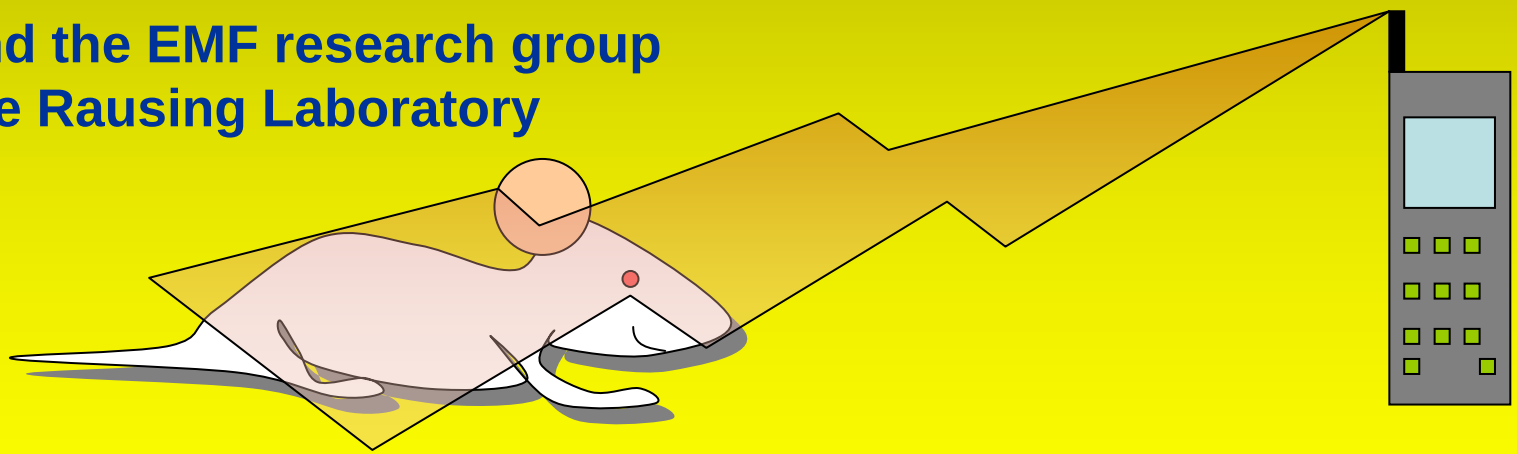
Effects of mobile phone radiation upon the blood-brain barrier, neurons, gene expression and cognitive function of the mammalian brain.

Professor Leif G. Salford
Dept. of Neurosurgery, Lund University, Sweden

and the EMF research group
the Rausing Laboratory



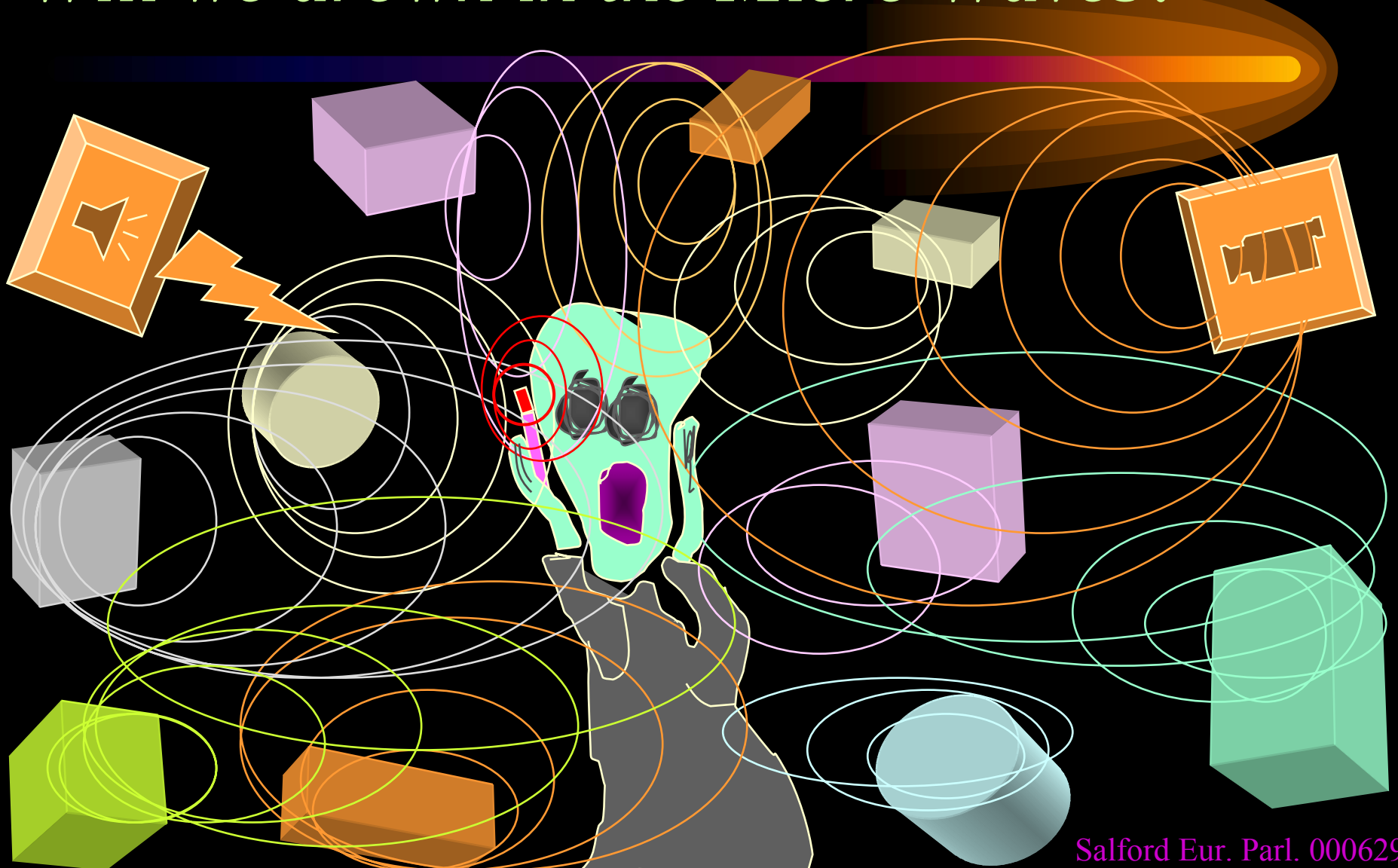
International NIR and Health Workshop
20090518-19 Porto Alegre - Rio Grande do Sul - Brasil

”25% of the world´s population soon volunteer as guinea-pigs in theWorld´s largest biological experiment”

Salford LG
European
Parliament
2000

Wireless Communication everywhere!

Will we drown in the Micro Waves?



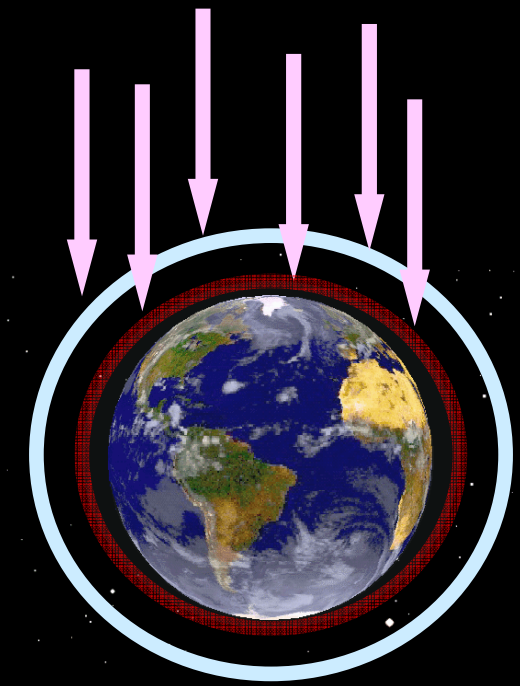
Salford Eur. Parl. 000629

Today half the world´s population volunteers as guinea-pigs in theWorld´s largest biological experiment

Porto Alegre 2009

A thin habitat

Only microwaves from Big Bang for 5 biljon years until 1940



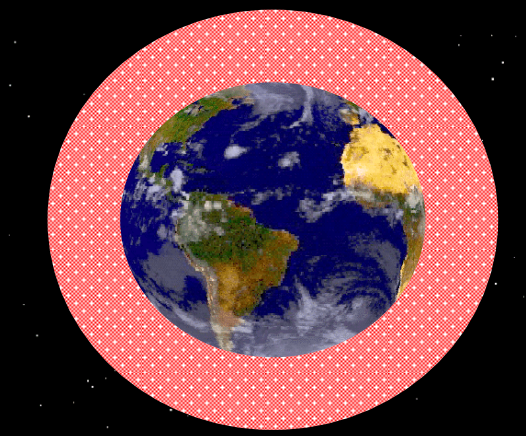
Sir Robert Watson-Watt created the first workable radar system 1930ies

Microwaves Today

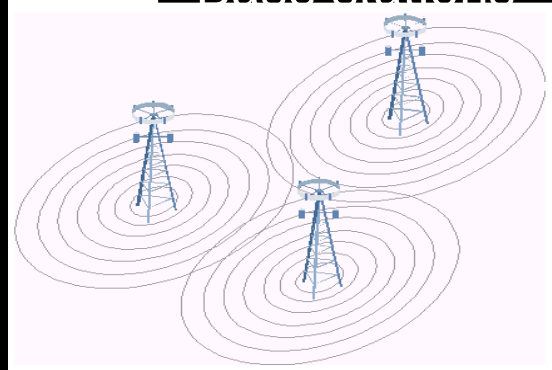
10^{11}-10^{18} times more

Dr. Percy Spencer Microwave oven 1946

The original mobile phone from SRA, Ericsson, 1956



Base stations



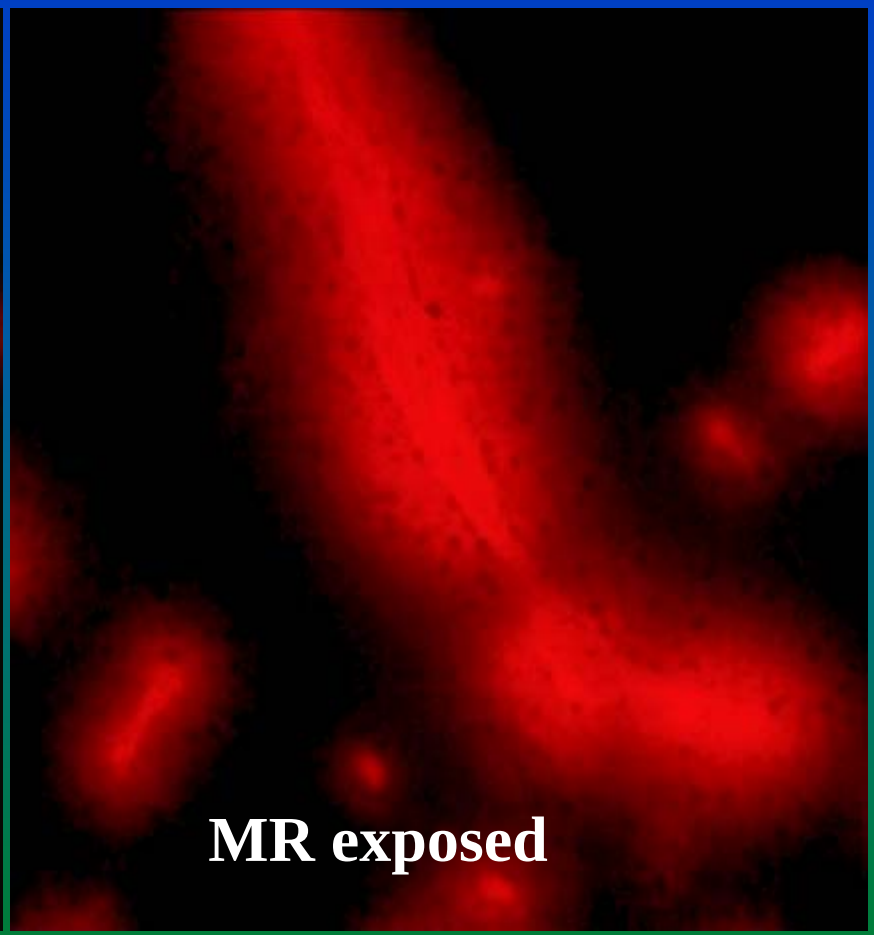
Mobile Phones 1980 -

EFFECTS UPON
the
BLOOD-BRAIN BARRIER

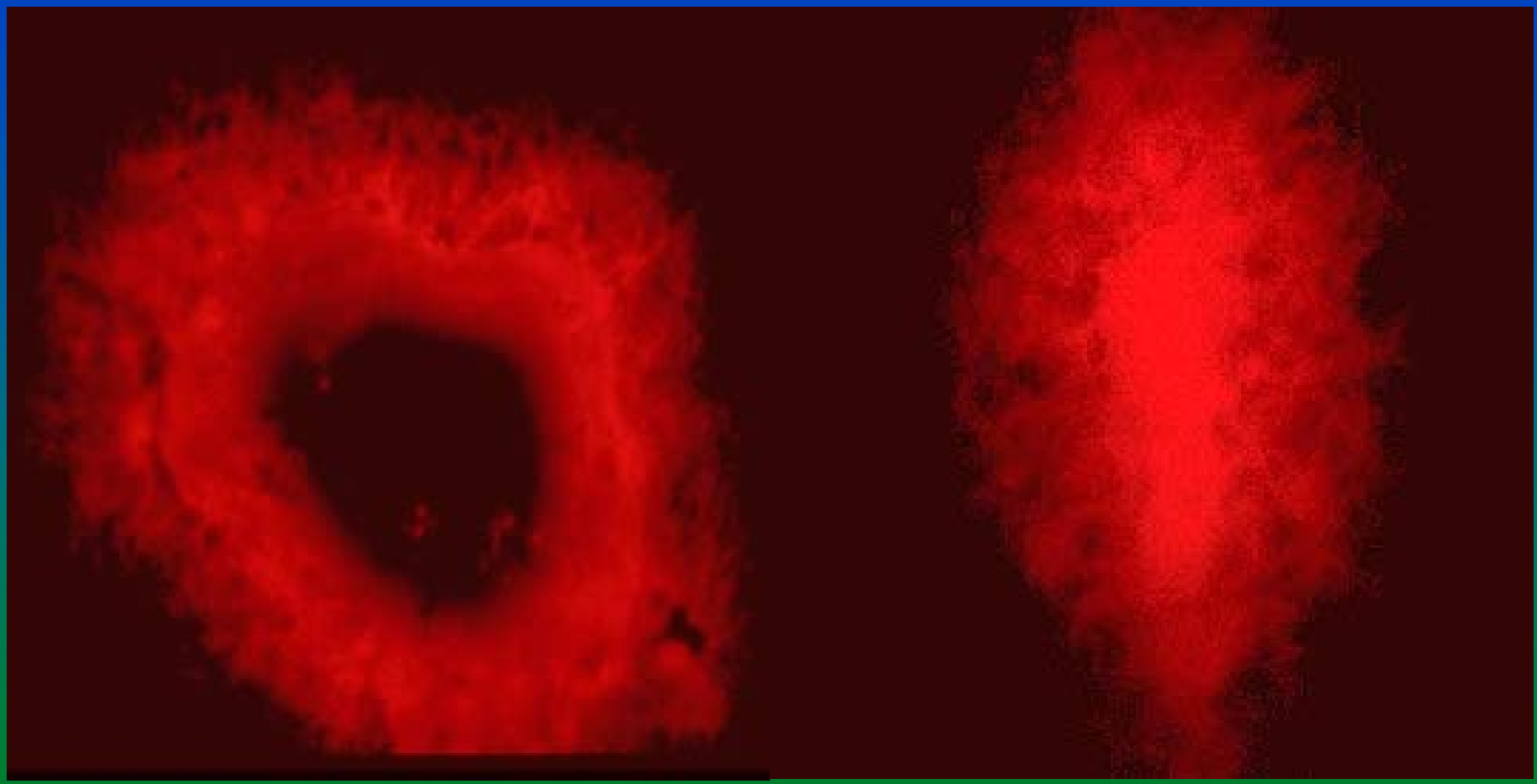
History of our BBB studies

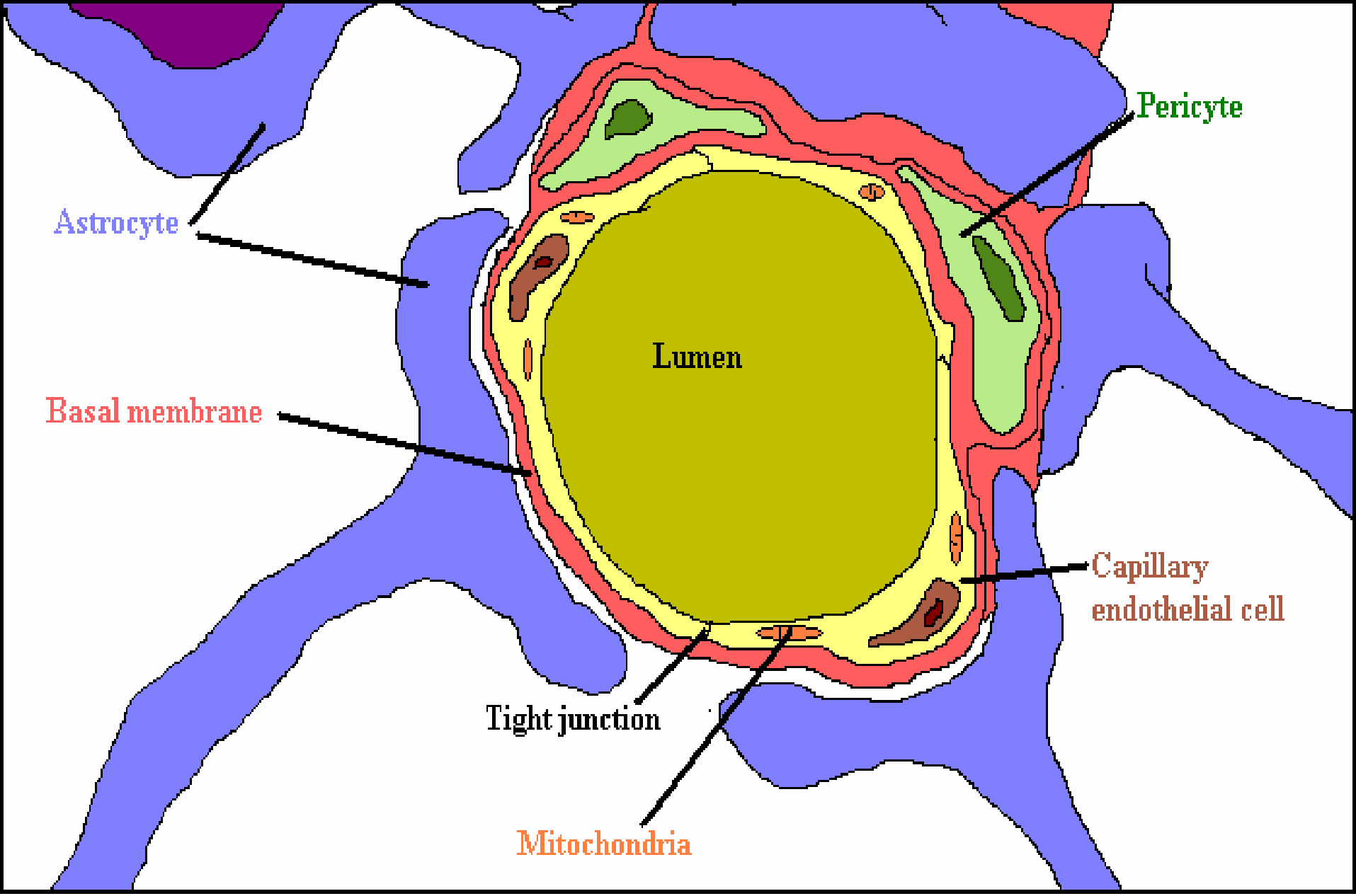
- **Shivers R et al., 1987 Visited 1988 in London Ontario**
- **1988 - blood-brain barrier (BBB) albumin leakage using Evans Blue after exposure for NMR imaging magnetic and RF fields.**
- **1989 – BBB leakage studies with immunostaining for albumin and fibrinogene using pulse modulated 915 MHz microwaves.**
- **1998 – BBB leakage of albumin using real GSM-900 and GSM-1800 exposure**

