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Effects of 60 Hz electromagnetic field exposure on APP695 transcription levels in differentiating human neuroblastoma cells

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Abstract

Epidemiological studies have suggested that workers with primary occupation that are likely to have resulted in the medium-to-high extremely low frequency (ELF) electromagnetic field (EMF) exposure are at increased risk of Alzheimer's disease (AD) pathogenesis. As a first step in investigating the possibility of an association between the ELF-EMF exposure and AD at the cellular level, we have used the differentiating IMR-32 neuroblastoma cells. In double-blind experiments, IMR-32 cells were exposed to the magnetic field intensities of 50, 100, and 200 μT at a frequency of 60 Hz for a period of 4 h at the three ages of differentiation (2, 10, and 16 days after incubation in differentiation medium). We used a custom-made Helmholtz coil setup driven by a 60-Hz sinusoidal signal from a function generator and an in-house built power amplifier. Total RNA extracted from the exposed cells was separated by the agarose gel electrophoresis and transferred to a nylon membrane for the northern hybridization. Digoxygenin-labeled APP695 RNA probes were used to detect changes in the APP695 mRNA levels in response to the ELF-EMF exposure. The results reported herein provided no support for any relationship between the APP695 gene transcription and IMR-32 differentiation age, as well as the magnetic field exposure. This study constitutes the first step towards investigating the possibility of an association between the ELF-EMF exposure and AD manifestations at the cellular level.

Keywords: ELF-EMF; Alzheimer's disease; Helmholtz coil; IMR-32

1. Introduction

There is a significant interest in the biological effects of the power frequency (60 Hz) electromagnetic fields (EMF). Health professionals, government administrators and regulators, scientists and engineers, and the general public are interested in this health issue. The focus of research in this area at the cellular level is to identify cellular responses to EMFs, to develop a dose threshold for such interactions and use such information to formulate and test the appropriate interaction mechanisms. Numerous studies have been undertaken during the past two decades to examine the biological effects in the cells exposed to the extremely low frequency (ELF)-EMFs, and the major interest has been to decipher the biological mechanism and site of interaction [1,2]. Several studies have demonstrated the possibility that a mechanism of interaction of the magnetic fields is through a direct reaction with DNA rather than through the generally accepted signal transduction cascade [3]. In these conditions, the cell is responding to magnetic field exposure in a manner analogous to that observed under the conditions of cellular stress, such as an increase in the transcripts for some heat shock genes [4,5]. Early studies in which different cells were exposed to EMFs pointed towards the general changes in the gene transcription [6–10] but did not address the more important issue of which specific genes were affected [11]. Specific mRNA level measurements in response to the ELF-EMF exposure showed an increase in the levels of histone H3 and p53 mRNA [12], IGF-II [13], histone H2B, v-*myc* [14], c-*fos* [15], and c-*myc* [16]. However, it is important to note that these experiments have been difficult to replicate [17–20].

In the epidemiological studies, it has been shown that the workers with primary occupations that are likely to have resulted in the medium-to-high ELF-EMF exposure are at an increased risk of Alzheimer's disease (AD) [21,22]. Alzheimer's disease is one of the most serious

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health problems in the US and its impact increases as the percentage of the elderly continues to increase. The etiology of the brain lesions associated with AD appears to be multifactorial [23]. Factors that are possible components in the etiologic process are either genetic [24–28], environmental [29–33], or related to aging [34,35]. AD is thought by many to be intimately, if not causatively, associated with the deposition of the short β-amyloid (Aβ) peptides in the cerebral cortex and hippocampus of affected individuals [36]. These Aβ peptides are liberated from the β-amyloid precursor proteins (APPs) after the cleavage of APPs in the membrane by the β- and γ-secretase enzyme [37]. Among the three major APPs, the APP695 isoform is predominantly expressed in the nervous tissue.

The concept that the ELF-EMF exposure might contribute to the AD pathogenesis merits attention [38]. As a first step in investigating the possibility of an association between the ELF-EMF exposure and AD at the cellular level, we have used the differentiating IMR-32 neuroblastoma cells. When differentiated, IMR-32 cells mimic large projection neurons of the human cerebral cortex. IMR-32 cells are of human origin, large in size, and have previously been used in studies related to the stability of APP [39]. Also, under certain tissue culture conditions, these cells have been shown to form intracellular fibrillary material [40], commonly observed in the brains of patients affected with AD. We reported herein on the APP695 gene transcription after exposure of the differentiating IMR-32 cells to ELF-EMF at intensities of 50, 100, and 200 μT.

