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Behavioural phenotypes in mice after prenatal and early postnatal exposure to intermediate frequency magnetic fields

Kajal Kumari[a,*], Hennariikka Koivisto[b], Capstick Myles[c], Naarala Jonne[a], Viluksela Matti[a,d], Tanila Heikki[b], Juutilainen Jukka[a]

[a] Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland
[b] A.I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland
[c] IT'IS Foundation, ETH, Zurich, Switzerland
[d] National Institute for Health and Welfare, Environmental Health Unit, Kuopio, Finland

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ABSTRACT

Electromagnetic fields are ubiquitous in the environment. Human exposure to intermediate frequency (IF) fields is increasing due to applications like electronic article surveillance systems, wireless power transfer, and induction heating cooking hobs. However, there are limited data on possible health effects of exposure to IF magnetic fields (MF). In the present study, we set out to assess cognitive and behavioural effects of IF MF in mice exposed during prenatal and early postnatal periods. Pregnant female mice were exposed continuously to 7.5 kHz MFs at 12 and 120 µT, from mating until weaning of pups. Sham exposed pregnant mice were used as a control group. A behavioural teratology study was conducted on the male offspring at two months of age to detect possible effects on the developing nervous system. Body weight development did not differ between the exposure groups. The exposure did not alter spontaneous motor activity when exploring a novel cage or anxiety in novelty-suppressed feeding or marble burying tests. Improved performance in the Rotarod task was observed in the 12 µT group, while the 120 µT exposure group swam more slowly than the sham exposed group in the Morris swim navigation task. However, indices of learning and memory (path length and escape latency during task acquisition and search bias during the probe test) did not differ between the exposure groups. Furthermore, the passive avoidance task did not indicate any impairment of long-term memory over a 48 h interval in the exposed groups. In a post-mortem histopathological analysis, there was no evidence for an effect of IF MF exposure on astroglial reactivity or hippocampal neurogenesis. The results suggest that the IF MF used did not have detrimental effects on spatial learning and memory or histological markers of tissue reaction. The two statistically significant findings that were observed (improved performance in the Rotarod task in the 12 µT group and decreased swimming speed in the 120 µT group) are likely to be chance findings, as they do not form an internally consistent, dose-dependent pattern indicative of specific developmental effects.

1. Introduction

Electromagnetic fields are pervasive in the environment of the modern world and are derived from many manmade sources. Health risks of extremely low frequency (ELF) magnetic fields (MF) produced by the generation, distribution, and utilization of electricity and radio frequency (RF) electromagnetic fields (EMF) from, *e.g.,* wireless communication devices, have been investigated in numerous studies over many decades. However, there is limited knowledge about the possible health effects of intermediate frequency (IF) fields between the ELF and RF ranges (Ahlbom et al., 2008). The IF frequency range covers from 300 Hz to 100 kHz (or up to 10 MHz; the upper limit depends on how RF is defined). Human exposure to IF MFs is increasing due to new applications such as electronic article surveillance (EAS) systems (Roivainen et al., 2014), wireless power transfer, induction heating cooking hobs, and magnetic resonance imaging (Floderus et al., 2002; ICNRP, 2008).

Our parallel findings provide evidence of impaired learning and memory in young adult mice exposed to 7.5 kHz MFs continuously for 5 weeks (Kumari et al., 2017b). The mRNA expression level of the pro-inflammatory cytokine TNFα was also elevated in the hippocampus of the high-exposure (120 µT) group. It is therefore possible that exposure to IF MFs during a sensitive period of an organism's life, specifically during development, can cause adverse effects in the developing brain.

* Corresponding author.
E-mail address: kajal.kumari@uef.fi (K. Kumari).

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Another recent study measured brain biomarkers and the morphology of the hippocampus in mice exposed to IF MFs (21 kHz MFs, 3.8 mT, 1 h/day) during prenatal to postnatal development (Win-Shwe et al., 2015). The results of the study indicate that developmental exposure to IF MFs may induce neuro-inflammation and changes in genes related to memory function. However, no effects were found in mice that were allowed to recover for one day after termination of exposure, which indicates that these effects were transient.

The developmental effects in animals exposed to IF MFs around 20 kHz, similar to those emitted by cathode ray tubes that were previously used as a computer displays (Huuskonen et al., 1993, 1998a, 1998b; Frölen et al., 1993; Juutilainen et al., 1997; Kim et al., 2004; Lee et al., 2009) have been addressed in several studies. However, these studies did not include behavioural teratology, which is considered a sensitive approach for detecting any effects on the development of the central nervous system (Bornhausen and Scheingraber, 2000; Satoh, 2003). There is mounting evidence that prenatal exposure to environmental toxicants may have a profound lifelong impact on the central nervous system (Sobolewski et al., 2014). The developing nervous system is more sensitive than the adult brain to radiation, toxicants, drugs, or any other changes in the external environment (Odacı et al., 2016; Kasten-Jolly et al., 2012). After birth, the development of rodents' brains continues until the age of 2 months with functional or structural refinements.

