

Article

An 1800 MHz Electromagnetic Field Affects Hormone Levels, Sperm Quality, and Behavior in Laboratory Rats (*Rattus norvegicus*)

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Abstract: In addition to natural electromagnetic fields (EMFs), so-called artificial electromagnetic fields exist in the biosphere, with mobile communications being one of their main sources. This study aimed to determine the impact of EMF at a frequency of 1800 MHz on the concentrations of selected hormones, sperm motility, viability, morphology and behaviors in laboratory rats. We used 28 rats divided into two equinumerous groups: control ($n = 14$) and experimental ($n = 14$). The rats in the experimental group were exposed to EMF for 12 weeks (for 10 min, four times daily); at the same time, the control specimens were kept in standard conditions. After 12 weeks, half of each group was killed, while the other half was maintained for another 4 weeks with no EMF emission. Elevated corticosterone levels and decreased thyroid-stimulating hormone levels were observed in the experimental specimens, which persisted for 2 weeks after the cessation of EMF emission. Exposure to EMF also resulted in decreased sperm motility and viability, as well as increased rat anxiety. This study shows that exposure to EMF (1800 MHz) may affect the endocrine status of the body and the behavior and reproductive functions of animals. However, hormonal disorders appear to be reversible.

Keywords: electromagnetic fields; hormones; behavior; sperm; rats



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1. Introduction

In addition to natural electromagnetic fields (EMFs), so-called artificial electromagnetic fields exist in the biosphere, the intensities of which exceed the natural background by several orders of magnitude. The main sources of artificial EMFs include power grids, mobile communications, electrical installations and devices, industrial inductive devices, radio/TV transmitters, and wireless Wi-Fi routers. Collectively, these fields are some of the largest energy pollutants on Earth [1]. Among the areas using electromagnetic fields, wireless communication technology deserves special attention because of its dynamic development. According to the International Telecommunication Union's 2023 report, 78 percent of the worldwide population (aged 10 and over) owns a mobile phone [2].

Interest in the impact of EMFs on living organisms, among other things, is due to their association with the generation of bioelectric phenomena; therefore, they may interfere with the functioning of biological structures. Electromagnetic fields generated in the bodies of mammals, including humans, are irregular (with different amplitudes) and non-stationary (that is, the mean, variance, and autocorrelation function are variable over time). EMF values measured at the tissue level range from 1 to 200 V/m, and the densities of generated currents do not exceed $100 \mu\text{A}/\text{cm}^2$ [3]. On a cellular scale, electrical fields with much higher intensities can be observed in the body. In each cell of the body, ion diffusion transport through the cell membrane and the action of the Na^+/K^+ pump form a cell membrane resting potential of 70 mV; combined with the thickness of the cell membrane (10 nm), this generates an electric field with an intensity of $7 \times 10^7 \text{ V/m}$ [4]. Studies on the effects of EMFs on living organisms have a very wide scope, from the electrical properties of organs and the absorption and processing of electromagnetic field energy in tissues to the mechanisms of their effects on biological structures. The interaction between electromagnetic radiation and a living organism depends on several parameters, including the length of exposure, the value of energy transferred, and the type of tissue acted upon. Studies on the impact of EMFs are very difficult to conduct, as changing even one field parameter can lead to a completely different effect on matter. Therefore, despite the numerous papers on EMFs published every year, questions concerning a full understanding of the mechanisms and identification of the effects of this factor on living organisms have not yet been answered [5–9].

Although still under investigation, quantum phenomena such as electron and proton tunneling have been proposed as possible mechanisms of EMF interactions with biological systems [10–17]. While beyond the scope of our study, these phenomena remain important in the broader landscape of biophysical research.

Experiments on the impact of EMFs on animals may reveal their health risks to humans and help biologists identify what biological processes and mechanisms are affected by them. Therefore, it is important to conduct complex experiments examining the impact of various environmental factors on living organisms to understand the overall effect of EMFs on the body; thus, the use of animal models is still necessary [18]. Rats are an example of model animals used in biological experiments. Their common use in laboratory studies is associated with the fact that about 90% of rat DNA is similar to that of humans. Furthermore, most human genes—the damage of which ends in disease—have equivalents in the DNA of this rodent [19].

This study aimed to determine the impact of electromagnetic fields at a frequency of 1800 MHz on the concentration of selected hormones, sperm motility, viability, morphology, and behavior in laboratory rats (*Rattus norvegicus*).

2. Materials and Methods

2.1. Biological Material

Male brown rats (*Rattus norvegicus*) of the Wistar strain were obtained as the biological material of the study from the Center of Experimental Medicine at Silesian Medical University in Katowice. The experiments used 28 rats, whose age at the beginning of the experiment was 12 weeks. Prior to the experiment, the rats were quarantined for seven days.

