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Interactions between radiofrequencies signals and living organisms

Effects of radiofrequency field on the blood-brain barrier: A systematic review from 2005 to 2009

Effets des radiofréquences sur la barrière hémato-encéphalique : Revue systématique de 2005 à 2009

Anne Perrin [a,*], Celine Cretallaz [a], Alice Collin [a], Christine Amourette [a], Catherine Yardin [b]

[a] *Radiobiology Department of the Health, Service Research Center for Defence (IRBA-CRSSA), BP 87, 38702 la Tronche cedex, France*
[b] *Department of Histology and Cytogenetics, EA3842, Limoges University Hospital, Faculty of Medecine, 87025 Limoges cedex, France*

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ABSTRACT

The published results available in 2005 were insufficient to draw firm conclusions concerning the possible non-thermal effects of radiofrequency fields on the blood-brain barrier (BBB). This critical review deals with 16 articles on this topic published since 2005. The methodological quality of these articles was not equivalent. We therefore analysed the underlying methodologies from both their biological and physical aspects. We conclude that recent studies provide no convincing proof of deleterious effects of RF on the integrity of the BBB, for specific absorption rates (SAR) up to 6 W/kg.

RÉSUMÉ

En 2005, les résultats publiés ne permettaient pas de donner une réponse claire quant à l'existence ou non d'effets non thermiques des radiofréquences (RF) sur la barrière hémato-encéphalique (BHE). Cette revue critique de la littérature scientifique porte sur 16 articles parus depuis. Les études étant de qualité méthodologique inégale, leur méthodologie a été analysée autant au point de vue biologique qu'au point de vue physique. En conclusion, les travaux récents n'apportent pas de preuve convaincante d'un effet des RF sur l'intégrité de la BHE avec des débits d'absorption spécifique (DAS) allant jusqu'à 6 W/kg.

1. Introduction

The brain is the central element of the nervous system. Its position within the skull makes it particularly exposed to the waves emitted by mobile telephones during conversations. Studies of the effect of radiofrequency fields (RF) on this organ are essential, given the massive scale of mobile telephone use in the general population, at increasingly young ages. In particular, the possible effect of RF on the blood-brain barrier (BBB) has been highlighted and became a source of public controversy. This possible effect also gave rise to a considerable debate in the scientific community.

The functioning brain is one of the tissues in the body most dependent on energy resources. Despite this need for large amounts of nutrients, it is essential to maintain the homeostasis of the cerebral microenvironment. This protection of the

* Corresponding author.
E-mail address: aperrin.crssa@gmail.com (A. Perrin).

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cerebral microenvironment requires the isolation of the brain from the external environment, blocking access to the brain of most substances circulating in the bloodstream, but allowing the passage of nutrients, metabolic substrates and waste products. This highly specialised function is carried out by a system called the blood-brain barrier (BBB), which regulates exchanges at the interface between the blood and the brain. This structure consists essentially of the endothelial cells lining the blood vessels and the projections of glial cells surrounding the capillaries. The histological organisation (with tight junctions between endothelial cells) and the properties of these cells render the BBB a real selective and metabolically active barrier controlling the passage of solutes [1]. Some specific localised zones of the brain are not protected by the BBB. The endothelial cells in these regions do not form tight junctions and remain fenestrated, allowing the free exchange of molecules between the blood and the adjacent neurons. These regions (the seven brain nuclei) are involved in the hormonal regulation of other organ systems. Free exchanges between the blood and neurons allow these regulatory centres to respond to changes in the concentrations of peptides and other substances in the blood.

Increases in BBB permeability might lead to changes in brain metabolism and in the synaptic activity of neurons. Such increases have been implicated in the mechanisms underlying a number of pathological conditions: chronic neurological autoimmune diseases (multiple sclerosis), infectious diseases (meningitis, malaria, AIDS) and have also been shown to occur during ischemia, hypertension, trauma and brain tumours. This has led to a number of investigations being carried out *in vivo* or *in vitro*, to examine the effect of RF on the integrity of the BBB.

