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# Interaction of Microwaves and a Temporally Incoherent Magnetic Field on Single and Double DNA Strand Breaks in Rat Brain Cells

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*The effect of a temporally incoherent magnetic field (noise) on microwave-induced DNA single and double strand breaks in rat brain cells was investigated. Four treatment groups of rats were studied: microwave-exposure (continuous-wave 2450-MHz microwaves, power density 1 mW/cm$^2$, average whole-body specific absorption rate of 0.6 W/kg), noise exposure (15 mG), microwave + noise-exposure, and sham-exposure. Animals were exposed to these conditions for 2h. DNA single- and double-strand breaks in brain cells of these animals were assayed 4h later using a microgel electrophoresis assay. Results show that brain cells of microwave-exposed rats had significantly higher levels of DNA single- and double-strand breaks when compared with sham-exposed animals. Exposure to noise alone did not significantly affect the levels (i.e., they were similar to those of the sham-exposed rats). However, simultaneous noise exposure blocked microwave-induced increases in DNA strand breaks. These data indicate that simultaneous exposure to a temporally incoherent magnetic field could block microwave-induced DNA damage in brain cells of the rat.*

**Keywords** Brain cells; DNA single and double strand breaks; Microwaves; Temporally incoherent magnetic field.

## Introduction

Nonionizing electromagnetic fields (EMF) are ubiquitous in the human environment. Electrical equipment and appliances emit extremely low frequency (ELF) EMF. Radiofrequency radiation is used in cellular phone and wireless communication transmission. Users of these devices are exposed to these fields.

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Litovitz and his colleagues have proposed that groups of receptors on the cell membrane could detect EMF in the environment. However, for a response to occur, an EMF has to be coherent, i.e., its characteristic parameters, such as frequency, amplitude, waveform, and pattern have to remain constant over a certain period of time (>~10 sec). Incoherent EMFs, i.e., those with changes in characteristic parameters over shorter periods of time, may be detected but do not trigger a biological response. Furthermore, superimposition of an incoherent EMF over a coherent EMF can make the sum field incoherent, thus blocking the biological effect of the coherent field. For details and theory of this EMF–detection model, readers are referred to the publications of Litovitz et al. [2, 10, 12]. Specifically, they have reported that a superimposed incoherent EMF could inhibit ELF EMF-induced abnormalities in developing chick embryos [11] and changes in ornithine decarboxylase (ODC) activity [3, 12, 14]. Litovitz et al. [13] also found that the same incoherent EMF superimposed on a microwave field blocked the microwave-induced increase in ODC activity in cultured cells. The hypothesis was further supported by the results of the experiments of Raskmark and Kwee [15] showing that an incoherent magnetic field could mitigate the effect of a coherent 50-Hz magnetic field on proliferation of human epithelial amnion cells, and of Lin and Goodman [9] that a superimposed incoherent magnetic field blocked the enhancement effect of a 60-Hz magnetic field on c-myc transcript levels in human leukemia cells.

In previous studies, we found that exposure to microwaves caused an increase in DNA single- and double-strand breaks in brain cells of the rat [6, 7]. Increases in breaks were quite uniform among the cells in the brain [7], even though different cell types were measured. Further research showed that the effects of microwaves on DNA could be blocked by pretreating rats before exposure with free radical scavengers [8], suggesting the involvement of free radicals. In the present research, we investigated whether simultaneous exposure to a temporally incoherent magnetic field (noise) could attenuate microwave-induced increases in DNA single- and double-strand breaks in brain cells of the rat.

## Methods and Procedures

### *Animals*

Male Sprague-Dawley rats (2–3 months old, 250–300 g) purchased from B & K Laboratory, Bellevue, WA, were used in the experiments. They were housed at a maximum of three in a cage in the exposure room for at least 24h before an experiment. The room was maintained on a 12h light-dark cycle (light on between 7:00 AM and 18:00 PM) and at an ambient temperature of 22°C and a relative humidity of 65%. Animals were provided with Purina rat chow (#5012) and water ad libitum. Animal-use procedures of this research were approved by the Animal Care Committee of the University of Washington.

