

# Biological Effects of Prolonged Exposure to ELF Electromagnetic Fields in Rats: III. 50 Hz Electromagnetic Fields

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Groups of adult male Sprague Dawley rats (64 rats each) were exposed for 8 months to electromagnetic fields (EMF) of two different field strength combinations: 5 $\mu$ T - 1kV/m and 100 $\mu$ T - 5kV/m. A third group was sham exposed. Field exposure was 8 hrs/day for 5 days/week. Blood samples were collected for hematology determinations before the onset of exposure and at 12 week intervals. At sacrifice, liver, heart, mesenteric lymph nodes, bone marrow, and testes were collected for morphology and histology assessments, while the pineal gland and brain were collected for biochemical determinations. At both field strength combinations, no pathological changes were observed in animal growth rate, in morphology and histology of the collected tissue specimens (liver, heart, mesenteric lymph nodes, testes, bone marrow), and in serum chemistry. An increase in norepinephrine levels occurred in the pineal gland of rats exposed to the higher field strength. The major changes in the brain involved the opioid system in frontal cortex, parietal cortex, and hippocampus. From the present findings it may be hypothesized that EMF may cause alteration of some brain functions. *Bioelectromagnetics* 19:57–66, 1998. © 1998 Wiley-Liss, Inc.

**Key words:** electric and magnetic fields; neurotransmitters; brain receptors; histology; hematology; serum chemistry

## INTRODUCTION

An increased health risk due to exposure to electric and magnetic fields (EMF) at 50 and 60 Hz has been reported by several authors. In vivo studies have dealt especially with increased tumor incidence, effects on reproduction and development, and neural and behavioral changes [Seegal et al., 1989; Svedenstål and Johanson, 1995; Stuchly et al., 1992; Coelho et al., 1995]. Recently several epidemiological studies reported an elevated cancer risk, especially for childhood leukemia and brain cancer, associated with exposure to residential and occupational EMF, whereas other studies have not confirmed these results [Peters et al., 1991; Feychting and Ahlbom, 1993; Olsen et al., 1993; Loomis et al., 1994; Theriault et al., 1994; Wertheimer et al., 1995; Savitz and Loomis, 1995]. A study conducted in textile factories described an increased inci-

dence of Alzheimer's disease in workers exposed to magnetic fields [Sobel et al., 1995].

Investigations were also conducted on the possible involvement of the melatonin circadian secretion profile in the EMF effects. In fact, it has been suggested that the alteration of the blood level of this hormone consequent to EMF exposure could result in a decreased protection against tumor growth [Wilson et al., 1986; Kato et al., 1993; Reiter, 1993; Rogers et al., 1995]. On the other hand, studies on CNS showed a depressed activity of different types of neurotransmitter

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system (acetylcholine, dopamine, serotonin, amino acids), and protein content in the brain of animals exposed to EMF [Vasquez et al., 1988; Seegal et al., 1989; Zecca et al., 1991; Hagino et al., 1992; Lai et al., 1993; Yu et al., 1993].

In previous studies in this laboratory [Zecca et al., 1991; Margonato et al., 1993; Margonato et al., 1995] have investigated the "in vivo" effects of separately applied electric or magnetic fields on body and tissue growth, hematology, and the tissue level of a number of neurotransmitters. To study possible synergistic effects of electric and magnetic fields, it was necessary to carry out similar experiments exposing the animals to electric and magnetic fields. Adult male rats were exposed for 8 months to EMF (5 $\mu$ T - 1kV/m, 100 $\mu$ T - 5kV/m) according to a protocol analogous to an occupational time schedule. After sacrifice, the animals underwent a macroscopic examination of the whole body, and the main organs and tissues were histologically tested. The central nervous system was assessed by measurements of concentrations of dopamine, norepinephrine, serotonin,  $\mu$ -opioid receptors, and D-2 dopamine receptors in various brain regions. Additional data (body weight, hematologic values, serum chemistry) were obtained throughout the study.

## MATERIALS AND METHODS

### Animals and Exposure Conditions

One hundred and ninety two Sprague-Dawley albino male rats, 10 week old, were randomly divided into three experimental groups according to Fisher's table for random distribution and then placed into three identical EMF exposure units for 8 months. Unit 1 was used for sham exposure, unit 2 was activated with 5  $\mu$ T - 1 kV/m and unit 3 was activated with 100  $\mu$ T - 5 kV/m. EMF exposure was 8 hrs/day (9 a.m. - 5 p.m.) and 5 days/week (Monday through Friday). Animals were under constant controlled illumination 24 h/day at a light intensity of  $150 \pm 30$  lux; temperature was  $22^\circ\text{C} \pm 2$  and humidity was  $55\% \pm 5$ . Animals had continual access to food (ad libitum), but could drink only during the field-off time. The rats were killed by decapitation after 8 month's exposure, according to a random sequence, between 9:00 and 12:30 A.M. At sacrifice, blood and organs were collected. The brain was removed and the pineal gland separated and immediately frozen in dry ice.

