

The in vivo effects of low-intensity radiofrequency fields on the motor activity of protozoa

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Abstract

Purpose: To analyze the direct and transgenerational effects of exposure to low-dose 1 GHz (mobile phone/wireless telecommunication range) and 10 GHz (radar/satellite communication range) radiofrequency electromagnetic fields (RF-EMF) on the motility of ciliates *Spirostomum ambiguum*.

Materials and methods: *S. ambiguum* were exposed to 1 GHz and 10 GHz RF-EMF with power flux densities (PD) ranging from 0.05–0.5 W/m² over a period of time from 0.05–10 h. The motility of directly exposed ciliates and their non-exposed progeny across 10–15 generations was measured.

Results: Exposure to 0.1 W/m² of either 1 or 10 GHz RF-EMF resulted in a significant decrease in the motility. The dose of exposure capable of altering the mobility of ciliates was inversely correlated with the flux density of RF-EMF. The motility of the non-exposed progeny of ciliates irradiated with 0.1 W/m² of 10 GHz RF-EMF remained significantly compromised, at least, across 10–15 generations, thus indicating the presence of transgenerational effects.

Conclusions: The results of our study show that low-dose exposure to RF-EMF can significantly affect the motility of irradiated ciliates and their non-exposed offspring, thus providing further insights into the unknown mechanisms underlying the in vivo effects of RF-EMF.

Keywords: Radiofrequency electromagnetic radiation, low-intensity electromagnetic fields, ciliate, motor activity

Introduction

In recent decades the growing exposure to radiofrequency electromagnetic fields has raised a considerable concern regarding its biological effects for humans and other organisms. Although the results of a number of epidemiological studies still remain inconclusive, the International Agency for Research on Cancer (IARC) has recently classified ELF-EMF exposure as possibly ‘carcinogenic to humans’ (Baan et al. 2011). Given this, the development of efficient and

reliable test-systems for monitoring the effects of radiofrequency electromagnetic fields on a wide range of organisms is clearly warranted.

The existing risk estimates of EMF radiation heavily rely on the results of numerous in vivo and in vitro studies in mammals (Funk et al. 2009). Although these studies have provided important information regarding the effects EMF radiation, quite often they are very laborious and, besides, their sensitivity may not be high enough for detecting the effects of low and, in some cases, intermediate doses. Given the fact that these are the main doses of concern, the development of more sensitive experimental models for evaluating germline effects of EMF radiation is clearly needed. The results of a number of previous studies show that the aquatic ciliate *Spirostomum ambiguum* may represent such a model (Tushmalova et al. 1998, Nałecz-Jawecki and Sawicki 1999, Bakhvalova et al. 2007). Using this model, here we have analyzed the effects of low-intensity RF-EMF radiation on the motility of *S. ambiguum*.

Materials and methods

Test organism and experimental design

The strain of *S. ambiguum* used in our experiments was originally collected in the pond of the Moscow Zoo and successfully reared for 10 years in our laboratory. Normally, these ciliates are relatively large (up to 1 mm in length and 30–50 µm in diameter) and can be observed at low magnification ($\times 2$). The ciliates were kept in a mass culture at $20 \pm 1^\circ\text{C}$ in glass tubes containing 15 ml of chlorine-free double-filtered tap water (pH 7.2–7.5, O₂ 8.4–11.8 mg/l at 19–22°C, total mineral content 6.8 mg/l, Fe 0.3 mg/l, Mn 0.1 mg/l) with the density of 30–50 ciliates per ml as previously described (Tushmalova 2007). Ciliates were fed weekly with yeast suspension (0.5 mg/ml). Mass culture was maintained in log phase by diluting 1:1 with water each week. All experiments were performed at Obninsk INPE NRNU ‘MEPhI’.

