

Effects of 171 MHz Low-Intensity Electromagnetic Field on Glucocorticoid and Mineral Corticoid Activity of the Adrenal Glands of Rats

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A sub-acute electromagnetic field (EMF) biological effect study was carried out on rats exposed in the Transverse ElectroMagnetic exposure chamber at 171 MHz Continuous Wave (CW). The experiments involved three exposure levels (15, 25, and 35 V/m) for 15 days with triplicate parallel sham-exposed controls in each series. All exposure conditions were simulated for the evaluation of the electromagnetic energy distribution and specific absorption rate (SAR) in the rat phantoms. Studies have shown a biphasic biological response depending on time and absorbed electromagnetic energy. Under low SAR, approximately 0.006 W/kg, EMF exposure leads to the stimulation of adrenal gland activity. This process is accompanied by an initial increase of daily excretion of corticosterone and Na⁺, which is seen as a higher Na⁺/K⁺ ratio, followed by a decrease of these parameters over time. It is possible that EMF exposure causes a stress response in animals, which is seen as an increased adrenal activity. *Bioelectromagnetics*. 2019;40:578–587. © 2019 Bioelectromagnetics Society.

Keywords: electromagnetic field; TEM cell; corticosterone; SAR; intensity-time dependence

INTRODUCTION

Safety standards to protect humans from adverse biological effects of electromagnetic fields (EMFs) span the electromagnetic spectrum from static to 300 GHz. The 30–400 MHz range is characterized by the whole and part human body resonant absorption. Identical absorption relationships were established in humans and animals as a result of determining the absorbed electromagnetic energy in computational models using numerical methods of theoretical dosimetry [Dimbylow, 1997; Durney, 1980]. Therefore, international and national standards, despite differences in maximum permissible levels (MPL), are characterized by a significant reduction in EMF MPL in the frequency band from 10 to 400 MHz, compared with neighboring frequencies. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines, the MPL is 61 V/m from 10 to 400 MHz [ICNIRP Guidelines, 2005]. In United States regulations for the band 30–300 MHz, the MPL is 61.4 V/m [IEEE Standard C95, 2005]. The Russian MPL is more stringent and mandates 15 V/m for frequencies 30–300 MHz [SanPiN 2.2.3359-16, 2016].

To harmonize the MPL in the 30–300 MHz frequency range, experiments should be conducted to research EMF biological effects in conjunction with

epidemiological studies. There are few biological studies in this frequency range, and they were mainly focused on short-term effects—radiofrequency (RF) current intensity in human limbs [Tofani et al., 1995] or temperature increases [Chen and Gandhi, 1988]. However, many television, radio broadcasting, and professional communication systems, unlike cellular communication systems and other household RF sources, operate in the meter wavelength frequency range. This is a good reason for conducting biological research in that band. In addition, it is possible that biological effects detected at meter wavelength EMF can be observed in another part of the RF spectrum [Sheppard et al., 2008].

Conflict of interest: None.

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EMF biological effect analysis shows that the most sensitive non-specific response of the neuroendocrine system, a stress reaction, appears during acute and chronic EMF exposure [Lu et al., 1980; Black and Heynick, 2003]. The concentration of the adrenal hormone cortisol in biological fluids (saliva and urine) is a sensitive and non-invasive biomarker of the neuroendocrine system response to EMF exposure [Vangelova and Velkova, 2014]. Cortisol in the saliva of eight adult subjects (average age 24.8 years) was studied after 4 h exposure of the head with a pulsed EMF at 900 MHz (1 W/m^2 , 10-g head averaged $\text{SAR} = 0.025 \text{ W/g}$) [Radon et al., 2001]. The results showed no change in neuroendocrine homeostasis, and the concentration in cortisol saliva after exposure was not significantly different from the control. The hormonal profile of cell phone users, or of people aged from 14 to 60 years living at a distance from 20–100 to 100–500 m from a base station, showed a significant effect on the pituitary-adrenal axis in the form of changes in cortisol and adrenocorticotrophic hormone (ACTH) plasma levels [Eskander et al., 2012]. Prospective studies of school children (average age 12.5 ± 1.5 years) revealed a change in cortisol saliva after talking 10–20 min on a cell phone ($\text{SAR} 0.571 \pm 0.2 \text{ W/kg}$) compared with the control group [Geronikolou et al., 2015]. These prospective studies have considered this hormone as a prognostic marker of the endocrine stress response. The results can be interpreted as showing that ACTH and cortisol concentrations show non-specific (stressor) reactions of the hypothalamic-pituitary-adrenal axis to electromagnetic exposure. The excretion of cortisol, adrenaline, and norepinephrine was studied in operators of radio and television stations (110 subjects), who during the working day were exposed to EMF in the frequency range 6–25 MHz ($3.1\text{--}137 \mu\text{W/cm}^2$) and 66.5–900 MHz ($1.89\text{--}5.24 \mu\text{W/cm}^2$) [Vangelova and Velkova, 2014]. A significant increase in cortisol excretion and norepinephrine compared with control persons (110 people) was found in workers exposed at $3.1\text{--}137 \mu\text{W/cm}^2$. In the group of subjects exposed at $1.89\text{--}5.24 \mu\text{W/cm}^2$, the excretion of all hormones was not significantly changed compared with the control group.