2. Materials and methods

2.1. Cell lines, reagents, and culture conditions

Freely dividing IMR-32 cells obtained from the American Type Culture Collection (Rockville, MD, USA) were routinely cultured in 25-cm^2 flasks (Costar, Cambridge, MA) in 10-ml medium in 95% air and 5% CO_2 atmosphere at 37 °C and fed (i.e., refreshed medium) at 2-day intervals. The growth medium comprised of the Eagle Minimum Essential Medium with 2 mM L-glutamine and Earle's Balanced Salt Solution (BSS) adjusted to contain 1.5 g/l sodium bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate, 10% heat-inactivated fetal bovine serum (FBS) [41]. When confluent, the cells were detached by washing with trypsin solution preheated to 37 °C. The suspension was centrifuged ($250 \times g$, 10 min) and the cells were replated at 1×10^6 cells per 60-mm plate. For the induction of differentiation, cells were plated 2 days prior to the addition of the differentiation medium. The differentiation medium comprised of the Eagle Minimum Essential Medium with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/l sodium bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate, 5% heat-inactivated FBS and 10 μM Bromo-deoxyuridine. The differentiation medium was refreshed once every 2 days.

2.2. Magnetic field exposure system

Details of the magnetic field exposure system are published elsewhere [42]. Briefly, the magnetic field was generated by a pair of symmetric Helmholtz coils, custom-manufactured in the laboratory. The coils have an inner diameter of 2.75 in. (to accommodate 60-mm petri dishes) and an outer diameter of 3.25 in. The vertical distance between the coils is 0.3125 in. The coils were driven by a sinusoidal signal from a function generator (LFG 1300S, Leader) and an in-house built class AB power amplifier. The magnetic field (B) at the center, between the coils, was measured with an F.W. Bell gaussmeter (Model 9550) and probe (Model T-99-253). B was adjusted by varying the coil current. A custom-made blinding switch box was included to eliminate experimenter bias. The switch box is composed of three 8-pole, 6-position rotary switches (Electroswitch, CA; Model #C4D0806N-A). Three front panel switch combinations were used to randomize the exposure, sham, and external coil activations.

2.3. Experimental conditions

The general experimental design involved differentiation age (three levels), magnetic field intensity (three levels), and exposure mode (field-exposed, sham-exposed, and external coil activation).

Based on an earlier study involving the characterization of IMR-32 with the neuron-specific enolase as a differentiation marker [43], three differentiation ages were selected as follows: 2, 10, and 16 days after incubation in differentiation medium. The second day represents the undifferentiated (young) cells, the 10th day represents the differentiating (maturing) cells, and the 16th day represents the fully differentiated (mature) cells. It is important to consider the differentiation age as a factor because previous studies in our laboratory have shown that the vulnerability of the differentiating neuroblastoma cells to the external stimuli is differentiation age-dependent [44].

Based on studies on the potential sources of the ELF-EMFs and exposure levels in the office and domestic environments, the predominant medium values of 0.2–1 μT and the high intermittent values of > 100 μT were stated as effective [45,46]. In this study, the ELF-EMF exposure levels up to 200 μT was considered well within practical limits. The choice of 60-Hz frequency was based on the fact that most exposure studies have focused on the possible adverse effects of the power frequency magnetic fields.

The duration of the magnetic field exposure has been stated as important in the maintenance of the steady state transcript levels [47]. Short-term ELF-EMF exposures (minutes) have been claimed to produce short-lasting responses attributable to the inhibitory effect of the resultant

nonspecific synthesized proteins [16]. Studies addressing the temperature as a stress factor have shown that the time required to cause an effect is reduced logarithmically with increasing temperature [48,49]. Most of these studies have considered the exposure periods of several hours (2–4 h) in order to maintain the transcript levels (e.g., Ref. [50]).

To minimize experimenter bias, a double-blind approach was used. A custom-made blinding switch box was used to expose cells to magnetic fields in an unbiased manner. An example of the blinding switch box setting is shown in Table 1. As revealed, three exposure modes (field-exposed, sham-exposed, and external coil activation) were made possible. After subjecting to different exposure modes, cells were washed twice with 1 ml of HEPES-BSS (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, HEPES 10 mM, D-glucose 10 mM, pH 7.4), and dislodged from the 60-mm dish using a cell scrapper. The cells were then centrifuged using the same buffer and the pellets were snap-frozen in liquid nitrogen and stored at −70 °C for later RNA isolation and processing.