Effect of MR examination on the BBB leakage of Evans Blue in rat brain

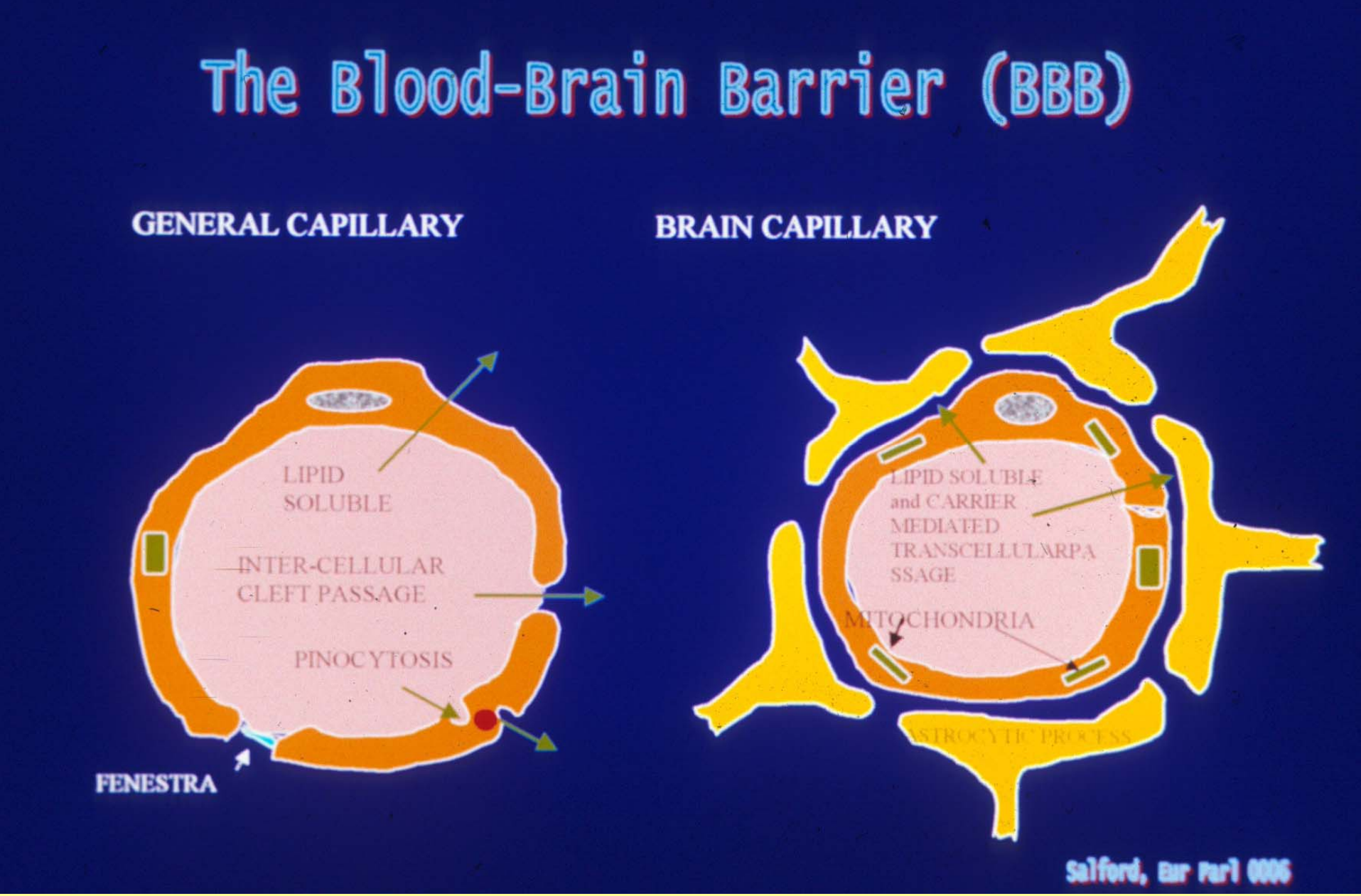


Evans Blue leakage through the BBB of rat brain After exposure to MR examination




Pericyte
Astrocyte
Lumen
Basal membrane
Capillary
endothelial cell
Tight junction
Mitochondria

The BBB



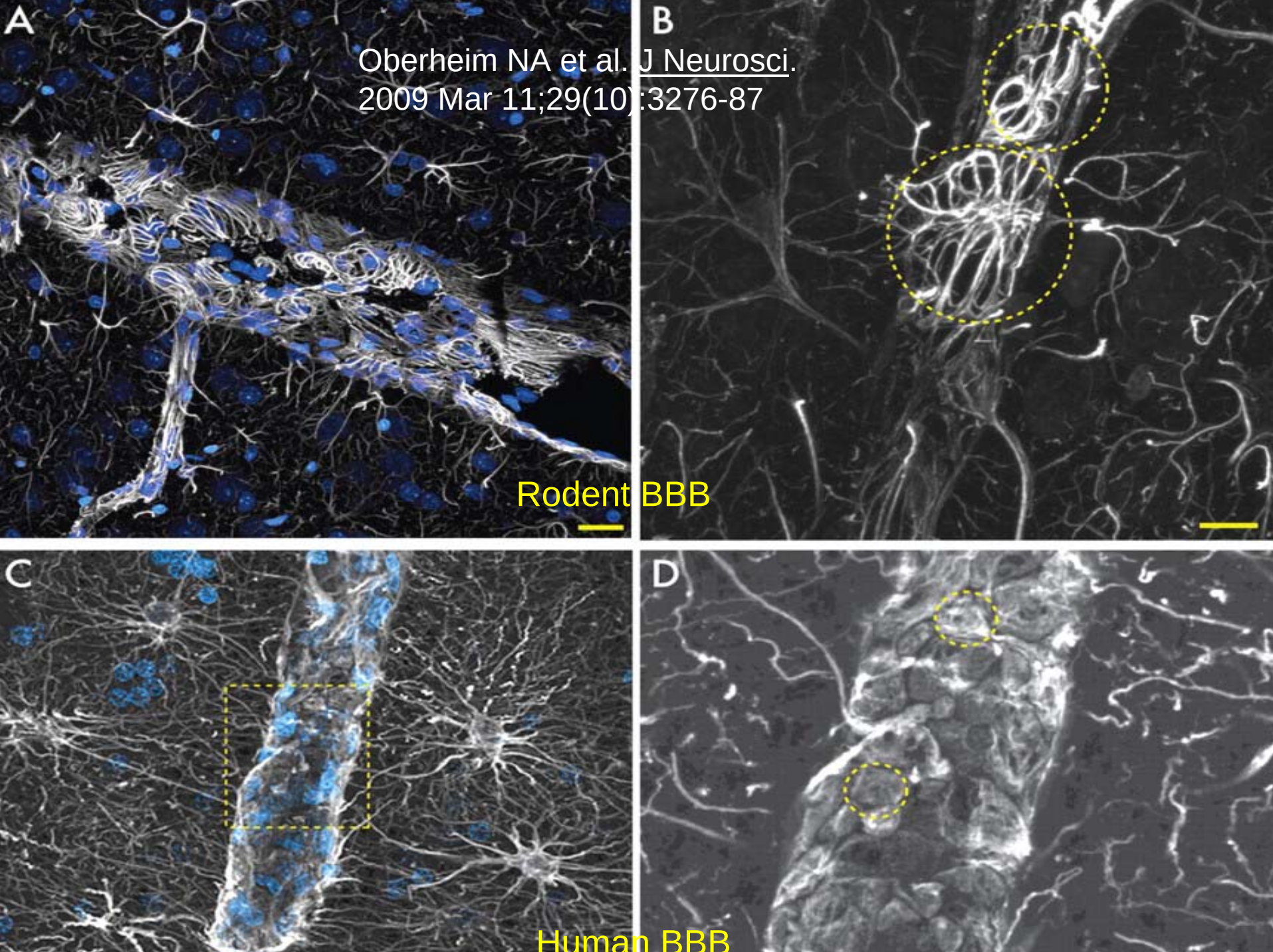
All mammals have a Blood-Brain Barrier. There are good reasons to believe that the BBB of a rat functions as the human BBB – But there might be differences which make results from animal experiments not directly translatable to the human situation!

Rodent BBB

=

Human BBB?

much in common but some difference!


A
B
C
D
Oberheim NA et al. J Neurosci. 2009 Mar 11;29(10):3276-87
Rodent BBB
Human BBB

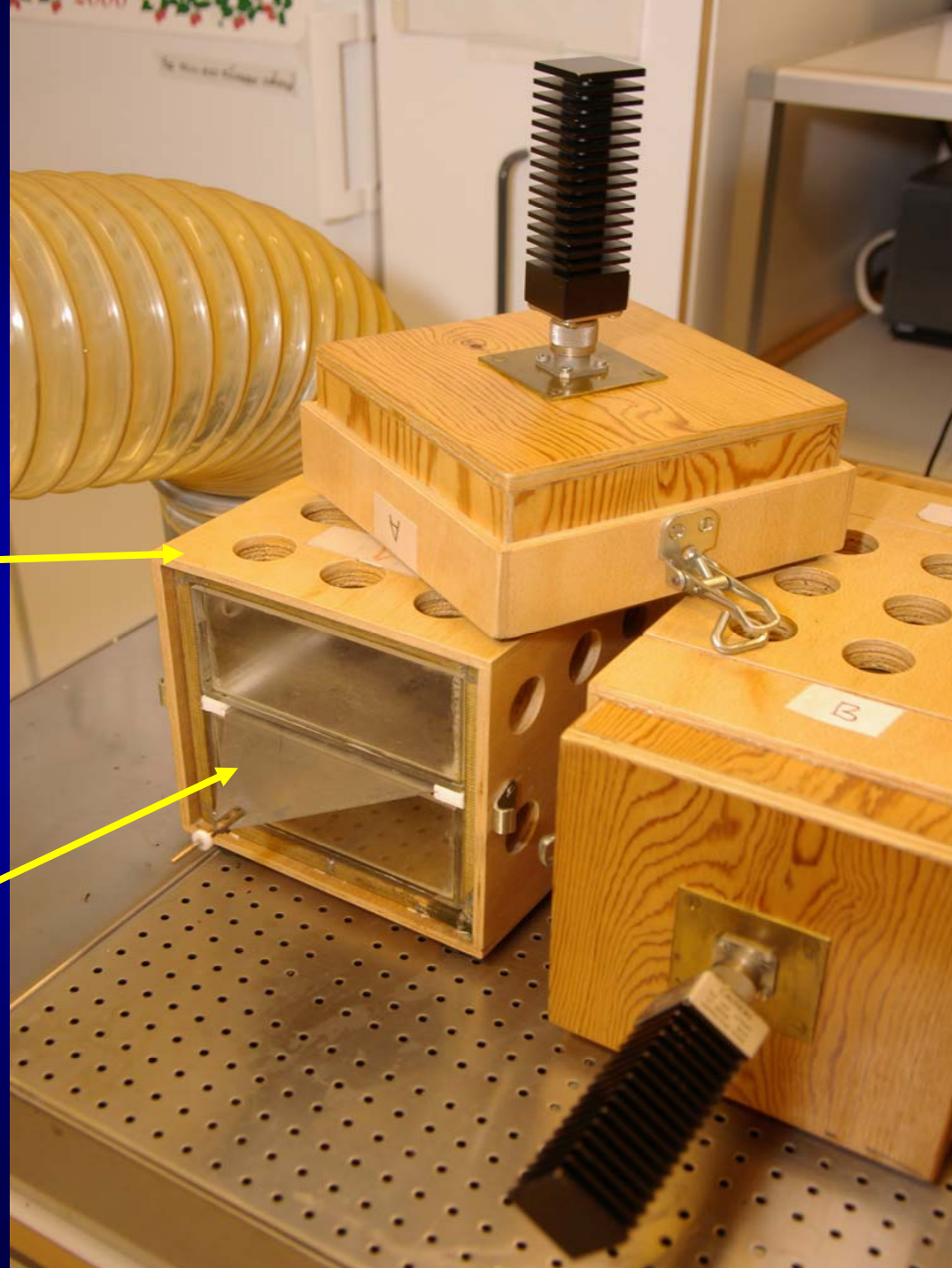
TEM-cell

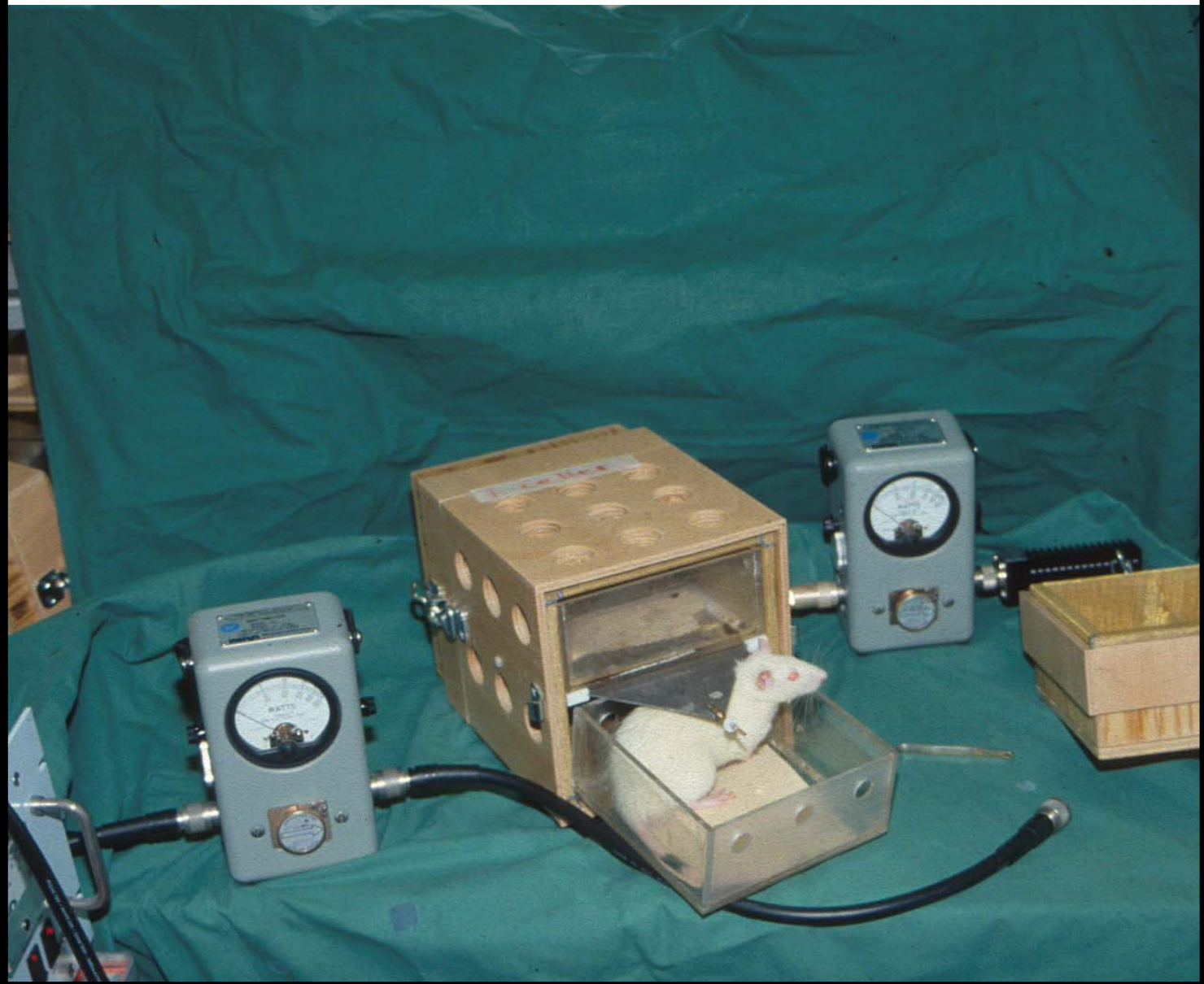
= Transverse electromagnetic transmission cell

Enclosed in a wooden box that supports the outer conductor (made of brass net)

The central plate, septum (made of aluminium)

