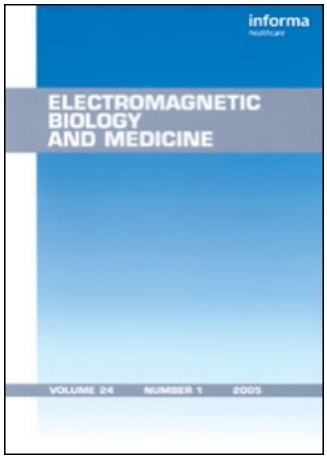

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Review

Radiofrequency and Extremely Low-Frequency Electromagnetic Field Effects on the Blood-Brain Barrier

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During the last century, mankind has introduced electricity and during the very last decades, the microwaves of the modern communication society have spread a totally new entity—the radiofrequency fields—around the world. How does this affect biology on Earth? The mammalian brain is protected by the blood-brain barrier, which prevents harmful substances from reaching the brain tissue. There is evidence that exposure to electromagnetic fields at non thermal levels disrupts this barrier. In this review, the scientific findings in this field are presented. The result is a complex picture, where some studies show effects on the blood-brain barrier, whereas others do not. Possible mechanisms for the interactions between electromagnetic fields and the living organisms are discussed. Demonstrated effects on the blood-brain barrier, as well as a series of other effects upon biology, have caused societal anxiety. Continued research is needed to come to an understanding of how these possible effects can be neutralized, or at least reduced. Furthermore, it should be kept in mind that proven effects on biology also should have positive potentials, e.g., for medical use.

Keywords Albumin; Blood-brain barrier; Mobile phones; MRI.

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Introduction

During the billions of years that organisms have existed on earth, they have been exposed to, and moulded by, the original physical forces: gravitation, the sun's rhythmically changing radiation, other cosmic irradiation, heat/cold, mechanical forces, and the omnipresent terrestrial static electric and magnetic fields. The existing organisms are created to function in harmony with these forces and have done so for 3.5 billion years.

This was the truth until the last century when mankind introduced the use of electricity, and the very last decades when the microwaves of modern communications spread around the world. The next step is the cordless society based upon microwave penetration in public as well as private surroundings. Today, one third of the world's population are owners of microwave-producing mobile phones, and even more, live in a milieu filled with microwave-emitting equipment such as base stations and other systems for wireless communication.

Is this only for good? Or, might this have effects in biology? Such effects we must anticipate and evaluate as far as possible, and if needed, reduce or avoid.

The questions might seem easily answered; there seems to be little evidence that the human organism is definitively damaged. However, during recent years, several scientific reports have shown significant, but often weak, effects on cells in vitro, experimental animals, and also humans (for reference, see Hyland, 2000).

The first studies on possible risks of microwaves for the living organism were reported in the 1970s, e.g., before the advent of mobile phones, when radar and microwave ovens posed a possible health problem. Frey et al. (1975) found increases in the blood-brain barrier permeability of rats to fluorescein after 30 min of exposure to both pulsed and continous waves at 1.2 GHz. Similar observations were made by Oscar and Hawkins (1977), who demonstrated that at very low energy levels, the fields in a restricted exposure window, caused a significant leakage of ^{14}C-mannitol, inulin, and dextran (with the same molecular weight as albumin) from the capillaries into the surrounding cerebellar brain tissue. These findings, however, were not repeated using ^{14}C-sucrose (Gruenau, 1982). In the following years, much attention was directed to MRI effects upon the blood-brain barrier. It was shown (Shivers et al., 1987; Prato et al., 1990) that combined exposure to RF, pulsed, and static magnetic fields resulted in a pinocytotic transport of albumin across the blood-brain barrier. In more recent years, in vitro models have been increasingly applied to investigate the blood-brain barrier; in one of these, it was shown that EMF at 1.8 GHz increases the permeability to sucrose through the blood-brain barrier (Schirmacher et al., 2000).

Our group has studied the effects of RF electromagnetic fields on the blood-brain barrier and upon tumor growth in the mammalian brain since 1988. Our studies on the effects of CW and pulsed modulated microwaves at 915 MHz have been revealed to cause significantly increased leakage of albumin through the blood-brain barrier of exposed rats as compared to non exposed animals (Persson et al., 1997; Salford et al., 1992, 1993, 1994, 2001, 2003). Recently, we have also examined the effects of long term exposure—55 weeks—upon brain morphology and cognitive functions (Nittby et al., 2008a). The effects of GSM RF upon gene expression have been studied (Nittby et al., 2008b) and 3G exposure studies are under way.

The Blood-Brain Barrier (BBB)

The mammalian brain is protected from exposure to potentially harmful compounds in the blood by the BBB. This is a hydrophobic barrier formed by the vascular

endothelial cells of the capillaries in the brain with tight junctions between the endothelial cells, leaving no fenestrae. The tight junctions are composed of tight junction proteins (occludin, claudin, and zonula occludens, where the zonula occludens is the intracellular peripheral membrane protein that anchors claudin and occludin to the actin cytoskeleton; Alberts et al., 2002). An important part is the binding of claudin proteins on opposing membranes, where claudin-5 in particular is crucial in the BBB (Daneman and Barres, 2005). Astrocytes are surrounding the outer surface of the endothelial cells with protrusions, called end feet, and are implicated in the maintenance, functional regulation, and repair of the BBB. The astrocytes form a connection between the endothelium and the neurons and constitute a second barrier to hydrophilic molecules.

Other periendothelial accessory structures of the BBB include pericytes and a bilayer basal membrane which surrounds the endothelial cells and pericytes. The basement membrane (basal lamina) supports the ablumenal surface of the endothelium and may act as a barrier to passage of macromolecules. The pericytes are a type of macrophages, expressing macrophage markers with capacity for phagocytosis but also for antigen presentation. In fact, the pericytes, which cover about 25% of the capillary surface (Frank et al., 1987), seem to be in a position to significantly contribute to central nervous system (CNS) immune mechanisms (Thomas, 1999). The pericytes also have other functional roles: with their capability for contractility they seem to serve as a smooth muscle equivalent, and through regulation of endothelial cells they maintain the stability of blood vessels (Thomas, 1999). Additionally, the astrocytes seem to be highly involved in many diseases, both infectious and autoimmune, and also in other diseases such as Alzheimer's by production of amyloid. Also, by regulating their vascular permeability, the pericytes are supposed to play an important role in inflammatory diseases (Thomas, 1999).

