UNIVERSITY OF ROSTOCK

Institute for Cell Biology and Biosystem Technology
Division of Environmental Physiology

Functional and Molecular Investigations after 1.8 GHz Radiofrequency Electromagnetic Fields Exposure in Different Immune Relevant Cells

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Idea - Goal

Appropriate endpoint - Functional study

- Changes in homeostasis
  - Free radical release
  - Cell cycle analysis
  - Apoptosis induction
- Cell system
  - Immune relevant cells

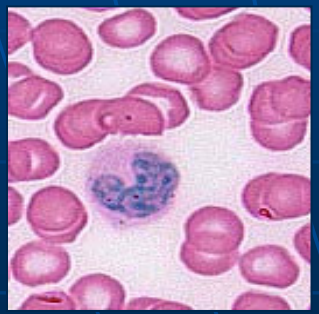
Molecular study

- Hsp70 - connection to redox status
- Protein screening
- Real time RT-PCR

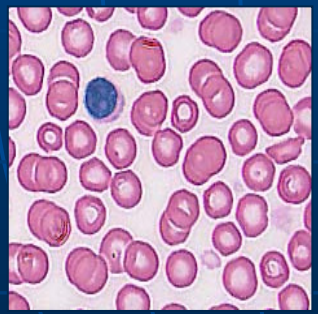
Cell systems

- **Human primary monocytes and lymphocytes:** isolated from human umbilical cord blood within 48 h after birth
- **Mono Mac 6 (MM6):** human acute monocytic leukemia cells
- **K562:** human chronic myeloid leukemia

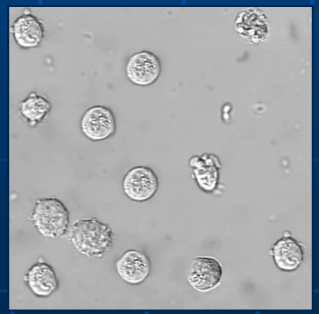
Monocytes



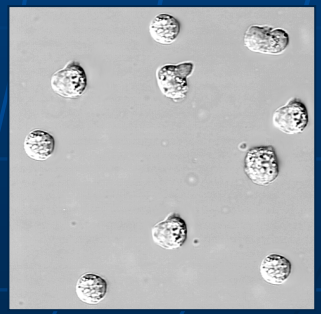
Lymphocytes



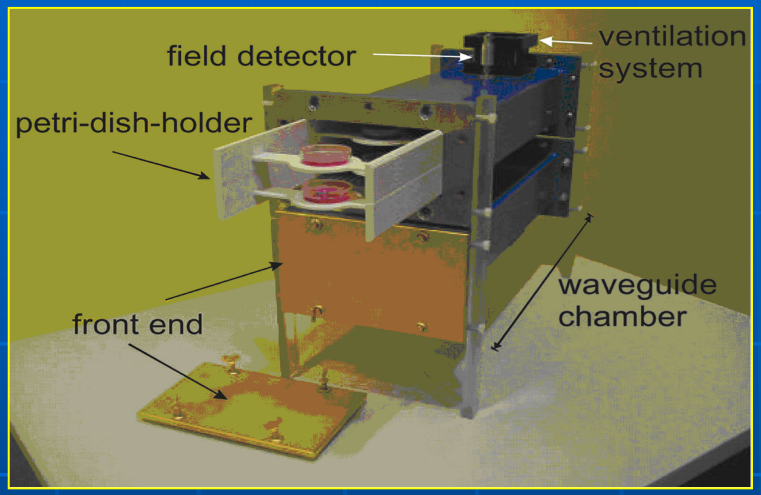
Mono Mac 6 cells

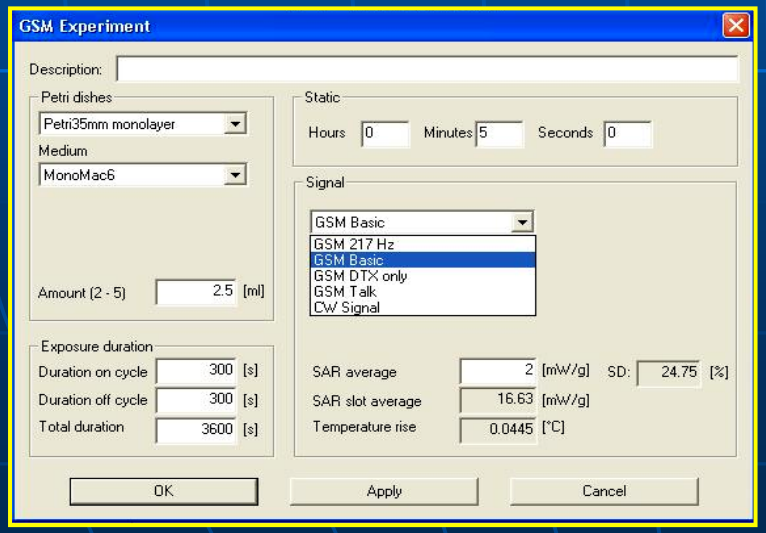


K562 cells



IT`IS Radio frequency setup





1.8 GHz:

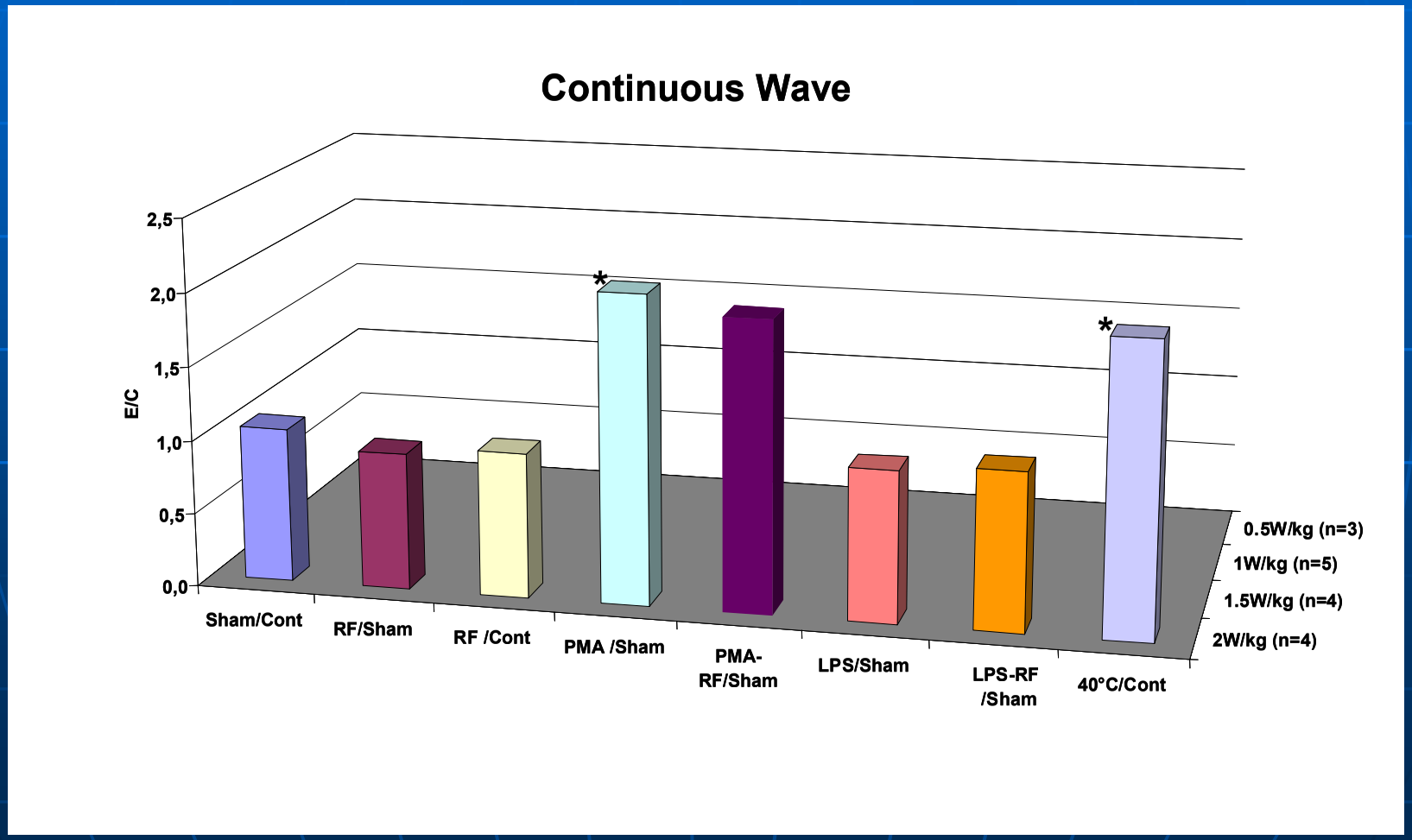
- CW
- GSM 217 Hz
- Hearing (GSM-DTX)
- Speaking (GSM-nonDTX)
- Talk (GSM-Talk)(70 : 30%)

