Bioelectromagnetics

Effects of Long-Term Exposure of Intermediate Frequency Magnetic Fields (20 kHz, 360 μT) on the Development, Pathological Findings, and Behavior of Female Mice

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The use of magnetic fields in the intermediate-frequency (IF) range to wirelessly charge electric cars with power transfer in the kilowatt range has become increasingly widespread, leading to unavoidable stray fields in the microtesla range. Only a handful of studies have assessed the potential biological risks associated with exposure to such fields. We exposed female mice ($n = 80$ per group) to either 20 kHz, 360 μT (rms), or sham in Helmholtz coils to conduct a blind design study. Exposure started at 3 months of age (24 h/day). Body mass was recorded every 1–2 weeks. At 10 months of age, three behavioral tests were performed on 24 animals per group. Three months later, the mice were sacrificed and organs (brain, liver, kidney, spleen, and lung) were removed and prepared for microscopic analysis. Our findings demonstrate no differences in the development of body mass and survival rates (96% and 89%, respectively). Similarly, no significant differences were observed in tumor incidence rates. When it comes to behavioral tests, the 8-arm maze results revealed no significant differences. In contrast, the Rotarod data were significantly ($P < 0.001$) different with longer retention times seen in the exposed mice. In the open field, the number of supported rears was significantly lower ($P < 0.01$), whereas the other endpoints did not show any differences. Overall, our data reveal no adverse effects of exposure to 20 kHz, 360 μT on the development and tumor incidences, while the significant differences in the behavioral tests may indicate higher levels of alertness in mice.

Keywords: magnetic fields; intermediate-frequency; mice; cancer; behavior

INTRODUCTION

Historically, research has predominately focused on investigating the biological effects of human exposure to extremely low-frequency magnetic fields (ELF-MF) and radiofrequency electromagnetic fields (RF-EMF). Studies on the potential health risks of magnetic fields in the intermediate-frequency (IF-MF) range (300 Hz to 10 MHz) remain limited [WHO, 2005]. The use of household appliances and electrical components that operate by utilizing IF-MF has grown significantly. This includes devices, such as inductive cookers, charging stations for mobile phones, and wireless charging systems for electric cars. A recent review sheds light on the large heterogeneity in research concerning endpoints, methods, and study designs to elucidate the biological impact of IF-MF exposure [Bodewein et al., 2019]. In 2017, the Scientific Committee on Emerging and Newly Identified Health

Conflicts of interest: None.

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Risks (SCENIHR) recommended additional research to identify potential risks on biological systems due to exposure to such fields [SCENIHR, 2015].

While the power transported by charging stations for mobile phones falls in the range of a few watts, the power required to charge electric cars wirelessly is in the two-digit kilowatt range. A higher power is needed to charge batteries of bigger vehicles, such as trucks or buses. The wireless transmission of electrical energy occurs by using two separate coils. The frequency is typically in the range of 20–150 kHz to yield better efficiency and keep the physical limitations, such as the coils' inductivity, in mind [Li and Mi, 2015]. However, even if the geometry of the coils and the positioning of the vehicle, e.g., the car is optimal, magnetic stray fields are unavoidable. Due to the comparatively high-power transfer, these stray fields are close to the legally defined exposure limits, which are often identical to those recommended by the International Commission of Non-Ionizing Radiation Protection (ICNRP). The stray fields fall in the frequency range between 3 kHz and 10 MHz, while the exposure limit for the general population is 27 μT [ICNIRP, 2010].

Due to the apparent lack of data concerning the potential health risks posed by exposure to IF-MF, the current study was performed to investigate the biological effects of IF-MF.

