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A story about effects of Microwaves from Mobile-phones, and Alzheimer's disease

Bertil RR Persson

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Chapter I
Prologue

1.1 Mobile phone radiation and Alzheimer's disease

A team of clinical researchers in Lund, Sweden, found that electromagnetic radiation, such as that used in mobile communications, at low non-thermal power values (<2 W/kg), causes users' blood albumin to leak through the blood-brain barrier "BBB" into in the brain tissue. The BBB is supposed to protect the brain from unwanted and toxic molecules that are potentially in the blood to transfer into the brain tissue. But after exposing rats to radiation from cell phones, albumin from the blood leaks into the brain and accumulates in neurons and glial cells

They exposed rats to different magnetic and electromagnetic fields as well as continuous and pulsed 915 MHz microwaves modulated with different repetition rates (50-200 pulses per s) GSM-900 and GSM-1800 (Persson et al., 1997, Persson, 2021).

The studies carried out at Lund University by:
The neurosurgeons: Leif Salford MD, PhD, professor emeritus,
Henrietta Nittby MD, PhD,
Neuropathologist: Arne Brun MD, PhD, professor emeritus.
Medical physicist: Bertil RR Persson PhD, MDhc, professor emeritus..
Jacob Eberhardt PhD,
Electrical engineer: Lars Malmgren Tech. Dr.

The detailed results are collected in the book:

"More likely than unlikely": A story about the blood-brain barrier and mobile communication Dedicated to Leif G. Salford on his 80th birthday 2021-12-07 (Persson, 2021).

Leif Salford spoke before the European Parliament on 29-06-2000;

"*Les effets possibles sur la santé des ondes électromagnétiques de hauates frequences (telefoni mobil)"*

In his conclusion at the presentation, he stated the following:

If mobile communication, even at extremely low SAR values, causes the users' own albumin to leak through the BBB, which is supposed to protect the brain, other unwanted and toxic molecules in the blood, it can also leak into the brain tissue and concentrate in the brain, brain neurons, and glial cells. It cannot be excluded that this

(especially after many years of intensive use) may promote the development of autoimmune and neurodegenerative diseases.

Figure 1-1
The future? - Is mobile phone radiation dangerous?
We do not know yet!

1.2 Mobile phone radiation and treatment of Alzheimer's disease

In late 2023, it came to my attention that Amyloid antibody drugs enter the brain poorly, with only one in a thousand administered antibodies getting through the BBB.

In my literature searches, I did not find anyone who tried to improve it with microwaves. However, it appeared that Arendash in the USA,used microwaves in order to treat patients with Alzheimer's disease. He used similar microwave pulse train and SAR levels that we used in our first studies of the BBB leakage of Albumin (Persson et al., 1997). Arendash and coworkers reported already in 2010 that treatment with electromagnetic fields protects against and reverses cognitive decline in transgenic mice with Alzheimer's disease (Arendash et al., 2010).

Arendash's report indicates that prolonged exposure to non-thermal levels of microwave radiation directly associated with GSM-900 cell phone use. confers cognitive benefits. They observed both cognitive protective and ameliorating effects of long-term exposure in both normal mice, and transgenic mice designed to develop Alzheimer's-like impairment.

Their results indicate that microwave exposure at ICNIRP-permitted non-thermal levels could safely apply as a non-invasive, non-pharmacological therapy for Alzheimer's disease (AD) that effectively improves memory (Arendash et al., 2010).

Zhi and co-workers of Arendash reported in 2023 that exposure to 900 MHz microwaves alleviated AD-like symptoms in APP/PS1 transgenic mice, potentially leading to a non-invasive strategy for AD treatment (Zhi et al., 2023).

Transgenic APP/PS1-mice and normal WT-mice were exposed 2 hours daily in 270 days to GSM 900 MHz microwave radiation at non-thermal SAR levels, and examined after 90, 180, and 270 days. They assessed cognition in the Morris water maze, Y-maze

and novel object recognition. Congo red staining, immunohistochemistry and ELISA used to analyze Aβ plaques, Aβ40 and Aβ42 content. The difference in expressed proteins in the Hippocampus between microwave-exposed and non-exposed AD mice identified by using proteomics.

The results show that spatial and working memory improved in AD mice after long-term 900 MHz microwave exposure compared to after sham exposure.

Microwave irradiation (900 MHz) during 180 or 270 days did not induce AP plaque formation in normal WT mice. In contrast, in 2- and 5-month-old APP/PS1 transgenic mice, showed inhibited Aβ accumulation in the cerebral cortex and Hippocampus after microwave irradiation.

In summary, the results indicated that long-term exposure to microwave radiation reduces the development of AD plaques and exerts a beneficial effect on memory (Zhi et al., 2023).

1.3 Clinical trial of microwave treatment of Alzheimer's disease

Based on a large amount of preclinical data, a pilot clinical trial planned to determine the safety and efficacy of 915MHz microwave therapy in patients with mild to moderate AD (Arendash, 2016).

In 2019, Arendash and coworkers reported the results of a first clinical trial of transcranial electromagnetic therapy (TEMT) in Alzheimer's disease, which showed cognitive improvement with associated changes in cerebrospinal fluid, blood, and brain imaging (Arendash et al., 2019).

Long-term exposure to 915MHz microwave radiation so-called TEMT administration to patients appears to be completely safe over a 2½-year period, with no harmful side effects. On six cognitive/functional tasks (including ADAS-cog13, Rey AVLT, MMSE and ADL), there was no decline in any measure during this period.

Long-term TEMT induced decreases in CSF levels of C-reactive protein, p-tau217, $A\beta_{1-40}$ and $A\beta_{1-42}$ while modulating the levels of CSF oligomeric Aβ. In plasma, long-term TEMT modulated and normalized levels of both p-tau217 and total tau.

Although only a limited number of AD patients were involved in this first study, the results suggest that TEMT can halt the cognitive decline of AD for a period of at least 2½ years and can do so without safety concerns

Although no control group was included, this study otherwise demonstrates widespread and sustained interruption of progressive AD cognitive decline up to 2½ years by a new biotechnology-based technology, called *Transcranial Electromagnetic Therapy* (TEMT).

Furthermore, no adverse effects were observed in the limited group of AD patients over the long time period of this study. Although the current results are encouraging for TEMT's ability to halt the progressive cognitive decline of AD, expanded placebo-controlled clinical trials are necessary to establish that ability and thus advance TEMT as perhaps the first AD therapeutic intervention that can halt or reverse this dreaded brain disease.

Arendash, G. and C. Cao argued in 2023 that TEMT has the potential to reduce the progress of several age-related diseases other than Alzheimer's (Arendash and Cao, 2023).

So the question arise if it time to apply non-ionizing radiation therapy (NIRT) to the treatment of age-related neurological diseases???

The following story will tell about how the fear of mobile radiation may be the savior of Alzheimer´s disease.

The second chapter will tell about the findings of the non-thermal effect of the microwave irradiation from GSM-mobile phones.

The following chapters will discuss possible mechanisms involved in the interaction with Alzheimers disease and summarize reported pre-clinical and clinical results.

1.4 References

ARENDASH, G. & CAO, C. 2023. Transcranial Electromagnetic Wave Treatment: A Fountain of Healthy Longevity? *International Journal of Molecular Sciences,* 24**,** 9652.

ARENDASH, G., CAO, C., LIN, X., ZHANG, X., ABULABAN, H., SMITH, A., BARANOWSKI, R., WISNIEWSKI, G., BECERRA, L., ANDEL, R., WITTWER, D., MOULTON, J. & ARRINGTON, J. 2019. A Clinical Trial of Transcranial Electromagnetic Treatment in Alzheimer's Disease: Cognitive Enhancement and Associated Changes in Cerebrospinal Fluid, Blood, and Brain Imaging. *Journal of Alzheimer's Disease,* 71**,** 57-82-82.

ARENDASH, G. W. 2016. Review of the evidence that transcranial electromagnetic treatment will be a safe and effective therapeutic against Alzheimer's disease. *Journal of Alzheimer's Disease,* 53**,** 753-771.

ARENDASH, G. W., SANCHEZ-RAMOS, J., MORI, T., MAMCARZ, M., LIN, X., RUNFELDT, M., WANG, L., ZHANG, G., SAVA, V., TANG, J. & CAO, C. 2010. Electromagnetic Field Treatment Protects Against and Reverses Cognitive Impairment in Alzheimer's Disease Mice. *Journal of Alzheimer's Disease,* 19**,** 191-210.

PERSSON, B. R. R. 2021. *More Probably than Unlikely, A Tale of the Blood-Brain Barrier and Mobile Communication* Chisinau-2068, Moldavia, Lambert Academic Publishing.

PERSSON, B. R. R., SALFORD, L. G. & BRUN, A. 1997. Blood-Brain Barrier permeability in rats exposed to electromagnetic fields used in wireless communication. *Wireless Networks,* 3**,** 455-461.

ZHI, W., ZOU, Y., MA, L., HE, S., GUO, Z., ZHAO, X., HU, X. & WANG, L. 2023. 900 MHZ electromagnetic field exposure relieved AD-like symptoms on APP/PS1 mice: A potential non-invasive strategy for AD treatment. *Biochemical and Biophysical Research Communications,* 658**,** 97-106-106.

Chapter II
Non-Thermal effects of Mobile phone radiation

2.1 BBB leakage after acute exposure to microwaves

On 25-27 May 1989, the Royal Academy of Sciences in Stockholm organized a conference on the Mechanisms for how electromagnetic fields affect living systems. They invited international expertise in the field such as Ross W. Adey, H.P Schwan, Carl F. Blackman, Abe R. Liboff and others.

The Salford group from Lund presented their studies of the effect of microwaves on albumin leakage in the blood-brain barrier in rats (Salford et al., 1992). Since then, extensive investigations demonstrated albumin leakage in the BBB at SAR values well below thermal levels. (Salford et al., 1993; Salford et al., 1994; Persson et al., 1997; Salford et al., 2000; Salford et al., 2001; Belyaev et al., 2006; Eberhardt et al., 2008; Salford et al., 2007a; Salford et al., 2007b; Nittby et al., 2008a; Salford et al., 2008; Nittby et al., 2009; Nittby et al., 2011; Persson et al., 2012; Salford et al., 2012; Persson et al., 2016).

In the studies, male and female Fischer 344 rats. Exposed to Microwaves at 915 MHz, either as continuous waves (CW) or GSM-pulse modulated at 217 Hz in a transverse electromagnetic transmission line chamber (TEM).

As shown in Figure 2-1, the opening of the BBB is most efficient at SAR values in the range 0.1-0.5 mW/kg and less efficient in the range 50-500 mW/kg. In this low SAR range, thermal effects are unlikely. Thus, there appears to be a non-thermal mechanism involved that triggers the opening of the BBB (Persson, 2021).

The results have created concern in the ranks of authorities and organizations who firmly believe that microwaves cause no other biological effects than those due to

thermal effects. Thus, the Salford group's observations of non-thermal effects judged very subtle without pathological relevance.

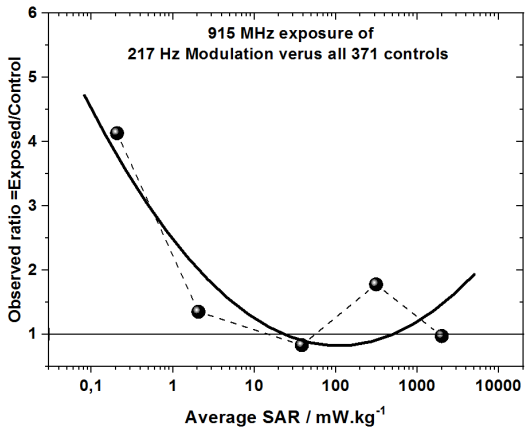


Figure 2-1

Second order polynomial fit of the observed relationship (OR) for the weighted mean score of BBB albumin leakage due to 217 Hz modulated 915 MHz exposure at varying SAR (mW.kg-1) resulting in the equation:

$$OR = 2.48 - 1.63 \times \log(SAR) + 0.40 \times [\log(SAR)]^2$$

Although with the naked eye one can see dots of brown colored Albumin in brain-samples of the rats exposed to microwaves. Despite the massive disbelief about Albumin leakage in the BBB, the Salford group continued the studies and thereby discovered dark shrunken neurons in the brain of the irradiated rats.

The pathological relevance of microwave exposure of humans must be determined by the outcome of "the world's largest biological experiment ever" as Leif Salford put it before the EU Parliament in 2000. However, he emphasized that the experiment will be difficult to evaluate, as there is no control group to compare it to, as nowadays all populations on earth more or less exposed to microwaves.

In previous generations the natural microwave exposure level has been limited to the exposure of cosmic microwaves at several thousand times lower level than nowadays.

The general opinion is that mobile telephone radiation does not increase mental illness and alleged allergic effects. So the expansion of mobile phone networks continues with new generation at even higher frequencies. In 2024, the fifth generation 5G base stations generally installing without caring about health effects. The research group in Lund did not get any resources to continue the investigations of biological effects in our "echo-free chamber" of new mobile phone generations at the higher frequencies. It seemed best not to know.

In Lund, however, studies continued to confirm the non-thermal effects of exposure with GSM mobile telephone microwaves on the blood-brain barrier and the brain's neurons. In 2003, Salford and colleagues presented in the journal Environmental Health Perspectives the results of a study of nerve damage in the mammalian brain after exposure to microwaves from GSM Mobile Phones (Salford et al., 2003).

That particular study included thirty-two Fischer 344 rats both male and female 12–26 weeks' old weighing 282±91 g, divided into four groups of eight rats each. Three groups exposed for 2 hours with GSM-900 microwaves in TEM cells, with average whole-body SAR values of 2, 20 and 200 mW/kg, while the fourth group was sham-exposed as control. After exposure, the rats allowed to stay in their cages for approximately 50 days, where we observed them daily for neurological and behavioral abnormalities. After perfusion, the rats brain cut into 5µm sections and stained with cresyl-violet to show dark neurons, and with Albumin antibodies to detect BBB leakage (Salford et al., 1994).

Controls and test animals all showed signs of albumin in the Hypothalamus, indicating that the albumin staining of the BBB leak also works. The results of the different groups' BBB leakage after 50 days were assessed by the neuropathologist Arne Brun as shown in Figure 2-2 according to the ranking from no leakage(0) to very strong leakage(3).

Figure 2-2 shows the same trend as in previous investigations, that the strongest BBB leakage occurs at the lowest SAR values.

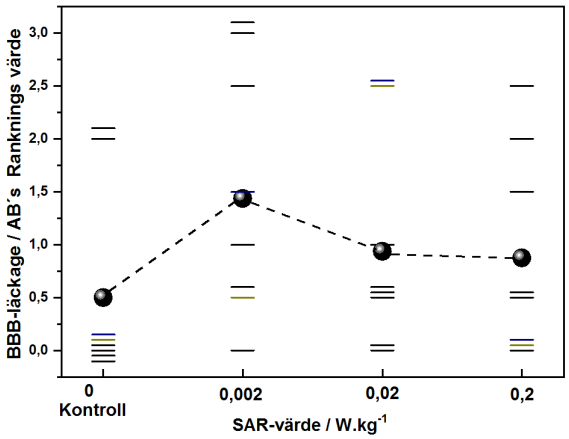


Figure 2-2
The different groups' BBB leakage after 50 days according to Arne Brun's assessment with the ranking 0= o leakage to 3= ery strong leakage.
● Indicates the mean value, and
— Marks the value for each individual animal.

Exposed animals typically showed multiple albumin-positive foci around finer blood vessels in both white and gray brain matter (Figure 2-3a).

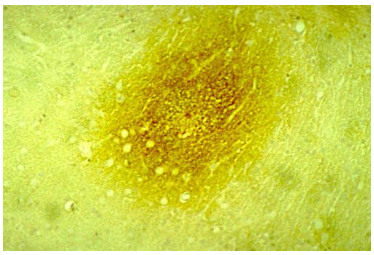


Figure 2-3a
Exposed animals usually showed albumin-positive foci around finer blood vessels in white and gray matter.

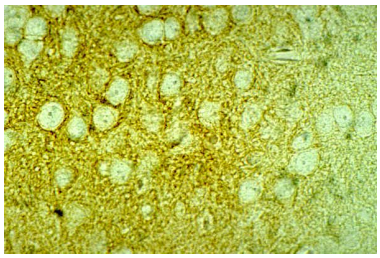


Figure 2-3b
Here the albumin has spread in the tissue

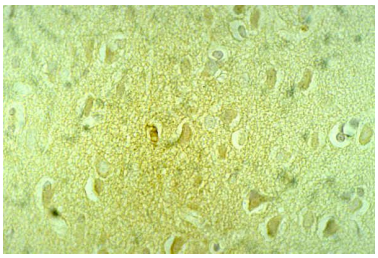


Figure 2-3c
Scattered neurons with albumin uptake

Figure 2-3b shows how the albumin has spread locally from the blood vessels into the brain tissue between the cell bodies. The neurons in Figure 2-3b did not contain any albumin, but as Figure 2-3c shows, some scattered neurons do contain albumin.

The presence of "dark nerve cells" stained with Cresyl-violet assessed by the neuropathologist Arne Brun completely without knowledge of the exposure. He ranked the occurrence of dark neurons according to the following gradation:

0 = no or single dark neurons,
1 = moderate presence of dark neurons,
2 = abundant presence of dark nerve cells.

Figure 2-4 show how Cresyl-violet staining revealed the presence of scattered and clustered dark neurons, which often shrunken and dark homogeneously stained without discernible internal cellular structures. Some of these dark neurons were also albumin positive or showed cytoplasmic microvacuoles indicating an active pathological process.

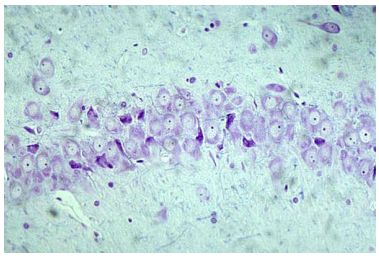
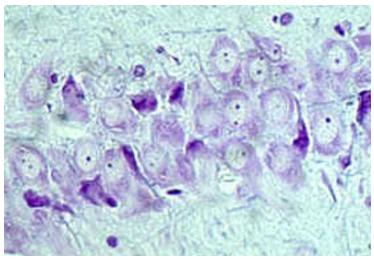
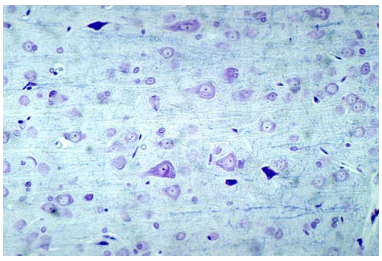


Figure 2-4a
The presence of dark neurons hippocampus



Figur 2-4b
Enlargement of area with dark neurons in the hippocampus



Figur 2-4c
The presence of dark neurons in the cortex

He observed no hemorrhages and no appreciable reactions of astrocytes, glia, or microglia located adjacent to neurons. Altered neurons appeared alongside normal neurons throughout the brain, but particularly in the cortex, hippocampus and basal ganglia. Arne Brun estimated the percentage of abnormal neurons to be approximately 2% on average, but with a larger percentage in certain limited areas.

Figure 2-5 shows the presence of dark neurons under different exposure conditions indicating a significant positive relationship with the SAR value of the microwave exposure. A combined non-parametric test with all four exposure situations simultaneously, showed that the distribution of rank scores was significantly different between the groups ($p < 0.002$).

It remains unclear whether the degree of albumin leakage observed in our previous experiments is sufficient to cause permanent damage. However, after a single exposure

with increasing SAR-values the occurrence of dark neurons after a 50-d recovery period, increased linearly up to 200 mW/kg (Salford et al., 2003).

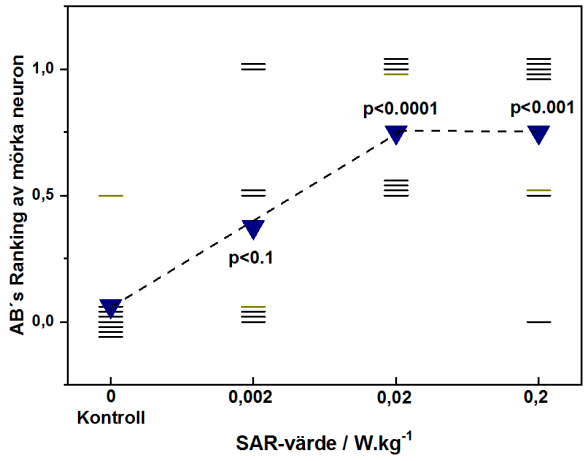


Figure 2-5

The presence of "dark neurons" was assessed blindly by the neuropathologist Arne Brun without knowledge of the exposure and ranked according to the following grading:

0 no or single dark neurons,

1 moderate presence of dark neurons

2 abundant occurrence. .

▼ Indicates the average, and

— Marks the ranking value for each animal

.

After more than 30 years of research into the non-thermal effects of electromagnetic fields from mobile phones and base stations, Salford claims:

It is more likely than not, that they have effects on the human brain (Salford, 2008).

Damaged neurons, so-called dark neurons, observed in the cortex as well as the Hippocampus and basal ganglia in the brains of rats exposed to GSM-900MHz microwaves at non-thermal SAR< 0.2 W/kg.

The presence of vacuoles in several of the observed dark neurons indicates the damage occurred in the living animal.

However, the dark neurons may also be associated with apoptotic cell death, derive from organelle damage due to release of hydrolytic lysosomal enzymes and substances that accumulate in the lysosomes.

The time between the last exposure and the perfusion fixation is of great importance for the detection of the BBB leakage around the blood vessels, because extravasated albumin diffuses quickly into the brain tissue and becomes increasingly difficult to detect with immune histology. Since albumin leakage appears even 8 weeks after the exposure, the initial albumin leakage in the brain tissue might initiate a secondary blood-brain barrier opening.

Twelve- to 26-week-old rats were included in the study so that the age-related results relate to human teenagers who are particularly frequent users of cell phones. Since the brain in its maturation process is particularly sensitive, the results of this study should seriously considered in children's' and adolescents' use of mobile phones. A neuronal injury of the kind described here may not show immediate consequences. If the exposure

repeat frequently, it can lead to a decrease in the brain's reserve capacity, with the risk of later neuronal disease or even premature aging.