### Exposure System

The facility for animal exposure was at Centro Elettrotecnico Sperimentale Italiano (CESI) in Milan, Italy. The rats were individually housed in 24 cm wide,

26.5 cm long, 30 cm high polycarbonate cages. These were placed on electrically grounded metal nets and placed in three identical exposure units. The electric field was generated by parallel electrodes with a 40 cm air gap. The magnetic field was generated by five pairs of vertically arranged rectangular coils (width 1.9 m, height 0.95 m). The external size of the system was  $1.9 \times 1.9$  m and the distance between two adjacent pairs of coils was 0.6 m. Magnetic coils were fixed to wooden frames, and the absence of significant vibrations was confirmed by acceleration measurements. Furthermore, the frame with coils was mechanically separated from that holding the animal cages. The coil design (number of turns, cross-section of conductor and rated current) was optimized in order to reduce Joule-heating losses to the level which did not affect the temperature in the exposure area. Sham exposed rats were held in a similar non-energized system. In the area where the sham exposed animals were housed, even after energization of the exposure systems, the magnetic field was equal to the background level (0.04  $\mu$ T). Details of this facility were described in previous papers [Margonato et al. 1993; Margonato et al., 1995].

## Investigated Variables

### Neurochemistry

*Collection of brains and dissection into different regions.* The animals were sacrificed by decapitation and the brains were removed immediately. The pineal gland was separated and frozen in dry ice. The brain was sliced in coronal sections (1.5–2.0 mm thickness) using the Bregma and Interaural Line as landmarks [Paxinos and Watson 1986], and the following areas were dissected out: frontal cortex, parietal cortex, striatum, hypothalamus, hippocampus, cerebellum. The samples were stored at  $-80^\circ\text{C}$  for neurotransmitter and receptor assay.

*Measurement of brain amines and metabolites.* Dopamine (DA) and its metabolites (homovanillic acid, HVA and 3–4dihydroxyphenylacetic acid, DOPAC), serotonin (5-HT) and its metabolite (5-hydroxyindoleacetic acid, 5-HIAA) and norepinephrine (NE) were determined in the brain areas and pineal gland by high pressure liquid chromatography (HPLC) with electrochemical detection. After weighing, the tissue was homogenized in 10–30 ml/g wet tissue of 0.1M HClO<sub>4</sub> containing 0.3–0.1  $\mu$ g/ml of ( $\pm$ )isoproterenol as internal standard and 10  $\mu$ g/ml of ascorbic acid, then centrifuged at 9,000 g for 10 min at  $+4^\circ\text{C}$ . The supernatant was stored at  $-20^\circ\text{C}$  up to the moment of injection into the chromatograph.

The HPLC system had a Series 10 Chromatography Pump (Perkin Elmer) connected to a LC-4B amp-

erometric detector (Bio Analytical System). Chromatograms were recorded and peak area calculated with a C-R4A integrator (Shimadzu). A column of 5  $\mu\text{m}$  particle size, 25 cm length, 4.6 mm i.d. (Beckman) was employed at room temperature and was connected to a pre-column C18 (C.P.S. Analitica). The mobile phase was 10% methyl alcohol and 90% 50 mM sodium acetate with 0.05 mM EDTA- $\text{Na}_2$ , 0.7 mM sodium heptansulfonate and adjusted to pH 4.50 with 2M phosphoric acid. The flow rate was 1.7 ml/min and a 0.75 V potential was applied to the glassy carbon electrode versus a silver chloride reference electrode. A 50  $\mu\text{l}$  volume of supernatant was injected into the chromatograph.

**Determination of dopamine D2 receptors.** The dopamine D2 receptors, in frontal cortex and striatum, were quantified using the D2 dopamine receptor antagonist [ $^3\text{H}$ ]YM-09151-2 (Dupont-NEN) as radioligand in saturation assay [Sugiyama et al., 1985]. Approximately 50 mg wet tissue were pooled for each assay to yield a final protein concentration of 20  $\mu\text{g}$  per tube. Membranes were prepared by homogenizing the tissue in a Dounce tissue grinder in ice-cold 5 mM HEPES buffer, supplemented with 118 mM NaCl, 2.5  $\text{CaCl}_2$ , 4.8 mM KCl, and 1.2 mM  $\text{MgSO}_4$ . The homogenized tissue was placed on ice for one hour, then was centrifuged at 40,000 g, because there were no dopamine D2 receptors in nuclei's fraction. The pellet was retained and washed two times in 20 mM HEPES, supplemented with the above ions, before final suspension in 20 mM HEPES.

The dopamine D2 assay was performed in triplicate in disposable glass test tubes, using 20 mM HEPES buffer with a final volume of 1 ml. Nine concentrations of [ $^3\text{H}$ ]YM-09151-2 were used, ranging from 3 pM to 300 pM at varied intervals, for determination of saturation curves and Scatchard analysis. Non-specific binding was determined using 10  $\mu\text{l}$  of 0.5  $\mu\text{M}$  (+) butaclamol (Research Biochemical, INC.).

Reaction mixture of radioligand and 50  $\mu\text{l}$  aliquots of tissue homogenate (to yield a final protein concentration of approximately 20  $\mu\text{g}$  per tube) was incubated at 37°C for 30 min. Separation of bound and unbound radioligand was accomplished by filtration under suction through Whatman GF/B glass microfiber filters, which were presoaked in 0.05% polyethylenimine, using three 5 ml rinses of ice-cold 5 mM HEPES buffer. The filtration unit was a Kimble Filter Funnels (Fisher Scientific). Filters were counted by a Packard 1900 CA Tri-Carb in Soluen R-350 scintillation cocktail (Packard Instrument) at an efficiency of 50%.

