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Acute Exposure to a 60 Hz Magnetic Field Increases DNA Strand Breaks in Rat Brain Cells

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Acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25, and 0.5 mT) caused a dose-dependent increase in DNA strand breaks in brain cells of the animals (assayed by a microgel electrophoresis method at 4 h postexposure). An increase in single-strand DNA breaks was observed after exposure to magnetic fields of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Because DNA strand breaks may affect cellular functions, lead to carcinogenesis and cell death, and be related to onset of neurodegenerative diseases, our data may have important implications for the possible health effects of exposure to 60 Hz magnetic fields. *Bioelectromagnetics 18:156–165, 1997.* © 1997 Wiley-Liss, Inc.

Key words: 60 Hz magnetic fields; DNA single-strand and double-strand breaks; brain cells; microgel electrophoresis

INTRODUCTION

In recent years, both the popular media and the science media have raised concerns about possible health hazards of environmental exposure to extremely-low-frequency (ELF) electromagnetic fields, especially to 50 Hz and 60 Hz [Abelson, 1989; Bridges and Preache, 1981; Brodeur, 1990; Florig, 1992; Morgan et al., 1990; Pool, 1990a–c]. With increased use of electricity and equipment emitting electromagnetic fields, many people are subjected to intermittent and chronic exposure to ELF fields of various intensities and forms. There are speculations that ELF magnetic fields can act as copromoter or promoter of cancer [see reviews in Holmberg, 1995; Loscher and Mevissen, 1994; Wrensch et al., 1993]. Epidemiological studies have suggested that ELF electromagnetic fields may increase the risk of various types of cancer, including leukemia and brain and breast tumors [e.g., Juutilainen et al., 1990; Loomis et al., 1994; Savitz and Loomis, 1995; see also review in Wrensch et al., 1993].

In the present study, we investigated the effect of acute exposure to a 60 Hz magnetic field on DNA strand breaks, a common form of DNA damage, in brain cells of the rat. DNA damage that accumulates in cells over a period of time could be the cause of slow-onset diseases, such as cancer. Indeed, DNA strand breaks have been correlated with carcinogenicity [Ames, 1989a,b; Cerutti, 1985; Tice, 1978], cell death [Onishi et al., 1993; Prigent et al., 1993; Walker et al., 1991; Ward, 1990], aging [Hart and Setlow, 1974; Tice, 1978], and neurodegenerative diseases [Mullaart et al., 1990b; Robins et al., 1983].

The method of Singh et al. [1995] was used in this research to measure DNA single-strand and double-strand breaks in individual brain cells of the rat. The method is one the most sensitive for assaying DNA strand breaks and can be used to evaluate breaks in a single cell. It has been used in numerous studies on DNA damage and genotoxicity [see reviews in Fairbairn et al., 1995; McKelvey-Martin et al., 1993]. The technique involves making a microgel on microscope slides, consisting 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 and in a neutral condition for dou-

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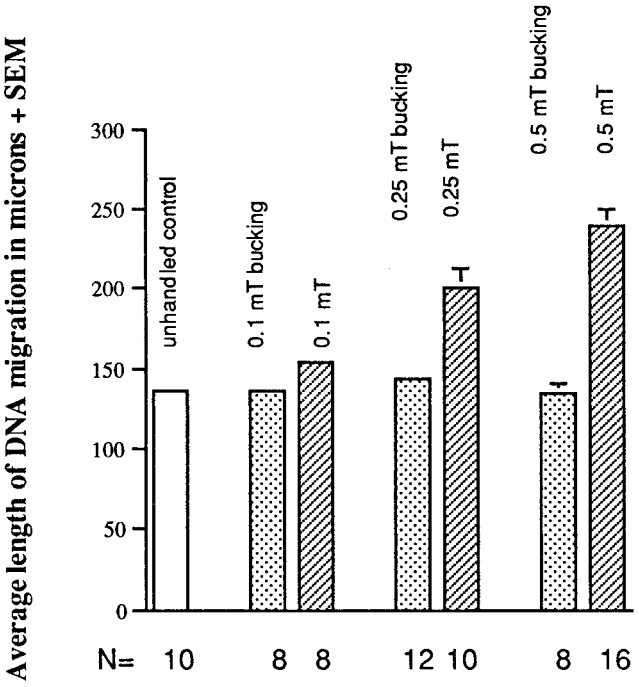

Fig. 1. Levels of DNA single-strand breaks (expressed in length of DNA migration) in the different treatment groups. The average of 0.1 mT magnetic field-exposed group is significantly different from its ''bucking'' control group at $P < .05$ (two-tailed Newman-Keuls test) and averages of 0.25 and 0.5 mT magnetic field-exposed groups are significantly different from their respective bucking controls at $P < .01$. N is the number of animals studied in each condition. Fifty cells from each animal were measured, and the mean migration length of the 50 cells was a data point used in data analysis.

ble-strand break measurements. 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 technique is more sensitive than other available methods in detecting DNA strand breaks. It can detect one break per 2×10^{10} daltons of DNA in lymphocytes induced by X-rays [Singh et al., 1995].

