magnetic field intensity in a 40-400 Hz bandwidth. Spot readings are taken in the center of the bedroom, kitchen and family room; a 2-3 hour time tracing (at 10s sample rate) is taken during the interview. Outdoor readings include a front door spot reading and wire code information.

A full residential history was obtained for prior residences in the last 15 years. To assure that all prior residences for at least 80% of subjects were included, the prior period was truncated to 7 years prior to diagnosis for cases or prior to interview for controls. In these homes measurements consist of wire code information and outdoor spot readings only.

Occupational exposure assessment: Occupational information came primarily from a lifetime job history obtained during interviews. Information included the job description, company name, city, starting and ending years of service. These occupation and industry data were coded using the 1980 Standard Occupational Classification (SOC) 1987 Standard Industrial Classification schemes. Average magnetic field exposures will be assigned based on a job matrix approach and summing over the cumulative person-years reported in the work history. Spot magnetic field measurements are obtained by visiting workplaces from a stratified sample of jobs from within industry categories in companies identified in or similar to those reported in the occupational histories. Coding categories where measurements are unavailable are assigned a numerical weight for average field based on literature values.

Population Based Job-Exposure Matrix for EMF: The following methods were used to develop a job-exposure matrix (JEM) to estimate occupational exposure to 60 Hz magnetic fields for study subjects. Personal exposure and spot measurement data for magnetic flux densities from five job exposure studies were coded using job title or description into the Standard Occupational Classification 1980 (SOC80) classification system. Exposure estimates for arithmetic mean, standard deviation, geometric mean, and geometric standard deviation were created for each 4-digit SOC80 job classification by combing exposure data from each study, weighted by the number of subjects.

Spot measurement data accounted for only a small fraction of the total exposure data (4 %). Spot measurement data within job classifications was grouped separately and compared to personal exposure estimates for the same categories and no significant differences were found. An effort was made to collect exposure data on jobs that are common within the brain cancer study population. Consequently, this JEM contains information on many jobs that have not previously appeared in a JEM for electromagnetic fields. Data was also aggregated into 2-digit SOC80 codes which cover broader classification groups in the study population.

Table 1. Distributions of residences By Wire Coding Status San Francisco Bay Area Adult Glioma Study 1991-1995

	Cases (n=492)	Controls (n=462)	All (n=954)
Total number of residences reported	1492	1503	2995
Residences not assigned	627 (42%)	645 (43%)	1272 (42%)
Subject lived at address 8 or more years ago or duplicate	547	551	1098
Out of California	80	94	174
Refusals, bad addresses, house gone (% of total)	169 (11%)	119 (8%)	288 (10%)
Completed (% of total residences)	696 (47%)	739 (49%)	1435 (48%)
Completed/Eligible within 7 year period (%)	697/865 (81%)	739/858 (86%)	1435/1723 (83%)

Table 2. Numbers and Percentages of Cases' and Controls' Residences with EMF Exposure Assessments, San Francisco Bay Area Adult Glioma Study, 1991-1995.

Wire Codes:	**Cases (n=492)**	**Controls (n=462)**
Index residence	484 (98%)	459 (99%)
All residences within 7 years prior to diagnosis or study entry	378 (77%)	375 (81%)
Front door Emdex spot measurements:		
Index residence	468 (95%)	448 (97%)
All residences within 7 years prior to diagnosis or study entry	362 (74%)	359 (78%)

Index residence is the diagnosis residence for cases or the residence at time of interview for controls.

Table 3. Average EMDEX front door spot-measurements in residences according to wire-code, San Francisco Bay Area Adult Glioma Study, 1991-1995.

	Wertheimer-Leeper wire code					Kaune-Savitz wire code		
Front door EMDEX (mG)	Underground (n=333)	VLCC (n=11)	OLCC (n=243)	OHCC (n=222)	VHCC (n=104)	Low (n=384)	Medium (n=342)	High (n=187)
mean ± s.d.	0.6± 0.8	0.3 ± 0.3	0.5 ± 0.6	1.0 ± 1.5	1.3 ± 1.5	0.6 ± 0.7	0.7 ± 1.2	±.4
% of residences with measures > 2 mG	3.6%	0.0%	6.6%	10.8%	17.3%	3.7%	8.2%	15.5%

Table 4. Odds Ratios and 95% Confidence Intervals for Wire Codes and Front Door Spot Measurements of Longest Held Residence, San Francisco Bay Area Adult Glioma Study, 1991-1995.

	Cases	**Controls**	**Odds Ratio**

Wire Code	# (%)	# (%)	95% CIWire Code # (%) # (%) 95% CI
(Wertheimer/Leeper)			
Underground & VLCC	170 (35)	150 (33)	
OLCC	128 (26)	130 (28)	0.9 (0.6-1.2)
OHCC	137 (28)	117 (25)	1.0 (0.7-1.4)
VHCC	52 (11)	63 (14)	0.7 (0.5-1.1)
(Kaune/Savitz)			
Low	239 (49)	235 (51)	
Medium	167 (34)	141 (31)	1.1 (0.9-1.5)
High	81 (16)	84 (18)	0.9 (0.7-1.3)

Front Door	Cases	Controls	Odds Ratio
Emdex Measure	**# (%)**	**# (%)**	**95% CI**
<=1 mG	377 (80)	355 (79)	
– 2 mG	62 (13)	60 (13)	0.97 (0.7-1.4)
– 3 mG	15 (3)	25 (6)	0.6 (0.3-1.1)
>3 mG	20 (4)	11 (2)	1.7 (0.8-3.6)

Odds ratios use lowest category for reference and are unadjusted.Adjustment for age at diagnosis or interview as a continuous variable, gender, race (w/nw), and residence owned versus not owned did not meaningfully alter the results.

Quality Assurance Measures

For the residential exposure assessment a sample of residences was recoded blind to the initial wire-coding. From this effort, we discovered that coding of thick versus thin primaries was problematic. To minimize any interpretative problems that would ensue from this potential source of misclassification, we recoded all wires according to the Kaune-Savitz scheme which does not depend on the thick/thin distinction. Analyses are conducted both for the Wertheimer-Leeper wire-codes and the Kaune-Savitz wire-codes.

Results

Residential Findings: Cases were not more likely than controls to have lived in residences with higher wire-codes than controls during the 7 years prior to diagnosis. Spot measurements also did not consistently or significantly indicate higher residential EMF exposures for cases compared to controls, but there was some suggestion of increased odds for cases in residences with measures above 3 mG. Although the study was not designed with adequate statistical power to detect effects at this high exposure level, since only 4% of cases' residences and 2% of controls' residences had measurements above 3 mG, residential EMF exposures above this level probably would not account for a very high proportion of adult glioma cases. These conclusions are preliminary pending further consideration of pertinent exposure period, other EMF exposure sources, potential effect modification, and additional potential confounders. These additional analyses are underway.

Occupational Exposures: The SOC80 coding system was derived for purposes other than EMF exposure grouping. However, the SOC80 codes appear to give some useful groupings at a 4-digit level. It is expected that the JEM will have a similar matching frequency with the general population as it did with the brain cancer study population. Therefore, the JEM will be useful to estimate EMF exposure for other studies or populations. Greater between

group variability than within group variability indicates the JEM provides reasonably good classification groups. Merging the EMF JEM with occupational histories of subjects is in progress.