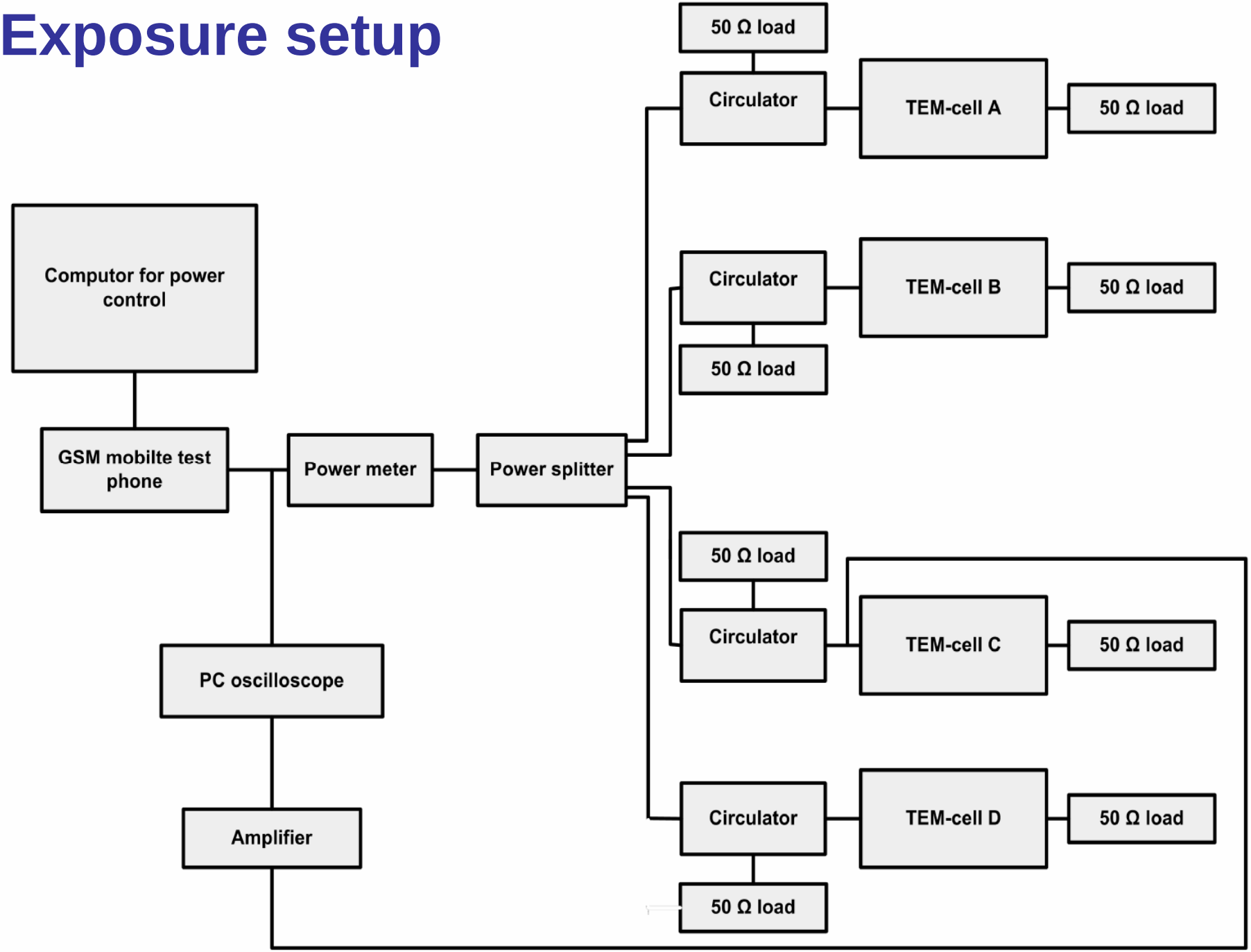
No stress-inducing restraint





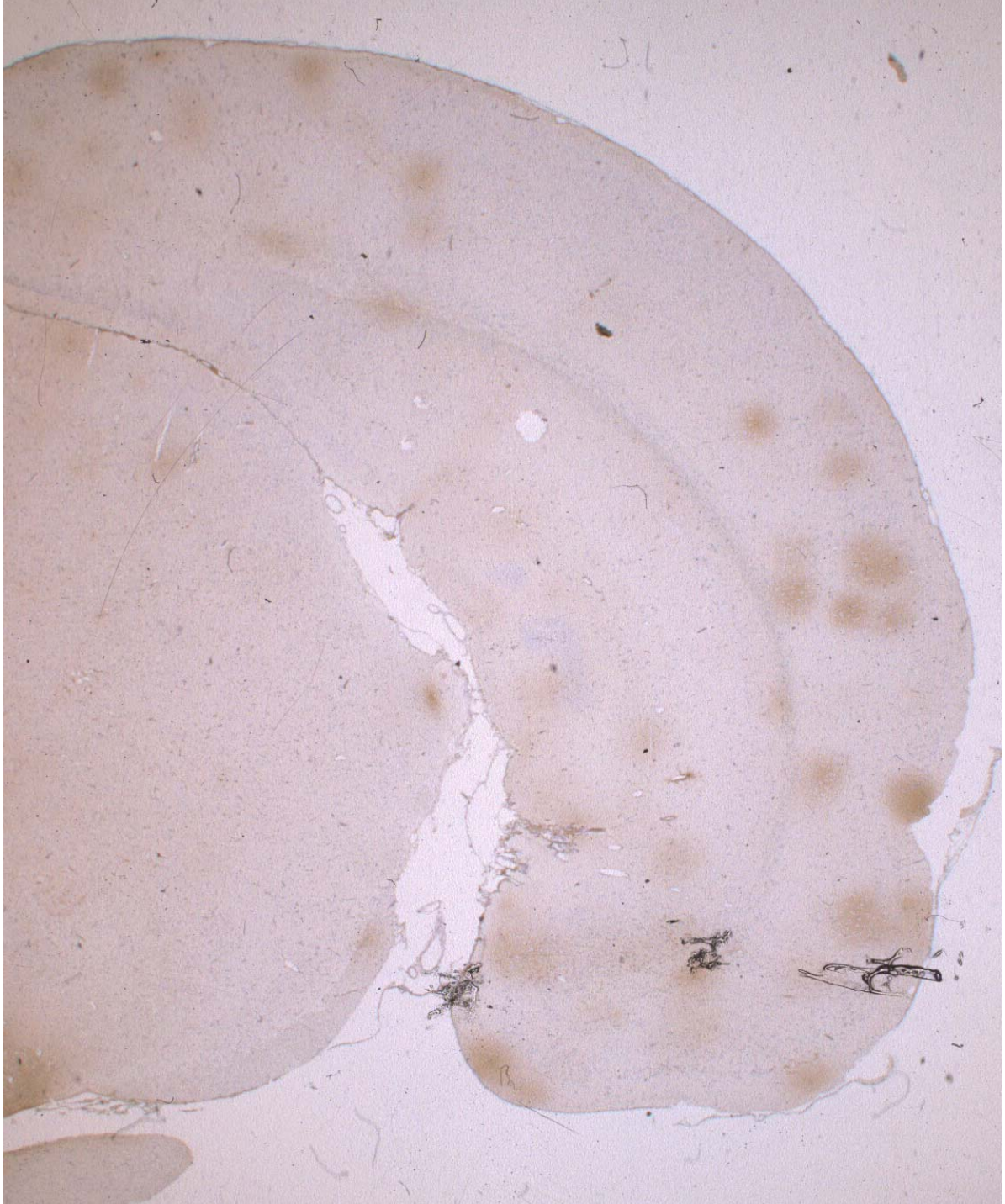
The TEM cell

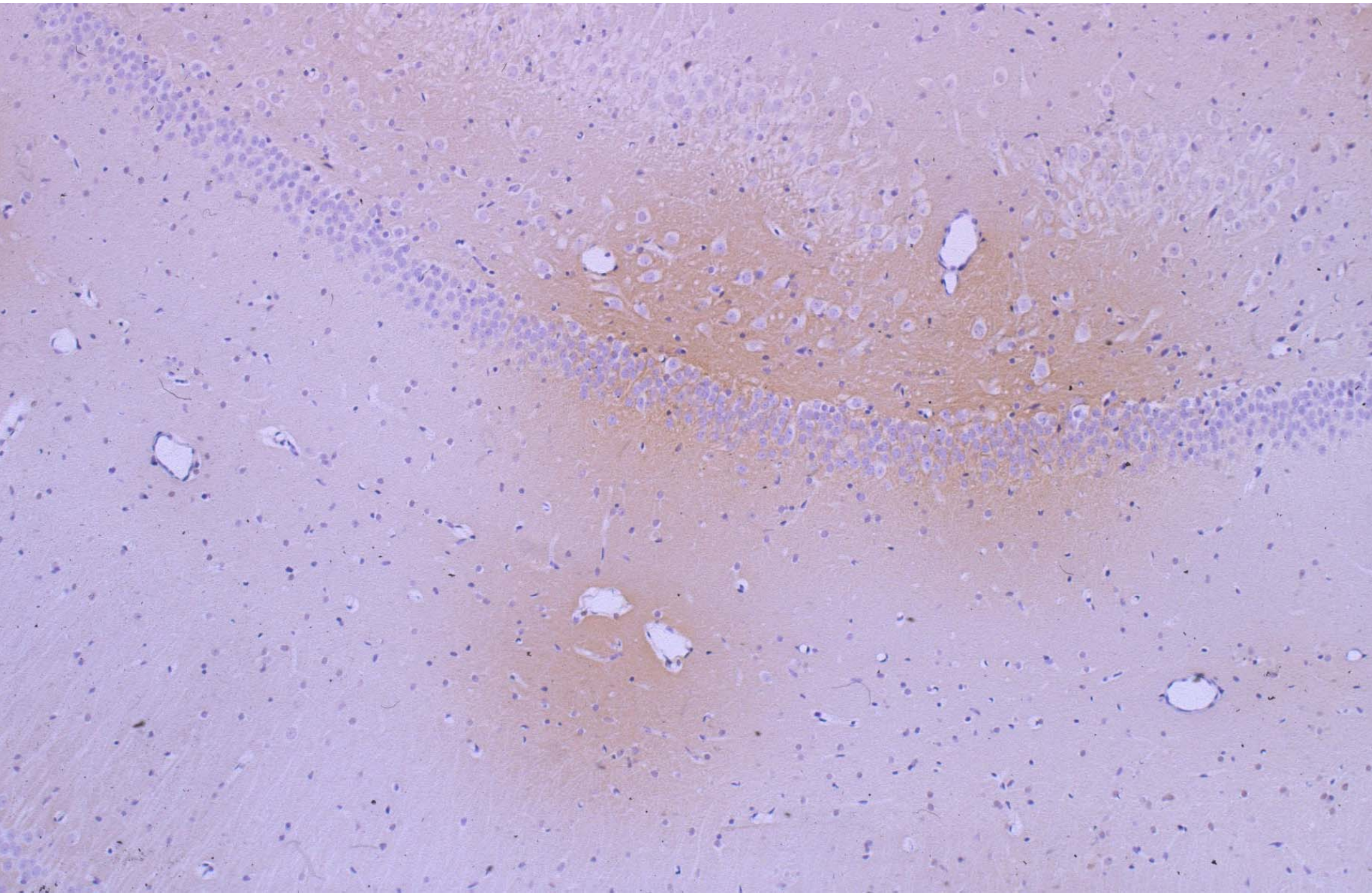
Exposure setup



Earlier experiments in The Rausing lab:

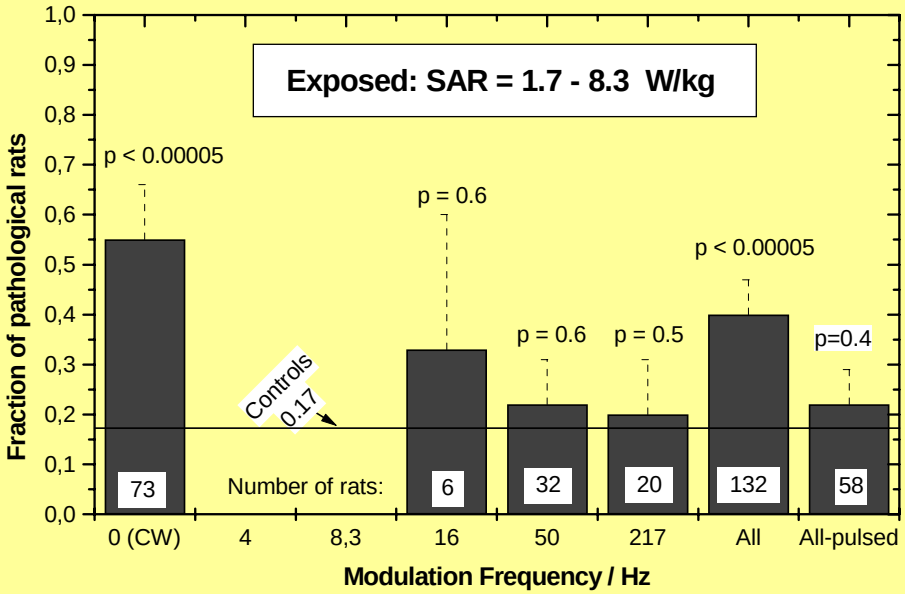
Albumin leakage through the BBB:
Fischer rats (>1600) exposed to EMF for 2 min - 16 hours (the absolute majority for 2 hours).
Examined within 30 minutes to 16 hours after exposure.

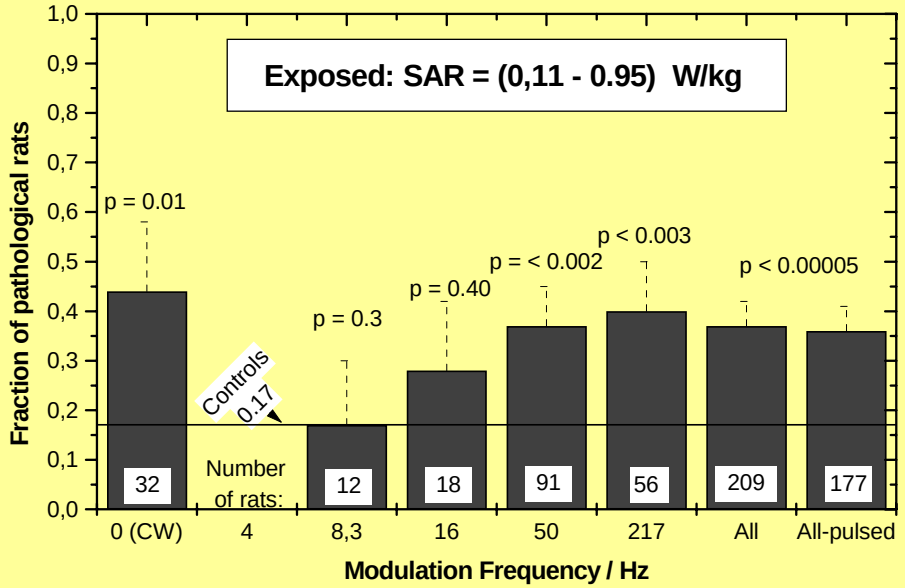


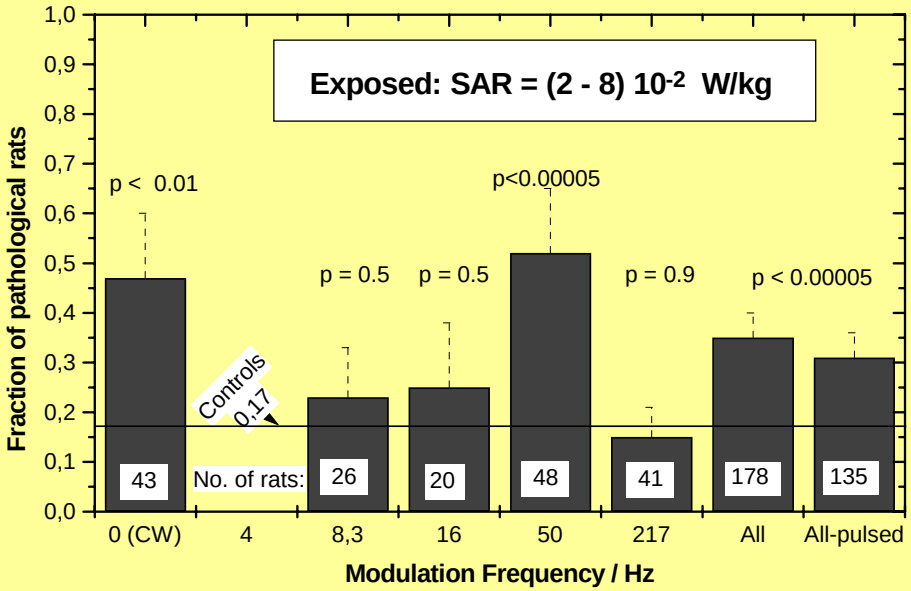


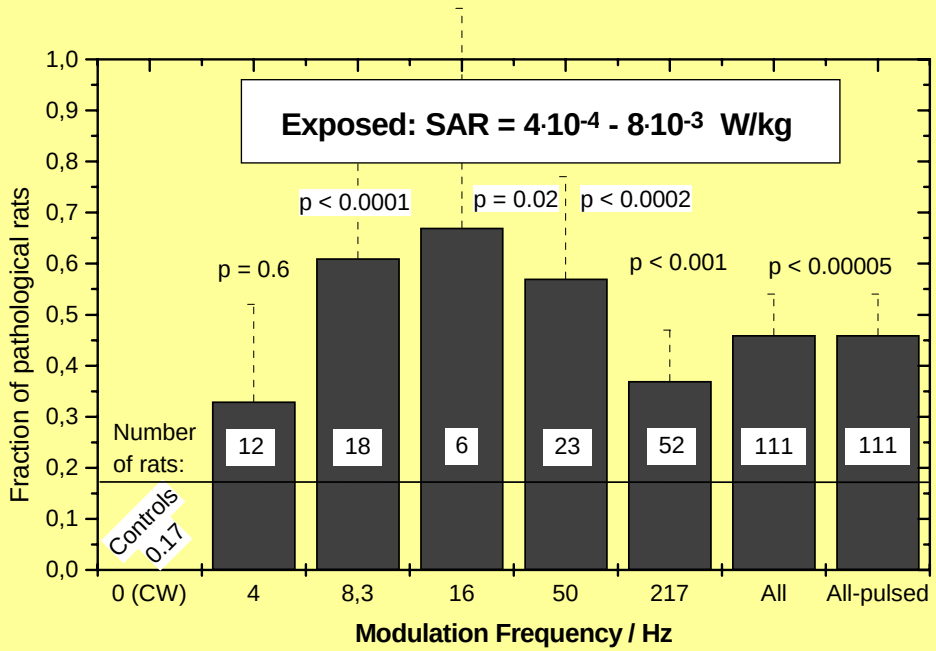
”Biological window”

1/1000 and 1/10000
of the energy at the antenna
of the mobile phone opens
the BBB more efficiently
than the energy at the antenna


Exposed: SAR = 1.7 - 8.3 W/kg
Fraction of pathological rats
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
p < 0.00005
p = 0.6
p = 0.6
p = 0.5
p < 0.00005
p=0.4
Controls
0.17
Number of rats:
73
6
32
20
132
58
0 (CW)
4
8,3
16
50
217
All
All-pulsed
Modulation Frequency / Hz