Physiologically, the microvasculature of the central nervous system (CNS) differs from that of peripheral organs. It is characterized not only by its tight junctions, which seal cell-to-cell contacts between adjacent endothelial cells, but also by the low number of pinocytotic vesicles for nutrient transport through the endothelial cytoplasm and its lack of fenestrations, and the five-fold higher number of mitochondria in BBB endothelial cells compared to muscular endothelia in rat (Oldendorf et al., 1977). All this speaks in favor of an energy-dependent transcapillary transport.

These above-described membrane properties of the BBB control the bidirectional exchange of molecules between the general circulation and the central nervous system. By at least four mechanisms, the endothelial cells directly control the flux of solutes into the brain parenchyma. First, the tight junctions and low number of pinocytotic vesicles guarantee that proteins cannot pass freely into the brain parenchyma. Second, solutes which are not highly lipid soluble, or which do not bind to selective transporters with high affinity, are excluded from free exchange. By means of this lipid solubility, carbon dioxide and oxygen, among many others, are able to enter the brain interstitial fluid passively, whereas the passage of, for example sugars and many amino acids, depends on other, active mechanisms. Third, the BBB has a capacity to metabolize certain solutes, such as drugs and nutrients (Ghersi-Egea et al., 1988). Fourth, active transporters maintain the levels of certain solutes at specific values within the brain interstitial fluid, made possible by active transport against the concentration gradients. These enzyme systems are differently distributed between the luminal and the ablumenal membranes of the endothelial cells, thus gaining the BBB polarity properties. For example, Na^+-K^+-ATPase is located on the

antilumenal membrane (Betz et al., 1980). It has been proposed that the active transport across the brain capillaries might be the most important mechanism for the regulation of the internal milieu within the brain parenchyma. Also, it has been proposed that this mechanism, requiring energy to function properly, might be the one most sensitive to disease and that interference with this active transport could play an important part in the neurological dysfunction seen in many metabolic disorders (Betz et al., 1980).

In summary, the BBB serves as a regulatory system that stabilizes and optimizes the fluid environment of the brain's intracellular compartment (Oldendorf, 1975; Rapoport, 1976; Salford et al., 2001).

The intact BBB protects the brain from damage, whereas the dysfunctioning BBB allows influx of normally excluded hydrophilic molecules into the brain tissue. This might lead to cerebral oedema, increased intracranial pressure, and in the worst case, irreversible brain damage. The normal selective permeability of the BBB can be altered in several pathological conditions such as epileptic seizures (Mihàly and Bozòky, 1984a,b) or extreme hypertension (Sokrab et al., 1988) and also transient openings of the BBB might lead to permanent tissue damage (Sokrab et al., 1988). Considering the ensuing leakage of substances from the blood circulation into the brain tissue, harmful substances might disrupt the cellular balance in the brain tissue and in the worst case, even carcinogenic substances might pass into the brain tissue. It has also been shown that an increased permeability of the BBB is seen in cases of oxidative stress (Parathath et al., 2006), where BBB dysfunction and neurodegeneration were shown to be mediated through an excitotoxicity mechanism by the serine protease tissue plasminogen activator, with NO and $ONOO^-$ as downstream mediators (Parathath et al., 2006).

Opening of the BBB thus can have detrimental effects and since it has been shown for a few decades that EMFs have the potency to increase the permeability of this barrier, a major debate is going on in society with increasing intensity. In the following, we try to clarify the actual status of the available evidence in the field.

Radiofrequency/Microwave Radiation

Early Studies

In early studies on the effects of low-intensity EMFs on the BBB, various compounds were injected intravenously, followed by EMF exposure and comparisons of the penetration into the brain tissue between sham and exposed animals.

Frey et al. (1975) found increases in the BBB permeability of rats to fluorescein after 30 min of exposure to both pulsed and continuous waves (CWs) at 1.2 GHz with average power densities of 0.2 mW/cm^2. Similar observations were made in a study with 180 animals by Oscar and Hawkins (1977). Exposure of anaesthetized rats for 20 min to 1.3 GHz of pulsed EMFs with average power densities of 0.3 mW/cm^2 resulted in leakage of ^{14}C-mannitol, dextran, and inulin into the cerebellar brain tissue, as well as inulin and dextran leakage from capillaries into hypothalamic and medullar tissue. Also, BBB permeability to mannitol was investigated in un-anaesthetised rats, which were exposed to pulsed radiation or sham exposed for 20 min. The animals were sacrificed at different time intervals after the exposure. BBB permeability was seen in the groups sacrificed 8 min and 4 h after exposure, but to a much lesser extent in those sacrificed after 8 h. Finally, the permeation of mannitol

through the BBB was found to be a very definite function of exposure parameters such as power density, pulse width, and the number of pulses per second. However, in later studies, Oscar et al. (1981) emphasised that changes of BBB permeability after microwave exposure partly could be explained by an increase of local cerebral blood flow. In accordance with this, they concluded that their initial findings (Oscar and Hawkins, 1977) might be of less magnitude than originally thought (Table 1).

In an attempt to repeat the findings of Oscar and Hawkins (1977), Preston et al. (1979) found no increase in the uptake of ^{14}C-mannitol in anaesthetised rats after 2450 MHz CW exposure for 30 min at power densities of 0.1 to 30 mW/cm^2. Preston et al. further concluded that the increased BBB permeability, which had been observed by Oscar and Hawkins (1977) in cerebellum and medulla, possibly had been misinterpreted and was not due to the EMF exposure. Rather, changes in blood flow and water influx or egress were supposed to be responsible for the BBB permeability in these caudal parts of the brain. Also, further attempts, made by Merritt et al. (1978), to replicate the findings of Oscar and Hawkins from 1977, resulted in the conclusion that no repetition of the initial findings could be made. Merritt et al. (1978) tried to replicate also the findings of Frey et al. (1975), but reported that no changes were seen. However, Frey commented upon this in an article in 1998, where he pointed out that, in fact, statistical analysis by the editor and reviewer of the data from the study by Merritt et al. provided a confirmation of the findings of Frey et al. (1975) (Frey, 1998).