Saturation and Scatchard analysis were performed using Packard Combicapt 2000 steroid receptor analysis software, adapted for membrane binding assay. Standard parametric statistical test were used to

analyze data. Protein concentrations were determined by the method of Lowry [Lowry et al., 1951]. For a single point assay of dopamine D2 receptors after completion of saturation and Scatchard analysis, a 50  $\mu\text{L}$  of suspended tissue solution was transferred to the reaction tube containing either 0.5 M (+) butaclamol or 20 mM HEPES. A final concentration of 20 pM of [ $^3\text{H}$ ]YM-09151-2 was added into the reaction tube. The suspended tissue with reaction mixture were placed in 37°C water bath for 30 min. After the reactions were completed, reaction solutions were filtered through Whatman GF/B glass microfiber filter, which were presoaked with 0.05% polyethylenimine. Bound radioactivity on the microfiber filters was determined by liquid scintillation counting.

**Determination of  $\mu$ -opioid receptors.** The number (B max) and the affinity of the  $\mu$ -opioid receptors for the specific ligand [ $^3\text{H}$ ]dihydromorphine (DHM) [Pfeiffer and Herz, 1982] were assayed in the frontal and parietal cortex, striatum, hypothalamus, hippocampus, and cerebellum according to a method previously described [Maggi et al., 1993]. The tissues were individually homogenized (glass-Teflon homogenizer) in 10 vol of sucrose 0.32 M. Homogenates were centrifuged at 1,400 g for 10 min; the resulting supernatants were decanted and preincubated for 30 min at 37°C, in order to eliminate the endogenous ligand which might interfere with the assay of  $\mu$  binding sites. At the end of the incubation, the material was centrifuged again at 48,000 g for 30 min. The pellets obtained (plasma membranes) were resuspended and homogenized in 3 vol of assay buffer (Tris-HCl 50 mM, pH 7.4) and submitted to the binding assay. The protein content of each plasma membrane preparation was determined according to the method of Bradford [1976]. Unlabelled DHM was synthesized in the authors' laboratory. [ $^3\text{H}$ ]-DHM (87Ci/mmol) was purchased from NEN-Dupont.

To evaluate the binding characteristics of  $\mu$ -opioid receptors of the investigated tissues, a series of homologous inhibition curves was performed on membrane preparations. These were obtained from two samples of each tissue under study, pooled and submitted to all the steps of the assay. This procedure makes it possible to establish the variability of the binding characteristics of DHM among the experimental animals. According to the optimization of the receptor assay performed by the DESIGN computer program [Rovati et al., 1988], aliquots from a pool of tissue membrane preparations (approximately 200  $\mu\text{g}$  of protein) were incubated with 0.1nM of [ $^3\text{H}$ ]-DHM and four increasing concentrations (1, 3, 6 nM, and 1  $\mu\text{M}$ ) of unlabelled DHM. Incubations were carried out at 25°C for 30 min. Tubes were then rapidly and individually

filtered through Whatman GF/B filters washed twice with 5 ml ice-cold assay buffer and counted in 7 mL Instagel scintillation cocktail (Packard Instruments). All samples were assayed in duplicate. In order to minimize the interassay variations, all inhibition curves were performed in the same assay. The data were analyzed by means of the LIGAND program [Munson and Robard, 1984] adapted to a MacIntosh computer.

**Behavior.** Every day, before and after energizing the exposure system, the excitability, motility, and preferential position (standing or horizontal) of rats in the cages were carefully observed.

**Body weight.** The rats were weighed upon delivery to the laboratory. The weight was then determined regularly at two weeks intervals, at the same time of the day. The last recording was obtained just before sacrifice.

**Necropsy Procedures.** After postmortem macroscopic examination of the whole body, six specimens of liver, heart, and mesenteric lymph nodes of the animals were dissected, fixed in 4% formalin, cut into 5  $\mu$ m slices and stained with hematoxylin-eosin for light microscopy. The testes were fixed in Bouin's solution; and a total of 40 specimens from the left and right organs (upper and lower pole ilus zone) were obtained, cut into 4  $\mu$ m slices and stained with hematoxylin-eosin, periodic acid-Shiff (PAS), Azan and Ponceaux 2R. Bone marrow specimens for cytologic examination were taken from the femur. The smears were air dried and stained with May-Grünwald-Giemsa.

**Hematology.** Three hundred microliters of blood were taken from the vein of the tail after 0, 12, 24, and 32 weeks, collected into tubes containing lithium heparinate and immediately stored in the refrigerator

(+4°C) until analysis. Red (RBC) and white (WBC) blood cells counts, hemoglobin concentration (Hb) and volume of packed red cells (VPRC) were determined using an electronic counter (Coulter Electronics).

