8 NEURODEGENERATIVE DISEASES

The possibility that exposure to RF EMF under the threshold of thermal effects potentially could affect neuropathological conditions such as Alzheimer disease (AD), Parkinson disease (PD), Amyotrophic Lateral Sclerosis (ALS) and Huntington disease (HD) is analysed in this chapter.

AD and PD are the most common neurodegenerative diseases, characterized by progressive loss of neurons in the cortex and hippocampus, and in dopaminergic neurons in the substantia nigra, respectively (McKhann et al., 1984; Pollanen, Dickson & Bergeron, 1993). Degeneration of the motor neurons in the spinal cord, motor cortex, and brainstem characterizes the ALS neurodegenerative disorder, whereas HD implies selective neuronal cell death in the striatum and cortex (Boillee, Vande Velde & Cleveland, 2006; Klepac et al., 2007; Sorolla et al., 2008; Tasset et al., 2012).

Despite the area of the brain affected, all these neurodegenerative diseases are characterized by oxidative damage as a key mediator in the onset, progression and pathogenesis, although with different and specific molecular determinants, often associated with an impairment in the folding, processing and ubiquitination of proteins and with specific genetic mutations (Bowling & Beal, 1995; Jellinger, 2009; Pal et al., 2014; Rao & Balachandran, 2002).

Moreover, there is also interest to investigate the possible effects of RF EMF exposure on neurodevelopment and behaviour related to general cognitive impairment that are not directly classified in the above mentioned pathologies. Breakdown of the normal blood-brain barrier with influx of blood-born molecules (plasma proteins) has been suggested to cause local damage as starting mechanisms of some neurodegenerative diseases; neuronal degeneration, associated to albumin leaks, is seen in areas with BBB disruption in several circumstances (Jellinger, 2010; Nittby et al., 2008).

8.1 Epidemiological studies

No studies on potential effects of radiofrequency fields on neurodegenerative diseases were available in 1993 when the previous WHO Environmental Health Criteria document was published. Since then, only one study, presented in two publications, has investigated effects on neurodegenerative diseases, as described below.

A Danish cohort study of neurological diseases included the 420 095 persons who started a mobile phone subscription from 1982 to 1995 (Schüz et al., 2009). The cohort was followed in the hospital discharge registry for identification of occurrence of migraine, vertigo, Alzheimer disease, vascular dementia, other dementia, Parkinson disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and epilepsy. (The Danish cohort is described in more detail in the Section 12.1.2 Mobile phone use). The Danish hospital discharge registry includes information on all hospitalizations from 1977, and from 1994 also outpatient hospital visits. For each disease, cohort members were followed from the date of their first subscription until their first hospital contact with the disease, the end of 2003, emigration or death, whichever occurred first. Standardised hospitalisation ratios (SHR) were calculated assuming a Poisson distribution of disease. The observed number of cases in the cohort was compared to that expected from age, sex and calendar year-specific population rates, where the subscriber cohort had been excluded. The income distribution in the cohort was compared to that in the general population. The majority of cohort members were male (85%) and only a small proportion was older than 60 years. Slightly increased risks of migraine (SHR=1.2; 95% CI 1.1–1.3) and vertigo (SHR=1.1; 95% CI 1.1–1.2) were observed. Risk estimates did not increase with duration since start of subscription. For Alzheimer disease, vascular and other dementia, and Parkinson's disease, slightly reduced risks were found, with the strongest risk reduction close in time to diagnosis, but for Alzheimer's disease and other dementia risk reductions were observed also with $\geq$10 years since first subscription (SHR=0.4; 95% CI 0.1–0.9 and 0.6; 95% CI 0.4–0.9, respectively). A slightly reduced risk was also observed for epilepsy in men. For ALS, MS, and epilepsy in women, no associations were found.

