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Final Report

Project reference: FSM A2021-01
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Project title: Brain in a dish – Effects of RF-EMF (5G) on brain development and neurodegeneration

1. State of Research.

1.1 Research activities performed, milestones and deliverables accomplished

The project was performed to improve the understanding of the impact of 5G NR FR1 (<6 GHz) RF-EMF exposure on neuronal development and related biomarkers known to be involved in neurodegeneration and associated diseases as well as related pathways. We investigated effects of 5G NR FR1 RF-EMF on distinct stages of brain organoids/mini brains during differentiation and development and identify molecular biomarkers to characterize the phenotype, maturity, and involved signaling pathways that play a role in neuronal differentiation and degeneration.

The organoids were developed from human induced pluripotent stem cells (iPSCs) and where either RF-EMF or sham-exposed during neural induction and differentiating and maturating of midbrain and cerebral organoids.

In this explorative project, we executed mainly research activities towards objectives of the proposal.

> Objective 1: Evaluation of the impact of 5G NR FR1 RF-EMF exposure on the development of midbrain and cerebral organoids on the neuronal phenotype and neuronal markers with and without induced neurodegeneration at different times during the development.
> Objective 2: Investigation of signaling pathways and key players (e.g., α-synuclein) involved in neurodegeneration.
> Objective 3: Analysis of electrical activity in the brain organoids using multi-electrode arrays (MEA).

While objectives 1 and 2 were studied in detail, only explorative work could be done on objective 3 because only one organoid provided electrical activity. This is in line with findings of other groups (Lancaster) who stated that only every 10^{th} brain organoid shows electrical activity. Another hurdle was the fact that the organoids need to grow on a chip with electrodes for a longer time, and we could not expose the chip in the waveguide due to interference with the RF EMF signal, and electrical activity can only be assessed after growing the organoid on the chip after exposure.

Instead, we also exposed human iPSC during differentiation and maturation into dopaminergic neurons to RF EMF (5G FR2) or sham. This was not foreseen in the proposed project. In parallel, iPSC-derived dopaminergic neurons differentiated as monolayer radiated or sham-exposed after 2 weeks of maturation were phenotypically characterized by immunofluorescence as well and signaling pathways, especially PI3-K/Akt/mTOR, Wnt/β-catenin and JAK-STAT and their respective key players, have been assessed by protein analyses, namely Western blot. These pathways are known to be critical in the development and maintenance of dopaminergic neurons and they were demonstrated to be altered in Parkinson's Disease. Most of the results were already presented in the intermediate report in May 2023.

1.2 Findings

Most experimental data and conclusions of this project were already provided in our intermediate report from May 2023, and the work resulted in a doctoral thesis by Selina Thomas, August 2023. This work will be published as soon as we finalized the quantification of the immunofluorescence analyses using the Cytation 10, a new device at our faculty respective at the Microscopy Center at the University of Bern. The work on proteins quantified by Western blotting has been finalized within the time of this project and the results are available in the doctoral thesis.

Key findings/conclusions of our projects are provided narratively below, listed in relation to our project tasks:

- Task 1: Morphological and molecular analysis of neuronal differentiation under RF-EMF exposure on the phenotype and maturation of neurons
    - No visible differences in the expression of all markers tested by immunofluorescence were detected in midbrain nor in cerebral organoids, and the different exposure times investigated (30 and 60 days, SAR 0.3 W/kg). The dopaminergic phenotype studied by the marker tyrosine

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hydroxylase (TH). no visual difference in TH immunoreactivity was detected between RF-EMF- and sham-exposed midbrain organoids at all timepoints studied. Similarly, no differences were found for the marker LIM homeobox transcription factor 1 alpha (Lmx1a), an early regulator of midbrain dopamine neural progenitor phenotype specification. Similar effects were obtain by Western blotting.

- Absence of evident morphological effects were found in the cerebral and midbrain organoids after RF EMF exposure versus sham-exposure.
- The neuronal marker class III β-tubulin (TUJ1) was used to identify immature/young neurons and MAP2 was used to identify mature neurons in midbrain and cerebral organoids. Neither immunofluorescence images nor analyses using Western blotting resulted in significant different changes in both markers. Western blot analysis was also used to examine the different isoforms of MAP2 in 30 days old midbrain organoids, a significant decrease in MAP2c (70 kDa) was observed in RF EMF-exposed compared to the sham controls, but no significant difference of this isoform was observed in the 60-day-old midbrain organoids when RF-EMF-exposed organoids were compared to sham-exposed organoids. MAP2a+b (280 kDa) protein levels were significantly higher in RF EMF-exposed midbrain organoids than in sham organoids with the effect depending on the age of the neurons.