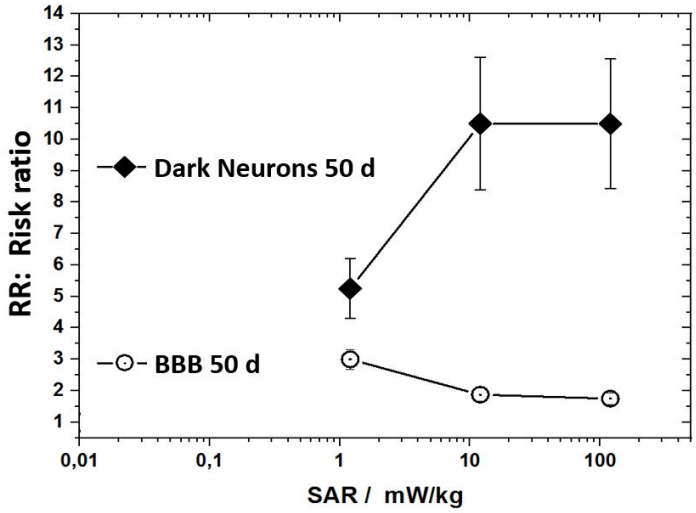


Figure 2-6

The risk ratio "RR" i.e. the number of exposed versus control animals classified as positive, at 50 days after exposure at varying SAR values

⊙ Albumin foci around blood vessels in the brain,
◆ Dark neuron

Arne Brun believes that it cannot be ruled out that after a few decades of daily use of mobile phones, a whole generation of users may suffer negative effects, perhaps already in middle age.

2.2 Other´s rat experiment concerning BBB leakage

Other rat studies of Albumin leakage into the brain parenchyma and neuron degeneration:

- Intra-carotid infusion of hyperosmolar solutions (Salahuddin et al., 1988, Salahuddin et al., 1990)
- Stroke-susceptible animals (Fredriksson et al., 1988) and
- Acute hypertension by aortic compression in rats (Sokrab et al., 1988a, Sokrab et al., 1988b, Sokrab et al., 1989, Sokrab et al., 1990b, Nordborg et al., 1991).

Furthermore, epileptic seizures cause leakage of plasma protein into the brain parenchyma, which may cause degenerated Purkinje cells in epileptic patients (Sokrab et al., 1990a, Tunkel et al., 1991, Eimerl and Schramm, 1991b, Eimerl and Schramm, 1991a)

Hassel et al. (1994) showed that injection of albumin corresponding to approximately 25% of the normal physiological serum concentration into the brain parenchyma of rats causes neuronal damage (Hassel et al., 1994).

de Gannes reported in 2009 the results of a study on blood-brain barrier permeability and neuronal degeneration in rats after head-only exposure to GSM-900 (de Gannes et al., 2009).

In the study, they exposed the heads of 16 Fischer 344 rats (14 weeks old) to the GSM-900 signal for 2 hours at different SAR values (0; 0.14; and 2.0 W/kg). Albumin leakage and neuronal degeneration were assessed 14 and 50 days after exposure and compared with cage or positive controls.

No statistically significant albumin leakage and no apoptotic neurons appear 14 days after the last exposure using the TUNEL method. Neuronal degeneration, assessed with Cresyl-violet or the more specific marker Fluoro-Jade B, was not significantly different among the groups tested (de Gannes et al., 2009).

Finnie and colleagues reported in 2001 on the effects of GSM-like radiofrequency fields on vascular permeability in mouse brain (Finnie et al., 2001). The whole mouse body exposed for 60 minutes with 898.4 MHz microwaves at SAR 4 W/kg. One group of control mice (BK) allowed to moving freely in their cages to exclude any stress-related effects while another group was sham-exposed (SK). Vascular permeability changes were detected using albumin immunohistochemistry and positive controls exposed to a toxin that increases vascular permeability in the brain.

The results of the number of animals with or without permeability changes reported in Table 2-1, and Table 2-2 show the p-values from Statistical analysis of exposed animals versus controls with Fisher's exact probability calculation and hPearson's Chi-square test.

Table 2-1

The number of observed permeability changes as reported (Finnie et al., 2001)

Experimental groups	Number of animals	Without leakage	With leakage
Exposed (EXP)	27	11	16
Cage Controller (BK)	10	8	2
Sham Exposed Controls (SK)	10	6	4
All Controls (AK)	20	14	6

Table 2-2

Statistical analysis of exposed animals versus controls using Fisher's exact probability calculation and Pearson's Chi square test

p-values of	Pearsons Chi2	Fisher Exakt one-sided	Fisher exakt two-sided
Exp versus BK		0.038	0.062
Exp versus SK		0.25	0.46
Exp versus AK	0.047	0.045	0.076

In November 2003, during the meeting at Reisensburg in Germany, **Pierre Aubineau** from France presented the results he previously presented at the Fifth International Congress of the European Bioelectromagnetic Society in Helsinki, Finland (Töre et al., 2001)

In his study, only the head exposed for up to 2 hours with 900 MHz GSM microwaves at SAR values between 0.2-3 W/kg. A qualitative analysis of albumin leakage in brain tissue slices shows leakage of plasma proteins in the rat brain and dura at non-thermal SAR levels. Figure 2-7a shows after two hours of exposure of a rat to 2 W/kg 900 MHz GSM microwaves plasma protein leakage in the BBB (Aubineau and Töre, 2003).

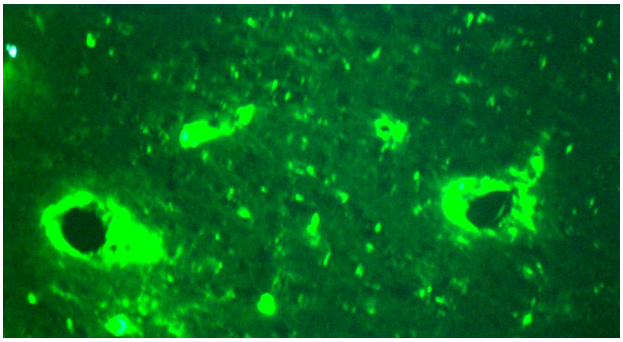


Figure 2-7a
Extravasation of Fluorescein labelled plasma proteins in the rat brain dura and BBB. (photo Pierre Aubineau 2003) (Aubineau and Töre, 2003).

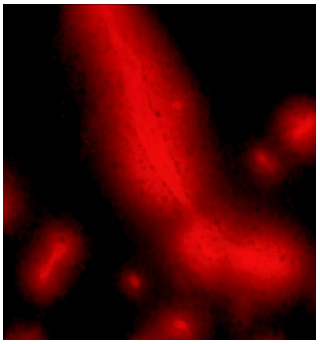


Figure 2-7b
Extravasation of Evans Blue stained plasma proteins in the BBB of rat brain (photo Arne Brun)

Figure 2-7 shows the similarity between the albumin leakages in the brain's vessels as observed with Fluororesin albumin by Pierre Aubineau in France, and that observed with albumin stained with Evans Blue by Arne Brun in Lund.

Tang and colleagues presented in 2015**,** a study, supported by the National Academy of Sciences of China (NSFC), regarding albumin leakage in the blood-brain barrier and the cognitive changes in rats exposed to 900 MHz microwaves (Tang et al., 2015 153).

A special device consisting of a round plastic tube cage (diameter: 5.5 cm, length: 12 cm) and a dipole antenna was used for continuous microwave exposure at 900 MHz with an average power density of 1±0.4 mW/cm^2. The specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head).

They exposed male Sprague-Dawley rats to 900 MHz microwaves for 3 hours per day. One-hundred-eight rats included in the study, with one group exposed for 14 days, another for 28 days and a group of sham-exposed controls.

Morphological changes examined by ultrastructural changes in the Hippocampus and cortex. Evans Blue assay assess blood brain barrier (BBB) damage. In rats exposed

for 28 days, cellular edema and neuronal cell organelle degeneration appeared in the rats.

In addition, BBB leakage of albumin in the Hippocampus and Cortex observed with immunostaining. They also found that microwave exposure for 28 days induced mkp-1 gene expression, resulting in ERK de-phosphorylation.

Taken together, their results showed that exposure to 900 MHz microwaves for 28 days partly impair spatial memory and partly cause BBB leakage of albumin in rats by activating the MKP-1/ERK signaling pathway (Tang et al., 2015 153).

Figure 2-8 shows quantification of the BBB permeability after microwave exposure as a ratio between the amounts of the dye Evans blue in the respective exposed group versus the control group. Evans blue ratio was significantly higher in the 14-day group than in the control ($p<0.05$). Furthermore, the ratio was even higher in the 28-day group with significance $p<0.01$. Thus, BBB permeability was maintained for at least 28 days.

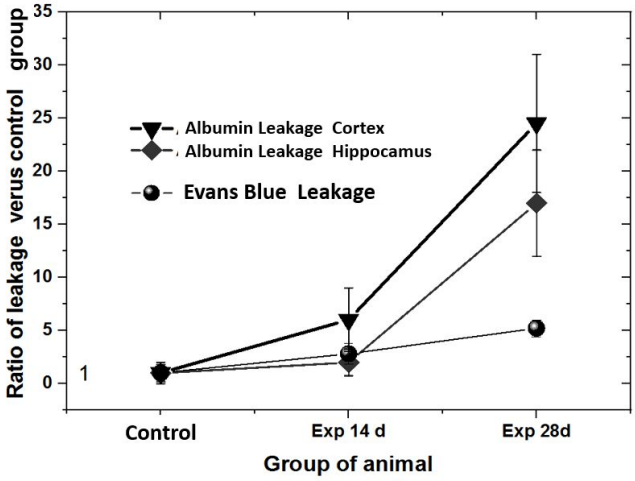


Figure 2-8
The relationship between the leakage of Evans blue and serum Albumin in the BBB in controls after 14d and 28 days of exposure 3 hours per day, respectively (Tang et al., 2015).

After exposure to 900 MHz microwaves 3 hours per day for 14 and 28 days, respectively, serum albumin had diffused into the neuropil between the cell bodies, which surround the neurons. Scattered neurons with positive albumin staining were present in the Hippocampus and Cortex. The results show that albumin uptake is higher in the cortex than in the hippocampus regions of rats in the 28 d group both in the 14 d group and in the sham-exposed control rats ($p < 0.01$) (Tang et al., 2015). These results are in line with the results published by Salford and colleagues in 2003 (Salford et al., 2003).

With the aim of elucidating the potential molecular pathways underlying the changes shown above, immunostaining of hemeoxygenase-1 (HO-1) carried out in neurons. Western blot used to determine HO-1 expression, phosphorylated ERK expression and mkp-1 expression. Thus, they found that microwave exposure for 28 days increased the

induction of the expression of MKP-1, resulting in increased ERK de-phosphorylation (Tang et al., 2015 121).

de Gannes and co-workers reported in 2017 the results of another study with 16 Fischer-344 rats (14 weeks old) where only the head was exposed to the GSM-900 signal for 2 hours at different SAR 0.026, 0.26, 2.6, and 13 W/kg (de Gannes et al., 2017).

Fifty days after repeated GSM or UMTS exposure, no change in neuronal degeneration detected at brain SAR values of 2.6 W/kg. However, at a SAR value of 0.026 W/kg, a statistically significant increase in degenerating neurons appeared in the medial cortex and the medial forebrain after GSM exposure and in the medial forebrain after UMTS exposure (de Gannes et al., 2017). This agree with the results of the Lund study that the lowest SAR-value result in largest effect (Persson, 2023)

2.3 Mechanism of Albumin's BBB-leakage

Shivers, and coworkers showed in 1987, that magnetic resonance imaging transiently alters blood-brain barrier permeability in rats (Shivers et al., 1987).

They showed that exposure to a short (23.2 min) standard clinical magnetic resonance imaging (MRI) procedure induces a temporary dysfunction of the blood-brain barrier in rats. Using freeze-fracture electron microscopy, they observed an increased vesicle-mediated transport of the protein tracer horseradish-peroxidase (eng. *Horse-Radish-Peroxidase* HSP) across micro-vessel endothelium in MR-exposed frontal cortex micro vessels of rat brains. This was the prelude to the studies of BBB-albumin-leakage carried out in Lund and referenced in previous chapters.

De Bock and coworkers presented in 2016**,** an overview of different transcellular transport pathways across the blood-brain barrier (De Bock et al., 2016).

Efficient neuronal signaling in the central nervous system strictly depends on a well-balanced microenvironment around glial cells, synapses and axons. Unique properties of the endothelium in the blood-brain barrier (BBB) largely determine the composition of its microenvironment with surrounding astrocytes and pericytes. BBB endothelial cells are equipped with a highly restrictive junctional complex that blocks the intercellular gap, thereby preventing para-cellular diffusion.

Para-cellular transport refers to the transfer of substances across an epithelium by passing through the intercellular space between cells. It is in contrast to transcellular transport, where the substances travel through the cell and pass through both the apical

membrane and the basolateral membrane. The para-cellular pathway is judged to be less likely concerning Albumin

However, trans-cytotic events, including (macro) pinocytosis, Clathrin-dependent and caveolin-dependent endocytosis of vesicles to the opposite membrane may contribute to increased BBB permeability, as the junctional complex remains intact between the endothelial cells.

De Bock summarizes the knowledge of transcellular BBB leakage observed in various pathological conditions and experimental studies.

In addition, it hypothesized that nonselective large-pore Connexin and Pannexin channels may contribute to direct transcellular transport through a direct diffusion pathway across the endothelial monolayer. However, the signaling ion Ca^{2+} involved in many steps of the vesicular pathway can indirectly control intracellular transport.

This is of particular interest because Baureus-Koch and coworkers in Lund in 2003 demonstrated an interaction between low-frequency magnetic fields and Ca^{2+} transport in cell membranes (Baureus-Koch et al., 2003). Time-varying magnetic fields with frequencies between 7 and 72 Hz and amplitudes between 13 and 114 μT are shown to interact with Ca-channel protein in cell membranes. The results agreed well with models of frequency-amplitude dependence of molecular biological effects of weak low-frequency magnetic fields (Viacheslav and Elena, 2023).

Stoll and coworkers presented in 2009 that transient widespread changes of the blood-brain barrier after cerebral photo-thrombosis observed with magnetic resonance imaging, using Gadofluorine-M (Gf) as contrast agent (Stoll et al., 2009).

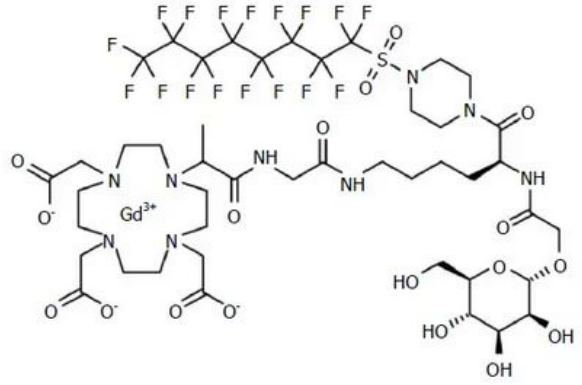


Figure 2-9
The MRI contrast agent
Gadofluorine M (Gf)
(MW 1530 Da)

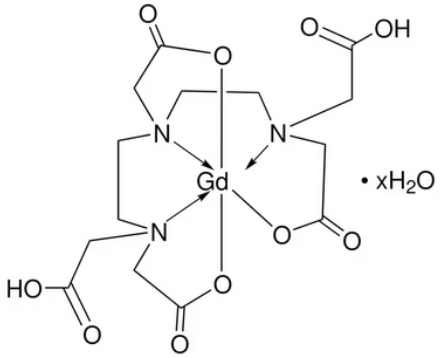


Figure 2-10 Gadolinium-DiethyleneTriamine-Pentaacetic Acid (Gd-DTPA)
(MW 938 Da)

After i.v. application, Gf led to bright contrast on T1-weighted MRI in the photo-induced lesions..

Leakage of *Gadofluorine M* (Gf) was limited in time to the first 24 hours after photo-thrombosis and corresponded to a transient breakdown of the BBB observed by leakage of the dye Evans blue.

In summary, their study demonstrates that Gf can visualize subtle disruptions of the BBB in three dimensions that not detected by most commonly used Gd-DTPA.

If changes in "Tight Junctions (TJ)" would contribute to increased BBB permeability, one would rather expect an opposite response, as smaller probes penetrate the TJ more easily than larger ones. Their results thus suggest that the transcellular pathway is the most likely explanation for the observed effects. In addition to Gadofluorine-M, free albumin and Evans Blue were also present in areas distant from the lesion site (Stoll et al., 2009).

Lim and **Gleeson** in 2011, discussed macro-pinocytosis as an endocytic pathway for internalizing large molecular complexes and microorganisms (Lim and Gleeson, 2011).

Macro-pinocytosis is a regulated form of endocytosis that mediates the non-selective uptake of solute molecules, nutrients and antigens. The Actin-dependent process initiate from surface membrane folds that give rise to large endocytic vacuoles called macro-pinosomes. Macro-pinocytosis is important in a range of physiological processes; it is highly active in macrophages and dendritic cells where it is an important pathway for capturing antigens. It is also relevant to cell migration and tumor metastasis and it represents a portal of cell entry exploited by a range of pathogens.

The molecular basis for formation and maturation of macro-pinosomes has only recently begun to be discussed (Lim and Gleeson, 2011).

Kabir, in 2003 discussed how hypermobile water accumulate around actin filaments (Kabir et al., 2003).

When certain molecules and ions (solutes) introduced into water, the hydrogen-bonded network of nearby water molecules reinforces them from thermal motion as those in the bulk-phase.

Using dielectric microwave spectroscopy measure the rotational mobility (dielectric relaxation frequency) of water-hydrating proteins and the volume of hydration shells, the hydration of Actin filaments (F-actin).

Measurements indicate that F-actin exhibits both structure-forming and structure-breaking effects. Thus, apart from the water molecules with reduced rotational mobility that make up a typical hydration shell, the water molecules around the F-actin that have a much higher mobility than bulk water.

Myoglobin studied as the representative example of globular proteins, however, shows no such double hydration.

The molecular surface of actin is rich in negative charges, which together with its filamentous structure provide a structural basis for the induction of a hypermobile water state (Kabir et al., 2003).

Zhao, and co-workers reported in 2009 effects of ionic liquid properties on lipase stabilization under microwave irradiation (Zhao et al., 2009).

The enzyme activity of immobilized Candida Antarctica lipase B (Novozyme 435), increased by microwave heating when the enzyme surrounded by a layer of water molecules. However, such enhancement decreased when the reaction system was dried.

This phenomenon due by the fact that the microwaves caused overheating of the water layer near the enzyme due to it´s high dielectric constant, ε=80.

Therefore, under microwave irradiation, the surface of the enzyme is likely to have a higher temperature than the bulk solvent due to superheating of the water layer.

Taken together, the results indicate that the effect of low level microwave irradiation on enzymes can explained by superheating of the water layer near the surface of the enzymes.

Horikoshi, and co-workers reported in 2015 the results of a study of enzymatic proteolysis of peptide bonds of a metal-endo-proteinase under precise temperature control with 5.8 GHz microwave radiation (Horikoshi et al., 2015).

The proteolysis of the peptide at 37◦C by 5.8 GHz microwave irradiation enhance about twice compared to conventional heating.

The results indicate that microwaves could exert non-thermal effects in biological systems (Horikoshi et al., 2015).

De Bock and co-workers in 2016, discussed transcellular transport across the blood-brain barrier. They concluded that trans-cytoplasmatic events at the BBB actively contribute to BBB leakage in many different pathologies.

Uptake of molecules from the extracellular environment involves pathways that rely on Clathrin-dependent endocytosis of molecules as well as Clathrin-independent macro-pinocytosis and caveolac-mediated uptake.

All these endocytosis pathways ultimately generate cytoplasmic vesicles identified according to their respective diameters. But also via the presence of surface markers such as small guanine-triphosphates (GTPases. Rab proteins) that function as key regulators of eukaryotic membrane trafficking.

Intracellular vesicles intend to release their cargo at the opposite membrane via endosomes or extracellular vesicles (exosomes), or fusing with the lysosomal compartment {De Bock, 2016 #154}.

Pinocytosis is the most likely process of albumin transport mediated by the formation of motile folds in the plasma membrane. Figure 2-11 shows how the folds arise from interaction with actin filaments that cause the cell membrane to fold and fuse with itself.

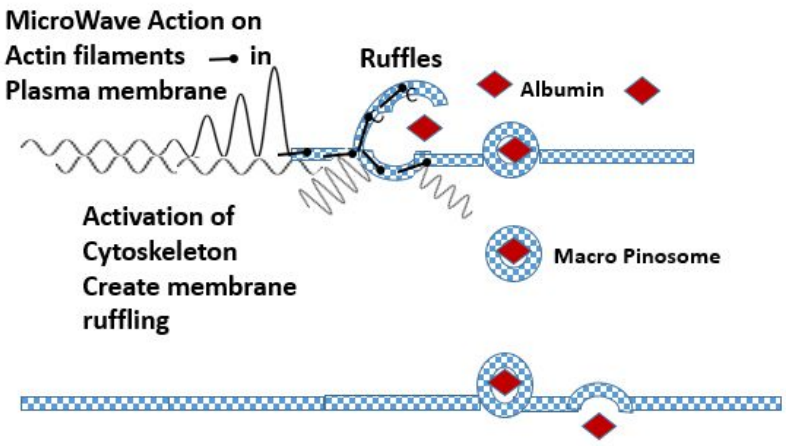


Figure 2-11
Folds in the plasma membrane formed by interaction with Actin filaments that cause the cell membrane to fold and fuse with itself (Low et al., 2016).

The fold's capture dissolved extracellular soluble substances such as blood albumin, which enclosed in vesicles migrate into the cell. These so-called macro-pinosomes have diameters from 200 to 500 nm in diameter. The uptake of the solutes via macro pinocytosis take place in a non-specific way in the cell.

Yamazaki, in 2020 discussed the effects of THz irradiation on cellular actin filaments, (Yamazaki et al., 2020 160).

The impact of terahertz (THz) radiation for deep tissues is usually neglected due to its strong absorption by water molecules on the surface of the skin. In this report, we found for the first time the demolishing effect of THz pulsed radiation on actin filaments lying in aqueous solution with a depth of a few millimeters. This result shows that the THz photon energy propagates in water several millimeters deep, possibly as a shock wave, and affected actin filaments. Thus, it THz waves seems to manipulate functions of proteins and cells in deep tissues.

Figure 2-12 illustrates how Actin filaments interact with microwaves and activate Macro-pinocytosis is the process of transporting albumin through the BBB.