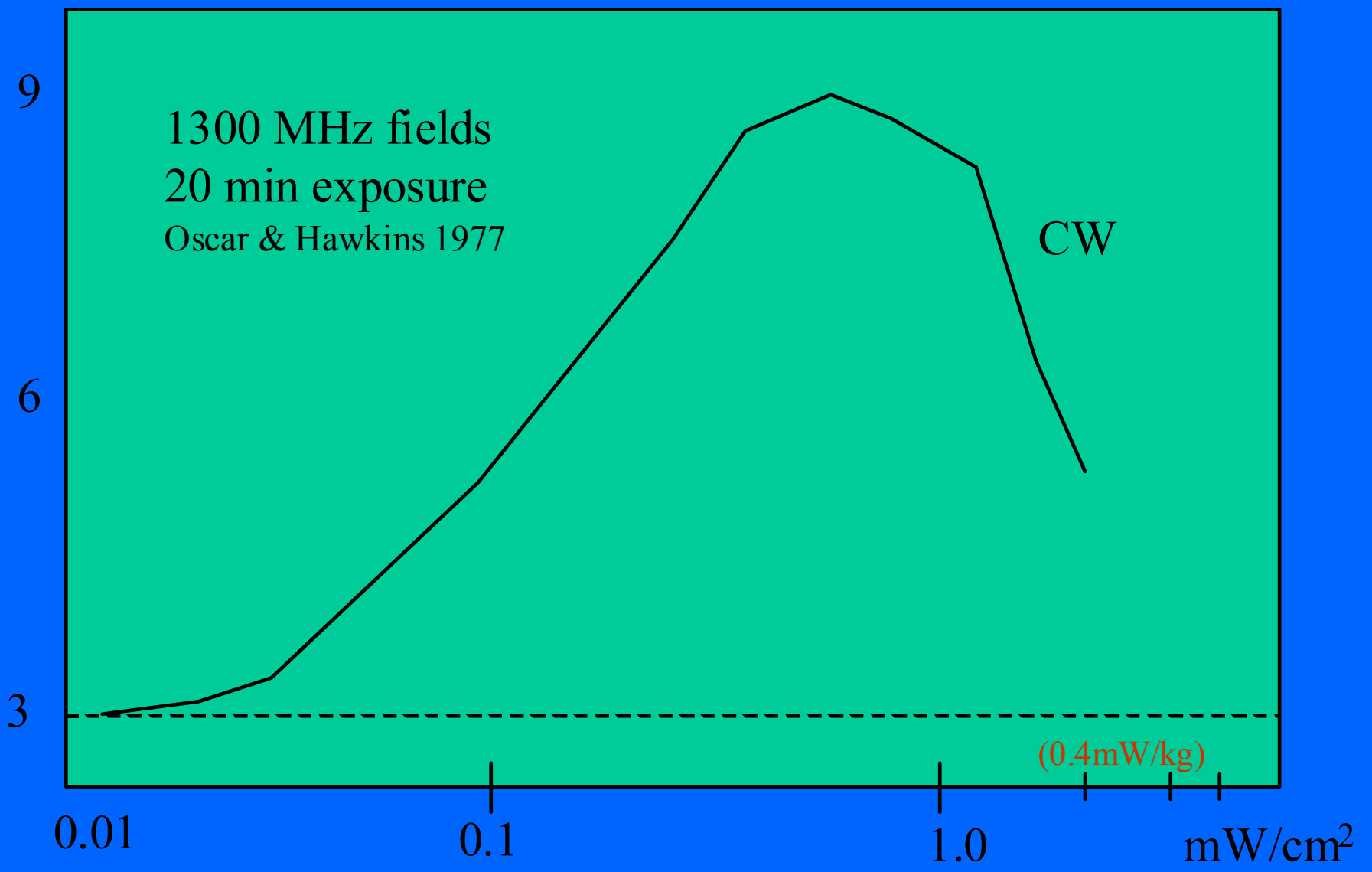

Exposed: SAR = (0,11 - 0.95) W/kg
Fraction of pathological rats
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
p = 0.01
p = 0.3
p = 0.40
p = < 0.002
p < 0.003
p < 0.00005
Controls
0.17
Number
of rats:
32
12
18
91
56
209
177
0 (CW)
4
8,3
16
50
217
All
All-pulsed
Modulation Frequency / Hz


Exposed: SAR = (2 - 8) 10-2 W/kg
Fraction of pathological rats
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
p < 0.01
p = 0.5
p = 0.5
p<0.00005
p = 0.9
p < 0.00005
Controls
0.17
43
No. of rats:
26
20
48
41
178
135
0 (CW)
4
8,3
16
50
217
All
All-pulsed
Modulation Frequency / Hz

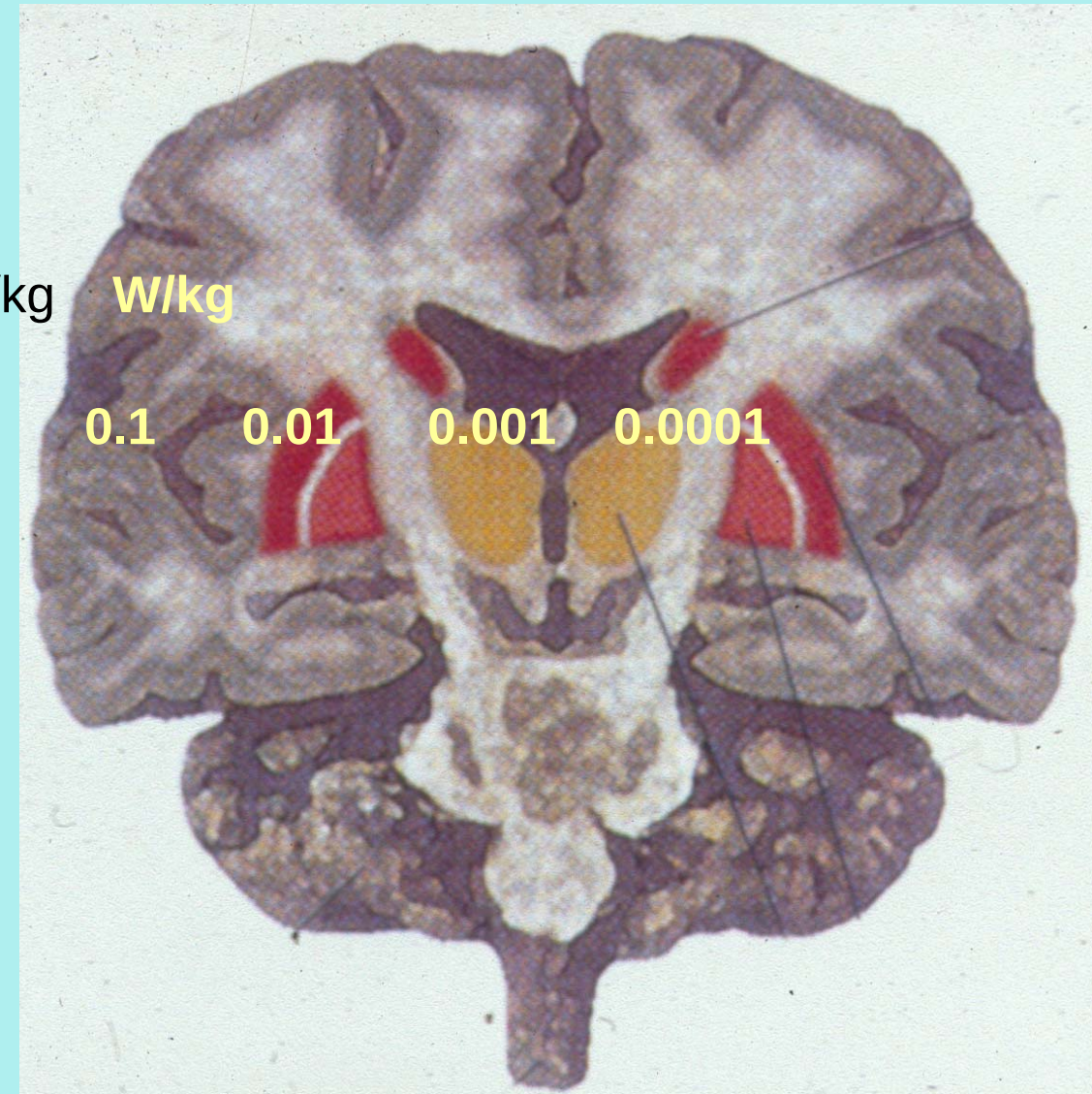

Exposed: SAR = 4·10-4 - 8·10-3 W/kg
Fraction of pathological rats
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
p = 0.6
p < 0.0001
p = 0.02
p < 0.0002
p < 0.001
p < 0.00005
Number
of rats:
Controls
0.17
12
18
6
23
52
111
111
0 (CW)
4
8,3
16
50
217
All
All-pulsed
Modulation Frequency / Hz

"WINDOWED" RELATION BETWEEN INTENSITY OF IRRADIATION AND BBB PERMEABILITY?

BUI

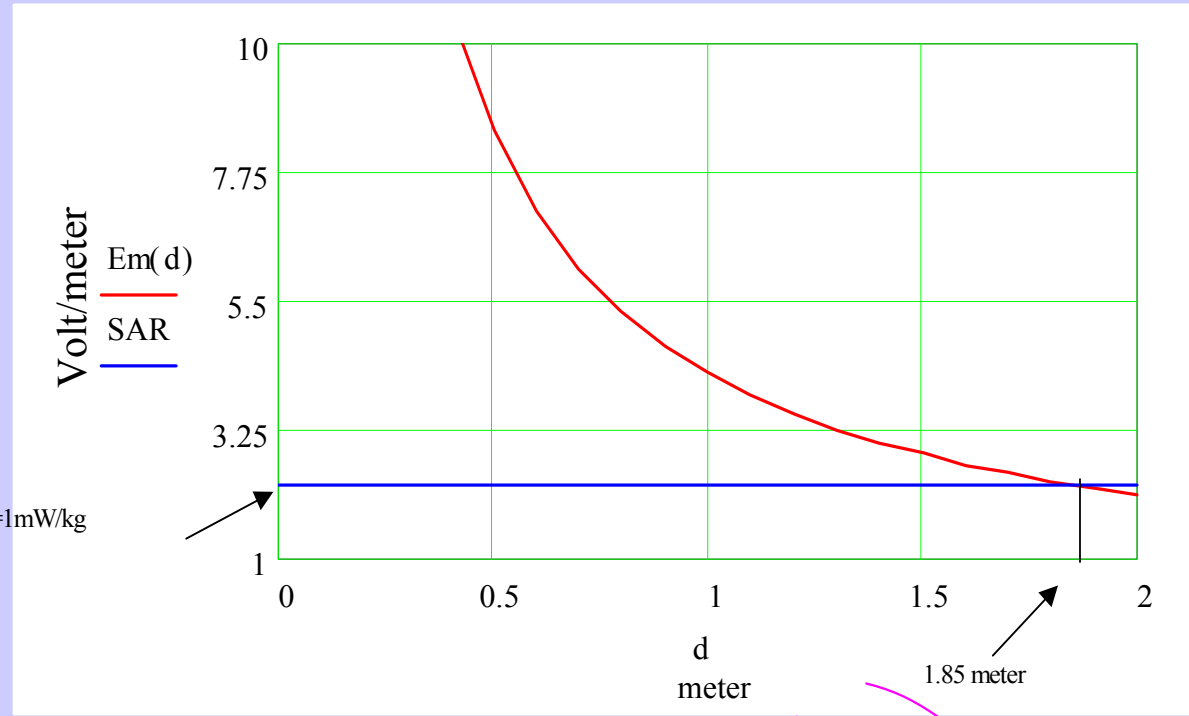


Antenna 1,4 cm from human head, 915 MHz, SAR values derived from Anderson and Joyer 1995 and Dimylow 1994

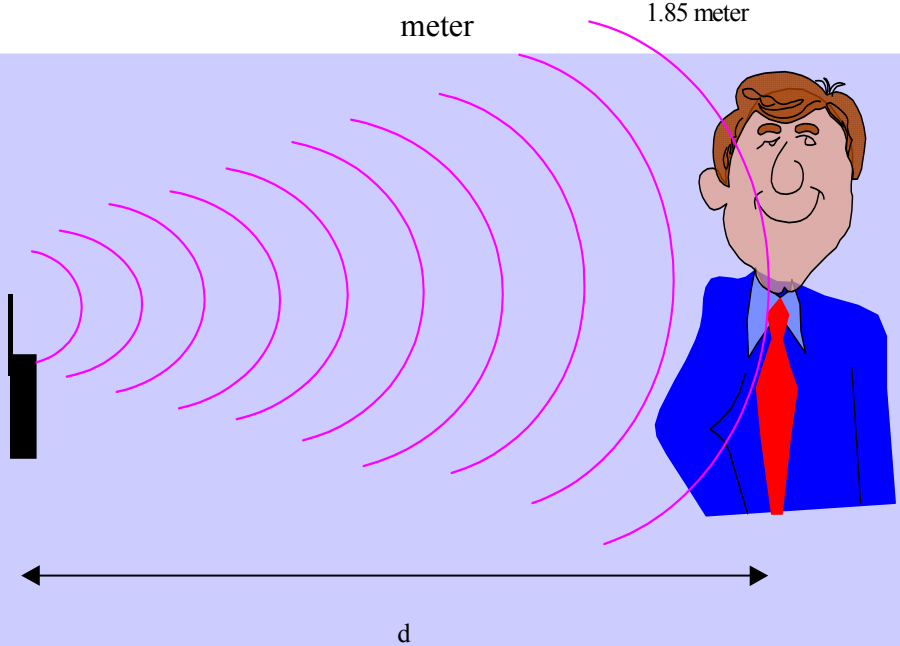


Salford and Persson

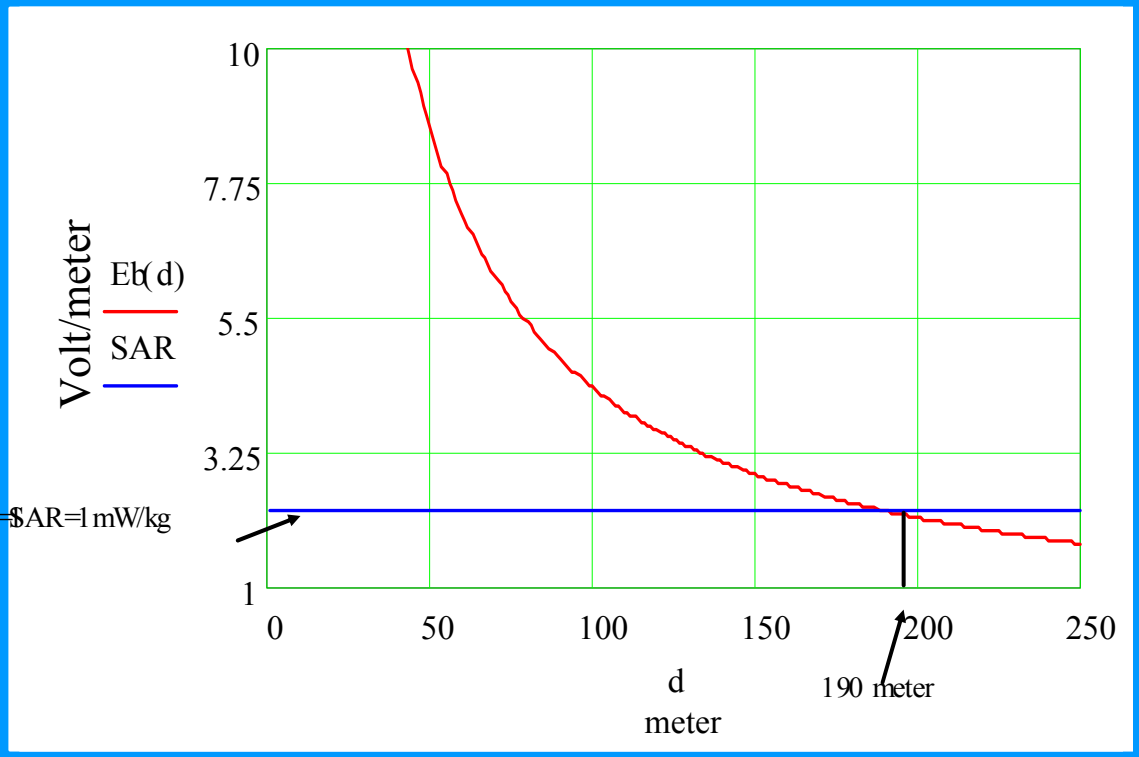
”Passive” mobile exposure ?

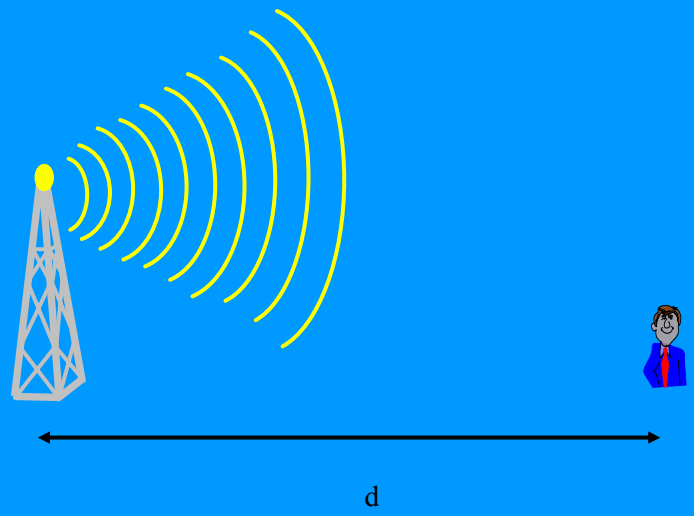


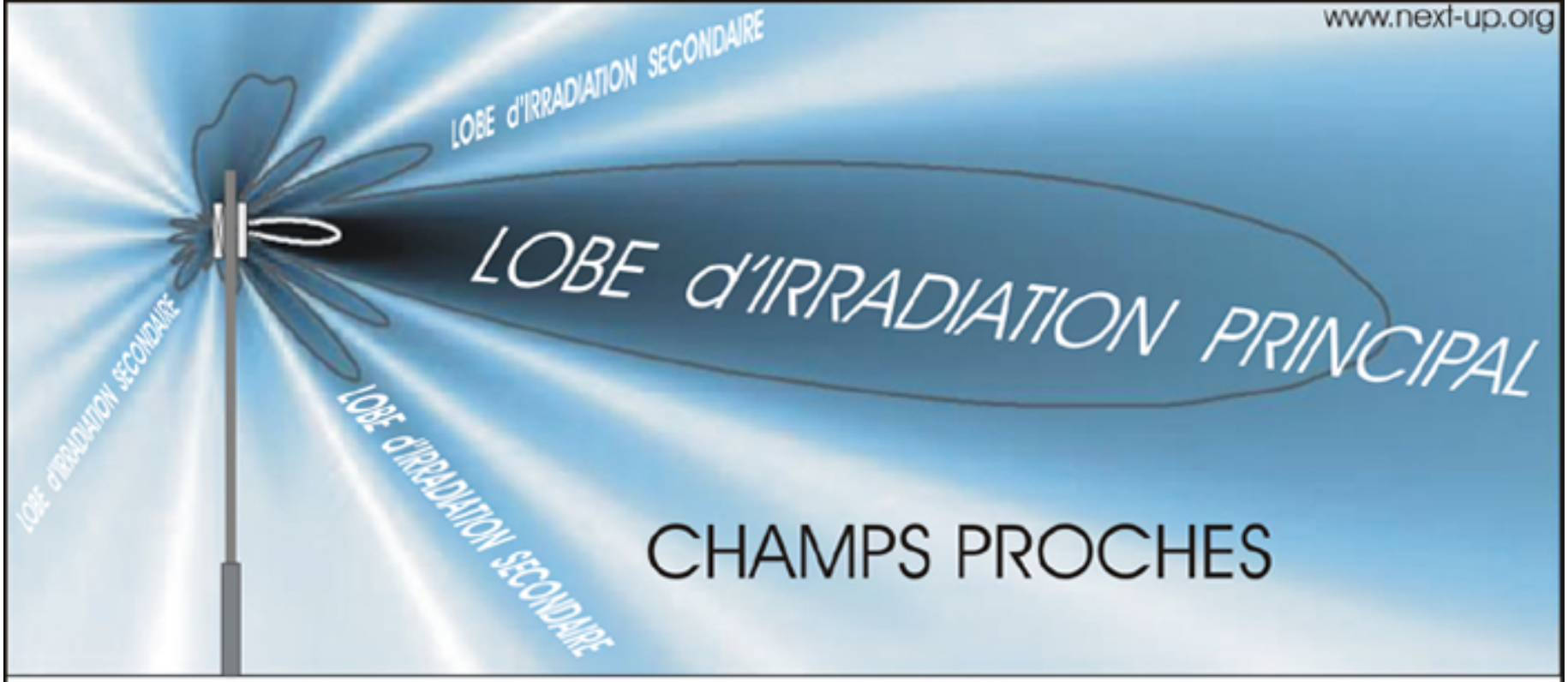
SAR = 1mW/kg
1.85 metres away
from the mobile
phone



