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The Effects of 900 Megahertz Electromagnetic Field Applied in the Prenatal Period on Spinal Cord Morphology and Motor Behavior in Female Rat Pups

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ABSTRACT

This study investigated the effect of a 900 megahertz (MHz) electromagnetic field (EMF) applied in the prenatal period on the spinal cord and motor behavior of female rat pups. Beginning of the study, female Sprague Dawley rats (180–250 g) were left to mate with male rats. Rats identified as pregnant were then divided into control (n=3) and EMF groups (n=3). The EMF group was exposed to 1-h 900 MHz EMF daily between days 13 and 21 of pregnancy. At 21 days old, rat pups were removed from their mothers and divided into two newborn rat groups, control (n=13) and EMF (n=10). The rotarod test was applied to the rat pups to assess motor functions and the open field test to evaluate locomotor activity. On day 32 of the study, the rat pups were decapitated, and the spinal cord in the upper thoracic region was removed. Following routine histological tests, they were stained with Cresyl fast violet. Rotarod test results revealed a significant increase in EMF group rat pups' motor functions ($p=0.037$). However, no difference was observed in the open field test results ($p>0.05$). In the EMF group' rat pups, we observed pathological changes in the spinal cord. On the basis of our results, 900 MHz EMF applied in the prenatal period affected spinal cord development. This effect was observed in the form of pathological changes in the spinal cord of rat pups, and it may be that these pathological changes led to an increase in rat pups' motor activities.

Key Words: female rat, electromagnetic field, spinal cord, motor behavior

NeuroQuantology 2013; 4:573-581

Introduction

The mobile phone has become an indispensible communication tool. It is also used as a means of entertainment and spending free time, particularly for children. However, how reliable mobile phones are in safety terms is still unknown. Mobile phones and health problems they may cause are therefore the subject of intense research among scientists (Barcal and Vozeh, 2007; Odaci *et al.*, 2008; Wigle *et al.*, 2008; Bas *et al.*, 2009a; 2009b; Sonmez *et al.*, 2010; Feychting, 2011). Studies have reported, for instance, that high-frequency EMF damages sleep quality (Borbely *et al.*, 1999), may lead to low birth weights (Mortazavi *et al.*, 2013), and to behavioral disorders in children (Divan *et al.*, 2008) and young people (Divan *et al.*, 2012) and may cause headache in children (Sudan *et al.*, 2012) and adults (Frey, 1998).

The spinal cord is the main pathway for information connecting the brain to the peripheral nervous system, and made from part of the neural tube during development. The neural tube caudal to the fourth pair of somites develops as the spinal cord. The lateral walls of

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This study was presented as poster presentation at the Controversies on Electromagnetic Field in Medicine and Biology Symposium, Turkey, Samsun, June 17 2013.
Received: September 13, 2013; **Revised:** October 20, 2013;
Accepted: December 1, 2013
***e*ISSN** 1303-5150

the neural tube thicken. Meanwhile the lumen slowly decreases in size until only a very narrow central canal is present in the human embryo spinal cord at 9 weeks (Moore and Persaud, 1993). Like other nervous system structures, the development of the spinal cord is influenced by various factors. Throughout development, a highly sensitive period, the normal morphology of the spinal cord may be compromised under the effect of various external and internal environmental factors, and associated function losses are highly probable (Adams, 1989; Au *et al.*, 2010; Wallingford *et al.*, 2013). Studies suggest that EMF may be one such factor, and that with other nervous system structures developing at the same time being affected by 900 MHz EMF (Odaci *et al.*, 2008; Bas *et al.*, 2009a; 2009b), the spinal cord may also be affected. Normal development compromise may therefore ensue. Since the spinal cord is the basic pathway for information connecting the brain to the peripheral nervous system (Moore and Persaud, 1993), pathology that may arise in the spinal cord may lead to behavioral changes by causing problems in the exchange of information between the brain and the peripheral nervous system.