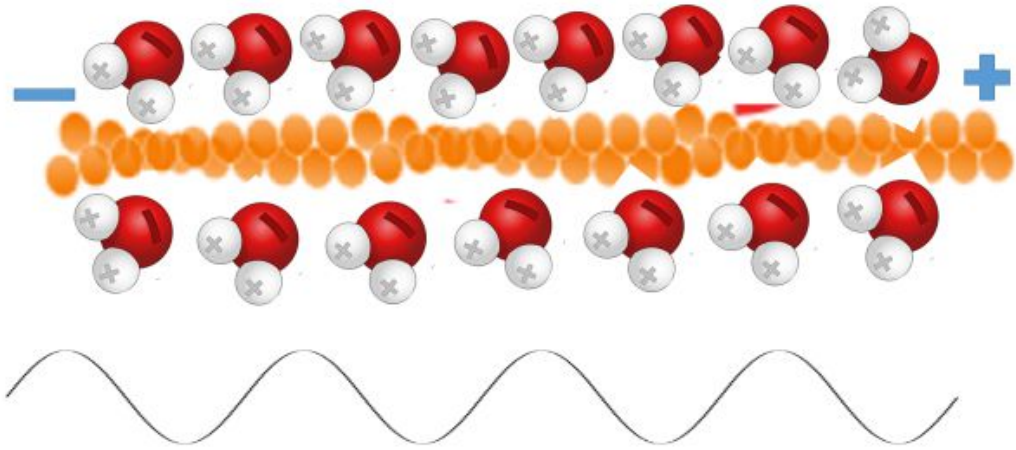


Figure 2-12

Microwaves interact with an actin filament surrounded by water molecules in the BBB membrane which stimulates macro-pinocytosis transfer of albumin

2.4 Effects after Long-term microwave exposure

Nittby and colleagues presented in 2008, the results of possible consequences for cognitive functions studied in a rat model after long-term exposure to 900 MHz (GSM-900) microwaves.

Of 56 Fischer-344 rats, 32 were exposed to microwaves from a GSM-900 test telephone at two different SAR levels of 0.6 and 60 mW/kg , Sixteen animals were sham-exposed and eight animals were cage-controls kept in the animal house. After 55 weeks of daily exposure for 2 hours each week, took place at 5-7 weeks after the last exposure.

In the episodic memory test, GSM-exposed rats showed impaired memory for objects and their temporal order, compared to sham controls ($P = 0.02$) (Nittby et al., 2008).

Grafström and coworkers presented in 2008, the results of histopathological examinations of the brain of the rats after the behavioural study above (Grafstrom et al., 2008).

To mimic the daily-life exposure to the electro-magnetic fields emitted by mobile phones, the effects of repeated exposures over a long period to GSM-900 radiation investigated in a rat model.

After the behavioral tests the brains evaluated for histopathological changes such as albumin extravasation, dark neurons. In this stud. Comparing the results of GSM-exposed animals to the controls showed no significant change in histopathological parameters (Grafstrom et al., 2008).

Regarding GFAP, lipofuscin aggregation (see Fig. 2-12), shown with Sudan Black B staining as an indicator of aging, was compared with a reference group of age-matched rats showed no signs of aging due to GSM-exposure.

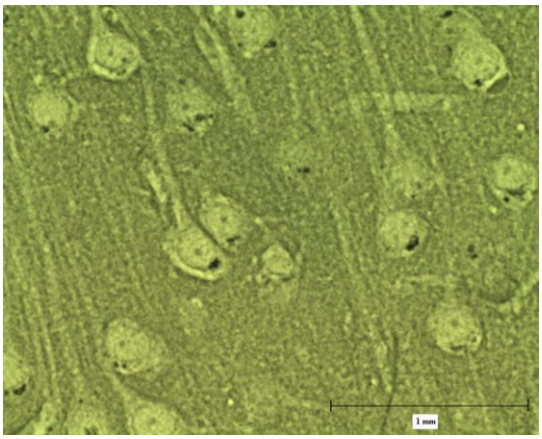


Figure 2-13

Cortex with neurons in animals exposed with SAR 60 mW/kg contains small amounts of lipofuscin that appear as black dots in the cytoplasm. Sudan Black B for lipofuscin. Magnification 100 (Grafstrom et al., 2008).

The signs of cytoskeletal and neuritic neuronal changes of the type seen in human aging visualized by the silver method of Gallyas. Figure 2-14 shows cortex with axons, visualized with the silver method of Gallyas in animals exposed with SAR 0.60 mW/kg. No differences appear between controls and exposed animals in terms of aging changes.

A few animals showed single neurons with a more conspicuous cytoskeletal tone compared to the majority of neurons in the same section. However, these were never convincingly pathological and showed no tangle formation.

In addition, there were no plaques and no granules or granulo-vacuolar bodies in the hippocampal neurons. The Kruskall-Wallis statistical test showed no significant differences between the exposure groups of high or low GSM- exposure, sham-exposed or cage-control animals.

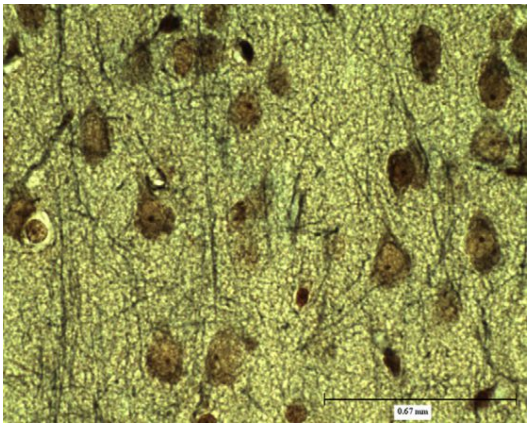


Figure 2-14.

Cortex with axons in animals exposed with SAR 0.60 mW/kg shown in black.
Gallyas silver method.
Magnification 150.
(Grafstrom et al., 2008)

In this long-term exposure study no Blood-brain Albumin permeability and no presence of dark neurons observed. which appearing in our previous BBB studies, as acute effects of short-term exposure (Grafstrom et al., 2008).

The conclusion of the study is thus that the long-term GSM-900 exposure did not result in any significant histopathological changes. Furthermore, the occurrence of aging parameters do not occur in this prolonged, almost lifelong, EMF exposure even though the parameters used are difficult to quantify and compare.

2.5 The Glymphatic system

The Glymphatic system is a novel discovered potential perivascular pathway for the elimination of solutes and waste from the brain's interstitial fluid (ISF).

Bradbury and coworkers in the 1980´s, showed that radiolabeled albumin injected into the brain parenchyma or cerebrospinal fluid (CSF) of a rabbit concentrated in neck lymph nodes (Bradbury et al., 1981).

Ichimura and co-workers studied in 1991 the distribution of extracellular tracers in perivascular spaces in the rat brain. They found that Evans-blue labeled albumin (EBA) accumulate in perivascular spaces after intracerebral injection (Ichimura et al., 1991).

High molecular weight tracers (ink or albumin labeled with colloidal gold, Evans blue, or Rhodamine) were microinjected into the perivascular space of an artery or vein on the brain surface, or into the cerebral cortex or subarachnoid space of anesthetized rats.

Subsequent distribution was followed both under intra-vital microscopy, to describe the pathways and direction of tracer movement, and in histological section, to describe the flow pathways at the light and electron microscopic level. The tracers remained largely in the perivascular spaces and in the interconnecting network of extracellular channels, including the sub-pial space and core of subarachnoid trabeculae.

Tracer also leaked across the pia into the subarachnoid CSE. Measurements of fluorescently labeled albumin indicated bulk flow of fluid in the perivascular space, around both arteries and veins, but this flow was slow, and its direction varied in an unpredictable manner.

These results confirm that perivascular spaces can act as conduits for fluid exchange between the brain and CSF, but do not support the idea that CSF circulates rapidly through brain tissue via perivascular spaces (Ichimura et al., 1991).

Iliff and coworkers presented in 2012, a para-vascular pathway that facilitates CSF flow through the brain parenchyma and elimination of interstitial solutes, including Amyloid-β (Iliff et al., 2012).

Because the brain lacks lymphatic circulation, it must clear extracellular proteins by an alternative mechanism. The cerebrospinal fluid (CSF) acts as a sink for extracellular solutes in the brain, but it is not clear how solutes from the brain interstitial fluid are moved from the parenchyma to the CSF.

Iliff and co-workers demonstrated that a significant portion of subarachnoid CSF cycles through the brain's interstitial space. On the basis of in vivo two-photon imaging of small fluorescent tracers, they showed that CSF enters the parenchyma along para-vascular spaces surrounding penetrating arteries and that brain interstitial fluid is cleared along para-venous drainage pathways.

Animals lacking Aquaporin-4 (AQP4) in astrocytes exhibit slower CSF influx through this system and a ∼70% reduction in solute clearance suggesting that bulk fluid flow between these anatomic inflow and outflow pathways is supported by astrocytic water transport.

Fluorescently labeled Amyloid-β, a peptide thought to be pathogenic in Alzheimer's disease migrate along this pathway. Deletion of the AQP4 gene suppresses the clearance of soluble Amyloid-β, suggesting that this pathway may remove Amyloid-β from the central nervous system.

Clearance by para-venous flow may also regulate extracellular levels of proteins involved in neurodegenerative conditions, its impairment perhaps contributing to the accumulation of soluble proteins (Iliff et al., 2012).

This system called "Glymphatic", given its association with Glia and its function analogous to lymphatic systems in other organs.

Iliff and coworkers, in 2013, reported the results of further study of cerebral arterial pulsatility driving para-vascular CSF-interstitial fluid exchange in the murine brain (Iliff et al., 2013).

CSF from the subarachnoid space moves rapidly into the brain along para-vascular pathways that surround penetrating cerebral arteries, mixes with brain interstitial fluid (ISF), and facilitates the clearance of interstitial solutes, such as Amyloid-β, in a pathway they have termed "Glymphatic" the system.

Iliff demonstrated that cerebral arterial pulsation is a key determinant of para-vascular CSF inflow into and through the brain parenchyma, and suggest that changes

in arterial pulsation may contribute to the accumulation and deposition of toxic solutes, including amyloid-β, in the aging brain (Iliff et al., 2013).

Wolf and coworkers presented in 2019 the results of quantitative and qualitative assessment of Glymphatic flow in rat brain using Evans blue labeled albumin (EBA) (Wolf et al., 2019).

Twenty-five µl of a 1% solution of Evansblue labeled albumin (EBA) injected into the intra-cisterna space of anesthetized postnatal rats. After injection EBA measured in serum collected at different time points. For 30 min after injection EBA was studied in vivo with a multiphoton microscope through a cranial window over the parietal cortex, The transfer of EBA from CSF to blood increased at higher intracranial pressure (ICP), which was considered to confirm the hypothesis of Glymphatic flow,

However, their experimental model disrupts Glymphatic flow through direct transit of EBA from brain to blood and reuptake of EBA from the blood into the brain via the damaged BBB (Wolf et al., 2019)

Studies of the of albumin leakage after acute microwave exposure might be used for quantitative assessment of Glymphatic flow. However, any asymmetry in the distribution of albumin around the leaking vessel that could indicate a directed flow do not occur (Eberhadt et al., 2008).

In addition, the albumin level around the vessels at different recovery times after termination of exposure to GSM-900 MHz microwaves at a SAR value of 100 mW/kg, indicates a half-life for albumin removal of 35 days. This is significantly slower than the times of less than 30 min reported by Wolf et al. for Evans Blue Labeled Albumin (Wolf et al., 2019).

2.6 Microwaves Induce Changes in Gene-expression

Alteration in the gene expression of GSM-900 microwaves

In 2006, Belyaev and coworkers conducted a study that aimed to investigate whether exposure of rat brain exposed to non-thermal levels of 900 MHz microwaves from GSM-900 mobile communication induces DNA strand breaks, changes in chromatin conformation and in gene expression (Belyaev et al., 2006).

The same exposure equipment described previously was used with a specific absorption level of SAR = 0.4 W/kg. The rats were exposed or sham-exposed for 2 hours. Immediately after the exposure, cell suspensions were prepared from different parts of the brain as well as from the spleen and thymus.

For analysis of gene expression patterns, total RNA was extracted from the cerebellum. Changes in chromatin conformation, indicative of stress response and genotoxic effects, were measured using the anomalous viscosity time dependence (AVTD) method. DNA double-strand breaks (DSBs) were analyzed by gel electrophoresis (PFGE).

The results show that GSM-900 microwaves did not induce PFGE-detectable DNA double-strand breaks or changes in chromatin conformation.

In contrast, the expression of genes in rat brain cells is affected. Gene expression profiles obtained by analysis of RNA. The results shown in Table 2-3 show the induced genes for proteins with various functions that include regulation of signaling substances, blood-brain barrier (BBB) and melatonin production.

In the cerebellum of all exposed animals, 11 genes in the table were up-regulated within a range of 1.34–2.74-fold, while the NAT1 gene was down-regulated 0.48-fold ($p < 0.0025$) (Belyaev et al., 2006). In Table 2-3, only those genes that showed statistically significant induced change are shown.

Tabell 2-3

Changes in rat gene expression in response to GSM 900 MHz microwaves. Functional annotation has been done using OMIM (http://www.ncbi.nlm.nih.gov) (Belyaev et al., 2006).

Gene symbol	Gene name	Function
Mss4	Mss4 protein	MSS4 encodes a phosphatidylinositol-4-phosphate 5-kinase that synthesizes phosphatidylinositol(4,5)-bisphosphate.
Qdpr	Quinoid dihydropteridine reductase	Co-factor in the Pterin-dependent aromatic amino acid hydroxylating systems. Involved in synthesis of monoaminergic neurotransmitters.
Natl	N-acetyltransferase 1	Detoxifies carcinogens and xenobiotics. Key role in the circadian rhythm of melatonin synthesis.
Slc6a6	Solute carrier family-6, member-6	Taurine transporter. Predominantly glial expression.
Mag	Myelin associated glycoprotein	Involved in myelination and glia-neuron interactions.
Cx3cll	Fractal ki ne (chemokine3X3C)	Activation and chemo-attraction of microglia.
GDN_RAT (Serpine2)	Glia-derived nexin-precursor	Serine protease inhibitor. Involved in synaptic plasticity.|
Rgc-32	Rgc32 protein	Cell cycle regulator)' factor.

ISI1_RAT (Insig-1)	Insulin induced growth response protein CL-6	Key regulator of cholesterol biosynthesis by interaction with IIMG-CoA Synthetase.
Collai	Collagen (I) alpha 1	Extracellular matrix structural protein. Its expression is regulated after sciatic nerve injury.
Gplbb	glycoprotein Ib	The gene for platelet glycoprotein Ib beta, a critical component of the von Willebrand factor (vWF) receptor. Blood coagulation.
Rac3	Ras-related C3 botulinum toxine substrate 1	Member of the RAS superfamily of small GTP-binding proteins involved in signal transduction .

The induced genes cover such functions as activation and chemoattraction of microglia into the infarcted tissue (Cx3cll fractalkine), cell cycle regulation (Rgc32 protein), and enzymatic hydroxylation (quinoid dihydropteridine reductase). Of particular interest is the altered expression of Slc6a6 and Nat1 genes. The Slc6a6 gene is involved in regulation of brain neurotransmitter regulation and BBB function.

The Nat1 gene that was downregulated (0.48;$P<0.0025$) encodes acetyltransferases that can acetylate endogenous arylalkylamines such as tryptamine, 5-hydroxytryptamine (serotonin), and 5-methoxytryptamine which is the immediate precursor of melatonin suggesting a possible reduced melatonin production in mobile phone users.

The observed gene expression differences for cytokines, growth factors, etc. that caurse inflammatory processes are relatively modest in the study (Belyaev et al., 2006).

In summary, the study showed that non-thermal levels of microwaves from GSM-900 mobile phones do not induce detectable DNA strand breaks in rat chromosomes and no changes in chromatin conformation,

In contrast, gene expression significantly affected in twelve genes with significance for the regulation of signaling substances, the function of the blood-brain barrier (BBB) and melatonin production.

Between the years 2005 and 2009, the study became the most referenced in the Bioelectromagnetics Journal and received the 2010 award for most influential publication.

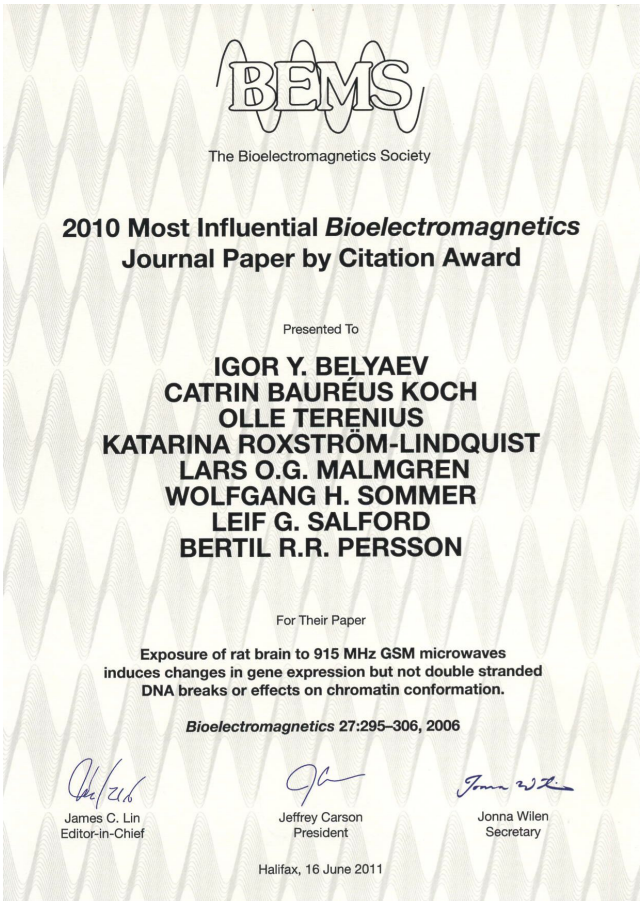


Figure 2-15

The Bioelectromagnetics Journal most Influential paper by Citation Award was established by the Bioelectromagnetics Society in 2007 to recognize scholarly contributions to the scientific community and to acknowledge and foster ongoing excellence in scientific discovery and achievement.

In 2010 our publication: *Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation.*" (Belyaev et al., 2006) received the 2010 award for most influential publication.

Alteration in the gene expression of GSM-1800 microwaves

In 2008, Nittby and coworkers reported the results of how exposure to non-thermal levels of GSM-1800 radiation changes gene expression in rat hippocampus and cortex (Nittby et al., 2008b).

The anechoic chamber used for RF exposure of the rats had a depth of 1m, width 1.1m, and height 2.0m (Malmgren 1998). The rats exposed to GSM-1800 MHz microwaves for 6 hours of exposure with a non-thermal level corresponding to a brain SAR value of 0.03W/kg.

One hour after the exposure, samples taken from the Cortex and from the underlying white matter as well as from the Hippocampus, which quickly frozen in liquid nitrogen.

Total RNA was isolated from the cortex and hippocampus after which microarray hybridizations performed on 31,099 rat genes, including splice variants in the samples from the four GSM-exposed Fischer 344 rats and four control animals.

Table 2-4 Significantly changed gene ontology categories in the hippocampus

Ratio downregulated / upregulated	Ratio
Transmembrane receptor activity	3,7
Integral to membrane	3,2
Intrinsic to membrane	3,2
Extracellular space	2,9
Extracellular region	2,8
Receptor activity	2,0
Extracellular matrix (sensu Metazoa)	2,0
Extracellular matrix	2,0
Organismal physiological process	1,6
Signal transducer activity	1,1
Hormone activity	2,0
G-protein coupled receptor protein	2,0
Signalling pathway	2,0
G-protein coupled receptor activity	2,0
Cell surface receptor linked signal	2,0
Transduction	1,2

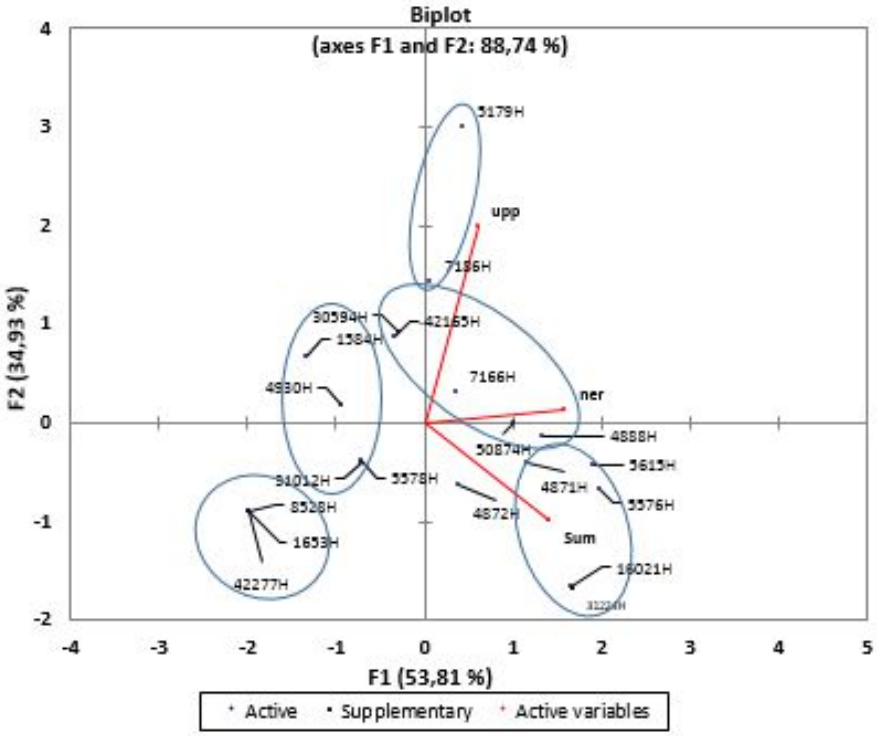
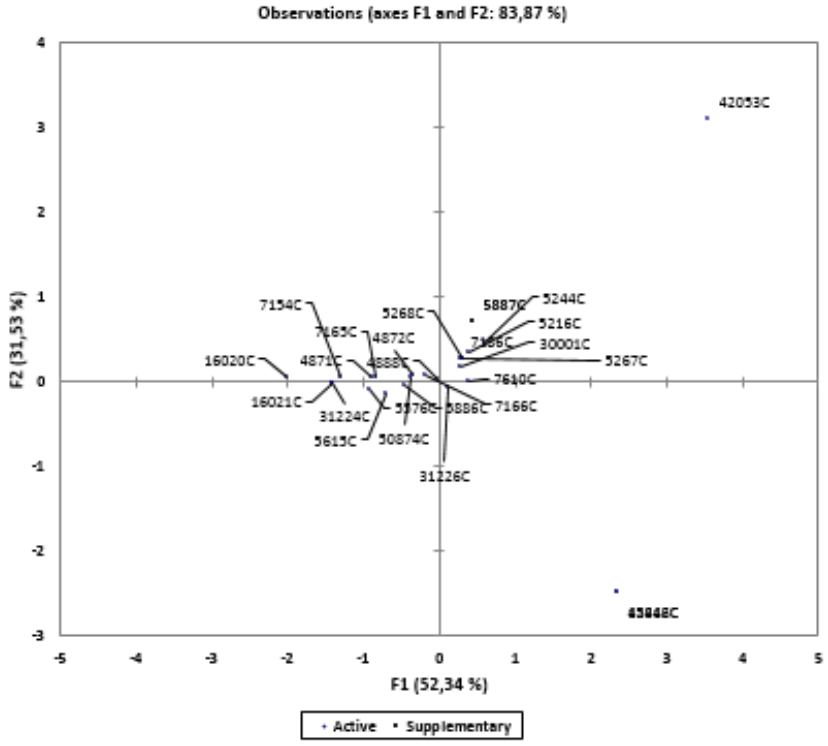


Figure 2-16

Biplot from Principal Compartment Analysis of Significantly Altered Gene Ontology Categories in the Hippocampus

Table 2-5 "*k-means clustering*" of Hippocampus PCA Factor scores: F1, F2 and F3

Class	Distance to centroid	GO-categorie	
1	0,732	5576	Extracellular region
1	1,177	5615	Extracellular space
1	0,934	4871	Signal transducer activity
1	0,870	16021	Integral to membrane
1	0,895	31224	Intrinsic to membrane
2	1,508	4888	Transmembrane receptor activity
2	0,948	4872	Receptor activity
2	0,702	50874	Organismal physiological process
2	0,996	42165	Neurotransmitter binding
2	1,002	30594	Neurotransmitter receptor activity
2	0,787	7166	Cell surface receptor linked signal transduction
3	0,264	4930	G-protein 00coupled receptor activity
3	1,029	1584	Rhodopsin-like receptor activity
3	0,639	31012	Extracellular matrix
3	0,643	5578	Extracellular matrix (sensu Metazoa)
4	0,823	7186	G-protein coupled receptor protein signaling pathway
4	0,823	5179	Hormone activity
5	0,010	42277	Peptide binding
5	0,005	1653	Peptide receptor activity
5	0,005	8528	Peptide receptor activity, G-protein coupled



Figur 2-17
Biplot from Principal Compartment Analysis of Significantly Altered Gene Ontology Categories in Cortex

Table 2-6 "*k-means clustering*" of Cortex PCA Factor scores: F1, F2 and F3

Class	Distance to centroid		GO-categorie
1	0,11	7154	Cell communication
1	0,55	5576	Extracellular region
1	0,49	4871	Signal transducer activity
1	1,09	5887	Integral to plasma membrane
1	0,22	16020	Membrane
1	0,20	3I224	Intrinsic to membrane
1	0,56	5267	Channel or pore class transporter activity
2	0,26	5886	Plasma membrane
2	0,50	31226	Intrinsic to plasma membrane
2	0,38	15842	Synaptic vesicle amine transport
2	0,12	42416	Dopamine biosynthesis
2	0,12	4872	Receptor activity
2	0,14	7I65	Signal transduction
2	0,50	42053	Regulation of dopamine metabolism
3	0,15	7186	G-protein coupled receptor protein signalling pathway
3	0,07	7l66	Cell surface receptor linked signal transduction
3	0,09	5268	Alpha-type channel activity
3	0,32	50874	Organ is ma I physiological process
3	0,36	5615	Extracellular space
3	0,05	7610	Behaviour
3	0,19	52l6	Ion channel activity
3	0,58	30001	Metal-ion transport
4	0,00	16021	Integral to membrane
4	0,00	4888	Transmembrane receptor activity
5	0,00	5244	Voltage-gated ion channel activity

Nittby's study shows that ten GO categories have changed markedly in both the Cortex and Hippocampus after the exposure (Nittby et al., 2008b). In the Hippocampus, these categories group into five quite distinct clusters. These include transmembrane receptor activity, receptor activity, cell surface receptor-linked signal transduction, and the G-protein coupled receptor-signaling pathway. In the Cortex, however, the grouping of the GO categories are more diffuse.