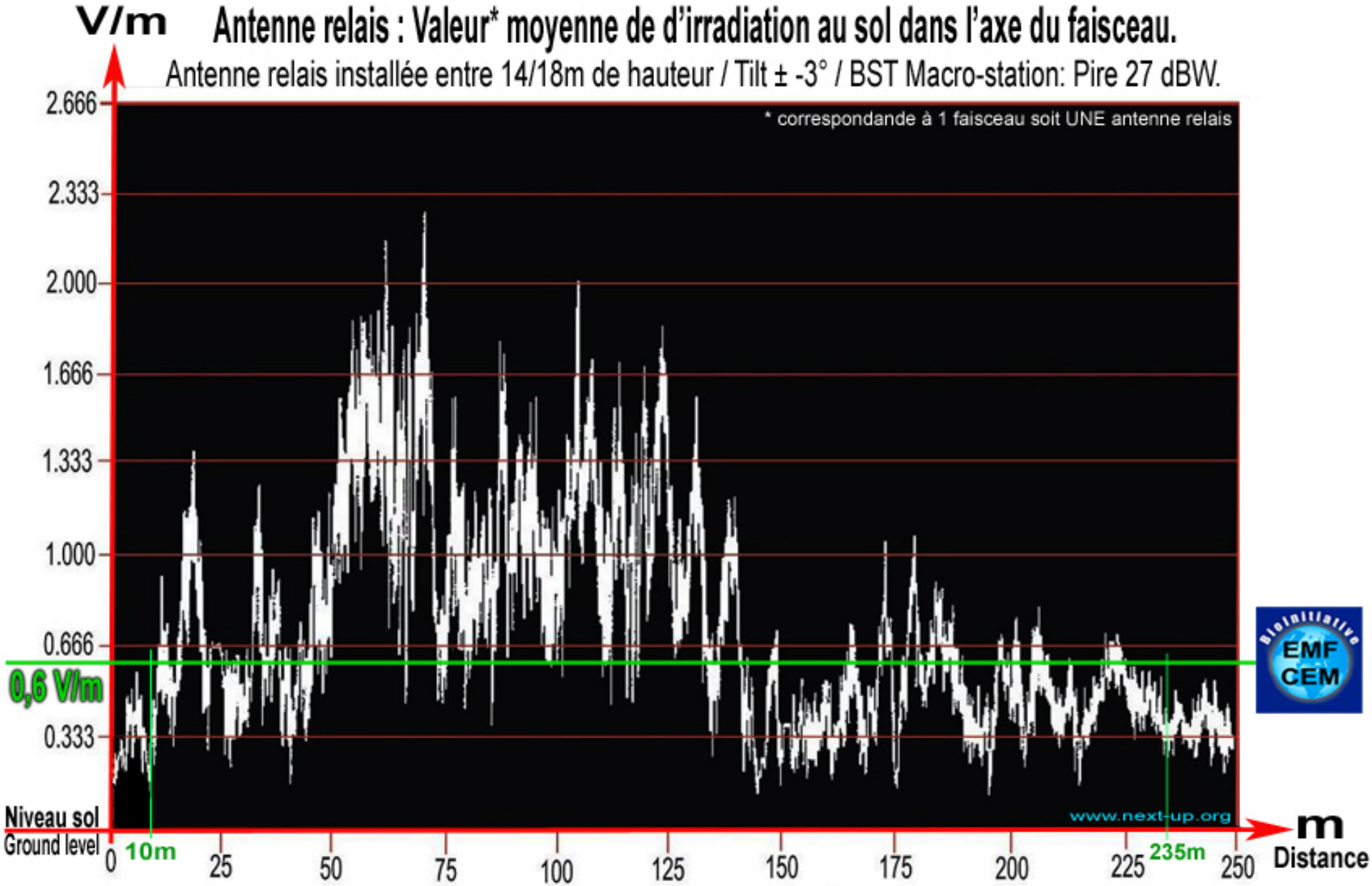
Effect from base stations ?


10
7.75
5.5
3.25
1
Volt/meter
Eb(d)
SAR
SR=SAR=1 mW/kg
0
50
100
150
200
250
d
meter
190 meter


d

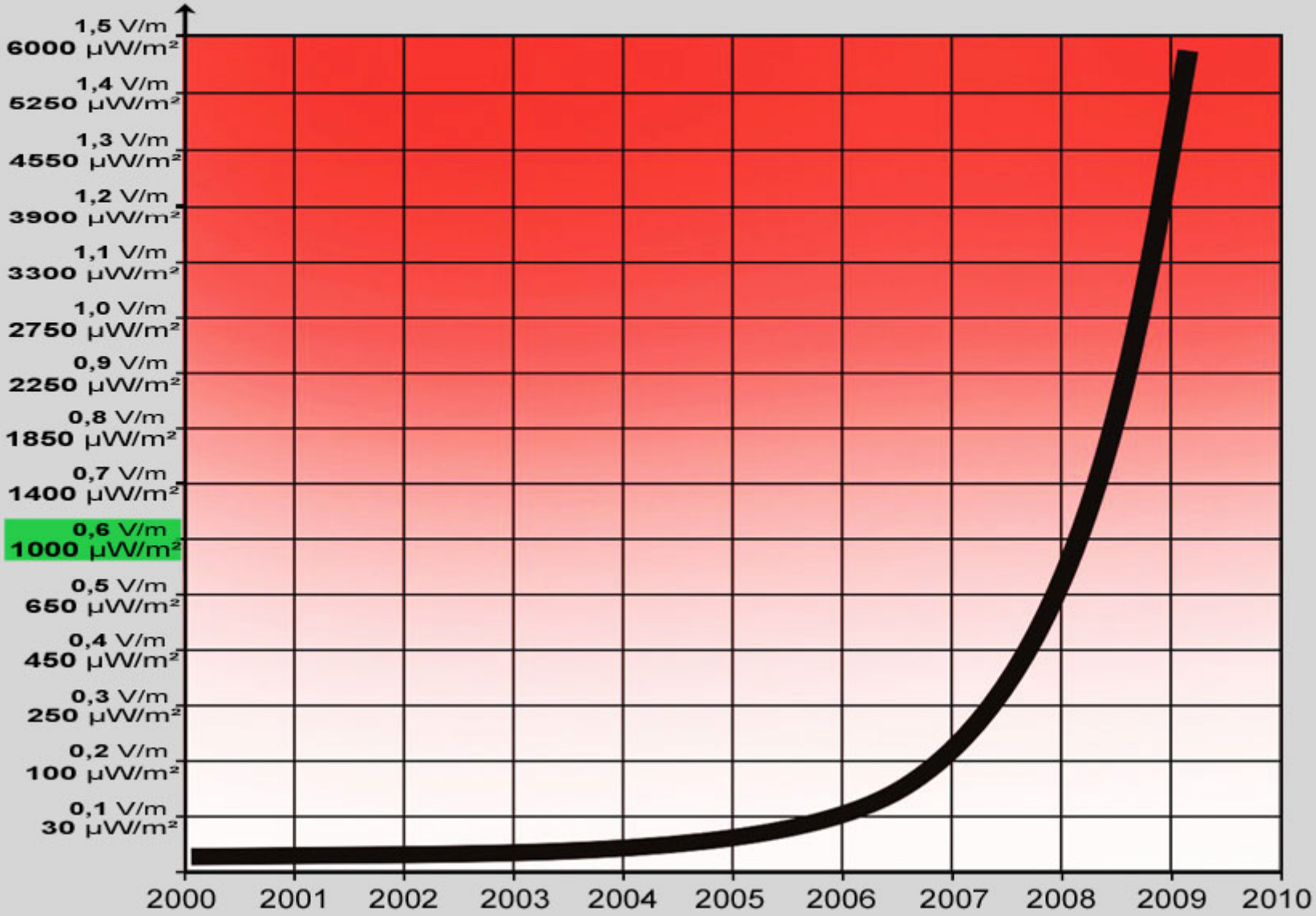


100 61 58 48 30 10 3 V/m COMPATIBILITÉ NORME APPAREILAGE 1 0,6V/m 0,3 0,1


V/m
Antenne relais : Valeur* moyenne de d'irradiation au sol dans l'axe du faisceau.
Antenne relais installée entre 14/18m de hauteur / Tilt ± -3° / BST Macro-station: Pire 27 dBW.
* correspondande à 1 faisceau soit UNE antenne relais
2.666
2.333
2.000
1.666
1.333
1.000
0.666
0,6 V/m
0.333
Niveau sol
Ground level
0
10m
25
50
75
100
125
150
175
200
225
235m
250
m
Distance
www.next-up.org
EMF
CEM

Average increase observed in urban area of artificial HF microwave radiation from 900 MHz - 2.5 GHz

Valeur moyenne constatée en milieu urbain de l'irradiation artificielle HF micro-onde de 900 MHz - 2,5 GHz



NEURONAL DAMAGE

Albumin in the Brain Parenchyma: Neuronal degeneration is seen in areas with BBB disruption:

*** Intracarotid infusion of hyperosmolar solutions in rats (Salahuddin et al. 1988)**

*** In the stroke-prone hypertensive rat (Fredriksson et al. 1988)**

*** In acute hypertension by aortic compression in rats (Sokrab et al. 1988)**

*** And epileptic seizures cause extravasation of plasma into brain parenchyma. The cerebellar Purkinje cells are heavily exposed to plasma constituents and degenerate in epileptic patients (Sokrab et al., 1990)**

Albumin is the most likely neurotoxin in serum (Eimerl et al. 1991)

Albumin in the brain

25 microlitres rat albumin infused into rat neostriatum.

10 and 30, but not 3 mg/ml albumin causes neuronal cell death and axonal severe damage.

It also causes leakage of endogenous albumin in and around the area of neuronal damage.

10 mg/ml is approx. 25% of the serum concentration

Hassel B et al. Neuroscience Letters 167:29-32, 1994

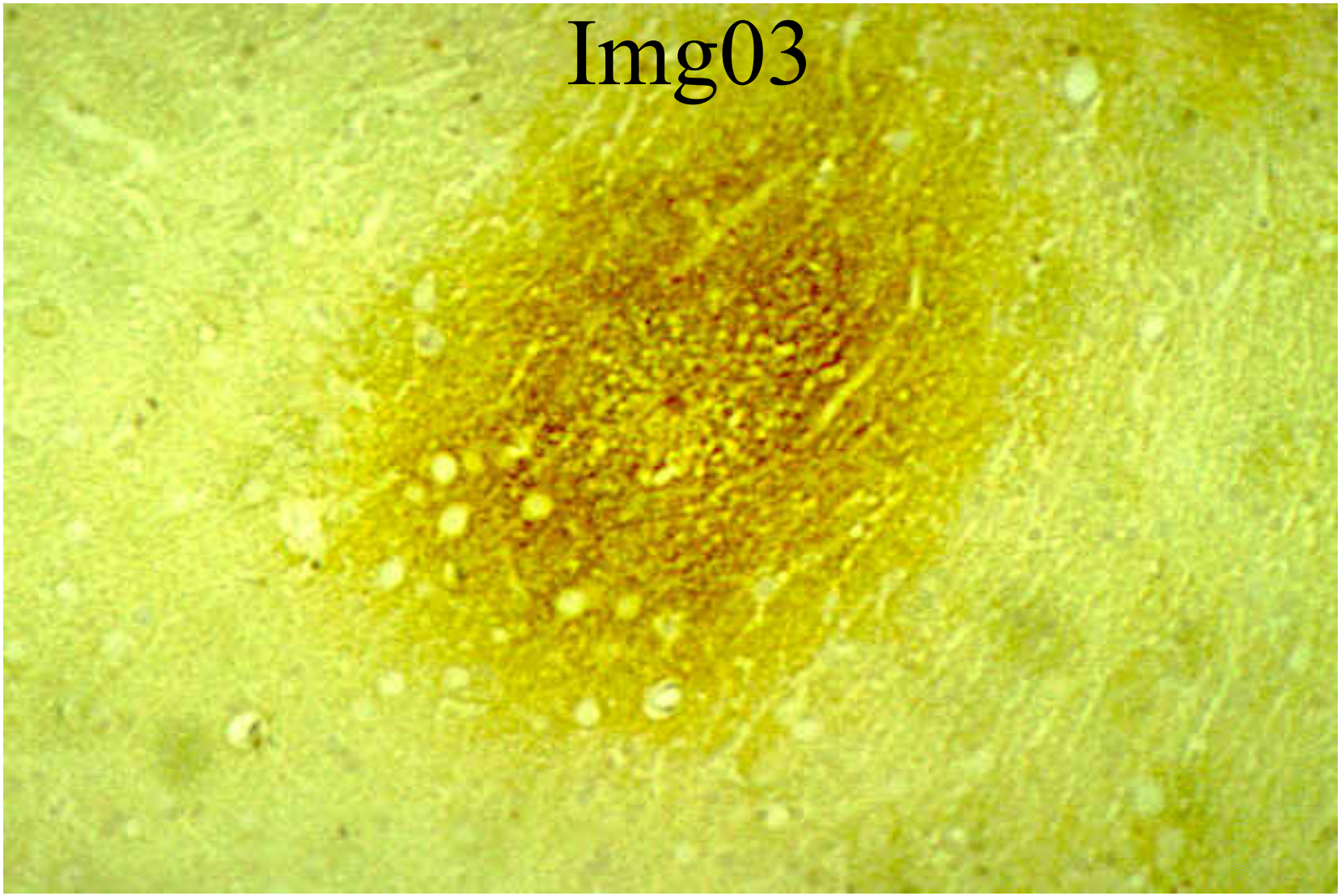
DAMAGE TO BRAIN CELLS LONG TIME AFTER ONE EXPOSURE FOR 2 HOURS TO MICROWAVES FROM A GSM MOBILE PHONE???

One exposure for 2 hours. Each exposure group: 8 rats (12-26 weeks old – comparable to human teenagers)

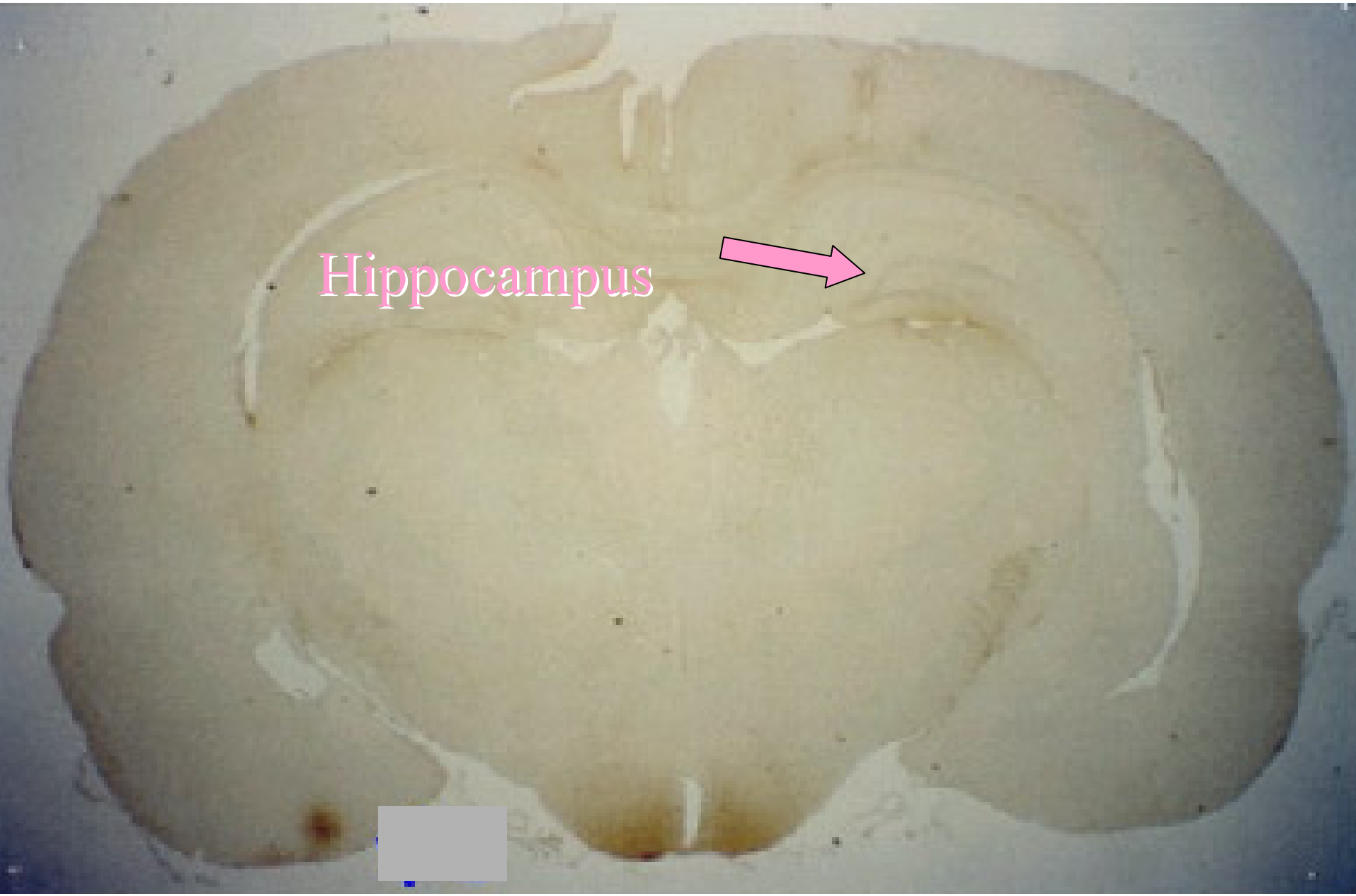
Exposure groups:
0,002 W/kg (1/1000 of the energy at the antenna)
0.02 W/kg (1/100 of the energy at the antenna)
0,2 W/kg (1/10 of the energy at the antenna
Control rats (8 animals in TEM-cell for 2 hours without GSM irradiation)