METHODS AND PROCEDURES

Animals and General Experimental Conditions

Male Sprague-Dawley rats (2–3 months old, 250–300 g) purchased from B & K Laboratory were used in this research. They were housed in a vivarium on a 12 h light-dark cycle (lights on 7:00–19:00 h) and were given food and water ad libitum. The experimental environment was kept at 22 °C with a relative humidity of 65%. Animal care was provided by the University of Washington Department of Comparative Medicine, which conforms to the NIH guidelines for the care and use of laboratory animals.

Rats were exposed to a 60 Hz magnetic field in a Helmholtz coil pair. The design and characteristics of this exposure system have been described previously [Lai et al., 1993]. Briefly, each Helmholtz coil is made of 80 turns of No. 6 wire wound in rectangular loops, with minimum internal dimensions of 0.86×0.543 m. During construction, epoxy was layered between loops to glue them together. This minimizes vibration noise when the coils are activated. The coils are completely shielded against emission of electric fields; they are wound on frames fabricated from wood and aluminum. The coils were designed with split windings terminated on multiterminal blocks so that they can be wired in various series or parallel combinations for impedance matching and connecting to multichannel or multifrequency sources. A switch can be used to put the coils ''in phase'' to generate magnetic fields or in the ''bucking mode.'' Because there are two sets of coils in each Helmholtz coil, in the bucking mode they are activated in an antiparallel direction to cancel mutually the fields generated by each of the coils. This condition controls for possible effects of heat and vibration generated when the coils are activated.

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

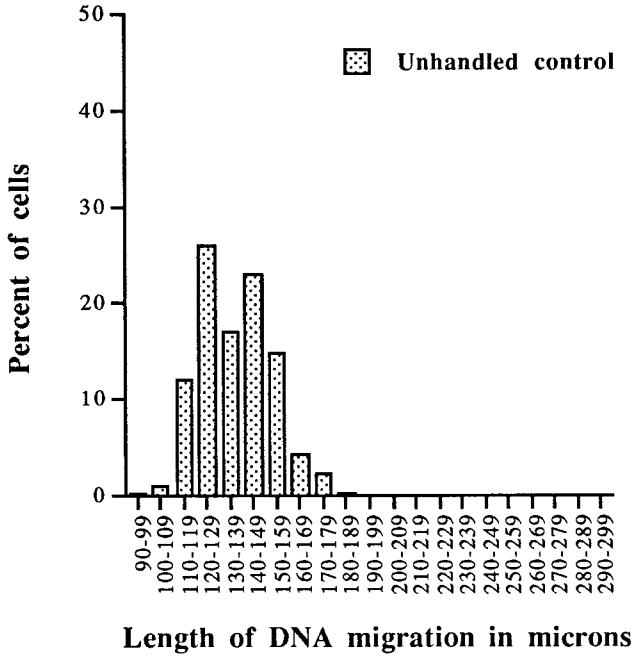

Fig. 2. Percentage distribution of brain cells vs. DNA migration length (single-strand breaks) of unhandled animals (N = 10). Fifty cells were scored from each animal. Therefore, data from the unhandled controls represent the distribution of 500 cells from 10 animals.

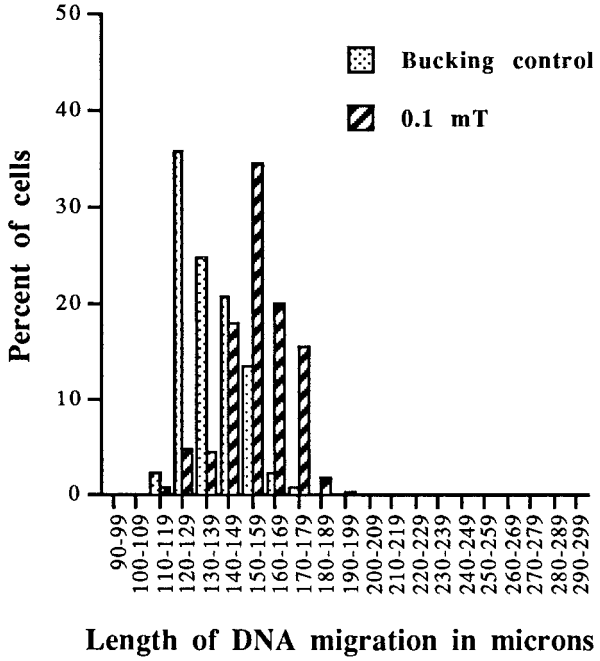

Fig. 3. Percentage distribution of brain cells vs. DNA migration length (single-strand breaks) of rats exposed to a 60 Hz magnetic field of 0.1 mT (N = 8; 400 cells) and rats exposed in the bucking mode (N = 8; 400 cells). Distribution patterns of the two treatments were significantly different from each other (χ^2 = 311.6, df = 7, $P < .001$).

(5.6 mT). With an exposure level set at 1 mT, the heat dissipation for each coil is less than 8 W. The heat generated is efficiently dissipated due to the large surface area of the coil and good ventilation in the exposure room. During exposure, animals were housed in a plastic cage enclosure (length 45 cm, width 21 cm, height 22 cm) placed in the center of the space between the coils. Three animals could be exposed in the cage at the same time. The ambient magnetic field (i.e., when the power supply to the coils is turned off) was 0.14 μT.

