

# Magnetic-Field–Induced DNA Strand Breaks in Brain Cells of the Rat

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In previous research, we found that rats acutely (2 hr) exposed to a 60-Hz sinusoidal magnetic field at intensities of 0.1–0.5 millitesla (mT) showed increases in DNA single- and double-strand breaks in their brain cells. Further research showed that these effects could be blocked by pretreating the rats with the free radical scavengers melatonin and *N-tert-butyl- $\alpha$ -phenylnitron*, suggesting the involvement of free radicals. In the present study, effects of magnetic field exposure on brain cell DNA in the rat were further investigated. Exposure to a 60-Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA single- and double-strand breaks. Prolonging the exposure to 48 hr caused a larger increase. This indicates that the effect is cumulative. In addition, treatment with Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field–induced DNA strand breaks. These data further support a role of free radicals on the effects of magnetic fields. Treatment with the iron chelator deferiprone also blocked the effects of magnetic fields on brain cell DNA, suggesting the involvement of iron. Acute magnetic field exposure increased apoptosis and necrosis of brain cells in the rat. We hypothesize that exposure to a 60-Hz magnetic field initiates an iron-mediated process (e.g., the Fenton reaction) that increases free radical formation in brain cells, leading to DNA strand breaks and cell death. This hypothesis could have an important implication for the possible health effects associated with exposure to extremely low-frequency magnetic fields in the public and occupational environments. *Key words:* apoptosis, DNA strand breaks, free radicals, iron, magnetic field, necrosis. *Environ Health Perspect* 112:687–694 (2004). doi:10.1289/ehp.6355 available via <http://dx.doi.org/> [Online 27 January 2004]

Use of electricity exposes people constantly to low-intensity, extremely low-frequency electromagnetic fields, particularly at the power frequencies of 50 and 60 Hz. In previous research (Lai and Singh 1997a), we found that rats acutely exposed to a 60-Hz sinusoidal magnetic field showed an increase in DNA single- and double-strand breaks in their brain cells as measured by the microgel electrophoresis assay. An increase in DNA single-strand breaks was observed after 2 hr of exposure to the magnetic field at flux density of  $\geq 0.1$  millitesla (mT), whereas an increase in double-strand breaks was observed at  $\geq 0.25$  mT. Using the microgel electrophoresis assay, Ahuja et al. (1997, 1999), Phillips et al. (1997), Svedenstal et al. (1999a, 1999b), and Zmyslony et al. (2000) have also reported an increase in DNA strand breaks in cells after magnetic field exposure. In studies by Ahuja et al. (1997, 1999), an increase in DNA single-strand breaks in human lymphocytes was observed after 1 hr of exposure to a 50-Hz magnetic field at 0.2–2 mT, whereas in the study by Phillips et al. (1997), an increase in single-strand breaks was observed in human Molt-4 cells after 24 hr of exposure to a 60-Hz magnetic field at 0.1 mT. Svedenstal et al. observed an increase in DNA double-strand breaks in brain cells of mice after 32 days of exposure to magnetic fields of 7.5  $\mu$ T (Svedenstal et al. 1999a) and after 14 days of exposure at 0.5 mT (Svedenstal et al. 1999b). Zmyslony et al. (2000) reported an increase in single-strand breaks in rat lymphocytes exposed to a

50-Hz magnetic field at 7 mT in the presence of iron cations. More recently, Ivancsits et al. (2002, 2003a, 2003b) reported an increase in DNA single- and double-strand breaks in human fibroblasts intermittently (5 min on/10 min off) exposed to a 50-Hz magnetic field at 1 mT, whereas continuous exposure produced no significant effect. Because the other studies reporting effects of magnetic fields on DNA were carried out under continuous exposure conditions, the results of Ivancsits et al. (2002, 2003a, 2003b) indicate that the interaction of magnetic fields with DNA is quite complicated and apparently depends on many factors. Furthermore, McNamee et al. (2002) reported no significant effect on DNA strand breaks in cerebellar cells of immature mice exposed continuously to a 60-Hz magnetic field at 1 mT for 2 hr. Miyakoshi et al. (2000) reported that a high-intensity (> 50 mT) 50-Hz magnetic field had no significant effect alone, whereas it potentiated X-ray–induced DNA single-strand breaks in human glioma cells. Thus, effects of magnetic fields on DNA may depend on factors such as the mode of exposure, the type of cells studied, and the intensity and duration of exposure.

In the present study, we further investigated the effect and mechanism of interaction of magnetic field exposure on brain cell DNA in the rat. In a previous experiment (Lai and Singh 1997b), we found that pretreating rats with melatonin and a spin-trap compound (*N-tert-butyl- $\alpha$ -phenylnitron*) blocked the effect of a 60-Hz magnetic field on DNA.

Because melatonin and spin-trap compounds are efficient free-radical scavengers, the data suggest that free radicals play a role in the effect of the magnetic field. In another study (Singh and Lai 1998), we found that acute magnetic field exposure induced the formation of DNA–protein and DNA–DNA cross-links in brain cells of rats, which could be the results of free-radical damage involving iron cations (Altman et al. 1995; Lloyd et al. 1997).

In this study, effects of exposure duration and treatments with the vitamin E analog Trolox (Forrest et al. 1994), the nitric oxide synthase inhibitor 7-nitroindazole (Kalisch et al. 1996; Moore and Bland-Ward 1996), and the iron chelator deferiprone (Fredenburg et al. 1996; Kontoghiorghes 1995) were investigated. In addition, incidences of apoptosis and necrosis in brain cells of rats acutely exposed to a 60-Hz magnetic field were studied.

## Materials and Methods

**Animals.** Male Sprague-Dawley rats (2–3 months old, 250–300 g), purchased from B & K Laboratory (Bellevue, WA), were used in this research. They were housed for at least 24 hr before an experiment in the room in which they would be exposed to magnetic fields. The laboratory was maintained on a 12/12-hr light/dark cycle (light on 0700–1900 hr), at an ambient temperature of 22°C and a relative humidity of 65%. Animals were provided with food and water *ad libitum* in their home cages and during exposure.