**Serum chemistry.** At sacrifice, free flowing blood was collected into 5 ml tubes. After centrifugation (600 g for 15 min) the serum was collected in Eppendorf vials and stored in the refrigerator (-20°C) until analysis. Blood chemistry analysis was started after blood sampling and completed in a week. Every day the same number of samples taken from the three experimental groups was analyzed. The samples were analyzed for serum alkaline phosphatase, cholesterol, and uric acid by Auto Analyzer Instrument (Technicon Instruments). Serum levels of luteinizing hormone (LH) were measured with a specific radioimmunoassay procedure [Niswender et al., 1968]. The sensitivity of the assay was 0.5 ng/ml. To estimate interassay variations, all samples were run in duplicate in a single assay. The intra-assay variation was 2.56%. The data are expressed in terms of NIH-S20 (standard).

**Statistical Analysis.** Statistical analysis for all the neurochemistry data was performed using the F-test. Other data were analyzed by using Student's t-test,  $\chi^2$  test (for proportions, see Armitage and Berry, 1987) and ANOVA when applicable. Differences were considered to be statistically significant at  $P < 0.05$ . Values are given as mean  $\pm$  S.E.M.

## RESULTS

### Neurotransmitters and Receptors

The results obtained are shown in Tables 1-4. No significant difference was found in the concentra-

TABLE 1. Neurotransmitters Concentration in Rats Exposed to 50 Hz EMF for 8 Months<sup>a</sup>

Brian areas	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Frontal cortex sham	0.343 $\pm$ 0.029	N.D.	N.D.	N.D.	0.678 $\pm$ 0.067	0.271 $\pm$ 0.05
1kV/m-5 $\mu$ T	0.344 $\pm$ 0.017	N.D.	N.D.	N.D.	0.824 $\pm$ 0.042	0.274 $\pm$ 0.01
5kV/m-100 $\mu$ T	0.315 $\pm$ 0.023	N.D.	N.D.	N.D.	0.687 $\pm$ 0.071	0.272 $\pm$ 0.01
Parietal cortex sham	0.555 $\pm$ 0.025	N.D.	N.D.	N.D.	0.777 $\pm$ 0.033	0.411 $\pm$ 0.03
1kV/m-5 $\mu$ T	0.554 $\pm$ 0.032	N.D.	N.D.	N.D.	0.805 $\pm$ 0.044	0.412 $\pm$ 0.02
5kV/m-100 $\mu$ T	0.530 $\pm$ 0.028	N.D.	N.D.	N.D.	0.819 $\pm$ 0.049	0.399 $\pm$ 0.01
Striatum sham	N.D.	4.648 $\pm$ 0.503	0.864 $\pm$ 0.044	1.084 $\pm$ 0.022	0.541 $\pm$ 0.042	0.356 $\pm$ 0.02
1kV/m-5 $\mu$ T	N.D.	4.310 $\pm$ 0.496	0.851 $\pm$ 0.059	1.064 $\pm$ 0.023	0.535 $\pm$ 0.044	0.361 $\pm$ 0.02
5kV/m-100 $\mu$ T	N.D.	4.135 $\pm$ 0.368	0.810 $\pm$ 0.046	1.043 $\pm$ 0.026	0.509 $\pm$ 0.040	0.355 $\pm$ 0.02
Hypothalamus sham	0.724 $\pm$ 0.077	0.161 $\pm$ 0.018	0.100 $\pm$ 0.017	N.D.	0.612 $\pm$ 0.077	0.325 $\pm$ 0.03
1kV/m-5 $\mu$ T	0.613 $\pm$ 0.070	0.133 $\pm$ 0.013	0.090 $\pm$ 0.011	N.D.	0.562 $\pm$ 0.061	0.288 $\pm$ 0.03
5kV/m-100 $\mu$ T	0.633 $\pm$ 0.067	0.166 $\pm$ 0.020	0.088 $\pm$ 0.013	N.D.	0.609 $\pm$ 0.046	0.300 $\pm$ 0.02
Cerebellum sham	0.225 $\pm$ 0.013	N.D.	N.D.	N.D.	0.274 $\pm$ 0.016	0.128 $\pm$ 0.00
1kV/m-5 $\mu$ T	0.261 $\pm$ 0.023	N.D.	N.D.	N.D.	0.290 $\pm$ 0.015	0.126 $\pm$ 0.00
5kV/m-100 $\mu$ T	0.236 $\pm$ 0.012	N.D.	N.D.	N.D.	0.287 $\pm$ 0.015	0.131 $\pm$ 0.00

<sup>a</sup>Concentration of norepinephrine (NE), dopamine (DA) and its metabolites 3-4dihydroxyphenyl acetic acid (DOPAC) homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain areas. Values are mean  $\pm$  S.E.M. as  $\mu$ g/g wet tissue and were obtained by analyzing brain areas from 10 rats in each experimental group. (N.D., not detectable).

**TABLE 2. D2 Receptors Concentration in Rats Exposed to 50 Hz EMF for 8 Months<sup>a</sup>**

Brain areas	sham	1kV/m-5 μT	5kV/m-100 μT
Striatum	182.0 ± 8.2	198.0 ± 11.4	185.1 ± 11.3
Frontal cortex	12.6 ± 2.5	10.2 ± 1.3	8.4 ± 0.7

<sup>a</sup>Concentration of dopamine D2 receptors in brain areas. Values are mean ± S.E.M. as fmol/mg protein and were obtained by analyzing brain areas from 21 rats in each experimental group.

in the frontal cortex and in the parietal cortex took place without any modification of the affinity of the receptors for the ligand.