Exposure was between 8:00 and 11:00 AM, to control for possible variation in responses due to circadian rhythm. All experiments were run blind; i.e, the experimenters performing the DNA strand break assay did not know the treatment (exposure) conditions of the animals. Controls for these experiments were animals placed for the same period of time in the Helmholtz coil pair activated in the bucking mode with the same electric current. Thus, a group of bucking mode controls was used for each flux density of the magnetic field-exposed experimental group. In addition, DNA strand breaks were also analyzed from brain cells of a group of unhandled rats to control for the possible effect of exposure procedures.

In the experiment, rats were exposed in the Helmholtz coil system to different intensities (flux densities of 0.1, 0.25, and 0.5 mT) of a 60 Hz magnetic field for 2 h. Four hours after exposure, rats were sacrificed and DNA single-strand and double-strand breaks in brain cells were assayed. This experimental schedule was used because in other research we observed an increase in brain cell DNA breaks in rats after 2 h of exposure to microwaves and assayed at 4 h postexposure [Lai and Singh, 1995].

An animal was sacrificed by placing it in a closed foam box containing dry ice (CO_2) for 65 s. (Cardboard was placed on top of the dry ice to prevent its direct contact with the animal.) This method of euthanasia minimizes red blood cell contamination of tissue samples. Rats were then decapitated with a small animal guillotine, and their brains were dissected out immediately. Cells from the brains were isolated for DNA single-strand and double-strand break measurements.

DNA Single-Strand and Double-Strand Break Assays

The microgel electrophoresis assay described by Lai and Singh [1996] was used to study DNA single-strand and double-strand breaks in brain cells. All

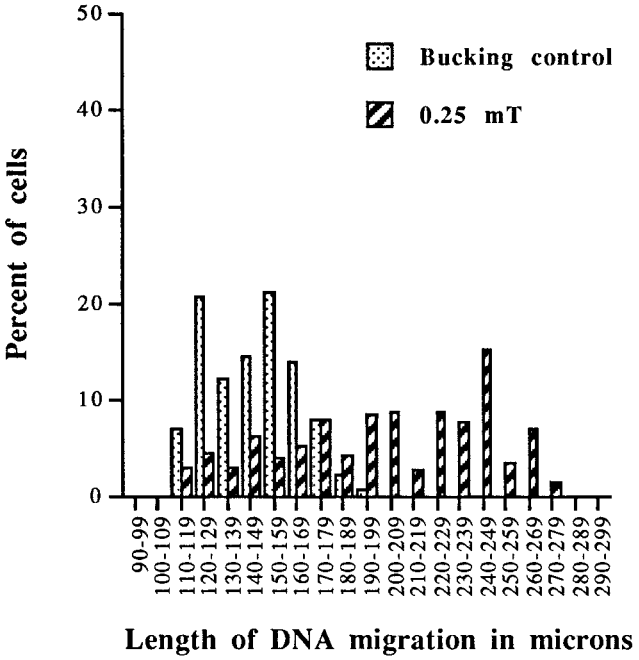

Fig. 4. Percentage distribution of brain cells vs. DNA migration length (single-strand breaks) of rats exposed a 60 Hz magnetic field of 0.25 mT (N = 10; 500 cells) and rats exposed in the bucking mode (N = 12; 600 cells). Distribution patterns of the two treatments were significantly different from each other (χ^2 = 569.9, df = 16, $P < .001$).

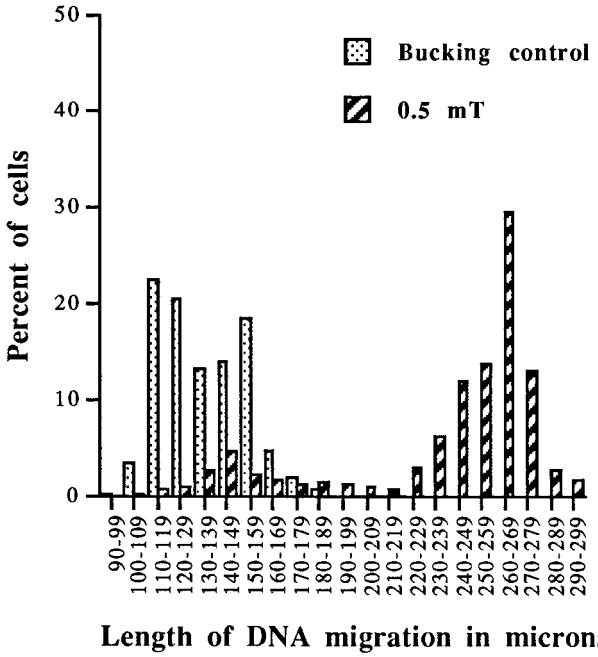

Fig. 5. Percentage distribution of brain cells vs. DNA migration length (single-strand breaks) of rats exposed a 60 Hz magnetic field of 0.5 mT (N = 16; 800 cells) and rats exposed in the bucking mode (N = 8; 400 cells). Distribution patterns of the two treatments were significantly different from each other ($\chi^2 = 851.3$, df = 19, $P < .001$).

chemicals used in the assay were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise noted. All procedures were carried out in minimum indirect light. Immediately after removal from the skull, the brain was immersed in ice-cold PBS (NaCl 8.01 g, KCl 0.20 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.20 g, per liter; pH 7.4) containing 200 mM of N-t-butyl-α-phenylnitrone, a spin-trap compound, and quickly washed four times with the PBS to remove most of the red blood cells. A pair of sharp scissors was used to mince (approximately 200 cuts) the tissue in a 50 ml polypropylene centrifuge tube containing 5 ml of ice-cold PBS to obtain pieces of approximately 1 mm^3. Four more washings with cold PBS removed most of the remaining red blood cells. Finally, tissue pieces were dispersed into single-cell suspensions in 5 ml of PBS by using a 5 ml Pipetman. This cell suspension consisted of different types of brain cells.