SAR: 0.5; 1.0; 1.5; 2.0; 5.0; 10.0 W/kg

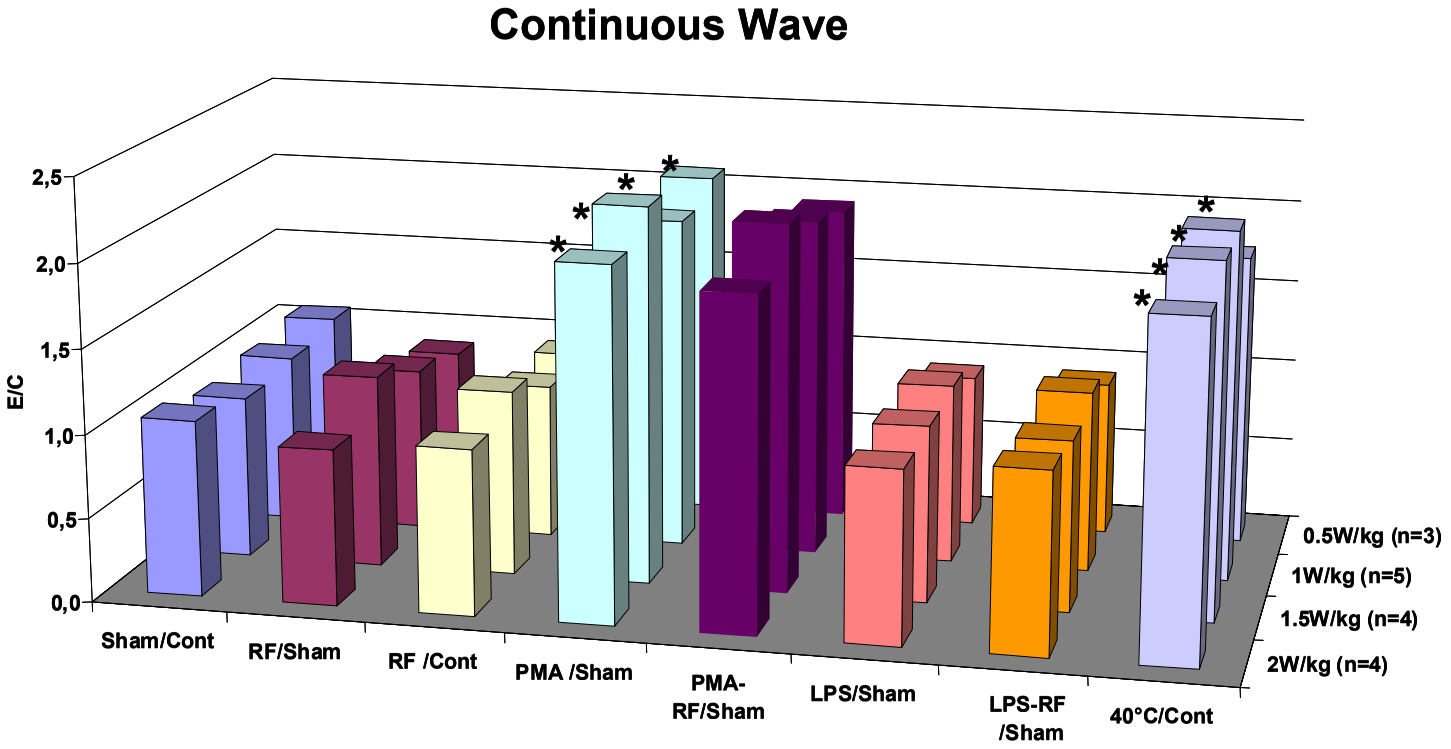
Exp. time: 10 min on/off
45 min cont.
60 min cont.
up to 12 h cont.

Statistics: 3-8 parallels;
>3 independent experiments
$p<0.05$;
ANOVA, Student's t-test