According to Russian RF safety guidelines, the substantiation of permissible limit values is based not only on acute biological effects but also on sub-chronic and chronic biological effects. Therefore, of special interest are the studies of sub-chronic or sub-acute (less than 1 month) effects of EMF with E-field values from 15 to 60 V/m, especially for exposure levels higher than Russian hygienic norms. It is possible that these intensities are of the most interest

in the investigation of sub-acute or sub-chronic exposure effects of the neuroendocrine system response. In accordance with this consideration, the objective of this study was the assessment of glucocorticoid and mineralocorticoid activity in animals by using 171 MHz sub-acute exposure. The results will be useful in clarifying the validity of MPL and establish compliance with international safety standards.

MATERIALS AND METHODS

A comprehensive assessment of EMF biological effects consisted of three interrelated and complementary parts: theoretical dosimetry (simulation), experimental dosimetry, and experimental studies; the two last were carried out on laboratory animals.

Animals

Three hundred twenty-four Wistar male rats weighing 200–220 g were housed in accordance with institutional animal care policies with access to water and food under standard laboratory procedures. All animal experiments were performed between 08:00 h and 14:00 h with simultaneous parallel sham exposure. All protocols were approved by the Institutional Ethical Committee. To compensate for differences in body mass, the rats were divided into two groups (108 and 216 rats) by a stratified random method. The first group of 108 rats was divided in 9 groups of 12 rats, which were used for the dosimetric assessment, and the second group (216 rats) was partitioned in 18 groups of 12 rats for the EMF biological effect investigations (Table 1). During the experiments, the animals were kept in individual Plexiglas metabolic cages. The size of the metabolic cage with urinal was $0.180 \times 0.140 \times 0.130 \text{ m}$, and the internal space for free placement of one rat was $0.178 \times 0.138 \times 0.100 \text{ m}$, which allowed the experimental animals to move freely in the horizontal plane (E-H orientation).

Exposure system and dosimetry

The exposure and dosimetric system utilized a Transverse ElectroMagnetic (TEM) Crawford cell [Crawford, 1974]. The TEM cell dimensions were $1.764 \times 0.636 \times 0.636 \text{ m}$, and the work area was $0.882 \times 0.636 \text{ m}$. In the work area of the TEM cell, electromagnetic plane waves propagated along the axis of the cell with E and H vectors orthogonal to each other and orthogonal to the direction of propagation. The exposure system equipment included: Agilent 8648 C generator (Agilent Technologies, Santa Clara, CA), R&S BBA100 broadband amplifier (Rohde & Schwarz, Munich, Germany),

TABLE 1. The Grouping of Animals

Series	Conditions	Number of rats					
		Dosimetry			Exposure		
		15 V/m	25 V/m	35 V/m	15 V/m	25 V/m	35 V/m
1	Exposure	12	12	12	12	12	12
	Sham	–	–	–	12	12	12
2	Exposure	12	12	12	12	12	12
	Sham	–	–	–	12	12	12
3	Exposure	12	12	12	12	12	12
	Sham	–	–	–	12	12	12
Total animals		36	36	36	72	72	72
			108			216	

Agilent 778D-011 couplers (Agilent Technologies), Agilent E4419B two-channel power meter (Agilent Technologies) with N8482A sensors (Agilent Technologies), and Huber + Shumer cable assemblies (Huber + Shumer, Pfäffikon, Switzerland). The exposure and dosimetry system block diagram is shown in Figure 1. Sham exposure of parallel groups was performed simultaneously in a second identical TEM cell located in the same room. During the experiments, all TEM cells had ventilation and air conditioning to keep the temperature at 20–24°C.

From measurements of input and reflected power in the TEM cell, it is possible to calculate the absorption of electromagnetic energy in all the objects in the TEM cell workspace. See the block diagram in Figure 1.

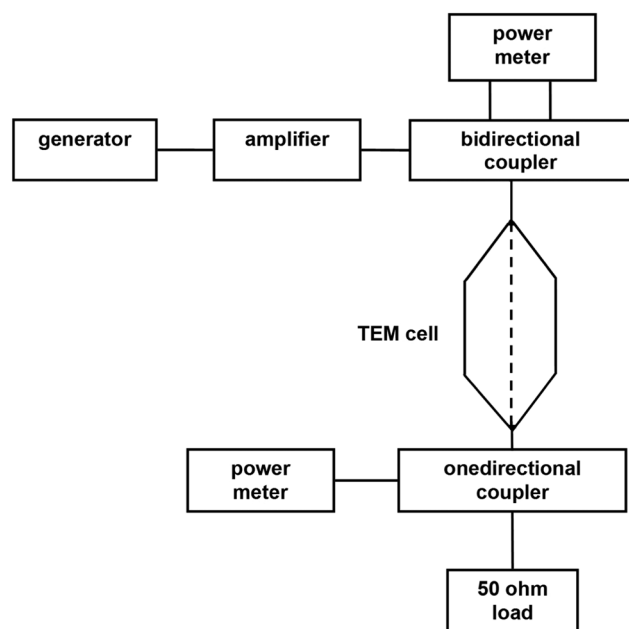


Fig. 1. Block diagram of exposure and dosimetry system.