In the light of studies reporting morphological changes in the cerebral structures of rat pups exposed to 900 MHz EMF throughout pregnancy and behavioral impairments in rat pups associated with these changes (Odaci *et al.*, 2008; Bas *et al.*, 2009a; 2009b; Hancı *et al.*, 2013; Hao *et al.*, 2013), it is probable that morphological changes and associated behavioral impairments will also occur in the spinal cords of pups of mothers exposed to the same effect. The purpose of this study was therefore to investigate whether any postnatal changes in spinal cord morphology and motor behavior occurred in pups obtained from mothers exposed to the effect of 900 MHz EMF between days 13 and 21 of pregnancy.

Materials and methods

Study design

Karadeniz Technical University School of Medicine Animal Ethical Committee was obtained before the study began. Animal experiments and procedures were compatible with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sixteen female rats (6-8 weeks old, weighing 180-250 g) were first obtained from the KTU Surgery Research Center (KTUSRC). These were housed in the KTUSRC in standard plastic cages on sawdust bedding in an air-conditioned room at 22 ± 1 ^{o}C under controlled lighting (12 h light/12 h dark cycle). Standard rat chow and tap water were provided ad libitum.

Animals and groups

Each of the 16 female rats exhibiting two regular cycles was then placed a cage with a male rat for mating purposes. Pregnant rats were identified using vaginal smears on the following day. Female rats with sperm in their smear specimens were classified as pregnant, and that day was taken as day 0 of pregnancy. Six pregnant rats were divided at random into two groups of three rats each. The first group was classified as the control group (CG). No procedure was performed on these animals throughout pregnancy. The second group of pregnant rats was classified as the EMF group (EMFG). These rats were placed inside a Plexiglas jar at the same time every day between the 13th and 21st days of pregnancy and exposed to 900 MHz EMF for 1 h. The groups were maintained in separate cages in the same room during the experiment, apart from during exposure to EMF, which took place in the EMF exposure room. Rats were placed in separate cages before giving birth, and pups were left to feed naturally with their own mothers in the same cages. No procedure was applied to the newborn rats postnatally. The study proceeded with 13 rat pups obtained from the control group mothers and 10 pups from the experimental group. Female pups obtained from control group mothers were classified as the newborn control group (NCG) and female pups from EMF group mothers as the newborn electromagnetic field group (NEMFG). Female rat pups were kept with their mothers in order to feed naturally in the same cages until the 21nd day postnatally. They were then removed from their mothers and placed in separate cages. For four days after separation from their mothers they were kept under the same laboratory conditions, and were given only rat chow and tap water. During this period rat pups' anxiety at separation from their mothers was allowed to subside (Savignac *et al.*, 2011), and no procedures were performed on the animals. When the rats were 26 days old, rotarod tests to assess motor functions and open field tests to evaluate locomotor activities were started.

EMF exposure system

A glass jar (made of Plexiglas with dimensions of 30 cm X 42 cm X 52 cm) was manufactured especially for the study. Pregnant rats were placed inside the jar and exposed to 900-MHz EMF for 1 h using an ultra-high-frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483). The oscillator was supplied with a constant power source (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903) with output power of approximately 300 mW and a frequency adjusted to 900-MHz. This was then secured to a half-wave dipole antenna made from a copper rod (1 mm x 15 cm) by means of a coaxial cable. The antenna was inserted into the central area, approximately 11 cm inside the open surface of the jar. Calculations established that rats were exposed to a mean electrical field intensity of 10 V/m inside the jar. This is equivalent to the intensity of the electrical field emitted by mobile phones in speak mode in their immediate surroundings (Bas *et al.*, 2009a;2009b; Odaci *et al.*, 2008; Sonmez *et al.*, 2010; Hancı *et al.*, 2013).