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Ciliates were exposed either to 1 GHz (mobile phones and telecommunication devices frequency) or 10 GHz (radars and satellite communication frequency) of RF-EMF in 3.5 cm plastic Petri dishes (Nuova, Aptaca, Canelli, Italy), suspended in 0.5 cm layer of chlorine-free double-filtered tap water. The 1 GHz exposure system consisted of a R2-52 generator equipped with a flat spiral antennae with diameter 11 cm (Priborelectro, Moscow, Russia). EMF generated by it possesses left-handed circular polarization (ellipticity coefficient at least 0.7) in which the vector of electrical field strength E is perpendicular to the direction of energy and wave propagation and rotates around it with frequency of radiation. The 10 GHz RF-EMF source comprised of G4-109 generator equipped with pyramid-shaped horn antennae (14.5 × 14.5 cm; Tetron, Moscow, Russia), which emitted EMF wave with linear polarization. For both types of exposure, the samples were placed under the sources of RF-EMF as previously described (Sarapultseva et al. 2009, Litovchenko et al. 2011).

For both frequencies, the ciliates were exposed to PD of 0.05, 0.1 and 0.5 W/m². The dose of exposure were within the Russian safety standards with the maximum allowed level (MAL) value of 0.1 W/m² for 900 MHz at a whole-body specific absorption rate (SAR) of 44 mW/kg (Russian Ministry of Health 2003). It should be noted the above-mentioned MAL value is substantially lower than those permitted in Europe (European Committee for Electrotechnical Standardization [CENELEC] 2010) and internationally (Repacholi et al. 2012).

In our experiments, the PD were measured by P3-18 (Rial, Nizhny Novgorod, Russia) dosimeter with sensitivity $3 \cdot 10^{-4}$ W/m² (error rate 12%) according to the guidance (Russian Ministry of Health 2002) and technical certificate. For the 1 GHz RF-EMF irradiation with PD 0.05, 0.1 and 0.5 W/m² the distance from the source was 30, 18 and 3.5 cm, respectively. According to the results of our previous work (Litovchenko et al. 2011), the design of a flat spiral antenna with diameter 11 cm provides the smooth decrease of PD from 0.50–0.05 W/m² within the distance from the source ranging from 3.5–30 cm and without spikes (Figure 1).

For all the 10 GHz RF-EMF irradiations the distance was 10 cm and PD was regulated by an integrated attenuator to

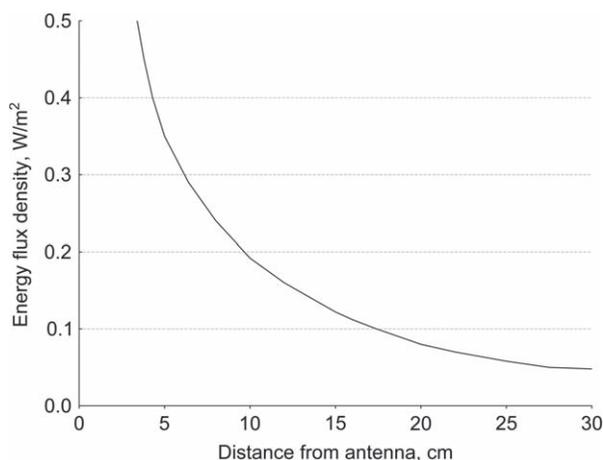


Figure 1. Power flux density for the 1 GHz experiment.

the G4-109 generator. Within the area of exposure, the PD values' decrease from the centre did not exceed 15%.

During irradiation, *S. ambiguum* were kept in the Petri dishes containing 4 ml of water with a density of 20–30 ciliates per ml. The chaotic movement of ciliates ensured the uniformity of exposure. The time of exposure ranged from 0.05–10 h. Background measurements of the power density in the laboratory did not exceed 0.0015 W/m² at all measurement points. The strength of electric field was 3 V/m, magnetic field – 0.08 μT, measured by ATT-8701 DC/AC Milligauss magnetometer (Tektronix, Inc., Oregon, USA).

The dose of exposure (J/m²) was estimated as $3600 \times \text{EFD (W/m}^2) \times \text{time (h)}$. During exposure, all corresponding sham-treated (control) groups were placed in the same room at the distance of 1 m from the irradiator, where the electric and magnetic components were kept on the background level. The temperature was monitored by an Infrared Thermometer (Precision Mastech Enterprises, China) with precision $\pm 0.1^\circ\text{C}$ each 15 min during the first hour of exposure and later each hour to the end of treatment. It was kept within $20.3 \pm 0.2^\circ\text{C}$ for all doses of exposure. To ensure the homogeneity of energy deposition in each experiment, the temperature was measured across all irradiated and sham-treated Petri dishes, which remained fairly constant ($\pm 0.2^\circ\text{C}$) for the whole period of exposure up to 10 h.