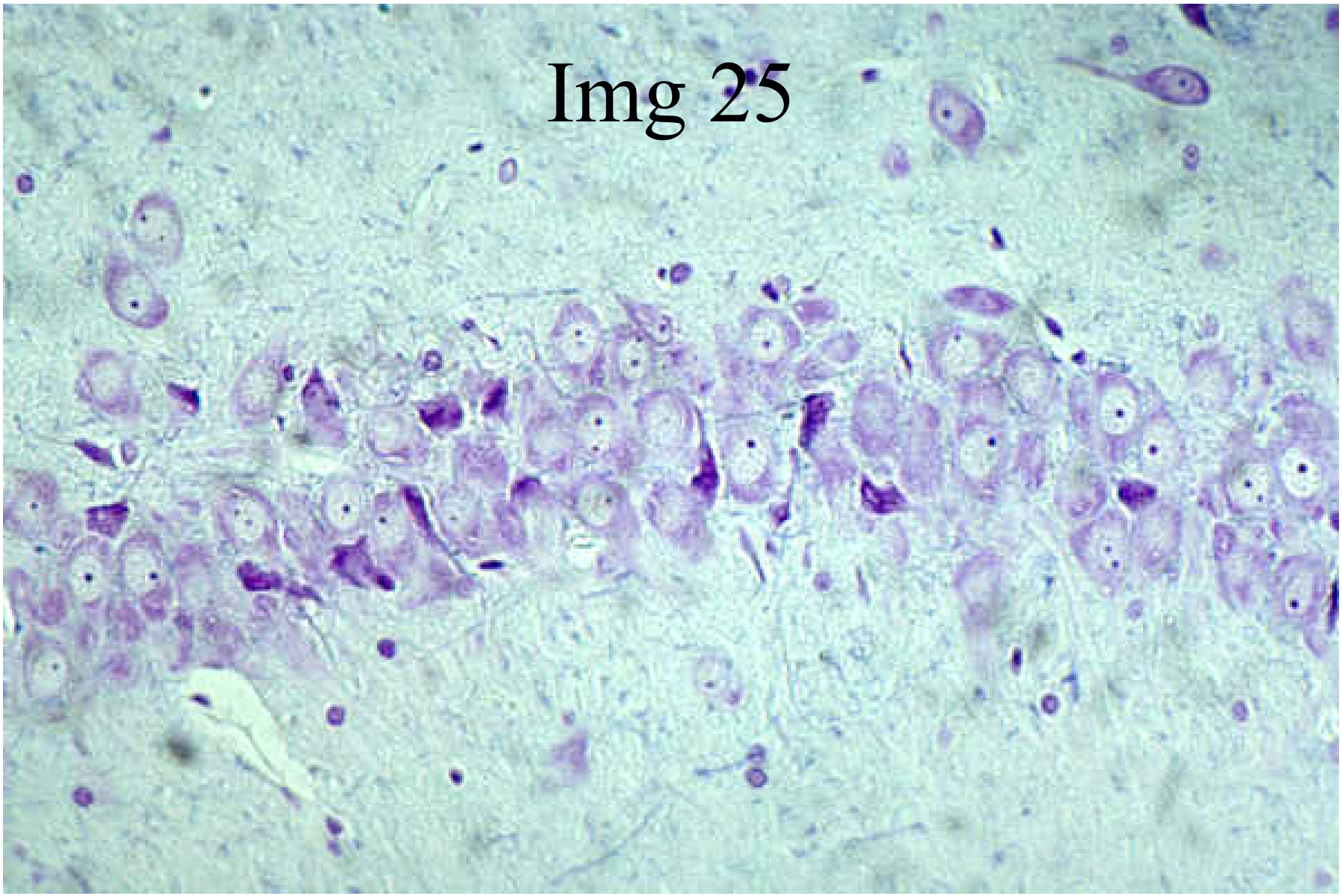
The animals were then allowed to survive for 50 days in standard cages. They were then anesthetised and sacrifized by perfusion-fixation followed by histopathological examination for neuronal damage and albumin leakage.

Result:
Albumin leakage also after 50 days!!

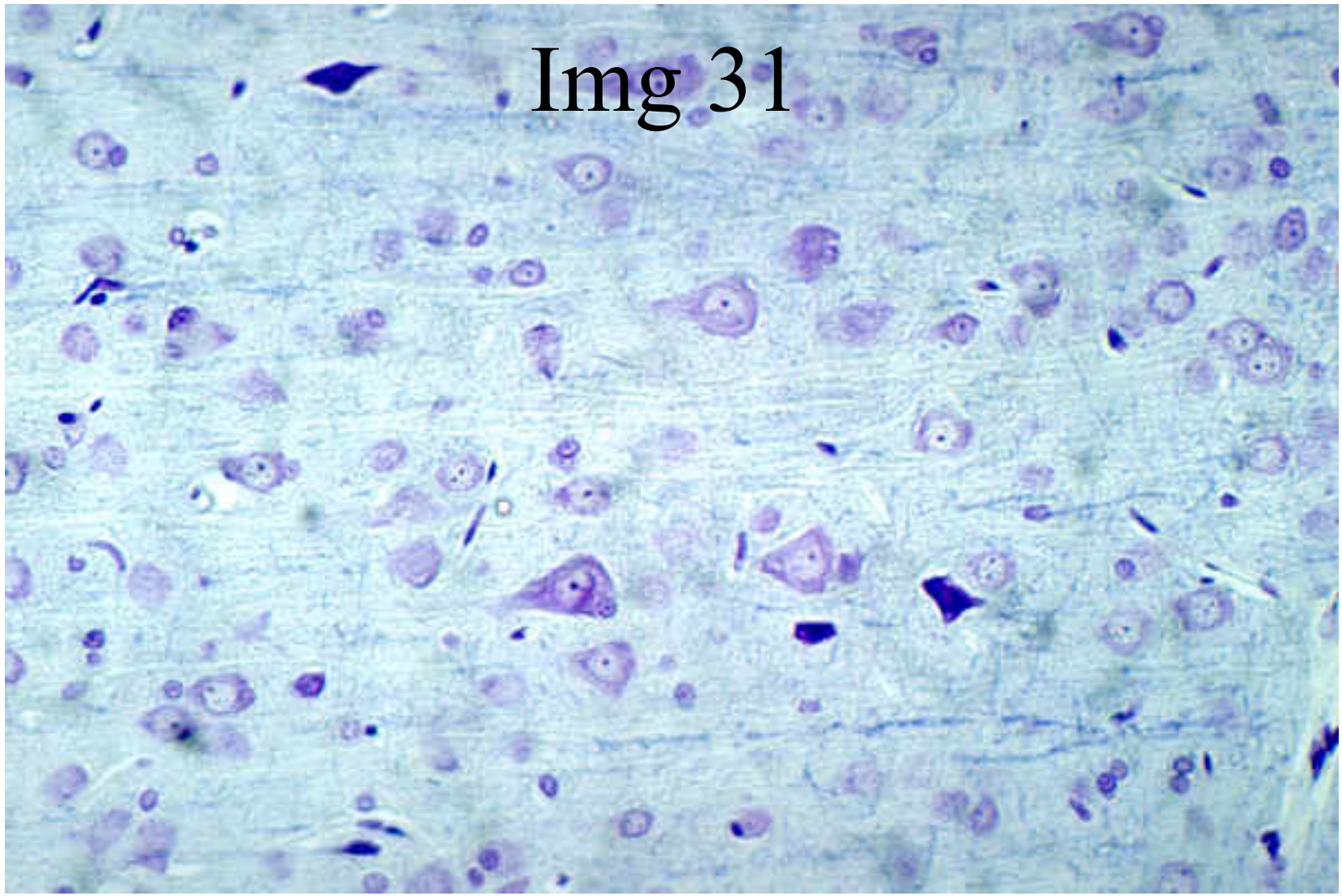

Img03

And
”Dark neurons”
50 days after
2 hours GSM-exposure!


Hippocampus


Img 25


Cortex


Img 31

Up to 2% of the neurons are damaged
50 days after a 2-hour GSM exposure
Significance p=0,002
(Kruskal Wallis)

Continued work, completed:

Connection albumin leakage – neuronal uptake - damage?

# rats				
1600	48	48	48	32
	48	48	48	32

Neuronal uptake and damage

Albumin leakage

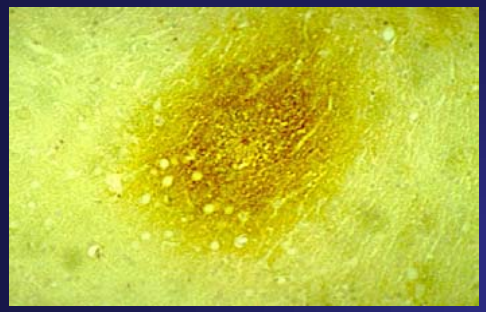
2 hours exposure

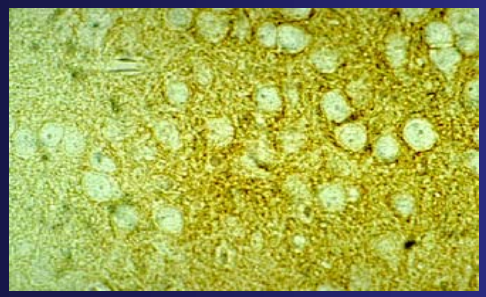
0 7 14 28 50 days

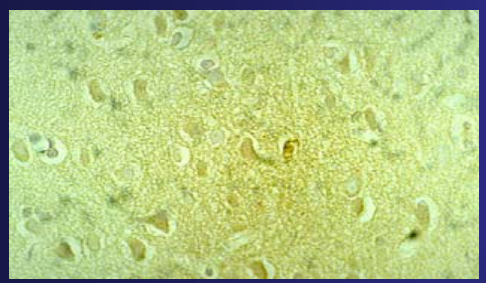
Exposure scheme, number of animals

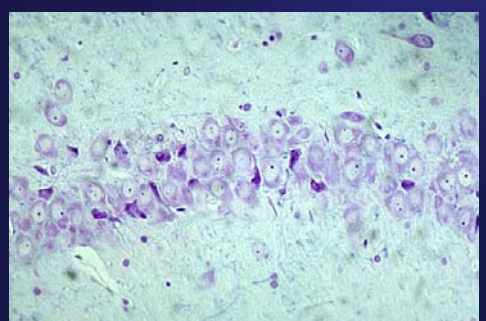
Recovery time (days)	sex	sham	SAR (mW/kg)			
			0.2	2	20	200
14	m	8	4	4	4	4
14	f	8	4	4	4	4
28	m	8	4	4	4	4
28	f	8	4	4	4	4
50	m	4		4	4	4
50	f	4		4	4	4
+						
7	m	8	4	4	4	4
7	f	8	4	4	4	4

Exposed vs sham









	7d	14 d	28 d	50 d
Albumin foci	**0.04**	**0.02**	**ns**	**0.04**
Diffuse albumin	**ns**	**ns**	**ns**	**ns**
Neuronal albumin	**0.02**	**0.005**	**ns**	**ns**
Dark neurons	**ns**	**ns**	**0.01**	**0.001**

Continued work

Connection albumin leakage – neuronal uptake - damage?

# rats						
	48	48	48	32	56	**Neuronal uptake and damage**
1600	48	48	48	32	56	**Albumin leakage**

2 hours exposure

0 7 14 28 50 386 + 46 days

Long term experiments

Fischer 344 rats were exposed for 2 hours to GSM 900, (of in average 0.6 and 60 mW/kg)
or sham exposed in our TEM-cells once a week for 13 months (386 days).
After this they were studied for cognitive functions and compared to cage controls and were sacrificed 46 days later and examined histopathol.

EFFECTS UPON

COGNITIVE FUNCTION

Exposure

2 hours weekly for 55 weeks
GSM-900 mobile phone

Number of Fischer 344 rats (Totally 56)	Exposure (at the initiation)
16 (8 ♀, 8 ♂)	0.6 mW/kg (5mW to TEM-cell)
16 (8 ♀, 8 ♂)	60 mW/kg (0.5W to TEM-cell)
16 (8 ♀, 8 ♂)	Sham
8 (4 ♀, 4 ♂)	Cage controls

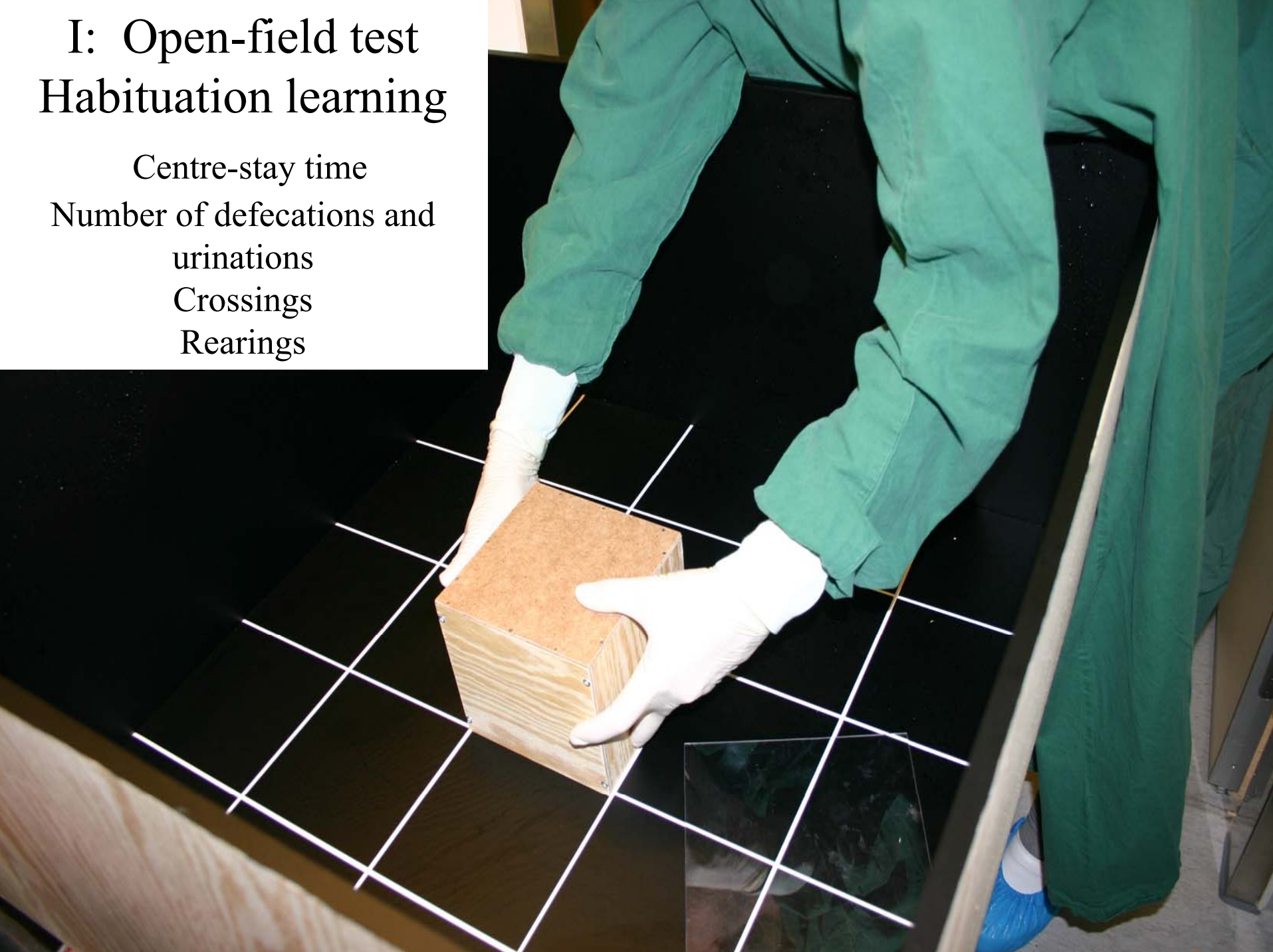
I: Open-field test
Habituation learning

Centre-stay time

Number of defecations and urinations

Crossings

Rearings



Results

- No difference due to GSM exposure
- Influenced by sex, day of training, being a cage control

Episodic memory test

- What, where and when

(Kart-Teke et al. 2006)

- Assessment of relative recency of two remembered objects

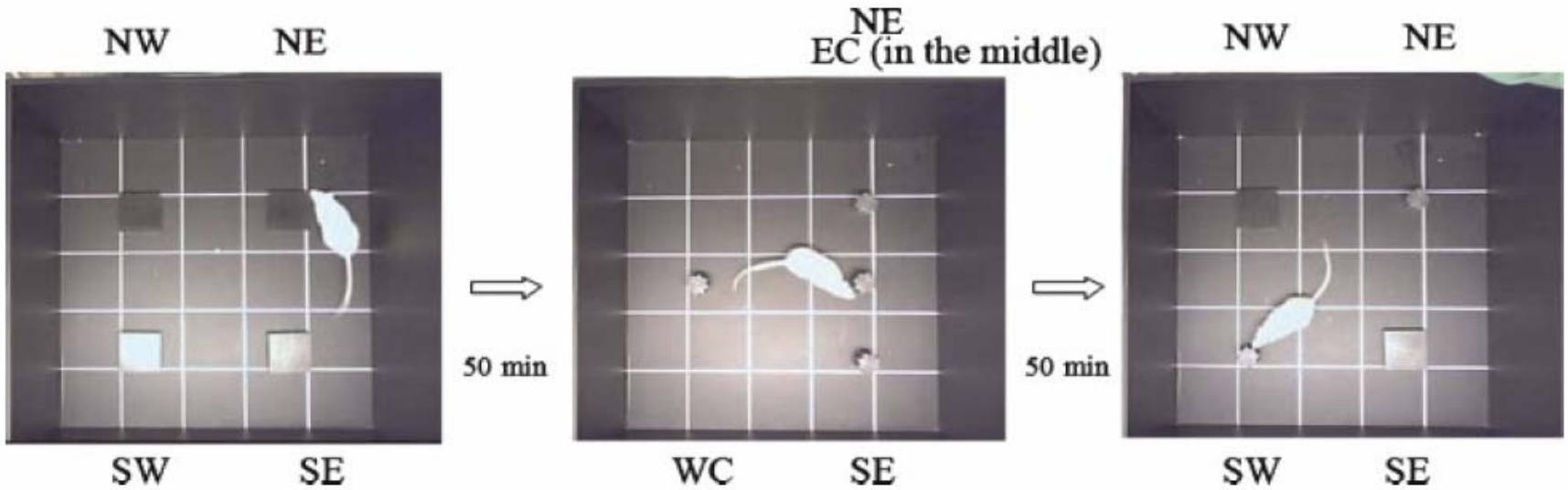
(Hannesson et al. 2004)

- Ability to discriminate based upon the novelty of an object location

(Ennaceur et al. 1997)

Episodic-Like Memory Test

Long-term memory of different objects



Results

GSM exposure vs sham

- Impaired episodic memory
- Impaired memory for objects
- Impaired memory for their temporal order of presentation
- Spatial memory not affected

Cage controls have more reduced performance than both sham and GSM exposed rats.