MATERIALS AND METHODS

IF-MF Exposure Units

As a consequence of magnetic field exposure, the induced currents inside the tissue, and not the magnetic exposure fields themselves, are responsible for the biological effects. Within the exposure experiments conducted for the presented study, the magnetic flux density had to be adjusted such that it induces the same maximum electric field strength—proportionally correlated with the induced current densities via the electrical conductivities of the tissues—inside a mouse as a magnetic flux density of 27 μT (rms value, reference level of ICNIRP inside a human body). Therefore, a detailed dosimetric analysis of the body's internal electric field strengths induced by magnetic fields in a tissue model of a mouse [Reinhardt et al., 2007] and also in a tissue model of a human body ("Duke" of the "The Virtual Family") [Christ et al., 2010] was conducted by the Chair of Electromagnetic Theory, University of Wuppertal, Germany [Zang et al., 2017]. It turns out that the rms value of the magnetic flux density needs to be set 13.3 times higher than the ICNIRP reference value of 27 μT. This factor includes uncertainties due to varying induced electric fields and currents in individual mice and humans, resulting from different complex dielectric structures. Accordingly, mice had to be exposed at 360 μT (27 μT × 13.3) to achieve comparable induced currents. Figure 1 shows the results of the dosimetric analysis in the mouse model.

The requirement of simultaneously exposing 80 mice per group in one large coil system at 20 kHz and 360 μT proved to be a technical challenge. Two Helmholtz coil pairs were calculated and set up to generate homogeneous magnetic fields of 360 μT (rms). The coils had a hexagonal shape (side length = radius = vertical distance = 0.6 m) (Figs. 2 and 3). Measurements of the magnetic fields (ELT-400 with 100 cm² isotropic probe; Narda, Pfulingen, Germany) at various locations (Fig. 3) revealed acceptable deviations from the calculated average value of 360 μT (Table 1). Because the measuring range of the meter was exceeded at full

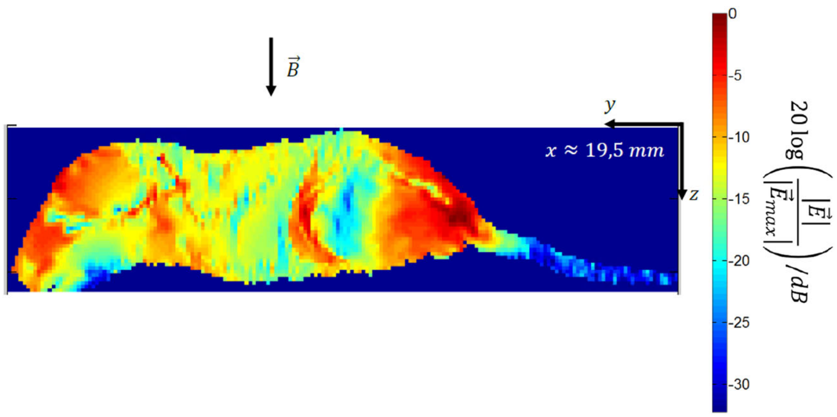

Fig. 1. Distributions in the body-internal electric field strength of a mouse model with a body mass of 40 g. The volume of the voxels was 0.674 × 0.674 × 0.674 mm³.

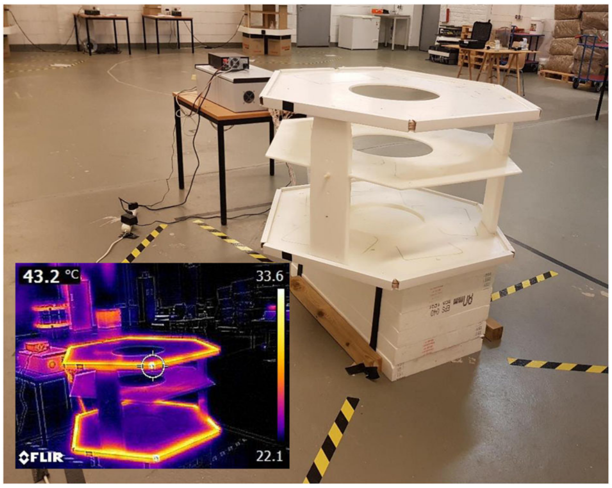

Fig. 2. One of the two Helmholtz coil systems. The insert shows the thermography of the same coil system.

current, the measurements were made at a quarter of the current (15 A).

