Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on “Biological responses to terahertz radiation with different power density in primary hippocampal neurons” by Li Zhao et al. in PLoS ONE (2023)

Category [radiofrequency, in vitro study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

Terahertz (THz) waves are electromagnetic fields in the frequency range from 100 Gigahertz (GHz) up to 3000 GHz and higher and are also sometimes called “submillimeter waves”. Such frequencies are not in use by current communication technologies but will be applied in the next generation of mobile communication, 6G. Potential application scenarios are, for example, holographic applications or extended realities (XR). Such applications need large contiguous frequency ranges with bandwidths of several GHz, which are only available in the THz range [2]. However, health effects and biological responses of THz waves have not been studied to the same extent as the currently used frequencies and further research is necessary.

2 Results and conclusions from the authors perspective

The authors investigated biological effects of THz waves on rat’s primary hippocampal neurons in a cell culture study. Primary neurons were isolated from newborn Wistar rats, cultured and randomly divided into different exposure groups. The neurons were THz exposed (0.12 THz at 10 mW and 0.157 THz at 50 mW for 10 or 30 min) and compared with a sham-exposed group. Immediately or 1 h after exposure the i) cellular activity, ii) cell apoptosis, iii) effects on cellular structure, iv) neuron function and the v) concentration of amino acid neurotransmitters in the culture supernatant were analyzed.

Cellular activity of primary neurons was statistically significantly decreased immediately and 1 h after exposure in the group exposed to 0.12 THz for 10 min. After 30 min exposure duration, the decrease was

only statistically significant 1 h after the end of exposure. In contrast, the group exposed to 0.157 THz showed a statistically significant increase in cellular activity immediately after 30 min exposure, but 1 h after exposure no difference in cell activity was observed. Also, after 10 min exposure duration (immediately or 1 h after end of exposure) cell activity was not changed. Because opposite effects on cellular activity were observed in the 0.12 and 0.157 THz-exposed group after 30 min, only this time point was investigated in the subsequent analyses.

The apoptosis assay showed a statistically significant induction of apoptosis immediately after 30 min exposure in the 0.12 and 0.157 THz-exposed groups, compared to the sham-exposed groups.

The ultrastructure of neurons was examined by transmission electron microscopy immediately after 30 min exposure. The 0.12 THz-exposed group showed swollen mitochondria, damaged cristae, swollen endoplasmic reticulum and an increased lysosome count. The 0.157 THz-exposed group showed similar changes but with fewer lysosomes.

The concentrations of 16 amino acids were measured in the culture supernatant immediately after 30 min exposure. The concentration of leucine, lysine, arginine, threonine, phenylalanine and serine was slightly lower after exposure to 0.12 or 0.157 THz. Proline was statistically significantly lower in the 0.157 THz group compared to the two other groups. Concentration of glutamic acid was increased after exposure to 0.157 THz. Opposite effects on the concentration of cysteine were observed, with lower levels in the 0.12 THz and higher levels in the 0.157 THz-exposed group, when compared to the sham-exposed group.

Effect on neuron function was examined by immunofluorescence staining of postsynaptic density protein (PSD)-95. Changes in expression and localization of PSD-95 were observed in both 0.12 and 0.157 THz exposed cells, but differences were not statistically significant.

The authors conclude that, generally, in vitro THz exposure of rat primary hippocampal neurons induced cellular apoptosis and altered cellular activity as well as regulated the release of amino acid neurotransmitters. They further state that the observed effects were closely associated with intensity, duration of exposure and wavelength. The authors hypothesize that the opposite effects on cellular activity at the two power intensities could be attributed to the response to acute stress, by upregulating calcium ions and enhancing enzyme activity in the group exposed to 0.157 THz at 50 mW.

3 Comments by the BfS

Research focusing on biological effects of terahertz radiation on neural cells and the nervous system and the underlying mechanisms of action has so far been limited. The possibility of environmentally altered neuronal activity and/or function could cause brain cancer and other neurological diseases, or affect memory and learning. This would be important for the assessment of health effects of THz waves. The authors thus take up a topic of high relevance and pursue it with an interesting and comprehensive experimental approach in which they investigate the viability and various functional activities of neuronal cells under THz exposure.

