

Influence of GSM and UMTS on the Blood Brain Barrier *in vitro* - additional results

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In vitro-Experiments on exposure to
RF-fields of mobile telecommunication
C. Blood brain barrier

- BBB in vitro (rat brain endothelial cell cultures)
- GSM 1800 exposure
- UMTS exposure

- differential gene expression (genechip arrays)
- selection of BBB related candidates
- verification of diff. gene expression (qRT-PCR)

goals

- Influence of RF-EMF on endothelial cells of the BBB ?
- reduction of BBB towards an in-vitro model
- investigations on cellular level
- identification of potential EMF-targets on molecular level
- no hypotheses on pathophysiological issues

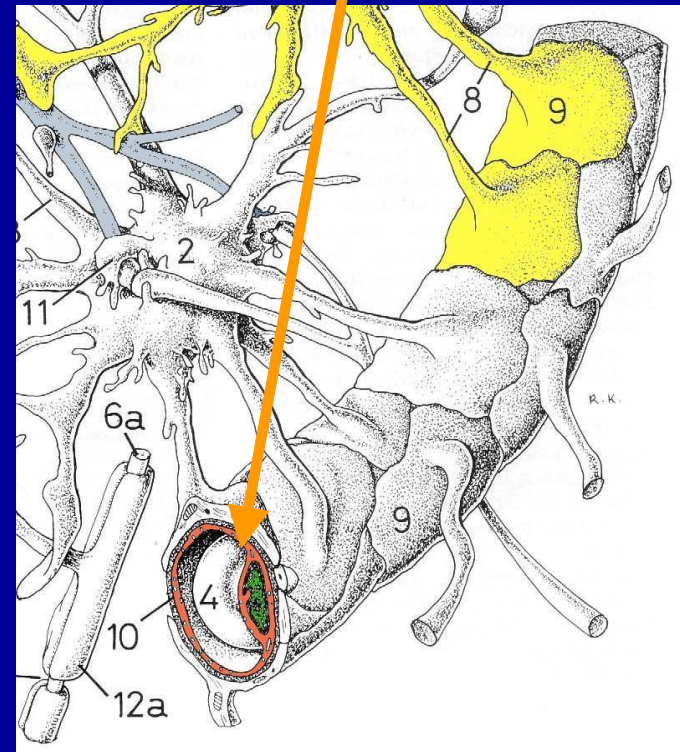
overview: project parts

- establishment and characterization of RBEC cultures as BBB in-vitro model
- design of exposure unit
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- qRT-PCR verification of gene regulation

The Blood-Brain Barrier (BBB)

- maintenance homeostasis of the CNS
- essential for proper brain function
- control of substance flow between brain tissue and circulating blood
- controlled import of nutrients into the CNS
- protection against toxins

Endothelial cells of the cerebral capillaries form the permeability barrier



Rat brain capillary endothelial cells (RBEC)



removal of meninges

homogenizing

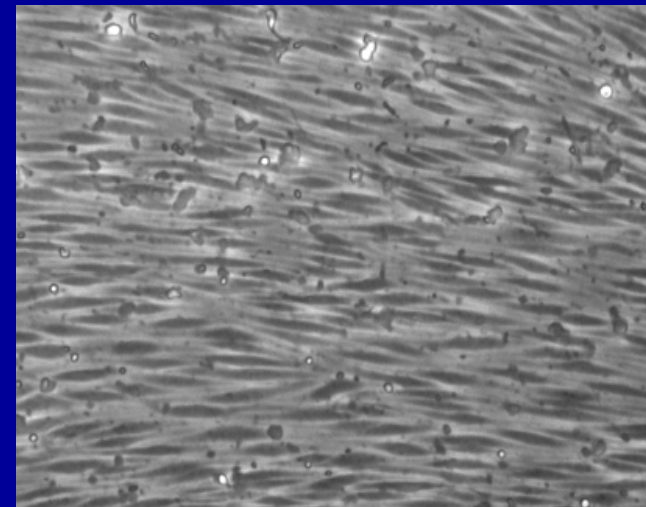
1st enzymatic digest

isolation of capillary vessels

2nd enzymatic digest

isolation of endothelial cells

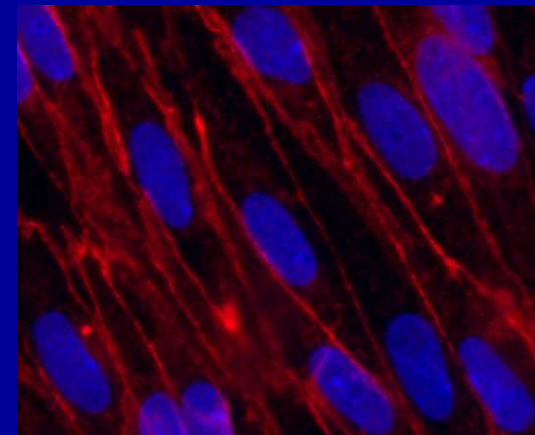
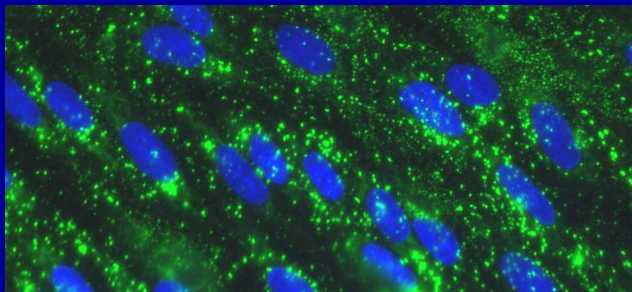
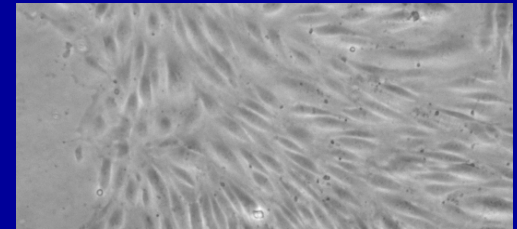
sowing and culturing