**In vivo magnetic field exposure system.** A Helmholtz coil pair system was used to expose rats to a sinusoidal 60-Hz magnetic field. This exposure system has been described in detail previously (Lai et al. 1993). Briefly, a computer program was used to design this Helmholtz coil pair system, which can produce a magnetic field with minimal heating and field variations over the exposure area. Each coil is made of two sets of 40 turns each of #6 wire wound in rectangular loops, with minimum internal dimensions of 0.86  $\times$  0.543 m.

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During construction, epoxy was layered between loops to glue them together. This minimizes vibration noise when the coils are activated. The coils are wound on frames fabricated from wood and aluminum and are therefore completely shielded against emission of electric fields. They are designed with split windings terminated on multiterminal blocks so that they may be wired in various series or parallel combinations for impedance matching and connecting to multichannel or multifrequency sources. It is wired such that a switch can be used to put the coils "in phase," to generate magnetic fields, or in the "bucking mode," in which the two sets of coil are activated in an antiparallel direction (with the same current as in the in-phase condition) to cancel the fields generated by each other. The bucking mode was used as a control condition in our research to control for the possible effects of heat and vibration generated by the coils. By varying the input current to the coils, exposure fields could be set anywhere from the ambient level to the maximum coil designed magnetic field strength of 5.6 mT. With an exposure level set at 1 mT, the heat dissipation from each of the Helmholtz coils is < 8 W of power. The heat generated is efficiently dissipated because of the large surface area of the coils and good ventilation in the exposure room. The magnetic field during exposure was monitored by input current to the Helmholtz coils and measuring the magnetic flux density with an EMDEX II magnetic field survey meter (EnerTech, Campbell, CA). The variation of the magnetic fields within the space between the coils as determined by theoretical calculation and actual measurement was  $\pm 3\%$  of the mean. The ambient magnetic field in our laboratory (i.e., when the power supply to the coils was turned off) was 0.14  $\mu$ T.

We have two similar exposure systems in two separate rooms in our laboratory. Thus, two exposure conditions could be run simultaneously. During exposure, rats were housed in a plastic cage (length, 45 cm; width, 21 cm; height, 22 cm) fitted with a Styrofoam cover. The cage was placed at the center of the space between the coils. During exposure, animals were provided with food and drinking water. Water was put in a plastic bottle fitted with a glass spout inserted through the Styrofoam cover. Up to three animals were exposed in a cage at a time.

**Experimental procedures for effects of magnetic field exposure on DNA strand breaks in brain cells.** Magnetic field exposure at 0.01 mT for 24 and 48 hr. In this experiment, rats were exposed in the Helmholtz coil system for 24 or 48 hr. Controls were exposed at the bucking mode for the same period of time. Immediately after exposure, one rat at a time was anesthetized by placing it in a covered foam box containing dry ice for 65 sec.

(A piece of cardboard was placed on top of the dry ice to prevent its direct contact with the animal.) The rat was then decapitated and its brain was dissected out immediately for DNA strand break assay. To allow time for tissue processing, there was a 5-min time gap between animals.

**Effects of drug treatments.** In drug treatment experiments, there were four treatment groups in each experiment: magnetic field/drug, bucking/drug, magnetic field/drug vehicle, and bucking/drug vehicle. Animals were exposed for 2 hr to the magnetic field at 0.5 mT or exposed to the bucking mode. At 4 hr postexposure, animals were sacrificed as described above and their brains removed for DNA strand break assay. The 2-hr exposure/4-hr waiting schedule was used in our previous studies (Lai and Singh 1997a, 1997b).

The drug treatment schedules were as follows: for Trolox (Sigma Chemical Co., St. Louis, MO), 100 mg/kg/injection, dissolved in 5% (wt/vol) propylene glycol, injected intraperitoneally at a volume of 2 mL/kg at 24 hr before exposure and immediately after exposure; for deferiprone (CP 20 L1, a gift from R.A. Yokel, College of Pharmacy, University of Kentucky, Lexington, KY), 15 mg/kg/injection, dissolved in physiologic saline, injected intraperitoneally at a volume of 1 mL/kg immediately before and after exposure; for 7-nitroindazole (Sigma), 50 mg/kg/injection, dissolved in 5% (wt/vol) propylene glycol, injected intraperitoneally at a volume of 2 mL/kg at 30 min before exposure and immediately after exposure.

Drug-injection controls were similarly injected with an equal volume of the appropriate vehicle. The drugs used in this study are hydrophobic and could easily pass through the blood-brain barrier.

**Assay methods for DNA single- and double-strand breaks.** The microgel electrophoresis assay for DNA single- and double-strand breaks in rat brain cells was carried out as described previously (Lai and Singh 1997b). The technique involves making a microgel on a microscopic slide. The microgel consists of a cell suspension imbedded in low-melting-temperature agarose and phosphate-buffered saline (PBS). The cells are then lysed in the microgel in high salt and detergents, treated with proteinase K, and electrophoresed in a highly alkaline condition for single-strand break determinations and in a neutral condition for double-strand break determinations. The DNA is then stained with a fluorescent dye to allow visual measurement of the extent of DNA migration, an index of DNA damage. This method is more sensitive than other available methods in detecting DNA strand breaks. It can detect DNA single-strand breaks induced by 0.01 Gy of  $\gamma$ -rays (Singh et al. 1995) or 0.032 Gy of X rays (Singh

et al. 1994), and double-strand breaks induced by 0.125 Gy of X rays (Singh and Stephens 1997) in human lymphocytes.