2.4. Isolation and analysis of total RNA

Total RNA was extracted by following the manufacturer's protocol for the monolayer cultures, using the TRIzol™ reagent [51]. The integrity of the extracted RNA was ascertained by the examination of 28S and 18S bands of the ethidium bromide-stained (1.2%) agarose–formaldehyde gels. Samples were considered to be degraded if the 28S band was not more intense than the 18S band. If any sample in a series was degraded, the experiment was repeated.

RNA samples (10–20 μg) were size-fractionated by electrophoresis in 1.2% agarose gels containing 6% formaldehyde and 1 × MOPS (40 V for 20 min; 60 V for 3 h), followed by the capillary blotting overnight from the formaldehyde gel to the nylon membrane (Roche Molecular Biochemicals, Indianapolis, IN). Air-dried membranes were cross-linked by UV irradiation to immobilize the RNA using a UV Stratalinker (Stratagene, La Jolla, CA) on the automatic setting. Membranes pre-hybridized in the Dig-Easy Hyb solution (Roche Molecular Biochemicals) were hybridized at 60 °C with digoxygenin-labeled APP695 and actin RNA probes. Conditions suggested in the kit for the hybridization and washing of membranes were followed (Roche Molecular Biochemicals). Intensity of the APP695 mRNA signal was obtained using the Digoxygenin chemiluminescent detection kit (Roche Molecular Biochemicals).

Table 1
An example of the front panel switch combinations with the corresponding exposure modes[a]

F1	F2	F3[b]	Exposure mode
0	0	0	EXP
0	1	0	SHAM
1	0	0	SHAM
1	1	0	EXP
X[c]	X	1	EXT

[a] Three internal rotary switches designated as RS1, RS2, and RS3 control the "meaning" of the three front panel switches F1, F2, and F3. By changing the RS switch setting, the front panel (F) switch meaning is altered. RS1 and RS2 control the polarity of the magnetic fields that are generated by the two coils to exposure (EXP) [current flowing in the same direction that the magnetic fields from both coils are additive], sham-exposure (SHAM) [current flowing in opposite directions that the magnetic fields from both coils cancel each other], and external coil activation (EXT) [current flowing to an identical coil located more than 1 m away from the biological specimen].

[b] The setting of RS3 determines which one of the three front panel switches is to be the controller of the "external coil/exposure coil" mode (in our combination, F3 is the controller).

[c] X implies that the switch can take either the value of 0 or 1 without affecting the outcome.

2.5. Hybridization probes

In preparation for the northern hybridization experiments, DNA segments corresponding to a segment of APP695 was amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR) reaction using the following pair of primers [52]; forward: 5′ AATACGACTCACTATAGGGAGA-CACCACAGAGTCTGTGGAAG 3′; reverse: 5′ CATACGATTTAGGTGACACTATAGAGGTGTCTCG-AGGTGTCTCGAGATACTTGT 3′; T7 RNA polymerase (underlined) and SP6 RNA polymerase (underlined) sequences were incorporated into the forward and reverse primers, respectively. These primers were synthesized at the Molecular Genetics Facility at the University of Georgia. RT-PCR was then conducted with Titan One tube RT-PCR system (Roche Biosciences) and evaluated by separation in a 1% agarose gel and staining with ethidium bromide.

The parameters for the RT-PCR were as follows: cDNA synthesis at 50 °C for 30 min, denaturation at 94 °C for 2 min; amplification cycle (denaturation at 94 °C for 0.5 min, annealing at 57 °C for 0.5 min, elongation at 68 °C for 0.75–4 min). PCR was run for 25 cycles with cycle elongation of 5 s for each cycle and a final elongation step at 68 °C for 7 min. The PCR product was purified by running the DNA in a 1.5% low melting point agarose gel at 50 V for 2 h and the DNA bands were excised from the agarose purified using a Supelco Wizard® minicolumn. The authenticity of the purified DNA was then verified by the sequence analysis at the Molecular Genetics Facility and digoxygenin-labeled APP695 RNA probes were prepared using the Digoxygenin labeling kit (Roche Biosciences). Genbank analysis showed that there was an overall homology with the existing sequences. Digoxygenin-labeled human actin probes were obtained from Roche Biosciences and were used as internal standards.

