

EFFECTS OF HEAD-ONLY EXPOSURE TO GSM-1800 OR UMTS ON THE BLOOD- BRAIN BARRIER *IN VIVO*

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Introduction

- There is growing evidence that mobile phone signals are unlikely to alter the integrity of the blood-brain barrier (BBB) in rodents.
- However, one group reported BBB permeation and dark neurons, up to 50 days after a single 2-hour exposure of rats to GSM-900 like signal.
- Few information available on new mobile phone signals

BfS Programme

- The German BfS project on the blood-brain barrier integrity and the occurrence of “dark neurons” in rats brains:
 - single and repeated exposures
 - GSM-1800 and UMTS signals
 - various SARs
 - various times after the end of the exposure

Exposure set-up

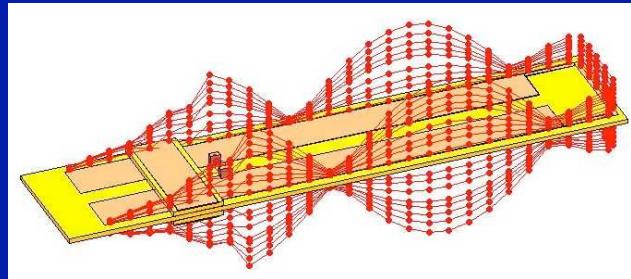
The loop antenna



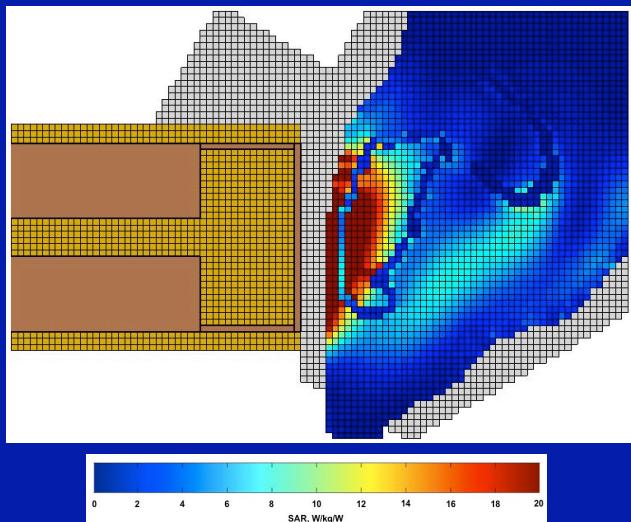
Characterised at 900 MHz
in Lévéque *et al.* IEEE
MTT, 2004.

Rats need to be restrained

Dosimetry



The fields induced in the head are produced by the neighbouring current element of the loop adjacent to the head



- Temperature measurements in fantome
- Numerical simulations (FDTD)
- Brain averaged SAR (BASAR)

Animal exposure

Male Wistar-Han rats

Signal: GSM-1800 or UMTS

Brain-averaged SAR=0.026, 0.26, 2.6, or 13 W/kg

Single exposure: 2 hours

Sacrifice immediately, 1 h, 1, 7 and 50 d after exposure

Repeated exposure: 2 hours /day, 5 days/week, 4 w

Sacrifice immediately and 50 d after exposure

Exposure protocol

Two series of 8 rats per exposure condition were performed



Controls

- Cage controls: animal facilities
- Positive controls: cold-shock 5' + 25' before sacrifice
- Sham-exposed rats : restraining

In all experiments, cage control and sham-exposed animals were handled in parallel