An updated analysis of MS in the Danish cohort was later performed with follow-up from 1987 (when handheld mobile phones became available) to 2004 (Poulsen et al., 2012). The Danish Multiple Sclerosis Registry was used to identify MS cases. In this registry, diagnoses are validated through medical records, and it includes the year and nature of first MS symptom(s). Incidence of MS was not associated with having a mobile phone subscription (incidence rate ratio (IRR)=1.06; 95% CI 0.96–1.18), and no consistent trend of increased risk with increasing time since first subscription was observed. For women, an increased risk estimate was observed among subscribers since $\geq$10 years (IRR=2.08; 95% CI 1.08–4.01), but also among women starting the first subscription <1 year before diagnosis (IRR=1.61; 95% CI 0.93–2.79), with reduced risk estimates in intermediate categories. The type of first symptom differed somewhat between mobile phone subscribers and

non-subscribers, with diplopia and fatigue being more common in subscribers and cerebellar symptoms among non-subscribers.

[The strength of the Danish cohort study is that exposure assessment is made independent of the disease. Limitations are the inability to identify corporate mobile phone users (but this is likely to only have a minor effect on the risk estimates) and that the amount of phone use was not available. Hospital admissions are likely to vastly underestimate the occurrence of migraine and vertigo, and cases captured may potentially be selective. Prodromal symptoms of dementia and Parkinson disease are likely to affect the likelihood that someone starts using a mobile phone, which may explain the reduced risk estimates observed. Confounding from education and socioeconomic status are other potential explanations.]

Table 8.1.1. Cohort study of mobile phone use and neurodegenerative diseases.

Country Time period	Outcome	Exposure	No. obs. cases	Hospitalisation/ incidence rate ratio (95% CI)	Comments	Reference
Denmark 1982-2003	Alzheimer disease	Ever subscriber	81	0.7 (0.6-0.9)	Hospital admissions unlikely to capture all cases of migraine and vertigo, potentially selective. Prodromal symptoms likely explanation for risk reductions. Confounding from education and SES may also contribute to risk reduction.	Schuz et al. (2009)
		1 year	1	0.2 (0.0-1.0)		
		1-4 years	25	0.8 (0.5-1.1)		
		5-9 years	50	0.8 (0.6-1.0)		
		≥10 years	5	0.4 (0.1-0.9)		
	Vascular dementia	Ever subscriber	68	0.7 (0.5-0.9)		
		1 year	2	0.5 (0.1-1.7)		
		1-4 years	19	0.7 (0.4-1.1)		
		5-9 years	34	0.6 (0.4-0.9)		
		≥10 years	13	1.1 (0.6-1.9)		
	Other dementia	Ever subscriber	383	0.7 (0.6-0.8)		
		1 year	21	0.5 (0.3-0.8)		
		1-4 years	131	0.7 (0.5-0.8)		
		5-9 years	198	0.8 (0.7-0.9)		
		≥10 years	33	0.6 (0.4-0.9)		
	Parkinson disease	Ever subscriber	237	0.8 (0.7-0.9)		
		1 year	10	0.5 (0.2-0.9)		
		1-4 years	82	0.8 (0.6-1.0)		
		5-9 years	110	0.8 (0.7-1.0)		
		≥10 years	35	1.1 (0.8-1.5)		
	ALS	Ever subscriber	104	1.0 (0.9-1.3)		
		1 year	11	1.2 (0.9-1.5)		
		1-4 years	42	1.0 (0.9-1.2)		
		5-9 years	44	1.0 (0.9-1.2)		
		≥10 years	7	0.9 (0.6-1.3)		
	MS	Ever subscriber	528	1.0 (0.9-1.1)		
		1 year	61	1.2 (0.9-1.5)		
		1-4 years	222	1.0 (0.9-1.2)		
		5-9 years	220	1.0 (0.9-1.2)		
		≥10 years	25	0.9 (0.6-1.3)		
	Epilepsy, men	Ever subscriber	1767	0.7 (0.7-0.7)		
		1 year	201	0.8 (0.7-0.9)		
		1-4 years	752	0.7 (0.7-0.8)		
		5-9 years	716	0.7 (0.7-0.8)		
		≥10 years	98	0.6 (0.5-0.7)		
	Epilepsy, women	Ever subscriber	337	1.1 (0.9-1.2)		
		1 year	41	1.1 (0.8-1.5)		
		1-4 years	156	1.1 (0.9-1.3)		
		5-9 years	135	1.0 (0.9-1.2)		
		≥10 years	5	0.7 (0.2-1.6)		