2.6. Evaluation of relative transcript levels

Our analysis allowed for the possible treatment-induced variation of the transcript levels based on the magnetic field strengths and differentiation time. In order to determine the

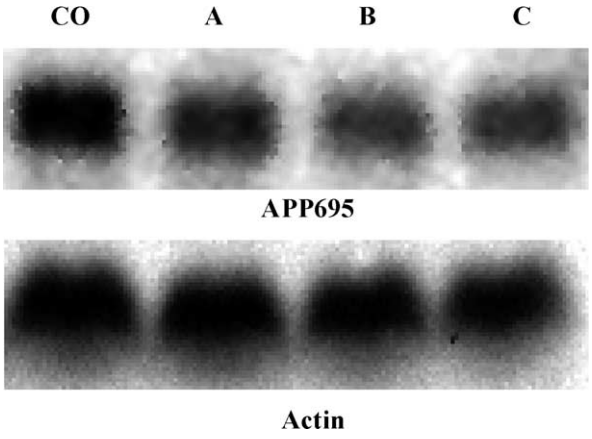

Fig. 1. Northern blots for expression of APP695 in 10-day-old BrdU-differentiated IMR-32 following a 4 h magnetic field exposure at 200 μT. CO = Control; A = Exposed; B = Sham; C = External Coil.

amount of hybrids formed between the APP695 transcript and its probe, the X-ray films were analyzed by densitometry using the Expression 636 (Epson, Long Beach, CA) and the Quantity One, version 2 (BioRad Laboratories, Hercules, CA). Measurements of APP695 transcript levels were expressed as the ratio of APP695 to actin (internal standard) for each experimental condition. Actin was used as an internal standard based on the earlier studies showing no changes in the actin transcript levels in response to the magnetic field exposure [53]. A typical example of a Northern blot output that is used for the processing and analysis is shown in Fig. 1. The top row shows the levels of the APP695 mRNA elicited by the hybridization with the APP695 RNA probe while the bottom row shows the levels of the actin mRNA elicited by the hybridization with the digoxygenin-labeled human actin RNA probe.

The data were represented as the ratio of the experimental (E) signal to the control (C) signal [13]. The relative signal (Rel. S) is a measure of the quantity according to the equation

$$\text{Rel. S} = (E/C) - 1 \quad (1)$$

where E/C is the mean ratio of the experimental (exposed/sham/external coil) to control the band density. Rel. S = 0 means no effect while the Rel. S is positive or negative for increased or decreased mRNA signal levels, respectively. All the treatments of the replicate ratios were examined with a two-tailed t-test to test the hypothesis that the ratio (E/C) is equal to unity.

3. Results and discussion

APP695 transcription level ratios, Rel. S, Eq. (1), for a magnetic field intensity of 100 μT as a function of the differentiation age are shown in Fig. 2. The largest variation among all the experimental conditions was a 23% decrease of the APP695 relative signal, observed on the 10th day of differentiation. In all the cases, there were no statistically significant ELF-EMF effects among the three experimental (exposed, sham, and external coil) and control conditions at the three culture ages of differentiation. The results also showed that there was no change in APP695 transcription level with differentiation age. APP695 transcription level ratios for all the experimental conditions in 16-day old differentiated IMR-32 cells as a function of magnetic field strengths are shown in Fig. 3. There were no statistically significant ELF-EMF effects between the three experimental (exposed, sham, and external coil) and control conditions at

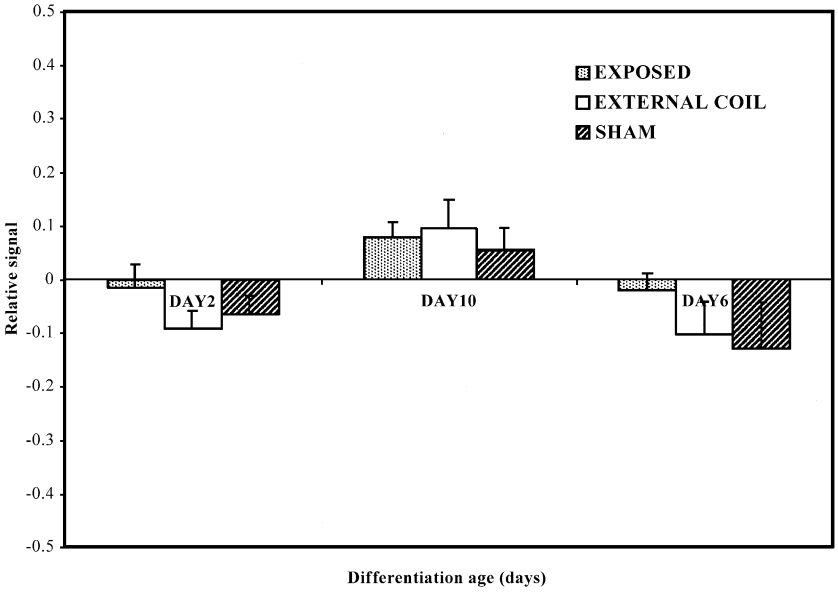

Fig. 2. Mean expression of APP695 relative transcription (n = 3) in BrdU-differentiated IMR-32 cells following 60 Hz magnetic field exposure (100 μT, 4 h).