The cellular activity measurement led to contradictory results in the 0.12 and 0.157 THz exposed groups. The cellular activity assay used is a cell viability assay that quantifies the number of living cells in the cell culture [3]. The authors state that the neurons were transferred after radiation to 96-well plates, however, no details about how the cells were transferred are given, and the reference to the manufactures protocol does not offer more information. This is critical since neurons are adhesive cells and can only be harvested by harsh methods. This may have influenced the viability of the cells and therefore the results.

Since the measurement of cellular activity or viability, respectively, was conducted in three independent experiments, possible effects of harvesting and transferring the cells might have balanced out after averaging the results. But this is difficult to judge, because the results of cell viability are only shown by optical density (OD) and not as ratio of viable cell numbers of sham and exposed cells. The shown OD differs a lot between the two frequency/power groups. For low power (0.12 THz, 10 mW) the measured

ODs are around 3, for high power (0.157 THz, 50 mW) measured ODs are around 0.6. This means that sham-exposed cells also show different OD values. The authors do not mention or discuss this discrepancy.

The observation that THz exposure at high power (0.157 THz, 50 mW) leads to more viable cells after exposure is in contrast to the results of the apoptosis measurement. Whilst a statistically significant increase in apoptosis was shown immediately after 30 min exposure in the 0.157 THz-exposed group (even higher than in the 0.12 THz group), the cellular activity assay showed an increase in viable cells in the same group after the same exposure time. These contradictory results were not considered in the discussion.

The authors chose to use only the 30 min exposure time for their subsequent analyses, because of the opposite results in the cell activity assay in the two different frequency/power groups. The decision is incomprehensible, especially as the 10 min exposure time led to different results as well (decrease in 0.12 THz group, no effect in 0.157 THz group).

The electron microscopy images are not very sharp and it is unclear on which structures some of the included arrows are pointing. Furthermore, the increased number of lysosomes in the 0.12 THz-exposed group is not shown in the figures. How damaged cellular structures were identified and classified is not indicated in the text.

For quantification of amino acid neurotransmitters in the supernatant, the authors chose 16 amino acids, but some of those serve primarily as precursors of neurotransmitters [4]. An altered concentration of these amino acids in the supernatant, therefore, does not necessarily mean an altered release of the corresponding neurotransmitters. Additionally, it is unclear, if and which of the observed changes in amino acid concentrations were statistically significant because three p-values are indicated with exactly the same value. Some of the amino acids show the same or even larger changes in concentration in the differently exposed groups but are not marked with a p-value.

No effect was found on neuron function by investigating the expression of PSD-95, although expected by the observed changes in cell viability, changes in ultrastructure and concentration of neurotransmitters. The authors suppose a PSD-95 independent modulation of neuron function. Alternatives are not further considered or investigated.

There are also some general details missing in the paper that would have improved the significance of the results: There is no information about blinding of experiments and their evaluations. The authors do neither describe why they chose the 0.12 THz and 0.157 THz frequencies, nor mention why different powers were used for them. In addition, the power density or the specific absorption rate (SAR) within the cell culture is not indicated.

In conclusion, the study pursued a very interesting approach, but in view of the contradictory results, its contribution to the assessment of biological effects in neural cells of THz waves is limited.

References

The first reference is always the manuscript at hand and the reference in the curly braces at the end of a reference {xx} correspond to a reference in the manuscript at hand and is consistent with the manuscripts reference style.

[1] Zhao L, Yi R, Liu S, Chi Y, Tan S, Dong J, Wang H, Zhang J, Wang H, Xu X, Yao B, Wang B, Peng R, “Biological responses to terahertz radiation with different power density in primary hippocampal neurons” PLoS One. 2023 Jan 20;18(1):e0267064. doi: 10.1371/journal.pone.0267064.

[2] Rohde & Schwarz White Paper “Fundamentals of THz technology for 6G”. PD 3683.6158.52, Version 01.02, November 2022 (ch)

[3] Kamiloglu S, Sari G, Ozdal T, Capanoglu E, "Guidelines for cell viability assays." Food Frontiers 1.3 (2020): 332-349. doi:10.1002/fft2.44

[4] Dalangin R, Kim A, Campbell RE, "The role of amino acids in neurotransmission and fluorescent tools for their detection." Int J Mol Sci. 2020 Aug 27;21(17):6197. doi: 10.3390/ijms21176197.

Impressum

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