In fact, it is very interesting that the GO categories show significant changes even with the low number of animals included in the study and with compensatory mechanisms in live animals compared to in vitro situations.

In summary, this work shows that changes in gene expression can occur upon exposure at non-thermal levels to radiofrequency fields (Nittby et al., 2008b).

Other´s Gene-toxicity studies of RF radiation exposure

Vijayalaxmi and **Prihoda**, reported in 2009 the results of a meta-analysis of data from 88 publications (1990-2011). Regarding genetic effects in mammalian somatic cells exposed to radiofrequency radiation (Vijayalaxmi and Prihoda, 2009).

In summary, their analysis indicated the following results:

- The magnitude of the difference between RF-exposed and sham/unexposed controls was small with a few exceptions.
- Under some RF exposure conditions, there was a statistically significant increase in gene-toxicity assessed from some endpoints: the effect was observed in studies with small sample sizes and was largely affected by publication bias. Studies conducted within the generally recommended guidelines for RF exposure showed a smaller effect.
- The multiple regression analyzes and heterogeneity goodness of fit data indicated that factors other than the five variables above and the quality of publications contributed to the overall results.
- More importantly, mean indices of chromosomal aberrations, micronuclei, and sister chromatid exchange endpoints in RF-exposed and sham/unexposed controls were within the spontaneous levels reported in a large database.

In conclusion, is the classification of RF as possibly carcinogenic to humans not supported by gene-toxicity evidence (Vijayalaxmi and Prihoda, 2009).

Lai and Singh presented in 2005, the results of a study of interaction between microwaves and a temporally disjoint magnetic field on single and double DNA strand breaks in rat brain cells (Lai and Singh, 2005).

They studied DNA single- and double-strand breaks in four treatment groups of rats:

1. Microwave exposure with continuous wave 2450 MHz microwaves, at power density 1 mW/cm^2, SAR value of 0.6 W/kg,
2. Magnetic incoherent noise exposure (45 mG), as well as
3. Microwave exposure + Magnetic noise exposure
4. Sham exposure.

Animals exposed to these conditions for 2 hours. DNA single- and double-strand breaks in brain samples of these animals were analyzed 4 hours later by a micro gel-electrophoresis assay

The results show that brain samples from microwave-exposed rats had significantly increased levels of DNA single- and double-strand breaks compared to sham-exposed animals.

Exposure to magnetic noise alone did not significantly affect levels (ie, they were similar to those of sham-exposed rats).

However, co-exposure to magnetic noise blocked microwave-induced increases in DNA strand breaks.

Their data indicate that simultaneous exposure to a temporally incoherent magnetic field can block microwave-induced DNA damage in rat brain cells. (Lai and Singh, 2005).

Kumar and coworkers reported in 2021, their results of cell phone signal radiation on epigenetic modulation in the Hippocampus of Wistar rats (Kumar et al., 2021).

A total of 96 male Wistar rats were exposed in groups for 2 hours per day for 1-month, 3-month and 6-month periods at SAR 0.6 mW/kg to microwaves with the frequencies 900 MHz, 1800 MHz and 2450 MHz.

After exposure, DNA methylation and histone methylation estimated by ELISA in samples of global hippocampus. In addition, DNA methyltransferase1 (DNMT1) and Euchromatic-histone-methyltransferase1 (EHMT1) expression were evaluated by real-time PCR, and Western blot.

The results indicated occurrence of significant ($p < 0.05$) changes of epigenetic modulation in the Hippocampus of animal's exposure to 900, 1800 or 2450 MHz for 2 hours daily. Global DNA methylation(5-Mc) decreased while histone methylation (H3K9) increased in the hippocampus. Furthermore, the magnitude of the changes increased with increasing EMF frequency and exposure time (Kumar et al., 2021).

Vijayalaxmi and **Foster i**n 2023, discussed the need for consensus guidelines regarding genetic damage assessments for radiofrequency fields (Vijayalaxmi and Foster, 2023).

Their review includes evaluation of the results of >300 articles published over the past three decades on possible genotoxic effects of exposure of human and animal tissues to radiofrequency electromagnetic fields (RF-EMF). They review the development of consensus guidelines for Geno-toxicity testing and the increasing emphasis on systematic reviews for the evaluation of scientific studies for use in health risk assessments. An appendix considers some issues in assessing the Bioeffects literature by examining a subset of Geno-toxicity publications that used the comet assay.

While most studies found no statistically significant effects of exposure, a significant minority of studies (mainly in vivo studies) reported statistically significant effects of exposure (Vijayalaxmi and Foster, 2023).

Singh and co-workers reported in 2023 that in vivo 8 hours exposure to 16 Hz digitally modulated 2115 MHz microwave radiation at a SAR level of 1.51 W/kg impairs neurogenesis and causes neuronal DNA damage in the young rat brain (Singh et al., 2023).

Increased levels of lipid peroxidation, carbon-centered lipid radicals and single-stranded DNA damage observed in the cerebral cortex (CC) and Hippocampus (HP) region in the rat brain.

Comet tail data show a statistical significant (t-test. $P \leq 0.05$) increase in fragmented and migrated tail DNA between sham and exposed groups ($n = 4$).

The exposure also induced degenerative changes and neuronal loss in DG neurons but had no effect on CA3 and CA1 neurons in the hippocampus and cerebral cortex (Singh et al., 2023).

Jeong and coworkers reported in 2015 that 1950 MHz electromagnetic fields improve Amyloid-β pathology in mice with Alzheimer's disease (Jeong et al., 2015).

They studied how exposure with 1950 MHz microwaves affected AD pathology in vivo using Tg-5xFAD mice as a model of AD-like Amyloid-β (Aβ) pathology. The transgenicTg-5xFAD and wild-type (WT) mice were exposed for 8 months to at SAR 5W/kg for 2 hours per day, 5 days per week with 1950 MHz microwaves modulated according to “Wideband Code Division Multiple Access”. WCDMA is a type of cellular-phone technology that developed as a third-generation (3G) mobile communications standard. That technology developed in the 1980s and used in second generation GSM modulation. But WCDMA uses a wider frequency band and provides higher data rates than previous versions of CDMA.

The 1950 MHz exposure of Tg-5xFAD mice caused a significant reduction of both Aß plaques, APP, and APP carboxyl terminal fragments (CTFs) throughout the brain, including the Hippocampus and entorhinal cortex. The ratio of $A\beta_{1\text{-}42}$ to $A\beta_{1\text{-}40}$ peptide also decreased in the Hippocampus.

They also found that parenchymal expression of β-amyloid precursor protein-cleaving enzyme-1 (BACE1) as well as neuro-inflammation reduced by 1950 MHz exposure in Tg-5xFAD. In addition, EMF exposure was shown to prevent memory impairment in Tg-5xFAD.

Gene profiling using micro-array data from hippocampus in Tg-5xFAD revealed after EMF exposure that 5 genes (TSHZ2, GM12695, ST3GAL1, ISX and TLL1), which are involved in Aβ, were significantly altered.

These findings indicate that chronic 1950 MHz microwave exposure directly affects AP pathology in AD but not in normal brain. Therefore, 1950 MHz microwave exposure may have a beneficial impact on AD (Jeong et al., 2015).

Son, and coworkers reported in 2023 that long-term exposure to 1950 MHz electromagnetic fields attenuates cognitive dysfunction in 5×FAD mice by regulating microglial function (Son et al., 2023).

Previous studies showed that long-term effects of radiofrequency electromagnetic field exposure in 5×FAD mice with severe late-stage Alzheimer's disease reduced both Amyloid-β deposition and glial activation, including microglial regulation of activated microglia (Jeong et al., 2015).

In their present study, microglia gene expression profiles and the presence of microglia in the brain of 5×FAD mice at 1.5 months of age were analyzed. A control group was sham-exposed and another group was exposed with 1950 MHz radio frequency electromagnetic fields with SAR values of 5 W/kg for 2 hours per day and 5 days/week for 6 months.

They showed that exposure to radiofrequency electromagnetic field for 6 months improved cognition and reduced Amyloid-β deposition. In addition, microglial proliferation in the hippocampus of 5×FAD mice treated with radiofrequency electromagnetic fields was reduced compared to those in the sham-exposed control group.

The expression levels of genes related to micro gliosis and microglial function in the exposed group compared with a CSF1R inhibitor (PLX3397)-treated group.

Both radiofrequency electromagnetic fields and PLX3397 suppressed the levels of genes related to micro gliosis (Csf1r, CD68 and Ccl6) and the pro-inflammatory cytokine interleukin-1. In addition, the expression levels of genes related to microglial function, including Trem2, Fcgr1a, Ctss, and Spi1, decreased after long-term radiofrequency electromagnetic field exposure, which was also observed in response to microglial suppression by PLX3397.

These results showed that radiofrequency electromagnetic field improved Amyloid-ß pathology and cognitive impairment by suppressing Amyloid-ß deposition-induced micro-gliosis and their key regulator, CSF1R (Son et al., 2023).

2.7 References

AUBINEAU, P. & TÖRE, F. 2003. Head exposure to 900 MHz microwaves induces plasma protein extravasation in the rat brain and dura mater at non-thermal SAR levels. . Workshop: The Blood–Brain Barrier—Can it be Influenced by RF-field Interactions?,, 2003. Schloss Reisensburg, Germany.

BAUREUS-KOCH, C., SOMMARIN, M., PERSSON, B. R. R., SALFORD, L. G. & EBERHARDT, J. L. 2003. Interaction between weak low frequency magnetic fields and cell membranes. *Bioelectromagnetics,* 24**,** 395-402.

BELYAEV, I. Y., BAUREUS KOCH, C., TERENIUS, O., ROXSTROM-LINDQUIST, K., MALMGREN, L. O. G., SOMMER, W. H., SALFORD, L. & PERSSON, B. R. 2006. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics,* 27**,** 295-306.

BRADBURY, M. W., CSERR, H. F. & WESTROP, R. J. 1981. Drainage of cerebral interstitial fluidinto deep cervical lymph of the rabbit. *Am. J. Physiol.,* 240**,,** 29–36.

DE BOCK, M., VAN HAVER, V., VANDENBROUCKE, R. E., DECROCK, E., WANG, N. & LEYBAERT, L. 2016. Into Rather Unexplored Terrain-Transcellular Transport Across the Blood-Brain Barrier. *Glia,* 64**,** 1097-1123.

DE GANNES, F. P., BILLAUDEL, B., TAXILE, M., HARO, E., RUFFIE, G., LEVEQUE, P., VEYRET, B. & LAGROYE, I. 2009. Effects of Head-Only Exposure of Rats to GSM-900 on Blood-Brain Barrier Permeability and Neuronal Degeneration. *Radiation Research,* 172**,** 359-367.

DE GANNES, F. P., MASUDA, H., BILLAUDEL, B., POQUE-HARO, E., HURTIER, A., LEVEQUE, P., RUFFIE, G., TAXILE, M., VEYRET, B. & LAGROYE, I. 2017. Effects of GSM and UMTS mobile telephony signals on neuron degeneration and blood-brain barrier permeation in the rat brain. *Scientific Reports,* 7.

EBERHARDT, J. L., PERSSON, B. R. R., BRUN, A. E., SALFORD, L. G. & MALMGREN, L. O. G. 2008. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. *Electromagnetic Biology and Medicine,* 27**,** 215-229.

EIMERL, S. & SCHRAMM, M. 1991a. Acute glutamate toxicity and its potentiation by serum-albumin are determined by the ca2+ concentration. *Neuroscience Letters,* 130**,** 125-127.

EIMERL, S. & SCHRAMM, M. 1991b. Acute glutamate toxicity in cultured cerebellar granule cells - agonist potency, effects of ph, zn2+ and the potentiation by serum-albumin. *Brain Research,* 560**,** 282-290.

FINNIE, J. W., BLUMBERGS, P. C., MANAVIS, J., UTTERIDGE, T. D., GEBSKI, V., SWIFT, J. G., VERNON-ROBERTS, B. & KUCHEL, T. R. 2001. Effect of global system for mobile communication (GSM)-like radiofrequency fields on vascular permeability in mouse brain. Pathology 33 (3):338-340.

FREDRIKSSON, K., KALIMO, H., NORDBORG, C., JOHANSSON, B. B. & OLSSON, Y. 1988. Nerve-cell injury in the brain of stroke-prone spontaneously hypertensive rats. *Acta Neuropathologica,* 76**,** 227-237.

GRAFSTROM, G., NITTBY, H., BRUN, A., MALMGREN, L., PERSSON, B. R. R., SALFORD, L. G. & EBERHARDT, J. 2008. Histopathological examinations of rat brains after long-term exposure to GSM-900 mobile phone radiation. *Brain Research Bulletin,* 77**,** 257-263.

HASSEL, B., IVERSEN, E. G. & FONNUM, F. 1994. Neurotoxicity of albumin in-vivo. *Neuroscience Letters,* 167**,** 29-32.

HORIKOSHI, S., NAKAMURA, T., KAWAGUCHI, M. & SERPONE, N. 2015. Enzymatic proteolysis of peptide bonds by a metallo-endoproteinase under precise temperature control with 5.8-GHz microwave radiation. *Journal of Molecular Catalysis. B, Enzymatic,* 116**,** 52-59.

ICHIMURA, T., CSERR, H. F. & FRASER, P. A. 1991. Distribution of extracellular tracers in perivascular spaces of the rat brain. *Brain Research,* 545**,** 103-113-113.

ILIFF, J. J., WANG, M., LIAO, Y., PLOGG, B. A., PENG, W., VATES, G. E., DEANE, R., GOLDMAN, S. A., NEDERGAARD, M., GUNDERSEN, G. A., NAGELHUS, E. A. & BENVENISTE, H. 2012. A paravascular pathway facilitates CSF flow through the brain

parenchyma and the clearance of interstitial solutes, including amyloid β. *Science Translational Medicine,* 4.

ILIFF, J. J., WANG, M., ZEPPENFELD, D. M., VENKATARAMAN, A., PLOG, B. A., LIAO, Y., DEANE, R. & NEDERGAARD, M. 2013. Cerebral arterial pulsation drives paravascular CSF–interstitial fluid exchange in the murine brain. *The Journal of Neuroscience,* 33**,** 18190-18199.

JEONG, Y. J., KANG, G. Y., KWON, J. H., CHOI, H. D., PACK, J. K., KIM, N., LEE, Y. S. & LEE, H. J. 2015. "1950 MHz Electromagnetic Fields Ameliorate Aβ Pathology in Alzheimer's Disease Mice." *Curr Alzheimer Res* 12 (5):481-92.

doi: 10.2174/1567205012051505261144448.

KABIR, S. R., YOKOYAMA, K., MIHASHI, K., KODAMA, T. & SUZUKI, M. 2003. Hyper-mobile water is induced around actin filaments. *Biophysical Journal,* 85**,** 3154-3161.

KUMAR, R., DESHMUKH, P. S., SHARMA, S. & BANERJEE, B. D. 2021. Effect of mobile phone signal radiation on epigenetic modulation in the hippocampus of Wistar rat. *ENVIRONMENTAL RESEARCH,* 192**,** 110297.

LAI, H. & SINGH, N. P. 2005. Interaction of Microwaves and a Temporally Incoherent Magnetic Field on Single and Double DNA Strand Breaks in Rat Brain Cells. *Electromagnetic Biology & Medicine,* 24**,** 23-29.

LIM, J. P. & GLEESON, P. A. 2011. Macropinocytosis: an endocytic pathway for internalising large gulps. *IMMUNOLOGY AND CELL BIOLOGY,* 89**,** 836-843.

LOW, P., MOLNÁR, K. & KRISKA, G. 2016. *Atlas of Animal Anatomy and Histology*

NITTBY, H., BRUN, A., EBERHARDT, J., MALMGREN, L., PERSSON, B. R. R. & SALFORD, L. G. 2009. Increased blood–brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. *Pathophysiology,* 16**,** 103-112.

NITTBY, H., BRUN, A., STROMBLAD, S., MOGHADAM, M. K., SUN, W., MALMGREN, L., EBERHARDT, J., PERSSON, B. R. & SALFORD, L. G. 2011. Nonthermal GSM RF and ELF EMF effects upon rat BBB permeability. *Environmentalist,* 31**,** 140-148.

NITTBY, H., GRAFSTROM, G., EBERHARDT, J. L., MALMGREN, L., BRUN, A., PERSSON, B. R. R. & SALFORD, L. G. 2008a. Radiofrequency and extremely low-frequency electromagnetic field effects on the blood-brain barrier. *Electromagnetic Biology and Medicine,* 27**,** 103-126.

NITTBY, H., WIDEGREN, B., KROGH, M., GRAFSTRÖM, G., BERLIN, H., REHN, G., EBERHARDT, J. L., MALMGREN, L., PERSSON, B. R. R. & SALFORD, L. G. 2008b. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. *The Environmentalist,* 28**,** 458-465.

NITTBY, H., GRAFSTROM, G., TIAN, D. P., MALMGREN, L., BRUN, A., PERSSON, B. R. R., SALFORD, L. G. & EBERHARDT, J. 2008c. Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics 29 (3):219-232. doi: 10.1002/bem.20386.

NORDBORG, C., SOKRAB, T. E. O. & JOHANSSON, B. B. 1991. The relationship between plasma-protein extravasation and remote tissue changes after experimental brain infarction. *Acta Neuropathologica,* 82**,** 118-126.

PERSSON, B. R. R., BRUN, A., MALMGREN, L., EBERHART, L., NITTBY H & SALFORD, L. G. 2016. Analysis and Modelling Blood-Brain Barrier Leakage of Albumin in Rats exposed to 915 MHz Microwaves at various Length of Time, Modulation frequencies and SAR

Acta Scientiarum Lundensia, 2016**,** 1-20.

PERSSON, B. R. R., MALMGREN, L., BRUN, A. & SALFORD, L. G. 2012. Non-Thermal" Effects on the Blood-Brain Barrier in Fischer rats by exposure to microwaves (ISSN 1651-5013). *Acta Scientiarum Lundensia,* 2012-006**,** 1-39.

PERSSON, B. R. R., SALFORD, L. G. & BRUN, A. 1997. Blood-Brain Barrier permeability in rats exposed to electromagnetic fields used in wireless communication. *Wireless Networks,* 3**,** 455-461.

PRIHODA, T. J. 2008. Genetic Damage in Mammalian Somatic Cells Exposed to Radiofrequency Radiation: A Meta-analysis of Data from 63 Publications (1990-2005). *Radiation Research,* 169**,** 561-574.

SALAHUDDIN, T. S., JOHANSSON, B. B., KALIMO, H. & OLSSON, Y. 1988. Plasma-protein extravasation in blood-brain-barrier injuries - an immunocytochemical and light microscopic study. *Clinical Neuropathology,* 7**,** 204-204.

SALAHUDDIN, T. S., JOHANSSON, B. B., KALIMO, H. & OLSSON, Y. 1990. Extravasation of plasma-proteins and structural-changes in the rat-brain after intracarotid infusions of hypertonic solutions. *Pathophysiology of the Blood-Brain Barrier: Long Term Consequences of Barrier Dysfunction for the Brain (Editos Johansson, B. B.Owman, C.Widner, H.)* 14**,** 131-134.

SALFORD, L. G. More probable than unlikely, that non-thermal electromagnetic fields from mobile phones and base stations do have effects upon the human brain. 1st Hellenic congress - the effects of electromagnetic radiation, 2008 Thessalonica, 24-25 May 2008.

SALFORD, L. G., BRUN, A., EBERHARDT, J., MALMGREN, L. & PERSSON, B. 1992. Electromagnetic field- induced permeability of the blood-brain barrier shown by immunohistochemical methods. *In:* NORDEN, B. & RAMEL, C. (eds.) *Interaction Mechanism, of Low-Level Electromagnetic Fields in Living Systems,.* Oxford Oxford University Press.