All chemicals used in the assay were purchased from Sigma unless otherwise noted. Immediately after removal from the skull, a brain was immersed in ice-cold PBS (NaCl, 8.01 g/L; KCl, 0.20 g/L;  $\text{Na}_2\text{HPO}_4$ , 1.15 g/L;  $\text{KH}_2\text{PO}_4$ , 0.20 g/L, pH 7.4) containing 200  $\mu$ M *N-tert-butyl- $\alpha$ -phenylnitron*. The brain was quickly washed four times with the PBS to remove most of the red blood cells. A tissue press was used to break up the brain tissue into small pieces ( $\sim 1 \text{ mm}^3$ ) in 5 mL ice-cold PBS (Singh 1998). Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 mL PBS, tissue pieces were dispersed into single-cell suspensions using a P-5000 Pipetman pipette (Rainin Instruments, Oakland, CA). This cell suspension consisted of different types of brain cells. Ten microliters of this cell suspension was mixed with 0.2 mL 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 45°C, and 50  $\mu$ L of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24  $\times$  50 mm rectangular #1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in a cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed and 200  $\mu$ L agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium *N*-lauroyl sarcosinate, 100 mM disodium EDTA, 10 mM Tris base, pH 10) containing 1% Triton X-100.

To measure single-strand DNA breaks, after lysing for 3 hr at 4°C in an ice bath, slides were treated with DNase-free proteinase K (1 mg/mL; Amresco, Solon, OH) in the lysing solution without detergents overnight at 37°C. They were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA) modified so that both ends of each electrode are connected to the power supply. One liter of an electrophoresis buffer [300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide (DMSO), and 10 mM tetrasodium EDTA, pH 13] was gently poured into the assembly to cover the slides to a height of approximately 6.5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0.4 V/cm,  $\sim 250 \text{ mA}$ , for 60 min) and the buffer was recirculated.

At the end of the electrophoresis, slides were removed from the electrophoresis apparatus and immersed in an excess amount of neutralization buffer (1 M ammonium acetate in ethanol, consisting of 5 mL of 10 M ammonium acetate in 45 mL absolute ethanol) in a Coplin jar (two slides per jar) for 30 min.

After neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar for 2 hr followed by 5 min in 70% ethanol and then air dried.

For double-strand breaks, microgel preparation and cell lysis were done as described above. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp., Indianapolis, IN; 10 µg/mL in the lysing solution) for 2 hr and then with proteinase K (1 mg/mL in the lysing solution) overnight at 37°C. They were then placed for 20 min in an electrophoretic buffer (100 mM Tris, 300 mM sodium acetate, and acetic acid at pH 9.0), and then electrophoresed for 1 hr at 0.4 V/cm (~100 mA). The slides were neutralized and dehydrated in 1 M ammonium acetate in absolute ethanol and 70% ethanol and then air dried as described above.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was pre-stained with 50 µL 5% DMSO in 30 mM NaH<sub>2</sub>PO<sub>4</sub> and 5% sucrose, and then stained with 50 µL 1-µM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50 mm coverglass. Slides were examined and analyzed with a Reichert vertical fluorescent microscope (model 2071) equipped with a filter combination for fluorescent isothiocyanate (excitation at 490 nm, emission filter at 515 nm, and dichromic filter at 500 nm). We measured the length of DNA migration by eye with a micrometer mounted in the eyepiece of the microscope. The migration length is defined as the length (in micrometers) from the beginning of the nuclear area to the last three pixels of DNA perpendicular to the direction of migration at the leading edge. It is used as the index of DNA strand breaks.

Two slides were prepared from the brain of each animal: one for assay of DNA single-strand breaks and the other for double-strand breaks. Fifty cells were randomly chosen and scored from each slide. However, cells that showed extensive damage with DNA totally migrated out from the nuclear region were not included in the measurement. These highly damaged cells probably resulted from the tissue and cell processing procedures, and they occurred equally in magnetic-field-exposed and bucking samples. Therefore, from each animal, 50 cells each were scored for single- and double-strand DNA breaks. The average migration length from 50 cells of a slide (an animal) was calculated and used in data analysis.

**Effects of magnetic field exposure on apoptosis and necrosis of brain cells.** In this experiment, rats were exposed to magnetic field (0.5 mT) for 2 hr or to the bucking mode. The method of Singh (2000) was used to study apoptosis and necrosis. This method

has been validated with two other methods of apoptosis measurement (morphologic estimation and DNA ladder pattern) using several known apoptosis inducers (Singh 2000).

At 4 hr postexposure, microgel from brain cells was made and processed as described above for the microgel electrophoresis assay to remove lipid and protein. Instead of electrophoresis, slides were immersed for 10 min in 0.3 M NaOH and 0.2% DMSO to reveal apoptotic and necrotic cells. Then they were immersed in 1 M ammonium acetate in 50% ethanol for 10 min and then in 100% ethanol with 1 mg/mL spermine for 2 hr to fix the DNA in agarose. Slides were then immersed for 5 min in 70% ethanol. Slides were dried at room temperature and, after staining with YOYO-1, observed under a fluorescent microscope for characteristics of apoptosis and necrosis. The percentage of cells undergoing apoptosis and necrosis was scored from each slide.

In general, apoptosis is caused by programmed cleavage of DNA into a unique size of approximately 186 bases and its multiples. After cells are lysed, DNA from apoptotic cells, in alkaline condition, would diffuse in agarose in a wider area than that of normal cells. Because of this diffusion, DNA is lightly stained. Cells in early apoptosis are easily lysed and show a dense, diffuse, lightly stained, and granular DNA. These are easy to observe because of their larger size and diffuse staining characteristics. Cells in late apoptosis show highly condensed chromatin (intensely stained), even after lysis, and diffused DNA around this condensed spot. In general, nuclear DNA outline in apoptotic cells is diffuse and fuzzy. However, necrotic cells appear different from apoptotic cells after lysis and staining. Because of DNA strand breaks at random and at fewer sites, the nuclear DNA outline is sharply defined but occupies significantly larger area compared with normal cells.

The experiment was run under blind condition; that is, the experimenters who prepared the slides and did the DNA strand-break, apoptosis, and necrosis measurements did not know the treatment conditions of the animals from which the slides were prepared.

**Data analysis.** Data from the DNA strand break assay were analyzed by the one-way analysis of variance (ANOVA), and difference between two groups was evaluated by the Newman-Keuls test. Data of apoptosis and necrosis were analyzed by the Mann-Whitney *U*-test. A difference at  $p < 0.05$  was considered statistically significant.