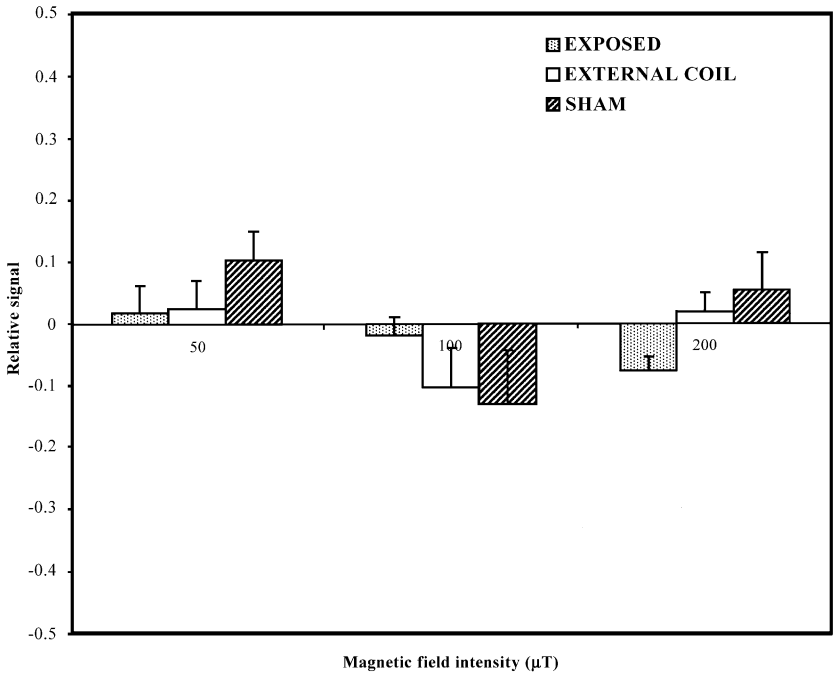

Fig. 3. Mean expression of APP695 relative transcription ($n = 3$) in 16-day-old BrdU-differentiated IMR-32 cells following 60 Hz magnetic field exposure (4 h).

the three different magnetic field intensities, suggesting no change in the APP695 transcription levels with increased magnetic field strength.

Table 2 summarizes 27 different *t*-tests. Each block in Table 1 represents a test of the null hypothesis H_o: the mean APP695 relative transcription levels for the respective experimental condition is equal to the value of unity (mean of the APP695 relative transcription level for the respective control condition) versus the alternative hypothesis H_1: the mean APP695 relative transcription levels for the respective experimental condition is not equal to the value of unity; *p*-values for each test are reported. A *p*-value greater than 0.05 indicated that the relative transcription levels for the experimental condition was not different from the control condition. Inspection of all the conditions show that all the 27 tests did not reject the null hypothesis at the 5% level, suggesting that there was no statistical difference between the experimental and control conditions at different magnetic field intensities and ages of differentiation.

The results reported herein provided no support for any relationship between the APP695 gene transcription and differentiation age, as well as the magnetic field exposure. This is in contrast with a study in which ELF-EMF blocked the differentiation of the erythroleukemia cells [54]. It

Table 2
Statistical comparison of the control (unexposed) and exposed IMR-32 human neuroblastoma APP695 relative transcription levels

Day 2			Day 10			Day 16		
EXP	SHAM	EXT	EXP	SHAM	EXT	EXP	SHAM	EXT
50 μT								
0.2613[a] (do not reject H_o)	0.1469 (do not reject H_o)	0.5466 (do not reject H_o)	0.3557 (do not reject H_o)	0.8595 (do not reject H_o)	0.9749 (do not reject H_o)	0.7289 (do not reject H_o)	0.5540 (do not reject H_o)	0.1458 (do not reject H_o)
100 μT								
0.7636 (do not reject H_o)	0.1968 (do not reject H_o)	0.1165 (do not reject H_o)	0.1140 (do not reject H_o)	0.3234 (do not reject H_o)	0.2179 (do not reject H_o)	0.5639 (do not reject H_o)	0.2660 (do not reject H_o)	0.2391 (do not reject H_o)
200 μT								
0.6842 (do not reject H_o)	0.6441 (do not reject H_o)	0.7298 (do not reject H_o)	0.2694 (do not reject H_o)	0.2471 (do not reject H_o)	0.4226 (do not reject H_o)	0.0735 (do not reject H_o)	0.4709 (do not reject H_o)	0.6446 (do not reject H_o)

EXP — field-exposed; SHAM — sham-exposed; EXT — external coil activation.

