# Effect of Extremely Low Frequency Magnetic Fields in Safety Standards on Structure of Acidophilic and Basophilic Cells in Anterior Pituitary Gland of Rats: an Experimental Study

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## Abstract

After discovery of magnetite in the brain of several mammalian species including humans, it was suggested that the human brain is sensitive to magnetic fields. The present study investigated the effect of 100 µT and 500 µT extremely low frequency magnetic fields (ELF-MF), established guidelines by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) for public and occupational magnetic field exposure limits at 50 Hz, on acidophilic and basophilic cells in the anterior pituitary gland of rats. 30 adult male Sprague Dawley rats were separated into three groups in this study (sham group: 10 and two study groups: 20). Rats in the study (experimental) groups separated equally into two groups that is 100 µT and 500 µT exposure groups. First experimental group rats exposed to 100 µT while second experimental group exposed to 500 µT ELF-MF for 2 hours per day (7 days in a week) during 10 months. For the sham group, the same experimental procedure was applied to the rats (2 hours / day / 7 days in a week for 10 months) except ELF-MF exposure. After ELF-MF and sham exposure, the pituitary gland were removed totally to examine the structure of acidophlic and basophilic cells in the anterior pituitary gland of rats as histomorphometric and histochemical. Affinity against dye in acidophilic and basophilic cells of first experimental groups' rat was found lower than sham group. It means that degranulation may occur in sitoplasmic granuls of these cells. Disappearing in the affinity of Periodic Acid-Shchiff (PAS)-Orange-G in acidophilic and basophilic cells of second exposure group has been indicating high disappearing of granuls in these cells. No significant differences were found between sham and exposure groups in terms of the number of acidophilic and basophilic cells in the anterior pituitary gland ($p>0.05$). Although degranulation observed in acidophilic cells, the results were not found to be significant between experimental groups ($p>0,05$). However, degranulated basophilic cells were found significant between experimental groups ($p<0,05$). In conclusion, it was suggested that long-term ELF-MF exposure, which are the limits for public environmental and occupational magnetic field exposure guidelines, can affect affinity of PAS-Orange G in acidophilic and basophilic cells of the anterior pituitary gland in rats.

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## Introduction

The possible association between exposure of humans to extremely low frequency magnetic fields (ELF-MF) and adverse health effects has attracted a great deal of scientific attention. Relation to the mammalian nervous system, exposure to 50/60 Hz electromagnetic fields (EMFs) has been implicated in a wide spectrum of effects[1]. Poole et al. reported an increased incidence of headaches and depression for individuals residing near transmission lines in humans[2]. In animal studies, alterations in central neurotransmitter turnover and pineal function have been observed after 60 Hz MF exposure. [3,4] These reports suggest that neural cells may be capable of transducing power line frequency EMFs into physiological responses[5]. Whether or not long-term ELF-MF exposure can increase risk for the adverse health outcomes is a central issue in the study of possible ELF-MF health effects.

It was reported that MF exposure can give rise to changes in neuroendocrine system function in a mammalian laboratory model[6]. Suppression of melatonin in the blood and pineal of the rat or hamster after magnetic field exposure have been reported by several studies[7-10].

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The anterior pituitary gland regulates the activity of the thyroid, adrenals, and reproductive gland[11]. Among other hormones it produces growth hormone, prolactin, thyrotropin, and corticotrophin. The pituitary also secretes endorphins that act on the nervous system to reduce sensitivity to pain[11,12]. The production and secretion of pituitary hormones can be influenced by factors such as emotions and seasonal changes. To accomplish this, the hypothalamus conveys information sensed by the brain to the pituitary[11,12]. All these features make the pituitary gland a suitable system to study the biological impact of non-ionising radiation[11].

Several organizations have established guidelines for public and occupational exposures to power frequency MF. At 50 Hz, magnetic field exposure limits have been set at 100 µT for public exposure and 500 µT for occupational exposure by the International Commission on Non-Ionizing Radiation Protection (ICNIRP)[13]. Thus, we chose 100 and 500 µT, with the idea that it is a more accurate reflection of the actual values in daily life.

The aim of the study was to ivestigate the effect of long term 100 and 500 µT ELF magnetic field exposure on acidophilic and basophilic structure of the anterior pituitary gland in rats.

## MATERIALS and METHODS

### Subjects and Animal Care

The experiments were performed on 30 male Sprague-Dawley rats obtained from Medical Science Application and Research Center of Dicle University, aged 4 months at the beginning of the study, weighing 300- 390g, and fed with standard pelleted food (TAVAS Inc. Adana, Turkey). The animals were kept in 14/10h light/dark environment at constant temperature of 22 ± 3°C, 45 ± 10% humidity. This protocol was approved by the local ethics committee.