RBEC monolayer

characterization of RBEC

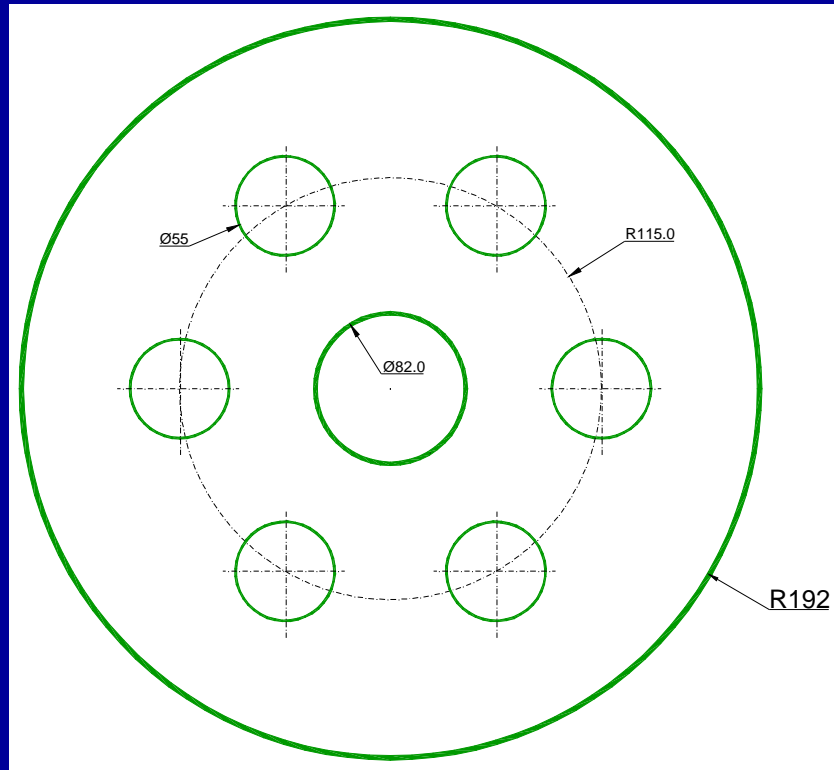
- + squamous morphology
- + von-Willbrand-Factor-VIII
- + vimentin
- + tight junction proteins: ZO-1, occludin
- - smooth muscle actin, GFAP, CD11b
- ----> minimal cell contamination



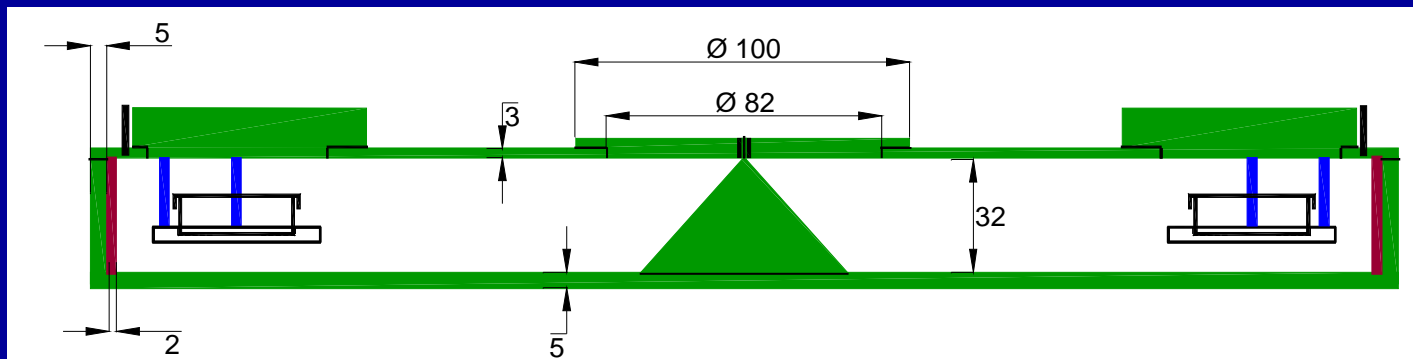
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assembly of radial waveguide



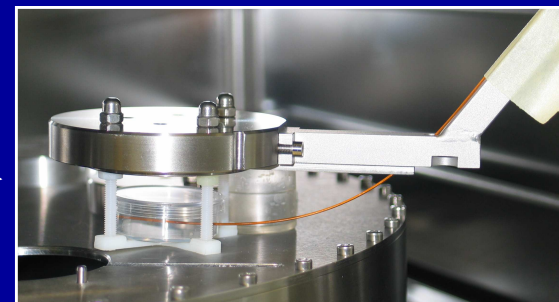
- 6 petridishes
- 40 cm diameter, 9 cm height
- sample holder centres petridishes
- temperature probe
- field probe





GSM-Exposure setup:

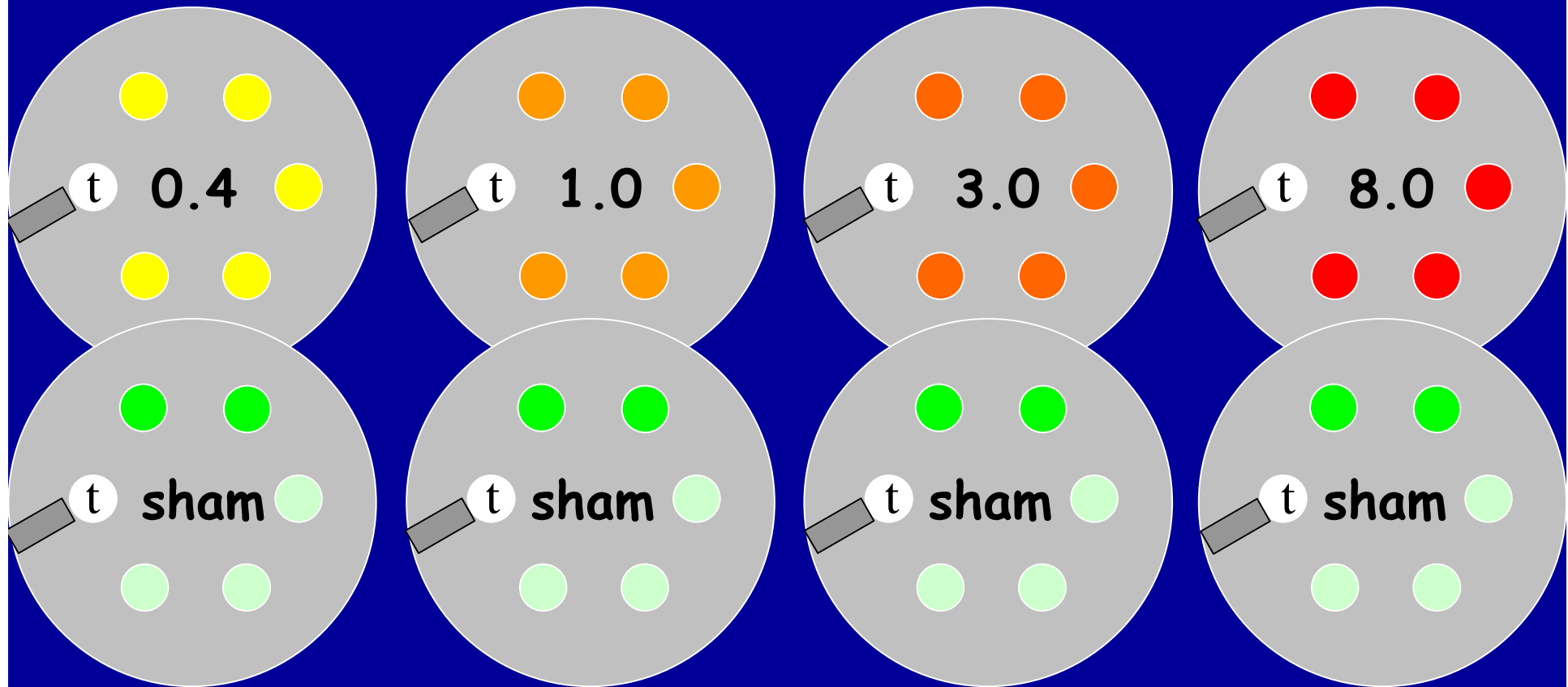
- amplifier
- signal generator
- wave guides
- fiberoptic temperature probes
- incubator



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exposure groups: GSM1800 / UMTS
@0.4-8.0 W/kg (72h)



biological replicates for gene chip analysis:

5+2 GSM

5+2 UMTS

5+2 GSM

5+2 UMTS

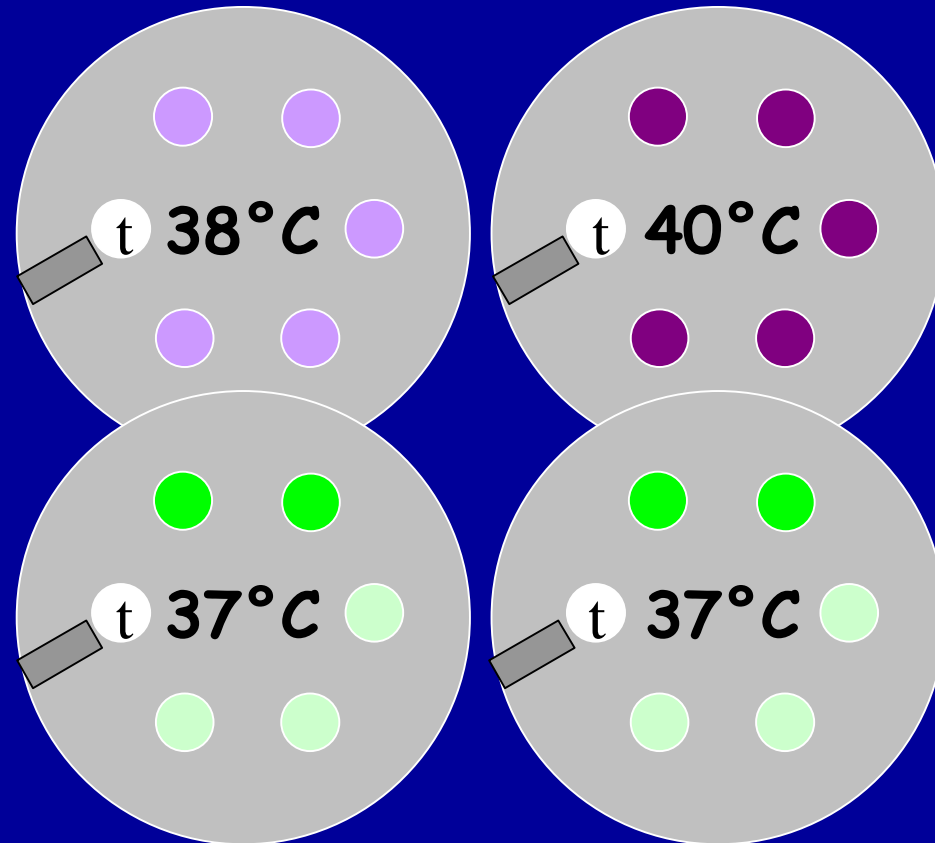
5+2 GSM

5+2 UMTS

5+2 GSM

5+2 UMTS

temperature control groups: 38°C / 40°C (72h)



max. temperature
@ 8W/kg: 38°C

biological replicates:

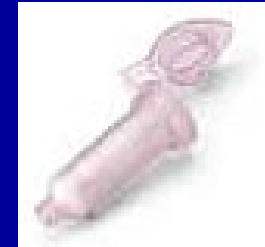
5+2 (38°C)

5+2 (40°C)

$\Sigma = 70$ Chip Arrays

RNA isolation protocol

- lysis of RBEC immediately after termination of exposure (< 5 min.)
- RNA isolation from RBEC:
Qiagen RNeasy Micro-Kit
store samples @ -70°C
- quality control: *Agilent Bioanalyzer*
- RNA-conc: min. $1\mu\text{g}/\mu\text{L}$



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Affymetrix GeneChip® Rat Genome 230 2.0 Array

31099 probe sets



- data tables showing the signal intensities of the various probe sets
- 28000 genes on the chip!
(=31099 probe sets)

filtering of ,absent calls'

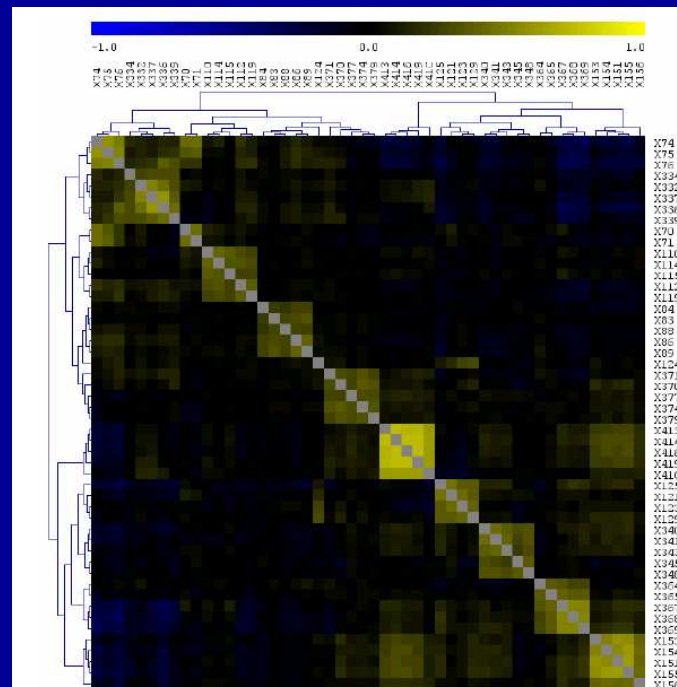
- MAS 5.0 (Microarray Suite, Affymetrix)
- normalization of signal levels
- of 31099 probe sets on the chip, 18663 could be detected reliably („present“ calls in ≥ 3 of 5 chips per experimental group)

18,663



filtering of genes with fold-change < 1.4

- of 18663 present probe sets, 14287 showed at least 1.4x change in gene expression compared to sham exposed RBEC