Open field test

The open field apparatus was made of white wood, with the floor divided into 16 equal squares, an open top and dimensions of 100 cm x 100 cm x 30 cm **(Figure 1A)**. Rats were placed in the apparatus from a predetermined corner and observed for 5 min **(Figure 1B)**. In order to assess each animal's motor activity, the number of squares crossed and numbers of rearings (inquisitive investigation of surroundings by rising onto its hind legs) were recorded. Number of defecations was also recorded in order to assess rats' anxiety. In order to prevent the scent of the previous animal affecting the behavior of the subsequent one, the device was cleaned with 30% alcohol. All data were recorded with a camera (Abbas *et al*, 2009; Gulec *et al.*, 2010; Franke *et al.*, 2001).

Rotarod Test

To analyze the effects of 900-MHz EMF exposure on motor coordination and balance skills we used the accelerating rotating rod test (Biopac, May RR 9701 Animal Rota Rod, India) **(Figure 1C)** (Kim *et al.*, 2012; Haghani *et al.*, 2013). A 7.2-mm-thick rotarod was used. The rotarod was applied to all groups at speeds of 4 rpm and 12 rpm. The test was performed in two stages. In the first stage, rats were familiarized with the rotarod device applied at 4 rpm for 1 min. This process was repeated three times at 10-min intervals. In the second stage, after rats had been placed on the rotarod moving at 4 rpm, the speed was raised to 12 rpm. The procedure continued until the rats fell off, and time to falling was recorded. For those rats that did not fall, the procedure was terminated after waiting 300 s. Falling time was then recorded as 300 s. This procedure was repeated three times at 15-min intervals (Kim *et al.*, 2012). After every procedure, the equipment was cleaned with 30% alcohol in order to eliminate such factors as animal scent, slipperiness and surface dirt. Rats were weighed before and after the procedure.

Histological procedures

At the end of the locomotor activity, anxiety and motor function tests (postnatal 32st day), NCG and NEMG rats were sacrificed on the same day by decapitation under deep anesthesia (Ketalar 50 mg/kg). The upper thoracic region of each animal's spinal cord was then removed and placed in 10% formaldehyde. After being stored in formaldehyde for 1 day, spinal cords were taken for decalcification. Tissues kept in decalcification for 4 days were subjected to routine histological tissue examination and fixed in paraffin after being kept in running tap water overnight. Spinal cords fixed in paraffin were sliced into sections 30 microns thick with the help of a fully automatic microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) and stained with cresyl violet. They were then investigated histopathologically under a research microscope (Olympus, BX51, Japan), and photographs were taken.

Statistical analyses

The non-parametric Mann-Whitney U test (Tunc *et al.*, 2007) was used to compare test results between the groups. Mean values are presented with their standard error means (SEMs), and they were considered to be significantly different when $p<0.05$. All statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SSPS Inc., Chicago, IL, USA).