## Results

Effects of 24- and 48-hr exposures to a 0.01-mT, 60-Hz magnetic field on DNA single- and double-strand breaks in rat brain cells are shown in Figures 1–4. Figures 1

and 2 show that magnetic field exposure increased single- and double-strand breaks, respectively, in brain cells. In addition, prolonging the duration of exposure from 24 to 48 hr significantly increased cumulative single- and double-strand breaks in cells: Single-strand breaks:  $F(3,28) = 28.66$ ,  $p < 0.01$ ; 24-hr vs. bucking,  $p < 0.01$ ; 48-hr vs. bucking,  $p < 0.01$ ; 24-hr vs. 48-hr,  $p < 0.01$ . Double-strand breaks:  $F(3,28) = 17.91$ ,  $p < 0.01$ ; 24-hr vs. bucking,  $p < 0.01$ ; 48-hr vs. bucking,  $p < 0.01$ ; 24-hr vs. 48-hr,  $p < 0.05$ . Figures 3 and 4 show, respectively, the distribution of cells according to their migration lengths of single and double DNA strand break measurements. Increase in cells with higher DNA strand breaks (longer DNA migration) shifts the distribution pattern to the right. The distribution patterns support the conclusion above from the data shown in Figures 1 and 2.

Effects of treatment with Trolox on magnetic-field-induced DNA single- and double-strand breaks are presented in Figures 5 and 6, respectively. ANOVA of the data shows significant treatment effect on both types of breaks:  $F(3,28) = 79.61$ ,  $p < 0.001$  for single-strand breaks, and  $F(3,28) = 49.59$ ,  $p < 0.001$  for double-strand breaks. Treatment with Trolox blocked the effects of the magnetic field on DNA strand breaks in brain cells.

Effects of deferiprone treatment are shown in Figures 7 and 8. Deferiprone treatment blocked the magnetic-field-induced increases in single- and double-strand breaks in brain cells [ANOVA shows significant treatment effects:  $F(3,26) = 33.53$ ,  $p < 0.001$  for single-strand breaks;  $F(3,26) = 49.02$ ,  $p < 0.001$  for double-strand breaks].

Similarly, the effects of 7-nitroindazole treatment are shown in Figures 9 and 10. 7-Nitroindazole treatment also blocked magnetic-field-induced increases in single- and double-strand breaks in brain cells [ANOVA shows significant treatment effects:  $F(3,26) = 50.52$ ,  $p < 0.001$  for single-strand breaks;  $F(3,26) = 22.57$ ,  $p < 0.001$  for double-strand breaks].

Data on apoptosis and necrosis of brain cells of rats after exposure to the 60-Hz magnetic field are shown in Table 1. Both apoptosis and necrosis were significantly increased by magnetic field exposure.

## Discussion

Taken together, results from this series of experiments and our previous research show that by prolonging the duration of magnetic field exposure, DNA strand breaks can be observed in brain cells of the rat at a lower flux density. In previous research (Lai and Singh 1997a, 1997b), we found no significant increase in DNA double-strand breaks in brain cells of rats exposed for 2 hr to a 0.1-mT 60-Hz magnetic field. In the present

experiment, a significant increase in double-strand breaks was observed at 0.01 mT after 24 hr of exposure. These data indicate an interaction between intensity and duration of exposure on biologic effects of magnetic fields. More interestingly, a significantly larger increase in DNA single- and double-strand breaks was observed after 48 hr of exposure compared with 24-hr exposure. This suggests a cumulative nature of the effects.

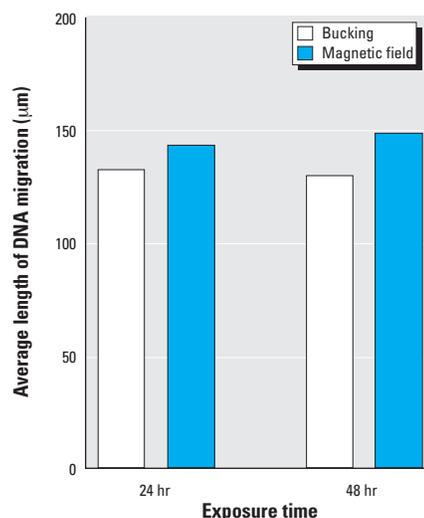
Results from the drug-treatment experiments indicate the following: *a*) Trolox treatment can block the effects of magnetic fields on DNA strand breaks. This further supports

the hypothesis that these effects of magnetic fields are mediated by free radicals, because Trolox is a potent free radical scavenger (Forrest et al. 1994). *b*) Nitric oxide may also be involved in the effects of magnetic fields on DNA (nitric oxide is also a free radical and plays important roles in cell functions). *c*) Data from the deferiprone treatment study suggest that iron may play a role in the effects of magnetic fields. This may also support the free radical hypothesis because iron is closely involved in free radical formation (e.g., via the Fenton reaction) in cells.

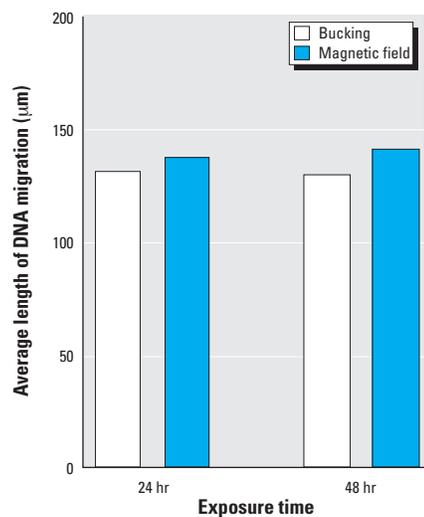
Relevant to our finding that magnetic fields can cause iron-dependent DNA strand breaks is that iron is present in higher concentration in the nucleus than in the cytoplasm because of the presence of an ATPase-related iron pump on the nuclear membrane (Meneghini 1997). Another study has reported iron atoms intercalated in DNA molecules, and DNA-ferrous iron complexes could enhance hydroxy radical formation from hydrogen peroxide compared with ferrous iron alone (Floyd 1981). These make DNA more vulnerable to iron-catalyzed free radical attack.