No alteration of BBB permeation of ^{14}C-sucrose and ^{3}H-inulin was found by Ward et al. (1982) after exposure of anaesthetised rats to CW at 2450 MHz for 30 min at power densities of 0, 10, 20, or 30 mW/cm^2 after correction for thermal effects. Similarly, Ward and Ali (1985) observed no permeation after 1.7 GHz exposure at SAR of 0.1 W/kg, using the same exposure duration and injected tracers as Ward et al. (1982). Absence of EMF induced BBB permeability was also reported by Gruenau et al. (1982), after injection of ^{14}C-sucrose in conscious rats and exposure 30 min pulsed energy (2.8 GHz at 0, 1, 5, 10, or 15 mW/cm^2) or continuous wave (2.8 GHz, 0, 10, or 40 mW/cm^2).

Proof of EMF-induced BBB permeability was put forward by Albert and Kerns (1981), who exposed un-anaesthetised Chinese hamsters to 2,450 MHz CWs for 2 h at SARs of 2.5 W/kg. In one-third of the exposed animals there was an increased permeability of the BBB to horseradish peroxidase (HRP) and the endothelial cells of these irradiated animals had a 2–3-fold higher number of pinocytotic vesicles with HRP than the sham animals. The mechanism of BBB permeability seemed to be reversible, since animals allowed to recover for 1 or 2 h after the EMF exposure had almost no HRP permeation. A total number of 80 animals were included in this study.

Temperature Dependence

In further studies, more attention was directed towards the effects of hyperthermia, resulting from exposure at high SAR-levels, on BBB permeability.

A study correlating changes of BBB permeability with the quantity of absorbed microwave energy (Lin and Lin, 1980), using Evans blue and sodium fluorescein as indicators of BBB permeation, showed that 20 min of 2,450 MHz exposure of anaesthetised Wistar rats caused no alteration of BBB permeability even at SAR-values of 80 W/kg. Notably, the same lack of alteration was observed also at lower SAR-values, down to 0.04 W/kg. In further studies by the same group (Lin and Lin, 1982), no permeation of Evans blue could be observed after exposure to 2,450 MHz

Table 1
BBB permeability after EMF exposure

Reference	EMF Frequency (MHz)	Modulation, pulses per second (pps)	Duration of exposure	SAR (W/kg)	Effect on BBB permeability?	Total number of animals included in the study	Tracer or studied effect	Remark
Findings by the Lund Group								
Salford et al. (1994)	915	CW and pulse-modulated with repetition rates of 8, 16, 50 and 200/s	2 h	0.016–5 W/kg	Yes	246 Fischer 344 rats	Albumin extravasation	
Persson et al. (1997)	915	217, 50 Hz and CW	2–960 min	0.0004–0.95 W/kg average whole-body	Yes	1002 Fischer 344 rats	Albumin extravasation	
Salford et al. (2003)	915	GSM	2 h	0.002–0.2 W/kg	Yes		Albumin extravasation and dark neurons	Effect was seen 50 d after the exposure
Eberhardt et al.	915	GSM	2 h	0.0002–0.2 W/kg	Yes	96 Fischer 344 rats	Albumin extravasation and dark neurons	Albumin extravasation 14 d after exposure, dark

(submitted 2007) Mobile phone exposure								neurons 28 d after exposure
Fritze et al. (1997)	900	GSM	4 h	0.3 to 7.5 W/kg	Yes		Albumin	Albumin extravasation only reported for SAR-values of 7.5 W/kg
Töre et al. (2001)	900	GSM	2 h	0.12; 0.5 and 2.0 W/kg	Yes	70 Sprague-Dawley	Albumin leakage, seen with fluorescein-labelled proteins	Albumin extravasation at SAR-values of 0.5 and 2.0 W/kg
Neubauer et al. (1990)	2450	100 pps	30–120 min	Average 2 W/kg	Yes		Rhodamine-ferritin complex	No leakage at 1 W/kg at short-term exposure of 15 min
Tsurita et al. (2000)	1439	TDMA	1 h daily, for 2 or 4 weeks	Average whole-body 0.25 W/kg; peak in the brain of 2 W/kg	No	36 Sprague-Dawley rats	Evans blue, albumin	
Kuribayashi et al. (2005)	1439	TDMA, 50 pps	90 min daily, for 1 to 2 weeks	Average brain power densities of 2 or 6 W/kg; average whole-body 0.29 or 0.87 W/kg	No	40 Fischer 344 rats	Three BBB-related genes; FICT-dextran and albumin extravasation	
Finnie et al. (2001)	898.4	GSM	1 h	Whole-body of 4 W/kg	No	60 mice	Albumin extravasation	
Finnie et al. (2002)	900	GSM	1 h daily, 5 d a		No	207 mice	Albumin extravasation	

(*Continued*)

Table 1 (*Continued*)

			week for 104 weeks	Average whole-body 0.25; 1.0; 2.0 and 4.0 W/kg				
Franke et al. (2005b)	1800	GSM	1 to 5 d	Average 0.3 W/kg	No	—	Sucrose permeation	In vitro model of BBB
Schirmacher et al. (2000)	1800	GSM	4 d	Average 0.3 W/kg	No	—	Sucrose permeation	In vitro model of BBB
Franke et al. (2005a)	1966	UMTS	1 to 3 d	Average 1.8 W/kg	No	—	Sucrose and albumin permeation	In vitro model of BBB
Cosquer et al. (2005)	2450	500 pps	45 min	Average whole-body 2 W/kg	No	Rats	Scopolamine methylbromide extravasation	Indirect investigation of BBB opening by performance in radial arm maze
RF exposure of other kinds								
Frey et al. (1975)	1200	1000 pps and CW	30 min	0.2 mW/cm^2	Yes	Rats	Fluorescein	
Oscar and Hawkins (1977)	1300	50–1000 pps	20 min	0.3 mW/cm^2	Yes	180 Wistar rats	Leakage of mannitol, dextran and inulin	
Preston et al. (1979)	2450	CW	30 min	0.1–30 mW/ cm^2	No	Rats	Mannitol	
Merritt et al. (1978)	1200 and 1300	1000 pps and CW	30 min	2–75 mW/cm^2 and 0.1–50 mW/cm^2	No	Sprague Dawley rats	Fluorescein, mannitol, serotonin	Tried to replicate findings by Frey et al. (1975) and Oscar and Hawkins (1977)
	2450	CW	30 min	10–30 mW/cm^2	No	Rats	Sucrose and inulin	