1.8 GHz - Free radical production (human Mono Mac 6 cells)



1.8 GHz - Free radical production (human Mono Mac 6 cells)



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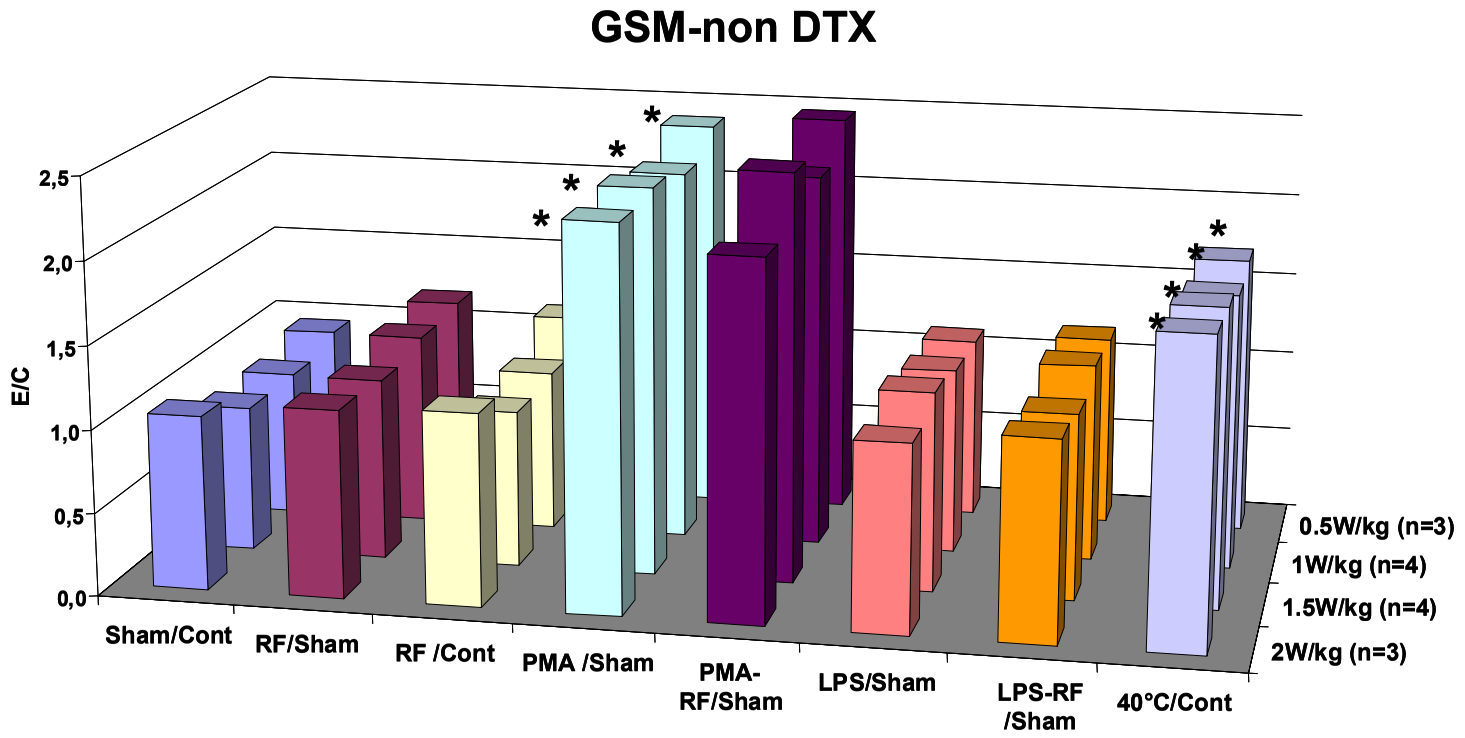
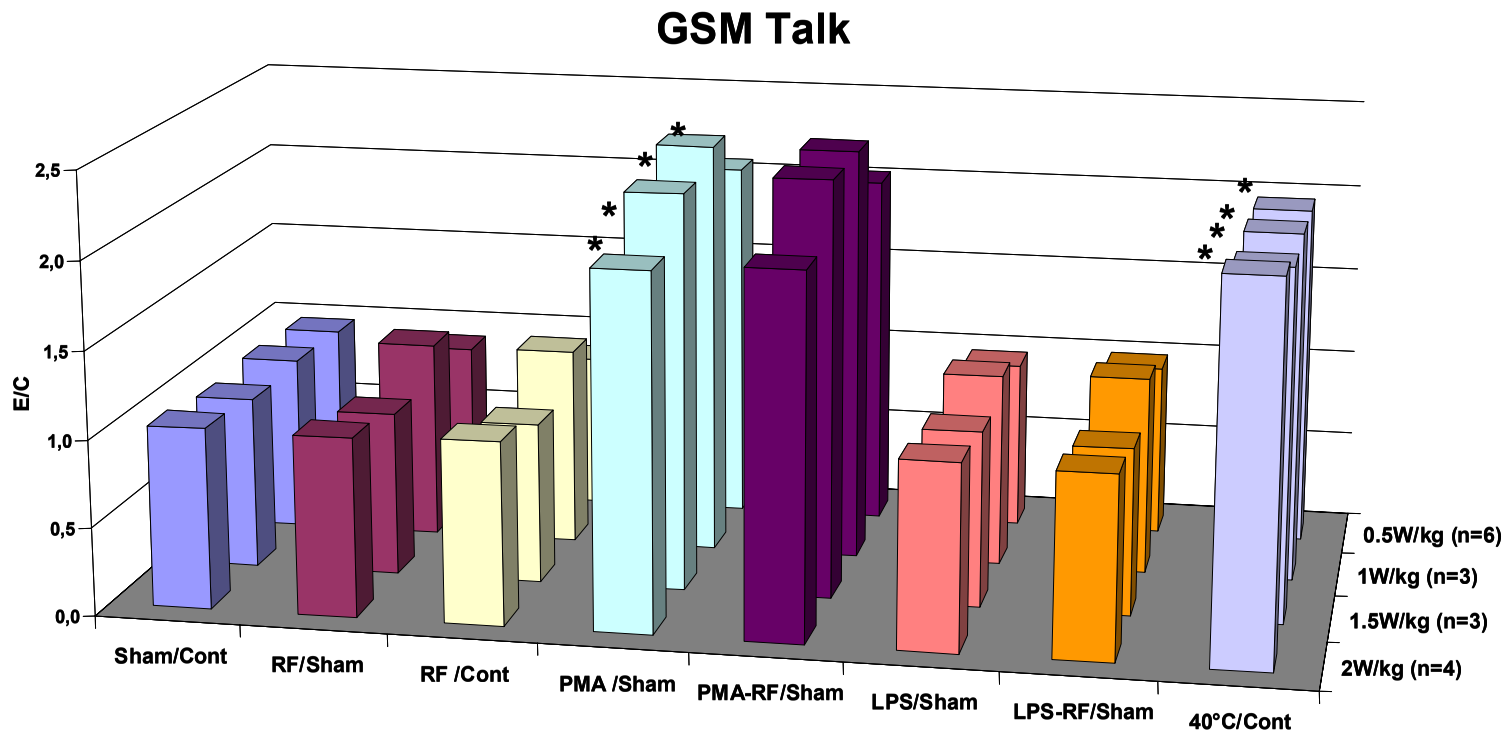
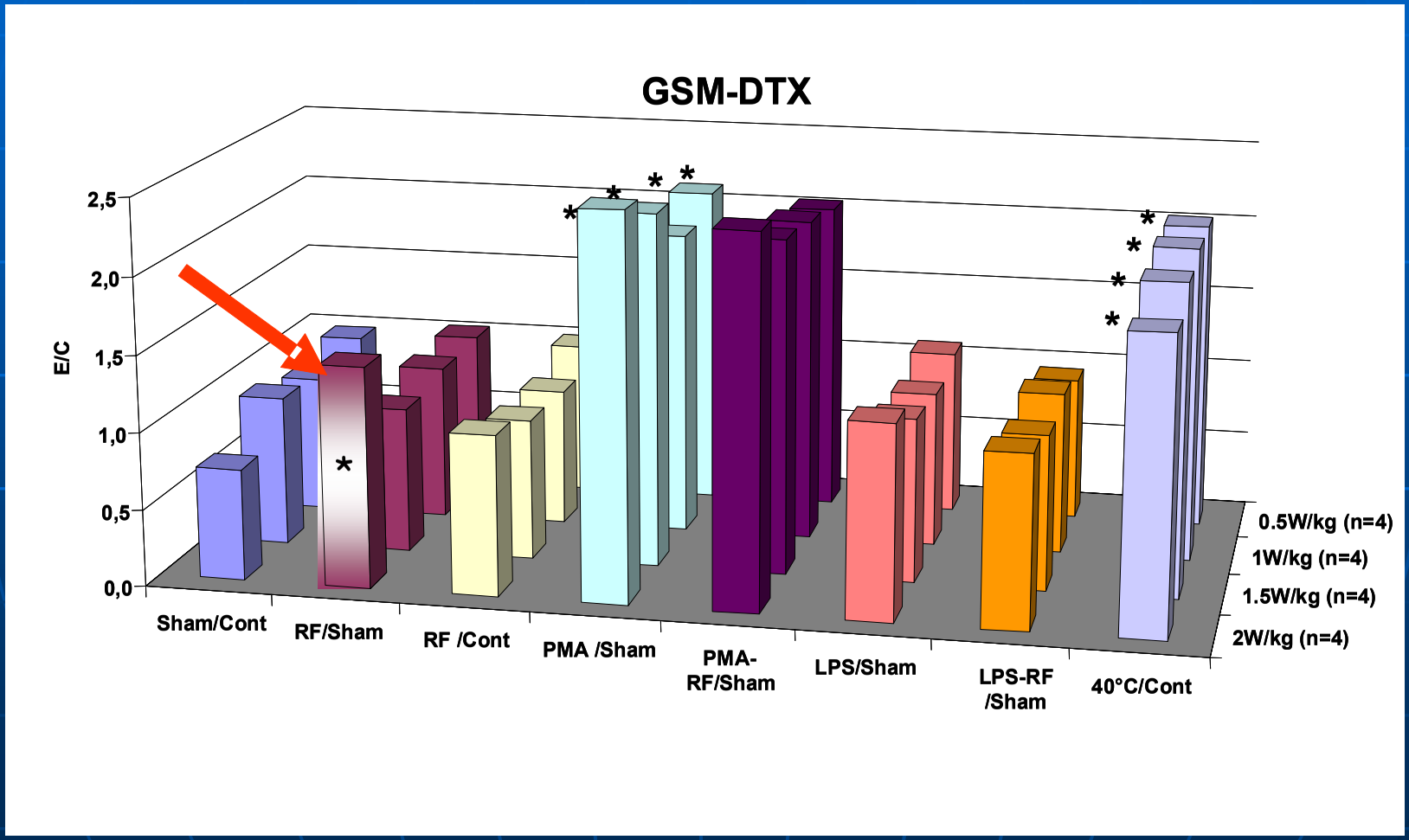