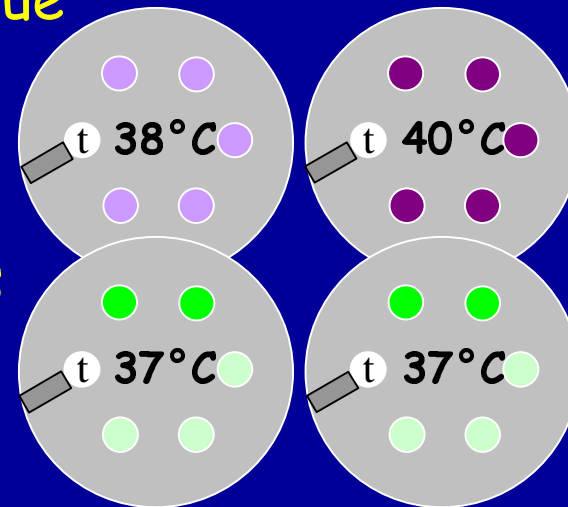


14,287

filtering by t-test vs. temperature controls

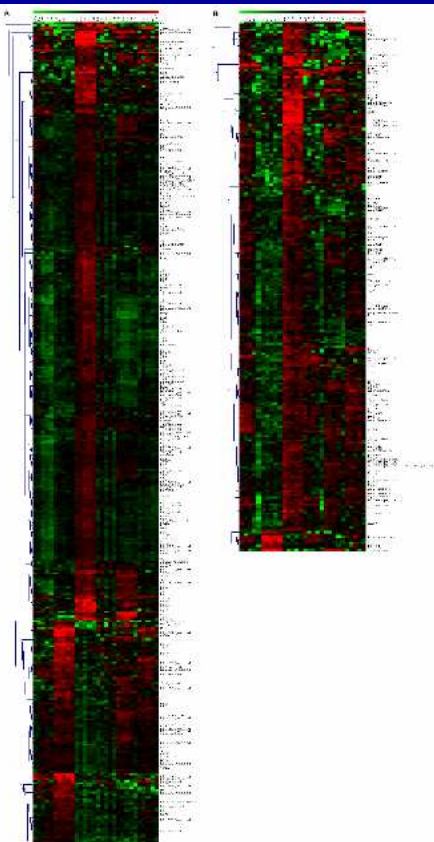
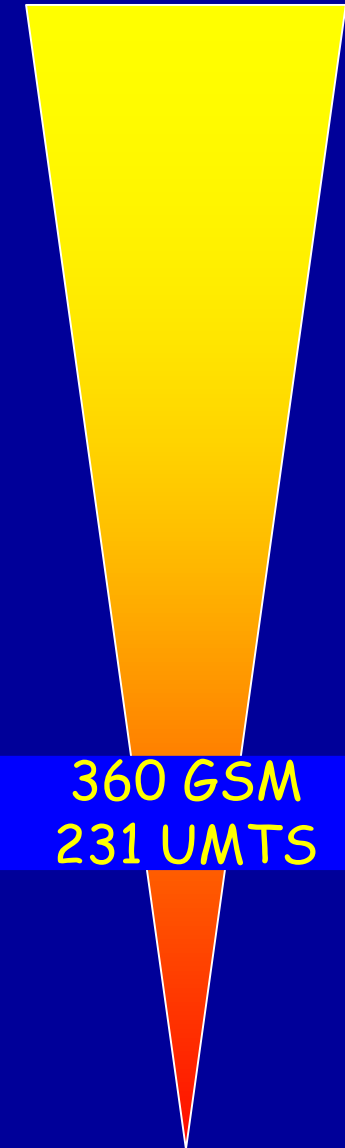
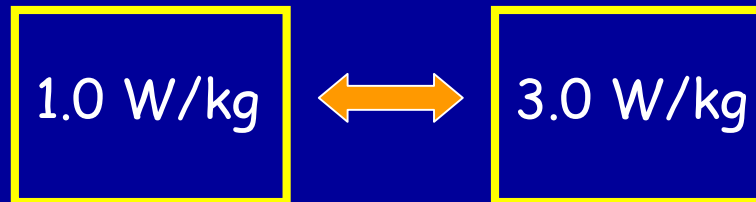
- of 14287 differentially expressed probe sets, differential expression of 11488 (GSM) or 8900 (UMTS) was not merely due to temperature increase ($p < 0.05$).

- manual selection of genes related to BBB



11,488 GSM
8,900 UMTS

Discriminatory Genes Analysis: SAM (Significance Analysis of Microarrays) 1W/kg vs. 3W/kg



- *GSM1800*: of 11488 genes, 360 genes were identified by SAM as differentially expressed between 1 W/kg group and 3 W/kg
- *UMTS*: 231 of 8900 genes
- parameter: no false positives

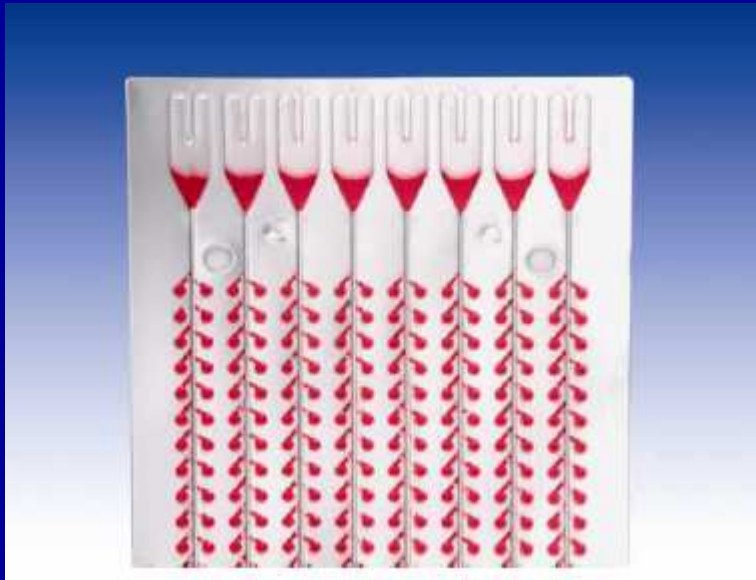
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Selection of genes for qRT-PCR validation

• SELECTION CRITERIA:

- BBB-Genes: 2x regulation
- SAM analysis 1W/kg vs. 3W/kg: 3x regulation
- other genes: 5x regulation