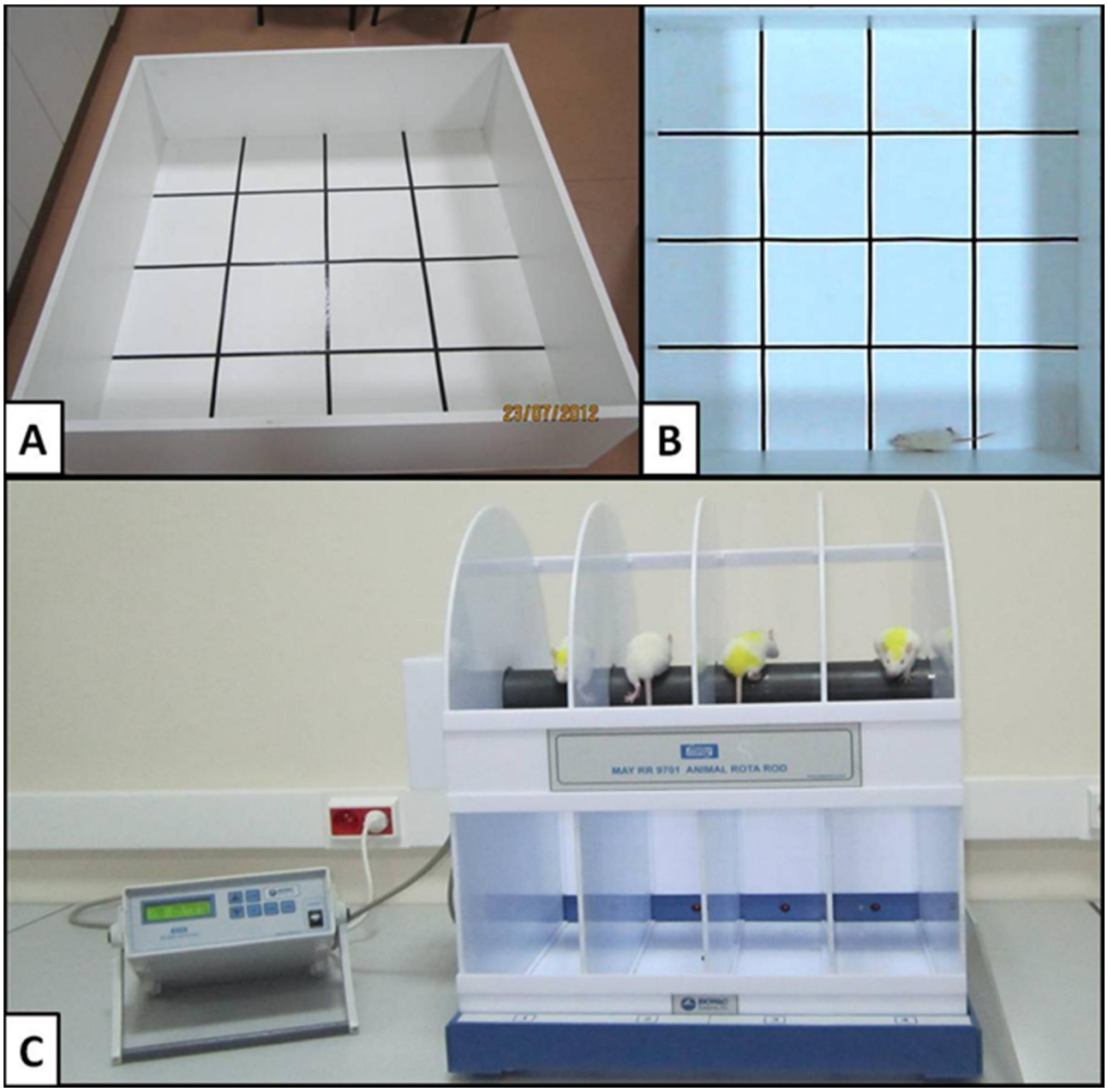


Figure 1. The open field apparatus (A and B) and rotating rod test device (C) are shown. In order to assess each animal's motor activity, the number of squares crossed in the open field apparatus and numbers of rearings were recorded. Number of defecations was also recorded in order to assess rats' anxiety. The accelerating rotating rod test was used to analyze study groups' motor coordination and balance skills

Results and Discussion

A statistically significant increase was observed in NEMFG group rat pup motor functions in the rotarod test applied under the same conditions to both groups of female pups (p=0.037) **(Figure 2).** The open field test was performed in order to assess the effect of EMF on locomotor activity. The number of squares crossed by rats in the test device and the number of rearings onto the hind legs were scored in order to examine motor efficacy and inquisitiveness. Increased defecation numbers during the experiment were regarded as anxiety findings. Statistical comparison of all the parameters obtained from the open field test revealed no significant difference between NCG and NEMFG (p>0.05) **(Table 1).**

No pathology was encountered in NCG at histopathological examination of the sections **(Figure 3 A, B and C)**. In NEMFG rats, however, we observed morphological impairment and atrophy in the spinal cord, vacuolization in gray matter and occasional myelin thickening, white matter infiltration

among the nerve fibers, marginal irregularity between white and gray matter and infiltration of gray matter inside white matter **(Figure 3 D and E)**. We also observed structural impairment in motor neuron pericaryon structure in the NEMFG rat spinal cords **(Figure 4 A and B).**

Table 1. The effects of electromagnetic field on open field test behaviors. Statistical comparison of all the parameters obtained from the open field test revealed no significant difference between newborn control group and newborn electromagnetic group (p>0.05). Values are expressed as mean ± SEM.