A previous review of 39 studies published between 1973 and 2005 was carried out by J. Lin [2]. The published results available in 2005 were inconclusive concerning the potential induction, by RF, of non-thermal effects on the BBB, at low levels of exposure. This review also involved a meticulous analysis of the methodologies used in these studies. Some of them reported effects for exposure levels below the authorised limits for mobile phones in particular (2 W/kg). Other studies failed to reproduce these results for specific absorption rates (SAR) between 0.3 and 1.5 W/kg. Moreover a large number of former studies provided no SAR value, giving the exposure level in W/m^2, making it impossible to determine the power effectively absorbed by the tissues. The results obtained by Salford's Swedish team raised particular attention, with effects reported down to SAR values three orders of magnitude lower than those authorised for cell phones [3]. Since 1997, this team has reported effects of low-power RF on the BBB (changes in permeability and/or the presence of so-called "dark" neurons) based on a series of experiments with frequencies between 900 and 915 MHz, either continuous or modulated GSM (Global System for Mobile communication), with SAR of 0.0016 to 5 W/kg. These studies demonstrated no dose-response effect. Some effects could be obtained at low SAR, but not at high SAR, for example. Within the scientific community, a debate occurred about possible confounding factors, focusing in particular on the protocols used for animal experimentation and sample treatment. Other considerations include the risk to human health if these results were to be confirmed. Only two studies have tried to reproduce the effects on BBB permeability to albumin initially reported by Salford's team [4,5]. Albumin extravasation was confirmed at 7.5 W/kg (clearly a thermal effect), but no significant modification was observed below this level (non-thermal effects). However, these studies were not identical replicas of the Swedish study. It was therefore considered indispensable to pursue such studies, to clarify the situation and remove the uncertainty. In 2006, given the possible repercussions for health of such an effect on the BBB, the WHO (World Human Organization) called urgently for the replication of these results in other laboratories, to confirm or invalidate these findings (RF research agenda, 2006, www.who.int/peh-emf/research/agenda/en/index.html).

This review article presents studies published on this topic between the start of 2005 and the end of 2009. These studies concerned the frequencies used by mobile phones, either continuous or with various types of modulation. The methodology used was analysed in detail, focusing on both biological and physical aspects. Altogether, 16 publications were subjected to critical analysis: three presenting results obtained *in vitro*, 11 presenting results obtained *in vivo* and two presenting results for humans.

2. Criteria used for methodological analysis

2.1. Dosimetry

A key prerequisite for these studies is the availability of an exposure system in which experiments can be carried out in conditions of controlled exposure (frequency, modulation, power, temperature, ventilation, etc.). The system used must be appropriate for the type of study and must be described in detail.

Specific absorption rate (SAR, expressed in W/kg) is the parameter characterising the level of exposure. It is therefore essential to determine this rate. Without it, the experimental conditions are not fully defined (the equivalent of carrying out toxicology tests on a product without knowing how much of the product had been administered). The SAR should be determined by at least two methods: numerical simulation and experimental determination (measurement of temperature and/or electrical field). Temperature should be measured with electromagnetically neutral probes (e.g. fibre optic probes).

As it is difficult to obtain reliable values for SAR, we analysed the dosimetric methodology presented in each study in detail, to ensure the validity of the SAR reported.

Several different situations emerged during our analysis of these research articles:

- The method used to obtain the SAR was well described: numerical simulations validated by the measurement of temperature and/or electrical field (validated dosimetry);

- The SAR was estimated from measurements (approximate calculations) or solely by numerical simulation. Either numerical or experimental validation was lacking (incomplete dosimetry);
- The method for obtaining the SAR was mentioned, but with no description or reference to another publication (undescribed dosimetry);
- The exposure conditions (SAR) were unknown and no dosimetry was carried out (dosimetry absent).

2.2. Biological aspects

Exposure is described as "acute" if it is of short duration (several minutes to several hours, depending on the model) and as "chronic" if it extends over a large proportion of the life of the animal or human. The notion of "semi-chronic" (or "subchronic") is used for *in vivo* exposure for several days to several months. For cell cultures, it is difficult to apply these notions of "chronic" and "semi-chronic" exposure, but acute exposure for several minutes or hours may be considered.

The quality criteria that must classically be taken into account for biological studies are:

- Relevance of the model,
- Validity of the techniques (reliability, sensitivity, etc.),
- Power of the study: number of cases (sample size) and number of replicates,
- Blind or even double-blind tests, particularly for studies on humans,
- Use of statistical tests.

For studies carried out *in vivo*, depending on the system used, the animals may be constrained (immobilised) during exposure, subjecting them to a major stress likely to modify the results. Handling stress has been reported to modify the permeability of the BBB [6,7]. For that reason, measures must be taken to limit this stress, notably by allowing the animals to get accustomed to this situation.

All studies carried out *in vivo* or *in vitro* should include a dummy or "sham" exposure group, making it possible to compare the results obtained with and without exposure to RF. This possibly ensures that any observed effect is genuinely due to the electromagnetic field and not to the exposure system itself. In practice, the experiment is carried out in two identical systems, differing only in terms of the presence or absence of the electromagnetic field. Sham exposure is included in most studies of this type.

Experiments are also conducted in parallel in the usual conditions of culture (for cells) or breading (for animals). In this case, we are dealing with "controls" (cage controls for the animals) or negative controls. The technique used should also be validated with positive controls, making it possible to demonstrate that the effect, if indeed there is one, is clearly detected with the used technique. A factor known to induce the studied effect is required for this (for example a mutagenic agent to detect DNA damage). In the absence of all these controls, it is not possible to conclude that any observed effect is specific to electromagnetic fields.