- Task 2: Analyses of biomarkers of signaling pathways related to neuronal differentiation and neurodegeneration in midbrain and cerebral organoids
  - Synaptophysin, a presynaptic marker for neurons, was studied in 30- and 60-day-old midbrain organoids. The staining of synaptophysin was found co-localized with the neuronal marker TUJ1, mainly in the zone between the VL areas. No visual difference was found between RF EMF- and sham-exposed midbrain organoids. Western blot analysis of synaptophysin showed a significant decrease in protein levels of RF EMF exposed organoids from 30-day-old midbrain organoids but not at day 60.
  - No difference of synaptophysin was observed between RF-EMF- and sham-exposed cerebral organoids at any time point examined regardless of the protein measurements performed.
  - The paired box protein 6 (Pax6), a nuclear marker for radial glial cells that coordinates cell differentiation and proliferation into neurons was not changed in immunostainings of RF EMF versus sham-exposed midbrains. However, Western blot analysis of RF-EMF-exposed midbrain organoids at 60 days of age showed significantly higher Pax6 protein levels when compared to sham-exposed organoids.
  - Nestin, a marker to distinguish neural progenitor cells from more differentiated cells, remained unchanged when RF EMF-exposed midbrain organoids were compared to sham-exposed organoids. The protein level of Nestin was significantly increased in radiated cerebral organoids at 30 days of age compared with non-radiated organoids, but not at 10 and 60 days.
  - Gial markers, S100beta and glial fibrillary protein (GFAP) both markers for astrocytes were investigated and no visual difference between RF EMF-exposed and sham organoids were obtained by immunofluorescence staining. In Western blotting, a significant increase in S100beta was found after RF EMF exposure in midbrain organoids but not in cerebral organoids. Analysis of GFAP expression in cerebral organoids showed that this marker was not observed until day 60. At developmental day 60, no significant difference between RF-EMF- and sham-exposed organoids was found.
- Task 3: Effects of RF EMF (5G FR2) on the differentiation and maturation of dopaminergic neurons
  - Tyrosine hydroxylase (TH), beta-3-tubulin, MAP-2, S100beta, synaptophysin, and NogoA expression do not show variations between sham and RF-EMF-exposed cell.
  - Analyses of Akt / P-Akt, and MAP-K / P-MAP-K expression also do not indicate significant differences but a trend toward an increase in P-MAP-K in radiated cells.

1.3 Conclusions

Overall, we conclude that the data obtained in our project provides little evidence for a substantial impact of RF-EMF exposure on neural differentiation. Yet, there are some observations of consistent but mostly not statistically

significant effects, for instance restricted to certain experimental conditions such as stress or cell type, which require further investigations.

Human iPSC-derived midbrain and cerebral organoids with neuronal, dopaminergic, and glial phenotypes were successfully generated. RF-EMF exposure at 1950 MHz at a SAR of 0.5 ± 0.12 W/kg for 48 h did not impair young/immature neurons and even promoted neuronal maturity. The data suggest that 5G radiation disrupts and/or slows the differentiation into neurons and may alter the synaptic activity. Protein levels of dopaminergic neurons were not affected by non-ionizing radiation, but altered reactivity was observed in astrocytes in response to the RF-EMF exposure. Our data show that midbrain organoids were more sensitive to RF-EMF exposure than cerebral organoids. The results of this study provided first insight into possible effects of 5G RF-EMF on neuronal development. Future work will include disease models, examinations at other times during brain organoid development, longer exposures, and other pathways related to neurodegeneration.

1.4 Outlook

In ongoing experiments, we will focus on midbrain organoids and will study the development of the organoids in chemically induced disease models as well as models that include genetically modified genes involved in neuronal development and differentiation. In addition, iPSCs under differentiation will be studied with and without genetically modified cells using the CRISPR-Cas9 technology.

1.5 Problems

In the end, most of our tasks could be successfully executed and finished. As described above, the electrical activity of exposed and sham-exposed brain organoids could not be completed due to methodological issues related to the interference of the RF EMF with the electrodes on the chip and the electrical activity being absent in many organoids as described by others as well.

2 Documents

Experimental data obtained are compiled in the doctoral thesis work of Selina Thomas entitled "*The role of RF-EMF (5G) on neuronal development and neuronal health using brain organoids*".
This work is currently for internal use only but will be submitted for publication as soon as the remaining analyses have been performed. We intend to continue investigating some observations beyond the scope of this project and perform a more in-depth analysis.

Date and Signature

26.01.2024

Meike Mevissen