Ward et al. (1982)								
Ward and Ali (1985)	1700	CW and 1000 pps	30 min	0.1 W/kg	No	Rats	Sucrose and inulin	
Albert and Kerns (1981)	2450	CW	2 h	2.5 W/kg	Yes	80 Chinese hamsters	Horseradish peroxidase	Reversible process with no HRP permeation after 1–2 recovery
Gruenau et al. (1982)	2800	CW and 500 pps	30 min	1–40 mW/cm^2	No	31 rats	Sucrose	
Lin and Lin (1980)	2450	500	20 min	0.04–80 W/kg	No	Wistar rats	Evans blue and sodium fluorescein	
Lin and Lin (1982)	2450	25–500	5–20 min	0.04–240 W/kg	No	51 Wistar rats	Evans blue	BBB permeability only at SAR of 240 W/kg, which is a thermal effect
Goldman et al. (1984)	2450	500		240 W/kg	No		Rubidium-86	Hyperthermia induced BBB permeability
Williams et al. (1984a)	2450	CW	30–180 min	4–13 W/kg	No	32 Fischer 344 rats	Fluorescein	BBB permeability only at hyperthermic levels >41°C
Williams et al. (1984b)	2450	CW	30–180 min	4–13 W/kg	No	20 Fischer 344 rats	HRP	
	2450	CW	30–90 min	13 W/kg	No	24 Fischer 344 rats	Sucrose	

(*Continued*)

Table 1 (*Continued*)

Williams et al. (1984c)								
Williams et al. (1984d)	2450	CW	30–180 min	4–13 W/kg	No	66 Fischer 344 rats	Fluorescein, HRP, sucrose	BBB permeability only at brain temperatures >40°C
Quock et al. (1986)	2450	CW	10 min	24 W/kg		Mice	Domperidone	BBB permeability due to temperature increase
Quock et al. (1987)	2450	CW	10 min	24 W/kg		Mice	Domperidone	BBB permeability due to temperature increase
Moriyama et al. (1991)	2450	CW				21 Sprague Dawley rats	HRP	BBB permeability due to temperature increase
Nakagawa et al. (1994)	2450	CW				Japanese monkeys		BBB permeability due to temperature increase
MRI exposure				Magnetic field				
Shivers et al. (1987)			23 min	0.15 T static magnetic field	Yes		HRP	Standard MRI procedure
Preston et al. (1989)			23 min	4.7 T static magnetic field	No	Rats	Sucrose	Standard MRI procedure
Prato et al. (1990)	65		23 min × 2	0.15 T static magnetic field	Yes	43 Sprague		Standard MRI procedure

					Dawley rats	Diethylenetriamine-pentaacetic acid (DTPA)	
Prato et al. (1994)		23 min × 2	1.5 T static magnetic field	Yes	50 rats		Standard MRI procedure
Garber et al. (1989)			0.3–0.5 T static magnetic field	Yes	Rats	Mannitol	Standard MRI procedure
Adzamli et al. (1989)				No			Standard MRI procedure
ELF exposure							
Öztas et al. (2004)	50	8 h daily for 21 d	0.005 T	Yes	34 Wistar rats	Evans-blue	BBB disruption in diabetic rats, but not in normoglycemic rats

RFs for 5–20 min when the SAR-values ranged from 0.04–200 W/kg. Not until a SAR-value of 240 W/kg, with ensuing rise in brain temperature to 43°C, was applied, the BBB permeability increased. These observations of demonstrable increases of BBB permeability associated with intense, microwave-induced hyperthermia were supported by another study by the same group (Goldman et al., 1984).

In a series of EMF exposures at 2,450 MHz CW, Williams et al. (1984a,b,c) concluded that increase of BBB permeability might not be explained by microwave exposure, but rather temperature increases and technically derived artefacts such as increase of the cerebral blood volume and a reduction in renal excretion of the tracer. Significantly elevated levels of sodium fluorescein (Williams et al., 1984a) were found only in the brains of conscious rats made considerably hyperthermic by exposure to ambient heat for 90 min or 2,450 MHz CW microwave energy for 30 or 90 min, but this was at high SAR values, 13 W/kg—far beyond the ICNIRP limit of 2 W/kg (ICNIRP 1998)—and not comparable to the experiments performed by, among others, our group, as described below.

With more research into the area of EMF induced BBB permeability, it became evident that with high-intensity EMF exposure resulting in tissue heating, the BBB permeability is temperature dependent (Williams et al., 1984d). Thus, the importance of differentiating between thermal and non thermal effects on the integrity of the BBB was realized. This is the reason why studies with increases of BBB permeability due to exposure to SAR-values well above recommended exposure levels (Quock et al., 1986; Quock et al., 1987; Moriyama et al., 1991; Nakagawa et al., 1994) need to be considered from another point of view, compared to those focusing on the non thermal effects of EMFs.

Continued Studies—MRI and BBB Permeability

Following the increasing use of magnetic resonance imaging (MRI), the effects of MRI radiation upon BBB permeability were investigated more thoroughly. MRI entails the concurrent exposure of subjects to a high-intensity static field, a radio-frequency field, and time-varying magnetic field. Shivers et al. (1987) observed that exposure to a short (23 min) standard (of those days) clinical MRI procedure at 0.15 Tesla (T) temporarily increased the permeability of the BBB in anaesthetised rats to horseradish peroxidase (HRP). This was revealed by electron microscopy (EM), to be due to an amplified vesicle-mediated transport of HRP across the microvessel endothelium, to the ablumenal basal lamina and extracellular compartment of the brain parenchyma. This vesicle-mediated transport also included transendothelial channels. However, no passage of the tracer through disrupted interendothelial tight junctions was present.