1.8 GHz - Free radical production (human Mono Mac 6 cells)

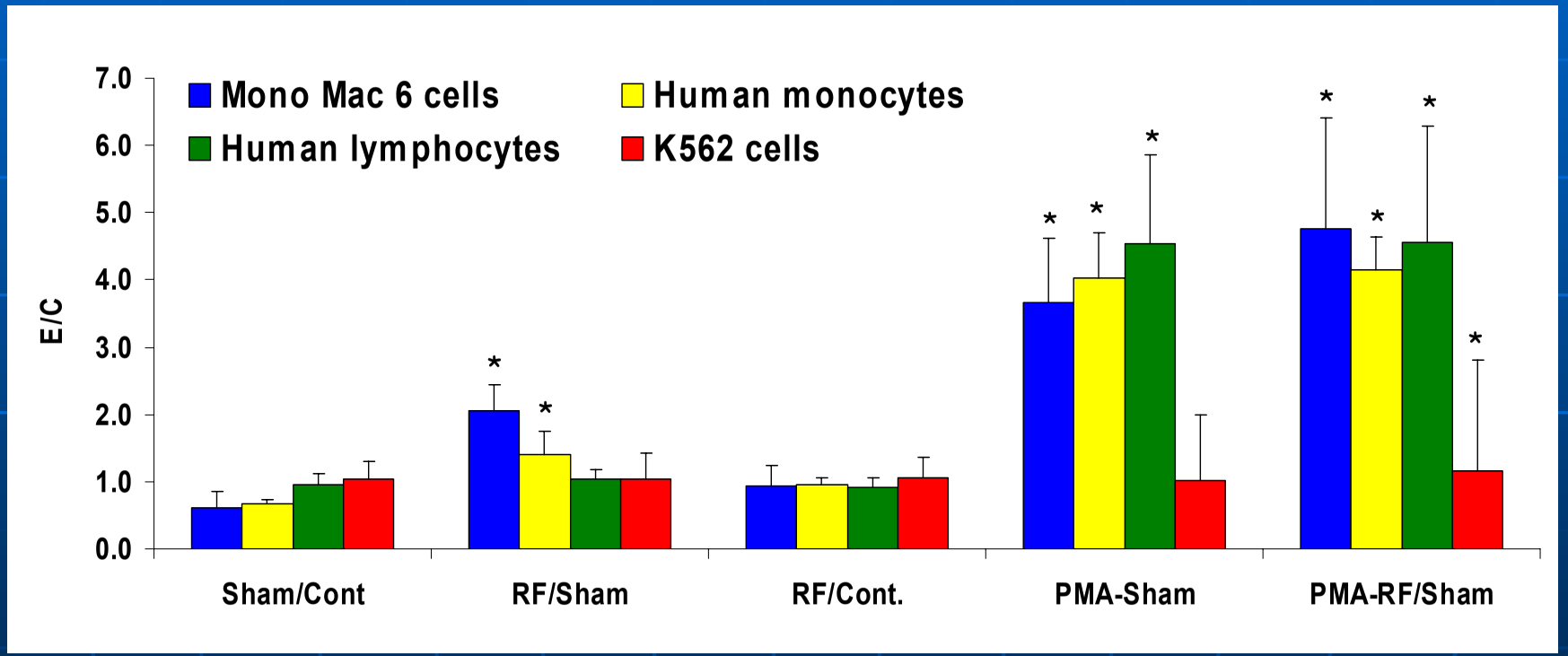


1.8 GHz - Free radical production (human Mono Mac 6 cells)



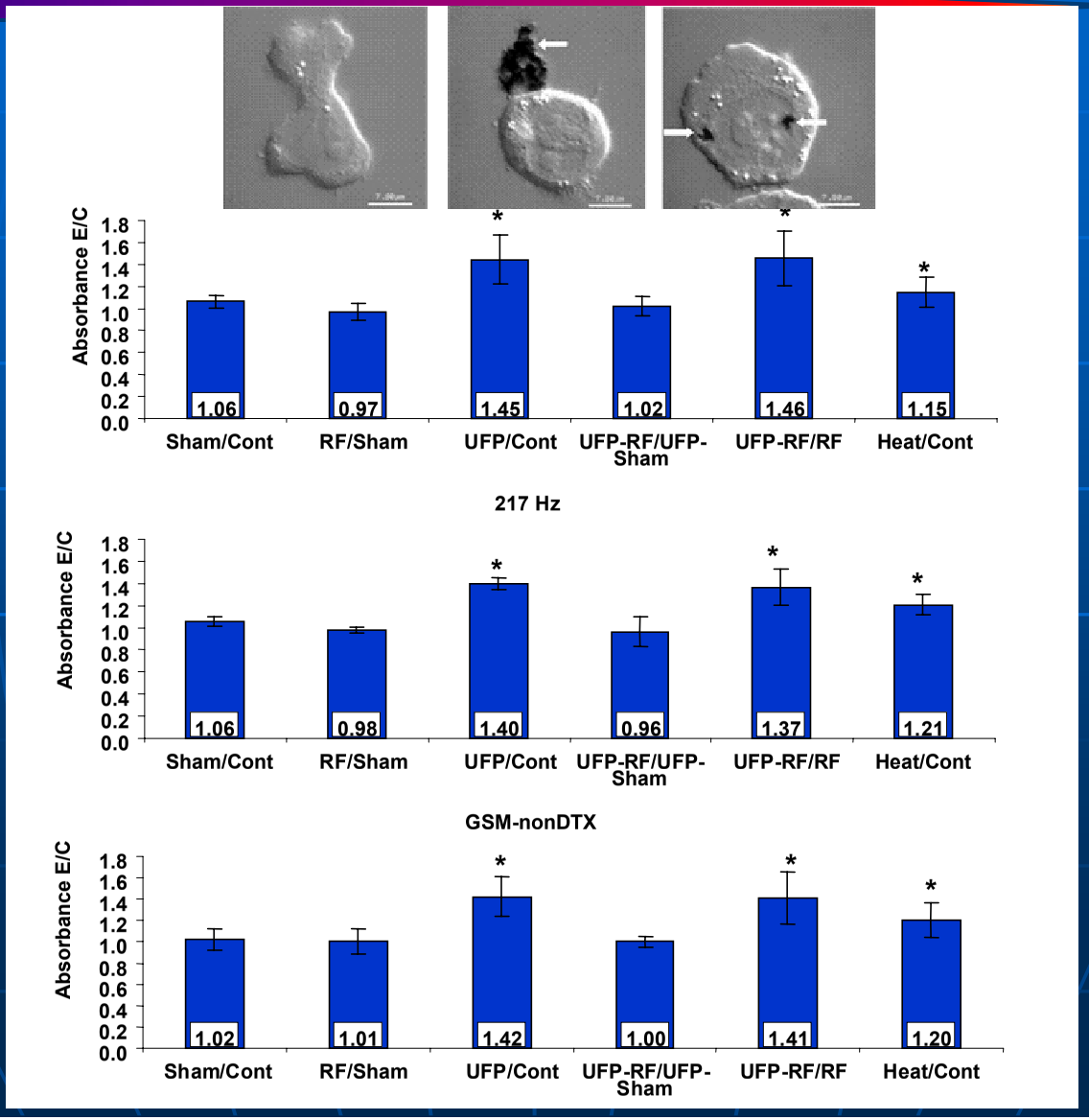
ROS production (DHR-assay)

GSM-DTX, 2 W/kg, for 45 min



n ≥ 3, p< 0.05

ROS / Phagocytic uptake – RF + Nanoparticles (Mono Mac 6)



Simkó's Lab

GSM-DTX signal
Influencing parameters

- Statistical differences between controls: negative
- Medium evaporation: negative
- Temperature increase: negative
- pH differences: negative
- Background ELF influence: negative

GSM-DTX-Sham-effect?

GSM-DTX

- active during hearing
- pulsed 1.8 GHz DTX signal (2, 8 and 217 Hz modulation)
- pulse maximum is variable, depending of average SAR

Cell type dependent decreased ROS production in sham:

- Mono Mac 6 cells and monocytes → sham effect
- K562 cells and lymphocytes → no effect

SAR dependency: 0.5, 1.0, 1.5, 2.0, 5.0, 10.0 W/kg

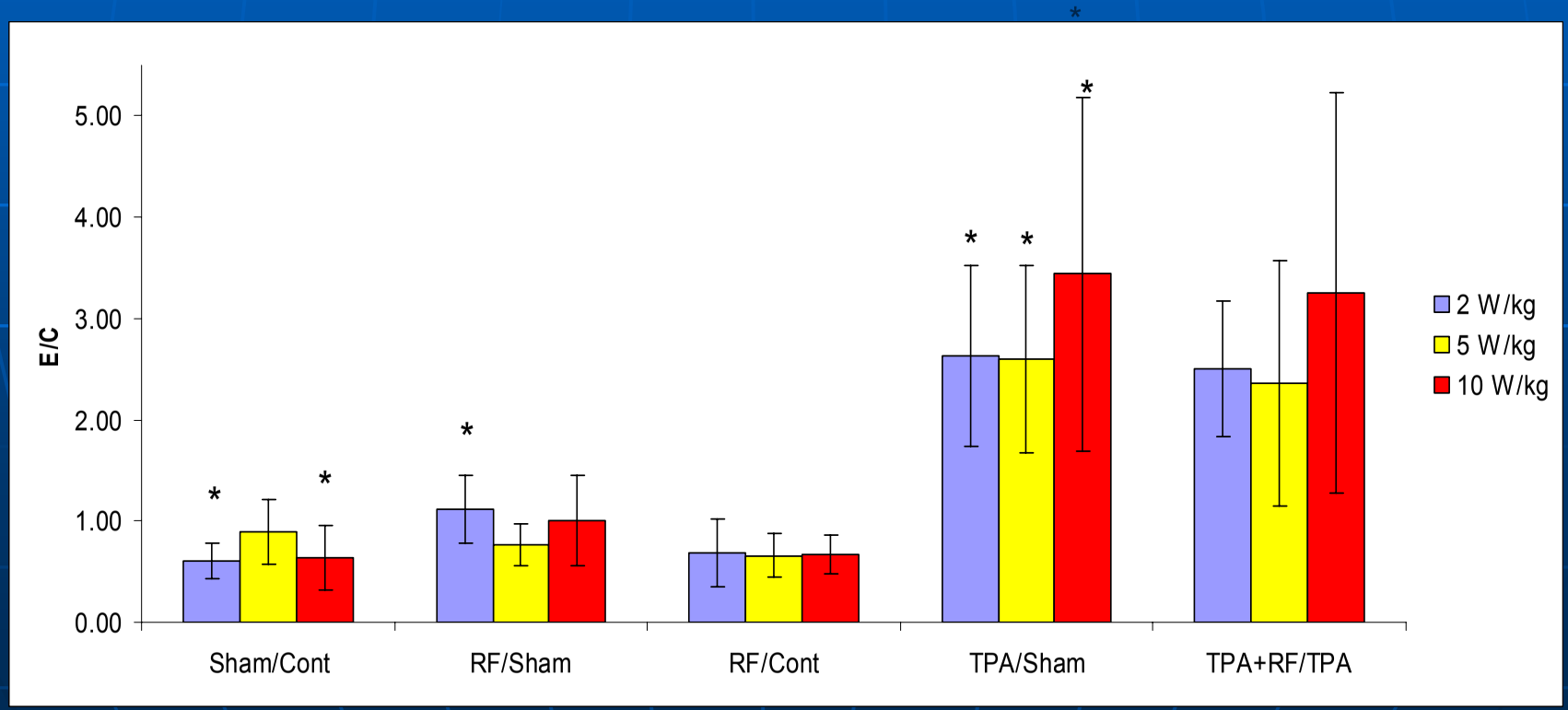
Frequency modulation of 1.8 GHz:

- 2, 8, 50 or 217 Hz moduliation
- fixed pulse maximum 500 W/kg and constant average SAR

GSM-DTX – SAR dependency
ROS production (Mono Mac 6)

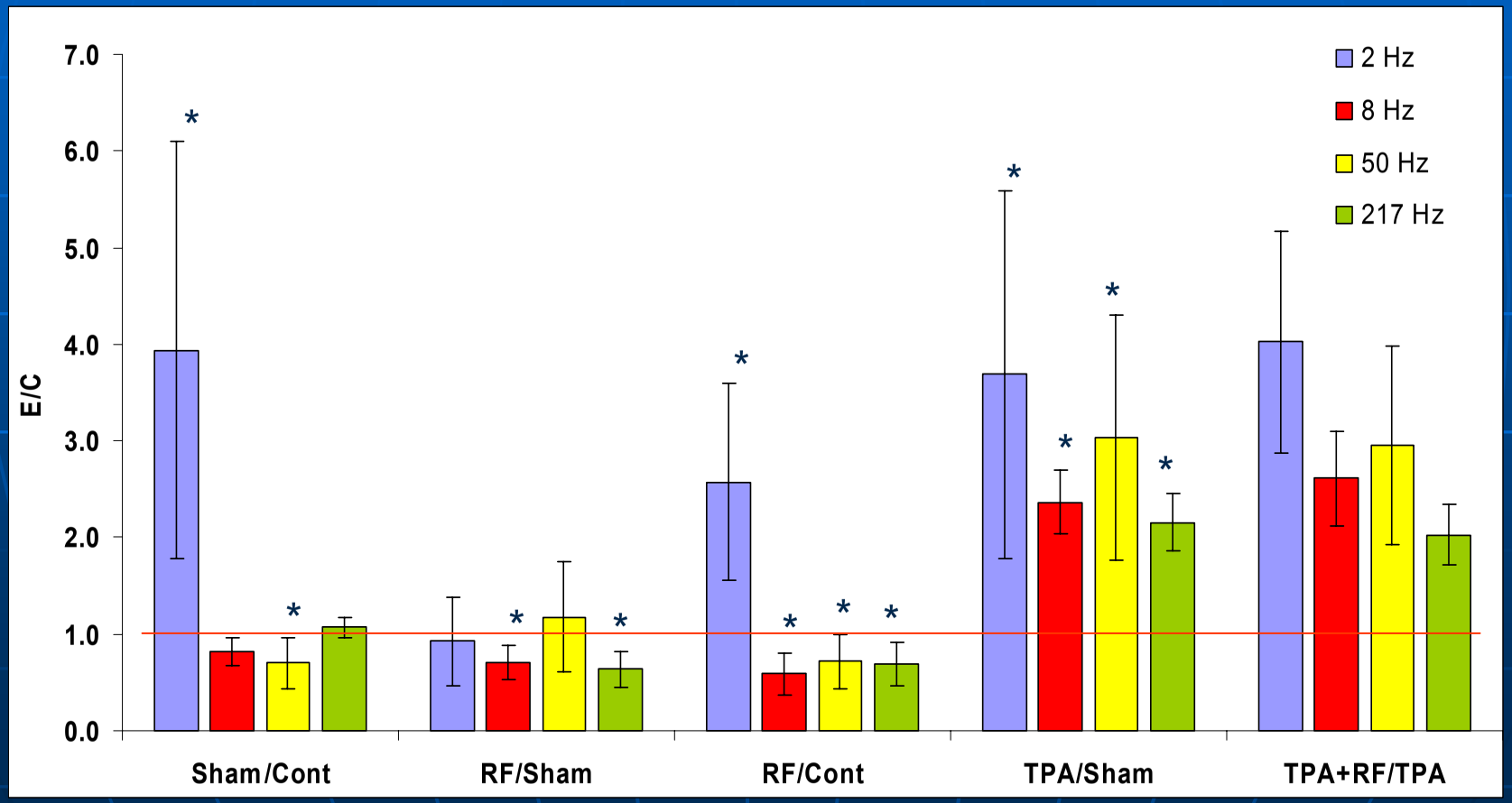
Average SAR: 2 W/kg (max. pulse: 140 W/kg)
5 W/kg (max. pulse: 350 W/kg)
10 W/kg (max. pulse: 700 W/kg)

Exposure time: 45 min



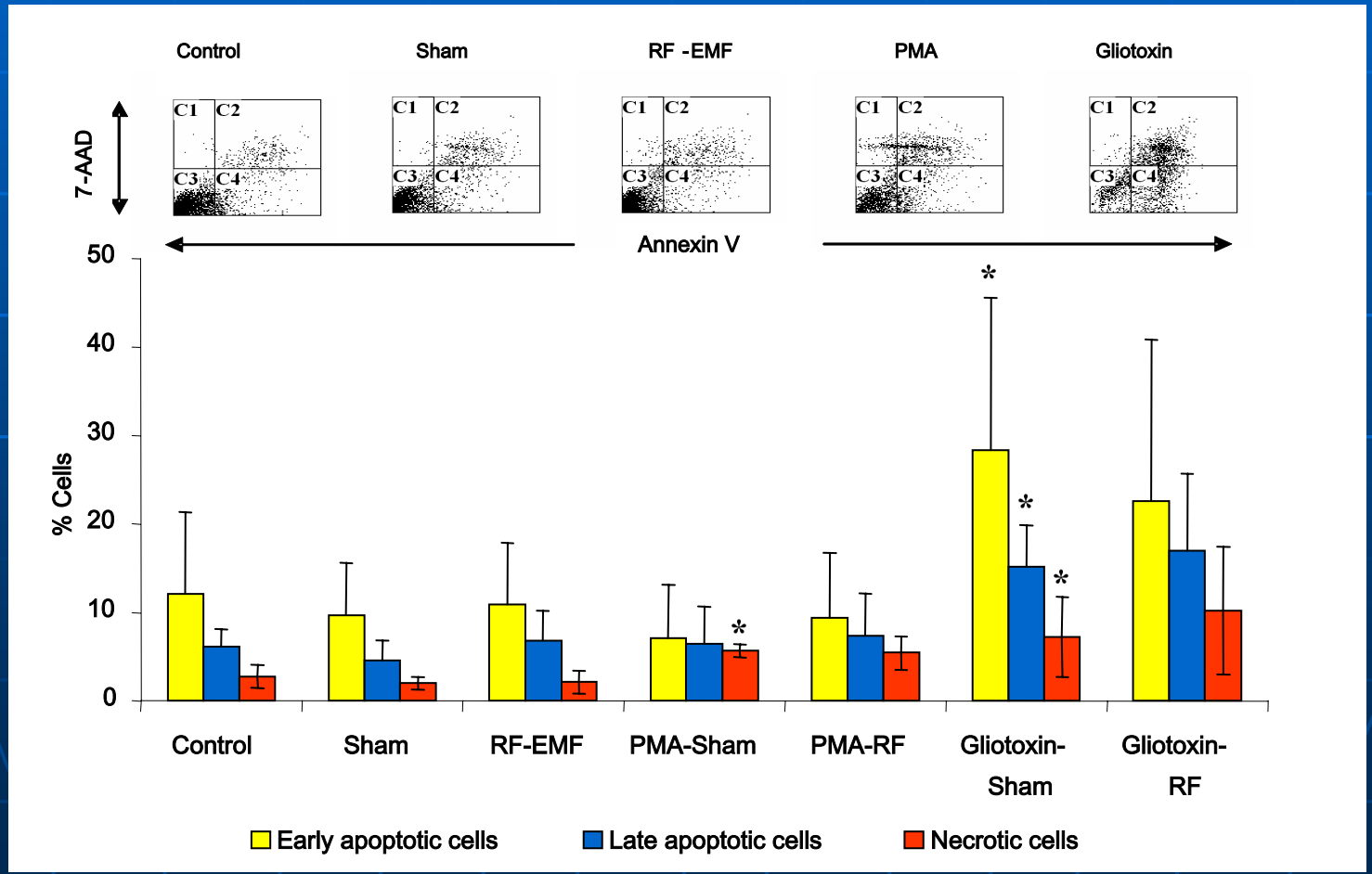
Frequency modulation of 1.8 GHz ROS production (Mono Mac 6)