Furthermore, ideally, experiments should be carried out blind, particularly if the analysis of the results depends on the subjective judgement of the investigator, as is the case for microscopic observations or the manual counting of events.

3. Methods for evaluating the integrity of the BBB

In vivo, various methods are used to evaluate the integrity of the BBB. All are based on an evaluation of BBB permeability. This permeability may be estimated by observation of the passage in the nervous system of molecules such as albumin, fibrinogen or immunoglobulins (or of cells such as lymphocytes), endogenous components of the vascular compartment, therefore not requiring injection into the animal. We can therefore speak, for example, of the extravasation of albumin. These molecules are visualised by immunohistochemical staining on sections of the brain or are determined in brain extracts (counting of events, measurement of fluorescence).

Other techniques involve injecting the animal with molecules labelled with a radioactive or fluorescent isotope or with a dye (Evans Blue) that would not normally cross the BBB. These molecules, such as ^{14}C-labelled or fluorescein-labelled sucrose, serve as tracers. These techniques can be coupled to intracerebral microdialysis.

Non-invasive techniques may also be used to detect the permeability of the BBB *in vivo*: magnetic resonance imaging (MRI) or positron emission tomography (PET).

One additional method involves evaluating changes in nervous system tissues through the detection of degenerating neurons – known as "dark" neurons – which can be detected by cresyl violet staining (which is not specific for neurons) or with a fluorescent molecule (Fluoro-JadeB) more specific for this cell type, thereby decreasing the frequency of false positive results.

During *in vivo* experiments in which animals are immobilised for the exposure period, the habituation step before exposure is particularly important, because handling stress may modify BBB permeability [6,7].

In vitro studies can be carried out on reconstituted BBB models, in which it is possible to measure permeability by studying the passage of molecules between two compartments or by measuring electrical resistance. These models consist of at least one cell type (endothelial cells), which may be co-cultured with other cells (astrocytes, glial cells, etc.), depending on the complexity of the model.

4. Literature review

For the 16 research articles analysed below:

- Three studies *in vitro* were carried out either over relatively long periods, with GSM signals at 1800 MHz [8], UMTS-1966 (Universal Mobile Telecommunication System) [9], or during short times corresponding to acute exposure to 915 MHz (continuous) or 20 Hz (modulated) [10];
- Eleven studies *in vivo* were carried out on rats or mice subjected to chronic [11], semi-chronic [12–16] or acute [17–21] exposure;
- Two studies were carried out on humans, by the same team [22,23].

This analysis is summarised in Table 1.

4.1. Studies in vitro

Franke et al. [8] observed no change in BBB permeability to sucrose in response to constant exposure, over a period of one to five days, to a GSM signal at 1800 MHz. The dosimetry of this study was well described. The same team [9] also reported an absence of effect of a UMTS signal in double-blind experiments involving measurements of the permeability of the BBB to various labelled tracers and electrical resistance. Temperature was controlled and numerical simulations for dosimetry were well described, but the method used for SAR calculation from the electrical field was not explained.

After an acute exposure to CW or PW-915, Kuo and Kuo [10] tried to get antiviral drugs that are active against HIV across the BBB, by administering them together with various types of molecules facilitating BBB permeabilisation. However, examination of the figures showed contradictory results. The SAR was unknown and the conditions of exposure were not clearly described. It is therefore not possible to interpret the results of this study.

4.2. Studies in vivo

All the *in vivo* studies were carried out on rodents (rats and mice).

The team of Salford performed three studies on Fischer rats in Sweden [11,18,21]. In the first, Eberhardt et al. [18] observed an effect on BBB permeability, detected by the passage of albumin and analysis of neuronal degeneration (by cresyl violet staining), following acute exposure of male and female rats at SAR of 0.12 and 1.2 mW/kg. The integrity of the BBB was assessed on days 14 and 28 post-exposure. The SAR tested were 0.1, 1, 10 and 100 mW/kg for the males and 0.13, 1.3, 13 and 130 mW/kg for the females (7 or 8 rats per group). Different SAR were used for males and females, because they generally have different weights. Significant effects on the passage of albumin across the BBB were observed and the number of dark neurons increased with decreasing SAR. This team previously reported similar effects in 2003 [24], but with maximal neuronal degeneration observed at another SAR (200 mW/kg). The authors provided no explanation for this. Similarly, they wondered how the observed extravasation of albumin, which was very slight, could possibly be related to neuronal degeneration, although a correlation was identified. They detected no effect of sex or position in the exposure chamber and there was no effect on other cells around the altered neurons.