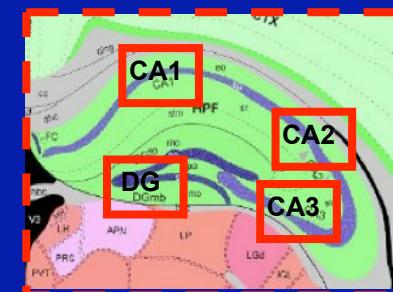
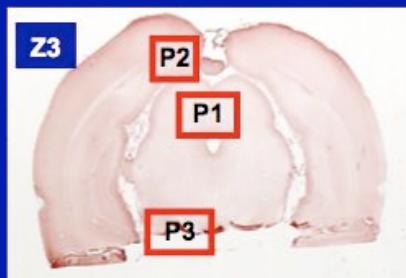
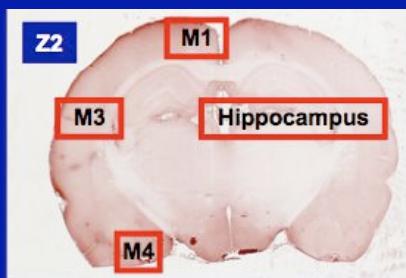
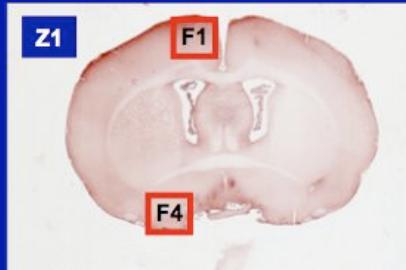
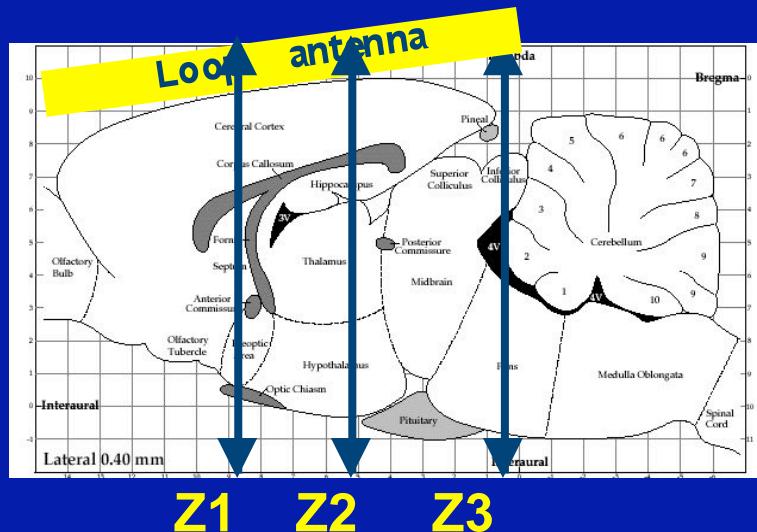
Samples

- At the time of sacrifice, rats are anesthetized using isoflurane inhalation (5% in air).
- Rat brains are fixed by intracardiac perfusion with paraformaldehyde (4% in PBS) and frozen (-80 °C).

Brains are coded at that step, so that the analysis is fully blind.

Samples

- Brain slicing ($10\text{-}\mu\text{m}$)
- 2 slices / rat / parameter
- 3 zones, 12 regions