Due to the skin effect, which is already relevant at 20 kHz, multistrand copper wire (HF-Litzwire, 630 single strands of 0.1 mm diameter each) was used (six turns per coil). As a matter of fact, skin depth in copper is approximately 500 µm at this frequency. A microcontroller-controlled inverter (half-bridge) generated a current of 60 A in the coils (Figs. 4 and 5). The wires had a temperature ranging between 44 °C and 47 °C during operation that was measured by thermography (camera model E6; FLIR Systems, Wilsonville, OR). To ensure a sinusoidal current and to reduce switching losses, the coils were operated resonantly by compensation capacitors. To prevent bias and conduct blinded experiments, two identical Helmholtz coils were constructed. The only difference between them was that in the control (sham) coil, the wire was wound in an antiparallel way, i.e., three turns clockwise and three turns counterclockwise. The rest of the parameters were identical, including the current and electronic box. The magnetic field strength in the control coil was less than 1% compared with the coil in which the mice were exposed. Both systems were located in the same room and were 4.75 m apart.

Due to the exposure limits for humans, warning signs and marks on the ground were made to indicate that entering the marked area was prohibited. When the cages had to be cleaned or the mice had to be inspected or taken out for behavioral tests, the coils were deactivated and later activated again. Both processes were controlled electronically and took approximately 5 s each. A light diode indicated whether the current was flowing.

Animals

The experiments were performed according to the German Animal Welfare and approved by local authorities (city state of Bremen). One hundred and sixty female mice (CD-1 IGS) were obtained from Charles River Laboratories, Sulzfeld, Germany at an age of 6 weeks. Males were not investigated due to their well-known aggressive behavior, which sometimes leads to fatalities [Robertson et al., 1996]. After arrival, animals were pseudo-randomized into two groups of 80 animals each with the aim of similar average body masses (Excel spreadsheet) and housed in groups of eight in trapezoidal polycarbonate cages (length 40 cm, width 17/30 cm, height 15 cm) covered with plastic lids with a sufficient number of holes to allow air exchange. All cages were lined with softwood bedding and were enriched with red polycarbonate houses (Ebeco, Castrop-Rauxel, Germany). Food (Altromin, Lage, Germany, type 1324) was available ad libitum. Water bottles with glass nipples were inserted from the top. Room temperature was 21 ± 1°C, and the light:dark cycle was 12 h:12 h, with lights being switched on at

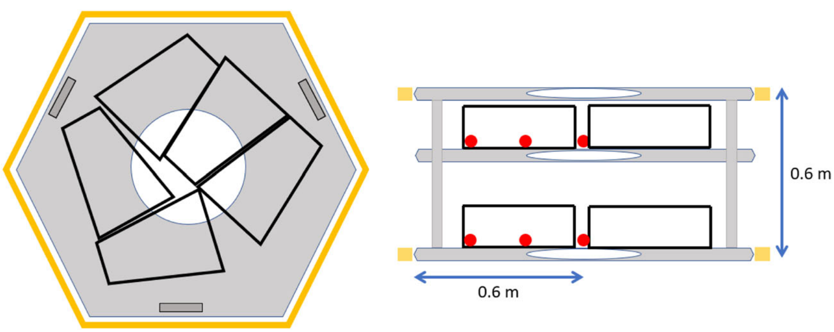

Fig. 3. Arrangement of the cages (five per stage) in the coils. **Left**: top view; **right**: side view. The red circles show the position of the probe for measurements of the field homogeneity (see Table 1). Yellow: coils.

TABLE 1. Results of Measurements of the Magnetic Fields at Different Positions Inside the Helmholtz Coil (Fig. 3)

	Distance from the center		
Height	**0 mm**	**200 mm**	**400 mm**
60 mm	352 μT	368 μT	460 μT
420 mm	360 μT	360 μT	368 μT

07:00 h. During the first 2 months of exposure, all mice were weighed every 2–3 days, thereafter once per week. Cages were cleaned twice per week, and fresh water and food were added. These procedures took approximately 30 min during which the exposure was interrupted.

Experimental Procedure

After acclimatization, IF-MF exposure started at the age of 3 months. Five cages were placed on the two layers of each coil so that 80 animals per group were simultaneously exposed (24 h/day, 7 days/week) or sham-exposed. Comparisons between the sham control group and the EMF-exposed group were conducted for several biologically relevant endpoints, including body mass, carcinogenic effects, and behavioral changes. To ensure a blinded study, the individuals carrying out the experiments or analyzing the data were unaware of which animals were exposed. The only parameter the individuals knew was whether the animal belonged to exposure system 1 or 2. All animals were inspected daily. After the evaluation of the histological examination and the completion of the statistical and behavioral tests, the blinding was lifted in exchange for the data.