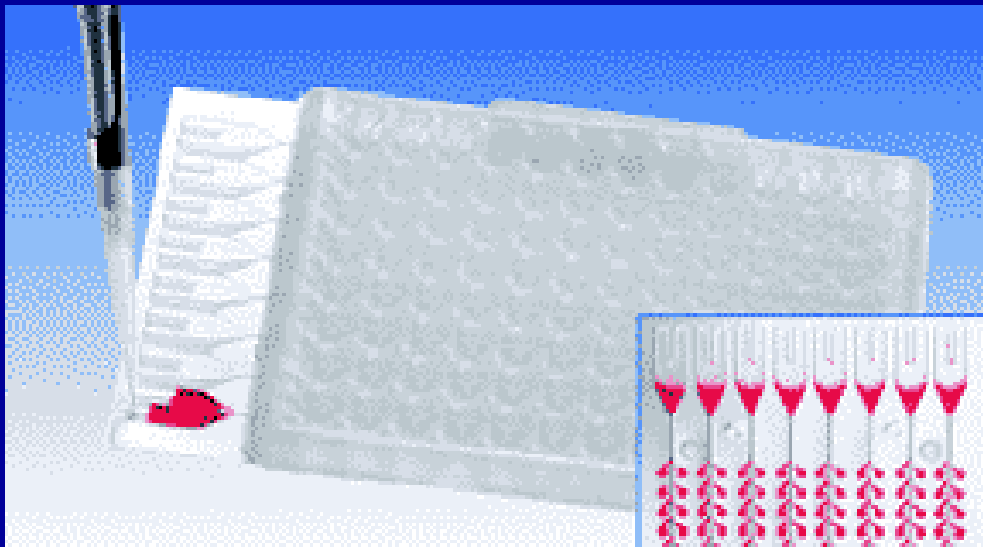


68 genes GSM
61 genes UMTS

Selected genes for qRT-PCR validation

• SELECTION CRITERIA:

- BBB-Genes: 2x regulation
- SAM analysis 1W/kg vs. 3W/kg: 3x regulation
- other genes: 5x regulation
- available for TaqMan® Low Density Arrays

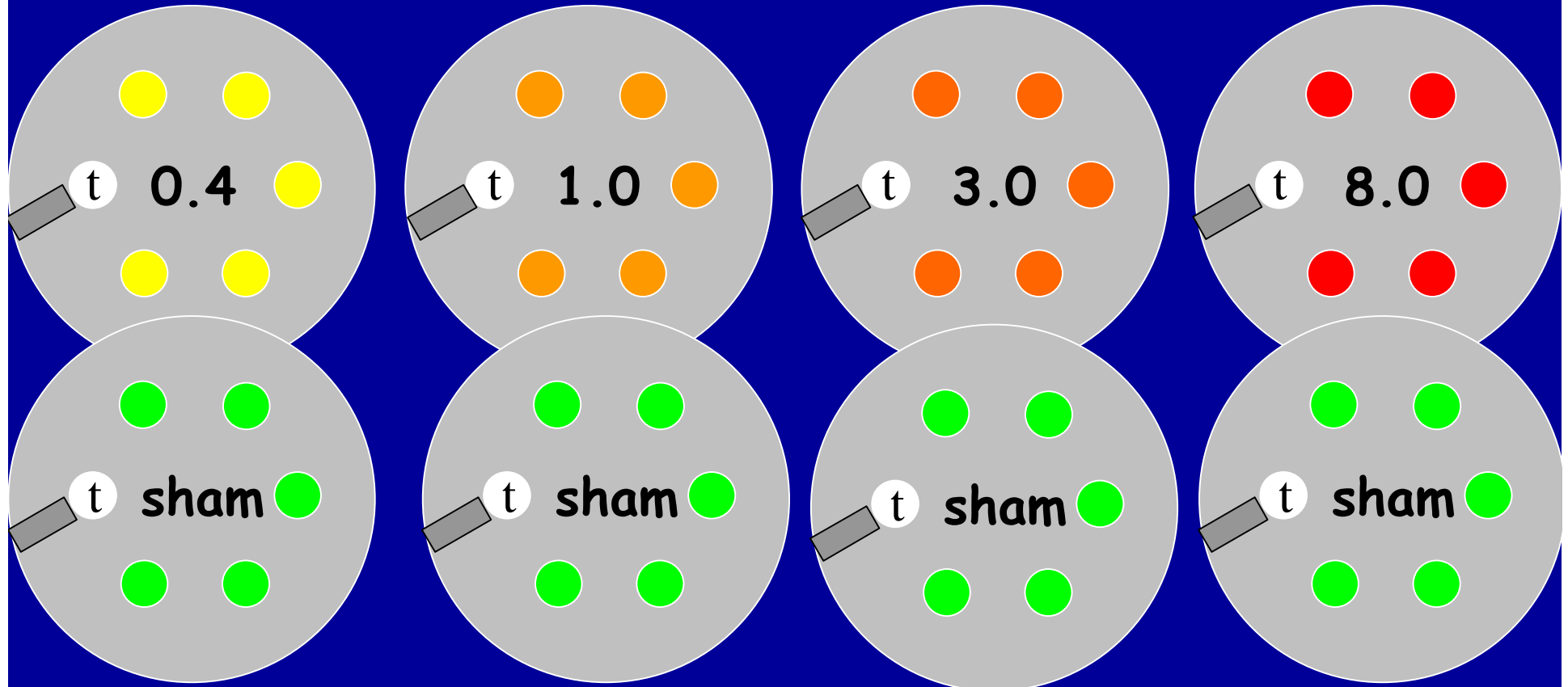


47 genes GSM
45 genes UMTS

selection of genes with BBB relevance (regulation to be validated)

- *Agtr1a* (angiotensin II receptor; vasoconstriction)
- *Col5a3* (procollagen V α 3; ECM)
- *Sdc2* (syndecan 2; monocyte migrat. brain endot.)
- *Slc33a1* (acetyl-CoA transporter)
- *Abcc8* (ATP binding cassette transp.: MRP)
- *Slc19a1* (folate & drug transporter)
- *Cldn1* (claudin1, ZO-1 & Occl. associated TJ-prot.)
- *Mmp2* (matrix metalloproteinase, involved in BBB regulation)