	Migraine	Ever subscriber	1401	1.2 (1.1-1.3)		
		1 year	148	1.3 (1.1-1.5)		
		1-4 years	611	1.2 (1.2-1.3)		
		5-9 years	586	1.2 (1.1-1.3)		
		≥10 years	56	1.1 (0.8-1.4)		
	Vertigo	Ever subscriber	2226	1.1 (1.1-1.2)		
		1 year	137	1.1 (0.9-1.3)		
		1-4 years	750	1.1 (1.1-1.2)		
		5-9 years	1148	1.1 (1.1-1.2)		
		≥10 years	191	1.0 (0.9-1.2)		
Denmark 1987-2004	MS	Ever subscriber	406	1.06 (0.96-1.18)	Diplopia and fatigue more common as first symptoms among subscribers	Poulsen et al. (2012)
		<1 year	31	1.09 (0.76-1.56)		
		1-3 years	96	1.05 (0.85-1.29)		
		4-6 years	128	1.08 (0.90-1.29)		
		7-9	117	1.04 (0.86-1.26)		
		≥10 years	34	1.09 (0.77-1.53)		

8.2 Animal studies

The search of the literature since 1992 on this subject provided a total of 11 animal experimental studies, seven of which cannot be included in the overall assessment because of a total or partial lack of proper exposure level assessment as well as of numerical and experimental dosimetry. The WHO (1993) report included about 15 *in vivo* studies on effects of RF on the nervous system, related to EEG measurements, Ca^{2+} mobility, choline uptake and interactions with neuroactive drugs, but no papers specifically dealt with neurodegenerative diseases.

A research group from Lund University has been actively investigating the effects of exposure to low-level 915 MHz fields on the integrity of the blood-brain barrier for many years. Some of these studies also included the analysis of so-called 'dark neurons', neurons that were darkly stained in the cresyl violet staining procedure used by these investigators. The dark neurons were considered to be dying, and thus indicative of neuronal damage. However, several of these publications suffered from a lack of adequate description of experimental data, including dosimetry, and are therefore listed under 'Studies not include in the analysis'. The effects on the blood-brain barrier have been more elaborately described in Section 5.3.3 Blood-brain barrier integrity.

Overall, this series of studies from Lund University provide some provocative and intriguing data, but despite regularly reporting field-related changes, they fail to provide compelling evidence for a consistent effect on blood-brain barrier function, largely because of omissions or unanswered questions regarding methodology or analysis. Nevertheless, the potential importance of these results prompted three independent attempts to replicate the key findings, two of which also assessed the occurrence of dark neurons. These investigations used the same strain of rat, similar exposure parameters, and two used the same type of exposure system as used previously. They also avoided some of the technical limitations in the original studies, which included using rats of both sexes and widely different ages, and poorly characterized dosimetry. In addition, the new studies habituated their animals to their exposure systems to reduce any effects of stress associated with the exposure condition.

Eberhardt et al. (2008) from the Lund group reported that exposure of Fisher 344 rats (exposed: n=8 per group; sham exposed: n=16) to a 915 MHz GSM signal for 2 h at whole body SARs of 0.12–120 mW/kg was associated with an increased albumin extravasation (indicating breakdown of the blood-brain barrier) measured 14 days after exposure (p=0.02) without an increase in dark neurons, while at 28 days after exposure no increased albumin extravasation was measured, but there was an increased occurrence of darkly stained neurons (p=0.02). There was an indication of an inverse dose-response relationship, although no explanation was offered for this result. [The quantification of the pathological effects in terms of numbers of dark neurons was very subjective and the numbers of brain slices scored per animal were not given. Also large weight variation of the animals (164–446 g) should be noted. This study is also discussed in Section 5.3.3 (Blood-brain barrier).]

A replication of the Lund studies was carried out by Masuda et al. (2009). They aimed to determine, using improved staining techniques, whether albumin leakage and dark neurons were present in rat brains 14 and 50 days after a single 2-h exposure to a 915 MHz EMF. Groups of 8 male Fisher 344 rats (12 weeks old) were exposed at an whole-body SAR of 0, 0.02, 0.2 or 2 W/kg in a TEM cell following the same protocol as the Lund studies. In this study the dose received by each rat was assessed in real time during the experiment through the power balance method. The SAR data showed rather large variations, mainly due to movement of the animal within the plastic holder used for the exposures. Dark neurons were rarely present, with no statistically significant difference between exposed and sham-exposed animals. Positive controls (injection of kainic acid (10 mg/kg) or cold injury) resulted in significant and large effects in terms of numbers of dark neurons. [This study is also discussed in Section 5.3.3 (Blood-brain barrier).]