Discussion and Contribution to Understanding Biological Effects of EMF

For the residential exposure assessment a sample of residences was recoded blind to the initial wire-coding. From this effort, we discovered that coding of thick versus thin primaries was problematic. To minimize any interpretative problems that would ensue from this potential source of misclassification, we recoded all wires according to the Kaune-Savitz scheme which does not depend on the thick/thin distinction. Analyses are conducted both for the Wertheimer-Leeper wire-codes and the Kaune-Savitz wire-codes.

Publications From This Grant

Wrensch M, Yost M, Miike R, Lee G, Touchstone J. "Adult glioma in relation to residential power frequency electromagnetic field exposures in the San Francisco Bay Area" Epidemiology, Submitted, 1998.

Other EMF Publications

1. Wrensch M, Bondy ML, Wiencke J and Yost M. "Environmental risk factors for primary malignant brain tumors: A review." J Neuro-oncology 17: 47-64, 1993.

The following papers are not directly related to EMF, but examine other risk factors for brain cancer in the study population being studied for EMF issues:

1. Lee M, Wrensch M and Miike R. "Dietary and tobacco risk factors for adult onset glioma in the San Francisco Bay Area (California USA)." Cancer, Causes & Control 8:13-24, 1997.
2. Wrensch M, Lee M, Miike R, Newman B, Barger G, Davis R, Wiencke J and Neuhaus J. "Familial and personal medical history of cancer and nervous system conditions among adult with glioma and controls." American Journal of Epidemiology;145:581-93, 1997.
3. Wrensch M, Weinberg A, Wiencke J, Masters H, Miike R and Lee M. "Does prior infection with varicella zoster virus influence risk of adult glioma?" American Journal of Epidemiology;145:594-7, 1997.
4. Wiencke JK, Wrensch MR, Miike R, Zheng-fa Z and Kelsey KT. "Population-based study of glutathione S transferease mu gene deletion in adult glioma cases and controls." Carcinogenesis;18(2):1431-1433, 1997.
5. Kelsey KT, Wrensch M, Zuo Z-F, Miike R and Wiencke JK. "A population-based case-control study of the CYP2D6 and GSTTT1 polymorphisms and adult glioma." Pharmacogenetics (In press), 1997.

In Preparation:

These two papers also are part of the dissertation of Sue Carozza, accepted at the University of North Carolina, in October, 1997.

1. Carozza S, Wrensch M, Miike R, Newman B, Savitz D and Olshan A. "Occupation and adult gliomas."1997.
2. Carozza S, Wrensch M, Miike R, Newman B, Savitz D and Olshan A. "Summary measures of occupational exposure in case-control studies of cancers: A comparison of lifetime job history with the last or unusual occupation."1997.

TITLE: **EM Fields Induced in the Human Body: A New Approach**

Principal Investigator: Tai Tsun *Wu*, Ph.D.
Harvard University
Cambridge, Massachusetts

Health Relevance: Cancer

Research Area: Calculation Of Induced Fields; and Currents; Exposure Situations; Risk Assessment

R01-ES-08051 **Start Date:** 9/01/96 **End Date:** 8/31/99

Abstract

Since there is biological evidence that exposure to electromagnetic fields (EMFs) may be related to some health effects at the cellular level, it is important to determine the current densities and EMFs induced in the human body in meaningful exposure situations. The objective is to develop a new tool for determining and understanding the currents and fields induced in biologically important organs on both the macroscopic and cellular levels. This involves their dependence on the frequency, magnitude and direction of the incident electromagnetic field. Achieving this objective will aid in the design of experiments by determining for a given EMF-field exposure to a person the EMF exposure causing comparable induced field and current components in animals and *in vitro* experiments.

The specific aims of the research include:

- The identification of exposure situations that permit the analytical determination of the electromagnetic field in which a body is immersed.
- The derivation of accurate formulas that relate the incident EMFs to the currents and fields induced in the exposed body and clearly display their dependence on the frequency, dimensions of the body, and magnitude and direction of the external field.
- The determination of the current density and the electric and magnetic fields in important organs of the body, the associated local field and its action at the cellular level.

During the first year of this grant, significant progress has been made. Correct, quantitatively accurate data on the directions and magnitudes of electric and magnetic fields and current densities in the organs of the human body when exposed to incident electric and magnetic fields have been obtained. The formulas for these quantities in a realistic model of a man with arms in any position provide the essential information needed by biomedical scientists working on this problem. The careful review of erroneous claims that thermal noise generates an electric field in the body that completely masks externally induced fields provides a meaningful assurance to biomedical scientists that their research is essential. Studies in progress to determine the induced electric fields in individual cells embedded in tissue will be continued. These include the spherical cell, spheroidal cell, and elongated cylindrical cell.

Experimental Design and Exposure Conditions

This is a wholly theoretical study, whose approach includes the following steps:

- Apply known accurate formulas for the complete EMF of horizontal and vertical electric dipoles to determine the EMFs generated by power lines, VLF transmitters, shipboard antennas, and cellular telephone transceivers.
- Using techniques developed recently, determine the average fields and currents in the body for the various configurations. For example, at low frequencies (50 Hz to 10 MHz), the body behaves like an electrically short receiving antenna, and the axial (circulating) current is due primarily to the electric (magnetic) field.
- Selected cross sections of the body containing biologically important organs act as conductances in parallel carrying a known axial current. With the known conductivities and dimensions of the organs, determine the current density and electric field in each organ.
- Study the effects of these at the cellular level and relate to *in vitro* measurements.

Quality Assurance Measures

The analytical results obtained will be compared with numerical and experimental results in the literature where these are available. Further numerical checks can be carried out if deemed necessary.

Results

During the past year, attention has been directed primarily to two areas that are well characterized by the titles of three papers written during this period. one of these has just been published, the other two have been accepted for publication.

- "Fields and Currents in the Organs of the Human Body when Exposed to Power Lines and VLF Transmitters" (IEEE Trans. Biomed. Eng. 45, 520-530, 1998) ; and "The interaction of Power-Line Electromagnetic Fields with the Human Body: An Examination of the Underlying Physical Principles" (IEEE Eng. in Med. & Biol. Mag., In press, 1998). The first of these papers reviews the extensive recent analytical studies of induced electric fields and currents that have appeared in mathematically oriented journals like the Journal of Applied Physics and Radio Science and relates the results to clinical and laboratory work. The purpose is to interpret the analytical results for the biologically oriented reader. The second paper is a critical review of recent, widely publicized technical, semi-technical and newspaper articles that reach some misleading, inaccurate, or incorrect conclusions. The topics examined are natural sources of exposure, exposure to 60-Hz electromagnetic fields and the calculation of induced currents and fields in the human body, thermal noise in the body, epidemiological studies, and the electric and magnetic fields in houses.