[a] *p*-values for *t*-test (*p*-value > 0.05 = do not reject H_o).

should be pointed out that the IMR-32 phenotype used in this study was differentiated with respect to the morphological and biochemical but not the electrophysiological end points [42].

Our data constituted the first step towards investigating the possibility of an association between the ELF-EMF exposure and AD manifestations at the cellular level. Absence of ELF-EMF effect on APP695 gene transcription levels provides incentives to explore the ELF-EMF effects on other factors implicated in the AD pathogenesis. For example, it would be useful to consider the effects of ELF-EMF exposure on the enzyme activities of β- and γ-secretases [55]. Previous studies have shown that a possible interaction mechanism is an electrochemical model that involves alteration in the enzyme activities that involve the ELF-EMF field induced changes [56–58]. Results from previous studies have suggested that the ELF-EMF fields could interfere or enhance enzyme activation by affecting the ion concentration available to the enzyme [59,60].

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References

[1] M. Blank, R. Goodman, Do electromagnetic fields interact directly with DNA? Bioelectromagnetics 18 (1997) 111.
[2] T.S. Tenforde, Biological interaction of extremely low frequency electromagnetic fields, in: S. Ueno (Ed.), Biological Effects of Electric and Magnetic Fields, Plenum, New York, 1996, p. 23.
[3] M. Blank, R. Goodman, Electromagnetic fields may act directly on DNA, J. Cell. Biochem. 75 (1999) 369.
[4] R. Goodman, D. Weisbrot, A. Uluc, A.S. Henderson, Transcription in *Drosophila melanogaster* salivary gland cells is altered following exposure to low frequency electromagnetic fields: analysis of chromosome 3R, Bioelectromagnetics 13 (1992) 111.
[5] H. Lin, M. Opler, M. Head, M. Blank, R. Goodman, Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells, J. Cell. Biochem. 66 (1997) 482.
[6] R. Goodman, C.A.L. Bassett, A.S. Henderson, Pulsing electromagnetic fields induce cellular transcription, Science 220 (1983) 1283.
[7] J.L. Phillips, L. McChesney, Effect of 72 Hz pulsed magnetic field exposure on macromolecular synthesis in CCRF-CEM cells, Cancer Biochem. Biophys. 12 (1991) 1.
[8] R. Goodman, A.S. Henderson, Some biological effects of electromagnetic fields, Bioelectrochem. Bioenerg. 15 (1986) 39.
[9] R. Goodman, A.S. Henderson, Sine waves enhance cellular transcription, Bioelectromagnetics 7 (1986) 23.
[10] R. Goodman, A.S. Henderson, Stimulation of RNA synthesis in the salivary gland cells of *Sciara coprophilia* by an electromagnetic signal used for treatment of skeletal problems in horses, J. Bioelectr. 6 (1987) 37.
[11] J.L. Phillips, Effects of electromagnetic field exposure on gene transcription, J. Cell. Biochem. 51 (1993) 381.
[12] M. Cantini, A. Cossarizza, F. Bersani, R. Cadossi, G. Ceccherelli, R. Tenconi, C. Gatti, R. Franceschi, Enhancing effect of low frequency pulsed electromagnetic fields on lectin induced human lymphocyte proliferation, J. Bioelectr. 5 (1986) 91.