2.2. Animal-Handling Conditions

The rats were kept under standard laboratory conditions suitable for these animals—two individuals in a cage, with access to water and standardized ad libitum breeding fodder. They were provided with enriching elements in the form of chewing toys and

tubes. The temperature in the rooms was 20–22 °C, with humidity at 50% ($\pm 10\%$). The animal house used a 12 h cycle of light and darkness, turning the light on at 7:00. The study was conducted in academic animal quarters, where all microclimatic parameters are continuously monitored using sensors placed in different parts of the rooms. The experiment was approved by the Local Ethics Committee in Krakow (Resolution No. 240/2019 of 27 February 2019). All methods were performed according to relevant guidelines and regulations, including the ARRIVE 2.0 guidelines and the 3Rs principles. Every effort was made to minimize the number of individuals used and the suffering of the animals. To familiarize the animals with the procedures conducted during the experiment, they were subjected to handling during the acclimatization period. Throughout both the quarantine and experiment, the animals' environment was enriched, and enrichment elements were regularly replaced with new ones. The health status of the animals was routinely monitored by a veterinarian. Rats were sacrificed individually in a separate room through an overdose of sodium pentobarbital (150 mg/kg) via intraperitoneal administration.

2.3. Scheme of the Experiment

The experiment lasted 16 weeks, using 28 rats divided into two equinumerous groups: control ($n = 14$) and experimental ($n = 14$). Apart from the experimental factor (EMF 1800 MHz), there were no other factors or procedures that could influence the results, so a sham group was not created. The experimental group was subjected to electromagnetic fields for 12 weeks, while the control group was kept in standard conditions (without EMF emission). After the field emission was complete, half of the animals ($n = 7$) from each group were killed, while the other half ($n = 7$) were kept for another 4 weeks without electromagnetic field emissions to determine whether that period was sufficient for the animals exposed for 12 weeks to return to a state of homeostasis. The number of animals (group size) used in the experiment was calculated based on the sample size justification procedure (assuming a standard deviation of $\sigma = 10$, a minimum detectable effect size of $\delta = 15$, a significance level of $\alpha = 0.05$, and a test power of 0.80 ($\beta = 0.20$)). The cages containing the rats were arranged next to each other in a star formation, with an omnidirectional antenna placed at the center, ensuring that the field emission was uniform across all cages, as per the EMF parameters provided below.

2.4. Generation and Measurement of Electromagnetic Field

The experimental group was exposed to electromagnetic waves for 10 min, four times daily: 8:00–8:10, 14:00–14:10, 18:00–18:10, and 22:00–22:10. The source of electromagnetic radiation was a generator emitting electromagnetic waves in the frequency range of the GSM cellular network (1100–2100 MHz). The average output power of the generator was 330 mW, with an instantaneous maximum power of 2 W. The load for the power stage was a Yagi GSM antenna with omnidirectional characteristics. The distance between the field generator antenna and the animals (depending on their position in the cage) was 30–40 cm.

A Tenmars TM-195 3-axis meter (Tenmars Electronics, Taipei, Taiwan) was used to control the electromagnetic field emitted by the generator and background radiation. Among other things, this enabled the isotropic measurement of electric field intensity (0.01–20.0 V/m), magnetic field intensity (0.1–532.6 mA/m), and power density (10.0–106.94 mW/m²) in a frequency range of 50 MHz to 3.5 GHz. The measuring device is characterized by an absolute error of 1 V/m and 2.45 GHz: ± 1.0 dB.

The device was used under the measurement conditions specified by the manufacturer.

2.5. Blood Collection and Analysis

Blood was collected (always in the morning) into previously heparinized test tubes through insertion into the caudal vein after applying local anesthesia (lidocaine, 10%).

Blood was collected at the beginning of the experiment (before the electromagnetic field generator was started), then every two weeks, and then after the emission was completed. The blood collection scheme was as follows: series I—before the experiment; series II—after 2 weeks of EMF emission; series III—after 4 weeks of EMF emission; series IV—after 6 weeks of EMF emission; series V—after 8 weeks of EMF emission; series VI—after 10 weeks of EMF emission; series VII—after 12 weeks of EMF emission; series VIII—2 weeks after the end of EMF emission; series IX—4 weeks after the end of EMF emission. Plasma was obtained from each blood series through centrifugation ($3000 \times g$, 20 min), and thyroid-stimulating hormone and corticosterone concentrations were analyzed. Thyroid-stimulating hormone (TSH) concentrations were determined in blood plasma using a Cusabio ELISA kit (Cusabio, Houston, TX, USA) according to the manufacturer's instructions, at a wavelength of 450 nm. Corticosterone (Cort) concentrations were determined in plasma using an Abcam ELISA kit (Abcam, Cambridge, UK) according to the manufacturer's instructions, at a wavelength of 450 nm. A BioTek Instruments EON microplate reader (BioTek, Winooski, VT, USA) was used to determine the concentrations of both hormones. Blood was collected after the animal was picked up and immobilized manually. No mechanical devices were used to immobilize the animals. Animal stress was minimized through prior handling. Moreover, blood was collected analogously for the experimental group and the control group, thus excluding the influence of stress related to the blood collection procedure on the results.