A further replication study of the Lund studies was performed by Poulletier de Gannes and co-workers (2009). They exposed 12-weeks old male Fisher 344 rats in groups of 8 at SAR levels averaged over the brain of 0.14 and 2.0 W/kg. Sham and cage-control animals were included, as well as positive control groups (n=10). The evaluation of dark neurons was assessed by cresyl violet staining (as in the Lund studies) and Fluoro-Jade B staining, which is more specific for degenerating neurons. DNA fragmentation indicative of apoptosis was detected in neurons in situ by the commercial NeuroTACS II kit based on the TUNEL (terminal deoxynucleotidyl transferase d-UTP nick-end-labelling) assay. The animals were restrained in order to allow local exposure of the brain, which was carried out using an exposure apparatus consisting of a printed loop antenna, so this differs from the Lund studies. The full dosimetry of this study was published in a previous paper (Leveque et al., 2004), that included a comparative analysis of human and rat brain exposure. The results were collected at 14 and 50 days after exposure. No degenerating neurons were detected after RF exposure; they only appeared in the positive controls. No apoptotic neurons were detected in any region of the brain with any RF exposure condition. [This study thus clearly failed to replicate the results of the Lund studies and highlighted the limitation of using cresyl violet as a marker for neuronal degeneration. Although the exposure conditions (restrained) differ from those of the Lund group (whole-body exposure in a TEM cell), the dosimetry in this study is more carefully performed and the highest exposure level was 10 times higher than the maximum level in the Lund studies. This study is also discussed in Section 5.3.3 (Blood-brain barrier).]

Studies not included in the analysis

Salford et al. (2003) investigated, in 12–26 weeks old Fisher 344 rats, whether 2 h exposure to a GSM mobile phone signal (915 MHz) can trigger leakage across the blood-brain barrier and in turn cause damage to neurons. Animals were exposed in a transverse electromagnetic transmission line (TEM) cell, at whole-body SARs of 0.002, 0.02 or 0.2 W/kg. Measurement of albumin extravasation and neuron staining were performed at 50 days after exposure. Scattered and grouped, often shrunken, dark neurons were observed. Some of these were also albumin positive or showed cytoplasmic microvacuoles indicating an active pathologic process. Changed neurons were seen in all locations, but especially in the cortex, hippocampus, and basal ganglia, interspersed among normal neurons. The occurrence of dark neurons at the two highest SAR levels was significantly increased ($p=0.01$ and 0.03). [There are a number of caveats with this study. These include wide age range of the rats used (12–26 weeks) and insufficient descriptions of the experimental procedures, exposure protocols and dosimetry in the TEM cells used. The exposure conditions were defined only through numerical dosimetry. The dosimetry is not described in Salford et al. (2003), but in Martens et al. (1993). However, SAR variations due to animal size, position and age were not dosimetrically analysed. The quantification of the pathological effects in terms of numbers of dark neurons was very subjective and the numbers of brain slices scored per animal were not given.]

To complement the previous studies of the Lund group, Nittby et al. (2009) examined the effects of exposure to GSM signals on the blood-brain barrier after an interval of 7 days. Groups of 8 (exposed) or 16 (sham) Fisher 344 rats were exposed to 915 MHz GSM signals at whole body SARs of 0.12–120 mW/kg and albumin extravasation and the occurrence of darkly stained neurons were assessed. The occurrence of darkly stained neurons was stated not to be increased. [No data are given in the paper to interpret these results.]