Motility of ciliates

The ciliates swim steadily at a rate of approximately 2.5 mm/s using a longitudinal row of small cilia. All measurements of motility were performed immediately after treatment. Twenty ciliates from each group were randomly taken from a Petri dish and individually placed on a plastic plate containing several holes in 5 mm in diameter and 1–2 mm in depth. The motility was measured by the MSB-10 microscope (Lyt-karino, Russia) with an eyepiece containing two crossed lines using the open-field technique (Brudzynski and Krol 1997, Drai et al. 2000), modified by Tushmalova et al. (1998). The motility of each ciliate was monitored by eye and expressed as a number of intersections of the lines per minute (Figure 2). If a ciliate at least half-crossed the line, we regarded this as an intersection.

To analyze the long-term effects of RF-EMF radiation, ciliates were kept in test tubes at $20 \pm 1^\circ\text{C}$ in 15 ml of chlorine-free double-filtered tap water for 4, 14, 21 and 30 days after treatment and their motility was measured as described above. According to our results, the duration of the life cycle of *S. ambiguum* remains practically unchanged over this period of time (Sarapultseva et al. 2010) and is approximately 2–3 days as previously reported by Wichterman (1986).

In the majority of experiments, the motility was measured for 20 ciliates per group in 2 or 3 replicates. All samples were double-coded. The data were analyzed using the non-parametric Kruskal-Wallis/Mann-Whitney test, corrected for multiple testing using the Bonferroni procedure.

Results

The results of our study are summarized in Tables I–III. For each sham-exposed and treated group, exposed to different

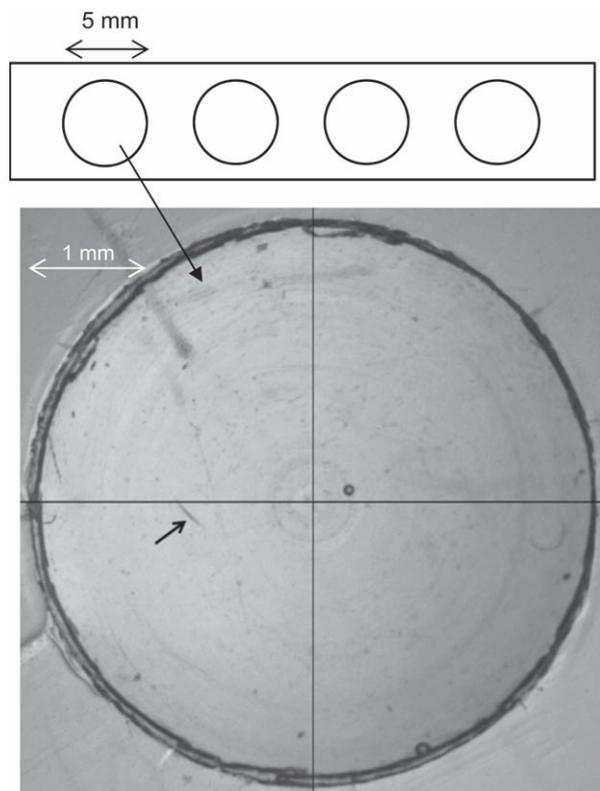


Figure 2. The open-field technique for analyzing motility of ciliate *Spirostomum ambiguum*. A plastic plate with holes 5 mm in diameter and 2 mm in depth is shown together with a photograph ($\times 2$) of ciliate at one of the crossed lines (arrowed).

doses of RF-EMF we first checked whether the motility of ciliates significantly differed between biological replicates. As within all groups the motility did not significantly differ (Kruskal-Wallis test, $0.09 < p < 0.99$), we therefore combined the data for each set of replicates. In all experiments, the motility of sham-irradiated ciliates was measured alongside the directly treated ciliates at the beginning and end of their exposure (Figure 3).

Given that in all groups the motility did not significantly differ, we concluded that the effects of confounding factors on the motility was negligible and therefore combined the control data.