Rats were separated into three groups in this study (sham group: 10 and two study groups: 20). Rats in the experimental groups separated equally into two groups that is 100 µT and 500 µT exposure groups. First experimental group rats exposed to 100 µT while second experimental group exposed to 500 µT extremely low frequency (ELF) magnetic field for 2 hours per day (7 days in a week) during 10 months in a plexiglas cage. For the sham group, the same experimental procedure was applied to the rats (2 hours / day / 7 days in a week for 10 months) except ELF-MF exposure.

### Magnetic field generation and exposure of rat to magnetic field

The MF was generated in a device designed by us that had one pair of Helmholtz coils of 25 cm in diameter in a Faraday cage (130×65×80 cm) that earthed shielding against the electric component. This magnet was constructed by winding 225 turns of insulated soft copper wire with a diameter of 1.0mm. Coils were placed horizontally as facing one another. The distance between coils was 25 cm. An AC current produced by an AC power supply (DAYM, Turkey) was passed through the device.

The current in the wires of the energized exposure coil was 0.12 A for 100 µT and 0.50 A for 500 µT, which resulted 50 Hz MF. The MF intensities were measured once per week as 100 µT and 500 µT in different 15 points of methacrylate cage by using digital teslameter (Phywe, 209101074, Göttingen, Germany) to ensure homogeneity of the field during the course of the experiment. Magnetic field measurements showed that, at the conditions of the experiment, the magnetic field exposure system produced a stable flux density of 100 µT, 500µT and stable frequency of 50 Hz with neglible harmonics and no transients. The 50 Hz stray fields in the sham-exposure system were 0,1 µT. The static earth magnetic field was measured with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL). The component parallel to the exposure field was 14 µT and the component perpendicular to the exposed field was 34 µT. All field measurements were performed by persons not involved in the animal experiments. Observers were not aware of which group of rats was ELF Magnetic Field-or sham-exposed, i.e. the whole study was done blind. No temperature differences were observed between exposure and sham coils during the exposure.

The first and second experimental groups were exposed to 100 µT and 500 µT ELF MF during 10 months, 2 h a day respectively. Third group was sham that were treated like experimental group except ELF-MF exposure (corresponding to first and second groups, respectively) in methacrylate boxes. The rats were free in methacrylate cage inside the coils. After ten months of MF exposure, the study was terminated. Immediately after the last exposure, blood of the animals was collected by cardiac puncture under ketamine anesthesia (100 mg/kg, intramuscularly) to kill rats and pituitary gland of the rat was removed for histopathological evaluation.

**Histological procedure**

Pituitary glands taken from the rats were fixed respectively in formaldehyde (10%) for histological assessment. After routine histological processing, the tissues were embedded in paraffin, sectioned, and stained with PAS-orange G. The histochemical stains used for specific chemical groups, such as the Periodic Acid-Shchiff (PAS) reaction for carbohydrates of glycoproteins[14].

Histological assessments were performed with a light microscope (Nikon eclipsse-400, colpix-4500), which has digital camera atachment. In histological evaluations, It was examined the structure of acidophil and basophil cells that were pars distalis of the anterior pituitary gland. Both normal and degranulated forms of acidophil and basophil cell were performed with an object lens[40] in 1 $mm^2$ field.

It was determined statisticaly significant alteration between exposure groups by counting degranulated cells.

**Statistical Analysis**

Data were analyzed by Kruskal–Wallis one-way analysis of variance ANOVA on ranks and post-hoc tests using Tukey and LSD and using Mann-Whitney U test. All hypothesis tests used a criterion level of $\alpha = 0.05$.

**Results**

Polygonal and round shaped acidophilic and basophilic cells were observed around of sinusoids of the anterior pituitary gland in sham group. In this group, It was examined granuls, which were stoplasm of acidophilic and basophilic cells, stained clearly with PAS-orange G. We observed that acidophilic cells were high affinity against PAS-orange G stains, stained as orange-G positive and basophilic cells was PAS positive (Fig. 1).

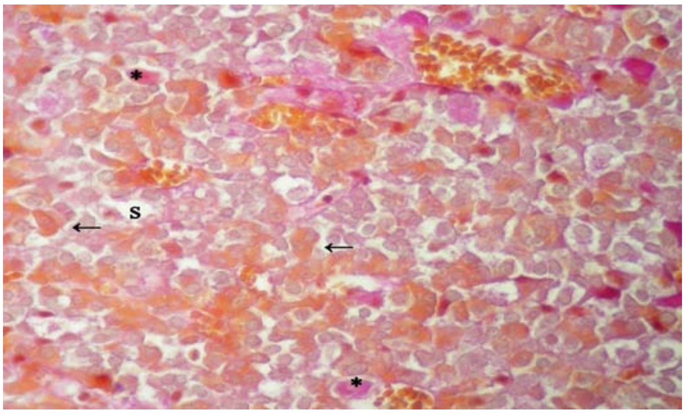

**Fig. 1** The appearance of the anterior pituitary gland in sham groups' rats. Acidophilic cells (→), Basophilic cells (*), sinusoid (s). (PAS-Orange G, ×40).