- "The Electric Field Induced in Celle in the Human Body When This is Exposed to Low-Frequency Electric Fields" (Physical Review E., In press, 1998). our analytical research began with the determination of electric currents and fields in the human body as a homogeneous conductor, first with arms in contact with the sides, then with arms raised. Next, the analysis was extended to determine the electric currents and fields in the individual organs of the body. Our attention has now advanced to the cellular level at which laboratory studies suggest a significant interaction between induced electric fields and cellular function can occur. The analysis determines the electric field in the interior of spherical and long nerve cells as well as in the enclosing membrane when the body is exposed to a 60-Hz axial electric field. Significant is the fact that, due entirely to the difference in geometry, the membrane of a spherical cell is an effective shield, whereas the membrane of a long nerve cell is not. At the same time, the field in the *membrane* of a spherical cell is found to be enormously greater than in its interior. Comparison with *in vitro* experiments on the effects of ELF fields on the cell membrane suggests that a 60-Hz electric field (400-1600 V/m) which induces in the body a field Ez-1 mV/m that acts on the cells is large enough to have an effect on signal transduction, cell division, and cell proliferation.

Discussion and Contribution to Understanding Biological Effects of EMF

The research carried out during the past year has led to simple analytical formulas that relate an incident electromagnetic field to the currents and fields induced in the organs of the human body with and without extended arms, both near to and far from the surface of the earth. It is these induced currents and electric fields that must be used by biomedical research workers to determine their effect on living cells. The formulas are accurate when the body is electrically very short (i.e., for wavelengths much longer than the body's dimensions), and clearly show their dependence on all variables and parameters.

A review of related work has led to the following conclusions: a) Wooden houses do not shield the interior from 60-Hz electric or magnetic fields. b) The attempt to determine the electric field in the body from a boundary condition with the normal component of the incident field alone in air is not valid. c) The assumption that only the magnetic field can enter the body, that the electric field is excluded, is incorrect and leads to the omission of the largest components of the electric field and current in the body. d) A new accurate basis for correct scaling with respect to frequency and dimensions has been provided. e) There is no electric thermal noise field in the human body. f) A review of laboratory and clinical studies of *in vitro* exposure of living tissue to electromagnetic fields and of dependable epidemiological studies leads to tentative safe limits on the magnitudes of induced 50û60 Hz electric fields in the organs of the body. When the same limits are applied to VLF fields, the conclusion is reached that if people living within 50 m of 220- or 440-kV power lines are at risk, those living within 46 km of a high-power VLF trans-mitter may also be at risk.

With regard to how these results may contribute to the overall goals of the RAPID Program, the following comment is offered. For low frequencies, it has been established that it is the electric field, not the magnetic field, that is potentially hazardous to health. In order to assess this potential danger, it is essential to determine the magnitude of the electric field that penetrates into the body, so that biologists can determine its possible effects on the various organs.

Clearly, more research is needed. Correctly focused research has the potential to discover not only whether the effects at the cellular level of induced ELF and VLF fields are harmful, but also to learn more about how life depends on electric fields and currents, e.g., across cell walls and along nerve axons.

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2. King RWP, “The interactioof power-line electromagnetic fields with the human body: An examination of the underlying physical principles, “ IEEE Engineering in Medicine and Biology Magazine, accepted for publication, 1998.
3. King RWP and Wu TT. “The electric field induced in cells in the human body when this is exposed to low-frequency electric fields.” Phys. Rev. E, accepted for publication 1998.
4. King RWP. “Electric field induced in a spherical cell in the human bocy when this is exposed to a 50-60-Hz electromagnetic field.” Radio Science, submitted for publication 1998.
5. King RWP and Wu TT. “Shielding by a house from the electric field of a power line.” IEEE Trans. Electromagn. Compatibil. Submitted for publication, 1998
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Other EMF Publications

1. King RWP and Wu TT. “The complete electromagnetic field of a three-phase transmission line over the earth and its interaction with the human body.” J. Appl. Phys. 78 (2): 668-683, 1995

2. King RWP and Sandler SS. “Electric fields and currents induced in organs of the human body when exposed to ELF and VLF electromagnetic fields.” Radio Sci. 31 (5): 1153-1167, 1996

3. King RWP “Currents and eletric fields induced in the human body when the arms are raised.” J. Appl. Phys 81 (11): 7116-7128, 1997.

TITLE: **60 Hz Magnetic Field -- Clocks and Reproductive Development**

Principal Investigator: Steven M. Yellon, Ph. D.
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Health Relevance: Cancer

Research Area: Calculation of Induced Fields; and Currents; Exposure Situations; Risk Assessment

R01-ES-06137 **Start Date:** 9/01/92 **End Date:** 8/31/95

Abstract

The proposal will determine whether 60 Hz magnetic field exposures have biological effects that are detrimental to reproductive development. The experimental plan, in part, will define the magnetic field exposure conditions that may influence a variety of physiological, endocrine, neural and molecular endpoints that are relevant to the central nervous system control of sexual maturation. The Djungarian hamster will be used as an experimental model because the process of puberty can be induced or arrested by environmental photoperiod. In long days, the critical period for the initiation of reproductive growth (15-25 days of age) is characterized by enhanced gonadotropin hormone secretion and increased numbers of neurons that produce gonadotropin hormone releasing hormone. By contrast, exposure to short days maintains each of these indices at prepubertal levels and blocks the onset of puberty. It is now recognized that these effects of photoperiod are mediated by the circadian rhythm in pineal melatonin production. Moreover, evidence in the Djungarian hamster and a variety of other species indicate that acute magnetic field exposure suppresses the nighttime rise in the melatonin rhythm in the pineal gland and in circulation. If the effect of acute magnetic field exposure on the melatonin rhythm is of physiological significance, then a disruption in the mechanism that conveys photoperiod information will result in an inappropriate neuroendocrine reproductive response to short days. Thus experiments will focus on acute and daily magnetic field exposures on the melatonin rhythm and reproductive development. The objectives are to determine whether exposure to magnetic field alters the biological clock in the suprachiasmatic nucleus of the hypothalamus that generates the melatonin rhythm or affects the production of melatonin by the pineal gland. Studies of the molecular responses to magnetic field treatment include transcriptional activation of immediate early genes. Therefore, the proposal's multi-level approach uses a strong animal model system to test hypotheses about the effects of magnetic field exposure on biological timekeeping and the neural mechanism that controls reproductive development.

Experimental Design and Exposure Conditions

Two identical coil systems were constructed and installed in a room that was 11 x 31 ft. Each coil system was located 5' from an exit door and separated by 14 feet. One coil system will be used for magnetic field exposure while the other for sham control. A countertop and ventilated hood were also present. This room is used only for the magnetic field exposure. Each coil system consists of six separate windings arranged as two modified Merritt coils to produce highly uniform fields along two perpendicular axes. Each axis has three equal-size square windings separated by a distance equal to one-half the length of a coil side. The outer two windings have 130 turns of #16 AWG PVC-insulated wire, and the inner winding has 65 turns. The horizontal windings are 52.3 inches (133cm) square and the vertical axis windings are 48 inches (122 cm) square. The vertical axis windings are nested within the horizontal axis windings. The coils are driven by the 60 Hz mains through a variable autotransformer (Superior Electric Powerstat model QI 16U) and can produce up to two gauss RMS. The magnetic field is uniform to within i 10% over a cubical volume nearly 36~ (90 cm) on a slide. AC magnetic field

measurements were made using a Monitor Industries model 42B-1 AC milligaussmeter and DC field measurements were made using a Bartington MAG-Ol fluxgate magnetometer.