	Open field test results	
	Newborn control group	Newborn electromagnetic group
Number of crossed squares	35 ± 8	27 ± 6
Number of rearings	21 ± 4	21 ± 5
Number of fecal boli	2 ± 0.4	2.2 ± 0.5

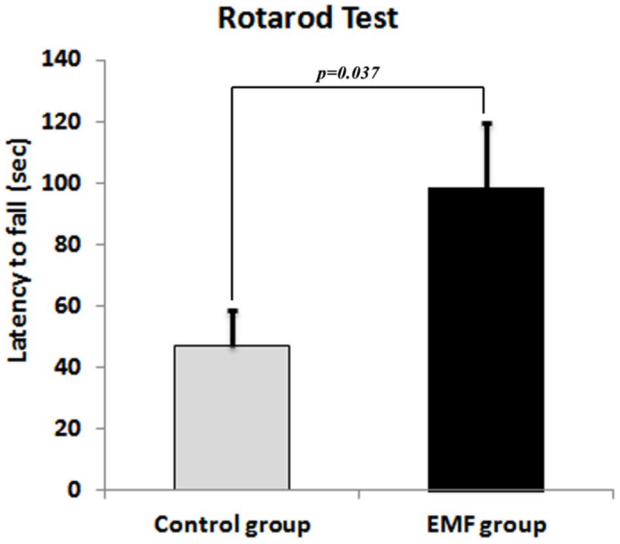


Figure 2. Results of the rotarod test applied to the newborn control and newborn electromagnetic field groups.

Various studies have investigated relationships between prenatal exposure to a range of environmental contaminants (such as chemicals, drugs and EMF) and fetal and child health (Ragbetli *et al.*, 2007; Wigle *et al.*, 2008; Aygün *et al.*, 2012). Experimental and clinical studies indicate that various pathological conditions, including fetal loss, prenatal growth restriction, preterm birth, birth defects and behavior deficits, are associated with fetal exposure to environmental contaminants (Adams, 1989; Wigle *et al.*, 2008; Au, 2010; Mortazavi *et al.*, 2013). Chemical toxicants in air, water and foods, and various consumer products are some of the most important such contaminants (Wigle *et al.*, 2008). One range of consumer products which may have a damaging impact on health are electromagnetic devices such as cell phone. Mobile phones and the effect of EMF emitted by these are a subject attracting considerable interest among researchers (Dubreuil *et al.*, 2002; 2003; Barcal and Vozeh, 2007; Ragbetli *et al.*, 2007; Aygün *et al.*, 2012; Hao *et al.*, 2013).

This study investigated behavioral and morphological changes in female rat pups exposed to 900 MHz EMF on days 13-21 in the prenatal period. Our results show that exposure to the effect of EMF emitted by mobile phones during pregnancy led to changes in newborn female rat pups' behavior and morphological changes in the spinal cord. Developmental periods of organisms from the same species are known to be capable of comparison irrespective of whether they are postnatal or prenatal (Rodier, 1980). On the basis of that information, the development of the rat nervous system in the neonatal period can be compared to the final period of the development of the human system. Our study results therefore suggest that long-term mobile phone use by mothers during pregnancy may affect babies' behaviors and spinal cord morphologies.