In 2009, the same team [21] carried out a study in similar conditions, with SAR of 0, 0.12, 1.2, 12 and 120 mW/kg, on 48 rats assigned to four groups of eight rats (exposed) and a group of 16 sham-exposed rats. The authors observed weak but significant permeability of the BBB to albumin at 12 mW/kg and for various time periods. By contrast, no effect on the number of dark neurons was observed. The sections were analysed blindly. The introduction took up about half this article and constituted a review essentially limited to the work of Salford's group.

However, Grafstrom et al. [11] detected no change in the permeability of the BBB to several types of markers and observed no dark neurons or neuronal damage after exposing rats to a GSM 900 signal with a SAR of 0.6 or 60 mW/kg, for two hours per week, over a period of 55 weeks. BBB integrity was assessed five to seven weeks after the end of the exposure period. In total, 56 four- to six-month-old rats were used at the start of the exposure period (initial weights of 200 g for the females and 365 g for the males, with equal numbers of male and female rats): 32 were exposed, 16 were sham-exposed and 8 were used as cage controls. The weight of the rats at the end of the experiment was 300 g for the females and 545 g for the males. Finally, the used sample was highly variable.

No dosimetry was performed in these three studies. A numerical simulation was mentioned for calculation of the SAR, but the method used was not described, and there was no evidence of experimental dosimetry.

Three teams [17,19,20], in France, the US and Japan, have attempted to replicate the results obtained *in vivo* by the Swedish team of Salford cited above [11,18,21], in comparable conditions. These three replication studies (on Fischer 344 rats) are presented below:

Masuda et al. [19] observed no passage of albumin across the BBB and no appearance of dark neurons in experiments aiming to replicate the results of Salford et al. [24] using an identical exposure system (cell transmitting electromagnetic radiation: TEM, with two compartments). Dosimetry was experimental, with SAR calculated from incident, reflected and transmitted power. No numerical simulations were carried out. The experiments were carried out blind. The animals were exposed to a continuous 915 MHz signal (SAR of 0.02, 0.2 and 2 W/kg) for 2 h. Eighty-two male rats were tested, in groups

Table 1
This table summarises the 16 articles analysed including their physical (signal modulation and frequency, mean SAR or power density, exposure set-up and dosimetry) and biological (biological model, exposure conditions, biological tests and/or measured parameters) aspects. The first paragraph concerns *in vitro* studies, the second *in vivo* studies and the third one human studies.

Tableau 1
Ce tableau présente les 16 articles analysés dans cette revue, en détaillant la partie physique (modulation, fréquence, DAS moyen ou densité de puissance, système d'exposition et dosimétrie) et la partie biologie (modèle biologique, conditions d'exposition, tests biologiques, et/ou paramètres mesurés). Le premier paragraphe concerne les études *in vitro*, le deuxième les études *in vivo* et le troisième les études humaines.

Reference	Physical part				Biological part				
Authors	Signal modulation and frequency (MHz)	Mean SAR (W/kg)	Exposure set-up and dosimetry	Dosimetry (validated, incomplete, undescribed, absent)	Biological model	Exposure conditions	*in vivo* exposure (chronic, semi-chronic or acute)	Biological tests and/or measured parameters	Effect (yes/no)
				IN VITRO STUDIES					
Franke H. et al. (2005) [8]	GSM-1800	0.3 W/kg	Rectangular waveguide (RG22). Numerical dosimetry. No temperature control.	validated	3 BBB cell culture models: co-culture system (rat astrocytes in co-culture with porcine brain microvascular endothelial cells or PBECs), and PBEC monocultures with or without serum	for 1–5 days	–	Permeation to radioactive tracers: 14C-sucrose, ^{3}H-glucose, ^{3}H-leucine, ^{3}H-alanine, or ^{125}I-bovine serum albumin on BBB models.	no
Franke H. et al. (2005) [9]	UMTS-1966	0.02 to 1.64 W/kg	Radial waveguide. Numerical dosimetry (E field distribution and SAR estimation). Temperature control.	incomplete	porcine brain microvascular endothelial cells (primary cultures)	for 84 h	–	Transendothelial electrical resistance (TEER) measurement, permeation to radioactive tracers: ^{14}C-sucrose, ^{3}H-glucose, ^{3}H-leucine, ^{3}H-alanine, or ^{125}I-bovine serum albumin. Immunocytochimy (occludin, ZO1), Western Blot.	no
Kuo Y.C. and Kuo C.Y. (2008) [10]	CW, PW-915	5, 10, 20 mW	Cylindrical copper coil.	absent	human brain-microvascular endothelial cells	90 min CW, modulation (20 MHz) with sinusoidal, square and triangular waves	–	Permeability to saquinavir (SQV) (antiretroviral agent) associated with 3 types of carriers: PBCA, MMA-SPM and SLN used to facilitate the transport of SQV. Measurement of resistance and permeability of [14C]sucrose but no given data for the concentrations of sucrose SQV measured by HPLC-UV.	yes
				IN VIVO STUDIES					
Eberhardt J.L. et al. (2008) [18]	GSM-900	Males 350 g: 0.1; 1; 10 and 100 mW/kg, Femelles 200 g: 0.13; 1.3; 13 and 130 mW/kg	TEM cell. Numerical dosimetry.	undescribed	Fischer 344 rats	2 h	acute	BBB permeability with albumin immunohistochemistry to detect albumin extravasation at day 14 and day 28: albumin foci (around vessels), diffuse albumin, neuronal albumin. Dark neurons (cresyl violet staining). Semi-quantification (score from 0 to 3). $n = 96$ (8×8 exposed, 2×16 sham rats).	yes