Magnetic field exposure parameters. Previous studies in the adult Djungarian hamster have demonstrated that acute exposure to a 60 Hz magnetic field (1 gauss for 15 in) 2h before lights off, suppressed the duration and delayed the peak in the nocturnal melatonin rhythm. Initially this same acute exposure paradigm will be used in the proposed studies of adults in short days and in juvenile hamsters in long or short days. Other exposure parameters need to be tested in order to determine the physiological significance of the acute magnetic field exposure effect during the critical period for reproductive development. *Time of day variables.* Hamsters in short or long days will be exposed to a 60 Hz MF (1 gauss) for 15 mm at 4 h before lights off or 2 or 4 h after lights off. *Field strength variables.* Hamsters in short or in long days will be exposed to a 60 Hz MF of either 0.1 or 2 gauss for 15 mm at 2 h before lights off. Controls will be simultaneously placed in the adjacent coil system but no current applied. Hamsters (6 in each group per time) will be killed as previously described at 1-3 h intervals to assess effects on the pineal and serum melatonin rhythm.

Replication Protocol

Plans are to repeat those experiments that meet the following criteria: 1) produce major magnetic field exposure effects on the melatonin rhythm or reproduction, 2) serve as a critical foundation for subsequent studies, 3) yield discrepancies in comparison with previous replicates or expected outcomes.

- Magnetic field intensity - 0.1,1 Gauss
- Duration of each MF exposure - 15 minutes
- Number of separate exposure periods - Single exposure
- Circadian time of exposure -4 or 2 h before lights off or 2 or 4 h after lights off
- Field oscillation frequency -60 Hz
- Harmonic content - N/A
- Polarization - linear
- Associated static (earth's) magnetic field - around 2 milliGauss
- Relative orientation of AC magnetic field - vertical
- Associated electric fields - N/A
- Intermittency - Continuous exposure
- Turn-on and turn~ff transients - Fields will be turned on after hamsters are positioned in the coil system and turned off before hamsters are removed. Field spatial homogeneity - uniform, less than 10% variation in volume.
- Field temporal homogeneity - no variations expected.
- Geometry of the cell culture system - N/A
- Geometry and movement of exposed animals - N/A
- Accessory non-EMF exposure - none
- Incidental EMF exposure - none

Results

The proposal was designed to determine whether 60 Hz magnetic fields (MF) had biological effects that interfere with the function and development of the reproductive system. A systematic and replicate investigation was conducted in the Djungarian hamster, a species in which the circadian melatonin rhythm is known to control the neural and endocrine mechanisms that initiate puberty and modulate reproduction. In every study, simultaneous sham-controls consisted of age and sex matched hamsters that were placed in an adjacent coils system but without current. As stated in the Specific Aims, the objectives of the proposal were:

1. To test the hypothesis that exposure to a 60 Hz MF produces biological effects that influence reproductive development.

2. To determine the mechanism through which the 60 Hz MF, acting at a cellular or molecular level, influences neuroendocrine reproductive development.

Progress was made on each of these aims. Various MF exposure paradigms were employed to assess effects on the photoperiod control of reproduction and the circadian melatonin rhythm during the continuum from puberty to senescence. These MF exposures are listed in Table I along with the citation where findings have been published or presented. The investigation has provided solid evidence to support the following conclusions:

- Acute exposure to MF, at dosimetry comparable to that of household appliances, did not interfere with the ability of long or short day lengths to regulate the duration of the nighttime melatonin rhythm in juvenile, adult or aged hamsters.
- In hamsters acutely exposed to a NIF, statistical differences in amplitude of the melatonin rhythm at any one time of night compared to that in sham-controls were, ostensibly, not replicated.
- Variations in amplitude appeared to reflect normal differences in the melatonin rhythm among replicate studies and are not a consequence of MF treatment.
- Acute and daily MF exposures did not produce physiologically significant consequences on reproductive maturation in juvenile hamsters.
- Daily MF exposures did not interfere with the photoperiod control of reproduction in adult or aged hamsters.
- Neither acute nor daily MF exposures interfered with the circadian clock mechanism that generates the melatonin rhythm in pineal gland production or circulation.
- Melatonin rhythms in male and female hamsters were not significantly different.
- Expression of Fos, an immediate early gene, varied in the retina and hypothalamus with respect to day and night; acute MF exposure did not affect Fos message or protein content in the retina or hypothalamus.
- Other molecular markers with relevance to the clock mechanism that generates the melatonin rhythm, i.e., message for S-AG in retina or AVP in the hypothalamus, were not affected by various acute MF exposures in juvenile and adult hamster.

Table 1. Specific Aims were addressed by experiments in which adult, aged or juvenile hamsters in long or short days were exposed, acutely or daily, to a 60 Hz MF of various doses, durations, modes (continous or intermittent), at specific clock times relative to lights off. Findings have been reported as indicated in the List of Publication.

Exp't	Age	Photo-period	MF Trrnt	Dose (G)	Duration (hr)	Mode	Hrs rel. Lts. Off	Pub. #
Exp 1	adult	LD	acute	1	0.25	cont	-2 h	1, 4, 7,10,14
Exp 1	adult	SD	acute	1	0.25	cont	-2 h	2, 7, 14
Exp 1.1	aged	LD	acute	1	0.25	cont	-2 h	16, 18
Exp 1.1	aged	SD	acute	1	0.25	cont	-2 h	16, 18
Exp 2	adult	SD	acute	1	0.25	cont	-4 h	6
Exp 2	adult	SD	acute	0.1	0.25	cont	-4 h	6
Exp 2	adult	SD	acute	1	0.25	cont	+4 h	6
Exp 2	adult	SD	acute	0.1	0.25	cont	+4 h	6
Exp 2.1	adult	DD	acute	1	0.25	cont	-2 h	6
Exp 2.1	adult	DD	dailv	1	0.25	cont	-2 h	6, 14
Exp 2.2	adult	LD	acute	1	0.25	mt	-2 h,	5,17,19
Exp 2.2	adult	LD	acute	1	0.25	mt	-1.25h	5,17,19
Exp 2.2	adult	LD	acute	1	1	mt	-2 h	5,17,19
Exp 3	juv	LD	acute	1	0.25	cont	-2h	3,12,15
Exp 3	juv	SD	acute	1	0.25	cont	-2 h	3,12, 15
Exp 4	juv	LD	acute	1	0.25	cont	-4h	3,12,15
Exp 4	juv	SD	acute	1	0.25	cont	-4h	3,12,15
Exp 4	juv	LD	acute	1	0.25	cont	+2 h	3,12, 15
Exp 4	juv	SD	acute	1	0.25	cont	+2 h	3,12,15
Exp 4	juv	LD	acute	1	0.25	cont	+4 h	***3,12,*** 15
Exp 4	juv	SD	acute	1	0.25	cont	+4 h	3,12,15
Exp 4	juv	LD	acute	0.1	0.25	cont	-2 h	3, 12
Exp 4	juv	SD	acute	0.1	0.25	cont	-2 h	3, 12
Exp 4	juv	LD	acute	0.1	0.25	cont	-4h	3,12
Exp 4	juv	SD	acute	0.1	0.25	cont	-4h	3,12
Exp 4	juv	LD	acute	0.1	0.25	cont	+2h	3,12
Exp 4	juv	SD	acute	0.1	0.25	cont	+2 h	3,12
Exp 4	juv	LD	acute	0.1	0.25	cont	+4 h	3, 12
Exp 4	juv	SD	acute	0.1	0.25	cont	+4 h	3, 12
Exp 5	juv	LD	dailv	1	0.25	cont	-2 h	3, 12,15
Exp 5	juv	SD	dailv	1	0.25	cont	-2h	3,12,15
Exp 6	juv	LD+M	daily	1	0.25	cont	-2 h	3,12,15