The effects of exposure to 1 GHz RF-EMF

Here we analyzed the effects of exposure to three different flux densities of 1 GHz RF-EMF on ciliates' motility, including 0.1 W/m^2 , which corresponds to the maximum allowed level of RF-EMF exposure in Russia (Russian Ministry of Health 2003). A highly significant reduction in ciliates' motility was detected following exposure to all flux densities (Table I). The analysis of dose-response curves revealed that a similar dose of $\sim 300 \text{ J/m}^2$ of relatively high-PD exposure (0.1 and 0.5 W/m^2) resulted in a highly significant 2-fold reduction of the motility. In contrast, low-PD to 0.05 W/m^2 was less effective and affected the motility only at much higher doses in excess of 1400 J/m^2 (Figure 4A). It should be noted that the dose-response curves for all three values of PD were clearly non-linear. Instead, above certain doses of exposure the magnitude of decrease was similar, whereas at smaller doses

Table I. The effects of exposure to 1 GHz RF-EMF on the motility.

Dose, J/m^2 (time, h)	n^*	Mean \pm SEM	Prob ⁺
0.05 W/m^2			
0 (sham-exposed)	60 (3)	2.10 ± 0.08	-
900 (5)	60 (3)	1.85 ± 0.11	0.3390
1260 (7)	60 (3)	2.10 ± 0.10	1
1440 (8)	60 (3)	1.20 ± 0.10	3.22×10^{-8}
1620 (9)	60 (3)	1.12 ± 0.11	7.64×10^{-9}
1800 (10)	60 (3)	1.15 ± 0.11	1.76×10^{-8}
0.1 W/m^2			
0 (sham-exposed)	60 (3)	2.15 ± 0.08	-
90 (0.25)	60 (3)	2.08 ± 0.09	1
180 (0.5)	60 (3)	2.02 ± 0.10	1
270 (0.75)	60 (3)	1.15 ± 0.11	1.78×10^{-9}
360 (1)	60 (3)	1.18 ± 0.08	6.07×10^{-11}
720 (2)	60 (3)	1.23 ± 0.11	1.12×10^{-7}
1080 (3)	60 (3)	1.15 ± 0.10	1.09×10^{-9}
1440 (4)	60 (3)	1.10 ± 0.10	2.22×10^{-10}
2160 (6)	20 (1)	1.20 ± 0.19	0.0001
2880 (8)	20 (1)	1.25 ± 0.14	4.28×10^{-5}
3600 (10)	20 (1)	1.15 ± 0.21	0.0002
0.5 W/m^2			
0 (sham-exposed)	60 (3)	2.12 ± 0.11	-
90 (0.05)	40 (2)	2.05 ± 0.13	1
150 (0.08)	60 (3)	2.10 ± 0.11	1
300 (0.17)	60 (3)	1.15 ± 0.10	1.15×10^{-7}
600 (0.33)	60 (3)	1.10 ± 0.11	3.16×10^{-7}
900 (0.5)	60 (3)	1.17 ± 0.11	1.69×10^{-6}
1350 (0.75)	20 (1)	1.15 ± 0.22	0.0021
1800 (1)	20 (1)	1.05 ± 0.15	5.92×10^{-5}

*The number of independent experiments is given in parentheses.

⁺Probability of difference from sham-exposed, Mann-Whitney test, Bonferroni corrected.

the mean mobility did not significantly differ from that in sham-controls.

The effects of exposure to 10 GHz RF-EMF

Table II summarizes the effects of exposure to 10 GHz RF-EMF on the motility of ciliates. Similarly to the 1 GHz data, a significant decrease in the motility was detected for all flux densities. The resultant dose-response curve (Figure 4B) was also remarkably similar to that for exposure to 1 GHz RF-EMF. Thus relatively high-PD exposure (0.1 and 0.5 W/m^2) to $\sim 300 \text{ J/m}^2$ equally affected the mobility of ciliates, whereas the same $\sim 50\%$ decrease in motility following exposure to 0.05 W/m^2 was observed at a much higher dose. The magnitude of decrease was similar across a wide range of doses.