Ten microliters of this cell suspension was mixed with 200 μl of 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 37 °C, and 30 μl of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24 × 50 mm square No. 1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed from the slide and 200 μl of agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium N-lauroyl sacosinate, 200 μM disodium EDTA, 10 mM Tris base; pH 10) containing 1% Triton X-100. After overnight lysing at 4 °C, slides were treated with DNAase-free proteinase K (Boehringer Mannheim Corp., Indianapolis, IN) in the lysing solution (pH 7.4, without Triton X-100) for 2 h at 37 °C.

For single-strand DNA breaks, slides were put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA), modified so that both ends of each electrode were connected to the power supply. One liter of an electrophoretic buffer (300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide, 100 mM Tris, and 10 mM tetrasodium EDTA; pH > 13) was gently poured into the assembly to cover the slides to a height of 6.5 mm above their surface. After 20 min, to allow for DNA unwinding, electrophoresis was started (0.4 V/cm, approximately 250 mA, for 60 min); the buffer was recirculated during electrophoresis. At the end of the electrophoresis, excess electrophoretic buffer was removed. The slides were gently removed from the electrophoretic apparatus and immersed for neutralization in 35 ml of 0.4 M Tris (pH 7.4) in a Coplin jar (two slides per jar) for 15 min. After two more similar steps of neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar for 30 min and then dried.

For double-strand DNA breaks, microgel preparation and cell lysis were performed as described above. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp.; 10 μg/ml in the lysing solution without Triton X-100; pH 7.4) at 37 °C for 2 h and then with proteinase K (1 mg/ml in the lysing solution without Triton X-100; pH 7.4) for 2 h at 37 °C. They were then placed for 20 min in an electrophoretic buffer (100 mM Tris, 300 mM sodium acetate and acetic acid; pH 9) and electrophoresed for 1 h at 0.4 V/cm (approximately 100 mA). The slides were treated with 300 mM NaOH for 15 min and neutralized as before with 0.4 M Tris (pH 7.4). Slides were dehydrated as described above for the single-strand break assay.

Staining and DNA migration measurement procedures were similar for both single-strand and double-strand breaks. One slide at a time was stained with 50 μl of 1 mM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50 mm coverglass. Two slides were prepared from each brain sample. One slide was