Histopathology

When an animal showed signs of a disease, or if it lost body mass considerably, it was sacrificed by an overdose of CO_2 and immediately dissected. At an age of 13 months, all remaining animals were sacrificed by an overdose of CO_2 and immediately dissected. At this time, already some animals had to be sacrificed before (see Table 2), and thus we decided to terminate the experiment in order to not lose animals during unattended times (i.e., during the night) and prevent organ decomposition. Organs (brain, liver, kidney, spleen, and lung) were removed and immersion-fixed overnight in Bouin's solution (HT 10132; Merck, Darmstadt, Germany).

After fixation, organs were dehydrated in concentrated ethanol and xylol and embedded in paraffin wax. One section per organ was prepared (5 μm) and stained with hematoxylin/eosin. Histological examination was done by morphological evaluation of sections and any pathological findings were recorded. Lymphomas were diagnosed as primary tumors in the spleens, or as metastases in the lung or kidney tissue. To ensure that our diagnoses were correct, a set of 73 sections was cross-checked by a professional pathologist (see Acknowledgments).

Behavioral Tests

In the intermediate frequency range, very few studies have investigated the behavioral effects of exposure, according to a recent systematic review [Bodewein et al., 2019]. Therefore, a set of long-established behavioral tests was conducted when the mice reached an age of 10 months. This time was chosen in order to have as long a duration of exposure as possible before the behavioral tests, on the one hand, and to have as many animals as possible still alive, on the other. For each test, 24 animals from the exposed and the sham-exposed groups were randomly selected (totally by chance). No animal, however, was used in more than one test in order to avoid any influence through handling. The remaining animals were not used for any behavioral tests. During the tests, the exposure was discontinued to prevent exposure above the legally defined limits of the persons handling the animals. Thus, there were also no stray fields present during these periods.

The 8-arm maze test was used to test whether exposure had an effect on memory. The identical arms (each with length 35 cm and width 5 cm) were built from coated chipboard and radiated from an orthogonal platform. A CCD camera (ABUS, Affing, Germany) was mounted above the maze for visual controlling on a computer screen. Hidden baits (linseed) were placed at the end of four arms as a reward. Each animal was put in the same arm as a starting point three times consecutively for a maximum duration of 120 s each. The time taken to find all the baits was monitored. The test was stopped when one of the following conditions was met: 120 s had elapsed, 10 arms were visited, or all 4 baits were found. This procedure was repeated every day for 1 week and then again for the last time 1 week later. The tests were performed during the light phase. To prevent odor cues, the maze was wiped using 70% ethanol. The following parameters were analyzed: time to complete the test, percentage of successful trials, and total errors.

To test the effect on motor ability and coordination, mice were put on rotating cylinders (Rotarod, Ugo Basile, Comerio-Varese, Italy) whose rotations increased from 4 to 20 rpm over a period of 2 min. The maximum time for each trial was 4 min. The time was

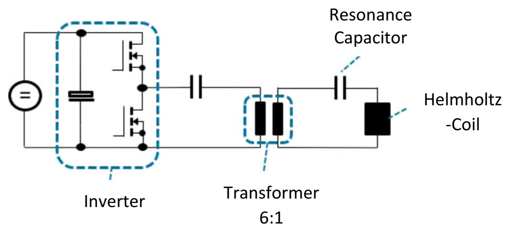

Fig. 4. Schematic circuit diagram of the electronic control of the Helmholtz coils.

automatically recorded when an animal could no longer stay on the cylinder and fell, causing a switch to close. The test was repeated twice for each animal, immediately after the preceding trial. The test was repeated on 2 consecutive days.

The anxiety or fear levels of mice were investigated by the open field test. Mice were put in the middle of an arena (50 × 50 cm) surrounded by 38-cm high walls that were divided into nine equal areas by thin lines. Video recordings were done for later analysis (CCD camera; ABUS). The animals were kept in the open field for 5 min each. The following behaviors were recorded: presence in the center and the peripheral squares, rearing (supported and unsupported), cleaning, and defecation.