SALFORD, L. G., BRUN, A., EBERHARDT, J. L. & PERSSON, B. R. R. 1993. Permeability of the blood-brain-barrier induced by 915 MHz electromagnetic-radiation, continuous wave and modulated at 8, 16, 50 and 200 hz. *Bioelectrochemistry and Bioenergetics,* 30**,** 293-301.

SALFORD, L. G., BRUN, A. & PERSSON, B. R. R. 2012. GSM 900 MHz exposure of Transgenic GFAP-Mice: A study of Histopathology, Tumour induction and BBB leakage. *Acta Scientiarum Lundensia,* 2012-003**,** 1-17.

SALFORD, L. G., BRUN, A., STURESSON, K., EBERHARDT, J. L. & PERSSON, B. R. R. 1994. Permeability of the blood-brain-barrier induced by 915 MHz electromagnetic-radiation, continuous-wave and modulated at 8, 16, 50, and 200 Hz. *Microscopy Research and Technique,* 27**,** 535-542.

SALFORD, L. G., BRUN, A. E., EBERHARDT, J. L., MALMGREN, L. & PERSSON, B. R. R. 2003. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. *Environmental Health Perspectives,* 111**,** 881-883.

SALFORD, L. G., NITTBY, H., BRUN, A., GRAFSTROM, G., MALMGREN, L., SOMMARIN, M., EBERHARDT, J., WIDEGREN, B. & PERSSON, B. R. R. The mammalian brain in the electromagnetic fields designed by man with special reference to blood-brain barrier function, neuronal damage and possible physical mechanisms. 22nd Nishinomiya-Yukawa Memorial Symposium, Oct 15-20 2007a Kyoto, JAPAN. Progress Theoretical Physics Publication Office, 283-309.

SALFORD, L. G., NITTBY, H., BRUN, A., GRAFSTROM, G., MALMGREN, L., SOMMARIN, M., EBERHARDT, J., WIDEGREN, B. & PERSSON, B. R. R. 2008. The mammalian brain in the electromagnetic fields designed by man with special reference to blood-brain barrier

function, neuronal damage and possible physical mechanisms. *Progress of Theoretical Physics Supplement***,** 283-309.

SALFORD, L. G., NITTBY, H., KROGH, M., GRAFSTRÖM, G., BERLIN, H., REHN, G., EBERHARDT, J., MALMGREN, L., PERSSON, B. R. R. & WIDEGREN, B. 2007b. Exposure to Global System for Mobile Communications at 1800 MHz Significantly Changes Gene Expression in Rat Hippocampi and Cortex *To be submitted 2007*.

SALFORD, L. G., PERSSON, B., MALMGREN, L. & BRUN, A. 2000. Mobile communication and the blood-brain barrier. *Journal of Vascular Research,* 37**,** 74-74.

SALFORD, L. G., PERSSON, B. R. R., MALMGREN, L., BRUN, A. & LANNOYE, P. 2001. Telephonie mobile et barrire Sang-cerveau (in french) Mobile Communication and the Blood-Brain Barrier. *Telephonie Mobile: Effets potentiels sur la sant, des ondes ,lectromagn,tiques de hautes fr,quences.* B-4053 Embourg, Belgique: MarcoPpietteur.

SHIVERS, R. R., KAVALIERS, M., TESKEY, G. C., PRATO, F. S. & PELLETIER, R. M. 1987. Magnetic-resonance-imaging temporarily alters blood-brain-barrier permeability in the rat. *Neuroscience Letters,* 76**,** 25-31.

SINGH, K. V., PRAKASH, C., NIRALA, J. P., NANDA, R. K. & RAJAMANI, P. 2023. Acute radiofrequency electromagnetic radiation exposure impairs neurogenesis and causes neuronal DNA damage in the young rat brain. *Neurotoxicology,* 94**,** 46-58.

SOKRAB, T. E. O., JOHANSSON, B. B., KALIMO, H. & OLSSON, Y. 1988a. A transient hypertensive opening of the blood-brain-barrier can lead to brain-damage - extravasation of serum-proteins and cellular-changes in rats subjected to aortic compression. *Acta Neuropathologica,* 75**,** 557-565.

SOKRAB, T. E. O., JOHANSSON, B. B., TENGVAR, C., KALIMO, H. & OLSSON, Y. 1988b. Adrenaline-induced hypertension - morphological consequences of the blood-brain-barrier disturbance. *Acta Neurologica Scandinavica,* 77**,** 387-396.

SOKRAB, T. E. O., KALIMO, H. & JOHANSSON, B. B. 1989. Endogenous serum-albumin content in brain after short-lasting epileptic seizures. *Brain Research,* 489**,** 231-236.

SOKRAB, T. E. O., KALIMO, H. & JOHANSSON, B. B. 1990a. Blood-brain-barrier and neuronal damage in acute experimental-hypertension and epileptic seizures. *Pathophysiology of the Blood-Brain Barrier: Long Term Consequences of Barrier Dysfunction for the Brain (Editors Johansson, B. B.Owman, C.Widner, H.),* 14**,** 159-163.

SOKRAB, T. E. O., KALIMO, H. & JOHANSSON, B. B. 1990b. Parenchymal changes related to plasma-protein extravasation in experimental seizures. *Epilepsia,* 31**,** 1-8.

SON, Y., PARK, H. J., JEONG, Y. J., LEE, H. J., CHOI, H. D. & KIM, N. 2023. "Long-term radiofrequency electromagnetic fields exposure attenuates cognitive dysfunction in 5×FAD mice by regulating microglial function." *Neural Regeneration Research* 18 (11):2497-2503-2503. doi: 10.4103/1673-5374.371379.

STOLL, G., KLEINSCHNITZ, C., MEUTH, S. G., BRAEUNINGER, S., IP, C. W., WESSIG, C., NÖLTE, I. & BENDSZUS, M. 2009. Transient widespread blood-brain barrier alterations after cerebral photothrombosis as revealed by gadofluorine M-enhanced magnetic resonance imaging. *Journal of Cerebral Blood Flow and Metabolism,* 29**,** 331-341-341.

TANG, J., ZHANG, Y., YANG, L. M., CHEN, Q. W., TAN, L., ., ZUO, S. L., FENG, H., CHEN, Z. & ZHU, G. 2015. Exposure to 900 MHz electromagnetic fields activates the mkp-1/ERK pathway and causes blood-brain barrier damage and cognitive impairment in rats. *Brain Research,* 1601**,** 92-101.

TÖRE, F., DULOU, P.-E., HARO, E., VEYRET, B. & AUBINEAU, P. Two-hour exposure to 2 W/kg, 900 MHz GSM microwaves induces plasma protein extravasation in rat brain. . In:

HIETANEN, M., JOKELA, K. & JUUTILAINEN, J., eds. 5th International Congress of the European Bioelectromagnetics Association, 6 September 2001, 2001 Helsinki, Finland. Helsinki:Finnish Institute of Occupational Health, 43–45.

TUNKEL, A. R., ROSSER, S. W., HANSEN, E. J. & SCHELD, W. M. 1991. Blood-brain-barrier alterations in bacterial-meningitis - development of an invitro model and observations on the effects of lipopolysaccharide. *In Vitro Cellular & Developmental Biology,* 27**,** 113-120.

VIACHESLAV, V. K. & ELENA, A. O. 2023. Molecular Biological Effects of Weak Low-Frequency Magnetic Fields: Frequency–Amplitude Efficiency Windows and Possible Mechanisms. *International Journal of Molecular Sciences,* 24**,** 10989-10989.

VIJAYALAXMI & FOSTER, K. R. 2023. The need for consensus guidelines to address the mixed legacy of genetic damage assessments for radiofrequency fields. *International Journal of Radiation Biology,* 99**,** 1016-1026-1026.

VIJAYALAXMI & PRIHODA, T. J. 2009. Genetic damage in mammalian somatic cells exposed to extremely low frequency electro-magnetic fields: a meta-analysis of data from 87 publications (1990-2007). *International Journal of Radiation Biology,* 85**,** 196-213.

WOLF, M. S., CHEN, Y., SIMON, D. W., ALEXANDER, H., ROSS, M., GIBSON, G. A., MANOLE, M. D., BAYıR, H., KOCHANEK, P. M. & CLARK, R. S. B. 2019. Quantitative and qualitative assessment of glymphatic flux using Evans blue albumin. *Journal of Neuroscience Methods,* 311**,** 436-441.

YAMAZAKI, S., HARATA, M., TSUBOUCHI, M., OGAWA, Y., ISOYAMA, G., OTANI, C. & HOSHINA, H. The Effects of THz Irradiation on Cellular Actin Filament. 2020 Buffalo, NY, Piscataway, NJ, USA, USA. IEEE.

ZHAO, H., BAKER, G. A., SONG, Z., OLUBAJO, O., ZANDERS, L. & CAMPBELL, S. M. 2009. Effect of ionic liquid properties on lipase stabilization under microwave irradiation. *Journal of Molecular Catalysis. B, Enzymatic,* 57**,** 149-157.

Chapter III
Alzheimer's disease and Microwave Interaction

3.1 Introduction

In 2016 the professor emeritus in neuropathology Arne Brun, presented a review of recent discoveries in Alzheimer´s disease, which widen the basis for future research (Brun, 2016).

The review intended to offer a general orientation on Alzheimer's disease (AD) with comments on the brain changes and clinical features. However, more importantly, it points out recent research findings, which may offer new alleys for AD research pertinent to the etiology and pathogenesis of AD. This new knowledge thus comes from various fields of research such as epigenetics, pointing to possible environmental etiologic factors. Further, exosomes may provide information on the state of the neuronal population for diagnostic purposes and might become useful as carriers of therapeutic substances. The newly disclosed protein complexity of the synapses may harbor a large yet unexplored field for neuro-chemical research pertinent to the early or likely initial loss of synapses in Alzheimer disease. The finding of a more generalized neuronal gene disturbance in Alzheimer's disease shifts the focus from age related changes to developmental disturbances and increased neuronal vulnerability. BBB incompetence with a start in the hippocampus, may initiate the degenerative process of Alzheimer's disease.

Finally, recent basic research findings on glial evolution, underscoring the distance between humans and rodents, our prime disease model animal, points to several new unexplored mechanisms, which may be relevant for the understanding of

neurodegenerative processes. Also stressed is the need to institute treatment at an early stage of the disease, necessitating research for markers, which will enable a diagnosis way ahead of the widespread damage present at the time of clinical debut (Brun, 2016).

The present story will present new ideas of microwave interactions with Amyloid plaques in Alzheimer's disease.

3.2 Amyloid-beta and Tau

Amyloid-beta precursor protein (APP) shown in Figure 3-1 is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. The origin of the APP protein and Amyloid formation probably goes back to about 500 million years ago.

The structure of APP includes an amyloid-β domain with cleavage sites for secretase enzymes. Two pathways compete for cleavage of the APP substrate, which leads to either amyloid genic or non-amyloid genic processing of the protein.

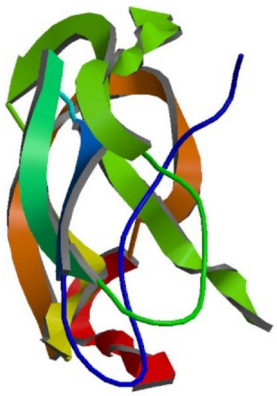


Figure 3-1
The APP protein coded by the APP-gene acts as a cell surface receptor involved as a regulator of synapse formation, neural, plasticity, antimicrobial activity and iron export.
https://en.wikipedia.org/wiki/Amyloid-beta precursor protein - Wikipedia

Amyloid-beta (Aβ) refers to peptides of 36–43 amino acids that are the main component of amyloid plaques found in the brains of people with Alzheimer's disease. The Aβ peptides derive from the amyloid-beta precursor protein (APP), which cleaved by beta-secretase and gamma-secretase form Aβ in a cholesterol-dependent process and substrate presentation. Amyloid-beta Aβ-molecules can aggregate to form flexible soluble oligomers that can exist in multiple forms.

Certain misfolded Aβ-oligomers (known as "seeds") can induce other Aβ molecules to adopt the misfolded form, leading to a chain reaction similar to a prion infection. The misfolded oligomers form tangles that are toxic to neurons.

Tau-protein is another protein implicated in Alzheimer's disease. This protein also forms prion-like misfolded oligomers, and there is some indication that misfolded Aβ also can cause misfolded Tau-protein.

Tau-protein primarily have roles in maintaining the stability of microtubules in axons and are abundant in the neurons of the central nervous system (CNS), with the highest abundance in cerebral cortex.

Alzheimer's disease is associated with Tau proteins in the form of hyper-phosphorylated insoluble aggregates.

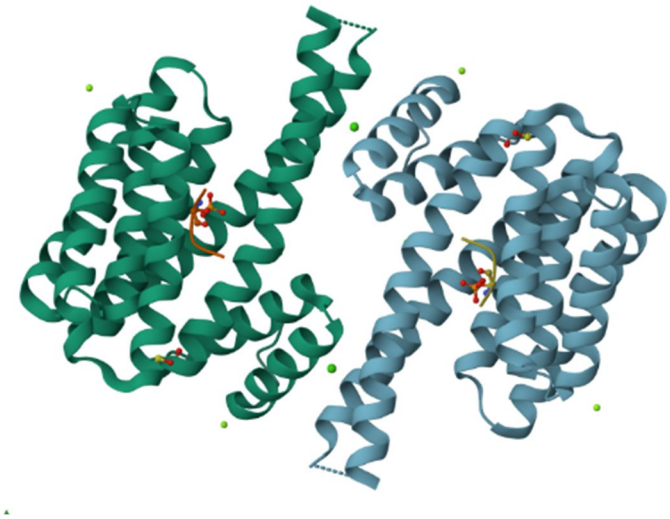


Figure 3-2

The Tau proteins, abbreviated Tau meaning from tubulin-associated unit, are a group of six highly soluble protein isoforms.
Tau protein - Wikipedia

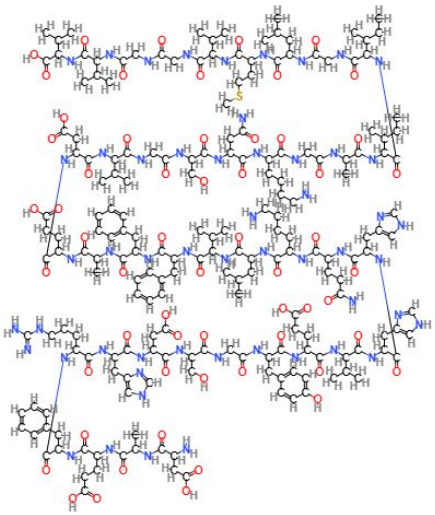


Figure 3-3a
Structural formula for Amyloid-beta peptide. (Moleview)

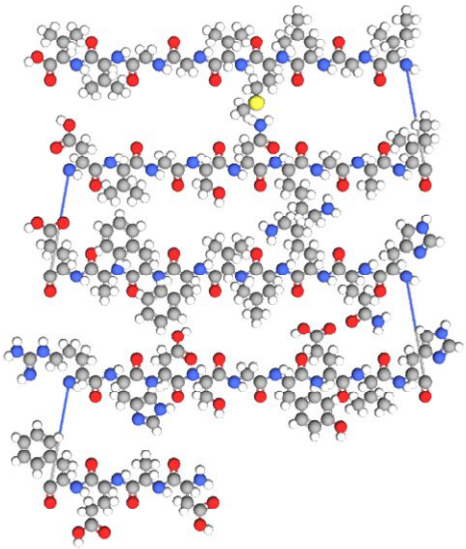


Figure 3-3b
Ball formula for Amyloid-beta peptide. H-white, C-green, N-blue, O-red (Moleview)

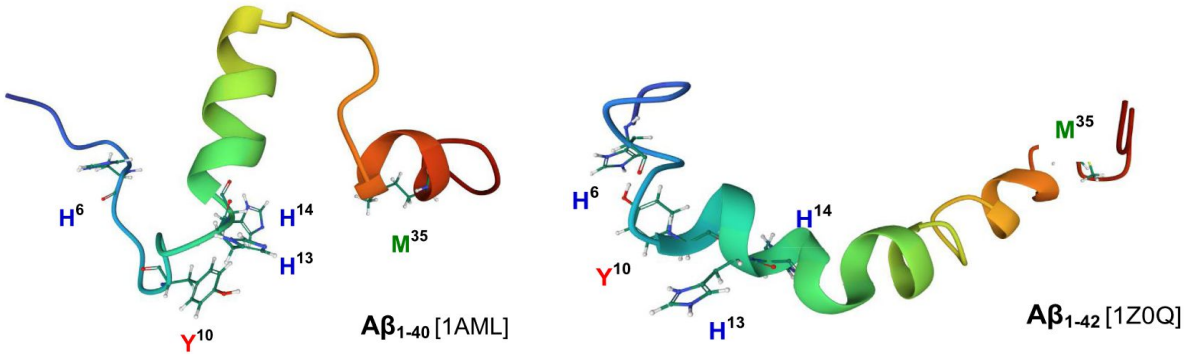


Figure 3-4

3D structures of human $A\beta_{1-40}$ and $A\beta_{1-42}$, , Aβ peptide sequences with the electro-active amino acid residues Y^{10}, H^{6}, H^{13}, H^{14} and M^{35} (Chiorcea-Paquim and Oliveira-Brett, 2022)

Amyloid beta peptide, Aβ, is a protease breakdown product of Amyloid-Precursor-Protein (APP), which forms the Amyloid fibrils that drive the pathological, insoluble and sticky Amyloid-beta plaques that cause Alzheimer's disease (AD). In the AD brain, APP is first cleaved by the β-secretase and then by the γ-secretase, leading to the formation of the predominant soluble monomers $A\beta_{1-40}$ (85-90%) and $A\beta_{1-42}$ (10-15%), This Aβ monomers production favors the formation of neurotoxic oligomers, which cluster as plaques around meningeal and cerebral vessels and brain grey matter (Chiorcea-Paquim and Oliveira-Brett, 2022).

Figure 3-5a shows the non-amyloidogenic pathway for the cleavage of APP in the amyloid-β domain by α-secretase, a complex that contains ADAM metalloproteases, releases the soluble ectodomain sAPPα and the C-terminal fragment CTFα. The subsequent cleavage of CTFα by γ-secretase produces a soluble extracellular p3 peptide and the APP intracellular domain (AICD) (Canter et al., 2016).

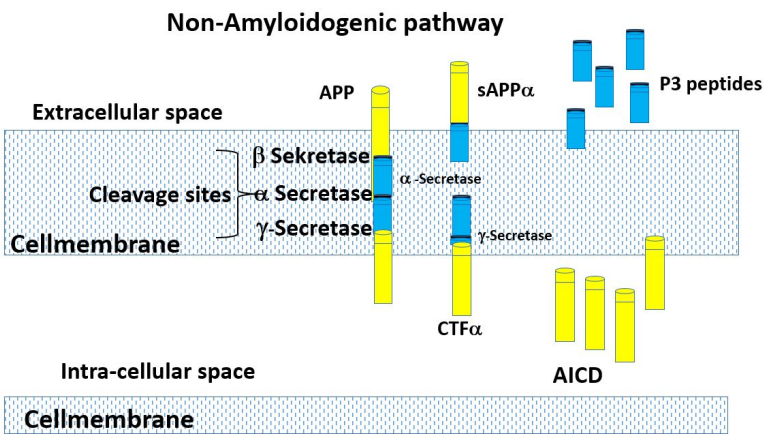


Figure 3-5a

In the non-amyloidogenic pathway, APP is first cleaved by α-secretase,

Then the C-terminal fragment further cleaved by γ-secretase to produce a 3-kDa peptide (p3) (Canter et al., 2016).

Figure 3-5b shows the Amyloido genic plaque formation pathway that involves APP cleavage by β-secretase (also known as BACE1), which releases the soluble ecto-domain sAPPβ and CTFβ.

Cleavage of CTFβ by γ-secretase yields Amyloid-β peptides of varying lengths as well as the AICD fragment. The pathogenic impact of amyloid-β peptides varies with length. The longer Amyloid-β(1–42) and Amyloid-β(1–43) species are more prone to aggregation and prion-like seeding, These structures seem to be more toxic than shorter Amyloid-β peptides, the ratio of Amyloid-β(1–42) and Amyloid-β(1–40) might predict the severity of AD (Canter et al., 2016).

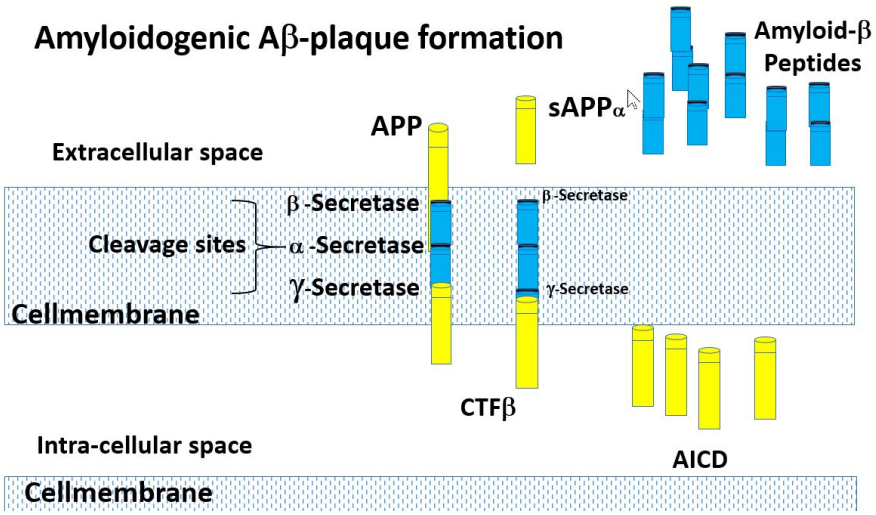


Figure 3-5b
In the amyloido genic pathway, the APP is first cleaved by β-secretase.
In the second step γ-secretase releases Amyloid-β (Canter et al., 2016).

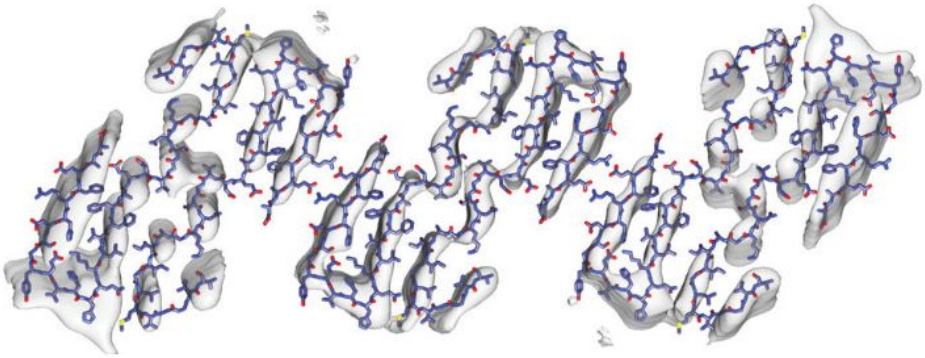
Aβ fibrils are arranged as tubular and paired helical filaments (Ferrari et al., 2003). Spiral symmetry can assemble and organize complex molecular structures from the nano-scale to the visible millimeter scale. The growth is associated with polymorphism, whose molecular structure constitutes a so-called C3 symmetry, implemented in a network (Antonijevic et al., 2023).