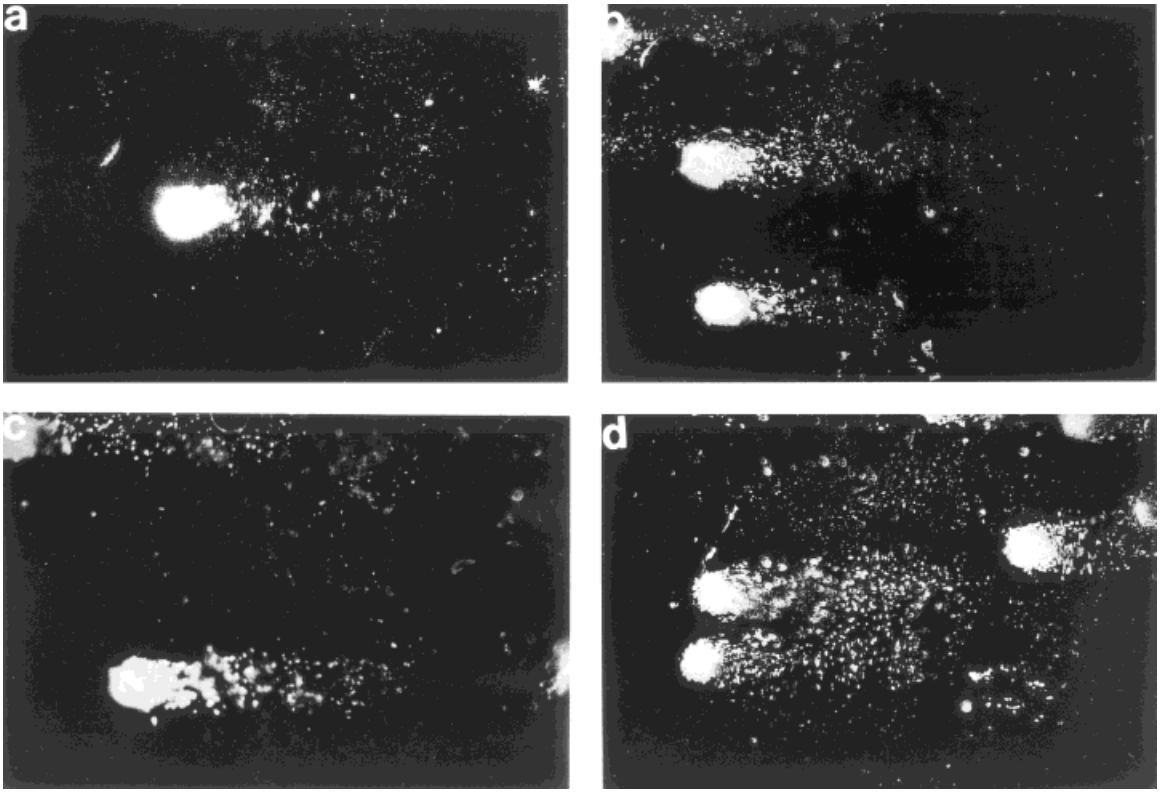

Fig. 6. Photographs of single-strand break DNA migration pattern of individual brain cells from rats exposed to bucking condition (0.1 mT) (**a**) or magnetic fields of 0.1 mT (**b**), 0.25 mT (**c**), and 0.5 mT (**d**). ×400.

assayed for DNA single-strand breaks and the other for double-strand breaks. The length of DNA migration (including the nucleus diameter to the last three particles of DNA visible perpendicular to the direction of migration) of each cell was measured. Fifty representative cells were scored from each slide. Therefore, from each brain sample, 50 cells each were scored for DNA single-strand and double-strand breaks.

Data Analysis

The averaged migration length (in micrometers) of the 50 cells scored from a sample was used as a data point in data analysis. Analysis of variance (ANOVA) followed by the Newman-Keuls test to determine significance of difference between two treatment groups was used in statistical analysis of averaged data. In addition, distribution of cells (in percentage of total) with respect to DNA migration lengths was also plotted. The χ^2 test was used to determine significant difference in patterns of migration between treatment groups. A difference at $P < .05$ was considered statistically significant.

RESULTS

Effects of acute magnetic field exposure on DNA single-strand breaks in brain cells of the rat are presented in Figures 1–6. Figure 1 shows the average length of DNA migration from the various treatment groups. One-way ANOVA of the data showed a significant treatment effect ($F[6,65] = 29.13$, $P < .005$). Data from magnetic field-exposed rats were compared with their respective ''bucking control'' by using the Newman-Keuls test. Data showed a significant increase in DNA single-strand breaks in brain cells of rats after exposure to a 60 Hz magnetic field at 0.1 mT ($P < .05$), 0.25 mT ($P < .01$), and 0.5 mT ($P < .01$). A dose-dependent effect, with longer average migration length at higher flux density of exposure, was observed. Plots of percentage of cells vs. length of DNA migration (in intervals of 10 μm) for each group of animals are presented in Figures 2–5. These data show a shift of the distribution to longer migration lengths as the flux density of the magnetic field increases. Figure 6 shows photographs of the DNA migration pattern (single-strand breaks) of individual brain cells from rats of the different treatment groups.

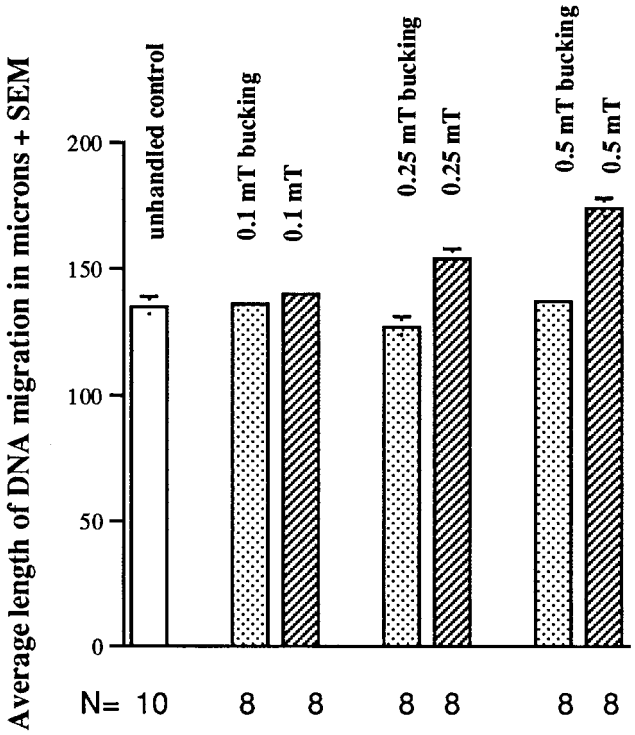

Fig. 7. Levels of DNA double-strand breaks (expressed in length of DNA migration) in the different treatment groups. A two-tailed Newman-Keuls test showed a significant difference at $P < .01$ between the groups exposed to 0.25 and 0.5 mT magnetic field and the respective bucking control groups. No significant difference was found between the 0.1 mT-exposed group and its bucking control.