Table 1 (*Continued*)

Reference	Physical part				Biological part				
Authors	Signal modulation and frequency (MHz)	Mean SAR (W/kg)	Exposure set-up and dosimetry	Dosimetry (validated, incomplete, undescribed, absent)	Biological model	Exposure conditions	*in vivo* exposure (chronic, semi-chronic or acute)	Biological tests and/or measured parameters	Effect (yes/no)
Nittby H. et al. (2009) [21]	GSM-915	0; 0.12; 1.2; 12; 120 mW/kg	TEM cell. Numerical dosimetry.	undescribed	Fischer 344 rats	2 h	acute	Same parameters and assays than Eberhardt et al., 2008 with J7 samples. Albumin extravasation at day 7: albumin foci (around vessels), diffuse albumin, neuronal albumin. Dark neurons (cresyl violet staining). Semi-quantification (score from 0 to 2). $n = 48$ (4 groups of 8 exposed rats, 16 shams).	yes
Grafstrom G. et al. (2008) [11]	GSM-900	0.6 and 60 mW/kg	TEM cell located in a wood box. The source is a test phone. Numerical dosimetry.	undescribed	Fischer 344 rats	2 h/week (once) for 55 weeks	chronic	BBB permeability with albumin immunohistochemistry to detect albumin extravasation, dark neurons (cresyl violet staining), lipofuscin aggregation (Sudan Black B), cytoskeletal and neuritic changes (silver method by Gallyas), astrocytes (GFAP). $n = 56$ (32 exposed rats, 16 sham and 8 control).	no
Masuda H. et al. (2009) [19]	CW-915	0; 0.02; 0.2; 2 W/kg	TEM cell. Experimental dosimetry.	incomplete	Fischer 344 rats	2 h	acute	Histology and immunohistochemistry of the brain. Albumin permeability. Dark neurons (hematoxylin and eosin staining). $n = 82$ (3 groups of 16 exposed rats, 16 sham and 16 cage controls).	no
McQuade J.M. et al. (2009) [20]	CW and GSM-915	0.0018 and 20 W/kg	TEM cell. Experimental dosimetry.	incomplete	Fischer 344 rats	30 min	acute	Albumin immunohistochemistry. BHE permeability. Blind experiments, two operators. Weighing of the fresh brain. $n = 512$ (exposed, sham, positive control, 27 to 42 rats per group).	no
Poulletier de Gannes F. et al. (2009) [17]	GSM-900	0; 0.14; 2 W/kg	Loop antenna. Numerical and experimental dosimetry.	validated	Fischer 344 rats	2 h	acute	BHE permeability for albumin. Dark neurons. $n = 98$ (5 groups of 8 exposed rats, 5×8 shams, 8 cage controls, 10 positive controls).	no

(*continued on next page*)

Table 1 (*Continued*)