Studies of the structure of Aß fibrils with high-resolution cryo-electron microscopy and with NMR technology show that there are several environment-dependent polymorphisms (Saxena et al., 2023).

Alzheimer's disease (AD) involves a progressive loss of memory and cognition, which according to genetic studies is associated with Amyloid-β. However, drug-based treatment strategies against AD aimed at reducing Amyloid-β have difficulty reducing cognitive symptoms. Clinical findings suggest that cognitive decline is the result of a complex pathophysiology and that targeting Amyloid-β alone may not be sufficient to treat Alzheimer's disease (Brun, 2016). A broader view of neural circuit-damaging processes expect to provide insights into new therapeutic strategies to cure amnesia in the disease (Canter et al., 2016).

Kollmer and co-workers, in 2019, reported on the structure and polymorphism of Aβ Amyloid fibrils extracted from the brain tissue of deceased Alzheimer patients (Kollmer et al., 2019).

They showed that Alzheimer's disease characterized by the presence of Aβ Amyloid fibrils and Tau protein neuro-fibrillary tangles. Kollmer used cryo-electron microscopy to structurally characterize brain-derived Aß-amyloid fibrils and show that they are polymorphic and dextrorotatory, which diffes from in vitro-generated Aβ fibrils (Kollmer et al., 2019).



Figur 3-7
Aβ-amyloid fibrils extracted from Alzheimer brain tissue (Kollmer et al., 2019).

The origin of multi-pathway pathogenesis has made Alzheimer's fatally contagious in the brain. Through polymorphism of the fibrils, a rapid replication triggered in large brain regions. The nucleation of the primary Aβ fibrils appears to be an extremely slow process. However, once a "seed" generated, the "crystallization" process appears to proceed deadly rapidly via multi-channel communication (Saxena et al., 2023).

Based on the mechanistic nucleation-based polymerization, researchers have attempted to develop a holistic approach to disintegrate Aβ plaques or stop their replication. However, different approaches such as removing Aβ fibrils or reducing the number of soluble Aβ have not yet yielded promising results.

Immunotherapy specifically targeting plaques can alleviate progressive plaque formation and prevent further aggregation, but without improvement in cognition. In human clinical trials, however, potential drugs often prove ineffective even after successful results in animal trials.

Söderberg and coworkers reported in 2023 how antibody drugs such as *Lecanemab, Aducanumab* and *Gantenerumab* in clinical trials for Alzheimer's disease generated binding profiles to different forms of Amyloid-beta (Söderberg et al., 2023).

Aβ exists in various forms, including monomers, oligomers, protofibrils, and insoluble fibrils in plaques. Oligomers and protofibrils have been shown to be toxic, and removal of these aggregates may represent an effective treatment for AD.

Söderberg and coworkers characterized the binding properties of Lecanemab, Aducanumab and Gantenerumab to different Aβ species using inhibition ELISA, immune depletion and surface plasmon resonance. All three antibodies bound monomers with low affinity. However, *Lecanemab* and *Aducanumab* had very weak binding to monomers, while *Gantenerumab* had slightly stronger binding.

Lecanemab was distinct because it had ten times stronger binding to soluble forms of Amyloid-beta, called protofibrils, compared to insoluble fibrils. *Aducanumab* and *Gantenerumab* prefer binding to insoluble fibrils over soluble protofibrils.

The results show different binding profiles for *Lecanemab*, *Aducanumab* and *Gantenerumab* that may explain clinical results observed for these antibodies regarding both efficacy and side effects (Söderberg et al., 2023).

Their study piqued my interest to use microwaves, which we found open the BBB for blood albumin, to try to promote the transport of the drugs through the BBB. But literature searches on this instead gave me links to how Amyloid-β deposits in the brain could be broken down by exactly the same type of pulsed microwave field we used in our BBB experiments (Persson et al., 1997; Arendash et al., 2019; Arendash, 2016).

3.3 Clathrin

Clathrin is a protein that plays a major role in the formation of coated vesicles. Clathrin firstly isolated and named by Barbara Pearse in 1976.

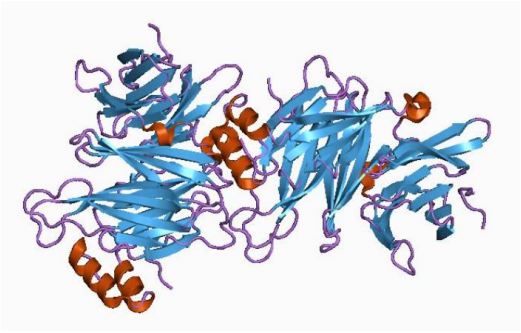


Figure 3-7
Clathrin protein
https://en.wikipedia.org/wiki/Clathrin

The Clathrin protein forms a triskelion shape consisting of three heavy chains and three light chains. When the triskelion interact, they form a polyhedral lattice that surrounds the vesicle. The protein's name, which in Latin *clathrum* means lattice.

Coat proteins, such as Clathrin, which used to build the small vesicles that transport molecules through the membrane out and into the cells. Endocytosis and exocytosis of vesicles allow cells to communicate, transfer nutrients, import signaling receptors,

mediate an immune response from the extracellular world, and excrete waste molecules (https://en.wikipedia.org/wiki/Clathrin).

Clathrin is like a molecular scaffold for vesicular uptake of agents at the plasma membrane, where its assembly into cage-like lattices lies below the Clathrin-coated pits in the cell membrane in classical endocytosis.

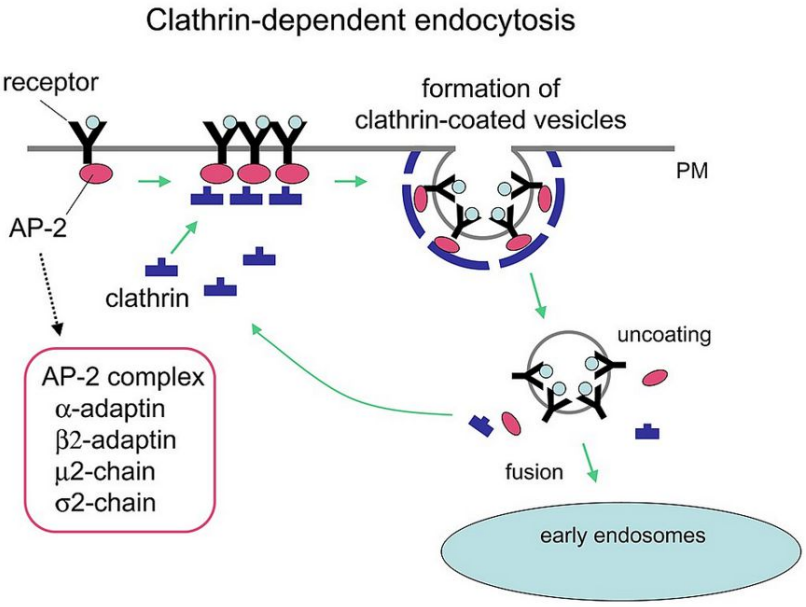


Figure 3-8
Plasma membrane with a pit formed by newly formed Actin filaments, and coated with Clathrin trapping the receptor binding the agent to be transferred (Grant, B. D. and Sato, M, 2006).

(from en.wikipedia)

http://www.wormbook.org/chapters/www_intracellulartrafficking/intracellulartrafficking.html

Clathrin and cargo molecules are assembled into clathrin-coated pits on the plasma membrane together with an adaptor complex called AP-2 that links clathrin with transmembrane receptors, concluding in the formation of mature clathrin-coated vesicles (CCVs). CCVs are then actively uncoated and transported to the endosomes (Grant, B. D. and Sato, M, 2006).

Kirchhausen and coworkers describe the structures of Clathrin, large cargo adapters, and other proteins that participate in forming a Clathrin-coated pit, loading its contents, pinching off the membrane as a grid-enclosed vesicle, and recycling the components. They integrate as much of the structural information as possible into a sketch of the main steps in coated pit and coated vesicle formation (Kirchhausen et al., 2014).

Clathrin protein covers breakdown products and foreign substances to vesicles, sorts and cleans the waste in the cells. The degradation product of the Amyloid precursor protein Aβ and its aggregated fibrils are associated with Alzheimer's disease. Why Clathrin fails to stop the rapid intertwining is debatable. In contact with a hexagonal

close-packed organic substrate, Aβ(1-42) fibrils form an electromagnetically sensitive fractal superstructure.

Using two independent experimental techniques with microwave and , we have discovered the electrical pulse-generating ability (i.e., beating/perturbation) of Aβ fractal networks that fine-tunes Clathrin-mediated disassembly by inducing a step-by-step morphogenesis.

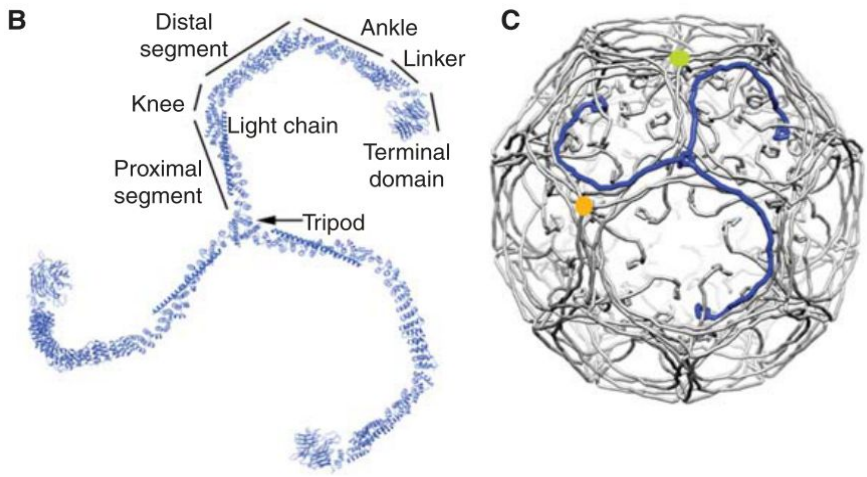


Figure 3-9

Clathrin triskelion consists of three Clathrin heavy chains that interact at their C termini, each ~190 kDa heavy chain has a ~25 kDa light chain tightly bound to it. The three heavy chains form the structural backbone of the Clathrin lattice, and the three light chains are thought to regulate the formation and disassembly of a Clathrin lattice (Kirchhausen et al., 2014).

Amyloid-β can form fractal-like antenna networks that can interact with electromagnetic pulses (EMPs).

These findings highlight how Amyloid-β deposits in the brain could be broken down by electromagnetic pulses (EMP) and prevent the rapid spread of Alzheimer's disease (Saxena et al., 2023).

3.4 Effect of microwaves on protein structure

In *vitro* studies

De Pomerai, and co-workers showed in 2000 that microwave radiation increases the growth of the nematode Caenorhabditis elegans (De Pomerai et al., 2000a).

They showed that long-term, about 8 h, exposure to continuous microwave fields can induce increased growth in the nematode worm Caenorhabditis elegans.

However, they found no detectable increase in temperature of either medium or worms during overnight exposure under these conditions, ruling out both generalized and localized (worm-specific) heating effects. They concluded that microwave exposure induced growth responses by one or more non-thermal pathways (de Pomerai et al., 2000b).

Thus, microwaves can exert non-thermal effects in biological systems, probably because of changes in the conformation of cellular proteins.

De Pomerai, and co-workers showed again in 2003 that microwave radiation can change protein conformation without bulk heating (de Pomerai et al., 2003).

Calabrò, E. and S. Magazù reported 2010 Non-thermal Microwaves Effects on Proteins Secondary Structure studied by Means of Fourier Transform Infrared Spectroscopy (Calabrò and Magazù, 2010).

In their study the effects of microwaves on the secondary structure of three typical proteins have been investigated. A set of samples of Lysozyme, Bovine- serumalbumin and Myoglobin in D_2O solutions were exposed for 8 hours to mobile phone microwaves at 900 MHz at SAR=0.85 W/Kg, lesser than the ICNIRP thermal limits of 2 W/kg. They studied the relative effects on the secondary structure of the proteins by means of Fourier Transform Infrared Spectroscopy.

Their results show an increase of the Amide-I band intensity in the secondary structure of proteins after the microwaves exposure. Furthermore, a weak shift of the Amide-I mode of Bovine-serum albumin and a heavier shift of the Amide-I of Myoglobin occurred after the exposure. In addition, they observed a clear increasing of the β-sheet components with respect to the α-helix content in the spectra of bovine serum albumin and myoglobin after the exposure, suggesting the hypothesis of the formation of aggregates.

The results led to conclude that non-thermal microwave exposure affect the secondary structure of proteins like Lysozyme, Bovine-serumalbumin and Myoglobin (Calabrò and Magazù, 2010).

3.5 The mechanism of EM destruction of AD plaque

Experiments by **Gosh** and co-workers in 2016 reveal that Aß in contact with a hexagonal close-packed Clathrin substrate forms a fractal-like antenna network. It allows interwoven Aβ bundles to respond to electromagnetic pulsed fields that may

explain the effect on treatment of Aβ plaques in brain regions of AD and AD mice treated with electromagnetic fields (Ghosh et al., 2016).

Clathrin transfers the nutrients inside and outside the cells, identifying and deforming the target protein and eliminating cell debris through endocytosis and exocytosis. Gosh and co-workers used two experimental techniques that could independently record the time-dependent disintegration processes of Aβ fibrils with the Clathrin chains.

With the help of imaging in a cryo-electron microscope, the time-dependent disintegration of Aβ fibrils could studied. Their study reveals that Clathrin light chains CLC disrupt Aβ aggregates and Clathrin heavy chains CHC alter structure and functionality of Aβ aggregates. Together, the Clathrin chains accelerate degradation.

During the breakdown of the Aβ fractals, the force of spontaneous pulsed oscillations (beats) interact with regions of the Aβ and light Clathrin chains.

Microwave exposure may thus assist the Clathrin chains.in breaking down Aß-aggregated plaques.

Based on these findings, if Aβ causes Alzheimer's via plaque formation, the plaque can adsorb the electromagnetic excitation at 500 MHz as shown by in vitro measurement on Aβ fibrils. In addition, in vitro measurements show that the Clathrin chains contribute to electromagnetic stimulation that contributes to plaque disaggregation in vivo.

The measurement of Aβ fibrils with a self-heterodyne Fabry Perot interferometer array revealed the action of the pulsatile signal-generating ability of Aβ networks. This means that the network communicates wirelessly by creating ordered signals from noise. Communication via electric field showed that the charged conductive surface of Aβ network reflects a strong light scatterer at 630 nm. Beat frequencies ensure that heavy and light Clathrin chains interact with Aβ in different ways. Thus, the Aß fibril network could respond to multiple input frequencies (Krzysztofik, 2017).

In the presence of a cell membrane or polar microtubules, fractal structures of Aβ could form a potential source of EM radiation, which transmits different frequencies depending on the length distribution in a domain. Other fractal networks such as the tau-filaments can also cause dynamic changes in the properties of neurons leading to tauopathy or inter-neuronal functional signaling (Wälti et al., 2015).

By studying the wireless signaling of Aβ fractal structures, one could study the effect of the microwave treatment and gain a deeper insight into mechanism behind.

3.6 References

Antonijevic, M., Rochais, C. & Dallemagne, P. 2023. "C3-Symmetric Ligands in Drug Design: When the Target Controls the Aesthetics of the Drug." *Molecules* 28 (2):679. doi: 10.3390/molecules28020679.

Arendash, G., Cao, C., Lin, X., Zhang, X., Abulaban, H., Smith, A., Baranowski, R., Wisniewski, G., Becerra, L., Andel, R., Wittwer, D., Moulton, J. & Arrington, J. 2019. "A Clinical Trial of Transcranial Electromagnetic Treatment in Alzheimer's Disease: Cognitive Enhancement and Associated Changes in Cerebrospinal Fluid, Blood, and Brain Imaging." *Journal of Alzheimer's Disease* 71 (1):57-82-82. doi: 10.3233/JAD-190367.

Arendash, G. W. 2016. "Review of the evidence that transcranial electromagnetic treatment will be a safe and effective therapeutic against Alzheimer's disease." *Journal of Alzheimer's Disease* 53 (3):753-771. doi: 10.3233/JAD-160165.

Brun, A. 2016. "Recent discoveries widen the basis for future research in Alzheimer´s disease. . ." *Acta Scientiarum Lundensia* 2016 (002):1-12.

Calabrò, E. & Magazù, S. 2010. "Inspections of Mobile Phone Microwaves Effects on Proteins Secondary Structure by Means of Fourier Transform Infrared Spectroscopy." Journal of Electromagnetic Analysis and Applications 2 (11):607-6017. doi: DOI: 10.4236/jemaa.2010.211080.

Canter, R. G., Penney, J. & Tsai, L.-H. 2016. "The road to restoring neural circuits for the treatment of Alzheimer's disease." *NATURE* 539 (7628):187-196. doi: 10.1038/nature20412.

Chiorcea-Paquim, A.-M. & Oliveira-Brett, A. M. 2022. Amyloid beta peptides electro.chemistry: A review. CURRENT OPINION IN ELECTROCHEMISTRY, 31, (100837): 1-10.

De Pomerai, D., Daniells, C., David, H., Allan, J., Duce, I., Mutwakil, M., Thomas, D., Sewell, P., Tattersall, J., Jones, D. & Candido, P. 2000a. "Microwave radiation induces a heat-shock response and enhances growth in the nematode Caenorhabditis elegans." *IEEE Transactions on Microwave Theory and Techniques, Microwave Theory and Techniques, IEEE Transactions on, IEEE Trans. Microwave Theory Techn.* 48 (11):2076-2081. doi: 10.1109/22.884198.

de Pomerai, D., Daniells, C., David, H., Allan, J., Duce, I., Mutwakil, M., Thomas, D., Sewell, P., Tattersall, J., Jones, D. & Candido, P. 2000b. "Non-thermal heat-shock response to microwaves." *Nature* 405 (6785):417-418. doi: 10.1038/35013144.

de Pomerai, D. I., Smith, B., Dawe, A., North, K., Smith, T., Archer, D. B., Duce, I. R., Jones, D. & Candido, P. M. 2003. "Microwave radiation can alter protein conformation without bulk heating." *Febs Letters* 543 (1-3):93-97. doi: 10.1016/s0014-5793(03)00413-7.

Dragicevic, N., Bradshaw, P. C., Mamcarz, M., Lin, X., Wang, L., Cao, C. & Arendash, G. W. 2011. "Long-term electromagnetic field treatment enhances brain mitochondrial function of both alzheimer's transgenic mice and normal mice: A mechanism for electromagnetic field-induced cognitive benefit?" *NEUROSCIENCE* 185:135-149. doi: 10.1016/j.neuroscience.2011.04.012.

Ferrari, A., Hoerndli, F., Baechi, T., Nitsch, R. M. & Götz, J. 2003. "β-Amyloid Induces Paired Helical Filament-like Tau Filaments in Tissue Culture." *Journal of Biological Chemistry* 278 (41):40162-40168. doi: 10.1074/jbc.M308243200.

Ghosh, S., Dutta, M., Ray, K., Fujita, D. & Bandyopadhyay, A. 2016. "A simultaneous one pot synthesis of two fractal structures via swapping two fractal reaction kinetic states." *Physical Chemistry Chemical Physics* 18 (22):14772-14775. doi: 10.1039/c6cp00447d.

Grant, B. D. and Sato, M. Intracellular trafficking (January 21, 2006), In; *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.77.1, http://www.wormbook.org.

Jeong, Y. J., Kang, G. Y., Kwon, J. H., Choi, H. D., Pack, J. K., Kim, N., Lee, Y. S. & Lee, H. J. 2015. "1950 MHz Electromagnetic Fields Ameliorate Aβ Pathology in Alzheimer's Disease Mice." *Curr Alzheimer Res* 12 (5):481-92. doi: 10.2174/1567205012051505261144448.

Kirchhausen, T., Owen, D. & Harrison, S. C. 2014. "Molecular structure, function, and dynamics of clathrin-mediated membrane traffic." *Cold Spring Harbor Perspectives in Biology* 6 (5). doi: 10.1101/cshperspect.a016725.

Kollmer, M., William, C., Leonie, F., Jay, R., Aref, B., Angelika, S., Matthias, S., Christina, J. S., Mathias, J. & Marcus, F. 2019. "Cryo-EM structure and polymorphism of Aβ amyloid fibrils purified from Alzheimer's brain tissue." *Nature Communications* 10 (1):1-8. doi: 10.1038/s41467-019-12683-8.

Krzysztofik, W. J. 2017. Fractals in antennas and metamaterials applications. . *In:* F, B. (ed.) *Fractal analysis-applications in physics, engineering and technology.* Rijeka, Croatia, : INTECH Open Science, .

Low, P., Molnár, K. & Kriska, G. Atlas of Animal Anatomy and Histology 2016.

Persson, B. R. R., Salford, L. G. & Brun, A. 1997. "Blood-Brain Barrier permeability in rats exposed to electromagnetic fields used in wireless communication." *Wireless Networks* 3:455-461.

Saxena, K., Singh, P., Dey, P., Wälti, M. A., Sahoo, P., Ghosh, S., Krishnanda, S. D., Riek, R. & Bandyopadhyay, A. Amyloid-β Can Form Fractal Antenna-Like Networks Responsive to Electromagnetic Beating and Wireless Signaling. Singapore: Springer Nature Singapore; 2023. 323-344 p. 10.1007/978-981-99-1916-1_251

Söderberg, L., Johannesson, M., Nygren, P., Laudon, H., Eriksson, F., Osswald, G., Möller, C. & Lannfelt, L. 2023. "Lecanemab, Aducanumab, and Gantenerumab — Binding Profiles to Different Forms of Amyloid-Beta Might Explain Efficacy and Side Effects in Clinical Trials for Alzheimer's Disease." *Neurotherapeutics* 20 (1):195-206. doi: 10.1007/s13311-022-01308-6.

Wälti, M. A., Orts, J., Vögeli, B., Campioni, S. & Riek, R. 2015. "Solution NMR studies of recombinant Aβ(1-42): From the presence of a micellar entity to residual β-sheet structure in the soluble species." *ChemBioChem* 16 (4):659-669-669. doi: 10.1002/cbic.201402595.

