Spotlight on EMF Research

Spotlight on "Acute exposure of microwave impairs attention process by activating microglial inflammation" by Jiang et al. in Cell & Bioscience (2024)

Category [radiofrequency, animal study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

Since mobile communications technologies have gained widespread use among the population, concerns about potential adverse effects of radiofrequency electromagnetic fields (RF-EMF) on brain function have been raised [2]. However, the scientific body of evidence is equivocal to suggest that RF-EMF exposures within the regulatory limits might have any detrimental impact on cognition. A recently published systematic review of animal studies, commissioned by the World Health Organisation, concluded that the evidence for adverse effects of RF-EMF exposure during pregnancy on offspring learning, memory and motor activity functions is of very low certainty, due to the low quality of the included studies [3].

2 Results and conclusions from the authors' perspective

Jiang et al. [1] conducted an experimental animal study to test their hypothesis that RF-EMF perturbs attention capacity by causing neuroinflammation and that this could be pharmacologically mitigated by administration of minocycline, a second-generation tetracycline class antibiotic.

To assess attention in the short-term after RF-EMF exposure, the authors exposed groups of nine male mice each to pulsed 2.856 GHz RF-EMF emitted by a horn antenna for a duration of 30 minutes. The average power densities of the RF-EMF were 8, 15, 30 and 50 mW/cm², which corresponds to whole body specific absorption rate (SAR) values of 2.1, 3.9, 7.8 and 13 W/kg, respectively. The exposed groups of mice were compared with a group of nine sham-exposed male mice. Behavioural tests were performed one day prior to (-1 d), one hour (0 d), one day (+1 d) and three days (+3 d) after a single RF-EMF exposure.

The authors used the five-choice serial reaction time (5-CSRT) task to test the attention of mice. Mice were trained for 60 days with the procedure prior to the RF-EMF exposure experiments. The 5-CSRT test was performed in a specialized apparatus with five touch-screen displays that flash pseudo-randomly for 1 second, followed by a 5-second hold. If a mouse touches the illuminated screen within this time interval, it receives a portion of sucrose solution as a reward. Incorrect or omitted responses are punished with a 2-

second flash of white light followed by a 5-second timeout. During the experimental phase, mice could complete an unlimited number of trials within 30 minutes.

Using two-way analysis of variance (ANOVA) and the Tukey's post-hoc multiple comparison test, Jiang et al. found statistically significant changes between the group of mice receiving the highest RF-EMF exposure of 50 mW/cm² and all the other RF-EMF- and sham-exposed groups. No other RF-EMF average power density elicited statistically significant differences in attention metrics compared to sham-exposure. Specifically, one hour after RF-EMF exposure (0 d), the number of correct 5-CSRT responses by mice in the 50 mW/cm² group dropped drastically compared to the sham-exposed group. The percentage of correct responses among the total number of trials, i.e. all trials including omissions and premature responses, decreased correspondingly, while the percentage of omissions increased sharply. At +1 d, these changes were still statistically significant, but quantitatively less pronounced. At +3 d, all attention metrics were indistinguishable between each group of mice. The authors conclude that exposure to high power RF-EMF impairs attention in a short-term and reversible manner, but does not affect cognition or learning ability, as evidenced by the unaltered accuracy rate.

The authors performed all follow-up experiments at the average power density of 50 mW/cm². To understand how RF-EMF might affect neurological function, they extracted the cerebrospinal fluid (CSF) from ten exposed and sham-exposed mice each, directly after exposure and three days later. They determined the identity and relative quantity of the proteins in the CSF by liquid chromatography mass spectrometry (LC-MS/MS). Among the proteins differentially upregulated directly after RF-EMF exposure, the pathways of "neuroinflammation", "activation of microglia" and "apoptosis" showed statistically significant over-representation. These findings prompted the authors to prepare and analyse brain sections using immunofluorescence and confocal microscopy. Acute RF-EMF exposure resulted in statistically significant increases in the percentage of microglia and the expression of several microglial activation markers. These effects were evident in brain regions relevant to attention and cognition, mainly the murine prefrontal cortex, but also the claustrum, striatum, nucleus accumbens and hippocampus. The M1 and M2 motor cortex regions were not affected. As minocycline has been reported to reduce neuroinflammation and microglial activation, Jiang et al. treated mice with this drug several times before RF-EMF exposure. Minocycline pre-treatment of RF-EMF exposed mice reduced almost all markers of microglial activation and inflammation to the levels of sham-exposed mice. Lastly, although minocycline pre-treatment did not alleviate the acute impairment of attention at 1 h after RF-EMF exposure, it improved the number of correct responses at +1 d.

Jiang et al. proposed that acute exposure to high average power density RF-EMF impairs attention by activating microglia. The resulting neuroinflammation acts upstream of cellular apoptosis, a form of programmed cell death. Although the anti-inflammatory drug minocycline prevented activation of microglia, it was ineffective at rescuing the acute deficit in attention at the day of RF-EMF exposure.