[13] R.J. Fitzsimmons, D.D. Strong, S. Mohan, D.J. Baylink, Low-amplitude, low-frequency electric field-stimulated bone cell proliferation may in part be mediated by increased IGF-II release, J. Cell. Physiol. 150 (1992) 84.
[14] R. Goodman, L.-X. Wei, J.C. Xu, A. Henderson, Exposure of human cells to low-frequency electromagnetic fields results in quantitative changes in transcripts, Biochim. Biophys. Acta 1009 (1989) 216.
[15] S. Rao, A.S. Henderson, Regulation of c-*fos* is affected by electromagnetic fields, J. Cell. Biochem. 63 (1996) 358.
[16] H. Lin, M. Blank, M. Jin, R. Goodman, Electromagnetic field stimulation of biosynthesis: changes in c-*myc* transcript levels during continuous and intermittent exposures, Bioelectrochem. Bioenerg. 39 (1996) 215.
[17] D. Krause, W.J. Skowronski, J.M. Mullins, R.M. Nardone, J.J. Greene, Selective enhancement of gene expression by 60 Hz electromagnetic radiation, in: C.T. Brighton, S.R. Pollack (Eds.), Electromagnetics in Biology and Medicine, San Francisco Press, San Francisco, 1991, p. 133.
[18] A. Lacy-Hulbert, R.C. Wilkins, T.R. Hesketh, J.C. Metcalfe, No effect of 60 Hz electromagnetic fields on *MYC* or β-actin expression in human leukemic cells, Radiat. Res. 144 (1995) 9.
[19] J.D. Saffer, S.J. Thurston, Short Exposures to 60 Hz Magnetic Fields do not alter *MYC* expression in HL60 or Daudi cells, Radiat. Res. 144 (1995) 18.
[20] G.H. Harrison, E.K. Balcer-Kubiczek, Z.-M. Shi, Y.-F. Zhang, W.A. McCready, C.C. Davis, Kinetics of gene expression following exposure to 60 Hz, 2 mT magnetic fields in three human cell lines, Bioelectrochem. Bioenerg. 43 (1997) 1.
[21] E. Sobel, Z. Davanipour, R. Sulkava, T. Erkinjuntti, J. Wikstrom, H.W. Henderson, G. Buckwalter, J.D. Bowman, P.-J. Lee, Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease, Am. J. Epidemiol. 142 (1995) 515.
[22] E. Sobel, M. Dunn, Z. Davanipour, Z. Qian, H.C. Chui, Elevated risk of Alzheimer's disease among workers with likely electromagnetic field exposure, Neurology 47 (1996) 1477.
[23] J.P. Blass, Metabolic alterations common in neural and non-neural cells in Alzheimer's disease, Hippocampus 3 (1993) 45.
[24] S.S. Mirra, M.N. Hart, R.D. Terry, Making the diagnosis of Alzheimer's disease: a primer for practicing pathologists, Arch. Pathol. Lab. Med. 117 (1993) 132.
[25] A. Goate, M.C. Chartier-Harlin, M. Mullan, Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease, Nature 349 (1991) 704.
[26] R. Sherrington, E.I. Rogaev, Y. Liang, E.A. Rogaeva, G. Levesque, M. Ikeda, H. Chi, C. Lin, G. Li, K. Holman, Cloning of a gene bearing missense mutations in early onset familial Alzheimer's disease, Nature 375 (1995) 754.
[27] E. Levy-Lahad, W. Wasco, P. Poorkaj, D.M. Romano, J. Oshima, W.H. Pettingell, C.E. Yu, P.D. Jondro, S.D. Schmidt, K. Wang, Candidate gene for the chromosome I familial Alzheimer's disease locus, Science 269 (1995) 973.
[28] C. Van Broeckhoven, Presenilins and Alzheimer's disease, Nat. Genet. 11 (1995) 230.
[29] W.A. Rocca, S. Bonaiuto, A. Lippi, Prevalence of clinically diagnosed Alzheimer's disease and other dementing disorders: a door-to-door survey in Appignano, Macerata Province, Italy, Neurology 40 (1990) 626.
[30] J.D. Birchall, J.S. Chappel, Aluminum, chemical physiology, and Alzheimer's disease, Lancet 2 (1988) 1008.

