Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on "Acute radiofrequency electromagnetic radiation exposure impairs neurogenesis and causes neuronal DNA damage in the young rat brain" by Singh et al. in Neurotoxicity (2023)

Category [radiofrequency, animal study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

Oxidative stress is often suggested as a possible mechanism of action of radiofrequency electromagnetic fields (RF-EMF) on the nervous system. The term oxidative stress describes an imbalance between the production of reactive oxygen species (ROS), and the cellular antioxidative defense system. ROS are naturally produced during cellular energy production or by immune cells for pathogen defense, but also function as a signal transducer. The level of ROS is normally controlled by antioxidative mechanisms, e.g. antioxidative enzymes. In this context it is important to distinguish between physiological oxidative stress (Eustress) that is necessary for cellular processes and harmful oxidative stress (Disstress) between which there is no clearly defined boundary [2]

2 Results and conclusions from the authors perspective

The authors assume that the interaction of RF-EMF radiation with the brain of adolescents could cause negative health effects via oxidative stress, DNA damage, inhibition of DNA repair, altered gene and protein expression, epigenetic changes, and altered intracellular calcium metabolism. The cellular mechanism behind these effects in the young brain are not known, but non-thermal effects are suggested.

To test their hypotheses and to clarify the impact of RF-EMF on the developing brain, the authors investigated if a single exposure with 1.51 W/kg for 8h can induce oxidative stress, DNA-damage, morphological and developmental changes in an adolescent rat brain.

For the detections of free radicals, Electron paramagnetic resonance (EPR) spectroscopy was applied using 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) as a spin trap. As further indicators of oxidative stress, lipid peroxidation, protein oxidation and total antioxidant capacity were measured in the cortex and the hippocampus. DNA damage was evaluated by comet assay.

Increased levels of oxidative stress and single-strand DNA breaks (SSB) were observed in exposed brains. The number of BrdU-positive (5'-bromo-2'-deoxy-uridin) cells in the dentate gyrus decreased in exposed rats, indicating reduced neurogenesis. RF-EMF exposure also induced degenerative changes and neuronal loss in dental gyrus neurons but had no effect on other regions of the hippocampus and cerebral cortex. The activity of Procaspase3 (precursor of caspase3, a cysteine protease, that finally leads to apoptosis (a form of programmed cell death)) did not increase upon exposure in any of the brain regions.

The authors conclude that short-term acute exposure to RF-EMF induces oxidative stress in young adolescent rat brain regions with a marked increase in carbon-centered radicals and lipid peroxidation. Rats exposed to RF-EMF showed SSB in the cortex and hippocampus, impaired hippocampal neurogenesis, and increased neuronal degeneration in the dental gyrus region of the hippocampus .

3 Comments by the BfS

The underlying question of the study is of scientific interest and of relevance for radiation protection. The topic of a possibly increased vulnerability of children and adolescents is still a matter of discussion and public concern. The study, however, is limited in answering this question because no controls were included and the methods chosen are only partially suited.

From the description of the exposure setup it can be concluded, that there was sufficient exposure contrast between exposed and sham exposed animals although there are several issues of concern. The formulas used for exposure calculation are not appropriate, because they were originally developed for a far field situation [3]. In a near field, as used in the present study, modelling a rat head as an ellipsoid is a very rough approximation. The presence of the body of the rat will influence the field distribution and the exposure of the head. In such a situation, the current state of the art is using voxelized anatomical animal models and simulating the brain exposure mathematically, e.g., using the FDTD method [4]. In addition to this, some details in the formulas used by the authors for exposure calculation are not given. Overall, it is not possible to reconstruct how the authors calculated exposure.

The authors provide no information whether the experiments and measurements were carried out in a blinded manner. Only the optical analyses (e.g., comet assay, BrdU, neuronal staining, and brain immunohistochemistry) were reported to be performed in a blinded fashion to the exposure group. Therefore, bias cannot be ruled out.

Moreover, the authors do not provide positive controls in their experiments. These are needed to evaluate the effects strengths (i.e. using DNA-damaging agents or ionizing radiation as a positive control for the comet assay or hydrogenperoxide as a positive control for the measurements of oxidative stress).

The presented EPR-spectra, exhibiting a single peak, are not typical. Under natural conditions usually several peaks are visible. The authors interpret their finding as an increase of carbon-centered radicals with adjacent oxygen in the exposed sample (g = 2.0035) and refer to [5]. However, in this reference neither RPM (rotations per minute) nor a g factor characterizing the magnetic moment, are mentioned.

Determining lipid peroxidation by measuring thiobarbituric acid reacting substances (TBARS) is unspecific, because there are too many non-oxidative stress related reactions that produce TBARS, including metabolism[6]. A positive control would help to classify the measured effect strength. Therefore, the extent of the change in lipid peroxidation or whether this can have an effect on the brain development cannot be determined from the presented data.

Also, the results from the comet assay are difficult to assess without a positive control. Concerning the estimation of proliferating (BrdU positive-) cells, a negative control, e.g. cage control, is missing, which makes it difficult to interpret the physiological relevance of this statistically significant effect. After a single injection of 300 ml BrdU, the number of BrdU positive cells should double within two to 24 hours, due to the normal rate of cell division in untreated young rats. In the present study, the number of BrdU positive cells increased in this period by a factor of 1.3 for the sham exposed and by a factor of 1.1 for the exposed

animals. This indicates that possibly also in the sham-exposed animals the cell division process was already unusually low.

Neither positive nor negative controls are available for quantification of the proportion of neurons with normal morphology, so the physiological or health relevance of the decrease in cells with normal morphology (reduced from approximately 95% to approximately 91%) are difficult to evaluate.

To estimate the number of mature neurons, the relative fluorescence intensity of the neuronal nuclear antigen NeuN was measured, so it remains unclear whether the number of neurons actually decreased or only the expression of NeuN decreased to a significant extent after exposure. The expression of NeuN is normally stable in mature neurons, except for certain (patho)physiological situations [7]. It remains unclear whether this was the case in the present study or whether the number of mature neurons was correctly determined. Again, a positive and negative control would have been helpful, although not mandatory, to interpret the results.

Overall, the study has major methodological and conceptual weaknesses. It's contribution to the scientific knowledge of a possible age-dependent sensitivity to RF-EMF is thus very limited.

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