RESULTS

Specific Aim I. Experiment 1. The objective of this study was to determine if acute MF exposure suppressed the nighttime melatonin rise such that a long-day melatonin rhythm was induced in hamsters in short days. Duration of the nighttime rise was found to be appropriate to the prevailing photoperiod whether hamsters were exposed to the 1 Gauss 60 Hz MF for 025 mm at 2 h before lights off or sham-treated. Significant differences in amplitude of the nighttime rise were found in MF- compared to sham-controls but possible NIF effects were not replicated at specific clock times when the study was repeated in hamsters in short or long days. All told, the experimental paradigm was performed twice in short-day adults and 5 times in long-day hamsters. In both MF and sham-exposed hamsters, statistically significant differences were found in the amplitude but not duration of the nighttime melatonin rise in pineal gland content and in circulation. Thus, apparently normal variability in the melatonin rhythm between replicate studies may account for statistical differences that have been reported in amplitude of the nocturnal melatonin rhythm. Other important outcomes from this study were that the melatonin rhythms in male and female hamsters were not statistically different, and time of year when a study was performed cannot account for the absence of MF expo sure effects on the pattern of melatonin production or secretion. These findings have been published. Since possible MF exposure effects appeared to be more prevalent in older hamsters, Exp 1.1 tested the hypothesis that MF exposure disrupted the melatonin rhythm in aged hamsters. Findings did not support this hypothesis. Rather the data suggested a lack of effect of acute 1 Gauss 60 Hz MF exposure on the nocturnal melatonin rhythm in aged hamsters (> 17 months of age) whether in long or short days.

Specific Aim 1. Experiment 2. The objective of this study was to determine if the sensitivity of the mechanism controlling the melatonin rhythm to MF exposure is acutely altered in adult hamsters by short days. Crucial MF exposure variables were tested including time of day for the 15 min I Gauss 60 *Hz* MF exposure (2 vs. 4 hr before or after lights off) and dose (1 vs. 0.1 Gauss). Derivatives of this study included Exp 2.1 which tested the hypothesis that photoperiod masked the effect of MF treatment. Thus, hamsters in constant dark (DD) were acutely or daily exposed to MF. In addition, Exp 2.2 tested the hypothesis that the 1 Gauss 60 *Hz* MF exposure mode, i.e., applied continuously for 0.25 hr or intermittently at intervals of I min on then I min off for 0.25 or 1 hr, modulated the nighttime rise on melatonin. The outcome of each of these studies support the same conclusion, MF exposures do not interfere with the circadian timekeeping mechanism that generates the melatonin rhythm in pineal gland production or in circulation (6). Moreover, daily MF treatments did not disrupt the photoperiodic mechanism that arrests reproduction in male or female hamsters (6, 14).

Specific Aim 1. Experiment 3. The absence of an acute or chronic effect of MF exposure(s) on the nocturnal melatonin rhythm in long- or short-day adult hamsters led to consideration of the possibility that the critical period for the initiation of reproduction (15-25 d of age) may be more sensitive to the effects of NIF exposure. To determine if MF exposures influenced the photoperiod control of sexual maturation, LD and SD reared juvenile hamsters were acutely exposed to the 1 Gauss 60 *Hz* MF for 15 min. at 2h before lights off. Findings suggest that juvenile hamster were resistant to the effects of acute MF exposure. Statistically significant differences in melatonin concentrations at specific clock times were found between hamsters that were acutely exposed to MF treatment and sham controls. However, effects were not replicated and comparable statistical differences were evident within the same treatment groups in the replicate study. Moreover, putative MF exposure effects on the melatonin rhythm failed to impact reproductive development in LD males or the arrest of puberty in SD hamsters. These results have been published (3, 12, 15).

Specific Aim I. Experiment 4. Other acute exposure conditions became the focus to test the hypothesis that *MF* treatment affects the nocturnal rise in pineal and circulating melatonin in juvenile hamsters at the onset of puberty. LD and SD reared juvenile hamsters were acutely exposed to a 60 *Hz* MF (0.1 or 1 Gauss) for 15 mm. at -4, -2, +2, or +4 h relative to lights off Melatonin rhythms in pineal gland content and in circulation were the same in MF- and sham-exposed hamsters. A portion of these findings have been presented (13).

Specific Aim 1. Experiment 5. In the absence of a replicable effect of an acute MF exposure on the melatonin rhythm, the effects of daily MF exposures on reproductive development in the juvenile hamster were studied. Evidence clearly indicated that daily exposure to MF did not interfere with the onset or progression of puberty in LD males or the inhibition of reproductive development in SD hamsters (3, 12, *15).*

Specific Aim I. Experiment 6. Daily MF exposure did not hinder the ability of melatonin injections to arrest puberty.

Specific Aim 2. Experiment I. Hamsters from the MF- and sham-exposed groups in Specific Aim I Experiment 3 were recruited to address the hypothesis that cellular expression of the nuclear protein Fos, an immediate early gene marker of enhanced cellular transcription, was increased following acute MF exposure. Basal Fos expression was similarly distributed in MF-treated and sham control hamsters; colocalization of Fos and GriRLI was rare, <10% of all GnRH cell bodies expressed Fos protein. Thus, MF effects on Fos protein expression in hypothalamic and rostral forebrain regions in juvenile hamsters were not found.