The EMF power density in the TEM cell workspace was measured with a NARDA NBM-550 broadband meter (Narda Safety Test Solutions, Pfullingen, Germany) connected to a PC via a fiber optic cable. In the TEM cell, the E and H vector orientation was evaluated using an IPM-101 M single-polarization probe EMF meter (Doza, Zelenograd, Russia).

Simulation of the animal exposure utilized the finite difference time domain (FDTD) method. The SEMCAD X v.14.8.6 platform (SPEAG, Zurich, Switzerland) was used to evaluate the electromagnetic energy distribution in the TEM cell and specific absorption rate (SAR) in the rat numerical phantoms (IT'IS Foundation, Zurich, Switzerland). The rat numerical phantom, which includes 52 tissues [Christ et al., 2010], is a Sprague–Dawley male, 185 mm length and mass 198 g.

Adrenal gland activity (glucocorticoid and mineralocorticoid functions)

Biological samples (rats' daily urine) for analysis were collected every day. Once a day, at 1 h after exposure, the contents of the urine jars (located under each animal cage) were merged by group, filtered from feces, divided in two samples, and used for the analysis of corticosterone and electrolytes [Pruett et al., 2008].

Corticosterone concentration and Na⁺ and K⁺ content were determined for each daily sample. Corticosterone was measured by enzyme-linked immunosorbent assay (ELISA) using a Corticosterone EIA test (Labor Diagnostika Nord, Nordhorn, Germany). The contents of Na⁺ and K⁺ ions were evaluated using the AAnalyst 400 flame atomic absorption spectrometer (PerkinElmer, Waltham, MA) with deuterium background correction for the standard method [The Perkin-Elmer Co., 1996].

The electrolyte results (Na^+ and K^+) are shown as Na^+/K^+ ratios.

Dosimetric Assessment

A. Dosimetric analysis. TEM cell size selection was based on preliminary calculations according to the high-frequency limit. The verification of TEM cell size applicability required the evaluation of input impedance, S parameters, EMF distribution, and EMF levels in the exposure space of the TEM cell.

In a simulated vertical TEM cell, the laboratory animal numerical (rat) phantoms were positioned orthogonally to the cell septum in 2/3 of the workspace and stacked horizontally, one under the other. The cell geometry is shown Figure 2. The EMF distribution preliminary analyses simulated the exposure of rat phantoms placed in the TEM cell with three electric field incident levels (15, 25, and 35 V/m).

Using the method of three power measurements, we evaluated the power loss in the TEM cell with the metabolic cages, with and without rats.

In the dosimetric simulations (Fig. 2), 12 animals, six rats in each side of the septum, were located in the workspace of the TEM cell. Special Plexiglas holders for the metabolic cages were mounted symmetrically with respect to septum; the metabolic cages were fixed to these holders. The TEM cells (sham and exposure) had a large hatch to place and remove the metabolic cages from the holders.

B. Dosimetric measurements. The rats' energy absorption measurements were carried out for three EMF levels in nine groups, three groups for each EMF level. The dosimetric rats were not used for the biological effect assessment.

The SAR assessments were carried out in three phases. First, power measurements of empty TEM cells were performed, then with 12 empty metabolic cages. Within the limits of the accuracy of the power meters, EMF absorption was not observed in the TEM cell in these two cases. Finally, power measurements were carried out with the experimental animals in their metabolic cages.

Knowing the number of rats, mass of all rats, input reflected, and transmitted power in the terminal load, it was possible to determine the total electromagnetic energy absorbed by all rats in the TEM cell workspace. The free movement of some animals in the metabolic cages did not affect the total absorption in a TEM cell. The body-averaged SAR of a single rat was estimated by dividing the total absorbed power by the mass of all rats and their numbers.

Biological Effect Assessment

As pointed out in the previous section, the rats were stacked horizontally with their individual metabolic cages, one under the other, in 2/3 of the workspace and positioned perpendicularly to the septum of the vertical TEM cells for the simultaneous real and sham exposures.

The animals were exposed every day to 171 MHz CW EMF in the TEM cell for 6 h/day for 15 days at three E-field amplitude levels: 15 V/m—3 groups, 25 V/m—3 groups, and 35 V/m—3 groups (Table 1). The background EMF of the room, where animals were kept and exposed, was 1.3 V/m and 2.1 A/m. During the entire exposure time, all animals (exposed and sham groups) were kept in metabolic cages with identical environmental conditions (light, noise, vibration, and temperature).