2.6. Analysis of Sperm Quality Parameters

Once the rats were killed, the epididymis tail was removed and placed on a sterile dish with physiological saline, and spermatozoa were obtained through an incision. The collected spermatozoa were transferred to a test tube with 4 mL of physiological saline. Spermatozoa suspended in solution were incubated in a water bath at 37°C for 4 min. After removing the test tube from the water bath, 4 μL of spermatozoa suspension was applied to a microscope slide and covered with a cover glass [20]. The preparations were evaluated for sperm motility using the Nikon Eclipse Ci light microscope at a magnification of $400\times$ (Nikon, Tokyo, Japan).

An eosin–nigrosine test was conducted to assess sperm viability. One drop of semen was mixed with one drop of eosin solution. After 30 s, three drops of nigrosine solution were added to the mixture, and it was stirred again. From one drop of the prepared suspension, a smear was made on a microscope slide and left to dry [20]. The preparations were evaluated using a Nikon Eclipse Ci light microscope with a magnification of $100\times$. In total, 200 spermatozoa in two replications were counted, distinguishing between pink-colored dead spermatozoa and colorless live spermatozoa.

Sperm morphology was also assessed based on the smears made to assess viability. The percentages of normal spermatozoa and spermatozoa with major and minor defects were assessed according to the methodology proposed by Bielański [20].

2.7. Behavioral Tests

Behavioral tests were carried out immediately after the end of EMF emission, before collecting blood from the rats.

2.7.1. Elevated Plus Maze

For the elevated plus maze test, we used a maze comprising four narrow crossed arms made of gray polyvinyl chloride board. Two arms opposite each other were surrounded by side walls (closed arms, $50 \times 10 \times 40\text{ cm}$). These arms were connected through a central square to two unprotected arms (open arms, $50 \times 10\text{ cm}$). The maze was placed 50 cm above the floor. The tests were conducted in a quiet room illuminated by low-intensity light, and a single test took 5 min. At the beginning of a test, a rat was placed on a central

platform facing the open arm. The evaluated rat behavior parameters were as follows: time spent in open arms, the number of entries into open arms, mictions, defecations, and hind leg stands.

2.7.2. Open Field Test

The open field test was carried out on a platform made of gray polyvinyl chloride board with the following dimensions: 100 × 100 cm, divided into 25 squares, with 40 cm high transparent acrylic glass walls. Each test lasted 20 min. At the beginning of a test, an animal was always placed in the same position, facing the right rear corner of the platform. The evaluated rat behavior parameters were as follows: time spent at the wall of the maze, time spent in the middle part of the board, locomotor activity at the walls and central part of the maze, mictions, defecations, and hind leg stands. The platform was illuminated with a 700 lx light source.

The behavioral tests were recorded on a graphics card using a Nikon Z6 camera, and the images were then transferred to a computer. The number of hind leg stands, defecations, mictions, and entries into open arms, as well as the time spent in open arms, at the wall of the maze, and in the central part of the board, were all evaluated by a human observing the recorded material. Locomotor activity in the open and closed arms of the elevated plus maze and near the walls and central part of the open field maze was analyzed using automatic image analysis. Rat activity time was determined using the automatic activity assessment method for very fast-moving animals described by Nieckarz et al. [21]. This method is easy to implement and allows one to not only observe the locomotion and the process of an animal's orientation in the environment, but also to detect subtle head movements associated with olfactory exploration in a given area. Activity in particular areas was studied separately by applying appropriate masks to the frames of the film being analyzed. The masks left the studied area intact and covered adjacent areas of no interest to a given test.

2.8. Statistical Analysis

All calculations were performed using the SAS 9.4 statistical package (SAS/STAT® User's Guide, 2021). The following parameters were considered: corticosterone concentration, thyroid-stimulating hormone concentration, sperm motility, sperm viability, percentage of morphologically abnormal sperm, and behavioral parameters.

The TABULATE procedure was used to characterize the distribution of the following parameters: corticosterone concentration, thyroid-stimulating hormone concentration, sperm motility, sperm viability, and percentage of morphologically abnormal sperm. For each parameter listed above, two tables were compiled. The tables contained means and standard deviations, as well as the smallest and largest values for groups, series, and group × series subclasses within each experiment. The analysis of variance was conducted using the GLM procedure. The linear model of the observations included the fixed effects of the experimental group series, as well as interactions between them. For the groups, series, and group × series subclasses, the least squares means (LSMEANS) were calculated and compared using the Tukey–Kramer procedure.