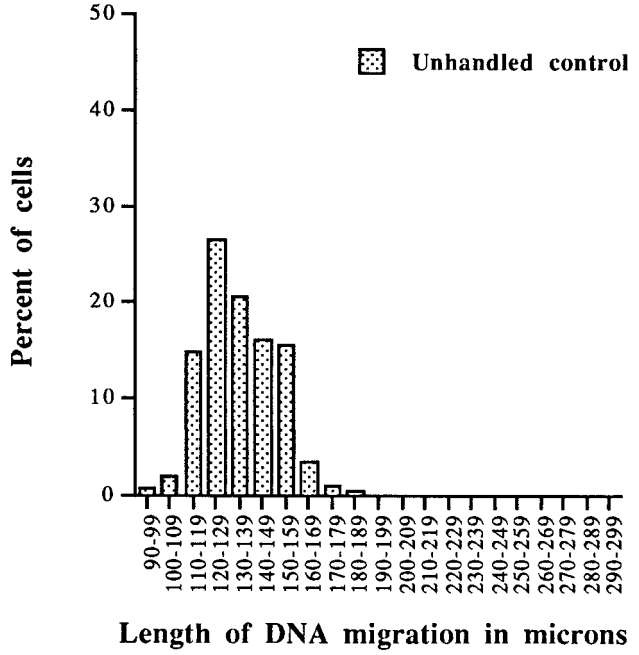

Fig. 8. Distribution of DNA migration length (double-strand breaks) of brain cells of unhandled rats (N = 10; 500 cells).

The effects of magnetic field exposure on DNA double-strand breaks in brain cells of the rat are shown in Figures 7–12. Figure 6 shows the average DNA migration lengths of the various treatment groups. One-way ANOVA of the data showed a significant treatment effect ($F[6,51] = 27.57$, $P < .005$). No significant effect on double-strand breaks was observed after exposure at a magnetic field flux density of 0.1 mT (compared to that of the respective bucking controls), whereas a significant increase was observed at flux densities of 0.25 mT ($P < .01$) and 0.5 mT ($P < .01$). Distributions of length of DNA migration in brain cells are shown in Figures 7–10. An increased shift of the distribution to longer migration lengths at higher flux densities of exposure was observed. Figure 12 shows photographs of DNA migration pattern (double-strand breaks) of individual brain cells from rats of the different treatment groups.

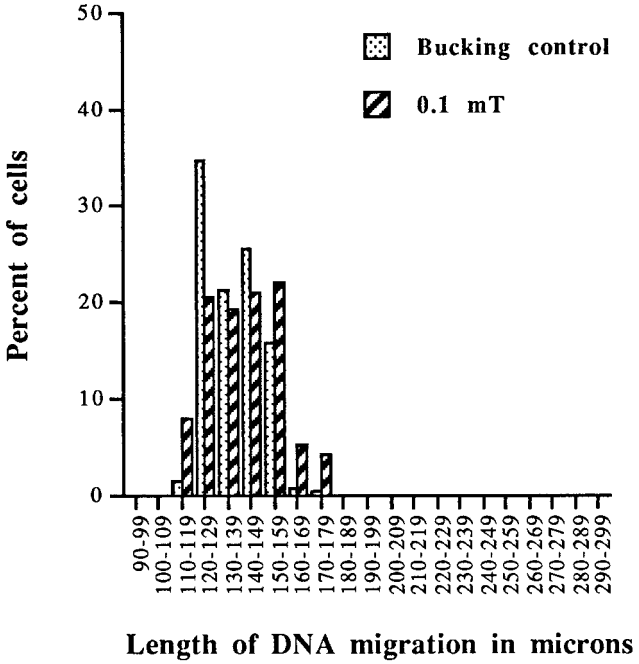

Fig. 9. Percentage distribution of brain cells vs. DNA migration length (double-strand breaks) of rats exposed to a 60 Hz magnetic field of 0.1 mT (N = 8; 400 cells) and rats exposed in the bucking mode (N = 8; 400 cells). Distribution patterns of the two treatments were significantly different from each other ($\chi^2 = 66$, df = 6, $P < .001$).

DISCUSSION

Our results show that acute exposure to a 60 Hz magnetic field causes an increase in both single-strand

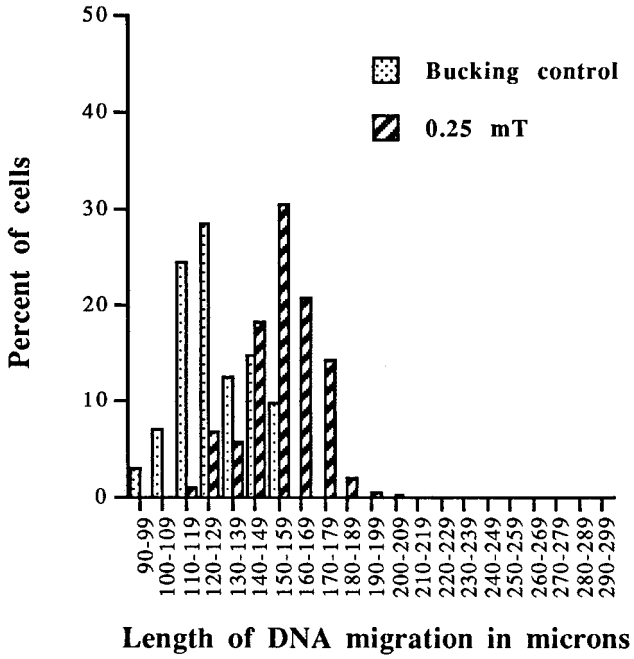

Fig. 10. Percentage distribution of brain cells vs. DNA migration length (double-strand breaks) of rats exposed a 60 Hz magnetic field of 0.25 mT (N = 8; 400 cells) and rats exposed in the bucking mode (N = 8; 400 cells). Distribution patterns of the two treatments were significantly different from each other (χ^2 = 390.8, df = 9, $P < .001$).

and double-strand DNA breaks in brain cells of the rat. ELF magnetic fields do not have enough energy to break chemical bonds directly in DNA molecules. A possible explanation of the present observations is that 60 Hz magnetic fields affect enzymatic processes involved in DNA repair, leading to an accumulation of DNA strand breaks. This hypothesis is supported by a recent report by Phillips et al. [1995] that acute exposure to a 60 Hz magnetic field significantly affected the activity of poly-ADP-ribose polymerization, an enzymatic activity involved in DNA repair. A similar effect on poly-ADP-ribose polymerization has also been observed by Behari et al. (personal communication) in brain cells of rats after chronic exposure to a 50 Hz magnetic field.