Banaceur et al. (2013) investigated the effect of 2400 MHz WiFi exposure in wildtype and 3XTg mice that are triple transgenic for three mutant human genes associated with AD. The mice were exposed for 2 h per day for 4 weeks. The average whole-body SAR was reported to be 1.6 W/kg. Several behavioural tests were performed. The Flex field test showed no differences between the sham controls and the exposed mice. In the Barnes maze test, the exposed 3XTg mice tended to escape faster in the maze than the sham-exposed ones ($p<0.05$), while no difference was observed in wildtype mice. In the two-compartment box test, the exposed 3XTg mice showed a reduced latency time to exit the dark box ($p<0.05$) and tended to decrease the number of

entries in the dark boxes (p>0.05). This globally indicated that the exposed 3XTg mice became less anxious. [Although whole-body SAR values are given, it is not clear how these values have been determined. No numerical and experimental dosimetric information is reported.]

Arendash and colleagues published several papers on studies using mice transgenic for APPsw (amyloid precursor protein) and PS1 (presenilin), both resulting in increased amyloid beta (Aβ) deposition in brain tissue, a hallmark of AD development. In the first paper (Arendash et al., 2010) they reported that long-term (7–9 months) exposure to a 918 MHz GSM-type signal for 1 h/day (SAR = 0.25 W/kg) protected adolescent/young adult transgenic mice from later cognitive impairment, reversed cognitive impairment and AD-like brain pathology in older transgenic mice, and increased cognitive performance of normal (non-transgenic) mice, thus indicating a potential noninvasive, non-pharmacological therapeutic strategy against AD. Interestingly, both cognitive-protective and cognitive-enhancing changes were associated with reduced brain Aβ deposition and increased cerebral blood flow, without increasing oxidative stress in the brain. Similar observations were made in young adult (2 months) and aged adult (15 months) animals.

In the second study (Arendash et al., 2012), they extended their investigations to very old (21–26 month) mice, bearing much heavier brain Aβ levels than the same animals used in their first publication. The exposure was for 2 months or 12 days for 2 h per day at whole-body SARs of 0.25 or 1.05 W/kg. In these aged mice, with advanced Aβ pathology, the findings after long term RF exposure further indicated a profound ability to reverse brain Aβ deposition, to induce changes in the regional cerebral blood flow, and to provide selected cognitive benefits, all without induction of brain hyperthermia and without increase in brain oxidative stress. The old transgenic mice and their normal littermates displayed an increased memory function in the Y-maze task, with regional cerebral blood flow in cerebral cortex reduced in both transgenic and normal mice after 2 months of EMF treatment. Brain temperature, assessed during the 2-month treatment, as well as in a separate group of transgenic mice during a 12-day treatment period, underwent no appreciable increases, whereas body temperature displayed no or only a slight increase during the EMF "ON" periods. [In both studies the assessment of the SAR levels is not clear. In the second study, the authors mention E-field levels from which they calculated the SARs, but where the field levels were measured is not stated. The animals were free roaming, so potentially there is a considerable variation in exposure levels.]

The same group (Dragicevic et al., 2011) observed reduced mitochondrial ROS generation and enhanced mitochondrial membrane potential in both cerebral cortex and hippocampus, but not in the striatum or amygdala, in RF-EMF exposed AD transgenic mice. In this study exposure was for 1 h per day during 1 month at the same SAR levels as used in Arendash et al. (2012). These findings are in contrast with what is stated in the other two publications (Arendash et al., 2010; Arendash et al., 2012), where they reported no change in the indices of brain oxidative stress. [Further studies, with a well-controlled dosimetric assessment, are needed to corroborate these findings, to elucidate the biological mechanism and to validate the therapeutic effect of RF fields, if any. In these papers SAR values are wrongly obtained by multiplying the external electric field with an average conductivity value. The calculation should use the internal electric field and the specific tissue conductivity and permittivity. Without a rigorous dosimetry the real delivered dose within the mice remains unknown.]

Seaman and Phelix (2005) investigated the effects of pulsed RF EMF and 3-Nitropropionic Acid (NP) on neuronal ultrastructure in the rat caudate putamen. Restrained Sprague Dawley rats (16–24 weeks old) were exposed to a 10 Hz pulsed 1.25 GHz field for 30 min per day for two days, 1.5 h following 3-NP injection. The whole-body SAR levels were 0, 0.6 and 6 W/kg. After exposure at 0.6 W/kg the effect of 3-NP on neurons was reduced. [For dosimetry the authors refer to an unpublished technical report. However, in that report the dosimetry of a CW sinusoidal signal at 1.2 GHz is provided. The animals in Seaman and Phelix (2005) were exposed to pulsed signals with a main frequency of 1.2 GHz and a wide frequency content. It is not appropriate to use the single frequency dosimetry for the exposures conditions reported in Seaman and Phelix (2005).]