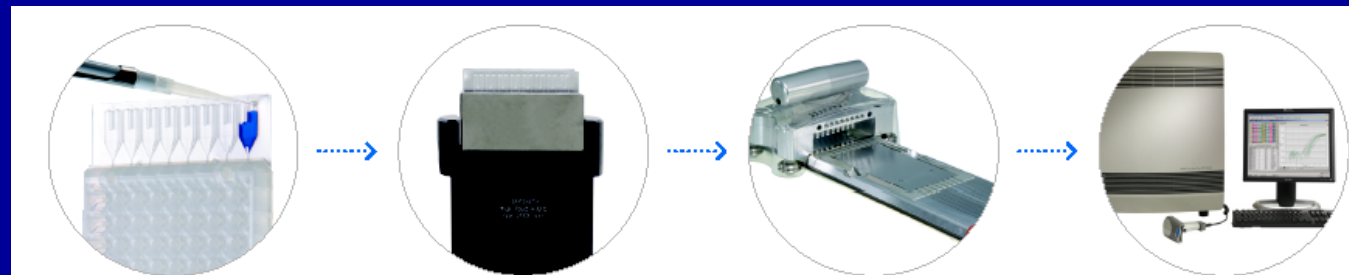
samples for qRT-PCR analysis



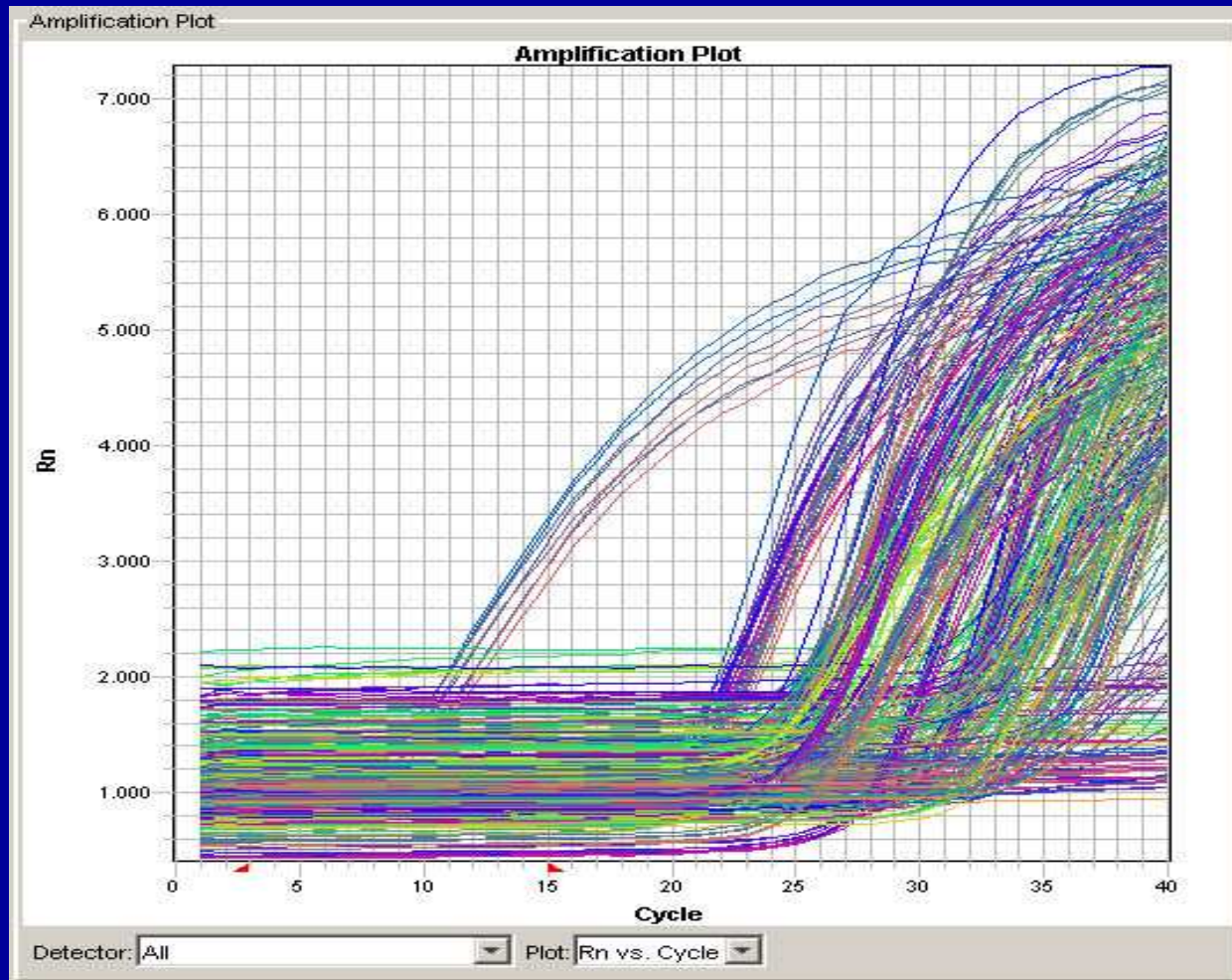
biological replicates for qRT-PCR: **5+5 GSM**
5+5 UMTS