Increases in apoptosis and necrosis in brain cells of rats exposed to magnetic fields may also be related to free radical formation. Both hydroxy radical and nitric oxide have been shown to cause apoptotic and necrotic cell death, especially in brain cells (Simonian and Coyle 1996). In addition to the present study, others have shown apoptosis in various other cell types after exposure to extremely low-frequency electromagnetic fields (Blumenthal et al. 1997; Ismael et al. 1998; Phillips et al. 1997; Simko et al. 1998; Singh et al. 1994).

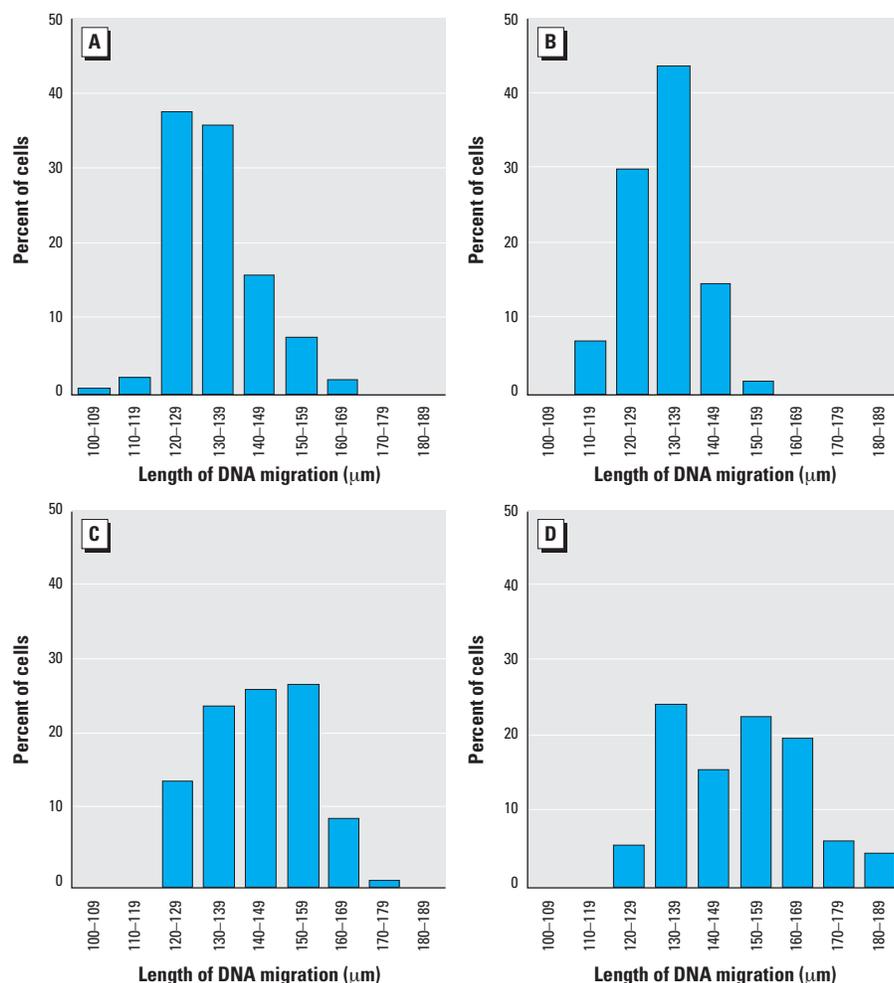
The free radical hypothesis that extremely low-frequency electromagnetic fields increase free radical activity in cells has been proposed by various researchers (Grundler et al. 1992; Reiter 1997). Effects of magnetic fields on cellular kinetics of free radicals (Eveson et al. 2000; Khadir et al. 1999; Roy et al. 1995) and free radical-related cellular processes (Fiorani et al. 1997; Katsir and Parola 1998) have been reported. Free radical-induced damage to DNA could have important effects on health (Beckman and Ames 1997). In addition to DNA damage, free radicals can cause damage in other biologic molecules,



**Figure 1.** Effects of 24 and 48 hr of exposure to a 0.01-mT, 60-Hz magnetic field on DNA single-strand breaks in brain cells of the rat. *n* = 8 for each treatment group. Magnetic field significantly different from sham at *p* < 0.01 for both 24- and 48-hr exposure.



**Figure 2.** Effects of 24 and 48 hr of exposure to a 0.01-mT, 60-Hz magnetic field on DNA double-strand breaks in brain cells of the rat. *n* = 8 for each treatment group. Magnetic field significantly different from sham at *p* < 0.01 for both 24- and 48-hr exposure.



**Figure 3.** Percentage distribution of cells as a function of DNA migration length (single-strand breaks) of the data shown in Figure 1. (A) Bucking, 24 hr. (B) Bucking, 48 hr. (C) 0.01 mT, 24 hr. (D) 0.01 mT, 48 hr.