During the next few years, more groups studied the effects of MRI exposure on the BBB permeability by injection of radioactive tracers into rats. One supported (Garber et al., 1989) while others contradicted (Adzamli et al., 1989; Preston et al., 1989) the initial findings made by Shivers et al. (1987). Garber et al. exposed rats to MRI procedures at 1.5, 0.5, and 0.3 T with RFs of 13, 21, and 64 MHz, respectively. Brain mannitol concentration was significantly increased at 0.3 T and 0.5 T but not at 1.5 T. No decrease in plasma mannitol concentration of MRI exposed animals was found and thus the authors concluded that effects of MRI associated energies on mannitol transport do not occur measurably in the body, and might be more specific to brain vasculature.

Preston et al. (1989) found no significant permeation of blood-borne ^{14}C-sucrose into brain parenchyma in anesthetized rats subjected to 23 min of MRI at 4.7 T and RFs at 12.5 kHz. However, the authors pointed out that if the MRI effect was focal and excess tracer counts were found only in restricted sites, there could have been MRI induced extravasation of sucrose that was not detected, due to the preponderance of normal tissue counts. When Preston et al. (1989) compared the lack of BBB leakage in their study to the MRI induced leakage which had been observed by Shivers et al. (1987), they also concluded that certain characteristics of electric and magnetic fields, which were present in the study by Shivers et al. but not in their own work, could have been critical to the observed effects.

In 1990, further studies by the Shivers-Prato group were presented (Prato et al., 1990) and the group could now quantitatively support its initial findings, in a series of 43 Sprague-Dawley rats. The BBB permeability to diethylenetriaminepentaacetic acid (DTPA) increased in rats after two sequential 23 min MRI exposures at 0.15 T. It was suggested that the increased BBB permeability could result from a time-varying magnetic field mediated stimulation of endocytosis. Also, the increased BBB permeability could be explained by exposure-induced increases of intracellular Ca^{2+} in the vascular endothelial cells. Since the Ca^{2+} is an intracellular mediator, increases of BBB permeability could possibly be initiated in this way. A few years later, in a series of 50 rats, the Shivers-Prato group also found that the BBB permeability in rats is altered also by exposure to MRI at 1.5 T for 23 min in 2 subsequent exposure sessions (Prato et al., 1994).

Our group started work on effects of MRI on rat brain in 1988 and found, by the use of Evans Blue, the same increased permeability over BBB for albumin (Salford et al., 1992).

This work was continued by separating the constituents of the MRI field: RF, undulant magnetic field, and static magnetic field. Since RF turned out to be the most efficient component of the MRI, the following studies focused mainly on the RF effects. Striving for investigating the actual real-life situation, endogenous substances, which naturally circulate in the vessels of the animals, were used. In line with this, albumin and also fibrinogen leakage over the BBB were followed after identification with albumin rabbit antibodies and rabbit anti-human fibrinogen.

The work by Blackman et al. (1985, 1989) and discussions with Prof A. R. Liboff, made the ground for studies on the frequency modulation 16 Hz and its harmonies 4, 8, 16, and also 50 Hz, of which the last is the standard voltage of the European power supply. A carrier wave of 915 MHz was used. Also, at an early stage 217 Hz modulation was added as this was the frequency of the then planned GSM system. The result of this work, with exposure to both CWs and pulsed modulated waves, in the most cases lasting for 2 h, showed that there was a significant difference between the amount of albumin extravasation in the exposed animals as compared to the controls. In the exposed group 35–50% of the animals had a disrupted BBB as seen by the amount of albumin leakage, while the corresponding leakage in the sham exposed animals was only 17%. The fact that sham-exposed control animals also show some amount of albumin extravasation, is most likely due to our very sensitive methods for immune histological examination. However, it is hard to explain the fact that although all animals in the 1997 series were inbred Fischer 344 rats, only every second animal at the most showed albumin leakage after EMF exposure. The question, what might protect the remaining 50% of the exposed animals from BBB disruption, is highly intriguing. It should be noted that in our large series, only in one single animal fibrinogen leakage has been observed.

In later studies, a significant ($p < 0.002$) neuronal damage is seen in rat brains 50 d after a 2 h exposure to GSM at SAR values 200, 20, and 2 mW/kg (Salford et al., 2003). This observation is corroborated in another study, where the animals were sacrificed 14 and 28 d, respectively, after an exposure for 2 h to GSM mobile phone electromagnetic fields at SAR values 0 (controls), 120, 12, 1.2, and now also 0.12 mW/kg (Eberhardt et al., submitted). Significant neuronal damage is seen after 28 d and albumin leakage through the BBB after 14 d. These findings may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 d.

In the majority of our studies, EMF exposure of the animals has been performed in transverse electromagnetic transmission line chambers (TEM-cells) (Salford et al., 1992, 1993, 1994, 2001, 2003; Van Hese et al., 1992; Martens and van Hese, 1993 and Persson et al., 1997). These TEM-cells are known to generate uniform electromagnetic fields for standard measurements. Each TEM-cell has two compartments, one above and one below the center septum. Thus, two animals can be exposed at a time. The animals are un-anaesthetized during the whole exposure. Since they can move and turn in the TEM-cells as they like, the component of stress-induced immobilization (described by Stagg et al., 2001) is effectively minimized. Through our studies, we have concluded that the amount of albumin leakage is neither affected by the sex of the animals, nor their placement in the upper or lower compartments of the TEM-cells.

Recent Studies on BBB Permeability, Focusing on the Effects of RF EMFs of the Type Emitted by Mobile Phones

With the increasing use of mobile phones, much attention has been directed towards the possible effects on BBB permeability, after exposure to the type of RF EMFs emitted by the different sorts of mobile phones.