By contrast, for statistical calculations regarding behavioral parameters, the TTEST procedure was used, and the means in the control group were compared with the means in the experimental group. This step was carried out in two stages. First, using the F test, the homogeneity of variance in both groups was examined. Later, depending on the result, a *t*-test for groups with similar variances or a *t*-test for groups with different variances was used to compare the means of both groups. The effect size was calculated according to Cohen [22]. Cohen's *d* is designed to compare two groups, taking the difference between

two means and expressing it in standard deviation units, thus demonstrating how many standard deviations lie between these two means.

Additionally, the *t*-test was used to compare the values obtained in electromagnetic field measurements.

The final stage of this part of the statistical calculations involved separately calculating Pearson's linear correlation coefficients between the above-mentioned variables in the experimental and control group. The CORR procedure was used to characterize the linear correlation between the variables, and *p*-values for correlation coefficients were corrected for multiple comparisons to control the overall type I error. The Bonferroni and Hochberg methods were used.

3. Results

3.1. Electromagnetic Field

The electromagnetic field emitted by the generator within the rat cages was characterized by the following parameters: the average electric field intensity ranged from 2.8 to 5.7 V/m (0.1 V/m), the average magnetic field intensity ranged from 7.2 to 14.0 mA/m (± 0.2 mA/m), and the average power density ranged from 17.7 to 63.6 mW/m² (± 0.5 mW/m²) at a frequency of 1800 MHz. Throughout the experiment, the EMF background was monitored, with an average value of 0.22 ± 0.07 V/m.

No significant differences were observed between the period without emission and the period with PEM emission.

3.2. Hormone Concentration

The statistical analysis of the corticosterone and thyroid-stimulating hormone concentrations in rat blood plasma (GLM test) showed the significance of all the main effects analyzed, i.e., groups ($p < 0.001$), series ($p < 0.001$), and group $\times$ series interactions ($p < 0.001$).

The post hoc test showed a significant increase in the concentration of corticosterone in blood plasma from the experimental group compared with the control values in series III–VIII ($p < 0.001$). The concentration significantly increased in series III compared with series II within the experimental group ($p < 0.01$), as well as in series VIII compared with series IX ($p < 0.001$) within the same group. No statistically significant differences between adjacent series within the control group were observed (Figure 1A).

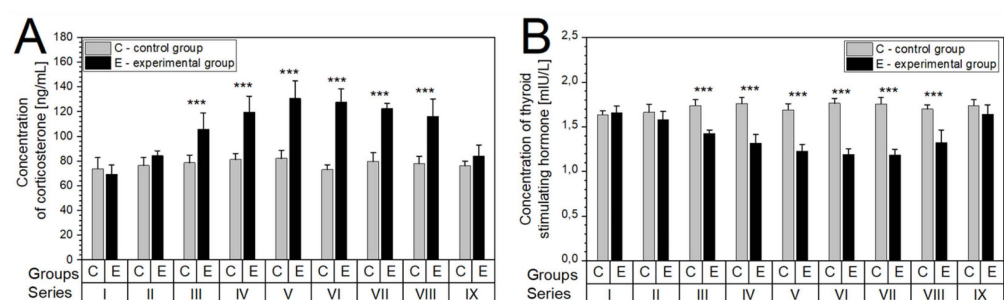


Figure 1. Mean concentration of corticosterone (A) and thyroid-stimulating hormone (B) in blood plasma of rats in the experimental and control group in individual series. C—control group (no EMF exposure); E—experimental group (8 weeks of EMF exposure at 1800 MHz). ***—statistically significant difference at $p < 0.001$ compared with the control value.

By contrast, the post hoc test of thyroid-stimulating hormone concentrations in rat blood plasma showed a significant decrease in TSH concentration from the experimental group compared with the control values in series III–VIII ($p < 0.001$). The concentration

significantly decreased in series III compared with series II within the experimental group ($p = 0.05$) and significantly increased in series IX compared with series VIII ($p < 0.001$) within the same group. Similarly to corticosterone, no statistically significant differences between adjacent series within the control group were observed (Figure 1B).

3.3. Semen Quality Parameters

In the evaluations of both sperm motility and viability, the GLM test showed the significance of the analyzed group effect ($p < 0.001$). However, no significance was recorded regarding the analyzed series effect ($p > 0.05$, sperm viability; $p > 0.05$, sperm motility) or the effect of the group $\times$ series interaction ($p > 0.05$, sperm viability; $p > 0.05$, sperm motility).