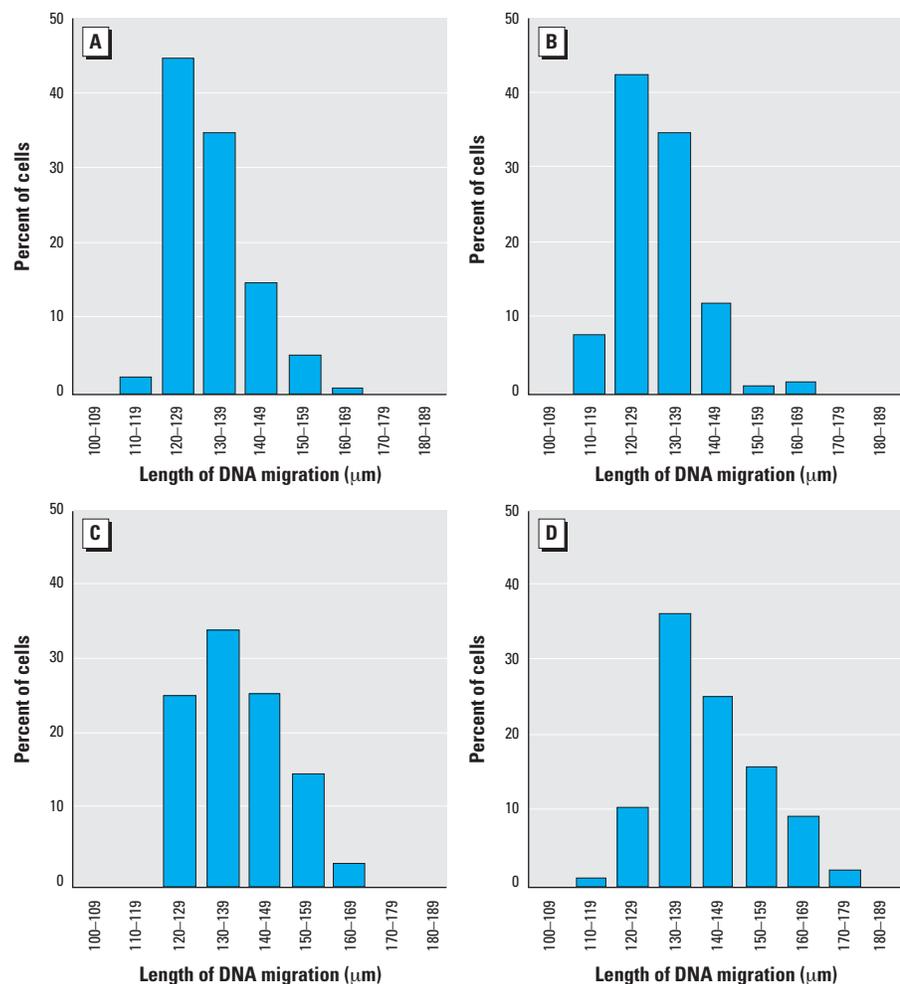
such as lipids and proteins, and can profoundly affect cellular homeostasis. In addition, under subtoxic conditions, free radicals are known to play an important role in cellular signal transduction processes (Suzuki et al. 1997). Disturbance in free radical metabolism could affect these biomolecular processes and cell functions.

Data from the present experiments suggest that magnetic-field-induced DNA strand breaks are caused by an iron-mediated free radical process, probably via the Fenton reaction, which converts hydrogen peroxide to the more potent and toxic hydroxy radical (Figure 11). Iron-induced oxidant formation is known to cause DNA strand breaks, DNA-protein cross-links, and activation of protein kinase C; increase the production of heat-shock proteins; and alter calcium homeostasis in cells (Altman et al. 1995; Farber 1994; Mello Filho and Meneghini 1984; Meneghini 1997; Stohs and Bagchi 1995). Other recent experiments have also implicated the involvement of iron/transition metals in the effects of electromagnetic fields. Zmyslony et al. (2000) reported an increase in DNA strand breaks in

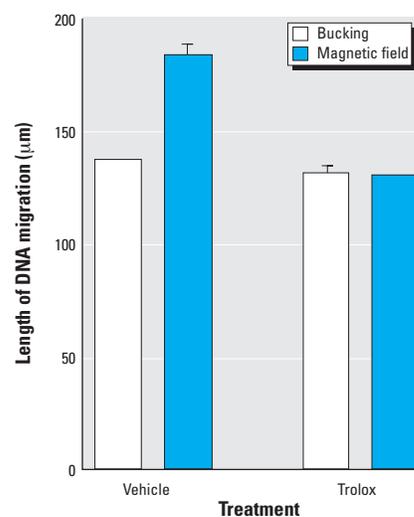
lymphocytes exposed to a 50-Hz magnetic field in the presence of ferrous chloride in the medium, whereas exposing the cells in the absence of ferrous ion had no significant effect. Further experiments from the same group of researchers (Jajte et al. 2001) showed that the effect was blocked by melatonin, suggesting the involvement of free radicals. An experiment by Lourencini da Silva et al. (2000) also implies that electromagnetic fields can cause damage in DNA plasmids in the presence of a transition metal (tin).

Our data show that inhibition of nitric oxide synthase by 7-nitroindazole can completely block the effects of magnetic fields on DNA. We propose that the effects of magnetic fields manifest through a two-stage process. In the first step, magnetic field exposure affects iron homeostasis in certain cells, leading to an increase in free iron in the cytoplasm and nucleus, which in turn leads to an increase in hydroxy radicals, via the catalytic reaction of the Fenton reaction, which damage DNA, lipids, and proteins. Damage to lipids (lipid peroxidation) in the cellular membrane in turn leads to an increase in calcium

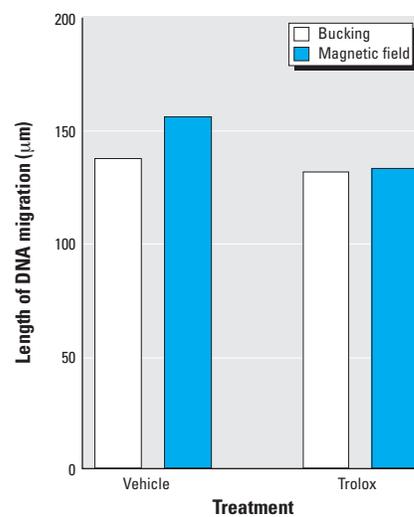
leakage from internal storage sites in the cell. This will trigger the second step, an increase in nitric oxide synthesis via the activation of calmodulin-dependent nitric oxide synthase. 7-Nitroindazole is an effective blocker of that enzyme (Kalisch et al. 1996). Involvement of nitric oxide in the biologic effects of magnetic



**Figure 4.** Percentage distribution of cells as a function of DNA migration length (double-strand breaks) of the data shown in Figure 2. (A) Bucking, 24 hr. (B) Bucking, 48 hr. (C) 0.01 mT, 24 hr. (D) 0.01 mT, 48 hr.