The transgenerational effect of exposure to 10 GHz RF-EMF

To establish whether the effects of RF-EMF irradiation can persist in the non-exposed progeny of irradiated ciliates, motility was measured 4, 14, 21 and 30 days after exposure to 0.1 W/m^2 of RF-EMF (Table III). As the duration of the cell cycle in *Spirostomum* is approximately 2–3 days (Wichterman 1986, Sarapultseva et al. 2010), we therefore analyzed the long-term effects of RF-EMF exposure up to 10–15 generations following the parental irradiation. The motility for all the progeny of treated ciliates remained highly significantly compromised up to 10–15 generations (30 days) after parental exposure (Figure 5). Meanwhile, for each parental dose, the effects of treatment were similar for parents and their offspring (Kruskal-Wallis test, $0.06 < p < 0.55$). We therefore conclude that exposure 0.1 W/m^2 of RF-EMF

Table II. The effects of exposure to 10 GHz RF-EMF on the motility.

Dose, J/m ² (time, h)	n*	Mean ± SEM	Prob ⁺
0.05 W/m²			
0 (sham-exposed)	60 (3)	2.15 ± 0.10	-
180 (1)	60 (3)	2.07 ± 0.13	1
360 (2)	60 (3)	2.17 ± 0.14	1
540 (3)	60 (3)	2.15 ± 0.11	1
720 (4)	60 (3)	2.07 ± 0.08	1
900 (5)	40 (2)	2.00 ± 0.13	1
1080 (6)	60 (3)	1.95 ± 0.11	1
1260 (7)	40 (2)	2.07 ± 0.15	1
1440 (8)	60 (3)	1.18 ± 0.12	8.42 × 10 ⁻⁷
1620 (9)	60 (3)	1.15 ± 0.10	1.47 × 10 ⁻⁸
1800 (10)	60 (3)	1.20 ± 0.13	4.48 × 10 ⁻⁶
0.1 W/m²			
0 (sham-exposed)	61 (3)	2.10 ± 0.09	-
90 (0.25)	61 (3)	1.97 ± 0.10	1
180 (0.5)	61 (3)	1.98 ± 0.08	1
270 (0.75)	61 (3)	1.34 ± 0.08	7.76 × 10 ⁻⁷
360 (1)	61 (3)	1.26 ± 0.08	5.35 × 10 ⁻⁸
720 (2)	61 (3)	1.16 ± 0.10	1.72 × 10 ⁻⁸
1080 (3)	61 (3)	1.08 ± 0.08	1.03 × 10 ⁻¹⁰
1440 (4)	61 (3)	1.05 ± 0.06	2.50 × 10 ⁻¹³
2160 (6)	61 (1)	1.23 ± 0.08	1.35 × 10 ⁻⁸
2880 (8)	61 (1)	1.24 ± 0.09	6.16 × 10 ⁻⁸
3600 (10)	59 (1)	1.29 ± 0.09	4.93 × 10 ⁻⁷
0.5 W/m²			
0 (sham-exposed)	60 (3)	2.18 ± 0.10	-
90 (0.05)	60 (3)	2.02 ± 0.12	1
150 (0.08)	60 (3)	2.08 ± 0.10	1
300 (0.17)	60 (3)	1.23 ± 0.11	3.24 × 10 ⁻⁷
600 (0.33)	60 (3)	1.17 ± 0.10	2.04 × 10 ⁻⁸
900 (0.5)	40 (2)	1.13 ± 0.12	4.23 × 10 ⁻⁷
1350 (0.75)	40 (2)	1.18 ± 0.12	1.39 × 10 ⁻⁶
1800 (1)	60 (3)	1.17 ± 0.13	7.43 × 10 ⁻⁷

*The number of independent experiments is given in parentheses.
⁺Probability of difference from sham-exposed, Mann-Whitney test, Bonferroni corrected.

equally affects the motility of directly exposed parents and their offspring.