The post hoc test showed a significant reduction in sperm motility in rats from the experimental group compared with the control value of animals euthanized after 12 weeks of field emission ($p < 0.001$) and 4 weeks after the end of the field emission ($p < 0.001$) (Figure 2A).

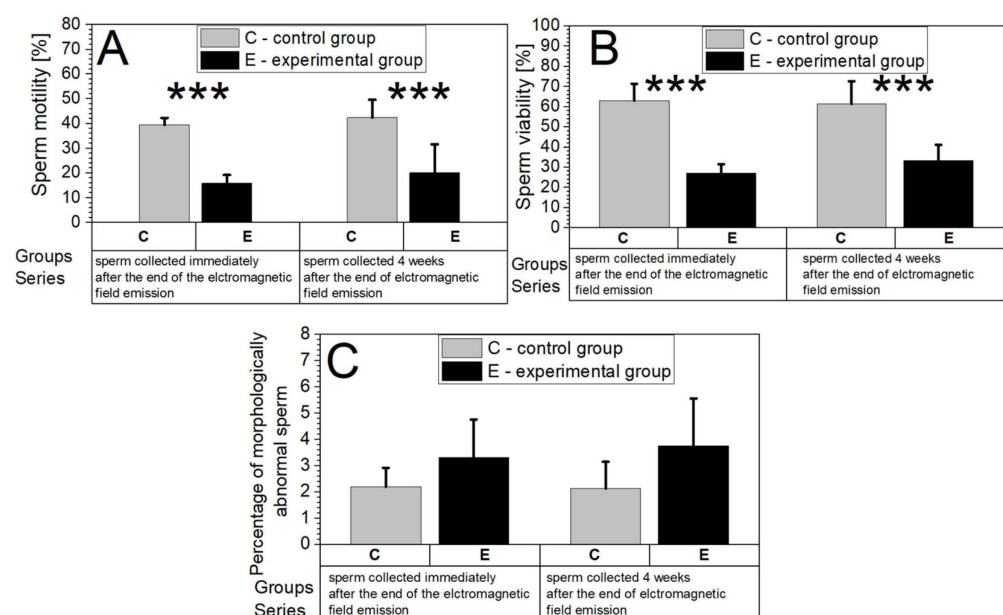


Figure 2. Sperm motility (A); sperm viability (B); mean percentage of morphologically abnormal sperm (C) in experimental and control rats. C—control group (no EMF exposure); E—experimental group (8 weeks of EMF exposure at 1800 MHz). ***—statistically significant difference at $p < 0.001$ compared with the control value.

Similar results were obtained when assessing sperm viability. The post hoc test demonstrated the significant reduction in sperm viability in rats from the experimental group compared with the control value of animals euthanized after 12 weeks of field emissions ($p < 0.001$) and 4 weeks after the end of the field emission ($p < 0.001$) (Figure 2B).

The statistical analysis (GLM test) concerning morphologically abnormal spermatozoa showed no significance in the analyzed main effects, i.e., groups ($p > 0.05$), series ($p > 0.05$), and group $\times$ series interactions ($p > 0.05$). The post hoc test showed no significant differences between the experimental and control group at the assumed significance level of 0.05 (Figure 2C).

3.4. Results of Behavioral Tests

Locomotor activity in the open arms of the elevated plus maze evaluated in experimental group rats was statistically significantly lower ($p < 0.001$) than activity in the control group specimens. Similarly, locomotor activity in closed arms was lower in the experimen-

tal group ($p < 0.01$). The time that rats spent in open arms was statistically significantly lower ($p < 0.001$) in the experimental rats than in the controls. The number of mictions and defecations in the experimental group was also statistically significantly lower than in the control group ($p < 0.05$ and $p < 0.01$, respectively). The effect sizes that have been applied did not affect the interpretation of the results (Table 1).

Table 1. Mean values of elevated plus maze parameters in rats from control and experimental group. Control group—no EMF exposure; experimental group—8 weeks of EMF exposure at 1800 MHz.

	Time Spent in Open Arms [s]	Locomotor Activity in Open Arms [s]	Time Spent in Closed Arms [s]	Locomotor Activity in Closed Arms [s]	Entries into Open Arms [n]	Mictions [n]	Defecations [n]	Hind Leg Stands [n]
Control group	52.00	44.38	248	161.97	6.14	1.71	1.57	6
Experimental group	11.44 ***	9.30 ***	288.56 ***	120.87 **	2.57 *	0.71 *	0.30 **	4.86
Effect size	1.88	1.86	1.88	1.33	1.08	1.06	1.34	0.34

*—statistically significant difference at $p < 0.05$ compared with the control value; **—statistically significant difference at $p < 0.01$ compared with the control value; ***—statistically significant difference at $p < 0.001$ compared with the control value. Effect size: An effect greater than 0.8 is considered large, an effect ranging from 0.2 to 0.5 is medium, and an effect less than 0.2 is small.