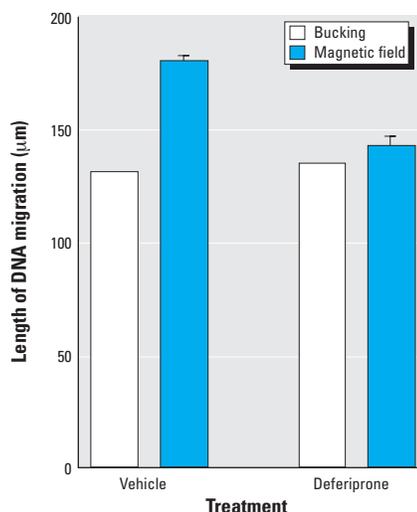
**Figure 5.** Effect of treatment with Trolox on magnetic-field-induced increase in DNA single-strand breaks in rat brain cells (mean ± SE). Trolox (100 mg/kg) was injected intraperitoneally at 24 hr and immediately before exposure to a magnetic field or the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (propylene glycol).  $n = 8$  for each treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in Trolox-treated animals.



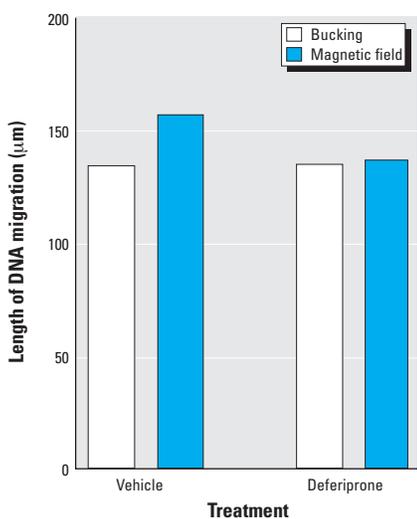
**Figure 6.** Effect of treatment with Trolox on magnetic-field-induced increase in DNA double-strand breaks in rat brain cells (mean ± SE). Treatment conditions were similar to those described for Figure 5.  $n = 8$  for each treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in Trolox-treated animals.

fields has been proposed by Adey (1997) and Yoshikawa et al. (2000).

In the second stage, DNA and other macromolecular damages are probably caused mainly by nitric oxide. Because the hydroxy radical has only a short distance of action

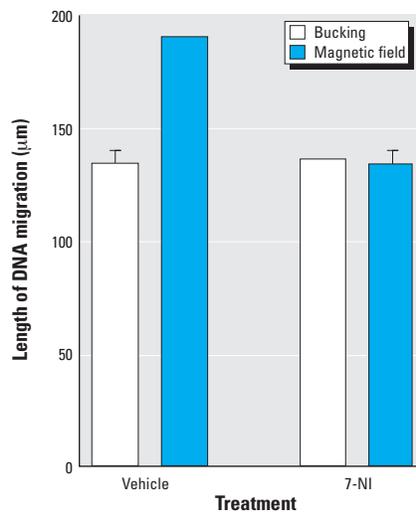


**Figure 7.** Effect of treatment with deferiprone on magnetic-field–induced increase in DNA single-strand breaks in rat brain cells (mean  $\pm$  SE). Deferiprone (5 mg/kg) was injected intraperitoneally immediately before and after exposure to a magnetic field or to the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (physiologic saline).  $n = 8$  for vehicle group, 7 for deferiprone treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in deferiprone-treated animals.

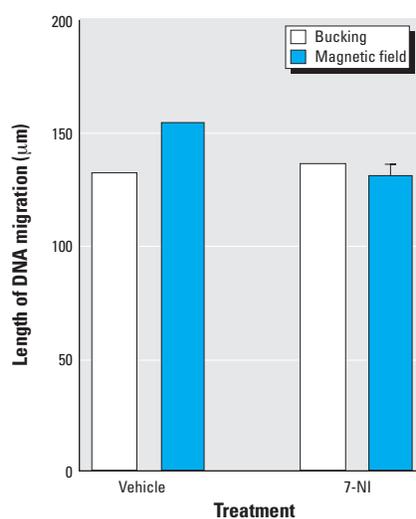


**Figure 8.** Effect of treatment with deferiprone on magnetic-field–induced increase in DNA double-strand breaks in rat brain cells (mean  $\pm$  SE). Treatment conditions were similar to those described for Figure 7.  $n = 8$  for vehicle group, 7 for deferiprone treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in deferiprone-treated animals.

( $\sim 40 \text{ \AA}$ ), whereas nitric oxide can diffuse the distance of several cell diameters, the transition from stage 1 to stage 2 changes the magnetic-field–triggered free radical damage from a localized event to a more widespread phenomenon. Nitric oxide can further amplify



**Figure 9.** Effect of treatment with 7-nitroindazole (7-NI) on magnetic-field–induced increase in DNA single-strand breaks in rat brain cells (mean  $\pm$  SE). 7-NI (50 mg/kg) was injected intraperitoneally at 30 min before and immediately after exposure to a magnetic field or to the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (propylene glycol).  $n = 8$  for each treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in 7NI-treated animals.

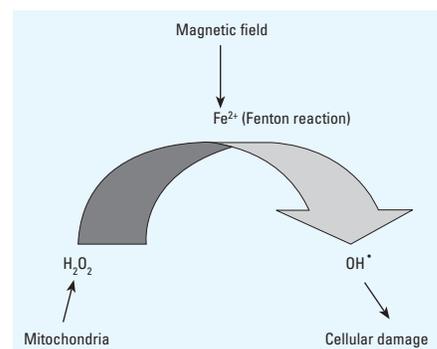


**Figure 10.** Effect of treatment with 7-nitroindazole (7-NI) on magnetic-field–induced increase in DNA double-strand breaks in rat brain cells (mean  $\pm$  SE). Treatment conditions were similar to those described for Figure 9.  $n = 8$  for each treatment group. Magnetic field significantly different from sham at  $p < 0.01$  in vehicle-treated animals. No significant difference between magnetic field and sham in 7NI-treated animals.