Discussion

This study was specifically designed to establish the long-term effects of low-intensity radiofrequency electromagnetic fields on the mobility of directly exposed ciliates and their offspring. The analysis of changes in motor activity has revealed that: (i) The motility of ciliates exposed to 1 and 10 GHz with low power flux density (non-thermal effects) of RF-EMF was significantly compromised; (ii) the efficiency of exposure depended on flux densities; (iii) the motility of non-exposed progeny at least across 10–15 generations of protozoa of irradiated parents was similarly affected; (iv) the magnitude of effects of irradiation on motility of directly exposed ciliates and their offspring did not increase with increasing of electromagnetic exposure within a certain range of doses.

We observed a significant abrupt reduction in motility of ciliates following exposure to a certain dose of 1 and 10 GHz RF-EMF. The magnitude of reduction following exposure from two different sources - flat spiral antenna (1 GHz) and pyramid-shaped antenna (10 GHz) - was practically indistinguishable. It would therefore appear that exposure either to circular-polarization wave in the vicinity of the source (1 GHz) or to plane-polarized wave in the remote zone (10 GHz) can equally affect the motility of ciliates.

Table III. The transgenerational effects of parental exposure to 10 GHz RF-EMF (0.1 W/m²) on the motility.

Dose, J/m ² (time, h)	n*	Mean ± SEM	Prob ⁺
Day 4			
0 (control)	60 (3)	2.10 ± 0.09	-
360 (1)	60 (3)	1.28 ± 0.09	3.16 × 10 ⁻⁷
720 (2)	60 (3)	1.18 ± 0.10	1.60 × 10 ⁻⁷
1080 (3)	60 (3)	1.12 ± 0.10	1.21 × 10 ⁻⁸
1440 (4)	60 (3)	1.10 ± 0.09	6.11 × 10 ⁻⁹
2160 (6)	60 (3)	1.20 ± 0.10	2.94 × 10 ⁻⁷
2880 (8)	60 (3)	1.17 ± 0.08	6.65 × 10 ⁻⁹
3600 (10)	60 (3)	1.15 ± 0.08	5.73 × 10 ⁻⁹
Day 14			
0 (control)	60 (3)	2.07 ± 0.10	-
360 (1)	60 (3)	1.22 ± 0.11	1.37 × 10 ⁻⁵
720 (2)	60 (3)	1.15 ± 0.11	1.06 × 10 ⁻⁶
1080 (3)	60 (3)	1.12 ± 0.12	3.03 × 10 ⁻⁶
1440 (4)	60 (3)	1.12 ± 0.11	9.98 × 10 ⁻⁷
2160 (6)	60 (3)	1.13 ± 0.13	5.69 × 10 ⁻⁶
2880 (8)	60 (3)	1.10 ± 0.12	8.71 × 10 ⁻⁷
3600 (10)	60 (3)	1.18 ± 0.10	1.94 × 10 ⁻⁶
Day 21			
0 (control)	60 (3)	2.13 ± 0.12	-
360 (1)	60 (3)	1.20 ± 0.10	4.18 × 10 ⁻⁷
720 (2)	60 (3)	1.05 ± 0.09	4.89 × 10 ⁻⁹
1080 (3)	60 (3)	1.15 ± 0.08	2.54 × 10 ⁻⁸
1440 (4)	60 (3)	1.10 ± 0.09	2.35 × 10 ⁻⁸
2160 (6)	60 (3)	1.30 ± 0.10	3.32 × 10 ⁻⁶
2880 (8)	60 (3)	1.22 ± 0.10	3.71 × 10 ⁻⁷
3600 (10)	60 (3)	1.08 ± 0.09	1.41 × 10 ⁻⁸
Day 30			
0 (control)	60 (3)	2.20 ± 0.09	-
360 (1)	60 (3)	1.47 ± 0.10	1.09 × 10 ⁻⁵
720 (2)	60 (3)	1.50 ± 0.11	6.55 × 10 ⁻⁵
1080 (3)	60 (3)	1.37 ± 0.10	5.62 × 10 ⁻⁷
1440 (4)	60 (3)	1.27 ± 0.09	1.51 × 10 ⁻⁸
2160 (6)	60 (3)	1.42 ± 0.12	2.22 × 10 ⁻⁵
2880 (8)	60 (3)	1.40 ± 0.10	1.61 × 10 ⁻⁶
3600 (10)	60 (3)	1.35 ± 0.10	5.36 × 10 ⁻⁷

*The number of independent experiments is given in parentheses.
⁺Probability of difference from controls, Mann-Whitney test, Bonferroni corrected.