The time spent at the wall of the open field maze wall was statistically significantly longer ($p < 0.001$) for the experimental group than for the control group. The locomotor activity time was statistically significantly lower in the experimental rats ($p < 0.001$). Furthermore, the time spent in the center of the open field maze was statistically significantly shorter in the experimental animals than in the control animals ($p < 0.001$), as was locomotor activity ($p < 0.001$).

An analysis of data obtained in the open field test showed that the number of hind leg stands in the experimental group was statistically significantly lower ($p < 0.05$) than the results obtained in the control group. The effect sizes that have been applied did not affect the interpretation of the results (Table 2).

Table 2. Mean values of open field test parameters in rats from control and experimental group. Control group—no EMF exposure; experimental group—8 weeks of EMF exposure at 1800 MHz.

	Time Spent in the Central Part of the Maze [s]	Locomotor Activity in the Central Part of the Maze [s]	Time Spent at the Walls of the Maze [s]	Locomotor Activity at the Walls of the Maze [s]	Mictions [n]	Defecations [n]	Hind Leg Stands [n]
Control group	110.40	92.35	1089.60	678.87	0.5	0.67	21.67
Experimental group	46.30 ***	32.40 ***	1153.70 ***	443.632 *	0.43	0.71	13.86 *
Effect size	1.63	1.54	1.60	1.63	0.14	0.06	1.12

*—statistically significant difference at $p < 0.05$ compared with the control value; ***—statistically significant difference at $p < 0.001$ compared with the control value. Effect size: An effect greater than 0.8 is considered large, an effect ranging from 0.2 to 0.5 is medium, and an effect less than 0.2 is small.

3.5. Correlation Results

Correlation analysis showed a very high positive correlation between corticosterone concentration and time spent in closed arms among the experimental rats. The analysis also showed a very high negative correlation between corticosterone levels and locomotor activity in open arms and between the concentration of this hormone and the time spent in

open arms (Table 3). By contrast, in the control group, calculations showed a high negative correlation between the concentration of this stress hormone and locomotor activity in open arms. The application of Bonferroni and Hochberg corrections did not significantly alter the statistical significance of the results (Table 3).

Table 3. Pearson’s correlation coefficients between corticosterone concentrations and rat activity in the elevated plus maze. C—control group (no EMF exposure); E—experimental group (8 weeks of EMF exposure at 1800 MHz).

	Time Spent in Open Arms		Locomotor Activity in Open Arms		Time Spent in Closed Arms		Locomotor Activity in Closed Arms		Entries into Open Arms	
	E	C	E	C	E	C	E	C	E	C
Corticosterone concentration	−0.889	−0.472	−0.854	−0.577	0.889	0.472	−0.215	0.054	0.176	−0.179
<i>p</i> -value	0.01	0.28	0.01	0.18	0.01	0.29	0.64	0.90	0.70	0.70
<i>p</i> -value with Bonferroni adj	0.05	1.00	0.05	0.90	0.05	1.00	1.00	1.00	1.00	1.00
<i>p</i> -value with Hochberg adj	0.03	0.87	0.03	0.87	0.03	0.87	0.70	0.90	0.70	0.90

The analysis of rat behavior conducted using an open field test showed a high positive correlation between corticosterone concentration and time spent at the maze wall among the experimental rats. There was also a high negative correlation between corticosterone concentration and time spent in the center of the maze, as well as between the level of this hormone and locomotor activity observed in the center of the maze (Table 4). No high correlations were found in the control animals. The application of Bonferroni and Hochberg corrections did not significantly alter the statistical significance of the results (Table 4).

Table 4. Pearson’s correlation coefficients between corticosterone concentration and rat activity, open field test. C—control group (no EMF exposure); E—experimental group (8 weeks of EMF exposure at 1800 MHz).

	Time Spent in the Central Part of the Maze		Locomotor Activity in the Central Part of the Maze		Time Spent at the Walls of the Maze		Locomotor Activity at the Walls of the Maze	
	E	C	E	C	E	C	E	C
Corticosterone concentration	−0.603	−0.132	−0.504	−0.339	0.603	0.132	−0.484	−0.105
<i>p</i> -value	0.15	0.78	0.25	0.46	0.15	0.78	0.27	0.83
<i>p</i> -value with Bonferroni adj	0.60	1.00	1.00	1.00	0.60	1.00	1.00	1.00
<i>p</i> -value with Hochberg adj	0.27	0.83	0.27	0.83	0.27	0.83	0.27	0.83