Methods

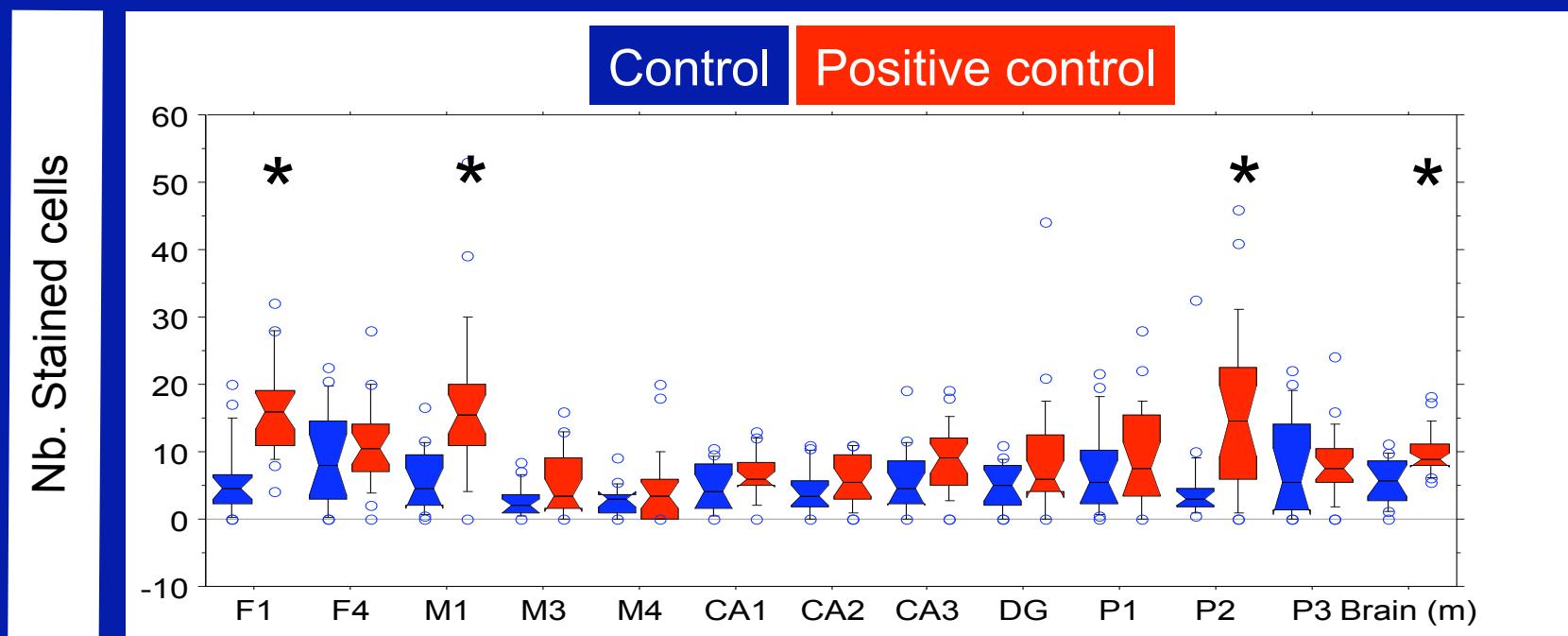
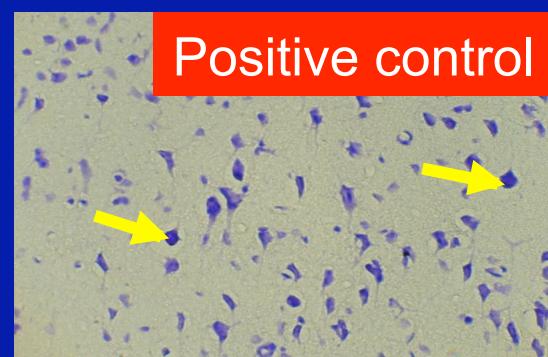
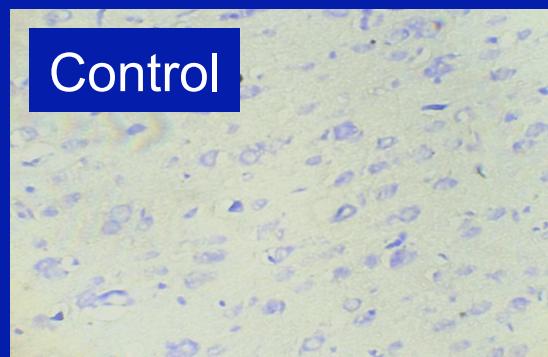
- **Dark neurons**, assumed to be degenerating neurons were identified using:
 - Cresyl-violet staining and visual analysis (counting)
 - Fluoro-Jade staining and visual analysis (scoring)
- **Albumin leakage** was identified using:
 - Albumin immuno-staining and visual analysis (counting)