Average SAR 2 W/kg (max. fixed pulse: 500 W/kg)
Exposure time: 45 min



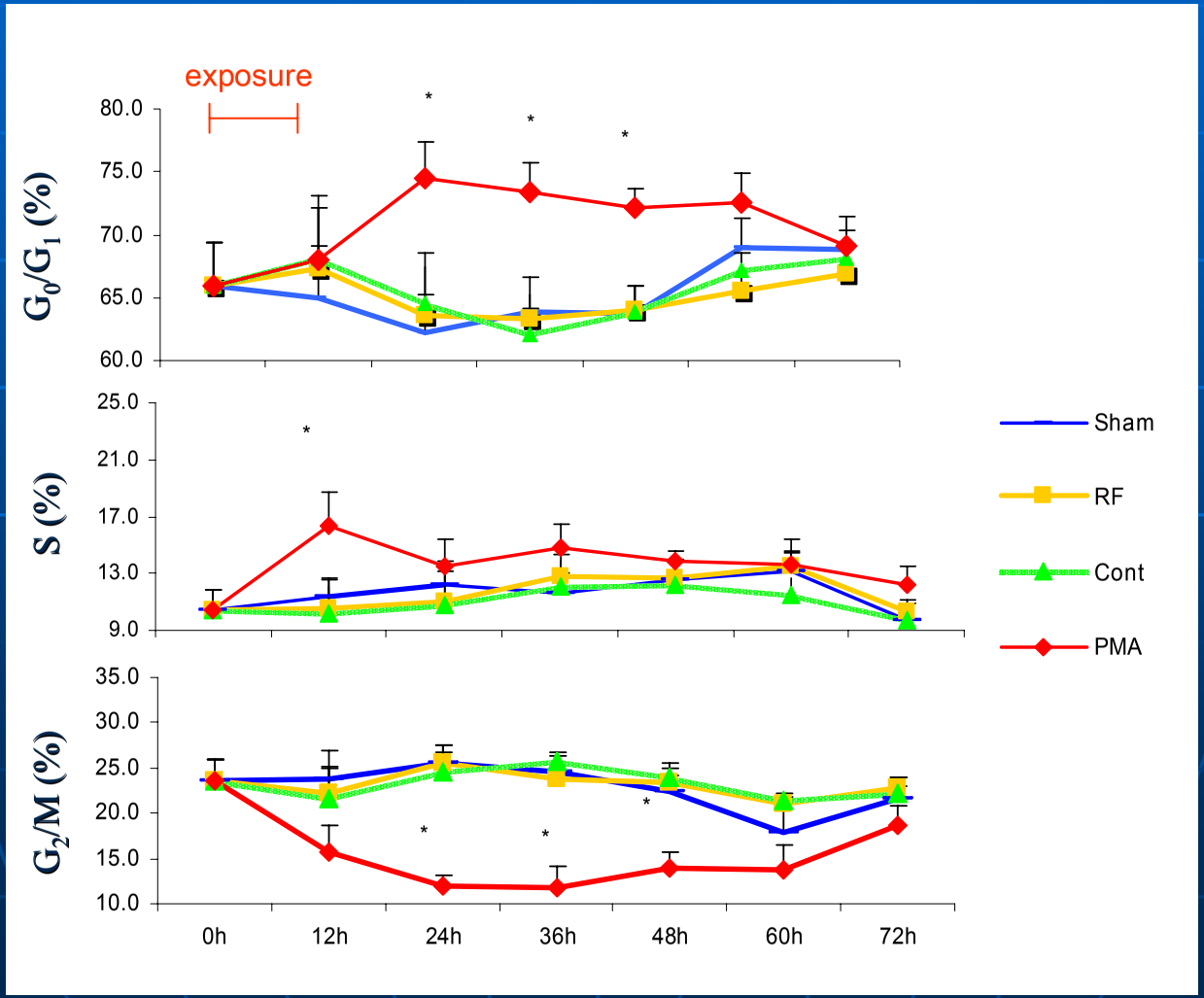
Apoptosis induction - DTX signal (Mono Mac 6)

Avarage SAR: 2 W/kg
Exposure time: 12 hours



Cell cycle control - DTX signal (Mono Mac 6)

Avarage SAR: 2 W/kg; Exposure time: 12 hours



Simkó's Lab

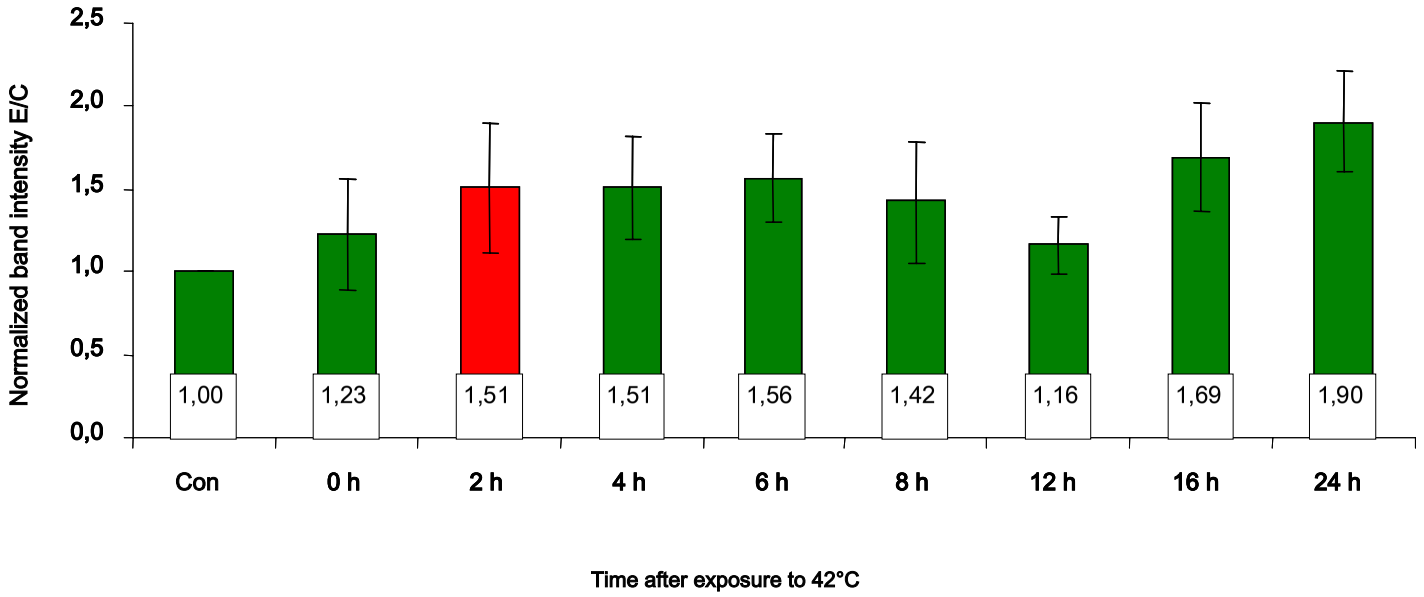
Functional studies

- "Sham effect" at 2 W/kg DTX signal
- Cell type dependency
- No apoptosis induction
- No changes in cell cycle distribution

Molecular study

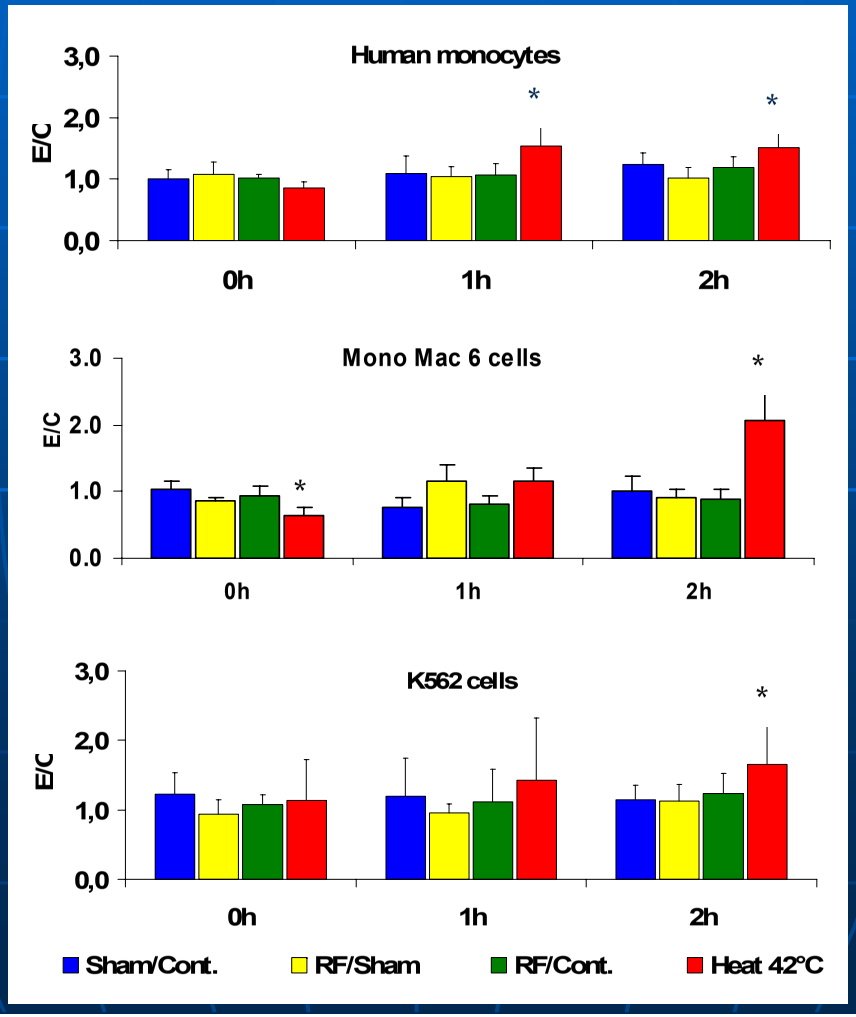
- Hsp70 - connection to redox status
- Protein screening