Repetitions of our initial findings of albumin leakage have been made (Fritze et al., 1997), with 900 MHz exposure of rats for 4 h at brain power densities ranging from 0.3–7.5 W/kg. Albumin extravasation into the brain tissue was seen, with significant difference between controls and rats exposed reported for 7.5 W/kg, which is a thermal level. However, Fisher exact probability test (two-tailed) performed on the reported results, reveals significant ($p = 0.01$, Fisher exact probability test) difference for the subthermal level group (SAR = 0.3 W/kg plus 1.3 W/kg, compared to sham exposed and cage control animals) where in total 10 out of 20 animals showed one or more extravasations direct after exposure (Salford et al., 2001).

Another group, working in Bordeaux, and led by Prof Pierre Aubineau, has also demonstrated evidence of albumin leakage in rats exposed for 2 h to 900 MHz at non thermal SAR-values, using fluorescein-labeled proteins. The results were presented at two meetings (Töre et al., 2001, 2002). The findings are very similar to those of our group, described above. At the BEMS meeting in 2002 in Quebec City in Canada, the Aubineau-Töre group presented results from exposure GSM-900 EMFs at SAR-values of 0.12, 0.5, and 2.0 W/kg. Seventy Sprague-Dawley rats were included in the study. In addition to normal sham and normal GSM exposed rats, also rats subjected to chronic dura mater neurogenic inflammation, induced by bilateral sympathetic superior cervical ganglionectomy, were included. Arterial blood pressure was measured during the exposure, and Töre et al. (2001, 2002) concluded that the pressure variations (100–130 mm Hg) were well below those limits, which are

considered to be compatible with an opening of the BBB of rats. In order to induce opening of the BBB in rats, arterial blood pressure needs to reach values of 170 mmHg, according to Töre et al. (2001, 2002). At SAR of 2 W/kg a marked BBB permeabilization was observed, but also at the lower SAR-value of 0.5 W/kg, permeabilization, although somewhat more discrete, was present around intracranial blood vessels, both those of the meninges and of the brain parenchyma. Comparing the animals, which had been subjected to ganglionectomy, to the other animals, Töre et al. made an interesting observation: as expected, albumin extravasation was more prominent in the sympathectomised sham-exposed rats as compared to normal exposed rats. This was due to the fact that the sympathectomised rats were in a chronic inflammation-prone state with hyper-development of pro-inflammatory structures, such as the parasympathetic and sensory inputs as well as mast cells, and changes in the structure of the blood vessels. Such an inflammation-prone state has a well-known effect on the BBB leakage. However, when comparing sham-exposed sympathectomised rats to GSM-exposed sympathectomised rats, a remarkable increase in albumin leakage was present in the GSM exposed sympathectomised rats compared to the sham rats. In the GSM-exposed sympathectomised rats, both brain areas and the dura mater showed levels of albumin leakage resembling those observed in positive controls after osmotic shock. Indeed, more attention should be paid to this finding, since it implicates that the sensitivity to EMF-induced BBB permeability depends not only on power densities and exposure modulations, but also on the initial state of health of the exposed subject.

In rats, uptake of a systemically administered rhodamine-ferritin complex through the BBB also has been observed, after exposure to pulsed 2.45 GHz EMFs at average power densities of 2 W/kg (Neubauer et al., 1990). The authors observed that the magnitude of BBB permeability depended on power density and duration of exposure. Exposure to a lower power density (1 W/kg) and shorter duration of the exposure (15 min) did not alter the BBB permeability, as compared to higher power densities (SAR 2 W/kg) and longer duration of exposure (30–120 min). The microtubules seemed to play a vital role in the observed BBB permeability, since treatment with colchicine, which inhibits microtubular function, resulted in near-complete blockade of rhodamine-ferritin uptake. The mechanism underlying the observed leakage was presumed to be correlated to pinocytotic-like transport.

In other studies, no effect of EMF exposure has been observed on the BBB integrity. With exposure to 1,439 MHz EMFs, 1 h daily during 2 or 4 weeks (average whole-body energy doses of 0.25 W/kg) no extravazation of serum albumin trough the BBB was observed in a series of 36 animals (Tsurita et al., 2000). However, in this small material only 12 animals in total were EMF exposed (6 rats exposed for 2 weeks and 6 rats exposed for 4 weeks).

Also, lack of interference with the BBB function of rats was found after 1,439 MHz exposure for 90 min/d for 1–2 weeks at average brain power densities of either 2 or 6 W/kg (Kuribayashi et al., 2005). A total number of 40 animals were included in the study.

Also, Finnie et al. (2001) came to the conclusion that no increase in albumin leakage over the BBB resulted from EMF exposure in a series of 60 mice. With whole body exposure of mice to GSM-900 EMFs for 1 h at a SAR of 4 W/kg or sham exposure, no difference in albumin extravazation was observed between the different groups. Also, free-moving cage controls were included in the study, and interestingly, there was no significant difference between these non restrained mice as compared to

the sham and EMF-exposed animals. Thus, the authors concluded that there were no stress-related exposure module confinement effects on the BBB permeability.

Finnie et al. (2002) also investigated more long-lasting exposure effects. In a series of totally 207 mice, they exposed the animals for 60 min daily, 5 d a week for 104 weeks at average whole body SARs of 0.25, 1.0, 2.0, and 4.0 W/kg. This lead to a minor disruption of the BBB, as seen by the use of endogenous albumin as a vascular tracer. However, it should be added that the authors performed no statistical analyses to evaluate the albumin leakage through the small vessels in the brain. In an answer under correspondence in the same journal (Finnie and Blumbergs, 2004), the authors presented the original data from the long-term study. From that table one can conclude that non leptomeningeal albumin leaking vessels were seen in few sham-exposed animals, and in one third of the animals in the 0.25 W/kg group and to a lesser extent in the higher SAR groups. However, now significant differences are present.

The integrity of the BBB has also been investigated indirectly. Cosquer et al. (2005) treated rats with the muscarinic antagonist scopolamine methylbromide, which is known to induce memory impairments, followed by EMF exposure at 2.45 GHz for 45 min at average whole-body SARs of 2 W/kg. Opening of the BBB after EMF exposure was hypothesised to affect the performance in a radial arm maze. However, no such alterations were observed and the authors concluded that no BBB opening seemed to have occurred. In agreement with this, no albumin extravasation was noticed.