Aldad et al. (2012) investigated the effects of RF EMF exposure on neurodevelopment and behaviour in prenatally exposed CD1 mice. Each experimental cage was equipped with a muted and silenced 800–1900 MHz cellular phone emitting continuously for 17 days during gestation. Memory deficit as well as decreased anxiety were observed in pups from exposed females. [The use of a cellular phone on top of the cage is an unacceptable exposure condition. It does not guarantee any control of the emitting power, and hence of the exposure of the animals. Therefore this study cannot be interpreted.]

Table 8.2.1 Animal studies on neurodegenerative diseases.

Endpoint, animals, number per group, age or weight at start	Exposure: source, schedule, level, freely moving or restrained	Response	Comments	Reference
Staining for endogenous albumin in brain by immunohistochemistry, dark neurons by cresyl violet 14 or 28 days after exposure Rat: Fischer 344 (n=8 (exposed) or 16 (sham)) 164–446 g	GSM 915 MHz in TEM cell 2 h WBA SAR 0.12–120 mW/kg Free	Increase in albumin extravasation after 14 days, but not after 28 days; increase in dark neurons only after 28 days.	No clear dose-response, lower SARs tended to give larger responses. Subjective quantification of dark neurons. Numbers of brain slices scored per animal not given. Also discussed in Section 5.3.3 (Blood-brain barrier).	Eberhardt et al. (2008)
Staining for endogenous albumin by IHC, dark neurons by cresyl violet, haematoxylin and eosin in brain 14 or 50 days after exposure Rat: male Fisher 344 (n=8) 12 weeks	GSM 915 MHz in TEM cell, 900 MHz GSM 2 h WBA SAR 0.02, 0.2, 2.0 W/kg Free	No effect.	Injection of kainic acid (10 mg/kg) or cold injury (positive controls) caused large effects. Also discussed in Section 5.3.3 (Blood-brain barrier).	Masuda et al. (2009)
Staining for endogenous albumin by IHC, dark neurons by cresyl violet, Fluoro-Jade B, apoptosis by NeuroTACS II in brain 14 or 50 days after exposure Rat: male Fischer 344 (n=8 or 10) 14 weeks	Pulsed 915 MHz GSM signal using head-only, loop-antenna system 2 h Brain-averaged SAR 0.15, 2.0 W/kg Restrained	No effect.	Acute cold injury and TACS-Nuclease (positive controls) caused large effects. Also discussed in Section 5.3.3 (Blood-brain barrier).	Poulletier de Gannes et al. (2009)

Abbreviations: GSM: Global System for Mobile Communication;TEM: transverse electromagnetic transmission line; WBA SAR: whole-body SAR

205

206 ### *8.3 In vitro studies*

207 Two types of glial cells, the astroglial and the microglial cells, are particularly interesting in the
208 context of biological effects of RF EMFs related to neurodegenerative disorders, because they are directly
209 involved in the response to brain damage as well as in the development of brain cancer. In particular, microglial
210 cells act as the primary immune effector cells in the brain, and play a pivotal role in the neuroinflammatory
211 processes which are critical components of neurodegenerative diseases.

212 In the previous WHO monograph (WHO, 1993), no in vitro studies on biological effects of RF EMFs
213 correlated with neurodegenerative disorders have been reported. In the present literature search, only six articles
214 have been identified on this topic. Two studies do not comply with the quality criteria for inclusion in the
215 analysis due to methodological issues related to the description of the exposure systems and dosimetry and are
216 only presented in the text. Unless specifically mentioned, papers did not report on blinding of the investigators to
217 the exposure conditions.