Statistical Analyses

Number of surviving animals and tumor incidences were compared by Fisher's exact test. The behavioral data were analyzed for normal distribution, and differences between the groups were tested by Bayesian unifactorial analysis of variance with groups (exposed and control), day, and trial (Rotarod) as independent variables (SPSS V. 27; IBM, Armonk, NY). A value of $P < 0.05$ was considered significant.

RESULTS

There was no significant difference in the development of the body mass between exposed and control animals. At the time of sacrifice, the average body mass was 47.6 ± 6.9 g (control) and 47.9 ± 7.0 g (exposed) (avg ± standard deviation, $P > 0.05$). The numbers of surviving animals were statistically not different (79 [control] vs. 75 [exposed], $P > 0.05$).

In the MF-exposed mice, the observed neoplastic lesions were mostly similar to those in the sham-exposed mice. We could identify a slightly higher incidence of malignant lymphoma and single cases of meningioma, bronchioalveolar carcinoma, hemangiosarcoma, and renal tubule carcinoma in exposed animals. Single cases of bronchioalveolar adenoma and bile duct adenoma could only be identified in the control group. However, the histopathological results show no significant differences between the control and exposed animals (Table 2).

The 8-arm maze revealed no significant differences in reference memory (entries into unbaited arms) and working memory (re-entries in baited arms) between exposed and control animals either. The number of successful trials and numbers of errors showed no trends over time, and no particular differences between the groups that were present (data are not shown). The same was true for the time needed to successfully complete a test (the values were always close to 120 s; Fig. 6), so almost all animals were unable to complete the test in time, i.e., they failed to learn.

The results for the Rotarod trials are shown in Figure 7. Here, the animals showed a marked increase in the time spent on the rotating cylinders over time. Overall, there is a significant ($P < 0.005$) difference between the control and exposed animals. This is especially true for the first day where the values for the exposed animals were double as compared with that for the controls ($P < 0.001$). Although these differences declined on the second day, they were still present, albeit smaller, and were only almost absent on the third day.

The open field trials revealed a significant difference in the number of supported rears of the animals, i.e., rears with contact to the wall. The numbers were significantly lower in exposed than in control animals (38.6 ± 2.6 vs. 50.4 ± 2.61; avg. ± SEM; $P < 0.01$). The other endpoints were statistically not different (data are not shown).

DISCUSSION

To the best of our knowledge, this is the largest study investigating the effects of exposure to intermediate frequency magnetic fields (IF-MF) in mice [Bodewein et al., 2019]. In fact, results from 80 animals per group (sham and control) were analyzed. Consequently, our findings are comparatively robust in terms of statistical reliability. This was possible due to the innovative exposure setup, which allowed us to simultaneously expose 80 mice in one Helmholtz-type exposure system. We hope that this system enables other researchers to fill the gap in knowledge concerning the biological effects of IF-MF in animals [SCENIHR, 2015].

Our study shows that continuous exposure to magnetic fields at 20 kHz and 360 μTesla does not cause significant changes in the body mass, the lymphatic tissue, and the incidence of tumors,

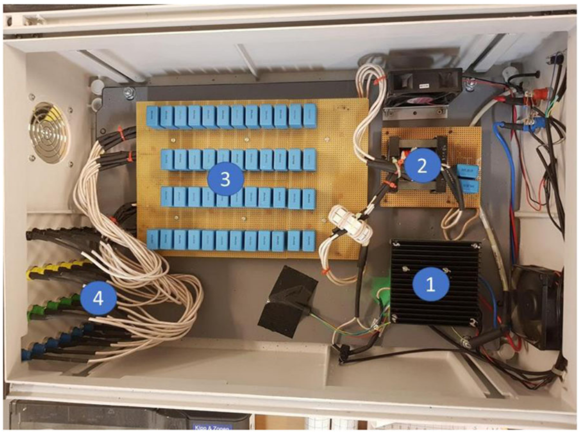

Fig. 5. Control box with the components for generating the current for the coils. 1, inverter; 2, matching inductance; 3, compensation capacitors; 4, coil connectors.