The analysis of ciliates exposed to 0.05, 0.1 and 0.5 W/m² of RF-EMF showed that the effects of exposure to these three PD reached a plateau at threshold doses. Our data therefore indicate that within a certain interval of doses, the effects of RF-EMF irradiation on ciliates' motility

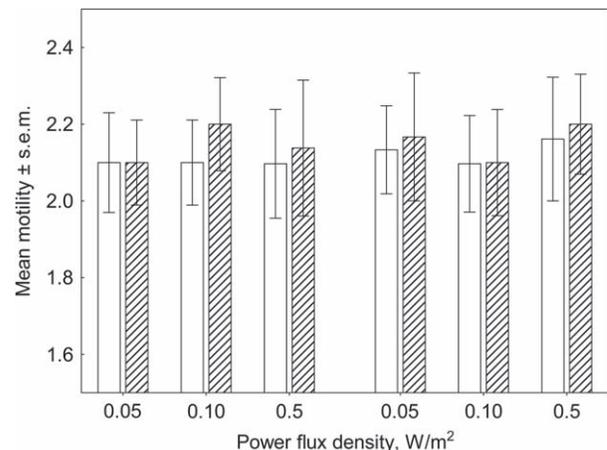


Figure 3. Mean motility of sham-irradiated ciliates at the beginning (open bars) and end (dashed bars) of sham treatment. Kruskal-Wallis test for all comparisons, 0.51 < p < 0.98. Error bars show the standard error of the mean (SEM) for three independent experiments.

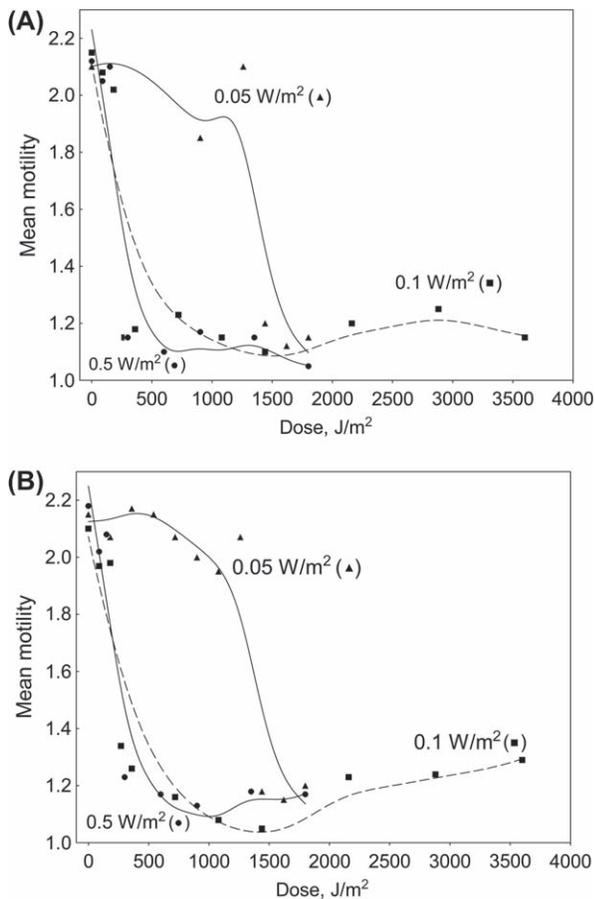


Figure 4. Dose-response curves for the effects of exposure to 1 GHz (A) and 10 GHz (B) RF-EMF on the motility of ciliates. The number of ciliates per each dose together with the values of standard error of the mean are given in Tables I and II.

may reach plateau. The negative effects of RF-EMF exposure including the frequency range analyzed in our study have recently been reviewed (Belyaev 2010). The author concluded that in many cases the dose-response curves are not linear. Instead, the effects of RF-EMF show sigmoidal depen-