## Methods of Microwave and Incoherent Magnetic Field Exposure

Animals were exposed to 2450-MHz microwaves in a cylindrical waveguide exposure system. The construction and characteristics of the system have been described in detail by Guy et al. [4]. Briefly, the system consists of individual

cylindrical waveguides connected through a power divider network to a continuous-wave microwave-power source (Hewlett Packard, HP-8616A signal generator). Waveguides were constructed of galvanized wire screen in which a circularly polarized $TE_{11}$-mode field configuration is exited. Each waveguide contains a plastic chamber to house a rat with enough space for it to move freely inside. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside the waveguide. In the present experiment, rats were exposed in the waveguide to 2450-MHz continuous-wave microwaves at a power density of 1.0 mW/cm$^2$, which gives a whole-body specific absorption rate in the rat of 0.6 W/kg [1]. For sham exposure, an animal is placed in a waveguide for 2h with no power input.

For incoherent magnetic field exposure, a waveguide was placed between a set of Helmholtz coils, which were positioned across the area where the plastic animal-holding cage was located. Thus, an animal could be exposed simultaneously with microwaves and noise, or the noise alone. The Helmholtz coils were constructed on a frame made of 3/4 in. flexible copper tubing. Each coil was wound with 100 turns using gauge 18 magnet wire. The nominal resistance of the coil pair was 5.8 ohms. E-field shielding was provided by connecting the coil frame to electrical ground. The incoherent magnetic field (noise) was generated using a signal recorded in an audiocassette tape. The tape was played back in a continuous-play cassette player connected to a power amplifier (Hewlett Packard, HP-467A) whose output was in turn connected to the Helmholtz coil. The recorded noise signal was provided by Dr. Miguel Penafiel of the Catholic University of America, Washington, D.C. This signal was specified as band-limited ELF-noise with nominal bandwidth between 30 Hz and 100 Hz. All exposures were carried out at an average magnetic field flux density of 45 mG that was monitored using an EMDEX meter (Enertech Consultants, Campbell, CA). The intensity of ambient magnetic fields (40–800 Hz) in the laboratory (i.e., when the power supply to the coils was turned off) was 1.4 mG.

## Experimental Procedures

There were four treatment groups: microwave-exposure, microwave + noise, noise alone-exposure, and sham-exposure. Animals were exposed to one of these conditions in waveguides for 2h and then returned to their home cage. (Times of exposure between animals were staggered by 10 min.) At 4h after exposure, one rat at a time was anesthetized by placing it in a covered foam box containing dry ice for 65 sec. (A 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. This 2h exposure/4h waiting schedule was used in our previous studies [6, 7]. The experiment was run under a blind condition, such that the person who did the DNA strand break assays did not know the treatment conditions of the animals. The experiment was carried out over several days. Two to three animals were exposed and assayed in each day. In each run, a sham-exposed animal was always included. Therefore, there are significantly more sham-exposed animals included in the study than the other three treatment groups.

The microgel electrophoresis assay for DNA single- and double-strand breaks in rat brain cells was carried out as described previously by us [8]. All chemicals used in the assay were purchased from Sigma Chemicals Company (St. Louis, MO) unless otherwise noted. Immediately after removal from the skull, a brain was

immersed in ice-cold phosphate-buffered saline (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 μM of N-t-butyl-α-phenylnitrone. It 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 (approximately 1 $mm^3$) in 5 mL of ice-cold PBS [16]. Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 mL of PBS, tissue pieces were dispersed into single-cell suspensions using a P-5000 Pipetman. This cell suspension consisted of different types of brain cells. Ten microliters of this cell suspension were mixed with 0.2 mL of 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 45°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 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 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, 100 mM disodium EDTA, 10 mM Tris base, pH 10) containing 1% Triton X-100.

To measure single strand DNA breaks, after lysing for 3h at 4°C in an ice-bath, slides were treated with DNAase-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% of 8-hydroxyquinoline, 2% dimethyl sulfoxide, and 10 mM tetra-sodium EDTA, pH 13) was gently poured into the assembly to cover the slides to a height of 6.5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0.4 volt/cm, approximately 250 mA, for 60 min) and the buffer was recirculated.

At the end of the electrophoresis, electrophoretic buffer was gently removed. Slides were then 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 of 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 2h followed by 5 min in 70% ethanol and then air-dried.

For double strand breaks, microgel preparation and cell lysis were done as described previously. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp., Indianapolis, IN) (10 μg/mL in the lysing solution) for 2h 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 1h at 0.4 volt/cm (approximately 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 previously.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was prestained with 50 μl of 5% DMSO in 30 mM $NaH_2PO_4$ and 5% sucrose, and then stained with 50 μl of 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 fluorescence isothyocynate (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. The migration length is defined as the length (in microns) from the beginning of the nuclear area to the last 3 pixels of DNA perpendicular to the direction of migration at the leading edge. It is used as the index of DNA strand breaks. This parameter can be easily and reliably measured using an inexpensive eyepiece micrometer due to the use of the intense fluorescent dye YOYO-1.