Reference	Physical part				Biological part				
Authors	Signal modulation and frequency (MHz)	Mean SAR (W/kg)	Exposure set-up and dosimetry	Dosimetry (validated, incomplete, undescribed, absent)	Biological model	Exposure conditions	*in vivo* exposure (chronic, semi-chronic or acute)	Biological tests and/or measured parameters	Effect (yes/no)
Cosquer B. et al. (2005) [12]	PW-2450	Whole body 2 W/kg ± 2 dB Brain 3 W/kg ± 3 dB	Cylindrical waveguide with numerical rat models (300 g).	validated	Sprague–Dawley rats	PW: 2 µs pulses, 500 Hz for 45 min during 10 days	semi-chronic	Maze performance of the animals after muscarinic antagonist injection (scopolamine MBR) that poorly crosses the BBB: Indirect assay to detect changes in BBB permeability: 12 arm radial maze test enabling demonstration of memory deficits during 10 consecutive days. Direct assay of BBB alterations by injections of Evans blue. $n = 36$ (12 exposed rats, 12 sham, 12 controls).	no
Kuribayashi M. et al. (2005) [16]	TDMA-1439	0; 2 and 6 W/kg	Carroussel in anechoïc chamber. Ventilation. Temperature and power control. Numerical and experimental dosimetry.	validated	Fisher 344 rats	90 min/day 6 days per week for 1 or 2 weeks	semi-chronic	Experiment 1: young (10 weeks old) rats: injection of 1,3-dinitrobenzene (positive controls). Experiment 2: four- and ten-weeks old rats (immature and young). Injection of FITC-dextran before the sacrifice. Immunohistochemical analyses of p-glycoprotein, aquaporin-4, claudin-5, von Willebrand-Factor-VIII related antigen, and albumin. Quantitative RT-PCR analysis of BBB related genes for p-glycoprotein, aquaporin-4 and claudin-5. $n = 40$ (experiment 1 : 10 and experiment 2 : 30).	no
Finnie J.W. et al. (2006) [13]	GSM-900	4 W/kg	Dipole antenna. Animals in plastic holders.	validated	BALBc mice	60 min/day 7 days after birth	semi-chronic	BBB permeability with albumin immunohistochemistry to detect albumin extravasation. $n = 30$ (10 exposed rats, 10 control, 10 sham) + positive control group (cadmium, $n = 10$).	no
Finnie J.W. et al. (2006) [14]	GSM-900	4 W/kg	Dipole antenna. Animals in plastic holders.	validated	Pregnant BALBc mice	60 min/day from day 1 to day 19 of gestation	semi-chronic	BBB permeability with endogenous albumin immunohistochemistry to detect albumin extravasation. $n = 90$ (30 exposed rats, 30 control, 30 sham) + positive control group (cadmium, $n = 10$).	no

Table 1 (*Continued*)

Reference	Physical part				Biological part				
Authors	Signal modulation and frequency (MHz)	Mean SAR (W/kg)	Exposure set-up and dosimetry	Dosimetry (validated, incomplete, undescribed, absent)	Biological model	Exposure conditions	*in vivo* exposure (chronic, semi-chronic or acute)	Biological tests and/or measured parameters	Effect (yes/no)
Kumlin T. et al. (2007) [15]	GSM-900	0 0.3 and 3 W/kg	RTL Numerical and experimental dosimetry.	validated	Wistar rats	2 h/day 5 days/week for 5 weeks	semi-chronic	Behavioural tests sensitive to neurobehavioural changes due to postnatal exposure to a number of environmental toxics. Cerebral immunohistology. $n = 72$ (24 high SAR, 24 low SAR ,24 sham).	yes
				HUMAN STUDIES					
Söderqvist F. et al. (2009) [22]	GSM-890	1 W/kg	Mobile phone in test mode (antenna located at 8.5 cm from the head). Experimental dosimetry.	incomplete	Human	30 min	acute	Questionnaire. Blood samples for serum transthyretin and S100b concentrations. $n = 41$.	yes
Söderqvist F. et al. (2009) [23]	ND-ND	ND	Blood samples taken from subjects (and survey on the use of mobile phone).	absent	Human	ND	chronic	Questionnaire. Blood samples for serum transthyretin concentration. $n = 314$.	yes

of 16 (or 8 for the analyses on days 14 and 50). Two positive controls were used, based on two different models of BBB rupture (cold, chemical).

McQuade et al. [20] observed no effect of 30 minutes exposure to modulated GSM 915 MHz (two types of modulation: 217 Hz and 16 Hz) or to a continuous signal (SAR of 0.0018 to 20 W/kg), in male rats weighing 125 to 300 g after several habituation steps (512 rats, in groups of 27 to 42). The permeability of the BBB to Evans Blue dye was examined on brain sections under the microscope. Two types of positive control (heating and chemical) were used. Sections were analysed blindly, by two investigators and, as a precaution, some of the slides were sent to Salford's team for counting. The same results were obtained. The exposure system was set up with the accord of the Salford team (after a visit), to ensure that the study was reproduced as faithfully as possible. The dosimetry was experimental, by calculation, as in the study by Masuda et al. [19], and by calorimetry. The temperature was measured with a probe compatible with radiofrequencies. A statistical method identical to that of Salford was used for analysing the results. The authors were surprised to see no change in the BBB at a SAR of 20 W/kg, but local measurements showed that the temperature did not exceed 40.6 °C and was therefore not sufficient to generate a thermal effect.

Poulletier de Gannes et al. [17] also failed to confirm the results of Salford, after exposing 16 rats to a GSM 900 signal (SAR of 0.14 and 2 W/kg) for 2 h after gradual habituation. They observed no effect on BBB integrity or neuronal degeneration. These authors also assessed neuronal apoptosis. Exposed animals were compared with the cage controls ($n = 8$) and with the positive controls (cold shock; $n = 10$). The authors used several methods to evaluate neuronal degeneration, including the more specific Fluoro-Jade staining technique. This study included complete numerical and experimental dosimetry.