Summary

- 55 weeks of GSM exposure
- No behavioural changes
- Significantly impaired episodic memory

Histopathological examinations after long-term exposure

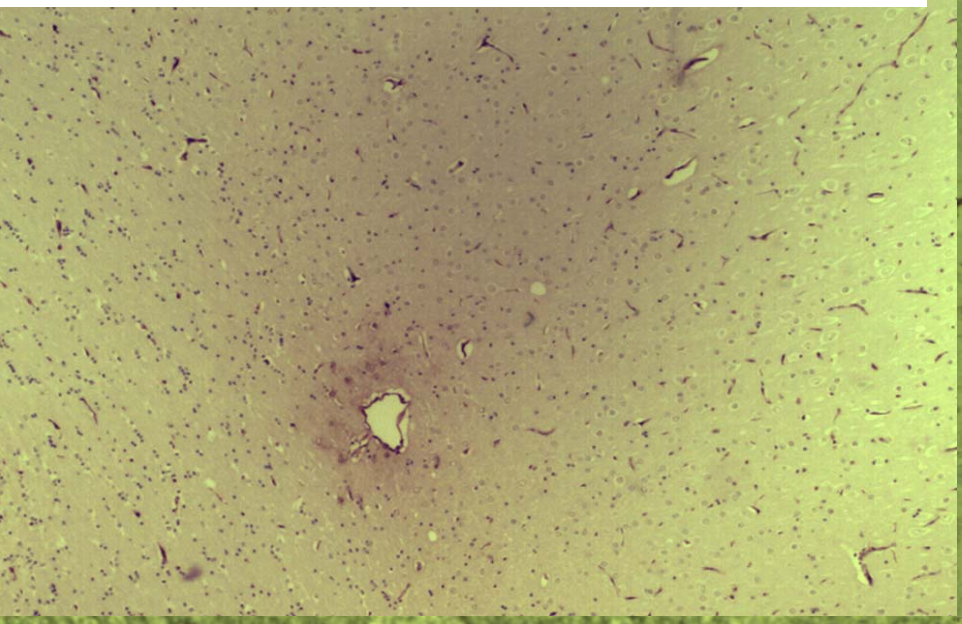
5-7 weeks after the GSM exposure

1) Albumin antibodies
2) Cresyl violet to detect damaged neurons

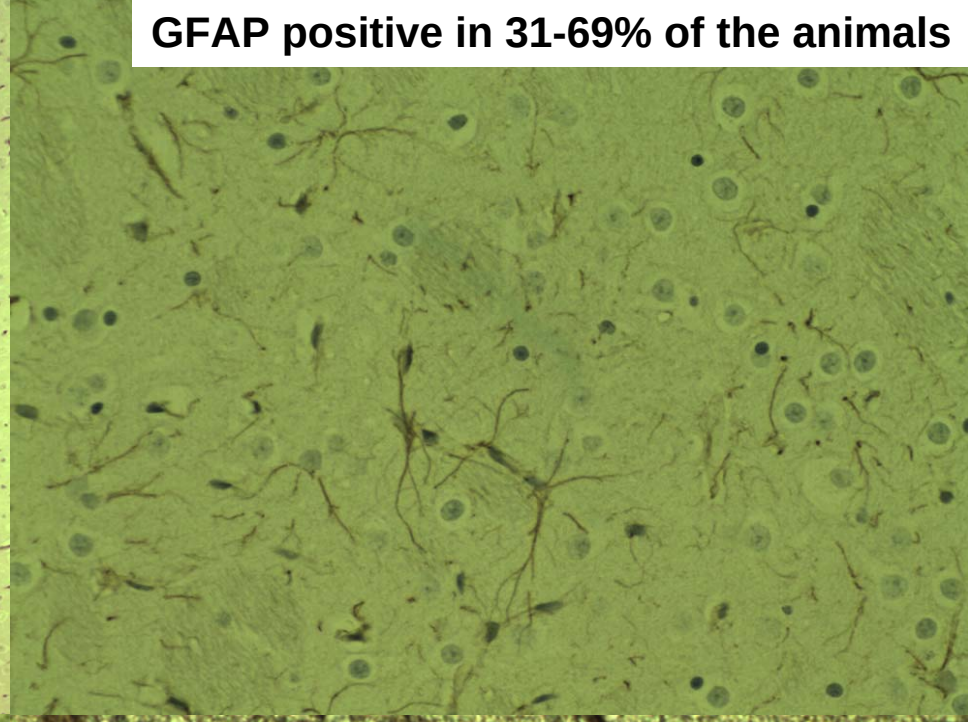
Indicators of accelerated ageing:

3) GFAP (glial fibrillary acidic protein) - glial reaction
4) Staining pigments in neurons with Sudan Black B to detect lipofuscin - a wear and tear product.
5) The silver method of Gallyas – to detect signs of cytoskeletal or neuritic changes

About 1 animal / group had albumin extravasation
About 40 % of the animals had dark neurons



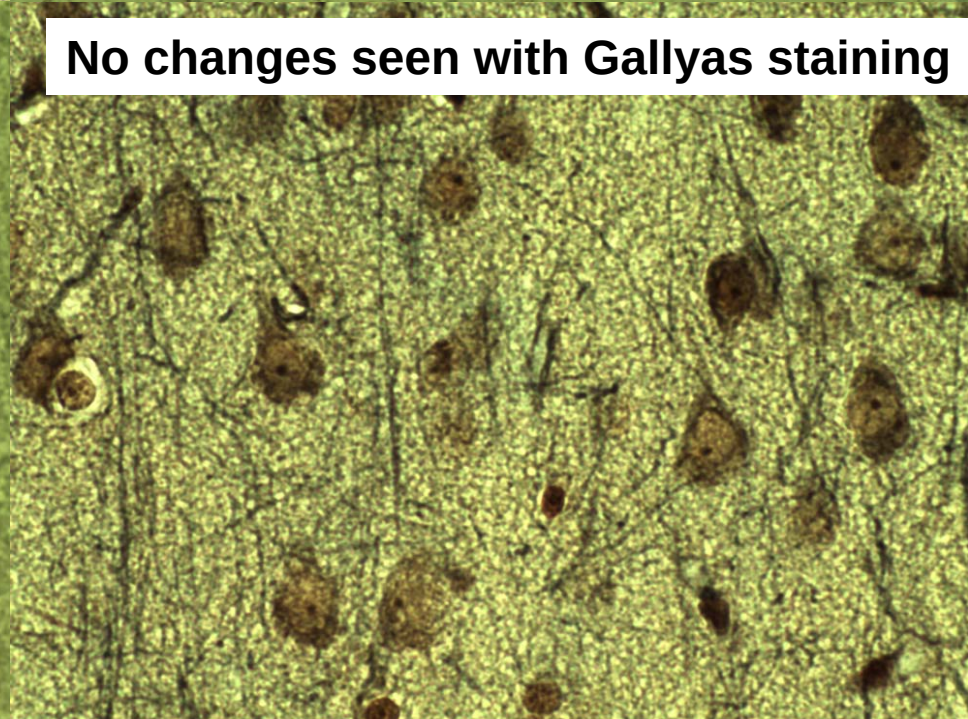
GFAP positive in 31-69% of the animals



Lipofuscin positive in 44-71% of the animals

Sudan Black B for lipofuscin

No changes seen with Gallyas staining



Results

- 5-7 weeks after the last exposure
- No significant difference between GSM and sham exposed rats
- Higher lipofuscin score -> impaired spatial memory
- Otherwise no correlation to episodic memory

Summary

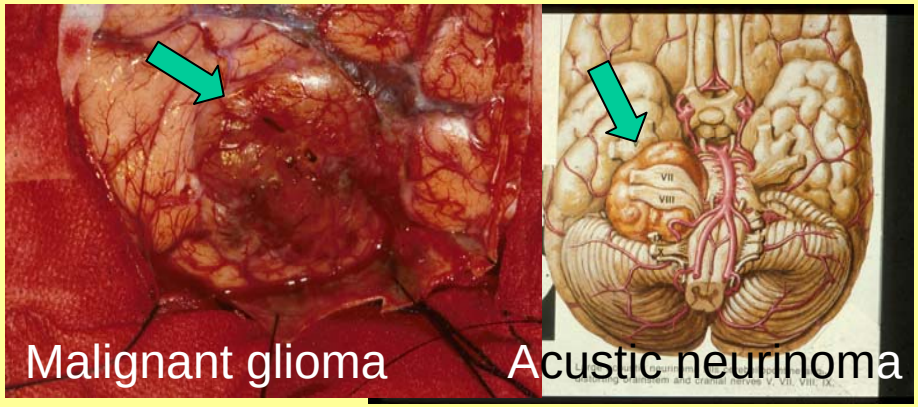
No significant histopathological differences between exposed and sham controls regarding:

- BBB permeability
- Neuronal damage
- Increased or accelerated ageing

Effects upon DNA?

Mobile phones and Brain tumours

Bioinitiative report July 2007



Lennart Hardell, MD, PhD, Dept of Oncology, Örebro University Hospital, Sweden
Kjell Hansson Mild, PhD, Dept of Radiation Physics, Umeå University, Sweden
Michael Kundi Ph.D., med.habil, Inst. of Env. Health, Vienna, Austria

”In summary we conclude that our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after > 10 years mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term brain tumor risk and needs to be revised”.

- Hardell et al. 2008 – metanalaysis
- No increased risk for brain tumours for all cases

BUT

- OR 2.0 for glioma after ipsilateral use > 10 years (CI 1.2-3.4)
- OR 2.4 for vestibular schwannoma after ipsilateral use > 10 years

EFFECTS UPON

GENE EXPRESSION

Previous Microarray Studies

In vitro

GSM exposure leads to altered gene expression in:

- **cultured human cells (Czyz et al. 2004, Lee et al. 2005, Pacini et al. 2002, Remondini et al. 2006)**
- **mouse embryonic stem cells (Nikolova et al. 2005)**

But not in:

- **human glioblastoma cells (Qutob et al. 2006)**
- **human neuroblastoma cell lines (Gurisik et al. 2006)**

In vivo

- **11 genes up-regulated 1.34-2.74 fold**
- **1 gene down-regulated 0.48 fold in rats**
- **Neurotransmittor regulation, BBB**
- **(Belyaev and the Lund group 2006)**

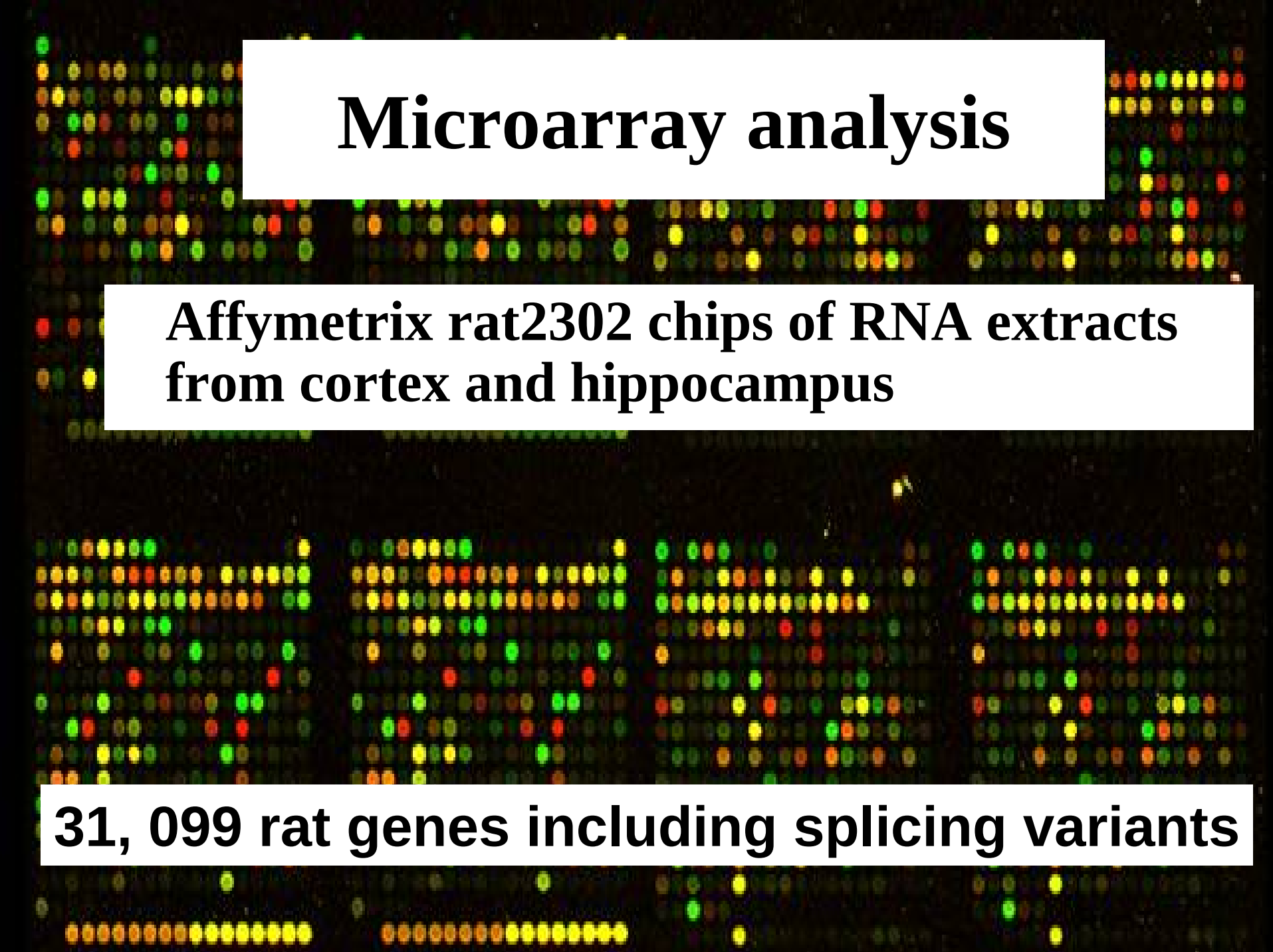
Effects upon DNA?

6 hours exposure to radiation from a GSM-1800 mobile test phone

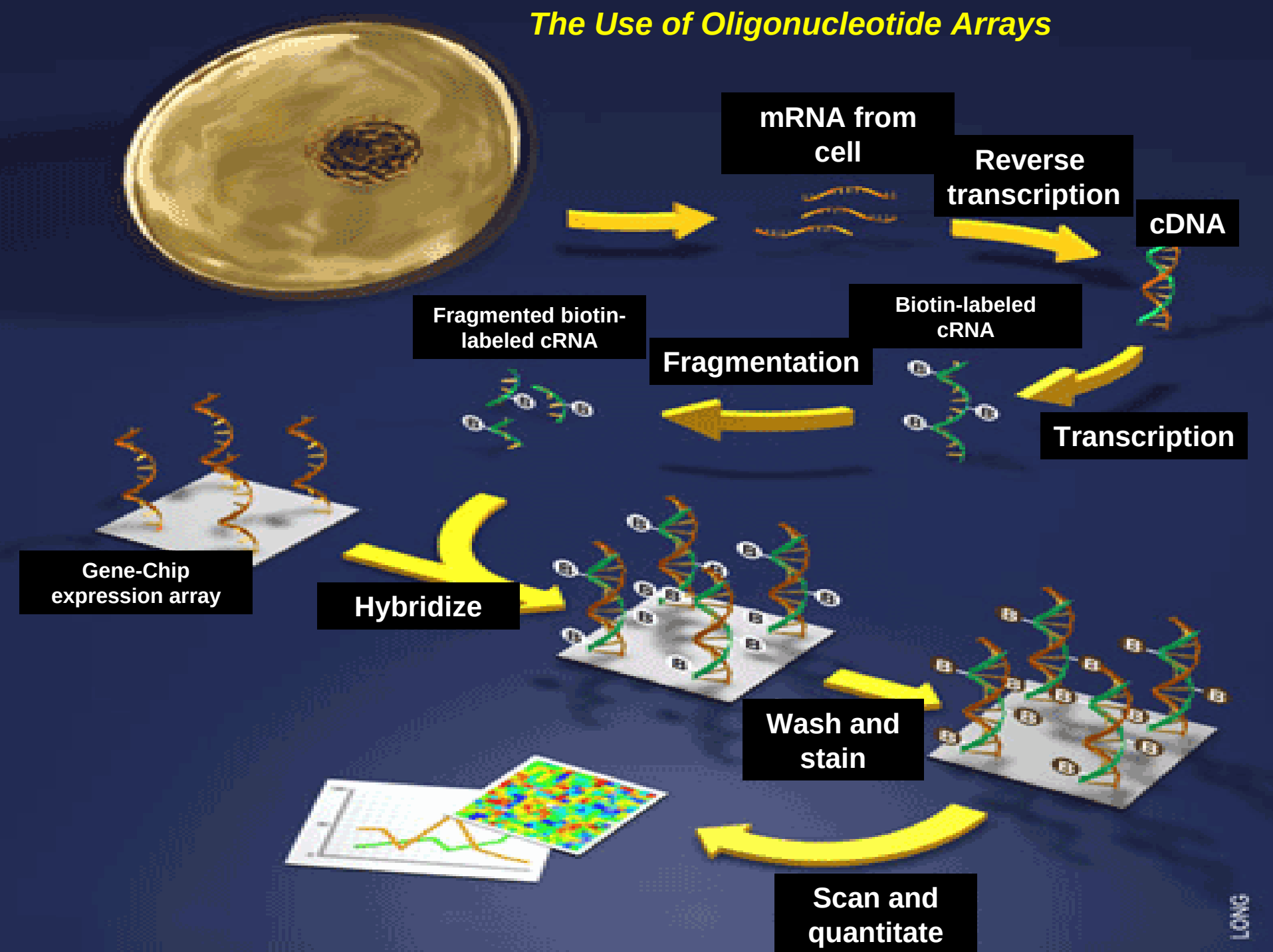
4 exposed Fischer 344 rats
4 sham controls

Analyses of gene expression in cortex and hippocampus

Anechoic chamber GSM-1800


Microarray analysis
Affymetrix rat2302 chips of RNA extracts from cortex and hippocampus
31, 099 rat genes including splicing variants

The Use of Oligonucleotide Arrays



Gene Ontology Analysis

- Predefined functional categories of genes
- Using GO categories biological processes, molecular functions, cell components

Results I

No significant difference at the single gene level when taking multiple hypothesis testing into account

Results II

- 25 GO categories altered in cortex
- 20 GO categories altered in hippocampus
  (with significances up to $p<10^{-23}$)

- Altered in both hippocampus and cortex
- (totally 10):
  extracellular region, signal transducer activity,
  intrinsic to membrane, integral to membrane
  (The cellular membrane seems to be an important target for the EMF effects)

- More genes are up-regulated than down-regulated

MECHANISMS??