218 Thorlin et al. (2006) exposed astroglial enriched cell cultures (primary astroglial cultures made from
219 new-born rat cerebral cortex, comprising at least 70% astroglial cells) to a 900 MHz GSM signal (SAR = 3
220 W/kg) for 4, 8 or 24 h, or to CW (SAR = 27 W/kg) for 24 h to evaluate the morphology and the release of the
221 pro-inflammatory cytokines interleukin-6 (IL6) and tumour necrosis factor-alpha (TNFα). After blind exposures,
222 no alterations in the examined targets were detected, as assessed by comparing RF-exposed and sham-exposed

samples. In a second set of experiments, astroglial enriched cell cultures were exposed to a 900 MHz CW signal for 4 or 24 h at SARs of 27 and at 54 W/kg. No differences in the levels of the astroglial cell-specific reactive marker glial fibrillary acidic protein (Gfap) were found in exposed samples compared to sham exposed ones. Moreover, the authors also exposed microglial cell cultures (purified from astroglial-enriched cultures, containing more than 90% microglial cells) to GSM-modulated 900 MHz (SAR = 3 W/kg) for 8 h and no effects in the microglial reactivity marker ED-1 (a macrophage activation antigen) were detected. Between three and eight independent experiments were performed for the different exposure conditions investigated. Cell cultures incubated at 38 or 42 °C were used as positive controls and gave positive findings. [This study has also been described in sections 10.3 and 12.3.2.3, where the results on immune system and cytokine expression are reported. Despite of the high SAR values applied in this study, thermal effects can be excluded due to the use of a water cooling system. Temperature was monitored through the RF EMF exposure by means of a fiber optic thermometer and temperature variation was maintained within ± 0.2 °C].

Del Vecchio et al. (2009) found that up to 144 h exposure to a 900 MHz GSM signal (SAR = 1 W/kg) did not change the response of SN56 neural cells and rat primary cultured neurons to glutamate toxicity (which is a final molecular mechanism in many neurodegenerative diseases) and to β-amyloid toxicity (a major toxic event in Alzheimer's disease), compared to sham exposed cultures. Two to four independent experiments with three replicates were carried out blinded. Treatments with glutamate or β-amyloid also served as positive controls. [This study is also reported in Sections 12.3.5 (Oxidative stress) and 12.3.6 (Cell proliferation).]

Hirose et al. (2010) exposed primary microglial cell cultures prepared from neonatal rats to 1950 MHz W-CDMA (SAR = 0.2, 0.8 or 2.0 W/kg) for 2 h. Assay samples obtained 24 and 72 h after exposure were processed in a blind manner. In three experiments they found that the percentage of cells positive for major histocompatibility complex (MHC) class II (the most common marker for activated microglial cells) was similar between RF- and sham-exposed cells. Further, no statistically significant differences in the production of TNFα, interleukin-1β (IL1β), and interleukin-6 (IL6) were detected. Treatments with lipopolysaccharide or interferon-γ as positive controls gave positive findings. [The SAR distribution in the exposed samples was quite inhomogeneous, and a 0.7 °C temperature increase occurred during 2 W/kg exposure. This study has also been reported in Section 10.4 (Immune system and haematology).]

Terro et al. (2012) exposed cultured primary cerebral cortical cells from embryonic Wistar rats to a 900 MHz GSM signal (SAR = 0.25 W/kg) for 24 h. In three independent experiments, by comparing RF exposed samples to sham exposed ones, they found that protein expression of α-synuclein (involved in Parkinson's disease, and substrate for chaperone-mediated autophagy (CMA), a cell defence response against stress) was decreased by ~24% ($p<0.01$) independently of CMA, whereas the expression of two other proteins involved in CMA, the heat shock cognate 70 (HSC70) and heat shock protein 90 (HSP90), were increased by ~26% ($p<0.01$) and decreased by ~10% ($p<0.05$), respectively. Serum-deprived cultures were used as positive controls and gave positive results. However, similar changes were detected in cell cultures subjected to 0.5 °C temperature increase in absence of RF EMF. [Since the temperature within the RF-exposed cultures increased by ~0.5 °C during the 24-h exposure period relative to the sham controls, the observed changes on protein expression are likely due to temperature elevations in the RF-exposed samples, as also concluded by the authors. This paper has also been reported in sections 12.3.3 (Gene and protein expression) and 12.3.4 (Apoptosis).]