Considering the results of the morphology and behavior tests together, EMF applied during pregnancy affected the development of the spinal cord, emerging as pathological changes in the spinal cord morphology, and these pathologies led to behavioral changes. Since the spinal cord is the main pathway for information connecting the brain and peripheral nervous system (Moore and Persaud, 1993), it is highly probable that pathologies that may appear in the spinal cord will emerge in the form of behavioral changes. Our histopathological evaluation of the NEMFG group revealed morphological impairment and atrophy in the spinal cord, vacuolization in gray matter and occasional myelin thickening, white matter infiltration among the nerve fibers, marginal irregularity between white and gray matter, infiltration of gray matter inside white matter, and impaired motor neuron pericaryon structure. The observation of such pronounced pathologies in the spinal cord may be associated with central nervous system development. The central nervous system development period is a critical and sensitive one. The development of

the central nervous system can therefore easily be affected in that period by drugs, inadequate nutrition and harmful environmental agents (Gokcimen *et al.*, 2007; Ozyurt *et al.*, 2011). For example, Odaci et al (2010) suggested that prenatal administration of diclofenac sodium, frequently used to treat dysmenorrhoea and menorrhagia in women of reproductive age, increases the number of Purkinje cells in the cerebellum of a developing female rat thru the 20th postnatal week (Odaci *et al.*, 2010).