Specific Aim 2. Experiment 2. Adult LD and SD hamsters from the MF- and sham-exposed groups in Specific Aim 1 Experiment 1 were recruited to address the hypothesis that Fos message or protein expression was induced by MF exposure. This work was performed by Dr. Sandra Hilliker in Dr. Haggren's laboratory or by Dr. Carlos Molina in the laboratory of Dr. Marino DeLeon. Three approaches were taken to determine whether an acute MF exposure induces a molecular response in the retina or hypothalamus, brain regions that represent components of the photoneuroendocrine system that regulate the pineal gland production of melatonin. A portion of this data has been presented (9, 11, 13). Immediate early gene responses to MF. Hamsters were MF- or sham-exposed and subsequently killed at various times following the acute treatment. The retina (right eye without lens) and hypothalamus (preoptic area to the pituitary stalk) were removed. cfos, S-AG and AVP niRNA levels and FOS protein concentrations were assessed over time and relative to treatment were determined by Northern or Western blot analysis. These genes are known to be regulated by light and are associated with the biological clock in the suprachiasmatic nucleus. GPDH served as a control for basal expression of transcript. Clock-related miRNA in retina and hypothalamus varied several fold over the 10 h study period in MF and sham controls. In consideration of this variability over time, the data failed to suggest that the concentration of message for S-AG in the retina or AVP in the hypothalamus were modulated by acute MF treatment. For c-fos message and FOS protein, as well concentrations were unaffected by MF treatment with respect to sham controls. AP- 1 binding Activity. Densitometric analysis of autoradiograms indicates that AP-1 binding was continuously prevalent throughout the late afternoon and night. Significant differences in AP-1 binding activity in the retina or anterior hypothalamus within MF- or sham-exposed groups or between treatments were not found. MF dose parameters and molecular responses in juveniles. To assess the effects of strength and time of day for MF exposure clock-related genes, the retina and hypothalamus were collected from juveniles in long day during the critical period for puberty (tissue from hamsters in Specific Aim 1 Exp 3). No consistent acute MF-exposure effect was found on the concentrations of cfos message in the retina or hypothalamus or in hypothalamic AVP mRNA content.

Specific Aim 2. Experiment 3. Since daily MF treatments were not found to alter reproductive development in juvenile hamsters, this study was not performed.

Specific Aim 2. Experiment 4. The hypothesis for this study was that daily MF exposures entrain the endogenous biological clock mechanism that generates the circadian melatonin rhythm. Since neither acute nor daily MF exposure (s) affect the melatonin rhythm or Fos expression or reproduction, it was unlikely that MF treatments would produce aftereffects on circadian rhythms in expression of immediate early genes. Thus, in the absence of a solid rationale for this study this specific experiment was not conducted.

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Other EMF Publications

1. Yellon SM and Truong HN. “Melatonin rhythm onset in the adult Siberian hamster: Influence of photoperiod, but not 60-Hz magnetic field exposure on melatonin content in the pineal gland and in circulation.” J Biol. Rhythm 13: (1) 52-59, 1998.
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Title: **Extended Analysis of EMF Exposure Metrics and Melatonin Levels in Utility Workers**

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Health Relevance: Cancer

Research Area: Studies Involving Humans, Epidemiological Research, Melatonin

RO3-ES08877 **Start Date:** 1/10/97 **End Date:** 1/09/99

Abstract

A variety of studies in animals and humans on the potential biological effect of power frequency electric and magnetic field (EMF) suggest that EMF exposures may suppress or alter nocturnal melatonin signaling. Alteration of melatonin signaling, although not necessarily a deleterious effect, could have a role in human disease outcomes and could serve as an important biomarker for EMF efer locations. While in bed at night subjects kept the dosimeter in a pouch located by their side of the bed. In addition, at the end of each work shift most subjects completed a work time-activity log noting various tasks they may have done during the day. From this work activity log an extensive job exposure-task analysis was collected for most electrical workers and a sample of administrative personnel (total of 121 subjects provided task analysis).

Experimental Design and Exposure Conditions

This data comes from a cohort of 192 utility workers studied over a 1 year period in northern Colorado. The study population included both workers expected to have elevated EMF exposures at work, and administrative (office) workers expected to have exposures similar to non-utility occupations. Continuous 24-hour EMF and light personal exposure data was recorded at 15 second intervals for a 5-day period by an EMDEX-C dosimeter; both daytime and night-time urine samples were collected to assess melatonin production during this same period. During data collection, subjects kept a daily time diary of activities, indicating if they were at home, work, in bed, or at othte.

Quality Assurance Measures

Our study aims to extend and perform a more detailed statistical analysis on an existing data set. The original study had a number of QA measures, including: (1) review of each individual data file for gaps and errors; (2) a check of the EMF meter operation prior to and after data collection; (3) review of time logs and meter motion data to check for protocol compliance; and (4) frequent calibration of the EMF meters. We are performing several tests to validate the quality and consistency of the data sets being derived from the original data files. Original files were provided for all subjects. We are recalculating a number summary metrics such as means, variances, and number of observations during time block of home, work and sleep to check for consistency with the original data; we will check that time card records agree with responses and dates from questionnaires and other documents; we will confirm the sample dates and urine volumes recorded on data collection forms. We also are closely examining the light data, which spans several orders of magnitude. Light data was concurrently recorded by the EMF dosimeter during personal monitoring and there is some evidence of non-linearity in the data at very high light intensities. This light data can be corrected by applying separate calibration adjustments derived for specific

combinations of light sensors and dosimeters. Since light is potentially an important conflunder for studies of melatonin production, we will perform analysis both with and without these calibration adjustments to determine the influence on the results.

Results

We have obtained from Drs. Reif and Burch a nearly complete copy of the data set for 192 workers. So far, we have only started coding the existing data for the job and task classification analysis. We have not started the analysis of alternative exposure metrics on the Emdex data files, which first must be converted to another format for processing. We also are adapting the daily time card records so they can be linked to the Emdex files to define residential or workplace exposure conditions. In addition, we are linking the work time-activity logs for 121 subjects to the exposure and melatonin outcome files.

The workers have a wide range of daily exposure to EMF; some exposures are comparable to typical residential values while others have levels > 50 fold higher. Comparison and generation workers show about a 2 fold increase for the TWA magnetic field, and about a 4 fold higher cumulative exposure above 2 mG. In terms of light exposures, the comparison and generation groups have about the same average levels, but the distribution group has levels about 2 fold higher. This reflects different work activities, since distribution workers (e.g. lineman) work more often outdoors while the generation workers spend their time indoors under conditions similar to office workers in the comparison group. In terms of light exposure above 500 lux (a threshold typically sufficient to suppress melatonin production) distribution workers have about a 4 fold higher exposure compared to other groups.

TABLE 1: Average magnetic field (in mG) by occupational group & time category

GROUP	WORK	HOME	OTHER	SLEEP
Comparison	1.5±0.1	1.4±0.1	1.5±0.1	1.04±0.1
Distribution	2.3±0.3*	1.6±0.2	1.6±0.1	1.3±0.2
Generation	3.2±0.6*	1.4±0.2	1.8±0.1*	1.2±0.1

(a) $p<0.05$ vs. Comparison workers

Table 1 Presents comparisons for the 3 occupational groups broken down by time card categories. These categories show that exposure differences between the groups mainly comes from the work and other exposure categories. For work exposures, the generation workers appear most different from the comparison group, while distribution workers appear different in terms of average exposure but otherwise similar to the comparison group. All groups appear similar with regard to home and bed time exposures.