Statistics

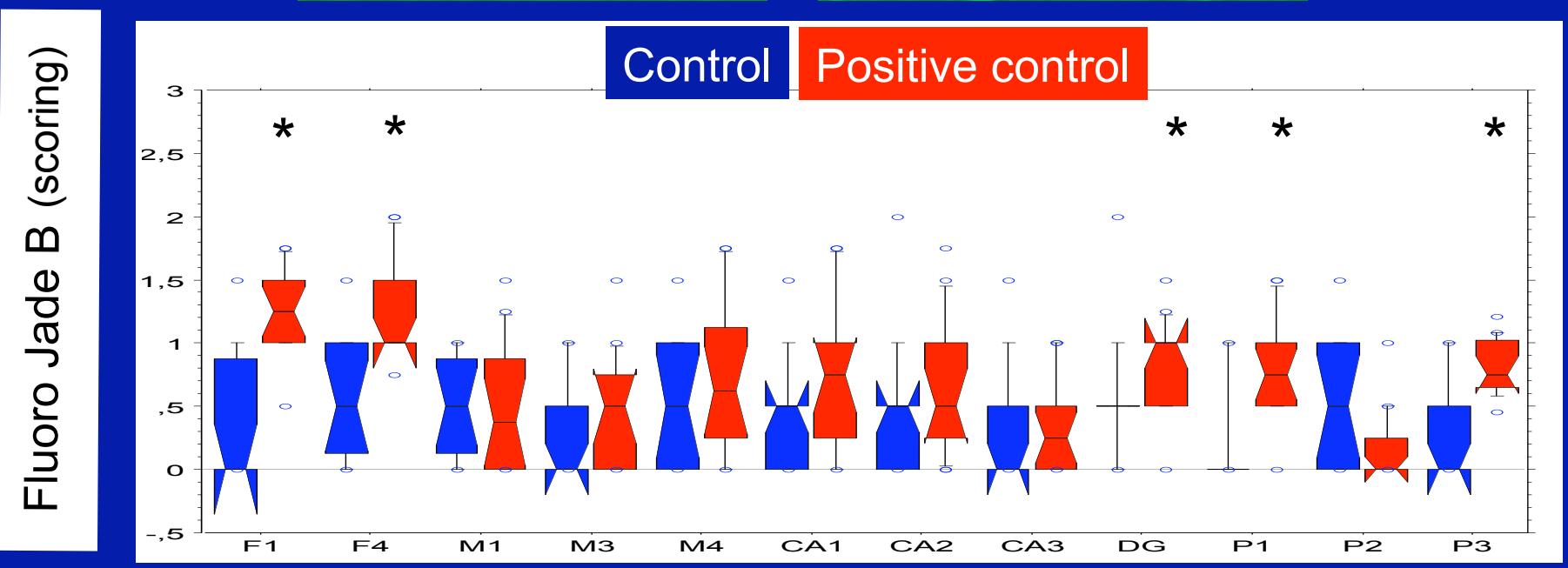
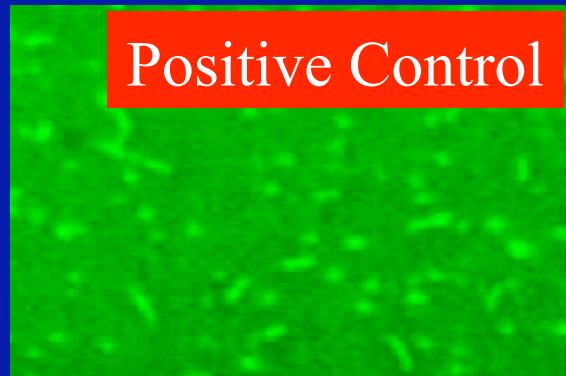
- For each exposure condition, groups of 16 rats were used, allowing for the detection of a significant variation of 35% ($p < 0.05$).
- Comparison positive vs cage- controls
- Comparison cage-control & exposed vs sham
- Statistical analysis was done using the Kruskal-Wallis test followed by adapted non-parametric post-tests.