Studies not included in the analysis

Hao et al. (2010) exposed murine N9 microglial cells to 2.45 GHz (SAR = 6.2 ± 1.5 W/kg, calculated at the bottom of the flask) for 20 min, and, compared to sham exposed samples, found an increase of STAT3 phosphorylation at 1, 6 and 12 h after exposure and in STAT3 DNA-binding ability (a specific DNA-protein complex appeared after 1 h and remained after 12 h after exposure). Phosphorylation of Janus Tyrosine kinase 1 and 2 (JAK1 and JAK2; both of them are able to phosphorylate STAT3) also increased. The phosphorylation of JAK1 increased at 1 h after exposure, and then returned to normal levels at 6 h and 12 h. The phosphorylation of JAK2 increased at 1, 6 and 12 h after EMF exposure, with similar kinetics as phosphorylated STAT3. In addition, after RF EMF exposure an increase was observed in the transcription levels of the inflammation-associated genes inducible nitric oxide synthase (iNOS) and TNFα, which contain STAT-binding elements in their promoter region, and an increase in nitric oxide (NO) release. When pyridone 6, a JAK inhibitor, was administered in addition to the RF EMF exposure, lower increases were seen in iNOS and TNFα, nuclear factor binding activity, activation of STAT3 and NO release. [This study has also been described in Section 12.3.2 (Intracellular and intercellular signalling).]

In a related study by the same group, Yang et al. (2010) investigated additional time-points and inhibitor conditions (three independent experiments). N9 microglial cells were exposed to RF EMF for 20 min at a SAR of 6 W/kg, then assessed at 1–24 h thereafter. The authors reported increased p-STAT3 immunoreactivity following RF exposure compared to sham exposed samples, when imaged by confocal microscopy ($p<0.01$). Western blot analysis demonstrated increased levels of both p-STAT3 and p-JAK2 at times ranging from 1–24 h after RF exposure ($p<0.01$), while p-JAK1 was only increased in expression at 1 h after exposure. [The results of these studies cannot be interpreted since the description of the exposure system and dosimetry is not adequate. Moreover, in both studies, the cultures were exposed to a relatively high RF field intensity of ~6 W/kg. Since culture temperatures were not monitored during or after RF exposure and SAR heterogeneity was not assessed within the culture flasks, thermal confounding in these studies cannot be excluded. Moreover, The latter study has also been described in Section 12.3.2 (Intracellular and intercellular signalling).]

Table 8.3.1. In vitro studies assessing effects of RF EMF exposure related with neurodegenerative disorders.

Cell type Number of independent experiments	Biological endpoint	Exposure conditions	Results	Comment	Reference
Primary cultures of rat astroglial and microglial cells n=3–8	Morphology and markers for damage-related processes: IL6, TNFα, Gfap, ED-1, total protein	900 MHz, GSM Average SAR 3 W/kg 4, 8 or 24 h 900 MHz, CW SAR 27 or 54 W/kg 4 or 24 h	No effects.	For immune system and cytokine expression see Section 10.3 and 12.3.2.3.	Thorlin et al. (2006)
Mouse SN56 neural cells Primary cultures of cortical neurons n=2–4	Glutamate toxicity β-amyloid toxicity	900 MHz, GSM Average SAR 1 W/kg up to 144 h	No effect.	For oxidative stress and cell proliferation see Sections12.3.5 and 12.3.6.	Del Vecchio et al. (2009)
Primary cultures of rat microglial cells n=3	Microglia activation	1950 MHz, W-CDMA SAR 0.2–2.0 W/kg 2 h	No effect.	Temperature increase at 2 W/kg. For immune system see Section 10.4.	Hirose et al. (2010)
Cultured primary cerebral cortical cells from embryonic Wistar rats n=3	α-synuclein; HSC70, HSP90	900 MHz, GSM Average SAR 0.25 W/kg 24 h	Increase in HSC70; decrease in HSP90; decrease in α-synuclein.	Thermal effect. For protein expression and apoptosis see Section 12.3.3 and 12.3.4. No information on blinding of the staff.	Terro et al. (2012)

"No effect" means no statistically significant effect.

Abbreviations: CW: continuous wave; Gfap: glial fibrillary acidic protein; GSM: Global System for Mobile Communication; Hsc: heat shock cognate; Hsp: heat shock ptotein; IL: interleukin; SAR: specific absorption rate; TNFα: tumor necrosis factor α; W-CDMA: Wideband Code Division Multiple Access.

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