Chapter IV
Pre-clinical Non-thermal AD-Therapy with Microwaves

4.1 Preclinical studies

Biological effects of radiofrequency electromagnetic fields (EMFs) on the blood-brain barrier (BBB) studied already in the 1990´s in Fischer 344 rats of both sexes (Persson et al., 1997). In the study, male and female Fischer 344 rats exposed to microwaves at 915 MHz both as continuous waves (CW) or pulse modulated at 217 Hz, in a transverse electromagnetic transmission line chamber (TEM). In total, 372 controls and 630 exposed rats examined with different modulation frequencies with different pulse power and at different time intervals. The specific absorbed power was non-thermal (< 2W/kg) and the time-length of exposure varied from 2 min to 960 min.

After exposure of the un-anesthetized rats, albumin and fibrinogen analyzed immunohistochemically in brain samples. The frequency of pathological leakage through the BBB increased significantly ($p < 0.0001$) from 17% for control rats, to 39% in all exposed rats. Grouping the exposed animals according to the level of specific absorbed energy (J/kg) gives a significant difference at all levels above 1.5 J/kg.

The frequency of pathological rats (0.17) is the same in all controls. While the frequency of pathological rats was 35% among rats exposed to pulse modulated (PW) and 50% among rats exposed to continuous wave (CW) exposure (Persson et al., 1997).

A polynomial fitting of the ratios of exposed to controls frequency, clearly shown a "bathtub" shaped SAR dependence (Figure 4-1). The opening of the BBB is most efficient at SAR values in the range 0.1-0.5 mW/kg and less efficient in the range 50-500 mW/kg. In this low SAR range, thermal effects are unlikely. Thus, there appears to be a non-thermal mechanism involved that triggers the opening of the BBB (Persson, 2021).

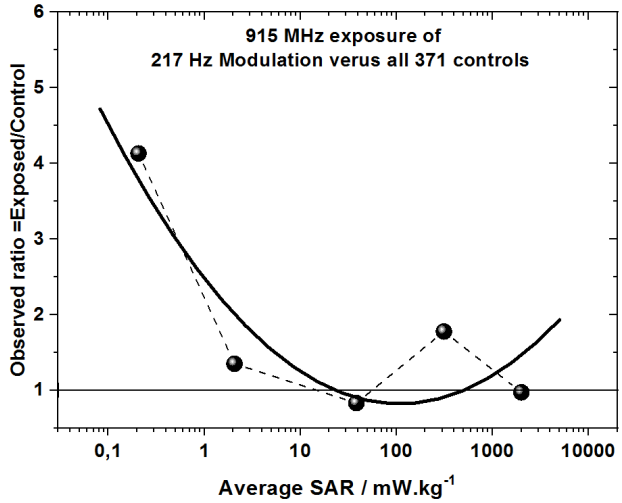


Figure 4-1

Second order polynomial fit of the observed ratio (OR) for the rats exposed at varying SAR (0.2-2000 $mW.kg^{-1}$) versus unexposed controls is expressed by the equation:

$$OR = 2.48 - 1.63 \times \log(SAR) + 0.40 \times [\log(SAR)]^2$$

Figure 4-1 shows how the observed ratios (OR) of BBB albumin leakage for 217 Hz modulated 915 MHz varies with the mean SAR values for each tenth SAR interval.

The highest and most significant OR values are at the low SAR values (< 2 mW/kg) while at higher SAR values between 10-1000 mW/kg the OR value is low but start to increase at SAR values above 2 W/kg. The effects on the BBB can thus benefit as an indicator of the presence of non-thermal effects of 900 MHz microwaves

4.2 Exposure of AD-transgenic mice to GSM microwaves

Recently, a number of preclinical studies applied similar GSM microwave pulse sequence as used in our BBB studies on AD transgenic mice. The results of their study show that in Alzheimer's transgenic mice, Aβ deposits decreased and some of the symptoms of AD reversed after daily, 2 hour exposure during a 7-9 month, (Arendash et al., 2010, Dragicevic et al., 2011).

Arendash and coworkers reported in 2010 that treatment with electromagnetic fields protects against and reverses cognitive impairment in mice with Alzheimer's disease (Arendash et al., 2010).

Arendash's results indicates that prolonged exposure to electromagnetic fields (EMF) directly associated with GSM-900 cell phone use (SAR=0.25 W/kg) confers cognitive benefits. Both cognitive protective and cognitive enhancing effects of GSM-EMF exposure detected for both normal mice and transgenic mice designed to develop Alzheimer's-like cognitive impairment. The cognitive interference task used in their study designed to be analogous to a human cognitive interference task.

In mice with Alzheimer's disease, long-term GSM-900 exposure reduced occurrence of Amyloid-β (Aβ) deposition through anti-aggregation action of Aβ-plaque during exposure periods. In addition the GSM-900 micrwave exposure increased neuronal activity and cerebral blood flow.

The results of these mouse studies indicate that electromagnetic field (EMF) exposure might be an alternative therapy regime for Alzheimer's disease with possible memory-enhancing effects (Arendash et al., 2010).

Zhi and co-workers reported in 2023 that exposure to 900 MHz electromagnetic fields alleviated AD-like symptoms in APP/PS1 mice, potentially leading to a non-invasive strategy for AD treatment (Zhi et al., 2023).

They exposed daily for 2 h per day, alternately for 270 days transgenic- (Tg myloid precursor protein APP/PS1) and WT- mice were to 900 MHz microwave radiation at non-thermal SAR levels between 0.25–1.055 W/kg. At 180 and 270 days, cognition was assessed by Morris water maze, Y-maze. In addition, tests with Congo red staining, immunohistochemistry and ELISA were performed to analyze Aβ plaques, Aβ40 and Aβ42 content in brain tissue.

Differential expression of proteins in the hippocampus between microwave-exposed and non-exposed AD mice identified by proteomics.

Spatial and working memory improved in AD mice after long-term 900 MHz microwave exposure compared to sham exposure.

The current results also indicated that long-term exposure to 900 MHz microwave radiation slows down the development of AD in transgenic mice. This effect occurred mainly in the late stage of the disease due to downregulation of apo-lipoprotein and synuclein alpha expression as well as excitatory/inhibitory neurotransmitters in the hippocampus.

This suggests that 900 MHz microwave exposure exerts a beneficial effect against AD and may be a potential therapy for AD (Zhi et al., 2023).

4.3 Exposure with GSM-900 2G affects Mitochondria

Dragicevic and coworkers reported in 2011 that long-term exposure to electromagnetic fields of GSM-900 mobile 2G-telephone prevents or reverses cognitive impairment in Alzheimer's transgenic "Tg-mice" (Dragicevic et al., 2011).

To elucidate the possible mechanism of EMF-induced cognitive benefits, they assessed brain mitochondrial function in aged Tg-mice and non-transgenic "NT-mice" after 1 month of daily EMF exposure. In Tg-mice, EMF treatment improved brain mitochondrial function by 50–150% across six established measures, which was greatest in cognitively important brain regions such as the cerebral cortex and hippocampus.

EMF treatment also increased the function of brain mitochondria in normal aged NT-mice, although the improvement was not as robust and less widespread compared to Tg-mice.

The EMF-induced improvement in brain mitochondrial function in Tg-mice was accompanied by 5-10-fold increases in soluble Amyloid peptide $A\beta_{1-40}$ within the same mitochondrial preparation. These increases in soluble Amyloid-β peptide (Aβ) in mitochondria were apparently due to the ability of EMF treatment to disaggregate Aβ oligomers, which are believed to be the form of Aβ- that causes mitochondrial dysfunction in Alzheimer's disease (AD).

Since brain temperature was either stable or decreased during/after EMF treatment, the EMF-induced mitochondrial improvement in both Tg-mice and normal NT-mice occurred through non-thermal effects. Collectively, these results suggest that brain mitochondrial enhancement may be a primary mechanism by which EMF treatment provides cognitive benefit to both Tg- and NT-mice.

Especially in this context is that mitochondrial dysfunction is an early and prominent feature of Alzheimer's pathogenesis. Thus, the GSM-900 EMH interaction at the mitochondrial level should be able to both prevent and treat the AD disease (Dragicevic et al., 2011).

4.4 1950 MHz 3/4G EMF exposure causes gene profiling

Jeong and Korean co-workers reported in 2015 that 1950 MHz electro-magnetic fields corresponding to 3/4G mobile telephone generation improve Aβ pathology in mice with Alzheimer's disease (Jeong et al., 2015).

They studied Tg-5xFAD mice as an *in vivo* model for how exposure with 1950 MHz-3/4G EMF affected AD-like Amyloid-β (Aβ) pathology. The transgenicTg-

5xFAD and wild-type “WT-mice” were exposed for 8 months to 1950MHz-3/4G EMF at SAR 5W/kg for 2 hours per day, 5 days per week.

They noted with chronic EMF exposure a significant reduction throughout the brain, including the hippocampus and entorhinal cortex, of both Aß plaques, APP, and APP carboxyl terminal fragments (CTFs). The ratio of $A\beta_{42}$ to $A\beta_{40}$ peptide decreased in the Hippocampus of Tg-5xFAD mice.

They also found that EMF-3/4G exposure of Tg-5xFAD mice inhibited parenchymal expression of β-Amyloid precursor protein-cleaving enzyme-1 (BACE1) and neuro-inflammation. In addition, EMF-3/4G exposure was also shown to prevent memory impairment in the Tg-5xFAD mice.

Gene profiling using micro-array data from Hhippocampus in Tg-5xFAD mice revealed after EMF-3/4G exposure that five genes (TSHZ2, GM12695, ST3GAL1, ISX and TLL1), which are involved in Amyloid-β, were significantly altered.

These findings indicate that chronic 3/4G EMF-exposure directly affects AP pathology in AD but not in normal brain. Therefore, EMF exposure has preventive effects against AD-like pathology in advanced AD mice with high expression of Aβ, suggesting that 3/4G EMF-exposure may have a beneficial impact on AD-patients (Jeong et al., 2015).

Son, and Korean coworkers reported in 2023 that long-term exposure to 1950 MHz-3/4G cell-phone formatted electromagnetic fields attenuates cognitive dysfunction in 5×FAD mice by regulating microglial function (Son et al., 2023).

Previous studies showed that long-term exposure with 3/4G-generation mobile-phone electro-magnetic field in 5×FAD mice with severe late-stage Alzheimer's disease, reduced both Amyloid-β deposition and glial activation, including microglial regulation of activated microglia (Jeong et al., 2015).

In their study, microglia gene expression profiles and the presence of microglia in the brain of 5×FAD mice at 1.5 months of age were analyzed. A control group was sham-exposed and another group was exposed with 1950 MHz radio frequency electromagnetic fields with SAR values of 5 W/kg for 2 hours per day and 5 days/week for 6 months.

They showed that exposure to radiofrequency electromagnetic field for 6 months improved cognition and reduced Amyloid-β deposition. In addition, microglial proliferation in the hippocampus of 5×FAD mice treated with radiofrequency electromagnetic fields was reduced compared to those in the sham-exposed control group.

The Son´s study focused on the expression levels of genes related to microgliosis and microglial function in the exposed group in comparison with a CSF1R inhibitor (PLX3397)-treated group.

Both 3/4G mobile-phone electromagnetic fields and PLX3397-inhibitor suppressed the levels of the genes CSF1R, CD68 and CC16 that relate to micro gliosis, and the pro-inflammatory cytokine Interleukin-1. In addition, the expression levels of genes related to microglial function, including TREM2, FCGR1A, CTSS, and SPI1, decreased after long-term radiofrequency electromagnetic field exposure, which also observed in response to microglial suppression by PLX3397.

These results showed that 3/4G mobile phone related electromagnetic fields ameliorated amyloid-ß pathology and cognitive impairment by suppressing amyloid-ß deposition-induced microgliosis and their key regulator, CSF1R.

4.5 Summary of preclinical studies

Both 2G (GSM) and 3/4G (WCDMA) cell phone modulated microwaves appear to affect the expression of genes that ameliorate amyloid-ß pathology and cognitive impairment in transgenic AD mice.

However, there are no studies of the dose-response of the effects, i.e. SAR levels and absorbed energy with variations in exposure time.

4.6 References

Arendash, G. W., Sanchez-Ramos, J., Mori, T., Mamcarz, M., Lin, X., Runfeldt, M., Wang, L., Zhang, G., Sava, V., Tang, J. & Cao, C. 2010. "Electromagnetic Field Treatment Protects Against and Reverses Cognitive Impairment in Alzheimer's Disease Mice." *Journal of Alzheimer's Disease* 19 (1):191-210. doi: 10.3233/JAD-2010-1228.

Dragicevic, N., Bradshaw, P. C., Mamcarz, M., Lin, X., Wang, L., Cao, C. & Arendash, G. W. 2011. "Long-term electromagnetic field treatment enhances brain mitochondrial function of both alzheimer's transgenic mice and normal mice: A mechanism for electromagnetic field-induced cognitive benefit?" *NEUROSCIENCE* 185:135-149. doi: 10.1016/j.neuroscience.2011.04.012.

Jeong, Y. J., Kang, G. Y., Kwon, J. H., Choi, H. D., Pack, J. K., Kim, N., Lee, Y. S. & Lee, H. J. 2015. "1950 MHz Electromagnetic Fields Ameliorate Aβ Pathology in Alzheimer's Disease Mice." *Curr Alzheimer Res* 12 (5):481-92. doi: 10.2174/1567205012051505261144448.

Mori, T. & Arendash, G. W. 2011. "Long-Term Electromagnetic Field Treatment Increases Brain Neuronal Activity: Linkage to Cognitive Benefit and Therapeutic Implications for Alzheimer's Disease." *Journal of Alzheimer's Disease & Parkinsonism* 1 (2):1-4.

Persson, B. R. R. More Probably than Unlikely, A Tale of the Blood-Brain Barrier and Mobile Communication Chisinau-2068, Moldavia: Lambert Academic Publishing; 2021 2021-12-07. 147 p.

Persson, B. R. R., Salford, L. G. & Brun, A. 1997. "Blood-Brain Barrier permeability in rats exposed to electromagnetic fields used in wireless communication." *Wireless Networks* 3:455-461.

Son, Y., Park, H. J., Jeong, Y. J., Lee, H. J., Choi, H. D. & Kim, N. 2023. "Long-term radiofrequency electromagnetic fields exposure attenuates cognitive dysfunction in 5×FAD mice by regulating microglial function." *Neural Regeneration Research* 18 (11):2497-2503-2503. doi: 10.4103/1673-5374.371379.

Zhi, W., Zou, Y., Ma, L., He, S., Guo, Z., Zhao, X., Hu, X. & Wang, L. 2023. "900 MHZ electromagnetic field exposure relieved AD-like symptoms on APP/PS1 mice: A potential non-invasive strategy for AD treatment." *Biochemical and Biophysical Research Communications* 658:97-106-106. doi: 10.1016/j.bbrc.2023.03.083.

Chapter V
Clinical Non-thermal Microwave Therapy

5.1 Clinical treatment of Alzheimer's disease

Arendash and colleagues in Tampa USA reported 2019**,** the results of a clinical trial of non-thermal microwave therapy in Alzheimer's disease. Their results showed cognitive improvement in treated AD-patients with associated changes in cerebrospinal fluid, blood and brain imaging (Arendash et al., 2019).

The common hypothesis of the cause of Alzheimer's disease (AD) is the appearance of small toxic aggregates (oligomers) of Amyloid-β (Aβ) and phospho-tau (p-tau). Treatment with GSM-900 electromagnetic fields (so-called Transcranial Electromagnetic Treatment TEMT) promotes disintegration of both Aβ- and p-tau-oligomers which promotes brain mitochondrial enhancement. These apparent "disease-modifying" actions of TEMT seems to both prevent and reverse memory impairment.

They conducted the clinical trial to evaluate the safety and initial clinical efficacy of TEMT against AD. The treatment applied a device called "*MemorEM™*" designed for daily treatment at home, allowing full mobility and comfort in performing daily activities during treatment (Figure 6-1a).

The device has a custom-made circuit board and a rechargeable battery inside the box housing, as well as a control panel on the outside for treatment control. This control panel and battery box worn on the upper arm,< is connected via a cable to eight specialized radiation-transmitters embedded between a double-layer head cover worn by the subject (Figure 5-1b). Emitters together provide global exposure in the forebrain via rapid sequential emitter activation.

By using that device two 1-hour treatments apply within a 24-hour period with at least a 7-hour interval between the two daily treatments.

When a treatment is in progress, the device transmits GSM-900 pulsed microwaves in a sequentially manner through each of eight transmitters antenna around the head.

Power levels (Specific Absorption Rate, SAR) for each emitter were set to an average of 1.6 W/kg. This exposure is similar to the one used in Lund to study the effect of GSM-mobile phone radiation on the BBB (Belyaev et al., 2006).

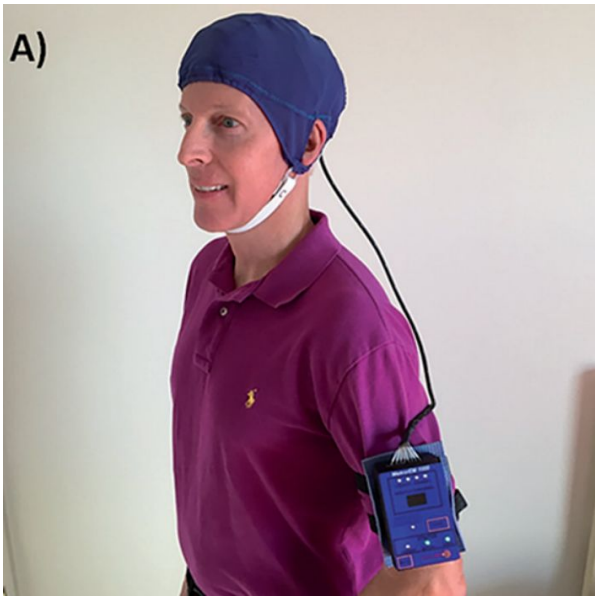


Figure 5-1a
A *MemorEM™* head unit worn by a person. (Arendash et al., 2019).
(with Arendash´ permission)

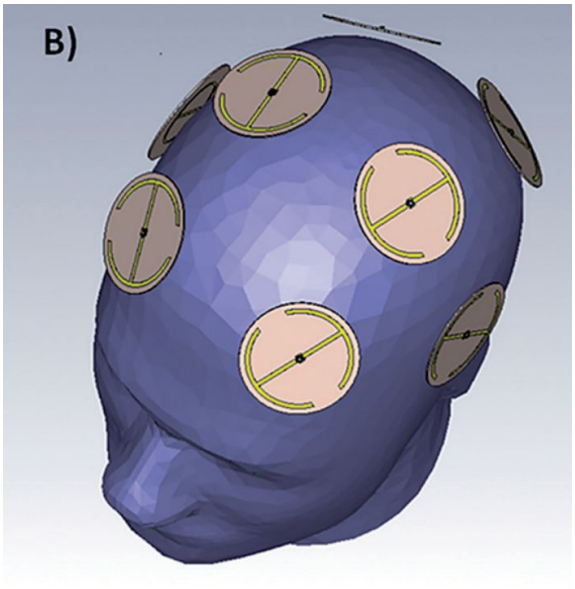


Figure 5-1b
Location of the eight electromagnetic transmitters embedded between the two-layer main casings of the device. (Arendash et al., 2019)

A FDTD human head computer simulations (IEEE Model 1528 phantom) show that the eight emitters' at this frequency and power level provide non-thermal microwave exposure to the human forebrain, including the cerebral cortex and underlying structures.

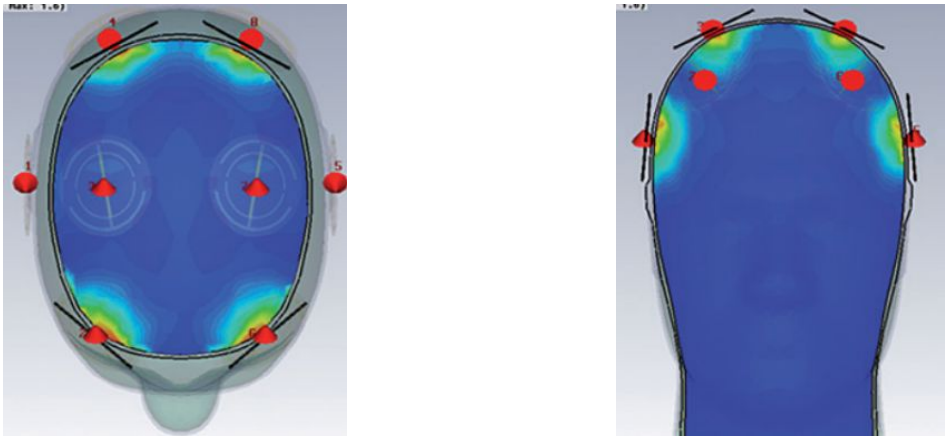


Figure 5-2
FDTD simulations showing electric field distribution under all 8 transmitters. Note that there is no overlap in the brain specific absorption rate (SAR) distribution between two transmitters, even when all are on at once (Arendash et al., 2019)-
(with Arendash´ permission)

A very similar calculated SAR distribution is obtained from actual electric field measurements taken under individual transmitters and in brain gel “in situ” (inside a human head phantom), using a robotic probe system and grid measurement pattern.

The Western Institutional Review-Board approved both the *MemorEM™* device and the clinical trial protocol as "non-significant risk".

The caregivers using the special head units (Figure 5-1) in treatment of eight AD patients with mild to moderate symptoms at their home. The TEMT treatment took place at two 1-hour periods each day during 2 months. Evaluation of the patients took place before the treatment, at the end of the treatment and 2 weeks after the last treatment.

The TEMT treatment caused no harmful behavioral effects, discomfort or physiological changes. Furthermore no signs of tumor induction or micro bleeding occur after the treatment..

TEMT treatment caused clinically important and statistically significant cognitive improvements in ADAS-cog, as well as in Rey AVLT.

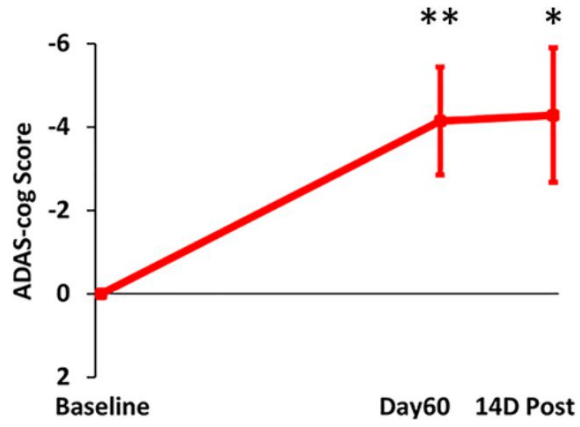


Figure 5-3a
Cognitive improvements in ADAS-cog, score (Arendash et al., 2019).