4. Discussion

Stress is a physiological and mental response to adverse stimuli, including environmental pollution. The neuroendocrine system is very sensitive to environmental changes, including electromagnetic fields. In neuroendocrine mechanisms of adaptation processes, adrenal glands play a leading role. The functional activity of the cortex of the adrenal gland is controlled by the pituitary gland hormone, adrenocorticotrophic hormone (ACTH), which regulates the level of corticosterone [23]. In the conducted experiment, after four weeks of exposing the animals to the field until the end of EMF (1800 MHz) emission, corticosterone

concentration in rats belonging to the experimental group was significantly higher than in the animals from the control group. This effect persisted for two more weeks after the end of field emission. A stress response was also activated by an 1800 MHz electromagnetic field in rat studies conducted by Bouji et al. [24] and Daniels et al. [25]. Moreover, Pawlak et al. [26] showed an increase in corticosterone concentration in the blood of chicks exposed to EMF (1800 MHz) during embryonic development. Chronic stress can suppress protective immune responses and intensify pathological ones; it may also increase susceptibility to some types of cancer [27]. In contrast, studies conducted by Daniels et al. [25] showed no significant differences between corticosterone concentration in rats exposed to electromagnetic fields at the frequencies used for mobile communications and those kept in control conditions. Moreover, Szemerszky et al. [28] found that low-frequency (50 Hz) fields did not affect corticosterone concentration in the experimental rats compared with the control rats. However, extremely low-frequency fields (50–60 Hz) have different properties from those of 1800 Hz fields, and their effects on living organisms may differ. The endocrine changes observed in the current study persisted for two weeks after the end of exposure, and after another two weeks, they were no longer observed. Thus, this article is one of the first reports indicating the transient nature of these changes.

The mechanism underlying the stress response to EMF exposure remains unclear [29]. Studies suggest that exposure to radiofrequency electromagnetic fields may result in adverse health effects, including structural changes in the brain and alterations in brain activity [30,31], which may disturb sleep patterns [32]. As electromagnetic fields affect the brain, it can be assumed that an increased stress hormone level may result from the influence of EMFs on the hypothalamus and/or pituitary gland.

TSH concentration was significantly lower during exposure (in series III to VII), as well as two weeks after the end of the EMF effect on the rats (series VIII). After another two weeks (series IX), the TSH concentration in the experimental group rats did not differ significantly from that recorded in the control group. Similarly, a study conducted by Koyu et al. [33] showed a statistically significant reduction in TSH concentration in the blood of the rats exposed to EMF (900 MHz) compared with control specimens. A decrease in the concentration of this hormone has also been recorded in people with the so-called electromagnetic hypersensitivity syndrome in people subjected to EMF [34]. Mortawazi et al. [35] showed a significant decrease in TSH concentration in the blood of students who used mobile phones intensively (field frequency, 900 MHz).

It appears that EMF may affect the pituitary gland by reducing the secretion of TSH, which is a typical response to various types of stress factors [36]. A significantly reduced amount of TSH may indicate hyperthyroidism. Hyperthyroidism as a result of EMF at 900 MHz frequency is suggested by the results obtained by Sechman et al. [37], who showed a significant increase in T3 and T4 concentrations in chicken embryos exposed to electromagnetic field. Notably, the results of the present study suggest that discontinuing EMF emission can normalize endocrine activity in the pituitary gland in terms of TSH secretion. It cannot be ruled out that the decrease in TSH secreted by the pituitary gland found in the current study may have resulted in secondary hypothyroidism and consequently led to a decrease in the rate of metabolic changes. However, verifying this conclusion would require additional determinations, i.e., the T3 and T4 concentrations, which may be good indications for further research.

The safety of human exposure to an ever-increasing number and diversity of EMF sources, both at work and at home, has become a public health issue. To date, many *in vivo* and *in vitro* studies have revealed that EMF exposure can alter cellular homeostasis, endocrine function, and reproductive function. Reproductive disorders that may be associated with EMF exposure include male germ cell death, changes in the estrous cycle, alterations in

reproductive hormones, variations in reproductive organ weights, reduced sperm motility, impaired early embryonic development, and decreased pregnancy success [38].

In our experiment, a statistically significant reduction in sperm motility was observed in the experimental group compared with the control group after 12 weeks of exposure and 4 weeks after the end of emission. These results may indicate the chronic impact of electromagnetic field on the tested parameter. In a study of rats exposed to EMF (900 MHz for 28 days), Narayanan et al. [39] observed statistically significantly lower sperm motility in specimens subjected to EMF exposure. In addition, Adebayo et al. [40] observed a significant reduction in sperm motility in rats exposed to 1800 MHz field emission for 5 weeks. Ghanbari et al. [41] also noted that radiofrequency electromagnetic field reduces sperm motility in rats. Bahaodini et al. [42] showed significantly lower sperm motility in rats exposed to 50 Hz EMF for 85 days. Moreover, other studies have shown that sperm motility in rats decreases with increasing magnetic field intensity [43]. Our study showed a statistically significant reduction in sperm viability in the group subjected to 12 weeks of EMF emission which persisted for 4 weeks after the end of emission. Furthermore, Ghanbari et al. [41] showed that radiofrequency electromagnetic fields significantly reduced the viability of rat sperm.