In a recent study (Ushiyama et al., 2007), the effects on the blood cerebrospinal fluid barrier after RF-EMF exposure were investigated for the first time. With a microperfusion method, cerebrospinal fluid from rat brain was collected in vivo. Fluorescent intensity of FITC-albumin in perfusate was measured. Rats exposed to 1.5 GHz RFs during 30 min at SAR-values of 0.5, 2.0, 9.5 W/kg for adult rats and 0.6, 2.2, 10.4 W/kg for juvenile rats, respectively, were compared to sham-exposed controls. Under these conditions, no increase in FITC-albumin was seen in the cerebrospinal fluid of exposed rats as compared to sham exposed controls. It was concluded that no effect on the function of the blood cerebrospinal fluid barrier was seen.

In Vitro Models

In recent years, there has been an increasing use of in vitro models in the search for BBB effects of EMF exposure.

In vitro models of the BBB have been studied, as by Schirmacher et al. (2000), with co-cultures consisting of rat astrocytes and porcine brain capillary cells. Exposure to GSM-1800 for 4 d with average SAR of 0.3 W/kg increased the permeability of ^{14}C-sucrose significantly compared to unexposed samples in the studied BBB model. These findings were not repeated in experiments performed later by the same group, after modifications of their in vitro BBB model (Franke et al., 2005b). The modified BBB model had a higher general tightness. It was specualted that at a higher original BBB permeability, which was present in the first study (Schirmacher et al., 2000), the cultures were more susceptible to the RF EMFs.

In the search after the mechanism underlying non thermal EMF effects, Leszczynski et al. (2002) observed human endothelial cells, with the interesting finding that GSM-900 exposure for 1 h with SAR-values of 2 W/kg resulted in changes in the phosphorylation status of many proteins. Among the affected pathways, the hsp27/p38MAPK stress response pathway was found, with a transient

phosphorylation of hsp27 as a result of the mobile phone exposure. This generated the hypothesis that the mobile-phone induced hsp27-activation might stabilize stress fibers and in this way cause an increase in the BBB permeability. Furthermore, it was also suggested that several brain damaging factors might all contribute to the mobile-phone induced effects observed in the brain and other structures as well.

Following the introduction of the 3G communication system, increasing attention has been drawn also to the effects of these RF fields. Using porcine brain microvascular endothelial cell cultures as an in vitro model of the BBB, no effects on barrier tightness, transport behavior, and integrity of tight junction proteins were observed after exposure to UMTS EMFs at 1.966 GHz for 1–3 d at different field strengths at 3.4–34 V/m, generating a maximum SAR of 1.8 W/kg (Franke et al., 2005a).

Low-Intensity, Extremely Low-Frequency Electromagnetic Field Radiation (ELF)

Only in a few studies have the effects of ELF exposure upon the BBB permeability been investigated. Öztas et al. (2004) found alteration of the BBB permeability in 33% of diabetic rats exposed to 50 Hz EMFs for 8 h at 5 mT. No effect was found in normoglycemic rats, leading to the conclusion that diabetes appears to increase the vulnerability of the BBB to the effects of EMFs.

Discussion

It has been suggested that BBB leakage is the major reason for nerve cell injury, such as that seen in dark neurons in stroke-prone spontaneously hypertensive rats (Fredriksson et al., 1988). Much speaks in favor of this possibility. The parallel findings in the Lund material of neuronal uptake of albumin and dark neurons may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 d. It should, however, be pointed out that the connection is not yet proven.

Also, other unwanted and toxic molecules in the blood may leak into the brain tissue in parallel with the albumin, and concentrate in and damage the neurons and glial cells of the brain. In favor of a causal connection between albumin and neuronal damage is a series of experiments performed in rats by another group at Lund University; albumin leaks into the brain and neuronal degeneration is seen in areas with BBB disruption in several circumstances: after intracarotid infusion of hyperosmolar solutions in rats (Salahuddin et al., 1988); in the stroke prone hypertensive rat (Fredriksson et al., 1988); and in acute hypertension by aortic compression in rats (Sokrab et al., 1988). Furthermore, it has been shown in other laboratories that epileptic seizures cause extravasation of plasma into brain parenchyma (Mihàly and Bozòky, 1984a,b), and in the clinical situation the cerebellar Purkinje cells are heavily exposed to plasma constituents and degenerate in epileptic patients (Sokrab et al., 1990). There are indications that an already disrupted BBB is more sensitive to the RF fields than an intact BBB (Töre et al., 2001; Franke et al., 2005b).

It has been stated by other researchers that albumin is the most likely neurotoxin in serum (Eimerl and Schramm, 1991). It has been demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 micro-liters of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ml albumin causes neuronal cell death and axonal severe damage (Hassel et al., 1994). It also causes leakage of endogenous albumin in and around the area of

neuronal damage. However, it is still unclear whether the albumin leakage demonstrated in our experiments locally reaches such concentrations.

The fact that some research groups observe albumin leakage/transport over the BBB after EMF exposure and others do not has led to a rather intense debate between the researchers but also in society, which is puzzled by the divergent findings. A major concentration of the involved research groups took place at Schloss Reisensburg in Germany in 2003, where the technical approaches in the studies of BBB effects especially were discussed. Two world-renowned researchers in the BBB field, Dr. David Begley of Kings College, London, and Prof. Olaf Poulsen of Copenhagen, Denmark, chaired the FGF/COST 281 Reisensburg, November 2–6 meeting. They made the final statement as a summary of the meeting: "It seems clear that RF fields can have some effects on tissues". The statement was made to a large extent on the basis of the concordant findings of the Bordeaux group, represented by Prof. Aubineau, and the Lund group, represented by Prof. Salford and Prof. Persson.

The biological effects of RF exposure depend on many parameters, such as mean power level and the time variations of the power (Bach Andersen et al., 2002) and whether in vivo or in vitro experiments are performed. In the in vivo situation, different kinds of animals, and also the same kind of animals but of different breeds, might react differently. It might not necessarily be the strongest RF fields that give rise to the most obvious biological effects. This has been observed by us (Persson et al., 1997; Salford et al., 2003). In many cases, the weak and precisely tuned EMFs have the most important biological function; two examples of this are cellular communication and protein folding. It seems quite likely that in different experimental set-ups, and in different living organisms, the signal has to be tuned to different properties in order to cause any effect. This could perhaps in some part explain why, in some cases, there are quite obvious effects of RF exposure, whereas in others, no such effects can be seen.