Histochemical staining of acidophilic and basophilic cells in the first experimental group was significantly found different than sham group. Affinity against dye in acidophilic and basophilic cells of first experimental groups' rat was found lower than sham group. It means that degranulation may occur in sitoplasmic granuls of these cells ( Fig. 2).

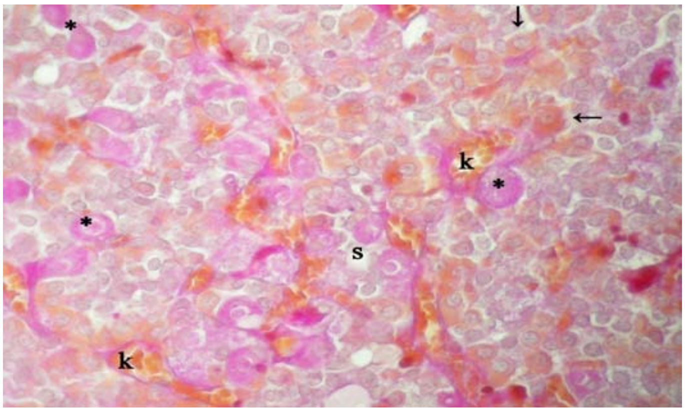

**Fig. 2** The appearance of the anterior pituitary gland in first experimental groups' rats. Acidophilic cells (→), Basophilic cells (*), sinusoid (s), capiller (k). (PAS-Orange G, ×40)

On the other hand, shrinking in sinusoids and disordering in cell arrangement was also observed in the first exposure group.

Disappearing in the affinity of PAS-Orange-G in acidophilic and basophilic cells of second exposure group has been indicating high disappearing of granuls in these cells (Fig. 3).

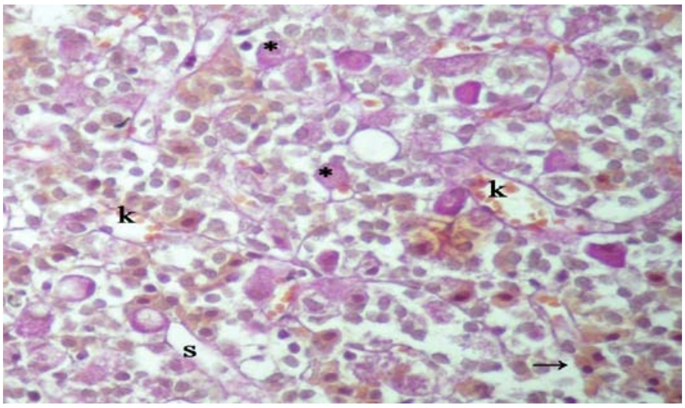

**Fig. 3** The appearance of the anterior pituitary gland in second experimental groups' rats. Acidophilic cells (→), Basophilic cells (*), sinusoid (s), capiller (k). (PAS-Orange G, ×40)

Acidophilic and basophilic cells of the anterior pituitary gland in the second experimental group were more affected than first experimental group. No granuls observed in the sitoplasm of the second exposure group. However, restricted sinusoids and only nucleus were observed in the sitoplasm of the second exposure group. The numbers of asidophilic and basophilic cells in the anterior pituitary gland were presented in the table 1. No significant differences were found between sham and exposure groups in terms of the number of acidophilic and basophilic cells in the anterior pituitary gland (p>0.05). Although degranulation observed acidophilic cells, the results were not found to be significant between exposure groups (p>0,05). By the way, degranulated basophilic cells were found significant between exposure the groups (p<0,05) (Tab. 1).

| Groups | Total Basophilic Cells Mean±SE | Total Asidophilic Cells Mean±SE | Degranulated Basophilic Cells Mean±SE | Degranulated Asidophilic Cells Mean±SE |
|---|---|---|---|---|
| Sham | 7,04±0,49 | 33,58±0,56 | - | - |
| First Experimental Group | 7,34±0,37 | 33,38±0,85 | 3,44±0,24[a] | 16,86±0,72 |
| Second Experimental Group | 8,82±0,86 | 33,22±1,16 | 5,90±0,13[a] | 18,50±0,54 |

**Tab. 1** The counts and degranulation situation of basophilic and acidophilic cells of pars distalis in the anterior pituitary gland. [a] *P*<0.05 as compare to exposure groups by Mann-Whitney-U test.

## Discussion

The brain and central nervous system are considered to be among the most likely sites of interaction between biological systems and power-frequency fields.[15] Although the mechanisms of the influence of these fields on nervous tissue remain mostly unknown, studies in relation to the effect of ELF-MF on brain and nervous system have been continuing to explain interaction mechanisms.