respectively, in female mice. Our findings are in accordance with the previously published data, including evidence of the absence of carcinogenicity of magnetic fields in the CD-1 IGS mouse model [Lee et al., 2007; Kumari et al., 2017; Herrala et al., 2018; Kumari et al., 2018; Nishimura et al., 2019]. Additionally, Nishimura et al. [2019] examined the carcinogenicity of a 20 kHz MF on a mouse model called Tg.rasH2 using shorter exposure times (26 weeks), again finding no evidence of carcinogenic effects. Another study in mice [Robertson et al., 1996] that exposed male and female animals to 10 kHz magnetic fields of up to 1 mT for 14 days or 90 days revealed no negative impact on the animals. In rats, studies at 20 and 60 kHz and flux densities of 6.25, 30, 100, and 300 μT, respectively, also did not reveal any substantial negative effects [Lee et al., 2006, 2010; Nishimura et al., 2016]. Taken together, the aforementioned studies do not indicate negative effects of acute or subchronic exposure to IF-MFs of various intensities and frequencies.

TABLE 2. Pathological Findings in the Animals

Organ/Diagnosis	Control	Exposed
Brain	$N = 80$	$N = 80$
Meningioma	0 (0%)	1 (1.3%)
Lung	$N = 79$	$N = 79$
Bronchiolo-alveolar carcinoma	0 (0%)	1 (1.3%)
Bronchiolo-alveolar adenoma	1 (1.3%)	0 (0%)
Spleen	$N = 80$	$N = 80$
Red pulp hyperplasia	6 (8%)	4 (5%)
White pulp hyperplasia	1 (1.3%)	2 (2.5%)
Liver	$N = 80$	$N = 80$
Bile duct adenoma	1 (1.3%)	0 (0%)
Kidney	$N = 80$	$N = 79$
Renal tube carcinoma	0 (0%)	1 (1.3%)
Hemangiosarcoma	0 (0%)	1 (1.3%)
Lymphatic tissue	$N = 80$	$N = 79$
Lymphoma	2 (2.5%)	6 (8%)

None of the differences were significant (Fisher's exact test).

Our results indicate that exposure to IF-MF does not increase the risk of pathological findings. However, in this study, only brain, liver, kidney, spleen, and lung tissue were analyzed for neoplastic lesions; the OECD guidelines recommend that some 40 tissues should be analyzed. Another potential limitation of our study is the relatively short exposure period of 10 months (OECD recommends 24 months of treatment). Yet another, but unavoidable, limitation was that for technical reasons the exposure could not be performed during the behavioral experiments. This is because interference of the strong magnetic field with the equipment (CCD camera or Rotarod) would inevitably have led to the person performing the experiment knowing whether or not the animals were being exposed.

It is also pertinent to understand the limitations of animal models as predictors of human biology. Therefore, the comparability and applicability of the results to humans need to be verified. Furthermore, this study only examined female mice and it is well-established that sex plays a crucial role in terms of cancer incidence, prognosis, and mortality [Zhu et al., 2019]. Therefore, further long-term studies that include male animals are needed to investigate the biological impact of IF-MF exposure.

The behavioral data are different as they showed significant effects in the Rotarod and the open field tests. The significantly decreased values of supported rears in the open field test indicate elevated stress levels, although this effect was only observed in male mice [Sturman et al., 2018]. The Rotarod data, originally developed to conduct tests that address motor coordination and neurological deficits [Dunham and Miya, 1957], are more difficult to understand because longer retention times are usually regarded as an indicator of a better condition of the animal. But in the context of the potentially elevated stress levels of the mice, as seen in the open field test, the longer retention times in the IF-MF-exposed animals may point to increased alertness. In other words, stress may lead to increased vigilance, leading the mice to do their best to stay on the wheels as long as possible, especially on the first day of the test. On the second and third days, this situation is no longer new for the animals, and thus the differences between the exposed and control animals disappear. At this point, we must leave this possible explanation as speculation since most studies involving the Rotarod test interpret shorter retention times as an indicator for adverse effects, e.g., decreased levels of time on the wheels