In the pineal gland a significant ( $P < 0.01$ ) increase in the content of NE was observed at the higher field strength (5 kV/m - 100 μT). At the lower field, no significant change was observed. Thus this appears to be a field strength dependent tendency toward an increase of NE (Table 4).

**TABLE 3. μ-Opioid Receptors Concentration in Rats Exposed to 50 Hz EMF for 8 Months<sup>a</sup>**

Brain areas	sham	1kV/m-5 μT	5kV/m-100 μT
Frontal cortex	86.72 ± 6.94	53.35 ± 11.29*	74.47 ± 11.26
Parietal cortex	75.72 ± 5.34	178.41 ± 12.52*	49.52 ± 6.68*#
Hippocampus	81.80 ± 11.91	129.92 ± 12.81*	111.23 ± 6.68
Hypothalamus	68.69 ± 6.27	73.92 ± 16.84	73.27 ± 20.13
Striatum	139.04 ± 16.90	125.03 ± 8.03	130.79 ± 6.20
Cerebellum	8.11 ± 2.58	4.64 ± 0.81	3.25 ± 1.04

<sup>a</sup>Concentration of μ-opioid receptors in brain areas. Values are mean ± S.E.M. as fmol/mg protein and were obtained by analyzing brain areas from 10 rats in each experimental group.

test-F: \* $P < 0.05$  sham-exposed vs exposed groups # $P < 0.05$  1kV/m-5 μT vs 5kV/m-100 μT.

Kd:  $1.1 \cdot 10^{-9}$  M.

**TABLE 4. Neurotransmitters Concentration in the Pineal Gland of Rats Exposed to 50 Hz EMF for 8 Months<sup>a</sup>**

Brain area	NE	5-HT	5-HIAA
Pineal gland			
sham	0.675 ± 0.086	48.16 ± 4.01	1.047 ± 0.114
1kV/m-5 μT	0.950 ± 0.124	37.17 ± 5.99	1.083 ± 0.101
5kV/m-100 μT	1.247 ± 0.137**	56.89 ± 11.76	1.012 ± 0.061

<sup>a</sup>Concentration of norepinephrine (NE), serotonin (5-HT) and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the pineal gland. Values are mean ± S.E.M. as μg/g wet tissue and were obtained by analyzing brain areas from 10 rats in each experimental group.

test-F: \*\* $P < 0.01$

tions of NE, DA and its metabolites, 5-HT and its metabolite in all investigated brain areas between sham-exposed and the experimental groups (Table 1).

No significant difference between sham and EMF exposed groups was found in the dopamine D2 receptor density in all tested brain areas (Table 2).

A significant increase of the μ-opioid receptor content was found in the hippocampus of rats exposed to both field levels ( $P < 0.05$ ). By contrast, a significant decrease ( $P < 0.05$ ) in the receptor density was observed in the frontal cortex only at the lower field strength. In the parietal cortex a significant increase ( $P < 0.05$ ) occurred at the lower field strength, while a significant decrease was observed at the higher field strength. No significant changes were found in striatum, hypothalamus, and cerebellum of exposed groups compared to sham (Table 3). The modification in the number of μ-receptors observed in the hippocampus,

**Body weight**

The mean body weight of the two exposed groups (1 kV/m - 5 μT and 5 kV/m - 100 μT) and of the sham exposed animals as a function of age is shown in Figure 1. From mean values of 360–380 g before exposure, body weight increased up to 700–740 g at the end of exposure. No statistical differences between the two exposed groups and the sham exposed rats were found at any time throughout the experimental period.

**Mortality**

Over the experimental time a total of 4 rats died in the sham-exposed group. In the experimental groups the deaths were 6 and 7 at the lower and higher field levels, respectively.

**Necropsy**

Macroscopic examination of the investigated organs did not reveal important alterations in exposed and

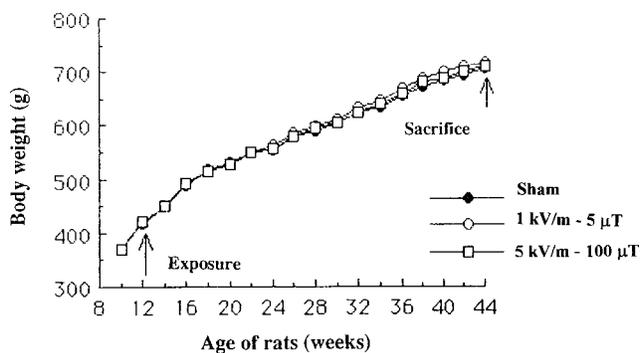


Fig. 1. Body weights of rats exposed to 1 kV/m - 5  $\mu$ T and 5 kV/m - 100  $\mu$ T electromagnetic fields. Values for sham-exposed animals are also shown.

sham-exposed animals. The occurrence of microscopic alterations in liver, heart and lymph nodes is summarized in Table 5. Its frequency was similar in the three groups and in no case the difference was statistically significant. For the testes, an evaluation of the fraction of the altered tissue was done by taking, from each of the organs, 20 slices for microscopic examination. In Table 6, the absolute number of altered testes together with the size of the lesion (as percent of the organ total mass) are given for both experimental and sham-exposed animals. No statistical significant differences were found between groups. Moreover, no neoplastic process was evidenced.