- Processes in the cell membrane reactive to the low energy of oscillating EMF -> leading to a change in membrane potential (Adey 1988)
- Low-level RFR as a stressor (Lai 1987)
- Formation of free radicals after RF exposure (Ilhan et al. 2004)
- Free radicals after MW exposure (Lai and Singh 2004)
- Alterations of protein conformation of serum albumin (De Pomerai et al. 2003)

EMF interaction with free ions; external oscillating fields -> forced vibrations of the ions -> increase of ic ion concentration -> osmotically driven entrance of water -> disruption of plasma membranes (Panagopoulos and Margaritis 2008)

EMF -> ROS -> rapid activation of ERK -> effects on transcription (Friedman et al. 2007)

ELF at 50 Hz -> SAPK (stress-activated protein kinase), inhibited when noise is applied (Sun et al. 2001 and 2002)

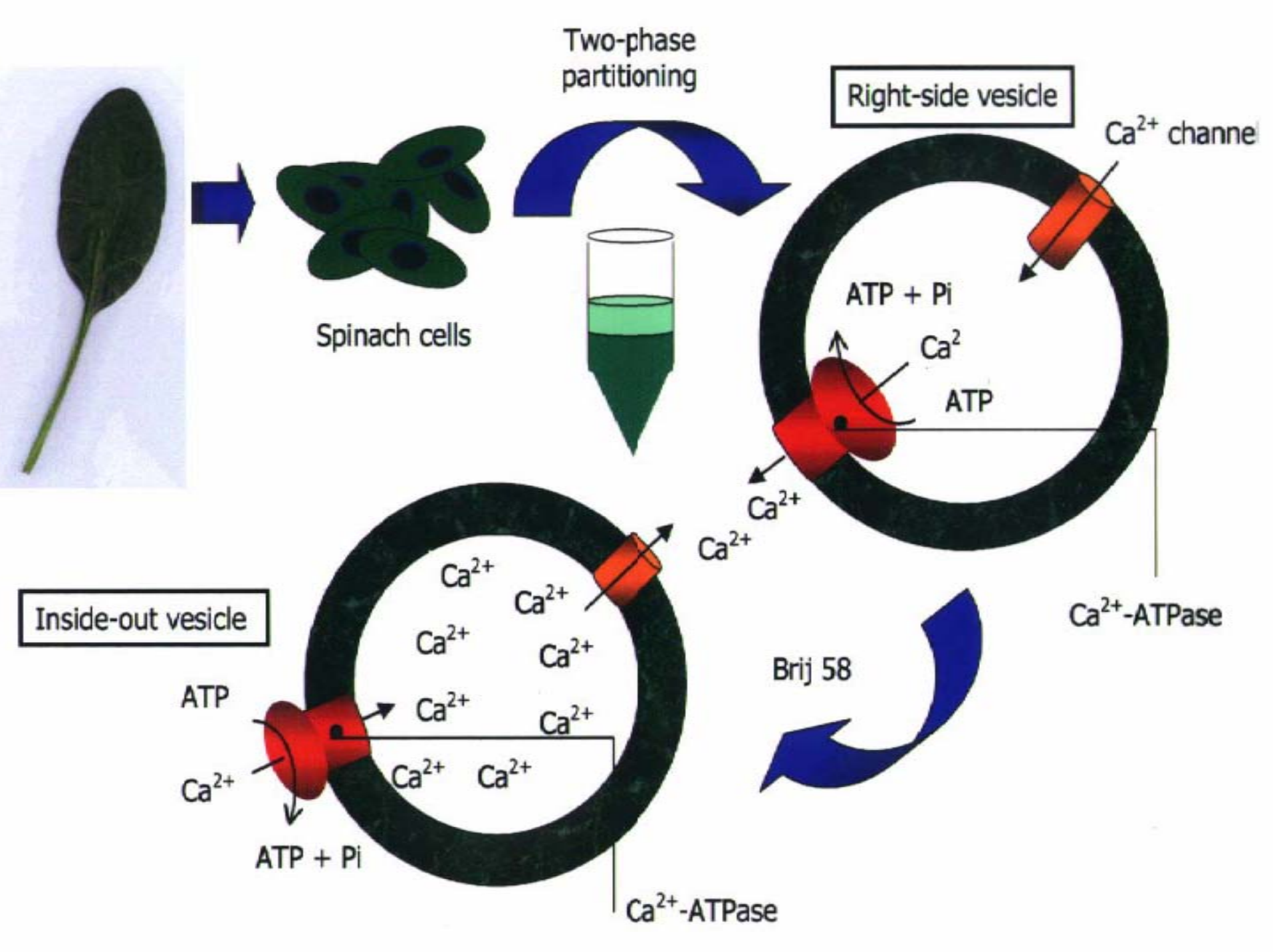
GSM exposure activated hsp27/p38MAPK stress signalling pathways -> possible stabilisation of endothelial stress fibres (Leszczynski et al. 2002)

Quantum-mechanical model for interaction with protein-bound ions; Ca2+-transport with resonances at certain frequencies

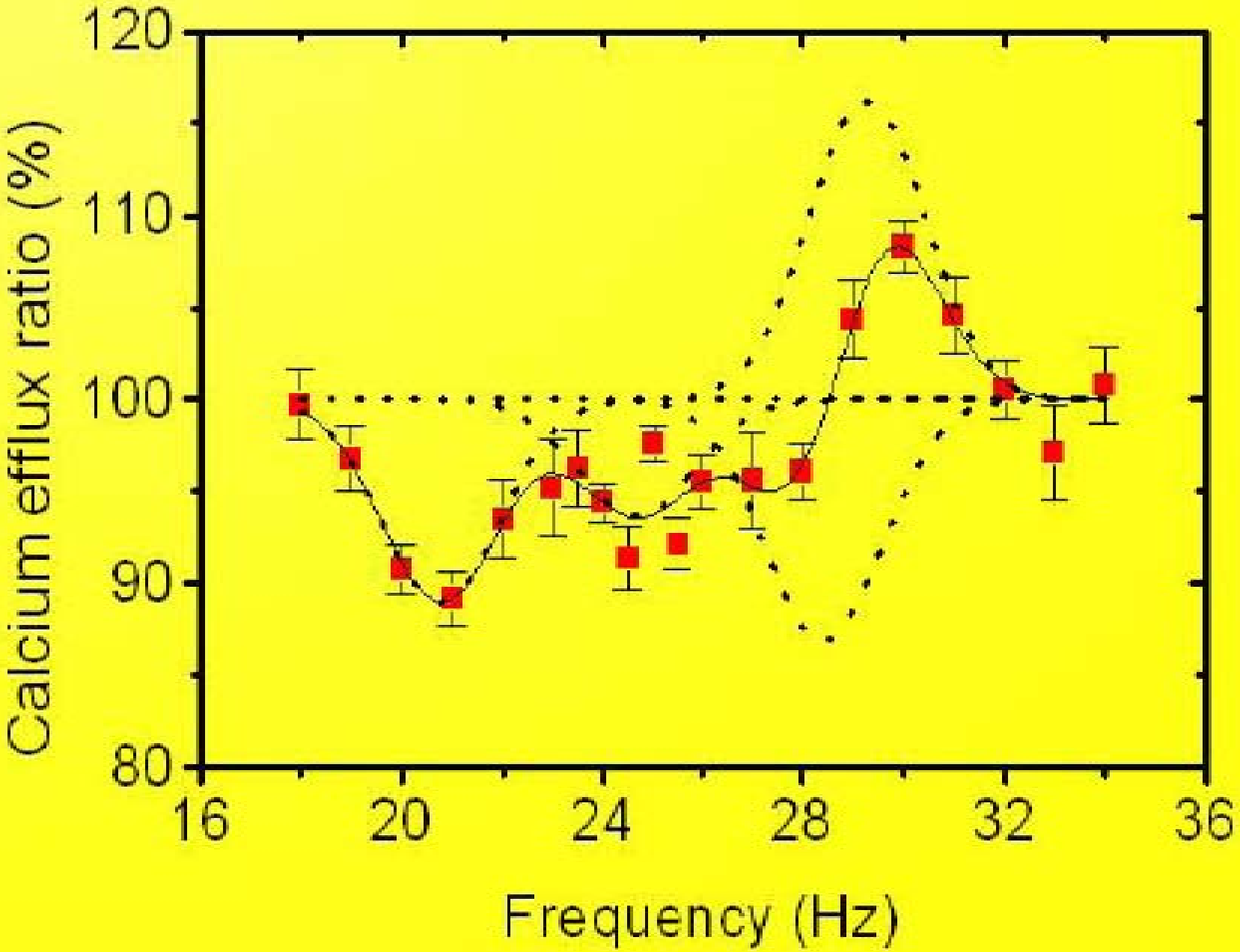
Bioelectromagnetics 24:395-402, 2003

C.L.M. Bauréus Koch, M. Sommarin, B.R.R. Persson,
L.G. Salford and J.L. Eberhardt

”We show that suitable combinations of static and time varying magnetic fields directly interact with the Ca2+ channel protein in the cell membrane, and we quantitatively confirm the model proposed by Blanchard”


Two-phase
partitioning
Right-side vesicle
Ca2+ channe
Spinach cells
ATP + Pi
Ca2
ATP
Ca2+
Ca2+
Ca2+-ATPase
Inside-out vesicle
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
ATP
Ca2+
ATP + Pi
Brij 58
Ca2+-ATPase

Blanchard


120
110
100
90
80
Calcium efflux ratio (%)
16
20
24
28
32
36
Frequency (Hz)

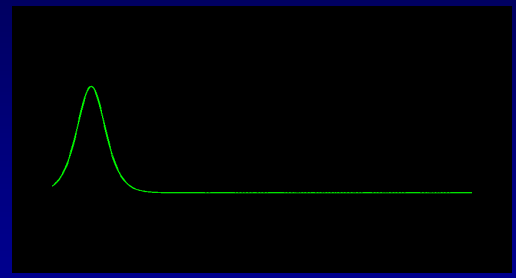
Continued work based upon studies by Bauréus-Koch et al. 2003

Studies on plasma vesicles from spinach with ELF and EMF from GSM

together with Dept of Plant Physiology, LU.

The Soliton Model

- A soliton is a non-linear wave



- Propagation in the lipids of biological membranes – vital role in the action potential propagation along nerve membranes (Heimburg and Jackson 2005)

- Generated and propagated along the microtubule protofilaments in neurons of the brain (Abdalla et al. 2001)

A new theory

Solitons instead of Hodgkin-Huxley?

Niels Bohr Institute Copenhagen

On soliton propagation in biomembranes and nerves Heimburg, T. and Jackson, AD. (2005) PNAS 102, 9790-9795:

The lipids of biological membranes and intact biomembranes display chain melting transitions close to temperatures of physiological interest. During this transition the heat capacity, volume and area compressibilities, and relaxation times all reach maxima. Compressibilities are thus nonlinear functions of temperature and pressure in the vicinity of the melting transition, and we show that this feature leads to the possibility of soliton propagation in such membranes.

The thermodynamics of general anesthesia. Biophys J. 2007 May 1;92(9):3159-65.

Anesthetics lower the temperature at which lipids become solid, making it difficult for the waves to form, thereby preventing nerves from sending pain signals.

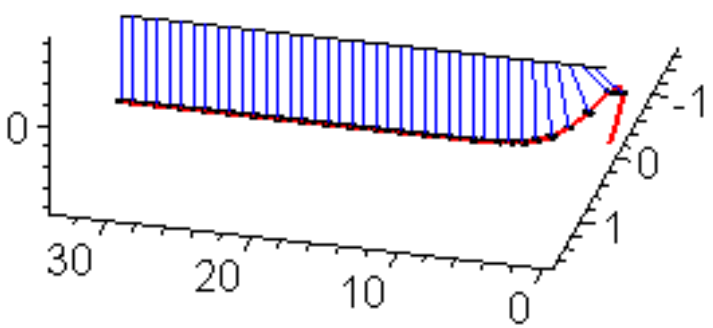
PHYSICAL REVIEW A VOLUME 38, NUMBER 3 AUGUST 1, 1988

Solitons hiding in DNA and their possible significance in RNA transcription

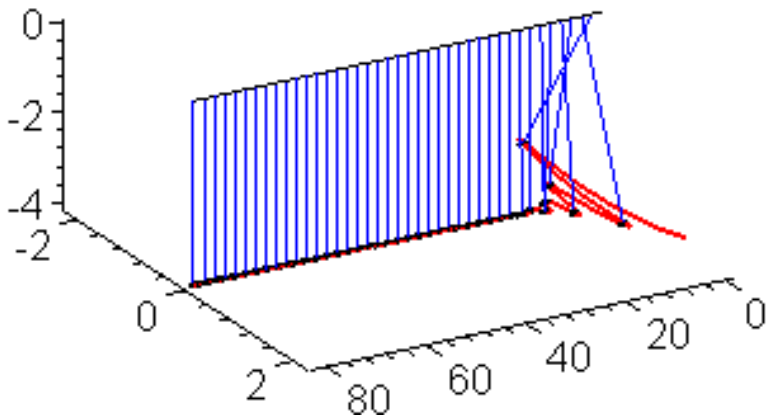
E. W. Prohofsky
Department of Physics, Purdue University, West Lafayette, Indiana 47907
(Received 16 February 1988)

We find that the hydrogen-bond–stretch bands of the double helix appear to be nonlinear enough to support solitary-wave energy concentration. Coupling this fact to predictions of our self-consistent theory of helix melting gives rise to speculations of a mechanism for base pair melting in RNA transcription which is consistent with the known energy needs of that process.

Large amplitude Breather



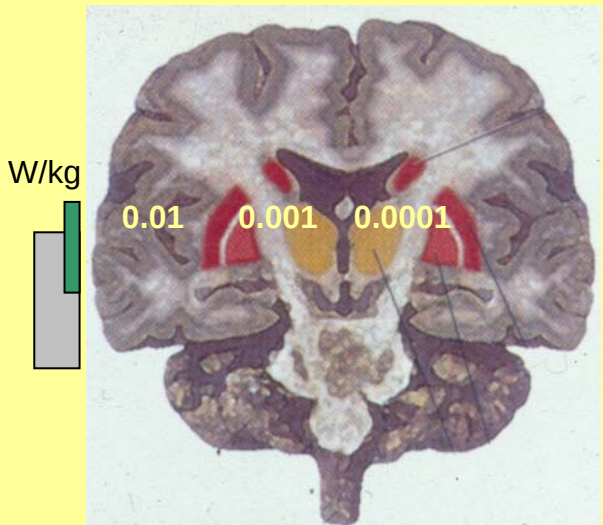
Small amplitude Breather

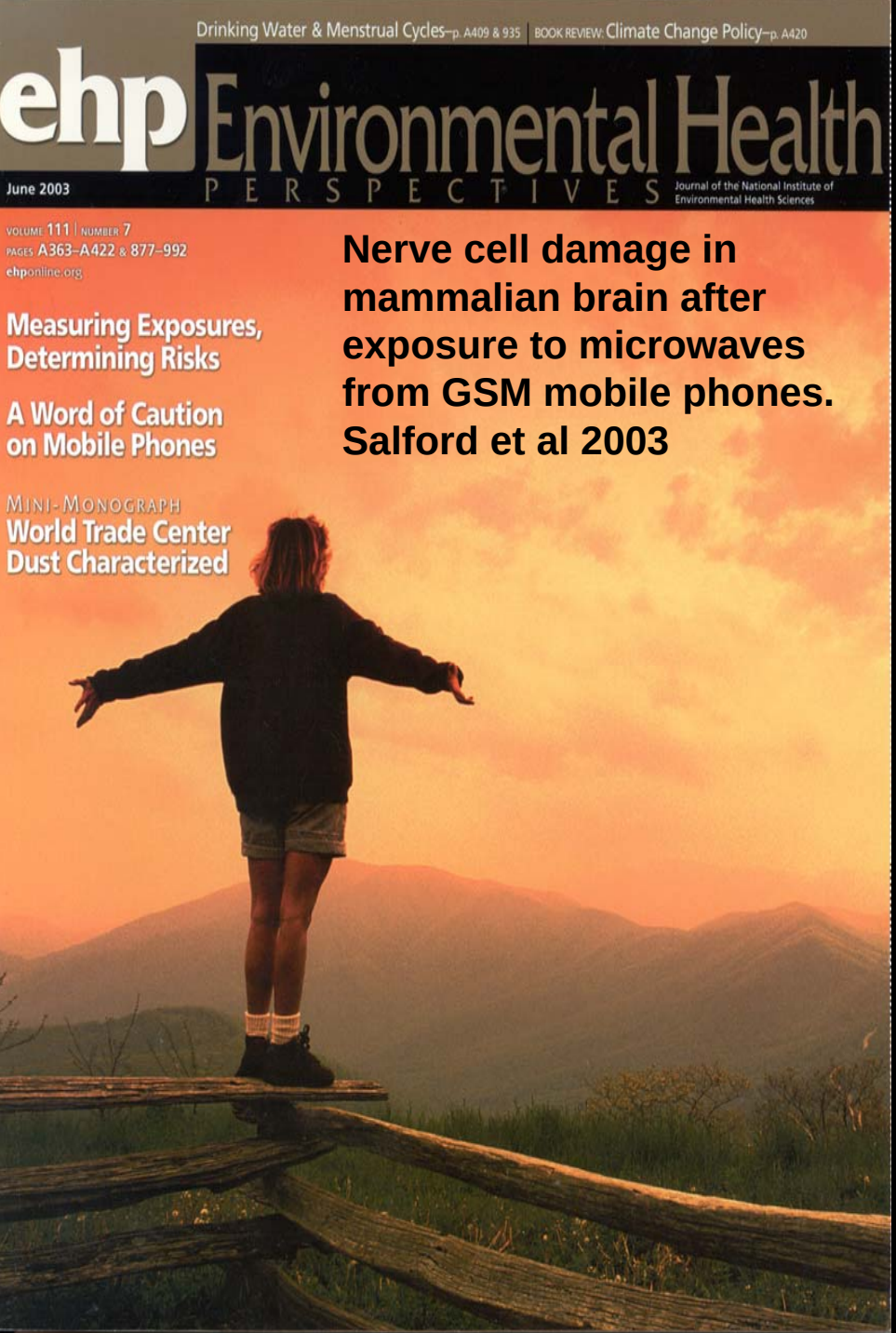


Conclusions

My opinion:

More probable than unlikely, that non-thermal electromagnetic fields from mobile phones and base stations do have effects upon the human brain





- "The intense use of mobile phones by youngsters is a serious memento. A neuronal damage of the kind, here described, may not have immediately demonstrable consequences, even if repeated.
- It may, however, in the long run, result in reduced brain reserve capacity that might be unveiled by other later neuronal disease or even the wear and tear of ageing.
- We can not exclude that after some decades of (often), daily use, a whole generation of users, may suffer negative effects maybe already in their middle age".

Muito obrigado

LUND
UNIVERSITY

My questions

Why not effects in all animals?
Why not in San Antonio - different animals?
Other studies – different exposure time, higher SAR etc
Why a window effect?
How to protect from the low SAR effects?
Why no significant findings after long term exposure?
Does it mean anything to humans?
Cf the BBB human – rodent – other species

If we find the mechanims – easier to judge danger

Search for the truth - combine efforts between labs