Two slides were prepared from the brain sample 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 all treatment groups. 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.

## Data Analysis

Data from the DNA strand breaks assay were analyzed by the one-way ANOVA and difference between two treatment groups was evaluated by the Newman-Keuls test. A difference at $p < 0.05$ was considered statistically significant.

## Results and Discussion

Results of single-strand DNA breaks are presented in Table 1. One-way analysis of variance showed a significant treatment effect ($F[3, 36] = 9.06$, $p < 0.01$). Comparisons of data from individual treatment groups by the Newman-Keuls test show that microwaves significantly increased single-strand breaks in brain cells of rats (microwave vs. sham, $p < 0.01$) and that simultaneous noise-exposure blocked the effect of microwaves (microwave vs. microwave + noise, $p < 0.01$, and no significant difference was found between sham and microwave + noise). In addition, there was no significant difference between sham and noise exposure alone, indicating that the noise has no significant effect on DNA single strand breaks.

Table 2 shows the results from the double-strand break assay. Similar conclusions can be drawn on the double-strand break data as for the single-strand

**Table 1**
Single strand DNA breaks in brain cells of rats (mean migration length in microns ± SEM)

| Treatment | Migration length | Number of animals studied |
|---|---|---|
| Sham | 141 ± 2.8* | 16 |
| Microwave | 163 ± 2.8 | 8 |
| Microwave + Noise | 137 ± 5.1* | 7 |
| Noise | 140 ± 3.8 | 9 |

*Significantly different from microwave at $p < 0.01$.

**Table 2**
Double strand DNA breaks in brain cells of rats (mean migration length in microns ± SEM)

| Treatment | Migration length | Number of animals studied |
|---|---|---|
| Sham | 141 ± 2.8* | 16 |
| Microwave | 151 ± 1.9 | 8 |
| Microwave + Noise | 135 ± 4.0** | 7 |
| Noise | 142 ± 4.1 | 9 |

*,**Significantly different from 'microwave' at $p < 0.05$ and 0.01, respectively.

break data. One-way analysis of variance showed a significant treatment effect ($F[3, 36] = 3.11$, $p < 0.05$). Microwave exposure significantly increased double-strand breaks in brain cells of the rats exposed (microwave vs. sham, $p < 0.05$) and that simultaneous noise-exposure blocked the effect of microwaves (microwave vs. microwave + noise, $p < 0.01$), whereas no significant difference was found between sham and microwave + noise, and between sham and noise exposure alone.

Thus, data from the present experiment show that acute exposure (2h) of rats to a continuous-wave 2450-MHz microwaves, at an average whole-body specific absorption rate of 0.6 W/kg, significantly increased the levels of DNA single- and double-strand breaks in brain cells of rats. This confirms our previous studies showing that acute microwave exposure increases single- and double-strand DNA breaks in brain cells. The data also show that simultaneous exposure to a temporally incoherent magnetic field can block these effects of microwaves. These data support the hypothesis of Litovitz et al. [13] that cellular response to electromagnetic (EM) fields occurs through a detection process involving temporal sensing. Superpositioning the incoherent magnetic field could have disturbed cellular sensing and thus responses to the microwave radiation.

A possible intermediate mechanism of interaction is via free radicals. Free radicals have been shown to play a major role in microwave-induced DNA strand breaks, since the effects could be blocked by pretreating the rats before microwave exposure with free radical scavengers [8]. However, because temporally incoherent EMF has been shown to block a variety of seemingly unrelated biological effects of coherent EMF, including chick embryo development [11], ornithine decarboxylase activity [3, 12, 14], cell proliferation [15], c-myc transcription [5], and behavior [5], it is likely that the primary site of interaction occurs at a more basic biological level, such as the cell membrane as proposed by Litovitz et al. [10, 12].

Since microwaves are emitted from cellular phones, users are constantly being exposed to the radiation. The possible health effect of exposure has been a concern. Particularly, DNA damage in cells can lead to serious health consequences. DNA damage can cause a disruption of normal cell functions, cell death, and carcinogenesis. Superimposing a temporally incoherent magnetic noise with the source of microwaves could be a possible mitigation of these potentially harmful effects.

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**Referenc**

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