In these three studies, the samples studied were homogeneous, with rats of the same age, same weight and same sex (all males, the use of females potentially leading to hormonal variations). In terms of exposure, some of the teams used higher SAR than those used in the studies by the group of Salford, or a broader range of powers.

Cosquer et al. [12] observed no effect of semi-chronic exposure to 2450 MHz pulses (2 µs, 500 Hz) for 45 minutes per day over 10 days, neither using indirect observations based on cognitive tests nor the passage of Evans blue dye across the BBB (study carried out on 36 male Sprague-Dawley rats). For the cognitive tests, the authors investigated whether RF modified the behavioural response of the animals to the injection of a muscarinic antagonist (scopolamine) that crosses the BBB only poorly. In contrast, the response of the cage control rats differed from those of the sham-exposed and exposed rats (effect of stress), despite habituation to handling before testing. On arrival at the laboratory, the rats were placed in individual cages, which is not desirable in terms of social behaviour. The dosimetry was complete and the SAR in the brain was 3 W/kg.

Four studies have been carried out on animals during periods of development, in which greater sensitivity might be observed [13–16]:

Kuribayashi et al. [16] exposed four- or ten-week-old Fischer rats for one to two weeks to a TDMA (time division multiple access)-modulated signal of 1439 MHz and studied the effects of this signal on the BBB. Satisfactory dosimetric data were provided. The various techniques used (administration of FITC-dextran before the animals were killed and observation of brain sections under a fluorescence microscope, immunohistochemical analysis of several proteins, quantitative RT-PCR) showed no effect on BBB, whereas a positive effect was observed for the positive control (injection of 1,3-dinitrobenzene). The authors concluded that exposure to electromagnetic waves at 1439 MHz had no deleterious effects on the BBB in young or immature rats.

Finnie et al. [13] observed no albumin extravasation in newborn mice exposed to a GSM 900 signal (4 W/kg) for 60 minutes/day for the first seven days after birth. In a previous study, these authors found no effect of the same exposure conditions on the brains of mouse foetuses exposed throughout gestation [14]. In this case, the foetal mice were exposed, throughout gestation (day 1 to day 19) to a GSM signal (900 MHz, SAR of 4 W/kg). Sham-exposed animals, positive controls (cadmium) and cage controls were included. The foetal mice were killed on day 19. An immunohistochemical study of endogenous albumin was carried out to check the integrity of the BBB. No albumin extravasation was observed in the cerebral cortex, thalamus, basal ganglia, brainstem, hippocampus, diencephalon or spinal cord.

In the study by Kumlin et al. [15], 21-day-old male Wistar rats were exposed for five consecutive weeks (2 h/day, 5 days/week) to modulated GSM 900 MHz signals (SAR of 0.3 and 3 W/kg). An immunohistochemical study of the brain was then carried out, in parallel with behavioural tests known to reveal changes in neurological behaviour after postnatal exposure to toxic compounds. No difference in weight was found between the exposed and unexposed rats. Histological studies found no evidence of an effect on general brain morphology, the number of dead or newly formed (dentate gyrus and hippocampus) neurons or changes in the BBB. By contrast, an improvement was observed in learning and memory in behavioural tests in rats exposed to RF.

4.3. Studies on humans

In these studies, the authors carried out determinations of the S100b and transthyretin (TTR) proteins concentrations on blood samples, as markers of BBB integrity [22,23]. These endogenous circulating proteins are not commonly used as markers, despite their value for this purpose.

Söderqvist et al. [22] carried out challenge tests in which they exposed 41 volunteers (17 men and 24 women between the ages of 18 and 30 years) to a GSM 890 MHz signal for 30 minutes. The SAR was given as 1 W/kg, but with no mention

of any numerical or experimental dosimetry. The subjects were seated in front of an LCD screen and watched a DVD during the exposure period. Four blood samples were taken from each volunteer: one on arrival at the laboratory, another after 30 minutes of rest, a third at the end of the exposure period and the last, 60 minutes after the end of the exposure period. According to the authors, the results showed no change in S100b protein levels after the challenge, whereas a significant increase in the concentration of TTR was observed right after the challenge (median values: 0.235 g/L versus 0.230 g/L before the challenge). However, there seemed to be an effect of sample storage conditions or an effect linked to the environment of the volunteer at the time of blood sampling. Furthermore, this variation was smaller than the coefficient of variation indicated by the authors: 5.5% for a concentration of 0.28 g/L. Significant differences in the concentrations right before and right after the challenge tests were also observed, and these differences alone may account for these results. These observations tend to modify the drawn conclusions.