In two previous studies [Fairbairn and O'Neill, 1994; Reese et al., 1988], no significant effect of ELF magnetic fields on DNA strand breaks in cells was reported. In the study by Reese et al. [1988], Chinese hamster ovary cells were exposed to a 60 Hz magnetic field at 0.1 and 2 mT for 1 h; no significant effect on DNA single-strand breaks was observed in these cells immediately after exposure as measured by the alkaline elution technique. In this study, cell samples were kept throughout the experiment under ''iced'' condition, which would preclude any effect due to change in enzymatic activity in the cells. In the Fairbairn and O'Neill [1994] study, human cells were first suspended in agarose on a slide before being exposed for 1 or 24 h to a 50 Hz pulsed magnetic field (peak flux density 5 mT, pulse duration 3 ms). Cells suspended in agarose are not in a good physiological environment. Thus, any possible effect of magnetic fields on enzymatic activity might not be revealed under such an experimental condition.

The ELF magnetic flux density in the environment varies over a wide range. For example, household and office levels can vary from 0.01 to 1 μT. Intermittently, levels can reach more than 10 μT. Levels near a power transmission line can be 10–30 μT, 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]. Recommended maximum levels of magnetic field exposure also vary. For example, the interim guidelines of the International Nonionizing Radiation Committee of the International Radiation Protection Association [IRPA/INIRC, 1990] for occupational situations are 0.5 mT

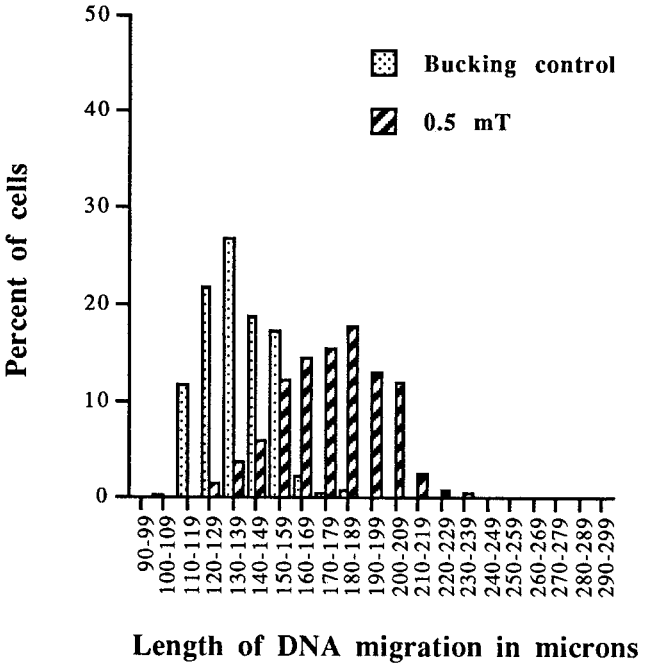

Fig. 11. Percentage distribution of brain cells vs. DNA migration length (double-strand breaks) of rats exposed a 60 Hz magnetic field of 0.5 mT (N = 8; 400 cells) and rats exposed in the bucking mode (N = 8; 400 cells). Distribution patterns of the two treatments were significantly different from each other (χ^2 = 492.5, df = 10, $P < .001$).