Barbier et al. studied the effect of exposure of single rat pituitary cells to 50 Hz sine wave magnetic fields of various strengths on the intracellular free $Ca^{2+}$concentration[16]. They have concluded that a 50 Hz, 50 µT magnetic field can markedly affect endocrine cell physiological processes[16]. Espinar et al. showed that static magnetic field can induce irreversible developmental effects on the processes of cell migration and differentiation of the chick cerebellar cortex[17]. Wilson et al. investigated the effects of 60 Hz magnetic field exposure on the pineal and hypothalamic-pituitary-gonadal axis in the Siberian hamster[18]. They indicate that both one-time and repeated exposure to a 0.1 mT, 60 Hz MF can give rise to neuroendocrine responses in the Siberian hamster[18]. Boland et al. have shown that neither continuous nor intermittent EMF exposure is able to modify cell death of cultured hipocampal cells[19]. Jelenkovic et al. investigated the possible effect of ELF-MF (50 Hz, 0,5 mT) on the brain of adult male Wistar rats following a 7-day exposure[20]. According to their results, they indicate that a 7-day exposure to extremely low-frequency magnetic field can be harmful to the brain, especially to the basal forebrain and frontal cortex due to development of lipid peroxidation[20]. Zecca et al. investigated the biological effects of prolonged exposure to ELF electromagnetic fields in rats[21]. In their study, groups of adult male Sprague Dawley rats were exposed for 8 months to electromagnetic fields (EMF) of two different field strength combinations: 5µT - 1kV/m and 100µT - 5kV/m[21]. According to their findings, they suggested that EMF may cause alteration of some brain functions[21]. It has been reported that prolonged exposure of extremely low frequency electromagnetic radiation attenuates the circadian nocturnal rise of melatonin in the pineal gland[22,23]. Canedo et al. showed that short MF exposures had a long term effect on cerebral cortex 5-HT and no long term effect on the pineal gland levels of 5-HT, a precursor of melatonin[24]. Cook et al. suggests that weak magnetic fields can affect the infiltration of immunologically responsive cells and the presence of mast cells in brain parenchyma[25]. Lisi et al. showed that exposure to 50 Hz ELF-EMF is responsible for the premature differentiation in pituitary corticotrope-derived AtT20 D 16 V cells[11]. In a toxicological studies, some authors investigated the effects of magnetic field on brain tissue. For instance, In a recent study, Kim et al investigateted the effect of 20 kHz triangular magnetic field on Sprague-Dawley rats. They found no histopathological alterations on rats' brain tissue after exposure to 20 kHz triangular magnetic field[26]. Robertson et al. investigated potential health effects of 10 kHz magnetic fields on mice in a toxicological study. However, they found no pathology in brain of mice after magnetic field exposure[27].

In the present study, the results describes the effect of long-term ELF-MF exposure on acidophilic and basophlic cells in the anterior pituitary gland in rats. It was determined dose-response interaction in relation to histopathologic alterations in acidophilic and basophilic cells. The results indicate that long-term 100 and 500 µT magnetic field exposure can affect affinity of PAS-Orange G in acidophilic and basophilic cells. It was also observed that 500 µT magnetic field was effective dose that affect affinity of PAS-Orange-G and granuls of cytoplasm in acidophilic and basophilic cells. However, acidophilic and basophilic cell counts were not alter after long-term 100 and 500 µT magnetic field exposure.

There are many studies in relation to the effect of ELF-MF on brain and electrophysiological interaction. However, no studies have been reported in the literature considering the effects of ELF-MF on acidophilic and basophilic cells of rat anterior pituitary gland. Therefore, we couldn't compare the results of the present study with other studies.

Although acidophilic cells secretes GH and prolactin hormones, basophilic cells secretes FSH, LH, TSH and ACTH hormones.[28] In the present study, it was determined significant difference in degranulated basophilic cells of rats that exposed to 100 and 500 µT ELF-MF (p<0,05). Degranulation in basophilic cells may cause deficiency in FSH, LH, TSH and ACTH hormones. This deficiency may cause functional disturbance in target organs depending on hormonal alterations.

## Conclusions

In conclusion, it was suggested that long-term ELF-MF exposure, which are the limits for public environmental and occupational magnetic field exposure guidelines, can affect affinity of PAS-Orange G in acidophilic and basophilic cells of the anterior pituitary gland in rats. It was also determined that 500 µT magnetic field was effective dose that affect affinity of PAS-Orange-G and granuls of stoplasm in acidophilic and basophilic cells. A detailed and molecular studies must be carried out to explain interaction mechanisms between ELF-MF and nervous system especially in relation to acidophilic and basophilic cells in the anterior pituitary gland.

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