Time dependent Hsp70 expression DTX signal (Mono Mac 6)



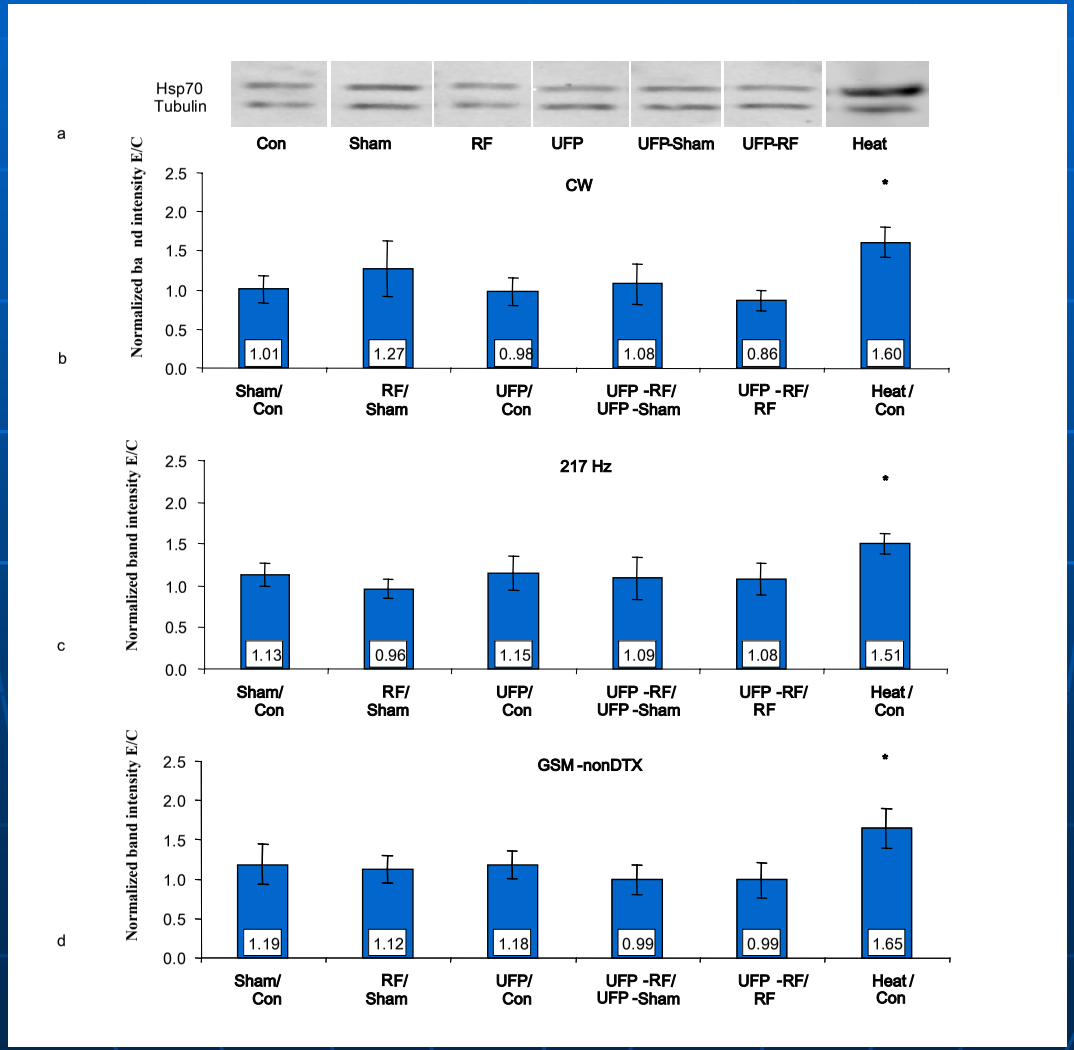
Hsp70 expression – DTX signal (2 W/kg)

Average SAR: **2 W/kg** (max. pulse: 140 W/kg)
Exposure time: 60 min



Simkó's Lab

Hsp70 expression – RF (2 W/kg) + Nanoparticles
Western blot (Mono Mac 6)

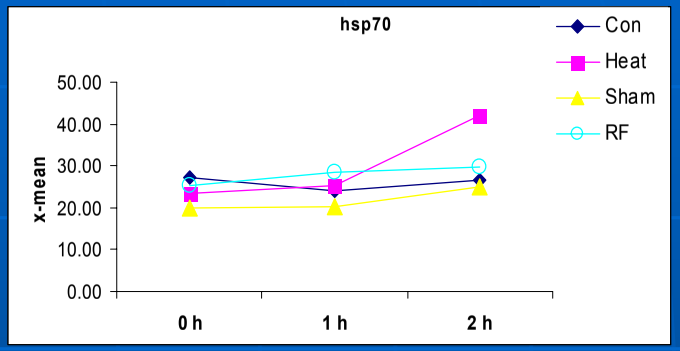


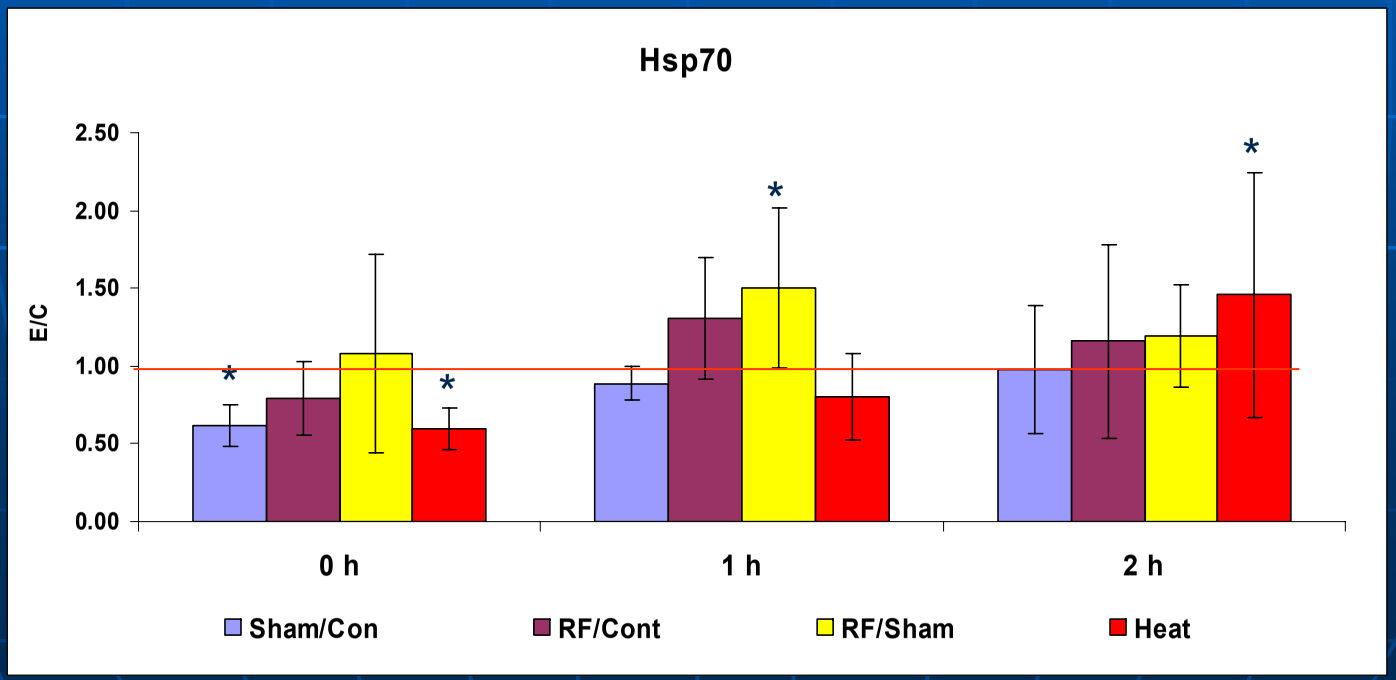
Simkó's Lab

Hsp70 expression – DTX signal flow cytomerty (Mono Mac 6)

Average SAR: **5 W/kg**
(max. pulse: 350 W/kg)