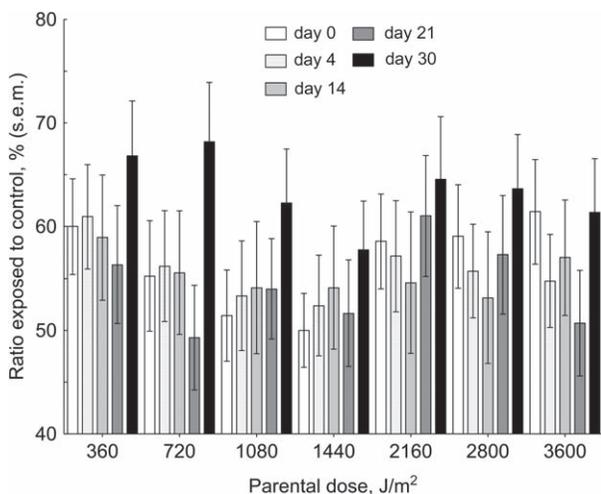


Figure 5. The long-term effects of exposure to 0.1 W/m² of 10 GHz RF-EMF on ciliates' motility. The ratio of mean values of exposed ciliates or their offspring to that in controls are shown. Error bars show the standard error of the mean (SEM) for three independent experiments.

dence and often reach a plateau at threshold doses. Our data are in line with these conclusions.

The results of our study, showing that low-PD RF-EMF irradiation affects the mobility of ciliates at much higher doses than following exposure to higher flux densities, are reminiscent of the data on the mutagenic effects of chronic exposure to ionizing radiation (Russell and Kelly 1982). According to the results of that study, chronic low-dose rate γ -irradiation produces fewer mutations in the male mouse germ line than are induced by an acute dose rate. Our data are therefore in line with these results.

The analysis of long-term effects of RF-EMF revealed that the mobility of non-exposed progeny of irradiated parents remained significantly compromised across at least 10–15 generations. Our data are in line with the results of a number of studies showing that low-dose exposure to γ -ray can compromise the viability of *Paramecium caudatum* and *Climacostomum virens* (reviewed in Bychkovskaya 2013). Their authors have also reported that the magnitude of effects of γ -irradiation on viability of these two species of unicellular organisms did not increase with increasing of exposure within a certain range of doses and manifested among the non-exposed progeny of irradiated parents. Our data are also in line with the results of previous studies on the transgenerational epigenetic effects of paternal exposure to ionizing radiation in mammals (reviewed by Barber and Dubrova 2006). The results of our work therefore provide important evidence for the putative role of epigenetic inheritance in evolution (Jablonka and Raz 2009, Dauer et al. 2010, Vandegehuchte et al. 2010). Taken together, the results of these studies and the data presented here imply that similar mechanisms may explain the above-mentioned long-term effects of exposure to RF-EMF and ionizing radiation.

One of the potential mechanisms underlying the observed effects of EMF on *S. ambiguum* may be attributed to the accumulation of low molecular weight toxic compounds in specialized membrane-bound extractable organelles extrusomes (Buonanno et al. 2012). It therefore remains to be established whether exposure to EMF can result in the accumulation of extrusomes and to what extent their presence can compromise the motility of *S. ambiguum*. It should be noted that the observed effects may also be attributed to the EMF-induced alterations in calcium metabolism as suggested by Deitmer et al. (1984). This possibility should be addressed in future studies.

It should also be noted that according to the results of our study significant changes in ciliates' mobility were detected following exposure to quite low dose RF-EMF with PD as low as 0.05 W/m² (corresponding to a whole body average SAR value of 22 mW/kg for 900 MHz exposure). As these PD values are within the range of the maximum allowed levels of human exposure to EMF recommended in Russia (Russian Ministry of Health 2003), our data therefore show that show that protozoa ciliates represent a useful model for the analysis of the effects of RF-EMF. We have previously shown that exposure to ionizing radiation can significantly affect the motility of *S. ambiguum* (Sarapultseva 2008). The analysis of morphological changes in *S. ambiguum* has also allowed establishing the effects of exposure to a number of organic

compounds (Nalecz-Jawecki and Sawicki 2002, 2003). Taken together, the results of these studies show that *S. ambiguum* represents a highly sensitive model organism which can be used as an in vivo test system/bio-indicator for the analysis of the effects of exposure to wide range of environmental factors.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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