A preliminary analysis was run on the data using repeated measures regression analysis with log transformed exposure data. A diurnal variation in urinary melatonin levels was found for day/night sample comparisons of melatonin metrics (6-OHMS/Cr and 6-OHMS concentration). The night/day ratios fall within the range of values noted in the literature as does the average night time total melatonin production of 17 ug. The effects of light-at-night based on questionnaire responses did not show a significant effect on melatonin, so further adjustment for nocturnal light may be unnecessary. Daytime light exposure seems an important co-variate with an effect on night time melatonin, but is closely linked to season; age shows a slight association and may remain as a co-variate. Nocturnal melatonin concentration (creatinine corrected) appeared to decreased with increasing geometric mean magnetic field exposures during work hours($p<.04$); a similar but weaker trend ($p<0.1$) was observed in association with 24 hour averaged magnetic field exposures. The results to date suggest that individuals with elevated EMF exposures during work may have decreased melatonin levels after accounting for age and light as possible confounders.

We plan to focus our analysis on (a) first completing the job/task coding and conducting a categorical analysis of melatonin levels in association with job duties and tasks (aim 3); (b) extracting the various time-series exposure coherence metrics from the raw Emdex files (aim 1); (c) analyzing these exposure metrics in association with melatonin within daily environments (home work, sleep) and by time of day (aim 2 and 1).

Discussion and Contribution to Understanding Biological Effects of EMF

Birch *et. al* [1] have reported an assiciation between certain EMF exposure conditions and lowered melatonin synthesis in a population of utility workers. This association persisted both for night time (home) and daytime (work) EMF exposures. Our analysis will use this same data set to test the influence of exposure timing and duration on reduction in melatonin biosynthesis, by examining which periods of the 24-hour day and exposure conditions contribute most to this effect. In particular, we will examine if exposure conditions found in the workplace or in the home are most influential. A second aim of our analysis is to test for decreased melatonin levels associated with particular job tasks or occupational groups which may indicate unusual EMF exposures. This analysis will focus on the subset of 121 workers for which job-task data are avaliable. We also will test for an association between melatonin produciton and alternative EMF exposure variables, such as the measures of exposure constancy and intermittency. All analysis will use a multivariate statistical model and consider the possibility of confounding with light, age, and season or other possible covariables.

This work will contribute to improving the understanding of biological effects of EMF by more sharply defining what exposure periods, tasks, and conditions can influence melatonin levels in this human population. This is potentially important for deciding if workplace or residential exposures should be the focus of investigation.

However, the laboratory studies of EMF and melatonin to date have not produced consistent results, perhaps in part because of uncertainty over the type of EMF exposures and timing of exposure that might produce a response. Recently, Reif, Birch, and co-investigators completed data collection in a study of EMF exposure and nocturnal melatonin levels in a population of 192 utility employees in Northern Colorado. Melatonin was assessed by analyzing 6-OHMS levels in urine samples collected over 3 consecutive nights and 3 work days. EMF and light exposures were assessed by personal dosimeters collecting data every 10 seconds for the entire 3 days. Subjects also completed an extensive questionnaire and kept diaries of work and non work activities. In addition, an extensive job exposure-task analysis was collected for most workers. These utility workers have a wide range of daily exposure to EMF; some individuals have exposures comparable to typical residential values, while others have levels more than 50 fold higher. The analysis to date indicates that individuals with elevated exposures to EMF during work have significantly lower melatonin levels after adjusting for confounders.

The aim of this proposal is to extend the analysis of this rich data set in three important ways. First, we will examine alternative exposure metrics such as the temporal coherence of exposure and the intermittence of exposure in relation to nocturnal melatonin. Second, we propose to study the importance of timing of exposures during the day relative to melatonin levels at night. Finally, we will analyze the job-task data to see if particular jobs or tasks show an association with altered melatonin levels, and if so, to try and determine if these tasks have distinctive exposure characteristics.

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None

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FUNDING

Electric and Magnetic Fields Research Projects

by Funding Mechanism

A. Projects Funded Under PUBLIC LAW 102 - 486 (SECTION 2118), RAPID Program

1.) Research Project Grants, R01s

Name	Project #	Total Costs BY 1994	Total Costs FY 1995	Total Costs FY 1996	Total Costs FY 1997	Total Costs FY 1998	Total Project Costs
Aaron	R01-ES07402	$0	$153,318	$179,314	$183,616	$60,257	$576,505
Astumian	R29-ES06620	$108,500	$108,267	$107,100	$107,100	$107,100	$538,067
Balcer-Kubiczek	R01-ES07120	$231,291	$194,936	$225,409	$0	$0	$651,636
Binninger	R01-ES07181	$127,389	$109,356	$134,368	$0	$0	$371,113
Byus	R01-ES06128	$249,418	$151,975	$0	$0	$0	$401,393
Graham	R01-ES07053	$280,953	$295,675	$354,351	$363,633	$0	$1,294,612
Hui	R01-ES07091	$169,090	$146,591	$170,460	$177,151	$0	$663,292
Lai	R01-ES6290	$172,475	$157,686	$186,550	$194,011	$0	$710,722
Liburdy	R01-ES06401	$283,235	$265,806	$282,721	$294,067	$0	$1,125,829
Litovitz	R01-ES06872	$0	$206,025	$177,115	$302,420	$0	$685,560
Luben	R01-ES06410	$234,517	$240,297	$237,530	$248,618	$60,270	$1,021,232
Mandeville	R01-ES07049	$151,027	$109,400	$77,947	$0	$0	$338,374
Marino	R01-ES05928	$0	$223,002	$166,802	$171,767	$0	$561,571
McCormick	R01-ES07093	$360,130	$213,542	$263,675	$274,219	$0	$1,111,566
McLeod	R01-ES06287	$179,306	$150,929	$0	$0	$0	$330,235
Mehta	R29 ES05970	$0	$0	$0	$0	$17,650	$17,650
Miller, R.	R01-ES07124	$226,193	$161,641	$181,449	$187,927	$0	$757,210
Miller, S.	R01-ES07127	$195,597	$198,742	$265,707	$356,786	$0	$1,016,832
Nuccitelli	R01-ES07133	$231,245	$192,863	$235,043	$235,135	$0	$894,286
Reiter	R01-ES07132	$206,102	$168,236	$198,195	$205,952	$0	$778,485
Rinehart	R01-ES07145	$231,340	$185,097	$221,756	$0	$0	$638,193
Saffer	R01-ES07122	$377,646	$323,753	$355,098	$0	$0	$1,056,497
Dibirdik	R01-ES07175	$0	$152,113	$144,617	$0	$147,665	$444,395
Weaver	R01 ES06010	$0	$23,166	$0	$0	$0	$23,166
Williams	R01-ES07076	$383,409	$299,158	$352,322	$359,120	$0	$1,394,009
Woloschak	R01-ES07141	$149,571	$128,001	$159,782	$0	$0	$437,354
Yellon	R01-ES06137	$156,745		$0	$0	$0	$156,745
	Sub-Totals	$4,705,179	$4,559,575	$4,677,311	$3,661,522	$392,942	**$17,996,529**