Every day, the urine samples from exposed and sham-exposed groups were collected and analyzed for corticosterone and electrolytes 1 h after exposure. The urine accumulated in a container located between the floor and bottom of the metabolic cages. The separation of boluses and urine was provided by a plastic mesh with a 2×2 mm cell size, which ensured the retention of feces and the passage of urine into the cuvette.

Statistical Analysis

The experimental data are presented as mean $\pm$ SD. Statistical differences were assessed using the unpaired two-tailed Student's t test. Differences at $P < 0.05$ were considered statistically significant.

RESULTS

Dosimetry

Initially, the exposure conditions were analyzed using the TEM cell numerical model to simulate meter wavelength EMF propagation. The EMF distribution in the exposure space of the TEM cell was homogeneous without rats in the 2/3 space from the central plate. The experimental measurements with the EMF meters confirmed the numerical results at several locations of the TEM cell workspace on both sides of the septum. These places were: near the first animal (top), between the third and fourth rats' location (middle), and near the sixth rat's location (bottom). The deviation between experimental and numerical RMS electric field strength was 15% less in $0.85 \times 0.41 \times 0.39$ m volume, which is about 2/3 size of TEM cell workspace. This volume was large enough for the placement of 12 rat phantoms and their

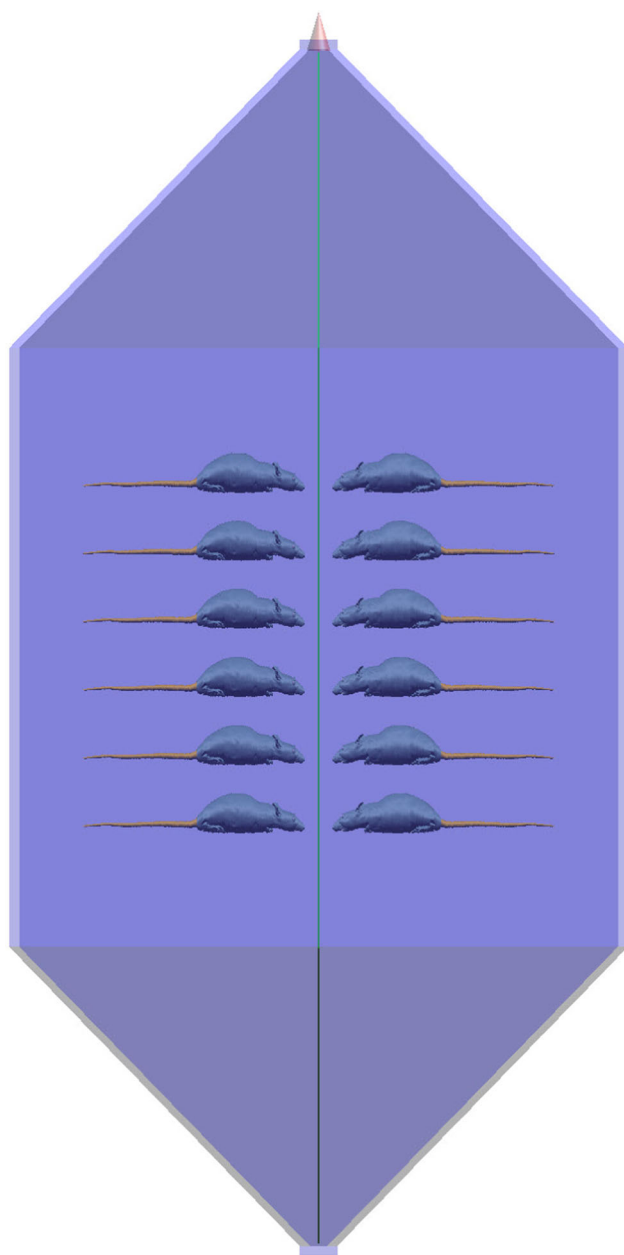


Fig. 2. Rat phantoms in simulated transmission electron microscopy (TEM) cells.

metabolic cages for the next phase of experimental dosimetry. The EMF preliminary analyses simulated the exposure of the numerical rat phantoms in the TEM cell, with three EMF exposure levels (15, 25, and 35 V/m). The numerical model with 12 rats is shown in Figure 2. This numerical model allowed us to investigate the relationship between electric field strength and SAR values. The simulations of three EMF exposure levels show distinctive SAR distributions in the rats (Fig. 3).

It is worth noting that the SAR distribution was very similar for all exposed rats. This is because the main mechanism of RF deposition in the animals was electromagnetic coupling with the RF currents and charges on the septum. This was due to the design feature of the TEM cell as an exposure system. The maximum tissue energy absorption was observed in the brain (hypothalamus, midbrain, cortex of the cerebrum, and cerebral spinal fluid) and adrenal glands. The general response of the hypothalamus-pituitary-adrenal cortex (HPA) axis is in the final product of this axis: the adrenal response. In the adrenal glands, the SAR levels were 0.009 W/kg for 15 V/m exposure, 0.026 W/kg for 25 V/m, and 0.051 W/kg for 35 V/m. For the hypothalamus, the SAR levels were 0.011 W/kg, 0.033 W/kg, and 0.065 W/kg for 15, 25, and 35 V/m, respectively. Experimental dosimetry, the second part of the study, used similar exposure conditions as in the simulation. Animals were placed in the TEM cell work area, where the field strength was homogeneous, as shown by simulation and measurements. The experimental data are in good agreement with the simulation results (Table 2).