The results of cytologic examination of bone marrow are summarized in Table 7a and 7b. In Table 7a the fractional incidence for a 3 level estimate (mild, moderate, high) of the content of fat tissue, megakaryocytes, eosinophils, and mast cells is given. Fractional distributions were similar in experimental and sham-exposed groups. The percent distribution densities of erythroid cells, myeloid cells, lymphocytes, and plasma cells are shown in Table 7b. The myeloid cell density was significantly higher in the exposed groups ( $P < 0.05$ ,  $\chi^2$ -test). No neoplastic changes were found.

### Hematology

The hematologic variables (mean  $\pm$  S.E.M.) of the two experimental and of the sham-exposed groups are shown in Table 8 as a function of the exposure duration. Statistical analysis of the data did not give evidence of any significant difference between groups.

### Serum chemistry

The mean values  $\pm$  S.E.M. of uric acid, cholesterol, and alkaline phosphatase in serum derived from blood samples collected at sacrifice from experimental and sham-exposed animals are shown in Table 9. These

parameters did not undergo changes in the experimental as compared to sham-exposed animals. Also serum LH was similar in sham-exposed and experimental animals. In sham-exposed and in animals with the lower field combination, LH exhibited large fluctuations among rats. The variation range in the animals exposed to higher fields was reduced and the average LH level was lower, than in the sham-exposed group. However such a difference was not statistically significant.

## DISCUSSION

All measurements were carried out in rats exposed for 8 months to EMF, that is about one-third of their life span. The field strength levels used for this study (5  $\mu$ T - 1 kV/m, 100  $\mu$ T - 5 kV/m) cover the range of values detected close to power lines and in some occupational settings [Mader et al., 1990; Kavet et al., 1992; Sobel et al., 1995]. Studies carried out with realistic field strength conditions and with prolonged exposure times like those in the present work are scanty [Kato et al., 1994; Smith et al., 1994; Margonato et al., 1995]. The condition of constant illumination was adopted in order to minimize the physiological role of melatonin and therefore to emphasize the possible effects due to EMF. Under these conditions the melatonin levels in serum were shown to be reduced to constant values in the order of 10 pg/ml [Brown et al., 1983].

Body weight, hematologic, and hematochemical determinations were carried out at day 0 and 12, 24, and 32 weeks thereafter. At sacrifice, blood, bone marrow, testes, liver, heart, mesenteric lymph nodes, brain, and pineal gland samples were collected for gross anatomy, histology, and biochemical determinations. Most emphasis was put into the evaluation of brain functional alterations as assessed by neurochemical measurements, in consideration of the growing interest for possible changes in brain activity generated by EMF exposure. The obtained results shall be discussed analytically.

### Neurotransmitters and Receptors

In the present study like in a previous one from this laboratory [Margonato et al., 1995] no changes in NE, DA, 5-HT and their metabolites were found in all investigated brain areas. The increase in NE concentration in the pineal gland appears to be characterized by a dose-effect response (see Table 4). This finding could be interpreted as a homeostatic response of the NE system toward the depression of the pineal activity generated by the EMF exposure. According to this hypothetical mechanism, the pineal function would be kept constant by an increased NE synthesis. This effect

**TABLE 5. Microscopic Alterations (Number of Rats Affected/Total)<sup>a</sup>**

Location	sham	1kV/m-5 μT	5kV/m-100 μT
Liver			
Biliary duct proliferation	11/41	14/45	10/43
Focal necrosis	1/41	3/45	3/43
Steatosis	1/41	0/45	1/43
Lymphocyte infiltrate	8/41	17/45	12/43
Heart			
Interstitial infiltrate	5/41	6/45	7/43
Mesenteric lymph nodes			
Edema	6/41	4/45	7/43
Histiocytosis	9/41	8/45	8/43

<sup>a</sup>Number of rats with microscopic alterations as a fraction of the total number of field- and sham exposed animals after a 32-week exposure.

**TABLE 6. Number of Testes with Microscopic Alterations<sup>a</sup>**

Size of lesion (%)	sham	1kV/m-5 μT	5kV/m-100 μT
1-5	12	17	15
25-30	1	1	0
50-80	1	0	2
Total	14/41	18/45	17/43

<sup>a</sup>The fraction of the altered tissue was monitored observing 20 slices from each testis. Values indicate the size of lesions as percentage of the whole organ and the relative incidence of the pathology in each group. The bottom line indicates the number of abnormal testes over the total within each group.

appears to be specific, since 5-HT was not influenced by this treatment.

The D2 receptor density was not affected by EMF exposure, however in a previous study [Hagino et al., 1992] a delay of the development of D2 receptors in newborn rats exposed to 50 μT magnetic field was found.