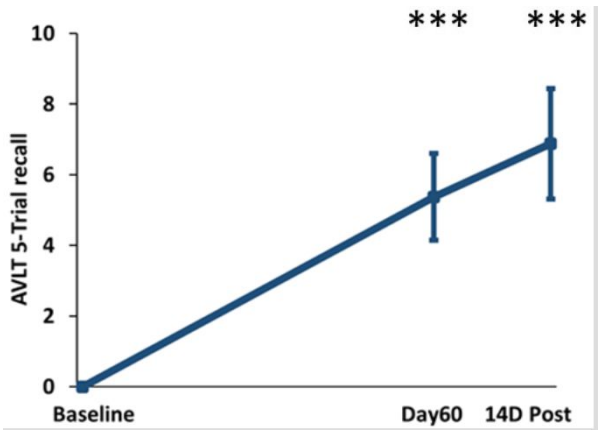


Figure 5-3a
Cognitive improvements in Rey AVLT.5-Trial recall (Arendash et al., 2019).

The TEMT treatment also affected the proteomics in cerebrospinal fluid (CSF) and plasma in respect to:

- Levels of soluble $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in CSF,
- Changes of Aβ-oligomer in CSF, related to cognition
- Decreased p-tau to $A\beta_{1\text{-}42}$ ratio in CSF and
- Reduced levels of Aβ-oligomer in plasma.

Pre- and post-treatment ^{19}FDG-PET brain scans revealed stable cerebral glucose utilization, with several subjects showing improved glucose utilization.

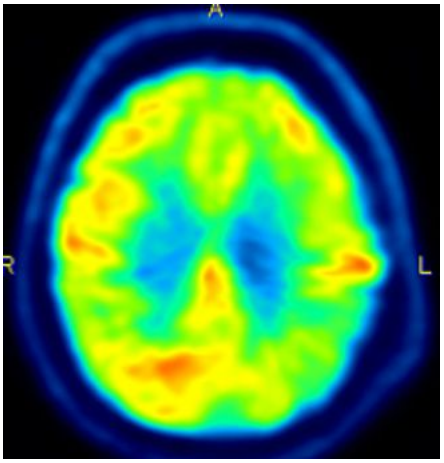


Figure 5-4a
^{19}FDG-PET scans taken before TEMT treatment (Arendash et al., 2019).

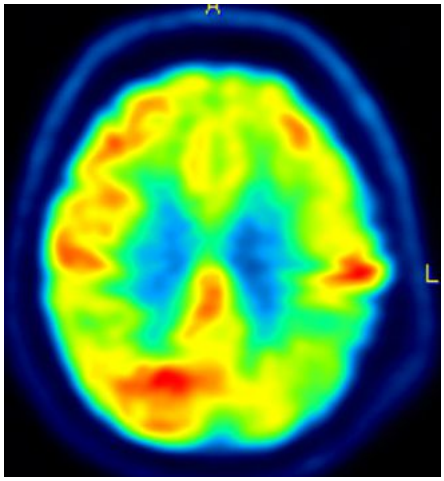


Figure 5-4b
^{19}FDG-PET scans taken after 2 months of TEMT treatment (Arendash et al., 2019).

Magnetic Resonance imaging and DTI diffusion tensor evaluation of FA fractional anisotropy in individual subjects provided support for TEMT-induced increases in functional connectivity within the cognitively important cingulate cortex.

In conclusion, TEMT treatment of AD patients appears to be safe, while producing cognitive improvement, changes of AD markers in CSF and blood, as well as evidence of stabilization or enhancement in brain connectivity (Arendash et al., 2019).

Cao and coworkers (including Arendash) reported in 2022 that the non-thermal microwave therapy (TEMT) "rebalances" blood and brain cytokine levels in the treated Alzheimer's patients. This could be a new mechanism to explain that the treatment reverses the cognitive impairment of the patients (Cao et al., 2022).

The immune system plays a critical role in the development and progression of Alzheimer's disease (AD). However, there is disagreement as to whether the development and progression of AD involves an over- or an under-activation of the immune system. In both scenarios, immune system cytokine levels are abnormal in AD and in need of rebalancing.

A pilot clinical study shows that 2 months of daily transcranial electromagnetic therapy (TEMT) at home was completely safe and resulted in reversal of the cognitive impairment of AD (Arendash et al., 2019).

For the eight mild to moderate AD-subjects treated in previous published work, this study seeks to determine whether TEMT administration had effects on blood or CSF levels of 12 cytokines.

Subjects received daily TEMT at home for 2 months by their caregivers, using premium *MemorEM*™ devices. For eight plasma cytokines, AD patients with lower

cytokine levels at baseline always showed increases in their cytokines, after both a single treatment or after 2 months of daily TEMT.

In contrast, AD subjects with higher plasma cytokine levels at both time points showed treatment-induced reductions in plasma cytokines. Thus, a rebalancing to reported normal plasma cytokine levels occurred with both acute and prolonged TEMT.

In CSF, TEMT induced a similar rebalancing for seven measurable cytokines, with the direction and magnitude of changes in individuals also being linked to their baseline CSF levels.

Their results strongly suggest that daily TEMT to AD patients for 2 months can "rebalance" levels of 11 out of 12 cytokines (GCSF, GMCSF, VEGF, IL-10, IL-17α, IL-15, IL-18, PDGF, INFγ, TGFα, IL-8, and NGF) in blood and/or brain, which is associated with reversing their cognitive decline.

TEMT is likely to provide these immuno regulatory effects by affecting cytokine secretion from brain microglia/astrocytes, choroid plexus, or neurons. This rebalancing of so many cytokines, and in both brain and systemic compartments, appears to be a remarkable new observation of TEMT action that may contribute significantly to its potential to prevent, halt, or reverse AD and other aging diseases (Cao et al., 2022).

Their study also showed that GSM-900 MHz microwave exposure could delay memory deficits and alleviate pathological changes in the brain of APP/PS1 transgenic mice.

Downregulation of apolipo-protein family, and expression of SNCA (Synuclein Alpha) as well as affecting the neurotransmitter balance, may play an important role in the observed effects.

These findings could provide a new direction for the development of effective non-invasive therapeutic strategies for AD (Cao et al., 2022).

Arendash and coworkers reported in 2022 that transcranial electromagnetic therapy halts cognitive decline over a 2½-year period in patients with Alzheimer's disease (AD) (Arendash et al., 2022).

The encouraging findings of cognitive improvements accompanied by ‘changes in AD markers in both the blood and cerebrospinal fluid (CSF), in the initial clinical trial was extended twice to cover a period of 2½ years. The current study reports on the resulting long-term safety, cognitive assessments, and AD marker evaluations from the five subjects who received long-term treatment.

The clinical trial included three separate but nearly identical clinical protocols (Figure 5-4), all involving AD patients in the first 2-month clinical trial and all approved in the United States by the Western Institutional Review Board (now WCG-IRB).

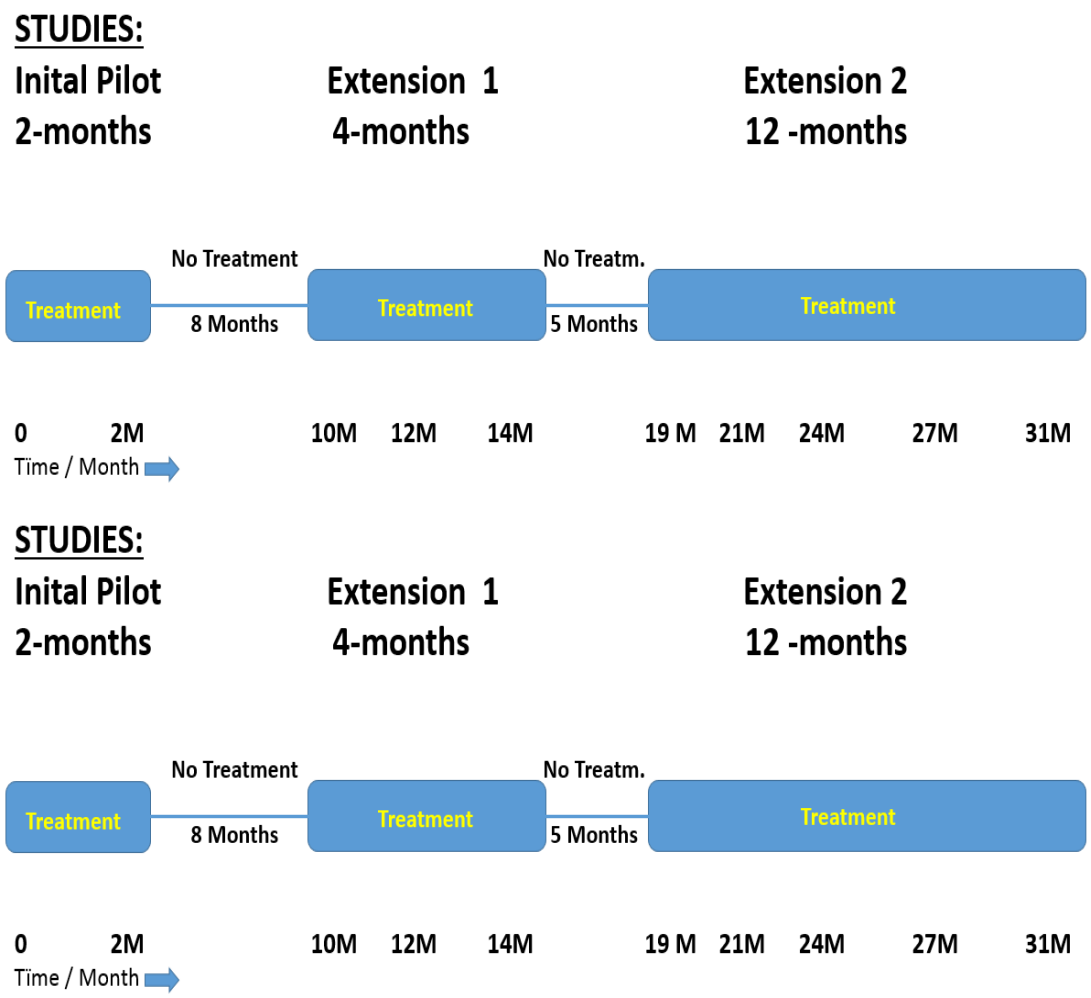


Figure 5-5

Scheme of the clinical trial included three separate but nearly identical clinical protocols

The first 2-month clinical pilot protocol (ClinicalTrials.gov NCT0295830) followed by a 4-month extension I protocol (ClinicalTrials.gov NCT03927040) and by a 12-month extension study II (ClinicalTrials.gov NCT04211633). Thus, the three clinical protocols combined in series as a single long-term study with a duration of 31 months, including two treatment-free periods.

The cognitive assessments at a given time point in any of these three protocols were identical, as were all blood and CSF sampling procedures in all three protocols; all blood and CSF samples were collectively analyzed at the final end of the 31-month

study for AD markers (see below). After the screening and baseline visits, follow-up visits occurred at 2, 10, 14, 19, 21, 24, 27, and 31 months post-treatment.

TEMT administration was completely safe over the 2½-year period, with no harmful side effects. On six cognitive/functional tasks (including ADAS-cog13, Rey AVLT, MMSE and ADL), no decline in any measure occurred during this period.

Long-term TEMT induced decreases in CSF levels of C-reactive protein, p-tau217, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ while modulating CSF-levels of Aβ-oligomer. In the plasma, long-term TEMT modulated/rebalanced levels of both p-tau217 and total tau.

Although only a limited number of AD patients were involved in their study, the results shown suggest that TEMT can halt the cognitive decline of AD for a period of at least 2½ years and can do so without safety concerns (Arendash et al., 2022).

5.2 Conclusions

Although no control group was included in the clinical study of eight patients treated by non-thermal microwave irradiation (TEMT), it demonstrates widespread and sustained interruption of progressive AD cognitive impairment up to 2½ years.

Furthermore, no adverse effects seems to occur in a limited group of five AD patients during the long time period of this study.

The results presented, demonstrate TEMT's ability to halt the progressive cognitive decline of AD. However, expanded placebo-controlled clinical trials are necessary to establish TEMT as a clinical AD therapeutic intervention that can halt or reverse this disease.

5.3 References

ARENDASH, G., ABULABAN, H., STEEN, S., ANDEL, R., WANG, Y., BAI, Y., BARANOWSKI, R., MCGARITY, J., SCRITSMIER, L., LIN, X., SHEN, N., ALJASSABI, A., LI, Y. & CAO, C. 2022. Transcranial electromagnetic treatment stops Alzheimer's cognitive decline over a 2 1/2year period: A pilot study. *Medicines* 9**,** 2-25.

ARENDASH, G., CAO, C., LIN, X., ZHANG, X., ABULABAN, H., SMITH, A., BARANOWSKI, R., WISNIEWSKI, G., BECERRA, L., ANDEL, R., WITTWER, D., MOULTON, J. & ARRINGTON, J. 2019. A Clinical Trial of Transcranial Electromagnetic Treatment in Alzheimer's Disease: Cognitive Enhancement and Associated Changes in Cerebrospinal Fluid, Blood, and Brain Imaging. *Journal of Alzheimer's Disease,* 71**,** 57-82-82.

BELYAEV, I. Y., BAUREUS KOCH, C., TERENIUS, O., ROXSTROM-LINDQUIST, K., MALMGREN, L. O. G., SOMMER, W. H., SALFORD, L. & PERSSON, B. R. 2006. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics,* 27**,** 295-306.

CAO, C., ABULABAN, H., BARANOWSKI, R., WANG, Y., BAI, Y., LIN, X., SHEN, N., ZHANG, X. & ARENDASH, G. W. 2022. Transcranial Electromagnetic Treatment "Rebalances" Blood and Brain Cytokine Levels in Alzheimer's Patients: A New Mechanism for Reversal of Their Cognitive Impairment. *Frontiers in Aging Neuroscience,* 14**,** 1-14.

Chapter VI
EPILOGUE

6.1 Epilogue

In recent years microwave treatment Alzheimer´s disease with microwaves at non-thermal SAR levels at frequencies and pulse modulation corresponding to mobile phone communication. Preclinical studies apply both the 2nd generation 900 MHz and GSM modulation, and the 3rd and 4th generation 1950 MHz with UMTS modulation.

In a clinical phase-1 study with eight subjects, Arendash and colleagues used GSM-900 MHz modulated microwaves at non-thermal SAR levels. Although they did not include a control group, their clinical study otherwise shows a widespread and sustained improvement in progressive AD cognitive impairment up to 2½ years after the first treatment (Arendash et al., 2019 23). Furthermore, they observed no adverse effects in the limited group of AD patients treated in their study.

However, extended placebo-controlled clinical trials are necessary to demonstrate that non-thermal microwave treatment can apply as a clinical therapeutic intervention for the treatment of Alzheimer's disease.

Their clinical results show that 900 MHz GSM mobile phone radiation has the ability to stop the progressive cognitive decline in AD patients.

It may seem strange that mobile phone radiation, which suspected of being harmful to normally healthy people,, has been chosen to treat serious disease. However, one sees an analogy in the use of ionizing radiation. which has very restrictive limit values for the normal population. in radiotherapy against tumor diseases.

However, there is need of extensive research to find the most effective treatment parameters for microwave treatment of neurologic diseases;

- Microwave frequency,
- Pulse length,
- Modulation frequency
- Applied SAR-value
- Absorbed microwave energy (dosimetry J/kg).

In our early studies of the effect of microwaves on the blood brain barrier, we found that the effect was dependent on the modulation frequency and pulse-length.

The average score values of albumin leakage in Fischer 344 rats (controls and exposed with 915 MHz microwaves at various modulation frequencies) are displayed in Figure 6-1

No pronounced difference appears between the various modulations frequencies other than the effect of CW seems to be most effective in opening the BBB. This is surprising since the common opinion is that pulse-modulated microwaves would be more biologically effective.

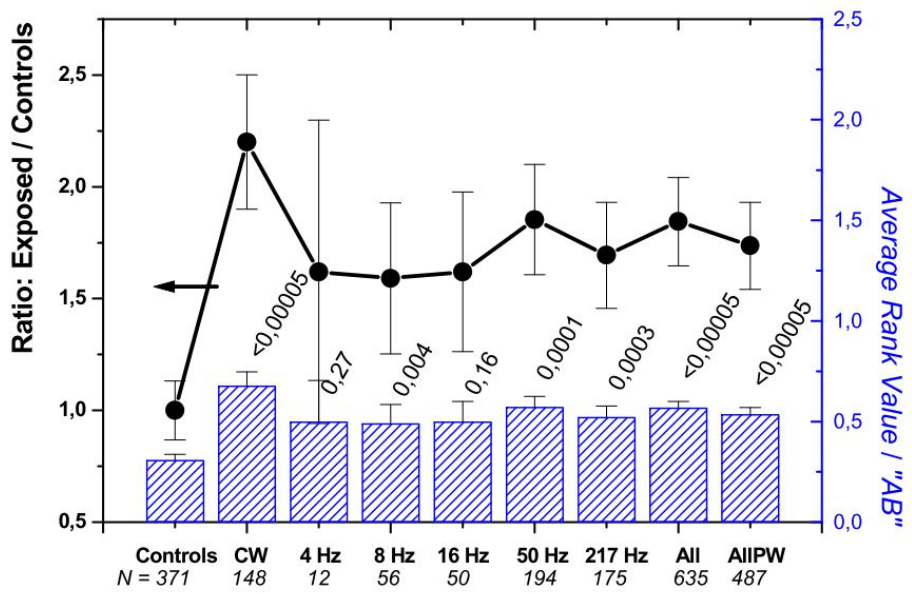


Figure 6-1

The histogram show the average rank values (”AB”) for BBB albumin leakage in Fischer 344 rats exposed to 915 MHz microwaves modulated at various frequencies (Hz). The p values represent the significance in difference from the controls. The line represents the ratios of the average rank values between exposed and controls. The number of rats in each group is displayed in the lowest line.

The purpose of this story is to spread the knowledge that non-thermal microwave effects exist and are potentially useful for treatment of Alzheimers disease.

I hope that research will start as soon as possible in Scandinavia that can confirm whether microwave treatment is a clinically useful method against Alzheimer's disease and eventually other Neurological diseases.

6.2 Suggestions for possible research projects:

1. With cryo-electron microscope, or MAX4 and ESS resources study the effects of microwaves with varying frequency, amplitude and modulation on Amyloid-β and Tau plaques.
2. In animal experiments with transgenic AD mice, study the cognitive effects of microwaves with varying frequency, amplitude and modulation.
3. With Finite Time Domain methods computer simulate the propagation of microwaves in the human brain.
4. Using imaging Nuclear Medicine and Magnetic Resonance methods to study the effects of non-thermal microwaves on the brain of AD patients.
5. Clinical studies should be easily allowed as there are no barriers to exposing humans to non-thermal levels of microwaves such as mobile phones.

In other words, we are facing a multifaceted research area with input from many different areas of expertise:

- Molecular biology and biochemistry
- Physical chemistry
- Nanophysics
- Pathology
- Neurology
- Psychology with cognitive research
- Psychiatry
- Medical Image Diagnostics
- Medical Radiation Physics
- Electrical engineering and electromagnetic physics
- etc.

Where is the spider in the web that can coordinate and manage such a project???

6.3 References

ARENDASH, G. & CAO, C. 2023. Transcranial Electromagnetic Wave Treatment: A Fountain of Healthy Longevity? *International Journal of Molecular Sciences,* 24**,** 9652.

ARENDASH, G., CAO, C., LIN, X., ZHANG, X., ABULABAN, H., SMITH, A., BARANOWSKI, R., WISNIEWSKI, G., BECERRA, L., ANDEL, R., WITTWER, D., MOULTON, J. & ARRINGTON, J. 2019. A Clinical Trial of Transcranial Electromagnetic Treatment in Alzheimer's Disease: Cognitive Enhancement and Associated Changes in Cerebrospinal Fluid, Blood, and Brain Imaging. *Journal of Alzheimer's Disease,* 71**,** 57-82-82.

My Story about the book:

A story about effects of Microwaves from Mobile-phones, and Alzheimer's disease

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1970 PhD, 2004 MD h.c.
since 1980-2005 professor of medical radiation physics
2005 – present: Professor emeritus at
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Published : >400 scientific publications, >20 extensive reports and books

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Summary of the book book:

A team of clinical researchers at Lund University found that electromagnetic radiation, such as that used in GSM-900 mobile telephones, at low non-thermal power values (<2 W/kg), causes users' blood albumin to leak through the blood-brain barrier "BBB" into in the brain tissue.

The neurosurgeons: Leif Salford MD, PhD, professor emeritus,
Henrietta Nittby MD, PhD,
Neuropathologist: Arne Brun MD, PhD, professor emeritus.
Medical physicist: Bertil RR Persson PhD, MDhc, professor emeritus..
Jacob Eberhardt PhD,
Electrical engineer: Lars Malmgren Tech. Dr.

The BBB is supposed to protect the brain from unwanted and toxic molecules that are potentially in the blood to transfer into the brain tissue. However, after exposing rats to radiation from cell phones, albumin from the blood leaks into the brain and accumulates in neurons and glial cells.

In late 2023, it came to my attention that Arendash in the USA, in order to treat patients with Alzheimer's disease, had used a similar microwave pulse train and non-thermal SAR levels as we used in our studies of the BBB leakage of blood-Albumin.

Concerns were raised that albumin leakage in the BBB after many years of intensive mobile phone use could promote the development of autoimmune and neurodegenerative diseases such as Alzheimer's.

At Christmas time in 2023 it came to my attention that Arendash and coworkers already in 2010 reported that treatment with GSM-900 electromagnetic fields protects against and reverses cognitive decline in mice with Alzheimer's disease (Arendash et al., 2010).

Arendash's report indicates that prolonged exposure to non-thermal levels of microwave radiation directly associated with GSM-900 cell phone use confers cognitive benefits. They observed both cognitive protective and ameliorating effects of such exposure in both normal mice and transgenic mice designed to develop Alzheimer's-like impairment.

Arendash and colleagues used similar 900 MHz GSM modulated microwaves at non-thermal SAR levels in a clinical phase-1 study with 8 subjects..

Although they did not include a control group, their clinical study otherwise shows a widespread and sustained improvement in progressive AD cognitive impairment up to 2½ years after the first treatment. Furthermore, they observed no adverse effects in the limited group of AD patients treated in their study.

It may seem strange that mobile phone radiation, which suspected of being harmful to normally healthy people, can treat Alzheimer´s disease.

The purpose of this story is to spread the knowledge that non-thermal microwave radiation effects exist, and are potentially useful for treatment of Alzheimer´s disease.

I hope that research will start as soon as possible in Scandinavia to confirm whether Microwave treatment is a clinically useful method against Alzheimer's disease and eventually other Neurological diseases.

Perhaps the story can be an inspiration for some younger student or researcher to work for improvement of the treatment of Neurological diseases.

Lund in August 2024, Bertil RR Persson