Biological Effects

In the third part of the study, EMF biological effects were evaluated using three exposure levels in the TEM cell, with triplicate parallel controls in each series using the setup of numerical and experimental dosimetry. The daily urine corticosterone concentrations for three exposure levels during 15 days are plotted in Figure 4.

The investigation probed the production of corticosterone as the terminal link in the HPA axis. The results of the experiments show that the highest increase of adrenal gland activity, measured by changes of corticosterone in daily urine of the animals, happens after the fourth ($P < 0.01$), fifth, and tenth ($P < 0.001$) day of exposure at the maximum SAR (0.038 W/kg); see Figure 4C. Corticosterone excretion after 1 day of EMF exposure showed no statistically significant change compared with controls. Thus, EMF exposure for 6 h per day caused adrenal gland activity increase at a SAR of 0.038 W/kg for all exposure times, whereas a SAR of 0.023 W/kg led to adrenal gland functional state changes after 10 days ($P < 0.001$), as shown in Figure 4B. The corticosterone excretion did not present any statistically significant changes at the lowest EMF exposure level (0.006 W/kg SAR) in all experimental groups (Fig. 4a).

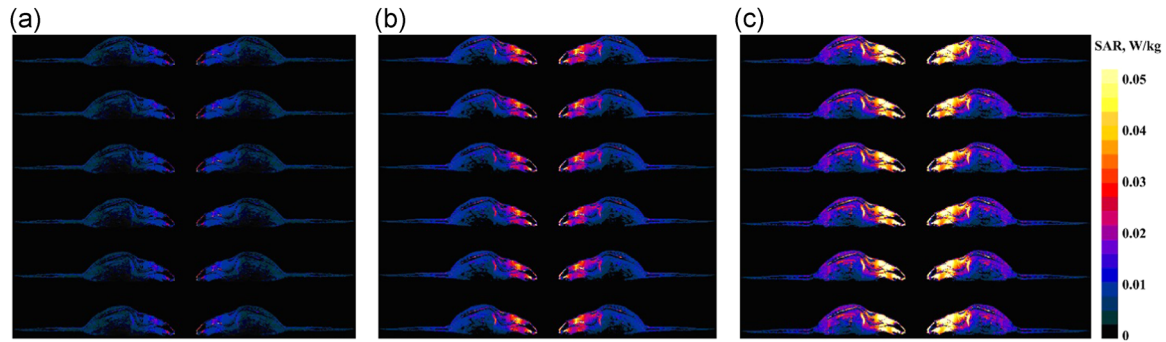


Fig. 3. SAR distributions in rat phantoms for three exposure levels in the transmission electron microscopy (TEM) cell (a) 15 V/m, (b) 25 V/m, (c) 35 V/m).

In the neuroendocrine system response, aldosterone is the primary adrenocortical hormone having mineralocorticoid activity. Its role is to maintain water-electrolyte homeostasis and regulation of sodium (Na^+) and potassium (K^+) excretion (Table 3). In these experiments, the mineral corticoid function of the adrenal glands was studied by the daily concentration levels of Na^+ and K^+ excretion and was estimated by the Na^+/K^+ ratio of rats exposed for 6 h per day for 15 days at the 15, 25, 35 V/m levels and of their shams.

Ion excretion changes were observed on the 10th day of EMF exposure (15 V/m, SAR 0.006 W/kg) due to a pronounced Na^+ concentration increase ($133.14 \pm 12.08 \mu\text{mol/L}$ shams, $197.28 \pm 12.18 \mu\text{mol/L}$ exposed). On the fifth exposure day at 25 V/m, the concentration of Na^+ excretion was $172.1 \pm 12.78 \mu\text{mol/L}$ for shams and $235.82 \pm 10.64 \mu\text{mol/L}$ for the exposed, and the concentration on 10th day was $219.23 \pm 17.73 \mu\text{mol/L}$ and $241.45 \pm 19.59 \mu\text{mol/L}$ for shams and exposed rats, respectively. On the contrary, K^+ excretion fell on the same days. On the fifth day at the highest exposure level (35 V/m), there was a Na^+ concentration increase ($167.98 \pm 11.14 \mu\text{mol/L}$ in shams, $366.54 \pm 14.13 \mu\text{mol/L}$ in exposed) with a slight K^+ excretion increase. On the 10th day of 35 V/m exposure, electrolytes showed the maximum Na^+ excretion for the exposed groups ($489.28 \pm 19.35 \mu\text{mol/L}$) and average

Na^+ excretion $180.71 \pm 16.18 \mu\text{mol/L}$ in shams, while the K^+ urine content of rats fell slightly.