The large variability in the brain distribution of μ-opioid receptors confirms previous reports [reviewed by Mansour et al., 1986]. On some of the brain areas under investigation the exposure to electromagnetic fields had some effects on the density of μ-opioid receptors. The low field level depressed the number of μ receptors in the frontal cortex, while inducing an increase of the receptors in the parietal cortex and in the hippocampus. The high field did not affect the number of μ receptors in the frontal cortex and the hippocampus, but induced a decrease (i.e. an effect opposite to that displayed by the low field) in the parietal cortex. These variations do not seem to be due to erratic values, considering the high number of animals tested for each field and the low levels of the standard errors of the means derived. A failure of the assay method utilized also seems to be ruled out, the internal standards uti-

**TABLE 7a. Bone Marrow<sup>a</sup>**

Variables	sham	1kV/m-5 μT	5kV/m-100 μT
Fat tissue			
Mild	5/45	4/43	7/41
Moderate	13/45	10/43	8/41
High	27/45	29/43	26/41
Megakaryocytes			
Mild	4/45	1/43	3/41
Moderate	37/45	37/43	35/41
High	4/45	5/43	3/41
Eosinophils			
Mild	26/45	22/43	18/41
Moderate	17/45	18/43	23/41
High	2/45	3/43	0/41
Mast cells			
Mild	22/45	22/43	19/41
Moderate	21/45	15/43	17/41
High	2/45	6/43	5/41

<sup>a</sup>Evaluation of the amount (mild, moderate, high) of fat tissue, megakaryocytes, eosinophils, and mast cells in bone marrow. Data are given as fractional value from each sham-exposed and experimental group.

**TABLE 7b. Bone Marrow<sup>a</sup>**

Cell type	sham	1kV/m-5 μT	5kV/m-100 μT
Erythroid cells			
5 < % < 25	0/45	2/43	4/41
25 < % < 50	45/45	41/43	37/41
Myeloid cells			
25 < % < 50	41/45	32/43*	28/41*
50 < % < 75	4/45	11/43*	13/41*
Lymphocytes & Plasma cells			
% < 5	4/45	4/43	0/41
5 < % < 25	39/45	39/43	37/41
25 < % < 50	2/45	0/43	4/41

<sup>a</sup>Distributions in bone marrow of the different cells observed in each group corresponding to each range of densities.  $\chi^2$ -test: \* $P < 0.05$ .

lized for the validation of the method did not show any alteration of the procedure during the analysis of the brain samples.

On the other hand different and even opposite effects of the two fields on the population of μ receptors present in the same brain area is not particularly surprising in the light of the results obtained in other laboratories. For example these results are reminiscent of the findings from the group of Kavaliers. These investigators have reported that short term exposure to low-intensity magnetic fields reduces the analgesic effects of opiates administered to molluscs and rodents [Kavaliers and Ossenkopp, 1991] The “hypoalgesic” effect of low-intensity magnetic fields reverts to an “analgesic” influence if experimental animals are submitted repeatedly to the same magnetic field [Kavaliers

TABLE 8. Mean Values  $\pm$  S.E.M. of Blood Variables<sup>a</sup>

Variable	0 weeks			12 weeks		
	sham	1kV/m-5 $\mu$ T	5kV/m-100 $\mu$ T	sham	1kV/m-5 $\mu$ T	5kV/m-100 $\mu$ T
n	16	14	18	54	56	56
RBC ( $10^6 \mu$ l)	10.2 $\pm$ 0.75	9.8 $\pm$ 0.75	10.2 $\pm$ 0.45	10.2 $\pm$ 0.23	9.4 $\pm$ 0.16	9.4 $\pm$ 0.12
VPRC (%)	43.5 $\pm$ 0.72	44.9 $\pm$ 0.59	44.2 $\pm$ 0.65	45.2 $\pm$ 0.35	45.8 $\pm$ 0.31	44.9 $\pm$ 0.65
Hb (g/dl)	15.1 $\pm$ 0.22	15.2 $\pm$ 0.32	15.4 $\pm$ 0.30	15.4 $\pm$ 0.50	15.5 $\pm$ 0.12	15.4 $\pm$ 0.15
WBC ( $10^3/\mu$ l)	15.2 $\pm$ 0.92	13.6 $\pm$ 0.90	14.9 $\pm$ 0.75	18.6 $\pm$ 0.67	16.4 $\pm$ 0.52	15.9 $\pm$ 0.49

<sup>a</sup>Red blood cell counts (RBC), volume of packed red cell (VPRC), hemoglobine concentration (Hb) and white blood cell counts (WBC) in exposed and sham exposed rats (n = number of animals) are shown at different times of exposure.

and Ossenkopp, 1993]. It is tempting to conclude that the opposite effects of the magnetic fields reflect a rearrangement of the binding characteristics of endogenous opioid populations according to the time length, i.e. the “dose” of exposure to the magnetic field. Also in line with this view are results derived from a different context. It has been reported that different doses of the same sex steroid have different effects on the number of  $\mu$ -opioid receptors in the same discrete brain areas of castrated female rats [Dondi et al., 1992].

If applicable to humans, the present results may present a hint in favour of the existence of a cause-effect relationship between EMF exposure and neurological disorders. Of course, a pre-requisite for being extrapolation of the present results to humans would be normalization for body mass of the effects of the fields. Moreover, it must be pointed out that at present, the mechanisms by which EMF might interfere with the functions of neurotransmitters and receptors of the CNS are unknown.