Cold-shock

Dark neurons detection: Cresyl violet



Cold-shock Dark neurons detection: Fluoro-Jade B



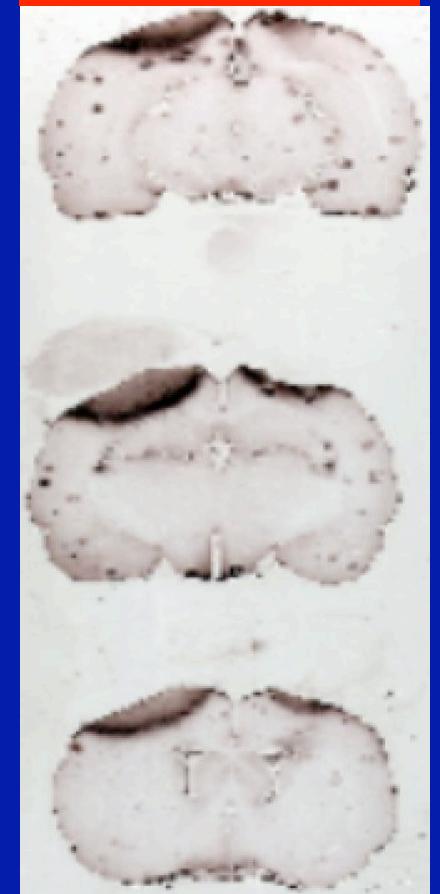
Cold-shock

BBB leakage detection: Endogenous albumin

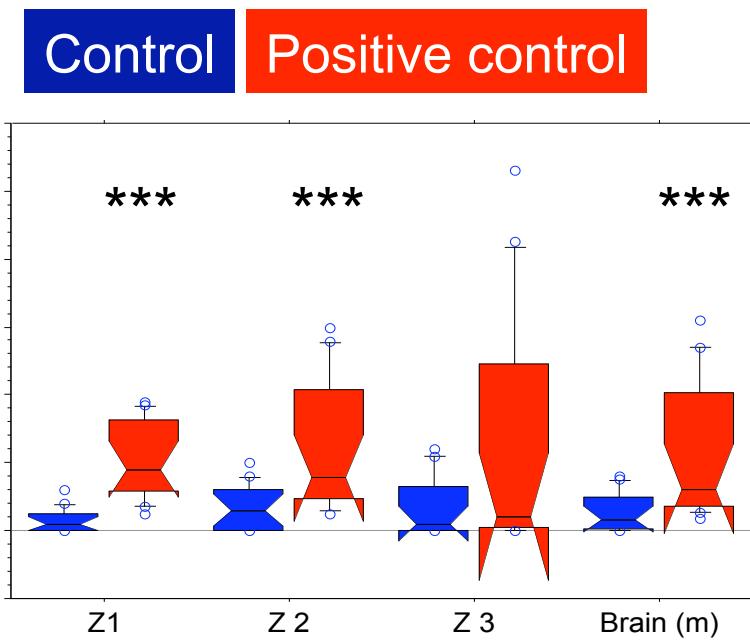
Control



Positive control



Albumin leakage (Nb. Spot)



Single exposure experiments

n = 15-16



Significant decrease ($p < 0.05$)

Significant increase ($p < 0.05$)

GSM : Dark neurons, Cresyl violet staining

Signal	SAR (W/kg)	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
GSM	0,026	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0,26	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2,6	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

GSM, Dark neurons : Fluoro Jade B staining

Signal	SAR (W/kg)	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2
GSM	0,026	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0,26	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2,6	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13	0h	ns	ns			ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns			ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns			ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns			ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns			ns	ns	ns	ns	ns	ns	ns

GSM : BBB leakage, endogenous albumin

Signal	SAR (W/kg)	Time	Z1	Z2	Z3
GSM	0,026	0h	ns	ns	ns
		1h	ns	ns	ns
		1d	ns	ns	ns
		7d	ns	ns	ns
		50d	ns	ns	ns
GSM	0,26	0h	ns	ns	ns
		1h	ns	ns	ns
		1d	ns	ns	ns
		7d	ns	ns	ns
		50d	ns	ns	ns
GSM	2,6	0h	ns	ns	ns
		1h	ns	ns	ns
		1d	ns	ns	ns
		7d	ns	ns	ns
		50d	ns	ns	ns
GSM	13	0h	ns		
		1h	ns	ns	ns
		1d	ns	ns	ns
		7d	ns	ns	ns
		50d	ns	ns	ns

UMTS : Dark neurons, Cresyl violet staining

Signal	SAR (W/kg)	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
UMTS	0,026	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0,26	0h	ns	ns	ns	ns	ns	ns	ns			ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns			ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	2,6	0h	ns	ns	ns	ns	ns	ns	ns			ns	ns	ns
		1h	ns		ns	ns	ns	ns	ns	ns			ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

UMTS, Dark neurons : Fluoro Jade B staining

Signal	SAR (W/kg)	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
UMTS	0,026	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0,26	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns			ns	ns			ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	2,6	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13	0h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		1d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		7d	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		50d	ns			ns			ns	ns			ns	ns

UMTS : BBB leakage, endogenous albumin

Signal	SAR (W/kg)	Time	Z1	Z2	Z3	
UMTS	0,026	0h	ns	ns	ns	
UMTS	0,026	1h	ns	ns	ns	
UMTS	0,026	1d	ns	ns	ns	
UMTS	0,026	7d	ns	ns	ns	
UMTS	0,026	50d	ns	ns	ns	
UMTS	0,26	0h	ns	ns	ns	
UMTS	0,26	1h	ns	ns	ns	
UMTS	0,26	1d	ns	ns	ns	
UMTS	0,26	7d	ns	ns	ns	
UMTS	0,26	50d	ns	ns	ns	
UMTS	2,6	0h	ns	ns	ns	
UMTS	2,6	1h	ns	ns	ns	
UMTS	2,6	1d	ns	ns	ns	
UMTS	2,6	7d	ns	ns	ns	
UMTS	2,6	50d	ns	ns	ns	
UMTS	13	0h				
UMTS	13	1h	ns	ns	ns	
UMTS	13	1d	ns	ns		
UMTS	13	7d	ns	ns	ns	
UMTS	13	50d	ns	ns	ns	