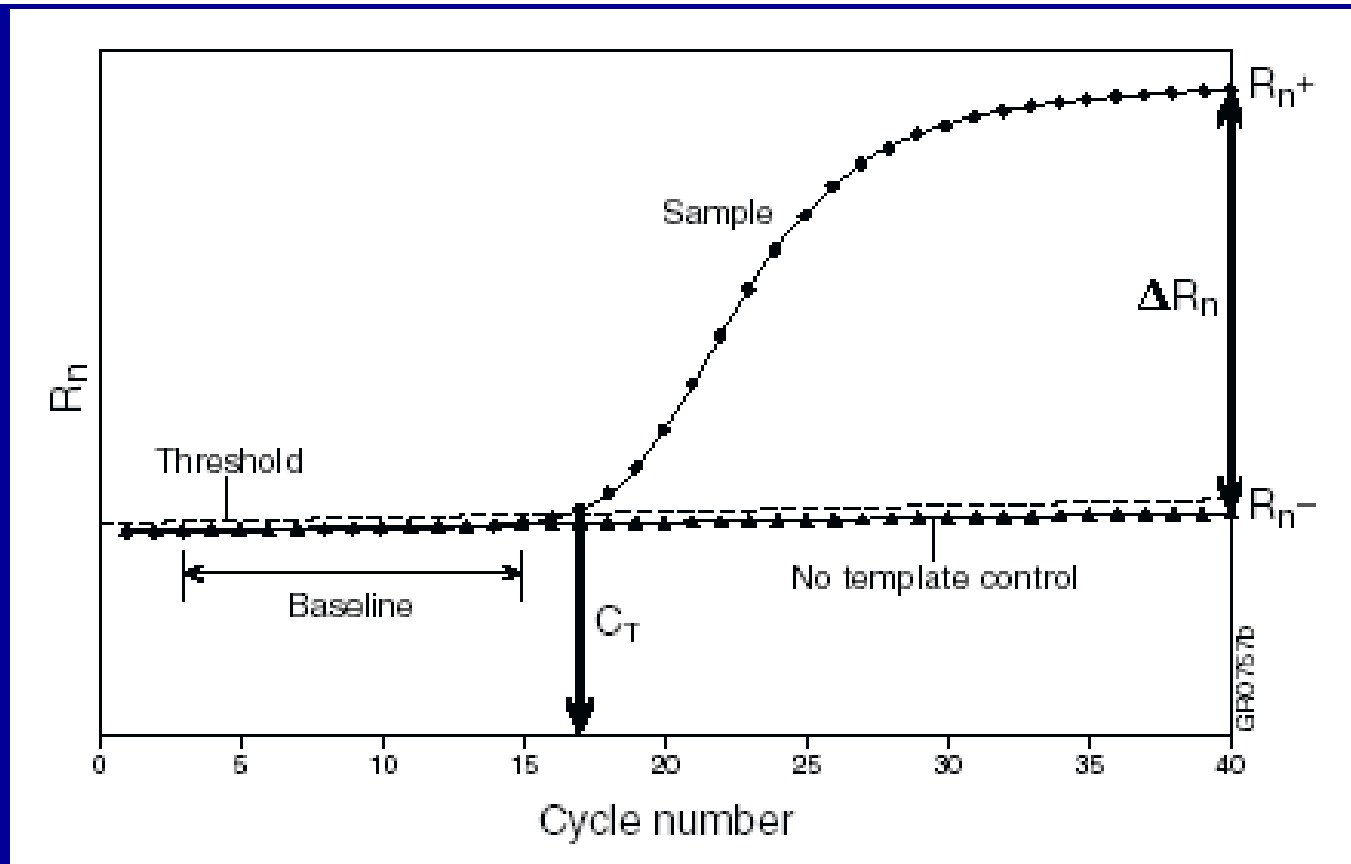
quantitative real time RT-PCR with TaqMan[®] Low Density Arrays

- reverse transcription of RNA to cDNA
- 200 ng cDNA in 100 μL per channel
- PCR reaction volume $\sim 1 \mu\text{L}$
- 2x 47 genes (UMTS and GSM)
- comparative quantification: $\Delta\Delta C_t$ -method
- 7680 individual qRT-PCR reactions



amplification plot for 384 qRT-PCR reactions (one TLDA)





$$C_{+}(\text{target gene}) - C_{+}(\text{endogenous control}) = \Delta C_{+}$$

$$\Delta C_{+}(\text{exposed}) - \Delta C_{+}(\text{sham}) = \Delta\Delta C_{+}$$

$$RQ = 2^{-\Delta\Delta C_{+}}$$

- ✓ establishment of an isolation method for RBEC
- ✓ characterization of RBEC
- ✓ installation of exposure device and determination of field parameters
- ✓ exposure of RBEC
- ✓ RNA isolation
- ✓ chip-arrays for differential gene expression
- ✓ bioinformatic evaluation of gene-chip data
- ✓ qRT-PCR experiments / evaluation of PCR data
- identification of potential protein targets

- **Dept. Neurology**
Dr. Helmut Franke, Prof. Dr. P. Young
Dir: Prof. E.B. Ringelstein, *University Hospital Münster*
- **Chair f. Electromagnetic Theory**
Dr. Joachim Streckert, Dr. Andreas Bitz
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- **Miltenyi Biotec**
Dr. Corinna Scholz, Dr. Jan Schäferkordt
- **Federal Office for Radiation Protection**
Dr. Monika Asmuß

thank you