In summary, statistically significant changes in the Na^+/K^+ ratio of the exposed in comparison with the sham groups were noted on the 5th and 10th days of exposure with SAR 0.023 W/kg ($P < 0.05$) and 0.038 W/kg ($P < 0.01$), respectively, as shown in Figure 5. The maximum increase of the Na^+/K^+ ratio, 2.99 ± 0.32 , was observed on the 10th day of the experiment with maximum EMF exposure (35 V/m, SAR 0.038 W/kg) as compared with the ratio 1.05 ± 0.13 for the sham exposure. For the first several days of exposure (1–3 days), there was no significant difference in Na^+/K^+ ratios.

DISCUSSION

Investigations of biological responses of the whole organisms exposed to EMF are important experimental studies. The main problem is the selection of biological responses, which could become sensitive indicators of biological effects caused by exposure.

In the present study, we used the analysis of the neuroendocrine system responses, which are highly sensitive to environmental changes, including EMF. Acting alone or in concert, the various components of

TABLE 2. Specific Absorption Rate (SAR) Mean Values of Simulation and Experimental Dosimetry Under 171 MHz EMF

Electric field strength, V/m	SAR mean values, W/kg (M $\pm$ SD)			
	Simulation		Experimental dosimetry	
	12 rats	1 rat	12 rats	1 rat
15	0.096 ± 0.005	0.008 ± 0.001	0.072 ± 0.011	0.006 ± 0.001
25	0.264 ± 0.013	0.022 ± 0.001	0.276 ± 0.041	0.023 ± 0.003
35	0.516 ± 0.026	0.043 ± 0.002	0.456 ± 0.068	0.038 ± 0.006

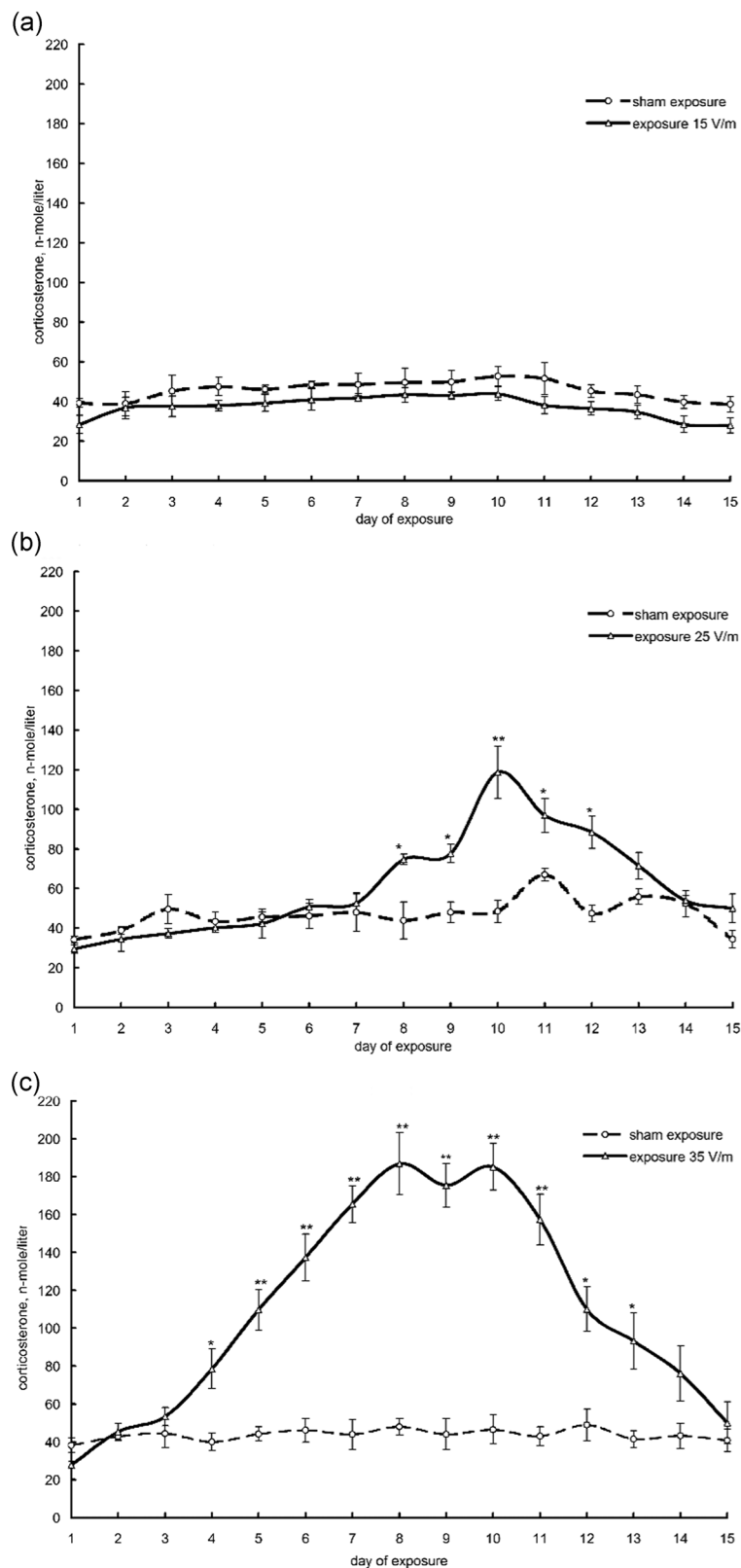


Fig. 4. Corticosterone concentration ($M \pm SD$) of daily urine in rats during 15 days of exposure (a) 15 V/m (body-averaged SAR = 0.006 W/kg), (b) 25 V/m (body-averaged SAR = 0.023 W/kg), (c) 35 V/m (body-averaged SAR = 0.038 W/kg) and in concurrent shams (* $P < 0.01$, ** $P < 0.001$).