Single exposures

- GSM-1800 and UMTS did not induce significant brain damage
- Results on BBB in agreement with the majority of papers published so far
- Results on degenerative neurons do not confirm the Salford's data obtained at 900 MHz

Repeated experiments

n = 15-16



Significant decrease ($p < 0.05$)
Significant increase ($p < 0.05$)

GSM : Dark neurons, Cresyl violet staining

	SAR	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
Cage control	0 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.026 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.026 W/kg	50 days	ns		ns	ns	ns		ns	ns			ns	
GSM	0.26 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.26 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2.6 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2.6 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

GSM, Dark neurons : Fluoro Jade B staining

	SAR	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
Cage control	0 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.026 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.026 W/kg	50 days	ns			ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.26 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	0.26 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	13 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2.6 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
GSM	2.6 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

GSM : BBB leakage, endogenous albumin

	SAR	Time	Z1	Z2	Z3
Cage control	0 W/kg	0 hour	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns
GSM	0.026 W/kg	0 hour	ns	ns	ns
GSM	0.026 W/kg	50 days		ns	ns
GSM	0.26 W/kg	0 hour	ns	ns	ns
GSM	0.26 W/kg	50 days	ns	ns	ns
GSM	2.6 W/kg	0 hour	ns	ns	ns
GSM	2.6 W/kg	50 days	ns	ns	ns
GSM	13 W/kg	0 hour	ns	ns	ns
GSM	13 W/kg	50 days			

UMTS : Dark neurons, Cresyl violet staining

	SAR	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
Cage control	0 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0.026 W/kg	0 hour	ns	green	ns	ns	green	ns	ns	ns	ns	ns	ns	green
UMTS	0.026 W/kg	50 days	ns	yellow	ns	ns	ns	yellow	ns	yellow	yellow	ns	ns	yellow
UMTS	0.26 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0.26 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	2.6 W/kg	0 hour	ns	ns	ns	ns	green	ns	ns	ns	ns	ns	ns	ns
UMTS	2.6 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13 W/kg	50 days	ns	yellow	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

UMTS, Dark neurons : Fluoro Jade B staining

	SAR	Time	F1	F4	M1	M3	M4	CA1	CA2	CA3	DG	P1	P2	P3
Cage control	0 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0.026 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0.026 W/kg	50 days	ns	ns								ns	ns	ns
UMTS	0.26 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	0.26 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	13 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	2.6 W/kg	0 hour	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UMTS	2.6 W/kg	50 days	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

UMTS : BBB leakage, endogenous albumin

	SAR	Time	Z1	Z2	Z3
Cage control	0 W/kg	0 hour	ns	ns	ns
Cage control	0 W/kg	50 days	ns	ns	ns
UMTS	0.026 W/kg	0 hour	ns	ns	ns
UMTS	0.026 W/kg	50 days	ns	ns	ns
UMTS	0.26 W/kg	0 hour			ns
UMTS	0.26 W/kg	50 days	ns	ns	ns
UMTS	2.6 W/kg	0 hour	ns	ns	ns
UMTS	2.6 W/kg	50 days	ns	ns	ns
UMTS	13 W/kg	0 hour	ns	ns	ns
UMTS	13 W/kg	50 days	ns	ns	ns

Repeated exposures

- Immediately after a 4-week repeated exposure
 - Globally GSM-1800 and UMTS did not induce significant brain damage
 - Results in agreement with the majority of papers published so far

Repeated exposures

- 50 days after a 4-week repeated exposure
 - UMTS did not induce significant brain damages
 - GSM-1800 increased BBB permeation at 13 W/kg

General conclusions

- The more convincing effect was found on BBB permeation 50 days after a 4-week repeated exposure to GSM-1800 at 13 W/kg
- This may be linked to the microwave heating effect
- Results to be independently confirmed

Dissemination

POULLETTIER DE GANNES F. Influence of mobile telecommunication fields on the permeability of the blood-brain barrier in laboratory rodents (in vivo), Workshop FGF Blood-brain barrier and RF, May 2006, Düsseldorf, Germany.

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Acknowledgements

- **I wish to thank**
 - The BioEM group, IMS laboratory
 - Dr Ruffié, Dr Lévêque, Prof Le Pape for their expertise
- **This work was supported by :**
 - the German Federal Ministry for Environment, Nature Protection and Reactor Safety,
 - the Aquitaine Council for Research,
 - and the CNRS, EPHE, ENSCP.