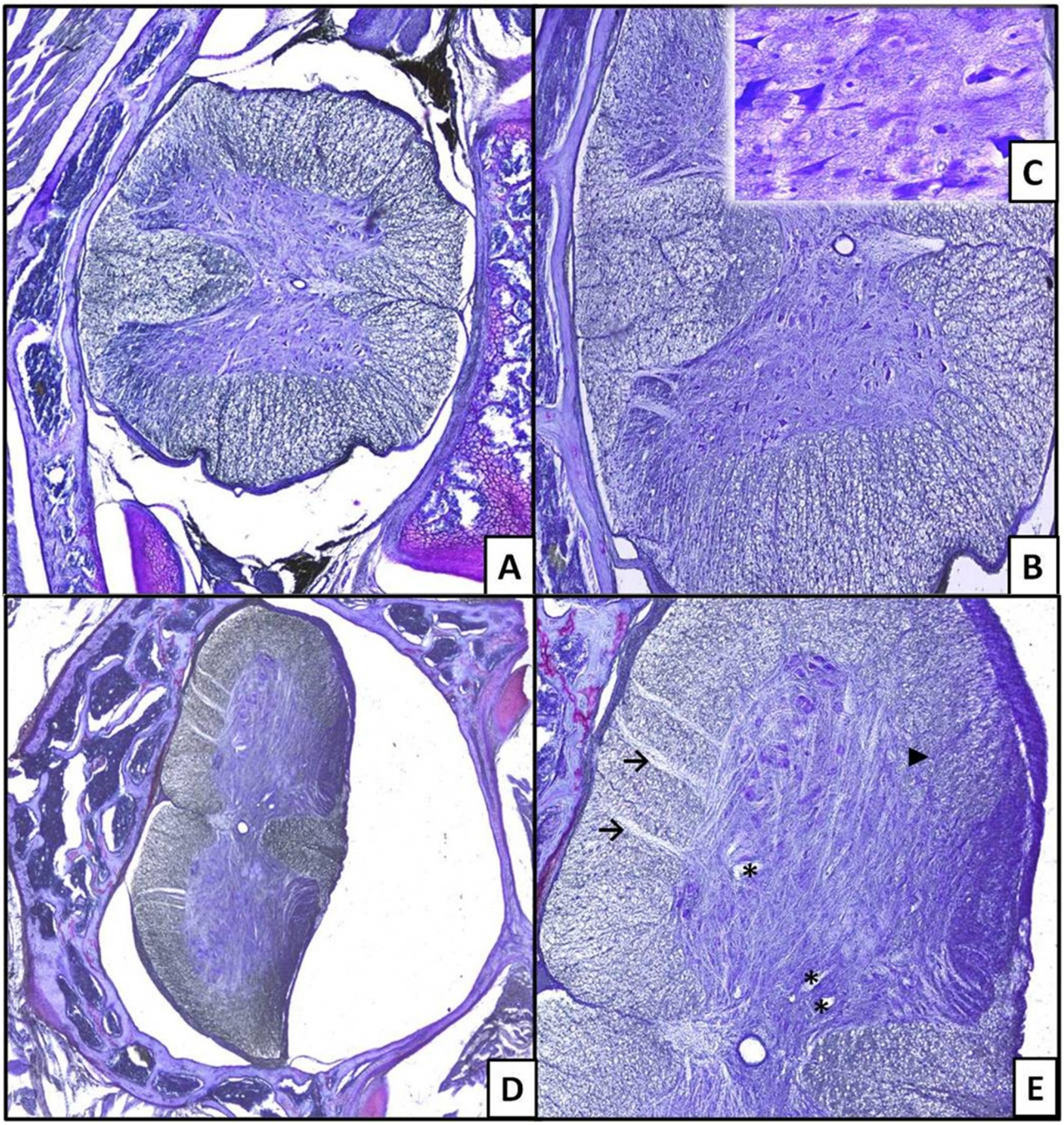


Figure 3. Normal spinal cord anatomical structure was not compromised in the newborn control group (A) and the neurons have a normal appearance (B and C) (Cresyl fast violet, A) X40, B) X100, C) X400). In the newborn electromagnetic field group, however, the anatomical structure of the spinal cord was compromised and atrophy (D), occasional vacuolization (star), infiltration (arrow) and marginal irregularities between gray and white matter (arrowhead) (E) can be seen (Cresyl fast violet, A) X40, B) X 100).

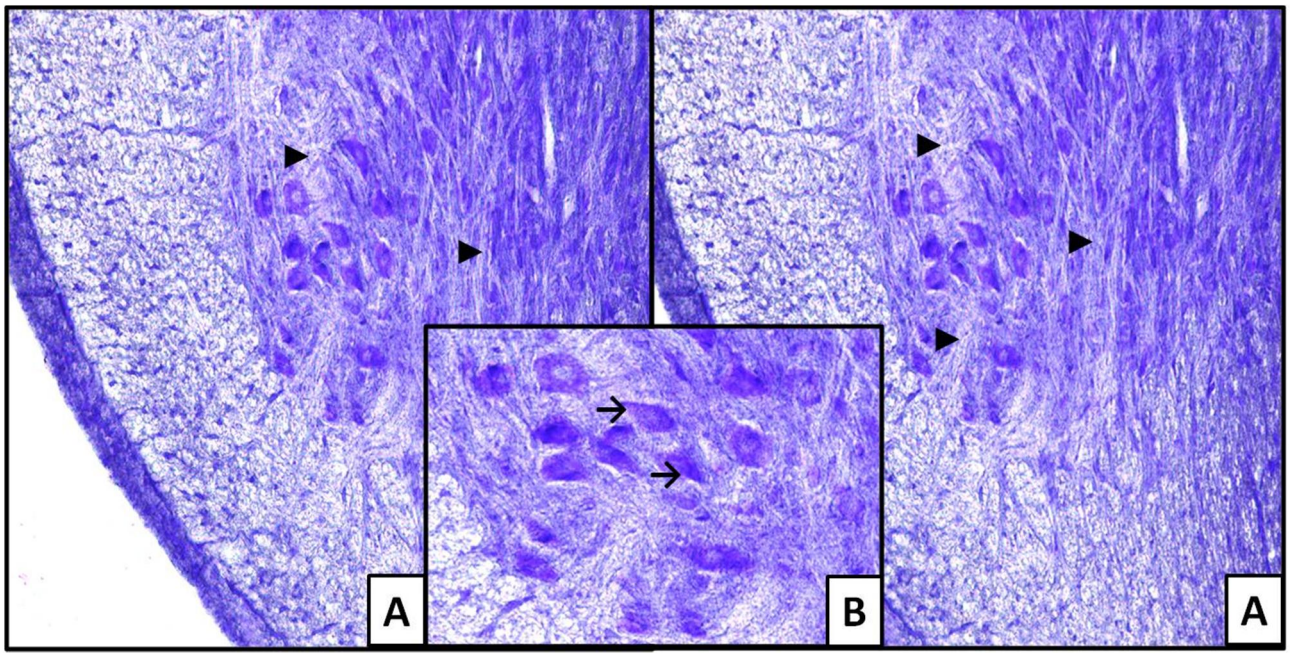


Figure 4. Myelin thickening (arrowhead)in the spinal cords of rat pups in the newborn electromagnetic field group (A) and impairment of motor neuron pericaryon structure (B) (Cresyl fast violet, A) X200, B) X400).