Exposure time: 60 min

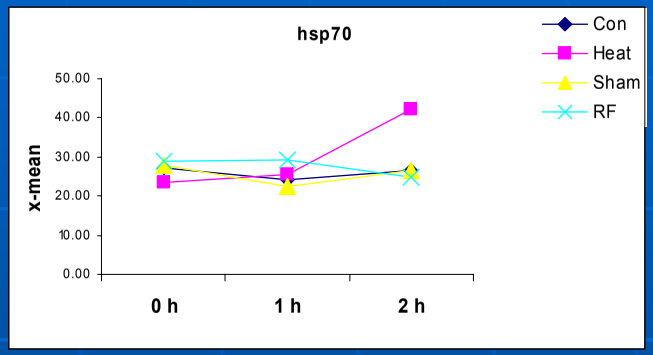


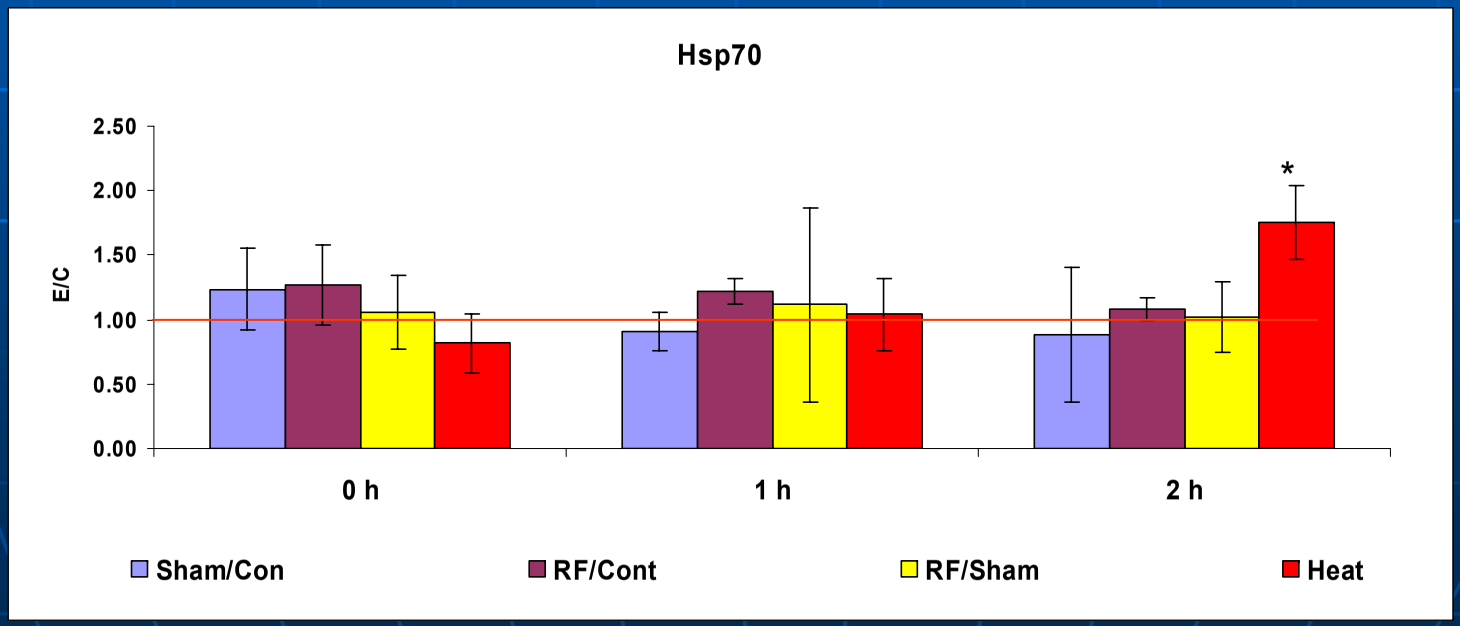


Hsp70 expression – DTX signal flow cytomerty (Mono Mac 6)

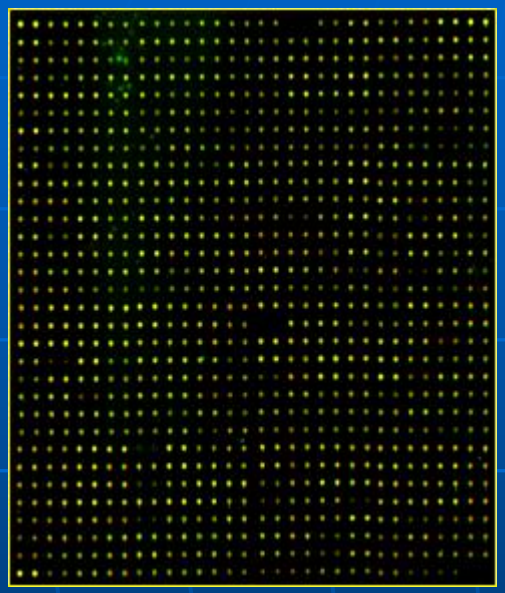
Average SAR: **10 W/kg**
(max. pulse: 700 W/kg)

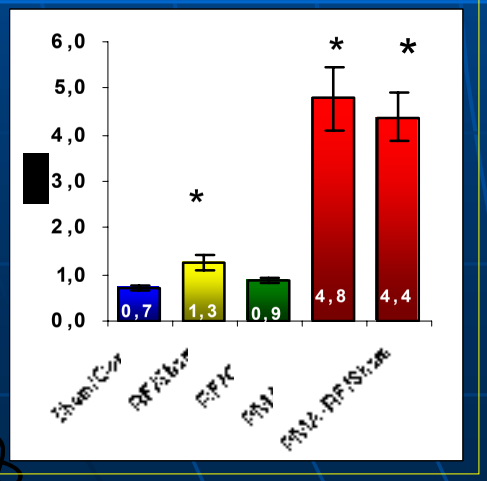
Exposure time: 60 min





DTX signal – Protein profiling arrays (human monocytes)





Human monocytes

Protein arrays:

- RF: 45 min, 2 W/Kg, DTX Exposition
- Sh: Sham
- C: Incubator control

Antibodies (Ab):

- 512 Ab dubble spotted (1024 spotts/array)
- Per array **Cy5** / **Cy3**
- RF-**Cy3** / Sh-**Cy5** and RF-**Cy5** / Sh-**Cy3**

Evaluation:

- 3 values of 4 must show the same direction of regulation (Ratio: > 0.7 and <1.3)

DTX signal – Protein profiling (human monocytes)

	Sham vs. control 10 up and 5 down regulations	RF vs. sham 23 up and 4 down regulations
Cell cycle regulation	2 up: Cyclin D1,Cyclin D3 2 down: Topoisomerase II b, Cdk7	5 up: Cyclin C, NPAT, XPA, TRF2, DDX1 2 down: Fos, Topoisomerase II a
Transcription factors	2 up: p63, PU.1(Spi-1)	2 up: Roaz, S III p15
Apoptosis	1 down: Bak	2 up: Caspase-9 / Apaf-3, DFF 45
Cell activation and cell signalling	2 up: MST3, ERp72 1 down: G alpha t	8 up: Inhibitor 2, cRAF1, AMPKb, MCP-1, PKCb, PI-3Kinase, AF6P, sme3/PA28-g
Other physiological processes	5 up: Integrin b1, Mint3, rSec8, Syntaxin 11, WT1	6 up: Acetylcholine Receptor, Amphiphysin, COMT, Endoglin Fatty Acid Synthase, g-Catenin 2 down: PMF-1, SCAR-1

- No conformity of regulated proteins between RF and sham
- Sham seem to down regulate cellular metabolism, whereas RF seems to activate or to compensate this effect

Summary

- Four cell types. 4 signals, 6 SAR values, different exposure times, 4 biological endpoints, and protein and gene analysis
- 1.8 GHz for any time and using different signal modulations do not induce free radical production or Hsp70 expression if data are compared to controls
- GSM-DTX signal at 2 W/kg induces a significant increase of free radicals if data are compared to sham
- The frequency modulation of the DTX signal induces “sham effect”
- Protein profiling showed 27 proteins which were regulated after RF
- Sham exposure seem to down regulate cellular metabolism, whereas RF seems to activate or to compensate this effect

Co-workers

Margareta Lantow

Christina Hartwig

Ronny Raasch

Publications

Lantow M, Viergutz T and Simkó M: Cell cycle analysis and apoptosis induction after exposure to radiofrequency radiation in human Mono Mac 6 cells Radiat Res. 166, 539-43. (2006)

Lantow M, Lupke M, Frahm J, Mattsson MO, Kuster N, and Simkó M: ROS release and Hsp70 expression after exposure to 1800 MHz radiofrequency electromagnetic fields in primary human monocytes and lymphocytes Radiat. Environ. Biophys. 45, 55-62 (2006)

Lantow M, Schuderer J, Hartwig C and Simkó M: Free radical release and Hsp70 expression in two human immune relevant cell lines after exposure to 1800 MHz radiofrequency radiation Radiat. Res. 165, 88-94 (2006)

Simkó M, Hartwig C, Lantow M, Lupke M, Mattsson MO, Rahman Q and Rollwitz J: Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultrafine particles in human Mono Mac 6 cells, Toxicol. Lett. 161, 73-82 (2006)

2 manuscripts in preparation

Thank you for your attention!

I am looking forward for a constructive discussion!