During the experiment, sperm motility and viability decreased both during EMF propagation and four weeks after emission stopped. This is likely related to the fact that spermatogenesis in rats lasts about 50 days, meaning that male reproductive cells were exposed to EMFs throughout their development [44].

It seems that the decline in sperm quality is associated with oxidative stress caused by increased levels of free radicals. Reduced sperm motility and viability have been observed at high superoxide anion concentrations [45]. Free radicals oxidize membrane phospholipids, decreasing cell membrane fluidity, which is associated with impaired motility and reduced sperm viability [46]. The current study showed that this experimental factor had no statistically significant effect on the percentage of morphologically abnormal sperm in the experimental group rats compared with the control group rats. Similar results were obtained by Dasdag et al. [47], who investigated the effect of radiofrequency EMF on testicular structure and sperm quality in rats, as well as by Sommer et al. [48], who subjected mouse genitals to electromagnetic fields throughout their entire lifespans. In contrast, Narayanan et al. [39] treated rats with a 900 MHz field for 28 days (for an hour a day), reporting a significantly higher percentage of abnormal sperm. Agrawal et al. [49] showed that increasing exposure time to mobile phones in men increases the number of morphologically abnormal sperm in their semen.

It is well known that radiofrequency electromagnetic fields can affect animal behavior [50]. In the current study, the elevated plus maze test showed statistically significant shorter time spent in open arms, fewer mictions and defecations, and lower locomotor activity in both open and closed arms in animals belonging to the experimental group compared with the controls. As in the above-described test, lower locomotor activity was observed in EMF-treated rats at both the central and wall parts of the maze during the open field test. The experimental rats also spent more time at the walls than in the central part of the field and performed fewer hind leg stands. During an open field test, Daniels et al. [23] also showed significantly reduced locomotor activity in rats exposed to EMF at frequencies used by mobile communications (840 MHz). In contrast, Szemerszky et al. [28] did not observe any significant differences in the behavior of rats exposed to a 50 Hz field during an elevated plus maze test. Decreased motor activity may indicate anxiety disorders, possibly related to a stress response [51]. Similar results were obtained by Hosseini et al. [52], who studied the effects of electromagnetic fields (frequency, 50 Hz) on stress levels in female rats. They established that corticosterone concentrations and anxiety behaviors increase in

animals subjected to EMF. The correlations we calculated seem to confirm this phenomenon, especially between stress hormone concentrations and the motor activity of rats in the open arms and central part of the open field maze.

5. Conclusions

Our results showed that electromagnetic fields emitted by mobile communications at 1800 MHz impacted the functioning of the rat body, demonstrating the effect of EMF on sperm motility and viability and rat behavior. The results may indicate the stress- and anxiety-inducing effects of this experimental factor, as well as its potential for changing the rate of metabolic changes and impairment in the reproductive functions of exposed individuals. Nevertheless, while the results of similar experiments have not yet provided a clear answer, they are an important clue in studying the mechanisms of this impact. According to reports indicating a significant decline in semen quality among men in industrialized countries, it is reasonable to consider a potential association between this phenomenon and increasing exposure to artificial electromagnetic fields. If further studies (including molecular research) confirm the existence of such a relationship, a discussion on the revision of current guidelines regarding permissible exposure levels to electromagnetic fields would be warranted (61 V/m in the EU). It should be noted that these threats may primarily concern people operating and servicing mobile phone antennas.

Limitations

The present study has several limitations, including a relatively small group size ($n = 7$), a limited range of biochemical parameters assessed, and the absence of in-depth analyses of the molecular mechanisms underlying the observed effects. In our opinion, the omission of a sham group does not represent a methodological shortcoming, as the animals were habituated to the presence of the generator antenna during the quarantine period. Moreover, all living conditions and procedures were identical in both groups, and the only variable distinguishing the control and experimental groups was the presence or absence of EMF exposure.

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Institutional Review Board Statement: This experiment was approved by the Local Ethics Committee in Krakow (Resolution No. 240/2019 of 27 February 2019). All methods applied in this study were performed according to relevant guidelines and regulations, including the ARRIVE 2.0 guidelines and the 3Rs principles. Every effort was made to minimize the number of individuals used and the suffering of the animals in the present study.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article. All materials are housed at the Department of Zoology and Animal Welfare, University of Agriculture in Krakow, Krakow, Poland. The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

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