The first group of spinal cord neurons in rats appears on the 10th embryonic day (ED), and very few emerge after ED 15. The earliest groups to form are motor neurons, arising from the basal plate. Somatic motor groups appear earlier than visceral groups. Motor groups form very quickly, in one to two days, while sensory groups appear more slowly (Rodier, 1980; Ozyurt *et al.*, 2011). Therefore, exposure to a harmful agent in these stages of pregnancy in rats may cause changes at the cellular level in the spinal cord. For example, in a stereological study Ozyurt et al. (2011) reported a decrease in total neuron numbers and spinal cord volume (C1-C4 spinal cord segments) at weeks 4 and 20 in male rat pups from pregnant rats injected with 1 mg/kg diclofenac sodium per day between days 5 and 20 of pregnancy (Kaplan *et al.*, 2012a; 2012b; İkinci *et al.*, 2013; Aktürk *et al.*, 2013). In agreement with our observations, their histopathological findings in the experimental group included cell loss, severe damage to motor and sensory neurons exhibiting chromatic and cytoplasmic condensation, Nissl substance dissolution, a vacuolization in both the ventral and dorsal horn, and vacuolar areas in the white matter of the drug-treated animals. They suggested in consequence that administration of diclofenac sodium affects development of neurons and volume of cervical spinal cord in prenatal animals (Ozyurt *et al.*, 2011).

The open field test was used to assess the effect of 900 MHz EMF applied in the prenatal period on locomotor activity (Abbas *et al.*, 2009; Gulec *et al.*, 2010; Franke *et al.*, 2011). Numbers of squares crossed were scored to assess motor efficacy and number of rearings were scored as inquisitive behavior. Increased defecation numbers during the experiment were regarded as an anxiety finding. No significant difference was observed between the EMF groups in any of the parameters obtained from the open field test. These results show that animals were not subjected to any stress during the tests. In the rotarod test performed under the same conditions on both groups (Kim *et al.*, 2012; Haghani *et al.*, 2013), a statistically significant increase was observed in the NEMFG rats' motor function test results. In contrast to our results, Haghani et al. (2013) recently reported that 900-MHz pulse-EMF irradiation for 6 h per day during the entire gestation period did not affect accelerated rotarod and open field test results. They suggested that chronic EMF radiation does not lead to any behavioral abnormalities in female and female newborn rat pups exposed to EMF during gestation (Haghani *et al*, 2013). It needs to be stated here that in Haghani et al.'s (2013) study, EMF was applied to pregnant rats throughout pregnancy for 6 h per day. In our study, however, EMF was applied between days 13 and 21 of pregnancy. However, their monitoring of newborn rats after pregnancy and

approximate times of separation from their mothers were similar to ours. Additionally, we began behavioral tests on day 26, while Haghani et al. (2013) performed behavioral tests on days 32-33. It should be borne in mind that the behavioral test results from the two studies may differ for these reasons.

Conclusion and Outlook

On the basis of our results, the development of the spinal cord was affected in female rat pups exposed to 900 MHz EMF on days 13-21 of the prenatal period. This effect persisted with pathologies in spinal cord morphology in the postnatal period, the pathological changes in the spinal cord compromised communication between the brain and the peripheral nervous system, and this emerged in the form of behavioral changes in 32-day-old female rat pups. Since developmental stages of animals from the same species can be compared irrespective of whether they are pre- or postnatal (Rodier, 1980), our study results suggest that the spinal cord development of babies of mothers engaging in long-term mobile phone use during pregnancy may be exposed to the effect of EMF emitted by such phones. However, further studies on the subject need to be performed in order to establish a definitive conclusion.

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