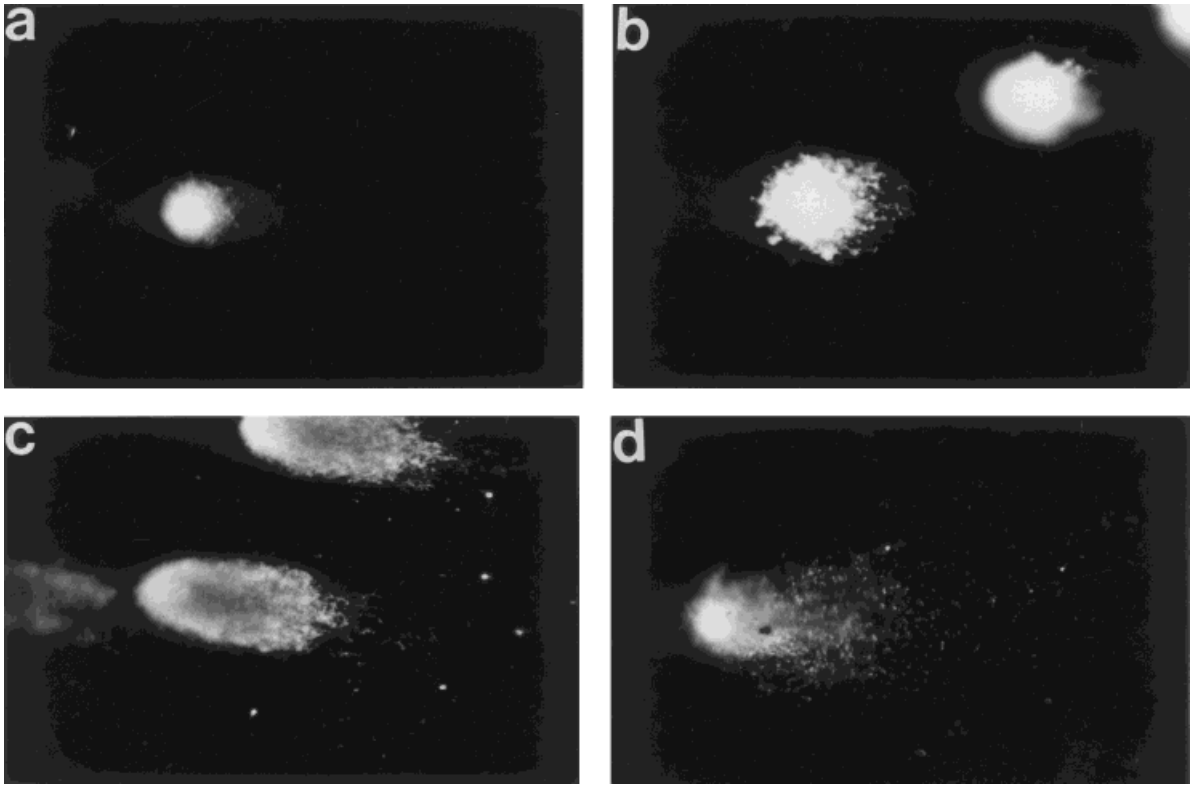

Fig. 12. Photographs of double-strand break DNA migration pattern of individual brain cells from rats exposed to bucking condition (0.1 mT) (**a**) or magnetic fields of 0.1 mT (**b**), 0.25 mT (**c**), and 0.5 mT (**d**). ×400.

for workday exposure and 5 mT for short-term (2 h) exposure, whereas for the general public they are 0.1 mT for 24 h/day exposure and 1.0 mT for exposure of a few hours per day. The National Radiological Protection Board (NRPB) of England recommends a limit of 2.0 mT for both occupational and general public exposure to ELF magnetic fields [NRPB, 1989]. In our study, we found that a 60 Hz magnetic field causes DNA single-strand breaks in rat brain cells at a flux density $\geq$0.1 mT and double-strand breaks at 0.5 and 1.0 mT. Magnetic fields of these intensities are within the limits of the IRPA/INIRC and NRPB guideline standards and exist in both the public and the occupational environments. Furthermore, the intensity of the magnetic field studied and found to have a significant effect on DNA is well below the level for producing the classical induced electric current effects [Bernhardt, 1985] and within the IRPA/INIRC and NRPB recommended magnetic field-induced current density threshold of 1 $\mu A/cm^2$. However, the effects we observed after magnetic field exposure are probably not caused by induced electric currents in the tissue by the oscillating magnetic field. In our research, we found that direct application of convulsive electric currents to the head (40 mA, 60 Hz, 2 s) did not significantly affect the amount of DNA single-strand breaks in brain cells of the rat [Khan et al., 1995].

An increase in DNA strand breaks in body cells could have an important implication for the possible health effect of exposure to ELF magnetic fields in the environment. According to the multistep and clonal model of the origin of cancer [reviewed in Goldberg et al., 1991; Stein, 1991], tumorigenesis is a multistage 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 has been shown also for aging [Ames et al., 1993; Chetsanga et al., 1977; Mullaart et al., 1990a; Targovnik et al., 1985; Wheeler and Lett, 1974]. Based on the suggestion of Alexander [1967], several investigators contend that accumulated DNA damage in neurons and other postmitotic cells is the primary factor in aging and death of an organism. Indeed, Wheeler and Lett [1974] have shown that DNA repair is age related in cerebellar neurons in beagle dogs, and Chetsanga et al. [1977]

showed that the rate of neuronal DNA single-strand breakage is higher in old than in young mice. Cumulative DNA damage in cells, particularly in neurons, has also been associated with Alzheimer's disease [Jones et al., 1989; Mullaart et al., 1990b; Robbins et al., 1983], Huntington's disease [Bridges, 1981; Scudiero et al., 1981], and Parkinson's disease [Robbins et al., 1983]. Aside from neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases, increases in DNA strand breaks are seen also in disorders of premature senility, such as xeroderma pigmentosum, Werner's syndrome, Cockayne syndrome, ataxia telangiectasia, and retinal dystrophies [Robbins et al., 1983]. 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 [Sobel et al., 1995] and amyotropic lateral sclerosis [Davanipour et al., 1995].

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 be carried out to characterize and understand the effect of ELF magnetic field exposure on DNA strand breaks in cells. In particular, interaction effects of exposure parameters, such as intensity and duration of exposure, intermittent vs. continuous exposure, etc., and the effect of magnetic fields on DNA metabolic enzyme activities should be investigated. It would also be interesting to investigate whether in vivo magnetic field exposure affects DNA in cells of other organs in the body.

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