Söderqvist et al. [23] analysed concentrations of TTR protein as a function of the recent use (in the last 10 to 400 minutes) of a mobile telephone or cordless telephone (DECT) in volunteers. The abstract of the article states that only 314 of the 1000 individuals recruited by telephone actually participated. This gives a low participation rate, of only 31.4%, entailing a risk of selection bias. The sample included more women than men and mean age was higher among the participants than among the non-participants. This sample appears to be non-uniform in terms of one of the parameters measured: the TTR concentration. Indeed, physiologically, men have a higher TTR concentration than women and individuals over the age of 47 have a higher concentration than younger people. TTR concentration is also higher in smokers than in non-smokers.

These results showed that S100b protein concentration is not affected by past or recent telephone use. The authors reported a significant variation in TTR concentration during the day. They suggested that high rates in men are associated with the use of mobile phones for a number of years and that the recent increase in mobile telephone use is leading to an increase in TTR concentration in women. However, these results do not appear to be statistically significant. Therefore a study validating these plasma parameters as criteria for BBB integrity appears to be necessary. The authors pointed out that more specific markers for the brain should be used. The TTR concentration in cerebrospinal fluid (CSF) should ideally also be analysed, but this is not possible for evident ethical reasons. The results of these studies are limited by all these biases.

5. In summary

This summary of effects on the BBB is based on 16 research articles, including:

- Three studies *in vitro*:
  Two of these studies [8,9] showed no effect of semi-chronic exposure to GSM or UMTS electromagnetic radiation for SAR values from 0.02 to 1.64 W/kg with validated dosimetry, but with incomplete dosimetry in one case.
  The third study [10] reported effects of RF at 915 MHz with modulation and an unusual exposure system, but without dosimetry to determine the SAR.
- Two studies in humans:
  Two studies have reported weak variation of circulating protein concentrations in humans, but the methodologies of these methods include several major flaws, particularly as concerns the choice of parameter, making it impossible to interpretate the results. Indeed, the parameter used has not been validated, varies between individuals and is determined in the blood rather than in the CSF [22,23].
- Eleven studies *in vivo*:
  Effects of exposure to RF on the permeability of the BBB and/or neuronal integrity have been sought *in vivo*, in conditions of acute, semi-chronic or chronic exposure, with a large range of SAR, extending from 0.0018 to 20 W/kg for GSM 900 or TDMA at 1439 MHz or 2450 MHz (continuous and modulated) signals.

Two of these studies reported effects. These two studies described heterogeneous effects of acute exposure to GSM 900 MHz, for a range of very low SAR values (0.12 to 130 mW/kg) [18,21]. There were flaws in the biological and physical aspects of the methodology used, making it impossible to validate the results.

Nine studies reported no effects. One study by the team of Salford reported an absence of effect of chronic exposure to a GSM 900 MHz signal, at SAR of 0.6 and 60 mW/kg [11]. The dosimetry was not described.

In two other studies, the experimental dosimetry was carried out correctly, but no mention was made of numerical simulations [19,20].

The other six studies included validated dosimetry (numerical and experimental): one for acute exposure to a modulated GSM signal at 900 MHz [17] and the other five for semi-chronic exposure to a modulated 2450 MHz signal [12], a TDMA signal at 1439 MHz [16] or at 900 MHz [13–15].

Four of these *in vivo* studies focused on early stages of development. Three studies (semi-chronic exposure) showed no change in the BBB in neonatal rats exposed to a TDMA signal at 1439 MHz (0.2 and 6 W/kg) [16], in gestating mouse foetuses exposed to a GSM 900 signal (4 W/kg) [13,14] and young rats exposed to the same frequency (0.3 and 3 W/kg) [15]. In this last study, an improvement in performance in cognitive tests was even observed after exposure.

Among these *in vivo* studies, three – in the United States, France and Japan [17,19,20] – tried to replicate the results obtained by the team of Salford et al. (900–915 MHz). These rigorous studies, carried out blind, did not confirm the results of the Swedish team, but did highlight a series of methodological biases that might account for them.

Note that, depending on the experiment, the team of Salford found different effects on BBB permeability or neuronal degeneration, for various SAR values in different experiments. These effects were, in some cases, greater for lower SAR values, but, in others, no effect of RF was observed. These results and those obtained by these authors in previous years appear contradictory. It would be a good idea for these authors to attempt to replicate these experiments in another laboratory or to allow an external team to repeat them on site, to resolve this question. It does not appear necessary to perform additional replication studies in other independent laboratories.

6. Conclusion

This review focuses on 16 research articles: three studies *in vitro*, 11 *in vivo* and two in humans. The methodological quality of these studies is highly heterogeneous. On detailed analysis, as far as their methodology is concerned, the studies carried out since 2005 provide no convincing proof of an effect of low level radiofrequency exposure on the integrity of the BBB. Instead, the studies with correct methodology tend to suggest that there is no effect for SAR of up to 6 W/kg, and even beyond (up to 20 W/kg in acute exposure conditions), on the biological models used.

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