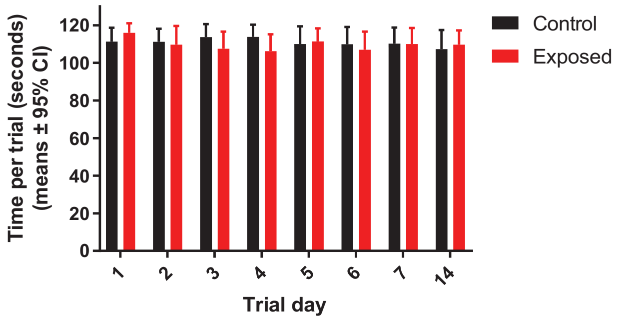

Fig. 6. Time spent in the 8-arm maze. No significant differences were noted between exposed and control mice, and no decrease during the test period was found. $N = 24$ per mean.

were seen in rats under stress conditions [Mizoguchi et al., 2002]. Alternatively, as one study reports, the observed effects may be caused by genetic/biochemical alterations in the brains of the mice [Win-Shwe et al., 2015]. However, this explanation is not supported by another study [Ohtani et al., 2019].

The results of the 8-arm test showed no differences between the exposed and control animals. The significance of this lack of an effect is, however, limited by the fact that the control animals obviously also failed to learn at all. The reasons for this failure may be the old age of the mice or the unappetizing bait [Shoji and Miyakawa, 2019]. Furthermore, it was demonstrated that although CD1 mice performed better in the water maze, they do not perform as well as C57BL/6J mice in land mazes (Barnes maze, the Multiple T-maze). These differences in performance indicate that CD1 mice strain may have deficits in spatial learning and memory [Patil et al., 2009]. Another explanation might be that we did not deprive the mice of food before testing, which is routinely done prior to such tests in order to increase the animals' motivation [Sharma et al., 2010]. We had done this on purpose, i.e., to avoid non-physiological stress for the animals. However, it was unexpected to see that the tradeoff would affect learning to such an extent.

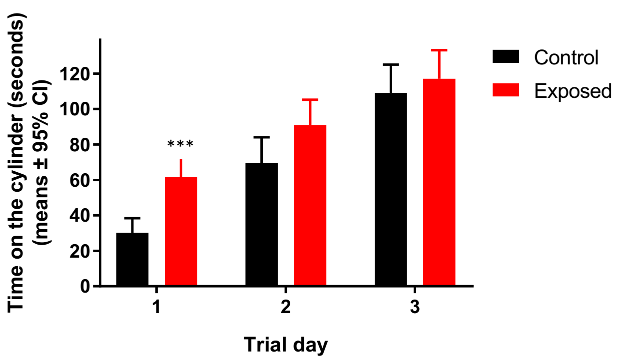

Fig. 7. Results from the Rotarod test. Overall, the exposed mice spent a significantly longer time on the rotating wheels than the control ($P < 0.005$; Bayesian univariate analysis of variance [ANOVA]). $^{***}P < 0.001$. The trend over time was highly significant as well ($P < 0.001$; Bayesian univariate ANOVA). $N = 24$ per mean.

Taken together, the data presented in this study indicate some behavioral effects in the exposed animals, while no differences were observed in growth and tumorigenesis between exposed and control mice. The potentially increased stress levels must be regarded as mild since they did not cause a drop in body mass, which is a very sensitive parameter for stress in rodents [Jeong et al., 2013]. Therefore, the overall results do not indicate that chronic exposure to 20 kHz magnetic fields at 360 μT poses a health risk. The overall conclusion of this study as well as of data from the literature is that there is a lack of evidence that IF-MF exposure contributes to any significant behavioral changes or influences growth or malignancy in the female mice model.

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AUTHOR CONTRIBUTIONS

A.L. is responsible for the study design, statistical analysis of the data, and the preparation of the manuscript. Experimental work was performed by K.D. (behavioral tests and tissue sampling) and I.G. (preparation of histological sections and staining). The ifak team was responsible for the design and building of the two exposure systems for generating the magnetic fields and for ensuring the operation during the study period.

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