[31] C.N. Martyn, D.J.P. Barker, C. Osmond, Geographical relation between Alzheimer's disease and aluminum in drinking water, Lancet 1 (1989) 59.
[32] A. Heyman, W.E. Wilkinson, J.A. Stafford, Alzheimer's disease: a study of epidemiological aspects, Ann. Neurol. 15 (1984) 335.
[33] J.A. Edwardson, J.M. Candy, Aluminum and the pathogenesis of senile plaques in Alzheimer's disease, Down's syndrome, and chronic renal dialysis, Ann. Med. 21 (1989) 95.
[34] W.A. Rocca, L.A. Amaducci, B.S. Schenberg, Epidemiology of clinically diagnosed Alzheimer's disease, Ann. Neurol. 19 (1986) 415.
[35] J.P. Blass, E.P. Cherniak, M.E. Weksler, Theories of aging, in: E. Calkins, P.J. Davis, A.B. Ford (Eds.), Practice of Geriatrics, 2nd ed., Saunders, New York, 1992, p. 10.
[36] D.J. Selkoe, The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease, Trends Cell Biol. 8 (1998) 447.
[37] D.J. Selkoe, The genetics and molecular pathology of Alzheimer's disease: roles of amyloid and the presenilins, Neurol. Clin. 18 (2000) 903.
[38] E. Sobel, Z. Davanipour, Electromagnetic field exposure may cause increased production of amyloid beta and eventually lead to Alzheimer's disease, Neurology 47 (1996) 1594.
[39] D.K. Lahiri, The stability of β-amyloid precursor protein in nine different cell types, Biochem. Mol. Biol. Int. 29 (5) (1993) 849.
[40] C.L. Masters, G. Simms, N.A. Weinman, G. Multhap, B.L. McDonald, K. Beyreuther, Amyloid plaque core protein in Alzheimer's disease and down syndrome, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 4245.
[41] D. Neill, D. Hughes, J.A. Edwardson, B.K. Rima, D. Allsop, Human IMR-32 neuroblastoma cells as a model cell line in Alzheimer's disease research, J. Neurosci. 39 (1994) 482.
[42] R.R. Rao, W.S. Kisaalita, Design and evaluation of a dual-purpose experimental system for exposure of mammalian cells to extremely low frequency (ELF) magnetic fields, Bioelectromagnetics (2001) submitted for publication.
[43] R.R. Rao, W.S. Kisaalita, Development of a distinct phenotype of a human neuroblastoma (IMR-32) cell line by chemical control of differentiation, Cytotechnology (2001) submitted for publication.
[44] W.S. Kisaalita, J.M. Bowen, Effect of culture age on the susceptibility of differentiating neuroblastoma cells to retinoid cytotoxicity, Biotechnol. Bioeng. 50 (1996) 580.
[45] P. Breysse, P.S.J. Lees, M.A. McDiarmid, B. Curbow, ELF magnetic field exposures in an office environment, Am. J. Ind. Med. 25 (1994) 177.
[46] A.S. Farag, M.M. Dawoud, S.Z. Selim, T.C. Cheng, A.M. Marcus, D. Penn, Electromagnetic fields in the home, Electr. Power Syst. Res. 45 (1998) 73.
[47] T.A. Litovitz, C.J. Montrose, W. Wang, Dose–response implications of the transient nature of electromagnetic field induced bioeffects: theoretical hypotheses and predictions, Bioelectromagnetics (Suppl. 1) (1992) 237.
[48] J.M. Lary, D.L. Conover, P.H. Johnson, J.A. Burg, Teratogenicity of 27.12 MHz radiation in rats is related to duration of hyperthermic exposure, Bioelectromagnetics 4 (1983) 249.
[49] M.A. Germain, W.S. Webster, M.J. Edwards, Hyperthermia as a teratogen: parameters determining hyperthermia-induced head defects in the rat, Teratology 31 (1985) 265.
[50] D.A. Rodeberg, J.G. Meyer, G.F. Babcock, Heat shock response: presence and effects in burn patient neutrophils, J. Leukocyte Biol. 66 (1999) 773.
[51] Gibco BRL Life Technologies, TRIzol™ Reagent. Doc. 3796, 1999.
[52] T.E. Golde, S. Estus, M. Usiak, L.H. Younkin, S.G. Younkin, Expression of beta amyloid protein precursor mRNAs: recognition of a novel alternatively spliced form and quantitation in Alzheimer's disease using PCR, Neuron 4 (1990) 253.
[53] G.H. Harrison, E.K. Balcer-Kubiczek, Z.-M. Shi, Y.-F. Zhang, W.A. McCready, C.C. Davis, Kinetics of gene expression following exposure to 60 Hz, 2 mT magnetic fields in three human cell lines, Bioelectrochem. Bioenerg. 43 (1997) 1.
[54] G. Chen, B.L. Upham, W. Sun, C.-C. Chang, E.J. Rothwell, K.-M. Chen, H. Yamasaki, J.E. Trosko, Effect of electromagnetic field exposure on chemically induced differentiation of friend erythroleukemia cells, Environ. Health Perspect. 108 (2000) 967.
[55] S.S. Sisodia, Alzheimer's disease: perspective for the new millennium, J. Clin. Invest. 104 (1999) 1169.
[56] M. Blank, L. Soo, Enhancement of cytochrome oxidase activity in 60 Hz magnetic fields, Bioelectrochem. Bioenerg. 45 (1998) 253.
[57] M. Blank, L. Soo, The threshold for Na, K-ATPase stimulation by electromagnetic fields, Bioelectrochem. Bioenerg. 40 (1996) 63.
[58] M. Blank, R. Goodman, An electrochemical model for the stimulation of biosynthesis by external electric fields, Bioelectrochem. Bioenerg. 19 (1988) 569.
[59] M. Blank, L. Soo, The effects of alternating currents on Na, K-ATPase function, Bioelectrochem. Bioenerg. 22 (1989) 313.
[60] M. Blank, L. Soo, The threshold for alternating current inhibition of the Na, K-ATPase, Bioelectromagnetics 13 (1992) 329.