**Body weight.** Average body weight increased rapidly during the first 7 weeks of exposure, then the growth rate leveled off in all animal groups. At the end of the experiment, the mean body weight was identical in experimental and sham-exposed animals. The results are in agreement with the data previously obtained in our laboratory in rats exposed to either electric [Margonato et al., 1993] or magnetic fields [Margonato et al., 1995]. In monkeys exposed to 0.2 mT and 20 kV/m at 76 Hz an enhanced growth rate was found [Grisset, 1979]. Alterations in growth rate were found by Marino [1990] in mice exposed to 60 Hz (0.5–100 kV/m).

**Necropsy.** Gross morphological alterations were equally distributed between exposed and non-exposed animals. Microscopic examination revealed alterations typical for rats of the strain used for this study. The present data are in substantial agreement with those from previous investigations by our group [Margonato et al., 1993; Margonato et al., 1995].

The microscopic examination of the testes evi-

denced no significant changes between groups. The altered fraction of the organ ranged from 1-5% of the whole mass. A low incidence of large size lesions was observed in experimental or sham-exposed animals.

The cytologic examination of bone marrow did not evidence abnormal differentiation. Only a slight increase of myeloid cells in exposed groups could be documented. Although the increase was statistically significant ( $P < 0.05$ ,  $\chi^2$ -test), the cell number may be considered within the physiological range [Jain, 1986]. Similar conclusions were drawn also in previous investigations from our laboratory [Margonato et al., 1993; Margonato et al., 1995]. It is noteworthy, however, that in the latter studies, the analysis of the bone marrow was performed by a different counting procedure.

**Hematology and Serum chemistry.** Previous epidemiological studies involving both electric and magnetic fields occupational exposure have shown an increased risk of leukemia [Garland et al., 1990; Bastuji-Garin et al., 1990]. In the present investigation no statistical significant difference was found between the two experimental groups and the sham-exposed animals in RBC, WBC, VPRC and hemoglobin. Moreover no leukemic cells were detected in blood, which is in agreement with our previous study [Margonato et al., 1995]. Uric acid, cholesterol, and alkaline phosphatase values in serum are not influenced by the two EMF exposure combinations. No changes in serum LH concentration were observed. The latter finding is compatible with the absence of plasma testosterone changes described by Kato et al. [1994] in rats exposed to magnetic fields of similar strength. Indeed, testosterone release is mainly controlled by LH.

## CONCLUSION

The present study shows that chronic EMF exposure of adult rats to electric and magnetic fields at an intensity comparable, after body mass scaling, with that to which humans are exposed in industrial settings may

TABLE 8. Continued

Variable	24 weeks			32 weeks		
	sham	1kV/m-5 $\mu$ T	5kV/m-100 $\mu$ T	sham	1kV/m-5 $\mu$ T	5kV/m-100 $\mu$ T
n	54	56	56	16	16	16
RBC ( $10^6$ $\mu$ l)	9.0 $\pm$ 0.16	8.4 $\pm$ 0.12	8.4 $\pm$ 0.93	9.4 $\pm$ 0.37	9.00 $\pm$ 0.35	8.60 $\pm$ 0.25
VPRC (%)	47.0 $\pm$ 0.43	46.1 $\pm$ 0.33	46.2 $\pm$ 0.49	45.6 $\pm$ 1.40	44.9 $\pm$ 0.65	45.6 $\pm$ 1.02
Hb (g/dl)	14.8 $\pm$ 0.19	14.9 $\pm$ 0.12	15.0 $\pm$ 0.12	15.5 $\pm$ 0.60	14.4 $\pm$ 0.42	14.9 $\pm$ 0.37
WBC ( $10^3$ / $\mu$ l)	15.8 $\pm$ 0.49	14.2 $\pm$ 0.44	14.0 $\pm$ 0.41	15.5 $\pm$ 0.50	14.7 $\pm$ 0.57	14.5 $\pm$ 0.44

TABLE 9. Mean Values of Serum Chemistry<sup>a</sup>

Serum chemistry	sham	1kV/m-5 $\mu$ T	5kV/m-100 $\mu$ T
n	16	16	16
Uric acid (mg/dl)	0.99 $\pm$ 0.10	1.06 $\pm$ 0.09	0.95 $\pm$ 0.08
Cholesterol (mg/dl)	86.0 $\pm$ 5.0	86.0 $\pm$ 3.0	92.0 $\pm$ 5.0
Alkaline phosphatase (IU/liter)	213.0 $\pm$ 18.0	212.0 $\pm$ 13.0	224.0 $\pm$ 12.0
LH (ng/ml)	0.682 $\pm$ 0.502	0.657 $\pm$ 0.511	0.425 $\pm$ 0.179

<sup>a</sup>Endpoints of uric acid, cholesterol, alkaline phosphatase and LH. Mean values of experimental and sham exposed rats (n = number of animals).

induce changes in the level of neurotransmitters and receptors both in the brain cortex and the pineal gland. Should future epidemiological studies confirm an increased risk for neurological disorders, particularly for Alzheimer's disease, in chronic EMF exposed individuals, a study like the present one should be extended to rats exposed for the whole life span and to primates with a variety of exposure protocols. This would allow to gain insight into the possible mechanisms of interaction of EMF and brain functions.

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