3 Comments by the BfS

There is no reliable scientific evidence to date that RF-EMF exposure within regulatory limits impairs cognitive function in the human population [4 5], although little data exists for the 2.856 GHz EMF frequency. Yet, some animal studies have reported detrimental effects [6].

The present paper has several strengths. Jiang et al. delivered a methodologically sound animal study with various follow-up experiments which were able to replicate and further elucidate their main finding of acutely impaired attention in male mice due to high power RF-EMF exposure. The group sizes ranged between 7 and 10 mice for the main experiments. The researchers were blinded to the exposure group allocation during the experimental procedures and data collection. The use of one-, two- and three-way ANOVA, depending on the number of tested experimental variables, followed by post-hoc tests and multiple hypothesis correction is appropriate for statistical hypothesis testing.

Potential limitations and shortcomings of the study concern data interpretation and exposure characterisation.

The authors do not provide values for the total number of trials completed by the different groups of mice during the 5-CSRT dose-finding experiment. The total number of completed trials can only be estimated from the presented graphs. All groups of mice show roughly 100 completed trials on average, except for the group with the highest 50mW/cm2 exposure which only amounts to about 40 at 0 d, leaving a difference of 60 completed trials. If the mice were inactive and unresponsive due to acute RF-EMF exposure stress, their trials should have been scored as omissions. According to the method description in the paper, the maximum length of an omission response trial is 13 seconds. Therefore, if a mouse omitted every presented opportunity to earn a sucrose reward within the 30-minute time window, it could still complete up to 138 trials. The reason for the sizeable discrepancy with the 40 trials, completed by the 50 mW/cm² group at 0 d, is not evident from the descriptions in the paper.

The authors write in their discussion that "*appropriate exposure parameters with rationale are needed to substantiate our study*". They provide minimal details regarding their exposure set-up, the method to calculate the SAR or possible thermic effects, and instead refer to a previous study of one of the corresponding authors [6]. In that study, however, rats were used as the experimental animal species instead of mice. Notably, at the end of an exposure to 50 mW/cm² RF-EMF for 6 min, Wang et al. [6] reported a peak temperature increase of 1.2 °C in the brain and of 0.6 °C in the rectum of rats, the latter reflecting an increase in core body temperature. In contrast, the present study investigated mice and exposed them to RF-EMF for 30 min, which is five times longer than in Wang et al. [6]. On the other hand, due to their smaller body size, mice have a higher surface area to volume ratio and lose heat faster than rats. Because the two studies differ regarding species and exposure time, it is not possible to extrapolate from the previous to the present study. Since hyperthermia can affect cognitive function [7-9], any confounding effects of heating should have been excluded in the present work by measuring brain and body core temperatures of mice during RF-EMF exposure. In addition, there is no information on the exact locations where the power densities were measured, or how they were averaged. It was also not stated how the SAR values for the mice were calculated.

The present study shows that "apoptosis" was the quantitatively and statistically most over-represented pathway among upregulated proteins in the CSF of mice acutely exposed to 50 mW/cm² RF-EMF. The previous study by Wang et al. [6] found an increase in body (part) temperature in rats at this average power density. Nevertheless, the current paper examined the hypothesis that high power RF-EMF primarily causes microglia activation. An alternatively formulated hypothesis with a reverse temporal order of effects would be that exposure to 50 mW/cm² RF-EMF for 30 min leads to heat-induced tissue injury and cellular apoptosis in the brain, which leads to secondary activation and recruitment of microglia, that subsequently clear up the cellular debris [10]. In line with such a hypothesis, the motor cortex was the only and the most superficially located region of the brain where no increase in microglia cells was observed. This could potentially be explained by more efficient heat dissipation from the motor cortex to the surroundings, compared to the more centrally located brain regions. On the same note, the authors describe minocycline as an inhibitor of microglial activation, providing a rationale for its use. However, minocycline is a pleiotropic agent and has also been reported to inhibit apoptosis as its major mode of action [11].

The molecular and cellular biology analyses in this paper were of high quality and the experimental animal groups were of adequate size. Yet, the paper failed to exclude the possibility that the reversible biological effects observed after RF-EMF exposure were due to tissue heating. The maximum exposure level of 50 mW/cm² corresponded to a whole body SAR of 13 W/kg. This is about 32 times higher than the basic restriction of 0.4 W/kg for occupational exposure, as recommended by the International Commission on Non-Ionizing Radiation Protection [12] to protect humans from rises in body core temperature of more than 1 °C. Exposures up to approximately 8 W/kg had no influence on the attention of mice. Therefore, this study suggests the existence of a threshold for an exposure-related attentional impairment and provides interesting insights into the effects of very high exposure levels, but is not suitable for testing the validity of the limit value recommendations.

Bundesamt für Strahlenschutz

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