TABLE 3. Results ($M \pm SD$) of Daily Electrolyte Excretion Na^+ and K^+ Concentration Assessments During Every Day of Electromagnetic Field (EMF) Exposures

Days of exposure	Exposure levels (SAR values)											
	15 V/m (0.006 W/kg)				25 V/m (0.023 W/kg)				35 V/m (0.038 W/kg)			
	sham-exposed		exposed		sham-exposed		exposed		sham-exposed		exposed	
	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺	Na ⁺	K ⁺
1	134.65 ± 11.02	132.01 ± 9.79	136.88 ± 6.24	134.20 ± 7.42	127.48 ± 12.15	121.41 ± 12.02	146.23 ± 13.16	139.27 ± 13.08	173.30 ± 10.18	168.25 ± 11.12	129.02 ± 9.28	124.06 ± 10.87
3	179.46 ± 13.45	170.91 ± 9.14	159.7 ± 1 ± 7.11	140.10 ± 8.16	164.38 ± 16.11	152.20 ± 13.18	222.6 ± 2 ± 19.67	149.41 ± 16.43	159.34 ± 9.71	147.54 ± 10.11	204.86 ± 12.73	167.92 ± 11.06
5	157.25 ± 15.19	145.60 ± 10.19	146.13 ± 5.14	122.8 ± 0 ± 7.74	172.10 ± 12.78	163.90 ± 15.86	235.82 ± 18.84	152.14 ± 15.14	167.98 ± 11.14	159.98 ± 9.16	366.54 ± 14.13	215.61 ± 13.94
10	133.14 ± 12.08	123.28 ± 14.17	197.28 ± 12.18	131.52 ± 4.16	219.23 ± 17.73	154.39 ± 11.63	241.45 ± 19.59	123.82 ± 13.24	180.71 ± 16.18	172.10 ± 12.19	489.28 ± 19.35	163.64 ± 11.24
15	183.96 ± 18.52	178.60 ± 18.52	176.34 ± 7.92	153.34 ± 7.24	192.26 ± 15.63	173.21 ± 15.87	207.36 ± 17.45	157.09 ± 16.79	147.92 ± 12.60	138.24 ± 11.85	232.94 ± 15.52	175.14 ± 12.57

the neuroendocrine system play a central role in the integrative activities of homeostasis support. One should not consider neuroendocrine alteration as necessarily pathological because the function of the neuroendocrine system is to maintain homeostasis, and hormone levels will fluctuate to maintain such organic stability and adaptation. These neuroendocrine relationships are distinguished by a hierarchy of activities, which consists of three levels of organization (hypothalamus, hypophysis, and endocrine gland), in which the secretion of hormones by the adrenal gland is controlled by the balance of signals, including non-specific response to external factors, for example, EMF [Lu et al., 1980]. In the neuroendocrine mechanisms of adaptation processes, the adrenal glands have a leading role. The functional activity of the cortex is controlled by the adrenocorticotrophic hormone of the pituitary gland, which regulates the corticosterone levels. The neuroendocrine response to EMF cannot be estimated by the absolute values of corticosterone; therefore, the dynamics of its concentration must be evaluated during exposure.

Considering the relationship between exposure levels and biological responses, we can note the proportionality between the corticosterone concentration and body-averaged SAR. The excretion of corticosterone on the first exposure day did not depend on the SAR from 0.006 to 0.038 W/kg. On the third and especially on the fifth day of exposure, a significant increase of corticosterone was noted only at the maximum SAR (0.038 W/kg). The highest excretion of corticosterone, and, consequently, glucocorticoid activity of the adrenal glands was detected on the 10th day of exposure. The activity of mineral corticoid function increased at the same time. Comparative analysis shows that these 15-day experiments provide the maximum excretion of corticosterone since the corticosterone level versus the SAR curve has an inflection point (Fig. 4b and c).

Neuroendocrine EMF effects are determined by reactions along the HPA axis. One of the most revealing criteria of biochemical shifts, which can be assessed in the dynamics of sub-acute effects, are changes in the adrenal cortex functional state as the final link of the HPA.