In this study, we set out to assess possible behavioural effects after prenatal to early postnatal exposure to IF MFs. The exposure was continuous during gestation and early postnatal development. Pregnant mice were exposed to 7.5 kHz MFs similar to those emitted by the EAS technology commonly used in supermarkets and other stores to protect merchandise against theft. Exposure to this kind of IF MF is common, and many of the exposed subjects are of child-bearing age (Roivainen et al., 2014). Two magnetic flux densities, 12 and 120 µT, were employed. The higher flux density exceeded the reference level of 100 µT in the frequency range 3 kHz to 10 MHz defined by the International Commission on Non-Ionizing Radiation Protection for occupational exposure (ICNIRP, 2010) and was higher than the maximum exposure levels (up to 60 µT) found near EAS systems used in supermarkets and libraries (Roivainen et al., 2014).

We applied a large battery of standard behavioural tests to cover possible effects on a variety of brain functions and brain regions. The assessment included spontaneous locomotion, special motor actions such as rearing, digging, and swimming, motor coordination and balance, fear and anxiety, appetite, learning, and short- and long-term memory. The tests are widely used in neuropharmacology and have been shown to be valid for prediction of drug responses in humans.

2. Materials and methods

2.1. Exposure setup

The details of the exposure system are described elsewhere (Kumari et al., 2017a). Briefly, the exposure system consisted of a wooden rack with five rectangular coils. Three identical coil systems were made for simultaneous exposure at three levels (sham exposure, 12, and 120 µT). The dimensions of the coils were 40 cm × 120 cm. The top and bottom coils had 6 turns of copper wire (diameter 1.8 mm), while the three middle coils had 4 turns. The vertical distance between the coils was 25 cm. There were 4 shelves for animal cages; each shelf was positioned 4 cm above one of the coils. The 7.5 kHz signal was generated by a Thandar TG501 function generator (Thurlby Thandar Instruments, UK) and amplified with a Behringer Europower EP 4000 amplifier (Music Group Services, USA) nominally capable of producing 1250 W of continuous power per channel. A 1.47 Ω resistor was connected in series with the coil system. As the inductive reactance of the coil system considerably resists the flow of current at 7.5 kHz, a capacitor (about 1.5 µF) was added to tune the system into series resonance at 7.5 kHz. Background MF in the sham-exposure coil system was measured with the calibrated coil described below. No MF exceeding the resolution of the measurement system (0.02 µT) was detected in the sham-exposure system when the exposure system was on.

Due to the low resistance of the coil system (about 0.5 Ω), the power dissipated in it is low, about 15 W at the highest MF strength (120 µT) used in this study. Furthermore, the coils, which were wound outside the wooden support structure, have a large surface area that ensures low-temperature operation. As wood is an excellent thermal insulator that provides no path for thermal conduction, radiant heating is insignificant given the low temperature differential. Therefore, given the effective air circulation in the animal room and the large volume of the coil system, heating of the animals was negligible. The combination of small animal size, low frequency and low field strength ensure that direct heating of the animal by the EMF is not a concern.

2.2. Evaluation of the exposure system and dosimetry

The homogeneity of the field was evaluated both numerically – with the Magneto-Static Solver in SEMCAD v14 (SPEAG, Switzerland) – and experimentally.

The MF strength was measured with a calibrated single-axis field-sensing coil (diameter 2.0 cm, length 1.3 cm). The sensing coil was shielded against electric fields and was connected to a multimeter (Agilent U1241B, Agilent Technologies, Malaysia) to measure voltage induced in the coil. The measured fields showed an overall homogeneity (SD) of 3.8%, with all measured points within ± 9% of the overall average. The average MF strength measured was 12.03 µT for the low exposure (12 µT) group.

For induced current densities and electric fields, SEMCAD v14 (SPEAG, Zurich, Switzerland) and the Magneto-Quasi Static solver were used to perform simulations. Tissue parameters were set according to Gabriel et al. (1996) dataset of dielectric body parameters. The validity of the condition $\omega\varepsilon < \sigma$ for the quasi-static approximation used was checked.

A pregnant female mouse voxel model having body weight of 29 g was used. The mouse model was exposed in the simulation to a homogeneous MF to determine the induced field quantities. Some limited dosimetry was also performed to estimate exposure of the fetuses. Uncertainties for the simulations and modeling were also estimated.

2.3. Animals and experimental design

In total, 22 female and 10 male C57BL/6 J mice (2 months of age) obtained from the Lab Animal Centre (Kuopio, Finland) were mated for 1–7 days, and the female mice were monitored daily for development of vaginal plugs during the following days. Upon appearance of the plugs, females were considered potentially pregnant, and this day was counted as gestational day 1 (GD-1). The mice were then randomly assigned to sham and exposed groups and exposed for 24 h/d, except for about one half-hour per week for animal care, to 7.5 kHz MFs at 12 or 120 µT from GD-1 until the pups were weaned. The pregnant females were weighed every week to check for increased weight as confirmation of pregnancy. Pregnancy was considered confirmed if the body weight increased more than 5 g in 10 d of after mating. Non-pregnant females were removed from the system and afforded subsequent mating attempts.

Immediately after mating, the female mice were housed for two weeks in transparent plastic cages (one animal per cage), with maximum dimensions of 28 × 19 × 14 cm. Thereafter, they were transferred to larger cages (39 × 28 × 14 cm at the cage top and minimum dimensions at the bottom of 33 × 23 × 14 cm). After delivery of the pups, the cages of the dams were changed only once per week to reduce the stress levels.

The dams gave birth on GD-19 to -21, which was counted as postnatal day 1 (PND-1) (Fig. 1). The offspring were weaned at PND-28.