iron-mediated free radical formation via its effects on iron metabolism (Richardson and Ponka 1997) and release of iron from ferritin (Reif and Simmons 1990). Thus, the effects will amplify. This damage can lead to two possible outcomes: *a*) Exogenous and endogenous cellular antioxidation processes will keep the damage in check by neutralizing free radicals, and eventually the cell will repair itself and survive. However, DNA damage and repair could lead to mutation and increase the chance of carcinogenesis. *b*) If the processes of free radical damage are not checked by cellular antioxidation and repair processes, the cell will die, because free radical peroxidation of lipids is a chain reaction. Both apoptosis and necrosis are possible. Increase in necrosis is probably a consequence of lipid peroxidative damage in cell membranes, especially that of mitochondria, whereas apoptosis is mainly triggered by DNA damage. The outcome of oxidative damage induced by magnetic fields will therefore depend on various factors, including the oxidative status of the cell, capability of endogenous antioxidation enzymes and processes to counteract free radical buildup, availability of exogenous antioxidants, iron homeostasis (a balance of iron influx, storage, and use), the parameters of exposure (e.g., intensity and duration of exposure and possibly the waveform of the magnetic field), and whether the oxidative damage is cumulative. Oxidative damage to DNA and its subsequent misrepair (i.e., mistakes in repairing the damage) are probably cumulative. To add to this, nitric oxide can be either mutagenic or cytotoxic (i.e., causing cell death) depending on intracellular conditions. It has been suggested that nitric oxide is

**Table 1.** Apoptosis and necrosis of brain cells of rats after exposure to magnetic fields.

	Percent	No.	$p$ -Value
Apoptosis			
Magnetic field	0.61	8	< 0.025
Bucking	0.28	8	
Necrosis			
Magnetic field	1.88	8	< 0.02
Bucking	0.99	8	



**Figure 11.** Schematic diagram of mechanism of effect of magnetic fields involving the Fenton reaction and free radicals.

mutagenic when the intracellular level of reduced glutathione is low, but cytotoxic (leading to apoptosis and inhibition of tumor growth) in a thiol-rich cellular environment that favors the formation of toxic nitrosothiols (Felley-Bosco 1998).

Summing up, we propose that magnetic fields initiate an iron-dependent free radical generation process in cells, which can lead to genotoxic changes and/or cell death. From this hypothesis, one can make the following speculation regarding the biologic effects of magnetic fields: Cells with high rates of iron intake (e.g., proliferating cells, cells infected by virus, and cells with high metabolic rates such as brain cells) would be more susceptible to the effects of magnetic fields because hydrogen peroxide, the substrate of the Fenton reaction, is a metabolic product of mitochondria. This may partially explain the negative results of two previous studies investigating the effect of magnetic fields on DNA. Reese et al. (1988) reported no significant effect of a 60-Hz magnetic field (0.1 and 2 mT for 1 hr) on DNA single-strand breaks in Chinese hamster cells. However, during exposure, in order to decrease DNA repair, their cells were kept under iced conditions. In a study by Fairbairn and O'Neill (1994), cells were first suspended in agarose on a slide before exposure to a 50-Hz pulsed magnetic field (peak flux density, 5 mT; pulse duration, 3 msec). Cells suspended in agarose are not in good physiologic conditions. Thus, in both of these studies, cells studied were probably not in a very active metabolic state.

A question is whether the DNA strand breaks induced by magnetic fields in our studies (Lai and Singh 1997a; present results) are biologically significant. The flux densities (0.01–0.5 mT) used in our studies are within the levels that one could encounter in the environment. Household and office levels of extremely low-frequency magnetic fields can vary from 0.01 to 1  $\mu$ T. Intermittent levels can reach more than 10  $\mu$ T. Levels near a power transmission line can be 10–30  $\mu$ T, whereas the magnetic flux density can vary between 0.1 and 1 mT near some electrical appliances (e.g., electric blankets, hair dryers). Much higher levels are expected in occupational exposures (Bernhardt 1985; Gauger 1984; Krause 1986; Tenforde and Kaune 1987).

To compare with the effect of ionizing radiation, we have exposed rats to 2 Gy of X rays and assayed DNA single-strand breaks in their brain cells. A peak increase of 76% was observed at 30 min after exposure (average length of DNA migration in nonexposed controls =  $133 \pm 2.2 \mu\text{m}$ ; in X-ray-exposed rats =  $234 \pm 2.2 \mu\text{m}$ ;  $n = 3$  in each treatment (unpublished data). It may not be appropriate to compare DNA damage caused by X rays with those by magnetic fields directly, because different

mechanisms may be involved. However, from the data presented in Figures 1 and 2, one can infer that the effect of environmental magnetic fields on DNA is relatively small compared with that of 2 Gy of X rays. It seems that cells may respond differently to high and low levels of DNA damage. A recent report by Rothkamm and Lobrich (2003) indicates a lack of DNA double-strand break repair in nondividing human fibroblasts exposed to very low X-ray doses ( $\sim 1$  mGy). Rothkamm and Lobrich speculated that “instead of repairing a DSB [double-strand break] in a particular cell with the risk of causing genetic alterations, it could be beneficial for an organism to remove the damaged cell and replace it by the division of an undamaged neighboring cell.” However, in the case of neurons that cannot divide and be replaced, such a response could lead to neurodegenerative diseases.

The human brain contains relatively high amount of nonheme iron, mainly in glial cells and myelin. It has been speculated that iron is used in the production and maintenance of myelin by oligodendrocytes (Francois et al. 1981; Gerber and Connor 1989). Thus, myelinated nerve fibers, such as those of motor neurons, could be more susceptible to damage by magnetic fields. Increased risk of neurodegenerative diseases due to magnetic field exposure could be a result of the death of neurons and glial cells or demyelination. Increased risks of amyotrophic lateral sclerosis (Davanipour et al. 1997; Hakansson et al. 2003; Johansen and Olsen 1998; Savitz et al. 1998), Alzheimer's disease (Feychting et al. 2003; Hakansson et al. 2003; Sobel et al. 1995), and Parkinson's disease (Noonan et al. 2002) have been reported in occupational exposure to extremely low-frequency electromagnetic fields.

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