1.) Research Project Grants, R03s

Principal Investigator	Grant Number	FY 1994	FY 1995	FY 1996	FY 1997	FY 1998	Total
Astumian	R03-ES08913	$0	$0	$0	$76,500	$76,500	$153,000
Balcer-Kubiczek	R03-ES08898	$0	$0	$0	$72,496	$72,496	$144,992
Blank	R03-ES08855	$0	$0	$0	$85,250	$85,250	$170,500
Costa	R03-ES08851	$0	$0	$0	$62,367	$63,650	$126,017
Craviso	R03-ES08903	$0	$0	$0	$72,150	$72,150	$144,300
Glickman	R03-ES08894	$0	$0	$0	$49,029	$89,860	$138,889
Goodman	R03-ES08867	$0	$0	$0	$85,250	$85,250	$170,500
Griffin	R03-ES08864	$0	$0	$0	$89,114	$90,367	$179,481
Grissom	R03-ES08896	$0	$0	$0	$72,122	$74,750	$146,872
Hahn	R03-ES08895	$0	$0	$0	$57,349	$57,595	$114,944
Hei	R03-ES08926	$0	$0	$0	$85,250	$85,250	$170,500
Kripke	R03-ES08930	$0	$0	$0	$70,548	$75,620	$146,168
Lai	R03-ES08865	$0	$0	$0	$72,627	$74,079	$146,706
Liberman	R03 ES08931	$0	$0	$0	$85,248	$85,248	$170,496
Loberg	R03 ES08921	$0	$0	$0	$106,400	$108,529	$214,929
McCormick	R03 ES08854	$0	$0	$0	$106,481	$0	$106,481
McDonald	R03 ES08911	$0	$0	$0	$105,869	$107,975	$213,844
Reiter	R03 ES08890	$0	$0	$0	$59,827	$64,380	$124,207
Sheppard	R03 ES08920	$0	$0	$0	$63,000	$62,962	$125,962
Sisken	R03 ES08878	$0	$0	$0	$72,560	$73,500	$146,060
Ryan	R03 ES08873	$0	$0	$0	$110,209	$0	$110,209
Yost	R03 ES08877	$0	$0	$0	$68,955	$71,099	$140,054
	Sub-totals	$0	$0	$0	$1,728,601	$1,576,510	**$3,305,111**

3.) Research Project Contracts, N01s and Interageny Agreements Y01s

Principal Investigator	Grant Number	FY 1994	FY 1995	FY 1996	FY 1997	FY 1998	Total
Anderson	N01-ES65397			$805,056	$1,003,603	$27,907	$1,836,056
Goldberg	PR702871				$83,639		$83,639
Hardisty	N01-ES35369				$10,430	$1,606	$12,036
Lotz	Y01-ES0032		$117,601	$184,239	$192,588	$83,400	$577,828
McCormick	N01-ES525351	$500,000		$221,327	$198,664		$919,991
Owen	Y01-1ES40272	$79,500	$222,000	$150,000	$131,300	$42,000	$698,000
	Sub-totals	$579,000	$321,601	$1,383,312	$1,609,794	$221,307	**$4,127,550**

4.) Intramural Projects, Z01s

Principal Investigator	Grant Number	Total Costs FY 1994	Total Costs FY 1995	Total Costs FY 1996	Total Costs FY 1997	Total Costs FY 1998	Total
Barrett	Z01-ES23019	$10,000	$120,000	$54,000	$70,000	$22,000	$276,000
Bilski	Z01-ES50138		$10,000				$10,000
Chignell	Z01-ES50136	$2,000	$73,000	$72,000	$76,000	$63,000	$286,000
Harry	Z01-ES21164	-	$6,000	$8,000	$6,000	$11,000	$31,000
Hong	Z01-ES90083			$59,000	$60,000	$15,000	$134,000
McMillian	Z01-ES90067	$15,000	$30,000				$45,000
Murphy	Z01-ES50156	$2,000	$68,000	$68,000	$47,000	$25,000	$210,000
Pennypacker	Z01-ES90066	$15,000	$30,000				$45,000
Portier	Z01-ES90602	-	$54,000	$41,000	$50,000	$80,000	$225,000
Putney	Z01-ES90087	$19,000	$43,000	$40,000	$46,000	-	$148,000
Travlos	Z01-ES21195		$5,000				$5,000
	Sub-totals	$63,000	$439,000	$342,000	$355,000	$216,000	**$1,415,000**

5.) Administration, Communication, Evaluation Activities

Project Leader:	Grant Number	Total Costs FY 1994	Total Costs FY 1995	Total Costs FY 1996	Total Costs FY 1997	Total Costs FY 1998	Total Costs FY1999	Total
Boorman	Administration / Communication	$94,000	$117,000	$113,000*	$154,000	$215,000	$25,000	$543,000
Portier	Evaluation	$0	$0	$155,000	$620000	$1,515,964	$278,100	$2,569,064
	Sub-totals	$94,000	$117,000	$268,000	$582,000	$1,688,000	$303,100	**$3,287,064**

* $8,000 was provided for a conference grant.

Grand Total Section 2118 **$30,131,254**

B. Projects Funded Under PHS Act. Section 301, NIH/NIEHS Research Programs

1.) Research Project Grants, R01s

Investigator	Project #	Total Costs FY 1994	Total Costs FY 1995	Total Costs FY 1996	Total Costs FY 1997	Total Costs FY 1998	Total Project Costs
Craviso	R01-ES07563	$0	$0	$0	$210,247	$198,467	$408,714
Ghandi	R01-ES06292	$0	$139,892				$139,892
Grissom	R01-ES05728	$169,149	$134,034	$0	$0	$0	$303,183
Henderson	R01-ES05735	$309,191	$369,402	$0	$0	$0	$678,593
Hunter	U01-CA62984	$41,667	$0	$0		$0	$41,667
Kirschvink	R01-ES06652	$194,514	$204,856	$0	$0	$0	$399,370
Leske	U01-CA62991	$0	$0	$0	$41,667	$0	$41,667
Liburdy	R01-ES07279	$200,449	$150	$203,115	$53,499	$0	$457,213
Mcleod	R01-ES07803	$0	$0	$0	$250,898	$260,058	$510,956
Mehta	R29-ES05970	$87,705	$103,425	$106,902	$0	$0	$298,032
Peters	R01-ES06912	$176,249	$147,904	$153,818	$303,334	$465,369	$1,246,674
Reif	R01-ES08117	$0	$0	$284,330	$276,893	$0	$561,223
Weaver	R01-ES06010	$86,637	$0	$0	$0	$0	$86,637
Wrensch	R01-ES06285	$201,547	$201,451	$205,590	$0	$0	$608,588
Wu	R01-ES08051	$0	$0	$0	$121,824	$125,357	$247,181
	Sub-totals	$1,467,108	$1,161,222	$953,755	$1,258,362	$1,049,251	**$5,889,698**

2. Research Project Contracts, N01s

Investigator	Project #	Total Costs FY 1994	Total Costs FY 1995	Total Costs FY 1996	Total Costs FY 1997	Total Costs FY 1998	Total Project Costs
McCormick*	N01-ES25351	$6,370,800	$ 1,000,000	$ 700,000	$ 1,048,500		$8,562,786
* FY1994 includes $2,147,741 in FY1992 and $708,159 in FY1993.							
Hardisty	N01-ES35369			$19,000	$17,345	$22,101	$58,446
				Total Funding for Program			**$44,583,738**