This is an adaptive-compensatory biological effect due to exposure, which caused a preferential EMF absorption in the brain (pituitary) of animals rather than in the adrenal glands. This assumption is supported by the larger average tissue SAR values in the rat brain—from 0.011 to 0.057 W/kg (from 0.012 to 0.065 W/kg in the pituitary gland)—than in the adrenal glands (from 0.003 up to

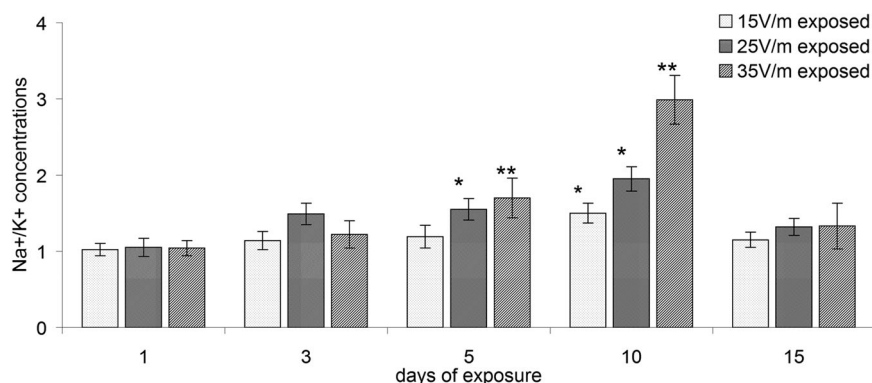


Fig. 5. Results of daily electrolyte excretion (absolute values of concentration shown as ratio Na^+/K^+) assessment after the electromagnetic field (EMF) exposure. * $P < 0.05$; ** $P < 0.01$.

0.017 W/kg). In our research, we investigated the HPA as a biological system exposed to EMF. The corticosterone concentration changes after 4–8 days of the experiment suggested a neuroendocrinological biological effect for all organs of HPA as a system. Therefore, we evaluated the final product of HPA—corticosterone.

The plot of the rats' corticosterone excretion for the maximum EMF exposure (35 V/m, SAR 0.038 W/kg) over 15 days exhibits an ascending and descending part (Fig. 4c). The ascending part can be considered as a permissive effect, directed to the activation of the body's defense mechanisms in response to the exposure. The subsequent descending part indicates the predominance of the suppressive effect of EMF biological exposure stress, as the adaptive-protective reactions of the body. The adrenal glands' mineralocorticoid activity (excretion of Na^+ and K^+) is time-shifted with respect to the change in the corticosterone concentration level in the animals' urine. The maximum excretion was observed on the 6–11th days of exposure at the maximum SAR (0.038 W/kg).

Evaluation of animals' behavior (control and experimental) was not carried out during our research. The animal behavior was observed only subjectively (*ad oculos*), and no noticeable difference was detected between the two sets of rats.

These results allow an assessment of non-specific adaptation mechanisms. These processes define the biological response to meter wavelength EMF exposure. In some publications, the EMF biological effect on adrenal gland hormones, involved in non-specific adaptation processes, was studied and showed increased glucocorticoid activity.

A hamster (*Mesocricetus auratus*) EMF exposure study (950 MHz for 3 h per day, SAR 0.66 W/kg)

was carried out for two experimental groups: the exposure of the first group lasted 10 days and the second for 60 days [Seyednour and Chekaniazar, 2011]. The results, as reported by the authors, showed a significant increase in serum cortisol in both animal groups compared with the control. Shorter EMF exposures of male rabbits at 950 MHz with 3 and 6 W, 2 h per day for 2 weeks, did not lead to a significant increase in blood serum cortisol of animals [Sarookhani et al., 2011]. The corticosterone concentration was evaluated in the plasma of Sprague–Dawley rats, EMF-exposed for a single 15-min session (900 MHz, SAR 6.0 W/kg) [Bouji et al., 2012]. Thirty minutes after exposure, the hormone level increased significantly, and within 12 h it returned to its original values.

The results of this experiment support the conclusion that EMF exposure has had no direct effect on the kidneys or ion excretion. It is probable that the increase of the exposure days and EMF levels led to a decrease in aldosterone secretion, which caused a delay in the excretion and accumulation of K^+ in exposed animals and an increased Na^+ excretion from 5 to 10 days of exposure. After 10 days of exposure, Na^+ urine excretion decreased in sham groups and K^+ concentration increased. The most changes in ion excretion were observed in Na^+ concentration, but the Na/K ratios showed a clear change in homeostasis.

It is likely that the observed corticosterone excretion enhancement is induced by the increase of both exposure parameters, duration, and SAR. This indicates a stress-limiting component of the adaptive response to the EMF action on the biological subjects.

Thus, adrenal gland activity in experimental rats, as measured by the hormone level in the blood, depends on the exposure time and level. The results of

corticosterone and ion excretion show the dependence of exposure levels and exposure time. These are biological effects of non-specific action of low-intensity EMF.

The observed EMF biological effects show adaptation to a maximum exposure level, which is lower than the MPL of ICNIRP. Also, biphasic processes produced activation of some biological mechanisms to exposure level higher than the Russian MPL. These results will be useful for future EMF biological effect investigations as a step to safety standard harmonization.

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