The search for the mechanisms behind the observed effects continues in several laboratories. As an example, studies in rats on long-term effects of GSM exposure in rats and electron microscopic examination of the brains from short-term GSM exposure can be mentioned (Salford et al., 2007).

Microarray analysis of the expression of all the rats' genes in cortex and hippocampus, after exposure to GSM RFs or sham exposure for 6 h, has shown interesting differences between exposed animals and controls (Nittby et al., 2008b). Genes of interest for membrane transport show highly significant differences. This may be of importance in conjunction with our earlier findings of albumin leakage into neurons around capillaries in exposed animals. It can be noted here that among the significantly altered genes from these evaluations, two variants of the gene RGS4 are upregulated in hippocampal tissue from exposed rats as compared to the sham-exposed rats (unpublished results). RGS is a regulator of G protein signalling, and it has been proposed that RGS4 might regulate BBB permeability in mammals, in a way corresponding to the role of its Loco homolog G protein coupled receptor (GPCR) in developing and maintaining the BBB permeability of Drosophila (Daneman and Barres, 2005).

It has also been suggested in other connections that manifestations of BBB disruption might also be mediated by the formation of free radicals, such as O_2^-, H_2O_2, and hydroxyl radical, which are supposed to oxidize cell membrane lipids by virtue of the high concentration of polyunsaturated fatty acids in these membrane constituents (Davson and Segal, 1996). As an example of this, it was reported by

Chan et al. (1983) that treatment of the brain of rats with a free-radical generating system resulted in lipid-peroxidation, and an increased permeation of Evans blue due to barrier breakdown.

Recently, a detailed molecular mechanism, by means of which mobile phone radiation exerts its effects, has been proposed (Friedman et al., 2007). By using Rat1 and HeLa cells, it was shown that EMF exposure resulted in rapid activation of ERK/MAPKs (mitogen-activated protein kinase). The activation of these ERKs was mediated by reactive oxygen species (ROS), resulting in a signaling cascade ultimately affecting transcription, by the central key role of ERKs in signalling pathways.

In the continued search for the mechanisms behind EMF mediated effects, their interaction with calcium-45 transport in biomembranes has been studied (Persson et al., 1992) and Ca^{2+}-efflux over plasma membranes has been observed in plasma vesicles from spinach exposed to ELF magnetic fields (Bauréus-Koch et al., 2003). With this model, quantum mechanical theoretical models for the interaction between magnetic fields and biological systems are tested. The model proposed by Blanchard and Blackman (1994), in which it is assumed that biologically active ions can be bound to a channel protein and in this way alter the opening state of that channel, could in this way be quantitatively confirmed. Thus, the membrane is one site of interaction between the magnetic fields and the cell, and more specifically, the Ca^{2+}-channels, are one of the targets. More recently, new models for the interaction between magnetic fields and hydrogen nuclei also have been proposed. We are presently investigating these experimentally. Also, application of RF fields will show whether EMF of this kind have the same possibility to affect Ca^{2+}-channels as the low-frequency fields applied in our study from 2003.

EMF-induced Ca^{2+}-efflux over plasma membranes, understandably, can have many different effects on the target cells. Some agents that increase the BBB permeability act through a contractile mechanism that widens the intercellular junctions of the capillary endothelium. An increase of free Ca^{2+} should mediate these changes, thereby resulting in measurable alterations of intracellular Ca^{2+}-levels in brain capillary cells after exposure to BBB-disrupting agents (Davson and Segal, 1996). Another hypothesis is that EMF-induced intracellular Ca^{2+}-alterations might affect Ets genes, which are transcription factors expressed in different tissues (Romano-Spica and Mucci, 2003). In this context, we could add that in our gene expression material from GSM-exposed rats vs., sham-exposed rats, one Ets variant gene is actually significantly up-regulated in hippocampus and one Ets1 gene is significantly up-regulated in cortex of the exposed animals.

In recent years, the GSM phones have been increasingly replaced by the 3G mobile phones, which emit EMFs of different frequencies and pulse modulations. Since these properties have a crucial role in the interaction between EMFs and living organisms, it is important to investigate what exposure to the new 3G fields might result in. Are the effects comparable to those of the GSM phones, or are there new unexpected effects? Or, in the best case, do the 3G fields not affect life at all? We presently study animals exposed to the new 3G fields, in the search of the answers to these questions.

Studies on EMF induced BBB disruption have shown contradictory results from different laboratories. Some groups demonstrate increased BBB permeability with their experimental conditions, whereas others do not. Many factors may contribute to this. One remarkable observation, which we have made in our studies throughout the years, is that exposure with whole-body average power densities below 10 mW/kg

gives rise to a more pronounced albumin leakage than higher power densities, all at non thermal levels. In many studies, the SAR exposure has been restricted to the peak power of 2 W/kg and average SAR-values of 0.25 W/kg (Neubauer et al., 1990; Tsurita et al., 2000 among others), since this is the value recommended as the maximum localized SAR-value for head and trunk exposure (ICNIRP 1998). The very low SAR-values, such as 1 mW/kg, which for example exist at a distance of more than one meter away from the mobile phone antenna and at a distance of about 150–200 m from a base station, have been extensively investigated by us and it is our conviction that continued research in this field should include SAR-values at these levels, not only for GSM, but also for systems such as 3G and others to be developed.

Demonstrated effects on the BBB, as well as a series of other effects upon biology (for reference see Hyland, 2000) have given rise to public anxiety. It therefore is up to the public and also the providers of the radiofrequency-emitting technologies to support continued research in order to understand the nature of the effects, thereby neutralizing or at least reducing them. Also, it should be kept in mind that proven effects on biology also means that positive potentials might be revealed. This might be useful in medical applications, for example a controlled opening of the BBB would enable previously excluded pharmaceuticals to reach their targets within the brain tissue.

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